The potential to improve pathogen removal in natural treatment systems with inclined planes

by

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SUMMARY

Water scarcity has increased the reliance on recycled wastewater to reduce the strain on global water supplies. High pathogen presence however, can be problematic with devastating impacts to public health a consequence. Reducing numbers are therefore essential.

Natural treatment ponds are becoming favourable using sunlight irradiance for microbial inactivation. Unfortunately in water light availability is restricted, lost through attenuation and non-microbial absorption. Increased exposure is exhibited in high rate algal ponds with shallow depths and paddlewheel rotation. To further improve removal strategies to enhance solar exposure are essential. Inclined planes have been proposed a possible solution with increased pathogen removal predicted when water is run down the sloped surface as a thin film. Pond walls are considered to be ideal inclined planes, formed with construction and often result with a large area of unused space. Utilising this redundant space can not only aid removal but assist in minimising costs.

In the current research the proposed theory was assessed is a series of laboratory and field based experiments. Examinations were performed using model and large scale high rate algal ponds with and without the addition of an inclined plane. MS2 and F-Specific phage were used as the test organism.

Results of the research demonstrated that phage inactivation rates could be improved with the inclusion of an inclined plane. In the model systems, the concept was proved in inclined planes of varying length under both optically clear and wastewaters when exposed to full sunlight or dark incubation and the inclined plane solely exposed. Operating the planes at the same hydraulic loading rate exhibited statistically similar removal rate. Results also confirmed water quality as an impacting factor as well as phage sensitivity towards sunlight irradiance. In the large scale system, mixed results were present with significantly improved inactivation only achieved once modifications to the system were performed. Nonetheless higher inactivation was observed whenever the inclined plane was in operation.

The current research has presented a novel and innovative approach to successfully improve pathogen removal in pond systems whilst maintaining the cost effectiveness of the system. The evaluations indicate the concept to be versatile with multiple disciplines with the water industry benefiting from the results. Furthermore, the concept paves a way for a more efficient treatment system to be realised. The research has also contributed to reducing the knowledge gap regarding phage removal in high rate algal ponds, which is currently lacking. This is the first time an inclined plane has been used for pathogen removal, with the concepts predicted adaptable to other pond systems.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Amy Hawley

4th March 2019

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1. INTRODUCTION

Elimination of microbial populations in water supplies is of global concern for their devastating effect on public health. The current conventional treatment practices although have improved immensely, increased pathogen removal is still critical (Hassani *et al.*, 1992; Benchokroun *et al.*, 2003). Therefore, the need to develop improved wastewater treatment strategies is of increasing importance to reduce global mortality and infection rates. The following review of literature examines some of the conventional treatment practices and the newer treatment methods. The proposal of a new disinfection technique to improve microbial reduction is also discussed.

1.1 Wastewater Reuse

The need to develop improved wastewater treatment strategies is global with infection by waterborne diseases high. Wastewater treatment is one of the components within the wider water, sanitation and hygiene (WASH) scheme aimed to prevent and limit disease spread (Cairncross *et al.*, 2010). Other strategies in WASH include excreta disposal, hand washing and adequate water quality (Cairncross *et al.*, 2010). Water is essential for life. Useable water however, is limited with water scarcity high. 71% of Earth's surface is covered by water (Sonune and Ghate, 2004) of which 1% is available for use. The remaining 70% is unattainable underground, frozen or salted (Corcoran *et al.*, 2010). Continual pressures of rapid overpopulation, political views and environmental and climatic changes; resulting in extensive periods of severe weather i.e. drought have put further strain on water supplies (Bouwer, 2000; Rijsberman, 2006; WHO, 2006b). An increase in global water shortages has resulted, threatening the well-being of entire communities from public health and agriculture to local business and tourism (Bouwer, 2000; Rijsberman, 2006; Pereira *et al.*, 2009).

The threshold of water scarcity as described by Pereira *et al.* (2009) is a water level less than 2000 m^3 $person^{-1}$ $year^{-1}$. Thomas and Durham (2003) identified from United Nation documents that 400 million people in 2003 were affected by low water availability. This number is predicted to increase by 2025 with approximately 3.0 billion people affected (Corcoran *et al.*, 2010).

To alleviate the strain, water management strategies have been devised, exploring and employing alternate water resources (Thomas and Durham, 2003). A favoured strategy is that of wastewater recycling, the application of taking water used from one source, treating it and reusing it for an alternate purpose. Common wastewater reuse applications include domestic and recreational use (i.e. toilet flushing, car washing and washing machine use), fire fighting, wetland construction, and agricultural, landscape, urban and industrial irrigation (i.e. mining-dust suppression) (Crook and Surampalli, 1996; Toze, 2006; NRMMC-EPHC-AHMC, 2009; Hamilton *et al.*, 2011).

Reuse not only provides a suitable water resource without hindering primary waters but reduces the need for artificial fertilisers for crop growth (Toze, 1997). Crops, such as maize and sunflower require nitrogen for increased growth, normally provided with the addition of nitrogen containing artificial fertilisers. Treated wastewater effluents is said to have elevated nitrogen concentrations therefore the need for fertilisers is reduced when reused for crop irrigation (Toze, 1997).

Wastewater is readily available with used water discharged from homes and industries regularly, many tasks can therefore be carried out without fresh or unused water required (Lee and Yigitcanlar, 2009). However, the high levels of chemical, physical and biological hazards make the water unusable. These levels must therefore be reduced before the water can be successfully released for reuse purposes (Lee and Yigitcanlar, 2009)

1.1.1 Guidelines for wastewater reuse

To ensure adequate treatment is achieved strict guidelines (WHO, 2004; NRMMC-EPHC-AHMC, 2006; WHO, 2006b; NRMMC-EPHC-AHMC, 2009; NHMRC-NRMMC, 2011) have been developed with health based targets and target $\log_{10}$ reductions outlined. Health based targets are usually expressed as loss of disability adjusted life years (DALYs) and are used to determine the risk and the burden of disease, with $\leq 10^{-6}$ DALYs person^{-1} year^{-1} the most commonly used (WHO, 2004). Using DALYs enables the comparison of different health outcomes and diseases to be achieved. Comparison between microbial and chemical risks can be determined also

DALYs are determined based on the quality and quantity of life and social magnitude (Equation 1.1) (Havelaar and Melse, 2003). This may also be determined based on the years of life loss (YLL) and years lived with the disability (YLD) (Equation 1.2).

$$Disability\ adjusted\ life\ years\ (DALYs) = N \times D \times S$$

Equation 1.1

Where,

N = number of people affected

D = life loss

S = severity of the unfavourable health conditions

$$DALYs = YLL + YLD$$

Equation 1.2

Where,

YLL = Years of life loss

YLD = years lived with disability

Log reductions are the percent removal or reduction of microorganism concentrations in a water sources by physical or chemical treatment. Reductions are determined on logarithmic or base 10 scale and calculated using the following equation (Equation 1.3) (NRMMC-EPHC-AHMC, 2006).

$$Log_{10}\ Reduction = Log\ (concentration\ in\ source\ water \times exposure \times N \times DALYd)$$

Equation 1.3

Where;

N = number of exposures per year

DALY d= dose equivalent to DALY of 10^{-6} which includes the ratio of dose response and illness infection. NRMMC-EPHC-AHMC (2006) indicated a DALYd of 1.6×10^{-2}, 3.7×10^{-2} and 2.5×10^{-3} for *Cryptosporidium*, *Campylobacter* and rotavirus respectively.

Finally, guidelines employ the aid of system validation to ensure treatment processes and systems can achieve required health-based targets. This is carried out prior to operation and whenever component upgrading is required. For validation a series of tests and analysis are conducted throughout different conditions, usually worst-case scenarios. A process may only be successfully validated when health based targets are achieved.

The required pathogen $\log_{10}$ reduction rates for reuse purposes is described throughout the Australian reuse guidelines (WHO, 2004; NRMMC-EPHC-AHMC, 2006; WHO, 2006b; NRMMC-EPHC-AHMC, 2009; NHMRC-NRMMC, 2011). For example recycled wastewater with the intent of being reused for crop irrigation: a 6.0, 5.0 and 5.0 $\log_{10}$ reduction of viruses, bacteria and protozoa respectively must be achieved in accordance to these guidelines (WHO, 2004; NRMMC-EPHC-AHMC, 2006; WHO, 2006b; NRMMC-EPHC-AHMC, 2009; NHMRC-NRMMC, 2011).

1.2 Wastewater Composition

Wastewater is the used water or liquid waste from domestic households, industries, commercial establishments and farms (i.e. piggeries) (Sincero and Sincero, 2003) and may include sewage effluents, sewer infiltration and inflow, storm water runoffs, groundwater and surface waters (i.e. water from lakes, reservoirs, and rivers) (Tchobanoglous *et al.*, 2003; Sonune and Ghate, 2004).

The composition of wastewater varies over time and between domestic and industrial wastewater, with the pathogenic load higher in domestic (Metcalf *et al.*, 2004). Domestic wastewater is comprised of the faecal waste matter of human (urine included) and the soiled water used in laundry and personal washing, toilet flushing, food preparation and other household chores requiring water (Mara, 2004; Shilton, 2005). There are five types of domestic wastewaters as

described in Table 1.1, categorised depending on origin and colour. Industrial wastewater is the water discharged from any industry or trade and often contains high concentrations of volatile and semi-volatile compounds, metals and metalloids (Quevauviller *et al.*, 2007; Qadir *et al.*, 2010).

Table 1.1: The five types of domestic wastewaters and their origin within the household. Table derived from (Otterpohl, 2002).

Wastewater	Origin
Black water	Toilet waste
Brown water	Sewage without urine
Grey water	Bathroom, laundry and kitchen water
Sewage	Both toilet waste (black water) and waste from household chores (grey water)
Yellow water	Urine (with or without flush water)

Elevated pathogen loads in domestic wastewater is correlated with the high microbial population within the human gut, making it more harmful than other wastewater sources (Qadir *et al.*, 2010). The concentration and type of microorganism present within a water source is dependent on the prevalence of the organism and infection within the community. This prevalence is influenced by the susceptibility and immune response of corresponding hosts, seasonal variation and the type of organism (Shilton, 2005; NHMRC, 2008).

Mara (2004) described fresh wastewater as turbid liquid comprised of both large and small-suspended solids (i.e. faecal matter, vegetable peel and maize or corn). This water is usually grey in colour with an odour not overpowering or unpleasant. Septic wastewater on the other hand has a very potent smell, as the water becomes anaerobic with the reduction of dissolved oxygen (DO) (Mara, 2004). This is more prominent in warmer, arid conditions. Wastewater is comprised 0.1% of solids and 99.9% liquid (Akpor and Munchie, 2011). The components in wastewater can be divided into nine categories as outlined in Table 1.2.

Table 1.2: Major components found in wastewater with common environmental effects. Table adapted from Henze *et al.* (2002), including information from WHO (2006b), Henze (2008) and Akpor and Munchie (2011)

Component	Contaminant of interest	Environmental and Health effects
Inorganic Material	Acids (i.e. hydrogen peroxide) Bases	Corrosion and toxic effects
Metals	Cd, Cr, Cu, Hg, Ni, Pb	Bio accumulation and toxic effects
Microorganisms	Bacteria and viruses Helminths and protozoa	Risk when bathing and eating seafood, communicable and heart disease and stomach ulcers
Nutrients	Ammonia Nitrogen Phosphorus	Eutrophication, oxygen depletion, toxic effects, Methaemoglobinaemia, stomach cancer
Odour and Taste	Hydrogen sulphide Volatile fatty acids	Aesthetic inconveniences and toxic effects
Organic material (Biodegradable)	Oxygen depletion (in rivers, lakes and fjords)	Fish death, odours
Organic Material (other)	Colouring Cyanide, phenol, solvents Detergents, fats, oils, grease Pesticides	Aesthetic inconvenience, bio accumulation and toxic effects i.e. carcinogenic, mutagenic disruption to hormone function
Radioactivity	Accumulation Toxic effect	Accumulation and toxic effects

1.3 Wastewater Pathogens

Enteric pathogens are abundant in both wastewater and the gut of infected hosts; human and warm-blooded animals. They can be found at a density of approximately 10-10^{10} microbes L^{-1} and 10^{12} microbes mL^{-1} luminal contents respectively (EPA, 2002; Sonnenburg *et al.*, 2004; WHO, 2006b; NRMMC-EPHC-AHMC, 2008). Entry into wastewater occurs via faecal excretion, organisms enter respective hosts generally via the faecal-oral route, pass through the gastrointestinal tract and are excreted in faecal waste (Amahmid *et al.*, 2002).

The range of microorganisms in wastewater is vast and includes species of virus, bacteria, helminth and protozoa. The most common are identified in Table 1.3. Their respective diseases, infectious dose and concentrations in wastewater are included in Table 1.3. The most common

response to infection with enteric pathogens is diarrhoea. Diarrhoea is one of the leading causes of global mortality with 1.5 million deaths reported in 2012 of which 622, 000 were children under the age of 5 years (WHO, 2014). 58% of the total diarrhoeal deaths reported were attributed to inadequate water, sanitation and hygiene (WASH) (WHO, 2014). In 2015, pneumonia, malaria and diarrhoea continued to be a leading cause of death in children under 5 years with the daily mortality rate 16, 000 children a day (UN and UNICEF, 2015).

Table 1.3: Common enteric pathogens isolated from wastewater samples. Corresponding diseases, infection dose and concentration in wastewater have been included. Table adapted from (EPA, 2002; WHO, 2006b; NRMMC-EPHC-AHMC, 2008).

Pathogen	Common Species	Associated Disease	Concentration in Wastewater	Infectious Dose
Viruses	Adenovirus Enteroviruses • Echovirus • Poliovirus Hepatitis A Rotavirus	Respiratory disease Gastroenteritis Paralysis Hepatitis Gastroenteritis	10^1-10^4 viruses L^{-1} 10^1-10^4 viruses L^{-1} 10^2-10^6 viruses L^{-1} 10^2-10^5 viruses L^{-1}	1-10
Protozoa	Cryptosporidium spp Entamoeba spp Giardia spp	Cryptosporidiosis Amoebic dysentery Giardiasis	1-10^4 oocysts L^{-1} 10^2-10^5 oocysts L^{-1} 1-10^2 oocysts L^{-1}	<10
Bacteria	Campylobacter spp Escherichia coli Mycobacterium spp Salmonella sp Shigella spp Vibrio spp	Gastroenteritis Gastroenteritis Johne's disease Typhoid & Gastroenteritis Dysentery Cholera	10-10^4 bacteria L^{-1} 10^5-10^{10} bacteria L^{-1} 10-10^4 bacteria L^{-1} 1-10^5 bacteria L^{-1} 10^1-10^4 bacteria L^{-1} 10^2-10^5 bacteria L^{-1}	10^6-10^{10} 10^4-10^6 180 10^3-10^7
Helminths	Ancylostoma spp Ascais spp Strongiloides spp Taenia spp Trichuris spp	Hookworm Roundworm Threadworm Tapeworm Whipworm	1-10^3 eggs L^{-1} 1-10^3 eggs L^{-1} 1-10^2 eggs L^{-1} 1-10^2 eggs L^{-1}	1-10

Enteric virus removal is regarded a priority due to their devastating effects on public health, high prevalence and strong resistance towards conventional treatment methods. It was identified that 10^5-10^{11} viral particles per gram of stool could be excreted by an infected host (Farthing, 1989). Host immunity in part is responsible for the low risk rating of viruses as indicated by the World

Health Organisation (WHO, 1989). Immunity against viruses is far greater and longer lasting than the immunity exhibited against bacteria, for instance (Toze, 1997; EPA, 2002).

1.3.1 Microbial Surrogates for Wastewater Studies

The use of indicator organisms has revolutionised the detection, identification and enumeration of faecal pathogens in wastewater. Wastewater treatment is heavily reliant on detection methods to establish microbial presence, faecal contamination and water quality monitoring. However, these methods are often tedious, time-consuming and inaccurate (i.e. the occurrence of false positives). In the case for detection of viral particles the processing and incubation required can take several days and the occurrence of inconclusive results frequent (Yousefi and Zazouli, 2008), hence the common use of indicators.

Multiple organisms share similar properties to each other however, not all are suitable for surrogacy. Therefore, to be considered a successful surrogate, an organism must comply within a strict criterion (Tchobanoglous *et al.*, 2003). This criterion is described in Table 1.4 and was devised to ensure the most appropriate indicator is used. It must be noted that several organisms are considered suitable fitting within majority of the set criterion but no organism existing complies by all the criteria (Horan, 2003).

Table 1.4: Criteria required for susceptible organisms to be classified successful surrogates for wastewater pathogens (Horan, 2003).

Indicator Organism Criteria	
1	Organism should be non-pathogenic
2	Organism should be suitable for all/most water types
3	Organism should be present when faecal contamination is
4	Organism should be present when the target organism is
5	Organism should be present in greater numbers than the target organism
6	Organism should be unable to grow or multiply within the environment
7	Organism should be detected by simple, quick and inexpensive detection methods
8	Organism should have ≥ resistance to disinfection, treatment and environmental stresses

Common surrogates include *E. coli,* bacteriophage, total and faecal coliforms (Craggs *et al.*, 2004a). These organisms, excluding bacteriophage are ideal for many organisms, but exhibit a

weak resistance towards several key disinfectants like chlorination (Tree *et al.*, 1997; Harwood *et al.*, 2005). This poor resistance makes them unsuitable indicators for protozoan parasites and enteric viruses, all characterised with high disinfection resistance (Havelaar *et al.*, 1993; Bonadonna *et al.*, 2002). Identification of a suitable surrogate for enteric viruses is often difficult given their greater persistence within the environment, low infectious dose, high infectivity and higher resistance towards disinfection by conventional means (Leclerc *et al.*, 2000; Campos, 2008). However, several indicators have been suggested, including other waterborne viruses and bacteriophage.

For other wastewater pathogens the World Health Organisation (WHO, 2006b) suggested *E. coli*, intestinal *Enterococci* and thermotolerant coliforms as suitable indicators for bacteria, *Ascaris ova* for helminths and *Clostridium perfringens* and aerobic (*Bacillus*) spores for protozoa. For surrogacy with *C. perfringens* validation must be done on particles of similar size to protozoa which are much larger (WHO, 2006b). A consensus on which indicator organism is ideal for a particular treatment or disinfectant is not always achieved throughout the literature, as is the case for ultraviolet light and photo-reactivation responses suggested by Lindenauer and Darby (1994).

1.3.2 Bacteriophage surrogates

Bacteriophages are non-pathogenic viruses that target and infect bacteria. These phage are highly abundant in influent sewage (2.2×10^7 mL^{-1}) and share similar properties to enteric viruses; including morphology, structure, functionality and composition (Grabow, 2004; Withey *et al.*, 2005). They also share similar resistance towards disinfection and water treatments, light (UV and visible), temperature, and pH, (Grabow, 2004; Withey *et al.*, 2005; Bolton, 2012). Phages are desirable surrogates as they can be rapidly and easily cultured, detected and analysed. Kott *et al.* (1974) identified wastewater as having a ratio of 1000:1 bacteriophage to enteric viruses. The difference in organism number is linked with the excretion pattern of each organism. Infected individuals excrete viruses for a short period whilst infected. Bacteriophage on the other hand, are continually excreted by a range of susceptible hosts, both animal and human (Grabow, 2004). Regarding phage to bacterial host ratios, if there is a low or insufficient concentration of respective host the ability of the phage to locate, attach and infect host cells is decreased (Grabow, 2004).

Goyal *et al.* (1987) suggested at least 10^4 bacterial host cell mL^{-1} is required for phage replication to be successful.

Infection by bacteriophage occurs through cell lyses; the triggering of the lytic cycle where bacterial host cells are damaged through the attachment and replication of the bacteriophage (Bitton, 2010). It must be noted that the infecting phage do not always initiate cell death, instead become integrated as prophage within the bacterial chromosome (lysogeny) (Bitton, 2010).

In general phages lack the ability to directly infect human hosts. However, some phage have the capability of transforming harmless bacteria into pathogenic bacteria It could therefore be perceived that an absence of phage can be just as desirable as an absence in viruses (Muniesa and Jofre, 1998; Muniesa *et al.*, 1999; Grabow, 2001).

The main faecal bacteriophage are categorised as F-RNA and F-DNA specific phage and somatic coliphage, with coliphage the term often given to *E. coli* infecting viruses (Bitton, 2010). The most common bacteriophage used throughout wastewater studies and faecal contamination is MS2.

MS2 is a F^+ specific RNA bacteriophage and the wider used indicator for viruses (Havelaar *et al.*, 1990). This phage can be easily detected through quantitative double layer agar phage assays and is non-pathogenic infecting only *E. coli.* Infection occurs via attachment to receptors on the F-pili of male *E. coli* and replicating within host cells (Zhang and Farahbakhsh, 2007; Wigginton *et al.*, 2012) (Figure 1.1). F-RNA phage production or synthesis of the F-Pili occurs only at temperatures above 30°C (Grabow, 2001). Therefore replication outside of an animal or human host which has a general body temperature of 37°C is unlikely (Grabow, 2001).

Figure was removed due to copyright restrictions.

Figure 1.1: Replication of F-RNA bacteriophage MS2. Figure sourced from http://faculty.washington.edu/jclara/301/M301lecOut/Phage.html

MS2 is a single stranded icosahedral virus with a 3.6kb genome and belongs to the Leviviradae family (Jolis, 2002). The similarities between MS2 and enteric viruses particularly enteroviruses (i.e. poliovirus) are strong. Grabow (2001) indicated the two organisms are almost indistinguishable when compared under an electron microscope. These phage, like viruses are strongly resistance to UV light inactivation (Nwachcuku and Gerba, 2004; Bolton, 2012), making them ideal for solar disinfection studies.

1.4 Microbial Inactivation

Reduction of microbial numbers in water is carried out via physical removal and inactivation, where treatment processes, disinfectants and environmental stresses are employed to remove or shut down microbial activity. Activities include replication and infectivity, killing the microorganisms. Factors have been identified influential against microbial inactivation, many related to the environment. (WHO, 2006b) outlined these as exposure to light and UV radiation, time, temperature, presence of intermediate hosts and moisture. Nutrient deficiency, salinity, pH, predation, acid, organic matter, solids and oxygen presence have also been identified to influence

inactivation (Sinton *et al.*, 2002; Chung *et al.*, 2006; Blaustein *et al.*, 2013). These processes have been widely studied throughout the literature, with *E. coli* inactivation well documented. The host organism and its metabolic processes also influence microbial inactivation (Grabow, 2001).

Throughout their life cycle microorganisms, encounter a variety of environmental pressures that need to be overcome for the successful survival of the organism. Alterations to the organism's environment can be detrimental: inducing stress and stress responses, inactivation and potential cell death. Osmotic shock can also contribute to phage inactivation (Jończyk *et al.*, 2011). These alterations may also be favourable prolonging cell survival. Pathogens existing within the human gut are passed through the stomach where conditions are acidic, into the neutral to alkaline conditions of the small and large intestines before being excreted, entering wastewater (Savage, 1977). Upon entry, organisms are faced with alterations to pH, temperature, exposure to UV light and chemical disinfectants. Understanding the response of microorganisms towards these different conditions is necessary to improve inactivation processes. However, it is the relationship between pathogen die-off and exposure to light that is of current interest and will be discussed in detail in Sections 1.6 and 1.7 respectively.

Temperature has a strong influence on microbial die-off. Exposure to high temperatures result in the elevated die-off of microorganisms and survival is prolonged for low temperatures (Ferguson *et al.*, 2003). Exposure to cool temperatures (i.e. below 10°C) causes the biochemical processes i.e. degradation to slow down (Olson *et al.*, 2004). In doing so, the infectivity of the organism is preserved. This preservation is essential if organisms are to be stored for extended periods. Azadpour-Keeley *et al.* (2003) identified a direct relationship between microbial inactivation and the rise in temperature. This was observed for viral inactivation and was supported by Bradaway *et al.* (1990). Bradaway *et al.* (1990) examined viral inactivation and temperature variations for poliovirus, rotavirus and MS2 (plus coliphage) for different seasonal temperatures. Inactivation was significantly higher with K_d values h^{-1} of 0.37, 0.20, 0.45 for poliovirus, rotavirus and MS2 respectively throughout summer when temperature averaged 36-41°C, compared to 0.06, 0.10 and 0.17 obtained throughout winter with cooler temperatures around 4-10°C. (Fong and Lipp, 2005)

suggested temperature can prevent the replication of viruses by damaging the viral capsid and prevent virus to host adsorption.

Microorganisms are sensitive to changes in pH. Inactivation is elevated when pH is increased to a level >9.0 (Craggs *et al.*, 2004b; Bitton, 2010; Hwang, 2012). Feng *et al.* (2003) identified microbial survival was prolonged for acidic conditions and shortened when water is more alkaline. This was evident for MS2 bacteriophage with inactivation 3.3 times greater for pH 9.0. This elevation in microbial die-off is associated with the increased uptake and transport of toxic chemicals and nutrients into microorganism cells in response to the chemical ionization by pH (Bitton, 2010; Hwang, 2012). Organisms exposed to a pH level lower than or equal to their isoelectric point (i.e. PI = 3.9 for MS2) will increase the ability of aggregation (Langlet *et al.*, 2007; Jończyk *et al.*, 2011).

1.4.1 Microbial Die-off Kinetics

The identification of microbial die-off and survival rates is important in establishing the extent of microbial contamination and the effectiveness of a disinfectant and treatment process at reducing microbial numbers. These rates are modelled based on first order kinetics where Chick's Law (1908) (Equation 1.4) is applied and plotted on a semi-logarithmic plot as a function of microbial mortality against time for an inactivation factor (i.e. temperature, chemical dose, radiation) (Hoff, 1986; Fallowfield *et al.*, 1996).However, it should be noted that when die-off rates are modelled in reactor systems using this pseudo-first order die-off rate constant the models may be influenced by the flow and mixing conditions within a reactor, with difference models required for plug-flow and continuous flow systems.

$$N_t = N_0 e^{-K_b t}$$

Equation 1.4

Where;

N = concentration of living microorganism at time t

N_0 = initial concentration of microorganisms

t = time (min, h, etc)

K_b = die-off rate constant

Microbial reduction: outlined by Peleg (2000) is often by several orders of magnitude and for the graphical representation of the observed die-off to be achieved successfully, a semi-logarithmic scale is applied. This is usually in the order of a base-10 logarithm (Peleg, 2003) with the resultant inactivation curve linear. Die-off rates are determined from the slope of the line obtained from the linear section of the resultant inactivation curve. However, the occurrence of non-linear survival curves is common with tailing and shouldering apparent. This tailing and shouldering signifies a lag in microbial die-off and has been suggested a function of mixed or subpopulations with different inactivation resistance that follow a different order of kinetics or the activation of dormant bacterial spores (Xiong *et al.*, 1999; Peleg, 2000; Teixeira and Rodriguez, 2010). This deviation from linearity has been strongly recognised throughout microbial inactivation in the food industry and only recently recognised in water studies (Simpson, 2010; Blaustein *et al.*, 2013).

Variation in non-linear inactivation curves is apparent throughout the literature. Studies by Blaustein *et al.* (2013) and Geeraerd *et al.* (2005) explored these inactivation curve differences. Blaustein *et al.* (2013) identified from a collaboration of data obtained from numerous studies, the occurrence of four curve patterns for logarithmic organisms against time. The four patterns are outlined in Figure 1.2. Curve type 2 was suggested the more common, apparent in 46% of the tested cases. The log-linear curve (type 1) normally associated with die-off was apparent in only 25% of the analysed data. These studies were largely carried out on then microbial survival in food, however the data presented is still applicable throughout water studies.

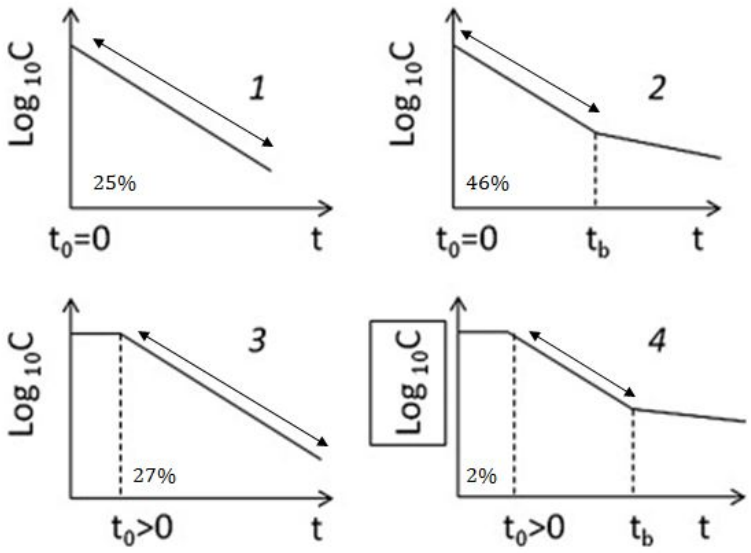

Figure 1.2: Microbial die-off curves: The four die-off curves types for log number of organisms against time observed from the Blaustein *et al.* (2013) study. Each type was apparent in 1) 25%, 2) 46%, 3) 27% and 4) 2% of cases. Inactivation rates were taken from the slope of the line (linear region) as indicated by the double-headed arrow.

Alternate mathematical models and amendments to Equation 1.4 have been characterised throughout the literature. The Weibull model was identified with a strong representative of non-linear survival curves (Peleg and Cole, 1998; Lambert and Johnston, 2000; Peleg, 2000; Buzrul and Alpas, 2007; Simpson, 2010). Xiong *et al.* (1999) identified suitable models for non-linear survival curves with both tail and shoulder incorporated. Similarly Mitchell and Akram (2017) acknowledged non-linear models reported within the literature for the persistence of different pathogens in various water sources. Geeraerd *et al.* (2005) acknowledged the development of GInaFiT; a Microsoft Excel add-on package to assess non-linear microbial survival curves and incorporates many of the established kinetics models.

1.5 Wastewater Treatment and Disinfection

Many treatment practices have been established and employed worldwide to reduce the microbial content in wastewater. These differ slightly depending on wastewater composition, microbial load, the extent of disinfection required and resource availability. The most economical and efficient treatment approaches are preferred.

1.5.1 Conventional Wastewater Treatment

Large wastewater treatment plants implemented across the globe use physical removal and both chemical and biological disinfection to treat influent wastewater. Treatment is not restricted to these plants with an increasing percent undertaken in specially designed treatment ponds (Section 1.8).

Wastewater is treated in multiple stages (Figure 1.3). Each stage is designed to target and remove different compounds in the water. The extent of treatment decreases with the progression of the treatment process. Traditionally three processes are undertaken; preliminary, primary and secondary treatment, but tertiary and quaternary treatments may be used also.

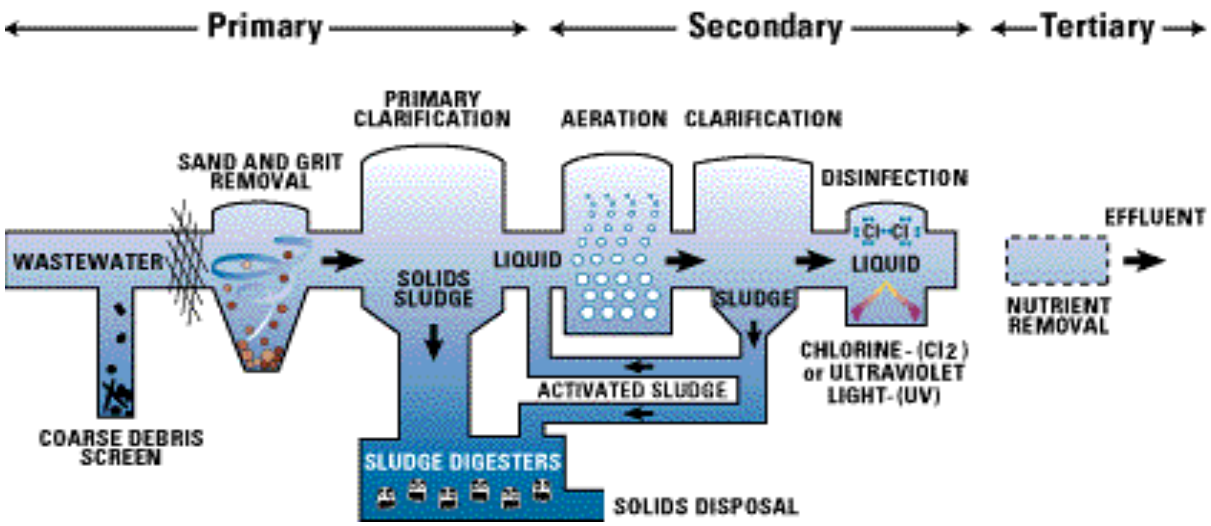

Figure 1.3: Outline of the preliminary, primary, secondary and tertiary treatment processes undertaken in large treatment plants. Figure sourced from http://www.sheffy6marketing.com/index.php?page=test-child-page

Preliminary treatment is the removal of solid and sludge material from incoming influent water. Materials such as oil, grease, gravel, sand, plastic and faecal matter are removed by screening, filtration and pre-aeration (Sonune and Ghate, 2004). Pre-aeration is the addition of compressed air to the water keeping solids in suspension before sedimentation (Mittel, 2006).

Further removal of suspended solids occurs throughout primary treatment. Floatation and sedimentation process are used, extracting waste material from the bottom of the sedimentation tank. Filtration and chemical coagulation may be employed also (Okoh *et al.*, 2007).

Secondary treatment is essentially the last major process throughout conventional treatment and is generally regarded as the biological stage; utilising microorganisms to remove 90% of remaining contaminants (Sonune and Ghate, 2004; Mittel, 2006). Remaining solids, nutrients and organic matter are removed via biological and chemical processes. Phosphorus and nitrogen are extracted from wastewater using both aerobic and anaerobic microbial processes (Tchobanoglous *et al.*, 2003; Mittel, 2006). Nitrogen substrates; nitrite, nitrate and ammonia are also removed.

Tertiary and quaternary treatments are employed as an additional disinfection step where removal of remaining pathogens, organic material, salts, nutrients and metals is achieved. These processes may include chemical or physical disinfection, and filtration. (Hijnen *et al.*, 2006; Okoh *et al.*, 2007).

The World Health Organisation (WHO, 2006b) identified the log reduction rates that could be obtained throughout primary, secondary and tertiary treatments. These values are depicted in Table 1.5, which include the predicted reduction rates for treatment ponds also.

Table 1.5: Summary of log pathogen removal rates achievable throughout conventional wastewater treatments. Log reductions were estimated for primary, secondary and tertiary treatments. Estimated values for treatment ponds were established from the collaboration of log reductions achievable in constructed wetlands, treatment reservoirs and waste stabilisation ponds (WSP). Table adapted from (WHO, 2006b).

Treatment Process	Pathogen Log Reduction			
	Bacteria	Helminth	Protozoan	Viruses
Primary Treatment	0-2	0.5-3	0-2	0-2
Secondary Treatment	1-2	1-3	0-1	0-2
Tertiary Treatment	0->6	1->3	0->6	1->6
Treatment Ponds	0.5-6	0.5-4	1-3	1-4

1.5.2 Other disinfection methods

Disinfection is a common process employed in wastewater treatment to reduce microbial components. The three common disinfection processes are chlorination, chloramination, ultraviolet

(UV) radiation and ozonation and these have been extensively studied (Toze, 1997; Al-Juboori *et al.*, 2010).

Chlorination; an inexpensive disinfection process by which the addition of chlorine additives reduces enteric pathogens from wastewater and is the most commonly used form of disinfection across the world (Hijnen *et al.*, 2006). It is effective on all water sources with residual properties capable of reducing microbial re-growth and prolonging disinfection (Toze, 1997; Metcalf *et al.*, 2004). Common chlorine additives include chloramines (NH_2Cl), hypochlorite (HOCl) and gaseous chlorine (Cl_2) (Lazarova *et al.*, 1999). A chlorine dose of 5-20 mg L^{-1} is considered sufficient for removal in municipal wastewater with a contact time of 30-60 minutes (Lazarova *et al.*, 1999). A higher concentration is required for the inactivation of some microorganisms i.e. viruses and protozoa; notably *Cryptosporidium*; with resistance to chlorination evident (Lazarova *et al.*, 1999; Szewzyk *et al.*, 2000; Zia *et al.*, 2008). Long contact times, microbial resistance, presence of suspended solids (interfers with pathogen removal) and production of undesirable trihalomethane (THMs) by-products are some of the disadvantages identified with chlorination (Gibbons and Laha, 1999; Lazarova *et al.*, 1999; Hwang, 2012). These by-products are formed from the interaction of chlorine with dissolved organic matter and have been associated with the elevated risk of cancer (i.e. bladder, colorectal and kidney cancers) (Bull *et al.*, 1995; Cantor *et al.*, 1998; Hwang, 2012). Chloramines may be used instead to reduce production of these by-products due to their stability and less reactive nature (Al-Juboori *et al.*, 2010). Chlorination is relatively inexpensive, however often requires dechlorination; the removal of chlorine residuals from treated water to lessen toxicity in chlorinated effluents (Lazarova *et al.*, 1999). Dechlorination increases costs by 20-30% (Lazarova *et al.*, 1999).

UV disinfection utilises light radiation to disinfect water and reduce contaminants and is often carried out during tertiary and quaternary stages of treatment (Al-Juboori *et al.*, 2010) both natural and artificial UV irradiation is used. Sunlight is used throughout natural UV disinfection, predominantly used in natural treatment systems and will be discussed in Sections 1.6 and 1.7 respectively. Artificial UV disinfection is undertaken in conventional treatment systems. UV fluorescing lamps are used for irradiation. Two alternate types of UV lamp are used for disinfection;

polychromatic medium-pressure UV lamps (MPUV) emitting wavelengths across the entire UVC and UVB regions (~200-315 nm) and monochromatic low-pressure UV lamps (LPUV) emitting wavelengths solely within the UVC region, primarily at 265 nm (Eischeid and Linden, 2007; Al-Juboori *et al.*, 2010). Although, studies have used 253.7 nm also (Eischeid and Linden, 2007). Destruction of microbial DNA has been associated with this region (Jagger, 1985). Disinfection with UV lamps is effective and capable of disinfecting water without the addition of unwanted by-products. However, replacement and operational costs can be expensive with contact times limited (Toze, 1997). Microbial regrowth is possible in UV treated water, with no disinfection residual apparent to prevent regrowth in distribution systems post treatment (Toze, 1997). Unlike chlorination UV disinfection is practical for small volumes only, but still considered more favourable of the two for its non by-product release (Oppenheimer *et al.*, 1997).

The final disinfection process; ozonation is the least used. An advantage of ozonation is its ability to carry out disinfection without being influenced by suspended solids and other particles, resulting in the uninterrupted treatment of medium water volumes (Toze, 1997). Microbial reduction caused by ozonation is reasonable but the expenditure, high energy requirements and release of unwanted by-products (i.e. formaldehyde) makes this process undesirable in comparison to other disinfection measures (Toze, 1997; Von Gunten, 2003; NHMRC-NRMMC, 2011). Viruses have been identified as more superiorly inactivated with ozone compared with other faecal pathogens (Tyrrell *et al.*, 1995). Ozonation has also been adopted as a control method for reducing taste, odour and colour of treated water (Zhou and Smith, 2001). Particle destabilisation and organic compound (synthetic) oxidation is also achievable with ozonation (Zhou and Smith, 2001).

The NRMMC-EPHC-AHMC (2009) identifies variation between achievable log reductions for viruses, protozoan and bacteria for the disinfection processes mentioned. Greater disinfection is required to reduce both viruses and protozoa in comparison to bacteria. Table 1.6 outlines the indicative log reductions obtainable by the three disinfection methods.

