



# Comparing the performance of a High Rate Algal Pond with a Waste Stabilisation Pond in rural South Australia

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**Early Sanitation:- Latrines inside Housesteads Fort, Hadrians Wall. Northumberland, UK - circa 124 A.D.**

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# ABSTRACT

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This study compares the performance of two natural wastewater treatment systems; waste stabilisation ponds (WSP) and High Rate Algal Ponds (HRAP) in rural South Australia. The systems were located in similar geographic and climatic zones, East North East of Adelaide.

The WSP treated the domestic wastewater from the township of Lyndoch, with an approximate population of 1,750 inhabitants, and daily treatment plant influent of 165 kL. The HRAP treated domestic wastewater from the smaller township of Kingston-on-Murray, with an approximate population of 140 producing daily treatment plant influent of 12 kL. All households in both townships had domestic septic tanks connected to a reticulation system to harvest their overflow to a central sump and pump station that pumped to the treatment plant. The WSP treatment plant was a three cell system with gravity feed between ponds, and a theoretical hydraulic retention time of 36 days in pond 1 and 15 days each in pond 2 and 3, for a total of 66 days. This system was observed over a period of two years. The HRAP was a single raceway 30 m x 5 m with adjustable depth settings. The HRAP was run at 0.32 m, ( $\theta=4.7$  d), 0.42 m ( $\theta=6.6$  d) and 0.55 m ( $\theta=9.2$  d). The depth setting was altered regularly to encompass observation periods in all seasons at all depths. This system was observed for a year. A second period of 9 months of HRAP observations was made in a similar manner, this time using wastewater that had already spent approximately 36 days in a facultative pond.

Parameters measured at both sites in all ponds were:-

- Continuously logged water temperature, dissolved oxygen and pH
- Continuously logged weather data – temperature, wind speed & direction, total solar radiation, UV radiation, rainfall.
- Water samples collected at regular intervals from inlets and all ponds and returned to the laboratory for estimations of the following:-
  - *E. coli* enumeration



- Chlorophyll a
- Suspended solids
- Turbidity
- BOD<sub>5</sub>
- Nutrients:– NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, PO<sub>4</sub>-P

The results were analysed to compare both the disinfection performance of the two systems and the relative ability to remove nutrients. A comparison was also made of the algal productivity of the two systems.

A mathematical model to predict the *E. coli* concentration in the HRAP effluent was constructed and the model outputs were compared with eight separate periods of intensive observation of *E. coli* numbers over periods of two to five days at a time. There was good correlation between model output and *E. coli* concentration observations.

The study answered in the affirmative the question of whether a High Rate Algal Pond system could replace a Waste Stabilisation Pond system in rural South Australia. It also offers clear advice on the design and operation of a High Rate Algal Pond system in rural South Australia.



# DECLARATION

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I, Alan Neil Buchanan certify that this work contains no material which has been accepted for the award of any other degree or diploma in any University or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Signed

# ACKNOWLEDGEMENTS

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This project is dedicated to my grandchildren and all of their generation. It is the only way I know to contribute to their future well-being in a world reluctant to acknowledge the fast approaching end of boundless resources – primary amongst those, water and energy. Only my family really understand the personal sacrifices needed to complete this task. Their unquestioning support remains critical to the mission.

Many people contribute over the extended journey that is the candidature involved in the creation of a thesis of this size. Primary amongst those are the two academic supervisors, Professors Howard Fallowfield and Nancy Cromar. Their enthusiasm for the task was palpable and infectious and advisory in good measure. They jointly and gently taught me the process of research, knowledge of which I have hankered after for many decades now. Remarkably, they eased me past the University bureaucracy. When I first arrived, I still carried the scars of too much ‘other’ bureaucracy, and they have helped the slow healing with their gentle good humour. Their years of ‘hands-on’ experience cannot be overrated as a resource when taking on a project of this complexity.

Dr Simon Williams provided all the mathematical grunt to distill complex biological activity into a series of formulas able to accurately predict outcomes.

The project arose from a collaboration between the Local Government Association (LGA) of South Australia and Flinders University. Within the LGA, Rick Gayler played an outstanding role as project champion and keeper of the funds. He almost single-handedly kept the project afloat as many initial capital intensive changes had to be made. He really believes in what we are trying to achieve and that makes all the difference.

Michael Clark, wastewater treatment manager for Barossa Council also deserves special mention for his 'freeing-up' of access to the Lyndoch waste stabilisation ponds for a number of years as we deployed various instruments in and around them. His long experience in the wastewater treatment industry meant he was always good for practical advice. We could always count on Michael's help when required, otherwise he stayed in the background and left us to get on with the job. Loxton-Waikerie Council also contributed greatly with their willingness to let us get on with the job, and to offer practical assistance with pumping on the occasions that the site flooded. It is my fervent hope that good use will be made of the treated wastewater available to them.

Coming into a University laboratory as an older student with no particular lab skills creates interesting inter-generational dynamics. To their credit, the younger cohort taught me well and soon treated me as their colleague and peer. I value that greatly. Over the time, a number stood out for their willingness to engage, including (in no particular order), Guaxin Huang, Yu Lian, Lei Mai, Ryan Cheng, Jess Hall, Michael Taylor and in particular, Natalie Bolton, who was always available to discuss techniques, and interpretation of results. Great assistance was also received from the laboratory manager, Raj Indela.

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| EQUATION NUMBER | EQUATION  | PAGE |
|-----------------|---|------|
| 1-33            | $INB = PRD((2.5^T/75) I_z)$   | 79   |
| 1-34            | $NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O$   | 81   |
| 1-35            | $HCO_3^- \leftrightarrow CO_2 + OH^-$   | 82   |
| 1-36            | $\mu = \hat{\mu} \left[ \frac{S}{K_s + S} \right]$  | 83   |
| 1-37            | $I_z = I_0 e^{-kz}$   | 90   |
| 1-38            | $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$   | 95   |
| 1-39            | $k = k_{d,20} \theta^{(T-20)} + \frac{\alpha \cdot I_{0,avg}}{\eta \cdot z_e} [1 - e^{(-\eta z_e)}] + \frac{v}{z_e}$  | 100  |
| 1-40            | $\frac{\alpha \cdot I_{0,avg}}{\eta \cdot z_e} [1 - e^{(-\eta z_e)}] = k_i$   | 100  |
| 1-41            | $C_n H_a O_b N_c + \left( n + \frac{a}{4} - \frac{b}{2} - \frac{3}{4} c \right) O_2$<br>$\rightarrow nCO_2 + \left( \frac{a}{2} - \frac{3}{2} c \right) H_2O + cNH_3$ | 107  |
| 1-42            | $k' C = -\frac{dC}{dt}$   | 107  |
| 1-43            | $k \cdot t = \ln \left( \frac{C_i}{C_e} \right)$  | 109  |
| 1-44            | $C_e = \frac{C_i}{(1 + k \cdot HRT)}$   | 109  |
| 1-45            | $C_e = C_i \cdot e^{-k \cdot HRT}$  | 109  |
| 1-46            | $k = 0.3(1.05)^{T-20}$  | 109  |
| 1-47            | $k = 0.71(1.09)^{T-20}$   | 109  |

| EQUATION<br>NUMBER | EQUATION   | PAGE |
|--------------------|--|------|
| 1-48               | $k = 2.622 \times 10^{-3} \lambda s - 0.194$   | 110  |
| 1-49               | $\lambda s = 350(1.107 - 0.002T)^{T-25}$   | 111  |
| 1-50               | $NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$  | 112  |
| 1-51               | $N_2O_3 + H_2O \leftrightarrow 2H^+ + 2NO_2^-$   | 112  |
| 1-52               | $N_2O_5 + H_2O \leftrightarrow 2H^+ + 2NO_3^-$   | 112  |
| 1-53               | $2NH_3 + 3O_2 \rightarrow 2NO_2^- + 2H^+ + 2H_2O$  | 115  |
| 1-54               | $2NO_2^- + O_2 \rightarrow 2NO_3^-$  | 116  |
| 1-55               | $r_n = \frac{\mu_n}{Y_n} \left( \frac{NH_4}{k_1 + NH_4} \right) \left( \frac{DO}{k_2 + DO} \right) C_T C_{pH}$ | 116  |
| 1-56               | $k_1 = 1^{(0.051(T-1.58))}$  | 116  |
| 1-57               | $C_T = e^{\alpha(T-T_0)}$  | 116  |
| 1-58               | $C_{pH} = 1 - 0.833(7.2 - pH)$   | 117  |
| 1-59               | $r_d = R2_{20} \theta^{(T-20)} NO_3 - N$   | 117  |
| 1-60               | $r_v = \frac{NH_3 \times K_L}{d}$  | 117  |
| 1-61               | $r_s = R1(Org - N)$  | 117  |
| 1-62               | $r_1 = \mu_{max20} \theta^{T-20} \left[ \frac{NH_3 - N}{K_3 + NH_3 - N} \right] (Org - N) \times P1$           | 118  |
| 1-63               | $r_2 = \mu_{max20} \theta^{T-20} \left[ \frac{NO_3 - N}{K_4 + NO_3 - N} \right] (Org - N) \times P2$           | 118  |
| 1-64               | $Na_4P_2O_7 + H_2O \leftrightarrow 2Na_2HPO_4$   | 121  |

| EQUATION<br>NUMBER | EQUATION   | PAGE |
|--------------------|--|------|
| 1-65               | $pH = pK_2 + \log \left( \frac{CO_3^{2-}}{HCO_3} \right)$  | 122  |
| 1-66               | $3HPO_4^{2-} + 5Ca^{2+} + 4OH^-$<br>$\leftrightarrow Ca_5(OH)(PO_4)_3 + 3H_2O$   | 122  |
| 2-1                | $C_a = 11.85(OD664) - 1.54(OD647)$<br>$- 0.08(OD630)$  | 147  |
| 2-2                | $PROD \text{ (mg(dry wt.)/m}^2\text{/h)} = PRD - RES - INB$  | 152  |
| 2-3                | $RES = X_1((1.5^T - 0.54)/100)$  | 153  |
| 2-4                | $INB = PRD((2.5^T/75) I_2)$  | 153  |
| 2-5                | $Pr_{alg} = 10 \times \frac{E_t \times I}{J}$  | 154  |
| 2-6                | $Y = \beta_0 + \beta_1 X + \varepsilon$  | 155  |
| 2-7                | $Y = \beta_0 + \beta^T X + \varepsilon$  | 155  |
| 2-8                | $Y = \beta_0 + \beta^T X + \gamma XX^T + \varepsilon$  | 155  |
| 3-1                | <i>Algal Productivity 1</i><br>$= 3.617 \times \textit{Algal Productivity 2}$<br>$- 2.562$   | 181  |
| 5-1                | Suspended Solids Productivity (g/m <sup>2</sup> /d) = 0.73(5 day<br>avg. Total Solar Radiation)-7.83(THRT)-0.52(NH <sub>4</sub> -<br>N)+78.4 | 285  |











# Chapter 1

## INTRODUCTION AND PROJECT OVERVIEW

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### 1.1 PROJECT AIMS

1. To compare the effluent treatment performance of a Community Waste Management Scheme (CWMS) lagoon with a High Rate Algal Pond (HRAP) at Kingston on Murray, approximately 260 km North East of Adelaide.
2. To determine the optimum operating conditions to maximise HRAP performance.
3. To provide criteria for HRAP design and operation in South Australia.

As the project unfolded, some additional features emerged as by-products of the extensive records kept. These were

- a. analysis of the factors involved in algal productivity in WSPs and HRAPs.

- b. Development of a mathematical model to allow forecasting of *E. coli* LRVs in HRAPs.

## 1.2 BACKGROUND

Waste stabilisation ponds (WSPs) and HRAPs have been operated independently in various parts of the world. Fallowfield and Garrett (1986) performed a desk study using HRAP data from California and WSP data from Israel to compare the performance of mixed and unmixed systems. This demonstrated that the HRAP shallow mixed system offered the potential to significantly reduce area requirements by up to 75% where the minimum monthly treatment temperature was 12°C – similar to that at Kingston on Murray.

The performance of these systems has only been compared side-by-side in the same location treating the same effluent once, by Picot et al. (1992) in Meze in the South of France. This work did not directly compare a WSP system as operated in South Australia with a HRAP as firstly, the HRAP was preceded by a primary facultative pond (1.5 m deep and 8 day retention time) or a clarifier with a 2 hour retention time (removing significant bacterial load); and secondly the influent water in South Australia has always been through household anaerobic septic tanks, which was not the case in France. Thus this case should be viewed more like the second phase of the work reported here, where the HRAP treated effluent delivered from the facultative pond.

The elevated rates of photosynthesis brought about by gentle mixing, which improves light availability for algal photosynthesis, produce supersaturating DO levels and high diurnal shifts in pH with values as high as pH 11 being recorded. This combination, together with greater exposure of the pond volume to UV irradiation, results in the rapid death of indicator organisms such as thermotolerant coliforms and *E. coli* (Curtis et al., 1992). Fallowfield et al, (1996) determined die-off rate constants ( $K_b$ ) for *E. coli* of 0.3 – 10.26 d<sup>-1</sup> in HRAPs operating in Scotland at mean pond temperatures of between 14 and

19°C at surface irradiances of 85 – 356 Wm<sup>-2</sup>, for the treatment of piggery wastewater. These environmental conditions compare with those of HRAPs operated in South Australia where pond temperatures range from 14 - 25°C (Evans et al., 2003). The higher treatment reaction rates result in shorter retention times and consequently reduced area requirements compared to unmixed lagoons such as WSPs. HRAPs have other potential advantages: firstly the reduced surface area may reduce evaporative losses and improves the water balance for irrigation. Secondly, in addition to improving the photosynthetic potential the gentle mixing increases the rate of atmospheric oxygen diffusion into the pond at night compared with unmixed systems, further maintaining an aerobic environment. Thirdly, gentle day time mixing may assist the process of reducing the super-saturation induced by algal photosynthetic effort. Supersaturation with oxygen is known to impede algal metabolism.

### **1.3 RESEARCH HYPOTHESIS**

The hypothesis is that the HRAP will be able to treat domestic wastewater to a similar standard as a conventional WSP in a shorter time and on a significantly lower land area.

### **1.4 Wastewater disposal and local government in South Australia**

CWMS provide wastewater services for approximately 10% of the population of South Australia via 166 individual schemes in 45 Councils. Of these, 90 are WSPs and the balance utilise some form of mechanical treatment. (LGA SA, 2012) The first CWMS lagoon was constructed in Pinnaroo in the Upper South East district in 1962. Schemes vary in size from some for very small shack

settlements with 10 connections to quite large townships with in excess of 4,000 connections. The average size scheme is about 400 connections.

The design for the Community Waste Management System (CWMS) lagoons originates from research conducted by Marais (1974) in the early 1970s on WSP design. These systems are relatively shallow and unmixed and as a consequence experience thermal stratification, where temperature may differ by 12°C between the lagoon surface and bottom (Sweeney et al., 2005). This also results in dissolved oxygen stratification with aerobic and anaerobic conditions occurring at the surface and bottom of the lagoon respectively. These variations in conditions throughout the depth influence the reaction rates of key treatment processes such as nutrient and BOD removal (Sweeney et al., 2007) and pathogen die-off (Sweeney et al., 2003). These systems may also suffer hydraulic short-circuiting, producing retention times shorter than those designed, which may result in insufficient treatment of the effluent (Sweeney et al., 2003).

There is a paucity of data regarding the performance of CWMS lagoons in South Australia. What is available is largely compliance monitoring data from which treatment performance cannot be derived since wastewater input data is not routinely collected.

#### **1.4.1 Funding & Structure of the project – initial plan, modified plan**

The South Australian Local Government Association and Loxton Waikerie Council (LWC), following an approach by Flinders University, agreed to include both a WSP and an HRAP in the new CWMS constructed at Kingston on Murray to enable comparison of the performance of the two systems treating 60 m<sup>3</sup> of effluent per day (Fallowfield and Cromar, pers.com.).

The initial plan was to split the incoming effluent and send half to the WSP and half to the HRAP. However, following construction it soon became apparent that rather than delivering 60 m<sup>3</sup> of effluent per day, the scheme was delivering 12 m<sup>3</sup> of effluent per day. As this was insufficient to run either treatment train

properly, the decision was made to reduce the surface area of the HRAP by 60% and feed that with all the wastewater. The WSP for comparison was found at Lyndoch, approximately 150 km to the East of Kingston-on-Murray but at the same latitude (34°S), treating wastewater for about 1,650 inhabitants versus the 140 inhabitants of Kingston-on-Murray. This change whilst unfortunate did not materially alter the research or the statistical approach to comparing the two systems. A multi-function weather station was installed at each site so a continuous record of important environmental parameters was available, to ensure all comparisons were valid.

## 1.5 LITERATURE REVIEW

### 1.5.1 OVERVIEW OF WASTEWATER MANAGEMENT

Sanitation is a term primarily used to characterize the safe/sound handling and disposal of human excreta as well as other waste products. The relationship between humans and water and sanitation has seen substantial changes through the ages, due to the influences of cultural, social and religious factors. Throughout history wastewater management has presented people and governments with far reaching technical and political challenges. (Lofrano and Brown, 2010)

#### *1.5.1.1 History of wastewater management.*

The first human communities were scattered over wide areas and waste produced by them was returned to land and decomposed using natural cycles.

Disposal problems were limited primarily because the numerically small communities were nomadic hunter–gatherers. A new era started when mankind established permanent settlements about 10,000 years ago, adopting an agrarian way of life. There is archaeological evidence of sewerage systems in the early cities of the Mesopotamian, Indus, ancient Egyptian and Greek civilisations, often conducting waste outside the city walls to be used as farm fertiliser. (Lofrano and Brown, 2010)

The sewage system of ancient Rome was very complex and included the largest, the Cloaca Maxima and many smaller sewers. Cloaca Maxima is now considered one of the oldest monuments of Rome. The Cloaca Maxima, translates from the Latin as the “biggest sewer” in Rome, had enough capacity to serve a city of 1 million people.

Most of these sewers were originally built as drainage canals. Constructed in the sixth century BCE, the Cloaca Maxima ran through the Forum Romanum. Its’ construction is generally attributed to king Tarquinius Priscus. In the second century BCE, the canal was covered, so it became an underground sewer. Even

though the Romans used covered sewers 2,200 years ago throughout their empire, the effluent was merely discharged into the nearest stream, lake or ocean. (Maier et al., 2009) This strategy sufficed whilst populations were low, but had impacts as populations grew.

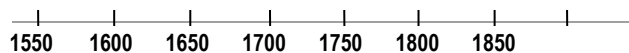
When the Roman Empire collapsed, the sanitary dark ages began and lasted for over a thousand years (approximately 476–1600 AD). During this period, households rarely had sanitary facilities, and the waste disposal practice was relatively simple - empty the chamber pot directly into the street below.

### EARLY WATER AND SANITATION SYSTEMS: 3000 BC to 1850

← Minoan Civilization

- 97 AD--Water Supply Commissioner for City of Rome- Sextus Julius Frontius
- Sewage Farms in Germany
- Sewage Farms in UK
- Legal use of sewers for human waste disposal London 1815; Boston 1833; Paris 1880
- Cholera epidemic in London 1848/9 & 1854
- Sanitary status of Great Britain – Chadwick Report (Rain to the River and Sewage to the Soil)

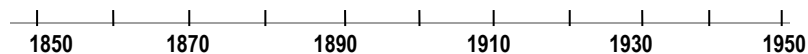
← 3000 BC



### GREAT SANITARY AWAKENING: 1850 - 1950

- Cholera epidemic linked to water pollution by Snow (London)
- Typhoid fever prevention theory developed by Budd (UK)
  - Anthrax – bacterial aetiology demonstrated by Koch (Germany)
  - Microbial pollution of water demonstrated by Pasteur (France)
    - Sodium Hypochlorite disinfection of water by Down (UK)
      - Chlorination of Jersey City water supply (USA)
      - Disinfection kinetics elucidated by Chick (UK)
        - Activated sludge demonstrated –Arden & Locket (UK)
  - Sewage for irrigation regulated in California (USA)

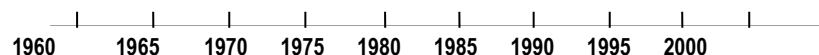
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### ERA OF WASTEWATER RECLAMATION, RECYCLING & REUSE: POST 1960

- Californian legislation to encourage wastewater reuse
- Secondary effluent used for crop irrigation in Israel
  - Research on direct potable reuse in Namibia
  - US Clean Water Act
    - Pomona Virus Study - California
    - WHO Guidelines for Agricultural & Aquacultural Reuse
    - US EPA Guidelines for Water Reuse

←





**Fig. 1-1 Key milestones in sanitary waste disposal and reuse. Adapted from (Asano and Levine, 1996)**

In London, wastewater was collected in cesspits beginning in 1189 and the contents conveyed to the countryside for land application. This was done by “rakers” or “gongfermors” who removed the foul sewage from cesspools and sold it to farmers just outside the city walls (Lofrano and Brown, 2010). This practice collapsed in 1847, when cheap guano from South America became the favoured fertiliser for farmers.

The trebling of London’s population from 1800 to 1860 outstripped both the water supply and sewage dumped into the Thames, culminating in the ‘Great Stink’ in the hot summer of 1858, forcing Parliament to empower the London Metropolitan Board of Works to build a unique and successful sanitary and water supply system. Validation of the new system was rapid – in the 1866 cholera outbreak, the only areas of London affected were those not yet connected to the new network (Solomon, 2010).

Prior to the mid-1800s, understanding the connection between routes of disease transmission and the causes of illness was greatly hampered by complete ignorance of the existence and role of pathogenic agents. Two centuries separated the seminal discoveries of the basic biological cell, including the existence of microbial entities, and the demonstration that certain microorganisms were at the root of disease promulgation.

Emergence from the sanitary dark ages with control and treatment of domestic wastewater really began once it was realised that these wastes were also linked to major human epidemic diseases such as cholera and typhoid. Diseases like cholera, typhoid, typhus, and dysentery were common in the United States, Europe, and other parts of the world prior to the 20th century. (Oswald, 1996, Unz, 2008).

The 20th century witnessed a revolution in wastewater management, associated with emerging awareness of environmental science and societal views towards pollution. A milestone was the Eighth Report (1912) of the Royal Commission on Sewage Disposal which introduced the concept of biochemical oxygen demand (BOD) and established standards and tests to be applied to sewage and sewage effluents which were copied by many other countries. Governments began to mandate waste treatment. Wastewater treatment facilities were constructed in the main cities of Europe until the First World War interrupted their installation.

### **1.5.2 Primary Treatment**

People excrete 100-500 g wet weight of faeces and 1-1.3 litres of urine per person per day (Bitton, 2005). This material is gathered by various mechanisms for the purpose of collective treatment to render it safe and reduce noxious odours.

Primary treatment is defined as the removal of heavier solids by gravity sedimentation. The earliest form of primary treatment was trenches and pits used for many centuries to remove heavier solids with the objective of reducing the load prior to application on the land to avoid clogging.

In the 1860s, L.H. Mouras designed a cesspit in which inlet and outlet pipes dipped below the water surface thus forming a water seal: the “fosses Mouras”. Septic tanks improved on this design and were patented by Donald Cameron in 1895. The Imhoff tank, designed by Karl Imhoff in 1906 was a further advance and it is still in worldwide use.

### **1.5.3 Secondary Treatment**

Secondary treatment uses micro-organisms to convert the carbonaceous (organic) materials in the wastewater to carbon dioxide, water and energy for re-growth. There are two basic types of secondary treatment: attached growth (biofilms) and suspended growth (activated sludge). Attached growth systems have a fixed substrate such as rock or plastic on which micro-organisms can

attach and grow. The wastewater flow is directed over this aerated biofilm resulting in reduction of biological oxygen demand (BOD). In a suspended growth system, a biologically active sludge (biomass) and wastewater are constantly mixed resulting in BOD reduction. The solids are then removed in a subsequent sedimentation step and the majority of the sludge is usually returned to the process.

#### **1.5.4 Prevention of Eutrophication**

Once secondary treatment and the reduction of carbonaceous pollutants was employed at most treatment plants, the prevention of eutrophication became the next goal for wastewater treatment. Depending on the receiving waters, many treatment plants are required to remove nitrogen, phosphorous or both. To achieve this, the first full scale commercial aerobic membrane bioreactors (MBR) processes appeared in North America in the late 1970s and then in Japan in the early 1980s. By 1993 external membrane bioreactor systems had been reported for use in sanitary application in Europe.

#### **1.5.5 Disinfection**

It wasn't until 1961 that the first chlorine residual controlled disinfection system was available. The first recorded use of ultraviolet light for disinfection was in France in 1916. In the early 21st century, chlorine gas continues to be widely used and ultraviolet disinfection is becoming the state-of-the-art.(Lofrano and Brown, 2010)

### **1.5.7 WASTE STABILISATION PONDS**

#### **1.5.7.1 The structure of a WSP system**

The idea of treatment ponds is to mimic nature's own processes as biological mechanisms. A WSP system consists of one or more in-series man-made earthen basins, with functional units achieving anaerobic, facultative and aerobic (maturation) roles. The WSP system offers simplicity and cheapness of design, construction and operation compared to the electro-mechanical systems

described above. However, they do require large areas of land – which is becoming a real constraint in the modern urban environment.

#### *1.5.7.2 Historical development of WSPs*

In a recent report the US EPA (2011) claimed that stabilisation ponds have been used for 3,000 years to treat wastewater. Probably more realistic is Gloyna's view (1971) noting that some ponds where effluent gathered were used as fish ponds by the ancient Greeks at Agrigantum, Sicily. Concurrent with the electro-mechanical processes developed for large cities, waste stabilisation ponds (WSPs) were being developed during the twentieth century for smaller communities and are now numerically one of the main natural wastewater treatment methods used worldwide. Initially, design criteria varied considerably from country to country, some concerned with micro-organism removal, some with suspended solids and some with BOD. These designs specified depth, surface area and organic loading. The preferred shape was rectangular and the most common depth was 1 to 2 metres.

There was little understanding and therefore little engineering design put into early ponds. As a result some failed. The decade between 1940 and 1950 saw considerable scientific effort placed on the better understanding of WSP design and operation criteria, for example, Gotaas (1948) published on the effects of temperature on sewage treatment. The scientific literature on these subjects expanded rapidly in the 1950's (Oswald et al., 1953, Oswald et al., 1957, Hermann and Gloyna, 1958) resulting in a worldwide design standardisation and a rapid phase of building in the decade 1960 to 1970 (Gloyna, 1971).

#### *1.5.7.3. WSP climatic zones suitability*

WSPs are particularly suited to tropical and subtropical countries since sunlight and ambient temperature are key factors in their process performance. Although widely used in warmer parts of the world for cities of up to 1 million inhabitants, some are installed in very cold climatic areas as well. Here, they are

typically used in smaller rural towns (up to 2,000 inhabitants) where the availability and cost of land is less problematic.

#### *1.5.7.4 World-wide use of WSPs*

The US EPA note the first recorded WSP in the USA was built in 1901, and that by 1983 there were more than 7,000 waste stabilisation ponds in use in the USA, ranging from tropical to Arctic areas (US EPA, 1983). By 2011, there were more than 8,000 wastewater treatment ponds in the USA, comprising more than 50% of wastewater treatment facilities, usually serving populations of up to 5,000 (US EPA, 2011). Canada had over 500 operational WSP systems by 1966. (Gloyna, 1971)

Mara and Pearson (1998) report widespread use of WSP's throughout Europe including over 2,500 WSPs in France, 1,100 in Germany, 64 in Portugal, 9 in the province of Almeria in Spain, 40 in the UK, 13 in Greece, 43 in Turkey, over 200 in Israel, 6 in Jordan, 2 in Egypt, 3 in Tunisia, 1 in Algeria, 9 in Morocco. They observe that WSPs service a wide range of populations from less than a thousand in rural France to large urban centres of up to 1.4 million people in Turkey. However, they also note that there are many examples of poorly operated and poorly maintained WSP systems in need of serious rehabilitation. Further, they note the limited re-use of treated water despite chronic water shortages and excellent suitability for crop irrigation of treated water.

Racault and Boutin (2005) report that in France the use of WSPs in small rural communities, with an average size of 600 person equivalents, has increased greatly since the end of the 1970s. These now represent 20% of sewage treatment plants in France, even though they handle only 1 – 2% of wastewater treated.

Mara (1997) reports on a long history of WSP use in India including a Kolkata (Calcutta East) system of 3,000 ha of ponds developed around 1917 by local fishermen. The system receives 550,000 m<sup>3</sup> per day of raw, untreated sewerage, and yields 4 tonnes of carp per hectare per year for local

consumption. He also notes the State of West Bengal has the highest number of WSPs in India.

Archer and Mara (2003) quoting Fitzmaurice (1987) report that 100 of 160 sewerage communities in New Zealand of more than one thousand people used WSPs as the predominant treatment method, ranging in size from 0.1 to 500 ha. Most were built in the period 1960 to 1985. In Australia, WSPs have been used extensively for wastewater treatment in smaller communities in rural areas in all States and Territories. (Power and Water Corporation, 2011, EPA Tasmania, 2012, LGA SA, 2012, Palmer et al., 1999)

Kayombo et. al. (2005) report many countries in tropical Africa use WSPs for wastewater treatment including Tanzania, Kenya, Malawi, Botswana, Zambia, Zimbabwe and South Africa. No numerical details are available.

Von Sperling (2005) performed a detailed study on the performance of 186 WSPs in many climatic zones – mostly in South America. He included 156 ponds in Brazil, 5 in Argentina, 2 in Columbia, 2 in Chile, 6 in Venezuela, 3 in Mexico, 4 in Spain, 1 in Belgium, 4 in Morocco, and 4 in Palestine.

#### *1.5.7.5 Recent replacement of WSPs*

In some cities, larger stabilization ponds have been replaced in the early 2000's by activated sludge waste water treatment plants, such as in Amman (Jordan) and in Adelaide (Australia) in 2004.

#### *1.5.7.6 WSP Principles*

In simple terms, waste stabilization ponds are impoundments into which wastewater flows in and out after a defined retention period. Treatment relies solely on the natural processes of biological purification that would occur in any natural water body. No external energy, other than that derived from sunlight, is required for their operation. Compared to other technologies WSPs appear to be simple, however, they contain a complex ecological system, which consists of algae, bacteria, virus, fungi, protozoa, rotifers, insects, crustaceans and often

chordate animals. Treatment is optimized by selecting appropriate organic loadings, retention periods and pond depths, to promote the maximum growth of organisms beneficial to the treatment process (Mara et al., 1992).

The WSP system typically consists of a series of continuous flow anaerobic, facultative, and maturation ponds (Babu et al., 2010, Mara, 2005). The anaerobic pond, which is the initial treatment reactor, is designed to reduce suspended solids and some of the soluble organic matter. The residual organic matter is further removed through the activity of algae and heterotrophic bacteria in the facultative pond. The final stage of pathogens and nutrients removal takes place in the maturation pond. These three types of pond when used in series, have demonstrated up to 95% removal of BOD and faecal coliforms. (Hamzeh and Ponce, 2007, Mara, 2005). The prime mechanisms for pathogen removal in maturation ponds are known to be: high UV light irradiance together with a significant amount of dissolved oxygen, temperature, and high pH (> 9), (Hamzeh and Ponce, 2007).

Waste stabilization ponds are designed to provide a controlled environment for wastewater treatment. Stretching back over seven decades, there are many versions of pond design in the literature (Marais, 1974, Power\_and\_Water\_Corporation, 2011, US EPA, 1983, US EPA, 2011, Mara and Pearson, 1998, Mara, 1997, Gloyna, 1971, Kayombo et al., 2005, Juanico and Shelef, 1994, Mara, 2005, Shilton and Mara, 2005, Thirumurthi, 1969, von Sperling, 1996, Gawasiri, 2003, Hermann and Gloyna, 1958, Marais, 1966) to name but a few of the many.

Pond size is established from theoretical and empirical relationships that give, directly or indirectly, an estimate of the hydraulic retention time needed to achieve a given effluent quality. Although in appearance and function wastewater stabilization ponds seem to be straightforward, in reality, they display considerable variability in performance because of highly complex physical, chemical and biological factors interacting with one another. These

interactions have proven resistant to mathematical modelling as there are many subtle interactions between each of the parameters.

Sah et al. (2012) comprehensively examined 23 peer reviewed models developed so far, including models developed for WSPs, HRAPs and aerated facultative lagoons (AFLs) incorporating hydraulics, water quality or both aspects of pond functioning. They conclude that although they might be helpful in understanding the processes in the pond or in pond design, they do not completely describe the processes and interactions in the pond. They also conclude that computational power no longer limits effective modelling, but rather a lack of detailed data for calibration and validation.

What is generally reported in the literature is a detailed study of single or two to three element interactions which are appropriate for the pond in which they are measured but difficult to generalise beyond that location and pond configuration. Every pond has slightly different size, depth and shape. The daily rate and quality of influent varies between ponds and between days in the same pond. The weather is always subtly different between locations, even in the same district. For example wind speed and direction is easily influenced by surrounding topography, and sunlight can be influenced by shading that is site specific.

#### *1.5.7.7 Solar Radiation*

Solar radiation is the only energy input into the WSP system. It is the powerhouse that drives algal growth, and causes heating of the surface layers. The more energetic solar rays at the ultra-violet end of the solar spectrum are responsible for considerable pathogen die-off, at least in the surface layers where they can penetrate (Jagger, 1985, Bolton et al., 2010, Benchokroun et al., 2003b, Calkins et al., 1976, Crane and Moore, 1986, Davies-Colley et al., 2000, Davies-Colley, 2005, Fujioka et al., 1981, Maïga et al., 2009, Moeller and Calkins, 1980, Reed, 1997, Reed et al., 2000, Sarikaya and Saatci, 1987, Sinton et al., 1999, Sinton et al., 2002). The more energetic ultra-violet rays are more rapidly



absorbed, particularly in the turbid waters of WSPs.(Williamson and Neale, 2009, Stefan et al., 1983)

#### *1.5.7.8 Design and Analytical Hydraulic Models for WSPs*

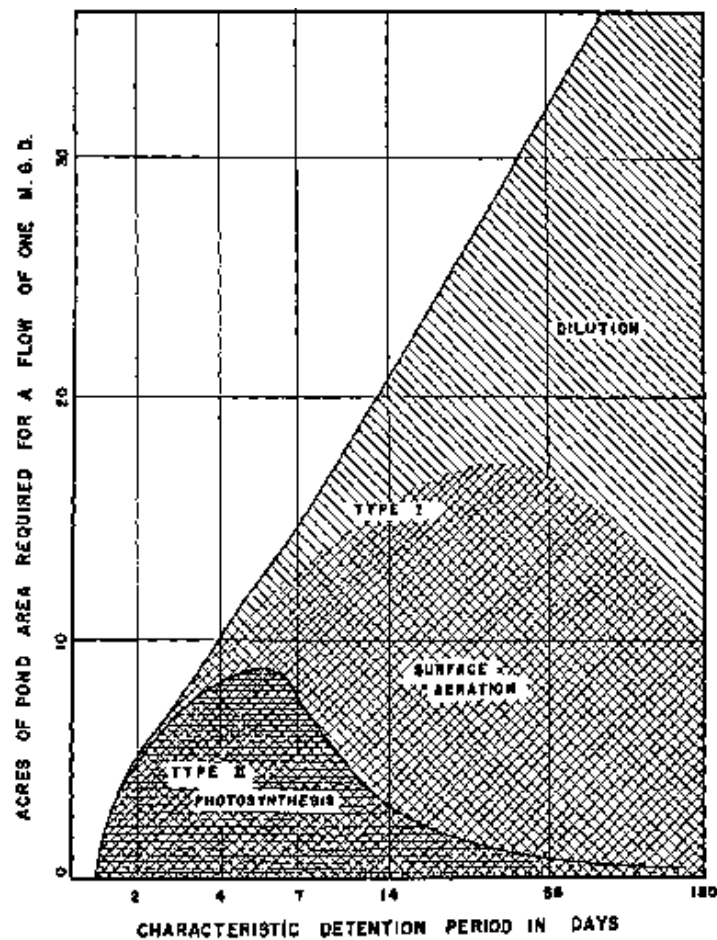
One of the major drawbacks to the design of WSPs is the lack of a rational approach that takes into account all the chemical, physical and biological processes governing the purification kinetics of the system.

The reactor's hydraulic behaviour is one such factor of prime importance, since it controls the residence time and the dispersion of the wastes in the reactor. The best knowledge of this hydraulic flow patterns comes from tracer experiments. Unfortunately, these experiments are rather lengthy and costly, especially when conducted on large basins such as WSPs and aerated lagoons. This is why, despite the major influence of ponds hydraulic characteristics on their removal efficiency, very few tracer studies have been conducted on real-size facilities or reported in the literature.

The knowledge of pond hydraulic flow patterns will always be critical information needed for rational design. Therefore, many ways have been introduced for estimating the macro-mixing conditions which occur in these large biological reactors.

In the early years of WSP design and construction much of the emphasis was on delivering a treated effluent that had most of the influent BOD removed. The early papers on WSP design concentrated on designing for natural oxygen production to allow rapid degradation of the BOD in wastewater (Oswald et al., 1953, Oswald et al., 1957, Hermann and Gloyna, 1958). This was seen as the most important feature of treatment of wastewater that was to be discharged into a natural waterway. The effluent BOD concentration needed to be low enough to not result in de-oxygenation of the receiving waterway. Even today this is still the case in much of Europe.

Oswald et al. (1955) pursued the idea of WSP design through laboratory and pilot plant investigations of sewage treatment in open ponds by photosynthetically produced oxygen. These studies provided some basic principles which were utilized for the engineering design of the process as well as for the prediction of the operational performance of new or existing oxidation ponds. The chemical, biological, operational, and economic factors which affect the use of engineered photosynthesis were explored.



**Fig. 1-2. Schematic Representation of General Types of Oxidation Ponds – as published by Oswald et. al (Oswald et al., 1955)**

These authors introduced the somewhat arbitrary division of Type 1 & 11 oxidation ponds based on pond area and detention time (see Figure 1-2) and concluded that “for most conditions, detention periods should not be less than one day for summer conditions nor more than six days for winter conditions. A

pond having a detention period of about three days and a depth of 12 inches should, for example, satisfactorily produce adequate oxygen by photosynthesis more than 80 percent of the time in latitudes up to 40°N.” They also noted that it is probable that some artificial vertical mixing must be considered an essential feature for Type 11 (shallow) oxidation ponds. This topic will be returned to under discussion of the development of High Rate Algal ponds.

Clearly, the path of WSP design being pursued by Oswald and colleagues had some serious limitations as there was a six-fold difference in the recommended treatment times between summer and winter. This disparity is not consistent with easy-care low technology solutions required for many remote installations. Most other authors pursued WSP design by invoking various forms of pond hydraulics as outlined below.

#### *1.5.7.9 Design Process excluding algae*

One other theoretical alternate design pathway was taken by James (1987), who made the observation that from his viewpoint the conversion of organic carbon to methane in anaerobic ponds was the main contributor to reducing oxygen demand in treated effluent; and therefore to his mind, the conversion of carbon to algal cells in facultative ponds was of doubtful benefit.

He also observed that the growth of algae in facultative ponds can contribute significant amounts of suspended solids (SS) to the effluent so that the overall change in unfiltered SS is small. His view that the efficiency of waste stabilization ponds in removing nutrients was also poor since much of the nitrogen and phosphorus leaves the pond system in the form of algae. If the nitrogen and phosphorus content of the algae are included in the effluent then removal efficiencies were much lower than conventional treatment. Whilst this statement is technically accurate, it ignores the fact that these nutrients have indeed been “stabilised” in the form of algal cells.

James (1987) further noted that it was in the removal of pathogens that stabilization ponds appear to be pre-eminent. However, he claimed that many

pond systems were designed on the basis of pathogen removal, but that in fact, they were relatively slow and inefficient (compared to energy intensive techniques), at this task as well. He presented the data shown in Table 1-1 to reinforce this assertion.

**TABLE 1-1. Comparison of Removal Rates in Different Wastewater Treatment Processes. Adapted from (James, 1987)**

| Process                                       | Retention Time (hours) | % Removal | Removal Rate |
|---|------------------------|-----------|--------------|
| Primary Sedimentation plus Activated Sludge   | 4 + 6 + 2 = 12         | 95        | 20% per day  |
| Primary Sedimentation plus percolating filter | 4 + 2 + 1 = 7          | 95        | 20% per day  |
| Waste Stabilisation Pond                      | 30                     | 99.999    | 20% per day  |

James then described a design system based on two deep anaerobic ponds in series followed by a single facultative pond, as a solution to the design issue. His view was that there was no single optimum design of waste stabilization pond to suit all conditions and it therefore appeared worthwhile to approach pond design in a variety of ways.

This alternative approach outlined below is an attempt to approach pond design by emphasising the importance of mixing and organic content in controlling the bacterial die-off. In contrast to all the other design approaches discussed in this review, the importance of methane production in BOD removal is emphasised. The undesirability of algae is also stressed both in encouraging the survival of bacteria and in creating an oxygen demand in the pond effluent.

To emphasise the merits of his unique design approach, James (1987) highlighted the major known causes of bacterial death in wastewater as follows

a) **Light-induced mortality** - in some aquatic environments such as the sea, it is apparent that light is responsible for rapid die-off of bacteria (Gameson and Gould, 1986). But light penetration in stabilization ponds is limited to the top 10-15 cm and as the highest bacterial concentrations rarely occur in the surface layer it is unlikely that light is a major cause of death in ponds. This is confirmed

by typical values for die-off rates in ponds ( $T_{90}$  values of 20 - 30 hours for *E. coli*) which approximate to dark removal values in freshwater and seawater experiments. This theme will be returned to in discussion later in this thesis.

b) **pH - induced mortality** – James (1987) noted that in his thesis (Smallman, 1986) suggests that bacterial death in ponds is due to algal photosynthesis causing periods (6-12 hours per day) of high pH. Results from his dialysis experiments showed significantly higher bacterial mortalities at pH levels above 9. He also showed that pH rose to 9 - 10.3 during periods of intense photosynthetic activity.

c) **Starvation-induced mortality** - experiments on bacterial die-off in fresh and marine waters (Gameson, 1985) have shown that  $T_{90}$  values of 1 to 2 days occurred in the presence of low levels (< 20 mg/L) of organic matter in the absence of light, predators or other sources of mortality. When the concentration of organic matter was increased the  $T_{90}$  values increased to 2 to 3 days. Similar results from (LeMoynes, 1982 ) indicate that at BOD levels above 20 - 30 mg/L growth of coliforms can occur at appropriate temperatures.

It would therefore appear to be important to maintain low organic concentrations (BOD <20 mg/L) if starvation is to cause a rapid die-off.

d) **Sedimentation** - the primary stages in any pond system, especially where they are anaerobic, act in a similar manner to a primary sedimentation tank and remove around 50% of the incoming bacteria. It is doubtful whether this mechanism is significant in subsequent stages.

The James (1987) design then consists in the first part of a pond system with a two-stage anaerobic reactor, preferably plug flow (i.e. high length: breadth ratio) which would remove the majority of the BOD as methane. In this way it is possible to obtain BOD removals that are sufficient to ensure starvation conditions in the subsequent facultative stage. The overall aim of the anaerobic stage would be to reduce the BOD to less than 40 mg/L. As long as this could be

achieved the second 'facultative' stage would then operate at the short retention time of 3 to 4 days. Whilst still providing the low BOD concentration to cause starvation, this short retention time would theoretically prevent the development of a significant algal population, but would still be designed to be aerobic.

Since algal photosynthesis would not contribute to the oxygen budget the design would be based upon the BOD loading not exceeding the surface re-aeration capacity. Obviously wind velocities and frequencies will largely determine re-aeration rates but the values of  $K_2$  (re-aeration coefficient) in quiescent conditions vary between 0.2 and 0.9 per day and suggest that the allowable depth for aerobic conditions would be at least 1 metre.

James (1987) claimed the relative shallowness of this facultative stage would have the following secondary benefits:

- a. **Increased rate of bacterial mortality due to light.** Because of the low algal levels, deep light penetration would give significantly enhanced die-off.
- b. **Maximise mixing due to wind action** which would reduce any tendency to short-circuiting, although this last point does not necessarily follow logically.

von Sperling put this design process in context in Chapter 3 of "Waste Stabilisation Ponds" (von Sperling, 2007) by stating that pond series of this sort are only needed for industrial wastewaters with high BOD concentrations, such as slaughterhouses, piggeries, dairies and beverage industries. The recommendation by James (1987) to use sequential anaerobic ponds when better bacterial removal is required was not borne out in a practical sense and is therefore not widely used.

#### *1.5.7.10 Hydraulic models for WSP design - either completely mixed or plug flow conditions*

Measurements of residence time distributions (RTDs) via tracers in full-scale WSPs have shown that individual ponds behave in a manner unique to their

circumstance. Some results show the pond behaving in what is effectively a completely mixed manner, that is, with a maximum tracer concentration at the outflow after only a fraction of the theoretical residence time (Frederick and Lloyd, 1996, Moreno, 1990). Other ponds show behaviour approaching plug flow conditions (Arceivala, 1983). Early analytical hydraulic models of WSP operation assumed either completely mixed or plug flow conditions (Juanico, 1991). These models, whilst physically simplistic, have the advantages of practical convenience and tractable mathematics.

The design process starts with the knowledge that there is a prescribed effluent BOD concentration that must be met and will be monitored for on a regular basis. There is also assumed prior knowledge of the rate of the reaction under pond conditions. Like all biochemical reactions, this rate needs to be adjusted for temperature. To achieve the desired effluent concentration from a known influent concentration, further knowledge is required of how fast the average particle moves through the pond system. A range of hydraulic models have been advocated and used over the past sixty years to attempt to predict in-pond behaviour, with each model having various useful and less useful features. The models assume various types of flow/mixing from the idealised completely-mixed and plug-flow conditions to the non-ideal mixing associated with dispersed-flow model. The scientific debate in the literature supporting various models is extensive and at times has been quite robust on this point.

The last step in the design process is undertaken once the total time required to achieve the desired outcome in the pond effluent has been estimated. Armed with this time requirement and estimating the daily flow into the system it is then relatively simple to calculate the volume of the treatment pond(s).

The formulas for completely-mixed and plug-flow conditions, which assume first order kinetics, steady state conditions and no evaporative or seepage losses are shown as Equations 1-1 and 1-2 respectively (Polprasert and Bhattarai, 1985)

$$\frac{C}{C_0} = \frac{1}{1 + k_T \theta} \dots \dots \dots (1 - 1)$$

$$\frac{C}{C_0} = e^{-k_T \theta} \dots \dots \dots (1 - 2)$$

In which  $k_T$  = the first order reaction rate – varies with temperature

$C_0$  = initial input concentration

$C$  = treated outlet concentration

$\theta$  = hydraulic detention time (days)

The completely mixed model as shown in Equation 1 has been the form used by most WSP designers and engineers as it is relatively simple to use without the need for complicated mathematics. The real problem for this equation is that the nominal or theoretical hydraulic detention time, ( $\theta$ ), as defined in Equation 1-3, is never the same as the actual residence time, even under stationary flow conditions.

$$\theta = \frac{V}{Q} \dots \dots \dots (1 - 3)$$

where  $V$  = pond volume

$Q$  = flow rate

The actual residence time, defined as the Mean Hydraulic Residence Time (MHRT) is always less than the nominal residence time ( $\theta$ ), (Persson and Wittgren, 2003). The residence time of a pond can be analysed with a tracer test, which produces a tracer concentration versus time distribution. Under plug flow conditions, the concentration versus time distribution is simply a spike with a very small standard deviation about the MHRT. The MHRT is defined as the centroid of the Residence Time Distribution (RTD), where the RTD function, is represented by the concentration or mass. This suggests that all individual



parcels of tracer entering the pond experience a similar period of residence. However, pure plug flow conditions never occur in natural systems, instead concentration versus time distributions have more or less deviation, i.e., show more or less dispersion.

To improve understanding of the relation between mean and nominal residence times, one can multiply the residence times by the flow, which gives us the relation between two volumes, the effective and total volume (Equation 1-4). The effective volume ratio, (*e.v.r*), is linked to length and width, but is also affected by factors, such as wind. Thackston *et al.* (1987) carried out a series of experiments on large shallow ponds between 60 to 600,000m<sup>3</sup> and developed a model to calculate the effective volume ratio (*e.v.r*) (Equation 1-5).

$$e.v.r = \frac{V_{eff}}{V_{total}} = MHRT/\theta \dots \dots \dots (1 - 4)$$

$$e.v.r = 0.84 \left\{ 1 - e^{(-0.59(L/W))} \right\} \dots \dots \dots (1 - 5)$$

Despite the limitations noted above, and usually without adjustment for *e.v.r.*, in Australia, a version of the Marais model (Equation 1-4) that estimates residual bacteria in the effluent water has been very popular with WSP designers. This is because WSP systems are generally installed in rural areas Australia. These are frequently areas of periodic and/or absolute water scarcity. As such they are in great need of treated wastewater for amenity and/or agricultural purposes. Under these circumstances, it has been more important to design ponds that will deliver known numbers of indicator organisms. In cases where reuse of treated wastewater is considered further treatment, or risk management measures may be required for human health safety reasons.

The Marais model (Marais, 1974) equation (Equation 1-6) has been widely used in Australia for estimating the effluent coliform concentration according to the complete-mix flow principles. In this case, it is used in the form

$$N = \frac{N_0}{1 + \theta K_b} \dots \dots \dots (1 - 6)$$

In which, N = the effluent coliform concentration (MPN/100 mL);

N<sub>0</sub> = the influent coliform concentration (MPN/100 mL);

K<sub>b</sub> = the coliform die-off coefficient (d<sup>-1</sup>);

θ = the hydraulic detention time (d)

Marais (1974) found the value of K<sub>b</sub> for faecal bacterial reduction to be dependent on temperature alone as shown in Equation 1-7.

$$K_b = 3.6(1.19)^{T-20} \dots \dots \dots (1 - 7)$$

In which, T = pond water temperature in the coldest month (°C)

Mara et. al. (1979) reported that this equation was suitable for pond design with winter temperatures ranging from 2°C to 21°C, and maybe up to 30°C. Marais (1974) expanded a little further to say that to achieve maximal bacterial die-off a series of equal size ponds should be designed using Equation 1-8.

$$\frac{C}{C_0} = \frac{1}{(1 + k_T \theta)^n} \dots \dots \dots (1 - 8)$$

where, n = number of ponds in the series

However, Lloyd *et al.* (2003) disagree with Mara's conclusion and instead state. "The widely used Marais design equation is based on the following flawed assumptions

- (a) mixing in the pond is instantaneous and complete
- (b) die-off in a pond is strictly according to Chick's Law
- (c) the retention time is based on influent flow and pond volume."

Paradoxically, although Marais concluded correctly that, “the plug flow system is the most efficient”, he went on to recommend the system of “intermediate efficiency” being “the series of ponds of equal size”. He also mentioned that “a minimum value for the retention time is probably about 3 days; (because) short retention times tend to short-circuit”.

Marais’ recommendation of the series of ponds of equal size would, in many cases, probably produce the effluent quality required if design engineers built the number of ponds as recommended in design manuals. However, practical constraints, including budget and available land, result in sub-optimal design. Cost cutting results in fewer pond dividing embankments being constructed, and sometimes the very specific risk which Marais warned against i.e. “the most inefficient pond system – a single pond, being constructed.” It is interesting to note that as Lloyd *et al.* experimented with pond configurations to optimise coliform die-off they ended up with a serpentine channel system very similar to a HRAP in outline, but without the paddlewheel to keep the fluid moving.

During the 1990’s various authors commenced studies of scale model ponds and smaller full scale WSPs using tracer dyes and organisms. These studies highlighted that neither plug flow nor completely mixed behaviour properly describe the real world pond hydraulics. As well, calculations of mean residence time can be used to estimate the volume of short circuiting in the pond (Levenspiel, 1999).

#### **1.5.7.11 Dispersed flow hydraulic model for WSP design**

A further hydraulic design variation is the dispersed flow model derived by Wehner and Wilhem (1956) (Equation 1-9) for chemical reactors exhibiting first-order kinetics and non-ideal mixing conditions. This was adapted to WSPs by Thirumurthi (1969). Under certain conditions this model can be used to give a more efficient description of outflow age. The dispersion number  $d$  is used to describe the magnitude of longitudinal dispersion within the pond, on a scale ranging from 0 (plug flow) to  $\infty$  (completely mixed conditions). Dispersion

number analysis of WSPs was developed from retrospective analysis of pond hydraulic performance. A number of expressions have been empirically developed to predict dispersion number based on pond parameters such as length, width, depth, and flow-rate (Agunwumba, 1992, Arceivala, 1983, Polprasert and Bhattarai, 1985).

$$C_e = \frac{C_i 4a e^{0.5d}}{(1+a) e^{a/2d} - (1+a)^2 e^{-a/2d}} \dots \dots \dots (1-9)$$

where,  $a = \sqrt{1 + 4k\theta d}$  and  $d = \frac{D}{UL} = \frac{D\theta}{L^2}$

$C_e$  = effluent BOD (mg/L)

$C_i$  = influent BOD (mg/L)

$k$  = first order BOD removal coefficient (day<sup>-1</sup>)

$\theta$  = mean detention time (days)

$d$  = dispersion number (dimensionless),

$D$  = axial dispersion coefficient (sq. ft. per hour),

$U$  = fluid velocity (ft. per hour),

$L$  = characteristic length of travel path of a typical particle in the pond (ft.)

Many authors such as Thirumurthi (Thirumurthi and Nashashibi, 1967, Thirumurthi, 1969, Thirumurthi, 1974) and (Polprasert and Bhattarai, 1985) and (Arceivala, 1983) regularly and frequently advised WSP designers and regulators to use the Wehner and Wilhem (Wehner and Wilhem, 1956) equation (Equation 1-9) or at least either of the simplified versions (Equation 1-10 and 1-11) rather than either of the ideal mixing condition models for design purposes. These equations were originally devised for chemical reactions in ponds/tanks that exhibit non-ideal mixing property (that is to say, that neither exhibit plug flow nor a completely mixed system).

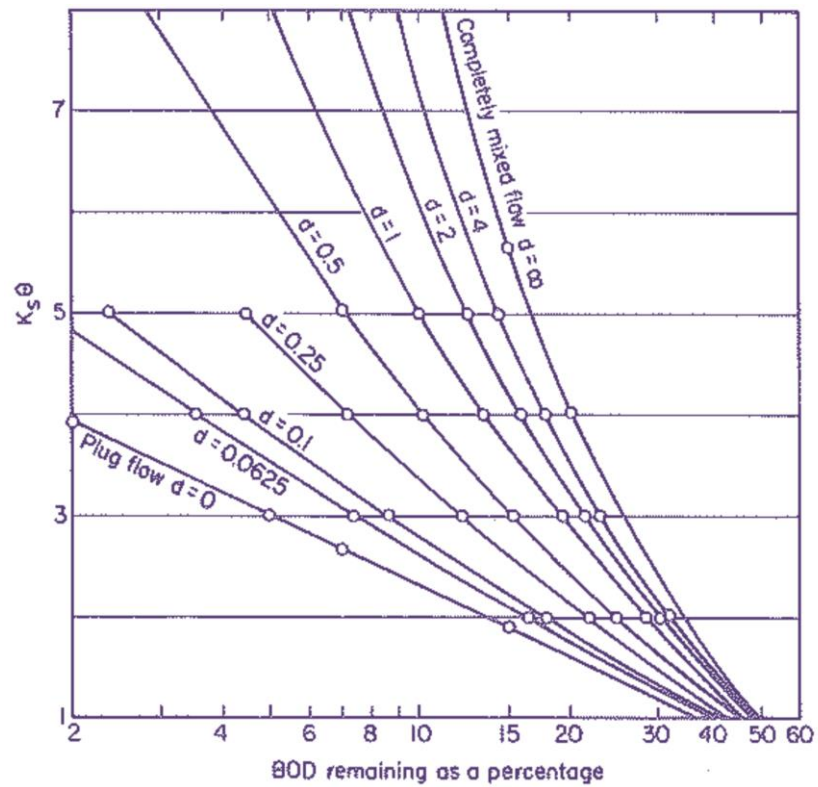
$$\frac{C_e}{C_i} = \frac{4ae^{1-a/2d}}{(1+a)^2} \dots \dots \dots (1 - 10)$$

$$\frac{C_e}{C_i} = e^{-K\theta} \dots \dots \dots (1 - 11)$$

It is clear that these three dispersed-flow design equations depend on two coefficients ( $K$  and  $d$ ). In an existing pond,  $d$  can be determined by tracer or dye studies, as suggested by Levenspiel (1999). For design purposes however, an estimate of  $d$  can be made using some hydraulic parameters of the pond, such as length, width, and hydraulic detention time ( $\theta$ ).

The value of  $K$  can be estimated by analysing experimental data, but not surprisingly, was found to be subject to variation due to a range of characteristics of the influent water and environmental conditions, such as temperature, solar energy, organic load, industrial wastes, pond shape and depth and mixing characteristics in the pond. Thirumurthi (1974) provided a range of solutions to overcome these stumbling blocks. Firstly, he supplied a large armoury of correction factors - all dependent on local conditions. Of course, these correction factors are highly unlikely to be known or understood in advance.

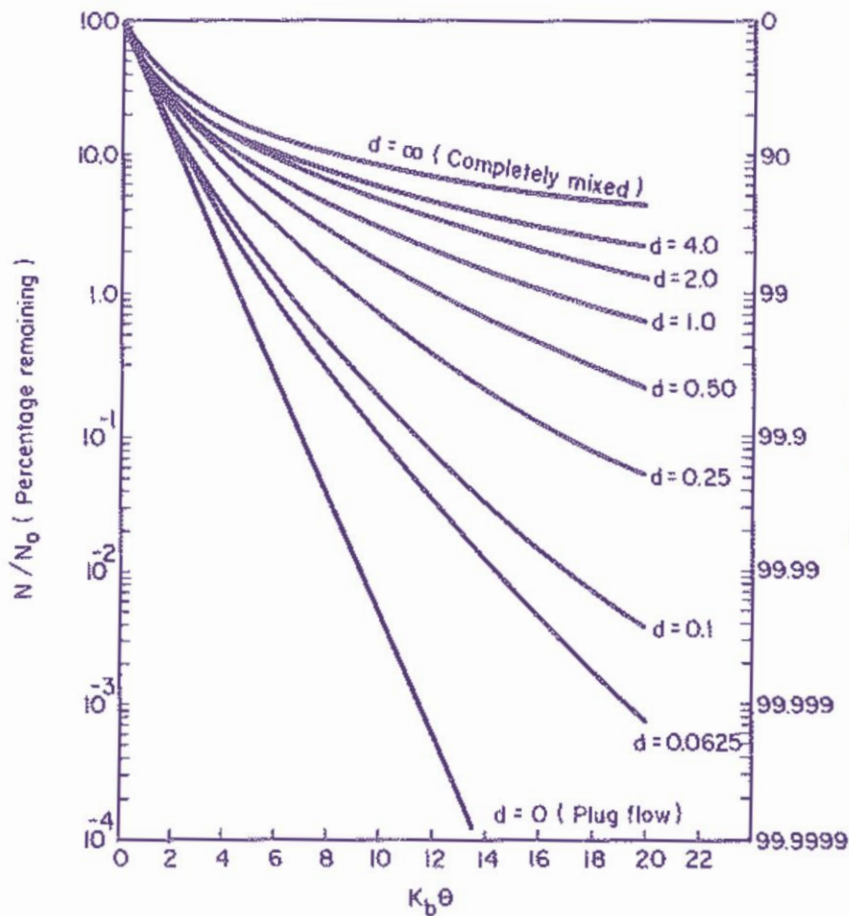
Secondly, he supplied the design formula charts relating  $K$ ,  $d$  and  $\theta$  as shown in Figs. 1-3 and 1-4. The importance of flow dispersion on pond performance can be seen in Figs 1-3 & 1-4. For example in Fig. 1-3, at  $K_s\theta = 5$ , and  $d$  value is 0.1, 98.5% of the BOD is removed, compared to 89% removal with a  $d$  value of 1.0. In ponds with high length to width ratios the  $d$  value is low ( $<0.5$ ), the internal flow approaches plug-flow conditions and less short circuiting occurs and more time is available for chemical reactions. The opposite is true for ponds with high  $d$  values as they approach completely mixed flow conditions.



**Fig. 1-3 A Wehner & Wilhem BOD design formula chart, adapted from (Polprasert and Bhattarai, 1985)**

As the required BOD or *E. coli* count is already understood from the regulatory guidelines, the values of  $K_s \theta$  or  $K_b \theta$  for a pond with a selected  $d$  value can be determined by reading from the graph in Figure 1-3 or 1-4. To find  $\theta$ ,  $K_s$  or  $K_b$  has to be evaluated. Once  $\theta$  is calculated, the required pond volume is determined by multiplying the influent flow volume by  $\theta$ , and the other size parameters can then be determined.

Despite these short cuts designed to make the design process more user-friendly, there are still considerable obstacles with this technique, so it is not surprising that Polprasert and Thirumurthi's advice was largely overlooked during the following decades in pond design and analysis.



**Fig. 1-4 Design formula chart for bacterial reduction in WSPs; adapted from (Polprasert and Bhattarai, 1985)**

In recent times von Sperling has done considerable work to both unify the various hydraulic design equations (von Sperling, 2002) and to advocate design using dispersed-flow models via a number of robust simplifications (von Sperling, 2005, von Sperling, 2003, von Sperling, 1999).

In the 1999 paper, von Sperling proposed a simplification of the Yanez, 1993 calculation (Equation 1-12) of the dispersion number ( $d$ ).

$$d = \frac{(L/B)}{-0.261 + 0.254(L/B) + 1.014(L/B)^2} \dots \dots \dots (1 - 12)$$

Where  $L$  = pond length (m)

$B$  = pond breadth (m)

The simplification (Equation 1-13) proposed by von Sperling was

$$d = 1/(L/B) = (L/B)^{-1} \dots \dots \dots (1 - 13)$$

Design equations using the coefficients  $d$  and  $K$  proposed in this work proved very reliable in predicting the performance of 66 tropical and sub-tropical ponds in Brazil.

Further to this work, von Sperling (2002) quantified the relationship between the first-order removal coefficients  $K$  from the three above-mentioned hydraulic regimes (CSTR, plug flow and dispersed flow) used in both design and performance evaluation of ponds. Based on theoretical considerations and regression analyses, the relationship between the  $K$  values was investigated, quantified and modelled. Two equations were then postulated to allow conversion of  $K$  values obtained for dispersed flow to (a)  $K$  for CSTR and (b)  $K$  for plug flow, based on the hydraulic detention time  $t$  and the dispersion number  $d$ . These coefficients, when applied in the CSTR or plug-flow equations yield approximately the same prediction of the effluent concentration as that obtained when using the dispersed flow model with its proper coefficient.

#### 1.5.7.12 Peclet Number

Nameche and Vasel (1998) attempted to consolidate design concepts based on a dispersed plug-flow system, which could be described as an "intermediate" hydraulic model, covering all the existing situations from the ideal plug-flow to completely mixed conditions. They thought that the model was one of the most widely used in the field of Sanitary Engineering since it requires only one parameter, called "Peclet number", obtained from tracer curves or residence time distributions (RTD). But this is also its major drawback as Peclet number determination relies on tracer experiments. Clearly, the drawback here is that a tracer experiment cannot be conducted on ponds that do not yet exist. The Peclet number is the inverse of the dispersion number and offers very little by way of improvement for pond design.



Peclet numbers can be rewritten as:

$$Pe = \frac{Q \times L}{W \times Z \times D} \dots \dots \dots (1 - 15)$$

where: Q = Flow rate

L = Pond length

W = Pond width

Z = Pond depth

D = Axial Dispersion Coefficient

By retrospectively analysing tracer studies from 30 ponds (including a HRAP), Nameche & Vasel introduced two new empirical equations to calculate the Peclet number – Equation 1-16 for WSPs and Equation 1-17 for all basins:

$$Pe = 0.31 \times \frac{L}{W} + 0.055 \times \frac{L}{Z} \dots \dots \dots (1 - 16)$$

$$Pe = 0.35 \times \frac{L}{W} + 0.012 \times \frac{L}{Z} \dots \dots \dots (1 - 17)$$

These equations were quite robust in forecasting the Peclet number for other WSPs but unsurprisingly were inadequate for the one HRAP in the group.

### 1.5.7.13 Simplified and unified WSP design

In a later paper, von Sperling (2005) examined the treatment performance of 186 facultative ponds located in various parts of the world. From these data two equations were derived for estimating the die-off coefficient  $K_b$  to be used for design purposes (dispersed flow, 20°C) in facultative and maturation ponds. The first equation relates  $K_b$  with the pond detention time  $t$  and depth  $H$  ( $K_b = 0.682 H^{-1.286} t^{-0.103}$ ) and fits well with the observed effluent coliform (logarithm) concentrations. The second equation is even simpler, depending only on the

pond depth, ( $K_b = 0.549 H^{-1.456}$ ) and also fits well with observed data. von Sperling summarised all these deliberations in the “Waste Stabilisation Ponds” book (von Sperling, 2007) stating that for design purposes reactors modelled according to dispersed flow fit between the two extremes of infinite longitudinal dispersion (complete-mix models) and no longitudinal dispersion (plug-flow models), which gives a better approximation of how real reactors behave.

Validation of predictive models by application to other sites has not always been as successful as von Sperling found, and previously Dorego (1996) had found when residence time distributions of various pond systems have been analysed.

#### **1.5.7.14 Secondary factors not included in design**

This lack of success has led researchers, including proponents of the dispersed flow model, to conclude that other factors are of significant, if not primary, importance in determining pond dispersion number. Frederick and Lloyd (1996) attributed pond short circuiting mainly to wind action and pond orientation, while Salter *et al.* (2000) report on a poorly operating WSP in which thermal stratification created significant dead zones and affected flow hydraulics and ultimately treatment performance. These findings indicate that  $d$  is not the static variable traditionally suggested, but rather a dynamic variable which is a function of pond flows and environmental conditions as well as pond design and layout.

Algal photosynthesis and accompanying nutrient uptake, while driving facultative pond oxygen production, is limited to zones of suitable photosynthetically active radiation levels. The photic zone is typically the top 50 cm of the pond, excepting the surface level where photoinhibition may occur under extreme conditions. The limited motility of many common algal species prevents diffusion of cells vertically, except where hydraulically driven. Photo-recovery experiments by Ratchford and Fallowfield (2003) also indicate that recovery of algal cells to photosynthetic production following photoinhibition

may be optimised by a period of resting in dark conditions. This suggests that under conditions of photoinhibition algal damage may be minimised by cycling cells periodically through the light and dark zones in the pond.

Biological reaction rates depend on the concentration of dissolved oxygen (DO) produced by photosynthesis. Many authors such as Oswald *et al.* (1955) and Thirumurthi (1974) have noted the importance of dissolved oxygen levels in regulating biochemical oxygen demand (BOD) removal efficiency. Additionally, nitrogen removal via nitrification can only occur under conditions of adequate DO.

Excessive DO inhibits denitrification (Metcalf\_&\_Eddy, 2003). Algal activity also regulates pH equilibrium, which affects ammonia volatilisation rate and phosphorus cycling. Recent publications suggest that, along with pH, other significant factors in determining pathogen disinfection rates are solar radiation, temperature, and dissolved oxygen levels (Curtis *et al.*, 1992, Davies-Colley *et al.*, 2000, Fallowfield *et al.*, 1996). These all vary throughout the pond.

Incorporation of these factors into more general algal ecology models has resulted in a more holistic model of parameter interaction. Authors such as Jorgensen and Gromiec (1985) and Kayombo *et al.* (1999) have suggested models in which more complete balances of biological interactions have been used to estimate species transformation rates. To date, attempts to apply such models to full scale WSP systems have been hampered by the scale of such systems and the lack of understanding of conditions throughout.

Incorporating a more holistic understanding of biological interactions into WSP models requires knowledge of pond conditions at a local level. The degree of non-linearity of these models makes treatment efficiency prediction under different hydraulic regimes very difficult. Using first order decay models, as noted above, the optimum pollutant reduction occurs under plug flow conditions (Levenspiel, 1999). However this does not take into account changes in biological efficiency outside the photic zone, or rate limitation due to limited

substrate diffusion rates. In high rate algal pond operation, peak efficiency is achieved by using complete mixing to maximise algal productivity and nutrient uptake (Fallowfield et al., 1996). Analogously, optimum WSP operation may also be achieved under a completely mixed flow regime, despite the sub-optimal residence time. Under these conditions all algal matter is exposed to non-inhibitory levels of radiation, and distribution of water quality through the pond is optimal.

In the context of more complex, non-linear models of biological optimisation, a more complicated model of operation provides more detail about the effects of local pond conditions (Sweeney et al., 2003). The results of computational fluid dynamics (CFD) simulations demonstrate the limitations of existing dispersion number correlations by showing the very large effect that a previously unconsidered factor (i.e. wind speed and direction) has on longitudinal dispersion in the pond. Furthermore, in the context of the spatial biological significance discussed earlier, the dispersion number cannot be used to estimate the consequences of a particular flow regime. While RTD analysis can predict that short-circuiting is occurring, information about the region through which the flow is occurring and the in situ biological conditions is lost. CFD simulations can restore insight into that lost area of understanding.

#### **1.5.7.15 Computational Fluid Dynamics**

More recently, increased computing power has seen the popularisation of CFD techniques and enabled the simulation of Residence Time Distribution (RTD) studies on ponds of any configuration or scale and under any physical conditions. Wood et al. (1995), Salter *et al.* (2000) and Shilton (2000) have demonstrated the ability of CFD RTD analysis to theoretically predict hydraulic short circuiting in operational WSP systems. Salter *et al.* (2000) and Shilton (2000) have also shown that first order time dependent decay models (Equation 1-14) can be integrated over the distribution area to quantify removal rates and potential improvements available from the simulated modifications. These predict final concentration  $N$  in terms of:

$$N = N_0 e^{-kt} \dots \dots \dots (1 - 14)$$

where  $k = f(\text{temperature})$

$N_0 = \text{inlet concentration}$

$t = \text{time}$

However the interaction between the biology and the hydraulics in the system is more complex.

#### **1.5.7.16 Summary of performance prediction and the design of WSPs.**

Much research effort has been expended in the pursuit of understanding WSP performance. Overall, it is clear that WSPs have individual nuances that make predicting their performance somewhat difficult.

### **1.5.8 The role of facultative ponds in the WSP system**

These ponds are of two types: primary facultative ponds that receive raw wastewater (after screening and grit removal) and secondary facultative ponds that receive settled wastewater from the primary stage (usually the anaerobic ponds effluent).

#### **1.5.8.1 Zones in facultative ponds**

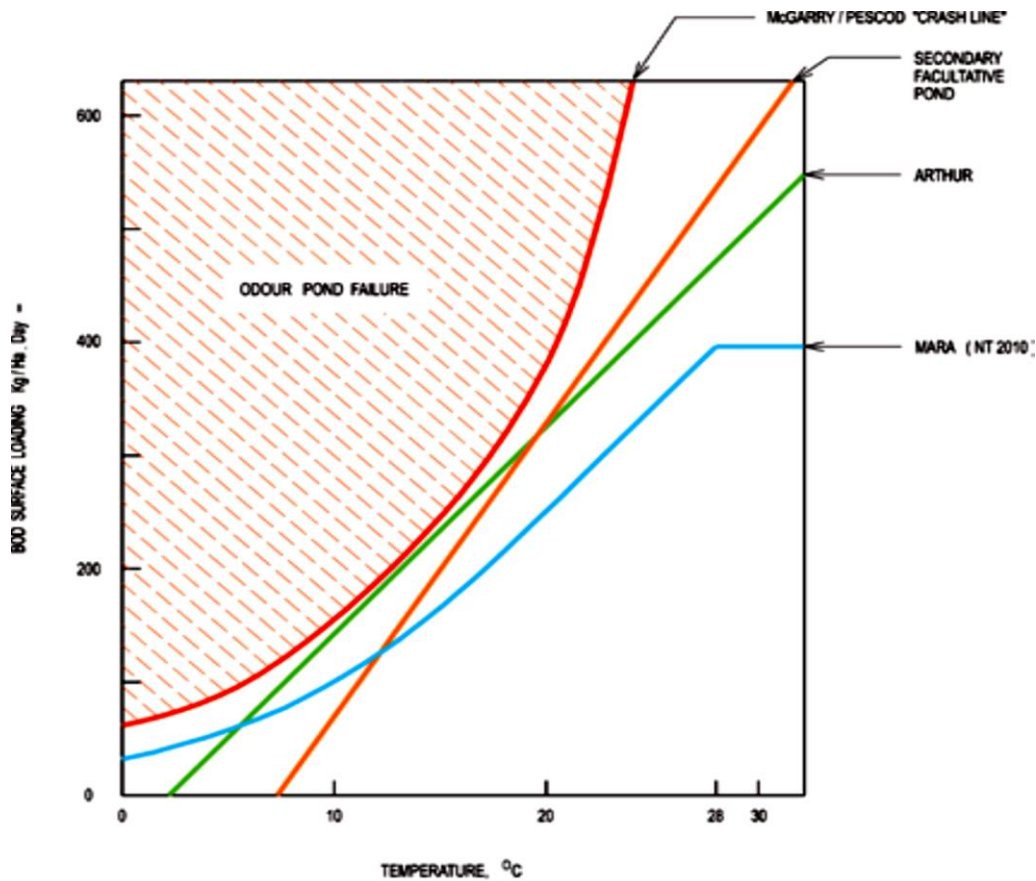
The treatment in facultative ponds occurs in three zones. In the upper, aerobic zone dissolved organic matter (soluble BOD) and finely particulate BOD is oxidised by aerobic respiration, with oxygen mainly supplied by the photosynthetic efforts of the algae growing in this zone. The suspended organic matter (particulate BOD) tends to settle to form the bottom sludge in the anaerobic zone. The sludge undergoes digestion by anaerobic organisms producing  $\text{CO}_2$ ,  $\text{CH}_4$  and a small amount of  $\text{H}_2\text{S}$  – which can cause malodour problems if the pond is overloaded to the point where no oxygen is available to oxidise the  $\text{H}_2\text{S}$  as it is released through the upper layers. After some time, the remaining sludge consists of the inert fraction only. Between these two zones there is a group of bacteria that can function in the presence and absence of oxygen called the facultative zone to achieve the oxidation of BOD (von Sperling,

2007). This zone is typically where the oxygen production by algae equals the oxygen consumption by algae and decomposing micro-organisms. This point is called the oxypause, and rises during the night when no photosynthesis occurs and falls during the day with algal photosynthesis.

#### **1.5.8.2 BOD removal from facultative ponds**

The depths of facultative ponds are usually in the range of 1–2 m, with 1.5 m being most common. Facultative ponds are designed for BOD<sub>5</sub> removal based on their "surface organic loading". The term refers to the quantity of organic matter, expressed in kilograms of BOD<sub>5</sub> per day (kg BOD<sub>5</sub>/ha/d). A relatively low surface organic loading is used (usually in the range of 80–400 kg BOD<sub>5</sub>/ha d, depending on the design temperature) to allow for the development of an active algal population. Depth, detention time and geometry (length/breadth ratio) are the other main design parameters.

The maintenance of a healthy algal population is very important as the algae generate the oxygen needed by bacteria to remove the BOD<sub>5</sub>. If the areal loading exceeds the algal oxygen generating capacity a pond 'failure' can be expected (see Figure 1-5). The algae give facultative ponds a dark green colour. Ponds may occasionally appear red or pink, due to the presence of anaerobic purple sulphide-oxidising photosynthetic bacteria (Mara and Pearson, 1986b). This change in facultative pond ecology occurs due to slight BOD<sub>5</sub> overloading, so colour changes in facultative ponds are a good qualitative indicator of pond function.



SOURCE : SAWYER et al (2002)

**Fig. 1-5 Facultative Ponds Areal Organic Loading Crash Lines as proposed by various authors (Power\_and\_Water\_Corporation, 2011)**

The concentration of algae in a well-functioning facultative pond depends on loading and temperature. It is usually in the range 500–1000  $\mu\text{g}$  chlorophyll-a/L (algal concentrations are best expressed in terms of the concentration of their principal photosynthetic pigment). The photosynthetic activity of the algae results in a diurnal variation of dissolved oxygen (DO) concentration and pH. The DO concentration can rise to more than 20 mg/L (i.e., highly supersaturated conditions) and the pH to more than 9.4 These are both important factors in the removal of faecal bacteria and viruses (Curtis et al., 1992).

BOD<sub>5</sub> removal in primary facultative ponds is about 70% on an unfiltered basis and more than 90 percent on a filtered basis (filtering the sample before BOD<sub>5</sub> analysis excludes the BOD<sub>5</sub> due to the algae in the sample; this "algal BOD<sub>5</sub>" is

very different in nature to ordinary wastewater BOD<sub>5</sub> or "non-algal BOD<sub>5</sub>"). Some regulators specify effluent BOD<sub>5</sub> requirements for WSPs in terms of filtered BOD<sub>5</sub> – for example, in the European Union WSP effluents are required to achieve ≤25 mg filtered BOD<sub>5</sub>/L (Council of the European Communities, 1991).

### **1.5.8.3 Thermal Stratification in Facultative Ponds**

Many factors may cause disturbances in the flow pattern of a pond with consequences to the actual treatment time. One of the factors is the thermal stratification, a natural phenomenon that is usually neglected in pond design. Stratification is best defined by the temperature distribution. Limnologists have divided the water by depth into three layers: (a) Epilimnion, the layer of isothermal water from the surface to the level where the temperature of the water begins to change rapidly with depth, (b) thermocline, the layer of water with rapidly changing temperature, and (c) hypolimnion, the layer of isothermal water between the thermocline and the bottom of the lake/pond. The hypolimnion water has higher density and viscosity than the epilimnion water.

In a stratified pond, non-motile algae settle into the dark zone of the pond, where they stop producing oxygen, and instead consume oxygen. Motile algae move down from the warmest surface layers (top 30 cm) and form a dense layer hindering the further penetration of solar radiation, leaving the upper layers relatively deprived of oxygen production and ability to stabilise nutrients (von Sperling, 2007).

The quality of water stored in each of the three layers varies with the season of the year. McEwen (1941) stated that, in general, a gradient of the concentration of any dissolved substance gives rise to forces tending by diffusion to equalize the concentration. Likewise, a temperature gradient tends to be eliminated by the resulting conduction of heat. However, such processes tending to produce a uniform distribution of heat and dissolved substances in still water are very slow (US\_Geological\_Survey, 1965).



The surface of the water is constantly undergoing changes in temperature because of the external agencies and the internal processes. These changes in temperature of the water surface are accompanied by corresponding changes in density. So long as the density of the water at the surface is less than that of the water below it, the water at the surface will remain in place. If the density becomes greater, however, particles of the water at the surface will descend until they reach a layer that is equal to their density.

The settling of particles of water due to an increase in density will cause a compensating upward displacement of warmer water. These particles of warmer water will move upward until they reach a layer of equal density. The vertical movement of particles of water that is caused by changes in density is considered to be a significant factor in the distribution of temperature with depth in a reservoir. The diffusion process of warmer water rising to the surface will continue as long as the temperature of the water is above 4°C, the temperature at which the density of water is at a maximum. When the temperature of the water is below 4°C, the colder water will float on the surface, and the warmer water will sink toward the bottom. The conduction of heat from the surface to the water below depends on the temperature gradient and the coefficient of thermal conductivity, which is approximately 0.0059 Watts per square centimetre multiplied by degrees Centigrade per centimetre for the normal range of water temperatures. Thus it can be deduced that the transfer of heat by conduction is slow.

According to Kellner and Pires (2002) thermal stratification is characterized by a high vertical thermal gradient, and is usually observed in deep lakes. However, although waste stabilization ponds have small depths, their high turbidity provides favourable conditions for the occurrence of this phenomenon, mainly during summer. During the warmer months, the layers nearest the surface concentrate a larger amount of thermal energy compared to the deeper layers, resulting in a temperature difference between the surface and the bottom of the pond. As a consequence a density profile appears, with the less dense

layers located at the surface of the pond and the densest layers lower down. This stratification in the water column induces alterations in the flow pattern and a decrease of the useful volume of the pond.

When a waste stabilization pond is thermally stratified, a density gradient exists and its internal vertical mixing is compromised (Chu and Soong, 1997). In this situation the pond behaves as a series of superimposed liquid layers with different densities, each layer being stable at a certain depth, with the densest layers close to the bottom.

The thermal stratification can be stable – persisting for months – or intermittent, appearing for a few hours in the day (Dor et al., 1993, Pedahzur et al., 1993, Torres et al., 1997, Sweeney et al., 2005).

As noted above, the main cause of thermal stratification in waste stabilization ponds is the heating of surface water by incident solar radiation; and in reverse destratification has been attributed mainly to the cooling of these surface layers.

#### *1.5.8.3.1 Effects of stratification*

Among the hydrodynamic and limnological problems that thermal stratification causes, the decrease in the volume of the active zone (useful volume), with consequences on the hydraulic detention time, is the main concern for design and operation of waste stabilization ponds. There are a number of reports in the literature describing stratification in very deep ponds (up to 8 metres deep), particularly in the South of Spain with a similar Mediterranean climate to South Australia (Llorens et al., 1992b, Torres et al., 1997).

Torres *et al.* (1997) studied the influence of the thermal stratification on the mixing efficiency of a pond located in the campus of the University of Murcia, south-east Spain. They found that during the winter, after the temperature of the surface layer had decreased, the active zone extended from the top to the bottom of the pond. During the summer, as a stable thermocline was formed,

the active zone extended from the surface to the depth where the effluent outlet was located. The volumes of the active zones were estimated as being 70% and 20% of the total volume of the pond during the winter and the summer, respectively. The consequence of this variation of the active volume is that the real hydraulic detention time varied from 70% to 20% of the theoretical hydraulic detention time. In a similar study, Moreno (1990), studied the hydraulic behaviour of a range of anaerobic (2.2 to 3.5m deep) and facultative ponds (1 to 2 m deep) spread across Spain and reported that the real hydraulic detention time varied from 10% to 42% of the theoretical value.

In these cases, it was found that in the cooler months, as long as there was no drop in atmospheric temperature to cool the upper layers of the water column, the active volume extended from the surface to the depth at which the outlet was situated. However, when the atmospheric temperature fell the active zone extended to the bottom of the pond. By contrast, in the hotter months, due to the formation of a stable thermocline, the active zone once again only extended from the surface to the outlet depth.

Although the dispersion number in waste stabilization ponds is influenced directly by the length-to-breadth ratio, the decrease in hydraulic detention time can mask its effects. In fact, Arceivala (1983) noted that some waste stabilization ponds located in hot climates had measured dispersion numbers greater than 4 when the bulk liquid was thermally stratified.

### **1.5.9 THE EFFECT OF WIND ON WSP PERFORMANCE**

The effect of wind in the distribution of temperature in the reservoir is considered an important factor in the diffusion process of vertical mixing. The frictional drag of winds upon the water surface results in a current directed with the winds. This upper layer in turn exerts a frictional drag upon the one underneath and so on to lower levels. Thus, particles of water are set in motion, and any tendency of the particles to move in the vertical directions is influenced by the different densities.

Horizontal currents, as induced by wind, cause the water to pile to the leeward side of the reservoir and to lower correspondingly at the windward side. When the density is equal throughout, a return gradient current due to this difference in pressure is generated in the deep strata, extending to a shearing zone near mid-depth which divides the water mass into two currents flowing in opposite directions. The horizontal currents are connected by one directed downward at the leeward side and upward at the windward side; thus a complete circuit is formed, and eventually the whole mass is thoroughly mixed.

The complete circulation of water in a reservoir as induced by the wind occurs only during the winter months or whenever the reservoir has an equal density throughout. For these periods of equal density, the lake water is overturned by the wind, and as the hypolimnion water is brought to the surface, a change in quality is easily detected. The complete overturn is usually noted in early and late winter for northern lakes. For southern lakes, one overturn can be detected in the late fall, and then the lake usually remains isothermal until the warming cycle is again resumed in the spring. During the other seasons, a circulation of this type is resisted by the different densities of water which restrict the circulation to the epilimnion and leave the thermocline and hypolimnion in a condition approaching stagnation. Also, the differences in viscosities corresponding to the water temperature contribute to the resistance to mixing (US\_Geological\_Survey, 1965).

Previous studies have demonstrated that significant variation in temperature can simultaneously exist throughout all three dimensions of large waste stabilisation ponds (Sweeney et al., 2002, Weatherell et al., 1999). This has important implications for the study of thermally induced hydraulic behaviour (e.g. stratified short-circuiting), and also for assessing the impact on expected treatment, given the established relationship between biological rate and temperature (Zhao and Zhang, 1991) in WSPs (Sweeney et al., 2005).

The US EPA (1983) established that wind generates a circulatory flow in bodies of water. To minimise short-circuiting due to wind, the pond inlet-outlet axis should be perpendicular to the prevailing wind direction (side wind). If for some reason the inlet-outlet axis cannot be orientated properly, baffling can be used to control, to some extent, the wind-induced circulation. It should be kept in mind that in a constant depth pond, the surface current is in the direction of the wind and the return flow is in the upwind direction along the bottom. These assertions were apparently based on limited field observations. There has been little systematic research during the past 20 years ago to prove this assumption.

There are many papers on the hydraulic behaviour of WSPs, of which only a few deal with the wind effect, and there are two distinct conclusions reached. One group considers that the wind is significant because it reduces the hydraulic performance by increasing short-circuiting, but it can improve the transverse mixing (Agunwumba, 1992, Sweeney et al., 2005).

Others consider that the wind is insignificant because the inertial force controlling the mixing is generated partially by the inlet discharge (Shilton, 1999). Within this group there are authors who conclude that wind effects on hydraulic efficiency are slight and that wind effects on dispersion and the overall residence time distribution are uncertain, but are probably significant. Based on studies of full-scale lagoons they suggested that the major effect of wind is to promote mixing and not reduction of Mean Hydraulic Retention Time (MHRT). According to Thackston *et al.* (1987) high wind-induced surface velocities and associated return underflows promote lateral and vertical mixing at the expense of low mixing and advective (short-circuiting plume) flow. Care is needed in interpreting this finding as Thackston's studies did not include analysis of flow paths under field conditions.

The published papers on hydrodynamic tracer studies and computational models are more extensive. Unfortunately, the majority of these did not consider the wind effect. As a result of the scarcity of reliable, well-documented

case studies the parameters which control mixing and hydraulic pathways in WSPs are not fully understood.

Wind action may play more than one role. Not only does it determine the velocity distribution and direction, but it also establishes the magnitude of the turbulence diffusion both in the surface layer and also lower down the liquid column (Banks, 1975). There is thus a need to know the impact varying wind directions and velocity distribution produces on MHRT. Aldana *et al.* (2010) demonstrated that even slight breezes can have very damaging effects on WSP performance.

Previous studies carried out by Aldana *et al.* (2004) examined the impact of wind on circulation patterns and suggested that the resulting flow paths are complex. A prototype lagoon at Lidsey in England with the prevailing wind blowing along the long axis opposite to the flow direction produced overturn circulation and significant short-circuiting during tracer studies. This resulted in an upward slope of 5 cm over the water surface in downwind direction (Bracho, 2003 ). Matthews *et al.* (1997) stated that wind-induced circulation patterns were prominent under low wind conditions, and wind speed and direction typically affected the results of the dye-tracing experiments, but they did not state the direction and velocity of wind.

Lloyd *et al.* (2003) demonstrated in a full-scale maturation pond at Ginebra, Colombia that the reduction of wind effects by wind breaks reduces mixing and hence dispersion, and significantly increases mean hydraulic retention time thus assisting in improving faecal coliform (FC) removal. They fenced the channel-maturation pond using a woven plastic wind break 2 m high to achieve these changes.

Arfi *et al.* (1993 ) also agree that particle re-suspension under certain conditions of fetch, wind velocity, bed roughness and bathymetry are induced by wind driven flow. They based their study in a shallow tropical lagoon (1m depth). The wind-induced surface currents are transmitted through an interface layer to the

bottom layer. This phenomenon was described by Chu and Soong (1997) as the entrainment law. As wind blows over the water surface the upper layer of the water body is mixed by the wind shear and deepens in the course of time as fluid from the lower moves in the opposite direction is entrained into the upper layer.

Since wind effects cannot be readily isolated in field studies, other methods such as calibrated computational fluid dynamic (CFD) (Guganesharajah, 2001) and physical models (Aldana, 2004) can be used to better understand the impact of wind on WSPs. These models including both laboratory, computational and pilot-scale ponds, allow tests to be conducted which would have been difficult with full scale WSPs. Guganesharajah (2001) used a calibrated model, HYDRO-3D, to define the mean hydraulic retention time (MHRT) distribution in WSPs including wind effects for a rectangular pond.

Sweeney et al (2002) used an uncalibrated computational model pond (FLUENT 5.5) for a trapezoidal pond. They simulated wind by using a shear stress equation and from the results of their simulation they concluded “the results indicate that a wind direction perpendicular to the direction of bulk flow (side wind) will produce the greatest degree of short-circuiting”. Although this contradicts the US EPA assertions, there is significant agreement with their FLUENT 5.5 model prediction and the age distribution profiles of HYDRO-3D. The two models agree that any increase in wind velocity reduces the mean hydraulic retention time of the pond and concurrently the delay time for tracer beginning to leave the pond is reduced.

### **1.5.10 THE ROLE OF MATURATION PONDS IN THE WSP SYSTEM**

Most of the discussion of design features of facultative ponds applies equally to maturation ponds. Maturation ponds are generally aerobic through their whole depth and their function is to remove excreted pathogens. The number and retention period (5-15 d) required in each are selected to satisfy faecal coliform (FC) discharge standards. Pathogen die-off is promoted by the high levels of pH

and dissolved oxygen generated in the ponds due to algal photosynthetic activity. Reductions of 4-6 log units of faecal coliforms, 2-4 log units for faecal viruses, and 100% removal of parasites are found (Mara et al., 1992).

### **1.5.11 HIGH RATE ALGAL PONDS (HRAPs)**

#### **1.5.11.1 Definition of HRAP**

HRAPs are an intensive biological wastewater treatment process which combines wastewater treatment, reclamation and algal biomass production (Oswald, 1972) (Shelef et al., 1980). In practice, HRAPs are shallow, mixed systems consisting of a series of interconnecting baffled channels. The process of mixing, using a paddlewheel to achieve a linear velocity of about  $0.2 \text{ ms}^{-1}$ , avoids thermal stratification and produces more a homogenous chemical environment throughout the pond. These conditions result in high rates of algal photosynthesis and consequently dissolved oxygen production. This leads to the potential for more rapid treatment ( $\text{BOD}_5$ , COD and nutrient removal) and /or higher organic loading rates (Fallowfield and Garrett, 1985a, Fallowfield et al., 2001, Cromar and Fallowfield, 1997a, Cromar et al., 1996).

#### **1.5.11.2 The History and Underlying Biology of High Rate Algal Ponds**

Green algae are classified with land plants under the Viridiplantae. This plant grouping includes a wide variety of organisms which differ greatly in cell organization, but all have the common characteristic of metabolic process initiation through photosynthesis. Algae differ from bacteria, fungi and protozoa in that the algal cell contains the light sensitive pigment chlorophyll and the biochemical mechanisms within the algal cell tend towards an



accumulation of organic materials rather than a breakdown of the organics (Helberger, 1964).

The capabilities that algae have for surviving in sewage environments have been known for some time. The alga, *Chlorella pyrenoidosa*, was isolated from sewage in 1903 by Chick (1903). Her research further revealed this organism's ability to metabolize ammonia and ammoniacal compounds. Based on these findings, Chick proposed that algae could possibly be effective as an active organism in the treatment of sewage polluted waters. Witt (1959) presents a list of many algal forms that can be found in sewage polluted waters. This list shows that the Chlorophyta are the most prevalent group of algae, while the Chlorococcales appear to be the dominant order of algae found in polluted waters. The occurrence of algae in waste stabilization ponds and their contribution to waste treatment in these facilities has been well documented. Golueke (1960) and Pipes (1961) have reported on the biological aspects of the symbiotic relationship between algae and bacteria in waste stabilization ponds.

The development of the HRAP began with the work of the team at UC Berkeley at their experimental plant at Richmond, California in the 1950's. At that time it was noted by Gotaas and Oswald (1954) that conventional secondary sewage treatment (either on trickling filters or by the activated sludge process) depended upon mechanical means to supply the oxygen necessary for bacteriological removal and stabilization of the organic material. For the preceding 25 years, engineers attempted to reduce the cost of providing this oxygen by using sewage oxidation ponds. These were customarily designed to detain the sewage long enough to allow sufficient oxygen for stabilization of the organic matter to enter by slow diffusion from the atmosphere.

Algae had often been observed near the outlet of oxidation ponds where the sewage was well oxidized, but at the time little information existed on their growth in such an environment, and considerable difference of opinion existed

as to whether algae would grow in relatively strong sewage or polluted waters in sufficient numbers to provide significant amounts of oxygen.

The possibility, that algae might supply oxygen to sewage less expensively or more efficiently than mechanical devices or diffusion can supply it from the atmosphere, led the Sanitary Engineering Research Laboratory of the University of California to initiate studies designed to determine whether algae could be effectively grown in sewage and other organic wastes and to explore the basic factors influencing algal growths on such mediums.

Work on the growth of algae was initiated at the University in 1950 with an investigation of the role of algae in sewage oxidation ponds (Ludwig et al., 1950). Until 1958, the pilot plant studies were concerned primarily with the development of design criteria for sewage treatment (Oswald et al., 1957); (Oswald et al., 1957). In 1958, the scope of the studies was expanded to include the production of algae (Oswald and Golueke, 1960).

Golueke et al. (1957) reported that in low-rate deep lagoons in which the approximate treatment rate was less than 11.3 mg of BOD<sub>5</sub> per litre per day, the disposal problem is either minor or non-existent, since algae were in concentrations rarely exceeding 20 mg/l. In high-rate shallow lagoons, sewage may be applied at several times the rate used in low-rate lagoons, and photosynthesis was practically the sole source of oxygen for aerobic biologic decomposition of wastes. Algal concentrations frequently reach 400 mg/l. Because of the high concentrations of algae characteristic of this process, suitable provision must be made for the ultimate disposal of the algal crop.

Their earliest published work focussed on WSP designs driven by the requirement to optimise the oxidation of BOD in wastewater (Oswald et al., 1955, Oswald et al., 1953, Oswald et al., 1957, Gotaas, 1948, Hermann and Gloyna, 1958). The earliest conceptions had a progenitor of a HRAP originally described as a small surface area oxidation pond (Type 2) with a low hydraulic

retention time requiring good algal growth to achieve the oxygen ratio necessary to oxidise influent BOD, see Figure 1. In 1963, Oswald (1963) published the first paper using the term High-Rate Pond, in which the ideas had progressed and matured to the point of requiring a separate descriptor, now commonly known as the High Rate Algal Pond (HRAP).

In the 1963 seminal paper, he offered the view that “The high-rate pond is a specialized form of waste stabilization system in which such objectives as nutrient recovery and waste water reclamation are added to the objective of sewage treatment which is normally accomplished in waste ponds.”

He noted that, “In a practical sense, the high-rate pond may be regarded as a specialized form of activated sludge plant in which the sludge is oxygenated through photosynthesis rather than through atmospheric re-aeration. In a conventional activated sludge plant, bacterial sludge is mixed and aerated with incoming sewage. The sludge absorbs nutrients and grows. Later in the process sludge is separated from the supernatant and discharged either into a digester or remains indefinitely in the aeration chamber. The supernatant, usually chlorinated, may be discharged from the plant. In the digester, sludge is decomposed anaerobically to methane, carbon dioxide, and other gases, and to a residue which, when drawn from the digester some 30 days later, is in the form of stable humus. This humus has some crude values as a soil conditioner.

When the sludge remains indefinitely in the aeration chamber, it undergoes aerobic oxidation within a time that is proportional to its stability. Sugars and proteins are decomposed in a matter of hours, whereas cellulose and lignin may require days and months, respectively. In any case, the sludge attains a relatively constant concentration in the aeration tank. The magnitude of sludge concentration reflects a balance between the rate of nutrient application and the rate of nutrient decomposition. Such an activated sludge plant is sometimes called a total oxidation plant.”

Oswald's concepts are embodied in Fig. 1-6, which depicts waste organic matter entering a cycle containing two groups of microorganisms, aerobic bacteria (as sludge) and micro algae. The bacteria oxidize the entering wastes and produce more sludge, carbon dioxide, and ammonia. The sludge returns to the system when the bacteria die, but carbon dioxide, ammonia, and other decomposition products are taken up by algae which in the presence of sunlight photosynthesize producing oxygen and more algal cells. Due to the close physical association of algae and bacteria within flocs (Cromar, 1994), oxygen is used immediately for bacterial oxidation, while excess algae are discharged suspended in a supernatant that is essentially exhausted of bacterial nutrients. Depletion of bacterial nutrients is one of the primary objectives of waste disposal, and when such nutrient depletion is accomplished the waste is said to be stable.

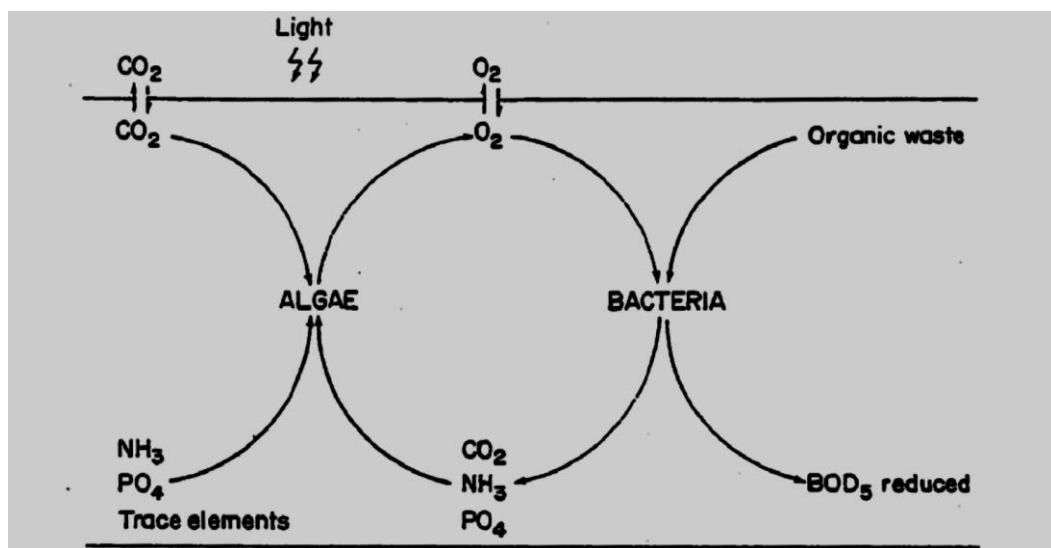


Figure 1-6 The major process occurring within an algal – bacterial wastewater treatment system (Fallowfield and Garrett, 1985a, Oswald, 1963, Oswald et al., 1957)

The high-rate pond, which is also a total oxidation plant, is designed and operated in such a manner that the single vessel (the pond) serves as a primary sedimentation tank, an oxygen generation plant, an activation and aeration chamber, and a final sedimentation chamber. In the design process, each

important operational feature of high-rate ponds is selected rationally to accomplish these specific processes economically. The major design features are detention period, depth, and mixing.

### **1.5.11.3 Mixing in the HRAP**

Mixing in the HRAP is the single largest factor distinguishing HRAPs from WSPs. The view expressed by Oswald in 1963 was that the objectives of controlled mixing were to aerate the sludge, control pH and dissolved oxygen, and thereby improve the algal-bacterial symbiosis. This rather limited perspective has been expanded as understanding has expanded to include exposure of both algae and pathogenic organisms to solar radiation on a regular basis.

At that time field studies had shown that bacterial sludge develops and settles rapidly in sewage after it enters the oxygen-rich environment in the conventional WSP. As is seen ubiquitously in facultative ponds (and to a lesser extent in maturation ponds) if the settled sludge is permitted to remain at the pond bottom for more than a day, it becomes anaerobic and malodorous. Mixing is the only obvious way to aerate such sludge.

Oswald deemed the most effective method of mixing was to move the entire body of liquid as a flowing stream through the use of a propeller or air-lift pumps in a baffled channel. The required flow velocity had to reach 1 to 1.5 feet per second (0.3 to 0.46 metres per second). More recent investigators have refined and standardised this velocity to between 0.1 and 0.3 metres per second, with 0.2 metres per second being commonly quoted (Dodd, 1986, Mihalyfalvy et al., 1998, Fallowfield and Garrett, 1985a, Picot et al., 1992, Oron and Shelef, 1982).

Interestingly, Oswald noted that some experimental results had shown that continuous mixing of a high-rate pond was almost as disastrous as no mixing at all. The reasoning given was that the turbidity imparted by the suspended sludge in continuous mixing shuts out light and halts photosynthetic oxygenation. In this event, a continuously mixed high-rate pond would become

a relatively inefficient but low-cost activated sludge pond. The conclusion was that it was best to mix a pond for approximately two or three hours each day. A program of mixing for a period of 2 to 4 hours beginning at midnight and again for a half-hour period beginning at 1:00 P.M. was indicated (Oswald and Golueke, 1960).

This conclusion was reinforced by Abeliovich (1980) who reported that the algae in the HRAP he was monitoring in Jerusalem were infected with a fungus of the *Chytridium spp.* By way of remediating the problem, they found the fungus could not complete its life cycle if they turned off the paddlewheel and allowed anaerobic conditions to develop for a few hours overnight. This technique prevented mass algal mortality from occurring. It is safe to say that all recent papers report experiments conducted in HRAPs undergoing continuous mixing with no serious adverse effects reported.

#### 1.5.11.4 Detention Period

Following Oswald's line of reasoning re HRAP design, the detention time is the average length of time a particle of liquid remains within a pond. The detention period for a high-rate pond is selected to permit accomplishment of one or all of the ponding objectives. When the pond objective is BOD removal, the problem is to provide sufficient time for oxygen production by algae to equal the oxygen demand of the waste. Oxygen production by algae is a function of the available light and the efficiency with which it is utilized, thus

$$W_{O_2} = KFS \dots \dots \dots (1 - 18)$$

Where  $W_{O_2}$  = weight of oxygen produced per unit of area daily.

F = percent of light energy conversion (whole number), and

S = quantity of sunlight energy available per unit area (solar energy flux).

K = constant

The values of S can be sourced from various tables for various seasons and latitudes, for example (Oswald et al., 1957).

Inasmuch as total oxidation of a waste is desired, it is essential to produce enough oxygen to meet the ultimate BOD of applied wastes on a continuing basis. The daily load of ultimate BOD on a pond is derived in Equation 1-19.

$$W_{O_2} = \frac{K_2 d L}{t} \dots \dots \dots (1 - 19)$$

Where,                      d = pond depth (m)  
                                   L = BOD load (mg/L)  
                                   t = retention time (d)

By equating oxygen produced to oxygen required, an approximation can be made of

$$t = \frac{d L}{F S} \dots \dots \dots (1 - 20)$$

Where, all of the terms were as previously defined.

### 1.5.11.5 HRAP Depth

If the HRAP is being designed to remove BOD by photosynthetically produced oxygen, the pond depth should be selected on the basis of availability of light to algae, which is determined mainly by intensity and the distance that sunlight penetrates into an algal culture.

Many studies have shown that light penetration follows the Beer-Lambert Law (Equation 1-21) (Falkowski and Raven, 2007), thus

$$d = \frac{(\ln I_0 - \ln I_d)}{C_a \alpha} \dots \dots \dots (1 - 21)$$

where,                       $I_0$  = light intensity at the pond surface ( $W/m^2$ ),  
                                    $I_d$  = light intensity at depth d, ( $W/m^2$ ),

$C_a$  is the concentration of algae (mg/l), and

$\alpha$  = is an absorption coefficient.

To design a pond on this basis, the values of sewage BOD and the amount of sunlight energy must be known or estimated. Furthermore, laboratory studies have shown that algae and oxygen are produced in a relatively constant weight proportion, as shown in Equation 1-22

$$O_2 = 1.67C_a \text{ (approx) } \dots \dots \dots (1 - 22)$$

where  $O_2$  and  $C_a$  are expressed in the same units.

Thus, if the ultimate BOD to be satisfied is known, a value may be found for  $C_a$  from Equation 1-22. If the value of  $I_0$  is considered to be  $158 \text{ W/m}^2$ , and the value of  $I_d$  is selected on the basis of prior knowledge regarding the light intensity at which photosynthesis compensates respiration – usually at about  $1.5 \text{ W m}^{-2}$ , Equation 1-21 may then be solved for  $d$ , which may, in turn, be substituted in Equations 1-20.

In 1973, Oswald (1973) explored the algal production potential from sewage and proposed Equation 1-23 as a design/productivity predictor. The method is based on estimating the algal concentration  $C_a$ , in milligrams per litre, as a linear function of the solar radiation  $S$ , and of a performance parameter given by the ratio between the retention time and effluent depth:

$$C_a = 0.1 \times \frac{F S}{h} \times \frac{\theta}{d} \dots \dots \dots (1 - 23)$$

where  $\theta$  = retention time (d)

$d$  = effluent depth, (m)

$F$  = photosynthetic efficiency, as a percentage (a 4% value is usually assumed for all conditions);

$S$  = solar energy flux, ( $\text{W/m}^2/\text{d}$ )



$h$  = heat of combustion of the algae,

As can be seen from Equation 1-23, the algal concentration  $C_a$  can be given in a general form as

$$C_a = \alpha_w x \dots \dots \dots (1 - 24)$$

where  $x$  = an independent variable,  $\theta / d$ , expressing the operational regime of the HRAP; and

$\alpha_w$  = a coefficient depending on the major environmental conditions as expressed by the

$S$  = solar energy flux (W/m<sup>2</sup>/d)

$h$  = energy status of the algal biomass. (W/kg)

However, this method was soon noted by Oron & Shelef (1982) to be inadequate for maximising algal yields as the linear expression for algal concentration does not consider the ambient temperature, and it assumes the photosynthetic efficiency is the same throughout the year. To correct for these deficiencies these authors proposed Equation 1-25

$$C_a = a \left( \frac{\theta}{d} \right)^\alpha S^\beta T^\gamma \dots \dots \dots (1 - 25)$$

And thence the algal productivity ( $\gamma$ ) by multiplying  $C_a$  by  $\frac{d}{\theta}$

where  $S$  = solar energy flux (W/m<sup>2</sup>/d)

$T$  = ambient temperature

$a, \alpha, \beta, \gamma$  = constants

Under the prevailing conditions in Israel, they determined values for the four coefficients  $a, \alpha, \beta$  and  $\gamma$  experimentally for each of the four seasons. They also reasoned that the detention time should not be less than 1.8 days, which is the minimal generational time span for the algae, or more than 8 days, as there

will be subsequent nutrient depletion. They also reasoned that for practical reasons the pond depth should not be under 0.2 m or over 0.9 m, the latter due to attenuation of light hindering algal growth. Their recommendation is that the ratio  $\frac{\theta}{d}$  should fall in the range of 6 to 12.

The practical outcome of this approximation is that if BOD removal is the target then for ponds operated at 0.32 m, the retention time should be between 1.92 and 3.84 days. For HRAPs operated at 0.42 m depth, the retention time should be between 2.52 and 5.04 days, and for HRAPs operated at 0.55 m depth, the retention time should be between 3.3 and 6.6 days.

#### **1.5.11.6 Balancing oxygen production and consumption in HRAPs**

Successful wastewater treatment and biomass production depends upon establishing an equilibrium between algal oxygen production and bacterial oxygen consumption. This equilibrium, together with the relative composition of the biomass may be controlled via the organic carbon loading rate (Cromar and Fallowfield, 1997b). Mineralisation of organic carbon provides inorganic carbon for algal photosynthesis this, together with the close physical association of algae and bacteria within flocs, reduces the potential for carbon dioxide limitation on algal photosynthesis (Schiefer and Caldwell, 1982). In addition to the mineralisation of organic carbon the maintenance of aerobic conditions within an HRAP also favour nitrification (Cromar et al., 1996).

### **1.5.12 ALGAL GROWTH**

#### **1.5.12.1 Reporting Conventions**

The term “growth rate” is not used consistently in the literature. It may be used casually to refer to biomass productivity, which is expressed as mass per unit area (or volume) per unit of time. In a continuously harvested system at steady state (or a semi-continuous system at near-steady state), the harvest rate, the

dilution rate (i.e. introduction of medium) and mean biomass productivity are approximately equal. “Growth rate” is also used to describe the exponential or logarithmic growth constant, which is the natural logarithm of the ratio between the number of cells (or other measure such as mass, volume, or optical density) at the end of a unit of time (e.g. a day) to that at the beginning of the time period ( $\ln [N_{t_1}/N_{t_0}]$ ). If the number of cells doubles in one day, the growth constant ( $k$ ) would be  $\ln [2/1]$  or 0.69. Other investigators use “growth rate” to mean the number of doublings in biomass per unit of time (e.g., doublings per day). The reciprocal, referred to as doubling time or generation time (time to achieve one doubling), is also a common expression. Most algae exhibit 1 to 2 doublings per day ( $k = 0.69$  to  $1.39$ ,  $T_d = 0.5$  to  $1.0$  (Burlew, 1953) (Sheehan et al., 1998) (Griffiths and Harrison, 2009).

#### **1.5.12.2 Algal Productivity vs Biomass Productivity**

The biomass produced in a HRAP is a complex mixture of green algae, aerobic bacteria, zooplankton (small quantities of rotifers, protozoans, amoebae and fungi) and detritus resulting in the term ALBAZOD being proposed by Carl Soeder (1980). The development of separation techniques (Cromar and Fallowfield, 1992) enabled quantification and determination of the influence of operational and environmental parameters on the relative composition of the biomass (Cromar et al., 1992).

Production of this biomass is through either, aerobic degradation of organic substances through bacterial respiration and new bacterial cell synthesis, or photosynthesis leading to new algal cell formation.

Algal productivity describes the mass of algae produced per day as a function of either area or volume. This may also be referred to as yield, although technically, yield takes into account harvesting parameters in addition to growth parameters. The overwhelming factor that controls biomass productivity is the area illuminated. The same productivity per unit area can be obtained with any combination of volume, depth, and concentration as long as the depth and/or

concentration are enough for optical extinction to occur (Burlew, 1953). Similar results were found in the SERI/NREL studies (Sheehan et al., 1998). In mass cultivation systems (especially flat ponds), algal productivity is typically measured in terms of mass per unit area ( $\text{g}/\text{m}^2/\text{d}$ ), as the surface area of the water is assumed to be equal to the area illuminated.

#### **1.5.12.3 Importance of Algal Growth in HRAPs**

As noted throughout this section, algal growth is integral to the performance of a High Rate Algal Pond, and needs to be understood as one of the key operational parameters. Through their production of photosynthetic oxygen, the algae create an environment enriched to the point of super-saturation in oxygen. This oxygen is then utilised by the aerobic bacterial population to “stabilise” the carbon material coming in and thus reduce the BOD levels. The concurrent use of  $\text{CO}_2$  in photosynthesis creates an alkaline environment that helps remove (along with the solar radiation) many of the harmful bacteria.

#### **1.5.12.4 Control of Algal Growth Rate**

Goldman (1979) summarised the important environmental parameters influencing algal growth rates ( $\mu$ ) such as light intensity (I), temperature (T), nutrients (S) and pH. The response to these parameters are distinctly species specific. The response to the three main parameters of nutrients, light intensity and temperature can be quantified by examining the shape of the response curve to each parameter with the other two held constant as in figure 1-7.

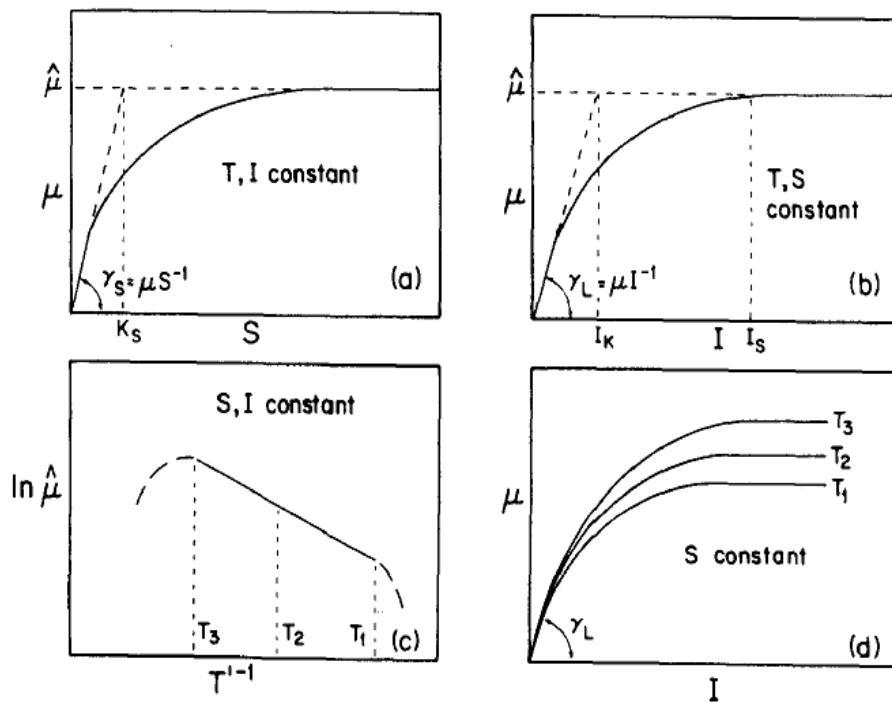
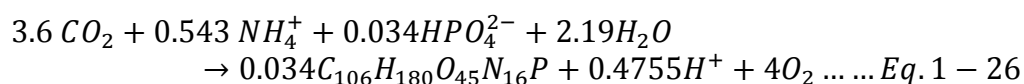


Fig. 1-7 General relationship between algal growth rates ( $\mu$ ) and environmental parameters (a.) limiting nutrient ( $S$ ), (b.) light intensity ( $I$ ), (c.) temperature ( $T$ ) and (d.) light intensity for varying temperatures. Adapted from (Goldman, 1979)

### 1.5.12.5 Modelling Algal Growth Rates and Biomass productivity

After Oswald produced his algal productivity model in 1973, (Equation 1-23) there have been many other authors with increasingly complex models.

Hill and Lincoln (1981) produced a mathematical model to describe the conditions for algal growth. In this model, algae are considered to require four substrates  $PO_4\text{-P}$ ,  $NH_4\text{-N}$ ,  $CO_2$  and light; all of which could therefore limit growth. The substrates were amalgamated into a single simplified overall equation (Eq. 1) describing the final algal cell constituents in terms of the Redfield ratio of  $C_{106}H_{180}O_{45}N_{16}P$ .



The influence of each of the substrates on growth kinetics is first calculated by a Monod function and then combined into an overall specific growth rate

equation for the algae. The basic Monod equation used for each individual growth substrate was

$$SUB^{\mu} = \frac{SUB^{\hat{\mu}} \times [SUB]}{SUB^{KS} + [SUB]} \dots \dots \dots Eq. 1 - 27$$

where:

$SUB^{\mu}$  = specific growth rate ( $day^{-1}$ )

$[SUB]$  = concentration of substrate (moles litre $^{-1}$ )

$SUB^{KS}$  = 'half-velocity' constant (moles litre $^{-1}$ )

$SUB^{\ddot{\mu}}$  = maximum specific growth rate ( $day^{-1}$ ).

All the authors noted above also treated radiation as a substrate, its influence on growth is introduced by the use of equation 1-28:

$$RAD^{\mu} = \frac{RAD^{\hat{\mu}} \times [RAD]}{RAD^{KS} + [RAD]} \dots \dots \dots Eq. 1 - 28$$

$RAD^{\mu}$  = specific growth rate as influenced by radiation ( $day^{-1}$ )

$RAD^{\ddot{\mu}}$  = maximum specific growth rate ( $day^{-1}$ )

$[RAD]$  = incident solar radiation (Cal/cm $^2$ /min)

$RAD^{KS}$  = 'half-velocity' constant (Cal/cm $^2$ /min).

In developing their model of algal growth rate Hill and Lincoln (1981) determined the 'half-velocity constants'  $K_s$  at 25° C for CO $_2$  as 0.103 mg C /L, NH $_4$  as 1.0 mg N /L, PO $_4$  as 0.3229 mg P /L and total solar radiation as 723 W/m $^2$ . The latter value for total solar radiation is well above other reports in the literature. For example, it is an order of magnitude higher than that reported by Myers (2009) for photosynthetic saturation by light intensity of 500 footcandles (21.8 W/m $^2$ ). Mihalyfalvy et al. (1998) report photoinhibition beginning at 65.7 W/m $^2$ , suggesting their 'half-velocity' constant would be about 33 W/m $^2$ . It seems most likely that a true 'half-velocity' constant for irradiance would be in the vicinity of 30 W/m $^2$ . Using these constants Hill and Lincoln were able to predict algal production within 5% of actual production. They noted that to obtain this predictive accuracy, there was considerable deviation from

theoretical stoichiometry, probably due to the complex algal, bacterial interplay in the wastewater systems.

Grobbelaar (1991) noted that algal growth is influenced by many factors, such as the supply of nutrients, CO<sub>2</sub>, temperature, light and turbulence. However the previous year, Grobbelaar et al. (1990) produced a model that accepts only two input variables, temperature and light energy. They wrote this in a generalized form as:

$$\text{PROD (mg(dry wt)/m}^2\text{/h)} = \text{PRD} - \text{RES} - \text{INB} \dots \dots \dots (1-29)$$

where        PROD = 'productivity',  
                  PRD = 'gross productivity',  
                  RES = 'respiration', and  
                  INB = 'photo-inhibition'.

They calculated the PRD component from inputs of biomass concentration present in the culture, culture temperature, and light impinging on the surface of the culture. This component becomes zero when no light is present and is given by the following equation:

$$\text{PRD} = (A_1 X_1 (A_2^T)) \left( \frac{I_z I_s (A_3^T)}{I_z + I_s (A_3^T)} \right) \dots \dots \dots (1-30)$$

where        A<sub>1</sub>-A<sub>3</sub> are constants,  
                  X<sub>1</sub> is the biomass concentration in mg (dry wt)/litre,  
                  I<sub>z</sub> is the irradiance in Einst./m<sup>2</sup>/h at the depth z in meters,  
                  I<sub>s</sub> is the light half saturation constant in Einst./m<sup>2</sup>/h, and  
                  T is a temperature factor calculated from

$$T = (T_t - 10) / 10 \dots \dots \dots (1-31)$$

where        T<sub>t</sub> is the measured temperature in °C.

Equation (1-30) has two components, i.e. a temperature/biomass term which could be regarded as the temperature response of growth and a second component which is the light/temperature response of growth.

The constant  $A_1$  can be interpreted as the efficiency of light utilization,  $A_2$  as the  $Q_{10}$  of photosynthesis,  $A_3$  as the  $Q_{10}$  for light half saturation and the factor  $I_s(A_3)$  as the temperature dependence of  $I_s$  (light half saturation constant) in the P versus I curves of photosynthesis) The component RES is interpreted as a total loss factor, i.e. losses due to respiration and exudation of organic compounds from the cells, and is calculated from the following equation:

$$RES = X_1((1.5^T - 0.54)/100) \dots\dots\dots(1-32)$$

RES increases exponentially with an increase in temperature. This loss factor continues unabated in the light and dark and the reassimilation of produced  $CO_2$  and excreted organics are not considered. It should be noted that RES does not take grazing by invertebrates or losses due to parasite attacks into consideration.

### 1.5.13 Limitations to Algal Growth

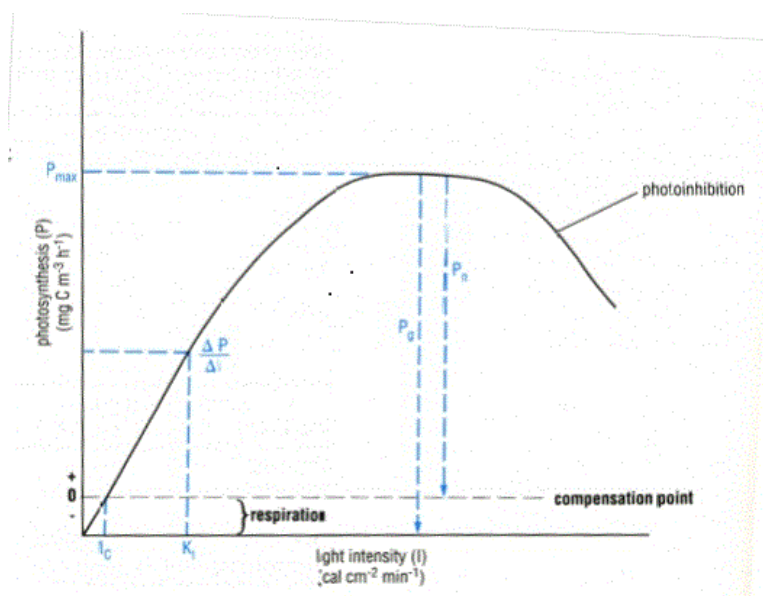
#### 1.5.13.1 Photo-inhibition limitations to Algal Growth

Oswald (1985) stated that the plant chloroplast is a photochemical system which works efficiently only at low light intensity. Photo-inhibition is a well-documented phenomenon that starts to operate from as little as 10% of natural sunlight. (Fig. 1-8) and depends on the intensity, quality and duration of irradiance.

Ratchford and Fallowfield (2002), (Ratchford and Fallowfield, 2003) showed that with both *Chlorella vulgaris* and *Synechococcus* the onset of photoinhibition occurred at irradiances  $>300 \mu\text{mol}/\text{m}^2/\text{s}$  ( $65.7 \text{ W}/\text{m}^2$ ) at temperatures  $>15^\circ\text{C}$ .



They showed that oxygen evolution decreased rapidly when cells were continuously irradiated at 65.7, 109.5 & 164 W/m<sup>2</sup>. However *Chlorella vulgaris* irradiated at the same irradiances on a light:dark cycle of 60s:20s, 30s:60s, and 60s:120s respectively maintained a constant rate of oxygen evolution over a 24 hour incubation period suggesting that the adverse effects of photoinhibition could be ameliorated by periods in the dark. Exposure time rather than the total light dose appeared to determine the effect of light:dark cycle times on photosynthesis. Mixing in HRAP's creates turbulent flow offering the potential of moving algae in and out of the 'light zone' and 'self-shaded zone' and therefore improving total algal productivity. However, these data point to the probability that photoinhibition is still an issue despite the potential for light dark cycling in the HRAP.



**Fig. 1-8 stylised representation of a P-I (photosynthesis-irradiance) curve, demonstrating the calculation of parameters such as  $K_{max}$  (notated as  $P_{max}$  on the y axis), half-velocity constant ( $K_i$  on the x axis) and photoinhibition.**

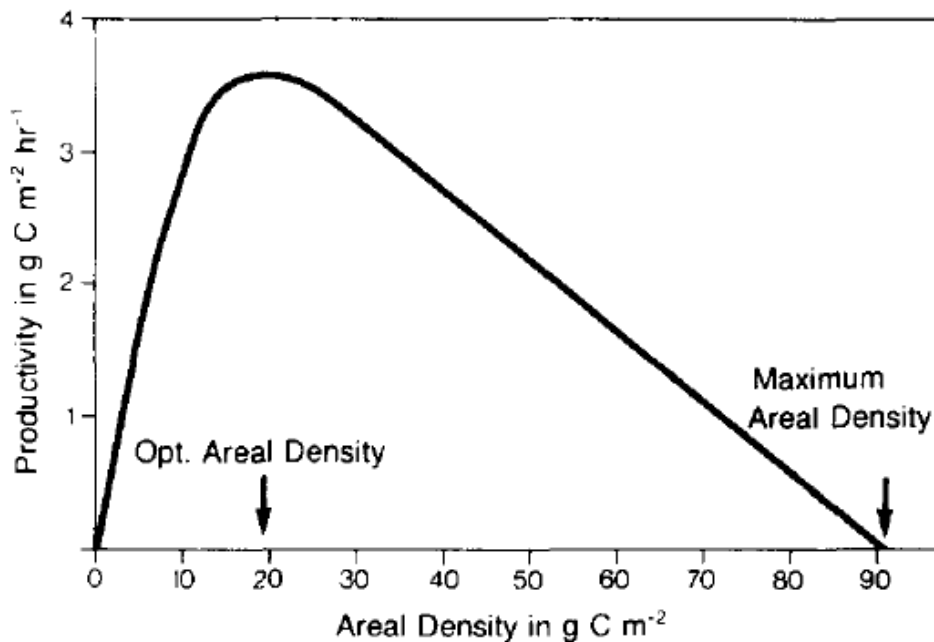
Sorokin and Krauss (1958) have shown that photo-inhibition is also temperature-dependent. Equation 1-33 describes the magnitude of photo-inhibition in the Grobbelaar et al. (1990) model:

$$INB = PRD((2.5^T/75) I_z) \dots\dots\dots(1-33)$$

Eq. 1-30 suggests that photo-inhibition increases linearly with increases in irradiance, but that the overall rate is determined by temperature.

### 1.5.13.2 Areal density limitations to Algal Growth

Soeder (1980) was the first to suggest that the areal density of a culture would be important in determining overall productivity and he suggested that cultures should be operated at areal densities of 50 to 150 g(dm)/m<sup>2</sup> for the optimal exploitation of incident solar radiation. Using a mathematical model which predicts algal productivity from input variables of light energy, temperature and biomass concentration, Grobbelaar (1981) determined the optimal areal density for average conditions in outdoor mass algal cultures to be about 40 to 45 g(dm)/m<sup>2</sup>. Vonshak et al. (1982) determined the biomass yield of *Spirulina platensis* as affected by the population density and season, under outdoor conditions. According to them the highest output rate was achieved at biomass optical densities (540 nm) of 0.3 to 0.35 (this represents an areal density of approximately 52 to 75 g (dm)/m<sup>2</sup>). (Hartig et al., 1988)



**Fig. 1-9 Productivity against areal density as calculated by the Grobbelaar et al. (1990) model for temperatures ranging from 5 to 35°C and irradiances from 0 to 8 Einst. /m<sup>2</sup>/h.**

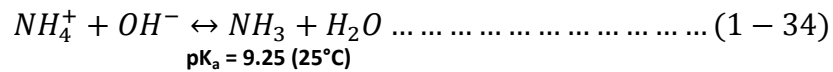
### **1.5.13.3 Possible sunlight limitation to algal biomass production**

During photosynthesis and growth, there are concurrent respiration and cell death occurring. Dark respiration and photorespiration are not mutually exclusive in algae. At low light intensity dark respiration is relatively more important than photorespiration, but with increasing light and oxygen and decreasing carbon dioxide, photorespiration increases. Thus photorespiration is probably more important in HRAPs. The point at which the combined effect of these influences equals the photosynthetic growth rate is called the compensation point, and this point varies widely with environmental conditions. At the far end of the growth curve there is a significant tapering due to the effects of photoinhibition, which may start at as little as 10% of natural sunlight (Goldman, 1979). Light energy is dissipated over the full depth of a HRAP so an individual algal cell sees varying light intensities, therefore its instantaneous specific growth varies depending on its vertical position.

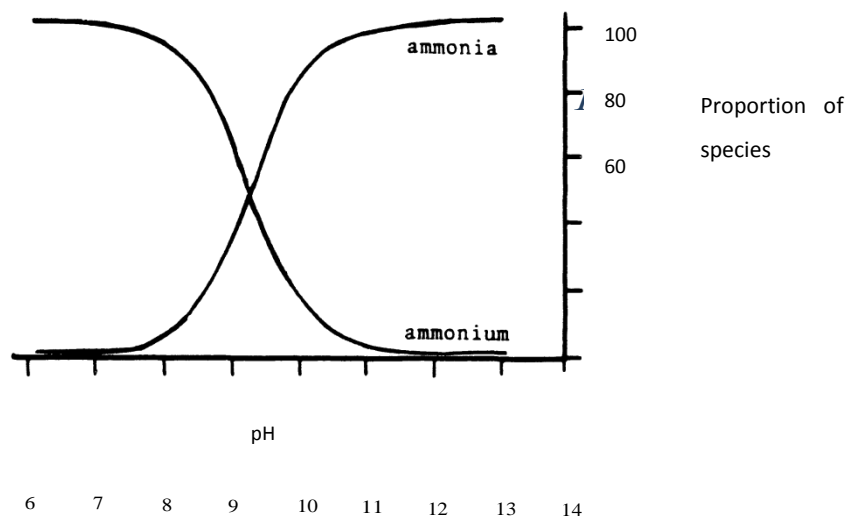
### **1.5.13.4 Possible Ammonia toxicity to algae**

Some authors have noted sudden temporary algal pulses and die-offs as a repeated and consistent pattern in algal ponds, and have speculated on the possible cause(s) (van der Post and Toerien, 1974, Shillinglaw and Pieterse, 1977, Shillinglaw and Pieterse, 1980, Garcia et al., 2000). Whilst unable to reach definitive conclusions on the cause of these periods of waxing and waning, some common features emerged. Usually, only one species was involved in a growth pulse and this species also died off to a greater extent in the following crash. As predator numbers are unlikely to reach massive proportions in the short time frames involved it is thought that predation by zooplankton or other grazers like *Daphnia* spp. was unlikely to be the cause of crashes. Toxic pond conditions

were thought to be the most likely source of the crashes. The most likely toxin being free ammonia, which becomes the dominant form in ponds of high pH as described in Equation 1-31 and Fig. 1-10. Shillinglaw and Pieterse (1977) were unable to rule out other endogenous or exogenous toxins, but they appear less likely.



Because the equilibrium in Equation 1-34 is shifted towards increased NH<sub>3</sub> formation with increasing temperature, the possibility of NH<sub>3</sub> toxicity in outdoor ponds is magnified considerably during the summer. For example, at 25°C only one third of the total ammonia is required to produce the same free NH<sub>3</sub> as at 10°C.



**Fig 1-10. Relative proportion of ammonia and ammonium ion as a function of pH at 25°C (Adapted from (Konig et al., 1987)).**

Abeliovich and Azov (1976) concluded that the impact of ammonia on the algal cell was probably due to inhibition of photosynthesis and that ionised ammonium was unable to penetrate across the cell membrane. Hence the inhibitory effect is pH dependant in the sense of creating more free ammonia to

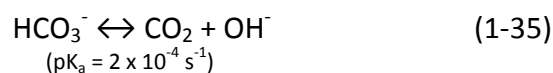
enter the algal cell and inhibit photosynthesis. The concentration at which ammonia becomes toxic varies greatly with individual species and pond growth conditions (Tam and Wong, 1996). For example Przytocka-Jusiak (1976) reported 50% and 100% inhibition of *Chlorella vulgaris* cell growth at 330 mg NH<sub>3</sub>-N/L and 700 mg NH<sub>3</sub>-N/L at pH 8-9. König et al. (1987) also showed that both *Chlorella* and *Euglena* exhibited no ammonia toxicity at 560 mg NH<sub>3</sub>-N/L at pH 6.8 (100% ammonium ion). In this study *Euglena* grew well at 17 mg NH<sub>3</sub>-N/L and pH 9.0, but was completely inhibited with 170 mg NH<sub>3</sub>-N/L and pH 9.0.

Additionally, Azov and Goldman (1982) demonstrated a 50% and 90% inhibition of *Scenedesmus obliquus* photosynthesis at 34 and 51 mg NH<sub>3</sub>-N/L at pH 9.5 and 20 – 25°C. These reports would suggest that some species of sewage-associated algae, such as *Scenedesmus*, are sensitive to the levels of ammonia and pH often encountered in HRAPs; others such as *Euglena* are tolerant of higher ammonia levels and *Chlorella* would not be affected by the levels found in these ponds.

#### 1.5.13.5 Possible Carbon limitation to algal growth

For some considerable time, carbon has been suspected of being a growth limiting factor in HRAPs treating wastewater, due to the high algal demand for it, whilst its concentration and bio-availability to algae is relatively low compared to other nutrients (Azov et al., 1982b).

According to Azov et al. (1982b), about 48% of the incoming carbon will be in an inorganic form and 52% in organic form. The form of carbon preferred by most algal species for photosynthesis is unionised, dissolved CO<sub>2</sub>. In the HRAP this will mostly come from daytime bacterial respiration. The degradation of bacterial biomass releases the main nutrients NH<sub>3</sub> and CO<sub>2</sub> for algal photosynthesis (Azov et al., 1982b). At high pH values free CO<sub>2</sub> is derived from bicarbonate according to Equation 1-35.



This is quite a slow reaction rate, but has been calculated to proceed fast enough to supply CO<sub>2</sub> demand for algal photosynthesis in alkaline HRAP wastewater. Azov et. al.(1982b) determined that the conditions under which carbon could become limiting to algal productivity were low inlet water organic carbon, high algal concentrations when the inlet water has low alkalinity and long retention times.

Goldman et. al. (1974) identified the Monod model as the most successful kinetic model for identifying limiting nutrients for algal growth, as defined in Equation 1-36.

$$\mu = \hat{\mu} \left[ \frac{S}{K_s + S} \right] \dots \dots \dots (1 - 36)$$

Where

$\mu$  = specific growth rate, d<sup>-1</sup>

$\hat{\mu}$  = maximum specific growth rate, 1/d

$S$  = limiting nutrient concentration, mg L<sup>-1</sup>

$K_s$  = half-saturation coefficient (limiting nutrient concentration at  $\hat{\mu}/2$ ), mg L<sup>-1</sup>

In practical terms, the  $K_s$  value marks the upper nutrient concentration at which growth rate ceases to be proportional to that nutrient. Thus for a nutrient to be limiting, its concentration must be equal to or less than the  $K_s$  value. Using this approach in a continuous-flow stirred tank reactor and two green algae, *Selenastrum cornutum* and *Scenedesmus quadricornum* at three pH ranges from 7.05 to 7.61, they demonstrated that the  $K_s$  values for both species were so low that carbon would not be a limiting nutrient in natural waters until the pH reached very high levels. This is consistent with Hill and Lincoln (1981) who, as noted earlier, found the  $K_s$  for CO<sub>2</sub> in their model was only 0. 105 mg inorganic-C/L. They further state that at such high pH levels, precipitation of other essential nutrients such as phosphorous, iron and trace elements, and metabolic inhibition would become major factors limiting algal growth.

Oswald (1985) reported that *Chlorella* absorbs carbon dioxide principally in the undissociated forms ( $\text{CO}_2$  or  $\text{H}_2\text{CO}_3$ ) and little if any as  $\text{HCO}_3^-$  or  $\text{CO}_3^{=}$ . Early studies on effect of carbon dioxide concentration on photosynthesis indicate that carbon dioxide saturation is achieved at or below 0.1 per cent. Above about 5 per cent, toxic effects become operative, although the upper limit is not definitely known. He therefore expected that growth rate will be independent of carbon dioxide concentration between 0.1 and 5 per cent.

Other authors (Park and Craggs, 2010, Park et al., 2011, Craggs et al., 2011, Craggs et al., 2012) argue that the carbon:nitrogen (C:N) ratio of typical wastewater as limiting to algal growth, based on the stoichiometry of wastewater and algal biomass. Three of these four papers reference Benemann (2003) for their wastewater and algal stoichiometry. Considerable confusion surrounds these claims as unfortunately, there is no such stoichiometric data in the Benemann reference. To add to the confusion, even though they reference the same paper, they quote quite wide variations in stoichiometry. Park and Craggs (2010) argue that typical domestic wastewater has a C:N ratio of 7:1, while algal biomass is typically 15C:N. Park et. al. (2011) state that domestic sewage is typically between 3 to 7C:N and algal biomass 6 to 15C:N. Craggs et. al. (2011) state that facultative pond wastewater is 2C:N and algal biomass between 5 and 10C:N. Meanwhile Craggs et. al. (Craggs et al., 2012) state that domestic wastewater is typically 3C:N and algal biomass 6C:N. The most widely quoted stoichiometry for algal elements is (Harris, 1986) using the Redfield ratio

C 106: H 263: O 110: N 16: P 1: S 0.7 - by atoms

C 47: N 7: P 1 - by weight

which converts to a 6.6 C:N ratio.

#### **1.5.14 BACTERIAL AND VIRAL DIE-OFF/REMOVAL**

It is generally accepted that the majority of microbiological health hazards associated with water consumption originate from faecal contamination (George et al., 2002, Dean and Lund, 1981, Amahmid et al., 2002). *E. coli*, *Shigella* sp., *Salmonella* sp. and *Vibrio cholerae* causing respectively diarrhoea, dysentery, typhoid fever and cholera are some of the pathogenic bacteria that may occur in water with some faecal contamination.

Viruses, causing diseases such as meningitis and hepatitis, as well as parasitic protozoans and helminths, are also usually present in domestic wastewater.

According to Dean & Lund (1981) primary treatment can eliminate between 40 and 70% of the bacteria, whilst biological processes such as trickle filters and activated sludge are effective at removing up to 99% of the pathogenic microorganisms. However, in order to make wastewater safe for reuse, further disinfection is necessary (Maynard et al., 1999a).

#### **1.5.14.1 INDICATOR ORGANISMS**

The routine examination of environmental samples for the presence of intestinal pathogens is often a tedious, difficult, and time-consuming task. Thus, it has been customary to tackle such examinations by looking first for certain indicator microorganisms whose presence indicates that pathogenic microorganisms may also be present. Developed at the turn of the twentieth century for assessing faecal contamination, the indicator concept depends on the fact that certain non-pathogenic bacteria occur in the faeces of all warm-blooded animals. These bacteria can easily be isolated and quantified by simple bacteriological methods. Detection of these bacteria in water means that faecal contamination has occurred and suggests that enteric pathogens may also be present (Gerba, 2009).

Some pathogens are present in very low numbers in wastewater and, because of this, or because effective isolation techniques have not yet been developed,



are difficult to detect. Microorganisms that are more numerous and more easily tested are, therefore, commonly used as indicators of faecal contamination. The human digestive system contains a large population of rod-shaped bacteria known collectively as coliform bacteria and each individual may discharge between 100 and 400 billion coliform bacteria per day (Metcalf\_&\_Eddy, 2003).

Because coliform bacteria are generally hardier than disease-causing bacteria, their absence from water is an indication that the water is bacteriologically safe for human consumption. Conversely, the presence of the coliform group of bacteria is indicative that other kinds of microorganisms capable of causing disease may also be present and that the water is potentially unsafe to drink.

Thus, since their first isolation from faeces towards the end of the 19<sup>th</sup> century (Rompré et al., 2002), the presence of the coliform group in water has been taken as an indication that pathogenic organisms associated with faeces may also be present.

In 1914 the U.S. Public Health Service adopted the coliform group as an indicator of faecal contamination of drinking water. Many countries have adopted coliforms and other groups of bacteria as official standards for drinking water, recreational bathing waters, wastewater discharges, and various foods. Indicator microorganisms have also been used to assess the efficacy of food processing as well as water and wastewater treatment processes (Gerba, 2009).

However, it has been learned that a number of deficiencies in the use of this indicator exist (Maier et al., 2009). All members of the coliform group have been observed to regrow in natural surface and drinking water distribution systems (Gleeson and Gray, 1997, Maier et al., 2009) The die-off rate of coliform bacteria depends on the amount and type of organic matter in the water and its temperature. If the water contains significant concentrations of organic matter and is at an elevated temperature, the bacteria may increase in numbers. This phenomenon has been observed in eutrophic tropical waters, waters receiving

pulp and paper mill effluents, wastewater, aquatic sediments, and organically enriched soil (i.e., sewage sludge amended) after periods of heavy rainfall.

Coliform bacteria may originate from a variety of sources and can for instance grow in soil (Metcalf\_&\_Eddy, 2003). Their presence does not necessarily, therefore, mean contamination with faecal waste. Tests have thus been developed that distinguish faecal coliform and, specifically *Escherichia coli*, which is the most common coliform among the intestinal flora of warm blooded animals and therefore more indicative of faecal contamination (Rompré et al., 2002). Other organisms such as faecal streptococci, enterococci and *Clostridium perfringens* have also been proposed for use as indicators (Metcalf\_&\_Eddy, 2003) but coliform, faecal coliform and *E. coli* remain the most commonly reported organisms in the literature and legislation.

Of greatest concern is the growth or recovery of injured coliform bacteria in a distribution system because this may give a false indication of faecal contamination. Coliforms may colonize and grow in the biofilm found on the distribution system pipes, even in the presence of free chlorine. *Escherichia coli* is 2400 times more resistant to free chlorine when attached to a surface than as free cells in water (LeChevallier et al., 1988). Still, the coliform group of bacteria has proved its merit in assessing the bacterial quality of water. Three methods are commonly used to identify coliforms in water. These are the most probable number (MPN), the membrane filter (MF), and the presence-absence (P-A) tests. A commercial version of the MPN test (IDDEX Ltd, Colilert™) was used exclusively for all *E. coli* assays in this work.

#### *1.5.14.1.1 The Impact of the Viable but Non-Culturable Cells (VBNC) state on Indicators*

It is suspected that indicator organisms such as *E. coli* often exist as viable but non-culturable cells (VBNC), meaning that the organism is in a state of metabolic shutdown that prevents its growth on a culture medium, but is not actually dead (Edwards, 1999, George et al., 2002). There are two conflicting schools of

thought interpreting the VBNC state: one is that it is a survival strategy and, as such, cells should be able to reverse the process when conditions become favourable. Conversely, it may be a moribund condition in which cells become progressively debilitated until death finally occurs (McDougald et al., 1998). Bloomfield et al. (1998) give a possible explanation for the inability to culture cells in such a state.

Bacteria undergo both biochemical and physiological adaptations that enable them to survive environmental stress such as nutrient limitation or UV exposure (Edwards, 1999). An important consequence of this is a reduction of growth rate to near zero. When such organisms are transferred to rich culture media, the metabolic pathways are rapidly switched on and flooded. The oxidation of substrates leads to overproduction of superoxide and free radicals, resulting in cell death (Bloomfield et al., 1998). The inability to detect VBNC by traditional culture methods, usually following membrane filtration, has led to the development of alternative analysis techniques such as the detection of the enzymes,  $\beta$ -D- glucuronidase and  $\beta$ -Dgalactosidase, produced by *E. coli* (Rompré et al., 2002) and the use of viability dyes (Edwards, 1999). Although the existence of VBNC is widely accepted, the extent to which inactivated pathogens remain virulent is still under investigation (McDougald et al., 1998, Rompré et al., 2002, George et al., 2002)

#### *1.5.14.1.2 Bacteriophages as Indicator Organisms*

Most of the work on pathogen removal in lagoons has concentrated on the removal of the bacterial indicator organisms, *E. coli* and faecal coliforms, as they can be rapidly and reliably identified and enumerated, but there has been very little work to investigate whether these results will be the same for pathogenic organisms such as viruses and intestinal parasites (Maynard et al., 1999b).

Because of their constant presence in sewage and polluted waters, the use of bacteriophage (or bacterial viruses) as appropriate indicators of faecal pollution

has been proposed. These organisms have also been suggested as indicators of viral pollution. This is because the structure, morphology, and size as well as the behaviour in the aquatic environment of many bacteriophage closely resemble those of enteric viruses. For these reasons, they have also been used extensively to evaluate virus resistance to disinfectants, to evaluate virus fate during water and wastewater treatment, and as surface and groundwater tracers. The use of bacteriophage as indicators of faecal pollution is based on the assumption that their presence in water samples denotes the presence of bacteria capable of supporting the replication of the phage.

Two groups of phage in particular have been studied: the somatic coliphage, which infect *E. coli* host strains through cell wall receptors, and the F-specific RNA coliphage, which infect strains of *E. coli* and related bacteria through the F+ or sex pili. A significant advantage of using coliphage is that they can be detected by simple and inexpensive techniques that yield results in 8-18 h.

The F-specific coliphage (male-specific phage) have received the greatest amount of attention because they are similar in size and shape to many of the pathogenic human enteric viruses. Coliphage f2,  $\Phi$ X174, MS2, and PRD-1 are the ones most commonly used as tracers and for evaluation of disinfectants. Because F-specific phage are infrequently detected in human faecal matter and show no direct relationship to the faecal pollution level, they cannot be considered indicators of faecal pollution (Havelaar et al., 1990). However, their presence in high numbers in wastewaters and their relatively high resistance to chlorination contribute to their consideration as an index of wastewater contamination and as potential indicators of enteric viruses. (Davies-Colley et al., 1999) also noted that some of the bacteriophages are sometimes useful indicators as they signify the presence of important human viral pathogens.

### **1.5.15 DISINFECTION IN NATURAL WASTEWATER TREATMENT SYSTEMS**

Disinfection refers to the partial destruction of pathogens to acceptable limits which differs from sterilisation, where all living organisms are destroyed or removed. Although sterilisation is possible with small quantities of water in a laboratory environment, for all practical purposes it is impossible when dealing with large flows in a wastewater treatment plant.

#### **1.5.15.1 BACTERIAL MORTALITY IN WASTE STABILISATION PONDS**

One of the prime functions of WSPs is the removal of pathogenic organisms, and factors affecting their decay are of concern to pond designers. In spite of extensive worldwide research and efforts to model the factors that affect the viability of coliform organisms in ponds, there still remains considerable debate in the literature about the true causes, and their relationship to each other. There are of course, some well recognised candidates. Starting in the 1970's a number of temperature dependant models were developed – the most widely used in pond design being that of Marais, (Marais, 1974). Others to develop temperature dependant models included Klock (1971), (Mancini, 1978) and Mills et al. (1982).

The real situation appears to involve a complex range of factors expanded on below, including sedimentation (Gannon et al., 1983),(Auer and Niehaus, 1993), starvation (Gann et al., 1968), pH (Parhad and Rao, 1983), dissolved oxygen (Curtis et al., 1992b), microbial antagonism (Polprasert et al., 1983), possibly algal toxic products (Merz et al., 1962), pond dispersion (Polprasert et al., 1983), pond depth (Mayo, 1989, Mayo, 1995), adsorption to particles (Ohgaki et al., 1986) and solar radiation (Calkins et al., 1976, Gameson and Saxon, 1967). Obviously in the real WSP situation one or many of these factors could be having an effect at any one time and the effects may be interdependent.

##### *1.5.15.1.1 Sunlight*

Solar irradiation is widely recognised as a major contributor to WSP water disinfection. UVB (280–320 nm), UVA (320–400 nm) and photosynthetically active radiation (PAR) (400–700 nm) all contribute to micro-organism removal

(Muela et al., 2000, Davies-Colley et al., 1997, Davies-Colley et al., 1999, Curtis et al., 1992). Many studies have concluded that sunlight is the most important factor causing disinfection in WSPs, e.g. Mayo (1995). This conclusion is largely based on observations of rapid die-off in the uppermost regions of WSPs and may ignore the slower contribution of 'dark' die-off observed in the lower regions of the water column where light is unable to penetrate.

*1.5.15.1.2 LIGHT ATTENUATION IN POND WATERS*

Curtis et al. (1994) pointed out that light is greatly attenuated in WSP effluent according to the light attenuation function (Equation 1-37).

$$I_z = I_0 e^{-kz} \dots \dots \dots (1 - 37)$$

where  $I_0$  is the subsurface irradiance,  
 $I_z$  the irradiance at depth  $z$  and  
 $k$  is a light attenuation coefficient

However, Heaven et al. (2005) pointed out the difficulties of accurately measuring attenuation in ponds, calling for a need to standardise a method. A huge body of work in the fields of limnology and oceanography concerns values and expressions for  $k$  (Kirk, 1994). For many purposes  $k$  is assumed to be a linear function of one or more components such as suspended solids (SS), dissolved solids or chlorophyll a. Numerous expressions have been proposed for conditions similar to those in WSPs, such as eutrophic lakes and estuaries (Tsirtsis, 1995); (Lonin and Tuchkovenko, 2001).

A definitive study of light penetration in WSPs, looking at both photosynthetically active radiation (PAR) and monochromatic light, was carried out by Curtis et al. (1994). Absorbance played a far more important role than scattering for all ponds in the study, pond-to-pond variation was mainly attributable to differences in algal biomass, and variations in attenuation were observed at different wavelengths and depths. A survey of WSPs in New

Zealand found a median euphotic depth of 0.35 m corresponding to a  $k$  value of  $13 \text{ m}^{-1}$  (Davies-Colley et al., 1995).

Heaven et al. (2005) found that at concentrations above 50 mg per litre the relationship between  $k$  (light attenuation coefficient) and suspended solids is non-linear;  $k$  also varied with depth. This could be modelled by a single equation, suggesting similarity of response in different cultures. At shallow depths and low suspended solids concentrations  $k$  values are variable and hard to measure reliably. The results highlight the need to standardise on a method for the measurement and reporting of  $k$  values if these are to be widely applicable in the development of pond models. To date this call for standardisation appears not to have been met.

#### *1.5.15.1.3 Light-induced mortality*

In some aquatic environments such as the sea, it is apparent that light is responsible for rapid die-off of bacteria (Gameson and Gould, 1986). James (1987) and Bolton et al. (2010) are in agreement that it is unlikely that light is a major cause of death in WSPs, as they note that light penetration in stabilisation ponds is limited to the top 10-15 cm and as the highest bacterial concentrations rarely occur in the surface layer. This is confirmed by typical values for die-off rates in ponds (T90 values of 20 - 30 hours for *E. coli*) which approximate to dark values in freshwater and seawater experiments.

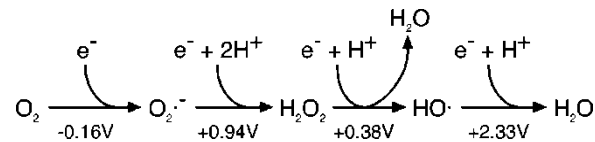
#### *1.5.15.1.4 UV light*

UVB, UVA and photosynthetically active radiation (PAR) have all been shown to contribute to the inactivation of micro-organisms in water. The highest energy (shortest wavelength) form, UVB in particular, can directly damage RNA, DNA and other cell constituents of micro-organisms, in processes termed direct photoinactivation. Due to the differences in energy for the different wavelength regions of the solar spectrum, inactivation mechanisms vary (Jagger, 1985, Whitlam and Codd, 1986, Sinton et al., 1999, Sinton et al., 2002, Kohn and Nelson, 2006, Davies-Colley et al., 1997, Curtis et al., 1992, Muela et al., 2002,

Bolton et al., 2010). In addition to processes of direct photoinactivation, UV light and to a lesser extent photosynthetically active radiation (PAR) are able to indirectly inactivate and damage micro-organisms via photo-oxidation.

#### 1.5.15.1.5 Photo-oxidation

Photo-oxidation occurs with the formation of highly reactive oxygen species (ROS), which react with and damage/inactivate micro-organisms.



**Figure 1-11 Reduction potentials for oxygen species. 1 M dioxygen is used as the standard state for the first step. Adapted from Imlay (2003)**

Absorption of visible and UV-A wavelengths by the brown substances present in humic waters results in the generation of superoxide anions -  $O_2^{\cdot -}$  and singlet oxygen -  $O_2^1$  (Whitelam and Codd, 1986).

Much of the behaviour of molecular oxygen and its partially reduced species derives from their reduction potentials (Figure 1-11) and molecular orbital structures. Molecular oxygen itself is a rarity, a stable di-radical, with two spin-aligned, unpaired electrons in its pi anti-bonding orbitals. An important consequence of this structure is that organic molecules with spin-paired electrons cannot transfer more than one electron at a time to oxygen. Because oxygen is a relatively weak univalent electron acceptor (and most organic molecules are poor univalent electron donors), this restriction ensures that oxygen cannot efficiently oxidise amino acids and nucleic acids. However, the unpaired electrons of dioxygen readily interact with the unpaired electrons of transition metals and organic radicals.

In contrast, the reduction potentials of  $O_2^{\cdot -}$ ,  $H_2O_2$ , and hydroxyl radical dictate that in thermodynamic terms they are much stronger univalent oxidants than dioxygen is (Figure 1-11). However, the anionic charge of  $O_2^{\cdot -}$  inhibits its



effectiveness as an oxidant of electron-rich molecules, while the reactivity of H<sub>2</sub>O<sub>2</sub> is diminished by the stability of its oxygen-oxygen bond. Neither of these features applies to the hydroxyl radical, and indeed HO· reacts at virtually diffusion limited rates with most biomolecules (Imlay, 2003).

In aquatic environments such as in a WSP, ROS can be produced by endogenous and exogenous sensitizers as well as by other reactions such as Fenton's reaction (Gracy et al., 1999, Curtis et al., 1992, Jagger, 1985, Whitlam and Codd, 1986). Sensitizers are light absorbing compounds that transfer their energy to other molecules leading to the formation of ROS. Endogenous sensitizers are found inside the cells of microbes, e.g. flavins and porphyrin derivatives while exogenous sensitizers are found outside the cell in the aquatic environment e.g. humic substances, photosynthetic pigments and dissolved organic matter (Kohn and Nelson, 2006, Jagger, 1985)

Potential sensitizers can be found within the cell (endogenous) (Webb and Brown, 1979) or outside the cell (exogenous) (Whitlam and Codd, 1986). These toxic forms are able to kill bacteria by damaging a vital cellular constituent. The cytoplasmic membrane is the most usual target for both endogenous (Peak et al., 1987) and exogenous (Tuveson et al., 1988) sensitizers in *Escherichia coli*.

#### **1.5.15.2 OTHER ENVIRONMENTAL CAUSES OF MICROBIAL MORTALITY IN POND WATERS**

Most authors agree that the main causes of bacterial mortality in WSPs are the four factors mentioned above in the discussion of the James (1987) WSP design approach (para 1.5.7.9). It is currently unclear to what extent other environmental factors such as pH, DO and the presence of photo-sensitizers contribute to disinfection in WSPs (Kohn and Nelson, 2006, Kohn et al., 2007, Fallowfield et al., 1996). The long retention times in WSPs enables factors such as sedimentation, predation, competition and sunlight to all contribute to the die-off of pathogens. While these ponds may not be able to achieve the same level and speed of disinfection as the chemical methods discussed below, they

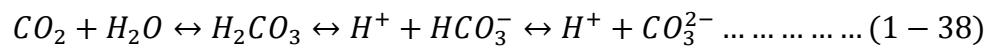
have no anthropogenic energy input requirement and are consequently low operation and maintenance systems.

#### *1.5.15.2.1 Dissolved oxygen (DO)*

High levels of DO occur in aquatic systems due to the photosynthesis of algae and macrophyte organisms. Sweeney et al. (2007) reported DO levels in the upper layers of a WSP reaching over 30 mg/L in summer. Due to light attenuation, however, DO stratification can vary significantly through the water column, with nearly all effective light being absorbed in the surface layer (Haag and Hoigne, 1986). Maturation ponds are generally photosynthetically oxygenated due to the relatively high optical clarity of the effluent received from the facultative pond. It is hypothesised that an increase in DO would result in an increase in ROS formation which may then lead to a corresponding increase in photo-oxidation.

#### *1.5.15.2.2 pH induced mortality*

Significant diurnal changes in pH occur frequently within WSPs due to algal photosynthesis, which consumes and removes CO<sub>2</sub> from the water. This in turn affects the carbonate/bicarbonate buffering system (Equation 30) leading to a decrease in hydrogen ions and a corresponding increase in pH (Paterson and Curtis, 2005). Assimilation of NO<sub>3</sub> may contribute to further increases in pH (Fallowfield et al., 1996).



Consequently, high pH values are often observed in WSPs, with values varying diurnally within the range of 7 – 10.5 (Kayombo et al., 2000, Sweeney et al., 2007, Arauzo, 2003, Benchokroun et al., 2003a, Botero et al., 1997, Craggs et al., 2004, Davies-Colley et al., 1999, Oswald, 1988a, Sebastian and Nair, 1984). It is hypothesised that an increase in pH would result in a decreased stability of the micro-organism cell with a subsequent increase in solar inactivation.

Parhad and Rao (1983) reached a number of conclusions after studying *E. coli* growth in algal broths with sterilised wastewater. They were

1. The growth of different algae in sterilized wastewater results in an increase of pH from 7.5 to more than 10.
2. *E. coli* could not grow in wastewater when the pH was greater than 9.2.
3. Both *E. coli* and algae can grow together when wastewater is buffered at pH 7.5.
4. *E. coli*, when grown in association with algae, is eliminated because of the high pH produced as a result of algal growth.
5. The decrease in *E. coli* observed in stabilization ponds need not be attributed to the presence of antibacterial substances; to the production of the toxic, extracellular products of algae; or to microbial antagonism.

In his thesis (Smallman, 1986) suggests that bacterial death in ponds is due to algal photosynthesis causing periods (6-12 hours per day) of high pH. Results from his dialysis experiments showed significantly higher mortalities at pH levels above 9. He also showed that pH rose to 9 - 10.3 during periods of intense photosynthetic activity.

#### *1.5.15.2.3 Temperature & Starvation-induced mortality*

Much of the earliest work on bacterial removal assumed that temperature was the most important factor controlling the removal mechanism, as described by the equation developed by Marais and Shaw (1961). Many subsequent workers, such as Bowles et al. (1979) and Ferrara and Harleman (1980) also concentrated on first order kinetics in which the removal rate is temperature dependent. More recent work has considers bacterial removal as a much more complex mechanism involving interactions between the physical, chemical and biological systems present in the lagoon (Maynard et al., 1999a), although temperature clearly remains an important parameter. For example, Polprasert et al. (1983), Pearson et al. (1987), Mara et al. (1992), and Mezrioui et al. (1995) all found

that removal of faecal coliforms increased with increasing temperature. To put this in perspective, Mara and Pearson (1986b) pointed out that the relationship between die-off and increasing temperature must be indirect, as higher levels of removal were found in tertiary lagoons in comparison to anaerobic and facultative lagoons operating at the same temperature.

Some investigators have suggested that a relationship between temperature and nutrients may mask the temperature effect on death rates in laboratory experiments. For example, Lessard and Sieburth (1983) noted a death rate dependence on temperature in studies of *Escherichia coli* in seawater using diffusion chambers. By contrast, a response was not observed in adjoining bath culture experiments. Sjogren and Gibson (1981) and (Klock, 1971) proposed a starvation mechanism for coliform mortality where enteric organisms persist until endogenous nutrient reserves are depleted.

Sjogren and Gibson (1981) found that in dilute lake water members of the tribe Klebsielleae (genera *Klebsiella*, *Enterobacter*, and *Serratia*) have a prolonged survival rate (40% or better after 24 hours), whereas other genera labelled 'non-survivors' were not viable for much longer than 24 hours under the same conditions. The non-survivors belonged to the genera *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Erwinia*, *Escherichia*, *Flavobacterium*, and *Pseudomonas*.

Sjogren and Gibson (1981) hypothesised that the mechanisms involved in surviving a stressful environment is to elevate levels of ribonuclease that would permit mobilization of internal carbon and energy resources. To support this they noted that *K. pneumoniae* and *Enterobacter cloacae*, which belong to the tribe Klebsielleae survive for extended periods of time (more than 5 days), have the highest ribonuclease levels when stressed in distilled water. On the other hand, the so-called non-survivors have very little ribonuclease. They went on to attempt to experimentally elucidate by measuring differences in ribonuclease and adenosine triphosphatase levels between *Escherichia coli* (non-survivor)

and *Klebsiella* (survivor) cells. At pH 7.5, stressed *E. coli* cells contained 14% of the adenosine triphosphatase activity detected in the control, whereas at pH 5.5, in the presence of calcium ions, these same cells contained 50% of the control adenosine triphosphatase levels. At pH 7.2, *E. coli* cells were strongly inhibited by the adenosine triphosphatase inhibitors, bathophenanthroline (a lipophilic metal chelator that inhibits by chelating ferrous iron in buried nonheme iron proteins) and oligomycin (an inhibitor of electron transport phosphorylation).

This led them to conclude that at least two mechanisms operate within a bacterium that allows it to persist in a nutrient depleted aquatic environment. The first mechanism involves conversion of cellular macromolecules such as ribonucleic acid into essential cellular components by means of an active ribonuclease i.e. to survive by scavenging internal endogenous reserves of protein, ribonucleic acid, and glycogen. The second mechanism involves the ATPase complex and an electrochemical gradient. In this way certain enteric bacteria are capable of utilizing acidic conditions (pH 5.5) as an electrochemical gradient to generate necessary high-energy intermediates for prolongation of survival beyond that possible in environments of near-neutral pH. As pond environments are typically even higher pH (8 – 10), this mechanism is unlikely to play a significant role most of the time.

Temperature effects on the rate of nutrient utilization and variability in nutrient (Mayo and Noike, 1996) availability in natural systems may explain the observed scatter in temperature-death relationships. In addition, batch culture conditions may present a more favourable environment than that encountered in natural systems.

Gann et al. (1968) found that coliform removal was closely associated with the removal of BOD, and suggested that coliforms are unable to compete with other bacteria for nutrients. They also concluded that the principal site of bacterial activity was located within the immediate vicinity of the influent. From 88 to 92

percent of the ultimate BOD removal and 88 to 93 percent of coliform reduction occurred in this area. Saqqar and Pescod (1992) also found that the faecal coliform removal rate increased with decreasing BOD. Mayo and Noike (1996) found that at high temperatures, the number of heterotrophic bacteria decreased in lagoons because of increased competition for glucose by *Chlorella vulgaris*.

Experiments on bacterial die-off in fresh and marine waters (Gameson, 1985) have shown that T90 values of 1 - 2 days occurred in the presence of low levels (< 20 mg/l) of organic matter in the absence of light, predators or other sources of mortality. When the concentration of organic matter was increased the T90 values increased to 2 - 3 days. Similar results from (LeMoyne, 1982 ) indicate that at BOD levels above 20 - 30 mg/l growth of coliforms can occur at appropriate temperatures. From all of the above work it is reasonable to conclude that starvation will occur more rapidly at warm to hot temperatures (20 - 35°C) with high metabolic activity compared to temperatures below 5°C when little metabolic activity will be occurring.

It would therefore appear to be important to maintain high temperatures and low organic concentrations (BOD <20 mg/l) if starvation is to cause rapid die-off.

#### *1.5.15.2.4 Sedimentation*

Bacteria are small and their settling rates are extremely slow. However, when they become attached to particulate matter, the sedimentation process can become more significant in overall disappearance. Gannon et al. (1983) found sedimentation (via attachment to particles from 0.5µm to 100µm) to be an important element in the overall faecal coliform disappearance in the upper end of the lake system they were studying. Concurrent rooftop studies the showed light level affected daytime disappearance.

Auer and Niehaus (1993) demonstrated that the majority of the faecal coliform bacteria were found to be associated with two particle classes out of seven classes originally used for classification: 0.45-1.0 and 6-10  $\mu\text{m}$ . The authors decided to simplify the analysis, and assigned all particles to one of two groups: small (0.45-10  $\mu\text{m}$ ) and large (> 10  $\mu\text{m}$ ).

On average, 90.5% of the faecal coliform bacteria were found to be associated with small particles and 9.5% were associated with large particles. Size class specific sedimentation velocities, are 1.17 and 2.40 m/d for the small and large particle classes, respectively.

Auer and Niehaus (1993) authors prepared an overall die-off rate constant  $k$  in Equation 1-39 that incorporated a dark die-off rate, a depth integrated die-off rate due to sunlight and a sedimentation rate

$k = k_d + k_i + k_s$  which expands into

$$k = k_{d,20}\theta^{(T-20)} + \frac{\alpha \cdot I_{0,avg}}{\eta \cdot z_e} [1 - e^{(-\eta z_e)}] + \frac{v}{z_e} \dots \dots \dots (1 - 39)$$

where  $k_{d,20}\theta^{(T-20)} = 0.73 \text{ d}^{-1}$  = dark death rate coefficient developed through in situ experiments, and reflects the response of faecal coliform bacteria to natural, environmental conditions.

$\alpha$  = The light proportionality constant ( $0.00824 \text{ cm}^2 \text{ cal}^{-1}$ )

$$\frac{\alpha \cdot I_{0,avg}}{\eta \cdot z_e} [1 - e^{(-\eta z_e)}] = k_i = \text{sunlit die-off coefficient depth} \dots \dots (1-40)$$

averaged and representative of conditions over the entire epilimnion. Death rate coefficients range from 0.5 - 4.57  $\text{d}^{-1}$

$I_{0,avg}$  = average irradiance immediately below the water surface over the incubation period ( $\text{cal cm}^{-2} \text{ d}^{-1}$ ). Measured directly for the system under study, as are

$\eta$  attenuation in the water column ( $\text{m}^{-1}$ )

$z_e$  = the epilimnion depth in metres

$\frac{v}{z_e} = k_s$  = sedimentation loss rate coefficient, and

$v$  ( $\text{m d}^{-1}$ ) is the sedimentation velocity mentioned above  
weighted average =  $1.38 \text{ m d}^{-1}$

In this study at least, it can be seen that sedimentation plays a significant role in determining the overall die-off rate coefficient, with the sedimentation component contributing more than the dark die-off and often as much as the irradiance die-off.

#### 1.5.15.2.5 Predation

There is very little work in the literature related to the effects of predation on bacterial removal in tertiary lagoons. Much of what does exist is contradictory and confusing. Pretorius (1962) found no evidence that coliphage play an important role in *E. coli* removal from lagoons, despite Maynard's 1999 review claiming that Pretorius found coliphage to be significant removers of *E. coli* from pond wastewater. Quite extraordinarily, the Maynard et al. (1999a) review (despite appearing in the highly regarded journal, *Water Research*), also claimed that Fernández et al. (1992) and Fernández et al. (1992b) both concluded that predation and competition were extremely important in the removal of faecal coliforms. In fact, neither of these papers mention predation. Furthermore, Maynard tells the reader that work by Skerry and Parker (1979), Mills et al. (1982) and Mayo (1995) concluded that predation was not important in removal of *E. coli* from pond wastewater. Once again, none of these papers even mention predation. Finally, some of the references quoted by Maynard *et. al.* were erroneous.

Loedolff (1965) examined the removal of *E. coli* through predation by micro invertebrates in ponds at Pretoria (South Africa). Two members of the Cladocera family were found to predominate in WSPs; *Moina dubia* and *Daphnia magna*. In-vitro studies showed that an individual *Moina* could remove



up to 93 *E. coli* cells per hour and the individual *Daphnia* up to 55 per hour. Loedolff concluded that at these rates of predation Cladocera do not contribute significantly to bacterial removal in ponds, as their numbers never reach a level sufficient to impact  $10^6$  per 100 ml of bacteria cells.

Chabaud et al. (2006) found grazing by protozoa could be an important biological mechanism for bacterial elimination in wastewater treatment systems. They found endogenous bacterial mortality rates were 10 times lower in wastewater treated with cyclohexamide (a protozoan inhibitor) ( $96 \text{ CFU mL}^{-1} \text{ d}^{-1}$ ) than in untreated wastewater ( $1100 \text{ CFU mL}^{-1} \text{ d}^{-1}$ ). They also found protozoa in the presence of a biofilm were responsible for 60% of bacteria removal. Biofilm without protozoa and a clean surface each removed similar quantities of bacteria. Rozen and Belkin (2001) found light appears to be the critical abiotic factor in affecting *E.coli* survival on exposure to seawater. As well, previous growth history plays a major part in preadaptation of the cells, and stationary phase cells are generally more resistant than exponentially growing ones. In the context of the current theme they noted predation, mostly by protozoa, is probably the most significant biotic factor.

Troussellier et al. (1986) used path analysis and ridge regression to model the removal of faecal coliforms from the Meze WSPs. This study included rotifers as known grazers on bacteria in ponds. It found that in no case do the temperature, biochemical oxygen demand ( $\text{BOD}_5$ ), Rotifers, or oxygen saturation ( $\%O_2$ ) variables have a significant direct influence on faecal coliform concentrations, as had been hypothesized, although the corresponding total covariances (equal to the correlation coefficients, since all variables were standardized) were significant in most cases. Even the indirect effects of temperature and irradiance on faecal coliforms were found to be negligible, despite significant correlations. The authors stress that this illustrates the dangers of a simplistic interpretation of correlation coefficients, outside the framework of causal modelling.

#### 1.5.15.2.6 Algal toxins

Some researchers such as Davis and Gloyna (1972) and Mezrioui et al. (1994) have suggested that certain algae produce substances which are toxic to bacteria. Mezrioui et al. (1994) suggested that Cyanobacteria secrete a substance that is toxic to *E. coli*, and *Chlorella* secrete a substance that is toxic to *Vibrio cholerae*. However, very little work was done to identify what the toxins secreted by the algae actually were. Toms et al. (1975) also investigated the possible secretion of toxins by algae by incubating samples of lagoon influent water with either water rich in algae or with relatively pure water in the dark or light. They found that bacteria in the samples mixed with purer water were killed more rapidly than the samples where algae were present, presumably due to the shielding of the bacteria from the light by the algae. They concluded that there was no evidence of the production of bacterial toxins by algae, and this was supported by the work of Mayo and Noike (1996) on the survival of heterotrophic bacteria in lagoons.

### 1.5.16 High Rate Algal Pond Disinfection

All the foregoing discussion on disinfection mechanisms discussed in the context of WSPs applies to HRAPs, perhaps with the notable exception of sedimentation. The reaction environment in HRAPs is more 'extreme' and the various disinfection processes may proceed more rapidly due to mixing through the UV surface disinfection zone, higher algal biomass (and possibly pH and DO) and ROS production.

High rate ponds provide potentially the most effective disinfection within the constraints of sustainability requirements (El Hamouri et al., 1994, Fallowfield et al., 1996, Bahlaoui et al., 1997, Davies-Colley et al., 2003). The disinfection mechanisms active in these ponds are high pH, sunlight, high oxygen production as well as some of the other factors, such as predation, present in conventional

ponds. The disinfection capability of high rate ponds is the focus of a portion of this thesis and will be discussed in greater detail in Chapters 4, 5 and 6.

The high levels of dissolved oxygen and pH as a consequence of algal culture, together mixing through surface layers exposed to high levels of UV irradiation, lead to high rates of disinfection (Fallowfield et al., 1996). The elevated rates of photosynthesis brought about by gentle mixing, which improves light availability for algal photosynthesis, produce supersaturating DO levels and high diurnal shifts in pH with values as high as pH 11 being recorded. This combination, together with greater exposure of the pond volume to UV irradiation, results in the rapid death of indicator organisms such as thermotolerant coliforms and *E. coli* (Curtis et al., 1992).

Fallowfield et al, (1996) determined die-off rate constants ( $K_b$ ) for *E. coli* of 0.3 – 10.26  $d^{-1}$  in HRAPs operating in Scotland at mean pond temperatures of between 14 and 19°C at surface irradiances of 85 – 356  $Wm^{-2}$ , for the treatment of piggery wastewater. These environmental conditions compare with those of HRAPs operated in South Australia where pond temperatures range from 14 - 25 °C (Evans et al., 2003). The higher treatment reaction rates result in shorter retention times and consequently reduced area requirements compared to unmixed lagoons such as WSPs.

#### **1.5.16.1 Summary of HRAP disinfection**

There is much debate in the literature on the mechanisms of bacterial removal, and there are conflicting results on the contribution of algal toxins, predation, starvation, temperature, light, pH and retention time, as discussed earlier. The use of first order reaction rate equations to describe bacterial die-off also requires careful scrutiny, despite it being widely used in many papers on the topic. This will be considered at some length later in the thesis.

These are therefore areas where this thesis seeks to shed further light and add to the knowledge bank. More work is needed to investigate the relationship between pH, dissolved oxygen concentration, light and lagoon depth. This area is particularly important, as tertiary lagoons are built with the idea of being primarily for pathogen removal. There is also the potential for conflict between the need for long retention times and shallow pond depths to ensure good bacterial removal, and the resulting increase in algal biomass leading to high concentrations of BOD and suspended solids in the effluent. The importance of light also raises questions about the suitability of lagoons in temperate and cold regions for providing pathogen removal.

### **1.5.17 CHEMICAL DISINFECTION**

For the sake of completeness, brief mention will be made of chemical disinfection as it is the method employed in the majority of urban wastewater treatment plants.

#### **1.5.17.1 Chlorine**

Since its introduction at the end of the 19th century, chlorine has remained the principal disinfectant for water, both wastewater and drinking water (Dean and Lund, 1981); (Lazarova et al., 1999). The key factor that makes it such a useful disinfectant is that it is a strong oxidant and halogenating agent. Some of the advantages of chlorine are that its use is now well understood; it has a residual effect, therefore continues to work for a relatively long period after dosing and, as an oxidant, it destroys odours such as those produced by hydrogen sulphide, mercaptans and other products of anaerobic decay. There are, however, a number of disadvantages to the use of chlorine. These include it being a hazardous chemical to work with, potentially toxic to the biota in the receiving environment, increasing the total dissolved solids (TDS) of the effluent and it may be consumed by oxidising inorganic compounds such as iron and

magnesium (Metcalf\_&\_Eddy, 2003). Chlorination of sewage effluent led to large scale localised destruction of riverine macroinvertebrates in a study on two rivers in Kwazulu-Natal, South Africa (Williams et al., 2004). Chlorine also has the potential to form carcinogenic substances known as trihalomethanes e.g. chloroform, bromoform, by its action on a variety of oxygenated organic compounds such as acetone (Lazarova et al., 1999). Due to these negative effects dechlorination plants are often necessary, which add a significant cost increase to water treatment.

#### **1.5.17.2 Chlorine Dioxide**

Chlorine dioxide is also an effective disinfectant with a good residual value and capability to oxidise odorous sulphides (Metcalf\_&\_Eddy, 2003). Apart from also affecting receiving water quality by increasing TDS and forming toxic substances such as chlorite when reacting with organic matter, the main disadvantage is that it is unstable and must be produced on site, greatly increasing operating cost (Dean and Lund, 1981).

#### **1.5.17.3 Ozone**

Ozone is another effective option (van Leeuwen, 1996); (Ernst and Jekel, 1999). It has the added benefits of oxidising sulphides and contributing dissolved oxygen. However, it carries with it many safety concerns as it is both highly corrosive and toxic (Metcalf\_&\_Eddy, 2003)). It is also energy intensive and expensive, with high maintenance and operational requirements.

#### **1.5.17.4 Ultraviolet**

This refers to UVC – usually delivered by mercury vapour lamps at 254 nm. The main advantage of UVC over the chemical techniques is that it is safer to work with and does not have any residual toxicity or negative effect on the effluent quality. It is however energy intensive and relatively expensive. Another concern with UV disinfection is that while it is very effective in reducing the culturable faecal coliform count it does not necessarily eliminate the micro-organisms. Studies by George, *et al.* (2002) found that  $\beta$ -D- glucuronidase

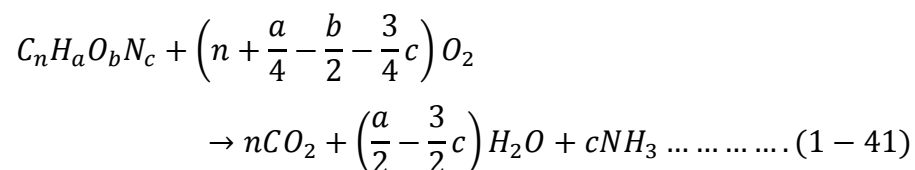
activity was not reduced by UV disinfection suggesting that the faecal coliform continued to exist in a viable but non culturable state.

## 1.5.18 NUTRIENT AND BOD<sub>5</sub> REMOVAL FROM NATURAL WASTEWATER TREATMENT SYSTEMS

### 1.5.18.1 Biochemical Oxygen Demand (BOD) Biology and BOD Removal

BOD is commonly defined as the amount of oxygen required by bacteria while 'stabilising' decomposable organic matter under aerobic conditions. The term 'decomposable' can be interpreted as referring to the organic matter that can serve as food for bacteria with energy derived from its oxidation (Sawyer et al., 2003). The test is commonly applied to domestic and industrial wastes as a measure of the pollution strength in terms of oxygen required if discharged into natural watercourses. It is a major test for regulatory purposes and for research evaluating the purification capacity of treatment plants.

Since this is a bioassay, it is essential that conditions are suitable for living organisms to function unhindered, i.e. there are no toxin substances and bacterial nutrients are all present. The quantitative relationship can be represented by the general equation 1-41.



Thus BOD can be interpreted as organic matter as well as amount of oxygen used. Temperature effects are held constant by performing the test at 20°C, and through experience it has been determined that a reasonably large percent of the total BOD (70 – 80%) is exerted in 5 days, therefore the test (BOD<sub>5</sub>) has been developed on the basis of a 5 day incubation period. This has the added

advantage of minimising the amount of ammonia oxidised by nitrifying bacteria. As these organisms are slower growing their effect is generally not seen until about Day 7 or 8.

BOD kinetic studies show that for practical design purposes, the BOD reactions are first-order in character. That is, the rate of the reaction is proportional to the amount of biodegradable matter remaining (Equation 1-42), as modified by the organism numbers. The reaction rate is controlled by the amount of food available to the organisms. This can be expressed as

$$k' C = - \frac{dC}{dt} \dots \dots \dots (1 - 42)$$

where  $C$  = concentration of biodegradable organic matter at

$t$  = time

$k'$  = rate constant for the reaction

For many years, and after much measuring in the US and UK of domestic waste and polluted rivers, the BOD reaction rate constant ( $k'$ ) was thought to be equal to 0.23 per day. More detailed examinations showed considerable variation from this number as techniques became more refined. For example,  $k'$  for domestic waste varies considerably from day-to-day, and averages closer to 0.40 per day (Sawyer et al., 2003). Two factors appear to be involved: 1) the nature of the organic matter and 2) the abilities of the organisms present.

Domestic and industrial wastewater can vary greatly in chemistry and availability to microbial attack. The part in solution is readily available, in contrast with the part in colloidal and coarse suspension, which generally needs the activity of hydrolytic organisms to become truly soluble and available.

In many cases the exertion of carbonaceous BOD has been observed to be bi-phasic, with the second phase being attributed to protozoa (Bhatla and Gaudy, 1965) predated the bacteria which have consumed the original carbon source.

The other major criticism of the BOD test is that it represents an overall value of the respiration of a numerically and taxonomically unknown population of micro-organisms, in a medium about which little information exists (Okafor, 2011). In reality, neither of these criticisms are of major significance for the majority of practical purposes the test is used for.

### 1.5.18.1.1 THE USE OF BOD IN POND DESIGN

Much has already been written earlier in this review on the plethora of design equations that have arisen – many of them using BOD removal efficiency as a key productivity parameter. As late as 1987, in a retrospective comparison of BOD removal performance against possible design equations Middlebrooks (Middlebrooks, 1987) found very few design equations capable of predicting the real BOD removal performance as measured in two series of data from ponds in USA. He found that the best fit of data for one of his series was the simple plug flow model encapsulated in Equation 1-43. The fit was not so good for his other data set: –

$$k \cdot t = \ln \left( \frac{C_i}{C_e} \right) \dots \dots \dots (1 - 43)$$

- where  $k$  = reaction rate constant
- $t$  = hydraulic retention time
- $C_i$  = influent BOD<sub>5</sub> concentration, and
- $C_e$  = effluent BOD<sub>5</sub> concentration

Analytical models for the design of primary facultative ponds are based on first-order kinetics. An ideal hydraulic pattern is assumed that may be either completely mixed (Equation 1-44) or plug-flow (Equation 1-45) which is simply a rewrite of Equation 1-43 (US EPA, 1983, von Sperling, 2002, Preul and Wagner, 1987, Mara, 1976).

$$C_e = \frac{C_i}{(1 + k \cdot HRT)} \dots \dots \dots (1 - 44)$$



$$C_e = C_i \cdot e^{-k.HRT} \dots \dots \dots (1 - 45)$$

where: HRT is the mean hydraulic retention time (days) and,

Other symbols - as defined above.

The accuracy of these equations may vary substantially with actual pond conditions and therefore their application is limited. The  $k$  value is temperature (T) dependent and the appropriate correction is obtained through Arrhenius style equations. For completely mixed conditions, Mara (1976) suggested Equation 1-46, while for plug-flow Equation 1-47 is recommended by US EPA (1983).

$$k = 0.3(1.05)^{T-20} \dots \dots \dots (1 - 46)$$

$$k = 0.71(1.09)^{T-20} \dots \dots \dots (1 - 47)$$

Thirumurthi (1974), Mara et al. (1979) and Uhlmann (1979) observed that reaction rates also varied with organic loading and decreased as loading was lowered. Ellis and Rodrigues (1995) reported  $k$  values ranging from 0.22 to  $k$  value was 0.168 day<sup>-1</sup> for unfiltered samples from full sized ponds at 20°C. They also found a first-order removal rate of 0.327 day<sup>-1</sup> in samples for algae removal. These authors recommended that a  $k$  value of 0.3 day<sup>-1</sup> at 20°C, from Equation 1-42, would be more appropriate for filtered BOD. For unfiltered samples, a more realistic  $k$  value would be 0.201 day<sup>-1</sup>. They also suggested an estimate of  $k$  (day<sup>-1</sup>) as a function of organic loading applied to the pond ( $\lambda_s$  in kg BOD/ha.day) according to the equation 1-48.

$$k = 2.622 \times 10^{-3} \lambda_s - 0.194 \dots \dots \dots (1 - 48)$$

where:  $\lambda_s$  is the maximum allowable surface loading applied to the pond (kg BOD/ha.day)

Actually, pond flow is neither completely mixed nor plug-flow. The dispersed flow is more adequate to represent the hydraulic pattern, as initially observed by Thirumurthi (1969) using the Wehner-Wilhelm kinetic equation.

The use of the dispersion based model is debatable because extensive investigation would be required to obtain reliable figures. Broad application is limited by a number of factors such as unsteady flow, wind, and inlet and outlet structures that may significantly influence dispersion in ponds (Silva et al., 2010).

According to Juanico (1981), the plug-flow model is more representative of bacterial removal compared to BOD removal, which is more likely to approach completely mixed conditions. Accepting the logic of this argument helps explain why investigations of the influence of hydraulic pattern have focused with good outcomes on coliform removal (von Sperling, 2003, Shilton and Harrison, 2003, Lloyd and Vorkas, 1999, Bracho et al., 2006, Shilton and Mara).

Mara and Pearson (1986a) and Mara et al. (1992) stated that limitations of “rational” methods based on first-order kinetics led to empirical procedures based on ambient temperature. Mara (1987) proposed a commonly applied model for the computation of the maximum allowable organic loading in facultative ponds (Equation 1-49).

$$\lambda_s = 350(1.107 - 0.002T)^{T-25} \dots \dots \dots (1 - 49)$$

where: T is the average temperature of the coldest month (°C), and

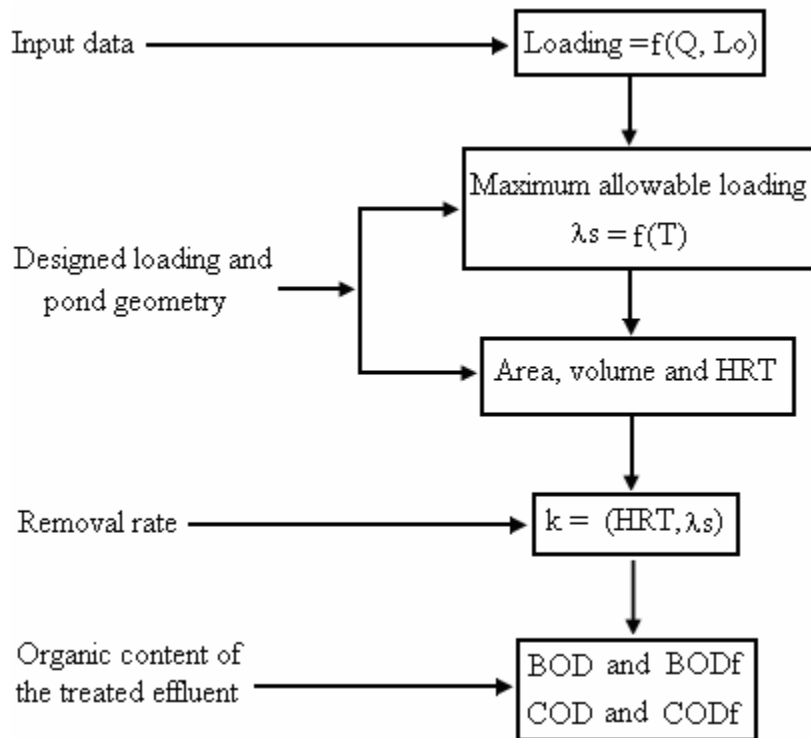
$\lambda_s$  is as above

According to Mara (1997) a properly designed primary facultative pond has a performance for BOD removal ranging from 70 to 80% for unfiltered samples and about 90% for filtered samples.

Looking at the BOD removal performance of six full-scale facultative ponds in Brazil, Silva et. al. (2010) decided that high HRT and consequent low surface

loading resulted in smaller first-order removal rates compared to the adjusted values from usual Arrhenius-style equations. Also, surface removal rates decreased as loading decreased and HRT increased.

As the traditional design procedure based on the first-order removal rate provides unrealistic figures they proposed a design approach based on the maximum allowable loading rate as outlined in Figure 1-12.



**Fig. 1-12 A proposed stepwise approach for the design of primary facultative ponds (Silva et al., 2010)**

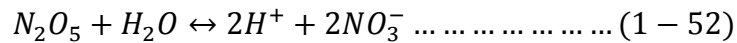
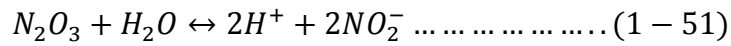
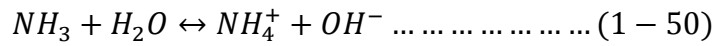
### 1.5.18.2 Nitrogen Biology and N removal

Compounds of nitrogen are of great importance in water resources. The chemistry of nitrogen is complex because of the multiple oxidation states that can be assumed, and that changes in oxidation state can be brought about by living organisms. These bacterial changes can be either positive or negative in aerobic and anaerobic conditions respectively.

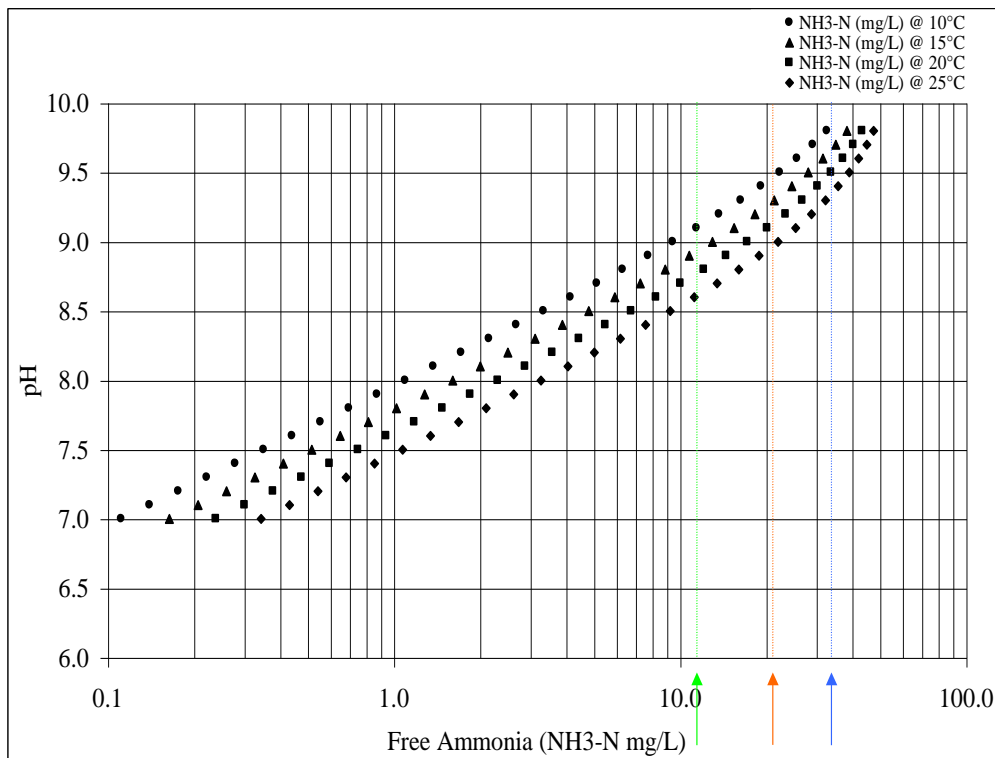
**Table 1-2 Oxidation states of nitrogen**

| Oxidation State | -111            | 0              | 1                | 11 | 111                           | 1V              | V                             |
|-----------------|-----------------|----------------|------------------|----|-------------------------------|-----------------|-------------------------------|
| Species         | NH <sub>3</sub> | N <sub>2</sub> | N <sub>2</sub> O | NO | N <sub>2</sub> O <sub>3</sub> | NO <sub>2</sub> | N <sub>2</sub> O <sub>5</sub> |

Three of these species combine with water to form inorganic ionised species – with the potential to be at very high concentrations,



These water soluble species – ammonium, nitrite and nitrate are known to be of long standing concern to the environment if released, and their concentration in surface and drinking water is heavily regulated. The reduced form  $N^3-$  is a major structural element in amino acids and thence proteins, and in nucleic acids. Nitrogen is readily changed between oxidation states and chemical form through biological, chemical and photo-chemical processes. Obviously nitrogen is an essential component of all living things, but excess of some forms in some environmental compartments can cause significant environmental disruption. These concerns range from health issues with nitrite in drinking water causing methemoglobinaemia in infants and the formation of carcinogens in the nitrosamine group (Choi and Valentine, 2002, Andrzejewski et al., 2005).



**Fig. 1-13 Free ammonia concentration variation with temperature and pH – assuming combined  $\text{NH}_4^+$  and  $\text{NH}_3$  level of 50 mg/L in *Scenedesmus obliquus*. Arrows indicate photosynthetic inhibition levels of 10% (green), 50% (red) and 90% (blue). (Azov and Goldman, 1982)**

Nitrogen, particularly in the form of ammonia is an essential algal nutrient and can lead to massive blooms in discharge waterways as the algae die-off and decompose in the process known as eutrophication. Depletion of oxygen also happens with bacterial autotrophs converting ammonia to nitrite and nitrate in slow moving water bodies. It is also well established that free ammonia is toxic to fish (Sawyer et al., 2003).

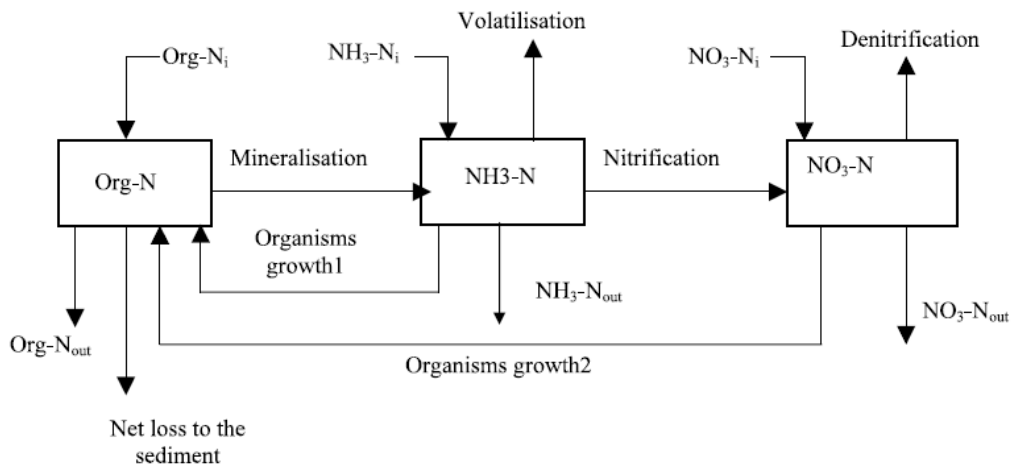
The pH conditions under which free ammonia predominates can be traced by following any of the temperature curves in Figure 1-13 (Azov and Goldman, 1982). The traces can be summarised as ‘the higher the pH and higher the temperature the more free ammonia is present’. Both high temperature and high pH occur regularly in treatment ponds in summer conditions in South Australia, therefore it is not unreasonable to expect ammonia volatilisation to play a role in these ponds. See also para 1.5.13.4

#### *1.5.18.2.1 FATE OF NITROGEN IN WASTEWATER*

The possible routes of nitrogen transformation in a pond are through nitrification, nitrification followed by denitrification, ammonia volatilisation, net loss to sediments, uptake by microorganisms and mineralisation of organic-N Senzia et al. (2002), (Reed, 1985).

Senzia et al. (2002) developed a dynamic rational model for nitrogen removal and transformation in facultative ponds, and compared their predicted outcomes with actual outcomes from an experimental system in Tanzania. They assembled a series of equations to predict all the variables and nitrogen flow routes reproduced in Figure 1-14. They considered this approach superior to the empirical model developed by Pano & Middlebrooks (1982), and to the Reed (1985) model (which was limited to a maximum temperature of 28°C) because both these models were limited by being based on total Nitrogen only, instead of considering all the possible species of N in the wastewater system. The key groups and flow routes and their calculation are considered below.

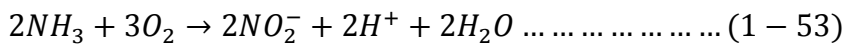
van der Linde et al. (2010) suggest that nitrogen removal mechanisms and pathways differ spatially and temporally on a global level. It is commonly known too that seasonality plays a significant role in nitrogen cycling within WSP - affecting ammoniacal nitrogen removal in particular. The principal and most widely accepted basis for ammonia removal within WSP has been attributed to the volatilization of ammonia (Soares et al., 1996, Silva et al., 1995, Pano and Middlebrooks, 1982), microbial uptake and assimilation (Senzia et al., 2002) and subsequent sedimentation and deposition into the sludge layer (Zimmo et al., 2004, Reed, 1985). The mechanism for nitrification and denitrification requires aerobic and anaerobic conditions proximate to each other in the same pond. Some authors support this mechanism, (Hodgson and Paspaliaris, 1996, Zimmo et al., 2004, Gómez et al., 1995) , while others doubt its importance. On the basis of the low prevailing nitrate concentrations in pond systems, several studies concluded that nitrification does not take place and consequently denitrification does not play a major role in nitrogen removal (Reed, 1985).



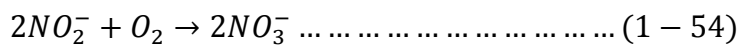
**Fig. 1-14 Diagrammatic representation of Nitrogen transformation and removal in WSPs (Senzia et al., 2002)**

*1.5.18.2.2 Nitrification*

In the wastewater environment, under aerobic conditions, ammonia is oxidised by autotrophic nitrifying bacteria in a two-stage process. The first (*Nitrosomonas* group) derive energy from the conversion of ammonia to nitrite as shown in Equation 1-53.



Nitrite is then further oxidised by another group of nitrifying bacteria, the *Nitrobacter* group, as shown in Equation 1-54.



The rate of nitrification  $r_n$ , is governed by the growth of chemoautotrophic nitrifying bacteria. In turn this growth depends on the pH, temperature, and concentration of ammonia and dissolved oxygen as described in Equation 1-55.

$$r_n = \frac{\mu_n}{Y_n} \left( \frac{NH_4}{k_1 + NH_4} \right) \left( \frac{DO}{k_2 + DO} \right) C_T C_{pH} \dots \dots \dots (1 - 55)$$

where:  $r_n$  = nitrification rate (mg/L/day)

$\mu_n$  = maximum growth rate of *Nitrosomonas* (approx. 0.008/day)

$Y_n$  = yield coefficient of *Nitrosomonas* (approx. 0.13)

$k_1$  = half saturation of  $\text{NH}_4$  for *Nitrosomonas* – also temperature dependant given by Equation 1-56

$$k_1 = 1^{(0.051(T-1.58))} \dots \dots \dots (1 - 56)$$

$k_2$  = half saturation of oxygen for *Nitrosomonas* (assumed to be 1.3 mg/L)

$C_T$  = temperature correction factor derived from an Arrhenius equation 1-57.

$$C_T = e^{\alpha(T-T_0)} \dots \dots \dots (1 - 57)$$

where:  $\alpha$  = an empirical constant (0.098/°C)

$T_0$  = reference temperature (15°C)

$C_{pH}$  = the pH growth limiting factor for *Nitrosomonas* – if pH  $\geq$  7.2 inhibition does not occur (Downing, 1996) and  $C_{pH} = 1$ ; if pH < 7.2 then  $C_{pH}$  is calculated via Equation 1-58

$$C_{pH} = 1 - 0.833(7.2 - pH) \dots \dots \dots (1 - 58)$$

### 1.5.18.2.3 Denitrification

Under anaerobic conditions, the denitrifying group of bacteria reverse the processes, generally only as far as nitrogen gas, which escapes to the atmosphere. However, a few bacteria reduce nitrite all the way back to ammonia for protein formation.

Denitrification in facultative ponds may occur near the sludge layer region if the pond is working properly (Fritz et al., 1979). Denitrification rate  $r_d$  was modelled using first-order Arrhenius kinetics as described by Equation 1-59



$$r_d = R_{20} \theta^{(T-20)} NO_3 - N \dots \dots \dots (1 - 59)$$

The Arrhenius constant  $\theta$  varies from 1.02 to 1.09 and denitrification constant  $R_{20}$  may vary from 0 to 1. The rate coefficient optimised from the model calibration gave  $\theta = 1.02$  and  $R_{20} = 0.1$  which is similar to the  $R_{20} = 0.07$  reported by Ferrara and Harleman (1980) for primary facultative ponds in the USA.

*1.5.18.2.4 Ammonia Volatilisation*

The rate of  $NH_3$ -N volatilisation,  $r_v$  depends on concentration of ammonia gas in the liquid  $NH_3$ -N (g), depth of the system (d) and mass transfer coefficient,  $K_L$  (also temperature dependent) as described by Equation 1-60

$$r_v = \frac{NH_3 \times K_L}{d} \dots \dots \dots (1 - 60)$$

*1.5.18.2.5 Net Loss to the Sediment*

The net loss of nitrogen to the sediments,  $r_s$  depends on the concentration of Org-N according to Equation 1-61.

$$r_s = R_1(Org - N) \dots \dots \dots (1 - 61)$$

They obtained a value for coefficient R1 from model calibration. A value of 0.015/d gave the best accordance between observations and model results.

*1.5.18.2.6 Conversion of mineralised forms  $NH_3$ -N and  $NO_3$ -N to biomass*

Ammonia and nitrate are assimilated into microorganisms at rates calculated as shown in Equation 1-62 and Equation 1-63

$$r_1 = \mu_{max20} \theta^{T-20} \left[ \frac{NH_3 - N}{K_3 + NH_3 - N} \right] (Org - N) \times P1 \dots \dots \dots (1 - 62)$$

$$r_2 = \mu_{max20} \theta^{T-20} \left[ \frac{NO_3 - N}{K_4 + NO_3 - N} \right] (Org - N) \times P2 \dots \dots \dots (1 - 63)$$

where: P1 and P2 are preference factors for  $NH_3$ -N and  $NO_3$ -N, respectively. It is assumed that microorganisms consume  $NO_3$ -N only when  $NH_3$ -N is not available. Both Neel et al. (1961), (Fritz et

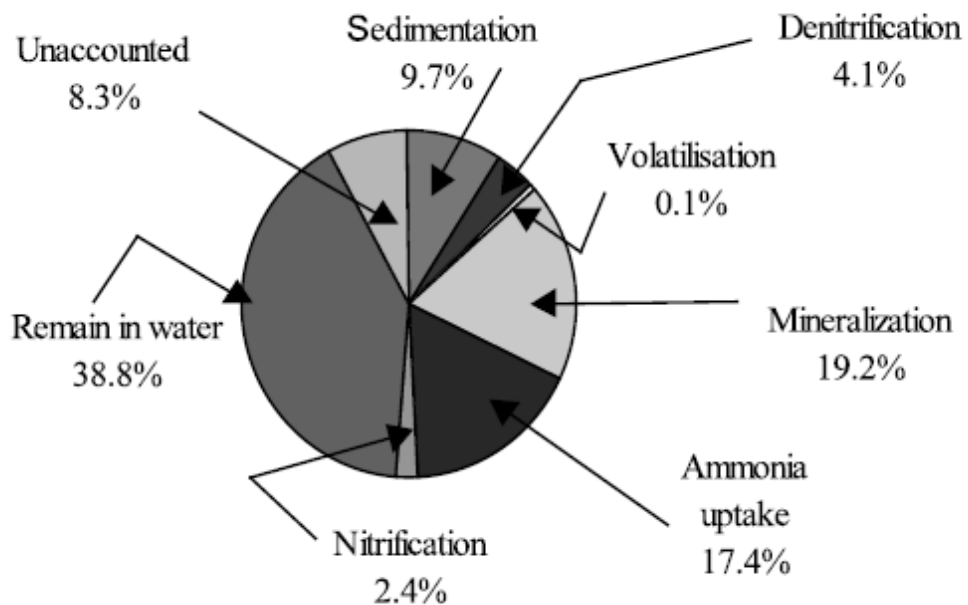
al., 1979) reported that  $\text{NH}_3\text{-N}$  is a preferred source of nitrogen by algae and bacteria for their growth as compared to  $\text{NO}_3\text{-N}$ .