Table 1.6: Indicative pathogen log reduction rates for common disinfection processes. Table adapted from (NRMMC-EPHC-AHMC, 2006)

Pathogen	Disinfection Process		
	Chlorination	**UVC Radiation**	**Ozonation**
Bacteria E. coli Campylobacter	2.0-6.0	2.0->4.0	2.0-6.0
Protozoa Cryptosporidium spp. Giardia spp.	 0-0.5 0.5-1.5	 >3.0 >3.0	 N/A N/A
Viruses Adenovirus Enterovirus Rotavirus	1.0-3.0	>3.0 >3.0 >1.0	3.0-6.0
Helminth	0-1.0	N/A	N/A
Bacteriophage	0-2.5	3.0-6.0	2.0-6.0

Many disinfection processes have been developed, all effective but limited. Limited in the sense that not all microorganisms are removed adequately, a consequence largely associated with microbial resistance (discussed in Section 1.7.4). Improvements to current disinfection processes are being sought continually with modifications to conventional treatment plants and ponds frequent.

1.6 Solar Irradiation

1.6.1 Sunlight Irradiance

Sunlight is a natural disinfectant with its irradiated wavelengths possessing germicidal properties. Of the wavelengths irradiated, those belonging in the UV and visible (Vis) regions are effective reducers of microorganisms. The UV region is the more energetic and superior inactivator, attracting majority of attention throughout disinfection studies. The UV spectrum is divided into three regions, shown in Figure 1.4. These regions are UVA (near-UV ~315-400 nm), UVB (mid-UV ~290-320 nm) and UVC (far-UV ~190-290 nm) (Jagger, 1985; Al-Juboori *et al.*, 2010), and vary in energy, inactivation ability and absorption. An additional UV wavelength; Vacuum UV (VUV ~100-190 nm) also lies within this UV region (Al-Juboori *et al.*, 2010). Germicidal properties against microorganisms have been identified within this region; however, the United States Environment

Protection Agency EPA (2006) outlined the impracticality of using this wavelength for microbial reduction claiming its rapid dissipation in water. Consequently, this wavelength will not be discussed further in this study.

UVC is the most germicidal of the UV regions, followed by UVB, UVA and Vis (400-700 nm) (Whitelam and Codd, 1986; Davies-Colley *et al.*, 1999) However, the short wavelengths of both UVB and UVC are rapidly lost to the atmosphere, with UVC absorbed completely (Alados-Arboledas *et al.*, 2003). This complete absorption means UVC has no involvement in solar disinfection, unless artificially generated in UV lamps. UVB is therefore regarded the most functional inactivating wavelength during solar disinfection.

Figure was removed due to copyright restrictions, but can be found on page 12 of VECCHIA, P., HIETANEN, M., STUCK, B. E., VAN DEVENTER, E. & NIU, S. (2007). *Protecting workers from ultraviolet radiation*, International Commission on Non-Ionizing Radiation Protection (ICNIRP).

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Figure 1.4: Ultraviolet light regions in the solar spectrum involved in microbial inactivation. Figure copied from Vecchia *et al.* (2007)

The interest in “natural” UV as a disinfectant is not a new phenomenon, but is becoming favoured. Its ability to successfully reduce *Cryptosporidium;* protozoan organisms strongly resistant against conventional disinfectants (i.e. chlorination), even when the UV dose is low (Clancy *et al.*, 2000; Choi and Choi, 2010) has excited interest within this area. Virus inactivation is also effective with low UV doses.

1.6.2 Solar Inactivation of Microorganisms

Cellular damage occurs in response to exposure to UV, resulting in inactivation. Inactivation can occur via one of two processes photoinactivation; direct inactivation and photo-oxidation; indirect inactivation (Muela *et al.*, 2002; Sinton *et al.*, 2002). Both processes are considered wavelength dependent.

Photoinactivation has been strongly associated with the absorption of UVB. When UVB is absorbed formations of photoproducts; primarily pyrimidine dimers, occur damaging the target organisms' nucleic acid, preventing replication and infectivity (Lindenauer and Darby, 1994; Kohn and Nelson, 2007; Kowalski, 2009). Pyrimidine dimers include thymine: thymine, cytosine: cytosine and thymine: cytosine, with thymine dimers the more common (Kowalski, 2009). Uracil dimers (uracil: cytosine and uracil: thymine) are formed in UV induced RNA damage (Kowalski, 2009). Dimer formation is a result of mutagenesis; a genetic information mutation, and cross-linking where corresponding carbon atoms (atoms 5 and 6 for thymine dimers) are covalently bonded and normal base pairing is prohibited (Oates *et al.*, 2003; Kohn and Nelson, 2007; Kowalski, 2009).

Alternately, photo-oxidation a highly oxygen dependent mechanism utilize the assistance of external 'organic' molecules (photosensitisers) to absorb UV light and inactivate microorganisms indirectly. Photosensitisers are light absorbing molecules that transfer the energy gained from the absorption of UV to other molecules and create reactive oxygen species (ROS) (Kohn and Nelson, 2007). Formation of ROS triggers photo-oxidation and microbial inactivation by oxidising enzymes, nucleic acids and other cellular components (Reed, 1997; McGuigan *et al.*, 1998). These sensitizers can be endogenous (found within cells), or exogenous (found in the water outside the cell). Examples of endogenous photosensitisers include flavins and porphyrin derivatives and exogenous photosensitisers include natural organic matter (NOM) such as humic substances (Curtis *et al.*, 1992; Silverman *et al.*, 2013). Hydrogen peroxides (H_2O_2), hydroxyl radicals (OH), singlet oxygen, ($^1O_2$) and superoxides (O_2-) are common ROS species (Oates *et al.*, 2003). Silverman *et al.* (2013) suggested microbial inactivation rates could be increased in the presence of NOM due to their ability to attenuate irradiated sunlight and produce ROS species. Davies-

Colley *et al.* (1999) has suggested a linear relationship between oxygen concentration and photo-oxidation and a synergistic relationship with pH.

An organism may be inactivated by either inactivation mechanism. Both Kohn and Nelson (2007) and Kohn *et al.* (2007) identified this. These studies found MS2 isolated from WSPs could be equally inactivated via photoinactivation (UVB) and photo-oxidation ($^1O_2$). Davies (2003) and Kohn and Nelson (2007) identified exogenous sensitizers and $^1O_2$ ROS species were dominant during the photo-oxidation of MS2. This was evident for enteric viruses also (Kohn and Nelson, 2007; Silverman *et al.*, 2013). Davies-Colley *et al.* (1999) suggested microbial inactivation was wavelength dependent with different wavelengths having greater or no effect on the organism. Organisms such as *Enterococci* and F-RNA phage were shown to be reduced by wavelengths across the entire spectrum. *E. coli* and F-DNA phage on the other hand were shown to be largely reduced by UVB Davies-Colley *et al.* (1999).

Snowball and Hornsey (1988) expressed the differences between microbial inactivation using solar irradiation (UV) and chemical disinfection. Both processes were shown to target different molecular components: affecting different biological processes. For instance, UV targets the organism's nucleic acids, which prevents replication. Alternatively chemical disinfection on the other hand inactivates microorganisms by targeting and destroying cellular components of the organism affecting metabolism and biosynthesis Snowball and Hornsey (1988).

1.6.3 Factors influencing solar radiation and disinfection of microorganisms

Solar UV disinfection is effective but limited, influenced by many limiting factors. These include the wavelength absorbed, the intensity and dose of the emitted wavelength and how susceptible the target organism is to inactivation (i.e. UV resistance), distribution of light in both water and atmosphere, composition of the water (i.e. turbidity, pH and DO), water type (i.e. wastewater, seawater and freshwater) and the exposure time (Al-Juboori *et al.*, 2010). Caslake *et al.* (2004) identified the relationship between these factors and the rate of pathogen removal. Data was obtained from river water and two wastewater treatment plants in Easton, Pennsylvania and Phillipsburg, New Jersey. Removal rates were shown to be elevated when pH, temperature and

intensity were increased and lower with the increase in pond turbidity and depth (Caslake *et al.*, 2004).

1.6.4 Light attenuation and absorption

Irradiated sunlight gets lost rapidly between emission and arrival at the earth's surface. This loss subsequently limits the amount of light available for disinfection and ultimately the disinfection ability against enteric pathogens. The short wavelengths of UV and Vis light make them susceptible to attenuation and absorption (Acher *et al.*, 1997; Caslake *et al.*, 2004). In both air and water, photons will encounter and collide with particle matter. Collision with these particles causes photons to scatter and attenuate. A gradual decrease in the intensity of the photons will result (Acher *et al.*, 1997; Caslake *et al.*, 2004).

Light absorption, is the complete uptake and transformation of light photons by particles, microorganisms, phytoplankton and algal matter into different forms of energy (Snowball and Hornsey, 1988). Absorption is different between wavelengths and absorbing particulates. Light attenuation (or light extinction) is the scattering of light where photons are not removed entirely, instead remain longer in the water and are dispersed into different directions, gradually reducing the intensity of the irradiated light and increasing the path length (Curtis *et al.*, 1994). Curtis *et al.* (1994) identified 98% of all light scattered will disperse in a forward direction. The remaining (2%) will be backward scattered. This is true for all turbid water.

Light attenuation is measured as a function of the Beer Lambert Law (Equation 1.5).

$$Ka = -\ln(\frac{I_z}{I_0})$$

Equation 1.5

Where:

Ka = attenuation coefficient

I_z = irradiance at depth z m

I_0 = irradiance at surface (0 m)

Water is comprised of many light absorbing and scattering particles causing a drop in light intensity. These include water particles, algae, suspended solids, tripton (inanimate particulate matter), gilvin (dissolved humic matter), and other organic and inorganic particulates (Curtis *et al.*, 1994). A strong correlation exists between light extinction (attenuation) and turbidity as well as the angle light enters the water. Turbidity is the measure of light scatter in water based on the cloudiness of water when light reflected at various angles collides with the suspended particulates in the water (Austin, 1974; Davies-Colley and Smith, 2001). NRMMC-EPHC-AHMC (2009) identified the reduction in disinfection ability within highly turbid water, water where the suspended solids concentration and light extinction is great. Davies-Colley and Smith (2001) also recognised this reduction suggesting the more turbid the water the less effective UV disinfection is. The angle light enters water also contributes to the reduction of light intensity and its disinfection ability.

Curtis *et al.* (1994) suggested that the more acute the angle of penetration the less depth the light could reach. Curtis *et al.* (1994) also identified light within water; specifically wastewater in pond systems was more absorbed than scattered. The study also suggested algal presence as a large contributor to light loss with a higher attenuation coefficient for downwards irradiance (K_d) observed in algal rich ponds. Algae was observed to predominantly affect shorter wavelengths (UVB), with the larger UVA and Vis used in photosynthetic reactions (Curtis, 1990; Curtis *et al.*, 1994).

1.6.5 Penetration Depths

Light has shallow penetration capabilities in turbid water such as wastewater. The distance light can penetrate in water is crucial, determining the degree of disinfection. This affects unmixed, stagnant ponds more so than continually mixed ponds. The penetration depth is wavelength dependent and heavily affected by attenuation.

The depth of water light is irradiated into has been shown to influence intensity loss, attenuation and penetration within pond systems and other water sources. Light is less likely to reach certain depths; such as the pond bed when pond depth is increased. This is especially true for highly turbid waters (Kirk, 1994; Fallowfield *et al.*, 1996)

UV penetration depths are determined using Equation 1.6 for any given depth (Lee and Rast, 1997).

$$I_{(z)} = I_{(O)} e^{-\eta z}$$

Equation 1.6

Where:

$I_{(o)}$ = light intensity at surface of water (100%);

$I_{(z)}$ = light intensity at depth z (%);

η = light-extinction coefficient or light-attenuation coefficient (m^{-1});

z = depth of euphotic zone (m)

The euphotic zone is the upper most layer of water where 99% of light is absorbed and more than 1% of the initial sunlight intensity is transmitted (Lee and Rast, 1997; Bolton, 2012). Given the large light absorption photosynthesis is largely carried out in this depth.

Bolton (2012) examined the penetration depths of pathogen inactivating UV wavelengths; UVA, UVB and Vis in turbid water (Figure 1.5). Penetration depths were examined in a 1 m deep highly turbid pond (46 NTU; Nephelometric turbidity units) with a suspended solid (SS) and Chlorophyll *a* (Chl *a*) concentration of 143 mg L^{-1} and 2.3 mg L^{-1}. The depths identified by Bolton (2012) confirmed the 99% UVB absorption within 0.03 m of water identified by Kohn and Nelson (2007) and therefore supported Haag and Hoigne (1986) who suggested majority of pathogen inactivating light was absorbed in the first 1 m of water. A similar study by Dias and von Sperling (2017) in Brazil (latitude 19°53'S) identified complete attenuation of UVA and UVB by 15 cm and PAR (Photosynthetically Active Radiation) by 30 cm. The Dias and von Sperling (2017) study also confirmed the link between attenuation and turbidity outlined by (Bolton *et al.*, 2010; Bolton, 2012),

finding that attenuation is largely affected by pond turbidity and that during the morning as solar intensity increases so did turbidity; a factor perhaps attributed to algal activity.

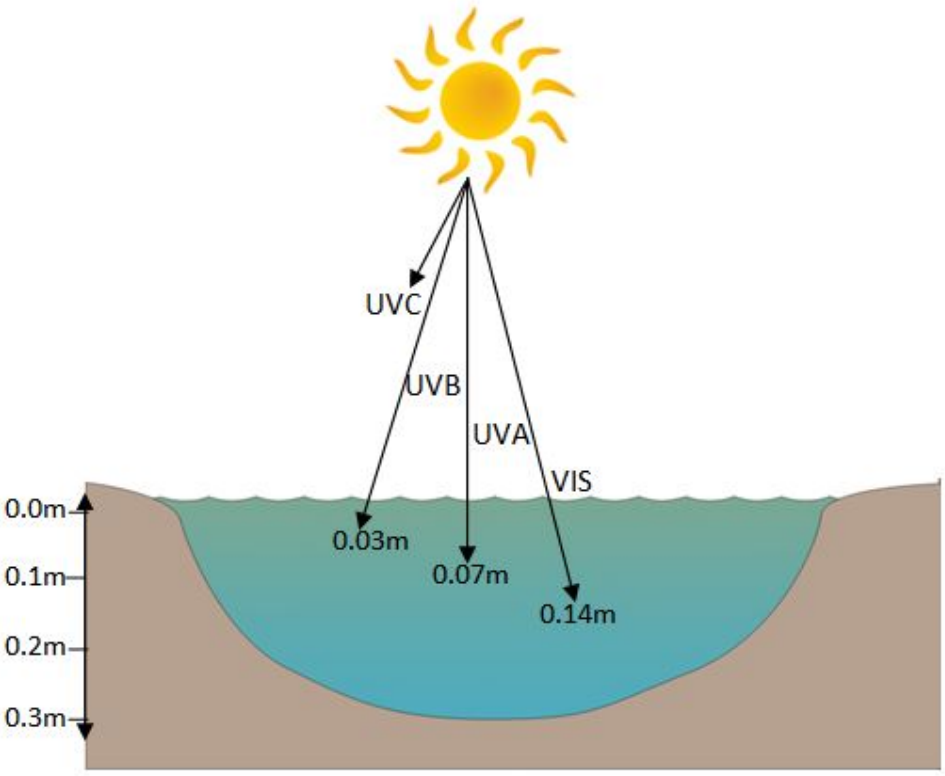

Figure 1.5. Maximum penetration depths observed for pathogen inactivating UV and Vis light in turbid water. Figure is adapt from work by Bolton (2012)

1.6.6 UV exposure, dose and microbial resistance

The exposure of pathogens to inactivating light is crucial for reduction. The quantity of light required to achieve maximum inactivation is variable, dependent on the microorganism with some requiring a dose rate higher than others (Toze, 1997). Chang *et al.* (1985) recognised this variance claiming the dose rate required to achieve a 3.0 log (99.9%) reduction of *E. coli* is at least three times lower than is necessary to obtain the same log reduction for viruses (3-4 x), bacteria spores (9 x) and ambiotic cysts (15 x) respectively. This information was obtained for rotavirus, poliovirus, *Bacillus subtillis* and *Acanthamoeba castellanii* when exposed to UVC at 254 nm. Chang *et al.* (1985) also acknowledged 30 mW s^{-1} cm^{-2} as the required UV dose rate to achieve a 99.9% removal of poliovirus (type 1).

This difference in dose rate is largely associated with the resistance of the pathogen towards radiation. A UV dose rate for inactivation of 200 MJ cm^{-2} (Eischeid *et al.*, 2009) for example is required for the successful removal of adenovirus; a highly UV resistant double stranded DNA virus persistent in faecal contaminated waste (Nwachcuku and Gerba, 2004; Shin *et al.*, 2005). The resistance of adenovirus is far greater than other enteric viruses and is predominantly against UVC radiation (Nwachcuku and Gerba, 2004; Shin *et al.*, 2005). Serotype 2 adenovirus has been shown resistant against all sunlight exposure (Love *et al.*, 2010). This is a dose nearly six times larger than that required to remove other enteric viruses, where 30-40 MJ cm^{-2} was found adequate (Meng and Gerba, 1996; Gerba *et al.*, 2002). The dose rate required for inactivation using UVA in comparison with UVB or UVC is much higher. This is largely due to the lower germicidal properties exhibited within this wavelength compared to the others (EPA, 2006). It is therefore of common belief that the higher the resistance, the higher the dose rate required. Treatment processes and operational costs are therefore placed under considerable strain with the increase in dose rate. This is of particular concern when funding is low and resources limited.

The elevation in required dose rates is believed a consequence of DNA repair mechanisms and the ability of the organism to photo-repair UV induced damage (Lindenauer and Darby, 1994; Eischeid *et al.*, 2009). The involvement of these repair mechanisms was supported by Love *et al.* (2010), however were suggested to be responsible for UV resistance instead. These repair mechanisms are evident in bacteria (i.e. *E. coli and Streptococcus faecalis*), viruses (i.e. adenovirus) and bacteriophage (i.e. PDR1 and T1) with a double stranded genome, but not in those with a single DNA or RNA strand (Love *et al.*, 2010; Rodriguez *et al.*, 2014).

The additional DNA strand in the double stranded genome acts as a template for replication allowing the undamaged complementary strand to be replicated and the damaged strand repaired (Eischeid *et al.*, 2011). This means that UV disinfection does not always kill the microorganism nor is it always permanent with the occurrence of photo-repair of UV induced damage in some organisms. This will often leave the organism viable but non-culturable with its infectivity status lost.

Love *et al.* (2010) also proposed genome length as being a contributing factor towards UV resistance. An increase in genome length was shown to increase resistance. This was evident within somatic coliphage. Similarly, enzymatic defence mechanisms have been identified within bacteria which act to protect against solar radiation (and other disinfectants) and repair any damage sustained to genetic material caused from exposure (Jagger, 1985; Davies-Colley *et al.*, 1997). Rodriguez *et al.* (2014) further explained that DNA repair mechanisms can occur under both light and dark conditions, with dark DNA repair has been linked with nucleotide excision repair and light mediated repair linked with photo-lyase enzyme (Bohrerova and Linden, 2007).

Sinton *et al.* (2002) recognised sunlight resistance in faecal coliforms observing slower inactivation in organisms isolated from the effluent isolated from WSPs, compared to raw sewage. Repair mechanisms are believed responsible with pre-exposure to sunlight a key contributor. This was not the case for *Enteroccoci* who had exhibited faster inactivation in the WSPs effluent a consequence of pre-exposure to sunlight in the pond rendering them 'sunlight sensitive' (Sinton *et al.*, 2002).

Exposure period and UV dose required for microbial inactivation is variable and disputed within the literature. Fisher *et al.* (2012) acknowledged these discrepancies identifying the reported exposure periods ranged from a few hours to over 24 hours with both complete and incomplete pathogen removal identified (Wegelin *et al.*, 1994; Oates *et al.*, 2003; Noble *et al.*, 2004; Dejung *et al.*, 2007; Boyle *et al.*, 2008; Fisher *et al.*, 2012). These findings were based on results obtained for pathogen removal using solar disinfection (SODIS). SODIS utilises solar irradiance to treat drinking water and is usually carried out in PET (polyethylene terephthalate) bottles (Oates *et al.*, 2003). Experimental setup and environmental conditions are likely responsible for this variation and should be considered for all solar exposure studies to restrict further discrepancies.

1.7 Wastewater Treatment Ponds

Treatment ponds or lagoons offer a simple means of treating wastewater effectively. They employ the use of natural treatment processes and reduce pathogen and nutrient concentrations, improving overall quality. Construction, operation and maintenance costs are reduced, ideal for resource-restricted areas; such as developing countries and rural regions (Araki *et al.*, 2001). They

are also suitable for all climatic conditions with the pond characteristics adjusted accordingly. The use of natural disinfection within these ponds means the release of unwanted hazards is limited making them environmentally desirable. These ponds operate in series, much like conventional wastewater treatment plants. They vary in pond type and number depending on the required disinfection level. A standard pond system such as the waste stabilisation pond (WSP) is comprised of a series of anaerobic, facultative and maturation ponds.

1.7.1 Waste Stabilisation Ponds (WSPs)

The configuration of WSPs is variable, dependent on treatment requirements and reuse intensions. Use of multiple ponds is common especially when high pathogen removal is required and the organic load is high, although single facultative ponds may be used (Gloyna, 1971).

The first pond in the WSP sequence is the anaerobic pond, although facultative ponds can be used in their absence. These ponds operate in the absence of oxygen and are designed to reduce DO levels (Mara *et al.*, 1992b). They are the deepest of all the WSPs with a depth approximately 2-5 m (Horan, 1989) and a high volumetric organic loading rate (100 - 300 g BOD m^{-3} day^{-1}) (Mara *et al.*, 2003). Depth is crucial in these ponds to accommodate the accumulation of sludge built up on the pond bed and to maintain the anaerobic conditions required (Mara *et al.*, 1992b). Anaerobic ponds have a short retention time ranging from 1.0 to 5.0 days (Horan, 1989). Horan (1989) identified the anaerobic pond removal rates for suspended solids (50-70%), biochemical oxygen demand; BOD (40-60%), helminth (70%), and faecal coliforms (1.0 log_{10}). Temperature has a positive influence on both the reduction of BOD and the organic loading rate in these ponds. A BOD reduction of 75% was observed when the temperature was increased to 25°C, opposed to the 60% achieved at 20°C (Okoh *et al.*, 2007). Sedimentation of suspended solids and pathogens occur, through the separation from floating material. This forms a removal layer of scum at the surface (WHO, 1987). Mineral and gas (methane (CH_4), Carbon dioxide (CO_2) and hydrogen sulphide (H2S)) formation occurs within these ponds through the decomposition of organic matter by anaerobic bacteria found in the sedimentation layer (WHO, 1987). Often additional treatment or aeration is required to remove these by-products (Hosetti and Frost, 1998).

The next pond is the facultative pond. Multiple facultative ponds may be used in parallel depending on treatment requirements and can be used in the absence of an anaerobic pond. This pond utilises both anaerobic and aerobic conditions for treatment and is shallower than anaerobic ponds, with a depth of 1-2 m (Horan, 1989; Hwang, 2012). Like the anaerobic pond, depth is essential for the maintenance of suitable conditions. These ponds are designed to reduce both BOD and pathogen loads, and is reliant on the symbiotic relationship between bacteria and algae for treatment. A reasonable growth of algae is therefore required. 50-70% BOD and 1.0 $\log_{10}$ faecal coliform reductions have been observed in facultative ponds (Hwang, 2012). The high surface area to volume (S/V) ratio of facultative ponds assists in the promotion of algal growth (Mara *et al.*, 1992b). Facultative ponds have a lower organic loading rate (80-400 kg BOD ha^{-1} day^{-1}) than anaerobic ponds but a higher retention time (Mara, 2003). The appropriate retention time is variable throughout the literature depending on the characteristics of the pond and climate, ranging between 4-50 days (Horan, 1989; Mara *et al.*, 1992b).

As a final step, the treated effluent from anaerobic and facultative ponds can be treated further in maturation ponds. These ponds offer secondary and tertiary treatment processes and are designed to reduce the microbial content from the wastewater (WHO, 1987). A single or multiple maturation pond may be used depending on the microbial concentration and the concentration permissible for the final effluent. All processes are carried out aerobically thus, a shallow pond depth is required, typically 1-1.5 m (Bolton *et al.*, 2010). Sunlight disinfection, specifically photo-oxidation is used to reduce the organisms. Hwang (2012) discussed the removal efficiencies obtainable throughout these ponds, identifying 30-60% BOD, 4 log faecal coliform, 100% parasite, 20-40% suspended solids and 40-60% nitrogen removal respectively. These ponds have a typical retention time of 3-10 days (WHO, 1987). Although Mara *et al.* (1992b) suggested 5-15 days was a suitable retention time and (Horan, 1989) indicated when multiple maturation ponds are used 12-18 days is ideal depending on loading rate.

Disadvantages have been identified for WSPs including sludge accumulation, odour release, long retention times and high nutrient, pathogen and algal concentrations (Senzia *et al.*, 2003; Wells, 2005).

1.8 High Rate Algal Ponds (HRAPs)

HRAPs have attracted attention across all areas of water treatment and algal production, exhibiting elevated disinfection and algal biomass production. This pond is of interest throughout the current study and can be found as a standalone HRAP or make up the mid-section of an advanced integrated wastewater pond system (AIWPS). Araki *et al.* (2001) described HRAPs as a combination of oxidation ponds and algal reactors designed to improve overall disinfection in treatment ponds. A large S/V ratio has been incorporated into this pond aiding in overall water treatment, pathogen removal and algal biomass.

Much shallower than WSPs with a depth of 0.2-1 m (Park *et al.*, 2011) the HRAP is capable of achieving a disinfection capability equal to other treatment ponds in $1/6^{th}$ of the time (Buchanan *et al.*, 2011a). The pond is set up in a raceway configuration segregated by narrow baffles (Figure 1.6). This configuration can be either single or multiple looped (Park *et al.*, 2011) and aids in the maintenance of a steady flow (Oswald and Golueke, 1960). The residence time for these ponds is approximately 2-8 days (Shilton, 2005).

Water is circulated through the pond via a paddlewheel, generally eight bladed. This is characteristic to HRAPs with the other treatment ponds remaining unmixed except from the wind. The paddlewheel enables mixing and circulation of the water to be continuous increasing exposure to inactivating sunlight and reducing thermal stratification; layer formation in the water varying in depth and temperature (Fallowfield and Garrett, 1985; Craggs *et al.*, 2004a; Craggs *et al.*, 2004b). Thermal stratification results in the short-circuiting of the pond and clumping of algae into 10-15 cm bands reducing pond performance (Tadesse *et al.*, 2004). The incorporation of baffles assists with the prevention of short-circuiting and keeps water flowing in the pond (Oswald and Golueke, 1960). Exposure and disinfection ability is increased by the rotation of water and pathogens in and out of UV exposed regions (Hu *et al.*, 1996). A mean velocity of 0.15 ms^{-1} is achieved (Craggs, 2005; Park *et al.*, 2011), with all pond regions experiencing a velocity of at least 0.05 ms^{-1} (Shilton, 2005).

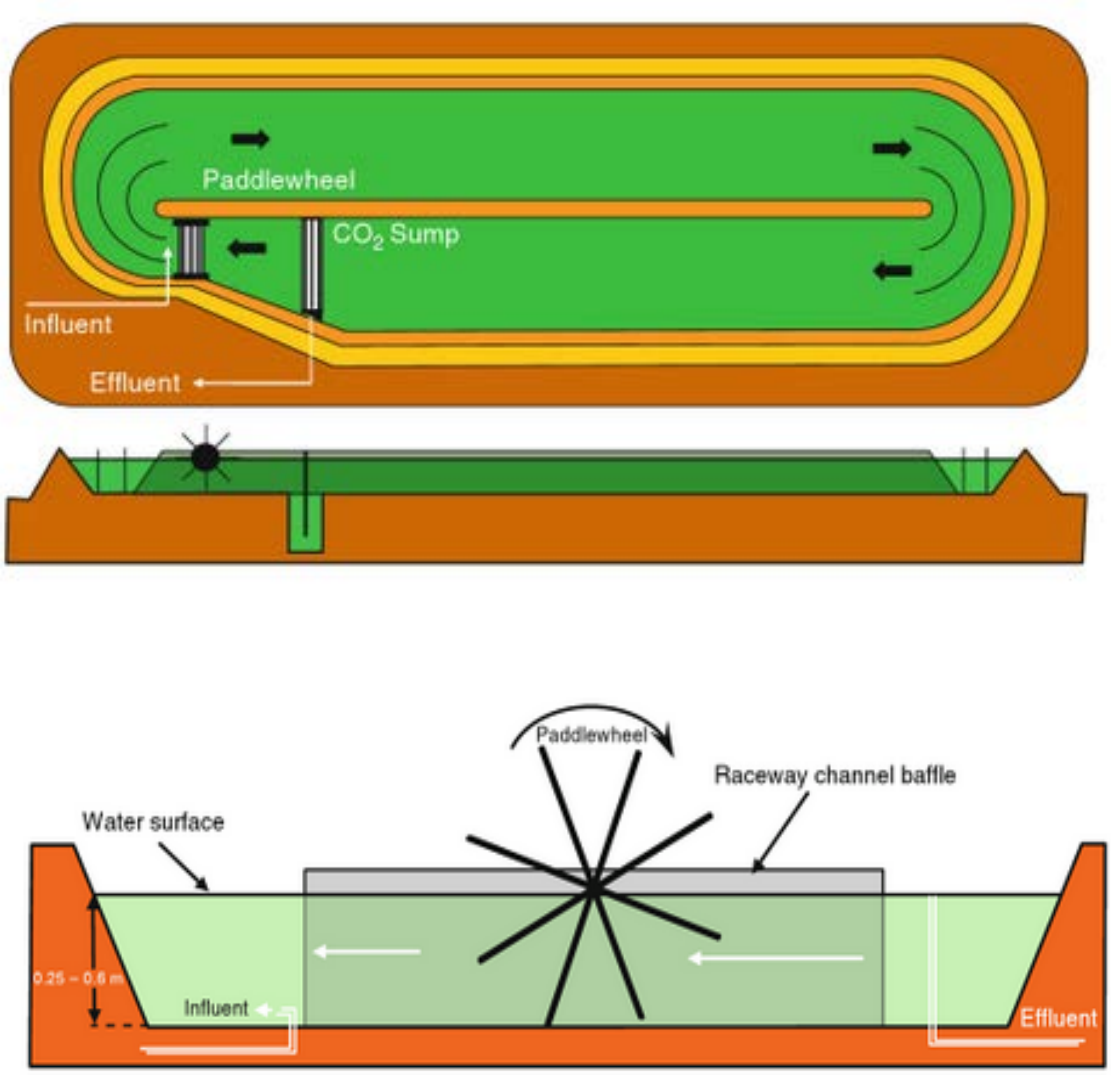

Figure 1.6: Schematic diagrams of a high rate algal pond. Diagrams include a) overview of the pond and b) side view including pond depth and paddle wheel rotation. The particular HRAP illustrated in (a) was designed to investigate the addition of CO_2 to wastewater and evaluate the effect this has on algal growth and production (Craggs *et al.*, 2012b; Craggs *et al.*, 2013).

1.8.1 Algal Biomass Production in HRAPs

Algal growth is highly abundant in these ponds. An annual algal-bacterial productivity of 12-40 g m^{-2} day^{-1} can be achieved in these ponds (Fallowfield and Garrett, 1985; Park *et al.*, 2011). Craggs *et al.* (2013) identified the annual algal productivity in HRAPs to be at least a threefold higher than those produced throughout conventional facultative ponds with 30 tonne ha^{-1} $year^{-1}$ produced in a New Zealand HRAP.

Oxygenation by non-pathogenic bacteria promotes the breakdown of organic matter such as CO_2, nitrogen and phosphorus. These nutrients as shown in Figure 1.7 are assimilated by algae and

utilised in photosynthetic processes, providing the required oxygen required (Craggs *et al.*, 2004a). Craggs *et al.* (2012a) suggested that up to 25 g m^{-3} of DO could be produced from the photosynthesis of algal in these ponds, and Craggs *et al.* (2013) identified the removal of nutrients with approximately 3 kg ha^{-1} day^{-1} phosphorus and 24 kg ha^{-1} day^{-1} nitrogen assimilated by the pond algae.

Figure was removed due to copyright restrictions, but can be found on page 76 of Oswald, W.J. & Gotaas, H.B. (1957). Photosynthesis in sewage treatment. *Trans. Am. Soc. Civ. Eng.* 122, 73-105

Figure 1.7: Relationship exhibited between algae and bacteria in wastewater treatment ponds for photosynthetic oxidation. (Oswald and Gotaas, 1957)

Sunlight is a key factor contributing to both algal production and photosynthesis in these ponds. However, if the algal biomass becomes too dense light exposure is restricted and the pond becomes shaded. Park *et al.* (2011) suggested that the majority of irradiated light is absorbed within the first 0.15m when algal biomass reached a concentration of approximately 300 g TSS/m^3. Curtis *et al.* (1994) identified longer UV wavelengths are absorbed less by algae than the shorter wavelengths, instead more commonly utilised for photosynthetic processes.

1.8.2 HRAP performance, solar radiation and pathogen removal within the pond

Many factors influence the overall performance of HRAPs and the disinfection ability of solar radiation. Factors include pond depth, hydraulic retention time, temperature, pH, nutrient availability, assimilation, and algal productivity.

The hydraulic retention time (HRT) or residence time for treatment ponds is variable, dependent on the season, composition and water clarity. The HRT is the time by which water is retained within a pond before being pumped into the next for further treatment or discharged reuse. A retention time of 7-9 days has been suggested for winter periods by Craggs *et al.* (2013) and halved (3-4 days) during summer where solar radiation is high. El Hamouri *et al.* (1994) also suggested this claiming a retention time of 6 days for cold weather and 3 days for hot based on the Mexican climate. El Hamouri *et al.* (1994) did acknowledge the potential need for area extension to achieve the similar disinfection during the winter period. The removal of pathogens, in particular protozoan cysts and oocysts is greater in ponds with longer HRTs, allowing sedimentation to be maximised.

Pond depth strongly influences the effect of sunlight irradiation and pond performance. Identifying a depth suitable for the required treatment condition is crucial. Shallow ponds are suitable for pathogen removal, as elevated sunlight exposure and aerobic conditions are achieved (Fallowfield *et al.*, 1996; Shilton, 2005). Alternatively, deeper ponds provide suitable storage reservoirs and anaerobic conditions (Shilton, 2005). Pond depth was shown to be inversely proportional to the microbial die-off constant (first order kinetics) (Sarikaya *et al.*, 1987; Sarikaya and Saatci, 1988). Oragui *et al.* (1986) identified a pond depth of 3 m has a removal rate for faecal indicator bacteria comparable to the removal identified in maturation ponds. Shilton (2005) identified 0.4 m as the minimum allowable pond depth where treatment is still practical and land usage is not extreme. However, the typical pond depth in many HRAPs is 0.3 m (Park *et al.*, 2011). Buchanan *et al.* (2011b) confirmed this identifying a pond depth of 0.32 m had exhibited a greater influence on *E. coli* die-off rates, in South Australia than a deeper pond depth of 0.43 m or 0.55 m. Therefore, a decrease in pond depth will result in an increase in sunlight exposure and with it an increase in microbial die-off rates (Fallowfield *et al.*, 1996). Solar exposure even at a shallow pond depth is still limited with UV penetration largely apparent in the surface area.

Water loss through evaporation is undesirable. Buchanan *et al.* (2011b) identified evaporative loss was about 5.4% less in HRAPs (5.2% evaporative loss for a surface area of 225 m^2) than in WSPs (10.6% evaporative loss for a surface area of 6,300 m^2), suggesting the amount of reuse water available is significantly higher when treated throughout HRAPs.

Pathogen removal, pH and DO levels are elevated within these ponds, nutrient concentrations; nitrogen, phosphorous and carbon however are often limited (Bahlaoui *et al.*, 1997). pH can be increased to >11.0 when the hydrogen ions and hydroxyl ions in the carbonate/bicarbonate buffering system of water is altered (Equation 1.7) (Paterson and Curtis, 2005). This is a consequence of CO_2 and HCO_3^- consumption by algae (Tadesse *et al.*, 2004).

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow H^+ + CO_3^{2-}$$

Equation 1.7

El Hamouri *et al.* (1994) claimed treatment could be enhanced in these pond systems with the addition of CO_2 improving algal growth and nutrient removal. Craggs *et al.* (2013) suggested CO_2 could increase wastewater treatment biomass to 60 t ha^{-1} year double the normal production rate. Oxygen is also essential throughout theses pond systems, facilitating the photosynthetic processes between microorganisms and algae, eliminating odour and enhancing both solar inactivation and microbial removal.

The organic loading rate in HRAPs is 150-600 kg BOD_5 ha^{-1} day^{-1} coupled with a retention time of 2-8 days (Azov and Shelef, 1982; Fallowfield and Garrett, 1985). This is comparable to the loading rate identified for WSP facultative ponds (200-600 kg BOD_5 ha^{-1} day^{-1}) (Fallowfield and Garrett, 1985). Azov and Shelef (1982) suggested the BOD_5 removal for the 2-8 day HRT was 93-96%. Cromar *et al.* (1996) who found the BOD_5 to be 60-99% depending on seasonal condition supported this.

Early studies regarded temperature as the primary factor responsible for microbial die-off within pond systems (Maynard *et al.*, 1999). Recent studies however, discovered microbial die-off was variable between pond systems of similar temperatures. Therefore, it was concluded that although

temperature was involved in die-off it was only indirectly involved in the removal assisting other processes; such as sunlight disinfection (Davies-Colley *et al.*, 1999). Temperature throughout HRAPs is uncontrollable but critical for the operation of biological processes and determining the scale of the pond (Tadesse *et al.*, 2004). Paterson and Curtis (2005) described variation in pond temperatures suggesting a range of <10°C to 30°C. Location of pond site is a contributing factor also. For instance in dry and arid regions where the temperature is high a smaller treatment pond is sufficient, to achieve the same disinfection performance as larger ponds used in cooler climates where temperatures are low (Paterson and Curtis, 2005).

The microbial removal in HRAPs is good Fallowfield *et al.* (1996), but limited information is available compared to WSPs. Majority of the literature has been centred on the removal of *E. coli* and faecal coliforms, with the quantification of virus and bacteriophage limited. El Hamouri *et al.* (1994) reported a yearly HRAP $\log_{10}$ removal rate of 2.27-3.19 $\log_{10}$ for faecal coliforms and nearly 100% helminth removal. The successful helminth removal was believed a factor of a 24-hour settling period before treatment. Craggs *et al.* (2004b) identified a 2.0 $\log_{10}$ reduction for *E. coli*, which complies with the $\log_{10}$ reductions outlined by NRMMC-EPHC-AHMC (2006) for microbial reduction in WSPs. NRMMC-EPHC-AHMC (2006) identified the indicative $\log_{10}$ removal rates for Microorganisms in WSPs: i.e. *E. coli* 1.0-5.0, viruses 1.0-4.0, bacteriophage 1.0-4.0 and bacterial pathogens 1.0-5.0 $\log_{10}$. Wells (2005) supported this 2.0 $\log_{10}$ removal of E. coli and further identified the complete *E. coli* (100%) reduction when two HRAPs at Rhodes University, South Africa were run in procession and the HRT was increased from 3 days to 6 days. This was complete removal was also apparent for faecal coliforms.

The die-off rate constants (K_b = 0.35-2.34 d^{-1}) for *E. coli* in HRAPs were identified by Fallowfield *et al.* (1996). These rates were established from two HRAPs in Scotland (55°29'19"N, 4°32'34"W) where *E. coli* were exposed to average temperatures of 9.5 – 23.4°C and solar irradiances of 85.0 – 365.0 W m^{-2} (Fallowfield *et al.*, 1996). Sinton *et al.* (2002) reported the solar inactivation rates (K_s) for faecal coliforms (0.086 m^2 MJ^{-1} and 0.084 m^2 MJ^{-1}), F-RNA phage (0.070 m^2 MJ^{-1} and 0.050 m^2 MJ^{-1}) and *E. coli* (0.078 m^2 MJ^{-1} and 0.073 m^2 MJ^{-1}) in WSP effluent throughout both summer and winter periods. Solar intensities ranged between 7-26 MJ m^{-2}. These K_s values were

shown to be double the inactivation rates identified during dark inactivation (i.e. 0.0162 h^{-1} faecal coliforms, 0.014 h^{-1} F-RNA phage and 0.0171 h^{-1} *E. coli*) thereby confirming the importance of light exposure to pathogen die-off within natural pond systems.