$\mu_{\text{max}20}$  is the maximum growth rate at 20 °C assumed to be 0.18/d (Ferrara and Harleman, 1980).

$\theta$  is the Arrhenius constant with a value 1.02; determined by model calibration.

$K_3$  and  $K_4$  are half saturation constants for ammonium and nitrate, respectively, with  $K_3$  set at 18 mg/L and  $K_4$  as 2.0 mg/L.

As noted, these equations were reasonably accurate to predict nitrogen transformations in an experimental pond in Tanzania. Figure 1-15 shows the breakdown of the percent of the total inflow nitrogen that emerged from the facultative pond in Tanzania, as part of the validation of the model (Senzia et al., 2002).



**Fig. 1-15 Where incoming nitrogen went in a facultative pond in Tanzania (Senzia et al., 2002)**

In contrast to the stable equatorial conditions, van der Linde et al. (2010) using rhodamine dye and  $^{15}\text{N}$  labelled  $\text{NH}_4$  in a WSP in the UK found marked seasonal variation in both hydrology and N transformations. They showed ammonium is rapidly taken up by the pond biomass (mainly algae) and assimilated into cell material, undergoing transformation from inorganic nitrogen to an organic fraction. This is subsequently released as soluble organic nitrogen as a by-product of cell metabolism, but it is mainly released through the degradation of cells through algal die-off.

More than double the amount of  $^{15}\text{N}$  was taken up in the suspended organic nitrogen fraction in summer compared to that in winter, and just under five times as much  $^{15}\text{N}$  appeared as soluble organic nitrogen in the summer than in winter. As temperatures and insolation are higher in the summer than winter, cell synthesis and metabolic functions are correspondingly faster. As expected, a high proportion of the influent  $^{15}\text{NH}_4\text{Cl}$  was assimilated quickly by the components of the pond biomass that use inorganic substrates as their nutrient source. This is reflected by both the higher  $\delta^{15}\text{N}$  and  $^{15}\text{N}$  concentration values for suspended organic nitrogen apparent in their summer data set.

In summer, a large proportion of the  $^{15}\text{NH}_4\text{Cl}$  left the pond unchanged, nevertheless pond biomass does play a very important role in the uptake of influent ammonium nitrogen within the system. The  $\delta^{15}\text{N}$  and  $^{15}\text{N}$  concentrations of suspended organic nitrogen and soluble organic nitrogen were lower in winter compared to summer, demonstrating that uptake and assimilation rates are lower in winter than in summer. In winter, the largest proportion of  $^{15}\text{N}$  left the pond in its unchanged  $^{15}\text{NH}_4\text{Cl}$  fraction as a result of lower temperatures and insolation.

### **1.5.18.3 PHOSPHOROUS BIOLOGY AND REMOVAL**

Both phosphorous and nitrogen are essential nutrients for algae and cyanobacteria, and environmental blooms of either can be a nuisance or a

definite health hazard to vertebrate life. The critical level of phosphorous below which blooms do not occur has been established as around 0.005 mg/L, (Sawyer et al., 2003) a very tiny amount considering wastewater may commonly contain 12 mg/L.

Prior to the development of synthetic detergents, the inorganic phosphorous in wastewater rarely exceeded 3 mg/L. Most of this was from human waste as a result of the breakdown of proteins and nucleic acids, and passage of the freed-up phosphorous in the urine. The amount of phosphorous is a function of dietary protein intake and is considered to be about 1.5 g/day on average in Western countries (Sawyer, 1954).

Most heavy duty synthetic detergents contain large amounts of polyphosphates as “builders”, typically amounting to 13% phosphorous. The very high use of these compounds instead of soap has greatly increased the phosphorous content of domestic wastewater.

All the polyphosphates (molecularly dehydrated phosphates) gradually hydrolyse in aqueous solution to the ortho form from which they were derived as per equation (1-64).



Apart from disodium phosphate, other orthophosphates include trisodium phosphate ( $Na_3PO_4$ ), Monosodium phosphate ( $NaH_2PO_4$ ) and diammonium phosphate ( $(NH_4)_2HPO_4$ ).

Phosphorus in wastewater is found in three principal forms (Metcalf\_&\_Eddy, 2003): orthophosphate, polyphosphates or condensed phosphates; and organic phosphorus compounds. Polyphosphates can be looked upon as polymers of phosphoric acid. Organic phosphorus compounds are mainly insoluble phosphoproteins, nucleic acids and polysaccharides. During biological treatment of sewage in HRAPs, complete hydrolysis of polyphosphates and the decomposition of organic phosphorus compounds results in formation of

orthophosphate which is about 80% of total phosphorus in an HRAP. There are a number of forms of orthophosphate in equilibrium as a function of pH. At the usual pH of municipal wastewater, the predominant form is  $\text{HPO}_4^{-2}$ .

There are two mechanisms for phosphorus removal in an HRAP; algal uptake and chemical precipitation. Phosphorus uptake by algae is lower than nitrogen uptake because the nitrogen content of algae is approximately ten times more than the phosphorus content which is approximately one percent of the 100 to 300 mg/L algal dry weight in a HRAP. If sewage contains 10 mg/L of phosphorus, algal phosphorus uptake would be only 1 to 3 mg/L. Precipitation of phosphates with polyvalent cations such as calcium and magnesium also occurs in a HRP due to the high pH. This precipitation is sometimes called "autoflocculation". which is often incomplete due to insufficient calcium and magnesium concentrations in the wastewater (Nurdogan and Oswald, 1995).

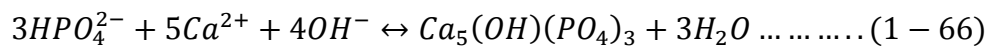
During active photosynthesis, the pH of a healthy culture in an HRAP may be as high as 11 in the afternoons of summer days and stays around 9 during winter (Nurdogan and Oswald, 1995). Inorganic-carbon-limited growth of algae causes a pH increase in the pond due to photosynthetic depletion of dissolved  $\text{CO}_2$ . The change in carbonate-bicarbonate ratio results in a shift in the HRAP pH as indicated by the Henderson-Hasselbach equation as in Equation 1-65:

$$pH = pK_2 + \log \left( \frac{\text{CO}_3^{2-}}{\text{HCO}_3} \right) \dots \dots \dots (1 - 65)$$

The value of  $pK_2$  is around 10.3 under ordinary conditions (Sawyer et al., 2003). Any increase in carbonate or decrease in bicarbonate or both will increase the magnitude of the carbonate-bicarbonate ratio and consequently the pH. Alkalinity species would be mainly carbonate and hydroxide at pH values over 10. Polyphosphates and organic-P are known to be removed by adsorption on  $\text{CaCO}_3$  crystals, which are formed in significant amounts in the pH range of HRAP operation. Supersaturation with respect to phosphate, hydroxide, and

carbonate salt of calcium, magnesium, and other metals in alkaline waters brings about the chemical precipitation of these salts at high pH values. As noted previously co-precipitation of microalgae with such chemical precipitates is termed "autoflocculation" (Sukenik and Shelef, 1984).

Both ortho-P and ammonia-N can be removed through precipitation of insoluble complexes such as  $\text{CaNH}_4\text{PO}_4$  and  $\text{MgNH}_4\text{PO}_4$ . The solubility of hydroxyapatite is so low that even at pH values as low as 9.0, a large fraction of ortho-P can be removed if sufficient calcium ions are present in solution. Using  $\text{HPO}_4^{2-}$ , an approximation of the chemical formation of hydroxyapatite can be written as in Equation 1-66



(Nurdogan and Oswald, 1995) were able to achieve significant removal of phosphorous and algal autoflocculation in a HRAP by adding small amounts of CaO to wastewater that already contained 25 mg/L of dissolved Ca. When dissolved Ca was increased to 60 mg/L phosphorous was removed almost completely. They maintained pond pH around 11 for over 1 month with no detrimental effects on algal growth or pond performance. It was noted that *Daphnia* did not graze on the high pH pond, but were very active in adjacent ponds with lower pH levels.

Mesplé et al. (1995) concluded that the main difficulty in modelling phosphate evolution in HRAPs was whether algae use the  $\text{PO}_4$  before precipitation, or whether they grow on the dissolved  $\text{PO}_4$  remaining after precipitation. In a later paper the same authors found that the  $\text{PO}_4$  precipitation process is very sensitive to nyctemeral variations (daily oscillations) in pH, as described above. Using their predictive model they simulated  $\text{PO}_4$  concentrations over a two-year period. The predictions of this model show that about 10% of total  $\text{PO}_4$  input is removed by precipitation whereas about 30% is removed by absorption (Mesplé et al., 1996).

### **1.5.19 COMPARISON OF PERFORMANCE OF HRAP AND WSPs**

The Land Area Requirement (LAR) of WSPs is large due to inefficient hydrodynamics. Short-circuiting and thermal stratification are often reported to occur (Herrera and Castillo, 2000, Shilton, 2000, Llorens et al., 1992a, Shilton et al., 2000). This is one reason why the shallow, mixed HRAP could offer significant advantages over the land hungry WSP system.

There are few direct comparisons of HRAP performance against WSP performance in the literature.

#### **a. Ouarzazate (30.92° N), Morocco**

In a comparison study reported by El Hamouri et al. (2003) the HRAP had an annual average  $k_{20^{\circ}\text{C}}$  (first order reaction rate constant) for COD removal of  $0.123 \text{ day}^{-1}$  for while the 3 associated facultative ponds had values of 0.097, 0.025 and  $0.003 \text{ day}^{-1}$ . Also, comparing nominal and tracer study hydraulic retention times showed large differences for the facultative ponds (indicating short-circuiting) but not for the HRAP.

Optimal chlorophyll-a concentration was found to be 3 mg/L for the HRAP and only 1.1 mg/L for the facultative ponds. The authors reported “pollutant specific removal rates” (SRR) that translate the hydrodynamic efficiency and the rate of COD biodegradation into pond performance per  $\text{m}^2$  and per day were calculated. They showed that the adoption of the HRAP in place of a series of 3 facultative ponds reduces the net land area requirement (LAR) by at least 40%.

#### **b. Meze, (43°25' N) France**

Picot et al. (1992) compared two HRAP systems with a standard 3 cell WSP at Meze in the South of France. Two experimental HRAPs were built on the same site. As can be seen in Fig. 1-20, the first pilot plant consists of a HRAP basin (Fig. 1-20, B35) with surface area 48 m<sup>2</sup>, depth 0.35 m, and detention time 8 days, preceded by a primary basin with depth 1.5 m and detention time 8 days. The second pilot plant consists of 3 HRAPs running in parallel, each one having a surface area of 100 m<sup>2</sup> and differing only in their depths: 0.30, 0.45 and 0.60 m (Fig. 1-21, B30, B45 and B60). They were supplied with two hour settled sewage. Detention time in the 3 ponds was fixed at 4 days in summer and 8 days in winter. Their findings are summarised in Table 1-1 and Figs 1-16 to 1-19.

**Table 1-3 Mean ± Std Dev of physico-chemical and bacteriological characteristics of influent and effluent WSP & HRAP wastewater over the years 1988 – 1990 as reported by Picot et al. (1992)**

|  |                         | Mean ± SD    |              |                    |
|--|-------------------------|--------------|--------------|--------------------|
|  |                         | Influent     | SP effluent  | HRAP(B35) effluent |
| <b>Physicochemical characteristics</b> |                         |              |              |                    |
| Water temperature                      | °C                      |              | 15.4 ± 6.99  | 17.2 ± 6.85        |
| pH                                     |                         | 7.85 ± 0.47  | 8.19 ± 0.27  | 8.53 ± 0.47        |
| Dissolved Oxygen                       | (mg/l)                  |              | 1.77 ± 1.57  | 11.88 ± 7.02       |
| Chlorophyll-a                          | (mg/l)                  |              | 0.59 ± 0.39  | 1.34 ± 1.34        |
| Suspended Solids                       | (mg/l)                  | 251 ± 109    | 136 ± 69     | 160 ± 120          |
| Chemical Oxygen Demand                 | (mg O <sub>2</sub> /l)  | 541 ± 408    | 216 ± 91     | 294 ± 200          |
| Dissolved COD                          | (mg O <sub>2</sub> /l)  | 249 ± 368    | 76 ± 26      | 78 ± 40            |
| Suspended COD                          | (mg O <sub>2</sub> /l)  |              | 140 ± 84     | 217 ± 194          |
| Kjeldahl Nitrogen                      | (mg N/l)                | 42.35 ± 5.98 | 33.70 ± 4.73 | 22.13 ± 9.83       |
| Suspended Organic Nitrogen             | (mg N/l)                | 11.16 ± 3.49 | 11.82 ± 7.40 | 12.4 ± 10.12       |
| Ammonia                                | (mg N/l)                | 27.25 ± 6.64 | 20.48 ± 9.48 | 6.93 ± 7.77        |
| Total Phosphorus                       | (mg P/l)                | 14.65 ± 3.34 | 11.35 ± 3.17 | 8.57 ± 2.45        |
| Suspended Organic Phosphorus           | (mg P/l)                | 3.47 ± 2.63  | 5.78 ± 4.8   | 2.96 ± 2.39        |
| Orthophosphates                        | (mg P/l)                | 10.21 ± 1.96 | 4.95 ± 3.56  | 4.54 ± 2.07        |
| Alkalinity                             | (meq/l)                 | 8.79 ± 1.71  | 10.07 ± 1.91 | 8.14 ± 1.32        |
| <b>Bacteriological characteristics</b> |                         |              |              |                    |
| Fecal coliforms                        | (log <sub>10</sub> /ml) | 5.04 ± 0.51  | 2.65 ± 1.01  | 2.92 ± 1.04        |
| Fecal streptococci                     | (log <sub>10</sub> /ml) | 4.04 ± 0.26  | 1.56 ± 0.87  | 1.71 ± 0.69        |
| <i>Pseudomonas aeruginosa</i>          | (log <sub>10</sub> /ml) | 1.70 ± 0.73  | 0.23 ± 0.32  | 0.22 ± 0.18        |
| <i>Aeromonas spp.</i>                  | (log <sub>10</sub> /ml) | 5.17 ± 0.48  | 3.25 ± 1.03  | 3.19 ± 0.42        |

These authors concluded that the HRAP process by generating algal growth reduces the required surface area by a factor of 5. They also considered that this



process is particularly interesting for nutrient removal, especially nitrogen, and could be useful in coastal areas susceptible to eutrophication. Importantly, they noted that environmental factors and pond depth play a more important role in HRAP efficiency than retention time of water in the basins. From a sanitary point of view the purifying efficiency of the HRAP is comparable to that of the WSP. Faecal coliform reduction was the same whether the HRAP was preceded by a primary pond or by a clarifier. HRAP depth had a marked effect; under specific load experimental conditions, a depth of 0.60 m results in a considerable decrease in purifying efficiency. In both the HRAP and WSP effluents, abundances of faecal pollution indicators showed seasonal fluctuations: low summer abundance, high winter abundance.

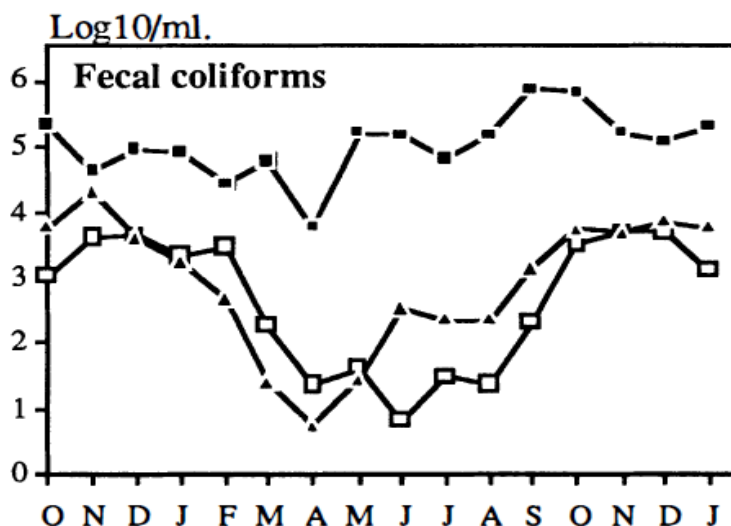


Fig. 1-16 Evolution of faecal coliforms in the influent (-), in stabilization pond (- □-) and HRAP (- Δ-) effluents. Picot et al. (1992)

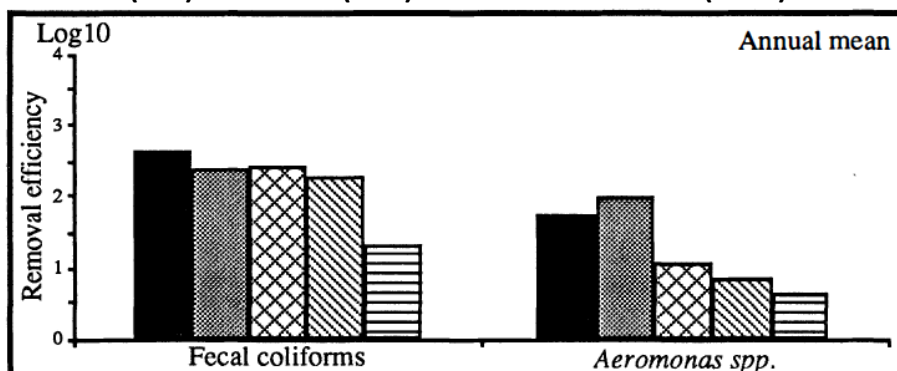


Fig. 1-17 Removal efficiency of faecal coliforms ( $\log_{10}$ ) in stabilization pond and HRAP pilot plants (B35, B30, B45 and B60). Picot et al. (1992)

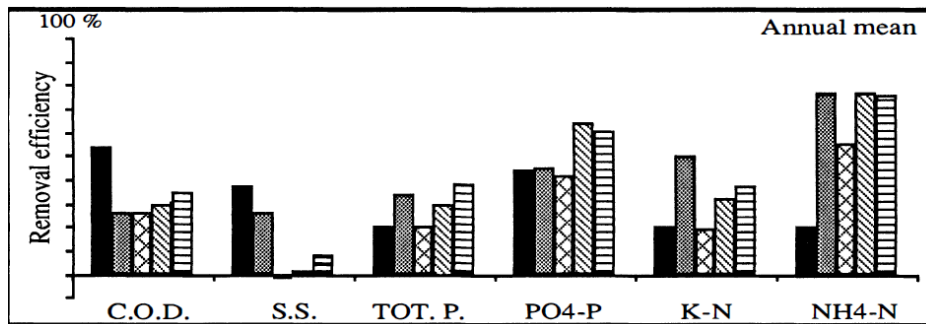


Fig 1-18 Removal efficiency of carbon, nitrogen and phosphorus pollution forms and suspended solids in stabilization pond ■ and HRAP pilot plants (B35 ▨, B30 ▩, B45 ▧ and B60 ▦). Picot et al. (1992)

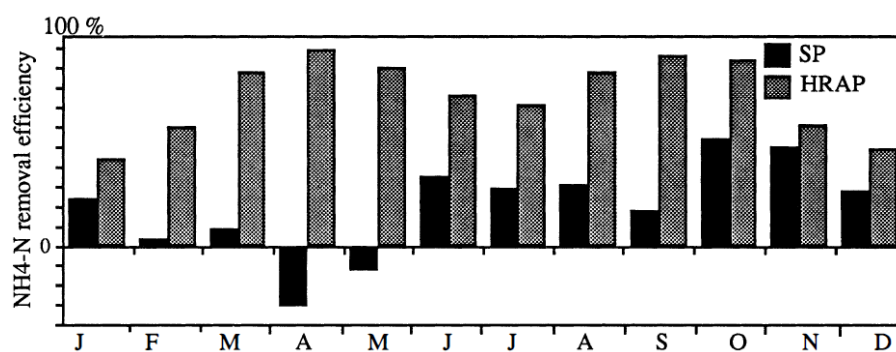
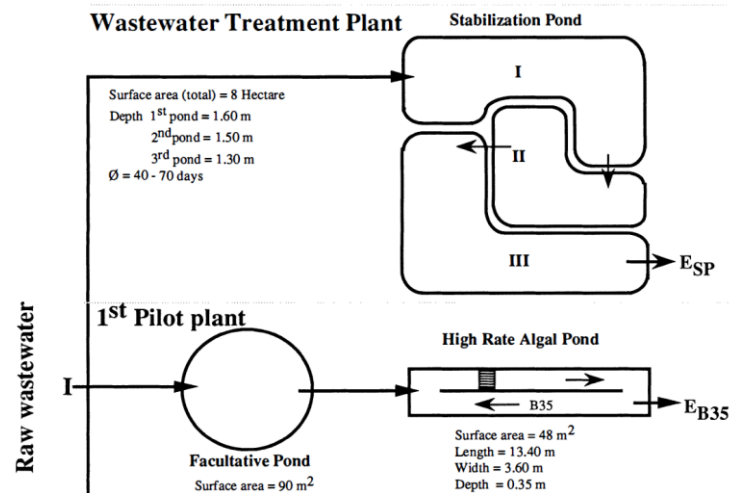
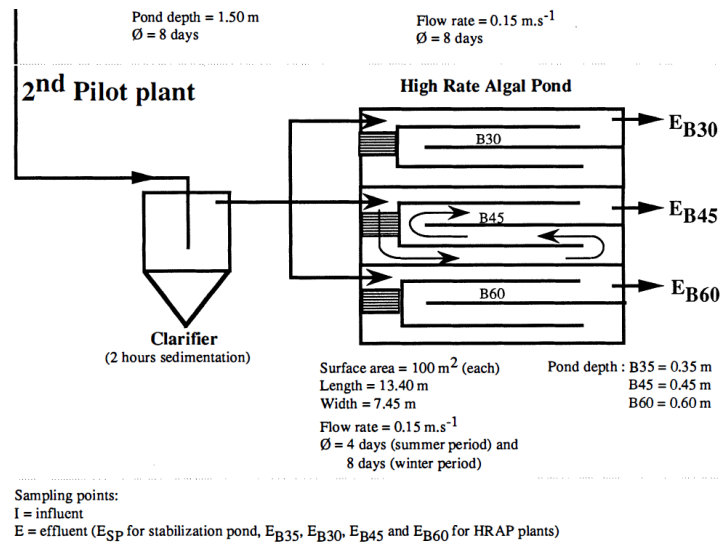


Fig. 1-19 Ammonia removal efficiency in Stabilization Pond (SP) and in High Rate Algal Pond (HRAP). Picot et al. (1992)

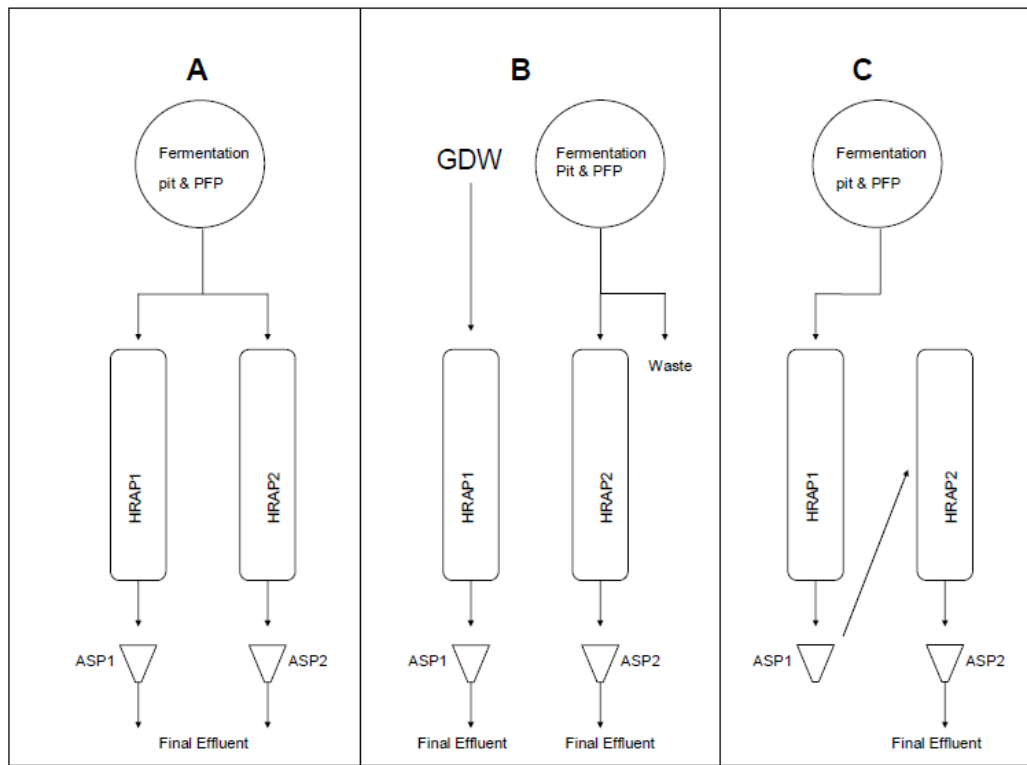




**Fig. 1-20 Plan view (not to scale) of the WSP:HRAP comparison ponds at Meze, France (Picot et al., 1992)**

Wells (2005) in his Masters' thesis presented observational results from a number of years of operation of an Advanced Integrated Algal Ponding System (AIAPS) at Grahamstown in South Africa. This system is at a similar latitude to the HRAP at KoM (33°18' S vs 34°13' S). Operation of the system varied during the course of the study period depending on the specific research objectives under investigation, see Figure 1-21. The hydraulic loading to the fermentation pit and PFP remained constant at 75m<sup>3</sup>.day<sup>-1</sup>. There was no control over the chemical oxygen demand (COD) in the raw sewage and the organic loading did, therefore, fluctuate. Experimental adjustments were only made with the HRAPs. During commissioning and the first 4 years of operation these two ponds were operated in parallel, each unit taking half of the PFP effluent i.e. 37.5m<sup>3</sup>.day<sup>-1</sup>. This equates to a 4 day hydraulic retention time (HRT).

In February 2000, the system was reconfigured and one HRAP was retrofitted to



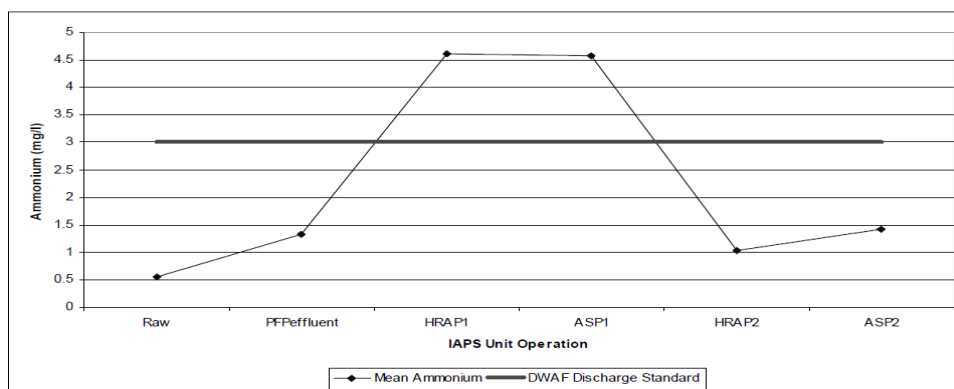
**Fig. 1-21 HRAP operation configurations used at Grahamstown (Wells, 2005)**

investigate the efficacy of the process as an independent unit operation, used as a tertiary treatment stage, polishing final effluent from a conventional sewage treatment facility. This application became known as the Independent High Rate Pond (IHRAP), with the final treated water being sourced from the Grahamstown Disposal Works (GDW). A THRT of 5 days was used for this study. During this time the primary facultative pond (PFP) effluent was split so that HRAP1 continued to operate as it did during the parallel, averaged configuration, i.e. receiving its design load (Fig. 1-21, B). The excess flow was wasted to drain. The ponds were operated in this manner until June 2003, when the IHRAP treating GDW final effluent was discontinued.

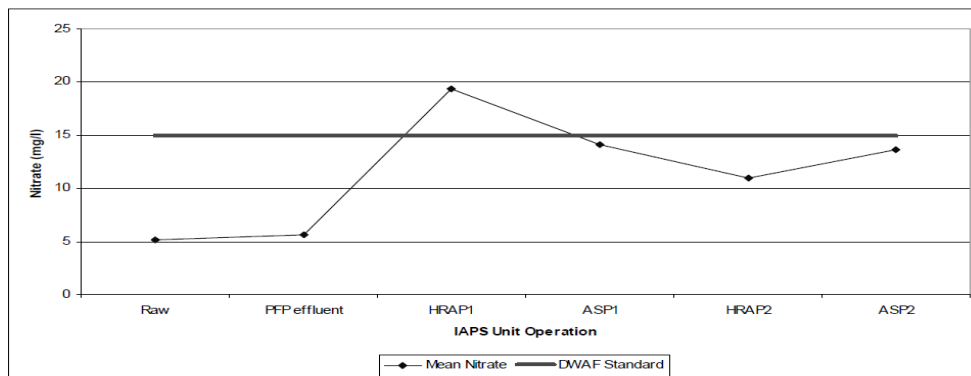
In June 2003 HRAP2 was reincorporated into the IAPS cascade but configured to operate in series with HRAP1 rather than in parallel as in the earlier phases of the project. The performance of the second HRAP as a polishing unit, receiving effluent from the first, after settling algae in the ASP, was thus evaluated. Retention times during this last period were varied between 3 and 6 days.

In all the scenarios above, the HRAPs were receiving water that had passed either through the GDW or the PFP, so the first level of treatment had already occurred before the water arrived at the HRAP. This was the equivalent of the second phase of HRAP investigation in the project being reported here, and in actuality, no true comparison between WSP and HRAP was reported in the Wells thesis.

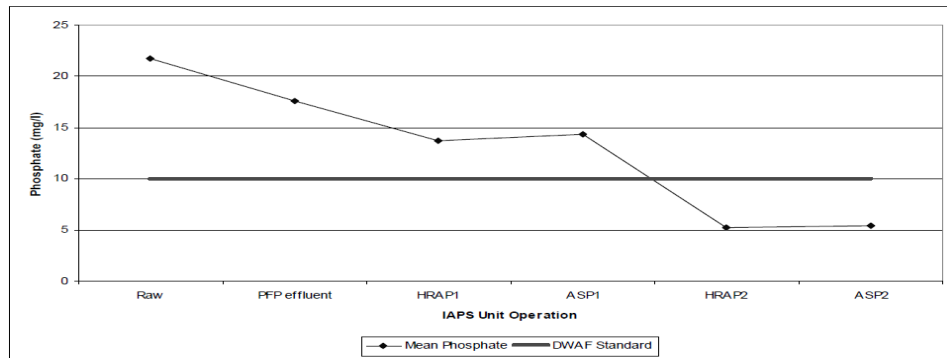
A summary of the results of the water passing through the PFP and then the two HRAPs in series (flow C in Figure 1-22) is contained in Figures 1-22 to 1-25.



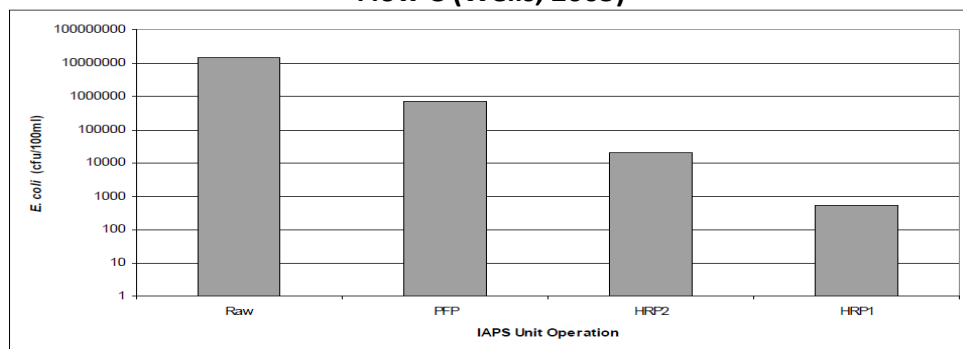
**Figure 1-22. Ammonium levels at discharge from the treatment elements in Flow C (Wells, 2005)**



**Figure 1-23. Nitrate levels at discharge from the treatment elements in Flow C (Wells, 2005)**



**Figure 1-24. Phosphate levels at discharge from the treatment elements in Flow C (Wells, 2005)**



**Figure 1-25. Log<sub>10</sub> E. coli levels at discharge from the treatment elements in Flow C (Wells, 2005)**

Of particular interest is the approximate 1.5 log reduction in *E. coli* counts at each treatment stage. Also, of note is the large drop in phosphate levels in the second HRAP. The reasons for this large removal are not adequately explained in the thesis. Finally, the ammonium level start low and increase in the first HRAP, which is not well explained, as the expectation would be for oxidation of ammonia to occur in the HRAP environment. Ammonia levels do decline again in the second HRAP.

#### 1.5.19.1 Summary of literature comparison of WSP & HRAP performance

The very limited comparisons available in the literature suggest the following key differences between WSP & HRAP performance

1. El Hamouri et al. suggest that the HRAP reduces the net land area requirement (LAR) by at least 40% compared to the WSP. Picot et al. suggest that the HRAP process by generating algal growth reduces the required surface area by a factor of 5.

2. Picot et al. note that from a sanitary point of view the purifying efficiency of the HRAP is comparable to that of the WSP. Faecal coliform reduction was the same whether the HRAP was preceded by a primary pond or by a clarifier.
3. El Hamouri et al. showed large differences between the nominal and tracer study hydraulic retention times for the facultative ponds but not for the HRAP. This indicated that short-circuiting happened in their facultative pond.
4. El Hamouri et al. demonstrated that the HRAP had an annual average first order reaction rate constant for COD removal consistently greater than the comparison facultative ponds.
5. Picot et al. reported a large range of similar nutrient removals from both systems (Table 1-1) with the key difference being the greater ammonia removal from the HRAP.

HRAPs have other potential advantages: firstly the reduced surface area may reduce evaporative losses and improve the water balance for irrigation. Secondly, as a consequence of the reduced areal footprint, the cost of construction of the HRAP is significantly lower than the WSP system.

## CHAPTER 2

# MATERIALS AND METHODS

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## 2.1 The Ponds

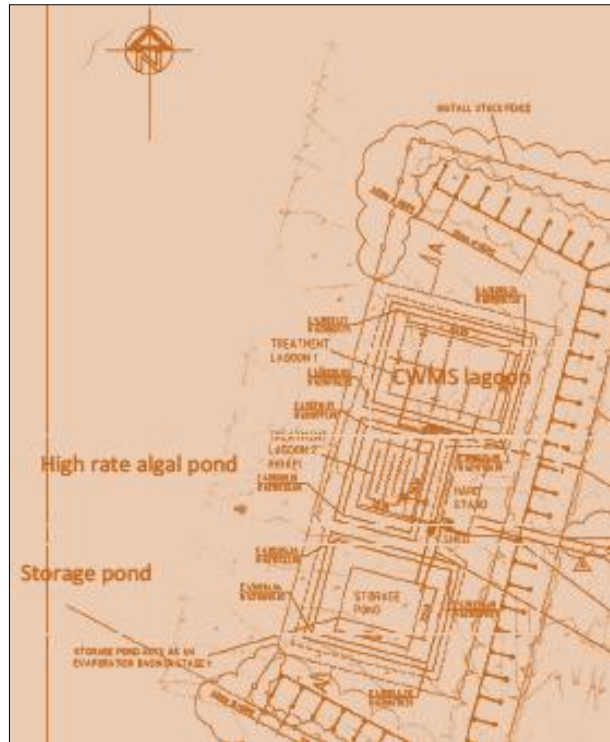
The study sites were located as follows:- the HRAP at Kingston-on-Murray (KoM) (34.2167° S, 140.3333° E), and the WSP at Lyndoch (34.6000° S, 138.8833° E). By road, the ponds are 156 km apart. The population of KoM is seasonally variable with a summer holiday and fruit picker boom. Population estimates range from 140 to 300, with 255 usually quoted. In the 2011 census the population of Lyndoch was recorded as 1,909.

The daily inflow comes from a central pumping station in the township which is activated and deactivated by float valves. At Kingston-on-Murray the height between the activating and deactivating float valves was set so that each pumping was of 2,000 litres delivered over 20 minutes by a pump that delivered 100 L/min. This was the smallest volume that could be pumped at each time as any smaller aliquot caused too frequent activation of the pump resulting in the thermal protection switch activating, turning off the pump and activating an alarm call to Council employees. Theoretically the pump was set to activate 6 times per day. In practice there was a cluster of pumpings, typically 2 pump activations in the morning, another in the early afternoon, 2 more activations in the evening and a final activation just after midnight. Treated wastewater then flowed by gravity into a balance storage lagoon prior to future horticultural re-use.

At Lyndoch, the pump volume was much lower, and was activated for about 5 minutes per pumping, spaced about 15 minutes between each pumping. The Waste Stabilisation Pond (WSP) system at Lyndoch was a three cell system with gravity flow between each pond (Plates 2-3 & 2-4). Pond dimensions are

detailed in para 2.1.5. Treated wastewater was then pumped on a daily basis to a storage dam nearby for subsequent re-use in local horticultural activities.

### 2.1.1 Original Design of HRAP and rebuilt design –



**Fig.2-1** Section taken from the site plan of the Kingston on Murray Community Waste Management Scheme incorporating a 5 cell waste stabilisation pond system and a high rate algal pond and a storage pond.

Figure 2-1 is taken from the original design plans showing the 5 cell waste stabilisation ponds in the Northern section, with the HRAP in the middle and a storage lagoon in the South. The CWMS, 5 cell lagoon and the HRAP were designed to enable a 50:50 or 60:40 split of wastewater water influent to each system. The CWMS lagoon had a total volume of approximately 2000m<sup>3</sup> and a surface area of 1600m<sup>2</sup> to achieve the designed combined theoretical hydraulic residence time (THRT;  $\theta$ ) of 66 days. The lagoon comprised of a 1 cell facultative pond ( $\theta$ , 36d) and four maturation ponds in series with a combined  $\theta$  of 30d. Knowing the pond dimensions allows an estimate to be made of the expected

faecal coliform removal efficiency or Log Removal Value (LRV). These were calculated for each pond from tables provided by von Sperling (2005) using  $K_b(\text{dispersed flow}) = 0.549 \times H^{-1.456}$  (average pond temperature of 20°C) (where H =pond depth) and an estimated dispersion number calculated from  $d = \frac{1}{L/B}$ , where L and B are the pond length and breadth respectively. The figures arrived at for the LRVs were 1.80 for the facultative pond and 0.75 for each of the maturation ponds giving an estimated total LRV of 4.80.

The HRAP was designed at a BOD<sub>5</sub> areal loading of 286 kg/ha/d using values for algal oxygen production rates and atmospheric oxygen diffusion derived by Evans et al. (2003). The initial HRAP was constructed with a surface area of 600m<sup>2</sup> (Fig. 2-2) at a maximum operational depth of 0.6m. The wastewater was circulated (0.2 m/s) through the meandering channels by an eight bladed paddlewheel driven by a geared reduction motor.

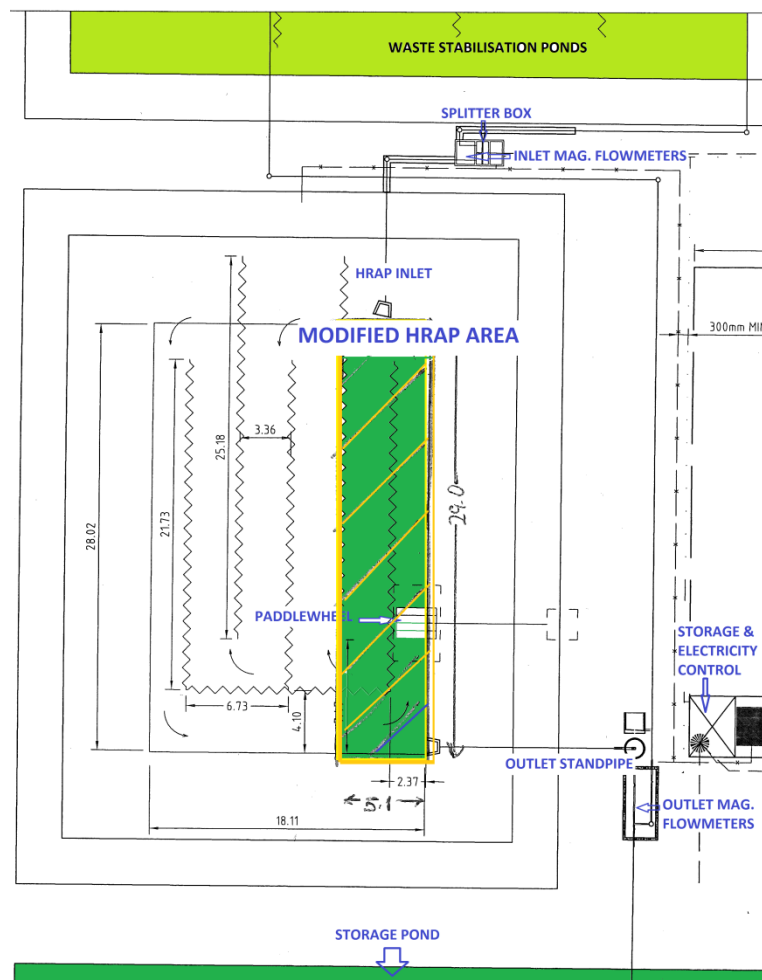
The original objective was to compare side-by-side the performance of the CWMS lagoon system and the HRAP, assuming a design flow of 60,000 L/d. However, when the site was commissioned the wastewater flow was measured at 12,000 L/d which was insufficient to run both plants simultaneously. The 600m<sup>2</sup> HRAP was also oversized for this flow rate. Substantial revision of the experimental plan by the research project team, and reconfiguration carried out by the LGA of the HRAP proved necessary, before the study could begin.

### **2.1.2 Revised site design**

The surface area of the HRAP was reduced by construction of an internal baffle enabling the system to be operated at depths yielding THRTs between 4 and 10 days. The HRAP became a single loop raceway, 30m long with a single channel width of 2.5m. The earthen ponds were lined with high density polyethylene sheet, from which the floating dividing wall between the channels was also constructed (Figure 2.2 & Plate 2-1). Wastewater within the HRAPs was circulated at a mean surface velocity of 0.2 m/s by an 8 blade, stainless steel

paddlewheel wheel driven through a reduction gearbox via a single phase 750 W electric motor (Plate 2-1 & 2-2). The performance of the HRAP 1 was evaluated when treating septic tank effluent reticulated to the wastewater treatment plant at Kingston-on-Murray.

Water depth was set by adjusting the height of the overflow pipe in the outlet standpipe (Fig. 2-2 & Plate 2-10).



**Fig.2-2 HRAP site plan with modified design overlain**



**Plate 2-1 & 2-2 Original HRAP configuration (left), modified HRAP configuration and weather station (right)**

### **2.1.3 HRAP study design (see also Table 2-1 & 2-2)**

Three water depths and two different strength influents were studied in the HRAP. An effort was made to study each factor in a “hot” and “cold” season (see para 3.1.1 for an explanation of the seasonal splits). The two influent strengths were septic tank derived (this part of the study will be referred to as HRAP 1) and facultative pond derived (referred to as HRAP 2) influents. The analyses of these influents are presented in Tables 3-2 to 3-6 and Figs. 3-4 to 3-6 for HRAP 1, and Tables 3-14 to 3-16 and Figs. 3-23 to 3-25 for HRAP 2 .

The three depths studied were 0.32 m (low), 0.43 m (medium) and 0.55 m (high). The influent volume remained constant throughout at  $12.35 \pm 3.1 \text{ m}^3$  per day. The THRT varied with depth from 4.5 days (low) to 6.4 days (medium) to 9.1 days (high). As the walls are sloped the surface area also varies with depth from  $192 \text{ m}^2$  (low),  $208 \text{ m}^2$  (medium) to  $226 \text{ m}^2$  (high). Pond volumes for the three depths were  $55.3 \text{ m}^3$  (low),  $78.6 \text{ m}^3$  (medium) to  $11.9 \text{ m}^3$  (high).

### **2.1.4 WSP study design**

No research changes were made to the operational conditions at the WSP. The ponds were monitored at approximately fortnightly intervals throughout the study period. The Council operating the WSPs introduced an alternative

treatment stream through a rotating biological contactor (RBC) in July 2011, which diverted 40 m<sup>3</sup>/d of inflow away from the WSPs. This changed the daily inflow from 165 m<sup>3</sup>/d to 125 kL/d.

### **2.1.5 The WSP at Lyndoch – size and retention times**

The Local Government Association of South Australia CWMS Program Manager, Richard Gayler, assisted in locating a CWMS lagoon system which could be used for the performance comparison. The Lyndoch system (Plates. 2.3 & 2.4) was selected. This three cell system with gravity flow between each pond, operated by Barossa District Council, was designed and constructed in 1979 to service a population of approximately 1,750. As noted in para 2.1.4, the wastewater influent flow varied within the study period between 125 and 165 m<sup>3</sup> per day. The lower flow rate was a consequence of the installation of a rotating biological contactor (RBC) during the project, through which some of the primary flow into the plant was diverted.



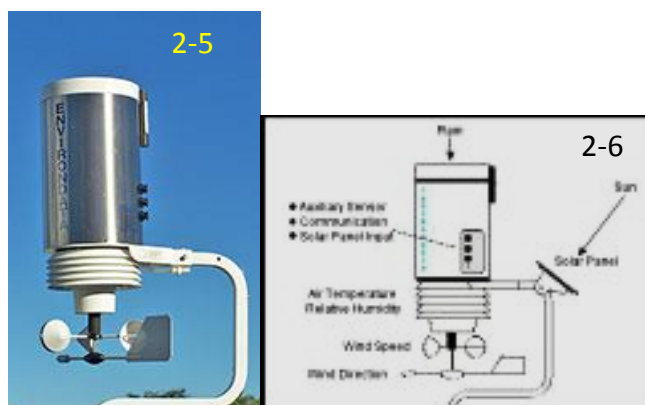
**Plate 2-3 & 2-4 Lyndoch Wastewater inlet to Pond 1, with running inlet water visible during a pumping period and all 3 ponds:- Pond 1 on left and 2 & 3 on right**

### **2.1.6 WSP Bathymetry**

As the ponds had not been desludged since construction in 1979, bathymetry for total depth and sludge depth using the white towel on a pole method (Lloyd, 2005) as adapted from Malan (1964) was conducted on a 10 metre by 10 metre grid for each pond. The facultative pond (WSP 1) was 180 m long and 35 m wide to give a surface area of 6,300 m<sup>2</sup>. The original depth was 1.2 m and now averages 0.68 m, effective volume of 4,533 m<sup>3</sup> and THRT of 27.5 days when inflow was 165 m<sup>3</sup>/d and 36 days when inflow was 125 m<sup>3</sup>/d. This was followed by two maturation ponds operated in series, each 85 m long and 30 m wide. Each pond has a surface area of 2,550m<sup>2</sup>, the effective volume, accounting for sludge, of the first maturation pond, WSP 2 (current effective average depth 0.62m) was 1581m<sup>3</sup> with THRT of 9.58 days at inflow rate of 165 m<sup>3</sup>/d and 12.65 days when inflow was 125 m<sup>3</sup>/d. The second maturation pond (WSP 3) has an effective volume 1,479m<sup>3</sup> (current effective average depth 0.58m) and a THRT of 8.96 days at inflow rate of 165 m<sup>3</sup>/d and 11.83 days when inflow was 125 m<sup>3</sup>/d.

### **2.1.7 Installation of automatic weather station, continuous logging devices for temperature, pH and DO in the pond**

Both sites were provided with a weather station, Envirodata WeatherMaster 2000™ (Plates 2-5 & 2-6) supplied by Envirodata Environmental Monitoring & Management, P.O. Box 395, WARWICK, Queensland, 4370, Australia, which continuously logged photosynthetically active radiation (PAR 400-700nm), total UV irradiation, air temperature, rainfall, humidity and wind speed and direction. Data was downloaded from the weather station to a laptop computer on each site visit.



**Plates 2-5 & 2-6 WeatherMaster 2000™ photograph and diagram of parts**

In all three WSPs, the water temperature, dissolved oxygen (DO) and pH were continuously monitored in situ by mid-pond position boxes with sensors placed 0.2 m below the surface (Plate 2-7). Data was recorded on to battery powered T-TEC A™ (Temperature Technology, 263 Gilbert St, Adelaide, 5000, SA) 4 – 20mA logger. pH probes used were Hach combination pH/ORP sensors connected to a Hach GLI Pro transmitter-controller. DO probes used were Danfoss Oxy 1100 connected to a Danfoss EMCO-1 transmitter-controller. Each transmitter-controller sent signals to T-TEC A loggers. Temperature Technology thermistor sensors were placed 0.2 m below the HRAP surface. The Temperature Technology thermistors operate in the range of 0 to 70°C± 0.2°C. These were operated continuously and data logged to T-TEC E & F™ loggers recording every 30 minutes.

To determine temperature stratification within the ponds at Lyndoch a chain of thermistors with sensors located at 0.1, 0.35 and 0.6 m below the surface (Plate 2-8) was placed in each pond; these were continuously logged to T-TEC E & F™ loggers recording every 30 minutes.

At the Kingston-on-Murray HRAP, identical equipment to that described above and in Plate 2-7 was placed on the bank with probes placed mid-stream, and loggers taking readings every 30 minutes.





**Plates 2-7 & 2-8. 2-7 Installed solar powered DO, pH and temperature monitoring box in mid-pond position. 2-8 Thermistor chain before installation mid-pond**

### **2.1.8 Measuring flow rates**

Barossa Council maintains a MagFLOW meter on the inlet side of the Lyndoch system and provided daily inlet data. Outlet data was measured by a privately owned meter as the treated water was sold to a local vineyard for irrigation purposes. MagFLOW meters (ABB, Electromagnetic Flowmeter & MagMaster recording box) were also installed on the inlet and outlet of the HRAP at KoM. These were not logged, but readings of total flows were taken on each visit.

## **2.2 Water sample collection and analysis**

### **2.2.1 Water sampling techniques**

For Lyndoch WSP 1, 1 litre inlet samples were collected as manual grab samples from the inlet pipe depicted in Plate 2-3 of the facultative pond. Manual 1 litre grab samples were collected from the outlet weir points for WSP 1, 2 & 3 (as an exemplar Plate 2-12 depicts WSP 1 collection point). Samples were refrigerated, transported to the laboratory and analysed within 24h of collection.



**Plates 2-9 & 2-10 HRAP - Sampling directly from the inlet splitter box as the wastewater is flowing; and from the stand-pipe in the outlet control pipe.**

For the HRAP, 1 litre samples were collected by 'grab sampling' directly from the inlet splitter box (Plate 2-9) during pumping or immediately after pumping had ceased. The timing of collection was to ensure no settling occurred as this would result in readings of BOD<sub>5</sub> and *E. coli* considerably lower than in the flowing sample.

Outlet water was collected from the riser controlling pond depth (Plate 2-10) in the outlet sump. A few samples were single manual grab samples from this riser. Most samples were collected by the refrigerated ISCO Avalanche auto-sampler (Fig. 2-11) as composite samples consisting of 400 ml collected at 0100 and another 400 ml at 1300 for each collection day. The samples were held at 1°C by the sampler until collection (maximum 12 days in the sampler, see Tables 2-1 & 2-2). They were then transferred to a car refrigerator and transported to the laboratory at 1°C and further refrigerated and processed within 24 hours.



**Plate 2-11 & 2-12** 2-11 ISCO Avalanche refrigerated multi sampler in-situ at the KoM HRAP site and 2-12 overflow weir sample collection site at the exit point of WSP 1

### **2.2.2 WSP Sampling Schedule**

Samples were collected approximately every fortnight from each of the WSP ponds at Lyndoch, with a total of 82 collections over the period April 2010 to February 2012.

### **2.2.3 HRAP Sampling Schedule**

For the HRAP, samples were collected according to the schedules in Table 2-1 & 2-2 below. The sampling schedule was interrupted from April 2011 to July 2011 whilst structural changes were made to allow inflow to come from the adjacent facultative pond effluent. Throughout the rest of the text, the two sampling periods will be referred to as HRAP 1 using septic tank fed influent, and HRAP 2 using facultative pond fed influent.

The scheduling was designed to ensure that each operational depth was sampled over three Theoretical Hydraulic Retention Times in a range of seasonal

conditions. After any change in depth 3 THRTs were allowed to elapse before sampling commenced to enable the new quasi steady state to be obtained prior to sampling.

Influent and effluent wastewater samples were analysed for turbidity (NTU), suspended solids (SS) and chlorophyll *a*. Filtered water (GFC Whatman, exclusion size 1.2µm) was used for the analysis of BOD<sub>5</sub>, and the nutrients NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N and PO<sub>4</sub>-P using methods described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). *E. coli* 100 mL<sup>-1</sup> was estimated using the Colilert® chromogenic MPN system( Iddex Ltd).

**Table 2-1: Sampling dates for HRAP system fed septic tank effluent (n, number of samples analysed)**

| Depth & THRT of HRAP                     |   |   |
|--|---|---|
| Low (0.32m)<br>THRT = 4.5 days<br>n = 57 | Medium (0.43m)<br>THRT = 6.5 days<br>n = 35 | High (0.55m)<br>THRT = 9 days<br>n = 31 |
| 5/5/10 - 9/5/10                          | 19/8/10 - 2/9/10                            | 11/7/10 - 19/7/10                       |
| 16/5/10                                  | 12/10/10                                    | 30/7/10 - 2/8/10                        |
| 31/5/10                                  | 15/11/10 - 16/11/10                         | 17/12/10 - 3/12/10                      |
| 4/6/10 - 9/6/10                          | 20/11/10 - 24/2/11                          | 29/12/10 - 10/1/11                      |
| 17/9/10 - 2/10/10                        | 3/3/11 - 7/3/11                             |   |
| 26/1/11 - 7/2/11                         |   |   |
| 14/2/11 - 18/2/11                        |   |   |
| 12/3/11 - 16/3/11                        |   |   |
| 20/3/11 - 28/3/11                        |   |   |

**Table 2-2: Sampling dates for HRAP system fed facultative pond effluent (n, number of samples analysed)**

| Depth & THRT of HRAP                     |   |   |
|--|---|---|
| Low (0.32m)<br>THRT = 4.5 days<br>n = 19 | Medium (0.43m)<br>THRT = 6.5 days<br>n = 22 | High (0.55m)<br>THRT = 9 days<br>n = 19 |
| 18/7/11                                  | 20/9/11 - 1/10/11                           | 21/8/11 - 8/9/11                        |
| 25/7/11                                  | 24/10/11                                    |   |
| 1/8/11 - 12/8/11                         | 9/12/11 - 17/12/11                          |   |
| 6/1/12 - 10/1/12                         |   |   |

## 2.3 Water Measurements

As noted in the installation comments in para 2.1.7, the probes in the WSPs were located mid-pond and the probes in the HRAP in mid-stream before the paddlewheel.

### 2.3.2 Water Dissolved Oxygen

Danfoss Oxy 1100 sensors were placed 12 cm below the surface and were logged hourly through a Danfoss EMCO -1 transmitter-controller and onto T-TEC A™ loggers. Information was downloaded to a laptop every visit to the site.

### 2.3.3 Water pH

Hach pH/ORP combination sensors were placed 12 cm below the surface and were logged hourly through a Hach GLI Pro transmitter-controller to T-TEC A™ loggers. Information was downloaded to a laptop every visit to the site.

### 2.3.4 Wastewater Ammonia (NH<sub>4</sub>-N)

The Foss Fiastar 5000 Analysis System (Foss Pacific Pty Ltd, North Ryde, NSW) with appropriate cassette was used for all the nutrient analyses. This system uses techniques that are automated forms of systems described in Greenberg et al. (1992).

The technique used for ammonia analyses was the Automated Phenate Method described in Test 4500-NH<sub>3</sub> H (Automated Phenate Method) on pages 4-84/5 of Greenberg et al. (1992) as performed in the Foss multi-analyser. Each test was performed in triplicate. The principle behind this test is alkaline phenol and hypochlorite react with ammonia to form indophenol blue in proportion to the ammonia concentration. The blue colour is intensified with sodium nitroprusside. Photometric measurement was made at 630 nm.

### 2.3.5 Wastewater Nitrate (NO<sub>3</sub>-N)

The technique used for these analyses was the Automated Cadmium Reduction Method described in Test 4500-NO<sub>3</sub><sup>-</sup> F (Automated Cadmium Reduction Method) on pages 4-91/2 of Greenberg et al. (1992) as performed in the Foss multi-analyser. Each test was performed in triplicate. The principle behind this

test is nitrate ( $\text{NO}_3^-$ ) is reduced to nitrite ( $\text{NO}_2^-$ ) in the presence of cadmium. The nitrite produced is then quantified by the method described in Section 2.3.6.

### **2.3.6 Wastewater Nitrite ( $\text{NO}_2\text{-N}$ )**

The technique used for these analyses was the Colorimetric Method described in Test 4500- $\text{NO}_2^-$  (Nitrogen (Nitrite)) on pages 4-85/6 of Greenberg et al. (1992) as performed in the Foss multi-analyser. Each test was performed in triplicate. The principle behind this test is the formation of a reddish purple azo dye at pH 2.0 to 2.5 by coupling diazotised sulphanilamide with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED reagent). Photometric measurement was made at 543 nm wavelength.

### **2.3.7 Wastewater Orthophosphate ( $\text{PO}_4\text{-P}$ )**

The technique used for these analyses was the Stannous Chloride Method described in Test 4500-P D (Stannous Chloride Method) on page 4-114 of Greenberg et al. (1992) as performed in the Foss multi-analyser. Each test was performed in triplicate. The principle behind this test is the formation of molybdophosphoric acid and subsequent reduction to intensely coloured molybdenum blue by stannous chloride. Photometric measurement was made at 690 nm.

### **2.3.8 Wastewater Suspended Solids**

For this purpose Suspended Solids (SS) was defined as the portion of total solids retained by a Whatman GFC filter with a nominal pore size of 1.2  $\mu\text{m}$  as described in Test 2540 D (Total Suspended Solids Dried at 103-105°C) on page 2-56 of Greenberg et al. (1992).

### **2.3.9 Wastewater Turbidity**

All samples were tested using the nephelometric method described in Test 2130 B (Nephelometric Method) on pages 2-9/10 of Greenberg et al. (1992) using a Hach DR/2000 direct reading spectrophotometer. The method is based on a comparison of light scattered by the sample under standard conditions compared with a standard reference suspension under the same conditions.

The higher the intensity of scattered light, the higher the turbidity. Formazin polymer was used as the reference turbidity standard suspension.

### 2.3.9 Wastewater chlorophyll *a*

All samples were tested using the spectrophotometric method described in Test 10200 (Chlorophyll – trichromatic method) on pages 10-18/19 of Greenberg et al. (1992) using a Shimadzu UV-1700 (UV-visible) spectrophotometer. Chlorophyll *a* was determined in triplicate by filtering a known volume (25 ml) of sample through a Whatman GF/C filter pad. Chlorophyll *a* was extracted from the algal cells trapped on the filter in the dark (4°C, 24 h) into 10 ml of 90% acetone and the concentration determined spectrophotometrically by measuring the optical density (absorbance) of the extract at three wavelengths – 664, 647 & 630 nm and then applying the trichromatic equation (Eq.2-1) of Jeffrey and Humphrey (1975).

$$C_a = 11.85(OD_{664}) - 1.54(OD_{647}) - 0.08(OD_{630}) \dots \dots \dots (Eq. 2 - 1)$$

### 2.3.10 Wastewater BOD<sub>5</sub>

The five day BOD test was performed to the same principles as described in Test 5210 B (5-Day BOD Test) on pages 5-2/6 of Greenberg et al. (1992), using the commercial apparatus - OxiTop®BOD measuring system. This respirometric system incorporates the OxiTop® OC 100 Controller, OxiTop®-C measuring heads, an inductive stirring system in a temperature controlled cabinet, and dark brown sample bottles.

In the system, a specified (according to expected result as in Table 2-3) volume of sample was placed in the sample bottle, along with a magnetic flea for induction stirring.

**Table 2-3 Sample volume (mL) for BOD range required using the OxiTop system**

| BOD <sub>5</sub> Range (mg/L) | Sample Volume (mL) |
|-------------------------------|--------------------|
| 0 – 40                        | 432                |
| 0 – 80                        | 365                |

|          |      |
|----------|------|
| 0 – 200  | 250  |
| 0 – 400  | 164  |
| 0 – 800  | 97   |
| 0 – 2000 | 43.5 |
| 0 – 4000 | 22.7 |

Two pellets of NaOH were placed inside a rubber quiver which was positioned inside the neck of the bottle to absorb CO<sub>2</sub>. The bottle sealed by a measuring head and measurement initiated with the OC100 controller. The bottles were placed in the temperature controlled light-sealed cabinet for 5 days. Micro-organisms present in the sample draw oxygen from the air remaining above the sample in the closed system to degrade organic substances. The CO<sub>2</sub> produced concurrently was absorbed by the NaOH pellets. As the O<sub>2</sub> level in the bottle decreased the pressure in the bottle decreased. This pressure reduction was recorded by strain sensors attached to a rubber diaphragm in the measuring head. The difference in pressure from start to finish was then converted to a BOD<sub>5</sub> value by the software in the OC100 controller.

### 2.3.11 Wastewater *E. Coli* Enumeration

*E. coli* /100 mL were enumerated by the Colilert “Quanti-tray” method (IDEXX Laboratories, USA) which compares well with MPN and other standard methods (Eckner, 1998).

Colilert uses Defined Substrate Technology® (DST®) to simultaneously detect total coliforms and *E. coli*. Two nutrient-indicators, ONPG and 4 methyl-umbelliferyl (MUG), are the major sources of carbon in Colilert and can be metabolized by the enzyme β-galactosidase present in coliforms and the *E. coli* enzyme β-glucuronidase, respectively. As coliforms, including *E. coli*, grow in Colilert, they use β-galactosidase to metabolize ONPG and change it from colourless to yellow. Total coliforms were not recorded in this procedure.

*E. coli* use β-glucuronidase to metabolize MUG and create fluorescence as per Figs 11 & 12. Since most non-coliforms do not have these enzymes, they are



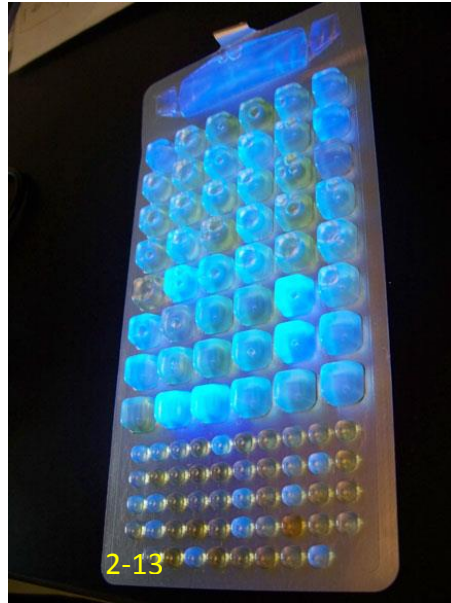
unable to cleave the substrate and produce fluorescent umbelliferone. The few non-coliforms that do have these enzymes are selectively suppressed by Colilert's formulated matrix.



**Figs. 11 & 12 Pictorial representation of the enzymatic metabolism of 4 methyl-umbelliferyl to the fluorescent 4 methyl-umbelliferone by  $\beta$ -glucuronidase (an enzyme found mainly in *E. coli*)**

#### **Quanti-Tray\* Enumeration Procedure**

1. Contents of one pack of chromogenic substrate added to 100 mL of the sample water or its dilution in a sterile vessel.
2. Vessel capped and shaken until chromogenic substrate dissolved.
3. Sample/reagent mixture poured into a Quanti-Tray or Quanti-Tray/2000 and sealed in an IDEXX Quanti-Tray Sealer.
4. Sealed tray placed in an incubator at  $35\pm 0.5^{\circ}\text{C}$  for 24 hours.
5. Number of positive wells counted (Plate 2-9) and reference made to the MPN table provided with the trays to obtain a Most Probable Number (MPN 100/mL).



**Plate 2-13** Incubated Quanti-Tray showing blue fluorescence in positive cells under a UV light source.

## **2.4 *E. coli* and MS2 dark die-off**

For a number of reasons it became important to understand the rate of die-off of the indicator organisms *E. coli* and MS2 if they were stored in the dark. This was because a refrigerated auto-sampler was used to collect samples over a period of 10 days at the remote location and these were collected and returned to the laboratory at the end of that period. It was important to know, what if any, adjustments were to be made to the numbers enumerated after different periods of storage. Later, this information was also used to study the die-off curve in an attempt to understand the principle mechanisms acting in dark die-off.

To achieve this aim on four separate occasions various inlet and pond waters were brought into the laboratory and held in plastic containers completely encased in aluminium foil to exclude light. Some were held in a refrigerator at 2.8°C and others were held in a cabinet at ambient laboratory air temperature (nominally 22°C but on occasion moving up to 28°C). Sub samples were removed from these jars at regular intervals and cultured for *E. coli* on all

occasions and MS2 on some occasions, using the enumeration techniques described in Sections 2.3.11 and 2.5 respectively.

## 2.5 Data Interpretation and Statistical Analysis

### 2.5.1 Comparing *E. coli* removal – Log<sub>10</sub> Reduction Values, inlet to outlet ratio, removal efficiency.

The inlet and outlet *E. coli* MPNs are reported in graphical format as time series in conjunction with various known predictors of die-off. Some estimation is then made of the importance of these predictors in influencing final pond system performance.

As tracer studies were not performed as part of this experimental program, no estimates could be made of dispersion coefficients. The hydrodynamics of WSPs and HRAPs are significantly different as WSP water moves essentially from inlet to outlet, whereas the HRAP treated water cycles past the outlet point numerous times during the treatment period. The two wastewater treatment systems in this study are quite different in other areas, such as influent and effluent flows, and pond volume and area.

The method chosen to report results and compare removal performance between the two systems are various permutations of mean, median and temporal log<sub>10</sub> inlet and outlet indicator values and the log<sub>10</sub> reduction value (LRV) over the Theoretical Hydraulic Retention Time (THRT) for performance comparison.

This study is characterised by the same wide variation in key descriptor areas. As noted in para 2.1.3 & 2.1.6 the pond dimensions, theoretical hydraulic retention times and flow rates were different for each pond and between systems. To achieve the most realistic comparison of these two processes, removal efficiency was estimated as the difference between raw wastewater (influent) and effluent bacterial concentrations in each system. The main tool

used to report these variations is time series graphs for each pond in both systems, displayed on the same graph. High frequency variations are controlled by applying the loess smoothing technique with 95% confidence intervals shadowing each curve. This allows visualisation of chronological clustering revealing successional steps of decreasing and increasing bacterial concentrations through time.

### **2.5.2 Predictive Models of Algal and Biomass Productivity**

No direct measurement of bacterial biomass has been made in this study to distinguish algal and bacterial biomass. Instead, algal biomass has been estimated as being represented by 50 times the mass of measured chlorophyll a. Biomass productivity has been reported as suspended solids (SS) productivity.

### **2.5.3 Comparison of Algal Productivity model predictions with this data**

Algal growth is influenced by many factors, such as the supply of nutrients, CO<sub>2</sub>, temperature, light and turbulence (Grobbelaar, 1991). Grobbelaar et al. (1990) produced a model that accepts only two input variables, temperature and light energy. They wrote this in a generalized form as:

$$\text{PROD (mg(dry wt.)/m}^2\text{/h)} = \text{PRD} - \text{RES} - \text{INB} \dots \dots \dots (2-2)$$

where            PROD = 'productivity',  
  
                    PRD = 'gross productivity',  
  
                    RES = 'respiration', and  
  
                    INB = 'photo-inhibition'.

The PRD component is calculated from inputs of biomass concentration present in the culture, culture temperature, and light impinging on the surface of the culture. Their PRD equation has two components, a temperature/biomass term which could be regarded as the temperature response of growth and a second component which is the light/temperature response of growth. It also contains

three constants which are not described in detail, and thus make the model impossible to replicate.

The component RES is interpreted as a total loss factor, i.e. losses due to respiration and exudation of organic compounds from the cells, and is calculated from the following equation:

$$RES = X_1((1.5^T - 0.54)/100) \dots\dots\dots(2-3)$$

RES increases exponentially with an increase in temperature. This loss factor continues unabated in the light and dark and the reassimilation of produced CO<sub>2</sub> and excreted organics are not considered. RES does not take grazing by invertebrates or losses due to parasite attacks into consideration.

The component INB (photo-inhibition) depends on the intensity, quality and duration of irradiance. Sorokin and Krauss (1958) have shown that photo-inhibition is temperature-dependent. The following equation describes the magnitude of photo-inhibition in the model:

$$INB = PRD((2.5^T/75) I_z) \dots\dots\dots(2-4)$$

Thus in the model, photo-inhibition increases linearly with increases in irradiance, but that the overall rate is determined by temperature.

As this complex model was impossible to replicate using the data obtained in this study, as many constant values are missing, a simpler model was used to allow comparison of predicted values against measured values. Other more complex models are available such as (Shelef, 1982), which account for temperature more thoroughly, but they were not explored here as they mostly rely on constants that need to be estimated from measured data specific to the site of measurement. In other words, their predictive value is limited as they rely on local variations that are not universally applicable.

Oswald (1973) proposed the following simple equation for gross algal productivity:-

$$Pr_{alg} = 10 \times \frac{E_t \times I}{J} \dots \dots \dots (2 - 5)$$

Where  $Pr_{alg}$  is gross productivity of the algal biomass (g/m<sup>2</sup>/day)

$E_t$  is total light conversion efficiency  $\approx 0.04$

$I$  is total solar radiation (cal/cm<sup>2</sup>/day)

$J$  is heat content of the algal biomass (kcal/g)  $\approx 6$

Equation 2-5 was used to calculate algal productivities for the study period using the estimates for  $E_t$  (0.04) and  $J$  (6) given above and the measured total solar radiation for the 24 hour period prior to the collection of the water sample. These calculated predictions were then used for comparison with measured productivities to test the accuracy of the predictions for suitability as a predictive model in the prevailing site conditions.

Two methods of calculating algal productivity were used and compared. The first was calculated by assuming that algae represent 60% of the suspended solids 'albazod'. The second was calculated by assuming that chlorophyll *a* represents 2% of the algal mass. The latter method was the preferred method as there was a more direct link between the measures value and the estimated value.

#### **2.5.4 Modelling *E. coli* removal**

In conjunction with Dr Simon Williams from the School of Computer Science Engineering and Mathematics, within the Faculty of Science and Engineering at Flinders University, Adelaide a novel approach to modelling *E. coli* removal from HRAP systems was developed, based on mass balance and solar disinfection. This is described in detail in Chapter 7.

### 2.5.5 Seeking possible causal relationships - Linear Regression, Regression Tree Analysis

Linear regression is a global model, where there is a single predictive formula holding over the entire data-space. The idea behind linear regression is as a way of making quantitative predictions. In simple linear regression, a real-valued dependent variable  $Y$  is modelled as a linear function of a real-valued independent variable  $X$  plus noise as in Equation 2-6:

$$Y = \beta_0 + \beta_1 X + \varepsilon \dots \dots \dots 2 - 6$$

In multiple regression, we let there be multiple independent variables  $X_1, X_2, \dots, X_p$ , as shown in Equation 2-7:

$$Y = \beta_0 + \beta^T X + \varepsilon \dots \dots \dots 2 - 7$$

This is all very well so long as the independent variables each has a separate, strictly additive effect on  $Y$ , regardless of what the other variables are doing. It's possible to incorporate some kinds of interaction as in Equation 2-8,

$$Y = \beta_0 + \beta^T X + \gamma X X^T + \varepsilon \dots \dots \dots 2 - 8$$

but the number of parameters is clearly getting very large very quickly with even two-way interactions among the independent variables, and stronger nonlinearities cause considerable issues.

When the data has lots of features which interact in complicated, nonlinear ways, assembling a single global model can be very difficult, and confusing even if successful. An alternative approach to nonlinear regression is to sub-divide, or partition, the space into smaller regions, where the interactions are more manageable. Further partitioning into more sub-divisions follows — this is called recursive partitioning — until finally the chunks of space are so 'tame' that simple models fit into them. The global model now has two parts: one is the recursive partition, the other is a simple model for each cell of the partition.

Prediction trees use the tree to represent the recursive partition. Each of the terminal nodes, or leaves, of the tree represents a cell of the partition, and has attached to it a simple model which applies in that cell only. A point  $x$  belongs to a leaf if  $x$  falls in the corresponding cell of the partition. Starting at the root node of the tree, a sequence of questions are asked about the features. The interior nodes are labelled with questions, and the edges or branches between them labelled by the answers. Which question we ask next depends on the answers to previous questions.

Decision trees used in data mining are of two main types:

- Classification tree analysis is when the predicted outcome is the class to which the data belongs.
- **Regression tree** analysis is when the predicted outcome can be considered a real number

The term Classification And Regression Tree (CART) analysis is an umbrella term used to refer to both of the above procedures. Trees used for regression and trees used for classification have some similarities - but also some differences, such as the procedure used to determine where to split.

There are many separate tools available in “R” packages which use different mathematical approaches to perform regression tree analysis. The standard R function for building tree models is ‘**rpart**’ (standing for recursive partitioning). This tool produces the classical regression tree as can be seen in Fig. 5-2. To achieve this, at any node a split was generated which maximally distinguishes the response variable in the left and right branches. Splitting continues until nodes were pure or the data were too sparse ( $< 6$  cases). Each explanatory variable was assessed in turn, and the variable explaining the greatest deviance in “ $y$ ” was selected. Deviance (variance) was calculated on the basis of a threshold in the explanatory variable; this threshold produces two mean values for the response – one above and the other below the threshold (Crawley, 2013).



There are also a number of diagnostic tools to check the relevance and accuracy of the tree chosen by the rpart software. These tools are generally used when the trees produced are too long and complex to be of value, and they guide the judicious pruning of branches. The tools were used in all the cases explored in this study, but the results are not reported here as there were no instances where pruning was required.

The report generated by the rpart software includes an estimate of the importance of each predictor in determining the rpart tree. This is another parameter which can be used as an adjunct to the visualisation provided by the tree output to aid the dissection of a large number of possible predictor variables in a scale of importance. These results are reported in the fourth column of Tables 5-1 to 5-7 inclusively. The order in which each variable is reported in these tables is based on the node improvement value generated by the boot-strapped randomForest software, so is not in order of importance as determined by rpart.

There are various refinements on tree-based methods that, by building multiple trees, improve on the performance of the single-tree methods. **The randomForest package** is so called because it takes multiple bootstrapped random samples, generating a separate tree for each such sample (i.e. a forest). The prediction is determined by a simple majority vote across the multiple trees. The prediction results for a randomForest run of bootstrapped values generating 5,000 trees are shown in Table 5-1 (Column 2). These predictions explain 64.3% of the variance of *E. coli* LRV in the 5,000 trees.

**cForest** is another computational tool using boot-strap generated variables for recursive partitioning. The core of the package is ctree, an implementation of conditional inference trees which embed tree-structured regression models into a well-defined theory of conditional inference procedures (R-Development-Core-Team, 2012). This implementation of the random forest (and bagging) algorithm differs from the reference implementation in randomForest with

respect to the base learners used and the aggregation scheme applied. As for randomForest, the output is not a single tree (as many trees are constructed to make the forest), but a graphical representation with the ranked predictors lined up on the y axis and the relative importance of each predictor marked on the x axis. A dashed red line is used to indicate the level below which predictors no longer have any significance. Obviously those to the right of the line are significant predictors.

The regression tree technique was used extensively in Chapter 5, to explore the relationships between key predictor variables and the key performance indicators, *E. coli* LRV, BOD<sub>5</sub> removal efficiency, NH<sub>4</sub>-N removal efficiency and biomass productivity, as exemplars.

It was specifically used to explore these relationships in the HRAP, not the WSP. The main reason for this distinction is that the relatively short retention time in the HRAP (~5 days) means the predictor variables are relevant for that period, particularly when 5 day averages are used for the predictor variable. Over the extended retention times in the WSPs, the daily records of each predictor variable lose relevance.

### **2.5.6 Comparing nutrient removal performances – daily removal rates, removal efficiency**

The removal of nutrients represented by BOD<sub>5</sub>, NH<sub>3</sub>-N, NO<sub>3</sub>-N, NO<sub>2</sub>-N and PO<sub>4</sub>-P are reported in time series graphical form as for *E. coli* and comparison of rates of removal between systems is reported as daily removal rates and removal efficiencies. Correlation with known predictor variables is included as well as linear regression where appropriate.

### **2.5.7 The R statistical and graphics tool**

The majority of the statistical analyses and graphical representations were prepared in the R statistical package (R-Development-Core-Team, 2012) unless otherwise specified. A number of specific processes were used with the aid of add-on packages.

### 2.5.7.1 Comparing distributions between groups

All of the known methods for comparing distributions between groups suffer from some problems. A one dimension scatterplot is only useful in cases where there are very few values per group. A boxplot uses quartiles, which are mathematically complex. As well, the detection of outliers is quite arbitrary, especially in case of non-normal underlying distributions. Even for normal distributions the number of outliers detected will grow if the number of observations grows, which makes individual outliers undetectable. In a violin plot the underlying distribution is more visible, but individual data points besides the minimum and maximum are not visible at all and no indication of the number of observations in a group is given.

A beanplot is a combination between a 1d-scatter plot and a density trace. In such a plot, outliers do not have to be detected, because all individual observations are visible in the scatter plot. Slightly complicated concepts such as quartiles are not used, but instead, the average is used to summarize the groups (Fig. 2-18b & 2-19right). As well, a density trace similar to the violin plot is used to summarize the distribution of the groups.

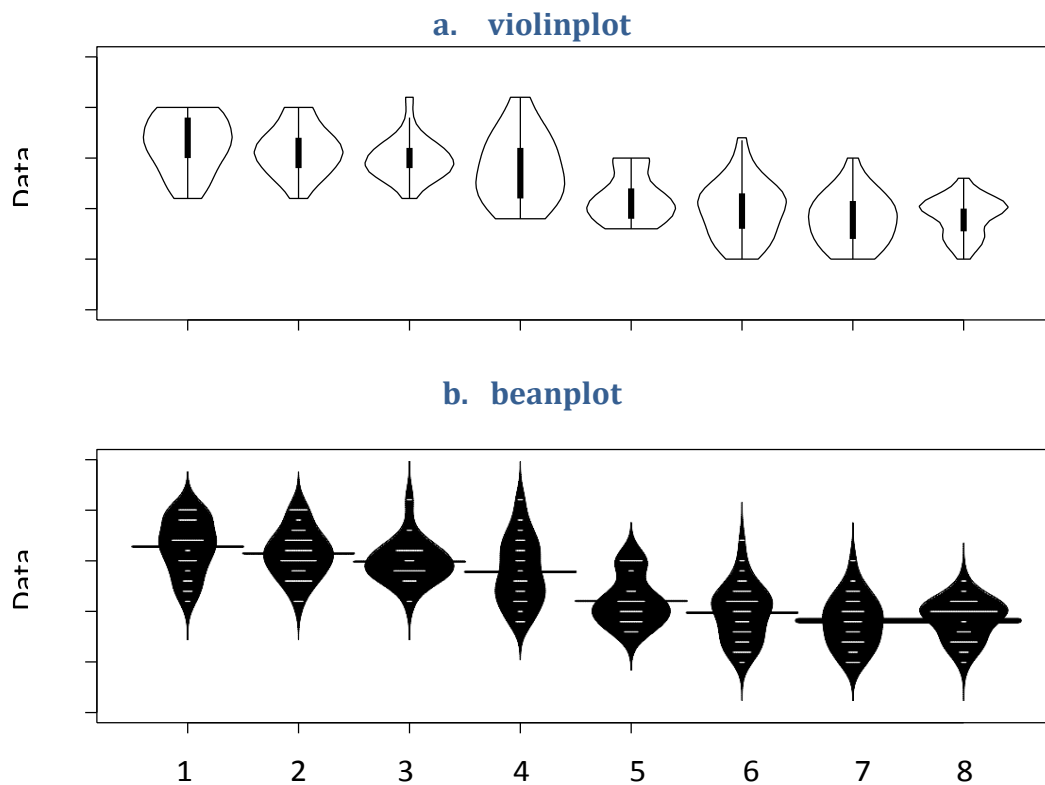
Various add-on “R” software were used to provide specific between-group visualisation as follows:-

#### *Caroline*

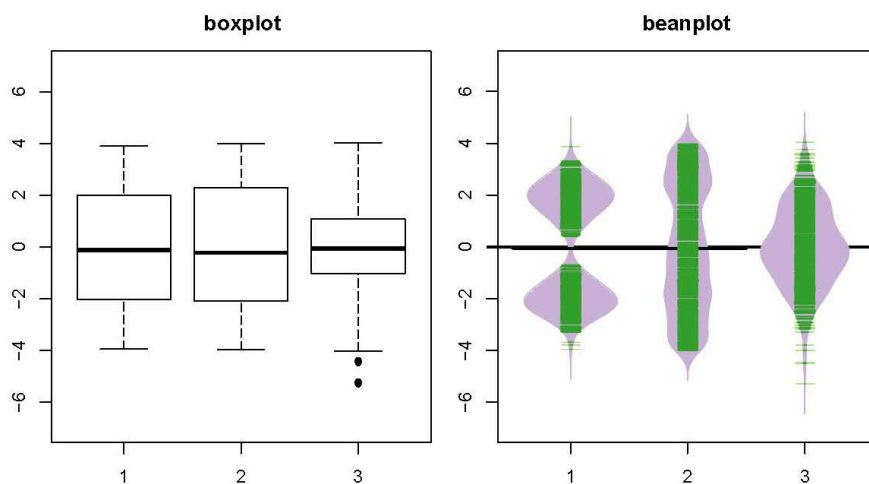
Caroline contains the software to construct violin plots. Much of the data is presented in tabular and box and whisker plot format from the base “R” package with violin plots from package “Caroline” used when necessary for greater clarity of presentation. A violin plot is a combination of a boxplot and a kernel density plot. The violin plot is similar to box plots, except that they also show the probability density of the data at different values (in the simplest case this could be a histogram). Typically violin plots (Fig. 2-18b) will include a marker for the median of the data and a box indicating the interquartile range, as in standard box plots. Overlaid on this box plot is a kernel density estimation.

### *Beanplot*

Beanplot contains the software to construct beanplots. A beanplot is a plot in which one or multiple groups ("beans") are shown. Each bean consists of a density trace, which is mirrored to form a polygon shape. As well, a one-dimensional scatter plot shows all the individual measurements, as is used in a stripchart. The scatter plot is drawn using one small line for each observation in a batch. If a small line is drawn outside of the density shape, a different colour is used to draw the line. This ensures that the density of a batch is still visible, even if there are many small lines that fall partly outside the density shape. To enable easy comparison, a per-group average and an overall average is drawn (Figs. 2-18b & 2-19right). (Kampstra, 2008)



**Figure 2-18 (a) Violinplots for a data set with eight groups. (b) beanplots for the same data set and groups.**



**Figure 2-19 Left: Boxplots for a bimodal, a uniform and a normal distribution. Right: In the beanplot the green lines show individual observations, while the purple area shows the distribution.**

### 2.5.7.2 Time series

#### *ggplot2*

ggplot2 is a plotting system for R, based on the grammar of graphics that makes it easy to produce complex multi-layered graphics. Most time series were fitted with a LOESS smoothing line encompassed with shading indicating the 95% confidence interval for that line. A LOESS function works by fitting simple models to localized subsets of the data to build up a function that describes the deterministic part of the variation in the data, point by point. The subsets of data used for each weighted least squares fit in LOESS are determined by a nearest neighbours algorithm. A user-specified input to the procedure called the "bandwidth", "smoothing parameter", "span" and designated  $\alpha$ , determines how much of the data is used to fit each local polynomial. The value of  $\alpha$  is the proportion of data used in each fit. The subset of data used in each weighted least squares fit comprises the  $n\alpha$  points (rounded to the next largest integer) whose explanatory variables values are closest to the point at which the response is being estimated (Cleveland and Devlin, 1988).

$\alpha$  is called the smoothing parameter because it controls the flexibility of the LOESS regression function. Large values of  $\alpha$  produce the smoothest functions that wiggle the least in response to fluctuations in the data. The smaller  $\alpha$  is, the closer the regression function will conform to the data. Using too small a value of the smoothing parameter is not desirable, however, since the regression function will eventually start to capture the random error in the data. Useful values of the smoothing parameter typically lie in the range 0.25 to 0.5 for most LOESS applications, and values in this range were used throughout this thesis.

### 2.5.7.3 Tree regression analysis

Three main packages were used in this study to produce one standard recursive partitioning output and two boot-strapped versions.

### *rpart*

The standard “R” function for building tree models is ‘rpart’ (standing for recursive partitioning). This tool produces the classical non boot-strapped regression tree as can be seen in Fig. 5-2. Further discussion on rpart is included in para 5.2.1.

### *randomForest*

The randomForest package is so called because it takes multiple bootstrapped random samples, generating a separate tree for each such sample (i.e. a forest). The prediction is determined by a simple majority vote across the multiple trees. Further discussion on randomForest is included in para 5.2.2.

### *Package “party” - cForest*

The core of the package is ctree, an implementation of conditional inference trees which embed tree-structured regression models into a well-defined theory of conditional inference procedures. This implementation of the random forest (and bagging) algorithm differs from the reference implementation in randomForest with respect to the base learners used and the aggregation scheme applied. Further discussion on cForest is included in para 5.2.3.

## **2.5.8 Dark Die-Off**

GlnaFIT, (Geeraerd et al., 2005) a freeware tool to assess non-log-linear microbial survivor curves was used to assess nine different types of microbial survival models on the experimental data. The authors claim that this program covers all known survivor curve shapes for vegetative bacterial cells. The nine model types are: (i) classical log-linear curves, (ii) curves displaying a so-called shoulder before a log-linear decrease is apparent, (iii) curves displaying a so-called tail after a log-linear decrease, (iv) survival curves displaying both shoulder and tailing behaviour, (v) concave curves, (vi) convex curves, (vii) convex/concave curves followed by tailing, (viii) biphasic inactivation kinetics, and (ix) biphasic inactivation kinetics preceded by a shoulder.

Next to the obtained parameter values, the following statistical measures are automatically reported: standard errors of the parameter values, the Sum of Squared Errors, the Mean Sum of Squared Errors and its Root, the  $R^2$  and the adjusted  $R^2$ .

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## CHAPTER 3

# HRAP RESULTS & DISCUSSION

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### **3.1 High Rate Algal Pond – Period 1, May 2010 to April 2011 – High Strength Influent (HSI)**

Throughout the body of the text, and in table and figure headings in this and other chapters, the shortened phrase HRAP 1 will be used to signify the HRAP that has septic tank treated overflow material fed in as inlet water. The term HRAP 2 will refer to the HRAP operated with facultative pond effluent as the inlet water. The microbial and nutrient concentrations in this HRAP 2 inlet water were significantly lower than the septic tank overflow water (Tables 3-2 and 3-14).

#### **3.1.1 Environmental Factors – air temperature, wind speed & direction, total solar radiation, UV radiation, rainfall**

##### **3.1.1.1 Prevailing weather**

The annual cycle of air temperature follows a typical semi-arid pattern (BSk in the Köppen climate classification) with hot dry summers and cold dry winters. The site experiences an annual average rainfall of 268 mm, reasonably evenly

spread throughout the year as can be seen in the long term Bureau of Meteorology data in Table 3-1.

Most importantly from a wastewater treatment perspective, daily sunshine hours and daily solar exposure ( $\text{MJ}/\text{m}^2$ ) vary from 5.2 hours and  $8.3 \text{ MJ}/\text{m}^2$  respectively in June and to 10.4 hours and  $28.1 \text{ MJ}/\text{m}^2$  in January (Table 3-1). To reinforce this point, there is only 30% of daily solar exposure in June that there is January. There is a rapid drop-off in daily solar exposure of 28% between the months of March and April. This is matched by a jump in daily exposure of 23% between the months of September and October. Likewise daily maximum air temperatures vary from  $31.8^\circ\text{C}$  in January, and daily minimum air temperatures reach only  $3.8^\circ\text{C}$  in July.

#### **3.1.1.2 Seasons used for comparative performance**

The rapid seasonal transition leads to a clearly defined bi-seasonal effect in pond temperatures. This effect was exploited for purposes of breaking down the results into two seasons rather than the traditional four seasons to establish patterns of indicator organism and nutrient removal. The dividing point was determined as the pond average daily water temperature either being above or below the median figure of  $17.6^\circ\text{C}$ . For this document, these 'seasons' have been labelled 'hot' and 'cold' throughout all subsequent reports and analyses. This technique ensured there was sufficient data in each split to allow comparisons with statistical significance as detailed in Tables 3-11 to 3-14 and Figures 3-3 to 3-10.

#### **3.1.1.3 Exceptional weather**

Over the summer months of 2010/11 there were three exceptional major rainfall events with rain totals exceeding 50 mm per day (Fig. 3-1). These events led to considerable flooding in the township of Kingston on Murray as the pump station in town is situated in a roadside hollow, so that when rainfall is intense, run-off water collects over, and enters, the sewage sump resulting in considerable volumes of rainwater being pumped to the treatment plant.

Over the 30 year period prior to 2012 there was on average 79 days of rain per year, according to historic rainfall data (Table 3-1) (Bureau-of-Meteorology (2012)). In 2010 there were 120 days of rain, which is 52% more wet days than normal, and 2011 had 116 days of rainfall (Fig. 3-1). Apart from the three massive events, the wet period had some influence on pond performance by increasing flows through the treatment plant as storm water seeped into the sewage reticulation system. However, this diluting effect was accounted for by the flow meter recordings of daily and total inflow and outflow from the system.

**Table 3-1. Historical ground weather station and satellite data, Moorook (5 km from study site) – 30 year climate data averages (Bureau-of-Meteorology, 2012)**

|               | Daily<br>sunshine<br>(hours) | Daily<br>Solar<br>Exposure<br>(MJ/m <sup>2</sup> ) | Rainfall<br>(mm) | Highest<br>Rainfall<br>(mm) | Lowest<br>Rainfall<br>(mm) | Max.<br>Temp<br>°C | Highest<br>Temp<br>°C | Min.<br>Temp<br>°C | Lowest<br>Temp<br>°C |
|---------------|------------------------------|--|------------------|-----------------------------|----------------------------|--------------------|-----------------------|--------------------|----------------------|
| <b>Jan</b>    | 10.4                         | 28.1   | 18.7             | 99.2                        | 0.2                        | 31.8               | 45.7                  | 13.6               | 3.8                  |
| <b>Feb</b>    | 10                           | 23.8   | 17.1             | 93.4                        | 0                          | 31.3               | 47.1                  | 13.4               | 6.1                  |
| <b>Mar</b>    | 9.3                          | 20.3   | 12.9             | 51.6                        | 0                          | 28.1               | 41.2                  | 12                 | 3.2                  |
| <b>Apr</b>    | 8.3                          | 13.9   | 16               | 53.8                        | 0                          | 23.9               | 37.9                  | 8.4                | -1.3                 |
| <b>May</b>    | 6.3                          | 10.4   | 22.8             | 80.8                        | 0.6                        | 19.5               | 29.6                  | 6.3                | -3.3                 |
| <b>Jun</b>    | 5.2                          | 8.3  | 25.2             | 69                          | 2.5                        | 16.3               | 25.7                  | 3.7                | -5.4                 |
| <b>Jul</b>    | 5.8                          | 9.2  | 28.3             | 61.8                        | 3.8                        | 15.8               | 23.9                  | 3.8                | -3.6                 |
| <b>Aug</b>    | 7.1                          | 12.5   | 27.4             | 76                          | 2.6                        | 17.5               | 31                    | 3.4                | -3.4                 |
| <b>Sep</b>    | 7.7                          | 16.7   | 26               | 63.8                        | 5.8                        | 20.7               | 35.4                  | 6.3                | -3.2                 |
| <b>Oct</b>    | 8.8                          | 21.7   | 26.2             | 71.6                        | 0.2                        | 24                 | 40.1                  | 8.4                | 0.4                  |
| <b>Nov</b>    | 9.3                          | 25.6   | 21.8             | 48.2                        | 3.8                        | 27.6               | 43.6                  | 11.3               | 2.7                  |
| <b>Dec</b>    | 9.7                          | 27.8   | 25.3             | 125.6                       | 3.2                        | 29.6               | 43.2                  | 13                 | 3.5                  |
| <b>Annual</b> | <b>8.2</b>                   | <b>18.4</b>  | <b>267.9</b>     | <b>440.8</b>                | <b>121</b>                 | <b>23.8</b>        | <b>47.1</b>           | <b>9</b>           | <b>-5.4</b>          |

### 3.1.2 HRAP Operational Conditions & Wastewater Physico-chemical Parameters

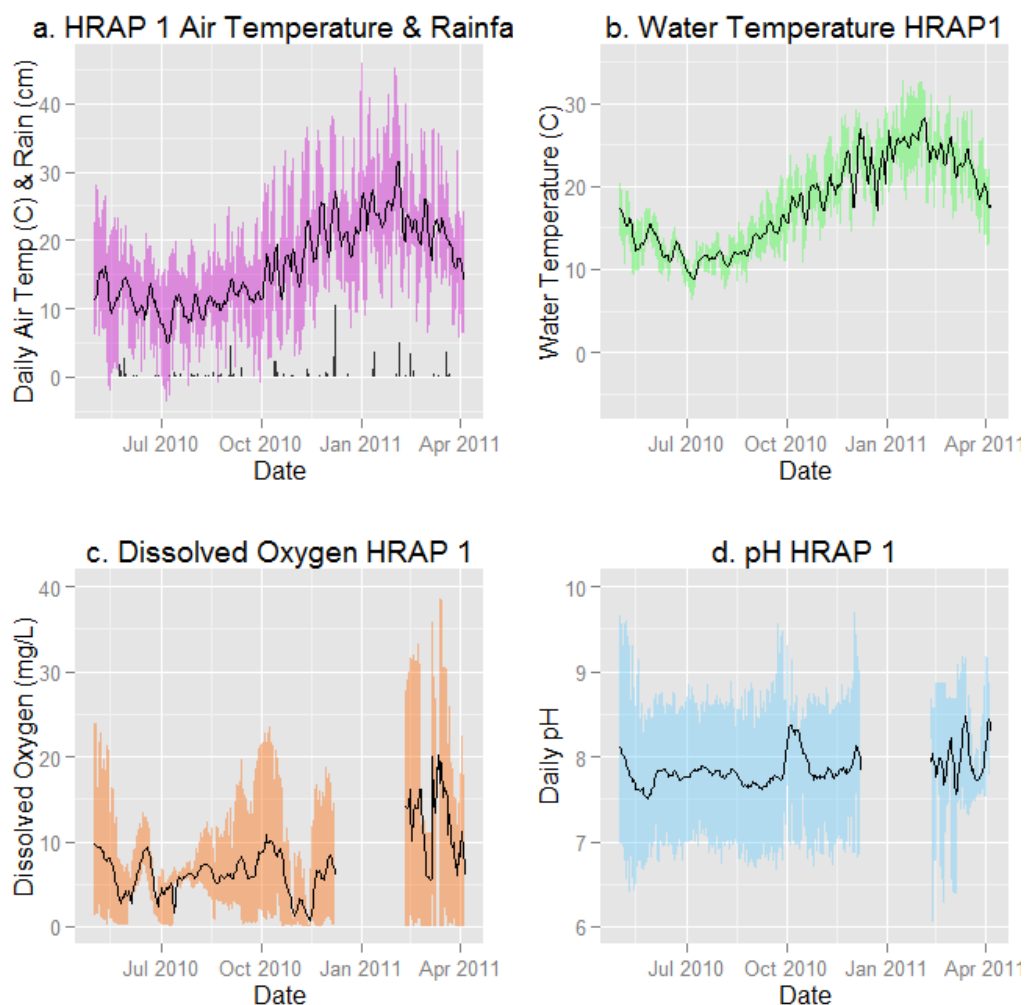
#### 3.1.2.1 Air & Water Temperatures

Conditions in the HRAP displayed seasonal temporal variations typically associated with variations in sunlight and ambient temperatures (Fig 3-1a). Air temperatures in mid-summer regularly exceed 40°C and mid-winter minima commonly drop below 0°C (Fig 3-1a). The average daily water temperature (Fig. 3-1b) commonly exceeded 30°C in summer and minima dropped below 5°C in

winter. The median pond temperature during the study period was 17.6 °C. This “binning” of otherwise continuous data is a device to allow readily understood and usable statistical analyses.

### 3.1.2.2 Dissolved Oxygen (DO)

The gap in the DO and pH record (Fig. 3-1 c&d) from the 8<sup>th</sup> December 2010 until 12<sup>th</sup> February 2011 was due to flooding on the 8<sup>th</sup> December which destroyed the instrument panels. It took some time to locate suitable replacements for the submerged and ruined instrumentation.



**Fig. 3-1 HRAP1 Daily maximum & minimum and 5 day average for:- a. air temperature and rainfall b. Water Temperature c. dissolved oxygen and d. pH recorded on-site at Kingston-on-Murray during the study period.**

Daily DO peaks of between 7 and 38 mg/L were observed throughout the year, with the higher peaks generally occurring during the warmer months when algal growth was at its greatest (Fig. 3-1 c). The lower peaks typically occurred when chlorophyll a levels were below 1 mg/L (see Fig. 3-6). However, the relationship between daily chlorophyll a and daily DO peaks was not as straightforward as may have been expected. Some of this lack of consistency may be due to difficulties in keeping the membrane of the DO probe clear of biofilm fouling. The overnight DO regularly dropped to 0 for significant periods of the year, but did not reach 0 for the colder months of the year, presumably because bacterial and algal respiration was limited by the cold water conditions. Daily average water temperature was below 15°C during this period.

### 3.1.2.3 pH

Among the many parameters which determine the performance of HRAPs, pH is one of the more complex. Daily pH peaks of between 8.0 and 9.8 were observed (Fig. 3-1d). Daily minimum pH occasionally fell as low as 6.5. Diurnal pH variations of 1.7 to 2 pH units were commonly observed. In general, the pH was not as high as reported by some other authors in HRAPs (Araki et al., 2001, Azov and Shelef, 1982, Azov and Shelef, 1987, El Hamouri et al., 1995a, El Hamouri et al., 2003, Picot et al., 1993). It is however consistent with the peak range found by other authors (Fallowfield and Garrett, 1986, Davies-Colley et al., 2000, Craggs et al., 2003a).