Disinfecting the ponds with UV prior to treatment has been explored and shown to yield both higher algal biomass production and elevated *E. coli* reduction (Santiago *et al.*, 2013). Santiago *et al.* (2013) examined a 3.1 log reduction in *E. coli*: 1.1 log units from the HRAP and 2.0 logs from the UV disinfection.

1.9 Inclined Planes for increased disinfection

Disinfection with UV is effective at reducing microorganisms in natural treatment systems. Exposure to sunlight and inactivating UV is reasonable throughout treatment ponds with greater exposure identified in HRAPs from the continual mixing and large S/V ratio. However, for complete pathogen removal exposure is still insufficient, a factor of shallow UV water penetration, high attenuation and absorption of irradiated wavelengths and shading by algal blooms (Park *et al.*, 2011). Increasing exposure is therefore critical and for improved disinfection, the impact from these factors must be lessened.

It is believed that increasing the surface area available for disinfection with reduced volume over that area will decrease UV attenuation and enable greater exposure and microbial die-off. The addition of an inclined plane with water running over it as a thin layer is therefore proposed to improve exposure.

This inclined plane already exists within the walls of HRAPs (Plate 1.1). The slope naturally falls to an angle of 45° and established with the construction of the pond. As of yet this inclined plane serves no great function or purpose in the actual treatment of wastewater, but is considered suitable for increasing solar exposure.

Inclined planes and thin films are not a new phenomenon. Solar disinfection and microbial irradiation have been examined in both areas, but combining both these concepts for improved

pathogen removal has yet to be explored, and thus will provide the overall focus of the current study.

Plate 1.1: The inclined plane surrounding the high rate algal ponds (HRAPs) at Kingston on Murray (KoM), South Australia.

A comparison of solar irradiation on a horizontal plane and solar irradiation on a tilted (inclined) plane was undertaken in a study by Navntoft *et al.* (2012). The study measured the amount of UV and global irradiation absorbed on both surfaces and identified a 3-4% increase in UV energy was observed when the disinfection surface was tilted 37°. Winter months were shown to yield global spectra and solar UV ratios of 1.70 and 1.25 respectively for the tilted surface compared to the 0.85 and 0.95 obtained throughout out summer. Increase in solar radiation was shown dependent on the orientation towards the sun, season and weather with heavy rains preventing accurate data readings. Iqbal (1983) and Duffie and Beckman (2013) both identified a 10% increase in solar radiation when the incline was in the direction of the sun and equal to the latitude.

The angle of inclination is critical if maximal exposure is to be achieved on the IP. However, this angle is varied and appears dependent on location. Majority, if not all the studies acknowledge the tilt angle should be equal to the latitude (φ); however they discuss that slight alteration to the angle might also be required. Ekpenyong *et al.* (2017) presented a review of the optimal tilt angles reported in studies carried out in South Africa, Turkey and India during winter including the modified tilt angles. It was found that an alteration value of up to 30° was reported and was dependent on location. The optimal tilt angle in its briefest form can be represented as described in Equation 1.8.

$$\textit{Optimal tilt angle} = \varphi \pm a^{\circ}$$

Equation 1.8

where φ is the angle of latitude and a is the degree of alteration.

For the development of the thin film a plastic membrane is required, enabling distribution and solar exposure of the water without loss to the environment (Craggs *et al.*, 2004a). A high-density polyethylene, HDPE membrane is commonly used. However, the hydrophobic properties of the plastic may restrict the distribution of the thin film and affect the overall disinfection ability of the

inclined plane. This would need to be considered when investigating thin film development on the inclined planes.

Investigation of water distribution down surfaces in thin films has been explored. Studies including Heredia and Duffy (2007) have explored the generation of thin films on hydrophobic surfaces with the inclusion of TiO_2 (titanium dioxide, titania); a photo catalyst that destroys organic particles and microorganisms in the presence of sunlight to enable water to travel smoothly down the medium, generally glass. This application has been applied to glass manufacturing, providing a self-cleaning glass. TiO_2 addition has also been explored in solar disinfection (SODIS) studies using PET bottles for treating drinking water. The current study however was looking at improving removal using thin films and inclined planes with the economical and environmental benefits associated with the ponds the addition of chemical products was not explored.

An initial investigation was undertaken to identify the potential of increasing solar exposure with the HRAP inclined plane (Hawley, 2012). This was carried out under both overcast (high cloud cover) and sunny conditions using model HRAP systems with MS2 bacteriophage the target organism. Results identified an increase in MS2 die-off for both conditions when exposed to sunlight via the plane. However, variations and inconsistencies within obtained results suggest the process is plausible but further study is required. It is therefore the aim of the current study to continue the exploration into the inclined plane disinfection

The current study aimed to further explore disinfection by inclined planes, by altering characteristics of the plane and subsequent flow rate to achieve the ideal model for disinfection that can be applied to working HRAP systems.

1.10 Aims and Objectives

The release of water suitable for reuse is crucial to many fields including public and environmental health, agriculture, and water industries. For this to be achieved significant research must be still undertaken for all aspects of treatment, but more importantly research into solar irradiation and natural treatment ponds where there is a current niche. Microbial die-off within these ponds is largely associated with the amount of solar radiation they are exposed to. This exposure is

increased in HRAPs but remains limited, lost through attenuation. It would be of advantage to examine pathogen die-off and solar exposure within these ponds and establish a mechanism to increase this exposure. Incorporation of an inclined plane is considered a beneficial addition to HRAPs, enhancing exposure through a reduced water depth over a larger area. An honours project (Hawley, 2012) began researching this addition examining the die-off of MS2 under both overcast (high cloud cover) and sunny conditions. Model HRAPs were used with the inclined plane included. Results identified the inclusion was plausible but greater research is required. It was therefore the aim of the current study to continue the exploration into inclined plane solar disinfection, as well as to provide relevant information regarding pathogen removal in HRAP, as a result of solar exposure.

The overall objectives of the study were to

- Examine pathogen removal in HRAPs with the addition of an inclined plane and establish 'proof of concept' in laboratory scaled model systems
- Evaluate different design parameters of the inclined plane system to improve performance and establish system efficiency.
- Establish the effectiveness of the inclined plane system when up-scaled into an already established pond system (i.e. Kingston on Murray).

2. MATERIALS AND METHODS

This chapter outlines the general materials and experimental methods undertaken throughout this research; including the incorporation of an inclined plane (IP) to a HRAP for improved pathogen removal. Investigations were carried out in South Australia using both model scale HRAPs at Flinders University (35.024267°S, 138.570551°E) and a larger scale HRAP at Kingston on Murray (KoM) (34.242641°S, 140.329529°E), where the IP was incorporated into a pre-existing pond. Methods have been separated into field and laboratory based for simplicity. Additional details specific to each pond system and research aims are described in their corresponding chapters.

2.1 The Inclined Plane (IP) System

An IP was designed and incorporated into both model and large scale HRAP systems. Both IPs are described separately in Sections 2.2 and 2.3 respectively. The role of the IP was to create a sloped surface for pond water to travel down at a reduced volume in a thin film. Figure 2.1 outlines the fundamentals and operation of the IP.

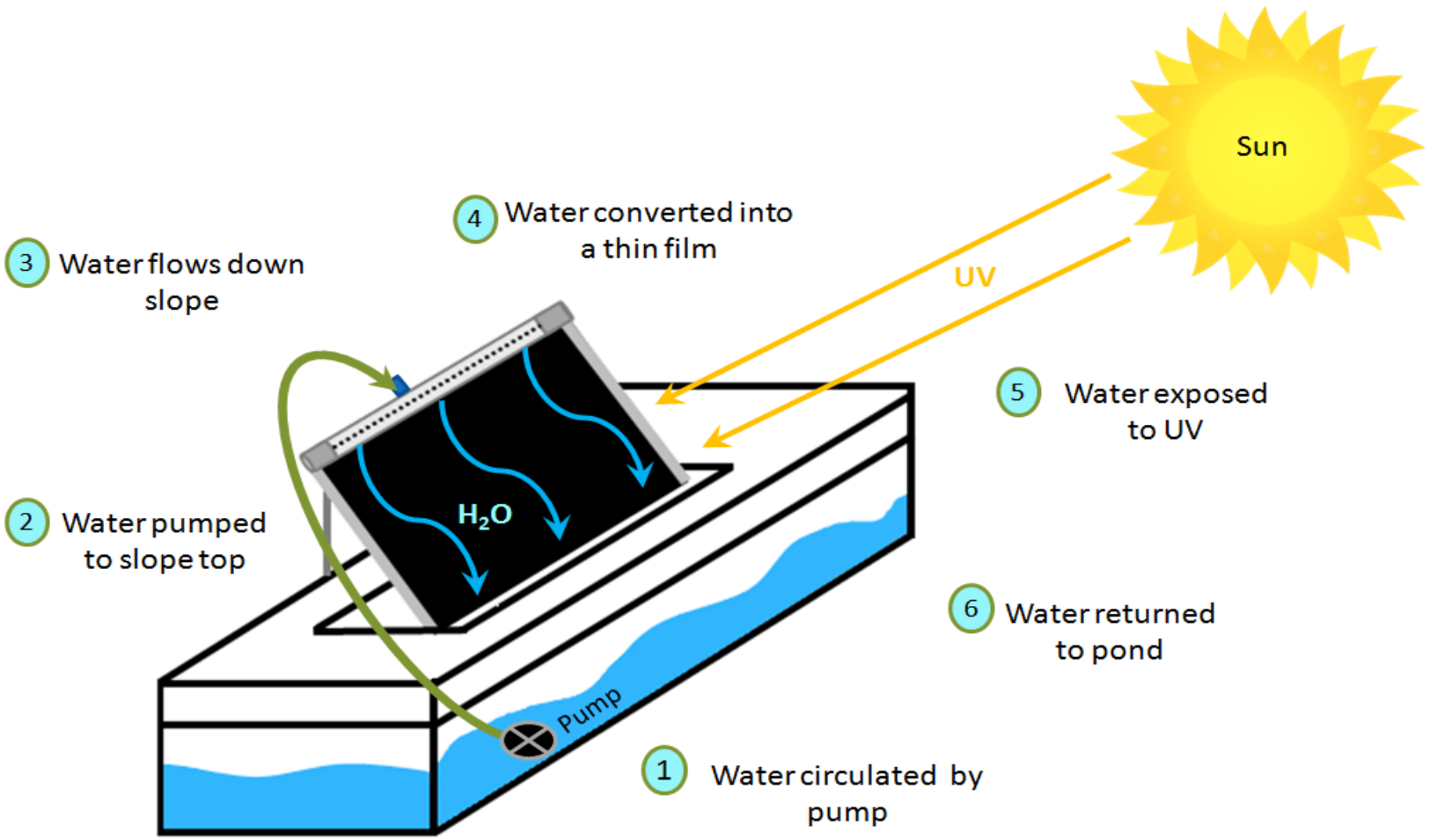

Figure 2.1: Fundamentals and operation of the inclined plane (IP). The system operated by circulating water through the system using a paddlewheel or aquarium pump. The water was then pumped to the top of the slope (i.e. pond wall) and through a manifold, comprising a pipe with evenly distributed holes. Whilst on the slope both water and pathogens were exposed to sunlight before being returned back to the pond. Figure adapted from Hawley (2012).

2.2 Laboratory Based Methods

2.2.1 Model HRAP+IP Systems

Model HRAP systems were constructed and setup outdoors at Flinders University. Two types of model HRAP were constructed; a control HRAP with a configuration that resembled normal pond operation in the field and a HRAP that included an IP (HRAP+IP). Model systems were batch operated. A line diagram is presented in Figure 2.2 which demonstrates the design and operation of the 'generic model IP'.

HRAPs were constructed from 100 L black storage containers. Plastic bin liners (240 L, GRUNT) (Plate 2.2) were used to line the vessels and prevent microbial adsorption to the boxes. Liners also assisted in providing a more accurate representation of the high density polyethylene (HDPE) used to line HRAPs. Wastewater was incubated in the dark by covering the box with a lid (Plate 2.1a). To accommodate the IP, a thin slit (0.08 m x 0.62 m) was cut out of the lid (Plate 2.1b). The HRAPs were filled to a depth of 0.30 m at a volume of 87 L. Systems were operated as either fully solar exposed; bulk water uncovered (open) or dark incubated (covered); with only the IPs solar exposed.

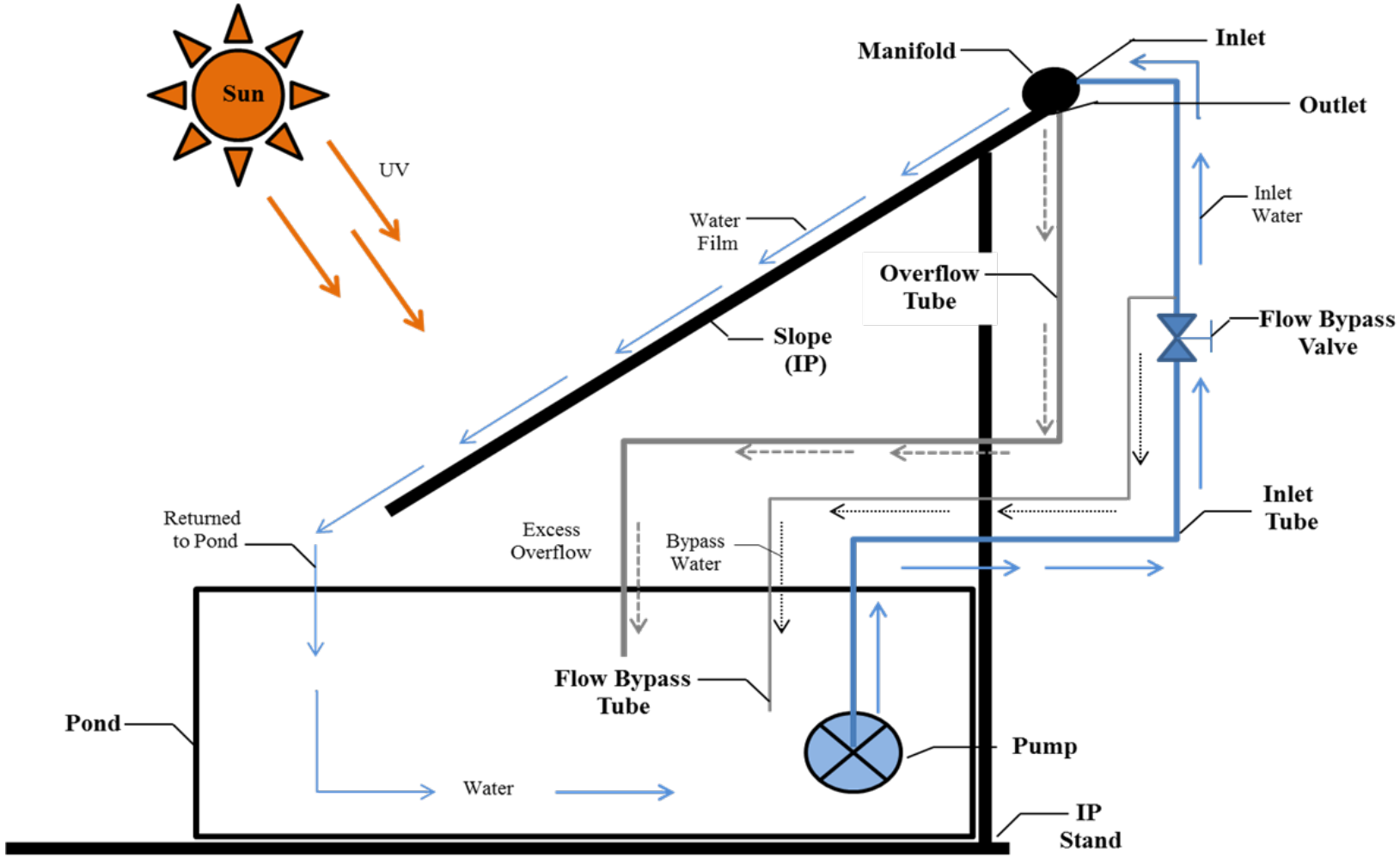

Figure 2.2: Line Diagram of model HRAP operation. Water from the pond gets pumped up to the top of the IP via an inlet tube. A bypass valve was incorporated to allow for water flow to be throttled when required

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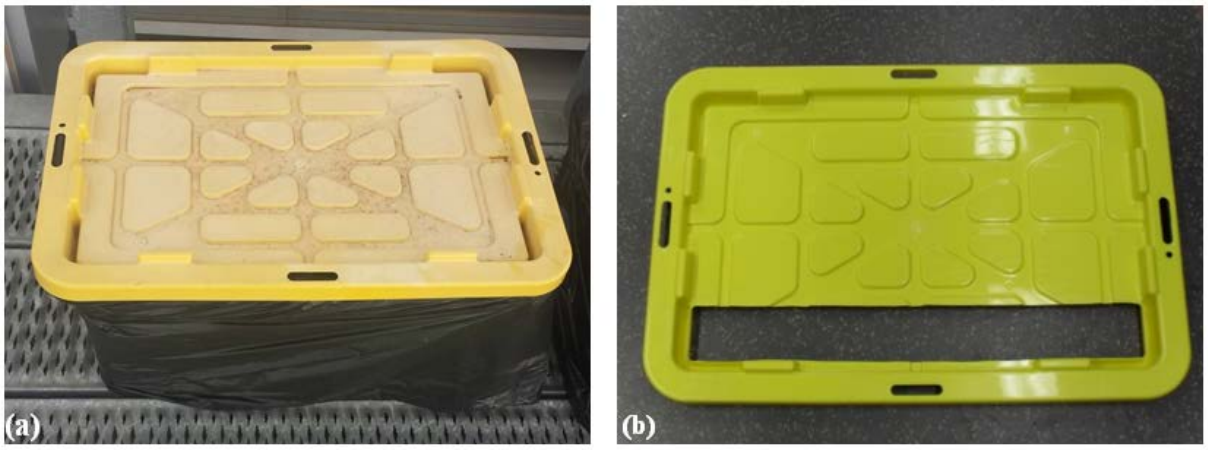


Plate 2.1: Model HRAPs. Photographs of the a) storage container and b) lid for inclusion of solar exposed IP with HRAP bulk water incubated in the dark.

Plate 2.2: Pond lining; Photographed example of a fully lined pond.

Three model IP systems were constructed; two with a surface area of 0.37 m^2 (Short-IP, SIP) and one with an area of 0.75 m^2 (Long-IP, LIP) (Plate 2.3). Plates 2.3 and 2.4 provide an example of the model HRAPs when dark incubated (bulk water covered) or solar exposed (bulk water uncovered). Black Perspex (5 mm and 6 mm) attached to a steel frame base (Plate 2.5a) formed the IP. The incline was set to 45° as this resembled the angle of the KoM pond wall. Black Perspex was used to mimic the HDPE lining of the HRAP embankment slope. The weight of the storage boxes was used as a counter balance to stabilise the IPs. Additional support was required for the longer IP, with the upper part of the IP rested against the surrounding barrier (as shown in Plate 2.3). Water was delivered down the slope using a 30 mm perforated pipe (2mm diameter holes, Plate 2.5c) attached to the Perspex top (Plate 2.5b). 10 mm reinforced nylon tubing attached to a 10 mm inlet valve (Plate 2.5d) was used to transport water from the storage reservoir (storage box) via the aquarium pumps to the IP. Surplus (overflow) water was redirected back into the storage reservoir via an outlet valve. This is demonstrated in Figure 2.2.

Paddlewheels used in HRAPs could not be incorporated into the model HRAPs due to size and practicality. Instead submersible aquarium pumps were employed for water circulation and transportation through the IP system. Two types of aquarium pumps were used; an Aqua One 102 maxi power which had a maximum flow rate of 500 L h^{-1} (Plate 2.6a) for the shorter-IP length (0.55 m) and an Aqua Pro™ AP1050 submersible pump; maximum flow rate of 1050 L h^{-1} (Plate 2.6b) for the longer-IP length (1.10 m). A valve system was incorporated to enable flow throttling (Plate 2.7). Specifications of both pumps can be found in Appendix 1.

$HRAP_D$ $HRAP_D$ + LIP $HRAP_D$ + SIP

Plate 2.3: Model high rate algal ponds (HRAPs) with an included inclined plane (IP). Systems included a HRAP without an IP, and an HRAP with a Long-IP (HRAP + LIP: 1.10 m, 0.75 m^2) and a Short-IP (HRAP + SIP; 0.55m, 0.37 m^2). Shown are the model systems when dark incubated with the bulk water covered with the IP exposed to sunlight

Plate 2.4: Example of the solar exposed model HRAPs. Show is the HRAP + SIP (Short-IP, 0.55 m and 0.37 m^2) when bulk water and IP are solar exposed.

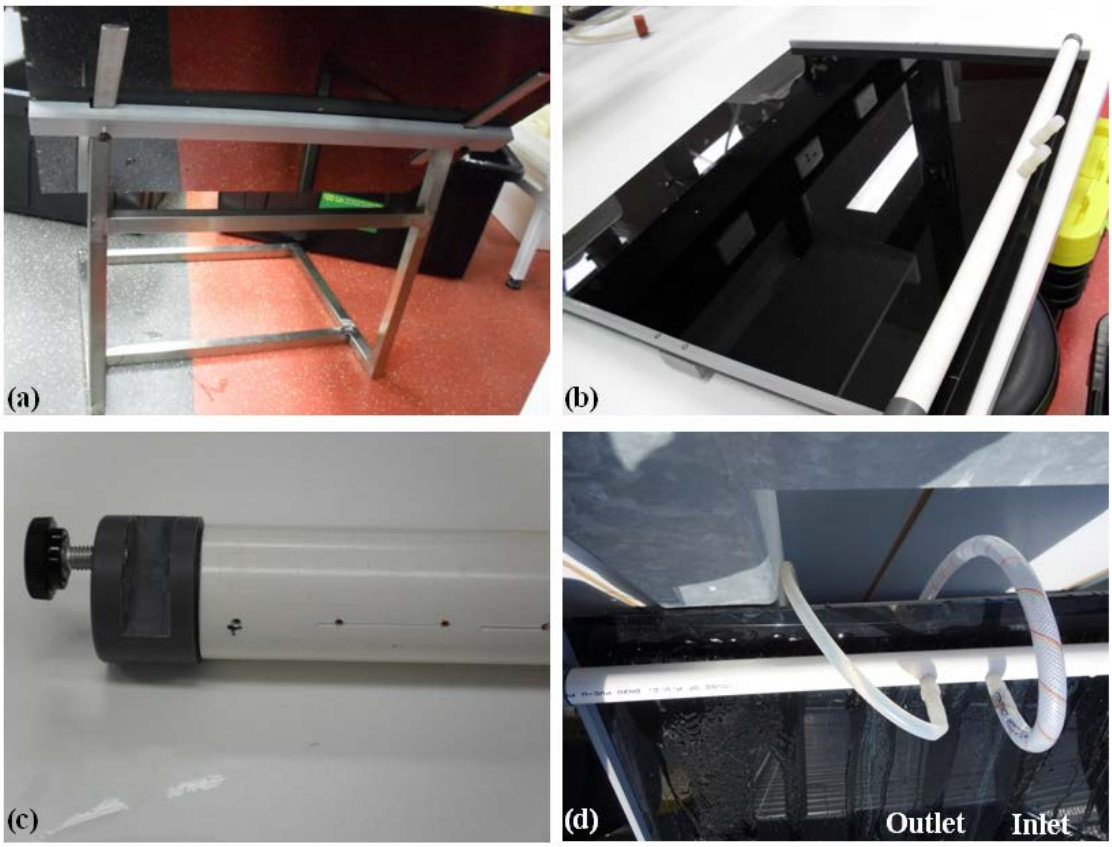


Plate 2.5: Components of the model inclined plane (IP) systems. Photographs of the a) back of the IP and stand b) Perspex with 30 mm pipe and c) 2 mm holes for thin film generation and d) the 10 mm reinforced nylon tubing and both inlet and outlet. Figure adapted from Hawley (2012).

a)

b)

Plate 2.6: Aquarium Pumps used in model HRAP systems for pond circulation and IP operation. Pumps included a) an Aqua One 102 Maxi Power head aquarium pump and b) an Aqua Pro™ AP1050 submersible pump.

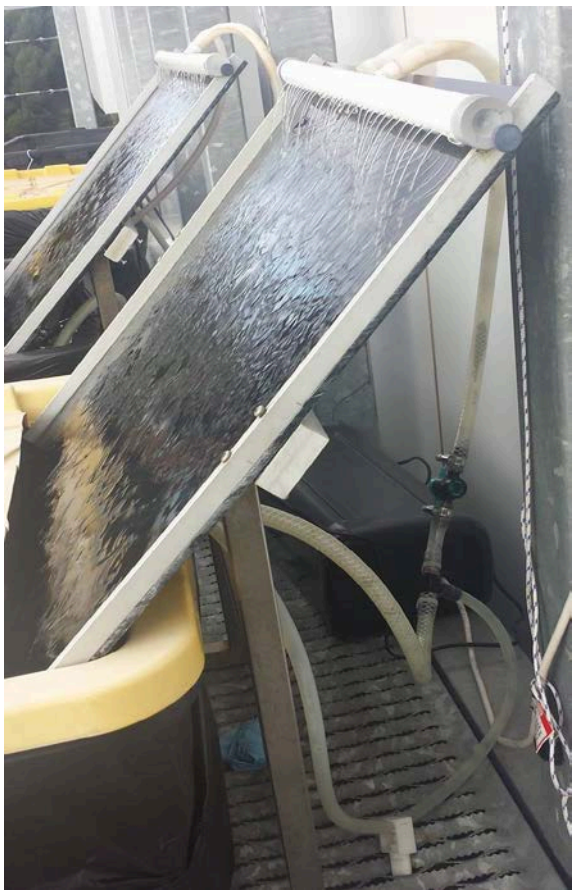


Plate 2.7: Valve system for flow throttling. Photograph of the valve system attached to the IP system.

2.2.2 Water Source

Two water sources were used throughout the investigation; optically clear water and wastewater. Tap water was used as the optically clear water source and only used in Chapter 3. Wastewater for the model systems was supplied from Mt Barker WWTP, South Australia (Chapters 4 and 5). Mt Barker WWTP is a community wastewater management system (CWMS) that treats domestic wastewater from septic tanks within the local and neighbouring communities. The wastewater had been lagoon treated prior to collection. Refer to Chapter 4 for additional details regarding the Mt Barker WWTP.

2.2.3 In situ water quality monitoring (Model HRAPs)

For model HRAPs, in situ water conditions were recorded during all collection periods. Measurements included dissolved oxygen; DO (YSI model 55, Xylem; MODEL, Jenway), pH (350 pH Meter, Jenway) and water temperature (YSI model 55, Xylem; MODEL, Jenway) A Solar Light

data logging radiometer – PMA 2100 was used to record UV radiation onsite fitted with a UVA (PMA 2110-WP, Solar Light) and UVB (PMA-2106-WP, Solar Light) sensor.

Environmental conditions were monitored continuously at both sites. In the absence of onsite monitoring, data made available by the Australian Bureau of Meteorology (BOM) (www.bom.gov.au/). BOM data was obtained from Adelaide Airport; station number 23034. This station was located approximately 9.58 km away (34.95°S 138.52°E) from the sampling site at Flinders University, South Australia. Parameters monitored included; solar irradiance (global, MJ m^{-2}), hours of sunshine (h) and ambient temperature (minimum and maximum, °C).

2.2.4 Sampling

Triplicate 10 mL samples were collected from each system at 1.5 h intervals, with sampling between 9:00 and 16:00 h. Sampling regime was adapted from the work by Hawley (2012). 1 L samples were collected at the start and end of each experiment, with the water analysed for changes in water quality from the starting Mt Barker water. Additional 100 mL samples were also collected in 3 h intervals for analysis of turbidity and chl concentrations.

2.2.5 Inactivation Experiments

Inactivation experiments were conducted using the model IP systems to determine the effectiveness of the IP. These experiments determined the reduction in MS2 and F-Specific bacteriophage numbers after being exposed to the various system configurations over a set time period. Experiments also looked at how inactivation was influenced when system operating conditions (i.e. incubation time) and hydraulics (i.e. flow rate and hydraulic loading rate (HLR) of the model systems were varied. For each configuration at least three experimental replications were performed. Initial incubation times were based on the findings reported by Hawley (2012).

2.3 Field Based Methods

2.3.1 Large Scale HRAP + IP System

An IP was introduced to the HRAP at KoM (Chapter 6). The embankment wall surrounding the pond was used as the slope. The pond wall was ideal as it already existed; formed with construction, and was pre-lined with HDPE plastic pond lining This simplified both the design and manufacture of the IP, meaning only the pipe work had to be retrofitted. This ensured construction costs were kept to a minimum.

The IP was located along one side of the pond wall and situated downstream of the paddlewheel (Plate 2.8). IP position was chosen based on 1) system accessibility and 2) availability to direct sunlight. Water was distributed through 25 mm diameter pipe with 3 mm diameter holes (Plate 2.9a). Later 5 mm diameter holes were used. Black PVC (polyvinyl chloride) flex tubing (25 mm) was used for all pipe work (Plate 2.9b) and like the model HRAPs, valve systems were incorporated to divert and throttle flow (Plate 2.9c). Figures 2.3 and 2.4 present a line diagram of the KoM IP and its functioning.

The IP was operated at two surface areas; 20 m^2 (4 m x 5 m) (Plate 2.10a and Figure 2.3) and 36 m^2 (4 m x 9 m) (Plate 2.10b and Figure 2.4). For the larger surface area; modification to the design was required. This modification is also outlined in Figure 2.4. Two submersible pumps were used: An Aqua Pro™ 7500 multi-use pump (Plate 2.11a) and a submersible pump (Plate 2.11b). Both pumps were required to operate the longer IP length; one attached at either end of the IP. This enabled a sufficient supply of water throughout the length of the conduit. Additional pump details for the Aqua Pro™ 7500 multi-use pump are outlined in Appendix 2.

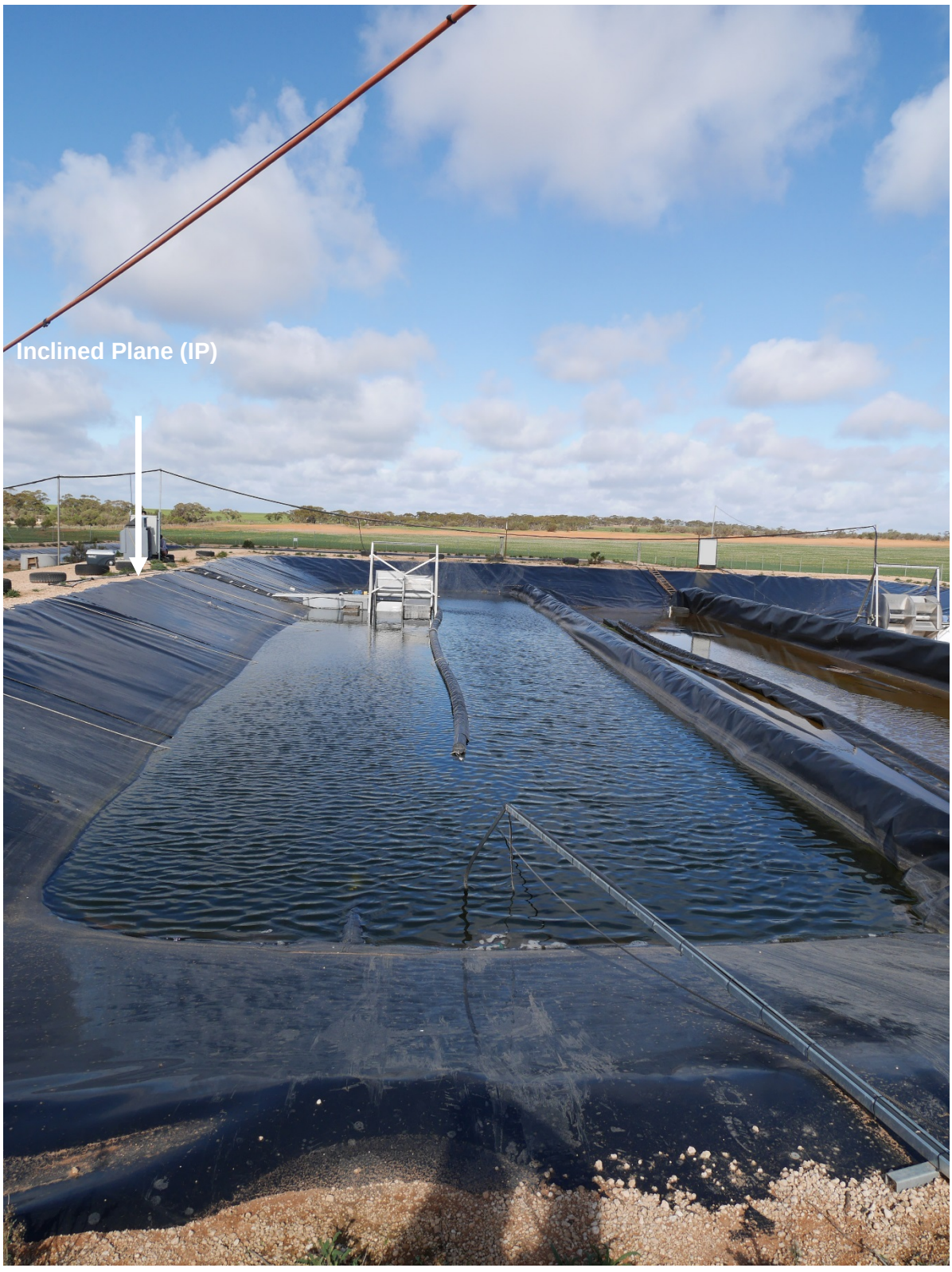


Plate 2.8: The large scale inclined plane (IP) at Kingston on Murray (KoM) HRAP.

Plate 2.9: Components of the large scale IP system attached to the KoM pond wall. System was comprised of a) 25 mm grey electrical conduit, b) 25 mm black PVC flex tubing and c) ball-valves for flow throttling.

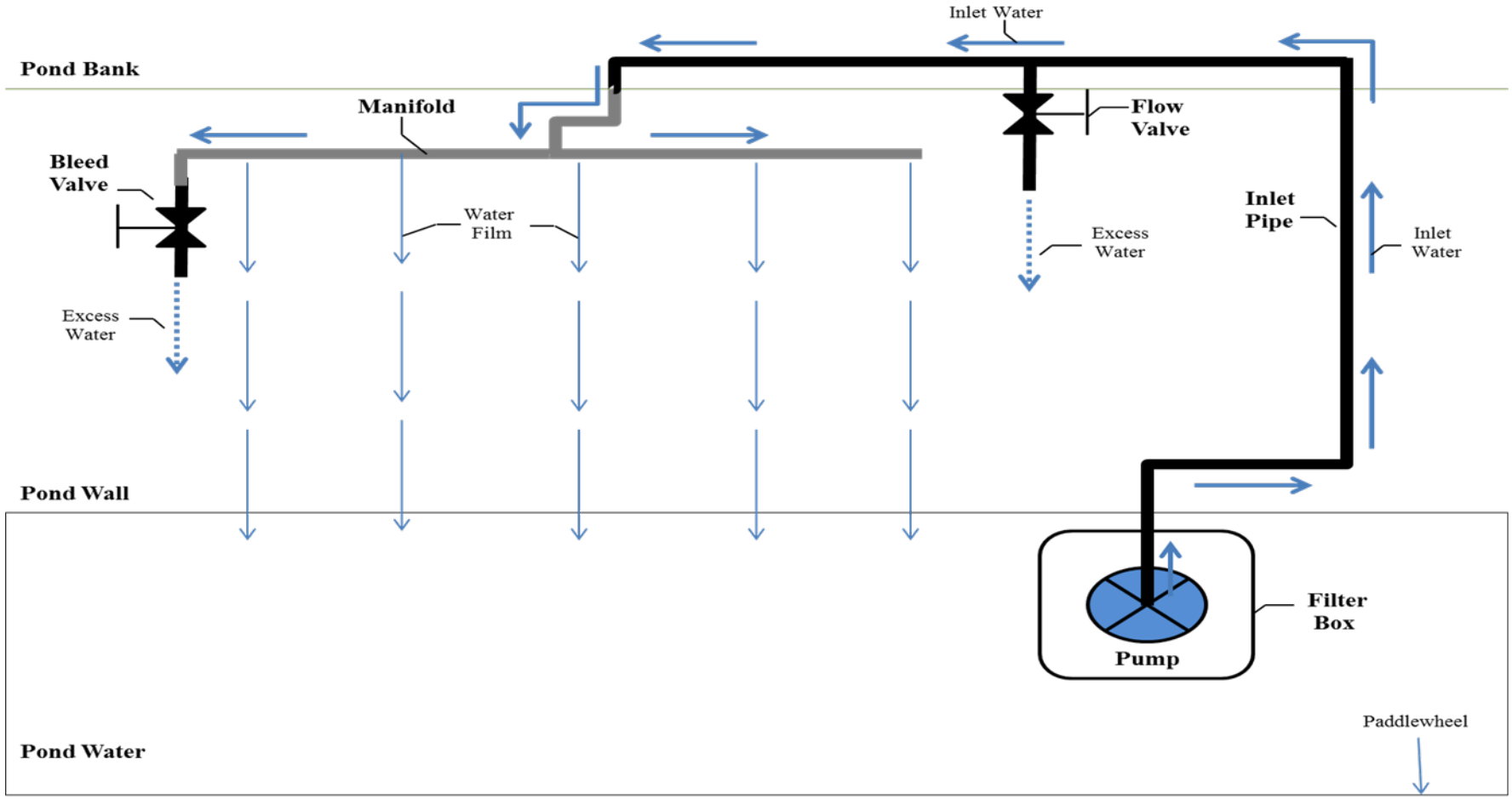


Figure 2.3: Line diagram of large scale IP operation. Water from the pond is pumped via an inlet pipe to the top of the embankment (pond wall). Water was then distributed through the manifold and returned to the pond down the wall. Flow and bleed valves were incorporated for flow throttling. Pump attached to IP was stored in a semi-submerged filter box. Filter box prevented large particles entering the system. Diagram is a representation of the 20 m^2 (5 m) IP.