There are other factors influencing pH, such as the innate alkalinity of the inlet water. This may explain the differences in HRAP pH reported by various authors from around the world, using differing influent waters. What was consistently reported by all these authors was the wide diurnal variation in pH as reported here, due to the well-known association with algal photosynthesis. Commentary on pH is of value as one of the more influential effectors on *E. coli* die-off in the analysis presented in Chapter 5, (Para's 5.2.2 and 5.2.3) is the 5-day-average pH. By contrast, the maximum and minimum daily pH values do

not rate as very influential, suggesting that it is sustained exposure to high pH that is required to damage *E.coli* sufficiently to influence the LRV figure.

#### *3.1.2.3.1 Indirect effects of pH on pond performance*

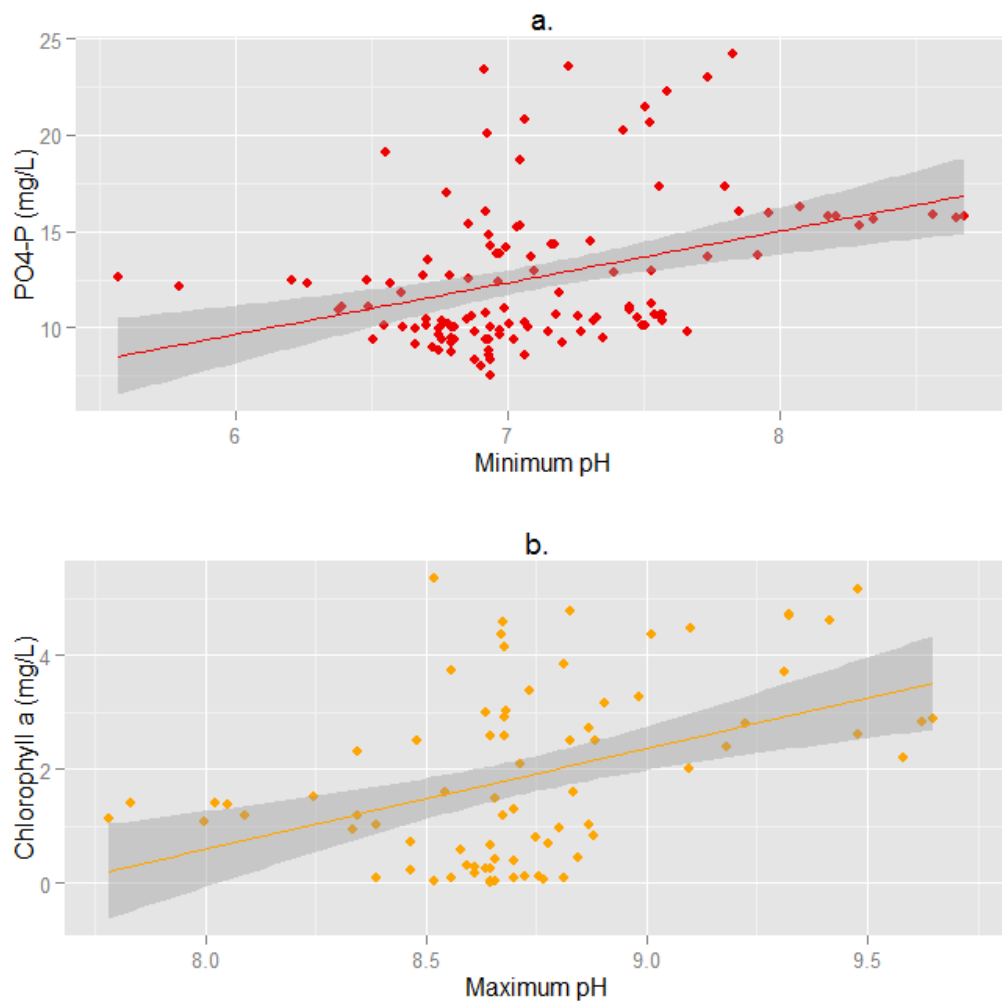
This study was not designed to explore indirect pH effects, so limited data is presented on the relationship between pH and PO<sub>4</sub>-P and chlorophyll *a* concentrations (Fig. 3-2 a & b).

According to Azov et al. (1982a) pH has many indirect effects, as well as the direct effects on *E. coli* die-off noted above. The first indirect effect is the capacity of pH to affect the availability of inorganic carbon to the algae via the ratios of the carbonate system species. However, it is probably more realistic to say that pH is influenced by algal photosynthesis and CO<sub>2</sub> uptake rather than it influencing algal growth.

The second indirect effect is via toxicity of unionised ammonia to the living biomass which is mediated by the ratio of free ammonia (NH<sub>3</sub>) to ammonium ion (NH<sub>4</sub><sup>+</sup>) (Azov and Goldman, 1982). Abeliovich and Azov (1976) found that ammonia and pH are dominant factors in determining the oxygen regime and growth rate in HRAPs when they are run at short detention times. There was no evidence of this found in this study.

Fig. 3-2a plots the PO<sub>4</sub>-P concentration against pH. The linear regression shown in Fig.3-2a of PO<sub>4</sub>-P concentration against daily minimum pH has a p-value of 2.148e-05 and R<sup>2</sup> of 0.132, so is regarded as statistically significant, with low predictive value. By contrast, linear regression of PO<sub>4</sub>-P removal efficiency against daily maximum pH has a p-value of 0.033 and R<sup>2</sup> of only 0.0424, so whilst regarded as statistically significant, pH has almost no predictive power for PO<sub>4</sub>-P removal efficiency.





**Fig. 3-2 Scatterplots with linear regressions and 95% confidence intervals in grey shade of (a.) PO<sub>4</sub>-P concentration against minimum pH and (b.) chlorophyll a concentration against maximum pH.**

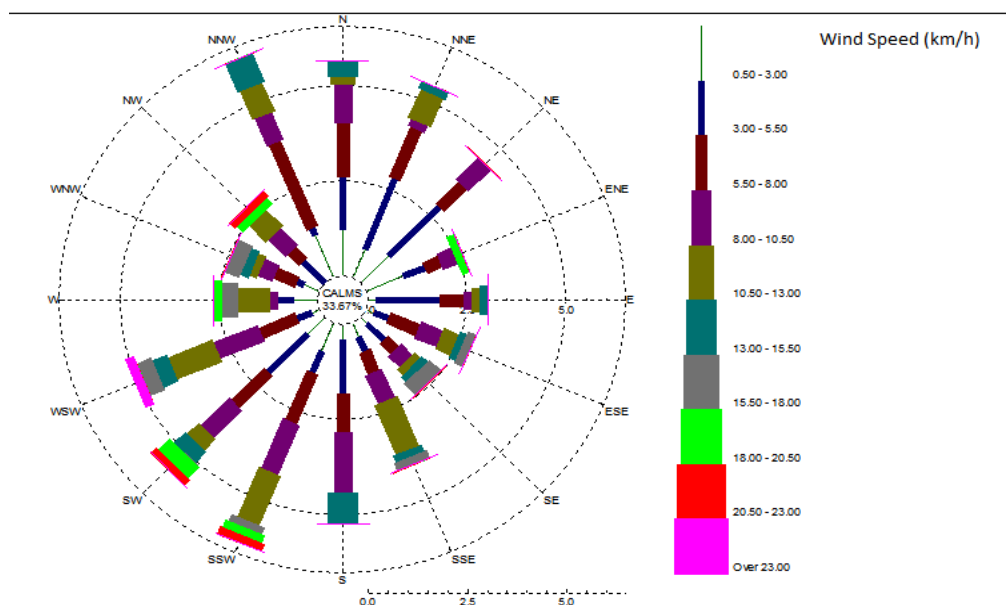
Fig. 3-2b plots chlorophyll *a* levels against daily maximum pH. The linear regression shown in Fig. 3-2b of chlorophyll *a* concentration against daily maximum pH has a p-value of 4.77e-05 and R<sup>2</sup> of 0.1818, so is regarded as statistically significant, with low predictive value. The choice of maximum pH or minimum pH in the plots was made on the basis of highest level of significance. In conclusion, measurable but small positive relationships existed between pH and HRAP algal concentration and PO<sub>4</sub>-P concentrations. No causal relationships were established.

Less significant indirect effects of pH in the HRAP include the availability of phosphorus to the algae (Bogan et al., 1960), precipitation of calcium and

magnesium salts, and in some cases, the determination of dominant algal species (Azov et al., 1980). This study did not address these issues.

### 3.1.3 Wind Speed & Direction

The daily average wind speed and direction is represented in Fig. 3-3 as a wind-rose for the 25 month period from April 2010 to May 2012. The prevailing winds came from the North and South quadrants with most of the strong winds also coming from those directions. As winds from these directions blow up and down the length of the HRAP they were not perceived to have any influence on HRAP performance. It might be argued that winds blowing towards the outlet may influence 'short circuiting', i.e. water driven into the outflow whereas wind in the opposite direction 'hold up' water to the outlet; although given loop raceway flow in one channel is up wind and one channel down wind they do cancel out, so the net effect is almost certainly not very important to HRAP performance.



**Fig. 3-3 Daily average wind speed and direction recorded on-site at Kingston-on-Murray for the period April 2010 to May 2012.**

### 3.1.4 Inlet Wastewater

The inflow rate from the septic tanks averaged 12.9 kL/d during the period of observation. This varied from troughs of 6 kL/d in winter to peaks of 18 kL/d in summer and the solitary extreme flooding event with over 90 kL/d. As the pond inflow was derived directly from household septic tank overflow, it had already had a period of two to three days settling in anaerobic conditions in the septic tanks. This period had a number of stabilising effects. Firstly, the inflow was of a uniform composition throughout the day and from week to week. This was reflected in the low variation in the observations of inlet water composition reported in Table 3-2.

**Table 3-2 HRAP 1 Inlet Wastewater septic tank effluent, volume & composition, where n = number of samples analysed.**

|               | Inflow<br>(kL/d) | BOD <sub>5</sub><br>(mg/L) | NH <sub>4</sub> -N<br>(mg/L) | NO <sub>2</sub> -N &<br>NO <sub>3</sub> -N<br>(mg/L) | PO <sub>4</sub> -P<br>(mg/L) | Log <sub>10</sub> E.coli<br>/100mL |
|---------------|------------------|----------------------------|------------------------------|--|------------------------------|------------------------------------|
| <b>Median</b> | 12.7             | 200                        | 87.8                         | 0.2  | 13.9                         | 6.384                              |
| <b>Mean</b>   | 12.9             | 204                        | 89.9                         | 0.4  | 13.1                         | 6.361                              |
| <b>SD</b>     | 3.0              | 39.6                       | 12.1                         | 0.5  | 3.8                          | 0.320                              |
| <b>n</b>      | 131              | 124                        | 121                          | 121  | 119                          | 124                                |

A further point to note in Table 3-2 is the highly reduced state of the incoming inorganic nitrogen – 99.6% being in the most reduced form - of ammonium. This reflects the anaerobic environment that the water had experienced in household septic tanks immediately prior to pumping to the HRAP.

The inlet nutrient composition is consistent with previously reported work in this area (Walmsley and Shilton, 2005, Metcalf\_&\_Eddy, 2003, Craggs, 2005a, Mara, 1997).

### 3.1.5 Areal and Volumetric Loading Rates

Both areal and volumetric loading rates have relevance in understanding pond performance in appropriate circumstances. The published literature on this

subject generally reports areal loading rates (Cromar and Fallowfield, 1997b, El Hamouri et al., 1995b). Volumetric studies have more relevance when comparing or describing processes that are sensitive to concentration, such as chemical reactions requiring close association between the participating microbiota and nutrient molecules. In static WSP ponds, stratification can seriously modify or reduce the reactive volume, in a manner that is not predictable. Thus, volumetric loading rates are harder to interpret, less relevant and less commonly used in WSP literature.

From a theoretical point of view, it is appropriate to use areal loading rates to describe and compare processes that are sensitive to inputs/outputs entering or leaving through the pond surface, such as incoming light or escaping gases.

However, in this study, volumetric BOD<sub>5</sub> loading rates theoretically offer greater insight to HRAP performance than areal loading rates, which are reported together with volumetric loading rates for comparative purposes in Table 3-3. The areal BOD<sub>5</sub> loading rates (Table 3-3) varied from 50 to 200 kg BOD<sub>5</sub>/ha/d. These are consistent with, or higher than, other reported HRAP loading rates. El Hamouri et al. (1995b) reported organic loading rates of 86 and 97 kg BOD<sub>5</sub>/ha/d, respectively in the hot and the cold season in Morocco, while the dilution rate was maintained at 0.22/d (THRT 3.5d).

Cromar and Fallowfield (1997b) operating small HRAPs in Scotland used COD loading rates of 100, 350 and 600 kg COD/ha/d while maintaining a constant retention time of either 5 or 7 days. They found that increasing loading rate increased dry matter production and resulted in a predominance of cyanobacteria over Chlorophyceae. Increased loading rate was also related to an increase in nitrogen removal, however more complete nitrification occurred at low COD loading rates. Phosphorus removal in the pond with 5-day retention time remained constant independent of loading rate, but in the pond with 7-day retention time phosphorus removal increased with increased COD loading. COD removal was independent of both retention time and loading rate. In the light

of these findings, it is not unexpected that loading rate differences between the various depths did not contribute significantly to pond performance. Further discussion of the influence of BOD<sub>5</sub> loading rates on pond performance in these ponds is contained in Chapter 5 (Para 5.3.2).

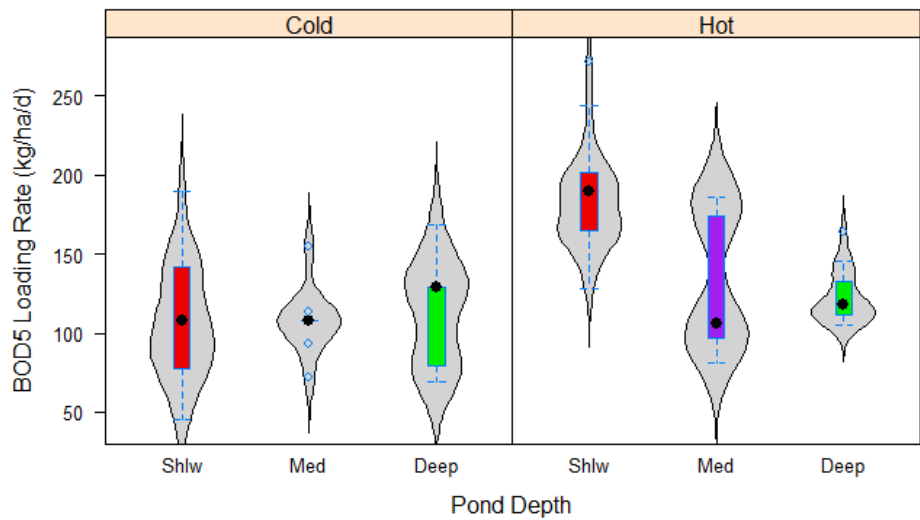
From Tables 3-3 and 3-4 it can be seen that the BOD<sub>5</sub> loading rate when the pond was operating at the shallow depth was significantly higher ( $p < 0.01$ ) than at both the medium and deep depths. However, when this is broken down further by temperature, it can be seen (Fig. 3-4) that in fact, the BOD<sub>5</sub> loading rate only increased in the shallow pond during the hot season, not the cold season. As this is inlet water, the reasons for this variation are beyond the scope of this study. Whilst this may not be directly explicable, it nevertheless, has performance implications as more detailed analysis in Para 5-3-2 shows that volumetric loading rate and pond temperature are the two most important parameters predicting HRAP performance with regard to removing BOD<sub>5</sub>. Otherwise, Fig. 3-4 shows that the BOD<sub>5</sub> loading rate was remarkably stable for most of the observation period.

**Table 3-3 HRAP fed septic tank influent; - Areal BOD<sub>5</sub> Loading Rates (kg BOD<sub>5</sub> /ha/d); n = number of observations**

| Depth     | Shallow<br>(0.32m) | Medium<br>(0.43m) | Deep<br>(0.55m) |
|-----------|--------------------|-------------------|-----------------|
| Median    | 165                | 108               | 118             |
| Mean±s.d. | 149±49             | 127±39            | 118±23          |
| n         | 58                 | 35                | 31              |
| Range     | 63.4 - 200         | 59.4 - 183.6      | 53.7 - 170      |

**Table 3-4 HRAP fed septic tank influent; - Volumetric BOD<sub>5</sub> Loading Rates (g BOD<sub>5</sub> /m<sup>3</sup> /d); n = number of observations**

| Depth     | Shallow<br>(0.32m) | Medium<br>(0.43m) | Deep<br>(0.55m) |
|-----------|--------------------|-------------------|-----------------|
| Median    | 40.0               | 28.1              | 21.0            |
| Mean±s.d. | 43.5±10.6          | 31.2±7.4          | 23.3±5.5        |
| n         | 58                 | 35                | 31              |
| Range     | 22.9 - 71.1        | 16.1 - 49.9       | 12 - 37.3       |

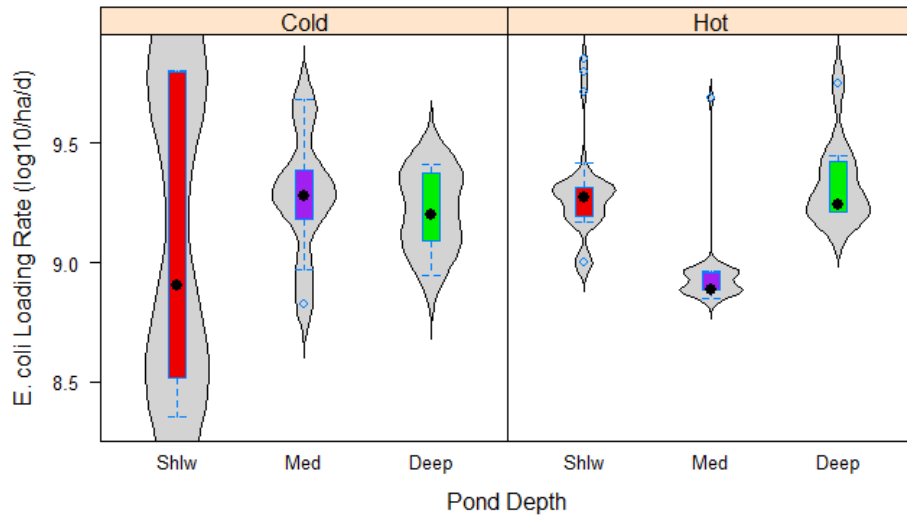


**Fig 3-4 Violinplots for HRAP1 fed septic tank effluent showing areal BOD<sub>5</sub> loading rate (kg BOD<sub>5</sub>/ha/d) by pond depth; 0.32m (Shlw); 0.43m (Med) and 0.55m (Deep) at wastewater temperatures <17.6 °C (Cold) or >17.6 °C (Hot) the median wastewater temperature throughout this study period.**

The literature does not refer to either *E. coli* loading rate or inorganic-N loading rate. They are reported in this results section (Tables 3-5 and 3-6 and Figures 3-5 and 3-6) as they are useful for understanding pond performance considered in more detail in the Chapter 5 analysis of pond performance. Based on prior knowledge of the main drivers of *E. coli* die-off (UV radiation), there was an expectation that areal loading rates would be more influential in predicting *E. coli* LRV in the ponds. In fact, as can be seen in Paras. 5.2.2 and 5.2.3 the areal and volumetric loading rates are roughly equivalent in predicting *E. coli* LRV.

**Table 3-5 HRAP1 fed septic tank effluent; Areal *E. coli* Loading Rates (log<sub>10</sub> *E. coli* /ha/d); n = number of observations**

| Depth     | Shallow<br>(0.32m) | Medium<br>(0.43m) | Deep<br>(0.55m) |
|-----------|--------------------|-------------------|-----------------|
| Median    | 5.313              | 3.961             | 5.215           |
| Mean±s.d. | 5.216±0.453        | 5.044±0.239       | 5.255±0.125     |
| n         | 58                 | 35                | 31              |
| Range     | 3.355 – 5.806      | 3.825 – 5.689     | 3.946 – 5.445   |

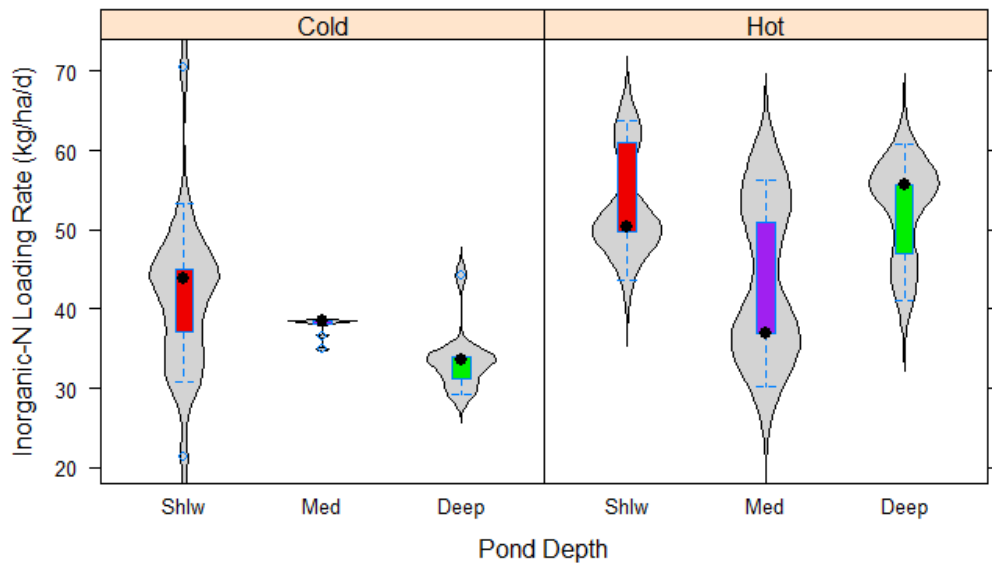


**Fig 3-5 Violinplots for HRAP1 fed septic tank effluent showing areal *E. coli* loading rate ( $\log_{10}$ /ha/d) by pond depth; 0.32m (Shlw); 0.43m (Med) and 0.55m (Deep) at wastewater temperatures  $<17.6^{\circ}\text{C}$  (Cold) or  $>17.6^{\circ}\text{C}$  (Hot) the median wastewater temperature throughout this study period.**

The areal *E. coli* loading rates (Table 3-5 and Fig. 3-5) were not consistently or significantly different at any of the operating depths or unduly influenced by temperature. As with BOD loading rates, the reasons behind the small differences in *E. coli* loading rates reported lie outside the scope of this study. Despite this, as mentioned above, either method of calculating loading rates (areal or volumetric) become one of the strong predictors of *E. coli* removal from the HRAP system (Paras 5.2.2 & 5.2.3, and Figs 5-1 & 5-2). The importance of *E. coli* loading rates is further emphasised in the model described in Chapter 7, which uses the concentration of *E. coli* in the incoming wastewater as the starting point from which losses due to dark die-off and light mediated die-off are deducted from the initial concentration.

**Table 3-6 HRAP 1; Areal Inorganic-N Loading Rates (kg Inorganic-N /ha/d); n = number of observations**

| Depth                           | Shallow<br>(0.32m) | Medium<br>(0.43m) | Deep<br>(0.55m) |
|---------------------------------|--------------------|-------------------|-----------------|
| <b>Median</b>                   | 49.8               | 38.2              | 46.0            |
| <b>Mean<math>\pm</math>s.d.</b> | 48.0 $\pm$ 9.6     | 42.0 $\pm$ 8.4    | 45.2 $\pm$ 10.8 |
| <b>Range</b>                    | 21.4 – 70.5        | 30.3 – 56.3       | 29.3 – 60.8     |
| <b>n</b>                        | 58                 | 35                | 31              |



**Fig 3-6 Violinplots for HRAP1 fed septic tank effluent showing areal Inorganic-N loading rate (kg Inorg-N/ha/d) by pond depth; 0.32m (Shlw); 0.43m (Med) and 0.55m (Deep) at wastewater temperatures <17.6 °C (Cold) or >17.6 °C (Hot) the median wastewater temperature throughout this study period.**

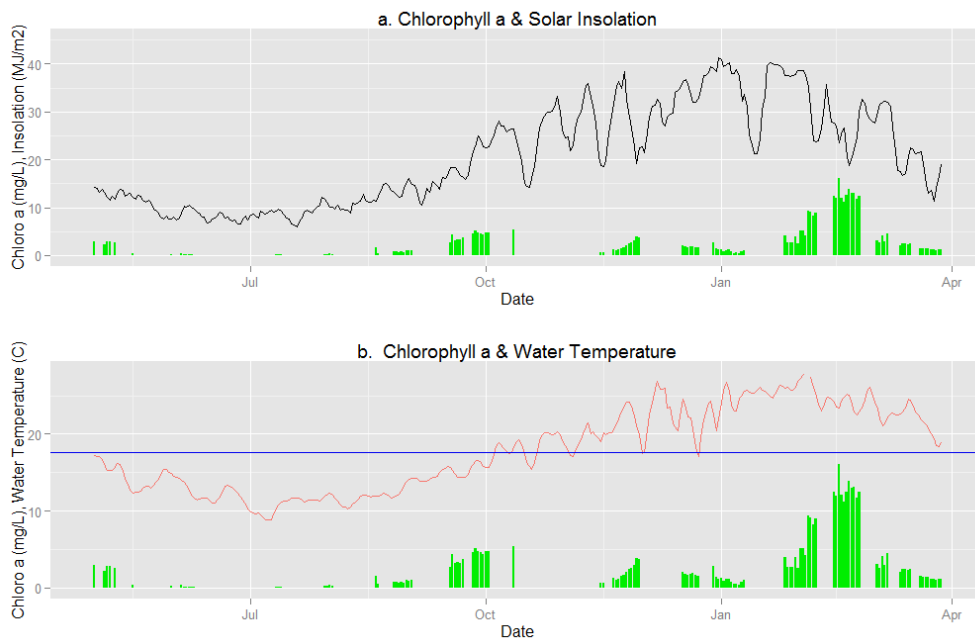
The inorganic-N loading rate (Table 3-6) for the shallow and deep operating depths was higher compared to the medium depth. For reasons that are not well understood, the inorganic-N areal loading rate was higher for the hotter period of the year (Fig. 3-6). The explanation may be that as nearly all of the inorganic-N in the inlet water is actually  $\text{NH}_4\text{-N}$ , it may be that the anaerobic reduction of organic-N components of the wastewater to the reduced form of  $\text{NH}_4\text{-N}$  proceeds at a faster rate in warmer conditions. Unfortunately, this could not be confirmed as total N measurements were not included as part of this study, which would confirm or refute if the nitrogen was simply partitioned between oxidation states.

### 3.1.6 Algal Growth in the HRAP fed septic tank effluent

Chlorophyll *a* concentration values (as a proxy for algal biomass concentration) peaked during the spring and autumn periods (Fig. 3-7). Previous work has



established that algae need both warmth and sunlight to grow (Fallowfield and Garrett, 1985a, Fallowfield and Martin, 1988, Fallowfield et al., 1992a, Abeliovich, 1980, Dalrymple et al., 2013, Grobbelaar et al., 1990, Oswald, 1988a, Van Straten and Herodek, 1982, Richmond et al., 1990). Goldman (1979) stated ‘...the chemical composition of phytoplankton is extremely variable under exacting laboratory conditions of nutrient limitation and approaches the ‘Redfield’ proportions (C:N:P of 106:16:1) when neither nitrogen nor phosphorus is limiting so that near maximal growth rates are attained.



**Fig. 3-7 Time series for the HRAP1 fed septic tank effluent showing the relationship between pond chlorophyll *a* concentration and (a) the daily total solar insolation, and (b) 5 day average pond temperatures with median temperature as blue line.**

There are many units used in the literature to report solar radiation levels. In this study, for consistency and comparison reasons, other units are converted to  $W/m^2$ . Conversions used for PAR (400-700nm) is  $1 \mu mol/m^2/s = 0.219 W/m^2$ , 1 footcandle =  $0.0428 W/m^2$  and  $1 Cal/cm^2/sec = 702.03 W/m^2$ .

In most further analyses the group of chlorophyll *a* and suspended solids concentrations for the month of February 2011 (peaks of 15.8 mg/L chlorophyll *a*) have been excluded or referred to alongside the same figures with the February figures excluded (Table 3-7, columns 1 & 2; and Table 3-8). This is because the values recorded appear to indicate algal productivity figures beyond the theoretical possible maxima for algal productivity. The maximum chlorophyll *a* concentration measured at all other times was 6 mg/L. It is not clear why this group were up to 2.5 times the otherwise consistent maxima, as these very high numbers had not been recorded prior to, or since that period in this pond.

On physical examination of the water containing this large amount of solid material, there was an amorphous matrix with strands of fibre and filamentous algae visible with large amounts of entrapped single celled algae and bacteria.

Confirmation of the large amount of algal material contained in the overall suspended solid was obtained from the concurrent chlorophyll *a* values. These were approximately 3 times ( $\geq 15$  mg/L) higher than the highest chlorophyll *a* observations (5 mg/L) at other times during the study (Fig. 3-7). The observations made in this period may in fact, represent a period of auto-flocculation, and/or a major period of growth of a filamentous algal form such as *Oedogonium* spp. Unfortunately, no calcium measurements or algal species observations were made during this study to shed light on these hypotheses.

### **3.1.6.1 Biomass & Algal Productivities**

Most reports of algal productivity in wastewater treatment systems are in fact referring to algal productivity as described in paras 1.5.6.5 and 1.5.7.2. Accurately separating the algal mass from the rest of the material present is technically achievable, but practically difficult (Cromar and Fallowfield, 1992). This study reports the algal productivity and two estimates of algal productivity, and compares those estimates with a predictive model (Table 3-7).

Two methods of estimating algal productivity were used and compared (Table 3-7). The first (1) was calculated by assuming that algae represent 60% of the suspended solids 'albazod'. The second (2) was calculated by assuming that chlorophyll *a* represents 2% of the algal mass.

Equation 2-6 was used to calculate algal productivities for the study period using the estimates for  $E_t$  (0.04) and  $J$  (6) given in para 2.7.2 and the measured total solar radiation for the 24 hour period prior to the collection of the water sample. The results are summarised in Table 3-7 and used for comparison with estimated algal productivities (Method 2) in Fig. 3.8. The relationship between the two methods algal productivity estimates could be described as equation 3-1.

$$\text{Algal Productivity 1} = 3.617 \times \text{Algal Productivity 2} - 2.562 \dots \dots (3 - 1)$$

The p-value for Eq. 3-1 is  $< 2.2e-16$ , and the  $R^2$  is 0.881. As the p-value is tiny it is safe to conclude that the two values can be described by Eq. 3-1 and that 88% of the variation in *Algal Productivity 1* can be explained by variation in *Algal Productivity 2*. However, it is recognised that neither of the assumptions hold true at all times, but the results allow some comparison of these with the forecasts from Oswald's equation.

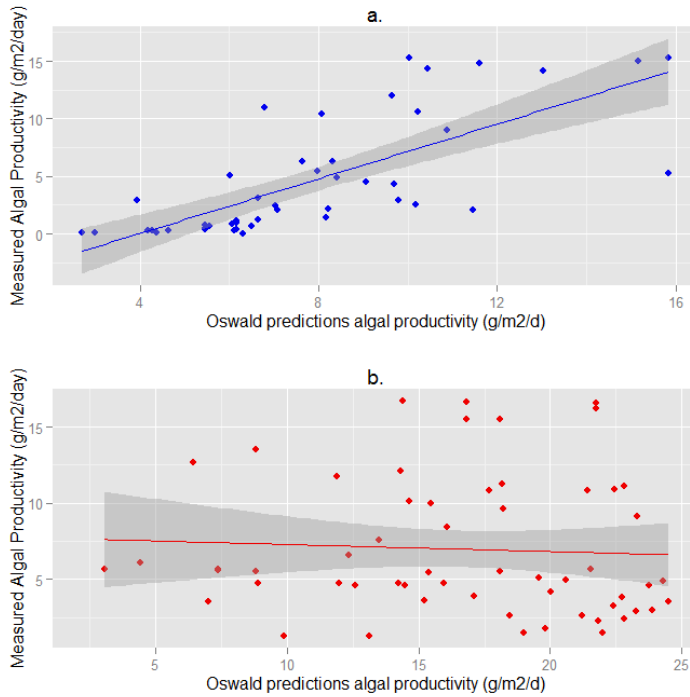
**Table 3-7. Albazod & Algal Productivity (g/m<sup>2</sup>/d) mean±standard deviations & ranges, as calculated by assuming – (1) Albazod including Feb.data (2) Albazod excluding Feb.data (3) Algae as 60% of albazod, (4) Algae containing 2% chlorophyll *a*, and (5) As predicted by the Oswald equation (Eq. 2-6) split by pond operating temperature and depth.**

|                  | <b>Albazod Productivity (with Feb)</b> | <b>Albazod Productivity (excl. Feb)</b> | <b>Algal Productivity 1</b> | <b>Algal Productivity 2</b> | <b>Oswald Predictions</b> |
|------------------|--|---|-----------------------------|-----------------------------|---------------------------|
|                  | <b>Mean±sd (Range)</b>                 | <b>Mean±sd (Range)</b>                  | <b>Mean±sd (Range)</b>      | <b>Mean±sd (Range)</b>      | <b>Mean±sd (Range)</b>    |
| <b>Deep-Cold</b> | 6.4±5.0<br>(1.52-13.9)                 | 6.4±5.0<br>(1.52-13.9)                  | 3.37± 2.92<br>(0.92-8.35)   | 0.80±1.44<br>(0.12-5.2)     | 5.91±3.37<br>(2.7-15.8)   |
| <b>Deep-Hot</b>  | 25.9±20.7<br>(9.09-97.8)               | 25.9±20.7<br>(9.09-97.8)                | 15.53±12.42<br>(5.45-58.7)  | 4.02±1.82<br>(2.81-9.1)     | 20.3±3.76<br>(9.8-24.5)   |
| <b>Med-</b>      | 15.3±8.7                               | 15.3±8.7                                | 9.18±5.21                   | 2.66±0.92                   | 8.09±2.12                 |

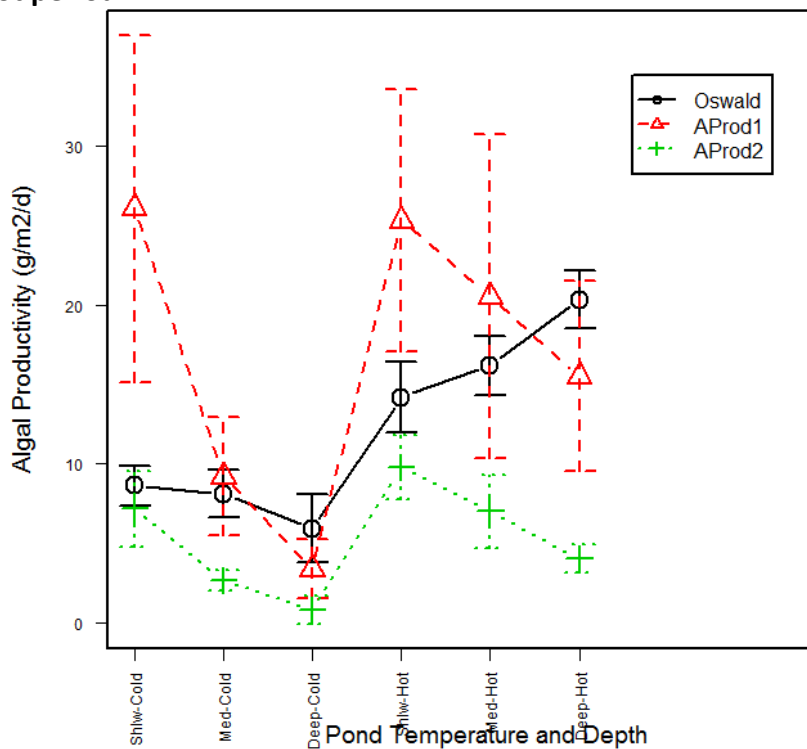
|                                    | <b>Albazod<br/>Productivity<br/>(with Feb)</b> | <b>Albazod<br/>Productivity<br/>(excl. Feb)</b> | <b>Algal<br/>Productivity<br/>1</b> | <b>Algal<br/>Productivity<br/>2</b> | <b>Oswald<br/>Predictions</b> |
|------------------------------------|--|---|-------------------------------------|-------------------------------------|-------------------------------|
|                                    | <b>Mean±sd<br/>(Range)</b>                     | <b>Mean±sd<br/>(Range)</b>                      | <b>Mean±sd<br/>(Range)</b>          | <b>Mean±sd<br/>(Range)</b>          | <b>Mean±sd<br/>(Range)</b>    |
| <b>Cold</b>                        | (7.8-37.8)                                     | (7.8-37.8)                                      | (4.68-22.7)                         | (2.13-4.8)                          | (3.9-11.4)                    |
| <b>Med-<br/>Hot</b>                | 133±183<br>(2.72-505)                          | 34.2±35.2<br>(2.72-127)                         | 20.52±21.14<br>(1.63-76.3)          | 7.00±4.76<br>(3.1-16.7)             | 16.2±4.46<br>(6.9-23.2)       |
| <b>Shlw-<br/>Cold</b>              | 46.6±44.6<br>(0.91-105)                        | 46.6±44.6<br>(0.91-105)                         | 26.08±26.48<br>(0.55-62.7)          | 7.15±5.72<br>(0.9-15.3)             | 8.60±3.09<br>(4.2-15.8)       |
| <b>Shlw-<br/>Hot</b>               | 133±150<br>(12-507)                            | 49.5±33.9<br>(12-113)                           | 25.31±17.71<br>(7.17-60.9)          | 9.80±4.35<br>(5.6-16.7)             | 14.2±6.26<br>(2.2-22.8)       |
| <b>Annual<br/>- All<br/>depths</b> | 79±127<br>(0.9-507)                            | 34.5±34.4<br>(0.9-127)                          | 20.7±20.6<br>(0.55-76.3)            | 6.33±5.34<br>(0.04-21.1)            | 13.2±6.4<br>(2.2-24.5)        |

The linear relationship between the Oswald equation predictions and the measured algal productivities (Method 2) are presented graphically in Fig. 3-8. In the cold period, the linear relationship had a p-value: 4.822e-09 and an R<sup>2</sup> value of 0.526. However, in the hot period the linear relationship had a p-value: 0.668 and an R<sup>2</sup> value of 0.003. In summary, the Oswald equation was a reasonable predictor of algal productivity in the cold period, and not a good predictor of algal productivity in the hot period.

A better understanding of the relationship can be gained from Fig. 3-9. A criticism of the simple Oswald equation is that it takes no direct account of temperature, although there is a close positive relationship between total solar radiation and temperature in some environments (including South Australia). The Oswald predictions do show a temperature effect (Fig. 3-8), but the main source of deviation of the predictions from the measurements is that the predictions do not show the effect of pond depth, which is clear in both the measured methods.



**Fig. 3-8 Scatterplots and linear regression lines with 95% confidence interval shading of – (a.) measured Algal Productivity 2 against Algal Productivity as predicted by the Oswald equation in the cold period, and (b.) measured Algal Productivity 2 against Algal Productivity as predicted by the Oswald equation in the hot period.**



**Fig. 3-9 Algal Productivities (g/m<sup>2</sup>/d) and 95% CI bars as calculated by (a.) Oswald equation predictions, black line (b.) Measured albazod & assuming**

algae as 60% of albedo, red dashed line (c.) Measured chlorophyll *a* & assuming algae containing 2% chlorophyll *a*, green dashed line.

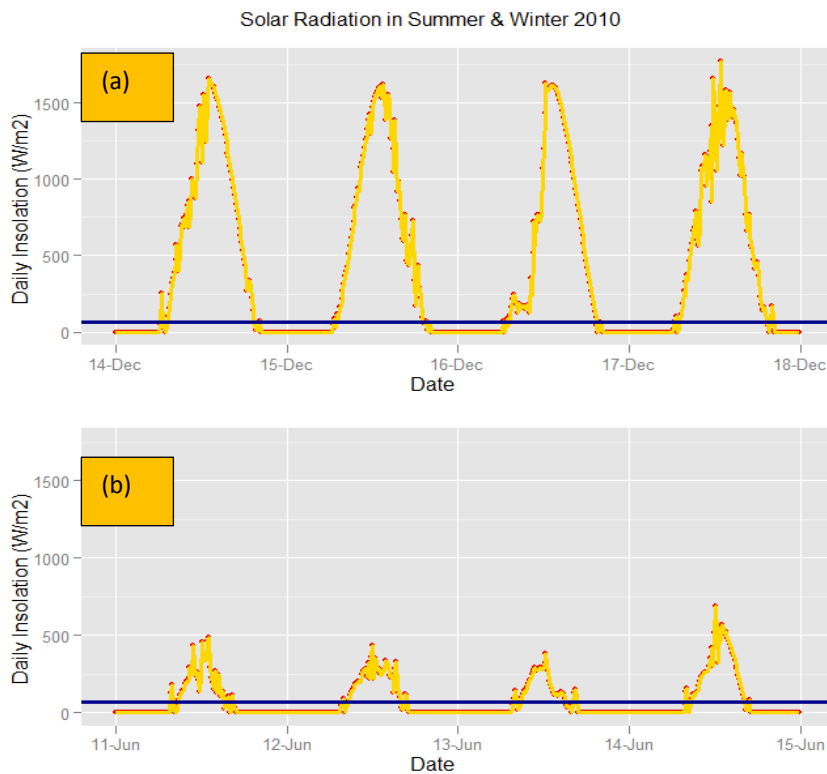
### 3.1.6.2 Possible Photo-inhibition

According to Mihalyfalvy et al. (1998), the onset of algal cellular photo-damage and oxidation (collectively called photoinhibition) occurs at a PAR irradiance of about  $300 \mu\text{mol}/\text{m}^2/\text{s}$  ( $65.7 \text{ W}/\text{m}^2$ ) - which is only 10% of full sunlight. Surface irradiance of  $65.7 \text{ W}/\text{m}^2$  was exceeded nearly all day in both summer and winter as illustrated in Fig. 3-10, where the dark blue line is set at  $65.7 \text{ W}/\text{m}^2$ .

The data presented graphically in Fig. 3-7 provide some limited evidence of possible photo-inhibition on algal growth in the HRAP during the peak summer months. This observation is made as the chlorophyll *a* levels during December and January are low, when solar irradiance levels are high, but peak in March when solar radiation levels decline, but water temperatures remain warm. Other possible reasons for the low chlorophyll *a* during December and January include grazing and fungal and viral attacks.

The results presented in Figs. 3-7 and 3-8 suggest that even with the mixing generated by the paddlewheel the Light:Dark cycle times suggested by Mihalyfalvy et al. (1998) were not sufficient to prevent significant photo-inhibition occurring in the summer months, but they may be sufficient in the winter months.

Rubio et al. (2003) developed an accurate mechanistic model of photosynthesis/photoinhibition. They were able to validate this model using laboratory simulation data from Marra (1978) of diurnal cycles of low and high irradiance similar to the data in Fig. 3-10, was able to show model-predicted photo-inhibition that matched closely their experimental data. They also determined that as per Mihalyfalvy et al. (1998) photo-inhibition occurred at around  $67 \text{ W}/\text{m}^2$ .



**Fig. 3-10 Typical 4 day periods of daily solar irradiance in (a) summer and (b) winter at the HRAP site compared to the irradiance known to initiate photoinhibition ( $65.7 \text{ W/m}^2$ ) drawn in as the horizontal dark blue line.**

### 3.1.6.3 Possible in-pond light climate inhibition to algal growth

Probably the major factor governing the in-pond light climate is the standing crop (areal density) as described in para 1.5.13.2 and Fig. 1-9. As reported in para 1.5.13.2 an average of the theoretical and measured estimates of the areal density at which optimal growth rates are achieved are between 45 and 55  $\text{g(dm)}/\text{m}^2$ . Table 3.8 shows the albedo areal densities (standing crop) recorded in this study by pond depth and temperature, all of which exceed the theoretical optimum for algal growth.

**Table 3-8 Standing crop (areal density) (g(dm)/m<sup>2</sup>) of albazod in HRAP 1 by pond depth and temperature.**

|                            | Albazod Standing Crop, including Feb. | Albazod Standing Crop, excluding Feb. |
|----------------------------|---------------------------------------|---------------------------------------|
|                            | Mean (g/m <sup>2</sup> )±sd (Range)   | Mean (g/m <sup>2</sup> )±sd (Range)   |
| <b>Deep-Cold</b>           | 50.9 ± 43<br>(14.4 – 120)             | 50.9 ± 43<br>(14.4 – 120)             |
| <b>Deep-Hot</b>            | 199 ± 154<br>(64.4 – 738)             | 199 ± 154<br>(64.4 – 738)             |
| <b>Med-Cold</b>            | 94.8 ± 53.8<br>(48.4 – 234)           | 94.8 ± 53.8<br>(48.4 – 234)           |
| <b>Med-Hot</b>             | 707 ± 902<br>(23.4 – 2,547)           | 220 ± 182<br>(23.4 – 645)             |
| <b>Shlw-Cold</b>           | 197 ± 192<br>(4.3 – 456)              | 197 ± 192<br>(4.3 – 456)              |
| <b>Shlw-Hot</b>            | 469 ± 532<br>(42.9 – 1,798)           | 176 ± 134<br>(42.9 – 451)             |
| <b>Annual - All depths</b> | 350 ± 543<br>(4.3 – 2,547)            | 171 ± 157<br>(4.3 – 738)              |

It would appear that under all operating conditions bar the deep, cold period that there was sufficient standing crop to reduce the algal growth rate below optimum.

### 3.1.6.4 Possible algal growth limitations by nutrients

Some attempt has been made to determine if any of the algal growth substrates (nutrients) were limiting algal growth in the HRAP. The concentrations of nutrients in the HRAP 1 was compared with the ‘half-velocity’ concentrations determined by Hill and Lincoln (1981) in developing their algal growth model. (Table 3-9)

**Table 3-9 Half-velocity constants for algal nutrients determined empirically after fitting to the Hill & Lincoln algal growth model. After Hill and Lincoln (1981), compared with the range of measured concentrations in HRAP 1.**

| Substrate              | SUB <sup>KS</sup> (moles/L)    | SUB <sup>KS</sup> (mg/L) | HRAP 1 (mg/L)             |
|------------------------|--------------------------------|--------------------------|---------------------------|
| <b>CO<sub>2</sub></b>  | 0.000081                       | 0.105                    | Not measured              |
| <b>NH<sub>4</sub></b>  | 0.000058824                    | 1.0                      | 2.5 – 78.0                |
| <b>PO<sub>4</sub></b>  | 0.000010417                    | 0.3229                   | 7.5 – 24.0                |
| <b>Solar Radiation</b> | 1.037 Cal/cm <sup>2</sup> /min | 728 W/m <sup>2</sup>     | 44 – 501 W/m <sup>2</sup> |



The first point to make is that the 'half-velocity' constant nutrient concentrations derived by Hill and Lincoln concur with those reported independently by other authors (Zabat (2009) for phosphorus, Shelef (2009) for ammonium and Goldman et al. (1974) for carbon-limited algal growth.

Referring back to Fig. 1-8 to define the 'half-velocity' constant, it seems unlikely that the 'half-velocity' constant value for radiation of  $1.037 \text{ cal/cm}^2/\text{min}$  ( $728 \text{ W/m}^2$ ) for  $\text{RAD}^{\text{KS}}$  reported by Hill & Lincoln (1981) is feasible. It is inconsistent with a number of other reports in the literature. For example, it is an order of magnitude higher than that reported by Myers (2009) for photosynthetic saturation by light intensity of 500 footcandles ( $21.8 \text{ W/m}^2$ ). Mihalyfalvy et al. (1998) report photoinhibition beginning at  $65.7 \text{ W/m}^2$ , suggesting their 'half-velocity' constant would be about  $33 \text{ W/m}^2$ . It seems most likely that a true 'half-velocity' constant for irradiance would be in the vicinity of  $30 \text{ W/m}^2$ .

Thirdly, from the data in Table 3-9 it is apparent that in the wastewater treated in HRAP 1, neither of the main nutrients ( $\text{NH}_4\text{-N}$  or  $\text{PO}_4\text{-P}$ ) or solar radiation were limiting to algal growth. Unfortunately, no comment can be made about the possibility of carbon becoming a limiting nutrient to algal growth as data for carbon concentrations were not recorded in this study.

### **3.2 Key Performance Indicators – nutrient removal, *E.coli* LRV, chlorophyll $\alpha$ .**

The data in Table 3-10 is provided as a broad overview summary of the key performance indicators averaged across the whole period of observation. It

encompasses operation at all three depths and all pond water temperatures, and as such is not valuable in determining the performance of the HRAP under the variety of environmental and pond depth conditions. This analysis will be demonstrated in Para 3.3 below. The HRAP treatment resulted in the removal of 92% of the BOD<sub>5</sub>, 70% of the NH<sub>4</sub>-N, 60% of total inorganic-N, 13% of PO<sub>4</sub>-P and about 1.7 log<sub>10</sub> of *E. coli*.

| <b>Table 3-10 HRAP1 inlet, outlet values and removal efficiencies at all depths for a range of performance related parameters</b> |              |               |                           |
|---|--------------|---------------|---------------------------|
| <b>BOD<sub>5</sub> (mg/L)</b>   |              |               |                           |
|   | <b>Inlet</b> | <b>Outlet</b> | <b>Removal Efficiency</b> |
| <b>Mean</b>   | 204          | 15            | 92.3%                     |
| <b>Median</b>   | 200          | 14            | 92.3%                     |
| <b>Std Dev</b>  | 39.6         | 8.7           | 3.2%                      |
| <b>Number</b>   | 124          | 124           |                           |
| <b>NH<sub>4</sub>-N (mg/L)</b>  |              |               |                           |
| <b>Mean</b>   | 89.9         | 27.1          | 69.1%                     |
| <b>Median</b>   | 87.8         | 23.9          | 73.6%                     |
| <b>Std Dev</b>  | 12.1         | 16.9          | 20.3%                     |
| <b>Number</b>   | 121          | 120           |                           |
| <b>Total Inorganic N (mg/L)</b>   |              |               |                           |
| <b>Mean</b>   | 91.2         | 40.1          | 53.5%                     |
| <b>Median</b>   | 88.9         | 37.5          | 60.5%                     |
| <b>Std Dev</b>  | 12.1         | 13.9          | 19.9%                     |
| <b>Number</b>   | 75           | 75            |                           |
| <b>PO<sub>4</sub>-P (mg/L)</b>  |              |               |                           |
| <b>Mean</b>   | 15.6         | 12.7          | 16.4%                     |
| <b>Median</b>   | 14.5         | 11.0          | 14.8%                     |
| <b>Std Dev</b>  | 5.2          | 3.8           | 12.4%                     |
| <b>Number</b>   | 119          | 118           |                           |
| <b>Chlorophyll <i>a</i> (mg/L)</b>  |              |               |                           |
| <b>Mean</b>   |              | 3.18          |                           |
| <b>Median</b>   |              | 1.95          |                           |
| <b>Std Dev</b>  |              | 3.60          |                           |
| <b>Number</b>   |              | 120           |                           |
| <b>Log<sub>10</sub> <i>E. coli</i> /100 ml and log<sub>10</sub> reduction value (LRV)</b>   |              |               |                           |
|   | <b>Inlet</b> | <b>Outlet</b> | <b>LRV<sup>1</sup></b>    |
| <b>Mean</b>   | 6.361        | 3.607         | 1.755                     |
| <b>Median</b>   | 6.384        | 3.670         | 1.639                     |
| <b>Std Dev</b>  | 0.320        | 0.538         | 0.479                     |

**Table 3-10 HRAP1 inlet, outlet values and removal efficiencies at all depths for a range of performance related parameters**

| Number   | 124   | 124    | 124     |
|--|-------|--------|---------|
| <b>NO<sub>3</sub> &amp; NO<sub>2</sub> -N (mg/L)</b> |       |        |         |
|  | Inlet | Outlet |         |
| <b>Mean</b>  | 0.4   | 13.2   | -12.8%  |
| <b>Median</b>  | 0.2   | 13.5   | -13.3%  |
| <b>Std Dev</b>                                       | 0.5   | 8.4    | -3.9%   |
| <b>Number</b>  | 121   | 120    | -120%   |
| <b>Suspended Solids (mg/L)</b>                       |       |        |         |
| <b>Mean</b>  | 107.3 | 909.5  | -802.2% |
| <b>Median</b>  | 101   | 250    | -149%   |
| <b>Std Dev</b>                                       | 37.5  | 1592   |         |
| <b>Number</b>  | 47    | 120    |         |

<sup>1</sup> LRV log<sub>10</sub> removal value

### 3.2.1 HRAP1 fed treated septic tank effluent: Performance at three operational depths

The overall pond performance data presented in Table 3-10 can be further broken down into the performance achieved at specific operating depths. The pond was operated at three depths; Shallow 0.32 m, medium 0.42 m and deep 0.55 m. In some cases pond depth did influence pond performance. The results are broken down by depth to report results of indicators relevant to wastewater treatment (Tables 3-11 to 3-13) and the removal efficiencies in Table 3-14. Two other measures of central tendency, median and geometric mean, are reported in Tables 3-11 to 3-13, as the mean value tends to overestimate the true population for a number of parameters such as suspended solids and chlorophyll a, due to a small number of extreme readings.

The beanplot series in Figs. 3-9 to 3-11 show the proportion of each of the major nutrients removed at each combination of temperature and pond depth.

**Table 3-11 HRAP 1 receiving septic tank treated influent operated at a depth of 0.32 m. HRAP treated effluent composition (n=58).**

|   | Mean ± SD     | Median | Geometric mean |
|---|---------------|--------|----------------|
| <b>BOD<sub>5</sub> (mg/L)</b>                 | 13.3 ± 8.6    | 11.7   | 10.5           |
| <b>NH<sub>4</sub>-N (mg/L)</b>                | 25.3 ± 16.7   | 20.4   | 21.4           |
| <b>NO<sub>2</sub>-N (mg/L)</b>                | 3.9 ± 7.8     | 3.2    | 2.3            |
| <b>NO<sub>3</sub>-N (mg/L)</b>                | 7.7 ± 3.2     | 7.7    | 5.9            |
| <b>PO<sub>4</sub>-P (mg/L)</b>                | 11.5 ± 2.8    | 10.4   | 11.3           |
| <b>SS (mg/L)</b>                              | 1260 ± 1688   | 584    | 540            |
| <b>Chlorophyll a (mg/L)</b>                   | 3.95 ± 3.71   | 2.82   | 2.41           |
| <b>Log<sub>10</sub> <i>E. coli</i> /100ml</b> | 3.768 ± 0.379 | 3.750  | 3.753          |

**Table 3-12 HRAP 1 receiving septic tank treated influent operated at a depth of 0.42 m. Composition of the HRAP treated effluent (n=35)**

|   | Mean ± SD     | Median | Geometric mean |
|---|---------------|--------|----------------|
| <b>BOD<sub>5</sub> (mg/L)</b>                 | 16.1 ± 9.4    | 16.9   | 12.5           |
| <b>NH<sub>4</sub>-N (mg/L)</b>                | 23.0 ± 12.3   | 19.3   | 19.7           |
| <b>NO<sub>2</sub>-N (mg/L)</b>                | 2.0 ± 1.7     | 1.0    | 1.2            |
| <b>NO<sub>3</sub>-N (mg/L)</b>                | 15.4 ± 3.5    | 16.2   | 13.5           |
| <b>PO<sub>4</sub>-P (mg/L)</b>                | 12.4 ± 2.0    | 12.3   | 12.3           |
| <b>SS (mg/L)</b>                              | 1407 ± 2139   | 510    | 651            |
| <b>Chlorophyll a (mg/L)</b>                   | 3.81 ± 3.32   | 2.09   | 2.22           |
| <b>Log<sub>10</sub> <i>E. coli</i> /100ml</b> | 3.316 ± 0.646 | 3.432  | 3.268          |

**Table 3-13 HRAP 1 receiving septic tank treated influent operated at a depth of 0.55 m. HRAP treated effluent composition (n=31)**

|   | Mean ± SD     | Median | Geometric mean |
|---|---------------|--------|----------------|
| <b>BOD<sub>5</sub> (mg/L)</b>                 | 21.8 ± 13.5   | 16.3   | 18.0           |
| <b>NH<sub>4</sub>-N (mg/L)</b>                | 35.6 ± 19.3   | 41.5   | 25.6           |
| <b>NO<sub>2</sub>-N (mg/L)</b>                | 2.2 ± 2.6     | 2.1    | 1.2            |
| <b>NO<sub>3</sub>-N (mg/L)</b>                | 6.9 ± 6.9     | 6.7    |                |
| <b>PO<sub>4</sub>-P (mg/L)</b>                | 15.2 ± 5.5    | 12.7   | 13.5           |
| <b>SS (mg/L)</b>                              | 304 ± 289     | 231    | 207            |
| <b>Chlorophyll a (mg/L)</b>                   | 0.96 ± 0.75   | 0.91   | 0.53           |
| <b>Log<sub>10</sub> <i>E. coli</i> /100ml</b> | 3.654 ± 0.473 | 3.627  | 3.631          |

**Table 3-14 HRAP1 removal efficiency performance parameters by pond depth shallow (0.32m), medium (0.42m) and deep (0.55m).**

|                | Removal Efficiencies<br>(% removed) |                    |                    | mg/L<br>increase <sup>1</sup>       | Log <sub>10</sub><br>removed |
|----------------|-------------------------------------|--------------------|--------------------|-------------------------------------|------------------------------|
|                | BOD <sub>5</sub>                    | NH <sub>4</sub> -N | PO <sub>4</sub> -P | NO <sub>3</sub> &NO <sub>2</sub> -N | <i>E. coli</i> LRV           |
| <b>Shallow</b> |                                     |                    |                    |                                     |                              |
| -Mean          | 93.4%                               | 69.8%              | 18.9%              | 12.2                                | 1.741                        |
| -StdDev        | 3.8%                                | 19.8%              | 11.5%              | 8.7                                 | 0.282                        |
| -Number        | 58                                  | 56                 | 54                 | 56                                  | 58                           |
| <b>Medium</b>  |                                     |                    |                    |                                     |                              |
| -Mean          | 92.5%                               | 73.5%              | 21.2%              | 17.0                                | 2.079                        |
| -StdDev        | 3.3%                                | 13.9%              | 13.6%              | 5.5                                 | 0.625                        |
| -Number        | 35                                  | 35                 | 35                 | 62                                  | 35                           |
| <b>Deep</b>    |                                     |                    |                    |                                     |                              |
| -Mean          | 90.2%                               | 61.1%              | 6.5%               | 9.1                                 | 1.977                        |

|         | Removal Efficiencies<br>(% removed) |                    |                    | mg/L<br>increase <sup>1</sup>       | Log <sub>10</sub><br>removed |
|---------|-------------------------------------|--------------------|--------------------|-------------------------------------|------------------------------|
|         | BOD <sub>5</sub>                    | NH <sub>4</sub> -N | PO <sub>4</sub> -P | NO <sub>3</sub> &NO <sub>2</sub> -N | <i>E. coli</i> LRV           |
| -StdDev | 3.1%                                | 23.8%              | 5.6%               | 8.4                                 | 0.426                        |
| -Number | 31                                  | 29                 | 29                 | 29                                  | 31                           |

<sup>1</sup>Increase due to nitrification in the HRAP when compared to influent concentrations

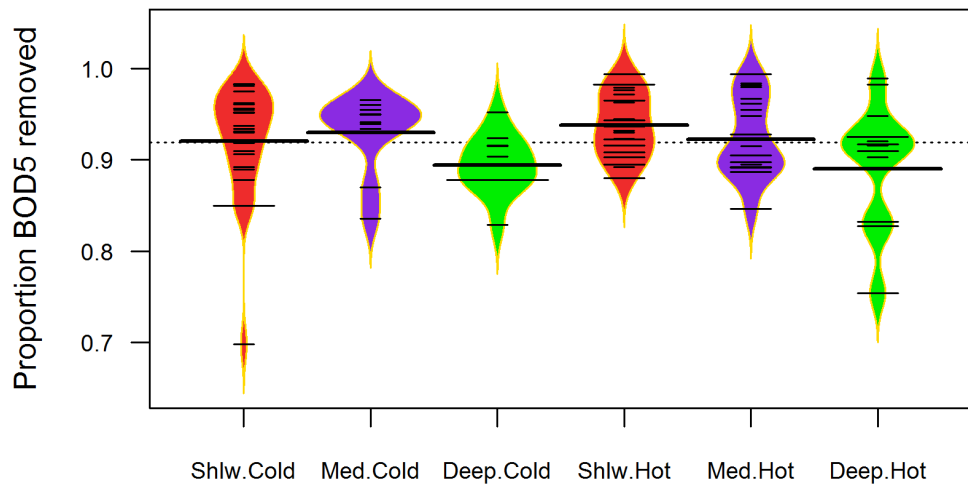
As there was little influence of pond depth in the nutrient removal efficiencies for NH<sub>4</sub>-N, NO<sub>3</sub> & NO<sub>2</sub>-N and PO<sub>4</sub>-P (Table 3-14) these were not considered in any further statistical analysis. They are, however, presented as complete data sets for each operational temperature range and pond depth in the beanplot series Fig. 3-9 to 3-14.

### 3.2.1.2 BOD<sub>5</sub> Removal

Around 90% of BOD<sub>5</sub> was removed under all combinations of operating depths and pond temperatures (Fig. 3-11). As is well known, BOD<sub>5</sub> removal efficiency is predominantly a measure of how effectively carbon is oxidised, although a smaller, slower reacting component is from nitrogenous sources. The high efficiency measured in this study can be directly attributable to the high dissolved oxygen environment created by algal photosynthesis. The deep ponds in both 'hot' and 'cold' periods were slightly less effective than the shallow and medium depth ponds during the same period, despite having longer retention times.

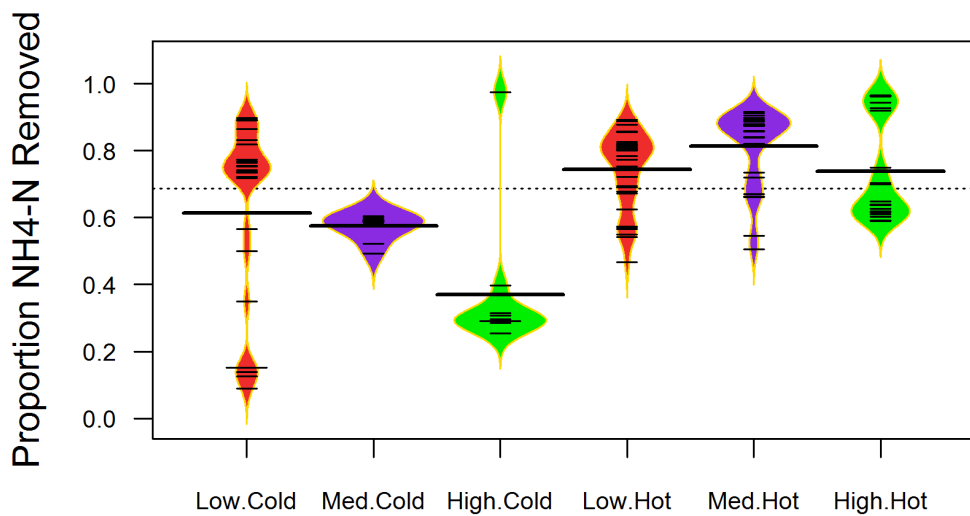
It is interesting to note that pond temperature was not important in determining the proportion of BOD<sub>5</sub> removed, despite the obvious temperature dependence of the rate of oxidation reactions. This suggests that the rate of the oxidation reactions is rapid enough at all temperatures encountered in this study such that by the end of the retention period, effectively all the carbonaceous material had been oxidised, and the remaining BOD<sub>5</sub> was largely from nitrogenous sources. It is not possible to confirm this hypothesis, as in the study, no attempt was made to distinguish carbonaceous from nitrogenous BOD<sub>5</sub> sources.

On the basis of these observations, HRAP 1 performance in removing BOD<sub>5</sub> can be expected to be consistent all year round.



Pond Depth & Pond Water Temperature

**Fig 3-11** Beanplot showing proportion of BOD<sub>5</sub> removed from the HRAP1 fed septic tank treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>17.6°C or <17.6°C, hot or cold respectively).



Pond Depth & Pond Water Temperature

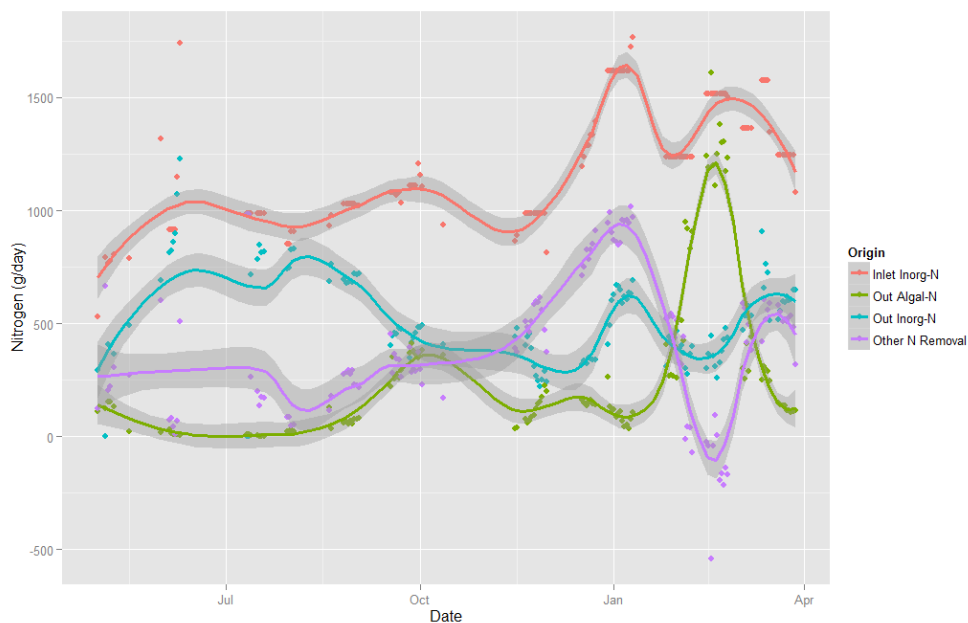
**Fig 3-12** Beanplot showing proportion of NH<sub>4</sub>-N removed from the HRAP1 fed septic tank treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>17.6°C or <17.6°C hot or cold respectively).

### 3.2.1.3 Inorganic-N Removal

The possible routes of nitrogen transformation in a pond are through nitrification, denitrification, volatilisation, net loss to sediments, uptake by microorganisms and mineralisation of organic-N (Senzia et al., 2002).

The principal and most widely accepted basis for ammonia removal within WSP has been attributed to the volatilization of ammonia (Soares et al., 1996, Silva et al., 1995, Pano and Middlebrooks, 1982), microbial uptake and assimilation (Senzia et al., 2002) and subsequent sedimentation and deposition into the sludge layer (Zimmo et al., 2004, Reed, 1985).

As with phosphorous some nitrogen assimilation into microbial biota will occur and consequently some nitrogen will exit the pond as discharged organisms. Through simple stoichiometry, it can be estimated that nitrogen is approximately 12.8% of the algal dry weight. By estimating the algal mass as fifty times the mass of chlorophyll a, an estimate of the mass of nitrogen exiting the pond per day as algal biomass by multiplying the algal mass estimate by 12.8%.



**Fig. 3-13 HRAP 1 loess fit and 95% confidence intervals for Inorganic-N incoming (red) and outgoing (blue), outgoing as algal-N (green) and outgoing as ammonia (purple) over time.**

On average, 1,200 g of inorganic-N enter the HRAP per day (red line in Fig.3-13). No measure was made of organic nitrogen entering the pond, so the following analyses do not account for nitrogen from this source. Of the incoming inorganic-N, 517 g inorganic-N (43%) exits the HRAP per day either in the original reduced form or in an oxidised form (green line in Fig. 3-13). A further 284 g (24%) exits in organic form as part of the algal biomass (blue line in Fig. 3-13). This line is highly variable and depends on periods of algal blooms as occurred in February 2012. As well, 400 g/day (33%) exit as ammonia (purple line in Fig. 3-13), and as explained below this is dependent on temperature, pH and pond depth.

As no sludge accumulates in the HRAP, deposition of dead organisms can be discounted as a nitrogen removal mechanism.

It is clear that nitrification occurs regularly in the HRAP (Table 3-12) in response to the high levels of dissolved oxygen imparted by algal photosynthesis. However, the oxidised forms ( $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$ ) that are so produced have no further means of exiting the HRAP, so again, this is not a feasible nitrogen removal pathway.

Considering all operating conditions, the average proportion of  $\text{NH}_4\text{-N}$  removed from the HRAP was 68% and total inorganic-N removed was 53%.

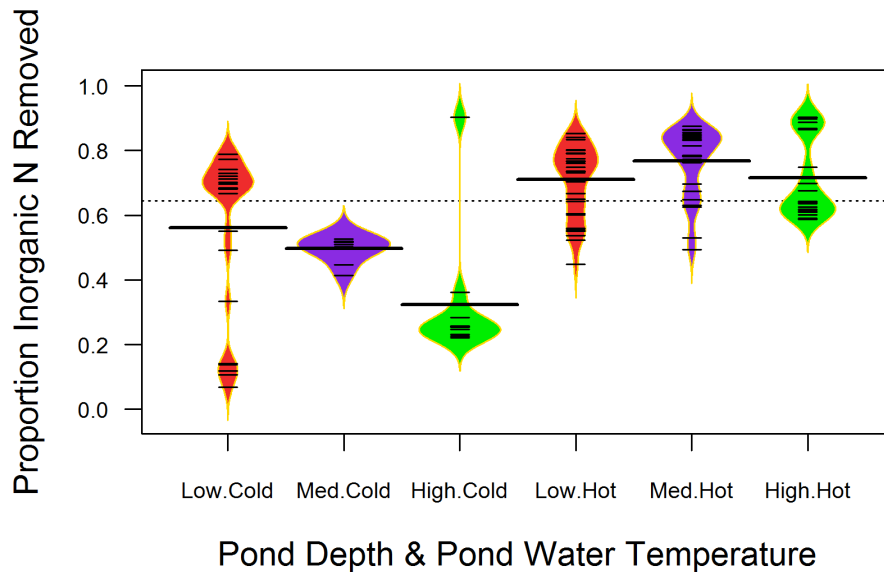
As described by Equation 1-51, the rate of  $\text{NH}_3\text{-N}$  volatilisation depends on the concentration of ammonia gas in the liquid  $\text{NH}_3\text{-N}$  (temperature and pH dependent, see Fig.1-12), depth of the system and a mass transfer coefficient, which is also temperature dependent. As expected, for all pond depths, the proportion of inorganic-N removed from 'cold' water ( $<17.6^\circ\text{C}$ ) was less than that achieved within 'hot' water ( $>17.6^\circ\text{C}$ ; Fig. 3-14). These differences were all statistically significant ( $p<0.05$ ) for all pond depths.

As the rate of volatilisation of ammonia is also inversely dependent on pond depth, it was expected that there would be an effect of pond depth observed in

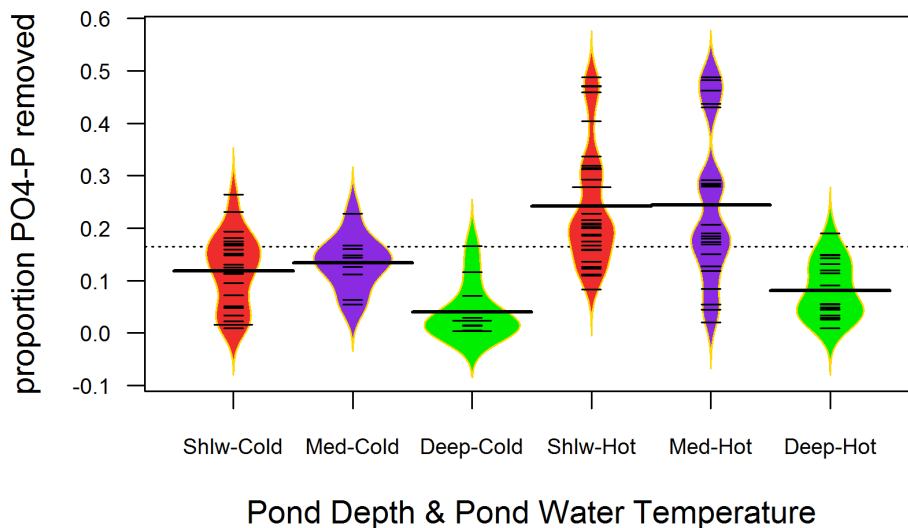


this study. This effect was observed in 'cold' water (Fig. 3-14), but not in 'hot' water. It is possible that in this case, the difference in pond depths was too small to have a measurable effect.

In summary, it appears that all three forms of removal of inorganic-N occur in the HRAP, with emphasis and relative importance shifting according to in-pond conditions and algal growth.



**Fig 3-14** Beanplot showing Inorganic-N removal by the HRAP1 fed septic tank treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>17.6°C or <17.6°C hot or cold respectively).



**Fig 3-15** Beanplot showing proportion of PO<sub>4</sub>-P removed from the HRAP1

fed septic tank treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>17.6°C or <17.6°C hot or cold respectively).

#### 3.2.1.4 PO<sub>4</sub>-P Removal

There are three possible mechanisms for phosphorus removal in an HRAP; exiting unchanged as an orthophosphate, algal uptake/assimilation and chemical precipitation. As no sludge accumulates in the HRAP, deposition of dead organisms can be discounted as a phosphorous removal mechanism.

The weight of phosphorous (in orthophosphate form) entering and leaving the pond on a daily basis was calculated from the orthophosphate concentrations (inlet and outlet) multiplied by the daily inflow. These are represented as the red and green lines respectively in Fig. 3-17 in g PO<sub>4</sub>-P/day. A number of assumptions (below) were made to estimate algal incorporated phosphorous levels. This was done as an attempt to indicate the extent to which the assimilation and precipitation mechanisms were effective in this study period.

The estimate of algal incorporated phosphorus was made through simple stoichiometry. The assumptions used were firstly, algal mass was assumed to be fifty times the mass of chlorophyll a. Secondly, algal phosphorous was assumed to be 1.7% of algal dry weight. This was based on the suggestions of Redfield (1958) and Redfield et al. (1963) that when nutrients are not limiting, the molar elemental ratio C:N:P in most phytoplankton is 106:16:1. This is the basis for the stoichiometry of the algal cell using the structural formula (CH<sub>2</sub>)<sub>106</sub>(NH<sub>3</sub>)<sub>16</sub>(H<sub>3</sub>PO<sub>4</sub>).

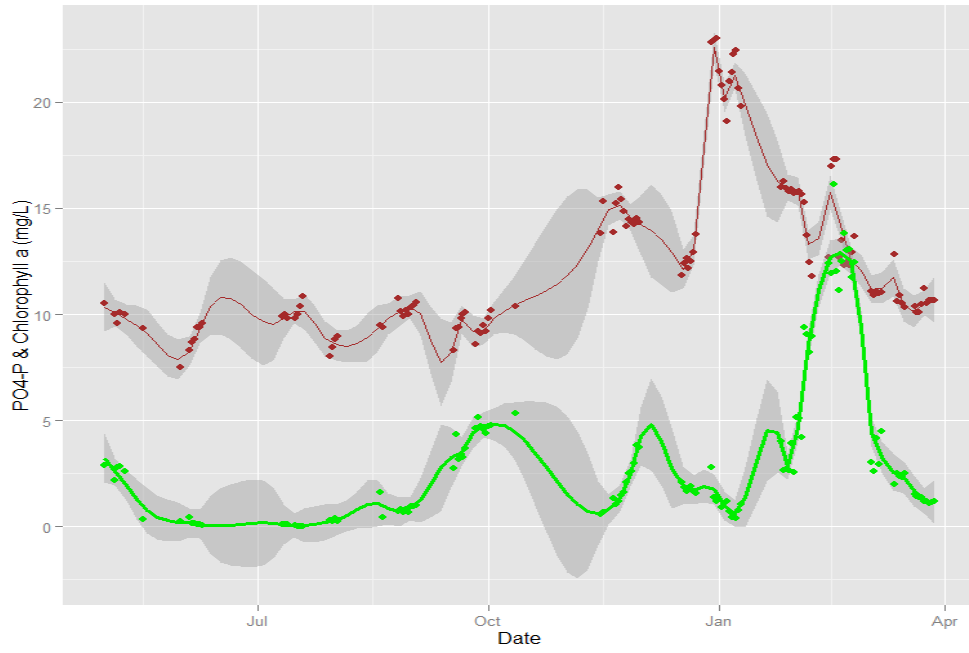
From these two assumptions an estimate of the mass of phosphorous (g PO<sub>4</sub>-P/day) exiting the pond per day in the algal biomass was made (blue line in Fig. 3-17).

Fig. 3-16 shows the concentration of PO<sub>4</sub>-P and of chlorophyll a in HRAP 1. Around the end of 2010 the concentration of PO<sub>4</sub>-P in the inlet began escalating and as a consequence the PO<sub>4</sub>-P levels in the HRAP remained 50 to 100% higher

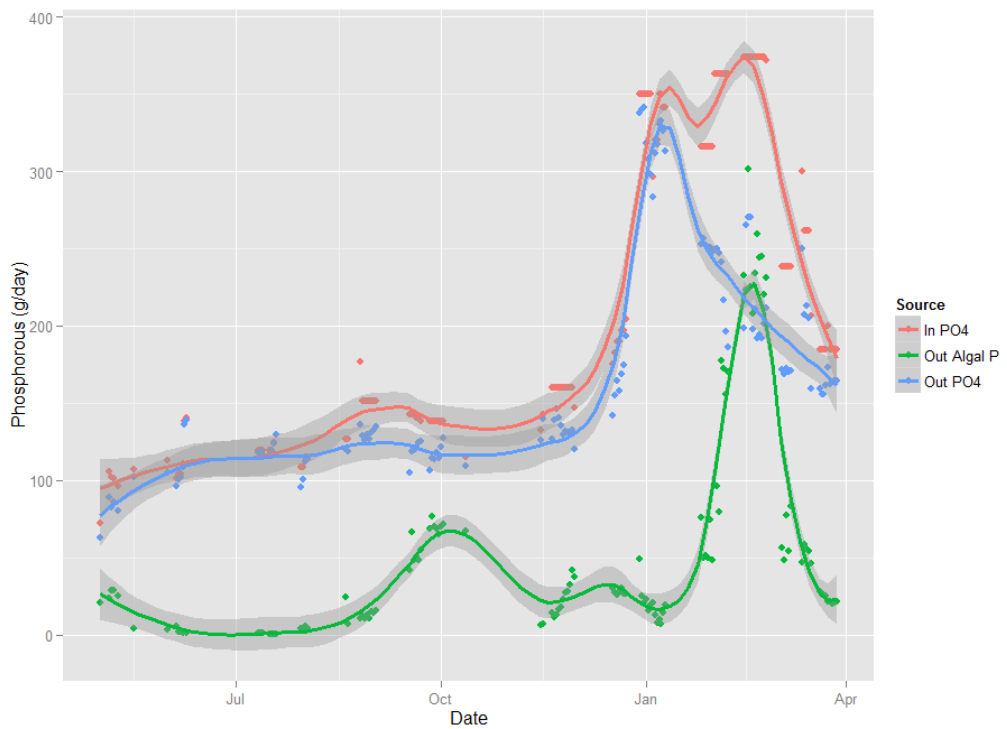
than previous nine month period. Whether by coincidence or by autoflocculation, the chlorophyll a levels rose dramatically about 4 to 6 weeks after the peak of PO<sub>4</sub>-P levels, but certainly during the period when PO<sub>4</sub>-P was around 50% higher than the previous nine months.

Firstly, the source of the high PO<sub>4</sub>-P in the inlet wastewater is unknown, but may be associated with industrial waste washing water entering the system. Secondly, during this period, even though the standing crop of algae was at exceptionally high levels, the amount of PO<sub>4</sub>-P removed per mg of algal standing crop was similar to all other periods, suggesting that in fact the algal growth recorded was real and perhaps growth was being aided by auto-flocculation improving the light climate in the HRAP, allowing further algal growth. As noted below, calcium and magnesium levels were not recorded, so it is not possible to confirm this hypothesis, but it must be considered a possible explanation for the extraordinary results recorded during the period of February 2011.

It can be seen in Fig. 3-17 that between 150 and 350 g/day (mean±sd = 215±97) of PO<sub>4</sub>-P entered the HRAP (red line) and most of the time that amount exited unchanged (green line). However, in September/October and January/February, significantly less PO<sub>4</sub>-P exited the HRAP than entered. During those periods, a compensatory amount of P exited the HRAP as algal-P. Algal-P exiting the pond (mean±sd = 38±48) reached a maximum of 214 g/day in February 2011.



**Fig. 3-16 HRAP 1 – loess fit and 95% confidence intervals for Chlorophyll a (green) & PO<sub>4</sub>-P (brown) over time.**

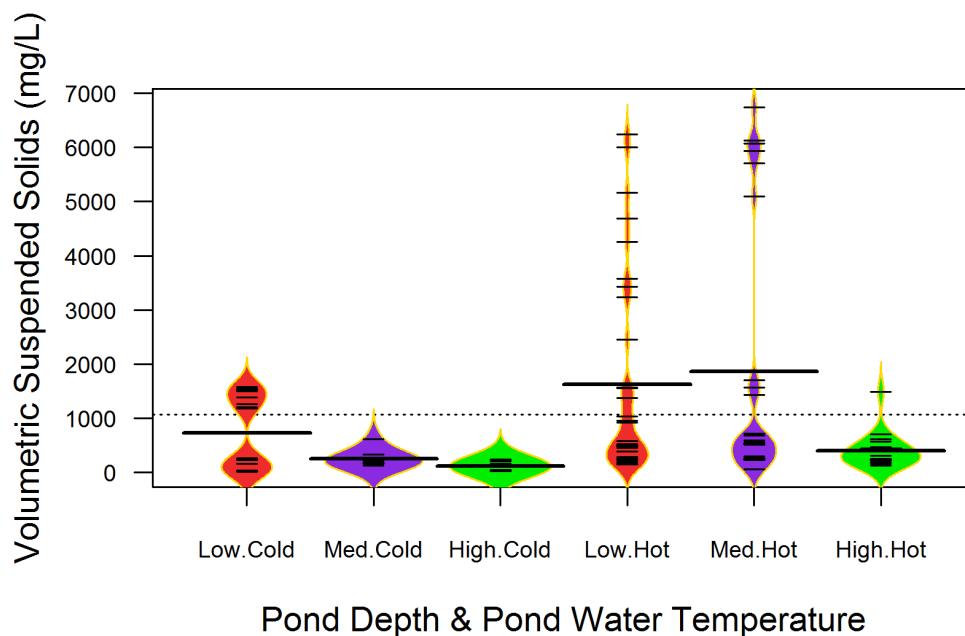


**Fig. 3-17 HRAP 1 – loess fit and 95% confidence intervals for PO<sub>4</sub>-P incoming (red) and outgoing (blue) and outgoing as algal-P (green) over time.**

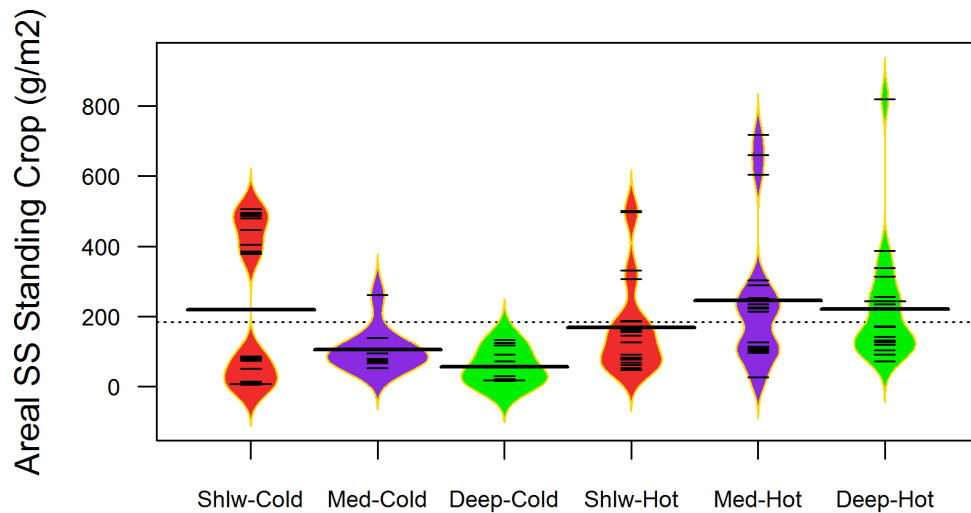
The overall average phosphorous removal efficiency was 16.4% under all operating conditions (Table 3-7 & Fig. 3-15). There was no significant difference in the PO<sub>4</sub>-P removal under most operating depths and temperatures, except

between the medium and deep ponds at wastewater temperatures  $<17.6^{\circ}\text{C}$  (cold). The standing algal crop during those two observation periods was the lowest of the whole period; hence the  $\text{PO}_4\text{-P}$  removal will have been the lowest, which may amplify small measurement errors.

Precipitation of phosphates with polyvalent cations such as calcium and magnesium also occurs in HRAPs in a process that also requires high pH. This precipitation is sometimes called "autoflocculation". which is often incomplete due to insufficient calcium and magnesium concentrations in the wastewater (Nurdogan and Oswald, 1995). This study did not measure calcium or magnesium levels so it is unclear whether precipitation of phosphorous occurred as well as algal uptake, although it is possible that a process like this occurred in the January & February period of this study.



**Fig 3-18** Beanplot showing the concentration of Suspended Solids (volumetric) exiting the HRAP1 fed septic tank treated effluent - displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature ( $>17.6^{\circ}\text{C}$  or  $<17.6^{\circ}\text{C}$  hot or cold respectively).



Pond Depth & Pond Water Temperature

**Fig 3-19** Beanplot showing the areal density or standing crop ( $\text{g}/\text{m}^2$ ) of suspended solids in the HRAP1 (excluding Feb. data) fed septic tank treated effluent - displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature ( $>17.6^\circ\text{C}$  or  $<17.6^\circ\text{C}$  hot or cold respectively).

### 3.2.1.5 Suspended Solids areal density or standing crop

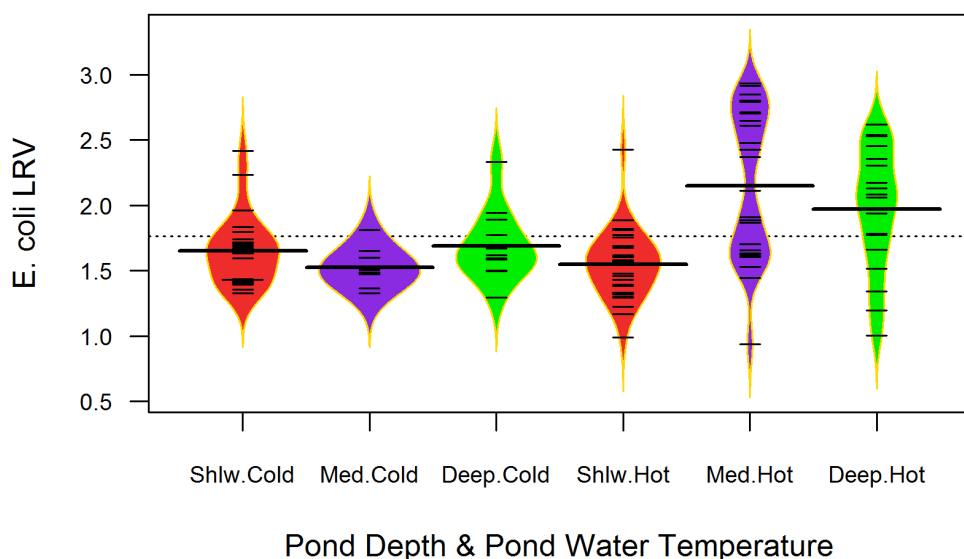
As can be seen in Figs. 3-18 and 3-19, showing graphical representation of concentration and areal density of suspended solids respectively for most of the period of observation, the measured areal density of suspended solids averaged  $185 \pm 174 \text{ g}/\text{m}^2$ . As explained in para 3.1.6, it was decided to exclude the 23 observations taken during February 2011.

As discussed in para 1.5.13.2 Soeder (1980) was the first to suggest that the areal density of a culture would be important in determining overall productivity and he suggested that cultures should be operated at areal densities of 50 to  $150 \text{ g}/\text{m}^2$  for the optimal exploitation of incident solar radiation. Richmond and Grobbelaar (1986) found that the standing crop at which maximal productivity occurred in an outdoor culture of *Spirulina platensis* varied with depth, at a depth of 150 mm it occurred at about  $90 \text{ g}/\text{m}^2$ , and at about  $60 \text{ g}/\text{m}^2$  in a culture 75 mm deep.

Hartig et al. (1988) found that algal productivity follows a 'wedge shaped' relationship with areal density and that maximal algal productivity occurred at

an areal density of 40 to 45 g/m<sup>2</sup>. They found that optimal areal density for maximal productivity was also influenced by factors such as culture depth, algal species, turbulence and available light. In their view, the establishment and maintenance of an optimal areal density is one of the most important operational procedures for the mass culture of algae. They also found that supersaturated concentrations of oxygen resulted in lower productivities due to photorespiration and/or oxidation.

All the above work was conducted in small algal cultures, using supplied nutrients, so the light environment was much more favourable than that found in wastewater. The albazod standing crop averaged 171±157 g/m<sup>2</sup> (Table 3-8), which is above the optimum quoted by the authors above. Higher densities may be advantageous in the relatively optically opaque wastewater. As mentioned, there were periods when the standing algal crop far exceeded the optimum for growth. It may well be that 'self-correction' by shading occurred to return the density back closer to optimum. It can be seen in Fig. 3-19 that the standing crop was generally higher in the 'hot' period compared to the 'cold' period.



**Fig 3-20** Beanplot showing *E.coli* LRV by the HRAP1 fed septic tank treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>17.6°C or <17.6°C hot or cold respectively).

### 3.2.1.6 *E. coli* LRV

There is significant interest in understanding the disinfection performance of the HRAP with regard to *E. coli* since this is a key performance indicator for both South Australian Department of Health and national regulators when validating new treatment systems where treated wastewater reuse is contemplated. The interaction of *E. coli* LRV with environmental conditions (temperature & irradiance) and pond depth, is crucial to the understanding of the performance of HRAPs for public health regulators and when making recommendations on pond design under Australian conditions. These detailed statistical breakdowns are provided in Tables 3-15 to 3-18 and Fig. 3-20, and a longitudinal overview can be seen in Fig. 3-22.

The overall removal of *E. coli* as determined by *E. coli* LRV over the period of the THRT, shows little difference in performance between pond depths and water temperatures. A mean value for *E. coli* LRV for all configurations was approximately 1.8. This is represented by the dotted line in Fig 3-20. When the wastewater was >17.6°C in the medium (0.42m) and deep (0.55m) ponds there was a difference in *E. coli* LRV from the other four reported operating conditions. Even though statistically significant at the 95% confidence level, these trends were not seen as overwhelmingly significant in the pond disinfection performance. This area will be discussed more fully in *Chapter 6 – Analysis*.

Table 3-15 shows the results of an Analysis of Variance model of *E. coli* LRV by pond depth and temperature. The p-value reported ( $1.82 \times 10^{-8}$ ) indicates significant differences between figures within the group. The differences in mean *E. coli* LRV are reported in Table 3-17 and 3-18. The results are summarised graphically in Fig. 3-20 & Fig. 3-21. In effect there are statistically significant differences in the *E. coli* LRV between the shallow and deep ponds in hot conditions, and the shallow and medium ponds under hot conditions. The medium depth pond also had statistically significant differences in the *E. coli*



LRV under hot and cold conditions. These results should be noted for the discussion in *Chapter 6 - Analysis* as noted above for a more complete analysis of the factors influencing *E. coli* LRV in the HRAP.

**Table 3-15 Summary of HRAP1 Anova Model of *E. coli* LRV by Pond Depth & Pond Temperature.**

|                   | Degrees<br>freedom | Sum<br>Squares | Mean<br>Square | F value | Pr(>F)    |
|-------------------|--------------------|----------------|----------------|---------|-----------|
| <b>Depth Temp</b> | 5                  | 7.387          | 1.477          | 10.657  | 1.82 e-08 |
| <b>Residuals</b>  | 118                | 16.357         | 0.139          |         |           |

**Table 3-16 HRAP1 Numerical Summary of *E. coli* LRV by Pond Depth & Pond Temperature.**

| Configuration       | Mean  | sd    | n  |
|---------------------|-------|-------|----|
| <b>Deep Cold</b>    | 1.632 | 0.185 | 11 |
| <b>Deep Hot</b>     | 1.988 | 0.463 | 20 |
| <b>Mid Cold</b>     | 1.522 | 0.140 | 10 |
| <b>Mid Hot</b>      | 2.151 | 0.576 | 25 |
| <b>Shallow Cold</b> | 1.649 | 0.270 | 24 |
| <b>Shallow Hot</b>  | 1.550 | 0.254 | 34 |

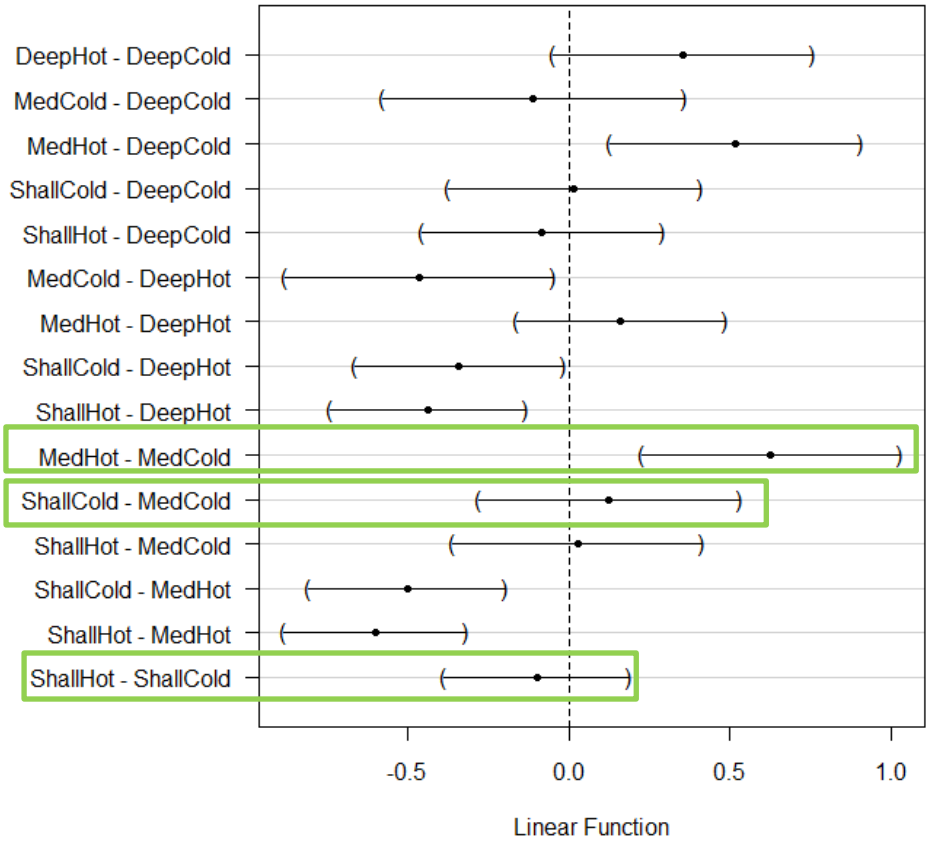
**Table 3-17 HRAP1 Multiple Comparisons of pairs of Means of *E. coli* LRV by Pond Depth & Pond Temperature: Tukey Contrasts.**

| Null Hypothesis                     | Estimate | Std.<br>Error | t value | Pr(> t )         |
|-------------------------------------|----------|---------------|---------|------------------|
| <b>Deep Hot – Deep Cold = 0</b>     | 0.356    | 0.140         | 2.548   | 0.115            |
| <b>Med Cold – Deep Cold = 0</b>     | -0.109   | 0.163         | -0.672  | 0.984            |
| <b>Med Hot – Deep Cold = 0</b>      | 0.519    | 0.135         | 3.850   | <b>0.002</b>     |
| <b>Shallow Cold – Deep Cold = 0</b> | 0.017    | 0.136         | 0.125   | 1.000            |
| <b>Shallow Hot – Deep Cold = 0</b>  | -0.082   | 0.129         | -0.634  | 0.988            |
| <b>Med Cold – Deep Hot = 0</b>      | -0.465   | 0.144         | -3.227  | <b>0.019</b>     |
| <b>Med Hot – Deep Hot = 0</b>       | 0.163    | 0.112         | 1.455   | 0.686            |
| <b>Shallow Cold – Deep Hot = 0</b>  | -0.339   | 0.113         | -3.009  | <b>0.036</b>     |
| <b>Shallow Hot – Deep Hot = 0</b>   | -0.438   | 0.105         | -3.174  | <b>&lt;0.001</b> |
| <b>Med Hot – Med Cold = 0</b>       | 0.628    | 0.139         | 3.507   | <b>&lt;0.001</b> |
| <b>Shallow Cold – Med Cold = 0</b>  | 0.126    | 0.140         | 0.900   | 0.944            |
| <b>Shallow Hot – Med Cold = 0</b>   | 0.027    | 0.134         | 0.204   | 0.999            |
| <b>Shallow Cold – Med Hot = 0</b>   | -0.502   | 0.106         | -3.715  | <b>&lt;0.001</b> |
| <b>Shallow Hot – Med Hot = 0</b>    | -0.600   | 0.098         | -6.121  | <b>&lt;0.001</b> |

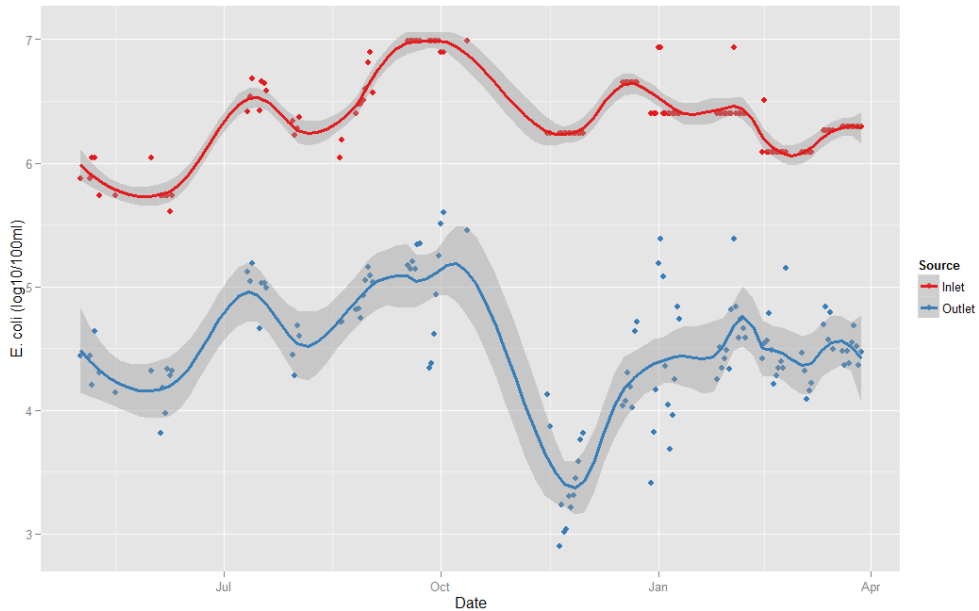
| Null Hypothesis                       | Estimate | Std. Error | t value | Pr(> t ) |
|---------------------------------------|----------|------------|---------|----------|
| <b>Shallow Hot – Shallow Cold = 0</b> | -0.099   | 0.099      | -0.995  | 0.916    |

**Table 3-18 HRAP1 95% family-wise confidence level of comparison of means of *E. coli* LRV by Pond Depth & Pond Temperature.**

| Null Hypothesis                       | Estimate | Lower  | Upper  |
|---------------------------------------|----------|--------|--------|
| <b>Deep Hot – Deep Cold = 0</b>       | 0.356    | -0.047 | 0.759  |
| <b>Med Cold – Deep Cold = 0</b>       | -0.109   | -0.578 | 0.360  |
| <b>Med Hot – Deep Cold = 0</b>        | 0.519    | 0.130  | 0.907  |
| <b>Shallow Cold – Deep Cold = 0</b>   | 0.017    | -0.374 | 0.408  |
| <b>Shallow Hot – Deep Cold = 0</b>    | -0.082   | -0.454 | 0.290  |
| <b>Med Cold – Deep Hot = 0</b>        | -0.465   | -0.881 | -0.050 |
| <b>Med Hot – Deep Hot = 0</b>         | 0.163    | -0.159 | 0.485  |
| <b>Shallow Cold – Deep Hot = 0</b>    | -0.339   | -0.664 | -0.014 |
| <b>Shallow Hot – Deep Hot = 0</b>     | -0.438   | -0.740 | -0.135 |
| <b>Med Hot – Med Cold = 0</b>         | 0.628    | 0.226  | 1.029  |
| <b>Shallow Cold – Med Cold = 0</b>    | 0.126    | -0.278 | 0.530  |
| <b>Shallow Hot – Med Cold = 0</b>     | 0.027    | -0.359 | 0.413  |
| <b>Shallow Cold – Med Hot = 0</b>     | -0.502   | -0.808 | -0.195 |
| <b>Shallow Hot – Med Hot = 0</b>      | -0.600   | -0.883 | -0.318 |
| <b>Shallow Hot – Shallow Cold = 0</b> | -0.099   | -0.385 | 0.188  |



**Fig 3-21 HRAP1 95% family-wise confidence level of comparison of means of *E. coli* LRV by Pond Depth & Pond Temperature.**

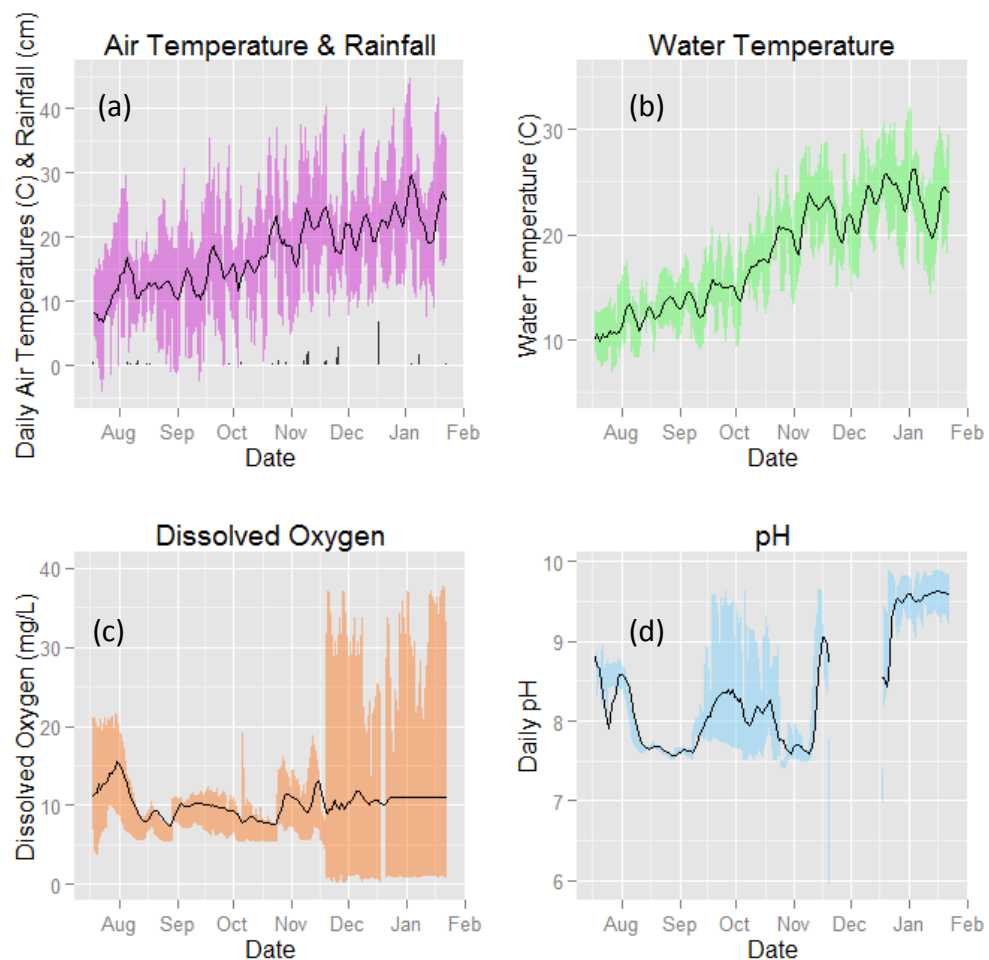


**Fig. 3-22 HRAP 1 – loess fit and 95% confidence intervals for *E. coli* concentration incoming (red) and outgoing (blue) over time.**

### 3.3 High Rate Algal Ponds – Phase 2, Inlet Water derived from adjacent facultative pond pre-treating septic tank effluent. July 2011 to February 2012

#### 3.3.1 Environmental Factors – air temperature, wind speed & direction, total solar radiation, UV radiation, rainfall

The data presented in Table 3-1 and Figures 3-1 and 3-23 show the most important climatic data for this period of study, reiterating that the annual cycle of air temperature follows a typical semi-arid pattern (BSk in the Köppen climate classification) with hot dry summers and cold dry winters.