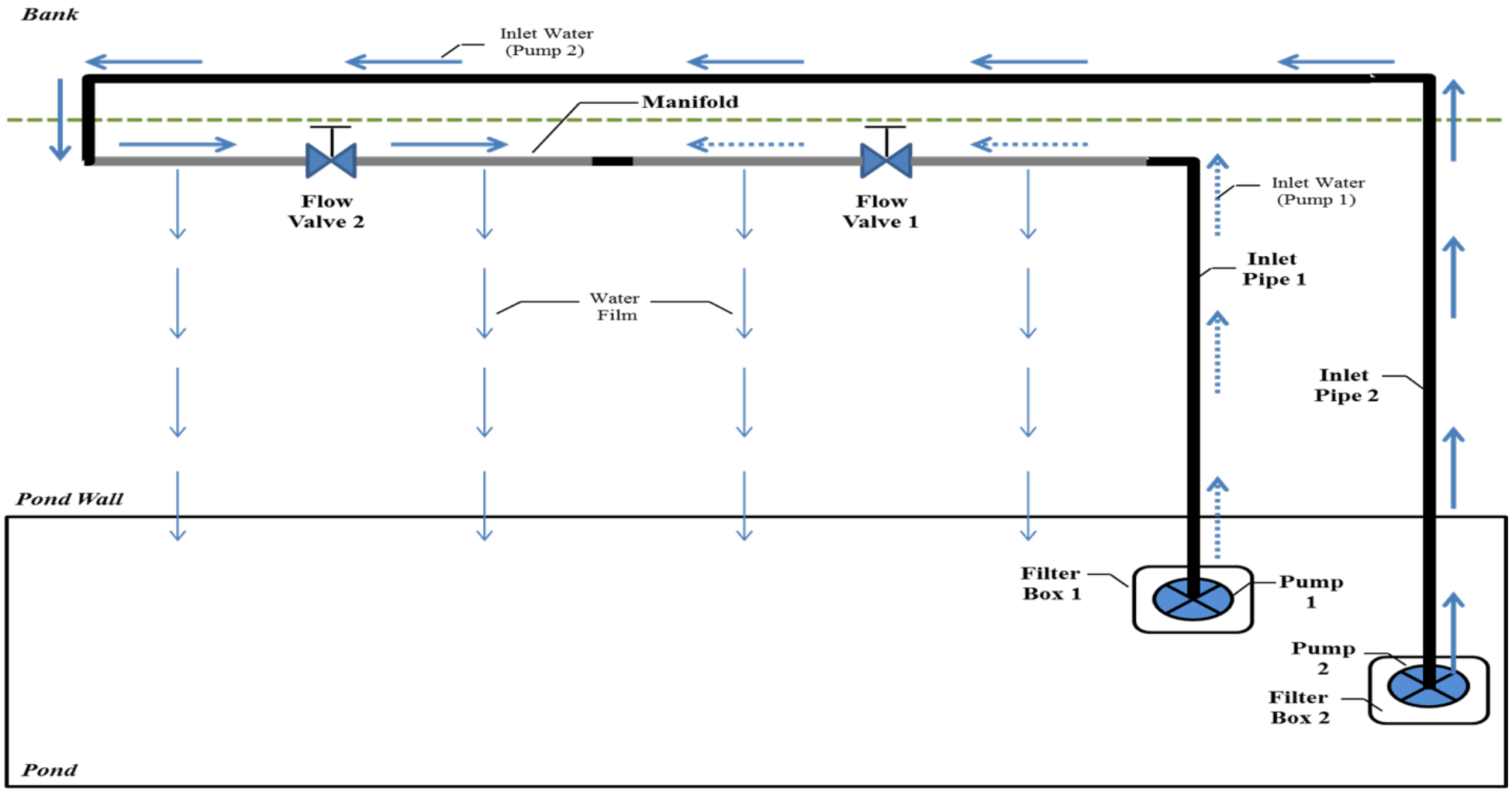


Figure 2.4: Line diagram of modified large scale IP. Pumps were connected to either end of the manifold with the water pumped to top of the pond wall via separate inlet pipes. Strongest flowing pump was connected to the furthest end of the manifold (i.e. inlet pipe 2). Flow valves provided partitioning of the flow. Diagram is a representation of the 36 m^2 (9 m) IP

Plate 2.10: The two IP surface areas and lengths used at KoM; a) 5 m (20 m^2) and b) 9 m (36 m^2).

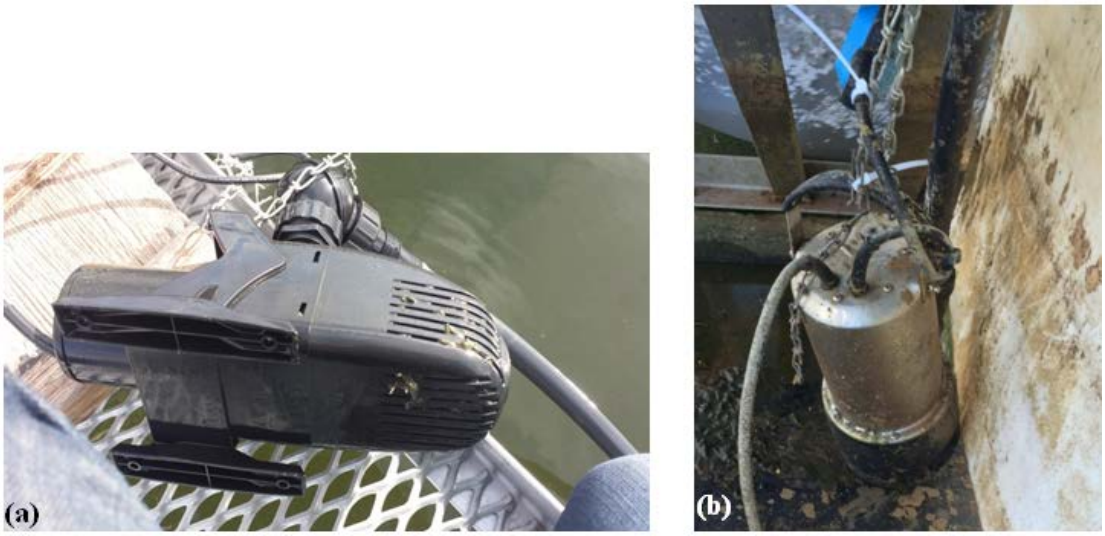


Plate 2.11: Submersible pumps used for IP operation at Kingston on Murray (KoM): a) Aqua Pro™ AP7500 multi-use pump and b) unspecified submersible pump

2.3.2 Water source and sampling

Like Mt Barker WWTP, the KoM HRAP is operated as a CWMS (refer to Chapter 6 for details). Septic tank effluents were pumped daily into the pond for treatment. The treated effluent was used for analysis of the field based system.

800 mL composite samples were collected at KoM using an auto sampler (Avalanche® Sampler, Teledyne ISCO Lincoln, NE). Composite samples were comprised of 2 x 400 mL aliquots collected at both 03:00 and 15:00. Samples remained onsite in the auto-sampler (~1°C) until they could be retrieved after 14 days. Collected samples were transported back to the laboratory within a sealed esky and chilled at 4°C until analysis. Analysis was undertaken within 24-48 h of retrieval. Figure 2.5 identifies the location of the KoM HRAP in relation to the university (Flinders University, South Australia) and the Mt Barker WWTP.

Figure 2.5: Map identifying wastewater (green) and tap water (red) sampling sites. Places included Mt Barker WWTP, KoM HRAP and Flinders University. Map is available from; http://umap.openstreetmap.fr/en/map/anonymous-edit/94168%3A1eYHCL8QbheWpvgUpPWh7PsiEKg.

2.3.3 In situ water monitoring in the field

In situ water temperature was logged onsite using T-Tec 6-3F: Temperature data loggers with duel temperature sensors (Temperature Technology). Weather and climate data at KoM were obtained from two BOM weather stations in the South Australian Riverland. Two stations were required due to the remoteness and monitoring capabilities of the weather stations. Table 2.1 identifies the stations used and the parameters measured at those stations. Location of each weather station is also outlined in Table 2.1.

Table 2.1: Weather stations used to monitor climate conditions at the Kingston on Murray (KoM) high rate algal pond (HRAP). Table includes station number, distance from the HRAP site and parameters measured at station. Weather and climate data included majority of measurable parameters. Data was obtained from the Australian Bureau of Meteorology (BOM) website (www.bom.gov.au/).

Station	Station Number	Coordinates	Distance from HRAP	Elevation	Parameters monitored
KoM	24006	34.22°S, 140.34°E	2.4 km	40 m	Solar Exposure Rainfall
Renmark	24048	34.20°S, 140.68°E	32.1 km	32 m	Weather and Climate Temperature

2.4 IP Operating Characteristics

Flow rates (Q), hydraulic loading rates (HLR) and cycle rates were characterised and calibrated for all IPs used. Q was calculated by quantifying the measured volume of water delivered by the pump to (large scale IP) or down the IP (model IP) during a specific time period. Rates were then calculated using Equation 2.1 and reported as litre per hour (L h^{-1}).

$$Flow\ Rate\ (Q)\ = \frac{V}{t}$$

Equation 2.1

Where

Q = flow rate (L h^{-1})

V = Volume (L) down inclined plane for time (t, h)

Hydraulic loading rates (HLR) for the IPs were calculated using Equation 2.2 and reported as litres per hour per square metres (L m^{-2} h^{-1}).

$$HLR = \frac{Q}{A}$$

Equation 2.2

Where

HLR = hydraulic loading rate (L h^{-1} m^{-2})

Q = flow rate (L h^{-1})

A = Surface area of IP (m^2)

The number of times the whole pond volume passed over the IP (i.e. cycle rate) during a specific time (t) was calculated from Equation 2.3. Units were reported as cycles per hour (cycles h^{-1}) or cycles per day (cycles d^{-1})

$$number\ of\ cycles, C = \frac{t}{\left(\frac{V}{Q}\right)}$$

Equation 2.3

Where

C = number of times passed over IP for time t (cycles h^1, cycles d^{-1})

t = time (h, d)

Q = flow rate down the IP (L h^{-1})

V = pond volume (L)

2.5 Stock Preparation, Quantification and Inactivation of MS2

Microbial analysis was carried out using both native F-RNA bacteriophage from the KoM wastewater and laboratory grown F-RNA bacteriophage; MS2. The laboratory grown MS2 was then spiked into the collected Mt Barker wastewater. Spiking was necessary due to the low numbers of native MS2 detected in wastewater following treatment at Mt Barker.

2.5.1 MS2 Stock Preparation

MS2 (ATCC # 15597-B1) stock was prepared through phage extraction. 9 mL half strength (0.5%) tryptone water (Oxoid) was added to a pre-existing plate with confluent plaque growth (at least 10^4 plaques). The plate and tryptone water were then incubated at 37°C for 45 minutes. During incubation the plate was gently agitated in 15 minute intervals, this ensured the plate was thoroughly washed. Phage-extracted tryptone water was syringed off the plate and filtered through a sterile 0.22 µm filter (Whatman, UK) into a 10% glycerol 90% (0.5%) tryptone water solution. The solution was then dispensed into 10 mL sterile tubes and swirled continuously, keeping the phage in suspension. Stock tubes were then frozen (~-20°C) until required. Concentration of MS2 stock used was 10^9 phage 100 mL^{-1}.

2.6 Microbial Analysis

2.6.1 Phage quantification

A double layer agar plaque assay adapted from Debartolomeis and Cabelli (1991) and Noble *et al.* (2004) was used to quantify the presence of phage within the systems. An antibiotic stock solution was prepared for microbial quantification. 0.30 g each of ampicillin sodium salt (Sigma) and streptomycin sulphate (Sigma) was dissolved in 200 mL reverse osmosis (RO) water and filtered through a sterile 0.22 mm filter (Whatman, UK). Filtered stock was dispensed into 10 mL sterile tubes and frozen (~-20°C). Antibiotics were defrosted when required and kept at 4°C for up to two weeks.

Water samples were serially diluted (ten-fold, 10X) in 9 mL sterile 0.5% tryptone water (Oxoid) as required plated and in triplicate.

For quantification *Escherichia coli* Famp (ATTC # 700891) was used as the bacterial host and was grown overnight (18-24 h) in 10 mL tryptone soya broth (TSB, Oxoid) + 1 mL ampicillin-streptomycin antibiotic stock. 5 mL neat (undiluted) or serially diluted water samples were dispensed into 10 mL sterile tubes. 5 mL of 100 mL molten tryptone soya agar (1.5% TSA: TSB + technical agar #3 (Oxoid)) + 10 mL *E. coli* host + 1 mL antibiotic stock was then added (aseptically) to each sample and mixed by inversion. Sample and agar mix was then poured onto a base agar

layer of 1.5% TSA + antibiotic stock. Plates were gently swirled and allowed to set for 10 minutes before being inverted and incubated at 37°C overnight (18-24 h). Plaques were then enumerated and reported as plaque forming units per 100 mL (PFU 100 mL^{-1}).

The quantification assay used was capable of detecting F-Specific coliphage, but could not select for F-RNA phage or MS2 specifically (Jebri *et al.*, 2017). Consequently, detected phage were reported as either MS2 in the optically clear water samples or as F-Specific phage in the wastewater where the presence of native phage was possible.

2.7 Wastewater quality analysis

American Public Health Association; (APHA, 1992) standard wastewater methods, outlined below were used to analyse the quality of collected wastewater samples. All analyses were performed in the Environmental Health laboratories, Flinders University.

2.7.1 Biochemical oxygen demand (BOD_5)

5 day biochemical oxygen demand (BOD_5) was determined using the OxiTop respirometric BOD method according to the manufacturer's instructions.

2.7.2 Chlorophyll *a* (Chl *a*)

Chlorophyll *a* concentrations were determined as a surrogate measure of algal biomass and were performed using Test 10200 (Chlorophyll trichromatic method) of the Standard methods for the examination of water and wastewater (Greenberg *et al.*, 1992). Duplicate 25 mL aliquots of wastewater samples were filtered through 47 mm glass microfiber filter papers (Labserv Filtration, LBS0GF 0.47). Filter papers were added to 10 mL McCartney bottles and fully submerged in 90% acetone-water solution. Bottles were refrigerated at 4°C for 24 h. 1.5 mL of acetone extract was removed and transferred into 1.5 mL Eppendorf tubes. Tubes were centrifuged for 10 minutes at 10,000 g. After centrifuging the supernatant was extracted and transferred into a glass micro-cuvette and absorbance was read. Absorbance was measured at three different wavelengths; 664.0 nm, 647.0 nm and 630.0 nm, using a Shimadzu UV spectrophotometer (UV-1800,

Shimadzu). 90% acetone-water solution used as the blank. Triplicate readings were taken for each sample at each wavelength. Chl *a* concentrations was determined from the mean absorbance using Equation 2.4 and Equation 2.5. Chl *a* concentrations were reported in micro grams per litre (µg L^{-1}).

$$Chl\ a\ absorbance = 11.85(OD_{664}) - 1.54(OD_{647}) - 0.08(OD_{630})$$

Equation 2.4

Where

OD_{664} = absorbance at 664 nm

OD_{647} = absorbance at 647 nm

OD_{630} = absorbance at 630 nm

$$Chl\ a\ (\mu g\ L^{-1}) = Chl\ a\ absorbance \times \left(\frac{V_{acetone}}{V_{sample}}\right)$$

Equation 2.5

Where

$V_{acetone}$ = volume of acetone (mL)

V_{sample} = volume of sample (L)

2.7.3 Nutrient concentrations

Wastewaters were analysed for total carbon (TC), inorganic carbon (IC), total organic carbon (TOC), and total nitrogen (TN) using a total organic carbon and nitrogen analyser (TOC-LCSH TNM-L analyser, Shimadzu).

2.7.4 Suspended solids (SS)

250 mL aliquot of wastewater sample was filtered through a pre-weighed and dried (105°C overnight) 90 mm glass fibre filter paper (Labserv Filtration, LBS0GF 0.90). Filter papers were then re-dried (105°C overnight) and re-weighed. Suspended solid concentrations (SS) were determined from the increase in initial paper weight (Equation 2.6).

$$SS\ (mg\ L^{-1}) = W_{final} - W_{initial} \times \left(\frac{1000}{V}\right)$$

Equation 2.6

Where

W_{final} = final weight (mg) of filter paper and residue

$W_{initial}$ = initial weight (mg) of filter paper

V = filtered volume of sample (L)

2.7.5 Turbidity

Turbidity was measured in the laboratory using a HACH DR/2000 direct reading spectrophotometer (HACH Company, Colorado, USA). Absorbance was read at 450 nm and turbidity reported in Nephelometric turbidity units (NTU).

2.8 Statistical Analysis

Enumerated F-Specific phage counts were log transformed and the corresponding log reduction values (LRV) were calculated (Equation 2.7). For the model HRAPs, inactivation rates were calculated from the slope of the linear regression of a semi-log plot of log reduction (log N_0-log N_t) against time exposed (h), with non-linear curves modelled using GInaFiT (Geeraerd and Van Impe Inactivation Model Fitting Tool); a Microsoft Excel add-in (Geeraerd *et al.*, 2005) (refer to 1.4.1). Inactivation rates (using the above method) were not calculated in the full scale system.

$$\text{Log Reduction} = \log_{10} N_0 - \log_{10} N_t$$

Equation 2.7

Where

N_0 = initial phage concentration

N_t = phage concentration at time t

Table 2.2 presents the models used to determine inactivation rates in GInaFiT. Examples of the corresponding die-off curves are shown in Chapter 1.4.1. K_D and K_L were used to report inactivation rates in the model systems under dark (K_D) or light (K_L) experimental conditions and were transposed from the resulting K_{max} values obtained from the linear part of the curve produced using the models in Table 2.2.

Table 2.2: Log linear inactivation models used in GInaFiT to determine non-linear inactivation rates.

	Inactivation Model	Reference
Log_{10} Linear	$N = N_0 \times \exp(-K_{max} \times t)$	(Bigelow and Esty, 1920)
Log_{10} Linear + Tail	$N = (N_0 - N_{res}) \times \exp(-K_{max} \times t) + N_{res}$	(Geeraerd *et al.*, 2000)
Log_{10} Linear + Shoulder	$N = \frac{N_0 \times \exp(-K_{max} \times t) \times \left(exp(K_{max} \times SL)\right)}{\left(1 + \left(exp(K_{max} \times SL) - 1 \times exp(-K_{max} \times t)\right)\right)}$	(Geeraerd *et al.*, 2000)
Log_{10} Linear + Shoulder + Tail	$\frac{(\mathrm{N_0} - \mathrm{N_{res}}) \times \exp(-\mathrm{K_{max}} \times \mathrm{t}) \times \left(\left(\exp(-\mathrm{K_{max}} \times \mathrm{SL})\right)\right)}{\left(1 + (\exp(\mathrm{K_{max}} \times \mathrm{SL}) - 1) \times \exp(-\mathrm{K_{max}} \times \mathrm{t})\right) + \mathrm{N_{res}}}$	(Geeraerd *et al.*, 2000)

Statistical analyses were carried out using statistical programs; R (R Core Team, 2014), SPSS (IBM Corp., 2011) and GInaFiT (Geeraerd *et al.*, 2005). Analyses undertaken included; data variability and normal distribution using histograms and quantile comparison (Q-Q plots), linear and non-linear regression, one-way analysis of variance (ANOVA) and analysis of covariance (ANCOVA) with Tukey HSD post hoc comparison and independent samples t-test. When appropriate, repeated measures (RM) ANOVAs were used. Statistical significance was considered

at 95% (P-value ≤0.05) and results were reported as mean ± standard error (SE).. Effect sizes were reported as 95% confidence intervals and when possible Cohen's *d* (t-tests; or partial Eta squared (η^2; ANOVA and ANCOVA) was provided. Equations 2.8 and 2.9 were used to calculate these effects sizes, respectively.

$$Cohen's\ d = \frac{\overline{X_1} - \overline{X_2}}{SD_{Pooled}},$$

$$where;\ \ SD_{Pooled} = \sqrt{\frac{(n_1 - 1){S_1}^2 + (n_2 - 1){S_2}^2}{n_1 + n_2 - 2}}$$

Equation 2.8

$$Partial\ ETA\ squared\ \eta^2 = \frac{SS_{effect}}{SS_{effect} + SS_{error}}$$

Equation 2.9

Relationships between LRVs and other variables (i.e. environmental parameters) were determined using linear regression and Pearson's correlation coefficient. A correlation matrix was performed. Strength of the correlation (r) was determined as outlined below.

- Strong correlation if r is between 0.85 and 1.00
- Moderate correlation if r is between 0.50 and 0.85
- Weak correlation if r is between 0.10 and 0.50
- No correlation if r is less than 0.10

3. EXAMINATION OF MICROBIAL REMOVAL EFFICIENCY OF AN INCLINED PLANE IN OPTICALLY CLEAR WATER

3.1 Introduction

Sunlight, crucial to microbial inactivation, is highly susceptible to decay in water (Kirk, 1994). This decay occurs through the rapid absorption and attenuation of light photons within the water column (Huovinen *et al.*, 2003; Caslake *et al.*, 2004; Paterson and Curtis, 2005). This loss is aided by the shallow penetration depths of UV and Vis light (Huovinen *et al.*, 2003; Caslake *et al.*, 2004; Paterson and Curtis, 2005). Disinfection performance; i.e. microbial reduction, within pond systems is therefore impaired.

Incorporation of an inclined plane (IP) to natural wastewater treatment ponds was proposed to improve microbial inactivation rates in HRAPs. The IP, as outlined in Chapter 2, provides a large surface area for which the water can be exposed in a thin film. This will assist in maximising light penetration without compromising other processes within the pond (Tredici, 2004),

However, before the IP can be successfully implemented within the field, an initial examination had to be undertaken to ensure effectiveness and derivation of optimal operating conditions. It was therefore essential that the system be first examined on a smaller, more controllable scale. Thus model HRAPs with and without an IP were employed.

Use of model HRAPs with IPs has only been examined once before by Hawley (2012), as part of an honours project. This study revealed that improved pathogen removal could be achieved under both overcast and sunny conditions from the inclusion of an IP. However, the author identified the need for additional and more in depth research before the concept could be considered plausible. This chapter therefore aims to expand from this previous study and provide relevant information that can enable the concept to be applied to real pond conditions.

Removal of MS2 and other F-RNA phage have been reported in both dark and illuminated waters; including clear water (i.e. drinking, tap, PBS and RO waters) (Governal and Gerba, 1997; Love *et al.*, 2010; Fisher *et al.*, 2011), seawater (Fujioka *et al.*, 1981; Love *et al.*, 2010), river water (Sinton

et al., 2002) and wastewater (Benchokroun *et al.*, 2003). As wastewater contains light attenuating properties, it was imperative that a more idealistic water type be chosen for this initial examination. Optically clear water (i.e. tap water) was considered ideal for its elevated light penetration properties (Paterson and Curtis, 2005). This water enabled easier characterisation of the IPs' removal abilities as it was not impaired by attenuation. Operating the HRAPs with only the IP solar exposed, whilst the pond surface is covered, will also aid in determining what proportion of inactivation can be attributed to the IP.

Specifically the aims for the current study were:

- To establish proof of concept of the proposed IP system and identify if its use is a viable addition to natural treatment ponds.
- To assess the removal capacity of the IP when operated under ideal disinfection conditions and how modifying the IP impacts removal capacity.
- To determine whether the IP inclusion has an impact on the characteristics of the water; i.e. pH, DO and temperature.

3.2 Methods

3.2.1 Experimental set-up and operational configurations

Set up of systems occurred the day prior to experimentation. Model HRAPs were filled with tap water (86.9 L) to a depth of 30 cm. Systems were covered on the first day of sampling using the box lids to prevent solar exposure. This allowed for any residual chlorine remaining in the water to be dissipated overnight. The investigation was carried out in two stages and thus two operational configurations were used. The configurations included;

1. $HRAP_D$+ SIP vs. $HRAP_D$ (Plate 3.1) and
2. $HRAP_D$vs. $HRAP_D$+ SIP and $HRAP_D$+ LIP (Plate 3.2).

Where, $HRAP_D$ indicates dark incubation with only the IP light exposed and SIP and LIP refer to the short and long IPs respectively. Operating parameters for each configuration are outlined in Tables 3.1 and 3.2 respectively. Eight experimental replicates were performed for configuration 1 and five for configuration 2. System calibration was carried out and flow rates were determined prior to set-up as described in Chapter 2.

Plate 3.1: Model HRAP experimental set-up Configuration (1) HRAP$_D$ + Short IP (SIP) vs. HRAP$_D$. Ponds were operated with optically clear (tap) water under dark incubation.

Table 3.1: Operating conditions for Configuration 1: HRAP$_D$ + SIP vs. HRAP$_D$

Operation	Pond Volume (L)	Pond Depth (m)	IP Length (m)	IP Surface Area (m^2)	Flow Rate ($L\ h^{-1}$)	Hours Run (h)
HRAP$_D$	86.9	0.30	-	-	-	24
HRAP$_D$ + SIP	86.9	0.30	0.55	0.37	175.5	24

Plate 3.2: Model HRAP experimental configuration 2: HRAP$_D$ vs. HRAP$_D$ + Short IP (SIP) (0.55 m) and HRAP$_D$ + Long IP (LIP) (1.10 m).

Table 3.2: Operating conditions for Configuration 2: HRAP$_D$ vs. HRAP$_D$ + SIP and HRAP$_D$ + LIP

Operation	Pond Volume (L)	Pond Depth (m)	IP Length (m)	IP Surface Area (m^2)	Flow Rate[a] ($L\ h^{-1}$)	Hours Run (h)
HRAP	86.9	0.30	-	-	-	24
HRAP$_D$ + SIP	86.9	0.30	0.55	0.37	129.6±5.09	24
HRAP$_D$ + LIP	86.9	0.30	1.10	0.74	131.1±1.27	24

[a] flow rates are reported as mean±SD

3.2.2 Experimental Design

Inactivation experiments were incubated for 24 h for both experimental configurations. Incubation time chosen was derived from Hawley (2012).

3.2.3 Sampling and System inoculation

1 mL MS2 stock (~ 10^9 PFU mL^{-1}) was inoculated into systems 5-10 minutes prior to the first sample collection (T_0). Systems were then mixed. Collection of samples were performed as described in Chapter 2, with triplicate 10 mL samples collected in 1.5 h intervals over a 24 h period, beginning at 9:00 am. Systems were operated continuously throughout the night. No sampling was conducted between 6:00 pm and 9:00 am. For each system, the initial (starting) MS2 concentration (N_0) was determined after inoculation at T_0.

3.2.4 Environmental conditions

In situ water measurements were recorded for water temperature, pH and DO. Weather conditions were also monitored onsite with UVA and UVB recorded whenever possible during daylight hours. Additional conditions were also obtained from the Australian BOM (Adelaide Airport, Station Number: 23034) (Chapter 2). These included; hours of sunshine (h), daily solar exposure (MJ m^{-2}), minimum and maximum ambient temperature (°C), and rainfall (mm). Sunshine hours as measured by the Australian BOM, is the duration of bright sunshine (lower than visible) recorded between midnight to midnight.

3.2.5 Statistical Analysis

Analysis was carried out on all collected data. Semi-log plots were used to illustrate $\log_{10}$ removal ($\log_{10} N_0 - \log_{10} N_t$) against time exposed. Log removal values (LRV) were reported as LRV_t, where t is the number of hours exposed. Inactivation curves were plotted of $\log_{10}$ MS2 against time exposed using GInaFiT (Geeraerd *et al.*, 2005). Inactivation rates (K_D) were determined from the corresponding K_{max} values derived from the linear part of the resultant curve with K_D representing inactivation under dark incubation. To analyse significant differences between removal with and without the IP an independent sample t-test was performed. Similarly a one-way ANOVA was

carried out to distinguish significance between the two IP lengths and the $HRAP_D$ control. A covariate analysis (ANCOVA) was also performed to detect differences between the models when the variation in different environmental conditions (i.e. solar exposure and water temperature) was controlled for. More specifically, how the IP influenced inactivation when the effects of these covariates were removed**.** Statistical significance was accepted at $p<0.05$. Normality testing was also performed to establish whether the obtained data was normally distributed (Shapiro-Wilks test for normality and Q-Q plots). To test for relationships between variables, linear regression and correlation (Pearson) was performed.

3.3 Results

3.3.1 MS2 inactivation: $HRAP_D$+ SIP vs. $HRAP_D$

Characterisation of the IP in Configuration 1: $HRAP_D$ + SIP vs. $HRAP_D$, was performed. Estimated hydraulic flow rate (Q, L h^{-1}) and cyclic rates (cycles h^{-1}) for the $HRAP_D$ + SIP are reported in Table 3.3. After operating the systems for an hour, the whole pond volume was estimated to pass down the slope approximately 2.02 times and 48.5 times a day (24 h). This equated to approximately 4214.65 L of water down the slope in a day.

Changes in pond volume in the model HRAPs after 24 h was calculated and the results presented in Appendix 3. The water loss observed in the $HRAP_D$ + SIP was minimal approximately 1.00±0.38 L. Loss observed in the $HRAP_D$ was believed to be a result of sampling loss.

Table 3.3: Characterisation of the $HRAP_D$ + SIP system in tap water. Included is the mean hydraulic flow rate and cycle rate; the number of times the whole pond volume passed over the IP in the 24h incubation period.

	IP Length (m)	IP Area (m^2)	Pond Volume (L)	Flow Rate (L h^{-1})	HLR (L m^{-2} h^{-1})	Cyclic Rate	
						Per hour (cycles h^{-1})	Per day (cycles 24 h^{-1})
$HRAP_D$ + SIP	0.55	0.37	86.9	175.5	474.3	2.02	48.5

A decline in the number of MS2 phage remaining in the $HRAP_D$ + SIP and $HRAP_D$ systems was evident after 24 h. A semi-log plot of mean MS2 log_{10} removal against time exposed illustrates the reduction in both systems (Figure 3.1).

The mean log_{10} removal was derived from eight experimental runs conducted throughout autumn and winter 2014. The highest removal was observed in the $HRAP_D$ + SIP, with a final log_{10} reduction value (LRV_{24}) of 4.54; 1.54 log_{10} higher than obtained in the corresponding control pond $HRAP_D$ (LRV_{24} 3.00). Hourly removal rates were determined as 0.11 log_{10} h^{-1} and 0.07 log_{10} h^{-1} for the $HRAP_D$ + SIP and $HRAP_D$ respectively. An independent samples t-test (Welch); identified the difference in mean LRV between the two systems over 24 h to be significant ($p<0.05$, actual $p=0.015$; 95%CI: 0.23 to 1.79, d = 2.01)

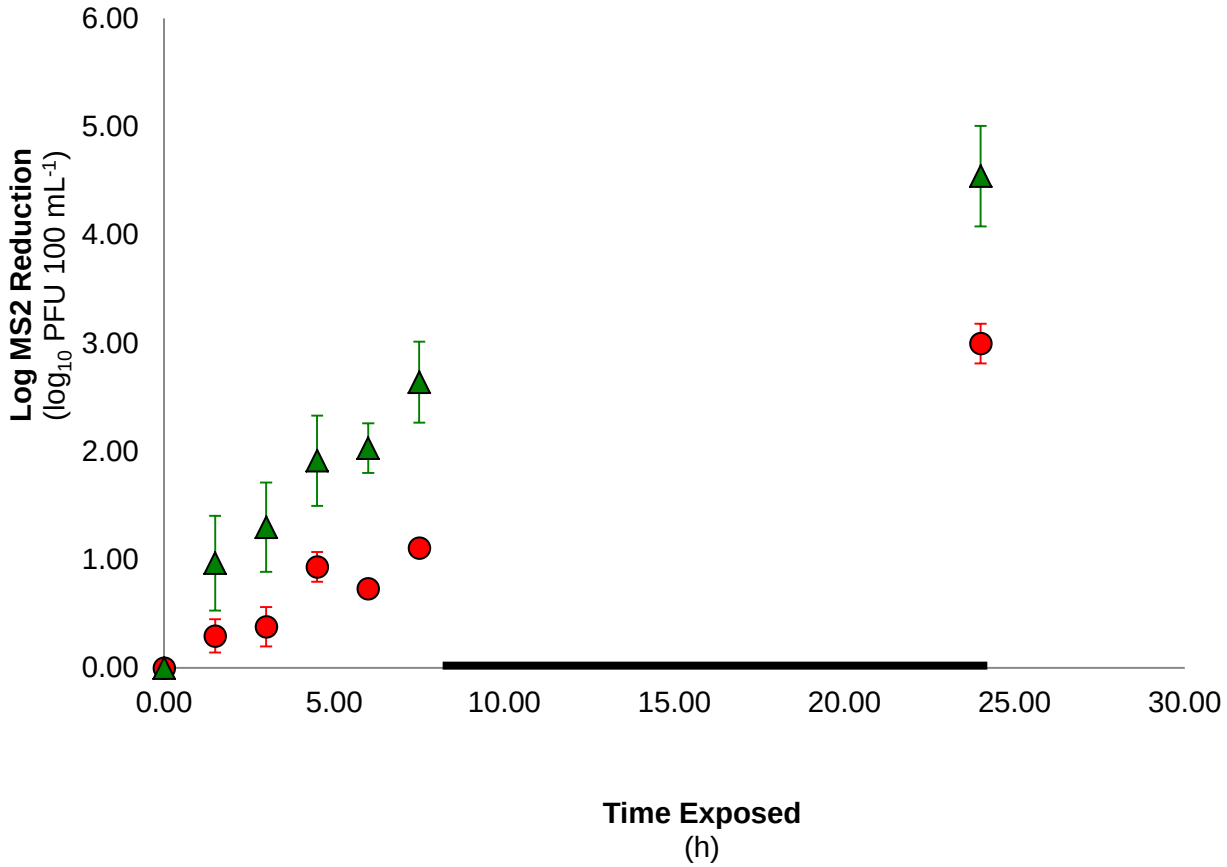


Figure 3.1: Semi-log plot of mean log_{10} MS2 removal (LRV, N_0-N_t) against time exposed (h) in tap water; for the Configuration 1: $HRAP_D$ + SIP (▲) and $HRAP_D$ control (●). Overnight incubation period is represented by (▪). Plot is comprised of the mean log_{10} MS2 removal obtained from eight experiments conducted throughout autumn-winter 2014. Solar exposure ranged from 4.8-26.2 MJ m^{-2}. Standard error of mean bars has been included.

Inactivation rates were determined from the slope of a semi-log inactivation curve generated in GInaFiT (log_{10} MS2 vs. time exposed). Inactivation was shown to deviate from linearity for both systems. A log-linear plus tail curve was then identified the best fit and was applied to the data (Figure 3.2). Log-linear plus tail model described by Geeraerd *et al.* (2000) was used to generate inactivation curve and determine inactivation rates (Equation 3.1).

$$N = (N_0 - N_{res}) \times \exp(-K_{max} \times t) + N_{res}$$

Equation 3.1

Table 3.4 presents the mean MS2 LRV and inactivation rates (K_D) obtained by the two HRAP systems after 24 h. To best represent the removal captured within the linear part of the die-off

curve, mean LRV over 7.5 h was calculated. 7.5 h was chosen as it was the last time period where a sample had been collected within the linear part of the decay curve shown in Figure 3.2. These removals are also reported in Table 3.4. To keep consistency, $LRV_{7.5}$ will be reported for the 24 h incubation periods throughout the subsequent chapters. Statistical comparison of the $LRV_{7.5}$ values is reported in Appendix 4.1.

An ANOVA was performed on the resulting K_D values, with Tukey's post hoc comparison indicating the difference in inactivation rates between the model systems was also significant ($p<0.05$, actual $p=0.008$); 95% CI: 0.20 to 1.05, d = 1.90) (Appendix 4.1).

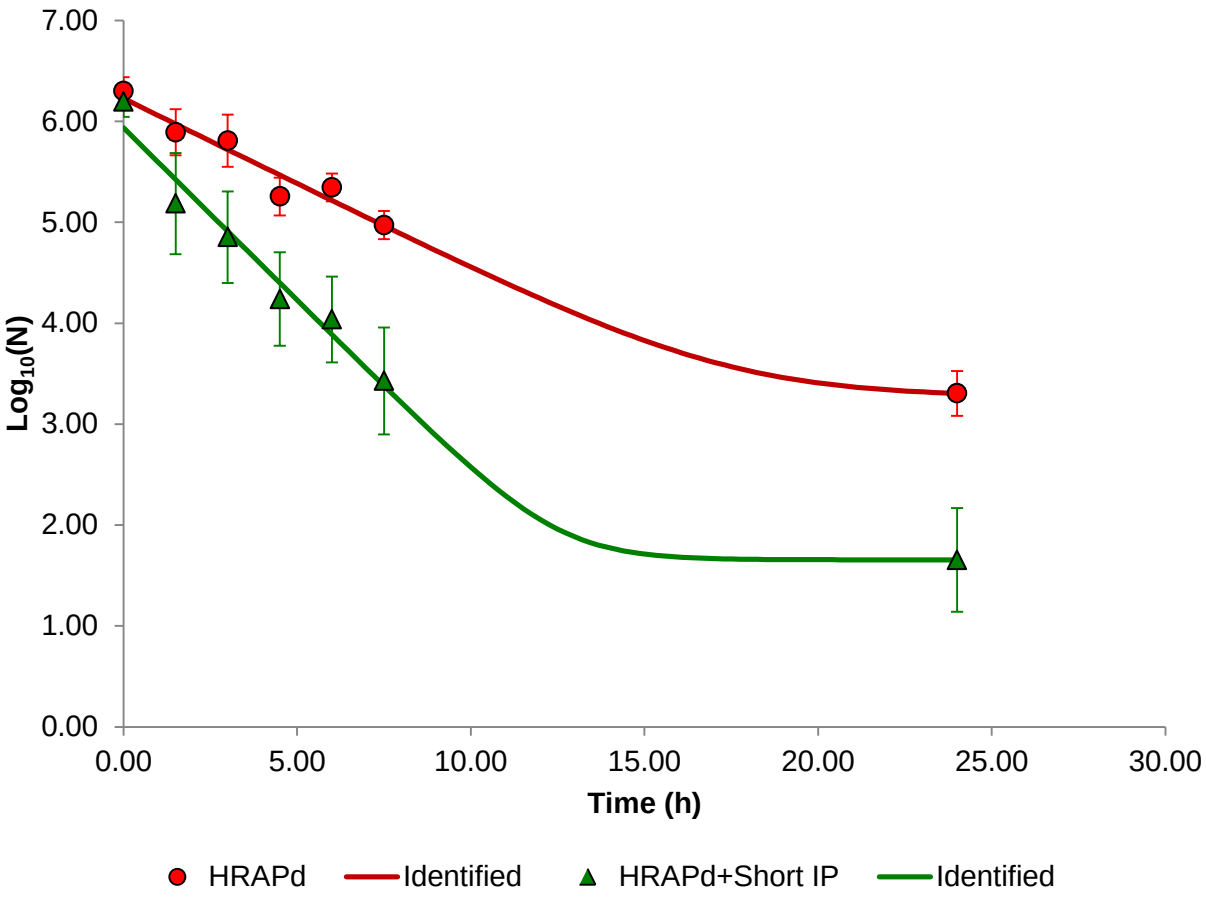


Figure 3.2: Example of a log-linear inactivation curve with tail for log_{10} MS2 (mean ± standard error, SE) remaining in the system after 24 h exposure. Curves were generated from the log mean MS2 detected in the model HRAPs over 8 experiments. Corresponding K_D values were $HRAP_D$ + SIP: 0.79±0.08, R^2 = 0.986 and $HRAP_D$: 0.39±0.03, R^2 = 0.986

Table 3.4: MS2 Inactivation rates (K_D, log_{10} MS2 h^{-1}) and log removal values; LRV (log_{10} PFU 100 mL^{-1}) in model HRAP systems after 24 h in tap water. Log-linear plus tail inactivation curve was used to model remaining MS2 in each system and was derived using GInaFiT (Geeraerd *et al.*, 2005). R^2 is an indication of goodness of fit for the log_{10} + tail model.

Model HRAP	Log Removal Rates (LRV)			Inactivation Rates (K_D)	
	n	$LRV_{7.5}$ ± SE	LRV_{24} ± SE	n	K_D ± SE
$HRAP_D$ +SIP	5	2.64±0.37	4.54±0.47	6	0.95±0.19
$HRAP_D$	5	1.11±0.03	3.00±0.18	6	0.35±0.03

Counts below the limit of detection (LOD; 20 plaques 100 mL^{-1}) were observed in both systems and always detected in the $HRAP_D$ + SIP system first. These values were omitted and treated as zeros and were shown to be in the tail of the log-linear curve.

In situ water parameters were monitored during each collection interval. These results are presented in Table 3.5. Overall, DO and water temperature was found to be 0.38 mg L^{-1} and 0.43°C lower in the $HRAP_D$ + SIP than the $HRAP_D$, respectively. pH, on the other hand was found to be 0.03 units higher (Table 3.5). An independent sample T-Test was performed for each parameter and the results presented in Table 3.6. Statistically, a significant difference was not identified for any of the analysed parameters between the $HRAP_D$ + SIP and $HRAP_D$ ($p>0.05$) (Table 3.6). Table 3.7 presents the recorded weather conditions collected from the Australian BOM over the eight experimental runs. Included in Table 3.7 are the onsite UV radiations measured for UVA and UVB. On average the SIP was exposed to 7.61±1.72 h of sunlight across the eight experiments. Global solar irradiance as recorded by the Australian BOM ranged from 4.8-26.2 MJ m^{-2}, the mean 15.45±2.87 MJ m^{-2} approximately. The reported values are the collective means across all eight experiments.

Table 3.5: In situ water parameters recorded for the $HRAP_D$ and $HRAP_D$+SIP. Systems were exposed for 24 h throughout five experiments conducted during autumn-winter using tap water.