**Fig. 3-23 HRAP2 receiving facultative pond effluent: Daily maximum & minimum and 5 day average for:- a. air temperature and rainfall b. Water Temperature c. DO and d. pH recorded on-site at Kingston-on-Murray during the study period.**

### 3.3.2 HRAP2 Operational Conditions & Wastewater Physico-chemical Parameters

HRAP2 displayed seasonal temporal variations typically associated with variations in sunlight and ambient temperatures (Fig 3-23a). As previously described, the summer maximum air temperatures regularly exceeded 40°C and mid-winter minima commonly drop below 0°C (Fig 3-23a). The average daily wastewater temperature (Fig. 3-23b) commonly exceeded 30°C in summer and minima dropped below 10°C for most of the winter/spring period. The median water temperature used to divide the data into “hot” and “cold” periods was 18.3°C.

The daily average DO remained close to 10mg/L throughout this study period. Daily DO peaks of between 8 and 38 mg/L were observed throughout the year, with the higher peaks generally occurring during the warmer months when algal growth was at its greatest (Fig.3-23c). The lower peaks typically occurred when chlorophyll *a* levels were below 1 mg/L (see Fig.3-27). However, again the relationship between daily chlorophyll *a* and daily DO peaks was not as straightforward as may have been expected. Once again, some of this lack of consistency may be due to difficulties in keeping the membrane of the DO probe clear of biofilm fouling. The overnight DO consistently dropped to 0 for the warmer periods of the year, but did not reach 0 during the colder months of the year, again presumably because bacterial and algal respiration was limited by the cold water conditions, but also the solubility of oxygen in water increases as the water temperature decreases, so the cooler winter water retains more dissolved oxygen.

Daily pH peaks of between 7.6 and 9.8 were observed (Fig. 3-23d). Diurnal pH variations of 1.7 pH units were observed in the spring period, but generally diurnal variation of pH was lower than in HRAP1 and more typically was only around 0.8 pH units. As noted in Para 3.1.2, regarding HRAP1, the pH was not as high as reported by other authors in HRAPs. This lower nutrient environment

was clearly less conducive to algal growth, and again some constraints on pH may have been due to the buffering capacity of the inlet water.

### 3.3.3 Inlet Wastewater Characteristics

The facultative pond effluent used as the inlet water for HRAP2 (Table 3-19) was significantly lower in all the major physico-chemical parameters by comparison with the inlet water used for HRAP1. Compared with the parameters in Table 3-2, the inflow volume to HRAP2 was similar, but there was approximately 12% of the BOD<sub>5</sub>, 21% of the NH<sub>4</sub>-N, 62% of the PO<sub>4</sub>-P, 58% of the log<sub>10</sub> *E.coli* and about 5 times as much NO<sub>2</sub>-N and NO<sub>3</sub>-N compared with the inflow to HRAP1. This significantly reduced nutrient load, particularly with regards organic carbon (BOD<sub>5</sub>) has obvious ramifications for the potential for algal growth.

**Table 3-19 HRAP 2 Inlet Wastewater - facultative pond effluent – Volume & Composition, where n = number of samples analysed.**

|                          | Inflow<br>(kL/d) | BOD <sub>5</sub><br>(mg/L) | NH <sub>4</sub> -N<br>(mg/L) | NO <sub>2</sub> -N & NO <sub>3</sub> -N<br>(mg/L) | PO <sub>4</sub> -P<br>(mg/L) | <i>E.coli</i><br>/100mL |
|--------------------------|------------------|----------------------------|------------------------------|---|------------------------------|-------------------------|
| <b>Median</b>            | 12.0             | 17                         | 22.35                        | 2.50  | 8.80                         | 3.544                   |
| <b>Mean</b>              | 12.1             | 24                         | 18.73                        | 1.92  | 8.75                         | 3.689                   |
| <b>SD</b>                | 3.0              | 15.8                       | 12.1                         | 0.91  | 3.20                         | 0.517                   |
| <b>Number of observ.</b> | 190              | 75                         | 75                           | 75  | 75                           | 75                      |

To further emphasise this point, the BOD<sub>5</sub> loading rate in both areal and volumetric terms was very low, particularly in the medium (0.43m) and deep (0.55m) depth configuration (Tables 3-20 & 3-21).

**Table 3-20 HRAP 2 fed facultative pond effluent: Areal BOD<sub>5</sub> Loading Rates (kg BOD<sub>5</sub> /ha).**

| Depth          | Shallow<br>(0.32m) | Medium<br>(0.42m) | Deep<br>(0.55m) |
|----------------|--------------------|-------------------|-----------------|
| <b>Median</b>  | 30.5               | 9.0               | 5.7             |
| <b>Mean</b>    | 23.4               | 9.1               | 7.2             |
| <b>Std Dev</b> | 18.1               | 2.9               | 1.6             |
| <b>Range</b>   | 3.2 – 53.2         | 6.3 – 12.6        | 5.7 – 8.8       |

Table 3-21 HRAP 2 fed facultative pond effluent: Volumetric BOD<sub>5</sub> Loading Rates (g BOD<sub>5</sub> /m<sup>3</sup>).

| Depth   | Shallow<br>(0.32m) | Medium<br>(0.42m) | Deep<br>(0.55m) |
|---------|--------------------|-------------------|-----------------|
| Median  | 9.5                | 3.1               | 2.1             |
| Mean    | 7.6                | 3.1               | 2.7             |
| Std Dev | 5.7                | 1.0               | 0.6             |
| Range   | 1.0 – 16.9         | 2.1 – 3.3         | 2.1 – 3.3       |

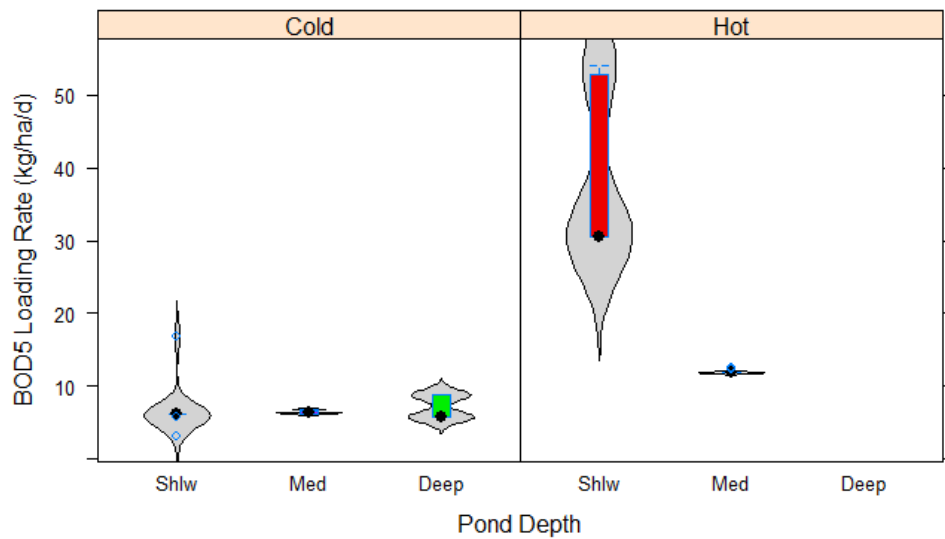
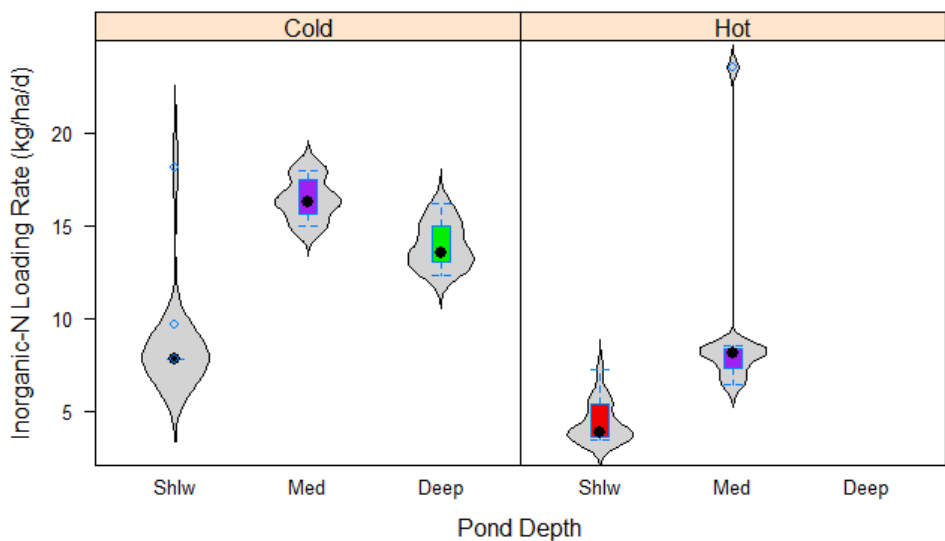
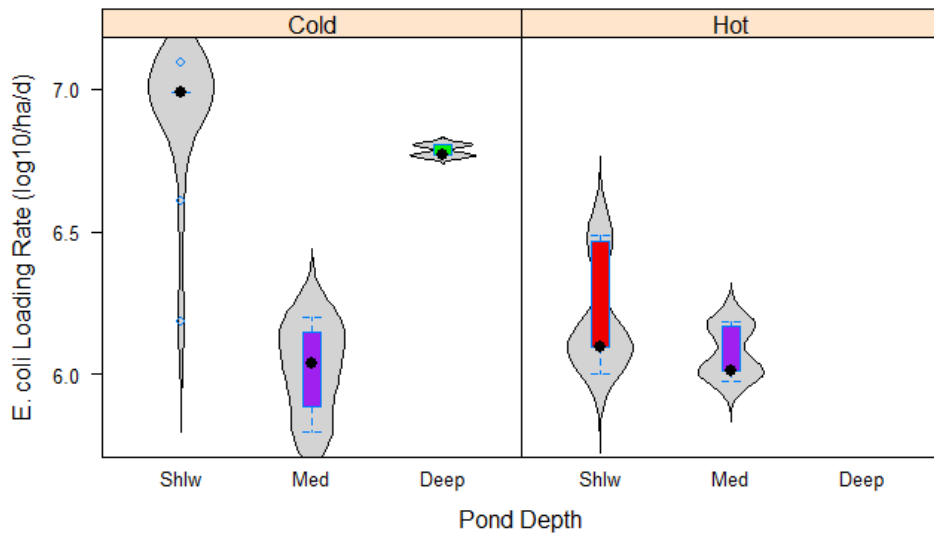


Fig 3-24 Violinplots for HRAP2 fed facultative pond effluent showing areal BOD<sub>5</sub> loading rate (kg BOD<sub>5</sub> /ha/d) by pond depth; 0.32m (Shlw); 0.43m (Med) and 0.55m (Deep) at wastewater temperatures <18.3 °C (Cold) or >18.3 °C (Hot) the median wastewater temperature throughout this study period.



**Fig 3-25 Violinplots for HRAP2 fed facultative pond effluent showing areal Inorganic-N loading rate (kg Inorg-N/ha/d) by pond depth; 0.32m (Shlw); 0.43m (Med) and 0.55m (Deep) at wastewater temperatures <math><18.3^{\circ}\text{C}</math> (Cold) or <math>>18.3^{\circ}\text{C}</math> (Hot) the median wastewater temperature throughout this study period.**



**Fig 3-26 Violinplots for HRAP2 fed facultative pond effluent showing areal *E. coli* loading rate ( $\log_{10} E. coli$  /ha/d) by pond depth; 0.32m (Shlw); 0.43m (Med) and 0.55m (Deep) at wastewater temperatures <math><18.3^{\circ}\text{C}</math> (Cold) or <math>>18.3^{\circ}\text{C}</math> (Hot) the median wastewater temperature throughout this study period.**

### 3.3.4 Algal Growth in the HRAP2 fed effluent pre-treated in a facultative pond.

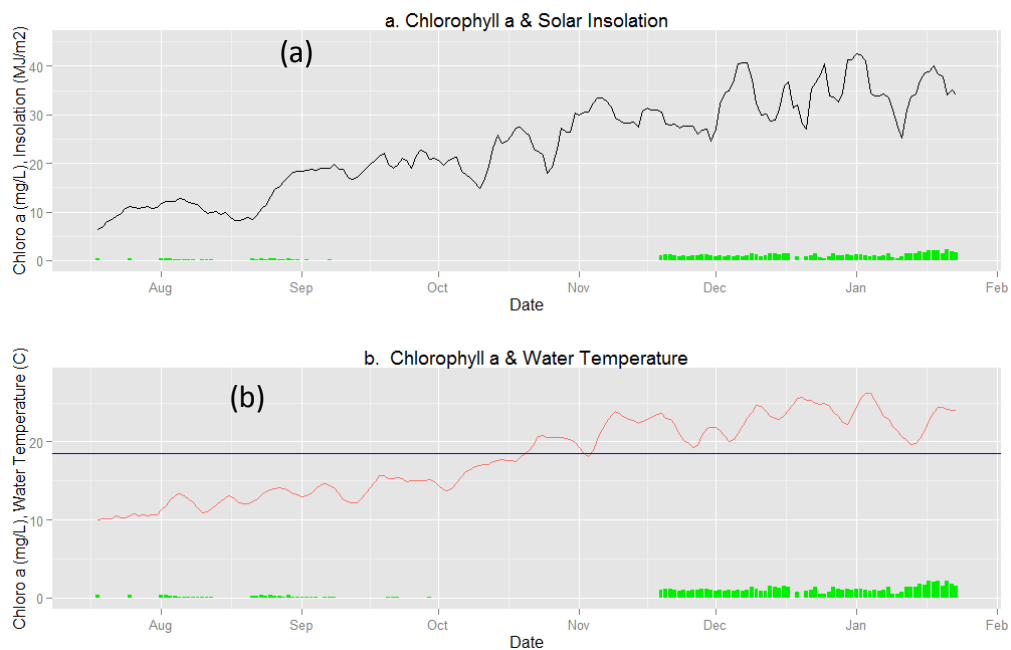
Chlorophyll *a* values were higher during the spring and summer periods (Fig. 3-27). Consistent with the reduced input of nutrients described above the chlorophyll *a* levels recorded were well below those found in HRAP 1. The overall average value was 0.769 mg/L with a maximum of 2.36 mg/L and a minimum of 0.03 mg/L

As presented in Table 3-7 and in *Section 3.1.4*, where the half-velocity constants for algal nutrients determined empirically using the algal growth model of Hill and Lincoln (1981) were considered, and from the data in Tables 3.22 to 3-25 it was apparent that the sole major nutrient which could limit algal growth in this system was carbon.  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$  levels are well above half velocity



concentrations of 1.0 and 0.3229 mg/L respectively. No direct measurement of carbon was made during this study, but it appears likely that the difference in chlorophyll *a* levels between the HRAP 1 and HRAP 2 was due to much lower available carbon since the inlet BOD<sub>5</sub> in HRAP 2 was 12% of that coming into HRAP 1.

The reduction in chlorophyll *a* production is obvious in Fig. 3-27 when the height of the green bars is compared with those in Fig. 3-7 (HRAP 1).



**Fig. 3-27 Time series for the HRAP2 fed facultative pond effluent showing a) chlorophyll *a* concentration and total solar irradiance and b) the chlorophyll *a* and HRAP2 wastewater temperatures (with blue 18.3°C median line) over the period 1 May 2010 to 1 Apr 2011.**

### 3.3.5 Key Performance Indicators – nutrient removal, *E.coli* LRV, chlorophyll $\alpha$ .

The data in Table 3-22 presents the key performance indicators averaged across the whole period of observation. It encompasses operation at all three depths and all pond water temperatures. As a broad overview, passage of facultative pond treated wastewater through the HRAP 2 resulted in the removal of 64% of

the BOD<sub>5</sub>, 66% of the NH<sub>4</sub>-N, 60% of total inorganic-N, and about 2.57 log of *E. coli*. However, a small amount of PO<sub>4</sub>-P accumulated in HRAP 2.

**Table 3-22 HRAP2 inlet & outlet values and removal efficiencies at all depths for a range of performance related parameters**

| <b>BOD<sub>5</sub> mg/L</b>   |              |               |                           |
|---|--------------|---------------|---------------------------|
|   | <b>Inlet</b> | <b>Outlet</b> | <b>Removal Efficiency</b> |
| <b>Mean</b>   | 23.5         | 6.8           | 63.8%                     |
| <b>Median</b>   | 16.8         | 7.0           | 59.1%                     |
| <b>Std Dev</b>  | 15.8         | 1.8           | 13.9%                     |
| <b>Number</b>   | 75           | 75            | 75                        |
| <b>NH<sub>4</sub>-N mg/L</b>  |              |               |                           |
| <b>Mean</b>   | 18.7         | 7.7           | 66.2%                     |
| <b>Median</b>   | 22.3         | 5.3           | 78.4%                     |
| <b>Std Dev</b>  | 12.1         | 6.9           | 26.1%                     |
| <b>Number</b>   | 75           | 75            | 75                        |
| <b>Total Inorganic N mg/L</b>   |              |               |                           |
| <b>Mean</b>   | 20.7         | 10.2          | 59.5%                     |
| <b>Median</b>   | 25.0         | 10.7          | 57.8%                     |
| <b>Std Dev</b>  | 11.5         | 8.2           | 25.4%                     |
| <b>Number</b>   | 75           | 75            | 75                        |
| <b>PO<sub>4</sub>-P mg/L</b>  |              |               |                           |
| <b>Mean</b>   | 10.1         | 9.4           | 7%                        |
| <b>Median</b>   | 10.0         | 9.7           | 5.4%                      |
| <b>Std Dev</b>  | 2.6          | 2.9           | 16.0%                     |
| <b>Number</b>   | 75           | 75            | 75                        |
| <b>Chlorophyll <i>a</i> mg/L</b>  |              |               |                           |
| <b>Mean</b>   |              | 0.65          |                           |
| <b>Median</b>   |              | 0.31          |                           |
| <b>Std Dev</b>  |              | 0.70          |                           |
| <b>Number</b>   |              | 75            |                           |
| <b>Log<sub>10</sub> <i>E. coli</i> /100 ml and log<sub>10</sub> reduction value (LRV)</b> |              |               |                           |
|   | <b>Inlet</b> | <b>Outlet</b> | <b>LRV</b>                |
| <b>Mean</b>   | 3.689        | 1.115         | 2.574                     |
| <b>Median</b>   | 3.544        | 0.954         | 2.735                     |
| <b>Std Dev</b>  | 0.517        | 0.785         | 0.813                     |
| <b>Number</b>   | 75           | 75            | 75                        |
| <b>NO<sub>3</sub> &amp; NO<sub>2</sub> -N (mg/L)</b>                                      |              |               |                           |
|   | <b>Inlet</b> | <b>Outlet</b> | <b>Added</b>              |

**Table 3-22 HRAP2 inlet & outlet values and removal efficiencies at all depths for a range of performance related parameters**

|                                |      |     |      |
|--------------------------------|------|-----|------|
| <b>Mean</b>                    | 2.5  | 1.9 | -0.6 |
| <b>Median</b>                  | 0.9  | 2.5 | 1.6  |
| <b>Std Dev</b>                 | 3.7  | 0.9 | 3.6  |
| <b>Number</b>                  | 75   | 75  | 75   |
| <b>Suspended Solids (mg/L)</b> |      |     |      |
| <b>Mean</b>                    | 91   | 269 | 178  |
| <b>Median</b>                  | 73   | 104 | 31   |
| <b>Std Dev</b>                 | 47.6 | 300 |      |
| <b>Number</b>                  | 75   | 75  |      |

### 3.4 HRAP2 Fed facultative pond effluent: Performance at three operational depths

The overall pond performance data presented in Table 3-22 can be further broken down into the performance achieved at specific operating depths. As with HRAP 1, HRAP2 was operated at three depths; shallow 0.32 m, medium 0.42 m and deep was 0.55 m. In some cases pond depth did influence pond performance. The results are broken down by depth to report statistical results of the significant indicators in Tables 3-23 to 3-25 and the removal efficiencies in Table 3-26.

**Table 3-23 HRAP2 receiving facultative pond treated influent operated at a depth of 0.32 m. HRAP treated effluent composition (n=32).**

|  | Mean ± SD     | Median | Geometric mean |
|--|---------------|--------|----------------|
| <b>BOD<sub>5</sub> (mg/L)</b>                | 7.7 ± 1.3     | 8.0    | 7.5            |
| <b>NH<sub>4</sub>-N (mg/L)</b>               | 5.4 ± 5.9     | 1.0    | 1.0            |
| <b>NO<sub>2</sub>-N (mg/L)</b>               | 0.9 ± 3.4     | 0.1    | 0.1            |
| <b>NO<sub>3</sub>-N (mg/L)</b>               | 0.4 ± 2.1     | 0.04   | 0.04           |
| <b>PO<sub>4</sub>-P (mg/L)</b>               | 7.8 ± 3.1     | 7.4    | 7.2            |
| <b>SS (mg/L)</b>                             | 294 ± 243     | 161    | 192            |
| <b>Chlorophyll <i>a</i> (mg/L)</b>           | 0.95 ± 0.61   | 0.97   | 0.69           |
| <b>Log<sub>10</sub> <i>E. coli</i>/100ml</b> | 1.270 ± 0.792 | 1.060  | 1.030          |

**Table 3-24 HRAP2 receiving facultative pond treated influent operated at a depth of 0.42 m. HRAP treated effluent composition (n=24).**

|                                | Mean ± SD  | Median | Geometric mean |
|--------------------------------|------------|--------|----------------|
| <b>BOD<sub>5</sub> (mg/L)</b>  | 5.4 ± 1.4  | 5.0    | 5.2            |
| <b>NH<sub>4</sub>-N (mg/L)</b> | 3.5 ± 2.1  | 3.3    | 2.6            |
| <b>NO<sub>2</sub>-N (mg/L)</b> | 1.4 ± 0.8  | 0.9    | 1.2            |
| <b>NO<sub>3</sub>-N (mg/L)</b> | 3.6 ± 2.4  | 3.7    | 3.4            |
| <b>PO<sub>4</sub>-P (mg/L)</b> | 11.4 ± 2.4 | 10.9   | 11.2           |
| <b>SS (mg/L)</b>               | 358 ± 423  | 57     | 151            |

|  | Mean ± SD     | Median | Geometric mean |
|--|---------------|--------|----------------|
| <b>Chlorophyll a (mg/L)</b>            | 0.8 ± 0.6     | 1.0    | 0.4            |
| <b>Log<sub>10</sub> E. coli /100ml</b> | 1.119 ± 0.629 | 0.900  | 0.937          |

**Table 3-25 HRAP2 receiving facultative pond treated influent operated at a depth of 0.55 m. HRAP treated effluent composition (n=19).**

|  | Mean ± SD     | Median | Geometric mean |
|--|---------------|--------|----------------|
| <b>BOD<sub>5</sub> (mg/L)</b>          | 7.0 ± 2.0     | 7.0    | 6.8            |
| <b>NH<sub>4</sub>-N (mg/L)</b>         | 17.0 ± 2.4    | 17.7   | 16.8           |
| <b>NO<sub>2</sub>-N (mg/L)</b>         | 0.8 ± 0.3     | 0.7    | 0.7            |
| <b>NO<sub>3</sub>-N (mg/L)</b>         | 0.6 ± 0.6     | 0.4    | N/A            |
| <b>PO<sub>4</sub>-P (mg/L)</b>         | 9.6 ± 0.4     | 9.6    | 9.6            |
| <b>SS (mg/L)</b>                       | 115 ± 55      | 100    | 104            |
| <b>Chlorophyll a (mg/L)</b>            | 0.2 ± 0.1     | 0.2    | 0.2            |
| <b>Log<sub>10</sub> E. coli /100ml</b> | 1.082 ± 0.271 | 1.079  | 1.051          |

**Table 3-26 HRAP2 removal efficiency performance parameters by pond depth; shallow (0.32m), medium (0.42m) and deep (0.55m).**

|                | Removal Efficiencies (percent removed) |                    |                    |             | Log <sub>10</sub> removed |
|----------------|--|--------------------|--------------------|-------------|---------------------------|
|                | BOD <sub>5</sub>                       | NH <sub>4</sub> -N | PO <sub>4</sub> -P | Inorganic-N | E. coli LRV               |
| <b>Shallow</b> |  |                    |                    |             |                           |
| -Mean          | 72±15%                                 | 72±26%             | 0.1±0.2%           | 75±25%      | 2.52±0.70                 |
| -Median        | 79%                                    | 85%                | 0.1%               | 75%         | 2.602                     |
| -Number        | 32                                     | 32                 | 32                 | 32          | 32                        |
| <b>Medium</b>  |  |                    |                    |             |                           |
| -Mean          | 59±7%                                  | 83±9%              | 0.1±0.1%           | 59±7%       | 2.12±0.66                 |
| -Median        | 61%                                    | 84%                | 0.1%               | 61%         | 2.46                      |
| -Number        | 24                                     | 24                 | 24                 | 24          | 24                        |
| <b>Deep</b>    |  |                    |                    |             |                           |
| -Mean          | 51±7%                                  | 35±9%              | 0.02±0.03%         | 34±4%       | 3.01±0.27                 |
| -Median        | 52%                                    | 34%                | 0.03%              | 35%         | 3.09                      |
| -Number        | 31                                     | 29                 | 29                 | 29          | 31                        |

As there was little difference in the nutrient removal efficiencies for NH<sub>4</sub>-N, NO<sub>3</sub> & NO<sub>2</sub>-N and PO<sub>4</sub>-P (Table 3-26) these are discussed below but not presented with further detailed statistical breakdown, other than presentation as

complete data sets for each temperature and pond depth in the beanplot series Fig. 3-28 to 3-33.

### **3.4.1. Nutrient Removal**

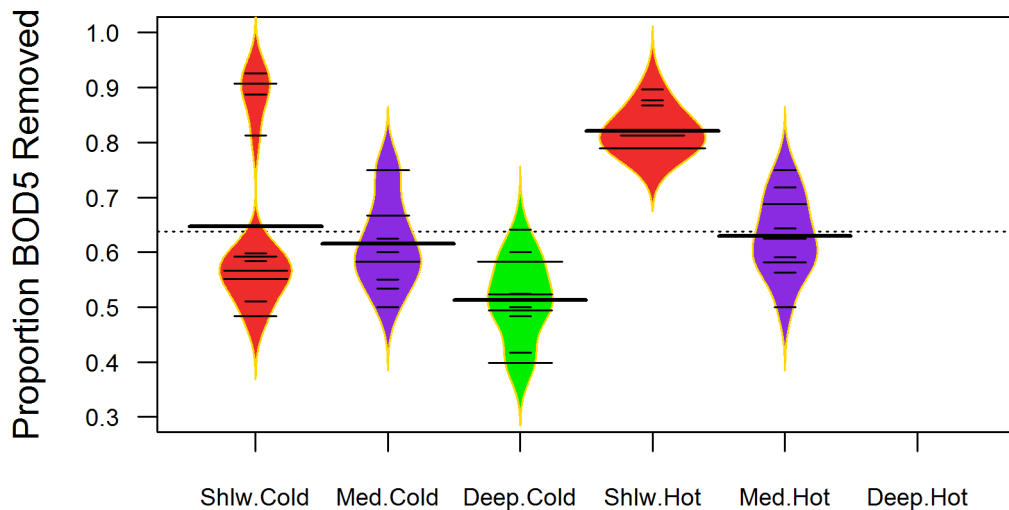
The beanplot series in Fig. 3-28 to 3-33 show the proportion of each of the major nutrients removed at each combination of temperature and pond depth.

### **3.4.2 BOD<sub>5</sub> Removal**

Around 64% of BOD<sub>5</sub> was removed under all combinations of operating depths and pond temperatures (Fig. 3-28), which is a considerably lower percentage than HRAP 1. This is probably a function of the incoming water having already undergone aerobic biological oxidation in the facultative pond, leaving the more resistant material for HRAP 2 to deal with.

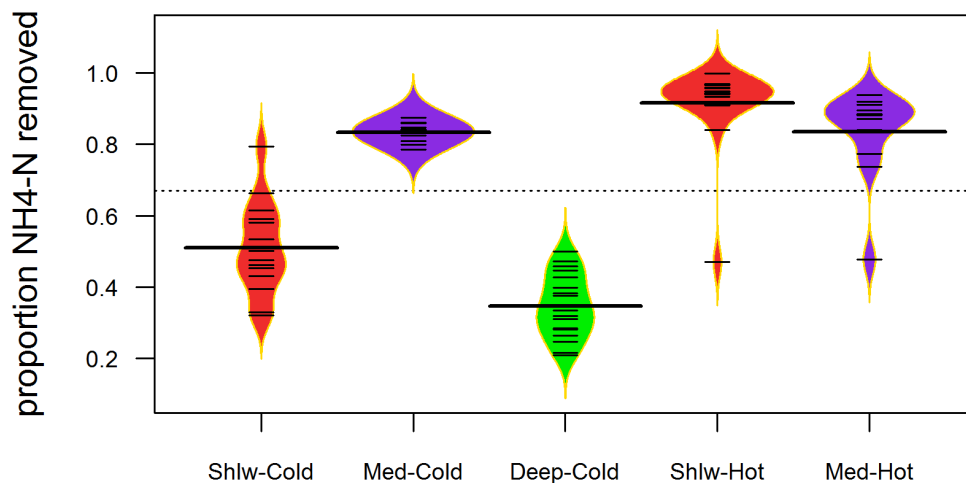
The deep operated HRAP 2 in the cold period was slightly less effective than the shallow and medium depth HRAPs, despite having a longer retention time. The shallow operated pond in the 'hot' period was the most efficient at removing BOD<sub>5</sub>.

As with HRAP 1, it is interesting to note that pond temperature was not of great importance in determining the proportion of BOD<sub>5</sub> removed, although the 'hot' ponds (>18.3°C) had slightly higher removal efficiencies, than their 'cold' counterparts. HRAP 2 performance for this parameter can be expected to be reasonably consistent all year round, with slightly lower efficiencies encountered during the colder months.



Pond Depth & Pond Water Temperature

**Fig 3-28** Beanplot showing proportion of BOD<sub>5</sub> removed from the HRAP2 - fed facultative pond treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>18.3°C or <18.3°C, hot or cold respectively).



Pond Depth & Pond Water Temperature

**Fig 3-29** Beanplot showing proportion of NH<sub>4</sub>-N removed from the HRAP2 - fed facultative pond treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>18.3°C or <18.3°C, hot or cold respectively).

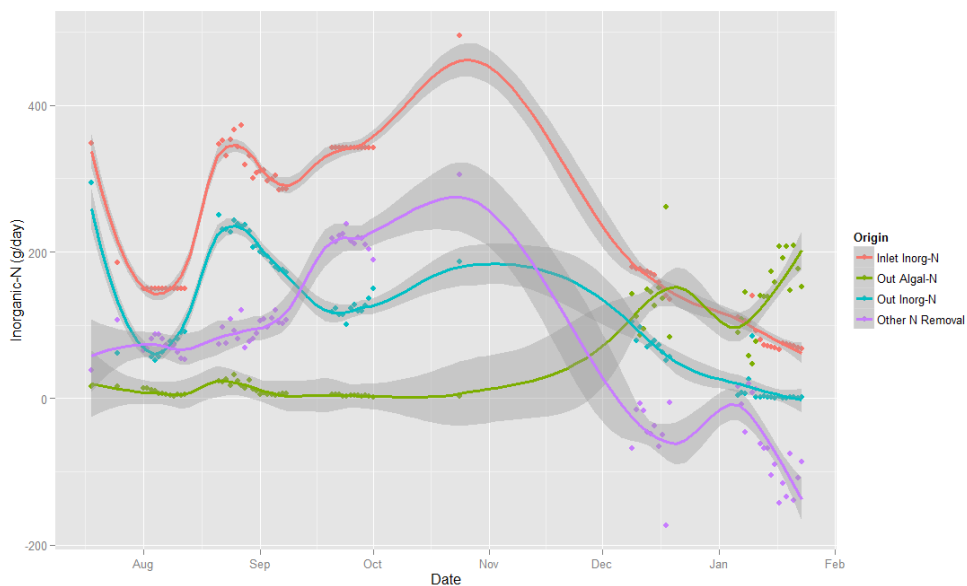
### 3.4.3. Inorganic-N Removal (including NH<sub>4</sub>-N)

The same possible routes of nitrogen transformation in a pond are volatilisation, nitrification, denitrification, net loss to sediments, uptake by microorganisms

and mineralisation of organic-N. As noted in Para 3.2.1.3, the principle and most widely accepted basis for ammonia removal within WSPs has been attributed to the volatilization of ammonia. Microbial uptake and assimilation into microbial biota will occur and some nitrogen will exit the pond as discharged organisms.

Again, as no sludge accumulates in the HRAP, this form of N removal is not possible. Nitrification certainly occurs, transforming the most reduced form of N ( $\text{NH}_3$ ) to one of the oxidised forms (Table 3-18).

As described earlier the rate of  $\text{NH}_3$ -N volatilisation depends on the concentration of ammonia gas in the liquid, which is in balance with  $\text{NH}_4^+$  ion concentration and dependent on temperature and pH, (see Equation 1-51 & Figs. 1-10 & 1-13) depth of the system and a mass transfer coefficient, which is also temperature dependent.



**Fig. 3-30 HRAP 2 loess fit and 95% confidence intervals for Inorganic-N incoming (red) and outgoing (green), outgoing as algal-N (blue) and outgoing as ammonia (purple) over time.**

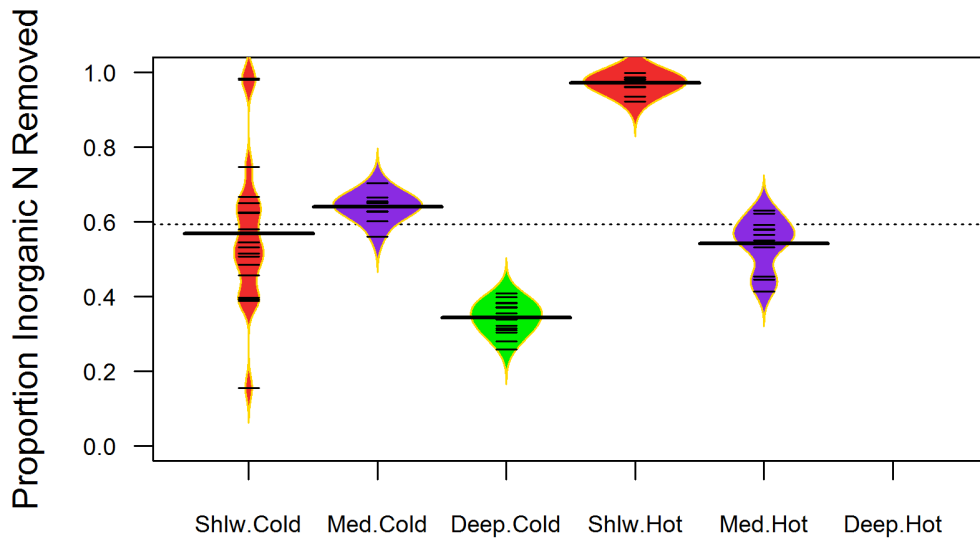
On average, 218 g of inorganic-N entered the HRAP per day (red line in Fig. 3-30). No measure was made of organic nitrogen entering the pond, so the following analyses do not account for nitrogen from this source. Of the incoming inorganic-N, 105 g inorganic-N (48%) exited the HRAP per day either in the original reduced form or in an oxidised form (green line in Fig. 3-30). A further 60 g (28%) exited in organic form as part of the algal biomass (blue line in Fig. 3-30). This line is highly variable and depends on periods of algal blooms as occurred in February 2012. As well, 53 g/day (24%) exited as ammonia (purple line in Fig. 3-30), and as explained above this is dependent on temperature, pH and pond depth.

Considering all operating conditions, the average proportion of  $\text{NH}_4\text{-N}$  removed (Fig 3-29) from the HRAP2 was 66% and total inorganic-N removed was 59%. It can be further noted that for all pond depths the proportion of inorganic-N removed from cold water is less than that achieved with hot water (Fig. 3-31). These differences are all statistically significant ( $p < 0.05$ ) for all pond depths. A relatively logical explanation for this is the greater rate of loss of  $\text{NH}_4\text{-N}$  due to volatilisation at higher temperatures and pH's (Fig. 3-30).

As the rate of volatilisation of ammonia is also inversely dependent on pond depth, there was some indication this effect was observed in both the 'hot' and 'cold' water (Fig. 3-31).

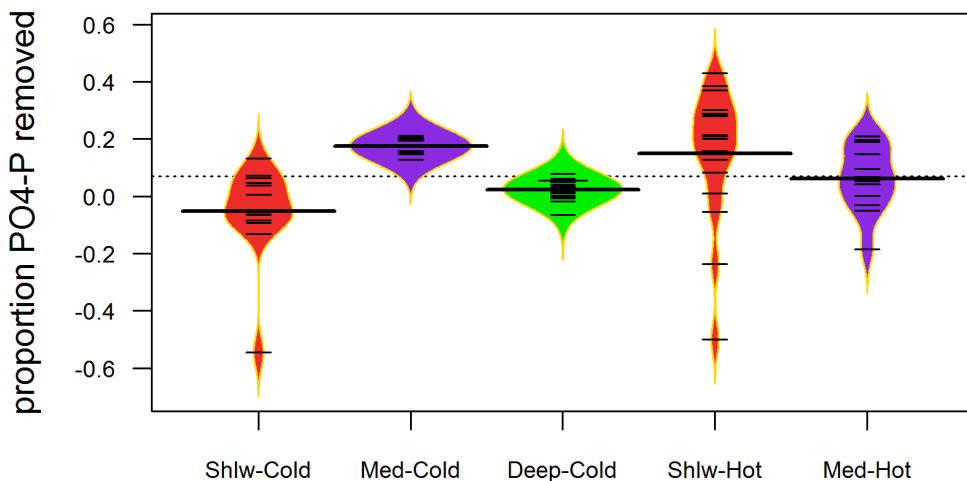
In summary, as with HRAP 1, it appears that all three forms of removal of inorganic-N occur in the HRAP 2, with emphasis and relative importance shifting according to in-pond conditions and algal growth.





Pond Depth & Pond Water Temperature

**Fig 3-31** Beanplot showing Inorganic-N removal by the HRAP2 - fed facultative pond treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>18.3°C or <18.3°C, hot or cold respectively).



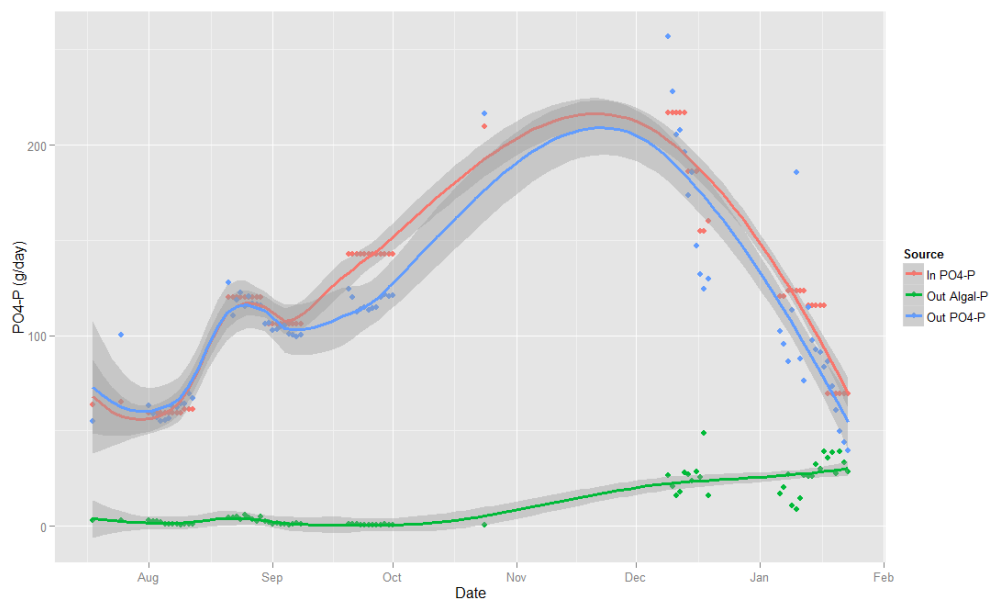
Pond Depth & Pond Water Temperature

**Fig 3-32** Beanplot showing proportion of PO<sub>4</sub>-P removed from the HRAP2 - fed facultative pond treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>18.3°C or <18.3°C, hot or cold respectively).

### 3.4.3. PO<sub>4</sub>-P Removal

As noted in para. 3.2.1.4, there are three possible mechanisms for orthophosphate removal in an HRAP; exiting unchanged as orthophosphate,

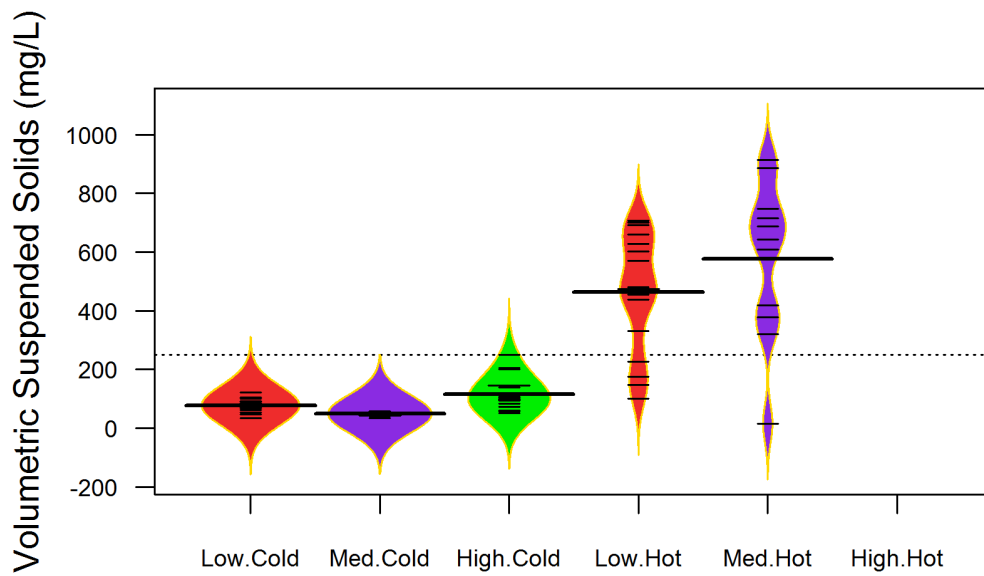
algal uptake/assimilation and chemical precipitation. As no sludge accumulates in the HRAP, deposition of dead organisms can be discounted as a phosphorous removal mechanism. The same analysis as used for HRAP 1 was used for HRAP 2 with the incoming and outgoing weight of phosphorous measured with the results shown in Fig. 3-33. Effectively, very little  $\text{PO}_4\text{-P}$  removal occurred under any operating conditions. The overall average removal was only 7% (Tables 3-22 Fig.3-32).



**Fig. 3-33 HRAP 2 – loess fit and 95% confidence intervals for  $\text{PO}_4\text{-P}$  incoming (red) and outgoing (green) and outgoing as algal-P (blue) over time.**

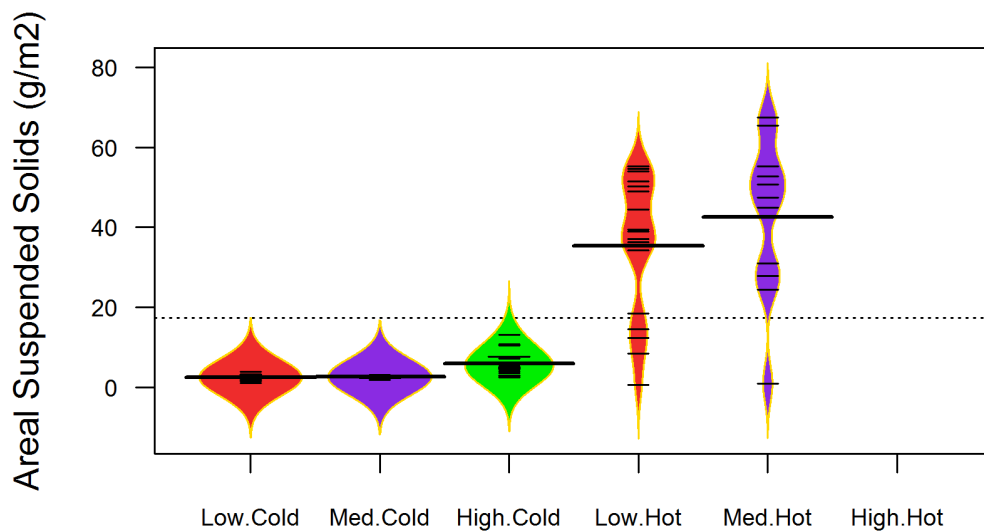
It can be seen in Fig. 3-33 that between 50 and 220 g/day of  $\text{PO}_4\text{-P}$  entered the HRAP (red line) and most of the time a similar amount exited unchanged (green line). However, from September to February, slightly less  $\text{PO}_4\text{-P}$  exited the HRAP than entered. From October, an increasing amount of P exited the HRAP as algal-P. Algal-P reached a maximum of 50 g/day exiting the pond in January 2012.

In summary, most orthophosphate that entered the HRAP exited unchanged as orthophosphate, with a very small amount exiting as algal-P.



Pond Depth & Pond Water Temperature

**Fig 3-34** Beanplot showing the volumetric amounts of Suspended Solids exiting the HRAP2 - fed facultative pond treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>18.3°C or <18.3°C, hot or cold respectively).



Pond Depth & Pond Water Temperature

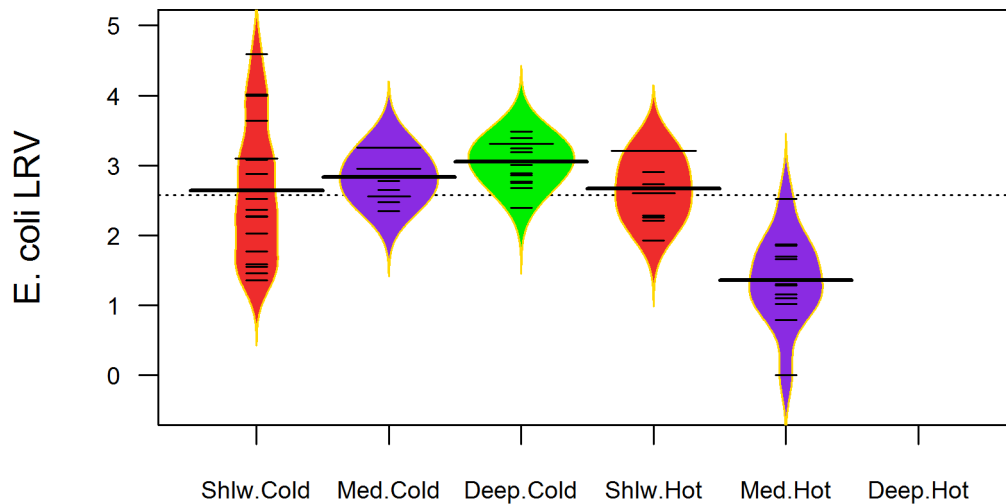
**Fig 3-35** Beanplot showing the Areal amounts of Suspended Solids exiting the HRAP2 - fed facultative pond treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>18.3°C or <18.3°C, hot or cold respectively).

### 3.4.5 Algal & Albazod Standing Crop and Productivity

As can be seen in Figs. 3-34 and 3-35 volumetric and areal suspended solids - for most of the period of observation, the measured areal suspended solids concentration averaged approximately  $18 \text{ g/m}^2$ , which is significantly ( $p < 0.005$ ) lower than the corresponding value for HRAP 1. Table 3-27 shows the albazod standing crop, algal and albazod productivities for HRAP 2. When compared to HRAP 1, the standing crop was 30%, the algal productivity was 0.3% and the albazod productivity was 9%. It is interesting to note that the albazod standing crop was still higher than the optimum level required for algal growth. Most of this material was actually brought in with the inlet water, as both the algal and albazod productivities are very low. This gives a very strong indication that even though the in-pond light climate was sub-optimal, for much of the time nutrients were limiting algal growth in HRAP 2.

**Table 3-27 HRAP 2 Mean  $\pm$  Standard Deviation and Median for Albazod Standing Crop ( $\text{g/m}^2$ ), Algal Productivity ( $\text{g/m}^2/\text{d}$ ) and Albazod Productivity ( $\text{g/m}^2/\text{d}$ ) for 0.32, 0.42 and 0.55 m depths and overall.**

|                         | Algal Productivity<br>( $\text{g/m}^2/\text{d}$ ) | Albazod<br>Productivity<br>( $\text{g/m}^2/\text{d}$ ) | Albazod Standing<br>Crop ( $\text{g/m}^2$ ) |
|-------------------------|---|--|---|
| <b>Shallow (0.32 m)</b> |   |  |   |
| - Mean $\pm$ SD         | <b>0.06 <math>\pm</math> 0.17</b>                 | <b>9.67 <math>\pm</math> 12.62</b>                     | <b>93.99 <math>\pm</math> 77.72</b>         |
| - Median                | <b>0.0</b>  | <b>0.2</b>   | <b>51.49</b>                                |
| <b>Medium (0.42 m)</b>  |   |  |   |
| - Mean $\pm$ SD         | <b>0.03 <math>\pm</math> 0.13</b>                 | <b>8.89 <math>\pm</math> 14.34</b>                     | <b>149 <math>\pm</math> 176</b>             |
| - Median                | <b>0.0</b>  | <b>0.08</b>  | <b>23.72</b>                                |
| <b>Deep (0.55 m)</b>    |   |  |   |
| - Mean $\pm$ SD         | <b>0.00 <math>\pm</math> 0.00</b>                 | <b>0.62 <math>\pm</math> 1.48</b>                      | <b>62.24 <math>\pm</math> 29.53</b>         |
| - Median                | <b>0.0</b>  | <b>0.0</b>   | <b>54.04</b>                                |
| <b>OVERALL</b>          |   |  |   |
| - Mean $\pm$ SD         | <b>0.04 <math>\pm</math> 0.14</b>                 | <b>7.13 <math>\pm</math> 12.1</b>                      | <b>104 <math>\pm</math> 116</b>             |
| - Median                | <b>0.0</b>  | <b>0.0</b>   | <b>53.5</b>                                 |



Pond Depth & Pond Water Temperature

**Fig 3-36** Beanplot showing *E.coli* LRV by the HRAP - fed facultative pond treated effluent- displayed by pond depth (Shlw, 0.32m, Med, 0.42 m & deep, 0.55m) and wastewater temperature (>18.3°C or <18.3°C, hot or cold respectively).

### 3.4.6 *E. coli* LRV

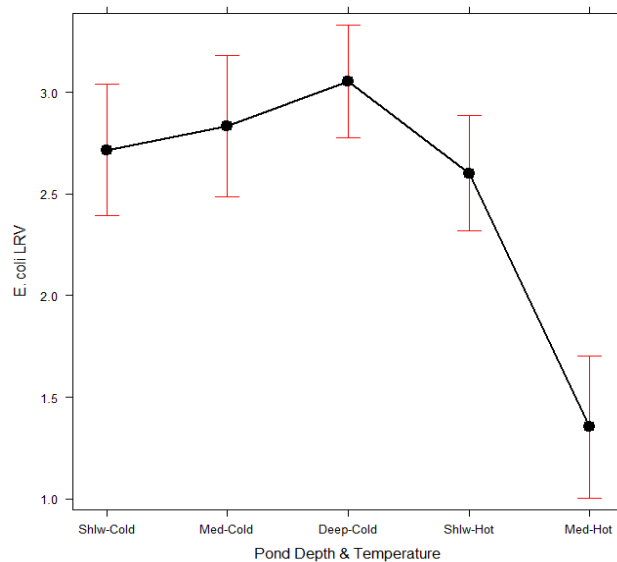
A mean figure for *E. coli* LRV for all configurations was approximately 2.6, and a longitudinal view of inlet and outlet *E. coli* concentration can be seen in Fig. 3-38. LRV data is summarised in Fig. 3-36 & 3-37 and Table 3-28. *E. coli* LRV showed little difference in performance between the HRAP 2 depths and wastewater temperatures, apart from a significantly lower LRV in the hot period at medium depth. It is not clear why this combination performed less effectively than the other combinations, as this was not a feature of HRAP 1, and is not predicted by the model described in Chapter 7.

It is clear that there was a higher LRV for *E. coli* in HRAP 2 compared to HRAP 1 (para 3.2.1.6). This may be due to lower suspended solids levels and therefore greater light penetration. Another possible factor is that the HRAP2 inlet water

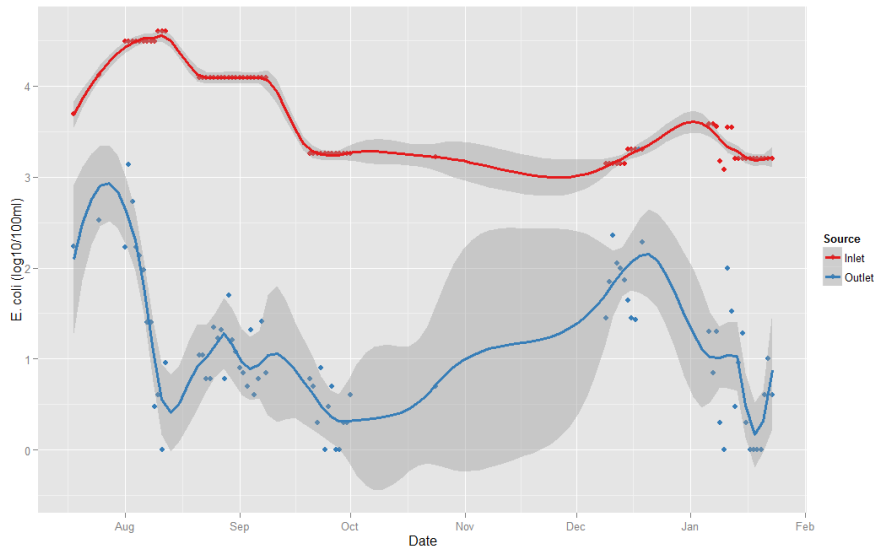
had already been in a facultative pond for at least 36 days before processing through the HRAP. This period may have induced starvation or otherwise weakened the *E. coli*, so they were more vulnerable to solar exposure in HRAP 2.

**Table 3-28 Numerical summary of HRAP 2 *E. coli* LRV at each pond configuration.**

| Configuration | mean±sd     | Range         | number |
|---------------|-------------|---------------|--------|
| Deep-Cold     | 3.052±0.295 | 2.390 - 3.487 | 19     |
| Med-Cold      | 2.832±0.320 | 2.351 - 3.254 | 12     |
| Med-Hot       | 1.353±0.637 | 0.003 - 2.519 | 12     |
| Shlw-Cold     | 2.716±1.044 | 1.358 - 4.597 | 14     |
| Shlw-Hot      | 2.602±0.502 | 1.549 - 3.204 | 18     |



**Fig. 3-37 HRAP 2:- Mean and standard deviation of *E. coli* LRV by pond depth and temperature.**



**Fig. 3-38 HRAP 2 – loess fit and 95% confidence intervals for *E. coli* concentration incoming (red) and outgoing (blue) over time.**

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## **CHAPTER 4.**

# **LYNDOCH WASTE STABILISATION PONDS – RESULTS & DISCUSSION**

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The results presented in this chapter are derived from the observations made at the Lyndoch wastewater treatment site. This is a typical waste stabilisation pond series constructed in the 1970s, consisting of 3 ponds in series.

The facultative pond (WSP 1) receives septic tank effluent from population of 1700 people and has a surface area of 6,400 m<sup>2</sup>, depth of 1.2 m, and effective volume of 4,533 m<sup>3</sup>, after accounting for accumulated sludge, allowing a theoretical hydraulic retention time (THRT) of 27.5 days with the original inflow rate of 165 m<sup>3</sup> per day, and 36 days with the diversion of 40 m<sup>3</sup> per day through the rotating biological contactor. The facultative pond is referred to as WSP 1 in the rest of this document. It is followed by two maturation ponds operated in series. These ponds will be referred to WSP 2 and WSP 3 respectively. Each of these ponds has a surface area of 2550m<sup>2</sup>. The effective volume, accounting for sludge, of the first maturation pond (WSP2) was 1581 m<sup>3</sup> with a working depth of 0.62 m and a THRT of 9.58 days at 165 KL/day, and 12.65 days at the lower flow rate 125 kL/d. The second maturation pond has an effective volume 1479 m<sup>3</sup> with a working depth of 0.58 m and a THRT of 8.96 days at 165 KL/day, and 11.83 days at the lower flow rate 125 kL/d. Thus the total THRT for this system is 46 days at 165 KL/day, and 60.5 days at the lower flow rate 125 kL/d.

The period reported on covers both periods of operation of HRAP 1 & 2 (as described in Chapter 3) and the gap between those two periods. The total period covered is from April 2010 to February 2012. The data for all three waste stabilisation ponds will be presented concurrently.

The rationale for this study was to compare the performance of this WSP system with that of a HRAP at Kingston-on-Murray in similar climatic locations. It is believed that this was the first time such a study has been performed and is unique in the literature. The statistical comparison of HRAP/WSP performance is presented in Chapter 6.

## 4.1 Environmental Factors – air temperature, wind speed & direction, total solar radiation, UV radiation, rainfall

### 4.1.1. Prevailing weather

As can be seen in Table 4-1 and Fig. 4-1, the annual cycle of air temperature and rainfall followed a typical Mediterranean pattern with hot dry summers and cold wet winters. There were some climatic events of note, particularly during the summer of 2010/11. These were three major rainfall events exceeding 50 mm per day in December 2010, February 2011 and March 2011 (Fig 4-1 and Table 4-2). These events led to considerable flooding in the township of Lyndoch and considerable volumes of water leaking into the septic tank reticulation system, and from there into the water pumped into the WSPs.

**Table 4-1. Historical Bureau of Meteorology data for Lyndoch – ground weather station & satellite climate data - Sixty year averages (Bureau-of-Meteorology, 2012).**

|               | Daily sunshine (h) | Daily solar exposure (MJ/m <sup>2</sup> ) | Annual Average Rainfall (mm) | Highest rainfall (mm) | Lowest rainfall (mm) | Days of rain | Max. temp. (°C) | Min. temp. (°C) |
|---------------|--------------------|---|------------------------------|-----------------------|----------------------|--------------|-----------------|-----------------|
| Jan           | 10.8               | 27.7                                      | 18.3                         | 119.6                 | 0                    | 3.6          | 30.5            | 15.2            |
| Feb           | 9.9                | 24.4                                      | 18.1                         | 98.5                  | 0                    | 2.8          | 30.5            | 15.6            |
| Mar           | 8.8                | 19.7                                      | 20.4                         | 97.3                  | 0                    | 4.3          | 27.3            | 13.3            |
| Apr           | 7.7                | 14.3                                      | 36.4                         | 208                   | 0                    | 6.8          | 23.1            | 10.7            |
| May           | 5.8                | 9.7                                       | 53.2                         | 159                   | 3                    | 11.1         | 18.7            | 8.2             |
| Jun           | 5                  | 7.7                                       | 56.6                         | 140                   | 3                    | 13.2         | 15.6            | 6.2             |
| Jul           | 5.3                | 8.5                                       | 58.9                         | 129.5                 | 12.2                 | 14.3         | 14.7            | 5.5             |
| Aug           | 6.5                | 11.2                                      | 57.3                         | 127.3                 | 8                    | 14.3         | 15.8            | 5.7             |
| Sep           | 7.1                | 15.5                                      | 53.4                         | 136.2                 | 4.8                  | 11.9         | 18.3            | 6.6             |
| Oct           | 8.4                | 20.6                                      | 44                           | 145                   | 0                    | 9.3          | 21.8            | 8.3             |
| Nov           | 9.4                | 24.9                                      | 28.4                         | 127.2                 | 0                    | 6.3          | 25.7            | 11.2            |
| Dec           | 9.9                | 26.7                                      | 24.4                         | 153.7                 | 0                    | 5.2          | 28.2            | 13.5            |
| <b>Annual</b> | <b>7.9</b>         | <b>17.6</b>                               | <b>468.5</b>                 | <b>814.4</b>          | <b>253.5</b>         | <b>103.1</b> | <b>22.5</b>     | <b>10</b>       |

**Table 4-2. On-site recorded data for the 2010/2011 portion of the study period; temperature and rainfall at Lyndoch.**

|  | Max | Min | Rainfall | Days of |
|--|-----|-----|----------|---------|
|--|-----|-----|----------|---------|

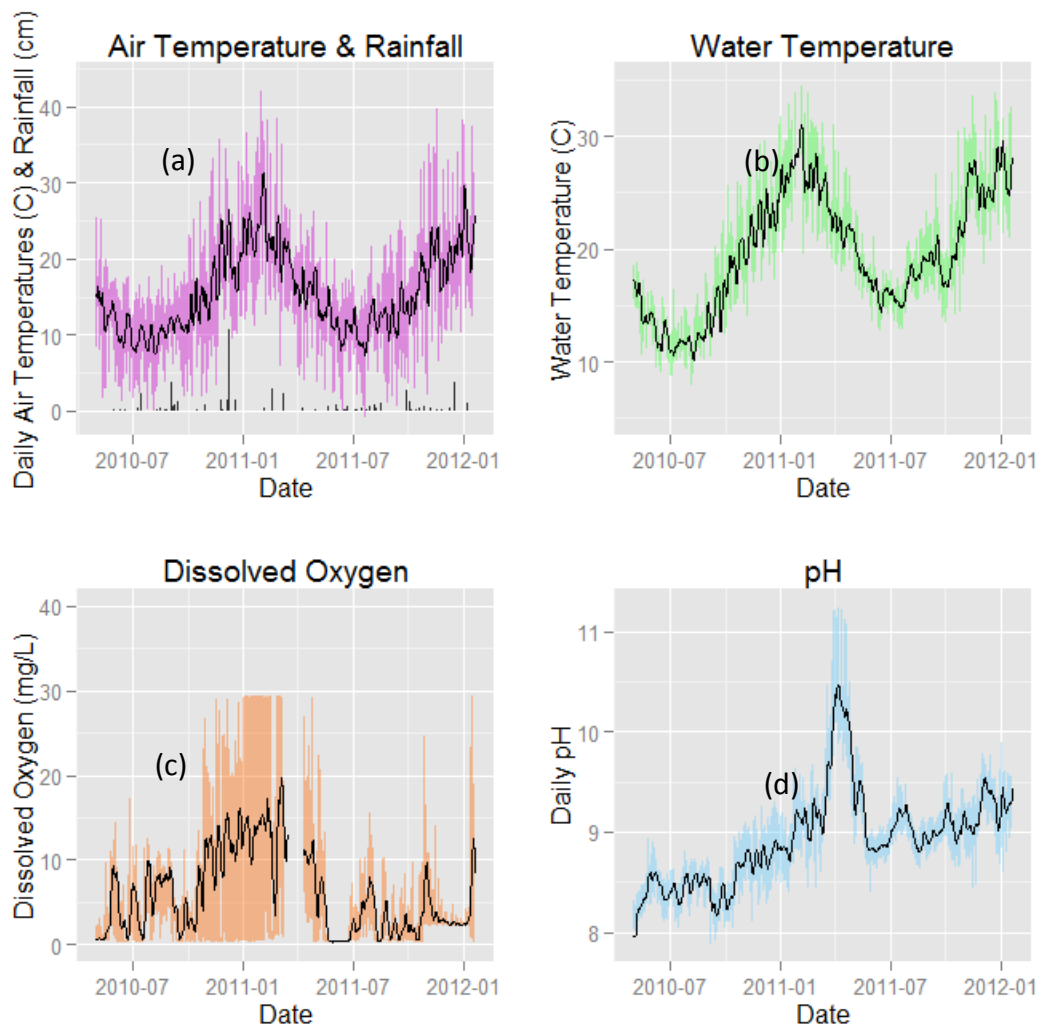
|                | Temp °C     | Temp °C    | (mm)       | rain       |
|----------------|-------------|------------|------------|------------|
| <b>Apr '10</b> | 22.5        | 11.2       | 56.8       | 14         |
| <b>May '10</b> | 17.9        | 6.2        | 46.4       | 11         |
| <b>Jun '10</b> | 13.6        | 3.7        | 37.6       | 13         |
| <b>Jul '10</b> | 13.3        | 3.8        | 38.6       | 13         |
| <b>Aug '10</b> | 13.0        | 5.3        | 69.8       | 23         |
| <b>Sep '10</b> | 14.6        | 6.5        | 89.8       | 16         |
| <b>Oct '10</b> | 20.0        | 7.9        | 31.0       | 8          |
| <b>Nov '10</b> | 23.3        | 10.7       | 27.0       | 10         |
| <b>Dec '10</b> | 25.5        | 12.8       | 153.7      | 14         |
| <b>Jan '11</b> | 30.8        | 15.2       | 3.8        | 2          |
| <b>Feb '11</b> | 27.9        | 15.7       | 98.8       | 7          |
| <b>Mar '11</b> | 22.5        | 12.5       | 110.6      | 6          |
| <b>Annual</b>  | <b>20.4</b> | <b>9.3</b> | <b>741</b> | <b>137</b> |

#### 4.1.2. Exceptional weather

The long term record shows there were usually 103 days of rainfall per year with an annual average of 468 mm. In the year, April 2010 to March 2011 there were 137 days of rainfall for a total of 741 mm (58% above average). The average daily maximum temperature for the year was 20.4°C, which is 2.1°C below the long term average, and the average daily minimum of 9.3°C was 0.7°C below the long term average minimum daily temperature.

#### 4.1.3 WSP Operational Conditions & Wastewater Physico-chemical Parameters

##### 4.1.3.1 Air & Water Temperatures



**Figure. 4-1 Environmental & Operating average, maxima and minima conditions for Lyndoch WSP1. (a). daily air temperature and rainfall (vertical bars), (b). pond water temperature, (c) dissolved oxygen and (d) pH.**

The facultative pond WSP1 displayed seasonal temporal variations typically associated with variations in sunlight and ambient temperatures (Fig 4-1a). Air temperatures in mid-summer occasionally exceed 40°C and mid-winter minima rarely dropped below 0°C (Fig 4-1a). During the study period the average daily water temperature (Fig. 4-1b) exceeded 30°C in summer for four days in January 2011. Daily minimum water temperatures reached just under 10°C in the June to August period in 2010 and remained above this in 2012. It is clear that this larger body of water was more strongly buffered against temperature variation than the smaller water body in the HRAPs.

#### 4.1.3.2 Dissolved Oxygen (DO)

The gap in the DO record (Fig. 4-1c) during March 2011 was due to membrane failure and the need to purchase a replacement. Daily DO peaks of between 5 and 40 mg DO/L were observed throughout the year, with the higher peaks generally occurring during the warmer months when algal growth was at its greatest (Fig.4-1c). The lower peaks typically occurred when chlorophyll *a* levels were below 1 mg/L (see Fig.4-3). However, the relationship between daily chlorophyll *a* and daily DO peaks was not as straightforward as might have been expected. Some of this lack of consistency may be due to difficulties in keeping the membrane of the DO probe clear of biofilm fouling. The overnight DO regularly dropped to 0 for significant periods of the year, but did not reach 0 for the colder months of the year, presumably because bacterial and algal respiration was limited by the cold water conditions. Daily average water temperature was below 15°C during this period.

#### 4.1.3.3 pH

Daily pH peaks of between 8.6 and 11.2 were observed (Fig. 4-1d). Daily minimum pH occasionally fell as low as 8. In contrast to the HRAP diurnal pH variations rarely exceeded 1.2 pH units. There was a significant rise in pH during the period of March to May 2011. This unexpected rise in pH occurred after the third major rainfall event when there was significant run-off from the saturated ground into the ponds. This run-off came from soil with much limestone through it, and the rise in pH was probably due to the addition of soluble lime from the limestone. This period of very high pH was notable for the large increase in *E. coli* die-off (Fig.4-7).

#### 4.1.4 Wind Speed & Direction

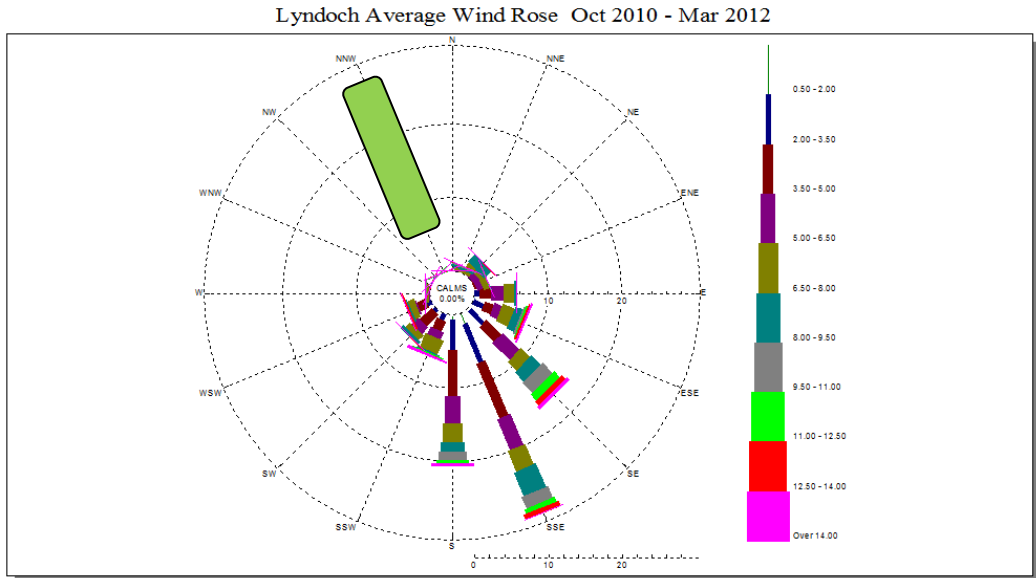
The daily average wind speed and direction is presented in Fig. 4-2 as a wind-rose for the 24 month period from April 2010 to April 2012. The prevailing



winds came from the southern quadrants with most of the strong winds also coming from those directions. This appears to be a local phenomenon.

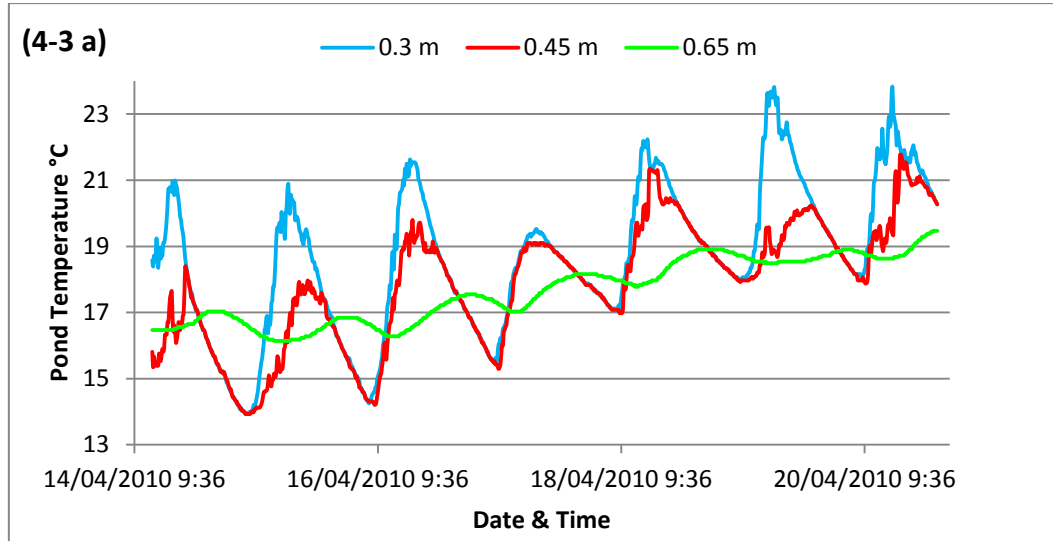
Over the two year period, April 2010 to April 2012 the average wind speed was 5.7 km/hour and ranged from 1 to 16 km/hour. It can be seen that the great majority of the daily average winds came from within an arc from the South to South East. This was a peculiar local effect attributed to the local topography. A katabatic wind forms most afternoons (sometimes blowing all day) descending from the surrounding hills, and funnels through the region of the treatment ponds frequently and very regularly. Katabatic wind (from the Greek: *katabaino* - to go down) is the generic term for downslope winds flowing from high elevations of mountains, plateaus, and hills down their slopes to the valleys or plains below. Most katabatic winds are the result of air in contact with upper level ground cooled by radiation, increasing in density, and flowing downhill and along the valley bottom.

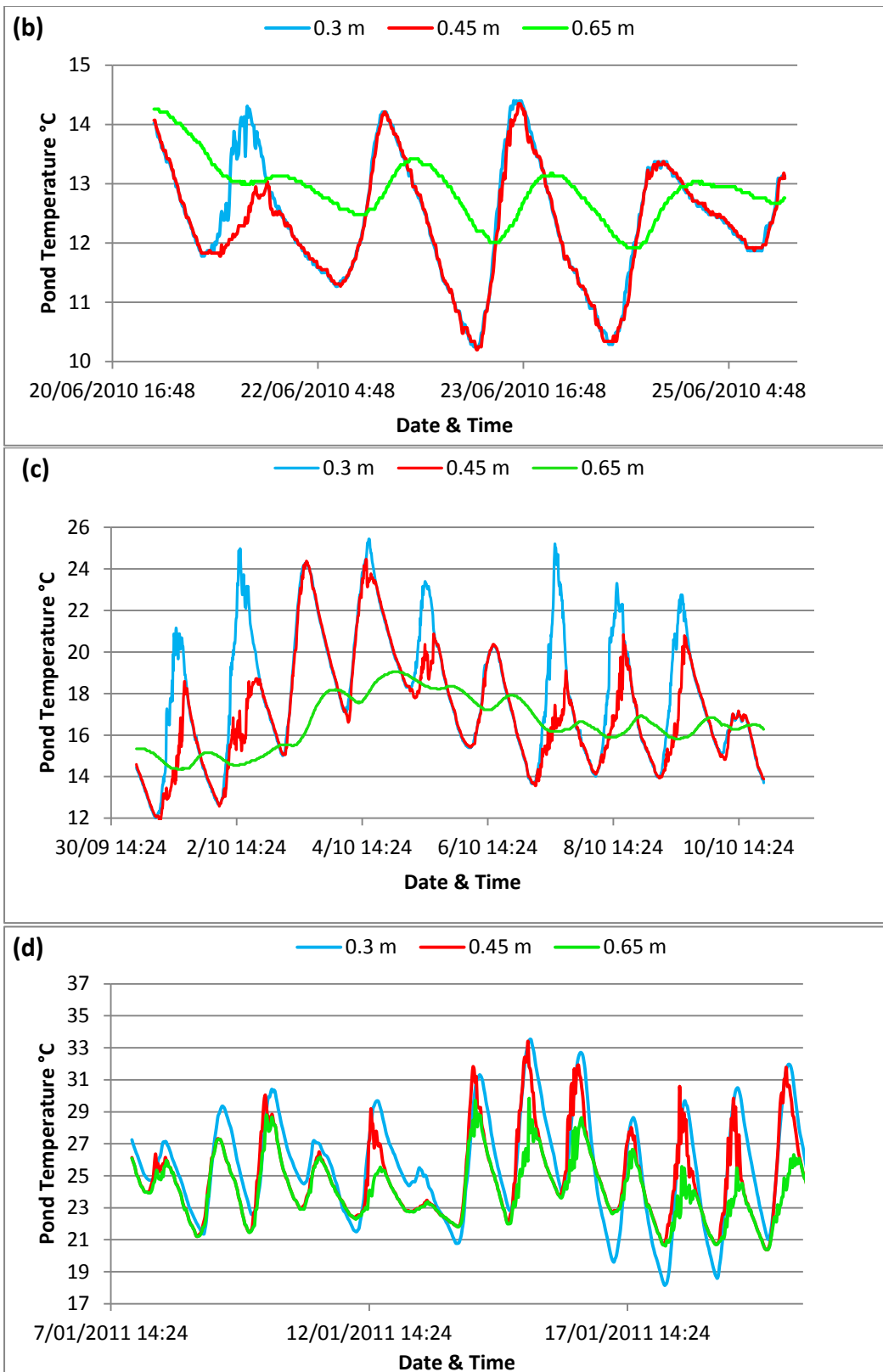
Winds from the South to South East arc blow directly along the length of the ponds - going against the direction of flow in the facultative pond (WSP 1) and with the direction of flow in the two maturation ponds (WSP 2 & 3). It is possible that this wind enhanced hydraulic short circuiting, particularly in WSP 2 & 3, as the wind pushed the incoming water rapidly toward the exit point (Sweeney et al., 2003, Sweeney et al., 2005). The other noticeable effect of this wind was to break down thermal stratification (Fig. 4-3 a-d) which frequently formed in the late night and in early morning periods as inversions (cooler water on top).



**Figure 4-2** Daily average wind speed and direction recorded on-site at Lyndoch during the study period. Wind strength indicated by colour coding and lower bar scale. Approximate orientation of WSPs indicated by green rectangle.

**4.1.5 Pond Temperature as measured by thermistor strings**  
**Some sample recordings are shown in Fig 4-3a-d for four different times of year of the pond temperature at 0.3m, 0.45m and 0.65m.**





**Fig. 4-3 WSP 1 Pond Temperatures (°C) at 0.3m (red), 0.45m (blue) & 0.65m (green) for time periods in (a) April (b) June (c) October and (d) January.**

The thermistor strings recorded pond temperatures every 30 minutes at depths of 0.3m, 0.45m and 0.65m. In April (Fig. 4-3a) there was a trend to prolonged daytime periods of stratification in the upper layers by as much as 4°C (between 0.3 m and 0.45 m) as the sun warmed the surface water. The deepest layer (0.65 m) maintained approximately the same temperature all day, and became the warmest layer by up to 2.5°C for prolonged periods at night.

In June (Fig.4-3b) this trend continued such that the deeper layer remained the warmest layer by 2°C for most of the day and all night. In October, the pattern was similar to April, with the sun warming the surface layer by as much as 6°C more than the middle layer for many hours during the day time.

By January, the lowest layers of the pond were responding much more to diurnal fluctuations and there appeared to be very little stratification occurring at this time of year.

Theoretically, this lack of stratification in summer should result in THRT being closer to their expected, and the periods of stratification at other times could result in more rapid transit of wastewater through the system resulting in irregular performance. It was not possible to associate any particular period of stratification with poor pond performance in this study. It may be that small ponds such as these are less prone to prolonged periods of strong stratification that result in rapid transit of wastewater through the system and cause noticeable irregularity of performance.

## **4.2 Inlet Wastewater**

### **4.2.1. Inlet flow volumes**

From the direct reading of the Barossa Valley District Council flow meters it was determined that the inflow rate averaged 165 kL/d during the period April 2010

to July 2011. After that time the Council operators installed a rotating biological contactor and diverted 40 kL/d through that process. This reduced the daily inflow to an average 125 kL/d from July 2011 onwards.

#### 4.2.2 Inlet wastewater composition

All the water for this treatment system was derived from septic tanks located at each household. In practical terms this meant there had been a period of two to three days for the waste to settle in an anaerobic environment. The other practical outcome was that the effluent entering the treatment system was remarkably stable in composition (Table 4-3) throughout the year in all seasons. Even after the short period of anaerobic settlement in the septic tanks, a reasonable amount of the organic nitrogen in the wastes had been converted to ammonia, the mean ammonia levels were about 75 mgNH<sub>4</sub>-N /L, with almost no oxidised forms of nitrogen entering the system.

**Table 4-3 Lyndoch WSP1 Inlet Wastewater Composition -Septic tank effluent, where n = number of samples analysed.**

|               | BOD <sub>5</sub><br>(mg/L) | NH <sub>4</sub> -N<br>(mg/L) | NO <sub>2</sub> -N &<br>NO <sub>3</sub> -N<br>(mg/L) | PO <sub>4</sub> -P<br>(mg/L) | <i>E. coli</i><br>Log <sub>10</sub> /100<br>ml |
|---------------|----------------------------|------------------------------|--|------------------------------|--|
| <b>Mean</b>   | 216                        | 75.3                         | 1.74   | 12.4                         | 6.266  |
| <b>SD</b>     | 42                         | 9.3                          | 4.53   | 2.7                          | 0.172  |
| <b>Median</b> | 220                        | 77.0                         | 0.00   | 12.1                         | 6.279  |
| <b>n</b>      | 73                         | 78                           | 62   | 78                           | 82   |

As with the HRAP influent the inlet nutrient composition is consistent with previously reported work in this area (Walmsley and Shilton, 2005, Metcalf\_ &\_ Eddy, 2003, Craggs, 2005a, Mara, 1997).

#### 4.2.3 BOD<sub>5</sub> Loading Rates

The Lyndoch facultative pond (WSP1; Table 4-4) was operated at the lower end of accepted permissible BOD<sub>5</sub> loading rates (Fallowfield and Garrett, 1986). The loading rates are considerably lower than those for the HRAP (Tables 4-3 and 4-4), even though the BOD<sub>5</sub> concentration was almost the same. The much larger

volume and surface area of the WSP means the loading rates were commensurately reduced.

**Table 4-4 Lyndoch Facultative Pond (WSP1) areal BOD<sub>5</sub> loading rate (kg BOD<sub>5</sub> /ha/d) & volumetric BOD<sub>5</sub> loading rate (kg BOD<sub>5</sub> /m<sup>3</sup>/d).**

|                | Areal BOD <sub>5</sub> Loading Rates<br>(kg BOD <sub>5</sub> /ha/d) | Volumetric BOD <sub>5</sub> Loading Rates<br>(kg BOD <sub>5</sub> /m <sup>3</sup> /d) |
|----------------|---|---|
| <b>Mean</b>    | 53.7  | 0.0076  |
| <b>Std Dev</b> | 11.1  | 0.0016  |
| <b>Median</b>  | 56.7  | 0.0080  |
| <b>Range</b>   | 29.1 – 90.8   | 0.0041 – 0.0128   |

**Table 4-5 Lyndoch Maturation Pond 1 (WSP2) areal BOD<sub>5</sub> loading rate (kg BOD<sub>5</sub> /ha/d) & volumetric BOD<sub>5</sub> loading rate (kg BOD<sub>5</sub> /m<sup>3</sup>/d).**

|                | Areal BOD <sub>5</sub> Loading Rates<br>(kg BOD <sub>5</sub> /ha/d) | Volumetric BOD <sub>5</sub> Loading Rates<br>(kg BOD <sub>5</sub> /m <sup>3</sup> /d) |
|----------------|---|---|
| <b>Mean</b>    | 14.6  | 0.0024  |
| <b>Std Dev</b> | 16.9  | 0.0027  |
| <b>Median</b>  | 8.0   | 0.0013  |
| <b>Range</b>   | 0.6 – 79.2  | 0.0001 – 0.0128   |

**Table 4-6 Lyndoch Maturation Pond 2 (WSP3) areal BOD<sub>5</sub> loading rate (kg BOD<sub>5</sub> /ha/d) & volumetric BOD<sub>5</sub> loading rate (kg BOD<sub>5</sub> /m<sup>3</sup>/d).**

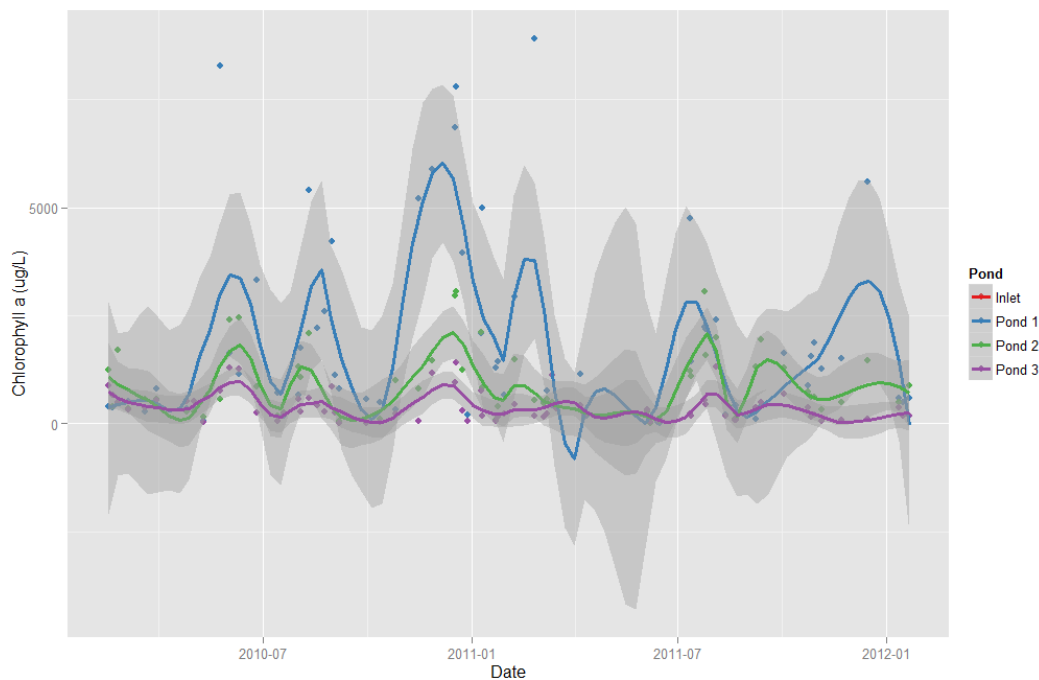
|                | Areal BOD <sub>5</sub> Loading Rates<br>(kg BOD <sub>5</sub> /ha/d) | Volumetric BOD <sub>5</sub> Loading Rates<br>(kg BOD <sub>5</sub> /m <sup>3</sup> /d) |
|----------------|---|---|
| <b>Mean</b>    | 12.9  | 0.0022  |
| <b>Std Dev</b> | 21.2  | 0.0036  |
| <b>Median</b>  | 5.3   | 0.0009  |
| <b>Range</b>   | 0.7 – 101   | 0.0001 – 0.0174   |

The BOD<sub>5</sub> loading rates for the two maturation ponds, WSP 2 & 3, (Tables 4-5 & 4-6) were approximately on quarter of those for the facultative pond, WSP1 (Table 4-4). This is consistent with the design parameters for WSP systems.

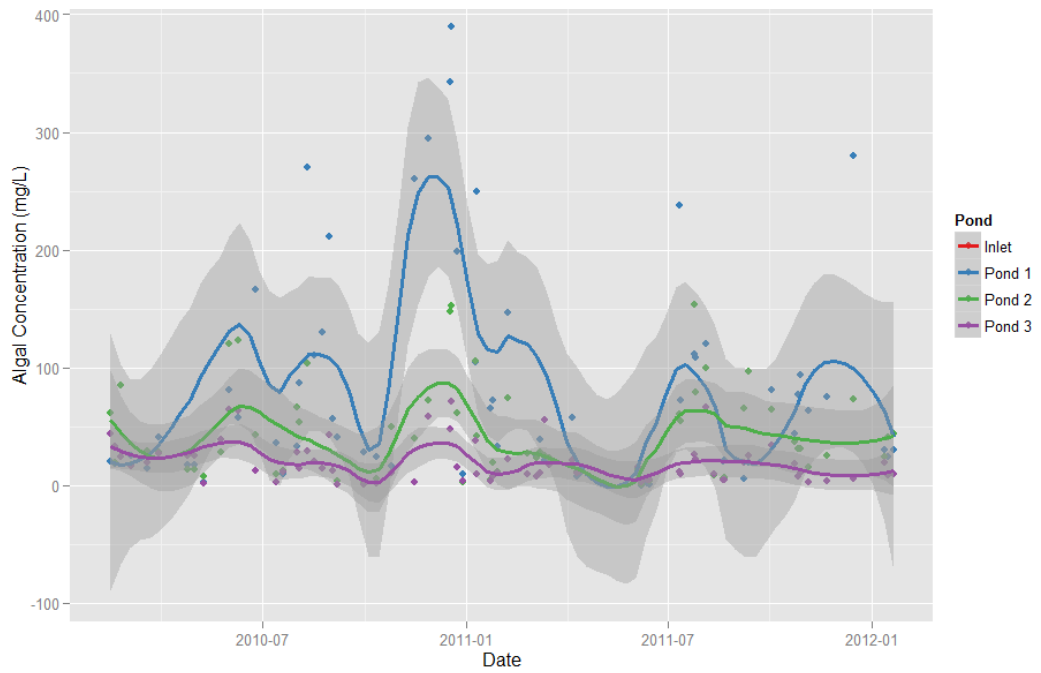
## 4.3 Pond Water Physico-chemical Parameters

### 4.3.1 Algal Growth in the Lyndoch WSP system fed septic tank effluent.

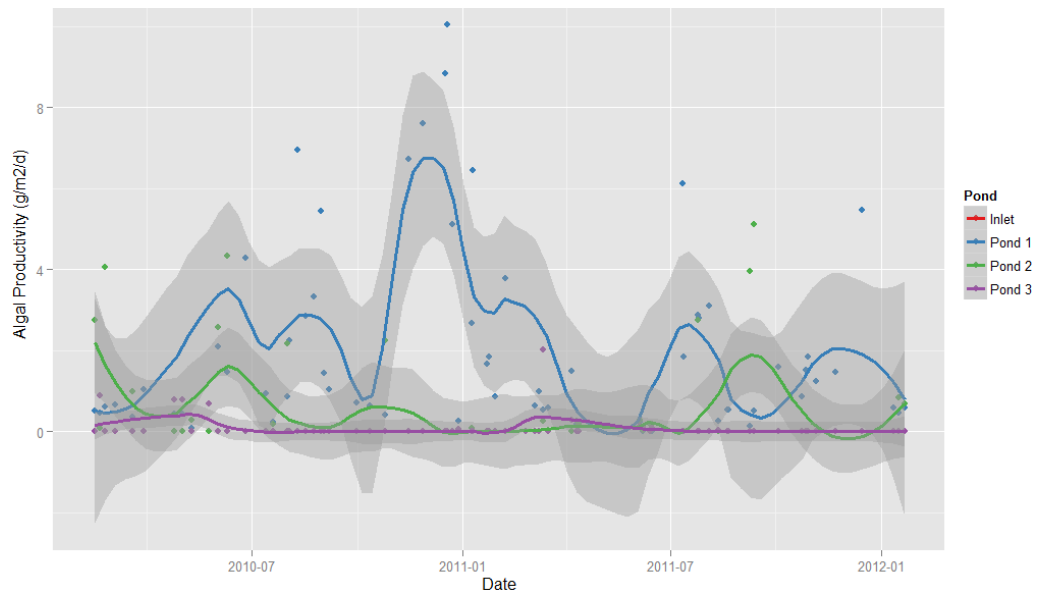
Algal growth in the three WSPs followed a cyclical path over time (Fig. 4-4) with each pond following approximately the same sequence of high and low algal populations. However, the fluctuations in algal population were of diminishing amplitude with each successive pond in the system. As algal growth is so important to the performance of WSPs, an effort has been made to understand the reasons behind the fluctuations. In the first series of graphs (Figs. 4-4, 4-5 and 4-6) chlorophyll a values, average water temperature and solar irradiation for each WSP are shown. The second series of graphs (Figs. 4-7, 4-8 and 4-9) the algal mass is graphed along with the key nutrients,  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$ . Table 4-8 shows the half velocity constants as determined by Hill and Lincoln (1981) for the key algal nutrients and the range of each of these nutrients seen in the WSPs.



**Fig. 4-4 Lyndoch WSP1, 2 & 3 (2010-2012) Scatterplot and loess-fit time line curves with 95% CI of chlorophyll a concentration for the three WSPs sequentially from top to bottom WSP1, WSP2 & WSP3.**



**Fig. 4-5 Lyndoch WSP1, 2 & 3 (2010-2012) Scatterplot and loess-fit time line curves with 95% CI of algal concentration levels for the three WSPs sequentially from top to bottom WSP1, WSP2 & WSP3.**

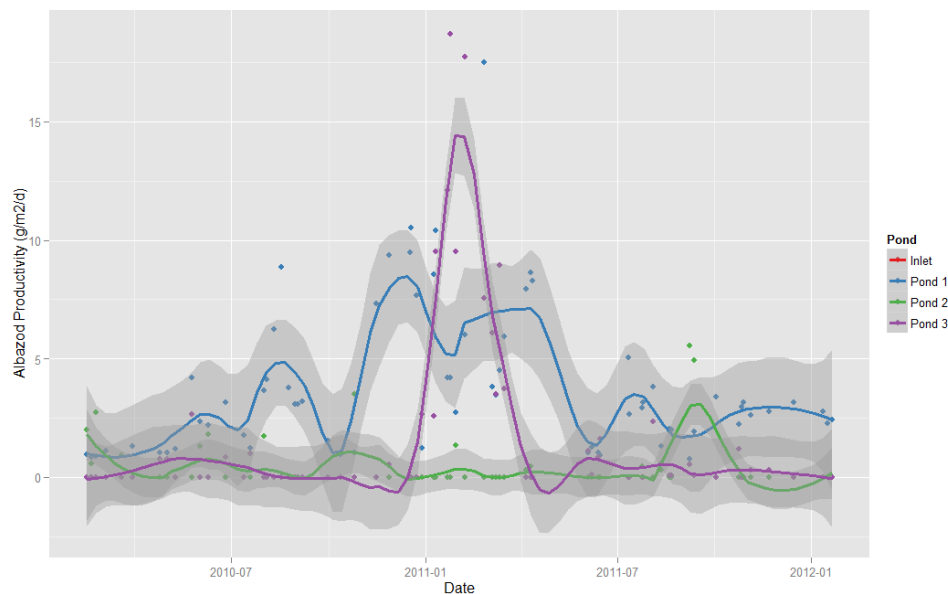


**Fig. 4-6 Lyndoch WSP1, 2 & 3 (2010-2012) Scatterplot and loess-fit time line curves with 95% CI of algal productivity (g/m<sup>2</sup>/d) for the three WSPs sequentially from top to bottom WSP1, WSP2 & WSP3.**

It is apparent from Fig. 4-4 that the level of algal productivity declines with the passage of wastewater through each pond in the WSP system. As the incoming light to the three ponds was identical, this must mean a diminishing supply of



nutrients was responsible for the declining algal growth pattern. Comparison of the nutrient concentration range in the ponds with the half velocity constants in table 4-8 shows there was sufficient nitrogen and phosphorous in the WSP 1 at all times. Unfortunately, no measure of inorganic carbon was available for this study. Examination of Figs. 4-5 & 4-6 shows that, in contrast to the HRAP at Kingston on Murray, there was no strong evidence of spring and autumn peaks of chlorophyll a in response to solar radiation and water temperature.



**Fig. 4-7 Lyndoch WSP1, 2 & 3 (2010-2012) Scatterplot and loess-fit time line curves with 95% CI of albazod productivity ( $\text{g}/\text{m}^2/\text{d}$ ) for the three WSPs sequentially from top to bottom WSP1, WSP2 & WSP3.**

Albazod productivity mirrors algal productivity (Fig. 4-7), i.e. most albazod activity occurred in WSP 1, with zero or little activity in WSP 2 & 3 for most of this period. There was one obvious exception in the January to March 2011 period, where albazod activity is recorded up to  $15 \text{ g}/\text{m}^2/\text{d}$ . This was silt from the banks after construction works and heavy rainfall and not true organic material.

In summary, there is no clear evidence of concurrent fluctuations in either environmental factors or supply of nutrients to explain the strong cyclic pattern of algal growth and death in WSP 1. It therefore may be hypothesised that

either grazing by protozoa and/or zooplankton, and/or disease (viral or fungal) processes were responsible for the frequent and sudden demise of a large proportion of algal populations. It also follows that subsequent ‘blooms’ of growth occurred when the ‘insulting factor’ itself died out, as there remains no other light, temperature or nutrient limitations to growth. This cycle fits a predator-prey model as described by Kretzschmar et al. (1993). To elucidate the answers to this question would require significant further research.

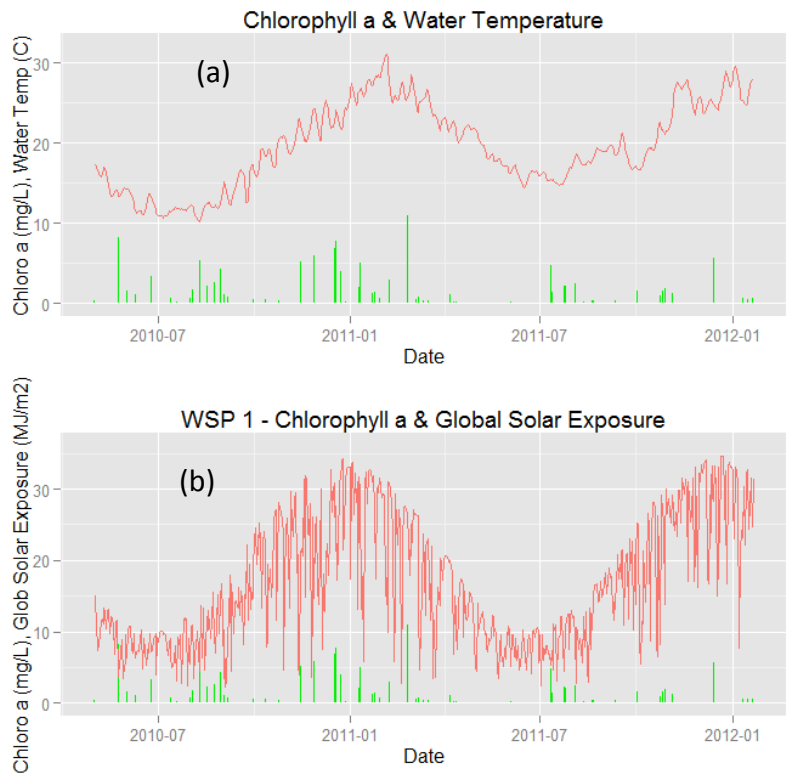
**Table 4-7 Half-velocity constants for algal nutrients determined empirically by Hill & Lincoln after fitting to their algal growth model (1<sup>st</sup> column) compared with the range of measured concentrations in WSP 1, 2 & 3; after Hill and Lincoln (1981).**

| Substrate              | SUB <sup>KS</sup><br>(mg/L) | WSP 1 (mg/L)                   | WSP 2 (mg/L)                   | WSP 3 (mg/L)                   |
|------------------------|-----------------------------|--------------------------------|--------------------------------|--------------------------------|
| <b>CO<sub>2</sub></b>  | 0.105                       | Not measured                   | Not measured                   | Not measured                   |
| <b>NH<sub>4</sub></b>  | 1.0                         | 6.7 – 72.0                     | 0.1 – 63.0                     | 0.003 – 62.0                   |
| <b>PO<sub>4</sub></b>  | 0.3229                      | 1.1 – 16.7                     | 1.1 – 17.4                     | 0.4 – 15.3                     |
| <b>Solar Radiation</b> | 728 W/m <sup>2</sup>        | 21.2 – 371<br>W/m <sup>2</sup> | 21.2 – 371<br>W/m <sup>2</sup> | 21.2 – 371<br>W/m <sup>2</sup> |

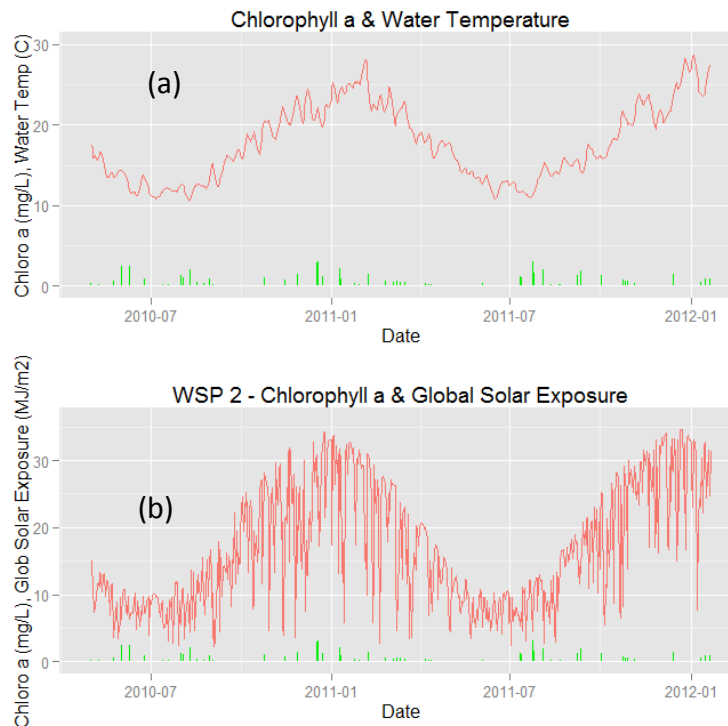
The algal populations of WSP2 and WSP3 followed a similar ‘boom and bust’ cycle, but in a reduced way. Data from Table 4-8 would suggest that nitrogen periodically become a limitation to algal growth. However, the lowest NH<sub>4</sub>-N and PO<sub>4</sub>-P levels measured in WSP 2 & 3 occurred during the months of January through to April (Figs 4-10 & 4-11). These months were not the periods of low algal concentration.

In summary, the second and third ponds in the series have short bursts of algal growth but not to the same level as the first pond (Fig. 4-4). A possible reason for the reduced prevalence of growth bursts was the reduction in concentration of all nutrients with passage from pond to pond (Tables 4-7, 4-8 & 4-9). The sudden reduction in algal concentration occurs in the second and third ponds at almost exactly the same time as in the first pond. A delay of a few days between these events in each pond is noticeable most of the time. This is consistent with an infectious agent passing from one pond to the next, having

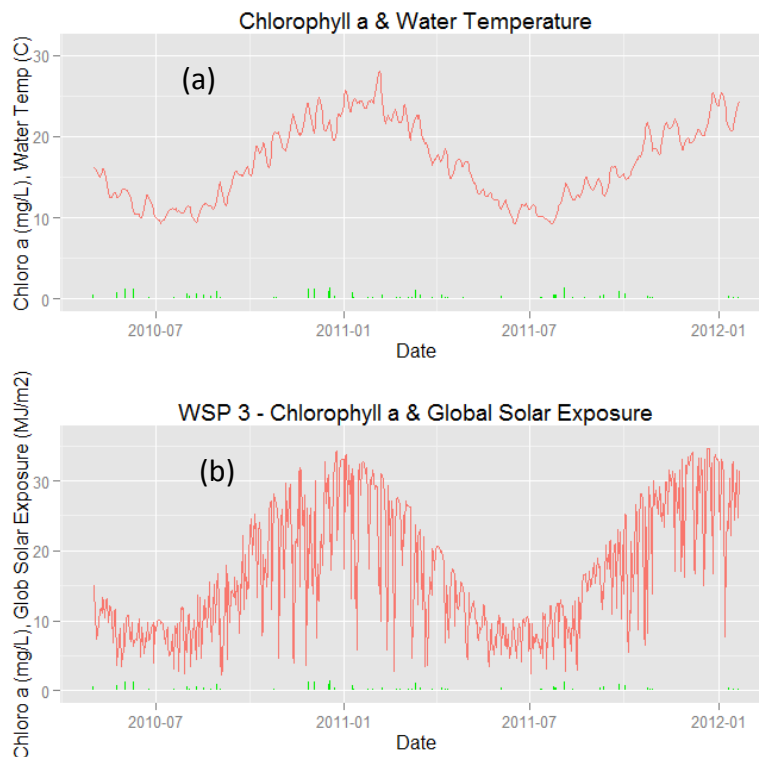
originally arrived in the first pond. It is also possible that the sudden reductions could be due to grazing by protozoa or zooplankton as noted above.



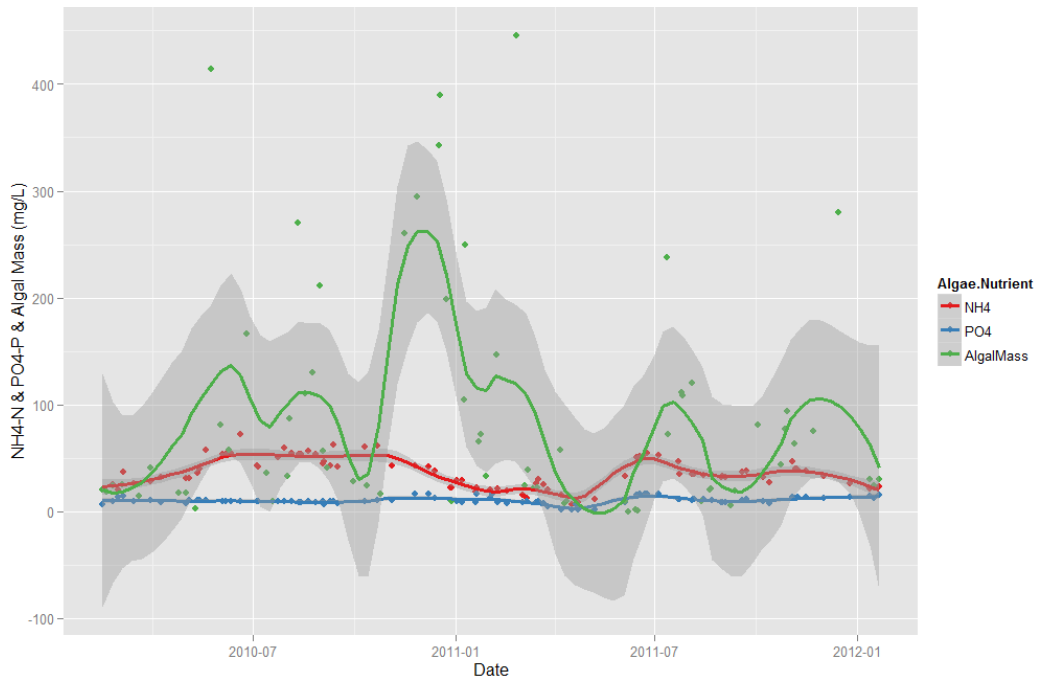
**Fig. 4-8 Time series for the Lyndoch WSP1 showing the relationship between pond chlorophyll *a* (green bar) and a) daily average pond temperatures (°C; red line), and b) the daily total solar radiation (MJ/m<sup>2</sup>; red line).**



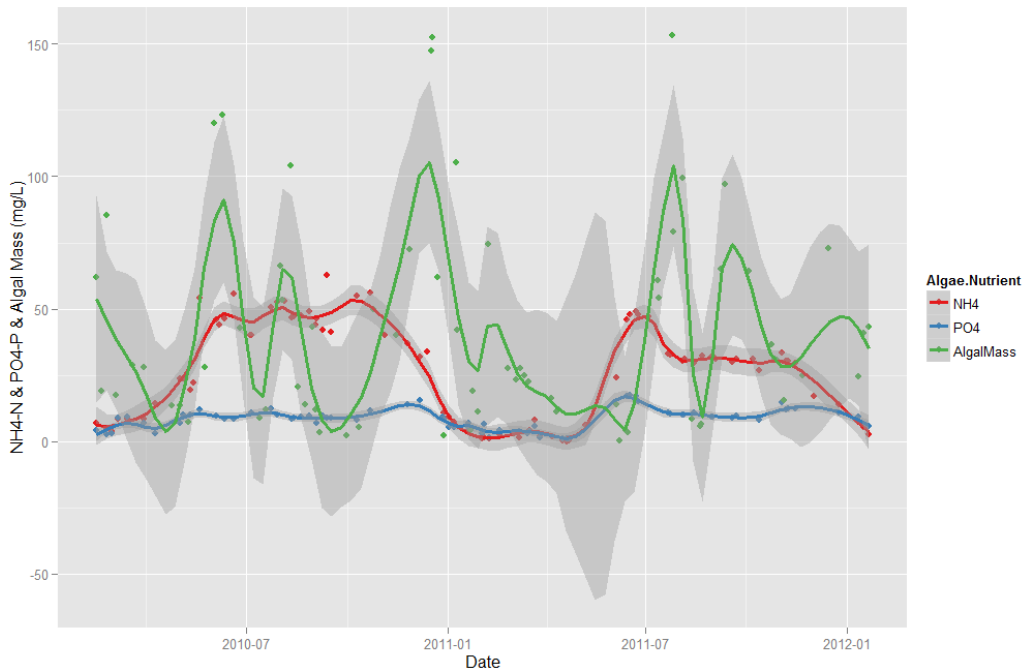
**Fig. 4-9 Time series for the Lyndoch WSP2 showing the relationship between pond chlorophyll a (green bar) measurements and a) daily average pond temperatures (°C; red line), and b) the daily total solar radiation (MJ/m<sup>2</sup>; red line).**



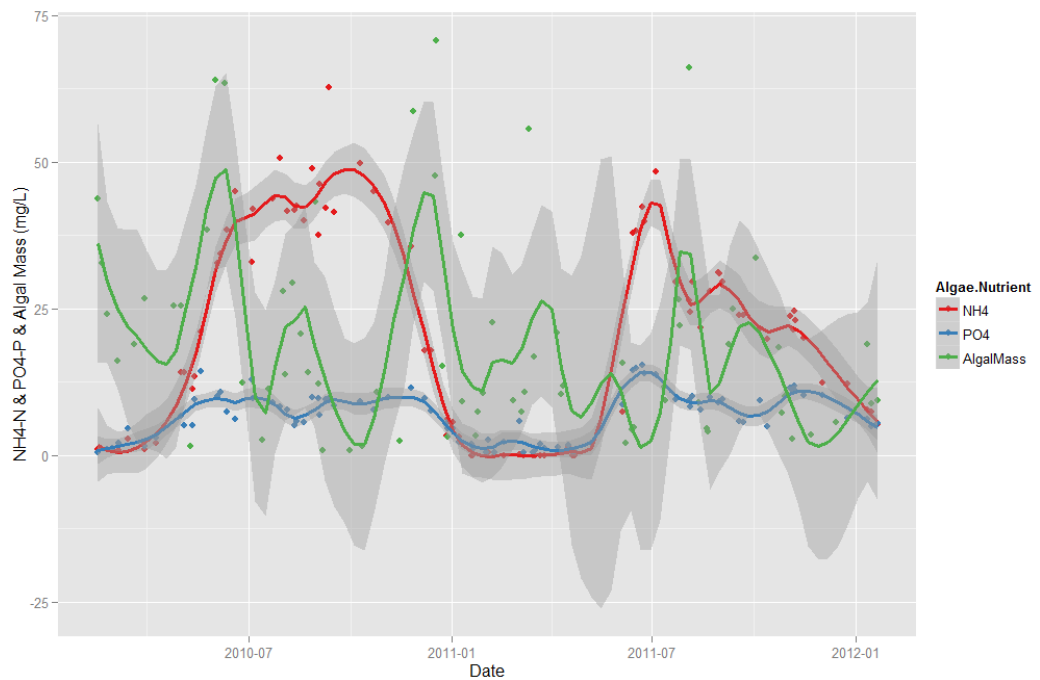
**Fig. 4-10 Time series for the Lyndoch WSP3 showing the relationship between pond chlorophyll a (green bar) measurements and a) daily average pond temperatures (°C; red line), and b) the daily total solar radiation (MJ/m<sup>2</sup>; red line).**



**Fig. 4-11 WSP 1. Time series for Algal Mass compared to two main nutrients, NH<sub>4</sub>-N and PO<sub>4</sub>-P.**



**Fig. 4-12 WSP 2. Time series for Algal Mass compared to two main nutrients, NH<sub>4</sub>-N and PO<sub>4</sub>-P.**



**Fig. 4-13 WSP 3. Time series for Algal Mass compared to two main nutrients, NH<sub>4</sub>-N and PO<sub>4</sub>-P.**

#### **4.4 Lyndoch WSPs: Key Performance Indicators – Nutrient Removal and *E. coli* Log<sub>10</sub> Reduction Value (LRV)**

The data shown in Table 4-8, 4-9 and 4-10 present the key performance indicators for each of the WSPs in turn across the whole period of observation. It encompasses operation over all seasons and pond water temperatures. As a broad overview, passage of wastewater through the WSP 1 on average resulted in the removal of 90% of the BOD<sub>5</sub>, 50% of the NH<sub>4</sub>-N, 47% of total inorganic-N, 17% of PO<sub>4</sub>-P and 2.115 log<sub>10</sub> of *E. coli*/100ml. These figures are consistent with previous studies. Published nitrogen removal efficiencies range from 5% to 95% (Mara, 1997, Pearson et al., 1996, Ferrara and Avci, 1982, Picot et al., 1992,

Pano and Middlebrooks, 1982). Published PO<sub>4</sub>-P removal efficiencies range from 20% to 50%, (Li et al., 1991, Mara, 1996, Racault et al., 1995, Racault and Boutin, 2005, Picot et al., 1992) so the results obtained in this study are at the low end of published range.

WSP 2 removed a further 21% of the remaining BOD<sub>5</sub>, 34% of the NH<sub>4</sub>-N, 28% of total inorganic-N, 33% of PO<sub>4</sub>-P and about 1.3 log<sub>10</sub> of *E. coli*/100ml, while WSP 3 removed approximately 26% of the remaining BOD<sub>5</sub>, 21% of the NH<sub>4</sub>-N, 0% of total inorganic-N, 19% of PO<sub>4</sub>-P and about 0.5 log<sub>10</sub> of *E. coli*/100ml.

To avoid confusion in reading Tables 4-8 to 4-10, it must be noted that the values included for inlet, outlet and removal efficiency are the means of the 62 to 82 observations made over the whole time period. The figure presented in each column is the mean of the whole tabular record (not presented).

**Table 4-8 WSP 1 inlet, outlet values and removal efficiencies for a range of performance related parameters, where n= number of samples analysed.**

| WSP 1 Key Performance Indicators |       |        |                    |
|----------------------------------|-------|--------|--------------------|
| BOD <sub>5</sub> mg/L            |       |        |                    |
|                                  | Inlet | Outlet | Removal Efficiency |
| <b>Mean</b>                      | 216   | 23     | 89.5%              |
| <b>Std Dev</b>                   | 42    | 26     | 11.4%              |
| <b>Median</b>                    | 220   | 14     | 93.2%              |
| <b>(n)</b>                       | 73    | 73     | 74                 |
| NH <sub>4</sub> -N mg/L          |       |        |                    |
| <b>Mean</b>                      | 75.3  | 36.8   | 50.4%              |
| <b>Std Dev</b>                   | 9.3   | 14.9   | 19.5%              |
| <b>Median</b>                    | 77.0  | 36.2   | 49.2%              |
| <b>(n)</b>                       | 78    | 78     | 78                 |
| Total Inorganic N mg/L           |       |        |                    |
| <b>Mean</b>                      | 75.3  | 39.1   | 47.3%              |
| <b>Std Dev</b>                   | 9.7   | 15.5   | 20.8%              |
| <b>Median</b>                    | 76.6  | 41.0   | 45.7%              |
| <b>(n)</b>                       | 62    | 62     | 62                 |
| PO <sub>4</sub> -P mg/L          |       |        |                    |
| <b>Mean</b>                      | 12.4  | 10.2   | 16.5%              |
| <b>Std Dev</b>                   | 2.7   | 3.3    | 25.5%              |

| WSP 1 Key Performance Indicators  |              |               |              |
|---|--------------|---------------|--------------|
| <b>Median</b>   | 12.1         | 9.9           | 13.4%        |
| <b>(n)</b>  | 78           | 78            | 78           |
| <b>Chlorophyll <i>a</i> mg/L</b>  |              |               |              |
| <b>Mean</b>   | 1.943        |               |              |
| <b>Std Dev</b>  | 2.225        |               |              |
| <b>Median</b>   | 1.144        |               |              |
| <b>(n)</b>  | 63           |               |              |
| <b>Log<sub>10</sub> <i>E. coli</i> 100 /ml and log<sub>10</sub> reduction value (LRV)</b> |              |               |              |
|   | <b>Inlet</b> | <b>Outlet</b> | <b>LRV</b>   |
| <b>Mean</b>   | 6.266        | 4.242         | 2.024        |
| <b>Std Dev</b>  | 0.172        | 0.626         | 0.646        |
| <b>Median</b>   | 6.279        | 4.160         | 2.162        |
| <b>(n)</b>  | 82           | 82            | 82           |
| <b>NO<sub>3</sub> &amp; NO<sub>2</sub> -N (mg/L)</b>                                      |              |               |              |
|   | <b>Inlet</b> | <b>Outlet</b> | <b>Added</b> |
| <b>Mean</b>   | 1.74         | 3.78          | 2.04         |
| <b>Std Dev</b>  | 4.53         | 5.85          | 1.32         |
| <b>Median</b>   | 0.00         | 0.53          | 0.53         |
| <b>(n)</b>  | 62           | 62            | 62           |
| <b>Suspended Solids (mg/L)</b>  |              |               |              |
| <b>Mean</b>   | 95           | 161           | 66           |
| <b>Std Dev</b>  | 22.8         | 120           |              |
| <b>Median</b>   | 110          | 132           | 22           |
| <b>(n)</b>  | 62           | 62            |              |

Table 4-9 WSP 2 inlet, outlet values and removal efficiencies for a range of performance related parameters, where n= number of samples analysed.

| WSP 2 Key Performance Indicator |              |               |                           |
|---------------------------------|--------------|---------------|---------------------------|
| <b>BOD<sub>5</sub> mg/L</b>     |              |               |                           |
|                                 | <b>Inlet</b> | <b>Outlet</b> | <b>Removal Efficiency</b> |
| <b>Mean</b>                     | 23           | 21.4          | 20.6%                     |
| <b>Std Dev</b>                  | 26           | 34.7          | 64.6%                     |
| <b>Median</b>                   | 14           | 7.9           | 38.9%                     |
| <b>(n)</b>                      | 73           | 62            | 62                        |
| <b>NH<sub>4</sub>-N mg/L</b>    |              |               |                           |
| <b>Mean</b>                     | 36.8         | 27.0          | 34.3%                     |
| <b>Std Dev</b>                  | 14.9         | 19.4          | 33.6%                     |
| <b>Median</b>                   | 36.2         | 30.5          | 18.1%                     |
| <b>(n)</b>                      | 78           | 59            | 78                        |
| <b>Total Inorganic N mg/L</b>   |              |               |                           |
| <b>Mean</b>                     | 39.1         | 32.0          | 27.5%                     |
| <b>Std Dev</b>                  | 15.5         | 19.2          | 30.0%                     |
| <b>Median</b>                   | 41.0         | 33.6          | 13.8%                     |
| <b>(n)</b>                      | 62           | 62            | 62                        |
| <b>PO<sub>4</sub>-P mg/L</b>    |              |               |                           |
| <b>Mean</b>                     | 10.2         | 8.8           | 33.2%                     |
| <b>Std Dev</b>                  | 3.3          | 4.1           | 43.3%                     |



| WSP 2 Key Performance Indicator  |       |        |       |
|--|-------|--------|-------|
| <b>Median</b>  | 9.9   | 9.3    | 14.5% |
| <b>(n)</b>   | 78    | 56     | 62    |
| Chlorophyll <i>a</i> mg/L  |       |        |       |
| <b>Mean</b>  | 1.943 | 0.852  |       |
| <b>Std Dev</b>   | 2.225 | 0.806  |       |
| <b>Median</b>  | 1.144 | 0.556  |       |
| <b>(n)</b>   | 63    | 62     |       |
| Log <sub>10</sub> <i>E. coli</i> 100 /ml and log <sub>10</sub> reduction value (LRV) |       |        |       |
|  | Inlet | Outlet | LRV   |
| <b>Mean</b>  | 4.242 | 2.911  | 1.302 |
| <b>Std Dev</b>   | 0.626 | 0.812  | 0.598 |
| <b>Median</b>  | 4.160 | 2.798  | 1.283 |
| <b>(n)</b>   | 82    | 62     | 62    |
| NO <sub>3</sub> & NO <sub>2</sub> -N (mg/L)  |       |        |       |
|  | Inlet | Outlet | Added |
| <b>Mean</b>  | 3.78  | 2.8    | 0.98  |
| <b>Std Dev</b>   | 5.85  | 5.0    | 0.85  |
| <b>Median</b>  | 0.53  | 0.5    | 0.03  |
| <b>(n)</b>   | 62    | 59     | 59    |
| Suspended Solids (mg/L)  |       |        |       |
| <b>Mean</b>  | 161   | 98.2   | 63    |
| <b>Std Dev</b>   | 120   | 66.5   |       |
| <b>Median</b>  | 132   | 87     | 49    |
| <b>(n)</b>   | 62    | 62     |       |

Table 4-10 WSP 3 inlet, outlet values and removal efficiencies for a range of performance related parameters, where n= number of samples analysed.

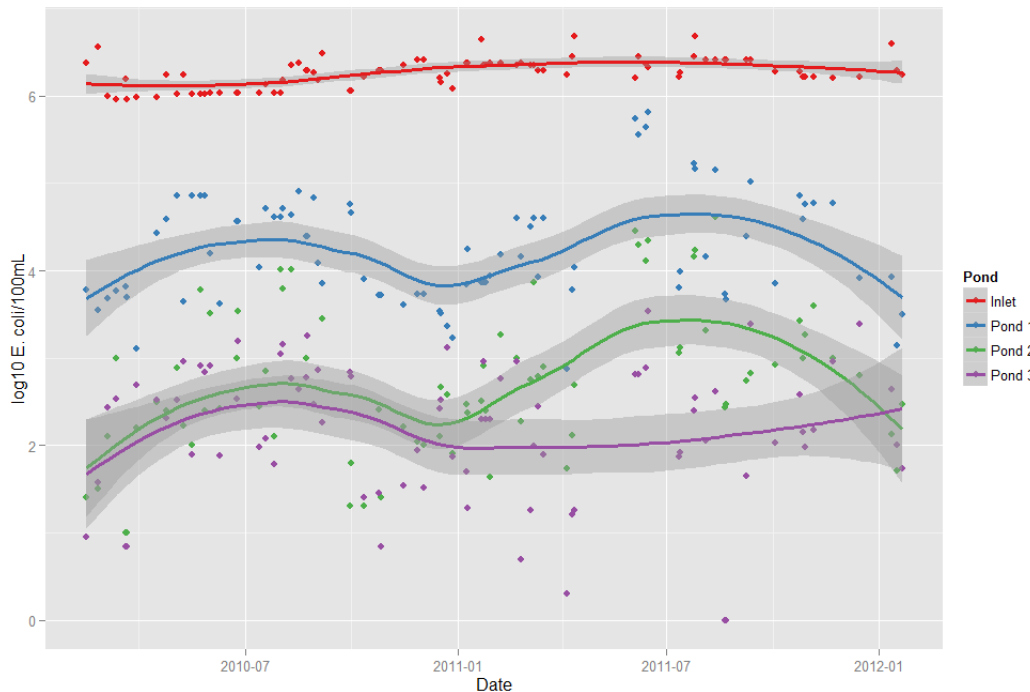
| WSP 3 Key Performance Indicators |       |        |                    |
|----------------------------------|-------|--------|--------------------|
| BOD <sub>5</sub> mg/L            |       |        |                    |
|                                  | Inlet | Outlet | Removal Efficiency |
| <b>Mean</b>                      | 21.4  | 11.5   | 25.6%              |
| <b>Std Dev</b>                   | 34.7  | 18.0   | 33.2%              |
| <b>Median</b>                    | 7.9   | 6.7    | 16.1%              |
| <b>(n)</b>                       | 62    | 73     | 74                 |
| NH <sub>4</sub> -N mg/L          |       |        |                    |
| <b>Mean</b>                      | 27.0  | 22.8   | 21.2%              |
| <b>Std Dev</b>                   | 19.4  | 17.3   | 87.2%              |
| <b>Median</b>                    | 30.5  | 23.8   | 19.9%              |
| <b>(n)</b>                       | 59    | 78     | 62                 |
| Total Inorganic N mg/L           |       |        |                    |
| <b>Mean</b>                      | 32.0  | 28.6   | -3.5%              |
| <b>Std Dev</b>                   | 19.2  | 18.0   | 78.6%              |
| <b>Median</b>                    | 33.6  | 27.4   | 8.7%               |
| <b>(n)</b>                       | 62    | 62     | 62                 |
| PO <sub>4</sub> -P mg/L          |       |        |                    |

| WSP 3 Key Performance Indicators  |              |               |              |
|---|--------------|---------------|--------------|
| <b>Mean</b>   | 8.8          | 7.27          | 18.8%        |
| <b>Std Dev</b>  | 4.1          | 4.01          | 26.6%        |
| <b>Median</b>   | 9.3          | 8.05          | 12.0%        |
| <b>(n)</b>  | 56           | 78            | 62           |
| <b>Chlorophyll <i>a</i> mg/L</b>  |              |               |              |
| <b>Mean</b>   | 0.852        | 0.371         |              |
| <b>Std Dev</b>  | 0.806        | 0.362         |              |
| <b>Median</b>   | 0.556        | 0.233         |              |
| <b>(n)</b>  | 62           | 63            |              |
| <b>Log<sub>10</sub> <i>E. coli</i> 100 /ml and log<sub>10</sub> reduction value (LRV)</b> |              |               |              |
|   | <b>Inlet</b> | <b>Outlet</b> | <b>LRV</b>   |
| <b>Mean</b>   | 2.911        | 2.188         | 0.540        |
| <b>Std Dev</b>  | 0.812        | 0.775         | 0.782        |
| <b>Median</b>   | 2.798        | 2.306         | 0.475        |
| <b>(n)</b>  | 62           | 82            | 82           |
| <b>NO<sub>3</sub> &amp; NO<sub>2</sub> -N (mg/L)</b>                                      |              |               |              |
|   | <b>Inlet</b> | <b>Outlet</b> | <b>Added</b> |
| <b>Mean</b>   | 2.8          | 5.5           | 2.7          |
| <b>Std Dev</b>  | 5.0          | 5.4           | 0.4          |
| <b>Median</b>   | 0.5          | 3.8           | 3.3          |
| <b>(n)</b>  | 59           | 62            | 62           |
| <b>Suspended Solids (mg/L)</b>  |              |               |              |
| <b>Mean</b>   | 98.2         | 108           | 66           |
| <b>Std Dev</b>  | 66.5         | 94.3          |              |
| <b>Median</b>   | 87           | 70            | 22           |
| <b>(n)</b>  | 62           | 62            |              |

#### 4.4.1 *E. coli* removal

As can be seen in Fig. 4-14 the inlet *E. coli* were remarkably stable in number. This was made obvious by the fact that the 95% confidence interval did not project beyond the loess best fit line. von Sperling (2005) in a study of 186 ponds worldwide found coliform removal efficiencies of 1.0 log units for secondary facultative ponds (90% removal) and 1.2 log units (94% removal) for each maturation pond in the series. In the Lyndoch ponds, the majority of *E. coli* removal occurred in WSP 1 (2.024±0.646) (Table 4-8 & Fig. 4-14), with some fluctuation in performance over the year; in particular more *E. coli* were removed over the warmer months in this study. WSP 2 *E. coli* LRV (1.302±0.598) tended to follow WSP1 mimicking the poor winter performance compared to summer (Table 4-9 & Fig. 4-14). The performance of WSP 3 *E. coli* LRV (0.540±0.728) was somewhat enigmatic as it followed WSP 2 closely for the first year, scarcely removing any further *E. coli*. It then began performing

independently in the second year. This change may be due to the installation and use of the rotating biological contactor (RBC) for this latter period. Chlorine was added to the effluent from the RBC system and the treated water returned to WSP 3. The broad 95% CI bands for WSPs 1, 2 & 3 indicate the highly variable nature of the WSP system in reducing pathogen indicator organisms.



**Fig 4-14 Lyndoch WSP1, 2 & 3 (2010-2012) Scatterplot and loess-fit curve with 95% CI of  $\log_{10} E. coli / 100\text{mL}$  as it passes through each of the three ponds sequentially from top to bottom Inlet (red), WSP1 (blue), WSP2 (green) & WSP3 (purple).**

The von Sperling (2005) paper provides a predictive resource by way of two tables of expected log reductions for coliforms in ponds of various dimensions. The key criteria required to interrogate the tables are the average pond temperature, length to breadth (L/B) ratio, depth and retention time. By these criteria, at 20°C and 30 day retention time, WSP 1 with a depth of 1m and an L/B ratio of 5 is predicted to remove approximately 1.6  $\log_{10}$  faecal coliforms. Given the annual variation in temperature and the two different THRTs experienced, this figure is not too dissimilar to the 2.0  $\log_{10} E. coli$  actually measured as removed during this study. The  $E. coli$  removal from WSP 2 & 3 with the same criteria except a L/B ratio of 3 would be predicted to remove 1.42  $\log_{10}$  faecal

coliforms each. This compares favourably with the 1.30 and 0.54 log<sub>10</sub> *E. coli* actually measured as removed during this study, with some loss of clarity when it came to WSP 3.

#### **4.4.2 Inorganic N (including NH<sub>4</sub>-N) removal**

As explained in para 3.2.1.3, a number of assumptions were made to arrive at a figure in grams per day of incoming and outgoing inorganic nitrogen, algal nitrogen and ammonia volatilisation. The activity largely centres around ammonia and the ammonium ion, and most of the removal happens in WSP1. Detailed description of removal is thus concentrated on WSP1 and ammonia/ammonium removal.

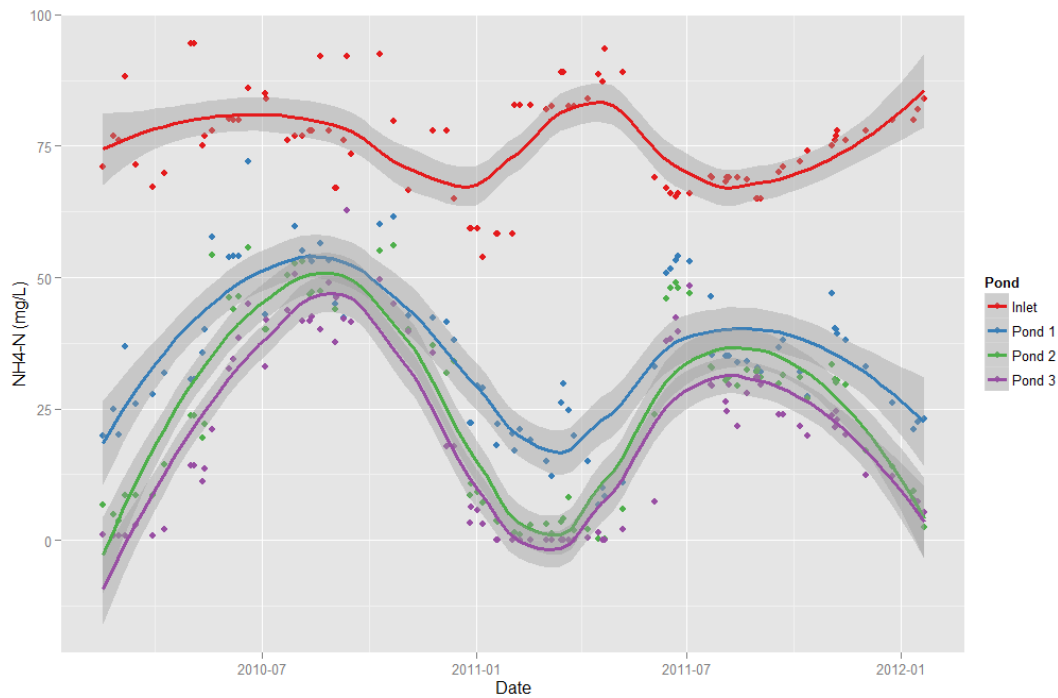
Between 10 and 14 kg of inorganic-N enters WSP 1 per day (red line in Fig. 4-16). No measure was made of organic nitrogen entering the pond, so the following analyses do not account for nitrogen from this source. Of the incoming inorganic-N, between 3 and 8.5 kg exited WSP1 as unchanged inorganic-N per day (30-60%) either in the original reduced form or in an oxidised form. A further 0.1 to 5.3 kg (<1-30%) exited in organic form as part of the algal biomass. The amount was highly variable and depended on periods of algal blooms as detailed in para 4.3.1. As well, 0.4-11 kg/day (1-80%) exited as ammonia. The timing and extent of this volatilisation is shown in detail in Fig. 4-17. As explained in para 3.2.1.3 the amount exiting via this method was dependent on temperature, pH and pond depth. In this study, most of this volatilisation occurred in the January to April period.

Comparisons with world literature removal efficiencies are provided in Para 4.4.

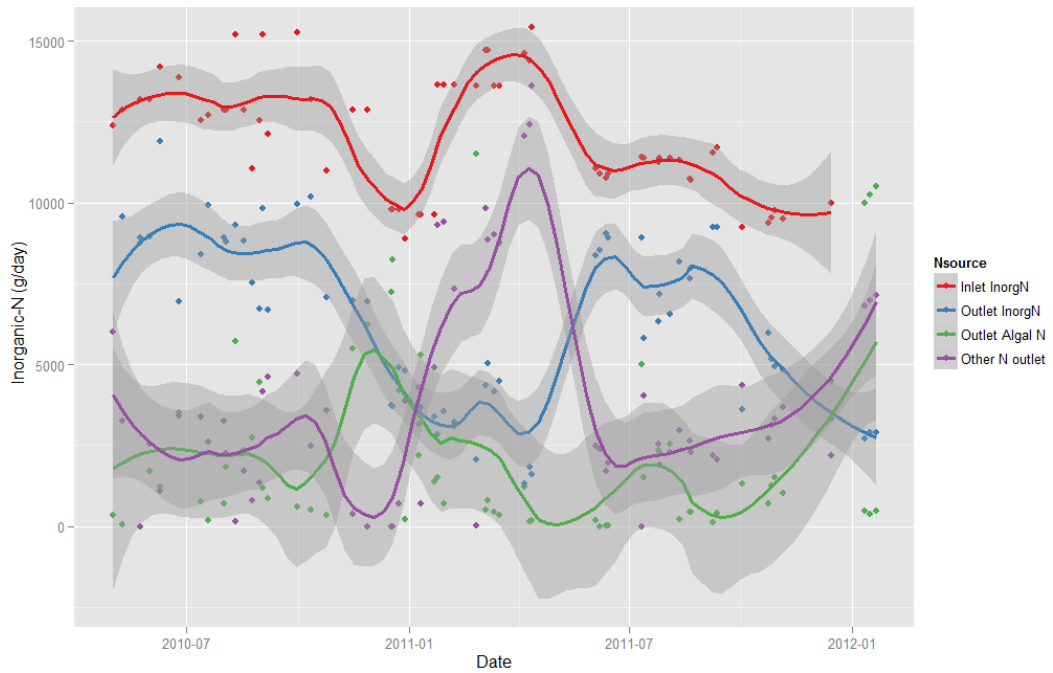
As for *E. coli* removal, the NH<sub>4</sub>-N inlet water was remarkably constant in NH<sub>4</sub>-N concentration over time. The bulk of NH<sub>4</sub>-N removal happened in WSP1, with the other two ponds contributing comparatively little to this process (Fig. 4-15).

As noted above, a feature was comparatively poor NH<sub>4</sub>-N removal in winter (~20%) compared to the summer and autumn months (up to 80%). As the pH in

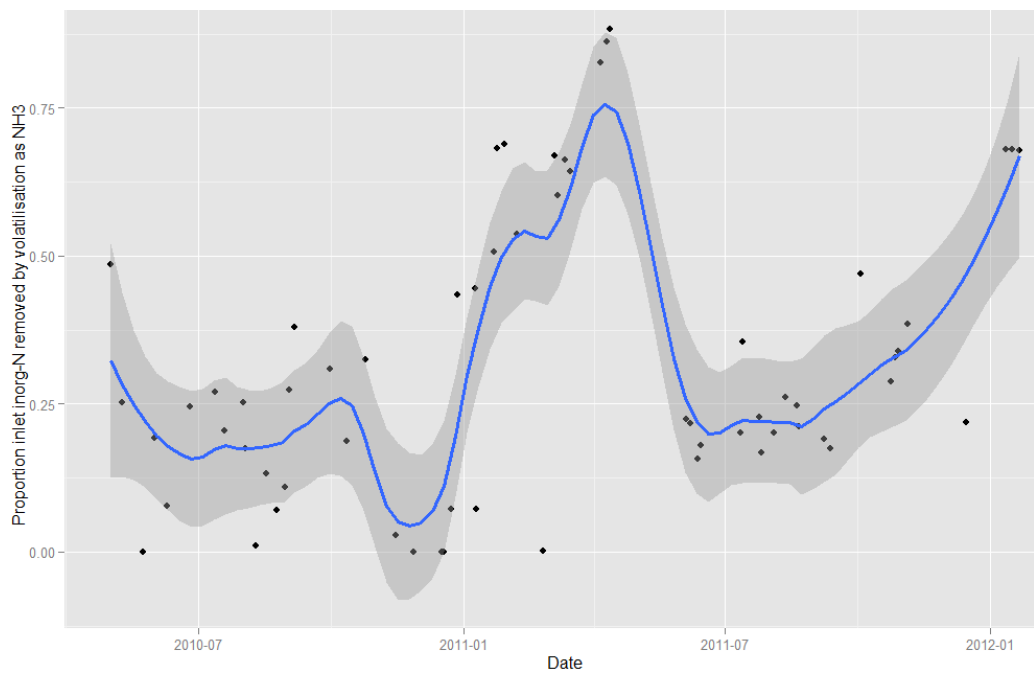
WSP1 regularly reached 9.5 (Fig 4-1d), and the water temperature also reached 30°C (Fig. 4-1b) in the warmer months, volatilisation of NH<sub>3</sub>-N in the warmer periods was enhanced.



**Fig 4-15 Lyndoch WSP1, 2 & 3 2010/12 Scatterplot and loess-fit curve with 95% CI of NH<sub>4</sub>-N as it passes through each treatment phase for the three ponds sequentially from top to bottom Inlet(red), WSP1 (blue), WSP2 (green) & WSP3 (purple).**



**Fig. 4-16 WSP 1 – loess fit and 95% confidence intervals for Inorganic-N incoming (red), outgoing (blue), outgoing as algal-N (green) and removed by  $\text{NH}_3$  volatilisation (purple) over time.**



**Fig. 4-17 WSP1 - loess fit and 95% confidence intervals for the percentage of inorganic-N removed via ammonia volatilisation over time.**

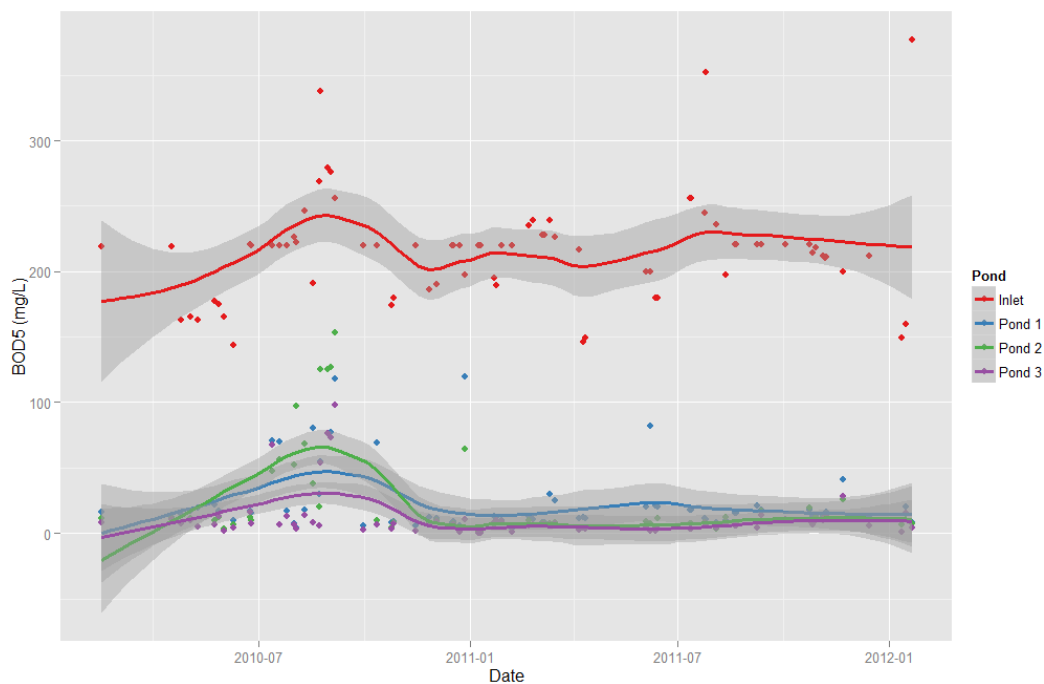
#### 4.4.3 BOD<sub>5</sub> removal

As with *E. coli* and  $\text{NH}_4\text{-N}$ , the inlet water was remarkably constant in BOD<sub>5</sub> concentration over time, see Fig. 4-18. The main features are that the majority

of BOD<sub>5</sub> removal happened in WSP1. As almost all the BOD<sub>5</sub> was removed by WSP1, there was very little opportunity for the other two ponds to contribute to this process. Walmsley and Shilton (2005) state that a properly constructed and run WSP system will have filtered effluent BOD<sub>5</sub> of less than 20 mg/L, and often less than 10 mg/L. In this system, the average filtered effluents BOD<sub>5</sub> were 23, 21 and 11.5 mg/L for WSPs 1, 2 & 3 respectively. Thus the BOD<sub>5</sub> performance of this system could be said to meet the previously reported standards.

It appeared that the strongest predictor of BOD<sub>5</sub> levels in any of the ponds was the inlet concentration of BOD<sub>5</sub>. A small rise in inlet BOD<sub>5</sub> in August/September 2010 was followed by a rise in BOD<sub>5</sub> concentration in the BOD<sub>5</sub> concentration in each of the three WSPs in succession. This trend was not repeated in the second year of the study.

It can be assumed that dissolved oxygen concentration in the ponds (Fig. 4-1 c) was always sufficient to oxidise the majority of the incoming organic carbon during the study period, despite the periodic fluctuations in algal concentration (Fig. 4-4) during that time.



**Fig 4-18 Lyndoch WSP1, 2 & 3 2010/12 Scatterplot and loess-fit curve with 95% CI of BOD<sub>5</sub> as it passes through each treatment phase for the three ponds sequentially from top to bottom Inlet(red), WSP1 (blue), WSP2 (green) & WSP3 (purple).**

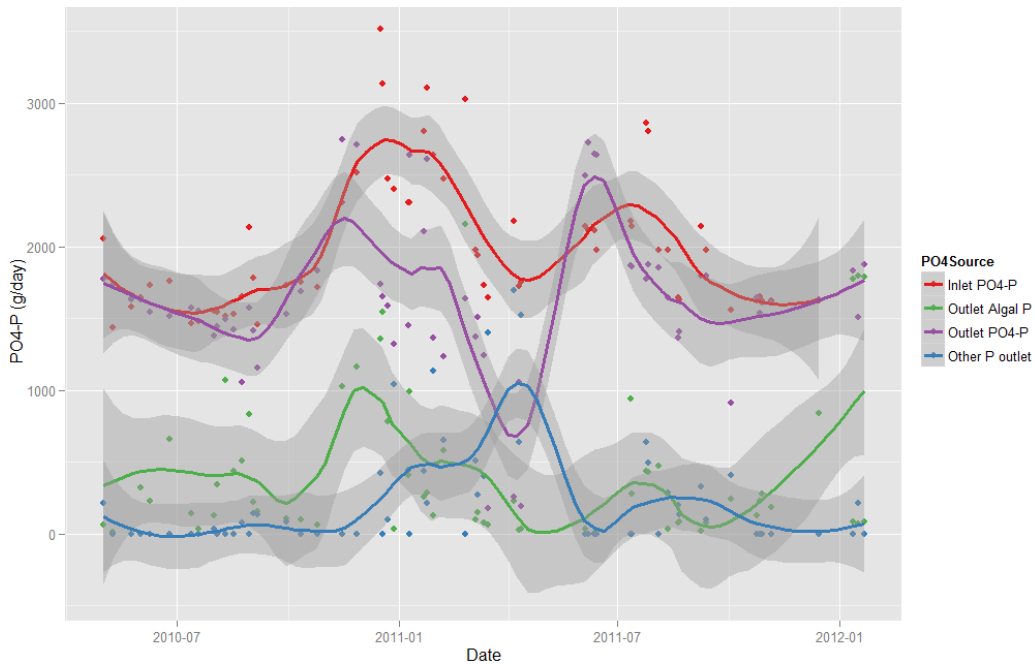
#### **4.4.4 PO<sub>4</sub>-P removal**

As noted in para. 3.2.1.4, there are three possible mechanisms for phosphorus removal in WSPs; exiting unchanged as an orthophosphate, algal uptake/assimilation/deposition and chemical precipitation. As sludge accumulates in the WSPs, deposition of dead organisms is certainly an active orthophosphate removal mechanism from the pond liquor.

Inlet PO<sub>4</sub>-P levels were far more variable than for the other nutrients, suggesting that there may be an industrial washing waste entering the system in the early summer period with levels of detergent laden with PO<sub>4</sub>-P exceeding normal domestic levels of PO<sub>4</sub>-P during that period (Fig. 4-19 and 4-20). Despite this higher summer inflow, in-pond PO<sub>4</sub>-P concentrations appeared to be at their highest in the winter/spring period and at their lowest in the summer/autumn period (Fig. 4-19)

The weight of phosphorous (in orthophosphate form) entering and leaving the WSP 1 on a daily basis was calculated from the orthophosphate concentrations (inlet and outlet) multiplied by the daily inflow. These are represented as the red and purple lines respectively in Fig. 4-19 in grams of PO<sub>4</sub>-P per day. A number of assumptions were made to estimate algal incorporated phosphorous levels. This was done as an attempt to indicate the extent to which the assimilation and precipitation mechanisms were effective in this study period. These assumptions are detailed in para 3.2.1.4. From the assumptions an estimate of the mass of phosphorous (g PO<sub>4</sub>-P/ day) exiting the pond per day in the algal biomass was made (green line in Fig. 4-19). The fourth line (blue) represents the residual unaccounted for, and must therefore represent the amount of phosphate chemically precipitated.

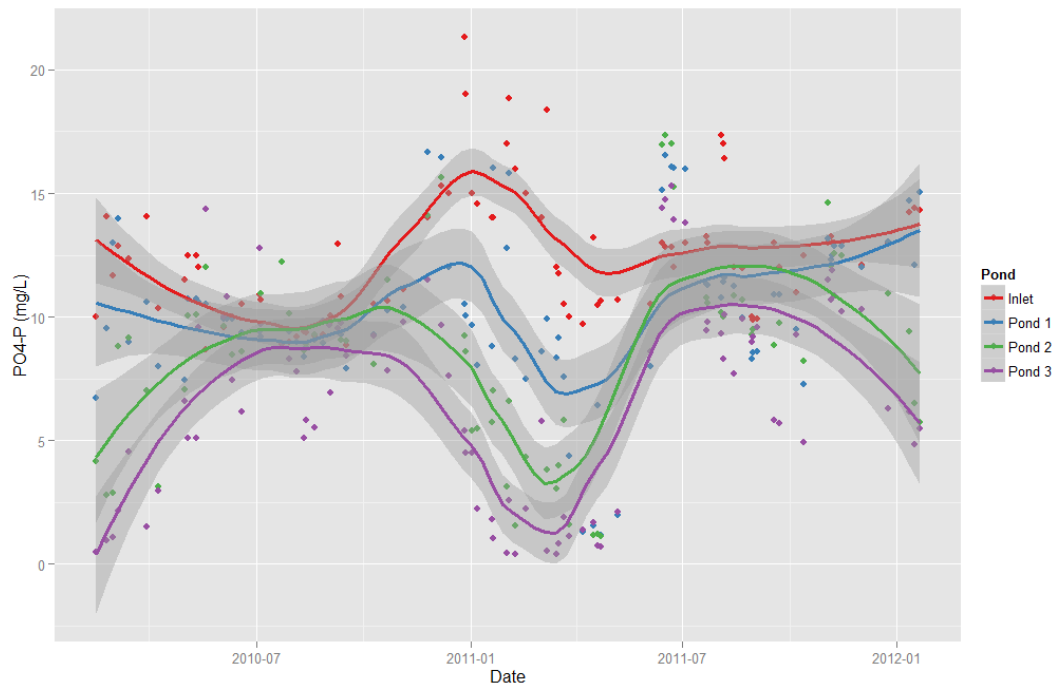




**Fig. 4-19 WSP 1 – loess fit and 95% confidence intervals for PO<sub>4</sub>-P incoming (red), outgoing (purple), outgoing as algal-P (green) and removed by internal precipitation (blue) over time.**

It can be seen in Fig. 4-19 that between 1.8 and 2.8 kg PO<sub>4</sub>-P /day entered WSP1. For half of the time that amount exited unchanged. However, from September 2010 to April 2011, significantly less unchanged PO<sub>4</sub>-P exited WSP 1 than entered. During this period, a compensatory amount of P exited WSP 1 as algal-P or precipitated, either in dead algal cells or in a chemical complex. Phosphorous exiting as algal-P reached a maximum of 1 kg/day in November 2010 and again in January 2012. Comparisons with world literature removal efficiencies are provided in Para 4.4.

Chemical precipitation of PO<sub>4</sub>-P can happen as a struvite complex in association with calcium and magnesium cations interacting with the phosphate anion to form an insoluble precipitate. Precipitation is favoured under conditions of high pH. No measure was made of calcium or magnesium levels in this study, and it is postulated that this occurred to some degree in the January to April period when pH levels peaked (Fig. 4-1 d).



**Fig 4-20 Lyndoch WSP1, 2 & 3 2010/12 Scatterplot and loess-fit curve with 95% CI of PO<sub>4</sub>-P concentration as it passes through each treatment phase for the three ponds sequentially from top to bottom Inlet(red), WSP1 (blue), WSP2 (green) & WSP3 (purple)**

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## CHAPTER 5

# PREDICTING HRAP POND PERFORMANCE

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### **5.1 The need to understand pond performance - for wastewater treatment management and for predicting biomass productivity**

To avoid critical operational malfunctions and for future design and planning purposes, the operators of the treatment plant need to understand the factors that influence the performance of wastewater treatment systems, and to be able to predict performance and potential malfunction. All natural treatment systems, such as ponds, rely for their efficacy on a range of environmental

factors impinging on the complex microbial communities and rich nutrient media in which they operate.

There are a number of factors considered important in the assessment of efficacy of wastewater treatment systems. Some can be managed operationally, such as HRAP depth, speed of mixing and hydraulic retention time. Others are the effect of the local environment such as air temperature, wind speed and direction, rainfall, total solar radiation and ultraviolet radiation. (Figs. 3-1 & 3-23) For this longitudinal study, these potential effectors have been continuously logged over the duration of the study and presented as longitudinal graphs in the Chapter 3, e.g. Figs. 3-1 (daily average air temperature & rainfall), 3-3 (wind speed and direction), 3-7 (daily solar radiation) and 3-10 (hourly solar radiation). For analysis and to help understand subtleties in daily performance, these effectors are introduced to the analytical process both in terms of an average daily value and/or a peak and/or minimum daily value. Finally, there are the “in-pond” characteristics that reflect both the incoming load of micro biota and physico-chemical changes induced in the HRAP by the photosynthetic activity of the ever present algal population, and the grazers and pathogens that disrupt the algal community. Some of these effectors were continuously logged, such as pH, DO and temperature (see Figs. 3-1 and 3-7) and others such as suspended solids, turbidity, chlorophyll *a*, *E. coli*, NH<sub>4</sub>-N, NO<sub>3</sub>-N, NO<sub>2</sub>-N, PO<sub>4</sub>-P and BOD<sub>5</sub> were analysed from water samples collected by the refrigerated auto-sampler, and returned to the laboratory. The last six on this list represent the target indicators of wastewater treatment performance.

From the operators’ viewpoint, the environment is not predictable on a day-to-day basis, but is generally more predictable at a seasonal level, so the available tools for control are quite coarse and limited to HRAP depth, speed of mixing and hydraulic retention time set on a seasonal basis, if adjusted at all.

Further complexity is added to the predictive systems as most of the key performance indicators are subject to control by a number of different predictor

factors interacting with each other, e.g. solar radiation and pond temperature are both significant predictors of many effects in the constantly moving HRAP water. However, as the total solar radiation rises and falls so does the pond temperature. It is often difficult to distinguish between the two effects.

### **5.1.1 Performance Prediction by regression tree analysis**

After considering many forms of analysis of the complex data sets and the interactions between effector factors, it was decided that multiple linear regression would not provide constructive answers and similarly, stoichiometric modelling since it was considered more appropriate to chemical reactants treated in abiotic systems. Furthermore, it was recognised that empirical equations developed for this HRAP system would not necessarily be transferrable to similar systems in other locations. It was decided that the most appropriate tool to use would be regression tree modelling and adding further diagnostic power by performing boot-strap analysis.

As noted in Chapter 2, regression tree modelling sub-divides, or partitions, the complex data space into smaller regions, where the interactions are more manageable. This is followed by further partitioning into more sub-divisions — called recursive partitioning — until finally the ‘chunks’ of data space are so ‘tame’ that simple linear models fit into them. The global model now has two parts: one is the recursive partition, the other is a simple model for each cell of the partition. As one method of reporting results, these two pieces of data can be combined into a prediction tree.

Prediction trees use the tree to represent the recursive partition. Each of the terminal nodes, or leaves, of the tree represents a cell of the partition, and has attached to it a simple linear model which applies to that cell only. A point ( $x$ ) belongs to a leaf if ( $x$ ) falls in the corresponding cell of the partition. Starting at the root node of the tree, a sequence of questions is asked about the features. The interior nodes are labelled with questions, and the edges or branches

between them labelled by the answers. Which questions are asked next depends on the answers to previous questions.

### **5.1.2 Performance indicators analysed**

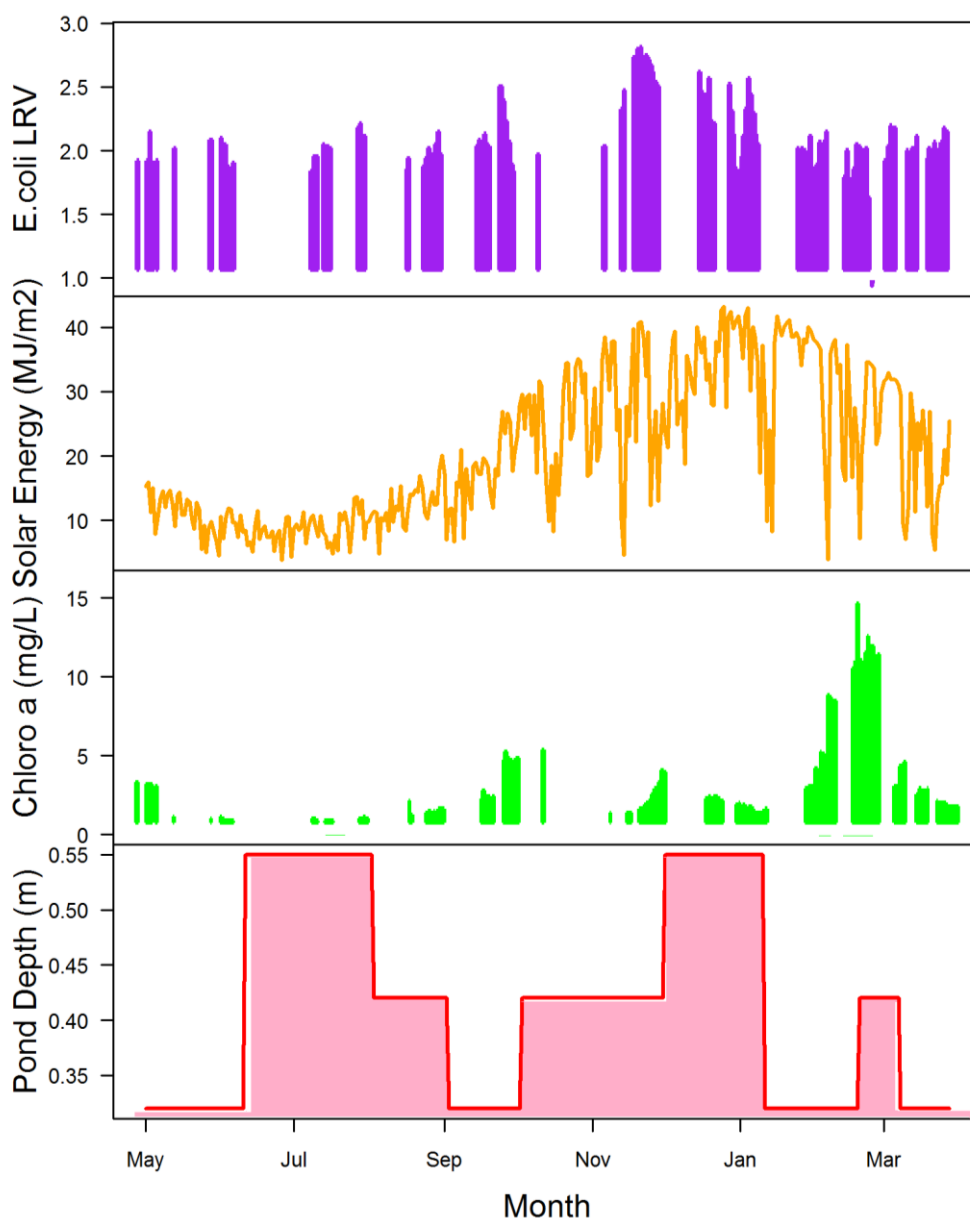
If the treated water is to re-enter a stream or river system, then removal of major nutrients that can cause eutrophication of the receiving water are very important. In many European countries this is considered the major factor in performance assessment. In this chapter, the factors influencing the removal of BOD<sub>5</sub> (as an indicator of available organic carbon) and total inorganic nitrogen and ammonia nitrogen will be considered. The factors influencing the removal of phosphate will not be considered as there was insufficient phosphate removed to warrant investigation.

In rural South Australia, there is a great need for treated water to be available for re-use for either horticultural, agricultural, woodlot activities or supporting town amenities, such as ovals and planted median strips. From the perspective of potential hazard to human health, there is a need to understand the microbiological nature of the water to be re-used, as a means of calculating any risk with the re-use of that water. As discussed earlier, a number of organisms have previously been identified as suitable to act as indicators of overall microbiological status. In general, these organisms have the characteristics of being easily enumerated, sourced exclusively from human waste material and being more resistant to environmental factors than the pathogenic organisms. This study considers the inter-relationship of factors involved in the removal of *E. coli* as an indicator of pathogenic microbial removal effectiveness of the HRAP systems considered.



## 5-2 Identification of predictors of *E. coli* Removal

From previous laboratory (Bolton et al., 2010) and pond (Curtis et al., 1992b, Curtis et al., 1992) studies much is known about the potential factors that could result in the death of key indicator organisms such as *E. coli* in HRAPs. Fig. 5-1 shows the measured *E. coli* LRV in HRAP1 fed wastewater pre-treated in a septic tank, with successively below it, total solar energy, measured chlorophyll *a* and finally the pond depth.



**Fig 5-1 HRAP 1 fed septic tank effluent: Time series showing, from the top the relationship between *E.coli* LRV (purple bars at top) and Global Solar Energy (orange line) , chlorophyll *a* (green bars) concentrations and the operational pond depth (pink columns).**

It can be recognised from Fig. 5-1 that the peak of *E. coli* LRV took place during November. At other times the *E. coli* LRVs were remarkably consistent, and certainly not subject to any period of abnormally poor performance. In particular, by observing the temporal relationships, it can be seen that pond depth was not consistently associated with either high or low *E. coli* LRV. Likewise, it can be noted that the peak *E. coli* LRV occurred before the absolute (maximum) peak of solar radiation at the end of December. The huge peak in chlorophyll *a* in late February was associated with a small dip in *E. coli* LRV. This did not appear to happen with the smaller peaks of chlorophyll *a*, for example the period in early October.

This series of complex events and interactions are consistent with the complex, rapidly changing ecological/biological mix happening in this natural wastewater treatment system. These findings reinforce the need to use a tool such as regression tree analysis to highlight the factors that were most significant in affecting the removal of *E. coli* from the HRAP.

### **5.2.1 The *rpart* tool for predicting *E. coli* LRV**

The standard R function for building tree models is '**rpart**' (standing for recursive partitioning). This tool produces the classical regression tree as can be seen in Fig. 5-2.

The report generated by the *rpart* software includes an estimate of the importance of each predictor in determining the *rpart* tree. This is another parameter which can be used as an adjunct to the visualisation provided by the tree output to indicate to aid the dissection of a large number of possible predictor variables in a scale of importance. These results are reported in the

fourth column of Tables 5-1 to 5-7 inclusively. The order in which each variable is reported in these tables is based on the node improvement value generated by the boot-strapped randomForest software, so is not in order of importance as determined by rpart.

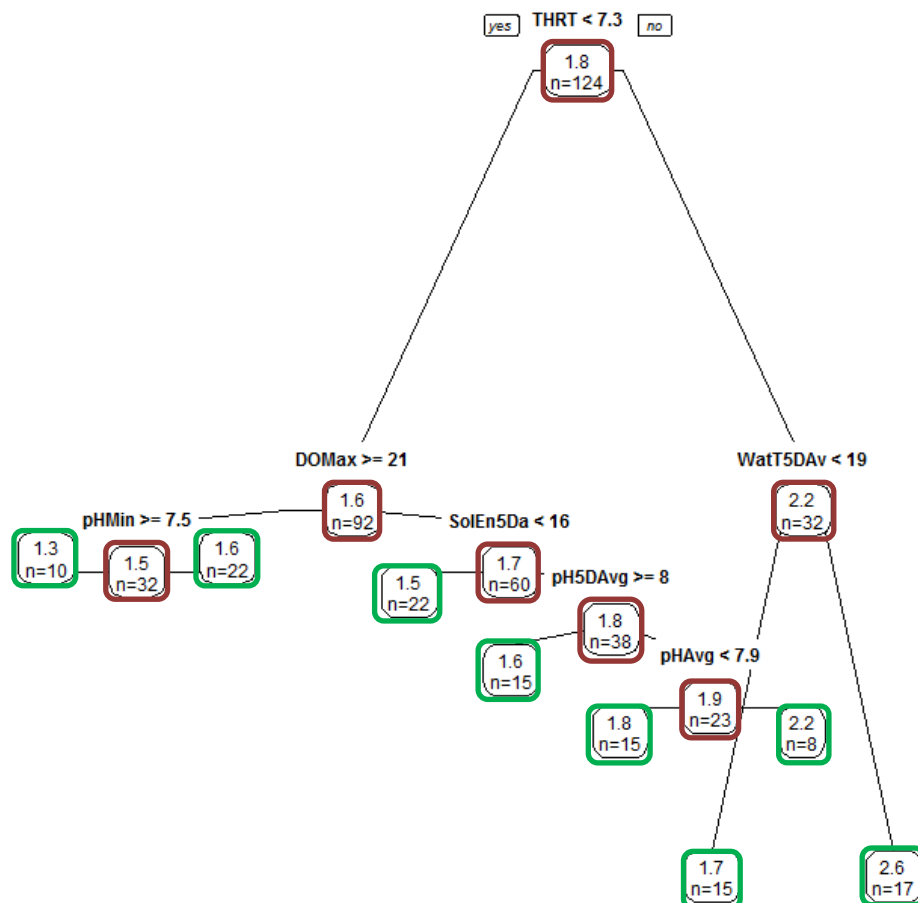


Fig. 5-2 HRAP1 fed septic tank effluent operated at 0.32, 0.42 and 0.55m, rpart Decision Tree for *E. coli* LRV, where the variables selected for analysis by rpart were, Node1 - theoretical hydraulic retention time (THRT, d), Node 2 - maximum daily dissolved oxygen (DOMax, mg/L), Node 3 - 5 day average water temperature (WatTemp5DAvg, °C); Node 4 - minimum water pH (pHMin); Node 5 - 5 day average Solar Energy (SolEn5Da, W/m<sup>2</sup>); Node 6 - 5 day average water pH (pH5DAvg); and Node 7 - average water pH (pHAv) The mean *E. coli* LRV for that group and the number of observations (n) analysed is presented inside the red rectangle for each node; and for each green rectangle at each leaf.

There are seven decision nodes in the rpart tree represented in Fig. 5-2 using the following abbreviations:-

Node 1 THRT = Theoretical Hydraulic Retention Time (days)

Node 2 DOMax = maximum daily dissolved oxygen (mg/L)

Node 3 WatTemp5DAvg = 5 day average water temperature (°C)

Node 4 pHMin = minimum water pH

Node 5 SolEn5Da = 5 day average Solar Energy (W/m<sup>2</sup>)

Node 6 pH5DAvg = 5 day average water pH

Node 7 pHAvg = average water pH

The vertical spacing of the nodes is proportional to the fit (in more precise statistical terminology the difference between a node's deviance and the sum of its two children's deviances).

There are eight leaves or end points (green rectangles in Fig. 5-2). These are added to the tree when further splitting would be of no advantage. Each has the number of observations that comprise that leaf and also the mean *E. coli* LRV associated with that leaf.

The first node divides the data into 2 groups (Fig 5.2), one set above 7.3 d THRT (taking the right branch), and the other set below 7.3 d THRT (taking the left branch). There were 124 observations taken into account initially, and the mean *E. coli* LRV for those 124 observations was 1.8. Most importantly, this node explained 29.9% of the deviance. In other words, whether the THRT was above or below 7.3 days explains nearly 1/3 of the variance in *E. coli* LRV in this data set. This is represented graphically by the length of the branch before the next node. It is clear this is the longest branch.

The right hand branch (Fig. 5.2; THRT > 7.3 days) then divided at another node where the division was made on whether the 5 day average water temperature

was above or below 19°C (right branch and left branch respectively). The 32 observations at this point had a mean *E. coli* LRV of 2.2, and this split explained a further 25.3% of the deviance. From this node two leaves are produced. On the left (Leaf 8) the 15 observations had a mean *E. coli* LRV of 1.7. The right leaf (9) had 17 observations with a mean *E. coli* LRV of 2.6.

The left hand branch (THRT > 7.3 d; Fig. 5.2) then bifurcated at another node where the division was made on whether the maximum daily dissolved oxygen was above or below 21 mg/L. The 92 observations at this point had a mean *E. coli* LRV of 1.6, and this split explained only a further 4.8% of the deviance. From this node, four further nodal bifurcations are produced by the software, but each of these nodes explain ever less of the variance, which is visually obvious by the short branches leading from each of these nodes.

The rpart tree in Fig. 5-2 explains in total 76.4% of the total deviance. Of this, a combined 29.9% and 25.3% (total of 55.2%) can be explained by the first two nodes alone. The rest of the tree only explains 21.2% of the variance in *E. coli* LRV. As noted above, the extra diagnostic test results will not be presented here as they do not contribute to the further understanding of the factors that influence *E. coli* LRV.

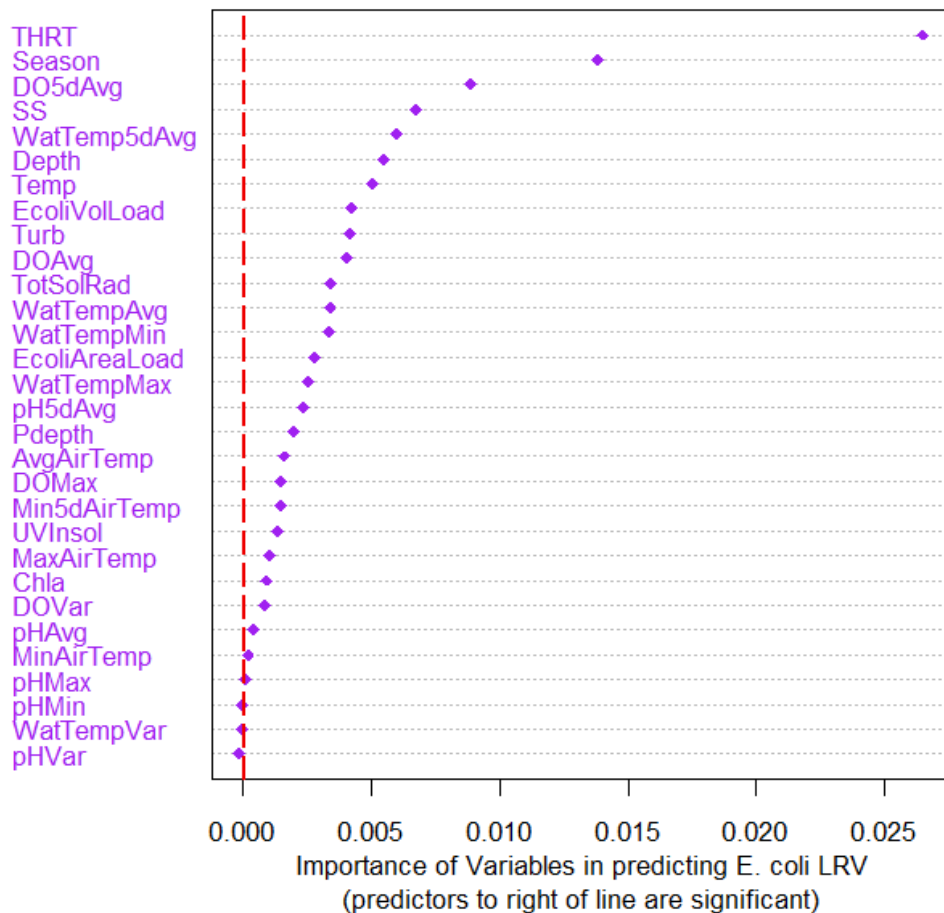
Also, as noted above, the software can be used to calculate the relative importance for each predictor. These are presented in column 4 in Table 5-1, along with the boot-strapped randomForest importance figures. Comparing the relative importance figure for all the predictor variables allows comparison of all the tree regression techniques used in this analysis and presented in para 5.2.4.

### **5.2.2 The randomForest tool**

The prediction results for a randomForest run of bootstrapped values generating 5,000 trees are shown in Table 5-1 (Column 2). These predictions explain 64.3% of the variance of *E. coli* LRV in the 5,000 trees.

### **5.2.3 The cForest tool**

Fig. 5-3 shows the cForest output for the *E. coli* LRV for HRAP 1, where once again Hydraulic Retention Time (0.026) was the most important followed by the season (0.015). Of interest, the 5 day average water temperature (0.006) was ranked fifth most important, compared to the second most important ranking allocated by randomForest (Table 5-1).



**Fig. 5-3 Chart showing relative importance of predictors used in a cForest bootstrap enhanced HRAP1 Decision Tree for *E. coli* LRV.**

### 5.2.4 Comparison of the relative importance of predictors by the three regression tree techniques

Table 5-1 shows the results for a randomForest run of bootstrapped values that produced 5,000 trees and explained 64.3% of the variance (Column 2), a cForest run of boot-strapped values producing 5,000 trees (Column 3), as well as the relative importance of predictor variables in the non-bootstrapped rpart tree (explains 76.4% of the *E. coli* LRV variance, Column 4).

**Table 5-1 HRAP1:- table of the ranking and relative importance of each predictor for *E. coli* LRV arranged in descending order of importance as ranked by the increase of node purity in randomForest (Column 2). Importance in cForest ranking is listed in Column 3 and importance in rpart is listed in Column 4. The top ten ranked variables in randomForest are highlighted in yellow for cForest and rpart.**

| Rank | Predictor Variable                   | randomForest & cForest<br>Boot-strapped |                       | rpart<br>Non boot-<br>strapped  |
|------|--------------------------------------|---|-----------------------|---------------------------------|
|      |                                      | Increase<br>Node<br>Purity              | Importance<br>cForest | rpart<br>variable<br>importance |
| 1    | Theoretical Hydraulic Retention Time | 3.887                                   | 0.026                 | 1.195                           |
| 2    | 5 day Avg. Water Temperature         | 2.249                                   | 0.0065                | 0.814                           |
| 3    | Season                               | 2.172                                   | 0.015                 | 0.249                           |
| 4    | 5 day Avg. DO                        | 1.241                                   | 0.009                 | 0.166                           |
| 5    | <i>E. coli</i> Volumetric Load Rate  | 1.144                                   | 0.0045                | 0.273                           |
| 6    | Total Solar Radiation                | 1.134                                   | 0.004                 | 0.127                           |
| 7    | <i>E. coli</i> Areal Load Rate       | 1.121                                   | 0.0035                | 0.273                           |
| 8    | Avg. Water Temperature               | 1.108                                   | 0.004                 | 0.416                           |
| 9    | Suspended Solids                     | 0.954                                   | 0.007                 | 0.429                           |
| 10   | UV Insolation                        | 0.877                                   | 0.002                 | 0.253                           |
| 11   | Max. Water Temperature               | 0.870                                   | 0.0032                | 0.193                           |
| 12   | Min. Water Temperature               | 0.761                                   | 0.004                 | 0.649                           |
| 13   | 5 day Min. Air Temperature           | 0.748                                   | 0.0022                | 0.657                           |
| 14   | Max. Air Temperature                 | 0.634                                   | 0.0018                | 0                               |
| 15   | DO Daily Variation                   | 0.631                                   | 0.0011                | 0.153                           |
| 16   | Chlorophyll a                        | 0.597                                   | 0.0012                | 0                               |
| 17   | Avg. Air Temperature                 | 0.577                                   | 0.0025                | 0.694                           |
| 18   | DO Daily Avg.                        | 0.530                                   | 0.0045                | 0.096                           |
| 19   | DO Daily Max.                        | 0.513                                   | 0.0021                | 0.102                           |
| 20   | 5 day Avg. pH                        | 0.508                                   | 0.0032                | 0.768                           |
| 21   | Turbidity                            | 0.471                                   | 0.0045                | 0                               |
| 22   | Min. Air Temperature                 | 0.467                                   | 0.0008                | 0                               |

|    |                                   |       |         |       |
|----|-----------------------------------|-------|---------|-------|
| 23 | Daily Variation Water Temperature | 0.454 | 0.000   | 0.194 |
| 24 | Pond Depth                        | 0.447 | 0.003   | 0.287 |
| 25 | Daily Variation pH                | 0.350 | -0.0007 | 0     |
| 26 | pH Max.                           | 0.317 | 0.000   | 0.139 |
| 27 | pH Min.                           | 0.290 | 0.000   | 0     |
| 28 | pH Avg.                           | 0.289 | 0.001   | 0.509 |

It is tempting to view the fact that the percent of variance explained by the non boot-strapped rpart technique (76.4%) is higher than that obtained by the boot-strapped technique (64.3%) as an indication that the rpart technique offers more insight in to the factors behind *E. coli* LRV in HRAP 1. It is more likely that the complex boot-strapped process amplifies and highlights the variability in all the possible predictor variables, without necessarily resolving their importance. The less complex rpart process to some degree overlooks the variability in the data, and may therefore provide an over-simplified answer.

The absolute value of the numbers reported is a function of the algorithms used and has no particular significance between columns. Within column the numbers represent the importance of each variable compared to all the other variables. The results are presented in order of descending importance of the randomForest predictors as defined by the relative increase in node purity.

Given that significantly different mathematical techniques are applied to the same data set, it is not surprising that the two boot-strap techniques arrive at a similar list of top ten most important predictors. They are however, quite different to the list generated by the non-boot-strap method, rpart.

The best predictor for *E. coli* LRV in HRAP 1 by all three regression tree techniques is the Theoretical Hydraulic Retention Time. It would appear that a longer hydraulic retention time is actually providing prolonged exposure to solar irradiation, high pH and DO and prolonged temperature exposure, all of which are known in-vitro contributors to *E. coli* die-off. It would appear that this prolonged exposure is necessary for *E. coli* die-off in HRAPs.



The average water temperature over the preceding 5 days was the next best predictor. The average water temperature on the same day was a less reliable guide. This is quite biologically plausible as it allows expression for the temperature effects to act over the full hydraulic retention time, not just on the day of the reading. In other words, the absolute value of a number of factors (including water temperature and DO) on any particular day is not a good predictor of what the *E. coli* LRV will be on that day, but that over a five day period the predictive value improves.

Following these two there was considerable divergence of results. In the bootstrapped techniques, the DO over the preceding 5 days was significant, as were the season and the volumetric loading rates (i.e. the incoming concentration of *E. coli*). It is interesting that the season as a whole is a better predictor than components that contribute directly to *E. coli* die-off in vitro, such as UV radiation and total solar radiation. Season can be seen as an indirect proxy for both sunlight and water temperature. As the season changes so do the sunlight radiation and the water temperature.

The non-boot-strapped rpart subsequently gives importance to pond and air temperature characteristics, but also to various measures of pH. pH factors have largely been ignored by the results of the two boot-strapped measures. As pH is a strong feature of in-vitro *E. coli* die-off it is interesting that other factors mentioned above appear to be of more significance in the *E. coli* die-off in the HRAP.

## **5-3 Identification of predictors of BOD<sub>5</sub> Removal**

The same predictive techniques that were applied to *E. coli* LRV were applied to BOD<sub>5</sub> removal.

### **5.3.1 The rpart tool for predicting BOD<sub>5</sub> Removal**

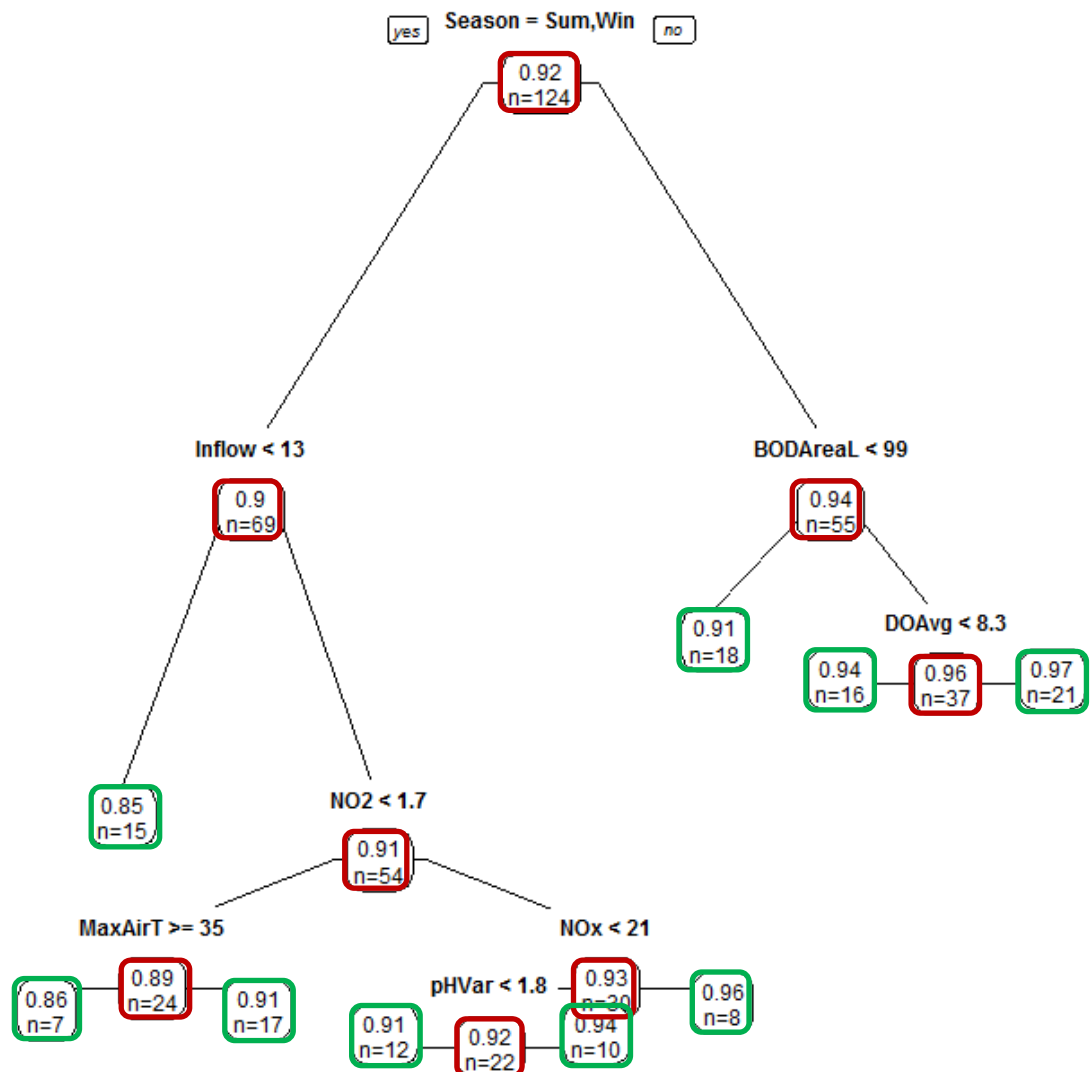


Fig. 5-4 HRAP1 rpart Decision Tree for predicting BOD<sub>5</sub> Removal Efficiency; inflow (kL/d), BOD Areal Load Rate (kg/ha/d); NO<sub>2</sub> (mg NO<sub>2</sub>-N /L); MaxAirT (maximum air temperature, °C), NOx (oxidised nitrogen, mg N/ L) and pHVar (diurnal variation in pH).

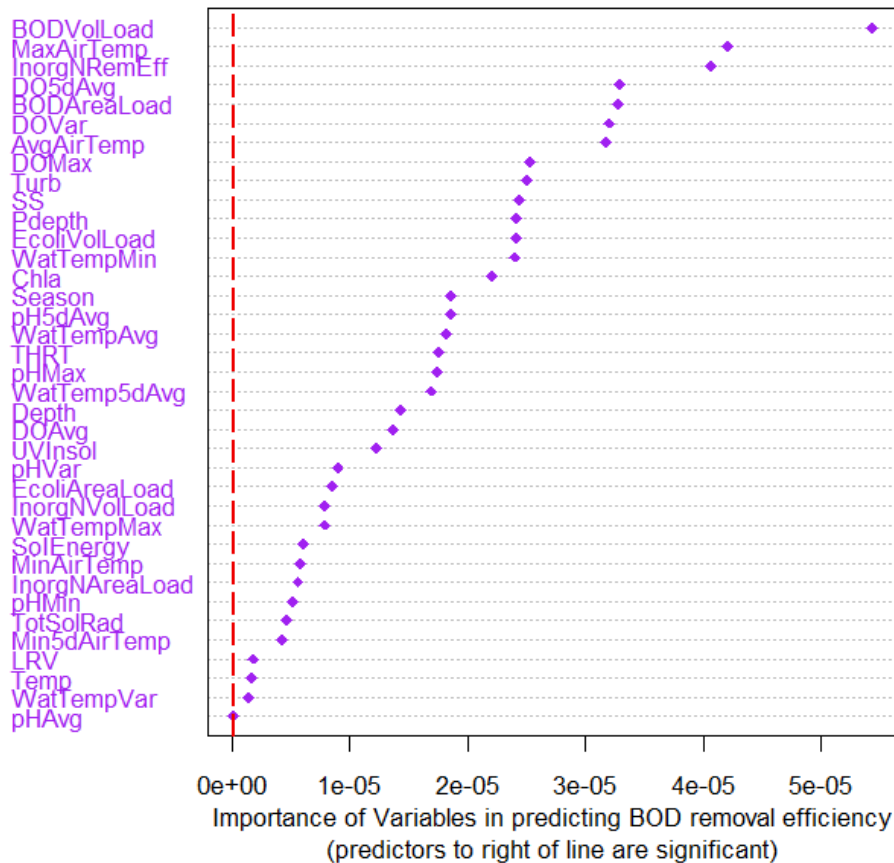
Using the rpart tool the resultant regression tree is quite complex (Fig. 5-4) with the season, areal BOD<sub>5</sub> loading rate and inflow rate being the most important predictors. This tree was only able to account for 56% of the variance in the BOD<sub>5</sub> removal efficiency in the HRAP 1.

### 5.3.2 The randomForest tool for predicting BOD<sub>5</sub> Removal Efficiency

The results of rating the importance of predictors for BOD<sub>5</sub> Removal Efficiency via the randomForest run are presented in Table 5-3. This was run for 2,500 trees, however rather than improving the predictive value, this technique has only explained 26.3% of the variance. The factors that are important in the rpart model are still important in the randomForest model, with a few notable additions, i.e. 5 day average water temperature, 5 day average DO and 5 day average pH have become important in this bootstrapped model. These logically should be ranked high in the predictors as BOD<sub>5</sub> removal is essentially an oxidative process that proceeds more efficiently at higher temperatures and DO levels. This also explains why the season is highlighted as important as DO is dependent on algal growth which is in turn dependent on warm, sunlit conditions.

### **5.3.3 The cForest tool for predicting BOD<sub>5</sub> Removal Efficiency**

The chart in Figure 5-5 shows the outcome of the cforest run to rank the predictors of BOD<sub>5</sub> removal from HRAP 1. These results reinforce the previously described importance values under rpart and randomForest, i.e. volumetric BOD<sub>5</sub> loading rate and five day average DO levels are important.



**Fig. 5-5 Chart showing relative importance of predictors used in a bootstrap enhanced HRAP1 Decision Tree for BOD<sub>5</sub> Removal Efficiency.**

### 5.3.4 Comparison of the relative importance of predictors by the three regression tree techniques

Table 5-2 shows the results for a randomForest run of boot-strapped values which produced 2,500 trees and explained 26% of the variance (Column 2), a cForest run of boot-strapped values producing 2,500 trees (Column 3), as well as the relative importance of predictor variables in the non-boot-strapped rpart tree (explains 56% of the BOD<sub>5</sub> removal efficiency variance, Column 4). Again, the percent of variance explained by the non-boot-strapped rpart technique (56%) is higher than that obtained by the boot-strapped technique (26.3%). The more complex boot-strapped process amplifies and highlights the variability in all the possible predictor variables, without necessarily resolving their importance, and the rpart process may be over simplifying the result as discussed above.

**Table 5-2 HRAP1:- table of the ranking and relative importance of each predictor for BOD<sub>5</sub> removal efficiency arranged in descending order of importance as ranked by the increase of node purity in randomForest (Column 2). Importance in cForest ranking is listed in Column 3 and importance in rpart is listed in Column 4. The top ten ranked variables in randomForest are highlighted in yellow for cForest and rpart.**

| Rank | Predictor Variable                          | randomForest & cForest<br>Boot-strapped        |   | rpart<br>Non boot-<br>strapped |
|------|---|--|---|--------------------------------|
|      |   | Increase<br>Node<br>Purity( $\times 10^{-2}$ ) | Importance<br>cForest<br>( $\times 10^{-5}$ ) | Importance<br>rpart            |
| 1    | <b>BOD<sub>5</sub> Volumetric Load Rate</b> | 1.20   | 5.4   | 0.174                          |
| 2    | <b>Maximum pH</b>                           | 1.18   | 1.9   | 0.529                          |
| 3    | <b>5 day Avg. DO</b>                        | 1.17   | 3.2   | 0.351                          |
| 4    | <b>5 day Avg. pH</b>                        | 1.16   | 2.0   | 0.301                          |
| 5    | <b>Maximum DO</b>                           | 1.13   | 2.6   | 0.268                          |
| 6    | <b>Maximum Air Temperature</b>              | 1.10   | 4.1   | 1.029                          |
| 7    | <b>5 day Avg. Water Temperature</b>         | 1.07   | 1.85  | 0.817                          |
| 8    | <b>Chlorophyll a</b>                        | 0.97   | 2.3   | 0.806                          |
| 9    | <b>Daily Variation DO</b>                   | 0.93   | 3.0   | 0.302                          |
| 10   | <b>Daily Avg. Water Temperature</b>         | 0.92   | 3.0   | 1.119                          |
| 11   | <b>Daily Avg. Air Temperature</b>           | 0.91   | 1.92  | 0.806                          |
| 12   | <b>Minimum Water Temperature</b>            | 0.89   | 2.4   | 0.529                          |
| 13   | <b>Daily Avg. DO</b>                        | 0.88   | 1.45  | 0.475                          |
| 14   | <b>Suspended Solids</b>                     | 0.73   | 2.45  | 0.536                          |
| 15   | <b>Turbidity</b>                            | 0.65   | 2.6   | 0.562                          |
| 16   | <b>Minimum pH</b>                           | 0.59   | 0.72  | 0                              |
| 17   | <b>BOD<sub>5</sub> Areal Load Rate</b>      | 0.58   | 3.2   | 0                              |
| 18   | <b>Daily Variation pH</b>                   | 0.54   | 0.9   | 0                              |
| 19   | <b>UV Insolation</b>                        | 0.52   | 1.0   | 0.190                          |
| 20   | <b>Minimum Air Temperature</b>              | 0.48   | 0.7   | 0.180                          |
| 21   | <b>Total Solar Radiation</b>                | 0.45   | 0.6   | 0                              |
| 22   | <b>Maximum Water Temperature</b>            | 0.44   | 0.9   | 0.467                          |
| 23   | <b>5 day Avg. Minimum Air Temperature</b>   | 0.43   | 0.6   | 0                              |
| 24   | <b>Season</b>                               | 0.40   | 2.0   | 0.569                          |
| 25   | <b>Daily Avg. pH</b>                        | 0.39   | 0.0   | 0.889                          |
| 26   | <b>Daily Variation Water</b>                | 0.35   | 0.1   | 0                              |

| Rank | Predictor Variable                          | randomForest & cForest<br>Boot-strapped        |   | rpart<br>Non boot-<br>strapped |
|------|---|--|---|--------------------------------|
|      |   | Increase<br>Node<br>Purity(x10 <sup>-2</sup> ) | Importance<br>cForest<br>(x10 <sup>-5</sup> ) | Importance<br>rpart            |
|      | <b>Temperature</b>                          |  |   |                                |
| 27   | <b>Theoretical Hydraulic Retention Time</b> | 0.32   | 1.9   | 0.196                          |
| 28   | <b>Pond depth</b>                           | 0.12   | 2.45  | 0                              |

As noted in para 3.2.1.2, it is logical to assume that the high BOD<sub>5</sub> removal efficiency measured in this study can be directly attributable to the high dissolved oxygen environment created by algal photosynthesis. The relatively high ranking of DO in the boot-strapped methods supports this contention. Many other parameters seem equally important, which may reflect more on the lack of discriminating power of the software employed.

## 5-4 Identification of predictors of NH<sub>4</sub>-N Removal Efficiency

The same predictive techniques that were applied to *E. coli* LRV and BOD<sub>5</sub> removal efficiency were applied to NH<sub>4</sub>-N removal efficiency. The results of the individual diagnostic techniques are not presented. Instead the summary table (5-3) is presented to highlight the similarity of the findings via all three techniques.

### 5.4.1 Comparison of the relative importance of predictors by the three regression tree techniques

Table 5-3 HRAP1:- table of the ranking and relative importance of each predictor for NH<sub>4</sub>-N removal efficiency arranged in descending order of importance as ranked by the increase of node purity in randomForest (Column 2). Importance in cForest ranking is listed in Column 3 and importance in rpart is listed in Column 4. The top ten ranked variables in randomForest are highlighted in yellow for cForest and rpart.

| Rank | Predictor Variable                      | randomForest & cForest<br>Boot-strapped        |  | rpart<br>Non boot-<br>strapped |
|------|---|--|--|--------------------------------|
|      |   | Increase<br>Node<br>Purity( $\times 10^{-2}$ ) | Importance<br>cForest ( $\times 10^{-4}$ ) | Importance<br>rpart            |
| 1    | Daily Variation DO                      | 122.76   | 21   | 1.258                          |
| 2    | Chlorophyll a                           | 94.65  | 32   | 1.705                          |
| 3    | 5 day Avg. Water<br>Temperature         | 60.18  | 11.5                                       | 2.173                          |
| 4    | Maximum DO                              | 50.80  | 10   | 0.603                          |
| 5    | Maximum Water<br>Temperature            | 34.83  | 9.5  | 0.893                          |
| 6    | Daily Avg. Water<br>Temperature         | 33.66  | 8  | 1.280                          |
| 7    | Turbidity                               | 14.71  | 4.2  | 0.754                          |
| 8    | Maximum pH                              | 11.02  | 6  | 0.254                          |
| 9    | Suspended Solids                        | 10.21  | 4.8  | 0.754                          |
| 10   | Minimum Water<br>Temperature            | 9.63   | 4.6  | 0.247                          |
| 11   | 5 day Avg. pH                           | 7.15   | 0.4  | 0.681                          |
| 12   | Total Solar Radiation                   | 5.37   | 3.8  | 0                              |
| 13   | 5 day Avg. DO                           | 5.32   | 3.7  | 0                              |
| 14   | Theoretical Hydraulic<br>Retention Time | 5.24   | 3.7  | 0.239                          |
| 15   | Depth                                   | 4.84   | 0.8  | 0.348                          |
| 16   | Daily Avg. Air<br>Temperature           | 4.60   | 0.4  | 0                              |
| 17   | Minimum 5 d Air<br>Temperature          | 4.41   | 0.75                                       | 0.281                          |
| 18   | UV Insolation                           | 4.34   | 0.6  | 0                              |
| 19   | Daily Avg. DO                           | 4.33   | 0.61                                       | 0                              |
| 20   | Maximum Air Temperature                 | 3.68   | 0.5  | 0.681                          |
| 21   | Inorganic-N Volumetric<br>Load Rate     | 2.99   | 0.78                                       | 0                              |
| 22   | Season                                  | 2.76   | 35   | 0.718                          |

| Rank | Predictor Variable                | randomForest & cForest<br>Boot-strapped        |  | rpart<br>Non boot-<br>strapped |
|------|-----------------------------------|--|--|--------------------------------|
|      |                                   | Increase<br>Node<br>Purity( $\times 10^{-3}$ ) | Importance<br>cForest ( $\times 10^{-4}$ ) | Importance<br>rpart            |
| 23   | Inorganic-N Areal Load Rate       | 2.66   | 0.57                                       | 0                              |
| 24   | Daily Avg. pH                     | 2.60   | 0.1  | 0                              |
| 25   | Daily Variation pH                | 2.50   | 1.1  | 0                              |
| 26   | Daily Variation Water Temperature | 2.31   | 0.3  | 0                              |
| 27   | Minimum pH                        | 1.77   | 0.08                                       | 0                              |
| 28   | Minimum Air Temperature           | 1.77   | 0.0  | 0                              |

The regression tree analyses for NH<sub>4</sub>-N Removal Efficiency are simpler and more diagnostic than either of the previous two examples. The rpart version (Table 5-4) effectively explains 90% of the variance and the randomForest version (Table 5-4), utilising 2,500 trees, explains 92% of the variance.