Model HRAP	Season	DO (mg L^{-1})				pH				Water Temperature (°C)			
		n	Mean	SD	SE	n	Mean	SD	SE	n	Mean	SD	SE
$HRAP_D$	Autumn	2	5.27	3.11	2.20	5	8.54	0.04	0.02	1	22.4	0.00	0.00
	Autumn	7	8.24	0.53	0.20	7	8.41	0.08	0.03	7	22.56	3.57	1.35
	Autumn	7	11.28	0.76	0.29	7	8.11	0.14	0.05	7	21.07	3.18	1.20
	Autumn	7	5.04	0.66	0.25	7	8.24	0.06	0.02	7	24.89	2.79	1.05
	Winter	7	2.33	1.09	0.41	7	8.10	0.16	0.06	7	18.31	2.44	0.92
	Mean	2	6.43	3.42	1.53	33	8.28	0.19	0.08	29	21.85	2.40	1.08
$HRAP_D$ + SIP	Autumn	2	3.82	0.03	0.02	5	8.54	0.04	0.02	1	22.40	0.00	0.00
	Autumn	7	8.05	0.45	0.17	7	8.41	0.08	0.03	7	23.31	4.22	1.59
	Autumn	7	11.03	1.04	0.39	7	8.11	0.14	0.05	7	22.50	3.98	1.50
	Autumn	7	4.86	0.82	0.31	7	8.24	0.06	0.02	7	23.50	3.57	1.35
	Winter	7	2.52	0.98	0.37	7	8.10	0.16	0.06	7	15.39	2.16	0.82
	Mean	2	6.05	3.45	1.54	33	8.28	0.19	0.08	29	21.42	3.41	1.52

Table 3.6: Independent samples t-test of in situ water parameters between the $HRAP_D$ + SIP and $HRAP_D$. Statistical significance was at p<0.05.

Parameter	HRAP	n	Mean±SE	SD	t	df	95% confidence intervals	p-value
DO (mg L^{-1})	$HRAP_D$+ SIP	30	6.43±0.61	3.33	0.23	58	-1.95 to 1.55	0.821
	$HRAP_D$	30	6.63±0.63	3.45				
pH	$HRAP_D$ + SIP	33	8.27±0.03	0.19	-0.63	64	-0.06 to 0.12	0.531
	$HRAP_D$	33	8.24±0.03	0.18				
Water Temperature (°C)	$HRAP_D$ + SIP	29	21.22±0.88	4.72	0.46	56	-2.74 to 1.71	0.645
	$HRAP_D$	29	21.73±0.68	3.68				

Table 3.7: Weather data collected from the Australian BOM. Onsite UV radiations collected for experimental Configuration 1: $HRAP_D$ + SIP and $HRAP_D$. BOM data was collected from Adelaide Airport station number: 23034 between February and June 2014.

	Season	Rain (mm)	Temperature (°C)		Sun Hours (h)	Solar Exposure (MJ m-2)	n	UVA (W m-2)	UVB (W m-2)
			Min.	Max.					
1	Summer	0.0	12.8	24.9	12.6	26.2	0	-	-
2	Autumn	0.0	13.0	24.3	11.3	24.2	0	-	-
3	Autumn	0.0	10.4	22.7	10.1	21.6	0	-	-
4	Autumn	0.6	19.0	23.1	0.0	4.8	8	5.00±0.96	0.16±0.04
5	Autumn	0.0	9.0	22.8	9.2	15.6	14	18.21±3.94	0.46±0.14
6	Autumn	0.0	7.0	20.7	8.2	14.8	7	16.12±3.78	0.43±0.14
7	Autumn	0.0	9.5	26.1	9.4	10.8	6	13.57±2.87	0.14±0.07
8	Winter	0.0	10.9	17.5	0.1	5.6	6	5.42±1.05	0.09±0.03
Mean±SE		**0.08±0.08**	**11.45±1.28**	**22.76±0.95**	**7.61±1.72**	**15.45±2.87**	**41**	**11.66±2.74**	**0.26±0.08**

Figure 3.3 demonstrates the relationship between solar exposure and MS2 die-off in the $HRAP_D$ + SIP, with solar exposure shown to have a positive impact on removal. Linear regression identified the relationship to be significant (R^2 = 0.693, p<0.05 (actual p=0.040)). A significant influence was not detected between K_D and the environmental parameters measured i.e. pH, temperature, DO, UVA and UVB (p>0.05) (Appendix 4.2). No significant relationships were detected in the $HRAP_D$ for any of the parameters measured.

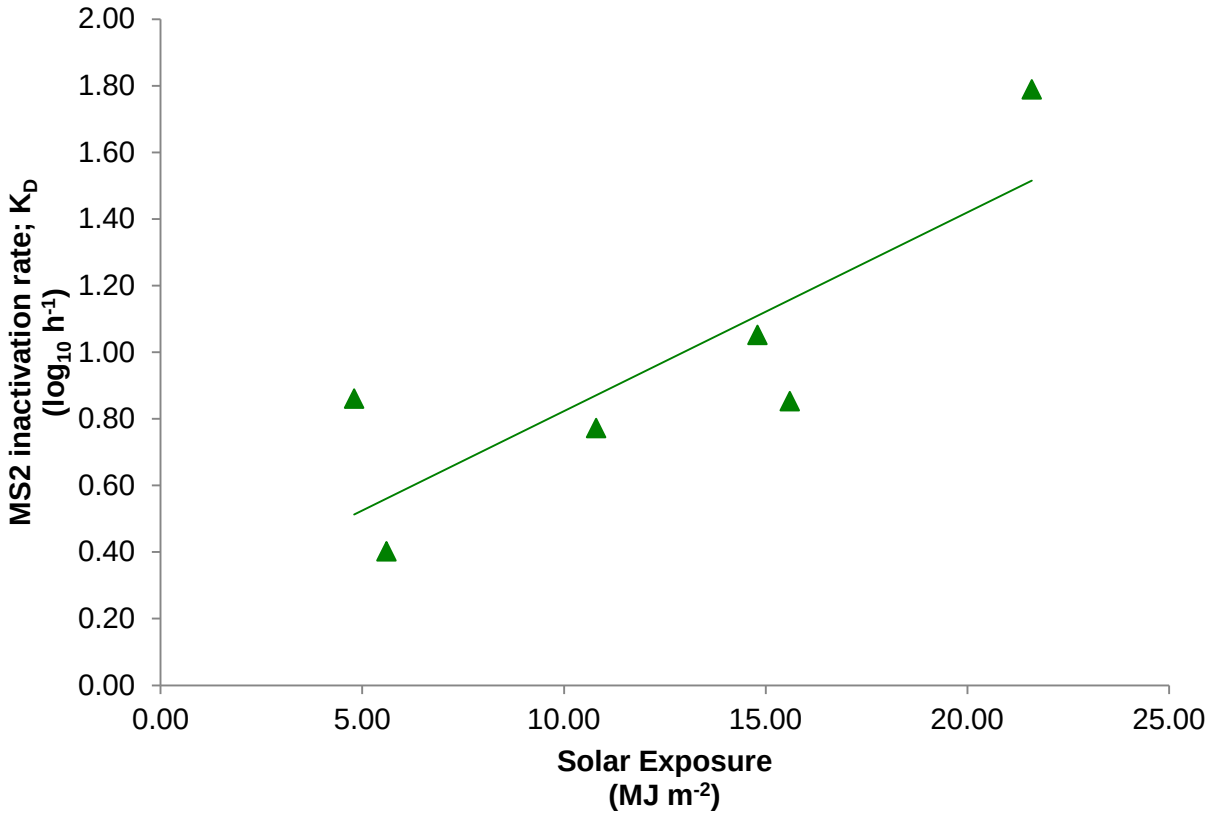


Figure 3.3: Influence of daily solar exposure (MJ m^{-2}) on MS2 inactivation rates (K_D, log_{10} h^{-1}) in the $HRAP_D$ + SIP. Significant relationship detected for IP_{ON} (R^2 = 0.693, p=0.04 (p<0.05))

An ANCOVA correcting for solar exposure was performed on the obtained MS2 K_D values. The results are presented in Appendix 4.3. A significant difference was still observed between the model HRAPs [F (1, 9) =14.69, p<0.05, actual p = 0.004; η^2 =0.62] when the effects of solar exposure were removed (Appendix 4.3: Table A4.4). This was also true, when water temperature was accounted for [F (1, 7) = 27.79, p=0.001: η^2 = 0.80] (Appendix 4.3: Table A4.5).

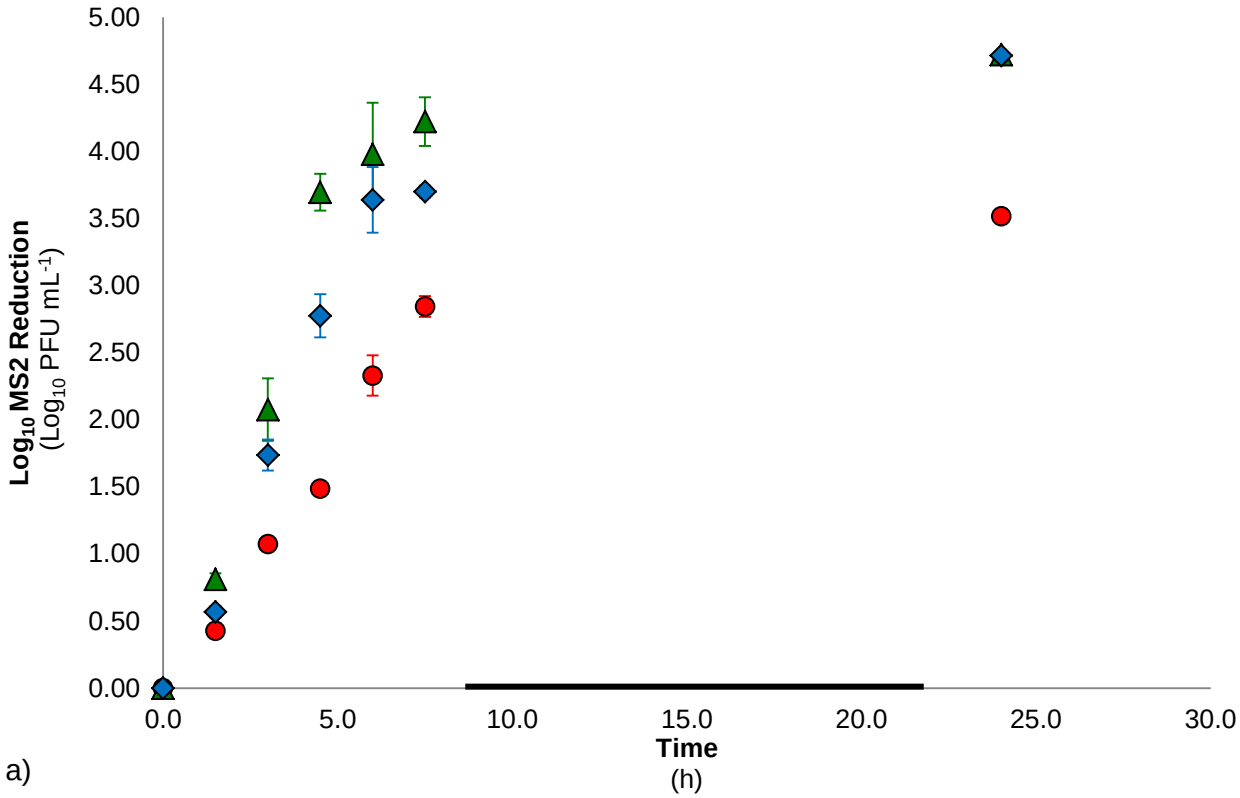
3.3.2 Microbial inactivation by Configuration 2, inclusion of Long IP (LIP)

Modification to IP length and operation was performed to determine whether MS2 die-off could be further improved and to what extent surface area, flow rate and areal loading rate influence inactivation with the IP. Length was extended to 1.10 m, increasing surface area to 0.75 m^2. Characterisation of the $HRAP_D$ + IP was performed and presented in Table 3.8. The SIP was re-characterised. Although the flow rates (L h^{-1}) and the whole bulk water volume cycling rates were the same the areal hydraulic loading rates (HLR) were different; SIP = 350.3 L^{-1} m^{-2} h^{-1}, and LIP =174.8 L^{-1} m^{-2} h^{-1}

Table 3.8: Characterisation of the Short and LIPs. Included is the mean hydraulic flow rate (Q, L h^{-1}), hydraulic loading rate (HLR; L m^{-2} h^{-1}) and cyclic rate for the $HRAP_D$+SIP and $HRAP_D$+LIP

Inclined Plane	IP Length (m)	IP Area (m^2)	Pond Volume (L)	Q (L h^{-1})	Hydraulic loading rate (L m^{-2} h^{-1})	Cyclic Rate	
						(cycles h^{-1})	(cycles d^{-1})
SIP	0.55	0.37	86.9	129.6	350.3	1.49	35.79
LIP	1.10	0.75	86.9	131.1	174.8	1.51	36.21

Concurrent operation of the $HRAP_D$ + SIP and $HRAP_D$ + LIP failed to identify an observable difference in MS2 die-off after 24 h (Figure 3.4); both IP systems produced a mean LRV_{24} of 4.72. Die-off in the model systems after 7.5 h is also shown in Figure 3.4. Comparison with the $HRAP_D$ (no-IP, LRV_{24} 3.52) confirmed inactivation to be higher with the IP; an increase of 1.2 LRV_{24} observed. Figure 3.5 presents a box plot of mean log_{10} MS2 removal for the three analysed systems. From this, a clear distinction between removal in the $HRAP_D$ and removal in the $HRAP_D$ + SIP and $HRAP_D$ + LIP can be seen.



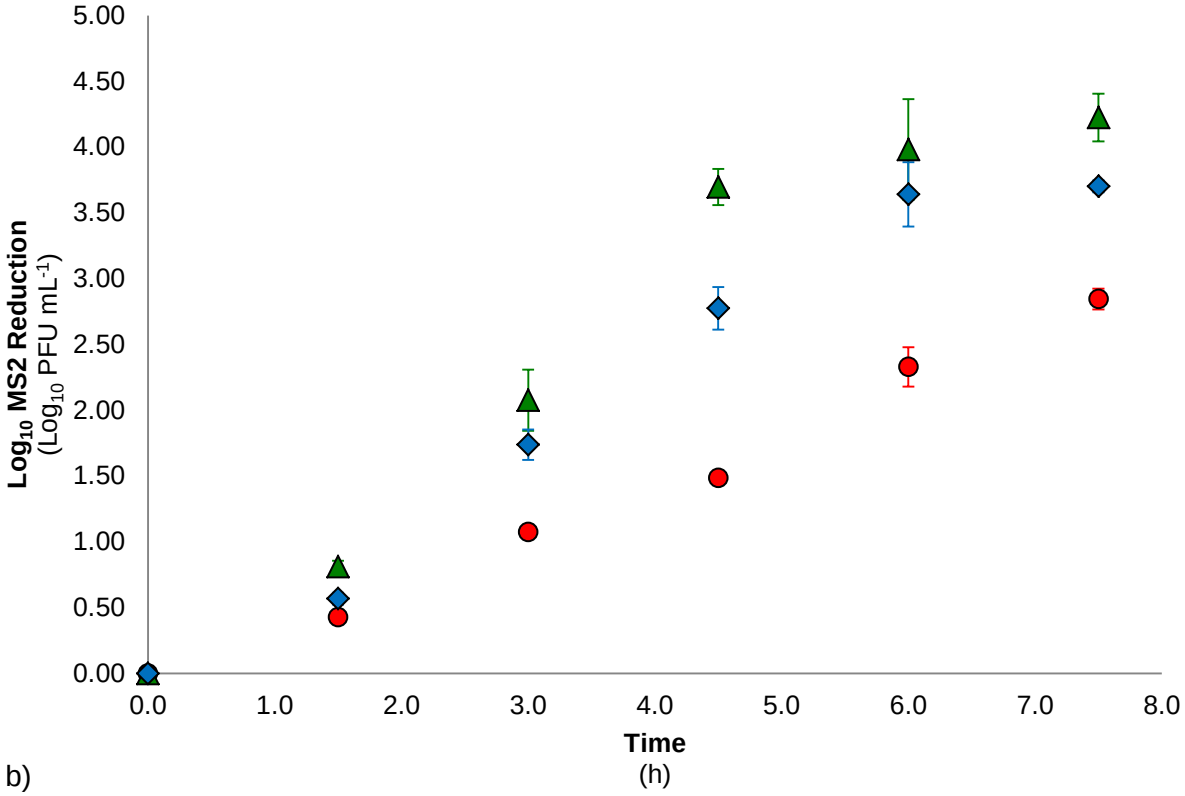


Figure 3.4: Semi-log plot of log_{10} MS2 removal (mean ± SE; log_{10} MS2 100 mL^{-1}) against time exposed for $HRAP_D$ + SIP (0.55 m, ◆) and $HRAP_D$ + LIP (1.10 m, ▲) together with the $HRAP_D$ (●) after a) 24 and b) 7.5 h. Night exposure is represented by (▪). Plot is comprised of the mean log_{10} removal obtained throughout November-December 2014. Global solar exposure ranged from 5.6 to 33.3 MJ m^{-2}.

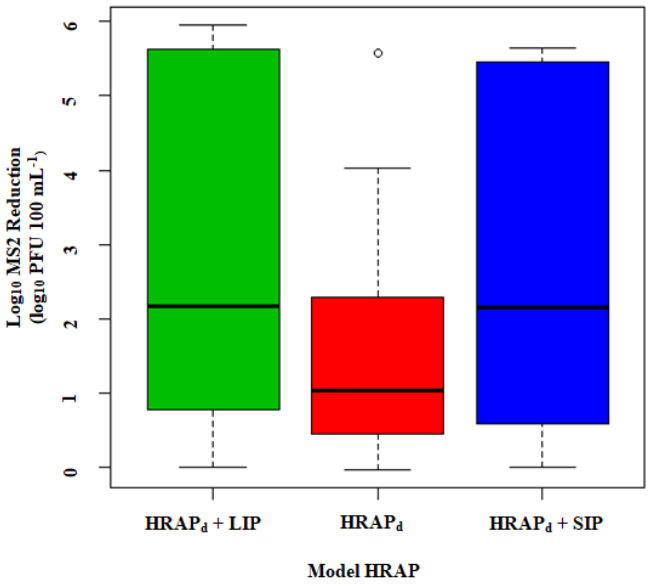


Figure 3.5: Box plot of mean±SD MS2 log_{10} reduction (LRV, log_{10} PFU 100 mL^{-1}) in model HRAPs with and without an IP, following 24 h incubation. Model HRAPs included $HRAP_D$ (no, IP), $HRAP_D$+Short - IP (SIP: 0.55 m, 0.37 m^2) and $HRAP_D$ + Long - IP (LIP: 1.10 m, 0.75m^2).

Table 3.9 presents the achieved LRVs and inactivation rates (K_D) for the three systems. A log_{10}-linear + tail inactivation model was again shown to best represent the removal in the systems. Like configuration 1, LRV captured within the linear part of the die-off curve was determined, with the mean LRV calculated between t=0 and t=7.5 h; the last time a sample was collected before the tail. Total LRV achieved in the systems was also calculated after 7.5 h incubation and are also reported in Table 3.9. Figure 3.4b also demonstrates the difference in LRV between the HRAPs after 7.5 h. Table 3.10 presents the results of a Tukey post hoc comparison that was performed on the obtained LRV and K_D values in the model HRAPs after 7.5 h and 24 h.

A covariate analysis (ANCOVA) was performed on the resultant K_D values to determine whether a statistical difference could be identified between the two IP lengths and the $HRAP_D$ when the effects of solar exposure and temperature were controlled. The results are presented in Appendix 4.3. A statistical difference however, was only detected between the $HRAP_D$ + LIP and $HRAP_D$ but only when controlled for both covariates (Appendix 4.3; Table A4.9).

Table 3.9: MS2 Inactivation rates (K_D, log_{10} MS2 h^{-1}) and log removal values; LRV (log_{10} PFU 100 mL^{-1}) in model $HRAP_D$, $HRAP_D$ + SIP and $HRAP_D$ + LIP after 24 h in tap water. K_D values were derived using log_{10} linear + tail model with R^2 an indication of goodness of fit for the model.

Model HRAP	Log Removal Rates (LRV)			Inactivation Rates	
	n	$LRV_{7.5}$ ± SE	LRV_{24} ± SE	n	K_D ± SE
$HRAP_D$	4	2.85±1.01	3.52±0.05	4	0.93±0.35
$HRAP_D$ + SIP	4	3.95±1.36	4.72±0.01	4	1.49±0.46
$HRAP_D$ + LIP	4	4.22±1.07	4.72±0.004	4	1.91±0.55

Table 3.10: Tukey's post hoc comparison of variance between obtained MS2 log reductions (LRV; log_{10} PFU 100 mL^{-1}) and inactivation rates (K_D; log_{10} h^{-1}) after 24 h for the modified IP lengths. Statistical significance was at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level								
Time (t; h)	Removal	Model HRAPs		Difference	Lower	Upper	p-value	Sig.
7.5	$LRV_{7.5}$	$HRAP_D$+SIP	$HRAP_D$	0.75	-0.11	1.61	0.082	-
		$HRAP_D$ +LIP	$HRAP_D$	1.10	0.24	1.97	0.018	*
		$HRAP_D$ +LIP	$HRAP_D$ +SIP	0.35	-0.51	1.22	0.483	-
24	LRV_{24}	$HRAP_D$ +SIP	$HRAP_D$	0.83	0.09	1.57	0.025	*
		$HRAP_D$ +LIP	$HRAP_D$	1.12	1.86	0.38	0.001	**
		$HRAP_D$ +LIP	$HRAP_D$ +SIP	0.29	-0.45	1.03	0.624	-
24	K_D	$HRAP_D$ +SIP	$HRAP_D$	0.99	-0.84	2.81	0.332	-
		$HRAP_D$ +LIP	$HRAP_D$	0.43	-1.39	2.25	0.794	-
		$HRAP_D$ +LIP	$HRAP_D$ +SIP	0.56	-1.26	2.38	0.681	-

Strength of statistical significance: * p<0.05, ** p<0.01, - p>0.05

In situ water conditions were monitored for the three systems (Table 3.11). The statistical analysis of the data is reported in Table 3.12. There was no significant difference in mean pH, DO and water temperature between any of the pond systems (p>0.05). However, the mean pH of the $HRAP_D$ (8.41±0.27) was significantly higher (p<0.05, actual p=0.021; 95% CI: 0.03 to 0.37) than the mean pH obtained in the $HRAP_D$ + LIP (8.08±0.07).

Table 3.13 reports the weather conditions both onsite and from the Australian BOM. On the IPs water and MS2 was exposed to approximately 7.6 h of sunlight at a solar exposure of 15.45 MJ m^{-2}.

Table 3.11: Monitored in-situ water parameters for the $HRAP_D$, $HRAP_D$ + LIP and $HRAP_D$ + SIP. Systems were exposed for 24 h dark incubated in tap water throughout spring-summer 2014.

System	Operational dates	Season	DO (mg L^{-1})		pH		Water Temperature (°C)	
			n	Mean±SE	n	Mean±SE	n	Mean±SE
$HRAP_D$	14/11/2014	Spring	5	3.90±1.06	1	8.72±0.00	5	22.36±0.92
	26/11/2014	Spring	7	3.97±0.74	0	-	7	23.63±1.81
	03/12/2014	Summer	7	3.07±1.11	7	8.29±0.01	7	31.11±1.85
	10/12/2014	Summer	7	0.58±0.05	7	8.22±0.01	7	21.23±0.31
		Mean	26	2.80±0.48	15	8.28±0.03	26	24.75±1.05
$HRAP_D$ +LIP	14/11/2014	Spring	5	3.90±1.06	1	7.14±0.00	5	20.38±0.73
	26/11/2014	Spring	7	3.97±0.74	0	-	7	24.29±1.77
	03/12/2014	Summer	7	4.24±1.27	7	8.21±0.03	7	27.91±1.95
	10/12/2014	Summer	7	0.54±0.08	7	8.10±0.06	7	20.43±0.52
		Mean	26	3.11±0.52	15	8.08±0.07	26	23.55±0.93
$HRAP_D$ + SIP	14/11/2014	Spring	5	4.11±1.19	1	8.64±0.00	5	20.38±0.85
	26/11/2014	Spring	7	4.04±0.76	0	-	7	22.86±1.96
	03/12/2014	Summer	7	3.90±1.26	7	8.21±0.02	7	27.73±2.06
	10/12/2014	Summer	7	0.54±0.08	7	8.19±0.01	7	20.89±0.37
		Mean	26	3.07±0.53	15	8.23±0.03	26	23.16±0.95

*data is a representation of the measurements recorded for each time interval within the individual experiment

Table 3.12: Tukey's post hoc comparison for establish differences in monitored water parameters for the $HRAP_D$, $HRAP_D$ + LIP and $HRAP_D$ + SIP Statistical significance was accepted at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level													
System		**DO**				**pH**				**Temp**			
		Difference	**Lower**	**Upper**	**p value**	**Difference**	**Lower**	**Upper**	**p value**	**Difference**	**Lower**	**Upper**	**p value**
$HRAP_D$ +LIP	$HRAP_D$	0.30	-1.42	2.03	0.908	-0.19	-0.37	-0.03	0.021*	-1.21	-4.50	2.09	0.656
$HRAP_D$ + SIP	$HRAP_D$	0.27	-1.45	1.99	0.926	-0.05	-0.23	0.12	0.760	-1.59	-4.89	1.70	0.483
$HRAP_D$ + SIP	$HRAP_D$ +LIP	-0.03	-1.76	1.69	0.999	0.15	-0.03	0.32	0.107	-0.39	-3.68	2.91	0.958

Strength of statistical significance: * p<0.05, ** p<0.01, *** p<0.001, - p>0.05

Table 3.13: Monitored weather conditions measured onsite and from the Australian BOM website.

	Operational dates	Season	Rain (mm)	Temperature (°C)		Sun Hours (h)	Solar Exposure (MJ m^{-2})	n	UVA (W m^{-2})	UVB (W m^{-2})
				Min	Max					
1	14/11/2014	Spring	0.0	18.8	22.8	0.4	5.60	7	9.16±2.84	0.35±0.13
2	26/11/2014	Spring	0.0	10.6	25.0	13.8	31.20	7	29.04±22.62	0.50±0.72
3	03/12/2014	Summer	0.0	19.0	30.0	12.5	31.10	7	37.69±20.11	1.13±0.94
4	10/12/2014	Summer	0.0	17.2	21.7	0.3	3.30	0	-	-
		Mean±SE	0.00±0.00	16.47±1.97	24.88±1.84	6.75±3.70	17.80±7.72	21	25.30±4.51	0.66±0.16

3.4 Discussion

The disinfection potential of inclined planes over which water is exposed to solar radiation was determined. Optically clear tap water inoculated with MS2 was circulated over inclined planes of different surface areas, only the IPs were exposed to solar irradiation; the bulk water in the HRAP reservoirs being incubated in the dark. The disinfection performance of HRAPs incorporating the IPs was compared to those incubated in the dark in the absence of an inclined plane.

This investigation was carried out in two parts, using two experimental configurations. The first, determined MS2 inactivation achievable by the $HRAP_D$ + SIP compared to the dark incubated $HRAP_D$ (Configuration 1: $HRAP_D$ + SIP vs. $HRAP_D$). The second examined whether increasing the IP length would have an even greater influence on removal rates (Configuration 2: $HRAP_D$ + LIP vs. $HRAP_D$). The impact the IP has on the in situ properties of the water was also analysed.

3.4.1 Microbial inactivation with and without IP inclusion

A statistically significant increase in inactivation was observed by incorporation of the SIP when compared to the dark incubated HRAP in the absence of an IP. The null hypothesis (H_0) that the removal of MS2 would be similar for all systems regardless of IP presence was therefore rejected and 'proof of concept' accepted.

The obtained results were found comparable to those previously reported by Hawley (2012), who discovered that MS2 could be reduced by 2.0 log reductions and 4.5 and 4.0 log reductions under overcast (11.4±2.1 MJ m^{-2}) and sunny (18.3±4.5 MJ m^{-2}) conditions for both a light exposed slope with a dark covered pond and a dark incubated pond, respectively. In the present study, a 4.54 and 3.00 log_{10} reduction was achieved for similar systems; $HRAP_D$ + SIP and $HRAP_D$, under a mean solar exposure of 15.5±8.1 MJ m^{-2}. This solar exposure included both sunny and overcast conditions.

In a similar study (without inclusion of an IP), Fisher *et al.* (2011) identified a die-off coefficient (K_{obs} h^{-1}) of 0.148±0.004 h^{-1} and 0.003±0.001 h^{-1} after 22 h exposed to simulated sunlight (no filter) and the dark in phosphate buffered saline (PBS) water (20 mM PBS, pH 7.5, temperature 20°C, 10 mm

beakers). These values were found to be lower than the K_D values reported here for the $HRAP_D$+ SIP (0.95±0.19 h^{-1}) and $HRAP_D$ (0.35±0.03 h^{-1}) (Table 3.4).

Inactivation was shown to deviate from the linear regression curve commonly associated with semi-log plots (Peleg, 2000). Instead a log-linear with tail curve was identified as the predominant fit (Figure 3.2). The presence of this curve type coincides with the deviation from linearity described by Simpson (2010) and Blaustein *et al.* (2013) for food and water microbiology. Blaustein *et al.* (2013) indicated the log-linear with tail curve as the most prevalent throughout the current literature (46%). The tailing exhibited in the curve is indicative of a lag in microbial die-off and is commonly associated with a sub- or mixed population (Peleg, 2000; Blaustein *et al.*, 2013). A sub-population is usually more resistant or out of reach of the treatment, receiving a lower dose (Bevilacqua *et al.*, 2015). The likelihood of a mixed population within this investigation is slim; given the MS2 that was inoculated into the model HRAPs was an isolated laboratory strain, and the only organism present within the system. High variability has been reported for survival and inactivation curves with a shoulder or tail (McKellar and Lu, 2003). Development of a secondary model to establish how this lag in die-off is influenced by its environment is difficult but important (McKellar and Lu, 2003).

3.4.2 Modification to the IP

With the IP found to be effective at achieving increased MS2 die-off (compared to the $HRAP_D$ without the IP), the next logical step was to identify the optimal and influential operating conditions to improve IP performance and further boost removal rates. Modifying the length of the IP was considered, with the aim to provide an even large surface area for exposure. Results reinforced the benefit of using an IP to enhance pathogen removal with the $HRAP_D$ + LIP also exhibiting greater removal than the $HRAP_D$. However, under the conditions tested removal was shown to be no better improved than the $HRAP_D$ + SIP, despite the longer-IP exhibiting higher inactivation 75% of the time (Table 3.9). Both exhibited a LRV_{24} 4.72.

Statistical analysis failed to differentiate an adequate difference in removal between the two analysed lengths ($p>0.05$) and thus the H_0 that identical removal in the model HRAPs irrespective of IP surface area was accepted.

At this stage however, it would be unadvisable to abandon the prospect of improved removal with a larger IP without further examination or experimentation under more realistic pond conditions; conditions where the additional surface area could be more beneficial. Further investigation would also be required to identify what role the hydraulic characteristics of the system have on inactivation performance.

Regardless of length, inclusion of the IP was still shown to exhibit inactivation higher than observed in its absence ($HRAP_D$) (Figures 3.4 and 3.5). Once again this difference was found to be significant ($p<0.05$) and further cements the plausibility of the proposed system as a potential addition to pond treatment.

The obtained inactivation rates for the three systems ($HRAP_D$; 0.93±0.35 h^{-1}, $HRAP_D$ + SIP; 1.49±0.46 h^{-1} and $HRAP_D$ + LIP; 1.91±0.55 h^{-1}) were again found to be higher than those reported by Fisher *et al.* (2011) under simulated sunlight (light; 0.15±0.04 h^{-1} and dark; 0.003±0.001 h^{-1}).

A number of limiting factors were identified as a potential cause for this indifference between the modified and original IP length. For instance, doubling the IP length was considered as a means to improving removal rates further by providing an even large surface are by which solar exposure can be achieved. However results indicated the LIP tended to achieve higher LRV and K_D values but not significantly better than was exhibited with the SIP. This similarity between the systems was still apparent even when the effects of temperature and solar exposure were removed (controlled using a covariate analysis, Appendix 4.3). It was possible that this extension was not large enough for a difference to be identified. Increasing the length and surface area further could be a potential solution. However, for this work modifying the length further was considered impractical due to the spatial restriction of the sample site as seen in Plates 3.1 and 3.2, and the confinement of the storage container and equipment used. Modifications to the base of the IP would also be required, to prevent the systems from toppling over from the added weight.

As mentioned above, light is influenced by the properties of the water (Kirk, 1994). Tap water is optically clear and free of particulate matter hence the decay of light through attenuation would be less prominent. As this water was also run down the IP at a reduced volume, it is likely that the water and MS2 received the incident light with minimal reduction by attenuation. If this was the case, the length of the IP may not have come into effect, with both IPs receiving maximal exposure on the slope. Substituting the tap water for a more turbid one; e.g. wastewater, could therefore result in the longer IP producing the higher inactivation.

The time required for the organisms to be exposed to sunlight is complex and continually disputed within the literature. Fisher *et al.* (2012) acknowledged this dispute deduced that inactivation of indicator organisms under natural sunlight could be rapid after a few hours of exposure or partial with microbial presence remaining after 48 h (Wegelin *et al.*, 1994; McGuigan *et al.*, 1998; Noble *et al.*, 2004; Dejung *et al.*, 2007). It is therefore possible that the exposure of 24 h used in this study was insufficient for a difference to be detected and that increasing the exposure time could be beneficial. This will be examined in the subsequent chapters in a wastewater matrix.

The quantity of light by which the IPs were exposed, could be another possible explanation. Solar radiation varies seasonally. Irradiance is typically higher during summer months as cloud cover is significantly less (Kirk, 1994). Greater and more direct exposure is therefore achieved (Kirk, 1994). Here, experiments were conducted throughout late spring-summer. Greater MS2 inactivation was expected. However, solar irradiance has been identified higher on tilted surfaces throughout winter (Navntoft *et al.*, 2012). Navntoft *et al.* (2012) indicated that the interception of UV irradiance on an IP during winter was 30-40% compared to the 10-15% typically received during summer. It is therefore possible, that if the two IP lengths were rerun during winter, the longer length would exhibit the superior inactivation that was predicted, a consequence of the larger area available to intercept the sunlight. It was observed that under the conditions examined inclusion of the IP regardless of IP length yielded a combined mean final LRV after 24 h of 4.7 $\log_{10}$ MS2 100 mL^{-1}; 4.1 $\log_{10}$ MS2 after 7.5 h.

Operation of the systems in the absence of light enabled removal by the IP to be established whilst limiting the amount of light available for both systems. Full solar exposure where both IP and pond are exposed to sunlight; i.e. pond left uncovered, should have a greater impact on removal and will be examined in Chapters 5.

3.4.3 Environmental conditions

In situ water conditions were found to be fairly consistent across all analysed pond systems, regardless of IP presence or absence. The variation amongst these parameters were minimal between the control $HRAP_D$ and the $HRAP_D$ + IPs ($p>0.05$) (Tables 3.6 and 3.12). Further, increasing the IP length had no impact on the *in situ* parameters. From this it can be concluded that, under the conditions tested, inclusion of the IP had a negligible impact on water composition. pH was found to be the only exception with the $HRAP_D$ + LIP exhibiting a significantly lower mean pH than the $HRAP_D$ + No-IP ($p<0.05$) (Table 3.12).

It has been commonly reported that the three parameters monitored; pH, DO and temperature, play a role in microbial inactivation. Alterations to these parameters outside of optimum levels can result in increased inactivation (Tchobanoglous *et al.*, 2003). In the absence of light however, these parameters were reported to have little to no impact on inactivation rates (Davies-Colley *et al.*, 1999). As no distinct difference was observed in pH, DO or temperature for the tested pond types, it could be concluded that enhanced solar exposure was the predominant cause of inactivation within these systems. This coincides with the information known about microbial removal within these treatment ponds.

Water temperature was shown to be consistently lower with the addition of the IP. This was unexpected, as elevated temperatures are typically associated with increased exposure to solar irradiance (Hwang, 2012). Evaporative cooling was believed to be responsible for these lower temperatures. During solar irradiation the emitted light heats up the water and raises the surface temperature, which leads to increased evaporation (Tadesse *et al.*, 2004). As water is evaporated this heat is removed from the system causing an overall drop in water temperature (Hondzo and Stefan, 1993).

Water loss by evaporation in the model HRAPs was considered to be small (<1% of the total pond volume) and consistent with the findings reported by Hawley (2012), who indicated that as this reduction was small it was unlikely evaporation had a significant impact on inactivation within these systems. Owing to this, evaporative loss in the model systems by evaporation will not be further discussed, but could provide a basis for future studies.

Exposing the IP to sunlight was the predominant aim. It was revealed that throughout the entirety of the study the HRAPs were exposed to mean solar radiations of 15.45±2.87 MJ m^{-2} (Table 3.7) and 17.80±7.72 MJ m^{-2} (Table 3.13). The relationship between MS2 inactivation and solar radiation is well documented (Davies-Colley *et al.*, 1999; Kohn and Nelson, 2007). In the model systems, Figure 3.3 demonstrated faster MS2 inactivation (K_D) when solar irradiance was increased.

3.4.4 General Observations

Running water down an IP has the potential to alter how water can be treated in the future, with a greater benefit predicted in areas of water, land and resource scarcity (Toze, 1997; Anderson, 2003; Hamilton *et al.*, 2011). In this study significant inactivation was achieved with the IP even though majority of the pond was operated in the dark (covered). Based on this, greater removal can be expected when both IP and HRAP are exposed to sunlight.

Solar disinfection (SODIS) has been adopted as a cheap and effective method for disinfecting drinking water (Oates *et al.*, 2003). This method has been widely used throughout developing countries and is a recommended method for water disinfection within the household (WHO and UNICEF, 2005; Sobsey *et al.*, 2008). This method exposes small volumes of water to direct sunlight to reduce microbial contamination. Water is poured, in small volumes, into polyethylene terephthalate (PET) bottles and placed into the sun for a time, usually 6 h (McGuigan *et al.*, 2012). Typically, the bottles are placed on a roof top at a slight incline. Whilst this approach is effective and inexpensive, it is restricted by the volume of water it can treat at any given time and the exposure time required for complete inactivation is variable. As outlined in Chapter 1 and by Fisher *et al.* (2012), after exposing water to SODIS for 24 h, microbial inactivation was often incomplete, whereas complete inactivation had been observed in the HRAP+IP systems after only 7.5 h of

exposure. Regarding pathogen removal, Sobsey *et al.* (2008) outlines the maximum and baseline LRVs obtainable by SODIS for bacteria (5.5+ and 3 LRV), viruses (4+ and 2 LRV) and protozoa (3+ and 1 LRV) when performed by skilled and unskilled persons, respectively. These LRVs are comparable to those reported in this chapter for the HRAP+IP. Thus, by adapting the model HRAP+IPs used here, a larger volume of water could potentially be disinfected in a reasonable amount of time, providing a more practical solution for water treatment in developing countries. Nonetheless, it would be advisable to assess how the HRAP+IP system manages bacterial and protozoan inactivation before the system could be considered for drinking water disinfection.

Limitations with the IP systems were detected. These limitations were largely associated with system operation and formation of the thin film. Instead of a uniform film developing as desired across the IP, a series of rivulets had formed. This limited the amount of water being exposed down the IP. After modification; such as removing the hydrophobic coating on the Perspex, the film was observed to develop successfully.

3.5 Conclusion

To enhance pathogen removal in natural treatment systems, increasing the area available to solar irradiation was examined. Operation of the systems in the absence of light, except on the IP and in optically clear water provided the basis of this investigation. Results showed that under the conditions examined inclusion of the IP was beneficial with elevated removal in all instances where it was present. From this work the following observations and conclusion can be derived;

- Increased MS2 removal was obtained whenever the IP was present
- Extension of the IP length failed to detect a positive increase in removal in optically clear water but was still shown to be higher than its corresponding control.
- Inclusion of the IP has a negligible impact on pH, DO and water temperature despite the obvious increase exposure to sunlight..
- Solar exposure was shown to have a positive impact on inactivation in the model HRAPs where the IP was present.