All three models rank chlorophyll a levels, daily variation in DO levels and the five day average water temperature as the key predictors of NH<sub>4</sub>-N Removal Efficiency. As discussed in para 3.2.1.3 and demonstrated in Fig. 3-13, incoming inorganic nitrogen (99+% is ammonia) is processed down three exit pathways. From the results reported in para 3.2.1.3, 43% of the inorganic-N exits the HRAP either in the original reduced form or in an oxidised form. A further 24% exits in organic form as part of the algal biomass. As well, 33% exits as ammonia, and as previously explained this is dependent on temperature, pH and pond depth. As described by Equation 1-51, the rate of NH<sub>3</sub>-N volatilisation depends on the concentration of ammonia gas, (temperature and pH dependent, see Fig. 1-10 and 1-13), depth of the system and a mass transfer coefficient, which is also temperature dependent.

The important prediction factors in all three regression trees, chlorophyll a levels, daily variation in DO levels and the five day average water temperature would support the view that other than that which leaves the system



unchanged (or oxidised), incorporation into algal biomass and volatilisation of ammonia are active in the HRAP.

## 5-5 Identification of predictors of Biomass Productivity

The same predictive techniques that were applied to *E. coli* LRV and BOD<sub>5</sub> removal efficiency were applied to biomass productivity. To avoid possible sources of misleading results, the data for the month of February 2011 were removed prior to commencing analysis, as previously described (para 3.1.6). The results of the individual diagnostic techniques are not presented here. Instead the summary table (5-4) is presented and it also highlights the similarity of the findings in the boot-strapped techniques. It must be noted that the experimental protocol was designed to answer the hypothesis that a HRAP could perform the same wastewater treatment process as a WSP system. The HRAP was operated at differing depths, which automatically changed the HRT. It was thus impossible to separate the effects of changing depth from changing HRT and no attempt was made to so in the analysis.

### 5.5.1 Comparison of the relative importance of predictors by the three regression tree techniques

As previously encountered, the two boot-strapped techniques produced results that are closely related (Table 5-4), compared to the non-boot-strapped technique. In this case, it is clear that boot-strapping improved the predictive performance, as primary nutrients NH<sub>4</sub>-N and sunlight were ranked very highly with boot-strapping, but not so highly without boot-strapping. Whilst turbidity is a useful guide to the in-pond light environment, and therefore may be expected to be an important predictor of suspended solids productivity, it cannot be incorporated in this analysis as it co-varies with suspended solids

concentration. A more detailed study would be required to determine the level at which turbidity becomes a clear impediment to photosynthesis.

As previously noted the percentage of variance explained by the rpart tree is quite high at 79.2% compared to the randomForest tree at 68.8%.

Of significant interest is the very high ranking of ammonia levels. This is consistent with the discussion in para 3.1.6.1 and 3.1.6.3, which highlight the importance of nutrient supply for algal growth. As noted in those paragraphs, the ammonium and phosphate ions are key algal nutrients along with inorganic carbon (not measured). It is significant that both are ranked highly by the bootstrapped measures as well as sunlight.

The other significant predictor is the theoretical hydraulic retention time. This implies that further increases of algal biomass are possible in the 4.5 to 9 day retention periods used in this study. It would be of interest to study the effect of algal harvest to see if accumulated algal biomass is a good indicator of total harvestable biomass

**Table 5-4 HRAP1:- table of the ranking and relative importance of each predictor for biomass productivity arranged in descending order of importance as ranked by the increase of node purity in randomForest (Column 2). Importance in cForest ranking is listed in Column 3 and importance in rpart is listed in Column 4. The top ten ranked variables in randomForest are highlighted in yellow for cForest and rpart.**

| Rank | Predictor Variable                   | randomForest & cForest<br>Boot-strapped |                       | rpart<br>Non boot-<br>strapped |
|------|--------------------------------------|---|-----------------------|--------------------------------|
|      |                                      | Increase<br>Node<br>Purity              | Importance<br>cForest | Importance<br>rpart            |
| 1    | NH <sub>4</sub> -N                   | 18886                                   | 110.2                 | 3.62                           |
| 2    | Theoretical Hydraulic Retention Time | 13023                                   | 158.6                 | 4.17                           |
| 3    | 5 Day Avg. Total Solar Radiation     | 10263                                   | 66.6                  | 10.23                          |
| 4    | Maximum DO                           | 9785.3                                  | 70.9                  | 5.24                           |
| 5    | Maximum pH                           | 9204.4                                  | 46.7                  | 0.63                           |
| 6    | Daily Avg. pH                        | 4196.4                                  | 21.8                  | 2.45                           |

|    |                                   |        |       |      |
|----|-----------------------------------|--------|-------|------|
| 7  | 5 Day Minimum Air Temperature     | 4083.5 | 12.6  | 7.25 |
| 8  | Inlet PO <sub>4</sub> -P          | 4000.2 | 31    | 4.61 |
| 9  | NO <sub>x</sub> -N                | 3815.1 | 13.38 | 7.16 |
| 10 | 5 Day Avg. pH                     | 3760.8 | 12.1  | 1.29 |
| 11 | 5 Day Avg. Air Temperature        | 3552.2 | 8.91  | 7.86 |
| 12 | Daily Variation DO                | 3054.2 | 21.2  | 5.72 |
| 13 | NO <sub>3</sub> -N                | 2941.7 | 16.2  | 2.02 |
| 14 | Inlet NO <sub>x</sub> -N          | 2941.5 | 9.67  | 0.84 |
| 15 | 5 Day Avg. W Temperature          | 2740   | 8.43  | 6.82 |
| 16 | Inlet Suspended Solids            | 2618.8 | 10.2  | 1.28 |
| 17 | PO <sub>4</sub> -P                | 2153   | 5.93  | 2.51 |
| 18 | NO <sub>2</sub> -N                | 2141.8 | 5.05  | 5.6  |
| 19 | Inlet NH <sub>4</sub> -N          | 2132.7 | 14.9  | 2.89 |
| 20 | Daily Avg. Air Temperature        | 2124.9 | 2.26  | 2.42 |
| 21 | Minimum DO                        | 1883.2 | 5.53  | 3.24 |
| 22 | 5 Day Avg. DO                     | 1661.8 | 2.28  | 0.92 |
| 23 | Maximum Air Temperature           | 1646.6 | 2.27  | 0.81 |
| 24 | Inlet BOD                         | 1570.4 | 3.16  | 0    |
| 25 | Daily Variation Water Temperature | 1401.9 | 8.19  | 0    |
| 26 | Maximum Water Temperature         | 1319.8 | 10.59 | 5.64 |
| 27 | Minimum Water Temperature         | 1261.1 | 2.66  | 2.5  |
| 28 | Daily Avg. Water Temperature      | 1259.7 | 5.92  | 0    |
| 29 | Minimum Air Temperature           | 1245   | 2.29  | 2.25 |
| 30 | Daily Avg. DO Avg                 | 1223.3 | 2.33  | 0    |
| 31 | Daily Variation pH                | 976.24 | 1.99  | 0    |
| 32 | Minimum pH                        | 647.56 | 0.05  | 0    |

A simple linear regression of the top three predictors yields the following relationship, which has a p-value of 5.2e-12, indicating strong statistical significance, but an R2 of only 0.39, indicating relatively poor predictive value.

$$\text{Suspended Solids Productivity (g/m}^2\text{/d)} = 0.73(\text{5 day avg. Total Solar Radiation}) - 7.83(\text{THRT}) - 0.52(\text{NH}_4\text{-N}) + 78.4 \dots\dots\dots \text{Eq. 5-1}$$

## 5.6 IDENTIFICATION OF PREDICTORS WHEN DATA IS COMBINED FROM BOTH HRAPs

There was insufficient data to allow meaningful tree regression analysis for HRAP 2 data. However, as the wastewater entering HRAP 2 was significantly lower in all major nutrient and pathogen indicator concentrations, it is of interest to understand the impact on performance prediction when the two sets of data are combined. Of main interest is to see whether the same predictors are still dominant despite the lower concentration of the analytes in the influent. The results of this section are reported in tabular form from a bootstrapped (randomForest) and non-boot-strapped (rpart) tree regression software output ranking each indicator in relative importance to the others in the set. It was decided to limit this part of the investigation to *E. coli* LRV and BOD<sub>5</sub> and NH<sub>4</sub>-N removal efficiencies. Little algae grew in HRAP 2, so this data was not used to try and further identify predictors for algal biomass production.

### 5.6.1 Identification of predictors of *E. coli* LRV - HRAP 1 & 2 combined

The *E. coli* organisms entering HRAP 2 from the facultative pond may simply be in a quiescent phase, or becoming increasingly unfit for survival. A response that may occur is the shut-down of major metabolic activity, due to the relatively hostile environment they have endured in the facultative pond compared to the gut from whence they emanated up to 36 days previously. Some of the conditions that may compromise *E. coli* fitness include high and fluctuating pH and DO, lower temperatures and depleted nutrient supplies. However, organisms existing near the bottom of the facultative pond at 1 metre depth may escape from these relatively hostile conditions. Unfortunately, this study will not have sufficient detail to determine the extent of these impacts on *E. coli* fitness.

Table 5-5 HRAP 1&2 combined:- ranking and relative importance of each predictor for *E. coli* LRV arranged in descending order of importance as ranked by the increase of node purity in randomForest (Column 3). Importance in rpart is listed in Column 4.

| Rank | Predictor                            | randomForest<br>Increase in<br>node purity | rpart<br>importance |
|------|--------------------------------------|--|---------------------|
| 1    | <i>E. coli</i> Areal Load Rate       | 10.06                                      | 18                  |
| 2    | Minimum DO                           | 7.51                                       | 13                  |
| 3    | Theoretical Hydraulic Retention Time | 6.60                                       | 8                   |
| 4    | <i>E. coli</i> Volumetric Load Rate  | 6.33                                       | 4                   |
| 5    | Maximum pH                           | 5.28                                       | 2                   |
| 6    | Daily Variation DO                   | 3.90                                       | 9                   |
| 7    | 5 Day Avg. Water Temperature         | 2.90                                       | 1                   |
| 8    | 5 Day Avg. pH                        | 2.87                                       | 3                   |
| 9    | Inflow Volume                        | 2.55                                       | 3                   |
| 10   | 5 Day Avg. Solar Energy              | 2.40                                       | 1                   |
| 11   | Minimum pH                           | 2.26                                       | 3                   |
| 12   | Maximum DO                           | 2.25                                       | 2                   |
| 13   | UV Insolation Rate                   | 2.14                                       | 2                   |
| 14   | Daily Avg. pH                        | 2.07                                       | <1                  |
| 15   | Daily Variation pH                   | 2.01                                       | 7                   |
| 16   | Season                               | 1.49                                       | <1                  |
| 17   | 5 Day Avg. DO                        | 1.44                                       | 2                   |
| 18   | Daily Avg. DO                        | 1.23                                       | 2                   |
| 19   | Total Solar Radiation                | 1.11                                       | 1                   |
| 20   | Suspended Solids                     | 1.03                                       | 1                   |
| 21   | Daily Avg. Water Temperature         | 0.96                                       | 1                   |
| 22   | Minimum Water Temperature            | 0.91                                       | 1                   |
| 23   | Maximum Air Temp                     | 0.85                                       | 1                   |
| 24   | Maximum Water Temperature            | 0.82                                       | <1                  |
| 25   | Minimum Air Temp                     | 0.77                                       | 1                   |
| 26   | Daily Avg. Air Temperature           | 0.71                                       | 1                   |
| 27   | Turbidity                            | 0.65                                       | <1                  |
| 28   | Daily Variation Water Temperature    | 0.61                                       | <1                  |

Table 5-5 shows the ranking and relative importance of the various predictors for *E. coli* LRV for the combined HRAP 1& 2 data. Comparing with the top 10 predictors for HRAP 1 (Table 5-1), there are a number of changes. Theoretical Hydraulic Retention Time changes from #1 to #3, 5 day Avg. Water Temperature changes from #2 to #6, Season changes from #3 to #16, 5 day average DO changes from #4 to #17, *E. coli* volumetric loading rate changes from #5 to #4, Total Solar radiation changes from #6 to #19, *E. coli* areal loading rate changes from #7 to #1, Daily average water temperature changes from #8 to #21, Suspended Solids changes from #9 to #20 and UV Insolation Rate changes from #10 to #13.

In general terms, the ranking of predictors is consistent with the die-off prediction model detailed in Chapter 7, which essentially argues that the loading rate and frequency govern the in-pond *E. coli* concentration, against a backdrop of consistent die-off due to solar radiation. Specifically, the rankings have shifted to emphasise loading rates and reduce emphasis on total solar and UV radiation. These changes are internally consistent if it is accepted that the amount of solar radiation is always in excess of needs under the conditions holding at Kingston-on-Murray.

The down-ranking of suspended solids is possibly due to the generally lower algal concentrations in HRAP 2 and thus a lower effect of shading influencing the *E. coli* die-off.

### **5.6.2 Identification of predictors of BOD<sub>5</sub> Removal – HRAP 1 & 2 combined**

Table 5-6 shows the ranking and relative importance of the various predictors for BOD<sub>5</sub> Removal Efficiency for the combined HRAP 1& 2 data. Comparing with the top 10 predictors for HRAP 1 (Table 5-2), there are a number of changes. These could be expected as the BOD<sub>5</sub> that entered HRAP 2 had already spent about 36 days in the facultative pond from which the water entering HRAP 2 was drawn. In this case the carbon has been exposed to oxidative microbial attack in the facultative pond to the point where the readily available carbon

has already been consumed and the remaining carbon forming the inlet BOD<sub>5</sub> is quite resistant to further microbial attack. It may be the case that some of the BOD<sub>5</sub> being recorded for HRAP 2 is actually microbial oxidation of nitrogen. This issue could be resolved by the incorporation of allylthiourea in separate BOD incubations to suppress the oxidation of nitrogen.

**Table 5-6 HRAP 1 & 2: - ranking and relative importance of each predictor for BOD<sub>5</sub> removal efficiency arranged in descending order of importance as ranked by the increase of node purity in randomForest (Column 3). Importance in rpart ranking is listed in Column 4.**

| Rank | Predictor                             | randomForest<br>Increase Node<br>Purity | rpart<br>importance |
|------|---------------------------------------|---|---------------------|
| 1    | BOD <sub>5</sub> Volumetric Load Rate | 1.56                                    | 22                  |
| 2    | Inlet BOD <sub>5</sub>                | 1.41                                    | <1                  |
| 3    | BOD <sub>5</sub> Areal Load Rate      | 1.32                                    | 20                  |
| 4    | Inlet NH <sub>4</sub> -N              | 0.528                                   | 14                  |
| 5    | Minimum DO                            | 0.419                                   | 16                  |
| 6    | Chlorophyll a                         | 0.086                                   | 1                   |
| 7    | Daily Variance DO                     | 0.081                                   | <1                  |
| 8    | 5 Day Avg. pH                         | 0.068                                   | <1                  |
| 9    | Inlet Suspended Solids                | 0.064                                   | 10                  |
| 10   | Inlet NO <sub>x</sub>                 | 0.063                                   | 3                   |
| 11   | Daily Variance pH                     | 0.060                                   | <1                  |
| 12   | Maximum DO                            | 0.059                                   | 1                   |
| 13   | NH <sub>4</sub> -N                    | 0.057                                   | 1                   |
| 14   | Hydraulic Retention Time              | 0.056                                   | <1                  |
| 15   | NO <sub>x</sub> -N                    | 0.050                                   | <1                  |
| 16   | PO <sub>4</sub> -P                    | 0.040                                   | 1                   |
| 17   | Maximum pH                            | 0.034                                   | 1                   |
| 18   | 5 Day Solar Energy                    | 0.033                                   | <1                  |
| 19   | Season                                | 0.028                                   | <1                  |
| 20   | Minimum daily pH                      | 0.025                                   | 1                   |
| 21   | 5 Day Avg. Water Temperature          | 0.025                                   | <1                  |
| 22   | Suspended Solids Productivity         | 0.025                                   | 5                   |
| 23   | Daily Avg. pH                         | 0.024                                   | 1                   |
| 24   | 5 Day Avg. DO                         | 0.021                                   | <1                  |
| 25   | Daily Avg. DO                         | 0.020                                   | 1                   |
| 26   | Minimum Water Temperature             | 0.018                                   | <1                  |
| 27   | Solar Energy                          | 0.017                                   | <1                  |
| 28   | UV Insolation                         | 0.014                                   | <1                  |

| Rank | Predictor                           | randomForest<br>Increase Node<br>Purity | rpart<br>importance |
|------|-------------------------------------|---|---------------------|
| 29   | Maximum Air Temperature             | 0.014                                   | <1                  |
| 30   | Daily Avg. Water Temperature        | 0.012                                   | <1                  |
| 31   | Daily Avg. Air Temperature          | 0.011                                   | <1                  |
| 32   | Daily Variance Water<br>Temperature | 0.010                                   | <1                  |
| 33   | Minimum Air Temperature             | 0.010                                   | <1                  |
| 34   | Maximum Water Temperature           | 0.009                                   | <1                  |
| 35   | Inflow Volume                       | 0.009                                   | <1                  |

BOD<sub>5</sub> Volumetric Load Rate remains #1, Maximum pH changes from #2 to #17, 5 day Avg. DO changes from #3 to #24, 5 day Avg. pH changes from #4 to #8, Maximum DO changes from #5 to #12, Maximum Air Temperature changes from #6 to #29, 5 day Avg. Water Temperature changes from #7 to #21, Chlorophyll a changes from #8 to #6, Daily Variation DO changes from #9 to #7, and Daily Avg. Water Temperature changes from #10 to #30.

Inlet BOD<sub>5</sub> and both volumetric and areal loading rates remain important predictors for BOD<sub>5</sub> removal efficiency. It is of note that the inlet NH<sub>4</sub>-N concentration has assumed an important ranking – presumably indicating that there is some oxidation of nitrogen by microbial activity. As noted above, this would need to be resolved by simultaneous BOD incubations with and without allylthiourea.

The other key feature is that chlorophyll a levels are now ranked slightly higher than in HRAP 1 alone. This is an indication that the lower algal concentrations (Fig. 3-7 and 3-27) in HRAP 2 have resulted in lower overall available dissolved oxygen and thus makes the algal concentration a more important predictor than when an over-abundance of algae and dissolved oxygen in HRAP 1 meant this resource was always available in abundant supply.



### 5.6.3 Identification of predictors of NH<sub>4</sub>-N Removal – HRAP 1 & 2 combined

Table 5-7 shows the ranking and relative importance of the various predictors for NH<sub>4</sub>-N Removal Efficiency for the combined HRAP 1& 2 data. Comparing with the top 10 predictors for HRAP 1 (table 5-3), there are a number of quite minor changes. These could be expected as the NH<sub>4</sub>-N that entered HRAP 2 had already spent about 36 days in the facultative pond from which the water entering HRAP 2 was drawn, which would result in much less NH<sub>4</sub>-N remaining. As described in para 3.5.3, 28% of the incoming ammonia exited as algal biomass and 24% exited as ammonia gas.

The rate of NH<sub>3</sub>-N volatilisation depends on the concentration of ammonia gas in the liquid, which is in balance with NH<sub>4</sub><sup>+</sup> ion concentration and dependent on temperature and pH, (see Equation 1-51 & Fig. 1-10 & 1-13) depth of the system and a mass transfer coefficient, which is also temperature dependent.

**Table 5-7 HRAP 1 & 2: - ranking and relative importance of each predictor for NH<sub>4</sub>-N removal efficiency arranged in descending order of importance as ranked by the increase of node purity in randomForest (Column 3). Importance in rpart is listed in Column 4.**

| Rank | Predictor                       | Increase in node purity | rpart importance |
|------|---------------------------------|-------------------------|------------------|
| 1    | Season                          | 2.225                   | 16               |
| 2    | Daily maximum DO                | 1.292                   | <1               |
| 3    | Chlorophyll a                   | 0.947                   | 4                |
| 4    | Daily maximum water temperature | 0.590                   | 11               |
| 5    | 5 day avg. Solar Radiation      | 0.559                   | 10               |
| 6    | Daily variation DO              | 0.515                   | 3                |
| 7    | Daily maximum pH                | 0.450                   | 1                |
| 8    | Daily avg. water temperature    | 0.345                   | 12               |
| 9    | 5 day avg. water temperature    | 0.328                   | <1               |
| 10   | Daily minimum pH                | 0.165                   | 1                |
| 11   | Suspended Solids Productivity   | 0.147                   | 1                |
| 12   | Inlet NH <sub>4</sub> -N        | 0.146                   | 2                |
| 13   | 5 day avg. pH                   | 0.145                   | 2                |

|    |  |       |    |
|----|--|-------|----|
| 14 | <b>Turbidity</b>                         | 0.145 | 2  |
| 15 | <b>PO<sub>4</sub>-P</b>                  | 0.144 | 1  |
| 16 | <b>Inorganic-N Load Rate</b>             | 0.139 | 2  |
| 17 | <b>Hydraulic Retention Time</b>          | 0.135 | 10 |
| 18 | <b>Daily minimum DO</b>                  | 0.123 | 1  |
| 19 | <b>Daily variation pH</b>                | 0.120 | <1 |
| 20 | <b>5 day avg. DO</b>                     | 0.109 | 1  |
| 21 | <b>Daily minimum water temperature</b>   | 0.101 | 11 |
| 22 | <b>Daily avg. pH</b>                     | 0.099 | 1  |
| 23 | <b>Daily avg. DO</b>                     | 0.094 | <1 |
| 24 | <b>Inlet Suspended Solids</b>            | 0.083 | <1 |
| 25 | <b>Inflow</b>                            | 0.058 | <1 |
| 26 | <b>Total Solar Radiation</b>             | 0.055 | <1 |
| 27 | <b>Inlet NO<sub>x</sub></b>              | 0.055 | 1  |
| 28 | <b>Daily avg. Air Temperature</b>        | 0.051 | <1 |
| 29 | <b>Daily variation water temperature</b> | 0.040 | <1 |
| 30 | <b>UV Insolation</b>                     | 0.040 | <1 |
| 31 | <b>Daily Maximum Air Temperature</b>     | 0.032 | <1 |
| 32 | <b>Daily Minimum Air Temperature</b>     | 0.028 | <1 |

Daily Variation DO changes from #1 to #6, Chlorophyll a changes from #2 to #3, 5 day Avg. Water Temperature changes from #3 to #9, Maximum DO changes from #4 to #2, Maximum Water Temperature changes from #5 to #4, Daily Avg. Water Temperature changes from #6 to #8, Turbidity changes from #7 to #13, Maximum pH changes from #8 to #7, Suspended Solids changes from #9 to #11, Minimum Water Temperature changes from #10 to #21

Chlorophyll a concentration is a very important predictor of NH<sub>4</sub>-N removal efficiency. This is consistent with the two removal mechanisms mentioned above, i.e. removal as algal biomass or conversion to ammonia gas which is dependent on high pH (Eq. 1-51).

The largest change in rankings is the season, which moves from #22 to #1. The influence of season may be through the temperature effects needed for ammonia volatilisation and for algal growth. Maximum and daily variation in DO are still very important predictors probably as a result of the link to algal growth. The other high ranking factors, pH and water temperature (as variously measured) are also factors that either drive algal growth (temperature) or are a consequence of algal growth (pH).

## **5.7 Summary of factors that influence the performance of HRAPs**

There are many overlapping, correlated and sometimes competing factors involved in the performance of the various wastewater treatment functions in the HRAP. These make it difficult, if not impossible to be dogmatic about which factors predominate. There are probably factors that are dominant at certain times of year and not others. The factors mentioned here are simply the highlights and represent the most well know pathways

It appears that the HRAP achieves *E. coli* disinfection mainly via solar radiation mechanisms, and these actions can be enhanced by retaining the wastewater for periods of up to 9 days. It also appears that *E. coli* disinfection is enhanced if the average water temperatures are above 25°C.

It appears that BOD removal is enhanced by high dissolved oxygen levels. These are often accompanied by high pH levels, but that high pH may be a correlated factor and not necessarily be contributing to the BOD removal. There appears to be plenty of reserve capacity (at least to 300 mg/L) for this activity, as the removal efficiency increases as the loading rate goes up.

NH<sub>4</sub>-N removal appears to be enhanced by conditions that increase the DO and pH levels. Indirect measurement in this study suggests that about 43% of NH<sub>4</sub>-N passes through the HRAP unchanged, 24% is assimilated into algal cells and 33% is volatilised off as NH<sub>3</sub>-N gas.

It appears that only 12% of PO<sub>4</sub>-P is removed from the HRAP and that all of this is removed via incorporation into algal cells.

It appears that there are initially plenty of dissolved inorganic nutrients in the HRAP to support algal growth, which means algal growth is then controlled by the pond temperature and solar radiation. The ammonia concentration does become an important predictor of algal productivity, apparently as much is used up in algal growth, and some volatilised.

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## CHAPTER 6.

# COMPARISON OF THE PERFORMANCE OF THE HRAP WITH A WSP SYSTEM

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The intent in this chapter is to consider the respective performances of the HRAP 1 operated with the same influent as the facultative pond in the WSP system, and to compare the performance in the removal of key nutrients, carbon (albeit organic carbon measured as BOD<sub>5</sub>), nitrogen (measured as NH<sub>4</sub>-

N), and phosphorous (measured as PO<sub>4</sub>-P) and the key pathogen indicator *E. coli*.

The same comparison (in section 6.8 onwards) was made between the performance of HRAP 2 and the two maturation ponds at Lyndoch combined using the same indicators. In that case the data was handled by treating the two maturation ponds as one continuous system. Comparison was made by comparing material entering WSP 2 and exiting WSP 3, with material entering and leaving HRAP 2. In some cases, (physico-chemical data) it was only feasible to present WSP 3 data (see para 6.8).

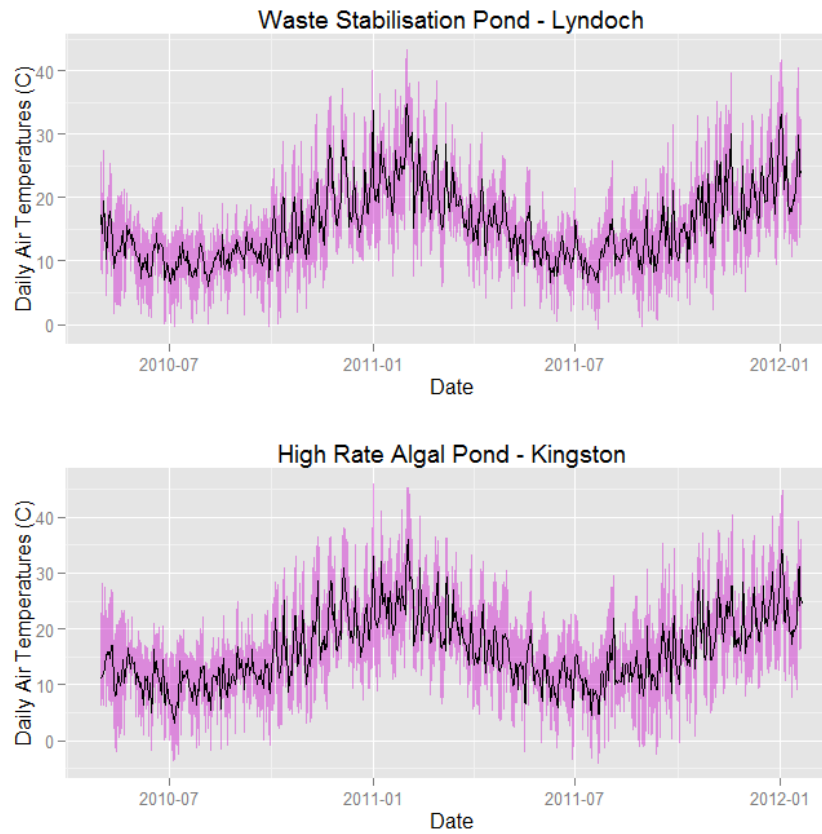
Initially, it was important to establish the comparability of the climate and the inlet water for both systems. These data have largely already been presented individually in Chapters 3 and 4, and will be represented here mainly in graphical format to allow ready visual comparison. This is followed by comparison of the physico-chemical data and the performance data.

Where possible a uniform colour scheme has been used in the graphical presentations. The HRAP data are presented in purple and the WSP data presented as green.

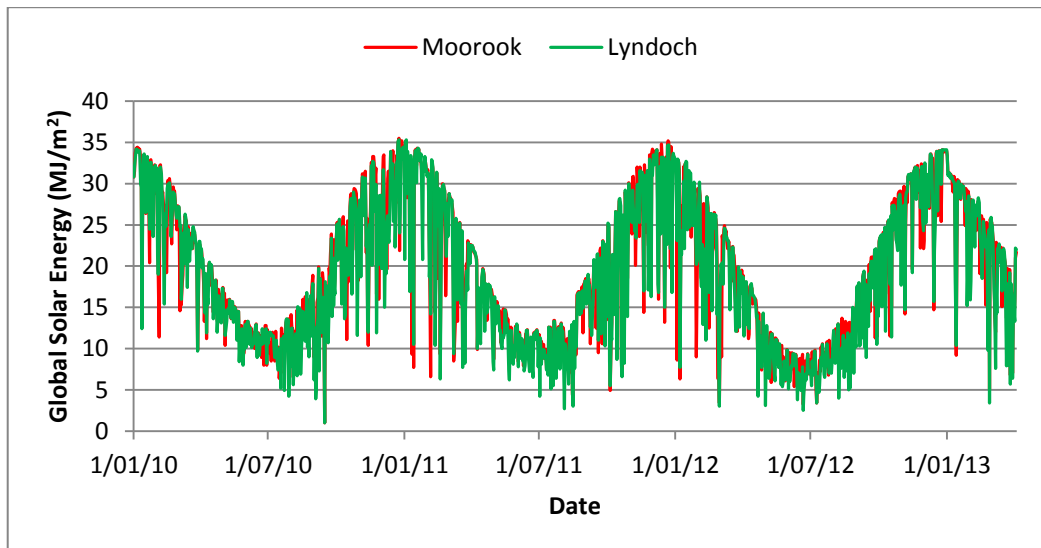
## 6.1 CLIMATE

### 6.1.1 Temperature and Global Solar Energy

From Fig. 6-1 it can be seen that the daily air temperatures were almost identical at the two sites. Small, but observable differences include the air temperature being 2-3°C hotter on the hottest days in summer and 2-3°C colder on the coldest mornings in winter at the more inland site at Kingston on Murray. These small differences in temperature *per se* would not be expected to have any influence on relative wastewater treatment performance at the two sites.



**Fig. 6-1 Time series comparison of daily air temperatures – maximum, minimum, and average of the WSP site at Lyndoch and the HRAP site at Kingston – recorded on site at the respective location over the study period.**



**Fig. 6-2 Bureau of Meteorology (2012) Global Solar Energy at Moorook (5 km from the HRAP at Kingston on Murray)) and Lyndoch proximate to and including the study period.**



Fig. 6-2 shows the Global Solar Energy as recorded by the Bureau of Meteorology for Lyndoch and Moorok (5 kms from the HRAP). The lines almost completely overlay each other, so it is reasonable to expect that any differences in Global Solar Energy reaching the respective ponds are so small as to cause no significant difference in wastewater treatment performance between the two sites. The Bureau of Meteorology data were used for convenience to demonstrate the close relationship over an extended period of solar energy reaching the ground at these two sites. The on-site weather stations showed the same relationship but over a smaller time period.

## 6.2 HRAP 1 AND WSP 1 (Facultative Pond) - both septic tank overflow fed:- PERFORMANCE STATISTICAL SUMMARY

Table 6-1 provides a statistical summary of the key features and performance of the Kingston HRAP 1 and the Lyndoch facultative pond (WSP 1). This table will be referred to in discussion of the relative performance of the two systems.

**Table 6-1 Summary of the physical and of performance related parameters (mean ± standard deviation) comparing the facultative WSP at Lyndoch with the HRAP at Kingston on Murray , both receiving wastewater pre-treated in on-site septic tanks, over the period May 2010 to March 2011.**

| Category            | Parameter  | Fac. Pond | HRAP 1     |
|---------------------|--|-----------|------------|
| Physical Parameters | Theoretical Hydraulic Retention Time (d)                         | 27.5      | 6.4        |
|                     | <sup>1</sup> THRT - post addition RBC (days)                     | 36.3      | N/A        |
|                     | Pond Surface Area (m <sup>2</sup> )                              | 6400      | 200        |
|                     | Pond Volume (m <sup>3</sup> )                                    | 6800      | 77         |
|                     | Sludge adjusted pond volume (m <sup>3</sup> )                    | 4533      | N/A        |
| Inflow Rates        | Inflow - pre addition RBC (m <sup>3</sup> )                      | 165       | 12         |
|                     | Inflow - post addition RBC (m <sup>3</sup> )                     | 125       | 12         |
| Pond Loading Rates  | BOD <sub>5</sub> Areal Loading Rate (kg/ha/day)                  | 53.7±11.1 | 135.3±43.1 |
|                     | BOD <sub>5</sub> Volumetric Loading Rate (g/m <sup>3</sup> /day) | 5.7±6.6   | 35.8±15.9  |

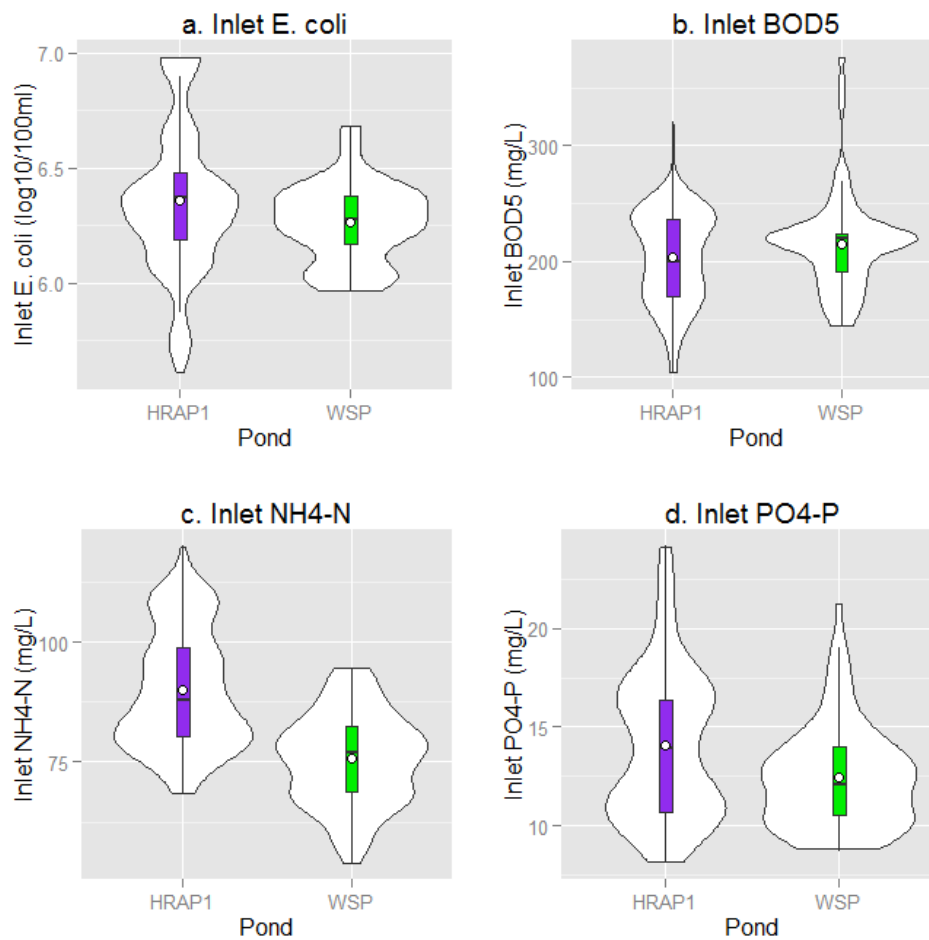
|                                       |   |                |                |
|---------------------------------------|---|----------------|----------------|
| <b><sup>2</sup>Incoming Radiation</b> | Total Solar Radiation 5 Day Avg. (W/m <sup>2</sup> )                | 177±90         | 210±125        |
|                                       | UV Radiation 5 Day Avg. (W/m <sup>2</sup> )                         | 4.6±1.4        | 4.8±1.4        |
|                                       |   |                |                |
|                                       | DO Daily Avg. (mg/L)  | 5.54±4.65      | 6.7±3.4        |
|                                       | DO Max (mg/L)   | 11.49±9.53     | 14.2±7.8       |
|                                       | DO Min (mg/l)   | 1.15±1.23      | 1.47±1.8       |
|                                       | DO Daily Variation (mg/L)   | 10.7±9.49      | 12.0±7.6       |
| <b>In pond Wastewater</b>             | pH Daily Avg.   | 8.93±0.47      | 7.89±0.28      |
|                                       | pH Max  | 9.18±0.55      | 8.7±0.33       |
|                                       | pH Min  | 8.69±0.42      | 7.1±0.5        |
|                                       | pH Daily Variation  | 0.49±0.22      | 1.61±0.6       |
|                                       |   |                |                |
|                                       | Temperature Daily Avg. (°C)   | 19.63±5.62     | 17.9±5.4       |
|                                       | Temperature Max (°C)  | 22.54±6.45     | 20.74±6.3      |
|                                       |   |                |                |
|                                       | Temperature Min (°C)  | 17.3±4.72      | 15.3±4.8       |
|                                       | Temperature Daily Variation (°C)                                    | 5.28±2.75      | 5.5±2.3        |
| <b>Microbial</b>                      | Inlet wastewater <i>E. coli</i> (log <sub>10</sub> /100 ml)         | 6.266±0.17     | 6.361±0.3<br>2 |
|                                       | Treated wastewater <i>E. coli</i> (log <sub>10</sub> /100 ml)       | 4.242±0.72     | 4.607±0.5<br>4 |
|                                       | <i>E. coli</i> Log Reduction Value (LRV;log <sub>10</sub> /d)       | 2.018±0.63     | 1.775±0.6<br>2 |
|                                       | 5 <sup>th</sup> percentile <i>E. coli</i> LRV(log <sub>10</sub> /d) | 1.047          | 1.301          |
|                                       | Chlorophyll a (mg/L)  | 1.943±2.23     | 3.18±3.56      |
| <b>Nutrients</b>                      | Inlet NH <sub>4</sub> -N (mgN/L)                                    | 75.3±9.3       | 89.9±12.1      |
|                                       | Treated wastewater NH <sub>4</sub> -N (mg/L)                        | 36.78±14.9     | 27.1±16.9      |
|                                       | NH <sub>4</sub> -N Removal Efficiency (%)                           | 50.5%±23.<br>1 | 68.4%±20.<br>7 |
|                                       | Inlet NO <sub>x</sub> -N (mg/L)                                     | 1.74±4.53      | 0.4±0.5        |
|                                       | treated wastewater NO <sub>x</sub> -N (mg/L)                        | 3.78±5.85      | 13.2±8.4       |
|                                       | treated wastewater NO <sub>2</sub> -N (mg/L)                        | 0.34±0.32      | 3.58±5.9       |
|                                       | treated wastewater NO <sub>3</sub> -N (mg/L)                        | 1.39±4.48      | 9.66±6.4       |
|                                       | Inorganic-N Removal Efficiency (%)                                  | 47.3%±21.<br>3 | 53.5%±19.<br>6 |
| <b>Other quality</b>                  | Inlet PO <sub>4</sub> -P (mg/L)                                     | 12.4±2.7       | 14.1±3.8       |
|                                       | Treated wastewater PO <sub>4</sub> -P (mg/L)                        | 10.2±3.3       | 12.0±2.8       |
|                                       | PO <sub>4</sub> -P Removal Efficiency (%)                           | 16.4%±26.<br>2 | 16.4%±14.<br>8 |
|                                       | Inlet BOD <sub>5</sub> (mg/L)                                       | 216±42         | 204±40         |
|                                       | Treated wastewater BOD <sub>5</sub> (mg/L)                          | 23.4±26.3      | 15.0±8.7       |
|                                       | BOD <sub>5</sub> Removal Efficiency (%)                             | 88.7%±13.<br>1 | 91.9%±5.1      |
|                                       | Inlet Suspended Solids (mg/L)                                       | 95±23          | 107±37         |
|                                       | Outlet Suspended Solids (mg/L)                                      | 161±120        | 910±1592       |

|                     |  |           |            |
|---------------------|--|-----------|------------|
| <b>parameters</b>   | Turbidity (NTU)                        | 148±139   | 773±989    |
| <b>Productivity</b> | Suspended Solids (g/m <sup>2</sup> /d) | 4.02±3.13 | 76.6±126.5 |

<sup>1</sup>Rotating biological contactor (RBC) installed only at Lyndoch, inlet wastewater diverted to it and returned to Lyndoch WSP 3 rather than the facultative pond consequently increasing the THRT of that pond.

<sup>2</sup>Incoming radiation recorded over a two year period for each system

### 6.3 Comparison of inlet wastewater composition to the Lyndoch facultative WSP and the HRAP at Kingston on Murray.



**Fig. 6-3 Violinplots of comparative inlet water for the Kingston on Murray HRAP 1 and the facultative pond at Lyndoch with internal boxplot showing the mean (open**

circle) and median (black line) of all respective data sets; A.)  $\log_{10}$  *E. coli*/100ml, b.) BOD<sub>5</sub> (mg/L) c.) NH<sub>4</sub>-N (mg/L) and d.) PO<sub>4</sub>-P (mg/L).

As can be seen in Fig. 6-3 and Table 6-1 the inlet water to each system was quite uniform, with the inlet *E. coli* numbers, BOD<sub>5</sub> and PO<sub>4</sub>-P being statistically identical ( $p < 0.005$ ). The HRAP inlet NH<sub>4</sub>-N was slightly higher than that going in to the WSP, but was judged to be an insignificant difference in terms of pond performance. Obviously because of the different size of populations serviced the daily volumes were quite different, but the microbial and chemical compositions were almost the same. The mean and median values for each parameter can be seen in the middle of each boxplot inside the violin plots in Fig. 6-3. These showed no differences between the two inlet waters. A point worth noting was that because of the much smaller surface area and volume of the HRAP, the BOD<sub>5</sub> (and other parameters) loading rates are significantly higher. The BOD<sub>5</sub> areal loading rate for the HRAP was 2.5 times higher, 135 kg/ha/day, than the Lyndoch facultative WSP, 54 kg/ha/day. Although these are low BOD<sub>5</sub> loading rates, they also need to be understood in the context of the WSP having a surface area 3.76 m<sup>2</sup> per person, whilst the HRAP had 1.2 m<sup>2</sup> surface area per person. This difference highlights the potential cost saving in future plant design.

## **6.4 TREATED WASTEWATER PARAMETERS FOR THE LYNDOCH FACULTATIVE WSP AND THE HRAP AT KINGSTON ON MURRAY EFFLUENT PRE-TREATED IN SEPTIC TANKS.**

The continuously logged parameters of DO, pH and temperature are shown in the time series Figs. 3-1 and 4-1 for the WSP 1 and the HRAP 1 respectively. The summary data in Table 6-1 shows that the largest and potentially the most significant difference operationally between the two systems was that of pH. In the HRAP the average daily pH was 7.89 with a daily maximum of 8.7 and a daily minimum of 7.1. The daily variation in the HRAP pH was 1.6. In contrast, in the WSP the average daily pH was 8.93 with a daily maximum of 9.18 and a daily minimum of 8.69. The daily variation in the WSP pH was 0.49. In Para 5.2.1 to 5.2.3 it was shown that the daily pH figures were not important predictors of *E. coli* LRV. However, the 5 day average pH figure was relatively important as a predictor of *E. coli* LRV.

However, direct comparison of the data is not possible as the pH probe was positioned in both pond systems 0.2 m below the pond surface. With constant mixing, the HRAP is homogeneous throughout all depths, whereas it is well known that WSPs stratify in terms of DO, pH and temperature (Sweeney et al., 2005, Sweeney et al., 2007). Due to limited resources, depth profiles were not available.

Nevertheless, as high pH is known to cause *E. coli* inactivation in vitro (Bolton et al., 2010, Parhad and Rao, 1983) the difference in pH noted between the two systems, at least in the top 0.2 m, may have been a source of the small difference in *E. coli* LRV between the two systems. (Fig. 6-4 a)

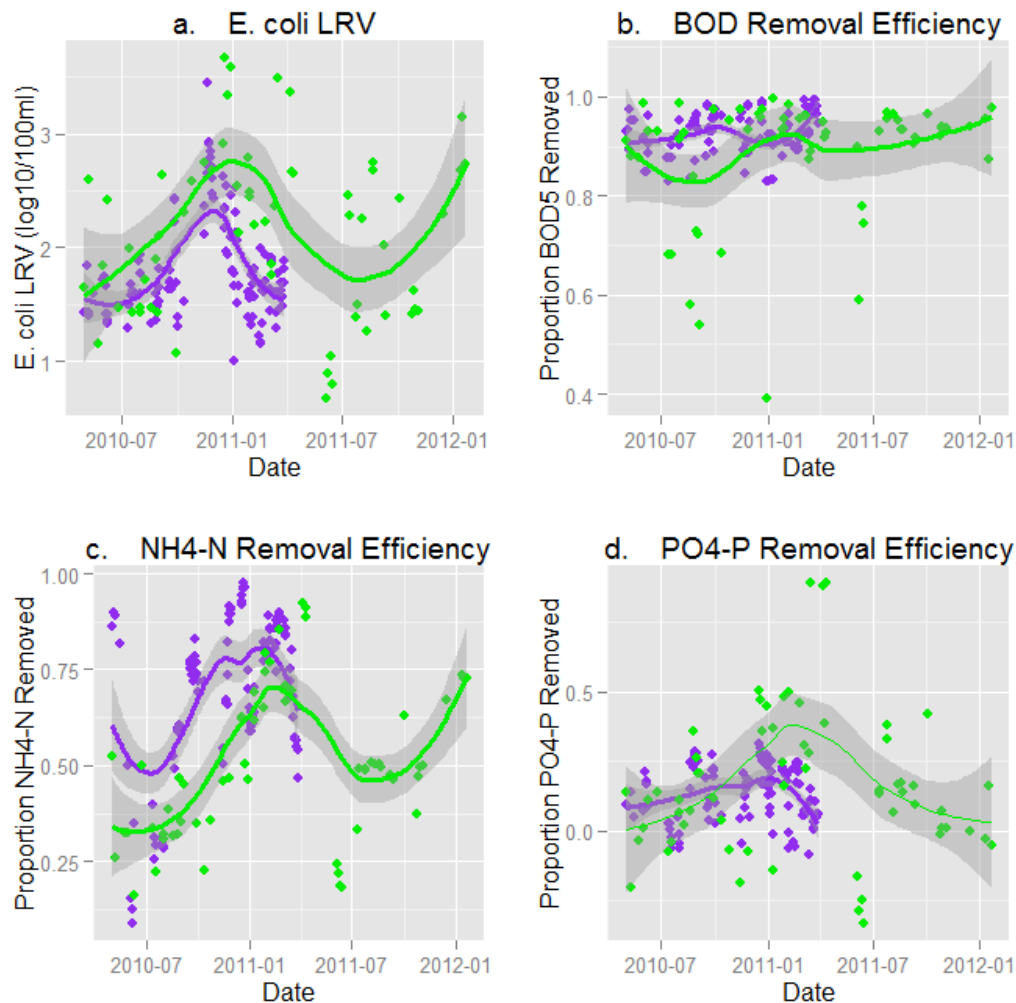
## 6.5 *E. coli* INACTIVATION

Further observations from the loess smooth line through the *E. coli* LRV data point in Fig. 6-4 a. are:-

- an annual sinusoidal cycle evident in both the HRAP and the WSP with peak *E. coli* LRVs in the spring for the HRAP and mid-summer for the WSP. This was consistent with the concept that both solar radiation

and/or temperature were important predictors of *E. coli* LRV in the HRAP as expressed in Table 5-1. It would also suggest the same mechanisms were as important in the WSP, although they were temporally different - *E. coli* LRV increases more sharply in winter and peaks two months later than the HRAP.

- Throughout most of the annual cycle the WSP had an *E. coli* LRV about 0.3 higher than the HRAP. Nevertheless, more importantly from a regulators point of view, there was much more variability in the performance of the WSP, which was evidenced in Fig. 6-4 a. by the broad spread of the 95% confidence interval (95% CI) band (grey shading either side of the green line) and the wide scatter of the individual points from a high of 3.65 to a low of 0.66. The 95% CI for the HRAP was much narrower and the points range from a high of 3.44 to a low of 1.0. The 5<sup>th</sup> percentile (i.e. the lowest 5% of data) for the WSP as reported in Table 6-1 was 1.047 and for the HRAP is 1.301. This inconsistent performance of WSP systems has been noted previously and has at least in part prompted the search for alternative approaches to wastewater management.



**Fig. 6-4** Loess smooth lines with shaded 95% CI comparing the wastewater treatment performance of Kingston on Murray HRAP 1 (purple) with the Lyndoch WSP 1 (green) both fed wastewater pre-treated in on-site septic tanks; a.) *E. coli* log reduction value (LRV  $\log_{10}$ ) b.)  $BOD_5$  removal efficiency c.)  $NH_4-N$  removal efficiency and d.)  $PO_4-P$  removal efficiency.

## 6.6 NUTRIENT REMOVAL

### 6.6.1 $BOD_5$ Removal Efficiency

As for the *E. coli* LRV, the  $BOD_5$  removal efficiency was similar for the WSP and the HRAP. For the WSP there was an annual sinusoidal cycle but it was relatively minor varying the  $BOD_5$  removal efficiency from 83% in late winter to 92% in late summer. Possible biological explanations for this are firstly that algal growth was greatest in late summer (Fig. 3-7) and DO levels were therefore higher (Fig. 3-1c.). As well pond temperatures are higher in summer, increasing

the metabolic rate of bacterial carbon oxidation. The HRAP BOD<sub>5</sub> removal efficiency had an even less pronounced seasonality, peaking in early spring and late summer, presumably under the same influence of algal produced DO levels (Fig. 4-1 c. and 4-6). Again the key difference in the performance was the far greater variability of the BOD<sub>5</sub> removal efficiency in the WSP compared to the HRAP, as expressed by the width of 95% CI band in Fig. 6-4 b and in the much larger standard deviation (SD) (14.5 vs 4.4) of the BOD<sub>5</sub> removal efficiency in Table 6-2. In numerical terms this can be seen in the range of BOD<sub>5</sub> removal efficiency in the HRAP only varying from 82% to 100%, whereas in the WSP the variability was from 40% to 100%.

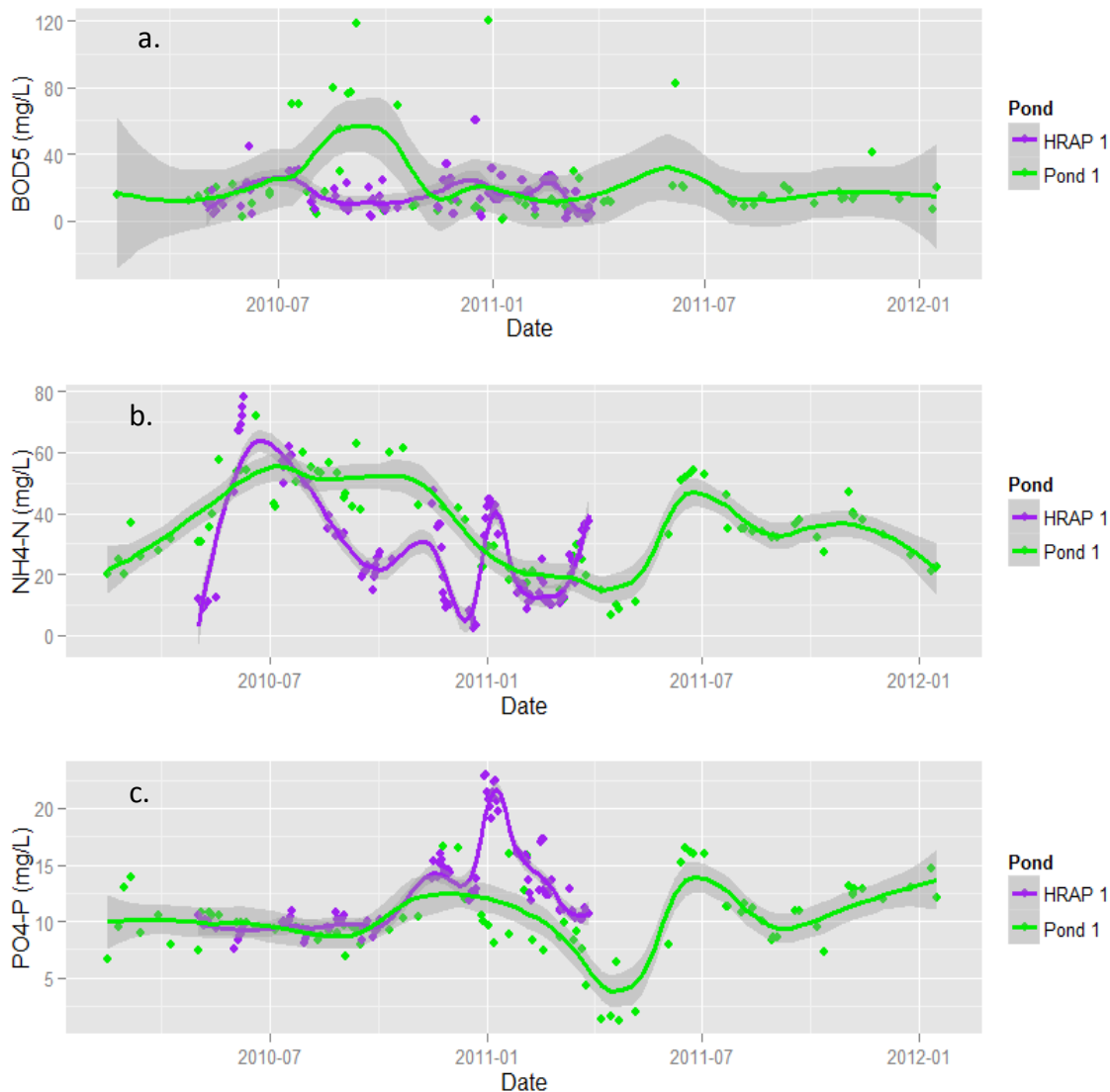
This makes the WSP a much less reliable treatment system than the HRAP if the treated water is to be returned to a stream, lake or ocean.

**Table 6-2 Standard statistical comparisons of the nutrient removal efficiency of the Kingston on Murray HRAP 1 and the Lyndoch facultative WSP 1 both fed septic tank treated effluent.**

| Nutrient                            | HRAP<br>(Mean ± S.D.) | WSP<br>(Mean ± S.D.) | p-value of<br>unpaired t-test<br>comparing the 2<br>means | Average<br>difference of<br>means |
|-------------------------------------|-----------------------|----------------------|---|-----------------------------------|
| <b>BOD<sub>5</sub><br/>Removal</b>  | 92.3 ± 4.4%           | 87.6 ± 14.5%         | 0.0458  | 5.3%                              |
| <b>NH<sub>4</sub>-N<br/>Removal</b> | 68.9 ± 20.8%          | 50.1 ± 19.7%         | 0.000000093   | 18.8%                             |
| <b>PO<sub>4</sub>-P<br/>Removal</b> | 12.1 ± 11.6%          | 20.8 ± 24.6%         | 0.0417  | -8.7%                             |

As all the p-values were less than 0.05, the alternative hypothesis is accepted, that there is a significant difference between the two means for each case. In the case of NH<sub>4</sub>-N there is clear evidence for significantly better removal efficiency from the HRAP. With BOD<sub>5</sub> and PO<sub>4</sub>-P removal efficiencies, the results are equivocal, and probably of no significance anyway.





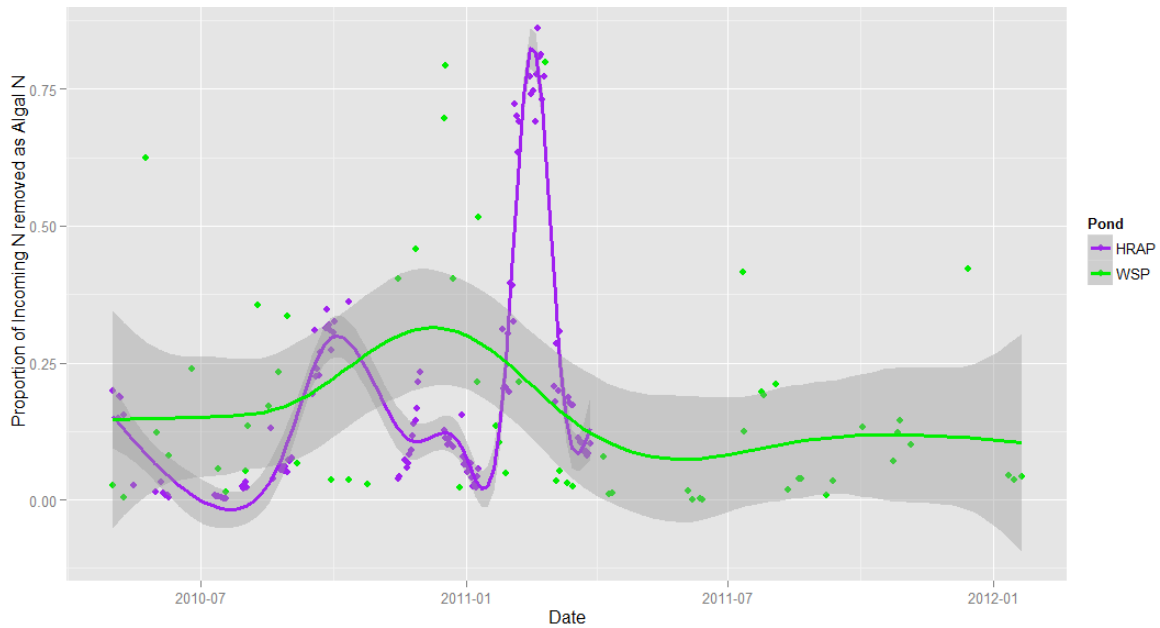
**Fig. 6-5** Loess smooth lines with shaded 95% CI comparing the wastewater treatment performance of Kingston on Murray HRAP 2 (purple) with Lyndoch WSP 2 & 3 (greens). (a.) BOD<sub>5</sub> concentration (mg/L) (b.) NH<sub>4</sub>-N concentration (mg/L) and (c.) PO<sub>4</sub>-P concentration (mg/L).

### 6.6.2 NH<sub>4</sub>-N Removal Efficiency

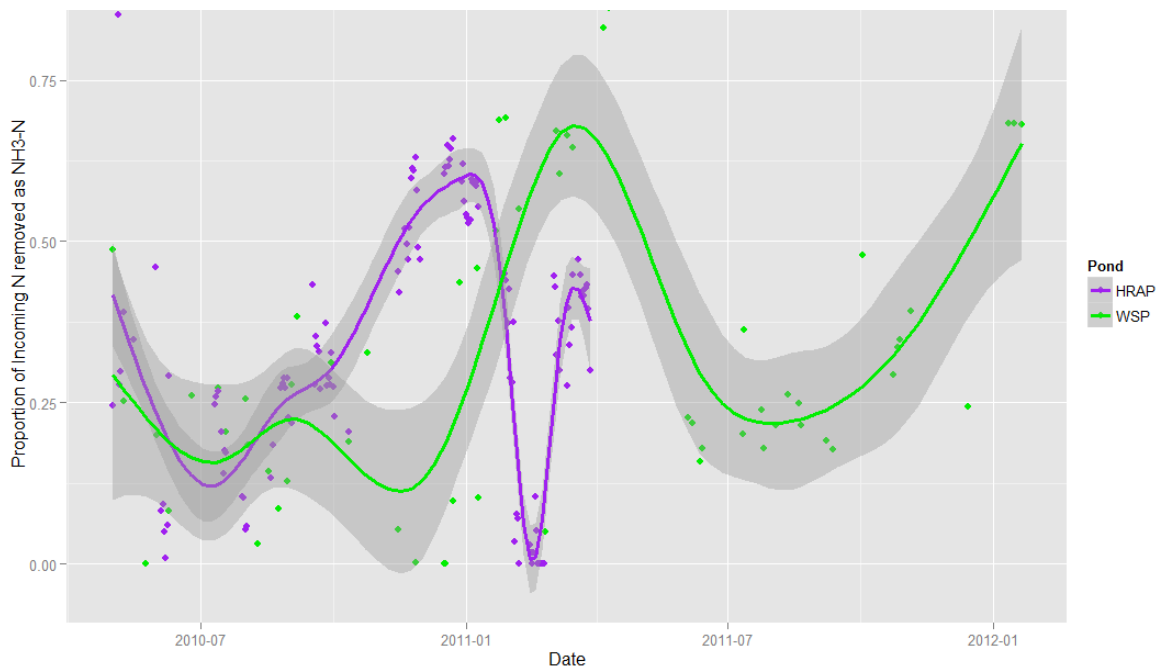
As for the *E. coli* LRV, the NH<sub>4</sub>-N removal efficiency was similar for the WSP and the HRAP, with a sinusoidal pattern reflecting annual seasonality, peaking in mid-summer and bottoming in mid-winter. As established in Section 5.4 & 5.6.3, and represented in Table 5-3, NH<sub>4</sub>-N removal efficiency was strongly mediated by chlorophyll a levels and DO levels. It was reasonable to assume that nitrogen was therefore removed from the ponds by both algal cell

incorporation, volatilisation of gaseous  $\text{NH}_3$  and perhaps in the WSP only some nitrification/denitrification releasing gaseous  $\text{N}_2$  to the atmosphere.

Both systems displayed considerable short term variations in  $\text{NH}_4\text{-N}$  removal efficiency, particularly in the colder winter months (Fig. 6-4 c. and Table 6-2). By comparing the loess smoothed lines in Fig. 6-4 c, it appears as though there is a difference between the  $\text{NH}_4\text{-N}$  removal efficiencies in the two ponds, with the HRAP  $\text{NH}_4\text{-N}$  removal efficiencies consistently 19% higher than the WSP for most of the year (Fig. 6-4 c. and Table 6-2). This difference can probably be attributed to the overall higher algal production and consequent N removal. The forms of removal are highlighted in Figs. 6-6 and 6-7. In the HRAP, there was a fluctuation between  $\text{NH}_3\text{-N}$  volatilisation in the October to January period, followed by a sharp change to algal removal in February, associated with the surge in algal growth in that period. There was even a very short period when algal growth was sufficient to remove all the incoming  $\text{PO}_4\text{-P}$ . The big difference between the two systems was that in the WSP,  $\text{NH}_3\text{-N}$  removal was low until December, and then escalated dramatically in the December to April period, before falling again by June. Removal through algal N remained at a fairly consistent level throughout the year. Of the remaining N, it was not possible to discern whether any was removed by nitrification followed by denitrification in the anaerobic zone of the facultative pond.



**Fig. 6-6** Loess smooth lines with shaded 95% CI comparing the proportion of incoming wastewater N removed as algal N by treatment at the Kingston on Murray HRAP 1 (purple) or the Lyndoch WSP 1 (green) - both fed wastewater pre-treated in on-site septic tanks.



**Fig. 6-7** Loess smooth lines with shaded 95% CI comparing the proportion of incoming wastewater N removed as NH<sub>3</sub>-N by treatment at the Kingston on Murray HRAP 1 (purple) or the Lyndoch WSP 1 (green) - both fed wastewater pre-treated in on-site septic tanks.

**Table 6-3 Standard statistical comparisons of the proportions of incoming N & P removed as algal N & P and NH<sub>3</sub>-N from the Kingston on Murray HRAP 1 and the Lyndoch facultative WSP 1 both fed septic tank treated effluent.**

| Nutrient                                      | HRAP<br>(Mean ± S.D.) | WSP<br>(Mean ± S.D.) | p-value of unpaired<br>t-test comparing<br>the 2 means | Average<br>difference of<br>means |
|---|-----------------------|----------------------|--|-----------------------------------|
| Percent N<br>Removed as<br>Algal N            | 20.7 ± 23.1%          | 16.4 ±<br>19.9%      | 0.186  | 4.3%                              |
| Percent N<br>Removed as<br>NH <sub>3</sub> -N | 33.3 ± 20.8%          | 32.4 ±<br>23.4%      | 0.803  | 0.9%                              |
| Percent P<br>Removed as<br>Algal P            | 15.3 ± 13.8%          | 13.3 ±<br>14.9%      | 0.374  | 2.0%                              |

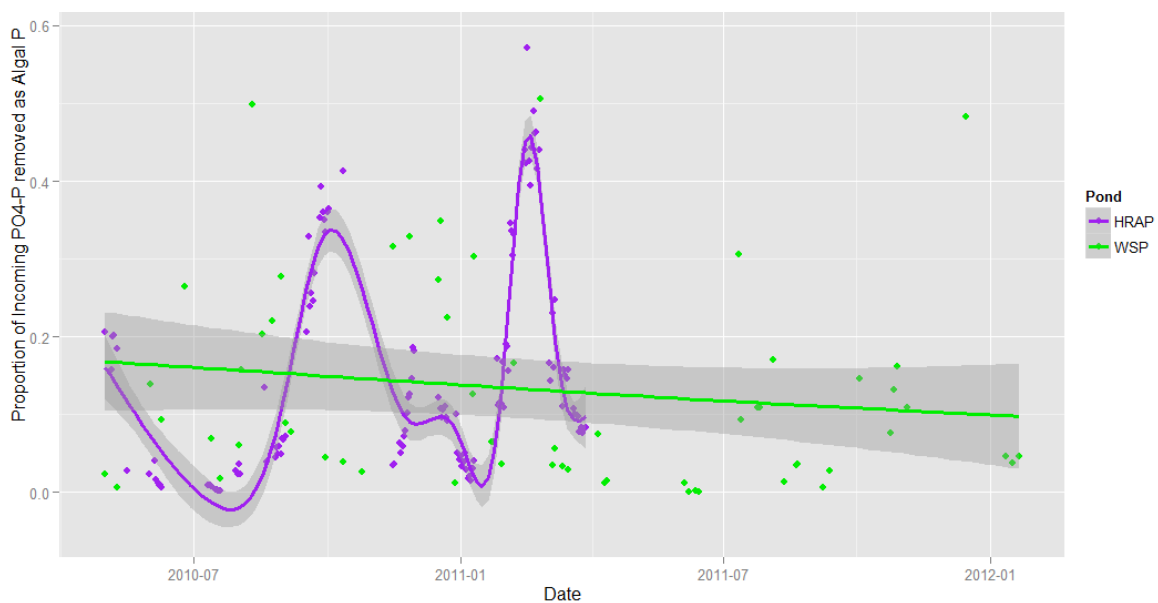
As all the p-values were greater than 0.05, we can accept the null hypothesis that there were no differences between the two means. In other words the HRAP and the WSP removed NH<sub>4</sub>-N in identical ways.

### 6.6.3 PO<sub>4</sub>-P Removal Efficiency

As noted in para 3.2.1.4, Redfield (1958) and Redfield et al. (1963) suggested that when nutrients are not limiting, the molar elemental ratio C:N:P in most phytoplankton is 106:16:1. Using the assumption that P represents 1.7% by weight of the total algal mass, it can be calculated that 48 ± 52 g algal-P/day exited the HRAP, which is 15.3% of the incoming PO<sub>4</sub>-P. By comparison, 2,006±481 g/day PO<sub>4</sub>-P entered WSP1 and 278±331 g/day exited as algal P, which is 13.3% (Table 6-3). As can be seen in Fig. 6-8, the pattern of removal of PO<sub>4</sub>-P as algal P is highly variable in the HRAP, changing rapidly with the growth and die-off of the algal biomass.

For the WSP there was an annual sinusoidal cycle but it was relatively minor varying the PO<sub>4</sub>-P removal efficiency from 5% in late winter to around 40% in late summer (Mean = 21% Table 6-2). By contrast, the HRAP removed around 12% of the PO<sub>4</sub>-P all year round with very little seasonal variability. As it is unknown for volatilisation of phosphorous to occur, removal must be either by incorporation into algal cells and removal as algae exit the system, or via precipitation with cations such as calcium and magnesium in a form of struvite.

The variable proportion removed from the HRAP via incorporation into algal cells is shown in Fig. 6-8. By contrast, the removal of  $\text{PO}_4\text{-P}$  as algal P in the WSP is steady throughout the year (Fig. 6-8), and the fluctuations in  $\text{PO}_4\text{-P}$  removal must be attributable to some form of precipitation taking place in the WSP over the summer period, but not in winter. Insufficient data were collected on the levels of calcium and magnesium ions in the system to determine what form this may have taken.

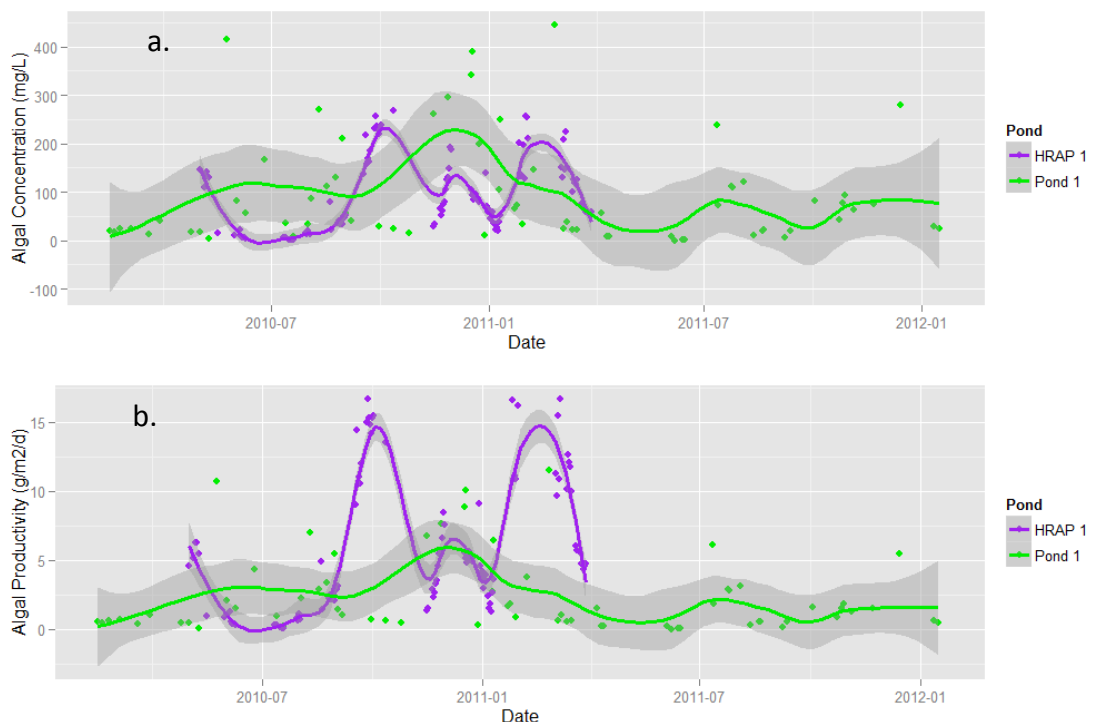


**Fig. 6-8** Loess smooth lines with shaded 95% CI comparing the proportion of incoming wastewater  $\text{PO}_4\text{-P}$  removed as algal P by treatment at the Kingston on Murray HRAP 1 (purple) or the Lyndoch WSP 1 (green) - both fed wastewater pre-treated in on-site septic tanks.

From an environmental regulatory standpoint, neither system removes much  $\text{PO}_4\text{-P}$ , but there is far greater variability in the  $\text{PO}_4\text{-P}$  removal from the WSP compared to the HRAP.

## 6.7 ALGAL CONCENTRATION & PRODUCTIVITY

As can be seen in Fig. 6-9a and Table 6-4 the algal concentration in the two systems was similar for most of the year, averaging 96 and 91 mg/L for the HRAP 1 and WSP 1 respectively. However, the algal productivity of the HRAP 1 averaged 5.9 g/m<sup>2</sup>/d whilst the WSP productivity averaged 2.3 g/m<sup>2</sup>/d. This extra productivity was concentrated in two bursts of activity in spring and autumn (Fig. 6-9b). This has implications if the harvesting of biomass becomes a priority, when a more even growth rate would be preferable. It is also important to remember that the HRAP was operating at varying depths throughout this study and no attempt has been made to adjust for depth in reporting these productivity figures, even though some effect is obvious, adjusting for that effect is not straightforward and probably fraught with error.



**Fig. 6-9** Loess smooth lines with shaded 95% CI comparing the wastewater treatment performance of Kingston on Murray HRAP 1 (purple) with Lyndoch WSP 1 (green). a. Algal concentration (mg/L) b. Algal productivity (g/m<sup>2</sup>/d).

**Table 6-4 Standard statistical comparisons of the algal concentration and productivity of the Kingston on Murray HRAP 1 and Lyndoch WSP 1.**

| Pond          | Algal Concentration (mg/L), n=102 |          | Algal Productivity (g/m <sup>2</sup> /d), n=102 |           |
|---------------|-----------------------------------|----------|---|-----------|
|               | Mean ± s.d.                       | Range    | Mean ± s.d.                                     | Range     |
| <b>HRAP 1</b> | 96 ± 74                           | 0.85-268 | 5.9 ± 4.9                                       | 0.04-16.7 |
| <b>WSP 1</b>  | 91 ± 108                          | 0.05-446 | 2.3 ± 2.8                                       | 0-11.5    |

WASSINK et al. (1953) concluded that the efficiency of light-energy conversion in both small and large scale cultures of *Chlorella* was 12 to 20 per cent, provided the intensity of the illumination was not too high. However, outdoor stirred mass cultures of *Chlorella* in full summer light show efficiency values of 2 to 3 per cent. Excessive illumination seems an important factor in producing low efficiency under natural conditions.

It should also be noted that grazing by *Daphnia* spp. during the winter period was particularly heavy in the HRAP. A feature of the algae in the HRAP appears to be their susceptibility to sudden growth increases followed by dramatic declines in population. As there was no change in the nutrient or light supply during these periods, it may be assumed that the sudden crash in algal populations was either due to heavy grazing or more likely infection with either fungi or viruses. This requires more research to elucidate, as it would appear to be a major risk factor if biomass harvesting was a secondary aim of wastewater treatment.

The algal populations in the WSP appear less vulnerable to massive population drops, but they nevertheless are overall more variable than in the HRAP as can be determined from the relative widths of the 95% CI lines for each system in Fig. 6-9a.

## 6.8 STATISTICAL SUMMARY OF THE KINGSTON ON MURRAY HRAP:- FED FACULTATIVE POND EFFLUENT AND LYNDOKH WSP 2 & 3 (MATURATION)

The data in this section has been the subject of debate and proven difficult to summarise accurately. The final choice was that the comparison should be made between the performance of a HRAP fed facultative pond water (HRAP 2), and two waste stabilisation ponds in series (WSP 2 & 3). WSP 2 was fed facultative pond water and WSP 3 was fed WSP 2 water. The WSP performance data recorded in Table 6-5 and beyond reflect facultative treated water that entered WSP 2 and exited through WSP 3 (the column heading states Mat. Pond 3), whereas the HRAP performance simply reflects facultative treated water that enters the HRAP and leaves it after treatment.

In contrast, the physico-chemical data in Table 6-5 and beyond for comparison are those recorded in WSP 3, not WSP 2, unless specified as in Fig. 6-10. The HRAP physico-chemical data simply represent data recorded in the HRAP 2. The largest single difference between the two treatments is the time spent in the treatment train. The HRAP was operated at three depths and subsequent Theoretical Hydraulic Retention Times, which averaged 6.4 days for the whole period. The WSP Theoretical Hydraulic Retention Time for the two ponds in series totalled 18.8 days for the initial 15 months and then 24 days for the final 15 months.

**Table 6-5 Summary of the physical and mean  $\pm$  standard deviation of performance related parameters comparing the physico-chemical and performance parameters of two maturation WSPs combined, with the HRAP 2 over the period Jul 2011 to Feb 2012.**

| Category            | Parameter                                      | Mat. Pond 2 & 3 | HRAP 2 |
|---------------------|--|-----------------|--------|
| Physical Parameters | Theoretical Hydraulic Retention Time (THRT; d) | 18.8            | 6.4    |
|                     | THRT - post addition RBC (days)                | 24              | N/A    |
|                     | Combined Ponds Surface Area (m <sup>2</sup> )  | 5000            | 200    |



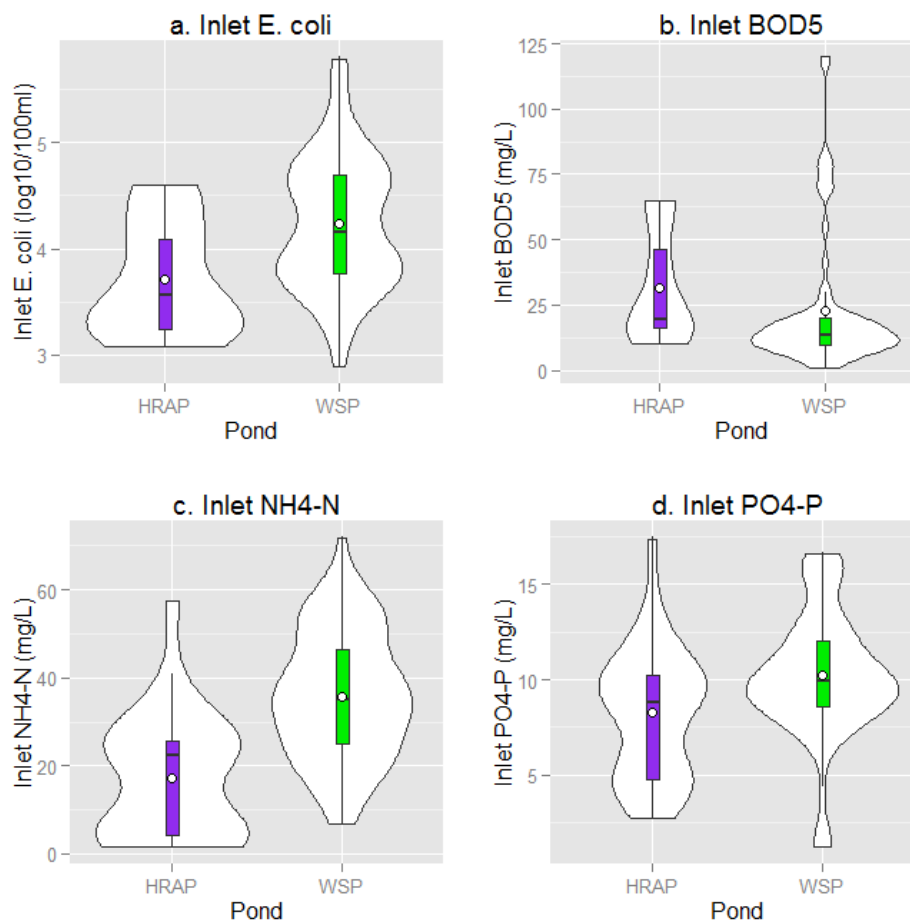
| Category                        | Parameter  | Mat. Pond 2<br>& 3 | HRAP 2      |
|---------------------------------|--|--------------------|-------------|
|                                 | Combined Ponds Volume (m <sup>3</sup> )                          | 4000               | 77          |
|                                 | Sludge adjusted pond volume (m <sup>3</sup> )                    | 3000               | N/A         |
| Inflow Rates                    | Inflow - pre addition RBC (m <sup>3</sup> )                      | 165                | 12          |
|                                 | <sup>1</sup> Inflow - post addition RBC (m <sup>3</sup> )        | 125                | N/A         |
| Pond Loading Rates              | BOD <sub>5</sub> Areal Loading Rate (kg/ha/day)                  | 7.6±1.6            | 15.2±14.4   |
|                                 | BOD <sub>5</sub> Volumetric Loading Rate (g/m <sup>3</sup> /day) | 0.8±0.93           | 4.3±4.7     |
| <sup>2</sup> Incoming Radiation | Total Solar Radiation 5 Day Avg. (W/m <sup>2</sup> )             | 177±90             | 210±125     |
|                                 | UV Radiation 5 Day Avg. (W/m <sup>2</sup> )                      | 4.6±1.4            | 4.4±1.7     |
| In pond wastewater              | DO Daily Avg. (mg/L)   | 5.54±4.65          | 10.2±1.7    |
|                                 | DO Max (mg/L)  | 11.49±9.53         | 18.9±10.5   |
|                                 | DO Min (mg/L)  | 1.15±1.23          | 6.5±2.9     |
|                                 | DO Daily Variation (mg/L)  | 10.7±9.49          | 12.4±13.0   |
|                                 | pH Daily Avg.  | 8.93±0.47          | 8.4±0.9     |
|                                 | pH Max   | 9.18±0.55          | 8.8±0.8     |
|                                 | pH Min   | 8.69±0.42          | 8.0±0.8     |
|                                 | pH Daily Variation   | 0.49±0.22          | 0.8±0.8     |
| Microbial                       | Temperature Daily Avg. (°C)                                      | 19.63±5.62         | 18.2±5.1    |
|                                 | Temperature Max (°C)   | 22.54±6.45         | 21.9±5.6    |
|                                 | Temperature Min (°C)   | 17.3±4.72          | 15.3±4.5    |
|                                 | Temperature Daily Variation (°C)                                 | 5.28±2.75          | 6.5±2.3     |
|                                 | Inlet wastewater <i>E. coli</i> (log <sub>10</sub> /100 ml)      | 4.242±0.72         | 3.689±0.52  |
|                                 | Treated wastewater <i>E. coli</i> (log <sub>10</sub> /100 ml)    | 2.188±0.78         | 1.008±0.79  |
|                                 | <i>E. coli</i> Log Reduction Value (LRV; Log <sub>10</sub> /day) | 1.84±0.93          | 2.681±0.801 |
|                                 | 5 <sup>th</sup> percentile <i>E. coli</i> LRV                    | 0.905              | 1.305       |
| Nutrients                       | Chlorophyll a (mg/L)   | 0.371±0.362        | 0.65±0.7    |
|                                 | Inlet NH <sub>4</sub> -N (mg/L)                                  | 36.78±14.9         | 18.7±12.1   |
|                                 | Treated wastewater NH <sub>4</sub> -N (mg/L)                     | 27.0±19.4          | 7.7±6.9     |
|                                 | NH <sub>4</sub> -N Removal Efficiency (%)                        | 47.8%±34.0         | 66.2%±26.1  |
|                                 | Inlet NO <sub>x</sub> -N (mg/L)                                  | 3.78±5.85          | 20.7±11.5   |
|                                 | Treated wastewater NO <sub>x</sub> -N (mg/L)                     | 5.5±5.4            | 10.2±8.2    |
|                                 | Treated wastewater NO <sub>2</sub> -N (mg/L)                     | 0.34±0.32          | 2.58±3.9    |
|                                 | Treated wastewater NO <sub>3</sub> -N (mg/L)                     | 1.39±4.48          | 7.66±6.4    |

| Category                 | Parameter                                    | Mat. Pond 2 & 3 | HRAP 2     |
|--------------------------|--|-----------------|------------|
|                          | Inorganic-N Removal Efficiency (%)           | 34.2%±30.4      | 59.5%±25.4 |
|                          | Inlet PO <sub>4</sub> -P (mg/L)              | 10.2±3.3        | 8.8±3.2    |
|                          | Treated wastewater PO <sub>4</sub> -P (mg/L) | 7.27±4.01       | 9.4±2.9    |
|                          | Inlet BOD <sub>5</sub> (mg/L)                | 23.4±26.3       | 23.5±15.8  |
|                          | Treated wastewater BOD <sub>5</sub> (mg/L)   | 11.5±18.0       | 6.8±1.8    |
|                          | BOD <sub>5</sub> Removal Efficiency (%)      | 88.7%±13.1      | 63.8%±13.9 |
| Other quality parameters | Inlet Suspended Solids (mg/L)                | 161±120         | 107±37     |
|                          | Outlet Suspended Solids (mg/L)               | 108±94.3        | 910±1592   |
|                          | Turbidity (NTU)                              | 148±139         | 773±989    |
| Productivity             | Suspended Solids (g/m <sup>2</sup> /d)       | 0               | 17.7±2.1   |

<sup>1</sup>Rotating biological contactor (RBC) installed only at Lyndoch, inlet wastewater diverted to it and returned to Lyndoch WSP 3 rather than the facultative pond consequently increasing the THRT of that pond.

<sup>2</sup>Incoming radiation recorded over a two year period for each system

## 6.9 INLET WATER



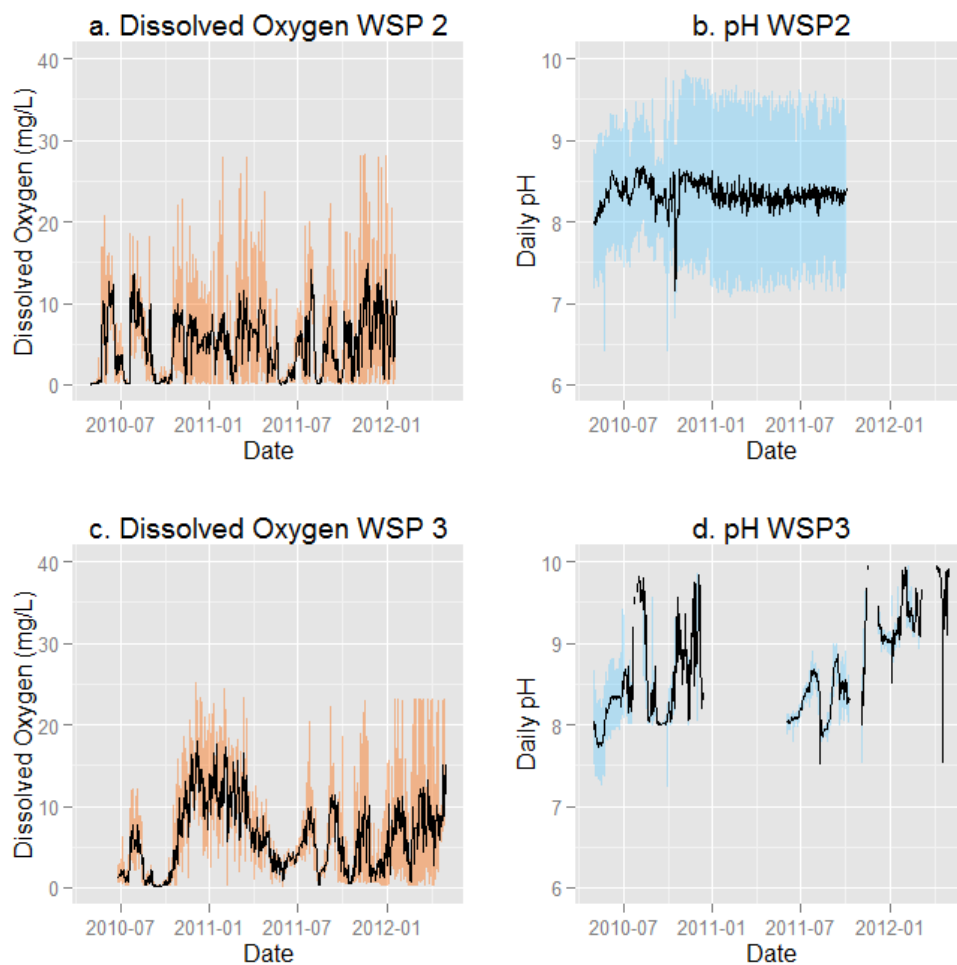
**Fig. 6-10** Kingston on Murray HRAP and the Lyndoch WSP 2 & 3 fed facultative pond treated effluent. Violinplots of comparing inlet wastewater composition, including internal boxplot showing the mean (open circle) and median (black line) of all data sets. a.) *E. coli* (log<sub>10</sub> /100ml), b.) BOD<sub>5</sub> (mg/L) c.) NH<sub>4</sub>-N (mg/L) and d.) PO<sub>4</sub>-P (mg/L).

As the inlet wastewater to both these pond systems was pre-treated in the respective facultative pond, there was greater variability in composition than there was for the septic tanks pre-treated inlet wastewater used in the previous comparison above (Section 6.2). Overall the inlet to the WSP was higher in most constituents, other than BOD<sub>5</sub>, but a judgement has been made that the differences were small enough to make no significant difference to pond performance. This was best ascertained by observation of the degree of overlap of the density plot component of the violinplots in Fig. 6-10. In all cases there

was significant overlap demonstrating the similarity of the two inlet waters. Of great significance was the point that both inlet waters were derived from facultative ponds with approximately 28 to 36 day retention times. This means the organisms will have been subjected to the same amount of stress, and the organic component of the waste been subjected to the same amount of oxidative stress.

## 6.10 TREATED WASTEWATER PARAMETERS FOR THE LYNDOCH FACULTATIVE WSP AND THE HRAP AT KINGSTON ON MURRAY FED EFFLUENT PRE-TREATED IN A FACULTATIVE WASTE STABILISATION POND.

The continuously logged parameters of DO, pH and temperature can be seen in the time series graphs in Fig. 3-23 for the HRAP 2 and Fig. 6-11 for WSP 2 and 3.



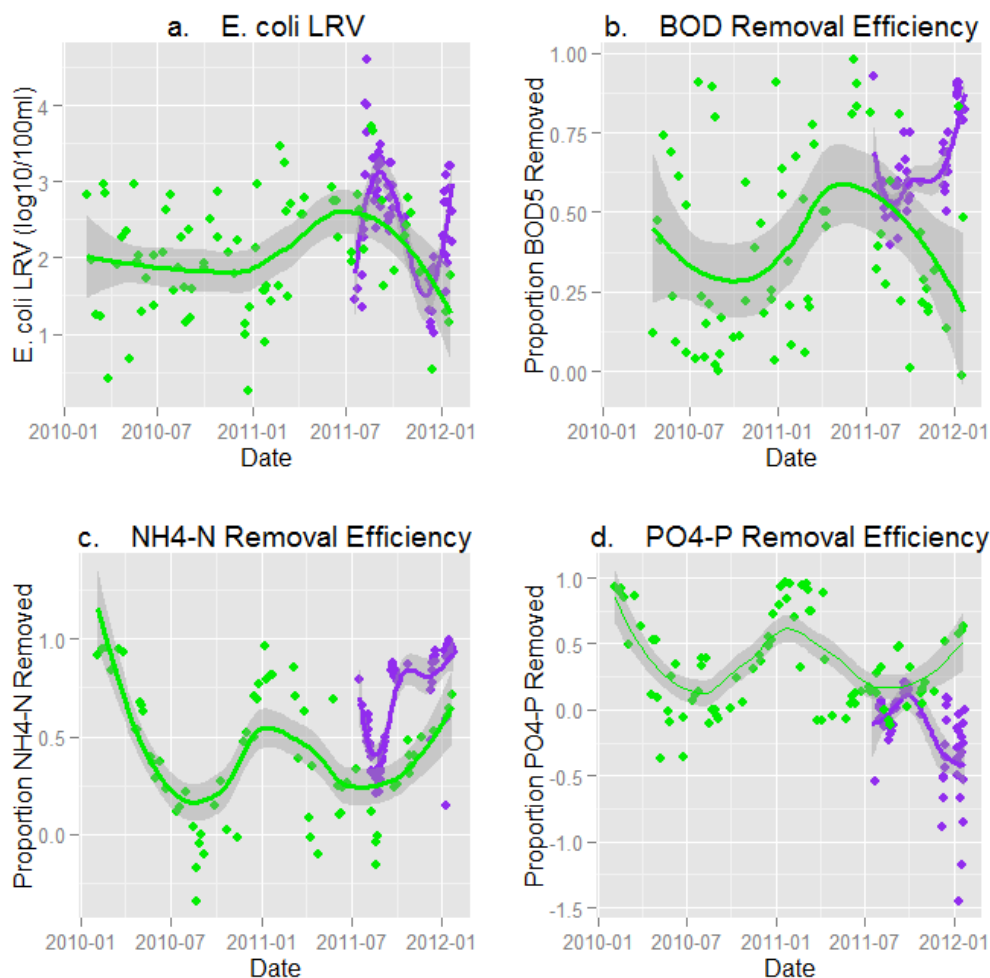
**Fig. 6-11 Time series for dissolved oxygen and pH in Lyndoch WSP 2 (a. and b.) and WSP 3 (c. and d.).**

The same caveat for WSP 1 about interpreting and comparing DO and pH probe data applies to WSP 3 data. That is, the probes were at a fixed depth of 0.22 m. Information about DO and pH at greater depths was not available. From

previous studies (Sweeney et al., 2007) it is known that there are significant changes in these parameters with depth. Nevertheless, the summary data in Table 6-5 shows that DO levels were the most significant physico-chemical difference between the HRAP and WSP systems (WSP 3). Graphically, this can be seen by comparing Fig. 6-11c. with Fig. 3-23c. In the HRAP, the average daily DO was 10.2 mg /L with a daily maximum of 18.9 mg/L and a daily minimum of 6.5 mg/L. The daily variation in the HRAP DO was 12.4 mg/L. In contrast, in the WSP the average daily DO was 5.5 mg/L with a daily maximum of 11.5 mg/L and a daily minimum of 1.15 mg/L. HRAP DO daily minimum of 6.5 mg/L implies that the pond did not reach anaerobic conditions overnight suggesting that the rate of respiration by both bacteria and algae was limited – probably by low nutrient levels.

In the HRAP the average daily pH was 8.4 with a daily maximum of 8.8 and a daily minimum of 8.0. The daily variation in the HRAP pH was 0.8. In contrast, in the WSP the average daily pH was 8.93 with a daily maximum of 9.2 and a daily minimum of 8.7. The daily variation in the WSP pH was 0.5. The daily variation in pH was greater in the HRAP 2 than in the WSP even though the absolute values in the WSP were higher.

## 6.11 *E. coli* INACTIVATION



**Fig. 6-12** Loess smooth lines with shaded 95% CI comparing the wastewater treatment performance of Kingston on Murray HRAP 2 (purple) with Lyndoch WSP 2 & 3 (green). a.) *E. coli* LRV ( $\log_{10}/100\text{ml}$ ) b.)  $\text{BOD}_5$  removal efficiency c.)  $\text{NH}_4\text{-N}$  removal efficiency and d.)  $\text{PO}_4\text{-P}$  removal efficiency.

Noteworthy observations from the loess smooth line through the *E. coli* LRV data point in Fig 6.12a were:-

- a minor annual sinusoidal cycle evident in the WSP with peak *E. coli* LRVs in the winter. By contrast, in the HRAP there was a sharp cycle that peaks in early spring and summer. These cycles are not consistent with known causes of *E. coli* die-off and may have more to do with the

metabolic state in which the *E. coli* arrive at the pond given they have been previously resident in a facultative pond

- The HRAP had an *E. coli* LRV about 0.8 higher than the WSP. The mean values are 2.68 and 1.84 respectively.
- Nevertheless, most importantly from a regulators point of view, there was much more variability in the performance of the WSP, which was evidenced in Fig. 6-4a. as the broad spread of the 95% confidence interval (95% CI) band (grey shading either side of the green line) and the wide scatter of the individual points from a high of 3.68 to a low of 0.24. The 95% CI for the HRAP was much narrower and the points range from a high of 4.6 to a low of 1.0. The 5<sup>th</sup> percentile (i.e. the lowest 5% of data) for the WSP as reported in Table 6-3 was 0.905 and for the HRAP was 1.305. This variability of performance of WSP systems may become an inhibitory factor for their future deployment.

## 6.12 NUTRIENT REMOVAL

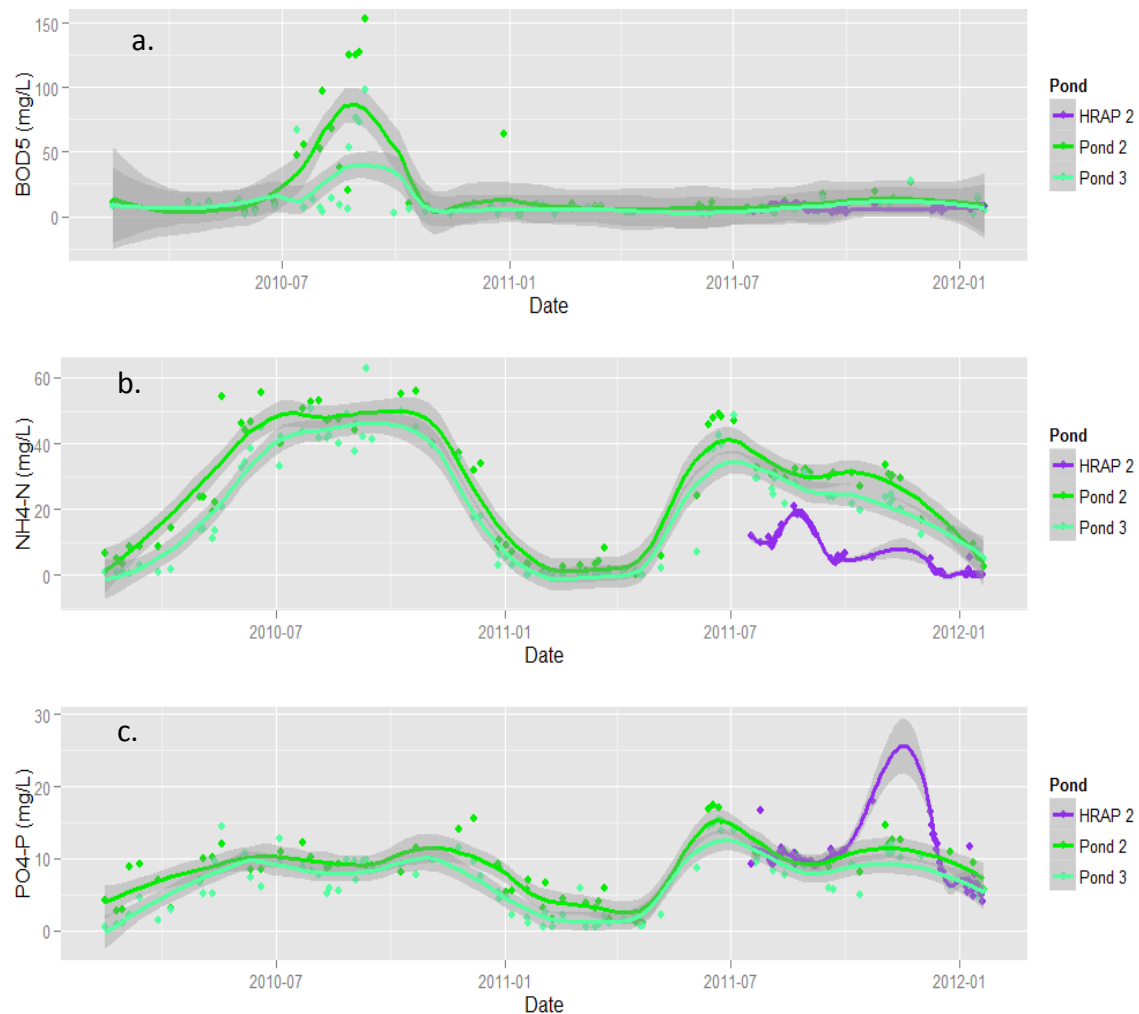
**Table 6-6 Standard statistical comparisons of the nutrient removal performance of the Kingston on Murray HRAP 2 and Lyndoch WSP 2&3.**

| Nutrient                            | HRAP<br>(Mean ± S.D.) | WSP<br>(Mean ± S.D.) | p-value of paired<br>t-test comparing<br>the 2 means | Average<br>difference of<br>means |
|-------------------------------------|-----------------------|----------------------|--|-----------------------------------|
| <b>BOD<sub>5</sub><br/>Removal</b>  | 63.3 ± 9.5%           | 57.1 ± 14.4%         | 0.0295   | 6.2%                              |
| <b>NH<sub>4</sub>-N<br/>Removal</b> | 66.1 ± 26.3%          | 48.1 ± 34.4%         | 0.0932   | 18.0%                             |
| <b>PO<sub>4</sub>-P<br/>Removal</b> | -14.1 ± 32.6%         | 28.0 ± 33.6%         | 0.363  | 42.1%                             |

As the p-value for the BOD<sub>5</sub> removal was less than 0.05, we can accept the alternative hypothesis that there was a significant difference between the two pond means. However, the p-value for the NH<sub>4</sub>-N and PO<sub>4</sub>-P removals are greater than 0.05, therefore we can accept the null hypothesis that there was



no difference between the two pond means for those two removal parameters. This is particularly true for PO<sub>4</sub>-P removal. There was a significant removal happening in WSP 2 over summer that did not appear to be related to algal activity and certainly did not occur in the HRAP.