Use of optically clear water provided the opportunity to assess IP performance without the presence of attenuates in the water. To ensure the IP is suitable for use in pond systems in the field identification of performance in more complex waters is required and will form the basis of research in the progressing chapters. Assessment of key operating conditions will also be performed, with the idea to establish which are influential to IP performance.

In addition, to ensure removal effectiveness of IP included systems is maximised, the following modifications were identified. These modifications will also be discussed in the progressing chapters.

1. Change the medium the systems are operated in
2. Extend the length of operation for all systems
3. Further extend the length and surface area of the IP
4. Examine the behaviour of the IP under different hydraulic conditions
5. Operation of the IP under full solar exposure and on a larger scale

4. IMPACT OF IP INCLUSION ON F-SPECIFIC PHAGE REMOVAL RATES IN DARK INCUBATED MODEL HRAPS OPERATED IN TURBID WASTEWATER

In this chapter, some of the data has been adapted and incorporated as part of an international conference preceding (Appendix 7.1) as well as a published paper in Water Science and Technology (Appendix 7.2).

Citations:

Hawley & Fallowfield (2016). Inclusion of pond walls to enhance solar exposure and pathogen removal. In: 11th IWA Specialist Group Conference on Wastewater Pond Technology, University of Leeds.

Hawley, A. & Fallowfield, H. (2018). Pond walls: Inclined planes to improve pathogen removal in pond systems for wastewater treatment? Water Science and Technology, wst2018269.

4.1 Introduction

In water environments the availability of light is highly variable; dependent on composition, clarity and depth of the water. The more complex the water the less likely light will be able to penetrate the surface and reach the pathogens (Paterson and Curtis, 2005) . This results in the pond being fairly unexposed and inactivation abilities restricted. Light availability will therefore be lower in turbid waters (i.e. wastewater) with the presence of algae, dissolved and suspended solids, particulate matter and nutrients contributing to light dissipation in water (Curtis *et al.*, 1994; Tchobanoglous *et al.*, 2003). Turbidity has a negative influence on sunlight irradiance by shielding pathogens and decreasing light transmittance within the water (Passantino *et al.*, 2004; Yu, 2015). Turbidity has also been described as the key predictor for light attenuation in pond systems (Bolton, 2012).

The literature shows majority of the light involved in inactivation is absorbed in the first 1 m of turbid water (Haag and Hoigne, 1986), UVB in the first 30 cm (Kohn and Nelson, 2007; Bolton, 2012). Balogh *et al.* (2009) indicated this to be much shallower than in clear waters, with UV light capable of penetrating several metres. Consequently, to improve disinfection in turbid waters increasing the exposure to sunlight whilst simultaneously restricting the impacts of attenuation is essential.

The application of an IP and thin film to model HRAPs was examined in Chapter 3 as a means of enhancing pathogen exposure to sunlight radiation over a larger surface area at a reduced volume. Under dark incubation elevated MS2 inactivation was achieved in optically clear water (tap) when the IP was incorporated and exposed to sunlight. More specifically in Chapter 3 it was identified that after 24 h incubation, inactivation could be increased by up to 53% with the IP included. However, pond systems such as HRAPs are designed to treat turbid wastewaters (Park *et al.*, 2011). Establishing disinfection performance of the IP in turbid water is therefore required.

In this work, the experiments and evaluations reported in Chapter 3 were repeated with the optically clear water replaced with wastewater collected from a local wastewater treatment plant (WWTP). Several other modifications to the experimental design and model systems were also made following the suggestions made in Chapter 3. Experiments were again performed in the absence of light with the IP solely solar exposed. Specifically this chapter aims to;

- Examine MS2 and F-Specific phage inactivation with the IP in turbid water
- Examine whether modification to the IP can improve inactivation
- Establish which operating parameters are crucial for inactivation with the IP
- Establish how the IP influences and is influenced by other aspects of the pond system; i.e. water quality, water type and pond conditions.

4.2 Methods

4.2.1 Water Source

Wastewater was collected from Mt Barker WWTP (35.068857°S, 138.876491°E) prior to experimental runs. Mt Barker WWTP is a community wastewater management system (CWMS) that treats septic tank effluent from local South Australian communities including Mt Barker, Nairne, Littlehampton and Brukunga. The plant is comprised of two lagoons; an aeration lagoon and a polishing lagoon, a dissolved air filtration system (DAF) and a continuous micro filtration unit (CMF). An aerial representation of the plant is shown in Plate 4.1. Water was collected from the inlet pipe to the DAF plant after it was pre-treated in the lagoon Plate 4.2. All water was collected in 25 L plastic containers and transported back to the onsite location at Flinders University. Collected water not used immediately was stored at ~4°C. Tap water collected from the Flinders University Health Sciences Laboratory was used as an optically clear comparison.

Plate 4.1: Aerial view of Mt Barker WWTP; including the a) aeration lagoon, b) polishing lagoon and c) dissolved air filtration (DAF) plant and sample collection site. Image available from https://www.google.com.au/maps/@-35.0699777,138.8749558,787m/data=!3m1!1e3

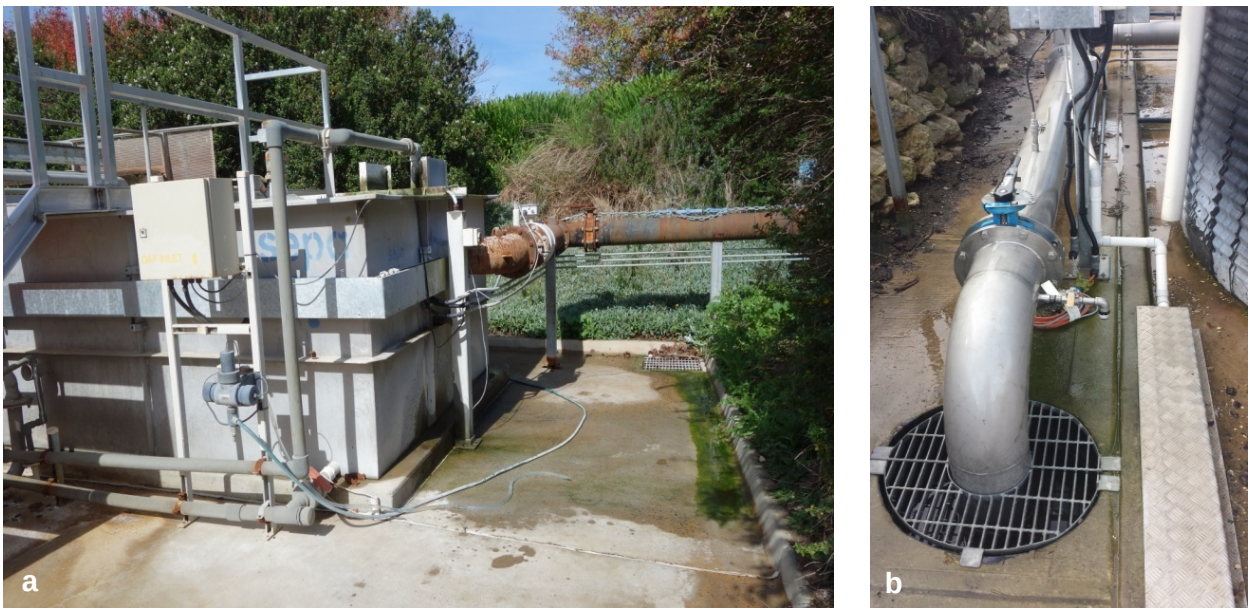


Plate 4.2: Pictures of the a) Dissolved air filtration (DAF) plant at Mt Barker wastewater treatment plant and b) inlet to DAF plant water sampling point

4.2.2 Model HRAP configuration

Model systems were set-up on site at Flinders University as described in Chapters 2 and 3, but with wastewater (Mt Barker) substituted for the tap water. Ponds were operated in the following configurations;

1. $HRAP_D$ + Short IP (SIP) vs. $HRAP_D$
2. $HRAP_D$ vs. $HRAP_D$ + SIP and $HRAP_D$+ Long IP (LIP) (**Error! Reference source not found.**)
3. $HRAP_D$ (1) and $HRAP_D$ + SIP (1) vs. $HRAP_D$ (2) and $HRAP_D$ + SIP (2) (Plate 4.4)

Where, $HRAP_D$ represents a covered HRAP with the bulk water operated under dark incubation and the IP solely exposed to sunlight. Operating conditions are outlined in Table 4.1 (configuration I and III) and 4.2 (configuration II).

$HRAP_D$ **$HRAP_D$+LIP** **$HRAP_D$+SIP**

Plate 4.3: Model HRAP arrangement for operational configuration II; No-IP vs. IPs of varying length. HRAPs included a) $HRAP_D$, b) $HRAP_D$ + Long IP (LIP; 1.10 m, 0.75 m^2) and c) $HRAP_d$ + Short IP (SIP; 0.55 m, 0.37 m^2).

$HRAP_D$ (2) **$HRAP_d$+SIP (2)**

Plate 4.4: Model HRAP arrangement for operational configuration II; No-IP vs. IPs of varying length. HRAPs included a) $HRAP_d$, b) $HRAP_D$ + Long IP (1.10 m, 0.75 m^2) and c) $HRAP_D$ + Short IP (0.55 m, 0.37 m^2).

Table 4.1: Model HRAP operating conditions for the Small – IP (SIP) when operated in configuration I; $HRAP_D$ + SIP vs. $HRAP_D$ and configuration III; $HRAP_D$ + SIP (1) and $HRAP_D$ (1) vs. $HRAP_d$ + SIP (2) vs. $HRAP_d$ (2). Parameters included flow rate (Q; L h^{-1}) and hydraulic loading rate (HLR; L m^{-2} h^{-1}).

Model HRAP	IP Length (m)	IP Area (m^2)	Q (L h^{-1})	HLR (L m^{-2} h^{-1})
$HRAP_D$ + LIP	1.10	0.75	131.1	174.8
$HRAP_D$ + SIP	0.55	0.37	129.6	350.3

Table 4.2: Model HRAP operating conditions for the Small – IP (SIP) and Long – IP (LIP) (Configuration II; $HRAP_D$ vs. $HRAP_D$ + SIP and $HRAP_D$ + LIP). Parameters included flow rate (Q; L h^{-1}) and hydraulic loading rate (HLR; L m^{-2} h^{-1}).

Model HRAP	IP Length (m)	IP Area (m^2)	1		2		3		4	
			Q	HLR	Q	HLR	Q	HLR	Q	HLR
$HRAP_D$ + LIP	1.10	0.75	262.7	350.3	130.8	174.4	174.6	232.8	225.0	300.0
$HRAP_D$ + SIP	0.55	0.37	129.6	350.3	62.1	167.8	87.7	232.7	111.0	300.0

4.2.3 Experimental design

Inactivation experiments were largely conducted as described in Chapter 3, with at least three experimental replicates performed, unless otherwise stated. All configurations were incubated for 24 and 49.5 h, with configuration II also incubated for 5 d; the HRT of the KoM HRAP (Chapter 6). For the experiments that assessed IP performance under different operating conditions; IP size, hydraulic loading rate (HLR) and different incubation times were used. These factors are known to influence inactivation in pond systems. HLR enabled the surface area of the IP to be manipulated without further alterations to the physical properties of the slope.

4.2.4 Sample inoculation and collection

Due to the low presence of native phage in the Mt Barker treated wastewater, 2 mL of MS2 stock (~ $x10^9$ PFU mL^{-1}) was inoculated into each system the morning of sampling. Prior to inoculation, 1 L samples of the Mt Barker wastewater was collected for analysis. Daily, 10 mL triplicates were collected between 9:00 am and 5:00 pm in 1.5 hour intervals. Every 3 hours an additional 120 mL samples were collected for analysis (i.e. chl a, turbidity and nutrient concentrations). At the completion of operation 1 L samples were also taken from each system for a more detailed analysis of water quality.

4.2.5 Water Analysis

Analysis was performed on the 1 L and 120 mL samples collected. Analyses included; chl a, nutrient concentrations (TOC, TC, IC and TN) and turbidity. SS and BOD_5 concentrations were also analysed for the before and after treatment samples (1 L). Analysis was performed using APHA (1992) standard wastewater methods and the BOD OxiTop - OxiTop$^{(R)}$ – C (WTW) method for BOD_5. Details regarding each analysis were presented in Chapter 2.

4.2.6 Environmental conditions

In situ water parameters and BOM environmental conditions were monitored as described in Chapter 2. Parameters included; water temperature, pH, DO (mg L^{-1}), UVA and UVB irradiance (W m^{-2}), sun hours (h), daily solar exposure (MJ m^{-2}), daily rain fall (mm) and both minimum and maximum ambient temperature (°C).

4.2.7 Microbial Quantification

Quantification of MS2 was carried out using the double layer agar quantification plaque assay detailed in Chapter 2. Tryptone water (5%) was used for sample dilution when required. Due to potential presence of native phage within the wastewater samples, detection of phage in the model systems was reported as F-Specific phage instead of MS2, despite MS2 being spiked into the system.

4.2.8 Statistical Analysis

F-Specific phage removal was determined as log removal values (LRV_t; $\log_{10}$ F-Specific phage 100 mL^{-1}) and inactivation rate constants (K_D; $\log_{10} h^{-1}$), with t and d representative of the incubation time and dark inactivation conditions. LRVs were determined from $\log_{10} N_0 - \log_{10} N_t$. These values were plotted on semi-log plots of LRV against time exposed. K_D was then derived from the corresponding linear regression of these plots. To establish linearity of the data, plots of $\log_{10}$ F-Specific phage ($\log_{10}$ (N)) against time exposed were assessed. In the event where the curves were identified as non-linear, $\log_{10}$ linear with tail or shoulder + tail die-off models were applied using GInaFiT (Geeraerd *et al.*, 2005). Use of these model; in particular the $\log_{10}$ linear with tail was based on the findings presented by Yu (2015). K_D was then taken from the corresponding $K_{max} \pm$ SE values Like Chapter 3; removal captured within the linear part of the die-off curve was taken into consideration for the 24 h incubation times. LRV determined after 7.5 h was therefore reported instead of the 24 h. Independent sample t-tests, ANOVAs and ANCOVAs were performed to establish the difference in mean removal with and without the IP. These tests were also performed to establish statistical significance in the monitored in situ parameters between the pond systems. Statistical significance was accepted at $p<0.05$. Relationships between variables were determined using linear regression and a correlation (r) matrix (Pearson's product moment correlation). Correlation was determined as positive or negative and classed as strong, moderate, weak or no correlation (refer to Chapter 2).

4.3 Results

This study examined the removal efficiency of the IP when model HRAPs were operated in wastewater. Systems were primarily dark incubated (covered) with the IP solely exposed. Both long (LIP) and short (SIP) IPs were used. The $HRAP_D$ and $HRAP_D$ + IP were operated contiguously throughout the investigation under the same environmental conditions (i.e. solar irradiances). Paired comparisons were therefore performed with the results presented as an aggregated summary across a number of experiments and repetitions. This enabled any differences in inactivation with the IP to be broadly determined. Data within this chapter has been adapted and incorporated as part of an international conference preceding (Appendix 7.1) as well as a research paper published in Water Science and Technology (Appendix 7.2).

4.3.1 F-Specific phage Inactivation: $HRAP_D$ vs. $HRAP_D$ + SIP

For configuration I, a decline in F-Specific phage numbers was observed in model systems when dark incubated in wastewater. Inclusion of the SIP achieved increased removal with total inactivation 1.3 times higher in the $HRAP_D$ + SIP ($LRV_{7.5}$ 0.86±0.08) than the $HRAP_D$ ($LRV_{7.5}$ 0.66±0.07) after 7.5 h (Table 4.3); 1.6 times after 24 h ($HRAP_D$ + SIP; LRV_{24} 2.35±0.26; $HRAP_D$ LRV_{24} 1.45±0.27) (Appendix.5.1).

As presented in Chapter 3, inactivation in the model systems was found to be best represented by the log_{10} linear + tail model when log_{10} F-Specific phage was plotted against time exposed. Removal will therefore be determined using this model henceforth. Table 4.3 summarises the obtained inactivation rates (K_D) derived from the resultant K_{max} values of the plot of log_{10} PFU against incubation time. Data presented in Table 4.3 is an indication of the mean removals collated over 5 experimental replicates.

Analysis showed the difference between the obtained K_D values in the $HRAP_D$ and $HRAP_D$ + SIP was not significant, even though the $HRAP_D$ + SIP exhibited faster inactivation ($p>0.05$, Table 4.4). When solar exposure and temperature were controlled the difference in inactivation between the

model HRAPs was found to be significant ($p<0.05$, Appendix 5.2: Tables A5.4-A5.6), indicating IP presence had an impact on inactivation.

Similarly, a statistical difference was also detected between mean removal ($LRV_{7.5}$) in the $HRAP_D$ and $HRAP_D$ + SIP after 7.5 ($p<0.01$, $p=0.010$, 95% CI: 0.04 to 0.21, d = 3.41) (Appendix 5.3; Table A5.14), with $HRAP_D$ + SIP again exhibiting elevated removal. On average it was estimated that the SIP accounted for approximately 29.1% of the removal observed in the $HRAP_D$.

Table 4.3: F-Specific phage inactivation in model systems; $HRAP_D$ and $HRAP_D$ + SIP after 24.0 h incubations. Inactivation included; log_{10} reduction values (LRV) and inactivation rate constants (K_{max}, mean ± standard deviation). SIP was operated at a flow rate of 129.6 L h^{-1}.

Incubation (h)	Model System	F-Specific phage Inactivation		
		n	K_D±SE	$LRV_{7.5}$±SE
24.0	$HRAP_D$	5	0.24±0.03	0.66±0.07
	$HRAP_D$ + SIP	5	0.30±0.03	0.86±0.08

[a] LRV were determined after 7.5 h incubation

Table 4.4: Independent samples t-test showing the comparison between mean F-Specific phage inactivation (K_D) in the $HRAP_D$ and $HRAP_D$ + SIP after 24 h incubated. Statistical significance was at $p<0.05$.

	Model HRAP	n	Mean ± SE	t	Df	Difference	Lower	Upper	p value	*d*
K_D	$HRAP_D$	5	0.24±0.03	1.26	8	0.06	-0.05	0.16	0.242	0.79
	$HRAP_D$ + SIP	5	0.30±0.03							

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01, Effect size (*d*) calculated using Cohen's *d*

Below outlines the global and onsite solar irradiances measured over an experimental series where the SIP was operated at a flow rate of 129.6 L h^{-1}. Irradiances ranged from 5.60 and 28.2 MJ m^{-2} (Global), 5.42 to 39.0 W m^{-2} (UVA) and 0.09 to 1.02 W m^{-2} (UVB) with means of 19.44±4.68 MJ m^{-2}, 23.82±8.44 W m^{-2} and 0.45±0.22 W m^{-2}, respectively. Water temperatures ranged from 10.8 to 32.6°C ($HRAP_D$) and 10.3 to 33.1°C ($HRAP_D$ + SIP), respectively.

Little variation in pH, DO and water temperatures was observed between the $HRAP_D$ and $HRAP_D$ + SIP systems. Mean concentrations were recorded at pH 7.83±0.07, 4.26±0.77 DO mg L^{-1} and

23.17±2.40°C for the $HRAP_D$ and pH 8.04±0.26, 5.64±1.43 DO mg L^{-1} and 23.02±2.40°C for the $HRAP_D$ + SIP. An independent samples t-test confirmed the difference between the means were not significant ($p>0.05$) (Appendix 5.3: Table A5.15).

4.3.2 Modification to IP length

Inclusion of the LIP resulted with an $LRV_{7.5}$ of 0.76 and a K_D value of 0.38±0.05 log_{10} h^{-1} after 24 h. Removal in the $HRAP_D$ + LIP was found to be 2.2 and 1.3 times higher than the $HRAP_D$ ($LRV_{7.5}$ 0.35) and $HRAP_D$ + SIP ($LRV_{7.5}$ 0.57), respectively (Table 4.5). 24 h LRV are reported in Appendix 5.1; Table A5.2). Increasing incubation time saw elevated inactivation in all systems. Again, the $HRAP_D$ + LIP achieved the highest removal, with the resulting $LRV_{49.5}$ (2.82±0.88), 1.2 and 1.8 times higher than the $HRAP_D$ + SIP ($LRV_{49.5}$ 2.33±0.69) and $HRAP_D$ ($LRV_{49.5}$ 1.53±0.61), respectively.

Tables 4.6 and 4.7 show the results from a Tukey's post hoc comparison performed on the resulting LRV and K_D values after 24 (7.5 h) and 49.5 h incubations. ANCOVAs correcting for solar exposure and temperature were also performed on the K_D values, with the results presented in Appendix 5.2. Unlike the tap water, a statistical difference was not detected between any of the analysed wastewater systems after 7.5 h. Similarly, the difference between the $HRAP_D$ + IP and $HRAP_D$ was only found to be statistically significant ($p<0.05$) when incubation time was increased to 49.5 h (Table 4.6). No difference was identified between the two IPs.

K_D values were also found to be statistically significant after 24 h between the $HRAP_D$ + LIP and the $HRAP_D$ but only after the effects of solar exposure were removed (Table 4.8 and Appendix 5.2: Table A5.7). This was also apparent when temperature was controlled (Appendix 5.2; Table A5.8). A significant difference however was not detected between any of the systems after 49.5 h, even after being controlled for the aforementioned covariates. Nonetheless, the $HRAP_D$ + LIP and $HRAP_D$ + SIP exhibited inactivation that was 1.5 and 1.2 times faster than the $HRAP_D$ on average across six experimental replicates (Table 4.5).

Table 4.5: Mean F-Specific phage die-off for model HRAPs with different IP lengths; short-IP (SIP; 0.55 m) and long-IP (LIP; 1.10 m) after 24 and 49.5 h incubation. Log removal values (LRV; log_{10} PFU 100 mL^{-1}) and inactivation rate constants (K_D; log_{10} h^{-1}) are reported.

Time (t: h)	Model System	F-Specific phage Inactivation Rates		
		n	$K_D \pm$ SE	$LRV_t \pm$ SE
24.0	$HRAP_D$	6	0.23±0.07	0.35±0.14[a]
	$HRAP_D$ + SIP	6	0.29±0.05	0.57±0.15[a]
	$HRAP_D$ + LIP	6	0.38±0.05	0.76±0.20[a]
49.5	$HRAP_D$	6	0.11±0.04	1.53±0.61
	$HRAP_D$ + SIP	6	0.14±0.06	2.33±0.69
	$HRAP_D$ + LIP	6	0.17±0.06	2.82±0.88

[a] LRV were determined after 7.5 h incubation

Table 4.6. Tukey's post hoc comparison of variance between the mean F-Specific phage log reduction values (LRV; log_{10} PFU 100 mL^{-1}) in the $HRAP_D$ and $HRAP_D$ + IP (SIP and LIP) model HRAP systems obtained after 7.5 and 49.5 h incubations. Statistical significance was at $p<0.05$.

Tukey multiple comparison of means 95% family-wise confidence level									
Model Systems		7.5 h				49.5 h			
		Difference	Lower	Upper	P-Value	Difference	Lower	Upper	P-Value
$HRAP_D$ + LIP	$HRAP_D$	0.23	-0.01	0.47	0.065	0.63	0.14	1.12	0.008**
$HRAP_D$ + SIP	$HRAP_D$	0.12	-0.12	0.36	0.419	0.37	-0.12	0.86	0.181
$HRAP_D$ + SIP	$HRAP_D$ + LIP	0.11	-0.35	0.13	0.491	-0.26	-0.75	0.23	0.426

Strength of statistical significance, * = $p<0.05$, ** = $p<0.01$

Table 4.7. Tukey's post hoc comparison of variance between the mean F-Specific phage inactivation rates (K_D; log_{10} h^{-1}) in the $HRAP_D$ and $HRAP_D$ + IP (SIP and LIP) model HRAP systems obtained after 24 and 49.5 h incubations. Statistical significance was at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level									
Model Systems		24 h				49.5 h			
		Difference	Lower	Upper	P-Value	Difference	Lower	Upper	P-Value
$HRAP_D$ + LIP	$HRAP_D$	0.15	0.05	0.25	0.005	0.06	-0.12	0.24	0.660
$HRAP_D$ + SIP	$HRAP_D$	0.06	-0.04	0.16	0.101	0.04	-0.15	0.22	0.861
$HRAP_D$ + LIP	$HRAP_D$ + SIP	0.09	-0.02	0.19	0.298	0.03	-0.16	0.21	0.933

Strength of statistical significance, * = p<0.05, ** = p<0.01

Table 4.8. Tukey's post hoc comparison of variance between the mean F-Specific phage inactivation rates (K_D; log_{10} h^{-1}) in the $HRAP_D$ and $HRAP_D$ + IP (SIP and LIP) model HRAP systems obtained after 24 and 49.5 h incubations when the effects of solar exposure were removed. Statistical significance was at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level									
Model Systems		24 h				49.5 h			
		Difference	Lower	Upper	P-Value	Difference	Lower	Upper	P-Value
$HRAP_D$ + LIP	$HRAP_D$	0.15	0.06	0.24	0.002**	0.06	-0.004	0.13	0.066
$HRAP_D$ + SIP	$HRAP_D$	0.06	-0.03	0.15	0.205	0.04	-0.03	0.10	0.337
$HRAP_D$ + SIP	$HRAP_D$ + LIP	0.09	-0.001	0.18	0.054	0.03	-0.04	0.09	0.193

Strength of statistical significance, * = p<0.05, ** = p<0.01

4.3.3 In situ water and environmental conditions

The $HRAP_D$ exhibited lower pH lower pH (7.31±0.02), DO (5.51±0.21 mg DO L^{-1}) and temperatures (20.40±0.71°C) compared to the $HRAP_D$ + SIP (pH 7.64±0.06, 6.58±0.23 mg DO L^{-1}, 20.66±0.98°C) and $HRAP_D$ + LIP (pH 7.73±0.06, 6.08±0.19 mg DO L^{-1}, 21.9±0.94°C). However, only pH, (and DO for the $HRAP_D$ + SIP) was found to be significantly lower ($p<0.05$) (Appendix 5.3: Table A5.16).

Figure 4.1 shows how the in situ water conditions varied throughout the 49.5 h incubation period. Data presented in Figure 4.1 is a representation of the mean levels recorded at each collection interval made within the 49.5 h incubation, i.e. every 1.5 h between 9:30 and 17:30. The data was collated over 6 experimental sets. Overnight measurements were not recorded.

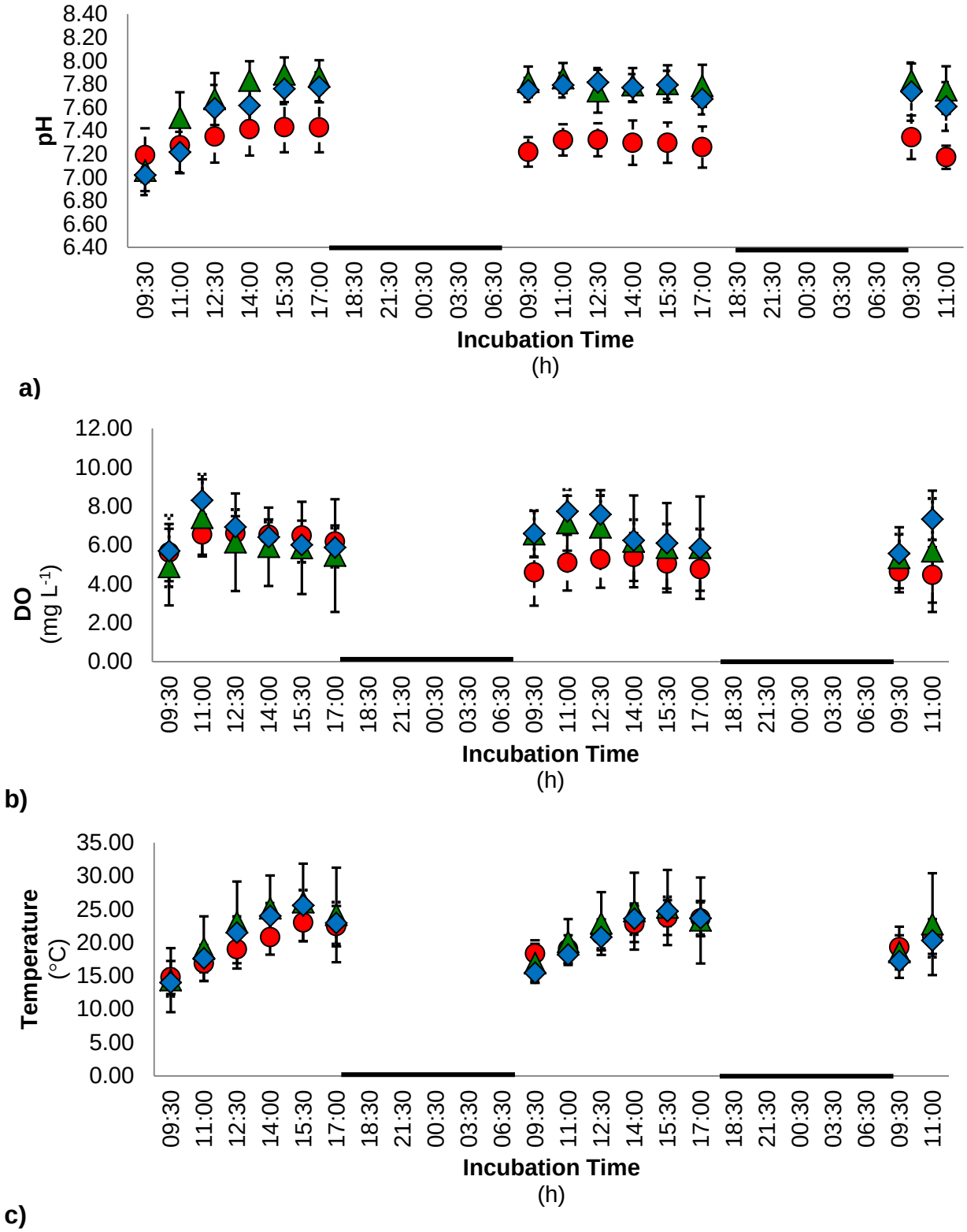


Figure 4.1: Monitored in situ pond conditions for the model HRAPs ($HRAP_D$ (●),$HRAP_D$ + SIP (◆) and $HRAP_D$ + LIP (▲)) recorded at each collection interval over a 49.5 h incubation period. Conditions monitored included a) pH, b) DO (mg L^{-1}) and c) temperature (°C). Collection intervals were between 09:30 and 17:00. No measurements were recorded overnight (17:00 – 09:30,▪). Global solar irradiance ranged between 4.40 and 28.40 MJ m^{-2}. Standard error bars have been included for each model HRAP.

Solar irradiance presented here is a representation of the two full day mean irradiance reported throughout the 49.5 h. Global irradiance (global) measured from the Australian BOM ranged between 4.40 and 28.40 MJ m^{-2}; 15.85±4.43 MJ m^{-2} the mean. Similarly UVA and UVB ranged from 8.96 - 38.71 W m^{-2} and 0.10 – 1.14 W m^{-2}, the means, 20.03±5.14 W m^{-2} and 0.45±0.15 W m^{-2} respectively.

Figures 4.2 and 4.3 show the relationship between inactivation (K_D) and solar irradiance (Global and UVA) across the six experiments with the $HRAP_D$, $HRAP_D$ + SIP and $HRAP_D$ + LIP. In general, inactivation was shown to be higher with increased solar irradiance, with the relationship significant ($p<0.05$) (Appendix 5.4). A significant relationship had not been identified with UVB for any of the model systems ($p>0.05$) (Appendix 5.4)

Elevated water temperatures associated with increased solar exposure was believed responsible for the increase in removal observed in the $HRAP_D$, with the water not directly exposed to sunlight. Figures 4.4 and 4.5 demonstrate the relationship between solar exposure, water temperature and F-Specific phage inactivation. Multiple linear regression indicated the relationship between the monitored parameters for the $HRAP_D$ i.e. F-Specific phage inactivation (K_D, log_{10} h^{-1}), solar irradiance (S, MJ m^{-2}) and water temperature (T, °C) was significant ($p<0.01$, actual $p=0.002$, $R^2=0.983$ and that approximately 98.3% of the variation can be explained by the model presented in Equation 4.1 for $HRAP_D$

$$MS2\ inactivation\ (K_d) = 0.00229\,S + 0.01404\,T - 0.21763$$

Equation 4.1

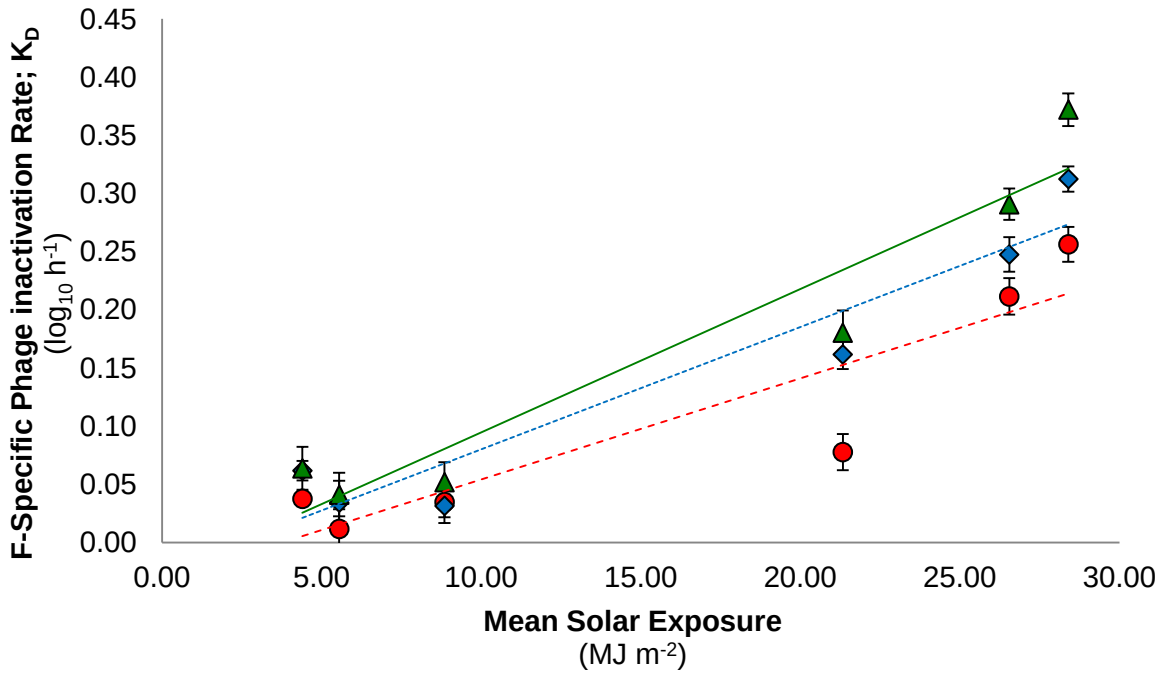


Figure 4.2: F-Specific phage inactivation rate constant, K_D (log_{10} h^{-1}) against daily mean solar (global) irradiance after 49.5 h incubation in the model HRAPs: $HRAP_D$ (●),$HRAP_D$ + SIP (◆) and $HRAP_D$ + LIP (▲). Liner trend line was incorporated with the corresponding R^2 and p values as follows; $HRAP_D$ + LIP: R^2 =0.9196, p=0.0073; $HRAP_D$ + SIP: R^2 = 0.916, p=0.003 and $HRAP_D$: R^2 = 0.836, p=0.011

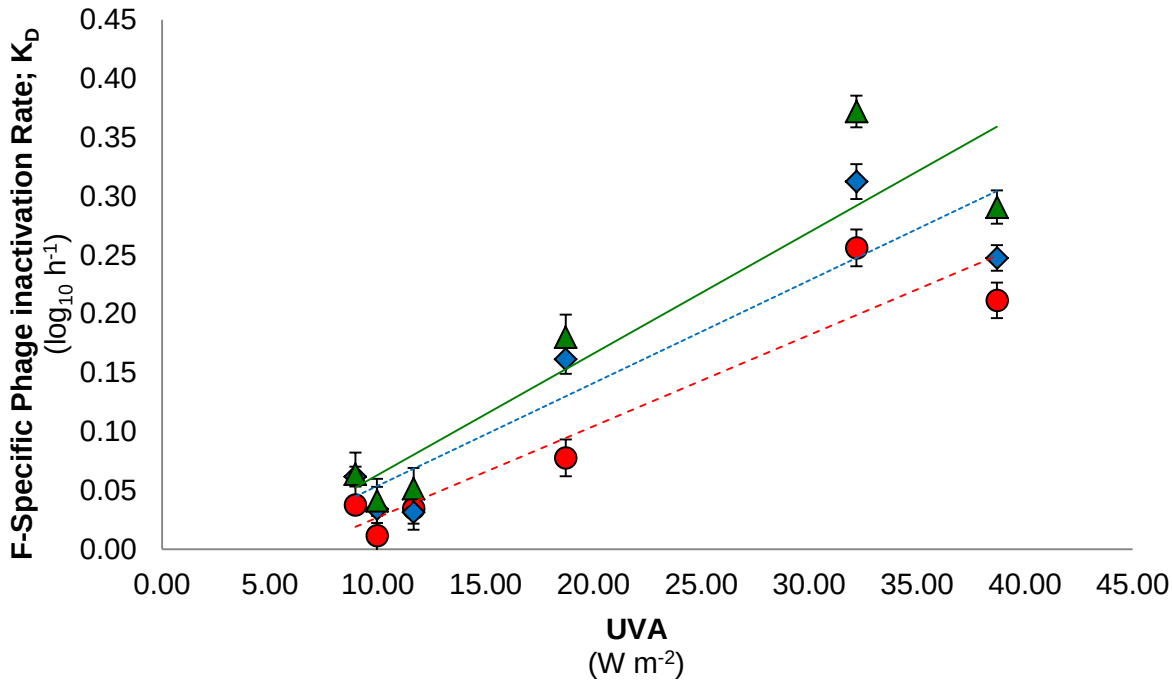


Figure 4.3: F-Specific phage inactivation rate constant, K_D (log_{10} h^{-1}) against UVA (W m^{-2}) after 49.5 h incubation in the model HRAPs: $HRAP_D$ (●),$HRAP_D$ + SIP (◆) and $HRAP_D$ + LIP (▲). Liner trend line was incorporated with the corresponding R^2 and p values as follows; $HRAP_D$ + LIP: R^2 =0.864, p=0.007; $HRAP_D$ + SIP: R^2 = 0.895, p=0.0086 and $HRAP_D$: R^2 = 0.895, p=0.004

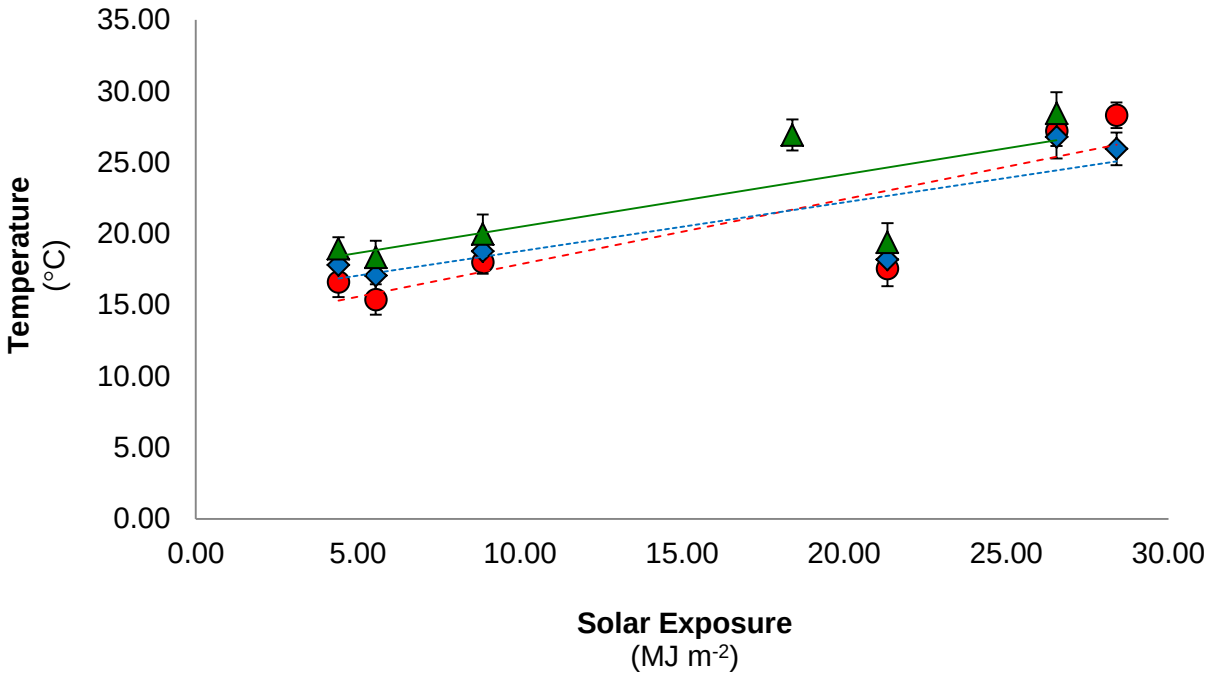


Figure 4.4: Relationship between water temperature (°C) and solar exposure (MJ m^{-2}) after 49.5 h incubation in the model HRAPs: $HRAP_D$ (●),$HRAP_D$ + SIP (◆) and $HRAP_D$ + LIP (▲). Liner trend line was incorporated the corresponding R^2 and p values as follows; $HRAP_D$ + LIP: R^2 =0.711, p=0.036; $HRAP_D$ + SIP: R^2 0.716, p=0.034 and $HRAP_D$: R^2 = 0.755, p=0.025.

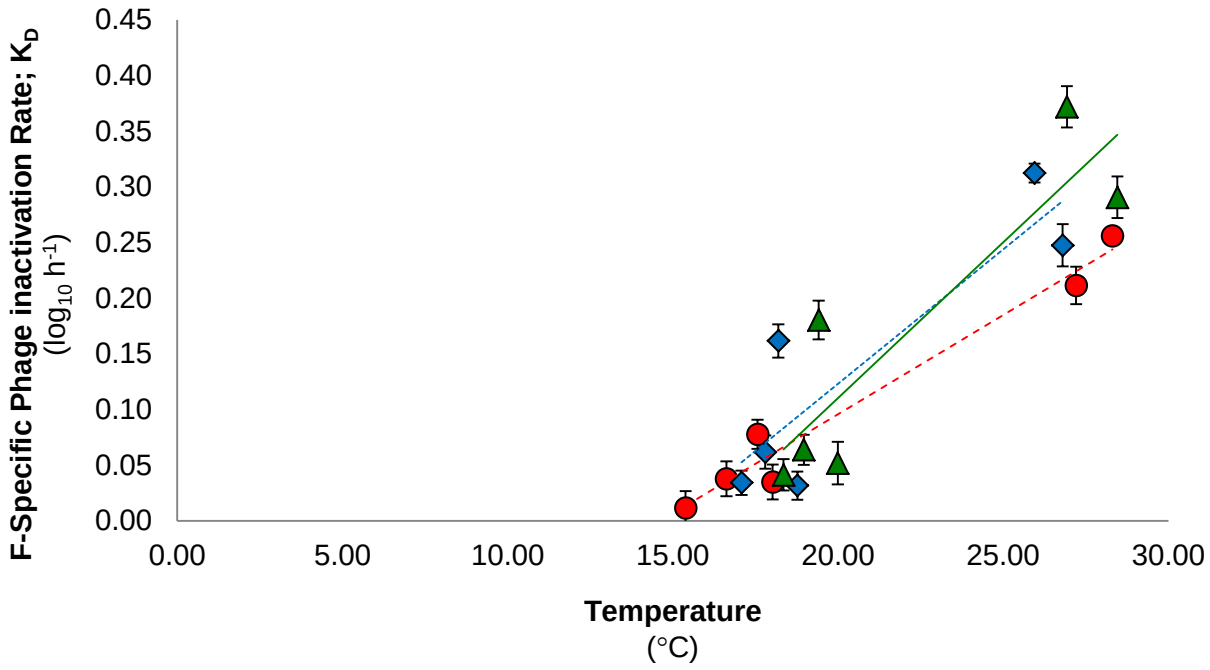


Figure 4.5: Relationship between water temperature (°C) and solar exposure (MJ m^{-2}) after 49,5 h incubation in the model HRAPs: $HRAP_D$ (●),$HRAP_D$ + SIP (◆) and $HRAP_D$ + LIP (▲). Liner trend line was incorporated the corresponding R^2 and p values as follows; $HRAP_D$ + LIP: R^2 =0.799, p=0.016; $HRAP_D$ + SIP: R^2 0.793, p=0.017 and $HRAP_D$: R^2 = 0.970, p=0.0004.

The physicochemical composition of the water at the completion of treatment was measured and the means reported in Table 4.9. Elevated chl *a* levels were observed in the $HRAP_D$ + IP with concentrations 1.6 and 1.4 times greater in the $HRAP_D$ + LIP (0.76±0.24 mg L^{-1}) and the $HRAP_D$ + SIP (0.71±0.17 mg L^{-1}) than the $HRAP_D$ (0.49±0.09 mg L^{-1}), respectively. Analysis indicated there were no significant differences between the model systems ($p>0.05$) (Appendix 5.3: Table A5.17). Similarly, there was no statistical difference observed between configurations for the other parameters tested, including SS and turbidity.

Furthermore, the concentrations reported in Table 4.9 were found to be lower, but not significantly from the starting concentrations reported in Table 4.10 for the Mt Barker treated wastewater (before F-Specific phage was inoculated) ($p>0.05$) (Appendix 5.3: Table A5.18). Table 4.11 reports the difference between final and starting concentrations.

Table 4.9: In situ water conditions in model HRAPs after 49.5 h incubation. Values are a representative from all experiments conducted, more specifically pH, DO and temperature are indicative of the mean levels across the pond systems

Parameter	$HRAP_D$				$HRAP_D$ + SIP				$HRAP_D$ + LIP			
	n	Mean±SE	Min.	Max.	n	Mean±SE	Min.	Max.	n	Mean±SE	Min.	Max.
pH	14	7.31±0.02	7.17	7.43	14	7.64±0.06	7.02	7.82	14	7.73±0.06	7.06	7.88
DO (mgL^{-1})	14	5.51±0.21	4.60	6.60	14	6.58±0.23	5.55	8.30	14	6.08±0.19	4.86	7.40
Temperature (°C)	14	20.40±0.71	14.77	23.75	14	20.70±0.98	13.98	25.57	14	21.92±0.94	14.37	26.03
Turbidity (NTU)	6	66.83±6.86	53.00	89.00	6	67.83±6.47	49.00	93.00	6	75.33±17.67	27.00	141.0
SS (mgL^{-1})	6	38.40±5.97	21.60	56.00	6	46.40±5.69	25.20	60.00	6	53.33±12.45	26.00	104.80
Chl *a* (mgL^{-1})	5	0.49±0.0.09	0.18	0.75	5	0.71±0.17	0.34	1.30	5	0.76±0.24	0.312	1.460
TOC (mgL^{-1})	6	27.20±4.80	15.59	43.22	6	27.15±5.54	15.44	44.15	6	33.27±7.77	11.44	63.04
TC (mgL^{-1})	6	39.60±15.29	25.13	60.26	6	44.77±7.76	22.63	71.30	6	47.01±8.50	24.34	71.31
IC (mgL^{-1})	6	12.40±2.24	5.35	19.58	6	17.62±7.56	3.54	53.94	6	13.74±2.47	8.26	23.37
TN (mgL^{-1})	6	33.61±1.37	28.00	37.65	6	36.24±2.38	27.97	44.20	6	35.07±1.61	29.06	41.01

Table 4.10: Composition of Mt Barker Inlet wastewater prior to treatment in model HRAPs

Parameter	n	Mean±SE	Min.	Median	Max.
Turbidity (NTU)	6	78.83±7.23	62.00	75.00	104.00
SS (mg L^{-1})	6	47.73±6.47	30.30	44.40	69.20
Chl *a* (mg L^{-1})	5	0.78±0.15	0.42	0.71	1.35
TOC (mg L^{-1})	6	29.91±4.88	15.39	30.07	46.10
TC (mg L^{-1})	6	50.66±7.30	24.52	50.91	71.00
IC (mg L^{-1})	6	20.74±3.94	9.13	19.65	33.62
TN (mg L^{-1})	6	31.78±2.77	23.54	32.11	38.99

Table 4.11: Difference between starting (Mt Barker inlet) and final in situ concentrations in the model HRAPs after 49.5 h. Arrows are indicative of the increase or decrease in concentrations.

Parameter	$HRAP_D$	$HRAP_D$ + SIP	$HRAP_D$ + LIP
Turbidity (NTU)	12.00 ↓	11.00 ↓	3.50 ↓
SS (mgL^{-1})	17.50 ↓	22.30 ↓	28.0 ↓
Chl *a* (mgL^{-1})	9.33 ↓	1.33 ↓	5.60 ↓
TOC (mgL^{-1})	2.72 ↓	1.09 ↓	3.36 ↑
TC (mgL^{-1})	11.06 ↓	5.89 ↓	3.65 ↓
IC (mgL^{-1})	8.35 ↓	3.13 ↓	7.00 ↓
TN (mgL^{-1})	1.83 ↑	4.46 ↑	3.29 ↑

4.3.4 Hydraulic loading rate

Alterations to the IP operating conditions were performed to further improve removal rates. Adjustment to the hydraulic loading rate (HLR) of the IP was achieved through flow rate

manipulation. Operation of the SIP and LIP at constant HLRs resulted in F-Specific phage $LRV_{49.5}$ that were statistically similar ($p>0.05$) (Appendix 5.3: Table A5.19). This observation was consistent across four examined HLRs. Table 4.12 provides details of the resultant inactivation rates and the in situ pond conditions associated with each HLR. Unfortunately, DO probe was unavailable throughout HLR 1 and HLR 2. It should also be noted that for HLRs 2 and 4 the log_{10} linear + shoulder and tail inactivation curve was used (refer to Table 2.2 Chapter 2 for the model), as it was identified to be a more appropriate fit.

The corresponding global solar irradiances were 8.85±7.55 (HLR 1; 167.8 L m^{-2} h^{-1}), 4.40±2.50 (HLR 2; 232.7 L m^{-2} h^{-1}), 5.55±4.35 MJ m^{-2} (HLR 3; 300.0 L m^{-2} h^{-1}) and 18.45±3.05 (HLR 4; 350.3 L m^{-2} h^{-1}). Similarly the onsite UVA and UVB irradiances were 11.66±2.42 W m^{-2} and 0.30±0.08 W m^{-2} (HLR 1), 8.96±1.93 W m^{-2} and 0.10±0.03 W m^{-2} (HLR 2), 9.97±1.68 W m^{-2} and 0.15±0.04 W m^{-2} (HLR 3) and 16.51±3.34 W m^{-2} and 0.41±0.13 W m^{-2} (HLR 4), respectively.

The best overall inactivation (LRV) was observed for HLR 4; 350.3 L m^{-2} h^{-1} with $LRV_{49.5}$ of 2.87 and 2.85 for the $HRAP_D$ + SIP and $HRAP_D$ + LIP, respectively (Table 4.12). Solar irradiance was believed responsible for the higher removal rates observed during HLR 4 (18.5±4.3 MJ m^{-2}) with the recorded global irradiance found to be 2.1, 4.2 and 3.4 times higher than was observed for the other HLRs.

Table 4.12: Corresponding F-Specific phage die-off rates (K_D; log_{10} h^{-1} and $LRV_{49.5}$; log_{10} PFU 100 mL^{-1}) and pond conditions for the $HRAP_D$ + SIP and $HRAP_D$ + LIP when operated at similar hydraulic loading rates (HLR; L m^{-2} h^{-1}), different flow rates (Q; L h^{-1}).

IP	HLR		Q	Inactivation		Pond Conditions		
				$LRV_{49.5}$	$K_D \pm SE$	pH	DO (mg L^{-1})	Temperature (°C)
SIP	1	167.8±2.4	62.1±0.9	0.82	0.03±0.13	7.41±0.07	-	18.77±1.16
	2	232.7±4.9	86.1±1.8	1.07	0.56±0.16	7.70±0.08	9.20± 0.25	17.80±0.79
	3	300.0±0.8	110.0±0.3	0.91	0.05±0.01	7.75±0.09	6.19±0.46	17.08±1.25
	4	350.3±8.4	129.6±3.1	2.87	0.16±0.02	7.07±0.11	-	17.57±1.01
LIP	1	174.4±1.4	130.8±1.2	0.98	0.05±0.02	7.43±0.07	-	19.99±1.34
	2	232.8±0.8	174.6±0.6	1.20	0.45±0.12	7.68±0.08	8.72±0.20	18.96±0.80
	3	300.0±0.8	225.0±0.6	1.16	0.08±0.01	7.87±0.08	5.78±0.37	18.35±1.16
	4	350.3±1.2	262.7±0.9	2.85	0.16±0.03	7.15±0.08	-	18.56±1.10

A correlation matrix (Pearson's product moment, r) was performed to see whether there was a statistically significant relationship between F-Specific phage die-off in the model systems and various operating conditions, including HLR, flow rate, IP length and IP cyclic rate (Table 4.13). A strong correlation was identified between HLR and F-Specific phage die-off for both LRV (r=0.993) and K_D (r=-0.986). LRV was also shown to be broadly correlated with IP length (r=-0.354). This correlation was not observed for K_D (r=-0.098).

Table 4.13: Correlation Matrix of inactivation in both IP systems and design parameters

	Pearson's Correlation Matrix					
	LRV	**K_D** ($\log_{10}$ h^{-1})	**IP Length** (m)	**Cycles** (h^{-1})	**Flow Rate** (L h^{-1})	**HLR** (L m^{-2} h^{-1})
LRV	1.000 [a]					
K_D	-0.890 [a]	1.000 [a]				
IP Length	-0.354 [b]	-0.098 [c]	1.000 [a]			
Cycles	-0.004 [c]	-0.421 [b]	0.929 [a]	1.000 [a]		
Flow Rate	-0.004 [c]	-0.421 [b]	0.929 [a]	1.000 [a]	1.000 [a]	
HLR	0.933 [a]	-0.986 [a]	0.006 [c]	0.357 [b]	0.357 [b]	1.000 [a]

[a] strong correlation; if |r| is between 0.85 and 1.00
[b] weak correlation; if |r| is between 0.50 and 0.85
[c] no correlation; if |r| is between 0.10 and 0.00

A stepwise multiple regression indicated a significant relationship existed between LRV, incubation time (t; h), HLR (L m^{-2} h^{-1}), pH and water temperature (T; °C) ($p<0.05$, actual $p < 2.2 \times 10^{-16}$, R^2 = 0.759). From this approximately 75.9% of the variation can be explained by the model outlined in Equation 4.2

$$LRV = -3.258 - 0.031\ t - 0.004\ HLR + 0.738\ pH - 0.066\ T$$

Equation 4.2

Where, $LRV = N_t/N_0$, where N_t is the number of phage at time t and N_0 is the starting concentration.

4.3.5 Incubation Time

The relationship between LRV and incubation time is shown in Figure 4.6. Inactivation was again shown to be increased in the $HRAP_D$ and $HRAP_D$ + IP with prolonged incubation. After four repetitions, with the IPs operated at HLR 300 L m^{-2} h^{-1}, the mean LRV_{5d} were 1.88±0.14 ($HRAP_D$), 2.85±0.14 ($HRAP_D$ + SIP) and 3.05±0.39 ($HRAP_D$ + LIP), respectively (Table 4.14). Again the systems incorporating IPs exhibited the greater removal of the two conditions tested.

Statistical analysis (Tukey's post hoc test) identified a significant difference in mean LRV between the $HRAP_D$ and both IP lengths after 5 d incubation ($p<0.05$). Corresponding p-values were; $p=0.039$ (95% CI: 0.03 to 0.93) for $HRAP_D$ vs. $HRAP_D$ + SIP and $p=0.007$ (95% CI: 0.26 to 1.16) for $HRAP_D$ vs. $HRAP_D$ + LIP, respectively (Table 4.15). A significant difference was again not observed between the two slopes ($p>0.05$, actual $p=0.341$: 95% CI: -0.22 to 0.65) (Table 4.15).

In addition, the similarity in removal observed with the IPs after 5 d suggests the influence HLR has on the outcome with the IP is unaffected by incubation time.

Both daily and hourly inactivation rates were found to be similar in the model HRAPs after the extended incubation (i.e. 5 d) ($p>0.05$) (Table 4.16). Nonetheless, inactivation was always observed faster with the IP present (Table 4.14). This was also true when solar exposure and water temperature were controlled (data not provided).

The in situ conditions after the 5 d incubation are presented in Table 4.17. Solar irradiances ranged between 10.4 and 21.2 MJ m^{-2} (mean; 16.36±1.79 MJ m^{-2}). Throughout the four experiments hours of sunshine (h) was recorded. On average the model HRAPs were exposed to 7.11±1.42 h of sunlight across the 5 d.

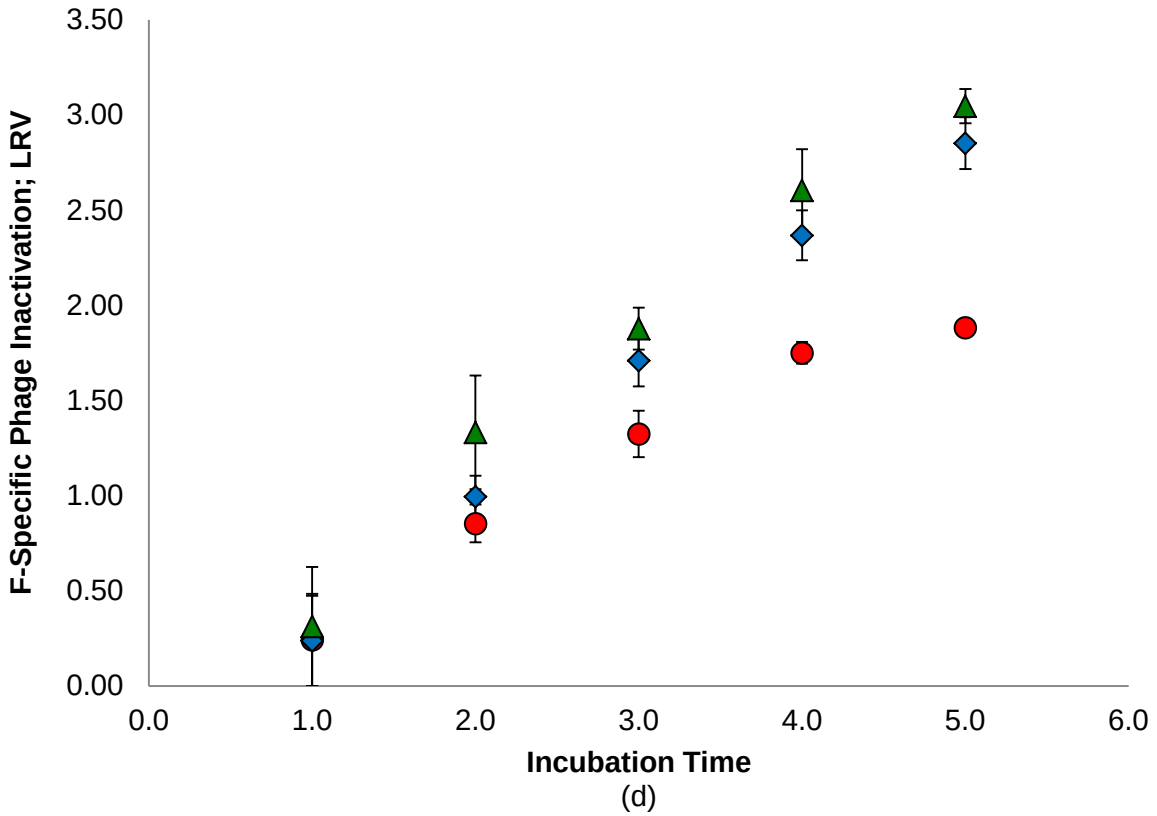


Figure 4.6: Relationship between inactivation and incubation time in the model HRAPs: $HRAP_D$ (●), $HRAP_D$ + SIP (◆) and $HRAP_D$ + LIP (▲) after 5 d. Standard error bars have been included.

Table 4.14: F-Specific phage die-off for model HRAPs; $HRAP_D$, $HRAP_D$ + SIP and $HRAP_D$ + LIP after 5 d incubation. Log removal values (LRV; log_{10} PFU 100 mL^{-1}) and inactivation rate constants (K_D; log_{10} h^{-1}) are reported, with both daily (log_{10} d^{-1}) and hourly (log_{10} h^{-1}) K_D values are presented.

Time	Model System	F-Specific phage Inactivation Rates			
		n	$K_D \pm SE$ (log_{10} h^{-1})	$K_D \pm SE$ (log_{10} d^{-1})	$LRV_{5d} \pm SE$
5 d	$HRAP_D$	6	0.06±0.00	1.00±0.34	1.88±0.14
	$HRAP_D$ + SIP	6	0.08±0.01	1.74±0.14	2.85±0.14
	$HRAP_D$ + LIP	6	0.08±0.01	1.82±0.14	3.05±0.39

Table 4.15: Tukey's post hoc comparison of variance between mean F-Specific phage log reduction values (LRV; log_{10} PFU 100 mL^{-1}) in the $HRAP_L$ and $HRAP_L$ +IP (SIP and LIP) model systems after 5 d incubation. Statistical significance at p<0.05

Parameter	Model HRAP		Tukey multiple comparison of means 95% family-wise confidence level				
			Difference	Lower	Upper	p-value	Sig.
LRV_{5d}	$HRAP_D$+SIP	$HRAP_D$	0.48	0.03	0.93	0.039	*
	$HRAP_D$+LIP	$HRAP_D$	0.71	0.26	1.16	0.007	**
	$HRAP_D$+LIP	$HRAP_D$+SIP	0.22	-0.22	0.65	0.341	-

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01

Table 4.16: Tukey's post hoc comparison of variance between hourly and daily inactivation rates (K_D: log_{10} h^{-1} and log_{10} d^{-1}) in the $HRAP_L$ and $HRAP_L$ +IP (SIP and LIP) model systems after 5 d incubation. Statistical significance at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level							
Comparison	Model Systems		Difference	Lower	Upper	p-Value	Sig.
K_D (log_{10} h^{-1})	$HRAP_D$+SIP	$HRAP_D$	0.01	-0.01	0.04	0.226	-
	$HRAP_D$+LIP	$HRAP_D$	0.02	-0.01	0.04	0.180	-
	$HRAP_D$+SIP	$HRAP_D$+LIP	0.001	-0.02	0.02	0.986	-
K_D (log_{10} d^{-1})	$HRAP_D$+SIP	$HRAP_D$	0.78	-0.11	1.64	0.087	-
	$HRAP_D$+LIP	$HRAP_D$	0.82	-0.08	1.71	0.074	-
	$HRAP_D$+SIP	$HRAP_D$+LIP	0.04	-0.86	0.93	0.993	-

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01

Table 4.17: Mean in situ water conditions in model HRAPs after 5 d incubation.

Parameter	$HRAP_D$				$HRAP_D$ + SIP				$HRAP_D$ + LIP			
	n	Mean±SE	Min.	Max.	n	Mean±SE	Min.	Max.	n	Mean±SE	Min.	Max.
pH	34	7.94±0.02	7.72	8.12	34	8.12±0.03	7.66	8.42	34	8.17±0.03	7.80	8.40
DO (mgL^{-1})	34	3.92±0.32	1.01	6.88	34	6.48±0.65	0.90	11.00	34	6.35±0.62	0.93	10.68
Temperature (°C)	34	17.34±0.78	9.70	27.10	34	16.78±0.77	9.60	24.80	34	17.32±0.76	9.60	25.9
Turbidity (NTU)	5	37.20±9.19	11.00	54.00	5	33.80±5.95	15.00	45.00	5	51.60±16.56	3.0	97.0
SS (mgL^{-1})	5	23.92±6.18	2.80	38.80	5	12.72±2.20	6.00	18.40	5	36.88±18.01	5.60	106
Chl *a* (mgL^{-1})	4	0.10±0.04	0.047	0.178	4	0.34±0.13	0.11	0.61	4	0.40±0.19	0.045	0.88
TOC (mgL^{-1})	3	16.03±1.93	12.43	19.01	3	20.94±1.12	18.72	22.34	3	25.29±1.24	22.83	26.72
TC (mgL^{-1})	3	36.30±5.69	24.97	42.89	3	33.22±7.85	24.71	48.89	3	44.87±8.03	32.55	59.95
IC (mgL^{-1})	3	20.27±7.36	5.96	30.46	3	12.28±7.46	3.71	27.14	3	19.58±7.21	9.73	33.62
TN (mgL^{-1})	3	54.99±10.02	36.21	70.42	3	42.98±5.65	37.08	54.28	3	28.56±6.68	15.97	38.73

4.3.6 Effect of water type

Concurrent operation of model systems in turbid (wastewater) and clear (tap) water indicated inactivation in the model systems was influenced by water type. For comparison under identical operating conditions, examination was performed using the SIP only. A second LIP was unavailable. Figure 4.7 shows the variation in removal for the different waters, with wastewater exhibiting the slower removal. For simplicity F-Specific phage was used to quantify die-off in both water types, despite MS2 being the only organism present in the optically clear water.

Faster inactivation was observed in both water types when the IP was present. However, the tap water operated $HRAP_D$ + SIP exhibited the highest removal overall with a $LRV_{7.5}$ 1.99±0.32 (Table 4.18). In general, inactivation was found to be highest in the order of $HRAP_D$ + SIP (TW) > $HRAP_D$ (WW) > $HRAP_D$ + SIP (WW) > $HRAP_D$ (WW).

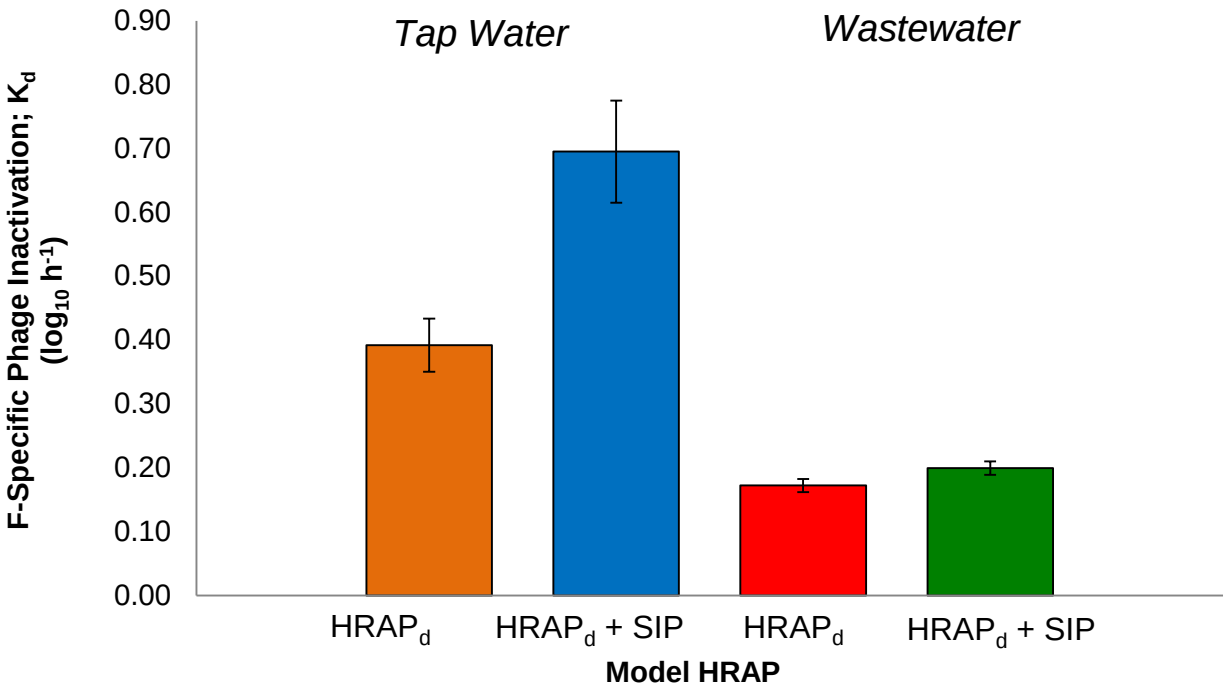


Figure 4.7: Inactivation rates (K_D, log_{10} h^{-1}; mean ± SE) for model systems operated in turbid (wastewater) and clear (tap) waters after 24 h. Solar irradiation ranged from 5.6 MJ m^{-2} to 10.8 MJ m^{-2}. Mean water temperatures ranged from 10.8 to 28.1°C in the tap water and 10.3 to 27.3°C in the wastewater.

Table 4.18: Comparison of F-Specific phage inactivation rates (K_D, log_{10} h^{-1}) and LRV (log_{10} PFU 100 mL^{-1}) achieved in the model HRAPs for optically clear (tap) and turbid (wastewater) waters. Incubation period was 24 h.

Water Type	Model System	Individual	
		$LRV_{7.5}$ ± SE	K_D ± SE
Tap Water	$HRAP_D$	1.20±0.13	0.39±0.06
	$HRAP_D$ + SIP	1.99±0.32	0.70±0.11
Wastewater	$HRAP_D$	0.51±0.06	0.17±0.02
	$HRAP_D$ + SIP	0.69±0.12	0.20±0.02

[a] LRV were determined after 7.5 h incubation

Table 4.19 presents the results of a Tukey HSD post hoc test comparing the mean inactivation rates (K_D; log_{10} h^{-1}) of the different treatment conditions. A significant difference was only detected between the HRAP + SIP (TW) and the other three conditions; $HRAP_D$ (TW) $p<0.05$, $HRAP_D$ + SIP (WW) $p<0.01$ and $HRAP_D$ (WW) $p<0.05$ (Table 4.19). Covariate analysis (ANCOVA) found inactivation was again significant in the model HRAPs when the effects of solar exposure were accounted for [$F(3, 3) = 85.59$, $p<0.001$, actual $p=0.0002$, $\eta 2 = 0.99$] (Appendix 5.2: Table A5.13). Based on the resulting R^2 of the model 99% of the variation in K_D can be attributed to model HRAP and solar exposure, with the former considered to be the most influential (Appendix 5.2: Table A5.13).

Similarly, when a Tukey HSD post hoc test was performed on LRV obtained over a 7.5 h incubation period the only comparison identified to be significant was the one between $HRAP_D$ + SIP (TW) and $HRAP_D$ (WW) ($p<0.05$, actual $p=0.027$; 95% CI: 0.11 to 1.25) (Appendix 5.3: Table A5.20). Again, the remaining comparisons were flagged to be insignificant ($p>0.05$) (Appendix 5.3: Table A5.20).

For the different test conditions the monitored pond conditions are summarised in Table 4.20. Levels were found to be consistent across all systems and waters, but were predominantly higher in the tap water systems. The $HRAP_D$ (WW) exhibited the lowest concentrations overall. Solar irradiances recorded ranged from 5.6 – 10.8 MJ m^{-2} for global irradiance (BOM), 0.68 - 23.0 W m^{-2}

for UVA and 0.01 - 0.48 W m^{-2} for UVB. The means were; 8.20±2.60 MJ m^{-2} (global), 9.50±1.91 W m^{-2} (UVA) and 0.11±0.04 W m^{-2} (UVB), respectively.

Table 4.19: Tukey's post hoc comparison of variance between F-Specific phage inactivation rates (K_D; log_{10} h^{-1}) obtained in model systems operated in different water types after 24 h incubated. Model systems examined included both a $HRAP_D$ and $HRAP_D$ + SIP operated in optically clear (tap) water or wastewater. Statistical significance was at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level						
Model Systems		**Difference**	**Lower**	**Upper**	**P-Value**	**Sig.**
$HRAP_D$ + SIP (TW)	$HRAP_D$ (TW)	0.30	0.02	0.59	0.042	*
$HRAP_D$ (WW)	$HRAP_D$ (TW)	-0.22	-0.51	0.07	0.111	-
$HRAP_D$ + SIP (WW)	$HRAP_D$ (TW)	-0.19	-0.48	0.09	0.158	-
$HRAP_D$ + SIP (TW)	$HRAP_D$ (WW)	0.52	0.24	0.81	0.006	**
$HRAP_D$ + SIP (TW)	$HRAP_D$ + SIP (WW)	0.50	0.21	0.78	0.007	**
$HRAP_D$ + SIP (WW)	$HRAP_D$ (WW)	0.03	-0.26	0.31	0.978	-

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01

Table 4.20: In situ pH, DO and water temperature for model systems operated in tap and wastewater under dark incubation. Onsite solar irradiances ranged from 0.68 – 23.0 W m^{-2} (UVA) and 0.01 – 0.48 W m^{-2} (UVB) with the means 9.50±1.91 W m^{-2} and 0.11±0.04 W m^{-2}.

Water Type	**Model System**	**pH**					**DO (mg L^{-1})**					**Temperature (°C)**				
		n	Mean	SE	Min	max	n	Mean	SE	Min	max	n	Mean	SE	Min	max
Tap Water	$HRAP_D$	14	8.17	0.04	7.80	8.36	14	3.69	0.44	0.19	5.78	14	21.6	**1.13**	13.1	28.1
	$HRAP_D$ + SIP	14	8.17	0.04	7.76	8.30	14	3.69	0.40	0.39	5.86	14	19.4	**1.36**	10.8	26.8
Wastewater	$HRAP_D$	14	7.76	0.05	7.23	8.03	14	2.92	0.44	0.18	5.34	14	18.9	**1.46**	10.8	26.8
	$HRAP_D$ + SIP	14	8.10	0.06	7.55	8.40	14	3.60	0.06	0.72	6.83	14	18.8	**1.60**	10.3	27.3

4.4 Discussion

This chapter continued to investigate MS2 inactivation in model pond systems where water was run over an inclined plane (IP); exposed to sunlight. Examination was undertaken in turbid wastewater and where the bulk water was dark incubated. IPs of different surfaces areas were used with die-off compared against those achieved in a dark incubated $HRAP_D$ with no IP. The results obtained could provide information crucial to the adaption of the system into the field. Operation of the ponds in the dark was considered ideal for establishing the contribution of the IP towards disinfection. Owing to the potential for native phage present within the wastewater MS2 was reported as F-Specific phage.

4.4.1 F-Specific phage inactivation in model HRAPs; $HRAP_D$ and $HRAP_D$ + SIP

Addition of an IP was found to have a positive impact on F-Specific phage die-off in optically clear water (Chapter 3). A similar relationship was observed when the IP was applied to model HRAPs operated with wastewater. Inclusion of the SIP resulted with a 30% increase in the LRV obtained following 7.5 h incubation (i.e. $LRV_{7.5}$ 0.86) compared to the corresponding $HRAP_D$ without the IP ($LRV_{7.5}$ 0.66). Although, the improvement was not always found to be significantly different from the $HRAP_D$ after 7.5 h it was enough to imply the plausibility of the system, with a significant difference predicted with modification to the operation time and slope size.

The F-Specific phage inactivation rates presented in Table 4.3 for the $HRAP_D$ (0.24 ± 0.07 $\log_{10}$ h^{-1}) and the $HRAP_D$ + SIP (0.30 ± 0.08 $\log_{10}$ h^{-1}) were identified as being either significantly higher than or equivalent to the wastewater F-RNA phage removal rates reported in the literature (no IP). For instance; Yu (2015) reported MS2 die-off rates of 0.04 h^{-1} and 0.30 ± 0.02 h^{-1} in raw wastewater when dark incubated or exposed to UVB at an irradiance of 1 W m^{-2}. The conditions in which the two studies were performed may of had an impact on the removal rates observed with the Yu (2015) study being performed in specialised light cabinets where the environmental conditions were controlled and maintained constant, opposed to the model systems used here which were subject to environmental and climatic variations brought about by the exposure to the elements.

Similarly, Sinton *et al.* (2002) reported F-RNA phage die-off from WSP effluent as 0.01 h^{-1} (dark), 0.05 h^{-1} (winter sunlight) and 0.07 h^{-1} (summer sunlight), which was again found to be significantly lower than those presented here.

Results demonstrated inactivation in the model systems to be best represented by the log_{10} linear + tail inactivation model. This model was also found to best describe phage inactivation in tap water (Chapter 3), reservoir water (Misstear and Gill, 2012) and wastewater (Yu, 2015).

4.4.2 Modification to IP length

To improve the removal performance with the IP, modification to the IP length and exposure surface area was examined. Previously, the longer IP length failed to further improve inactivation in tap water (Chapter 3). The absence of suspended solids in the tap water may be responsible since there was minimal attenuation and further light exposure did not increase inactivation. In wastewater however, suspended solids are more abundant and the effects of attenuation more prominent (Kirk, 1994; Fallowfield *et al.*, 1996). Consequently, it was thought that running the model HRAPs with the LIP in the turbid water would help to increase removal rates, as the volume of water exposed to sunlight at the reduced volume (thin film) is likely larger on the longer IP.

Results were found similar to Chapter 3, with no further improvement in F-Specific phage inactivation with the LIP inclusion despite the obvious increase in $LRV_{7.5}$ shown in Table 4.5. The increase in $LRV_{7.5}$ in the $HRAP_D$ + LIP ($LRV_{7.5}$ 0.76) was found to be 117 and 33% higher than the $HRAP_D$ ($LRV_{7.5}$ 0.35) and $HRAP_D$ + SIP ($LRV_{7.5}$ 0.57), respectively. The large difference in removals observed between the $HRAP_D$ and $HRAP_D$ + IP continue to provide evidence in support of the IP inclusion as a beneficial addition for enhanced treatment.

The same result was again observed when incubation time was increased to 49.5 h. This time however, the difference in removal between the $HRAP_D$ + IP and $HRAP_D$ was significant (Table 4.6); thereby suggesting that for turbid water, increased incubation duration was beneficial to IP performance.

Interestingly, the opposite was found to be true for the obtained inactivation rates, with significantly faster K_D values identified with the presence of the IP ($HRAP_D$ + LIP) after 24 h but not after 49.5 h. This similarity with the increased incubation time could be a consequence of the phage being rapidly decreased within the first 24 h however, as numbers declined inactivation slowed, potentially due to the presence of a more resistant sub population (as indicated by the tail in the respective die-off curves). This may have enabled removal in the non IP system to catch up.

Studies have found that viruses and phage are capable of adsorbing to particles (i.e. suspended solids) in wastewater, with this adsorption revisable (Sobsey and Cooper, 1973). In addition, the literature has also suggested that phage adsorption or aggregation may affect virus survival rate within the water column, with these process dependent on the amount of dissolved oxygen present (Ohgaki *et al.*, 1986; Mattle and Kohn, 2012). Consequently, this phage-particle association could provide a potential explanation as to why large die-off was also detected in the dark (covered) non-IP models. Such that it is possible that by covering theses systems (model HRAPs) other factors associated with the occurring microbiological processes were influenced as well as affecting the ability to detect phage concentrations using this double layer agar method.