**Fig. 6-13** Loess smooth lines with shaded 95% CI comparing the wastewater treatment performance of Kingston on Murray HRAP 2 (purple) with Lyndoch WSP 2 & 3 (greens). (a.) BOD<sub>5</sub> concentration (mg/L) ( b.) NH<sub>4</sub>-N concentration (mg/L) and ( c.) PO<sub>4</sub>-P concentration (mg/L).

### 6.12.1 BOD<sub>5</sub> Removal Efficiency

As for the *E. coli* LRV, the BOD<sub>5</sub> removal efficiency was similar for the WSP and the HRAP (Table 6-6 and Fig.6-12b, 6-13a). For the WSP there was an annual sinusoidal cycle but it was relatively minor resulting in a variation of the BOD<sub>5</sub> removal efficiency from 28% in autumn to 64% in spring. However, algal growth in these ponds (Fig. 6-11 a-d) was low enough to limit the dissolved oxygen levels, as noted in para 6.10 and Fig. 6-11c. With limited dissolved oxygen, BOD<sub>5</sub> removal efficiency would also have been limited.

The HRAP BOD<sub>5</sub> removal efficiency had a less pronounced seasonality, peaking in early spring and late summer, presumably under the same influence of algal produced DO levels (Fig. 3-23c).

The major difference in the BOD<sub>5</sub> removal efficiency in the WSP compared to the HRAP was the far greater variability in the WSP. This can be seen by the width of 95% CI band in Fig. 6-12b & 6-13a even though the standard deviations were almost the same (14.4 vs 14.1) as the BOD<sub>5</sub> removal efficiency in Table 6-4.

This further emphasises the lower reliability of the WSP as a treatment system than the HRAP if the treated water is to be returned to a stream, lake or ocean.

### **6.12.2 NH<sub>4</sub>-N Removal Efficiency**

As for the *E. coli* LRV, the NH<sub>4</sub>-N removal efficiency was similar for the WSP and the HRAP. In the WSP there was a typical sinusoidal pattern reflecting annual seasonality, peaking in mid-summer and bottoming in mid-winter. It has already been established that at the biological level NH<sub>4</sub>-N removal efficiency was strongly controlled by chlorophyll *a* levels and DO levels. There was no net algal growth in either of these systems, (Fig. 6-14 and 6-16b) therefore removal of N by incorporation into algal biomass and subsequent discharge is not possible as a pathway. A much more plausible pathway for a portion of the N removal in the WSP is death and sedimentation of algal and bacterial cells. This hypothesis can be considered feasible as there is considerably more algal

biomass in the influent than there is in the effluent (Fig. 6-14). The seasonal peak of  $\text{NH}_4\text{-N}$  removal supports the hypothesis that  $\text{NH}_3\text{-N}$  volatilisation was an important component of the N removal process in the WSP.

For the same reasons, it is likely that some N exited from the HRAP 2 as dead algal material as well as via  $\text{NH}_3\text{-N}$  volatilisation. It is not possible to apportion these numbers as no direct or indirect measurement was made in this study.

Overall, it is reasonable to assume that nitrogen was removed from the WSP and HRAP 2 by  $\text{NH}_3\text{-N}$  volatilisation and in the WSP by both algal cell incorporation and perhaps nitrification/denitrification releasing gaseous  $\text{N}_2$  to the atmosphere. The proportion leaving via the different pathways is not possible to estimate without further study.

The HRAP 2 consistently had 18% higher  $\text{NH}_4\text{-N}$  removal efficiencies than the WSP for most of the year (Fig. 6-12 c., 6-13b and Table 6-5), which can only be attributed to higher  $\text{NH}_3\text{-N}$  losses.

### **6.12.3 $\text{PO}_4\text{-P}$ Removal Efficiency**

For the WSP there is an annual sinusoidal cycle varying the  $\text{PO}_4\text{-P}$  removal efficiency from 7% in winter to around 55% in late summer (Mean = 28% Table 6-4), as happened in WSP 1. By contrast, the HRAP 2 appears to have either had no  $\text{PO}_4\text{-P}$  removal or even some additional  $\text{PO}_4\text{-P}$  entering the system. As this process appeared to only occur in the summer months, immediately post-harvest of the surrounding paddocks, it is possible this additional phosphorous was blown into HRAP 2 in dust from the surrounding farmland. This feature also emphasises that as there was no net algal growth in HRAP 2 (Fig. 6-16b), there was no uptake into algal biomass, so the  $\text{PO}_4\text{-P}$  remained largely unused in the treated wastewater.

It appears that unless there is strong algal growth, the removal of phosphorous from both the WSP and HRAP systems is severely limited to non-existent. This needs to be accounted for if systems are to be installed with the view of minimising nutrient levels in the treated wastewater.

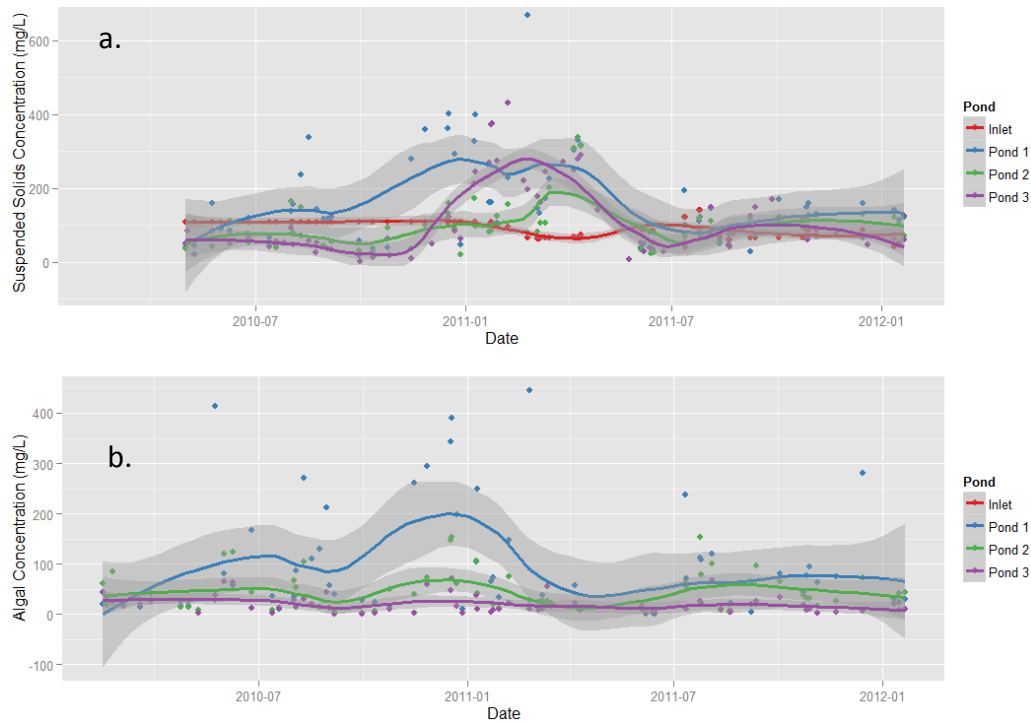
### **6.13 ALGAL & SUSPENDED SOLIDS CONCENTRATION**

In the ponds further down the treatment train such as WSP 2 & 3 and HRAP 2 it appeared that insufficient nutrients limits or completely stops algal growth in both systems. This effect can be seen in the algal productivity series in Figs. 6-16a and 6-16b. For nearly the whole period studied in both systems, the algal concentration is lower in the treatment pond than in the incoming partially treated water. This can be interpreted as the inlet water bringing all the algae into the ponds being studied and that there was almost no algal growth at all in these ponds.

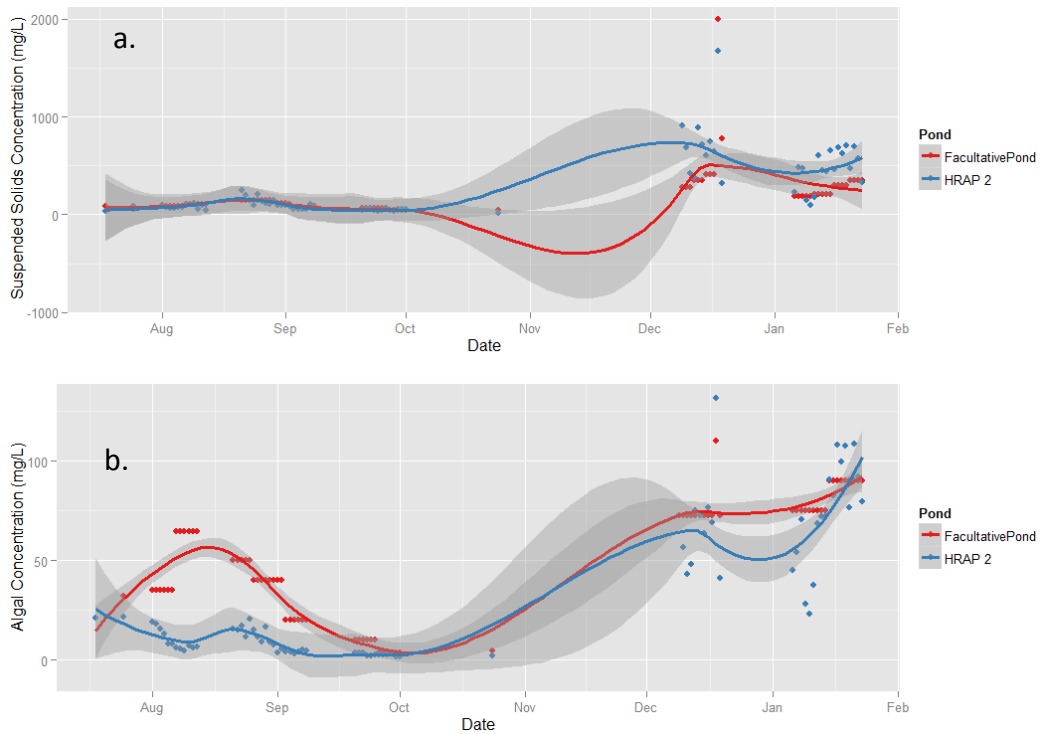
It is interesting to note that algal concentration was not always a good predictor of suspended solids concentration in the WSP system. In Fig. 6-14a, it can be seen that in WSP 2 and particularly WSP 3, from November 2010 to June 2011, there was a surge of suspended solids not due to algal growth (Fig. 6-14b). This was visible in the water as a colloidal clay material probably washed into the ponds from the surrounding earth following the periods of extraordinary rainfall during that period. Probably due to the continuous overflow outlet, the colloidal material cleared from WSP 2 much quicker than from WSP3, which was drained by intermittent pumping procedures.

By contrast, the algal concentration in HRAP 2 was a good indicator of suspended solids concentration (Fig. 6-15a&b).

It should also be noted that grazing by *Daphnia* spp. during the late winter/early spring period was heavy in the HRAP 2 (Fig.6-15b), and that managing that grazing could be vital if it is envisaged that the HRAPs will be used for biomass production. This is an area that requires further research as well.



**Fig. 6-14** Loess smooth lines with shaded 95% CI comparing the Lyndoch WSP 1, 2 & 3  
a. suspended solids concentration (mg/L) and b. algal concentration (mg/L).

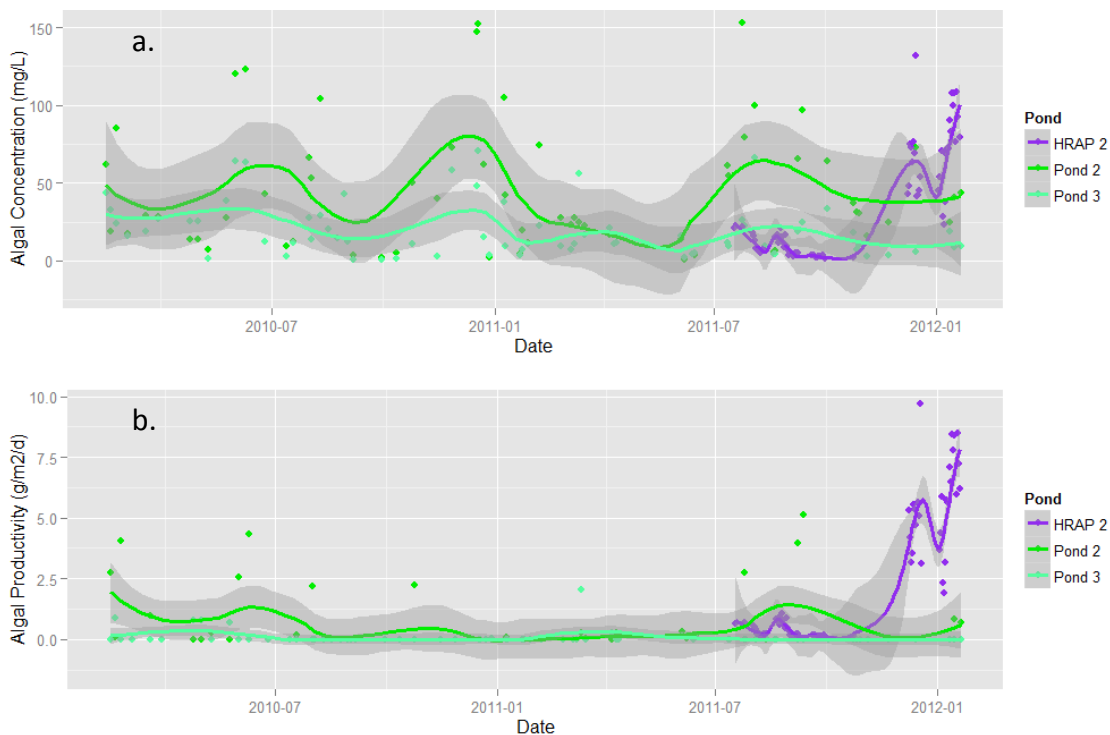


**Fig. 6-15** Loess smooth lines with shaded 95% CI comparing the Kingston-on-Murray HRAP inlet and outlet a. suspended solids concentration (mg/L) and b. algal concentration (mg/L).

It is also interesting to note that despite little or no new algal growth in either system, the algae coming in from their respective facultative pond sources were able to provide a suitable climate for both *E. coli* and nutrient removals as noted in the relevant sections above. From the perspective of wastewater treatment that continuity of treatment allows a great deal of confidence in the pond processes to achieve the desired outcomes. However, from the perspective of algal biomass productivity, the implication is clear. Algal biomass should be grown in and harvested from the first pond in the system. This study did not have an algal harvesting phase incorporated, so further investigation of the impact of harvesting would be a great advantage to improving biomass productivity performance.

**Table 6-7** Standard statistical comparisons of the algal concentration and productivity of the Kingston on Murray HRAP 2 and Lyndoch WSP 2&3.

| Pond   | Algal Concentration (mg/L), n=76 |           | Algal Productivity (g/m <sup>2</sup> /d), n=76 |          |
|--------|----------------------------------|-----------|--|----------|
|        | Mean ± s.d.                      | Range     | Mean ± s.d.                                    | Range    |
| HRAP 2 | 32.3 ± 34.5                      | 1.5-131.6 | 2.3 ± 2.8                                      | 0.07-9.7 |
| WSP 2  | 41.7± 38.8                       | 0.4-153.2 | 0.49 ± 1.2                                     | 0-5.1    |
| WSP 3  | 19.3 ± 17.5                      | 0.8-70.7  | 0.08 ± 0.30                                    | 0-2.02   |



**Fig. 6-16** Loess smooth lines with shaded 95% CI comparing the performance of the WSP 2&3 (greens) with HRAP 2 (purple) (both fed facultative pond outlet) a. algal concentration (mg/L) and b. algal productivity (g/m<sup>2</sup>/d).

## 6.14 COMPARISON OF CONSTRUCTION COSTS

One of the main advantages of natural wastewater treatment systems is that the initial capital costs and carbon footprint are low compared to complex, energy consuming electro-mechanical systems. When considering the HRAP as a possible replacement for all or part of a WSP system, construction costs will be

important. Costs can be considered in four categories. They are site, earth works, lining and plumbing, and electrical (including paddlewheel).

The site costs for the HRAP should be 40% of those for the WSP as equivalent sizing requires a 40% smaller footprint. Assuming excavation is the method of ponds construction, the surface area required for the HRAP is 40% and the depth is 30% of that required for the WSP. This means only 12% of the volume of earth is required to be excavated for construction of the HRAP system. Pond lining should also only be 40% of that required for the WSP, and the plumbing should be equivalent. The HRAP will require construction and installation of paddlewheels and also require electricity to drive the paddlewheels. As each of these costs is site dependent, it is not possible to give an accurate figure for the cost differences, other than to say that the HRAP could be expected to cost anywhere from 25% to 50% less to build than a WSP on the same site.

## **6.15 RELATIVE ADVANTAGES AND DISADVANTAGES OF WSP AND HRAP WASTEWATER TREATMENT SYSTEMS**

### **6.15.1 Advantages of the HRAP over the WSP**

#### **6.15.1.1 Land requirement**

Cost and availability of suitable land for wastewater treatment is often cited as a limitation to infrastructure investment (Picot et al., 1992, Oswald, 1996). If natural treatment systems are the preferred option, then the HRAP can treat the same volume of wastewater to the same level of pathogen and nutrient removal level as the WSP, using 40% of the area.



### **6.15.1.2 Construction Costs**

Construction costs for the HRAP can be considerably lower than for the WSP.

As described in para 6.14, the combination of total area reduction, smaller excavation and less pond lining required, more than offset the additional cost of the paddlewheel for the HRAP.

### **6.15.1.3 Performance consistency**

The HRAP provides a more consistent treated water output than the WSP.

Largely because of the continuous mixing in the HRAP, algal growth is greater and more reliable than in the WSP. As a consequence, conditions that enhance pathogen die-off and nutrient removal are more stable resulting in output of treated wastewater that is consistent in quality.

Additionally, WSP performance consistency is frequently made worse by the presence, or development over time, of hydraulic short-circuiting. If direct flow pathways between the inlet and outlet of each pond develop, then rapid transit of water from the inlet to the outlet can occur without adequate time for treatment processes. There are many ways hydraulic short-circuiting exists or can develop, including poor design with inadequate baffling, sludge accumulation creating narrow transit pathways, thermal stratification providing horizontal pathways without full mixing and persistent wind in an inlet to outlet direction. None of these problems occur in the HRAP.

Recovery time from an overwhelming event, such as flooding or organic overload is rapid in the HRAP compared to the WSP. The smaller treatment volume means the noxious influence is rapidly transited from the HRAP, and algal regrowth is rapid, leading to functional recovery in days. By contrast, the WSP can take months to recover normal functionality from an overwhelming event.

#### **6.15.1.4 Evaporative Losses**

Less water is lost to evaporation during treatment in a HRAP.

As the HRAP can achieve the same treatment results in 9 days compared to the 66 day treatment period for the WSP, time in the treatment process is reduced by 86%. The surface area of the HRAP is concurrently reduced by 60%. Overall, during the treatment evaporation of wastewater will be reduced by as much as 90% due to the combined effect of much shorter treatment time and the reduced surface area for evaporation.

#### **6.15.1.5 Desludging**

The HRAP does not require expensive desludging.

In the WSP system, depending on pond design, there are areas where the water movement slows down allowing particulate matter to sediment. Although this happens over the whole pond at a steady rate, sedimentation is accelerated at slow points such as edges and around the pond outlet. The rate at which this happens depends on many factors, such as pond design (particularly length to breadth ratio), and the organic load rates. Typically, sludge accumulates in the first (facultative) pond at a much greater rate than the latter ponds. By contrast the homogenous and continuously moving water in the HRAP does not allow for settling of dead algal and bacterial cells and other particulates that make up the sludge layer.

### **6.15.2 Advantages of the WSP over the HRAP**

#### **6.15.2.1 Paddlewheel and power supply**

The WSP does not require a mechanical paddlewheel and power supply.

The WSP is able to be constructed and left to operate without electrical power to the site. This can be invaluable in remote locations without a nearby reticulated power supply. In remote areas, a further advantage is that the WSP systems can operate with less frequent human checks to ensure they are functional.

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**CHAPTER 7.**

**A MATHEMATICAL MODEL FOR  
PREDICTING *E. coli* NUMBERS IN  
HRAPs RECEIVING  
WASTEWATER.**

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## 7.1 BACKGROUND

As part of the broader understanding of natural wastewater treatment systems, there have been many attempts to model the die-off of indicator organisms based on the established causes of microbial death in environmental conditions. Validated models are necessary if robust and reliable design criteria are to be defined. For waste stabilisation ponds these design related criteria have largely been met by the large number of models that have been promulgated since the 1960's. These were reviewed in Chapter 1 (pp 16 to 28). In Australia, the model used most frequently to design waste stabilisation ponds is found in Marais (1974). It remains popular because it is simple in concept and easy to use, and produces results that work. Essentially it allows calculation of a required theoretical hydraulic retention time with the only input required being that of an estimate of winter pond temperatures.

This simple model ignores the way that water moves through the system, and many authors have attempted to account for flow (hydrodynamics) with von Sperling summarising this work in two authoritative investigations (von Sperling, 2005, von Sperling, 2007). This area has been extended by the use of computational fluid dynamics (CFD) to more fully explain the way water moves through a pond system with the view to better design (Sweeney et al., 2003, Shilton, 2000, Salter et al., 2000).

Publications by Curtis et al. (1992) and Curtis et al. (1992b) have been influential in shaping thinking about wastewater pond disinfection. They suggested strongly that whilst UV radiation was an effective disinfectant, PAR was also important as a disinfectant in the presence of high concentrations of DO. They

report that the ability of light to damage faecal coliforms was highly sensitive to, and completely dependent on, oxygen. They noted that light-mediated damage of faecal coliforms was highly sensitive to elevated pH values, which also enabled light with wavelengths of >425 nm (in the presence of the sensitiser) to damage the bacteria. They concluded that humic substances, pH, and dissolved oxygen were important variables in the process by which light damages microorganisms in wastewater pond environments. This picture was enhanced by Bolton et al. (2010) and (Davies-Colley et al., 2000) who reported a very complex series of organism specific responses to interactions amongst the organism, (i.e.) the light, pH, temperature and DO environments.

Only a few authors have addressed the question of *E. coli* inactivation in HRAPs. Davies-Colley et al. (2003) reported on disinfection in a HRAP that was part of a more complex treatment train and focused on sunlight and dark processes in the HRAP and sunlight and sedimentation in the algal settling pond, as primary causative agents of disinfection. Craggs et al. (2004) operated a HRAP in batch mode so that removal of *E. coli* could be followed by sampling over time (2 days). They reported that *E. coli* removal was rapid during daylight hours and slow overnight. They fitted their data to a simple model, based on a complete-mix reactor equation, with a dark die-off term derived from night-time data, and a sunlight exposure term derived from day-time data. They also noted that dissolved oxygen and pH appeared to have little influence on inactivation rate over the measured ranges of pH (8.0–9.2) and DO (0–22 g/m<sup>3</sup>). Unfortunately, as their system was simplified dramatically by running the HRAP as a batch system to follow the decay of *E. coli*, it sheds little usable information on the functioning (and therefore the design requirements) for HRAPs run as continuous systems with constant rates of addition and removal of influent and treated effluent respectively. It is nevertheless instructive to note their conclusion that about 75% of the total *E. coli* inactivation in the HRAP was attributable to sunlight action.

The aim of developing the model presented here was to produce a robust and reliable way of predicting disinfection in HRAPs to facilitate their future design and implementation for wastewater treatment.

## **7.2 Development of a mathematical model for the prediction of *E. coli* inactivation within continuously fed HRAPs**

The model was developed in conjunction with, and relying totally on the mathematical skills of, Dr Simon Williams from the School of Computer Science Engineering and Mathematics within the Faculty of Science and Engineering at Flinders University, Adelaide.

### **7.2.1 Model Structure**

The model operates on a mass balance basis. To initiate a model run, the total *E. coli* load delivered at an initial inlet pumping is given based on historic data recorded in this study of the HRAP. The model then calculates the concentration of *E. coli* within the HRAP on an hourly basis, accounting for losses to pond discharge and inactivation and gains from each new wastewater addition. It is assumed that the *E. coli* concentration is uniform at all depths due to the mixing action in the HRAP.

The first effect is of dilution of the new wastewater addition in the total volume of the pond and then the effects of dark die-off and photo-inactivation are added. Photo-inactivation rates for each wavelength (UVA, UVB, PAR) are applied to the proportion of the pond which that particular wavelength can reach. For this model in-vitro *E. coli* inactivation rates in clear water for each irradiance (Bolton et al., 2010) are applied. These rates are constrained to



apply just to the specific inactivation depth, using previously published attenuation data for relevant wavelengths (Heaven et al., 2005, Curtis et al., 1994, Kohn and Nelson, 2006).

The three light inactivation rates for each wavelength are summed. The rate of inactivation measured in the dark is assumed to occur in the light at the same rate – this is added to the light inactivation rate and the overall inactivation rate calculated. The overall inactivation rate is used to calculate the numbers of *E. coli* inactivated and thus establish a new equilibrium pond concentration for each time interval with replenishment of *E. coli* occurring with each new wastewater addition at the pre-set time interval, and continuous losses to pond discharge.

### **7.3 Sunlight mediated *E. coli* inactivation**

The main driver of *E. coli* inactivation within the HRAPIN model was the well-established knowledge that the more energetic solar rays at the ultra-violet end of the solar spectrum are responsible for considerable pathogen die-off, at least in the surface layers where they can penetrate (Jagger, 1985, Bolton et al., 2010, Benchokroun et al., 2003b, Calkins et al., 1976, Crane and Moore, 1986, Davies-Colley et al., 2000, Davies-Colley, 2005, Fujioka et al., 1981, Maïga et al., 2009, Moeller and Calkins, 1980, Reed, 1997, Reed et al., 2000, Sarikaya and Saatci, 1987, Sinton et al., 1999, Sinton et al., 2002), but at the same time acknowledging that these more energetic ultra-violet rays were more rapidly attenuated with depth, particularly in the turbid waters of WSPs and HRAPs (Williamson and Neale, 2009, Stefan et al., 1983). The inactivation rates ( $\log_{10} \text{d}^{-1}$ ) and the respective effective inactivation depth for each range of wavelengths used in the model were derived from Bolton et al. (2010) and were  $25 \text{ d}^{-1}$  for UVB to 0.05m,  $1.4 \text{ d}^{-1}$  for UVA to 0.15m and  $0.4 \text{ d}^{-1}$  for visible light to 0.3m.

## 7.4 Dark (light independent) die-off

Although, the nature of dark die-off is poorly understood, *E. coli* dark die-off is included as continuous background die-off in the model. Some authors, such as Mara (2003b) prefer the term 'light-independent' rather than 'dark' die-off, as the putative mechanisms include protozoal grazing, *Daphnia* grazing, sedimentation, starvation and senescence, none of which depend on the presence or absence of light. In HRAPs, sedimentation is not possible due to the continuous mixing, and *Daphnia* are intermittent in their appearance. Of the other mechanisms, protozoan grazing may be the most influential. Stott (2006) reported a mesocosm study in New Zealand in which protozoan grazing accounted for the loss of up to 95% of *E. coli* and 61–82% of the viral indicators under dark conditions. It was further reported that protozoan grazing was responsible for up to 40% of the overall removal of bacterial and viral indicators during summer sunlight exposure. The role of dark die-off in the model is significant and continuous.

### 7.4.1 Establishing a figure for *E. coli* Dark Die-Off Rate

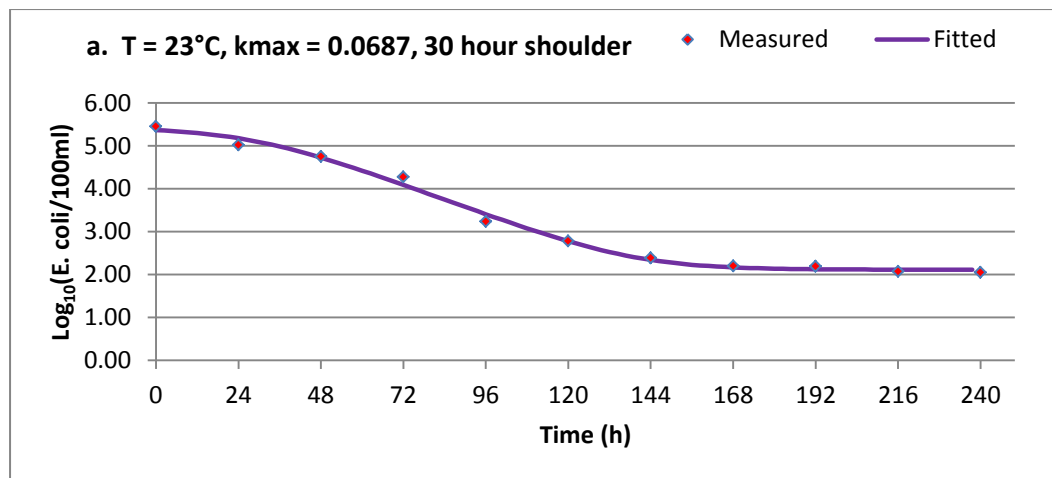
The problem lies in finding an appropriate rate to use in the model. There is a severe shortage of *E. coli* dark die-off rates reported in the literature. Only one estimate for *E. coli* dark die-off was found in Craggs et al. (2004). The estimation was performed by following *E. coli* numbers in a batch fed HRAP over a period of two days with an intervening night on two occasions. The dark die-off rate was reported as the night time rate calculated from just those two short time periods. Because of the limited experimental protocol no account is made for the effects of temperature variation on the dark die-off rate. The rate published in that report was  $0.02 \text{ h}^{-1}$ .

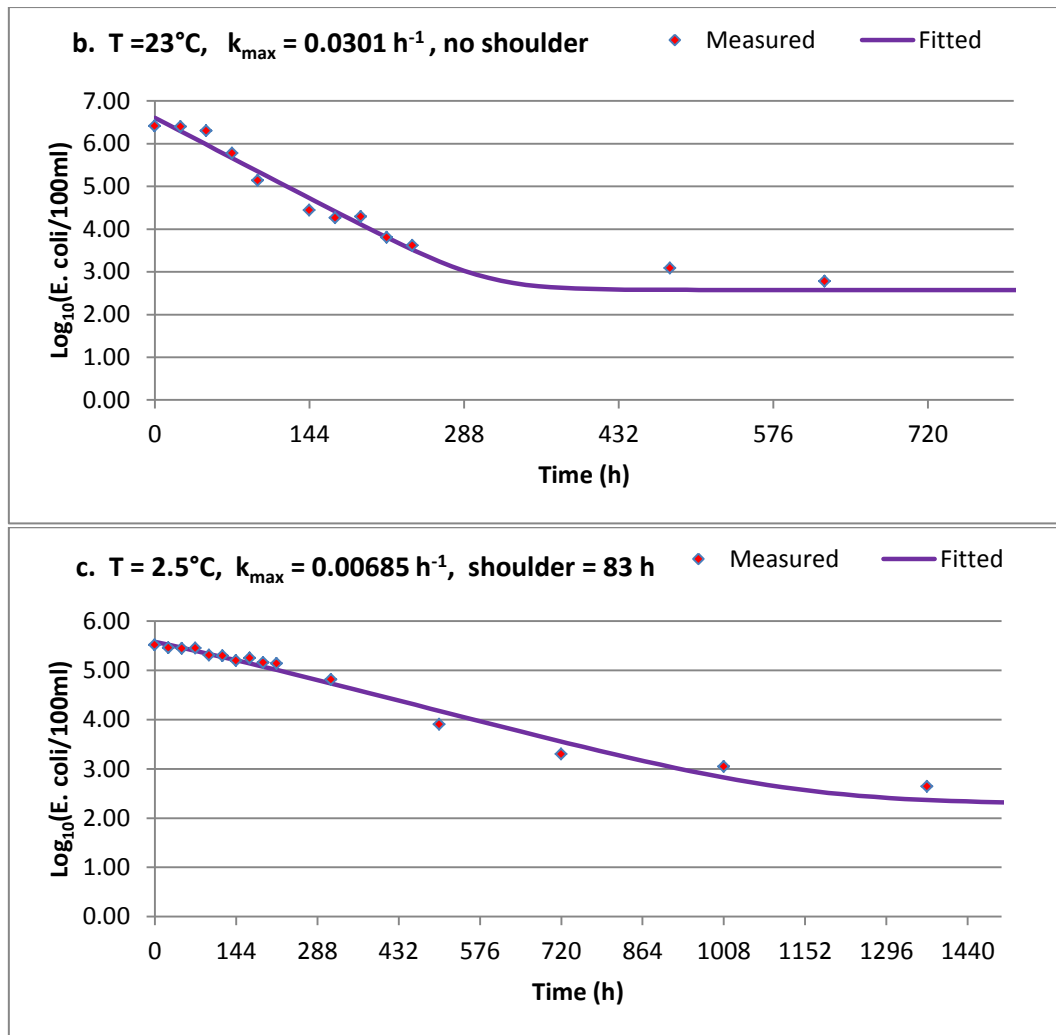
It was decided not to rely on that estimate as there were limitations in the way the rate was estimated. Prior experience with stored samples in this study had shown that the dark die-off rate varies over time in a log-linear plus tail and shoulder fashion as described in Para 2.5.8, and it also varies considerably with temperature. To establish some more statistically and biologically robust rate

figures, further studies on dark die-off of *E. coli* were undertaken as part of this study, as reported in Para 2.5.8

### 7.4.2 Results obtained from *E. coli* dark inactivation rate determinations in vitro

The *E. coli* dark inactivation rate data presented in Fig. 7-1 (a) was obtained from wastewater collected in winter and incubated in the dark at 23°C; the  $k_{\max}$  was  $0.0687 \text{ h}^{-1}$ , and the adjusted  $R^2$  was 0.991, indicating a very good fit of the simulation to the data (Table 7-2). A dark die-off  $k_{\max}$  of  $0.0301 \text{ h}^{-1}$ , (adjusted  $R^2$  0.969; Table 7-3)) was recorded for a different wastewater collected in summer and stored in the dark at 23°C (Fig 7-1b). Using the same water as used in Fig. 7-1, but decreasing the dark incubation temperature to 2.5°C (Fig 7-1c) decreased the *E. coli* inactivation rate constant by an order of magnitude to a  $k_{\max}$  of  $0.00685 \text{ h}^{-1}$  (adjusted  $R^2$  0.976; Table 7-3).





**Fig. 7-1** *In-vitro* determination of *E. coli* die-off rates in wastewater stored in the dark in the laboratory at either 23°C (a. & b.) or 2.5°C (c). A ‘shoulder’ showing there was a lag period before *E. coli* die-off commenced is visible in (a; 30h) and (c; 83h). Note the time scales are not the same for each graph.

This work established that dark die-off rate was temperature dependent. Thus for wastewater stored at 23°C a dark die-off rate of between 0.03 and 0.0687 h<sup>-1</sup> can be used for modelling HRAP performance. It is hypothesised that the two-fold difference between these two samples stored at the same temperature was the initial pond temperature at time of collection. The higher rate was collected in summer (Avg. pond temperature = 26°C) when protozoal activity was presumably high and the lower rate in winter (Avg. pond temperature = 11.4°C) when protozoal activity was presumably lower. Within the HRAP inactivation model (HRAPIN) the dark die-off rate can be varied to account for local prior

knowledge and/or differing environmental conditions of operation. If particularly cold water conditions are envisaged then a much slower dark die-off constant should be used, possibly as low as 0.0068/h.

**Table 7-1 *E. coli* dark inactivation at 23°C: Results of the statistical comparison between measured and fitted data (Fig 7-1(a)) using the method of Geeraerd et al. (2005).**

| Parameters                                  | Parameter values | Standard Error |
|---|------------------|----------------|
| SI (Shoulder length) (h)                    | 29.76            | 7.73           |
| $k_{max}$ ( $h^{-1}$ )                      | 0.0687           | 0.01           |
| $\text{Log}_{10}(E. coli/100ml)_{residual}$ | 2.11             | 0.07           |
| $\text{Log}_{10}(E. coli/100ml)_{initial}$  | 5.37             | 0.11           |
| <b>Mean Sum of Squared Error</b>            | 0.0155           |                |
| <b>Root Mean Sum of Squared Error</b>       | 0.1246           |                |
| <b>R-Square</b>                             | 0.9937           |                |
| <b>R-Square adjusted</b>                    | 0.9910           |                |

**Table 7-2 *E. coli* dark inactivation at 23°C: Results of the statistical comparison between measured and fitted data (Fig 7-1(b)) using the method of Geeraerd et al. (2005).**

| Parameters                                  | Parameter values | Standard Error |
|---|------------------|----------------|
| SI (Shoulder length) (h)                    | -                | -              |
| $k_{max}$ ( $h^{-1}$ )                      | 0.0301           | 0.003          |
| $\text{Log}_{10}(E. coli/100ml)_{residual}$ | 2.58             | 0.12           |
| $\text{Log}_{10}(E. coli/100ml)_{initial}$  | 6.60             | 0.15           |
| <b>Mean Sum of Squared Error</b>            | 0.0714           |                |
| <b>Root Mean Sum of Squared Error</b>       | 0.2673           |                |
| <b>R-Square</b>                             | 0.9730           |                |
| <b>R-Square adjusted</b>                    | 0.9685           |                |

**Table 7-3 *E. coli* dark inactivation at 2.5°C: Results of the statistical comparison between measured and fitted data (Fig 7-1(c)) using the method of Geeraerd et al. (2005).**

| Parameters                                  | Parameter values | Standard Error |
|---|------------------|----------------|
| SI (Shoulder length) (h)                    | 32.9             | 91.95          |
| $k_{max}$ ( $h^{-1}$ )                      | 0.00685          | 0.0008         |
| $\text{Log}_{10}(E. coli/100ml)_{residual}$ | 2.29             | 0.15           |
| $\text{Log}_{10}(E. coli/100ml)_{initial}$  | 5.58             | 0.12           |

|                                |        |
|--------------------------------|--------|
| Mean Sum of Squared Error      | 0.033  |
| Root Mean Sum of Squared Error | 0.1816 |
| R-Square                       | 0.9801 |
| R-Square adjusted              | 0.9759 |

## 7.5 Exclusion of other well-known die-off related factors (pH and DO) from the model

Included in the initial model were functions to account for the diurnal variation of pH and DO, as many authors (noted above) had considered these vital factors to understanding pond pathogen die-off. As the model was refined to reduce complexity, and using sensitivity analysis with the experimental results versus model results (Fig. 7-4), it became clear that retaining these two factors in the model did not contribute any greater degree of accuracy of prediction and at the same time added considerable complexity. This conclusion was in agreement with the findings of Craggs et al. (2004) who reported that pH and DO were “second order” factors, neither of which need be explicitly accounted for in simple modelling of disinfection in HRAPs unless (as yet undefined) extreme values are encountered. Guided by the principle of simplicity for the model produced here, the final working model no longer included pH and DO as factors contributing to *E. coli* inactivation within the HRAPIN model.

### 7.5.1 Other model inputs

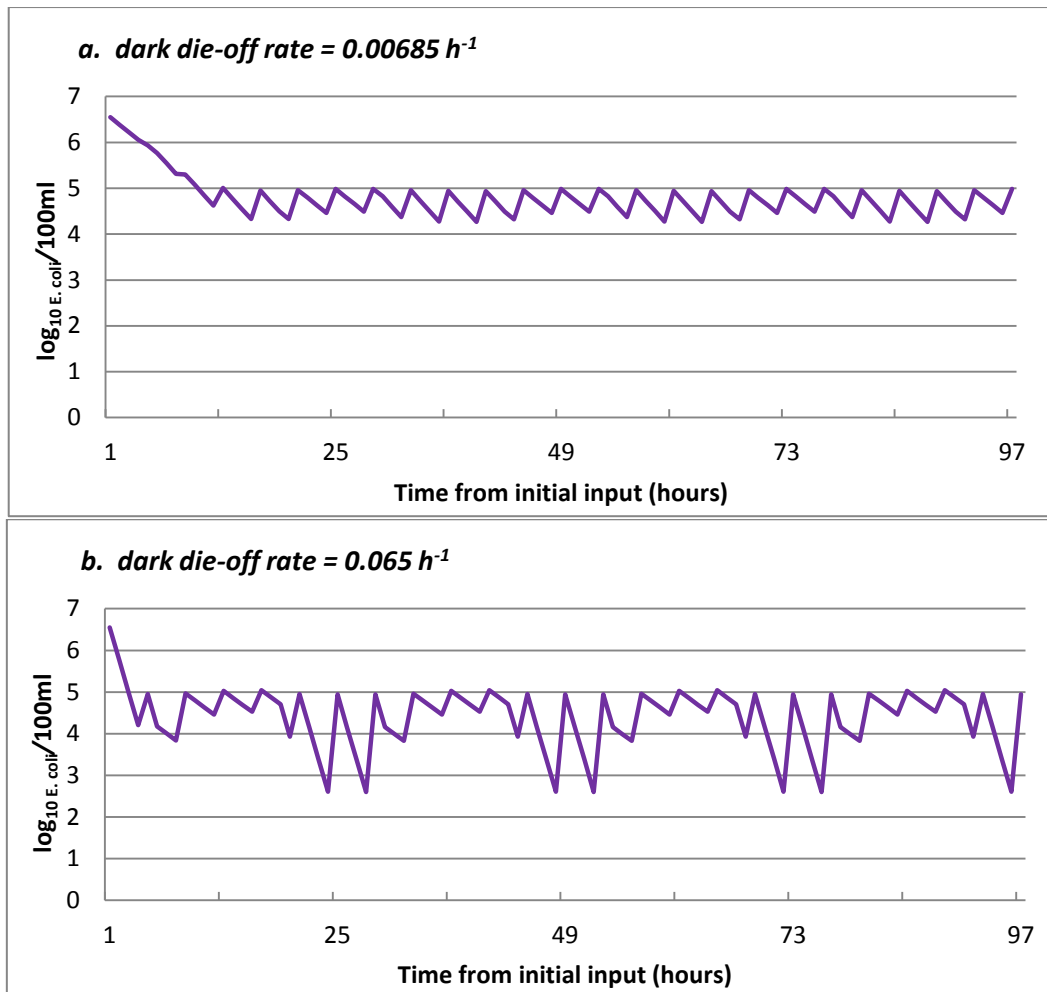
To initiate a “run” of the HRAPIN model it is necessary to input the following parameters:- physical dimensions of pond (surface area and depth), the initial concentration of *E. coli* in the inlet water, the theoretical hydraulic retention time (assumed knowledge of inflow rates and pond dimensions), and the frequency of adding influent wastewater. In the HRAP studied in this work the

values used for each of these inputs was as close as possible to the real dimensions. These are shown in Table 7-4 and the timing of intervals between addition of wastewater aliquots to the HRAP was generalised to 4 hours as specific time intervals could not be determined at Kingston-on-Murray.

### **7.5.2 HRAPIN output and the ability to observe effects of changing input parameters**

To conduct sensitivity studies various parameters can be adjusted such as frequency of input of wastewater, THRT, dark inactivation rate and depth of penetration of each wavelength. Altering the frequency of inputs results in a shorter or longer interval between peaks and the height of the peaks in *E. coli* concentration. The management message from this result is that continuous low volume input to the HRAP will result in more even concentration of *E. coli* over time, thus reducing variability in the HRAP output. Changing the depth of effective penetration of each light wavelength simply increased/decreased the final *E. coli* concentration band. This parameter is not really subject to control in the standard field operating environment, but rather it is determined by other factors such as suspended solids concentration.

The result of one modelling study is presented here to demonstrate the importance of understanding dark die-off rates. The output of the HRAPIN model using the influent *E. coli* input values and the light inactivation values presented above are shown for two different values of dark inactivation  $0.00685 \text{ h}^{-1}$  and  $0.065 \text{ h}^{-1}$  in Figs 7-2 (a) and (b) respectively.



**Fig. 7-2 HRAPIN model of the output of *E. coli* inactivation in HRAPs, comparing dark die-off set at (a) 0.00685 h<sup>-1</sup> and (b) 0.065 h<sup>-1</sup>. All other HRAP conditions were set at the same values: – depth = 0.32 m,  $\theta$  = 4.6 days, 4h interval between influent loadings.**

The difference between the two graphs (Fig. 7-2 (a) and (b)) shows how strongly dark die-off rates affect the simulated HRAP performance. With a very low dark die-off rate (Fig. 7-2 (a)) the *E. coli* concentration band is narrow (4.3 to 5.0) and regular, reflecting the fact that in this simulation the model is producing most of the *E. coli* inactivation via sunlight mediated mechanisms. By contrast, when the dark die-off rate is high (Fig. 7-2 (b)) the *E. coli* concentration band is broad (2.6 to 5.2) and irregular. Using standard statistical analysis:- the mean and standard deviation of the *E. coli* concentration band for the high dark die-off coefficient is 4.368±0.702 and the mean and standard deviation of the *E. coli* concentration band for the low dark die-off coefficient is 4.769±0.403. The



mean of the differences is 0.401 with a p-value of 4.572e-08 for the paired t-test of the differences of the means, implying that there is a significant difference between the means at the 95% confidence level. As well, the correlation coefficient between the two groups of simulated data is only 0.386 reflecting the large irregular *E. coli* concentration band in the high dark die-off coefficient simulation. In this simulation in the overnight period, there is significant *E. coli* inactivation occurring. This situation was not observed in the intensive study period, however the six months chosen for that study did not include any summer months. It would be of interest to repeat this work including summer months.

## 7.6 Intensive Study Periods

To support the development of the model, a series of intensive observations were made of the *E. coli* numbers in the HRAP and of the average UV radiation, pond temperature and hydraulic retention time (Table 7-4 and Figs 7-3 and 7-4). The observation interval varied from 2 hours to 8 hours. The timing of the observation periods was spread over six months to ensure there was a large range of the major parameters:- average daily UV radiation varied from 3.63 to 9.51 W/m<sup>2</sup>, average pond temperature varied from 10.8° C to 17.45° C, and hydraulic retention time varied from 3.72 days to 8.66 days.

### 7.6.1 Results of Intensive Study Periods

The general pattern for the recorded *E. coli* numbers during the day was that they remained in a regularly cyclic band varying between log<sub>10</sub> 4 to log<sub>10</sub> 5.5 (see Fig. 7-5). There was no discernible diurnal pattern to *E. coli* numbers, i.e. the values in the daytime were similar to those in the night time but the data were confounded by the 6 daily additions (including night additions) of influent (2kL)

containing *E. coli*. Smaller, shorter cycles appear within the larger rhythm if the observations were frequent enough (Fig. 7-3a.) Over the six month period there was a three-fold difference in UV radiation, and as can be observed in Fig. 7-3 and 7-4 and Table 7-4, this difference in UV radiation made no difference to the *E. coli* numbers observed or indeed to the pattern of change of numbers over the course of any particular single day. Thus, it was concluded that there was sufficient UV radiation on even the least sunny days in mid-winter to keep the disinfection process operating at a reliable rate in the HRAP.

**Table 7-4. The eight periods of intensive observation of the average UV radiation ( $Wm^{-2}$ ), pond temperature ( $^{\circ}C$ ), hydraulic retention time (d) and pond depth (m) recorded on those days.**

| Period      | Date          | Avg. UV Radiation ( $Wm^{-2}$ ) | Pond Depth (m) | Hydraulic Retention Time (d) | Avg. Pond Temp. ( $^{\circ}C$ ) |
|-------------|---------------|---------------------------------|----------------|------------------------------|---------------------------------|
| <b>May</b>  | 6/5/10        | 8.32                            | 0.32           | 6.07                         | 14.96                           |
|             | 7/5/10        | 8.84                            | 0.32           | 6.28                         | 16.77                           |
| <b>June</b> | 4/6/10        | 6.16                            | 0.32           | 4.67                         | 13.26                           |
|             | 5/6/10        | 5.88                            | 0.32           | 4.67                         | 13.21                           |
|             | 6/6/10        | 5.83                            | 0.32           | 4.67                         | 11.91                           |
|             | 7/6/10        | 4.73                            | 0.32           | 4.67                         | 11.25                           |
|             | 8/6/10        | 5.88                            | 0.32           | 3.72                         | 10.84                           |
| <b>July</b> | 11/7/10       | 5.91                            | 0.55           | 8.66                         | 11.63                           |
|             | 12/7/10       | 5.13                            | 0.55           | 8.66                         | 11.02                           |
|             | 16/7/10       | 3.63                            | 0.55           | 8.66                         | 12.57                           |
|             | 17/7/10       | 5.02                            | 0.55           | 8.66                         | 12.37                           |
|             | 18/7/10       | 4.03                            | 0.55           | 8.66                         | 10.80                           |
|             | 30/7/10       | 5.73                            | 0.55           | 8.66                         | 12.99                           |
|             | 31/7/10       | 6.19                            | 0.55           | 8.66                         | 12.40                           |
|             | 1/8/10        | 6.49                            | 0.55           | 8.13                         | 11.66                           |
|             | 2/8/10        | 6.95                            | 0.55           | 8.13                         | 10.43                           |
|             | <b>August</b> | 27/8/10                         | 7.53           | 0.42                         | 6.07                            |
| 28/8/10     |               | 6.15                            | 0.42           | 6.07                         | 13.80                           |

|                  |         |      |      |      |       |
|------------------|---------|------|------|------|-------|
|                  | 29/8/10 | 8.46 | 0.42 | 6.07 | 13.79 |
|                  | 30/8/10 | 9.41 | 0.42 | 6.07 | 14.34 |
|                  | 31/8/10 | 8.85 | 0.42 | 6.07 | 14.37 |
| <b>September</b> |         |      |      |      |       |
|                  | 18/9/10 | 7.17 | 0.32 | 4.26 | 14.56 |
|                  | 19/9/10 | 5.56 | 0.32 | 4.26 | 14.17 |
|                  | 20/9/10 | 5.15 | 0.32 | 4.34 | 13.36 |
|                  | 21/9/10 | 6.63 | 0.32 | 4.34 | 15.13 |
|                  | 22/9/10 | 6.07 | 0.32 | 4.34 | 14.60 |
| <b>October</b>   |         |      |      |      |       |
|                  | 27/9/10 | 8.88 | 0.32 | 4.34 | 17.45 |
|                  | 28/9/10 | 6.55 | 0.32 | 4.34 | 15.19 |
|                  | 29/9/10 | 7.19 | 0.32 | 4.34 | 14.20 |
|                  | 30/9/10 | 7.97 | 0.32 | 4.34 | 14.65 |
|                  | 1/10/10 | 9.13 | 0.32 | 4.34 | 16.67 |
|                  | 2/10/10 | 9.51 | 0.32 | 4.34 | 17.43 |

This study was unable to differentiate the disinfection performance at any of the three depths used during the period. The disinfection band at 0.55 m depth (Fig. 7-3(c)) was approximately the same amplitude and cyclic duration as the band at 0.32 m (Figs. 7-3 (a) and (b) and 7-4 (b) and (c)) and 0.42 m depth (Fig 7-4 (a)). This may reflect the short term nature of the study and the inability to compare multiple depths at the same time using the same inlet water.

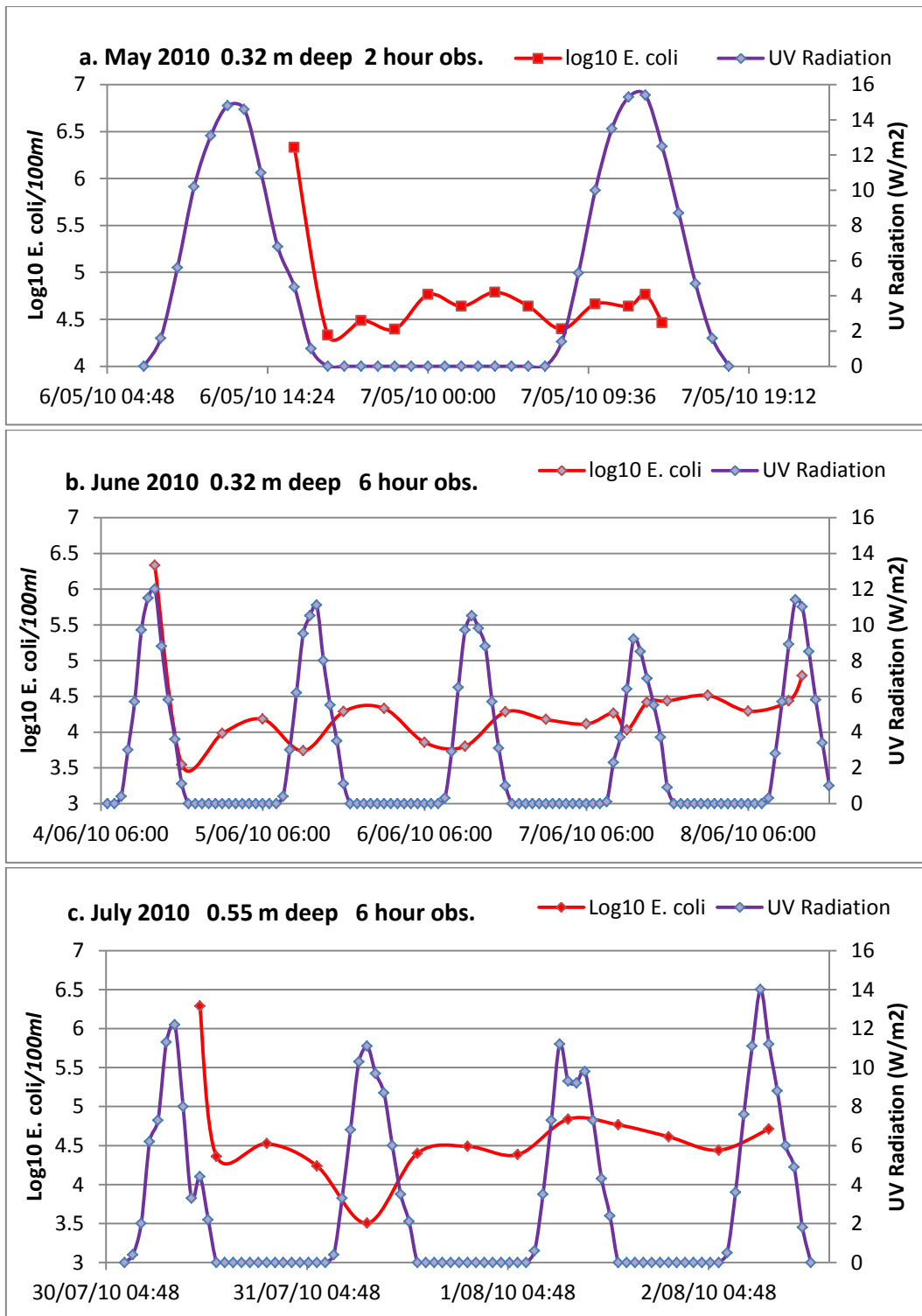
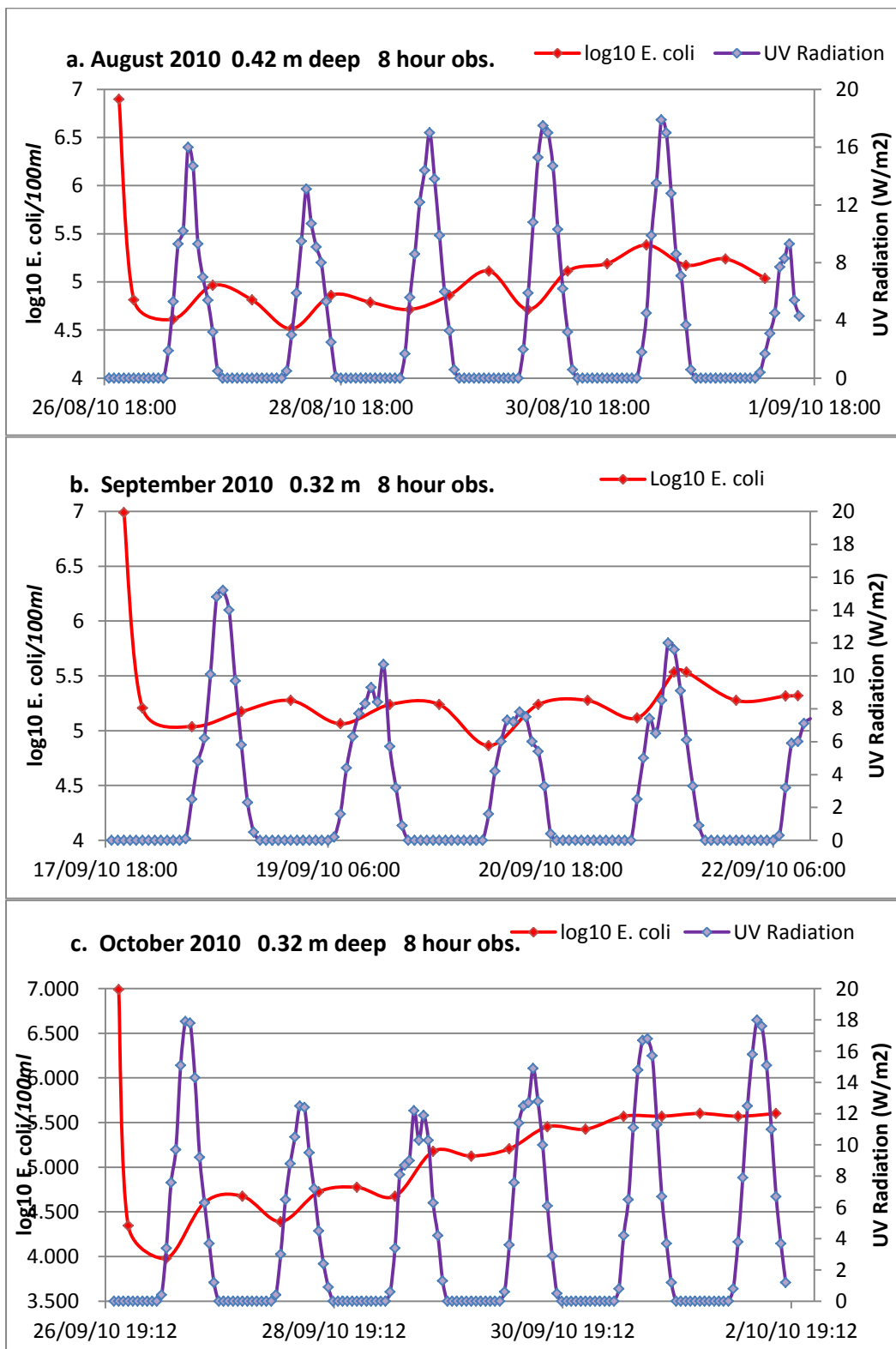


Fig. 7-3 Measured *E. coli* ( $\log_{10}$  MPN/100mL; in red) and hourly UV Radiation (in purple) recorded in the HRAP over three periods of intensive observation in the months of (a) May 2010 (2 hourly observations.) (b) June 2010 (6 hourly observations.) and (c). July 2010 (6 hourly observations.).



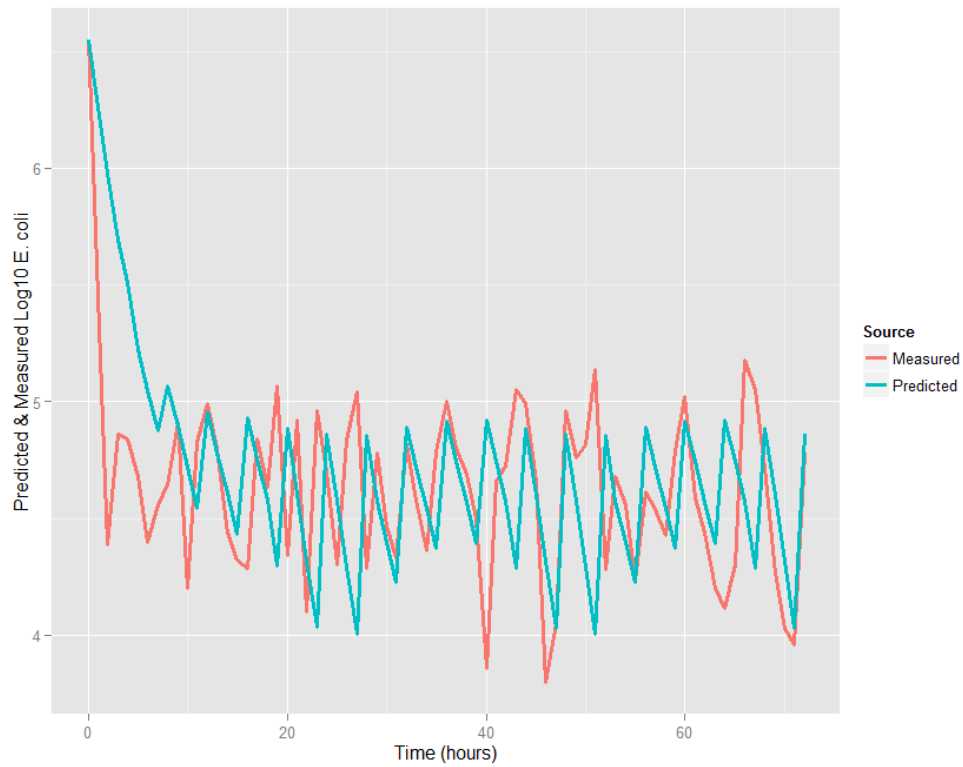
**Fig. 7-4 Actual *E. coli* numbers (log<sub>10</sub> MPN/100mL; in red) and hourly UV radiation (in purple) recorded in the HRAP over three periods of intensive observation in the**

months of a. August 2010 (8 hourly obs.) b. September 2010 (8 hourly obs.) and c. October 2010 (8 hourly obs.) . UV radiation on y-axis set to maximum of 20 W/m<sup>2</sup>

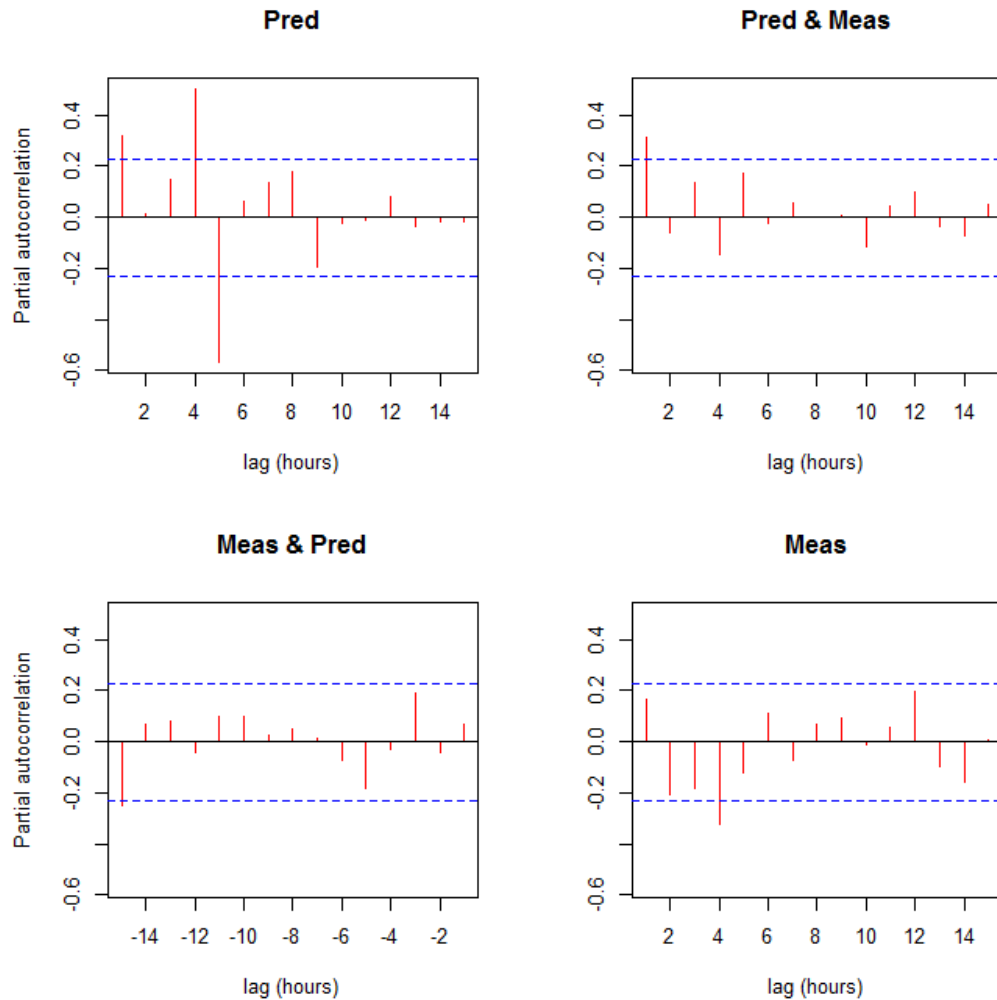
### 7.6.2 Comparing combined intensive study results to model predictions

The data from all eight intensive observation periods were combined to enable a meta-analysis of the results, since combining the data increased the number of observations post influent addition which gave a greater insight into the HRAP response. As the inlet load was always very consistent (see Table 4-2) the starting *E. coli* concentration point for the model simulations was similarly set at 6.549. The simulation used the mean values of the observation periods for each of the other preliminary settings, i.e. 0.40 m depth, 5.9 days retention time and 13.5 °C average temperature. This low pond temperature determined the use of a low dark die-off of 0.0118 h<sup>-1</sup>. The results are shown graphically in the time series graph in Fig.7-5.

Whilst there is not perfect symmetry between the predicted and the measured values, there is a large number of almost overlapping points. Statistically, the Pearson correlation coefficient is 0.40 (p=0.0004), indicating some degree of correlation. The mean and standard deviation are 4.611±0.340 for the measured and for the predicted values 4.673± 0.409. The p-value for the paired t-test of the difference between the means is 0.273 indicating that there is a small (0.06) and statistically insignificant difference between the means. Correctly interpreted, this means there is a 27% chance that a difference of this size in the means could have occurred by chance.



**Fig. 7-5 Comparing the HRAPIN model predicted *E. coli* concentration and an amalgam of *E. coli* concentrations measured during eight separate periods of intensive observation over six months from May to October 2010.**



**Fig.7-6 Correlograms for the HRAPIN predicted and measured dark die-off intensive study.**

The HRAPIN predicted values show strong autocorrelation at the four and five hour lag (Fig. 7-6). This merely reflects the programming, which allows for fresh wastewater to enter the system every 4 hours. There is a small autocorrelation for the measured values at four hours. It is not at all surprising that this level of autocorrelation is smaller than the autocorrelation for predicted as the actual timing of fresh wastewater delivery to the HRAP depends on the timing of use of household toilets, so although there are six pumpings per day, they are not necessarily spaced every 4 hours.



## 7.7 HRAPIN Model Summary

Visually, there is clear agreement in the general shape between the HRAPIN predicted and the *E. coli* concentration measured on eight separate occasions at a level of intensity designed to highlight the regular fluctuations noted when sampling the HRAP at any hour of the day or night, as seen in Fig.7-5. These can be summarised as ‘fluctuating in a band mainly between 4 and 5 log<sub>10</sub> *E. coli* on a four to five hourly basis’. The area of lesser clarity between the predicted and measured is in the synchronicity of the fluctuations. As noted above, this could be expected, as the exact timing of pumping to the pond from the septic tank reticulation system depends on the rate of fill of the septic tanks, that is to say on the rate of toilet usage, which is not steady throughout the day, whereas as the HRAPIN model, as set to match the operating conditions of the intensive study period, assumes pumping every four hours. The traditional statistics (correlation coefficient, paired t-test of means) support the case that says there are significant differences between the outputs at the 95% confidence level. Nonetheless, there is reason to have some confidence that the HRAPIN model will predict the range of outlet *E. coli* concentrations which a HRAP in South Australian conditions will deliver. This is a significant improvement over any previous attempt to model the disinfection performance of HRAPs.

Furthermore, the HRAPIN model provided evidence that the effluent *E. coli* concentrations are able to be simulated. It is then clear that the results of these simulations could be used to facilitate design and operation criteria for future HRAP implementations.

Of particular interest is the observation that even with a three-fold variation in daily UV irradiance, there was no discernible change in the size and range of the *E. coli* concentration band. This observation suggests that under South Australian conditions that there is always adequate UV radiation to maintain *E.*

*coli* inactivation at a consistent level. Higher UV irradiances do not improve this inactivation, presumably due to the rapid attenuation of UVB in wastewater.

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## CHAPTER 8

# REVIEW, SUMMARY AND CONCLUSIONS

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### PROJECT AIMS

1. To compare the effluent treatment performance of a Community Waste Management Scheme (CWMS) lagoon with a High Rate Algal Pond (HRAP) at Kingston on Murray, approximately 260 km North East of Adelaide.
2. To determine the optimum operating conditions to maximise HRAP performance.
3. To provide criteria for HRAP design and operation in South Australia.

## RESEARCH HYPOTHESIS

The hypothesis is that the HRAP will be able to treat domestic wastewater to a similar standard as a conventional WSP in a shorter time and on a significantly lower land area.

The aim of this chapter is to summarise the key findings as they relate to the hypothesis that the HRAP will perform an equivalent wastewater treatment as a conventional 3-cell WSP in a similar environment on a significantly less land area. There is no intention to further examine the performance of each system as it relates to previous reports in the literature, as this aspect is covered in earlier chapters. Instead, attention is drawn to results, (and factors that influence those results) that highlight the relative performance of each system and the environment in which they operate.

As WSPs have been extensively (possibly exhaustively) studied over many decades, the focus of this review of results is on the HRAP and the factors influencing its performance, particularly with regard pathogen surrogate removal.

Attention is drawn to the differences in performance when the HRAP is fed high-strength wastewater (HRAP 1) and low strength wastewater (HRAP 2).

Attention is drawn to the unique study and modelling of *E. coli* die-off in the HRAP.

As part of understanding the comparison between the HRAP and WSP, some focus is spent on understanding the factors influencing algal growth in the HRAP in particular.

Finally, attention is drawn to areas that would benefit from further research stemming from this study or areas not focussed upon in this study, which remain unexplored.



## 8.1 WSP Performance Summary

### 8.1.1 The environment & pond environment

The environment can be summarised as cold dry followed rapidly by hot dry interspersed with abnormally heavy rainfall periods. As anticipated, the DO, pH and pond temperatures all showed diurnal fluctuations consistent with algal photosynthetic activity during the day and algal and bacterial respiration at night in all three ponds. In WSP 1, the overnight DO typically dropped to 0, (except during the colder months of the year), and peaked mid to late afternoon between 5 and 40 mg/L with the mean DO daily variation of 10.9 mg/L, varying from 5.7 mg/L in winter to 18 mg/L in summer, the greater daily variation occurring during the warmer months when algal growth was at its greatest. Correspondingly, diurnal pH variations averaged 0.49 pH units, varying from 0.35 pH units in winter and 0.62 pH units in summer. The diurnal water temperature variations averaged 5.3°C, varying from 3.2°C in winter and 7.6°C in summer. From these physical seasonal variations it could be anticipated that the *E. coli* LRV would also show seasonal variations. Indeed the *E. coli* LRV averaged 2.024 overall, and varying from 1.675 in winter to 2.674 in summer, with the variation appearing to be synchronous with the seasonal physical pond variations.

As an observation, the great majority of the daily average winds came from within an arc from the South to South East. The prevailing wind along the length of the WSPs may have been expected to improve WSP 1 performance, although this study was not designed to test that hypothesis.

The mean (and range) areal and volumetric BOD<sub>5</sub> loading rates were 54 (29-91) kg/ha/d and 8 (4-13) g/m<sup>3</sup>/d respectively. These were low compared with other reports in the literature for WSP operations, and they were low compared to the HRAP BOD<sub>5</sub> loading rates noted in para 8.2.1. This suggests that this system should not suffer from organic matter overload. The mean (and range) areal and volumetric BOD<sub>5</sub> loading rates for the maturation ponds (i.e. the inlet to

WSP 2) were 15 (1-79) kg/ha/d and 2.4 (0.1-13) g/m<sup>3</sup>/d respectively. At times there was almost no reactive carbon material entering the maturation ponds.

### 8.1.2 Understanding WSP Performance Indicators

The 33 day average treatment period in WSP 1 resulted in the removal of 90% of the BOD<sub>5</sub>, 50% of the NH<sub>4</sub>-N, 47% of total inorganic-N, 17% of PO<sub>4</sub>-P and 2.024 log of *E. coli*. The next 11 days in WSP 2 removed a further 21% of the remaining BOD<sub>5</sub>, 34% of the NH<sub>4</sub>-N, 28% of total inorganic-N, 33% of PO<sub>4</sub>-P and about 1.3 log of *E. coli*, while the last 11 days in WSP 3 removed approximately 26% of the remaining BOD<sub>5</sub>, 21% of the NH<sub>4</sub>-N, 0% of total inorganic-N, 19% of PO<sub>4</sub>-P and about 0.5 log of *E. coli*.

The key to understanding the performance difference between the WSP and HRAP systems is in the THRT. All the key indicators highlighted above are similar to those reported for the HRAP, but the total average hydraulic retention time was 55 days compared to the 9 days for the HRAP. A possible way to highlight this difference is to report a key indicator, such as *E. coli* LRV, with a subscript designating the time required to achieve the result. Thus for WSP 1, the *E. coli* LRV would be designated *E. coli* LRV<sub>33</sub> = 2.024.

The *E. coli* LRV in each pond followed an annual sinusoidal variation as graphically demonstrated in Fig. 4-14. Thus, summer *E. coli* LRV<sub>33</sub> = 2.744 and winter *E. coli* LRV<sub>33</sub> = 1.675. This is consistent with the expectation that *E. coli* die-off in WSPs is temperature dependent. This study is unable to distinguish between the effect on die-off due to solar radiation levels and/or pond water temperature levels. Another important feature to notice with the WSP *E. coli* LRV was the high degree of variability. The 5<sup>th</sup> percentile *E. coli* LRV<sub>33</sub> = 1.047, and the 95<sup>th</sup> percentile *E. coli* LRV<sub>33</sub> = 3.363. The reasons for the high variability were not specifically identified, but could include intermittent hydraulic short circuiting and prolonged stratification.

The graph in Fig. 4-18 reinforces the statement that the vast majority of BOD<sub>5</sub> removal happened in WSP 1 (average 88.7% removal efficiency, at a consistent rate all year), to the extent that there was very little opportunity for the other two ponds to contribute to this process. It is reasonable to assume that the algal supply of DO was adequate at all times to achieve BOD<sub>5</sub> removal. On a few occasions BOD<sub>5</sub> actually increased in WSP 2 in particular, presumably due to algal die-off and release of nutrients back into that pond.

NH<sub>4</sub>-N removal was not as consistently effective as BOD<sub>5</sub> removal (average 68% removal efficiency, see Fig. 4-15). Most of the NH<sub>4</sub>-N removal happened in WSP 1, where the majority of the algae grew. There was strong seasonal variation with peak removals in autumn when algal growth and pH were at their maximum, and troughs in early spring. Three main mechanisms for NH<sub>4</sub>-N removal were occurring simultaneously, with the main removal mechanism varying from season to season. Between 30-60% exited WSP 1 as unchanged inorganic-N, 1-30% exited in organic form as part of the algal biomass and 1-80% exited as volatile ammonia. Most of the ammonia volatilisation occurred in the January to April period.

As can be seen in Fig. 4-20, only 6-10% of PO<sub>4</sub>-P was removed in the winter and spring periods, whereas up to 35% was removed in the autumn periods. Most exited as part of the algal biomass in the spring and summer (Fig.4-19), and most exited as assumed precipitates in the autumn period. The nature and initiation of these precipitates was not part of this study.

### **8.1.3 Algal Concentration and Productivity**

Algal concentrations (as estimated from chlorophyll a concentrations) were similar in the WSP and the HRAP systems. By contrast, algal productivity in the HRAP always exceeded algal productivity in the WSPs, and was often zero or negative (i.e. algal death) in the two maturation ponds. Algal productivities for WSP 1, 2 & 3 respectively averaged 2.3, 0.5 and 0.08 g/m<sup>2</sup>/d. Albazod

productivities for WSP 1, 2 & 3 respectively averaged 3.76, 0.40 and 1.69 g/m<sup>2</sup>/d. These productivities are significantly lower than HRAP productivities. As the sunlight and pond temperatures were the same in WSP 2 & 3 as in WSP 1, and there was still sufficient nitrogen and phosphorous in WSP 2 & 3 water, it can be concluded that carbon became a limiting nutrient for algal growth in WSP2 and WSP3.

#### **8.1.4 Overall Summary**

The WSP system used a larger area of land, took 55 days to treat to a similar standard and was significantly less productive of algal biomass than the HRAP. It was also more unpredictable in disinfection performance than the HRAP. Although, there has been widespread use of WSPs throughout the world over the last sixty years, as they come to the end of their working lives by clogging up with sediment, it would seem that replacement with HRAP systems offers wastewater treatment authorities clear advantages.

### **8.2 HRAP Performance Summary**

#### **8.2.1 The environment & pond environment**

As with the WSP, the environment can be summarised as cold dry followed rapidly by hot dry interspersed with abnormally heavy rainfall periods. The rapid transition of seasons was marked by a sudden drop-off in daily solar exposure by a factor of 28% between the months of March and April. This was matched by a jump in daily exposure by a factor of 23% between the months of September and October. To highlight the difference between the 'hot' and 'cold' seasons, in June there was only 30% of the January daily solar exposure.

Typically the DO, pH and pond temperatures all showed diurnal fluctuations consistent with algal photosynthetic activity (a rise in all three parameters) during the day and algal and bacterial respiration at night causing a fall in all three parameters. The extent of the fluctuations depended on the standing algal crop and algal growth rates, as well as intensity of solar radiation.

The pond inflow was derived directly from household septic tank overflow. After settling for two to three days in anaerobic conditions in the household septic tanks, there was little variation in the inlet water composition throughout the year. Inlet water for the HRAP 2 system was sourced from a facultative pond and was considerably less concentrated in all major nutrients and pathogens. This inlet water contained only 12% of the BOD<sub>5</sub>, 21% of the NH<sub>4</sub>-N, 64% of PO<sub>4</sub>-P and about 2.67 log<sub>10</sub> fewer *E. coli* compared to the inlet water for HRAP 1.

The areal and volumetric BOD<sub>5</sub> loading rates varied with pond operational depth and were consistent with other reports in the literature for HRAP operations. The mean HRAP1 areal loading rates (kg/ha/d) with range in parentheses were shallow depth (0.32m) 149 (63-200), medium (0.42m) 127 (59-184) and deep (0.55m) 118 (54-170 kg/ha/d). The mean volumetric loading rates (g/m<sup>3</sup>/d) with range in parentheses were shallow 44 (23-71), medium 31 (16-50) and deep 23 (12-37). At these comparatively low loading rates it was unlikely that organic matter overloaded the system during the study. The BOD<sub>5</sub> areal loading rates for HRAP 2 were 16%, 7% and 6% of the HRAP 1 loading rates for the shallow, medium and deep ponds, respectively.

### **8.2.2 Understanding HRAP Performance Indicators**

The 6 day average treatment period in HRAP 1 (considered over all three operating depths) resulted in the removal of 92% of the BOD<sub>5</sub>, 70% of the NH<sub>4</sub>-N, 60% of total inorganic-N, 13% of PO<sub>4</sub>-P and about 1.7 log of *E. coli*. The 6 day average treatment period in HRAP 2 (considered over all three operating depths) resulted in the removal of 64% of the BOD<sub>5</sub>, 66% of the NH<sub>4</sub>-N, 60% of total inorganic-N, 0% of PO<sub>4</sub>-P and about 2.57 log of *E. coli*. Even though these performance data are averaged over three operating depths they are comparable with performance data reported for the WSP in para 8.1.2, but occurring in 20% of the time required by the WSP. For HRAP 1, the *E. coli* LRV

could be designated *E. coli* LRV<sub>6</sub> = 1.8, and for HRAP 2 could be designated *E. coli* LRV<sub>6</sub> = 2.57. To emphasise the greater reliability of the HRAP 1 compared to the WSP the 5<sup>th</sup> percentile *E. coli* LRV<sub>6</sub> = 1.30, and the 95<sup>th</sup> percentile *E. coli* LRV<sub>6</sub> = 2.69, which is a significantly tighter range than the WSP reported in para 8.1.2.

The concept of *E. coli* loading rates was explored as a means of aiding understanding of *E. coli* removal performance. Predicting HRAP performance is explored further in para 8.3, but an observation on the use of LRV is worth noting here. When the data were boot-strapped both forms of expressing *E. coli* loading rate emerged as important predictors of *E. coli* LRV. Much emphasis is placed on the LRV by regulators as a means of determining and comparing treatment system performance. In this sense, regulators should understand that the *E. coli* LRV is strongly influenced by the concentration of organism in the inlet water. Further work is required to quantify the limitations of LRV and to find suitable alternatives when the inlet organism concentration is low.

Nutrient removal efficiencies for BOD<sub>5</sub>, NH<sub>4</sub>-N, NO<sub>3</sub> & NO<sub>2</sub>-N and PO<sub>4</sub>-P were largely independent of operational depth at the three depths studied although an influence of THRT cannot be discounted.

BOD<sub>5</sub> removal efficiency was consistently high throughout the year. The volumetric BOD<sub>5</sub> loading rate emerged as the most important predictor of BOD<sub>5</sub> removal efficiency when the data were boot-strapped for regression tree analysis. This would indicate that there was plenty of reserve capacity for BOD removal as the greater the concentration that went in, the more BOD was removed. At some point this relationship must break down, but under the conditions in this study (inlet BOD<sub>5</sub> ranged from 144 to 377 mg/L), the system could remove ever greater concentrations.

By contrast, neither areal nor volumetric inorganic-N loading rates (almost entirely NH<sub>4</sub>-N) were important predictors of NH<sub>4</sub>-N removal efficiency. NH<sub>4</sub>-N removal was not as consistently effective as BOD<sub>5</sub> removal (average 70% removal efficiency, see Fig. 3-13). Most of the NH<sub>4</sub>-N removal happened in

HRAP 1, where the majority of the algae grew. There was strong seasonal variation with higher removals occurring in the 'hot' period compared to the 'cold' period (Fig. 3-12). Three main mechanisms for  $\text{NH}_4\text{-N}$  removal were occurring simultaneously, with the main removal mechanism varying from season to season. Overall 43% exited WSP 1 as unchanged inorganic-N, 24% exited in organic form as part of the algal biomass and 33% exited as volatile ammonia. Most of the ammonia volatilisation occurred in the summer months.

87% of the incoming  $\text{PO}_4\text{-P}$  exited the HRAP unchanged and 13% exited as part of the algal biomass. Reflecting this,  $\text{PO}_4\text{-P}$  removal was generally higher in the hot' period compared to the 'cold' period (Fig. 3-15).

Pond depth played a role in the HRAP performance mainly through the effects of higher algal standing crops in the shallower ponds (Table 3-8). The shading effect of the denser standing crop may have affected the ability of sunlight to bring about disinfection; the *E. coli* LRVs for shallow, medium and deep ponds being 1.7, 2.1 and 2.0 respectively.

### **8.2.3 Algal Concentration, Algal and Albazod Productivity**

Including February 2011 data, the mean albazod standing crop was  $350 \text{ g/m}^2$  with a range of 4.3-2,547 (Table 3-8) and the mean algal productivity (chlorophyll a as 2% of algal mass) was  $11.2 \text{ g/m}^2/\text{day}$  (range 0.05-65), and albazod productivity was  $79 \text{ g/m}^2/\text{d}$ , with a range of 0.9-507 (Table 3-7). The ranges for these data are included to show how variable algal growth can be in HRAPs. At the same time, the key performance indicators are not compromised as the typical pattern involved a massive growth spurt followed by a crash followed by a quick recovery. Ideally, these growth fluctuations would be smoothed out. This would require more research to establish the exact causes and cures, particularly for the rapid declines.

Pond temperature and depth had some influence on algal growth patterns (Tables 3-7 & 3-8) as would be expected. Cold conditions resulted in lower algal growth and the deepest pond (0.55m) also restricted algal growth. Otherwise algal productivity had the following characteristics:

There appeared to be no nutrient limitation to algal growth in HRAP 1, while there was evidence for carbon limiting algal growth in HRAP 2.

Light appeared to be the main limitation to algal growth. In this regard, too much light may have been as limiting as too little light. Algal growth appeared to be limited by a combination of self-shading (particularly in the 0.55 m depth) and photo-inhibition for much of the year. This suggested that the mixing employed for the deeper ponds was not effective in getting all algae into the photic zone for sufficient time for photosynthesis to be effective. This may be a design feature for HRAPs operated for maximum biomass production, both in the operating depth and the length of raceway used. In reality, the effects of retention time and pond depth were not separable in this study. Further research would be required to study the effects of altering retention time without altering depth.

Algal growth appeared to be interrupted periodically by grazing or disease after a period of rapid growth in the spring and summer periods.

In HRAP 2, algal concentrations were about 22% of those in HRAP 1 at all depths and averaged 32 mg/L. The average algal standing crop was 104 g/m<sup>2</sup> which was 30% of the HRAP 1 average standing crop. However, algal productivity averaged only 0.04 g/m<sup>2</sup>/d, and for most of the time was zero, with algal death of incoming algae occurring as the predominating factor. These results provide indirect evidence that algal growth was subject to nutrient limitation in HRAP 2. It was possible that carbon became limiting in HRAP 2 after having 88% of the BOD<sub>5</sub> removed compared to the influent for HRAP 1. Further research would be required to determine the cause(s) of limited algal growth in HRAP 2.



### 8.2.4 Predicting HRAP Performance

For *E. coli* LRV predictive purposes, the Theoretical Hydraulic Retention Time combined with the inflow rate (volume and timing of additions) were the two most important factors. The 'mass balance' modelling for *E. coli* showed that the volume and timing of the addition required each day to achieve the desired THRT was very important for the HRAP disinfection process to be optimised. In effect, constant small volume addition would be ideal. In reality, most rural wastewater treatment plants will require intermittent additions, and the timing of these needs to be more carefully considered.

The five day averages of water temperature, DO, pH and Solar Radiation were the next most important predictors. The five day average data might well be anticipated to have greater predictive value than the single value measured on the day the inlet and outlet *E. coli* enumerations were determined. Conditions prevailing over the previous five days will have influence over the rate of disinfection as this is the THRT.

The knowledge gained from these statistical studies helped inform construction of the HRAPIN model to predict outlet *E. coli* concentration. Ultimately, this understanding is the major insight gained from this study.

Factors important for algal growth are important for BOD removal. Thus, the seasons, and five day averages for temperature, DO and pH all emerged as important BOD removal predictors. As it is well established that the oxygen produced by algal photosynthesis is essential for the activity of the heterotrophic bacteria responsible for BOD<sub>5</sub> removal, these findings would be exactly as anticipated.

It was estimated that 43% of the inorganic-N entering the HRAP per day, exited unchanged, 24% exited incorporated into algal biomass, and 33% exited via volatilisation as NH<sub>3</sub>-N gas. The latter two pathways require algal growth either

directly for biomass incorporation or indirectly for creating high pH conditions to allow NH<sub>3</sub>-N volatilisation. Hence, it is not unexpected that chlorophyll a, DO daily variation and five day average solar radiation were the most important predictors of NH<sub>4</sub>-N removal.

### **8.2.5 HRAPIN Model for *E. coli* removal from HRAPs**

A unique mathematical model to predict *E. coli* concentration in HRAP effluents was developed in conjunction with, Dr Simon Williams from the mathematics and computing sciences school within the Faculty of Science and Engineering at Flinders University, Adelaide.

The model was constructed and operated on a mass balance basis. The drivers of die-off in the model are, (in order of potency) UVB, UVA, visible light and dark die off. The die-off constants used for the light mediated forms were obtained from the literature and for dark die-off a combination of the literature and in-vitro studies as part of this study.

To validate the model, a series of eight periods of intensive collection of treated wastewater from the HRAP 1 were conducted over a six month period. The plots of measured *E. coli* numbers were superimposed over those predicted by the HRAPIN model. The model gives a remarkably accurate prediction of the measured *E. coli* concentration in the HRAP 1 for extended periods.

The predictions from this model have shown that optimum operation of the HRAP to achieve the greatest *E. coli* LRV requires continuous input of wastewater and an extended Hydraulic Retention Time.

In rural South Australia, it will be necessary to provide a gravity feed from a header or buffer tank to achieve slow continuous feed to the HRAP. Validation of such a system would be part of further research that would prove beneficial to the wastewater treatment industry. The actual treatment time required will

depend on the proposed use of the treated wastewater and the microbial reduction required to meet any particular regulatory standard.

### 8.3 Comparing HRAP & WSP performance

It was clearly demonstrated that the two systems had virtually identical climatic conditions, the main difference being greater rainfall at Lyndoch. It was also clearly demonstrated that the inlet water to both systems was nearly identical largely because both are sourced from settled household septic tank systems.

The BOD<sub>5</sub> areal loading rate for the HRAP is 135 kg/ha/day versus 54 kg/ha/day for the WSP. Although neither loading rates are extreme, this study demonstrates the ability of the HRAP system to handle what could be considered full strength inlet wastewaters.

Both systems showed an annual sinusoidal cycle of *E. coli* concentrations with peak concentrations in summer and lowest concentrations in winter. Throughout most of the annual cycle the WSP had an *E. coli* LRV about 0.3 higher than the HRAP. Nevertheless, most importantly from a regulators point of view, there was much more variability in the performance of the WSP. The 5th percentile *E. coli* LRV<sub>33</sub> (i.e. the lowest 5% of data) for the WSP was 1.047 and *E. coli* LRV<sub>6</sub> 1.301 for the HRAP. It must be repeated that the subscripts tell the real story that the HRAP achieved almost the same level of disinfection in less than one-fifth of the time taken by the WSP.

Statistical data such as the 5<sup>th</sup> percentile LRV are commonly used by regulators to decide on approval or pre-approval for building of new wastewater treatment systems, so this difference could have significance for that process. Further evidence of the greater variability in *E. coli* LRV is in the breadth of spread of the 95% confidence interval band around the loess smoothed LRV time series curves. The 95% CI for the HRAP is much narrower than that for the

WSP. This consistency of performance of the HRAP system was a strong feature when compared to WSPs.

Both systems were very effective at removing BOD<sub>5</sub>. Again the key difference in the performance was the greater variability of the BOD<sub>5</sub> removal efficiency in the WSP compared to the HRAP, most easily visualised by the width of 95% CI band for the respective loess smoothed curves for BOD<sub>5</sub> removal efficiency.

Both systems were quite effective at removing NH<sub>4</sub>-N. It has been established that the removal of this nutrient was modulated by algal concentrations (measured as chlorophyll a) and DO levels. It was established that inorganic nitrogen was therefore removed from the ponds by both algal cell incorporation and volatilisation releasing gaseous NH<sub>3</sub>-N to the atmosphere.

The HRAP consistently had NH<sub>4</sub>-N removal efficiencies 19% higher than the WSP for most of the year, which can probably be attributed to the overall higher algal productivity as previously noted.

For the WSP there was an annual sinusoidal cycle with varying PO<sub>4</sub>-P removal efficiencies from 5% in late winter to around 40% in late summer. By contrast the HRAP only removed around 12% of the PO<sub>4</sub>-P all year round with very little seasonal variability.

It is most likely that all the PO<sub>4</sub>-P removal from the HRAP was via incorporation into algal cells, whereas it appears that in the WSP over the summer period there was some additional form of removal, probably precipitation with calcium and magnesium. It is not clear if this extra PO<sub>4</sub>-P removal in summer was specific to the Lyndoch WSP system.

As noted in para 8.2.3, algal concentrations were very nearly equal in the WSP and the HRAP systems. However, algal and albazod productivity was always lower in the WSPs compared to the HRAP, and declined with the passage of wastewater through each pond in the WSP system. Equally algal and albazod productivities in HRAP 2 were significantly less than in HRAP 1. A possible

reason for this notable difference is that mixing of wastewater in the HRAP creates turbulent flow offering the potential of moving algae in and out of the 'light zone' and 'self-shaded zone' and therefore improving total algal productivity.

Constraints to algal productivity were probably similar in both systems. The light climate was almost certainly the major constraint, either through excessive illumination producing photo-inhibition, or particularly in the case of the WSPs, insufficient light penetrating below about 0.3 m depth for any photosynthesis to occur.

In WSP 2 & 3 and HRAP 2 there was indirect evidence that carbon became a limiting nutrient for algal growth.

Algal growth in the HRAP in particular, but also to some extent in the WSPs was characterised by periods of extremely rapid growth over a number of weeks when conditions were optimum, followed by remarkable crashes in algal population. The reasons for these events were not able to be elucidated in this study but they certainly require on-going research to clarify. Without full understanding it would appear to be a major risk factor if biomass harvesting becomes a major focus secondarily to wastewater treatment. It must be stressed however that these crashes had little adverse effect on wastewater treatment performance in the HRAP. It must also be stressed that the three days of massive flooding of the HRAP did not result in long term damage to the wastewater treatment. In fact, within 24 hours the HRAP system was treating wastewater in the same way as it had prior to the flooding.

It is possible to speculate that the sudden crashes in algal populations were either due to infection with either fungi or viruses or perhaps heavy grazing by protozoa or *Daphnia* spp. This is an area that requires further research. *Daphnia* spp. were obvious and visible during the winter period in both the HRAP & WSP, but vanished when the hot weather came in summer. The algal populations in the WSP appear less vulnerable to massive population drops, but

their overall lower productivities means they are unlikely to be used for biomass harvesting procedures, so this is not of any major practical significance.

#### **8.4 Optimising HRAP Design and Operating Criteria for South Australian conditions**

In response to the second and third aims of the project, key elements of design and operation of HRAPs in South Australian conditions emerged from this study. Obviously, each proposed site would require specific design according to the physical layout and population being serviced.

Firstly, if the sole purpose is to treat wastewater, then the HRAP should be operated in a way that maximises disinfection and nutrient removal. The most obvious design criteria are to build to the volume of effluent to be treated. This can be quite challenging in small 'holiday' destinations, where populations can increase five-fold for a few weeks each year, mainly over summer. The standard South Australian wastewater treatment design is based on 150 L per person per day. In South Australia, all of this will pass through home septic tanks with a 2 to 3 day residence time. In an extreme case such as a 'holiday town' situation, the HRAP volume capacity must enable a minimum 4 day residence time. To avoid over-building, flexibility should be built in to allow the HRAP to operate at up to 0.6 m depth for short periods. When the population pressure is removed the HRAP depth can be reset to 0.2 m depth allowing a three-fold increase in volume. Further flexibility can be achieved by adding a buffer tank before the HRAP to hold the equivalent of 5 days inflow. This can be filled during peak flow periods and drained slowly as demand drops. Doing this also allows a little flexibility with the retention time which can be set anywhere from 4 days to 8 days without altering the treatment performance by adjusting flow rates from the buffer tank. Clearly, the paddlewheel needs to be of sufficient dimensions to handle 0.6 m deep water as well as 0.2 m deep water. The paddlewheel also must be able to provide approximately 12 rpm turning speed and a water velocity of 0.2 to 0.3 m/s.

In the less extreme case, with stable year round populations, the HRAP can be built to treat the needs of the current population plus a percentage increase. In that case, the depth can be set to operate anywhere between 0.2 and 0.4 m without compromising treatment performance.

In all cases treatment performance will be enhanced by slow continuous feed of the influent water. In most situations, this will only be able to be achieved by having a pre-HRAP buffer tank, with an adjustable inflow rate via a gate valve. Adjustment to the outflow rate via another gate valve would provide even greater flexibility in setting the THRT. In cases where the inflow rate cannot be slow and continuous, the volume delivered at each inlet delivery should not exceed 4% of the total pond capacity.

A second HRAP in series with the first will provide significant extra disinfection performance. It should be built to the same dimensions as the first HRAP and receive treated wastewater from the first HRAP by gravity feed so the operation occurs automatically with no pumping required.

If it is proposed that the HRAP produce biomass from wastewater, then the design will be optimised by setting the operating depth between 0.3 and 0.4 m, and the retention time at 4 days. Harvesting the algae on a continuous basis will reduce the impact that high standing crops have on reducing algal growth. At the moment, harvesting technology is not well advanced, although continuously operating filter designs (such as the Z-filter™) offer potential for the future. Productivity will be light-limited until standing crops can be reduced to 40 to 50 g/m<sup>2</sup> on a continuous basis. Further studies of flocculation and auto-flocculation would offer some advantage to aid harvest and improve productivity. Further studies to reduce the catastrophic collapses in algal populations will also help future productivity. It may be that continuous harvesting and reducing the standing crop will reduce 'over-crowding' that may be allowing disease processes to run out of control.

A second HRAP in-series with the first offers no advantages for biomass productivity as nutrient depletion by the first HRAP makes algal growth in the second HRAP too slow to be worthwhile.

## **8.5 Has the hypothesis been proven?**

In para 1.2.1 the research hypothesis was stated as :

“The hypothesis is that the HRAP will be able to treat domestic wastewater to a similar standard as a conventional WSP in a shorter time and on a significantly lower land area.”

The simple answer is that the hypothesis has been proven. There is overwhelming statistical evidence found in this study that demonstrated that the performance of the HRAP against the key criteria was equal to or better than that achieved by a WSP in a similar climatic zone receiving essentially equivalent wastewater to treat. The comparison was made for the first or facultative part of the WSP and then again for the second or maturation part of the WSP system with and the same answer in both cases.

Possibly the two outstanding features that make the HRAP a more attractive model for future natural pond wastewater treatment system implementations are the much more rapid treatment time (6 days versus 33 days in this study) and the more consistent nature of the output compared to the WSP system.

The other key attraction for designers and builders is the 60% reduction in land area required to build a HRAP capable of treating the same volume of inflow, and consequently the much reduced capital cost of construction.



Thirdly, with a much reduced total treatment time (12 days versus 66 days) and a reduced evaporative surface area, the HRAP offers the opportunity to reclaim and reuse up to 90% more treated wastewater than the WSP.

Finally the HRAP does not require desludging at any stage. Many WSP facultative pond reach the end of their working life after forty years or so as the sludge accumulation reduces their effective volume to a critical level. Desludging is a slow, difficult and expensive procedure, and is best avoided.

## **8.6 Recommendations for further work**

Further work that would help facilitate HRAP design and construction in the future would include:-

1. For practical field implementations of HRAPs it would be desirable to have a proven design of an inflow buffering system to guard against flooding events.
2. The same buffering system could be used to implement a means of continuous input to the HRAP to produce the best disinfection outcome.
3. From a regulatory viewpoint further work is required to quantify the limitations of LRV as a measure of performance and then to find suitable alternatives when the inlet organism concentration is low.
4. Developing solar powered paddlewheel operation would allow even lower capital cost at the time of building and even lower maintenance and running costs.
5. If algal harvest is seen as a future requirement, greater understanding of the sudden die-off of algae would be essential.
6. Improving the understanding of flocculation and auto-flocculation would offer some advantages for harvesting and productivity.
7. To facilitate algal harvest it would be useful to investigate the ability of filamentous algae to withstand the poor light climate and continuous mixing in the HRAP.

8. To optimise algal growth, a more detailed study would be required to determine the level at which turbidity becomes a clear impediment to algal photosynthesis.
9. It would be of interest to study the effect of algal harvest to see if removing accumulated algal biomass improves the light climate to stimulate greater productivity.
10. For algal productivity it would be advantageous to explore altering retention times without altering depth.
11. Further research would be required to fully determine the cause(s) of limited algal growth in HRAP 2.
12. It would be of interest to repeat this work including summer months to try and replicate the period of massive algal growth. Understanding the conditions that induced that behaviour could yield great improvement in algal productivity.

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