4.4.3 In situ and environmental conditions

The physicochemical conditions of a pond such as pH, DO and temperature can have a strong impact on pathogen removal together with sunlight (Davies-Colley *et al.*, 1997). In the dark however, the influence of these parameters is less transparent and not as well documented (Curtis *et al.*, 1992; Davies-Colley *et al.*, 1999; Bolton *et al.*, 2010). Nonetheless, Davies-Colley *et al.* (1999) implied the harmful nature of these properties in WSPs with slower phage inactivation observed compared to sunlight. Other possible factors that influence dark inactivation processes include, predation, sedimentation, and lack of nutrients (Davies-Colley, 2005)

The relationship between pH and DO with Chl *a* is not uncommon in pond systems, with concentrations increased by the photosynthetic activity of the algae present in the water (Mara *et al.*,

1992a; Davies-Colley *et al.*, 1999; Tadesse *et al.*, 2004). What's more is these parameters, temperature included, are subject to diurnal variation with levels rising and falling with solar radiation, photosynthesis and bacterial respiration (Buchanan, 2014). Understandably, concentrations will be highest during the day when photosynthesis takes place and lower at night when photosynthesis stops but oxygen is still being consumed in algal and bacterial respiration (Pearson, 2005). Overnight conditions were not monitored during this investigation, however the trend observed in Figure 4.1 suggests this fluctuation took place in the model systems.

Similar to Chapter 3, faster F-Specific phage inactivation was observed in the model systems when solar irradiance was increased. This was found to be true for global and UVA irradiances measured onsite and from the Australian BOM, with a significant relationship identified for both (Figures 4.2 and 4.3). Temperature was considered to be responsible for the high removals observed in the dark incubated $HRAP_D$, with temperature known to both be increased with high solar irradiances and have a strong influence on solar inactivation of phage (Davies-Colley *et al.*, 1997; Davies-Colley *et al.*, 1999).

Chl *a*, turbidity and SS are known to have an impact on sunlight disinfection, contributing to light attenuation (Curtis *et al.*, 1994; Kirk, 1994; Bolton, 2012). Here, the highest concentrations reported were observed in the $HRAP_D$ + IP, but more specifically the $HRAP_D$ + LIP (i.e.; Chl *a*; 0.71±0.24 mg L^{-1}, SS; 53.33±12.45 mg L^{-1} and turbidity; 75.33±17.67 NTU. Interestingly, the highest F-Specific phage removal achieved was also observed in this model system. It is likely that the increased exposure to sunlight received on the IP resulted with greater algal growth which in turn may have contributed to the larger SS and turbidity concentrations.

4.4.4 The role of hydraulic loading rate

The hydraulics characteristics of a pond system have been reported to influence disinfection performance, including pathogen and pollutant removal (Herrera and Castillo, 2000; Von Sperling, 2005; Trang *et al.*, 2010; Dong *et al.*, 2011; Verbyla *et al.*, 2013; Verbyla and Mihelcic, 2015).

Hydraulic retention time (HRT), HLR and water depth were identified as important factors (Richardson and Nichols, 1985; Shuval, 1990; Mitsch and Gosselink, 1993; Kadlec and Knight, 1996), with HRT and depth the most broadly examined. Similarly, this study hypothesised IP HLR to influence F-Specific phage inactivation.

HLR refers to the volume of water that is passed over an exposed surface area during a set period of time. The time in which water and pathogens are exposed to sunlight is influenced by HLR, with HLR also impacting the contact time between water and slope. For instance, when the HLR is small the contact time and HRT will be fairly large, as the flow of water down the slope is reduced (Dong *et al.*, 2011). The opposite is true for larger HLR.

Results showed that for a given HLR, irrespective of IP length or surface area, IPs recorded inactivation rate that was statistically similar when operated under the same climatic conditions. Identifying this relationship is important for any subsequent up scaling to a larger field scale system or the adaption of the concept into another like pond system (i.e. WSP).

Equation 4.1 further supports the importance of incubation time, pH and water temperature on inactivation.

4.4.5 The relationship between inactivation and incubation time

The relationship between disinfection (solar and dark inactivation) and incubation time is strong, with the two correlated (Kirk, 1994; Al-Juboori *et al.*, 2010). The results from this study confirmed this relationship with increased die-off in the dark incubated $HRAP_D$ and solar exposed $HRAP_D$ + IP whenever incubation time was extended (Figure 4.6). This relationship was also confirmed in the model presented in Equation 4.1 (Section 4.3.4), which indicated the decrease in F-Specific phage numbers observed in the model systems was a function of time, HLR, pH and water temperature.

Earlier, it was observed that inactivation was increased in all systems when incubation time was increased from 7.5 h to 49.5 h. More specifically, Table 4.5 showed F-Specific phage die-off (LRV)

increased by 0.80, 1.18 and 1.42 log in the $HRAP_D$, $HRAP_D$ + SIP and $HRAP_D$ + LIP, respectively when for this incubation increase. The results also indicated a minimum of 49.5 h of treatment was required before removal was considered to be sufficiently higher than the non-IP system. Furthermore, complete inactivation was not observed in any systems for either incubation time. This was unlike the results presented in Chapter 3 which found 24 h sufficient enough for MS2 (F-Specific phage) to be undetectable in tap water. Figure 4.6 also identified increased inactivation in the systems, again complete removal was not observed.

For pond systems, incubation time or HRT refers to the amount of time the water is contained within the pond. In regards to inactivation mechanisms, HRT controls the time available for disinfection within the ponds (Davies-Colley, 2005). HRAPs typically have a HRT between 2-8 d (Oswald, 1996), with 5 d the HRT associated with the field based systems used in Chapter 6.

Increasing the incubation period to 5 d resulted with LRV_5 of 1.88±0.01 ($HRAP_D$), 2.85±0.14 ($HRAP_D$ + SIP) and 3.05±0.09 ($HRAP_D$ + LIP). These values were shown to agree with the recommended guidelines for virus and bacteriophage removal in treatment ponds (1.0 – 4.0 logs) as reported in the Australian guidelines for water recycling and by the World Health Organisation (NRMMC-EPHC-AHMC, 2006; WHO, 2006a).

By operating the systems at the 5 d incubation time, the likely removals expected for the field based system could be anticipated, and can also make comparison between the model systems and the field based systems possible. From these results it can be anticipated that when both pond and IP are solar exposed even larger inactivation rates could be observed with and without the IP.

Results for the 5 d continued to show the elevated inactivation with the IP inclusion, with a statistical difference identified between $HRAP_D$ + IP and $HRAP_D$. Differentiation between the two IP lengths was again not possible with similar removal rates observed. Based on the previous results, this outcome could be expected as both the IPs was again operated at the same HLR (300 L m^{-2} h^{-1}). This further supports the importance of HLR on F-Specific phage inactivation rates.

4.4.6 The effect of water type

Operation of the model systems in waters at opposite ends of the optical scale; i.e. optically clear (tap water, TW) and turbid (wastewater, WW) confirmed the influence water type has on F-Specific phage (MS2) inactivation rates. The latter having exhibited the slower inactivation rates of the two waters (Figure 4.7). A similar outcome was observed by Davies-Colley *et al.* (1999) and Kohn and Nelson (2007), who both reported higher inactivation rates for F-RNA phage in reverse osmosis (RO) water compared to WSP effluent.

Bolton (2012) examined light attenuation in different aquatic environments, reporting the variation to be large. Furthermore, Bolton (2012) indicated turbidity to be a key predictor of attenuation in pond systems with penetration depths found reduced with increased turbidity.

The slower inactivation observed in the wastewater systems was believed to be a consequence of the high turbidity and presence of algae, solids and particles in the water. These factors are all capable of manipulating light dispersion; contributing to attenuation and non-pathogen absorption (Curtis *et al.*, 1994).

Again, the presence of the IP was shown to have a positive impact on inactivation with elevated LRV and K_D values achieved in the two $HRAP_D$ + SIP (Table 4.18). The $HRAP_D$ + SIP (TW) however, exhibited the best removal overall. The resulting $LRV_{7.5}$ 2.9, 1.7 and 3.9 times higher than the $HRAP_D$ + SIP (WW), $HRAP_D$ (TW) and $HRAP_D$ (WW), respectively.

Furthermore, the success of the IP inclusion in both turbid and optically clear water implies the system as versatile with the capability of increased F-Specific phage inactivation across a broad spectrum of waters, thereby adding to the support of the IP as a beneficial addition to treatment strategies.

4.5 Conclusion

The inclusion of an IP to enhance pathogen removal in natural treatment systems was revisited in this chapter. Examination was again conducted in the absence of light, except on the IP. The results indicated an improvement in F-Specific phage inactivation was achieved in wastewater when the IP was present. This improvement supports the use of an IP as an effective treatment strategy for both optically clear and wastewaters. Overall, the following conclusions could be deduced

- Increased F-Specific phage inactivation was achieved whenever the IP was present for all conditions examined
- Increasing surface area of the IP failed to show a significant improvement to removal, but was still shown to be higher than when the system was operated without the IP
- Hydraulic loading rate was identified as an important factor that influenced inactivation rates and could be the key to adapting the system in the field.
- Incubation time was also found to be crucial for elevated inactivation with and without the IP
- Inclusion of the IP was found to be less effective in turbid water compared to optically clear water.

As the goal of this work was to apply the IP into the field, the next logical step would be to assess the IP performance when both pond and IP are solar exposed. This will be the focus for Chapter 5. It would also be worthwhile to gain an understanding of the removal efficiency of the IP when applied to more realistic pond conditions, including incorporation into a field based system. This will also give the focus for the progressing chapters.

5. IMPACT OF IP INCLUSION ON F-SPECIFIC PHAGE REMOVAL RATES IN FULLY SOLAR EXPOSED MODEL HRAPS OPERATED IN WASTEWATER

5.1 Introduction

Natural treatment ponds (HRAP, WSP) are large outdoor pond systems with the wastewater exposed to sunlight (Park *et al.*, 2011; Young, 2015); the primary disinfection processes of these systems (Mayo, 1995; Maynard *et al.*, 1999; Craggs *et al.*, 2004b). Diminished light availability with the water column however inhibits disinfection performance (Kirk, 1994; Bolton *et al.*, 2010; Dias and von Sperling, 2017).

In dark incubated model HRAPs, inclusion of a solar exposed inclined plane elevated inactivation of F-Specific phage and MS2. Specifically in wastewater where attenuation is more prevalent (Haag and Hoigne, 1986; Tchobanoglous *et al.*, 2003).

The eventual goal of this research is to incorporate the IP into a pond system in the field. Before this can be implemented, however, the contribution of the IP towards inactivation must first be assessed when both pond and IP are exposed to sunlight. This will provide a closer representation of the likely removals in the field as well as provide further information on F-Specific phage inactivation under natural sunlight, an area not as well represented as it is for simulated sunlight (Fisher *et al.*, 2011).

In this work, experiments with the model systems and IP were further examined in turbid wastewater. This time, however, the bulk water will be exposed to sunlight as well as the IP.

Specifically this chapter aims to

- Finalise examination using the model HRAPs
- Examine F-Specific phage removal with the IP when model HRAPs are also exposed to direct sunlight
- Examine inactivation performance in covered and uncovered HRAPs with and without the IP

5.2 Methods

5.2.1 Model HRAPs

Model systems were set-up as described in Chapters 2 and 4 with wastewater collected from Mt Barker Wastewater treatment plant (WWTP). This time however, the ponds were fully exposed to sunlight. Two pond configurations were used, with the second used to compare solar exposed and dark incubated systems

I. $HRAP_L$ vs. $HRAP_L$ + SIP and $HRAP_L$ + LIP

II. $HRAP_L$ vs. $HRAP_L$ + IP vs. $HRAP_D$ vs. $HRAP_D$ + IP (Plate 5.1).

Where, $HRAP_L$ represents a fully solar exposed HRAP (light) with both bulk water and IP exposed, and $HRAP_D$ represents a covered HRAP with the bulk water operated under dark incubation and the IP solar exposed. Operating conditions for Configuration I is outlined in Table 5.1. For Configuration II, the SIP was the only IP used due to the availability of a second IP. The two IPs were operated at the same HLR outlined in Table 5.1. A HLR of 300 L m^{-2} h^{-1} was used for all IP systems. Both configurations were operated for 24 and 49.5 h, with Configuration I also operated for 54 h.

Plate 5.1: Model HRAPs arrangement for operational configuration II; comparison between open and covered HRAPs; a) $HRAP_D$, b) $HRAP_L$, c) $HRAP_L$ + IP and d) $HRAP_D$ + IP, where IP of 0.37 m^2 (0.55 m) was used.

Table 5.1: Operating conditions for the Model HRAPs in configuration I.

Model HRAP	IP length (m)	Surface Area (m^2)	Flow Rate (Q, L h^{-1})	HLR (L m^{-2} h^{-1})
$HRAP_L$	-	-	-	-
$HRAP_L$ + SIP	0.55	0.37	225.0	300
$HRAP_L$ + LIP	1.10	0.75	111.0	300

5.2.2 Experimental Design

Inactivation experiments with the IP were operated as described in Chapters 2-4, with at least three experimental replicates performed for each investigation. Both LRV and K_L rates were used as an indication of phage removal in the model HRAPs and IP effectiveness. Incubation times were chosen based on the results obtained in Chapters 3 and 4, as well as for the KoM HRT (Chapter 6).

5.2.3 Sample inoculation and collection

Due to low native phage in the Mt Barker treated wastewater, 2 mL MS2 stock (~ $x10^9$ PFU mL^{-1}; Chapter 4) was inoculated into each system the morning of sampling. 1 L samples of the Mt Barker water was again collected prior to inoculation for analysis. Daily, 10 mL triplicate samples were collected between 9:00 am and 5:00 pm at 1.5 hour intervals. Additional 120 mL samples were collected every 3 hours for further analyses (i.e. Chl *a*, turbidity and nutrient concentrations). At the completion of operation 1 L samples were taken from each system for a more detailed analysis of water quality.

5.2.4 Water Analysis

Analysis was performed on the 1 L and 120 mL samples collected as presented in Chapter 4. Analyses included; Chl *a*, nutrient concentrations (TOC, TC, IC and TN) and turbidity. SS and BOD_5 concentrations were also analysed for the before and after treatment samples (1 L). Analysis was performed using APHA (1992) standard wastewater methods and the BOD OxiTop - OxiTop$^{(R)}$ – C (WTW) method for BOD_5. Details regarding each analysis were presented in Chapter 2.

5.2.5 Environmental conditions

In situ water parameters and BOM environmental conditions were monitored as described in Chapter 2. Parameters included; water temperature, pH, DO (mg L^{-1}), UVA and UVB irradiance (W m^{-2}), sun hours (h), and daily solar exposure (MJ m^{-2}).

5.2.6 Microbial Quantification

Quantification of MS2 was again carried out using the double layer agar quantification plaque assay described in Chapter 2. Tryptone water (5%) was used for sample dilution when required. Phages detected in the model HRAPS were reported as F-Specific phage as outlined in Chapters 2 and 4; due to possible presence of native phage in the pond, despite the inoculation of MS2.

5.2.7 Statistical Analysis

Statistical analysis was performed as outlined in Chapter 4. In brief; F-Specific phage inactivation was determined as inactivation rate constants (K_L; $\log_{10} h^{-1}$ or K_D; $\log_{10} h^{-1}$) and log removal values (LRV_t; $\log_{10}$ F-Specific phage 100 mL^{-1}) with t, L and D representative of the incubation time, inactivation in sunlight exposed conditions and inactivation under dark incubation respectively. LRVs were determined from $\log_{10} N_0 - \log_{10} N_t$, and plotted as semi-log plots of LRV against time exposed. K_L was derived from $K_{max} \pm$ SE corresponding to the plots of log F-Specific phage ($\log_{10}$ (N)) against exposure time with a $\log_{10}$ linear + tail model applied (GInaFiT, Geeraerd *et al.* (2005). Log_{10} linear + tail model had been identified in Chapters 3 and 4 as the best representation of the data, hence its use. LRVs for the 24 h incubation time were calculated after 6 h to take the data captured within the linear portion of the die-off curve into consideration. Shapiro-Wilks test of normality was used for assessing normal distribution of the data sets (results not included). Independent sample t-tests, one-way ANOVAs and ANCOVAs correcting for environmental covariates (i.e. solar exposure) were performed to establish the difference in mean removal with and without the IP. These tests were also performed to establish statistical significance in the monitored *in situ* parameters between the pond systems. Statistical significance was accepted at $p<0.05$. Relationships between variables were determined using linear regression and a correlation (r) matrix (Pearson's product moment correlation). Correlation was determined as positive or negative and classed as strong, moderate, weak or no correlation (refer to Chapter 2).

5.3 Results

This study examined the removal efficiency of the IP when model HRAPs were operated in wastewater. Investigation was undertaken in two sections. The first examined F-Specific phage removal when both bulk water and IP were exposed to sunlight. Systems were operated uncovered with both long (LIP) and short (SIP) IPs used. The second part compared inactivation under both solar exposure (open) and dark incubation (covered). Dark incubation; bulk water was covered and IP solely exposed (Plate 5.1 Section 5.2.1).

5.3.1 F-Specific phage Inactivation: $HRAP_L$ vs. $HRAP_L$ + SIP and $HRAP_L$ + LIP

When both pond and IP were solar exposed inclusion of the IP resulted in increased inactivation. The $HRAP_L$ + SIP (LRV_6 1.00±0.42, $LRV_{49.5}$ 3.25±0.26) and $HRAP_L$ + LIP (LRV_6 1.11±0.37, $LRV_{49.5}$ 3.83±0.23) achieved removals 1.6-2.2 and 1.9-2.4 times higher than the $HRAP_L$ (LRV_6 0.46±0.21, $LRV_{49.5}$ 2.02±0.37) after 6 and 49.5 h. Table 5.2 reports the obtained inactivation rates (LRV_t and K_L) for both exposure periods. LRV achieved after 24 h are reported in Appendix 6.1. The removals reported were collated from an experimental set where operating conditions (i.e. flow rate and HLR) were kept similar (refer to Table 5.1). This was to broadly assess the contribution of the IP on removal.

Table 5.2: F-Specific phage die-off in solar exposed model HRAPs with different IP lengths; short-IP (SIP, 0.55 m) and long-IP, 1.10 m) after 24 and 49.5 h. Inactivation rate constants (K_L; log_{10} h^{-1}) and solar exposed log_{10} reduction values (LRV_t; log_{10} PFU 100 mL^{-1}) are reported.

Time (t; h)	Model System	Inactivation		
		n	$LRV_t \pm SE$	$K_L \pm SE$ (log_{10} h^{-1})
24.0	$HRAP_L$	3	0.46±0.21[a]	0.26±0.05
	$HRAP_L$ + SIP	3	1.00±0.42[a]	0.46±0.15
	$HRAP_L$ + LIP	3	1.11±0.37[a]	0.46±0.15
49.5	$HRAP_L$	3	2.02±0.37	0.10±0.03
	$HRAP_L$ + SIP	3	3.25±0.26	0.14±0.02
	$HRAP_L$ + LIP	3	3.83±0.23	0.16±0.01

[a] LRV values were calculated after 6.0 h.

Comparison of the F-Specific phage decay rates (K_L) was performed with Table 5.3 presenting the results from a one-way ANOVA and Tukey's post hoc comparison after 24 and 49.5 h. The dataset was identified normally distributed (Shapiro-Wilks test for normality ($p>0.05$), data not shown). For both exposure times, K_L was found to be similar between the model HRAPs, despite faster inactivation observed in the IP included systems ($p>0.05$) (Table 5.3). Inclusion of the IP was found to have a significant effect on inactivation when solar exposure was controlled [$F(2, 5) = 7.20$, $p<0.05$ actual $p=0.034$, $\eta^2 = 0.74$] (ANCOVA; Appendix 6.2; Table A6.4). More specifically, the $HRAP_L$ + LIP and $HRAP_L$ + SIP demonstrated inactivation that was significantly faster than the $HRAP_L$ ($p < 0.05$) (Table 5.4).

Similarly, Table 5.5 presents the results from a one-way ANOVA with Tukey's post hoc comparison performed on the obtained LRV after 6.0 and 49.5 h. The dataset was also identified normally distributed (Shapiro-Wilks test for normality ($p>0.05$), data not shown). After 6.0 h, none of the comparisons between the model HRAPs was identified significant ($p>0.05$) (Table 5.5), despite the higher LRV achieved in the $HRAP_L$+ LIP and $HRAP_L$ + SIP (Table 5.2). However, when exposure time was increased to 49.5 h $HRAP_L$ + LIP and $HRAP_L$ + SIP were both found to be significantly different to the $HRAP_L$ ($p<0.05$) and from each other ($p<0.05$).

Table 5.3: Tukey's post hoc comparison of variance between the mean F-Specific phage inactivation rate (K_L; log_{10} h^{-1}) in the $HRAP_L$ and $HRAP_L$ + IP (SIP and LIP) model HRAP systems achieved after 24 and 49.5 h solar exposed. Statistical significance was at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level									
Model Systems		24 h				49.5 h			
		Difference	Lower	Upper	p-Value	Difference	Lower	Upper	P-Value
$HRAP_L$ + LIP	$HRAP_L$	0.20	-0.35	0.74	0.546	0.06	-0.19	0.30	0.499
$HRAP_L$ + SIP	$HRAP_L$	0.00	-0.54	0.55	1.000	0.01	-0.20	0.29	0.630
$HRAP_L$ + LIP	$HRAP_L$ + SIP	0.20	-0.35	0.75	0.536	0.04	-0.23	0.26	0.947

Strength of statistical significance, * = p<0.05, ** = p<0.01

Table 5.4: Tukey's post hoc comparison of variance between the F-Specific phage inactivation rates (K_L; log_{10} h^{-1}) in the $HRAP_L$ and $HRAP_L$ + IP (SIP and LIP) model HRAP systems when corrected for solar exposure after 49.5 h. Statistical significance was at p<0.05.

Tukey multiple comparison of corrected means 95% family-wise confidence level					
Model Systems		49.5 h			
		Difference	Lower	Upper	p-Value
$HRAP_L$ + LIP	$HRAP_L$	0.06	0.01	0.11	0.034*
$HRAP_L$ + SIP	$HRAP_L$	0.01	-0.01	0.09	0.085
$HRAP_L$ + LIP	$HRAP_L$ + SIP	0.04	-0.04	0.06	0.689

Strength of statistical significance, * = p<0.05, ** = p<0.01

Table 5.5: Tukey's post hoc comparison of variance between the mean F-Specific phage removal (LRV_t; log_{10} PFU 100 mL^{-1}) in the $HRAP_L$ and $HRAP_L$ + IP (SIP and LIP) model HRAP systems achieved after 24 and 49.5 h solar exposed. Statistical significance was at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level									
Model Systems		6 h				49.5 h			
		Difference	Lower	Upper	p-Value	Difference	Lower	Upper	P-Value
$HRAP_L$ + LIP	$HRAP_L$	0.28	-0.41	0.97	0.243	0.69	0.36	1.01	0.006**
$HRAP_L$ + SIP	$HRAP_L$	0.33	-0.27	0.92	0.148	0.87	0.59	1.15	0.002**
$HRAP_L$ + LIP	$HRAP_L$ + SIP	0.05	-0.05	0.15	0.172	0.18	0.13	0.23	0.002**

Strength of statistical significance, * = p<0.05, ** = p<0.01

As presented in Chapter 4, die-off in the model systems was also analysed over a 5 d period. However, F-Specific phage was not detected in any of the HRAPs incorporating IPs after 54 hours incubation. Values beyond 54 h were therefore not reported. Figure 5.1 shows the relationship between inactivation and prolonged solar exposure in the solar exposed ponds. LRV_{54} were 2.57±0.03 ($HRAP_L$), 3.86±0.62 ($HRAP_L$ + SIP) and 4.66±0.44 ($HRAP_L$ + LIP).

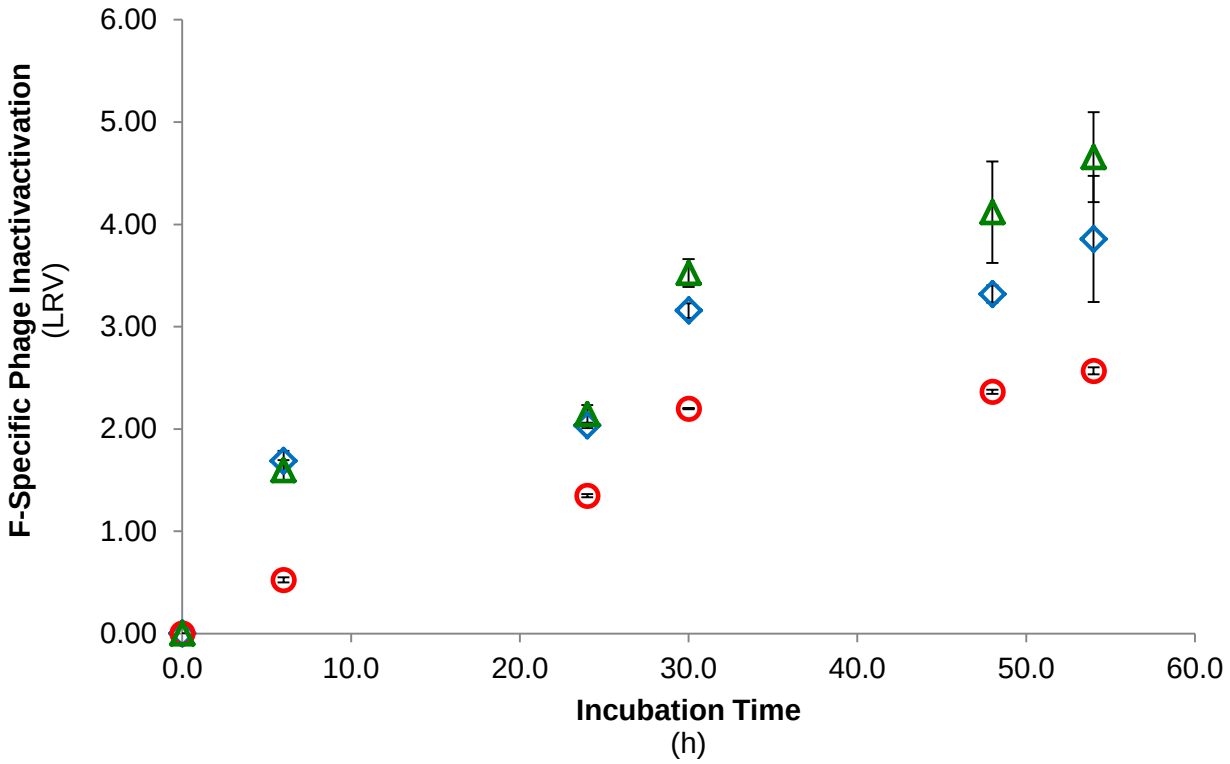
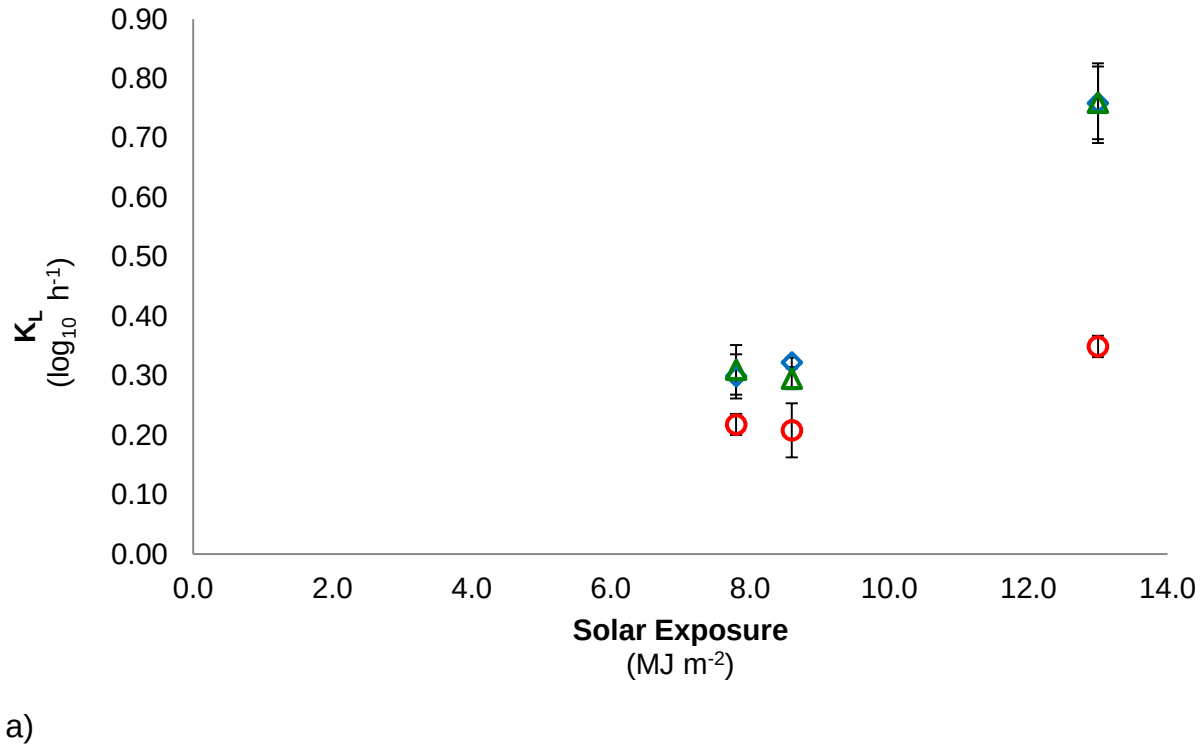


Figure 5.1: Relationship between inactivation and incubation time in solar exposed (uncovered) model HRAPs; $HRAP_L$: (○), $HRAP_L$ + LIP (△), $HRAP_L$ + SIP (◇) after 54 h exposed to sunlight. Daily solar exposure ranged from 18.2-25.3 MJ m^{-2} and water temperatures ranged from 23.20-35.40 °C. Standard error bars have been included

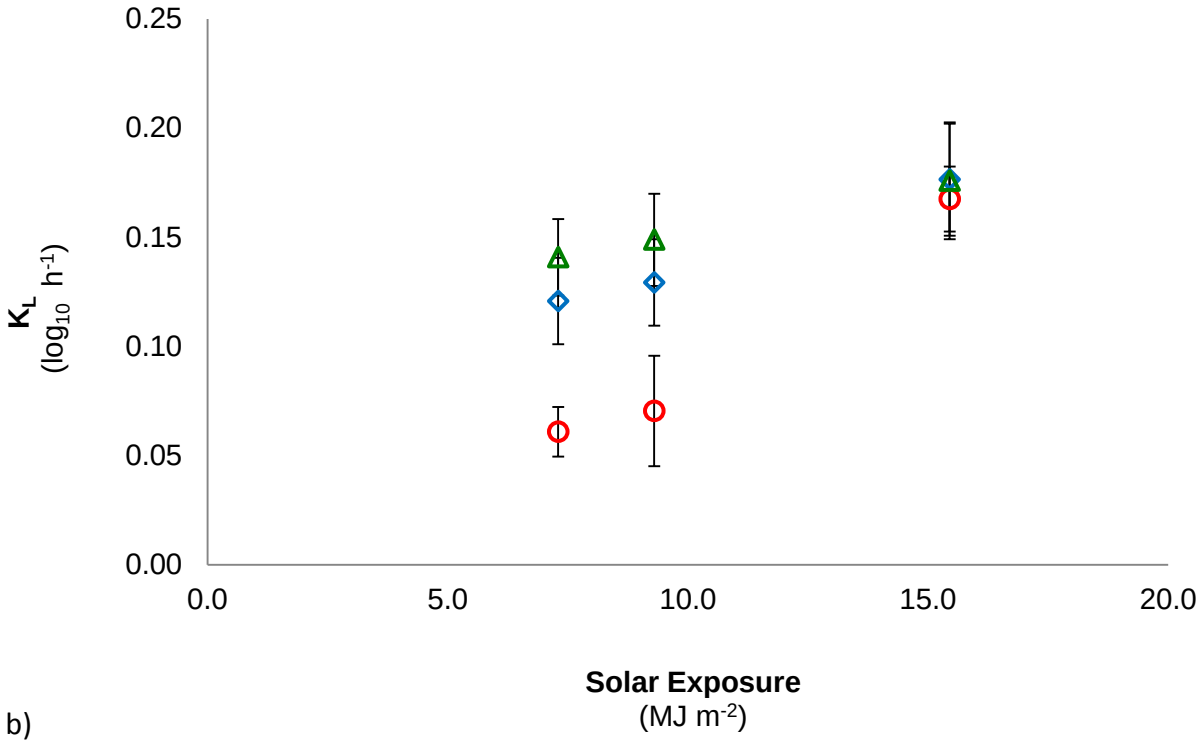
5.3.2 In situ water and environmental conditions and their effect on IP performance

Global solar irradiance, provided by the Australian BOM ranged between 7.80–18.20 MJ m^{-2} and 7.80–26.50 MJ m^{-2} for the 24 h and 49.5 h exposures across the three experiments. Mean irradiances were 9.80±1.62 MJ m^{-2} and 10.68±2.45 MJ m^{-2} for the 24 h and 49.5 h exposures, respectively.

The relationship between inactivation (K_L) and solar exposure is shown in Figure 5.2. A strong correlation (r= 0.804, R^2 = 0.646) was identified between inactivation rate and solar exposure, with inactivation shown to increase when irradiance was increased.



a)



b)

Figure 5.2: Observed F-Specific phage inactivation rates (K_L $log_{10}h^{-1}$) in open model HRAPs with and without an IP when exposed to sunlight (global solar exposure, MJ m^{-2}) after a) 24 and b) 49.5 h. Model HRAPs were: $HRAP_L$ (○), $HRAP_L$ + SIP (◇) and $HRAP_L$ + LIP (△). Inactivation rates were recorded for three experiments performed during, March, April and May. Standard error of mean bars has been included.

Higher pH (7.95±0.05), DO (6.28±0.44 mg L^{-1}) and temperatures (23.90±0.87°C) were recorded in $HRAP_L$ + LIP compared to $HRAP_L$ + SIP (pH 7.94±0.07, 5.52±0.44 mg DO L^{-1} and 23.73±0.77°C) and $HRAP_L$ (pH 7.89±0.06, 5.15±0.37 mg DO L^{-1} and 23.88±0.77°C) after 49.5 h (Table 5.6). For the measured parameters the differences in means were not significant between any of the systems ($p>0.05$), except for SS which was found to be significantly higher in the $HRAP_L$ ($p<0.05$) (Appendix 6.3: Table A6.11). Figure 5.3 shows the influence pH, DO and temperature had on $LRV_{49.5}$. Data presented shows the mean values obtained from three experimental runs. Similarly Figure 5.4 shows the influence of Chl *a*, turbidity and SS on F-Specific phage die-off.

Table 5.7 presents a correlation matrix of the measured water conditions (solar exposure, pH, DO, temperature, turbidity, SS, BOD and Chl *a*) and inactivation rates (LRV, K_L). SS was shown to have a strong negative correlation with LRV (r=-0.856).

Table 5.6: In situ pH, DO and water temperature for model systems operated in tap and wastewater under dark incubation.

Parameter	$HRAP_L$						$HRAP_L$+ SIP						$HRAP_L$ + LIP					
	n	Mean	SE	SD	Min	Max	n	Mean	SE	SD	Min	Max	n	Mean	SE	SD	Min	Max
pH	36	7.89	0.06	0.33	7.20	8.50	36	7.94	0.07	0.40	7.04	8.64	36	7.95	0.05	0.29	7.12	8.35
DO (mg L^{-1})	36	5.15	0.37	2.20	2.08	12.60	36	5.52	0.44	2.63	1.97	11.75	36	6.28	0.47	2.81	2.22	10.05
Temperature (°C)	36	23.88	0.77	4.65	16.30	33.10	36	23.73	0.80	4.82	16.20	35.20	36	23.90	0.87	5.21	16.00	35.20
Turbidity (NTU)	3	39.67	8.01	13.87	28.00	55.00	3	28.00	6.66	11.53	17.00	40.00	3	28.00	10.12	17.52	10.00	45.00
SS (mg L^{-1})	3	36.27	0.96	1.67	34.40	37.60	3	22.67	1.04	1.80	20.80	24.40	3	19.73	2.45	4.24	15.20	23.60
Chl *a* (mg L^{-1})	3	0.29	0.03	0.06	0.23	0.34	3	0.27	0.01	0.02	0.25	0.29	3	0.31	0.01	0.03	0.28	0.33

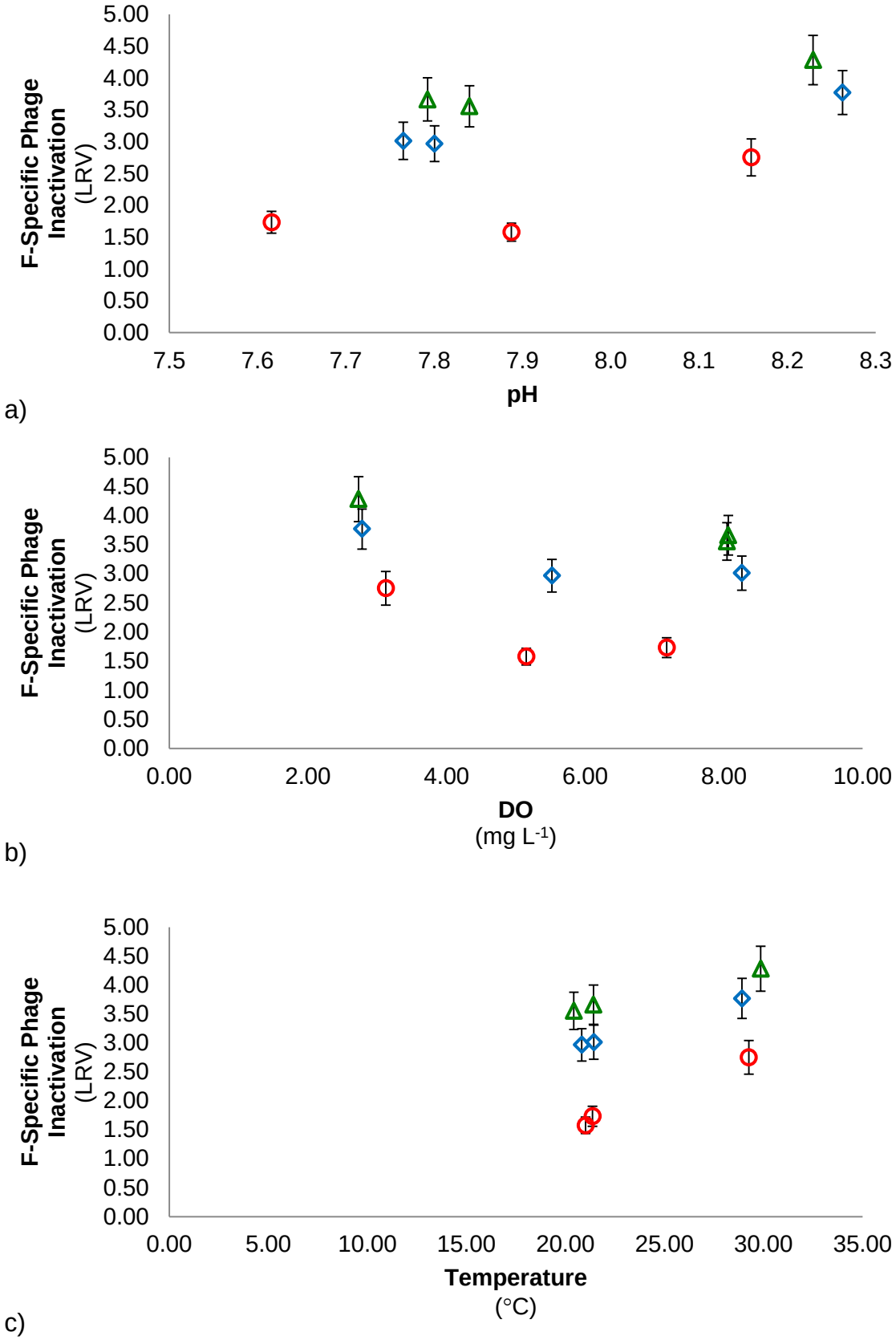


Figure 5.3: Influence of a) pH, b) DO and c) temperature on F-Specific phage log reduction values (LRV, log_{10} PFU 100 mL^{-1}, mean±SE) in solar exposed model HRAPs; $HRAP_L$ (○), $HRAP_L$ + SIP (◇) and $HRAP_L$ + LIP (△). Exposure time was 49.5 with solar irradiance ranging from 7.3-15.5 MJ m^{-2}).

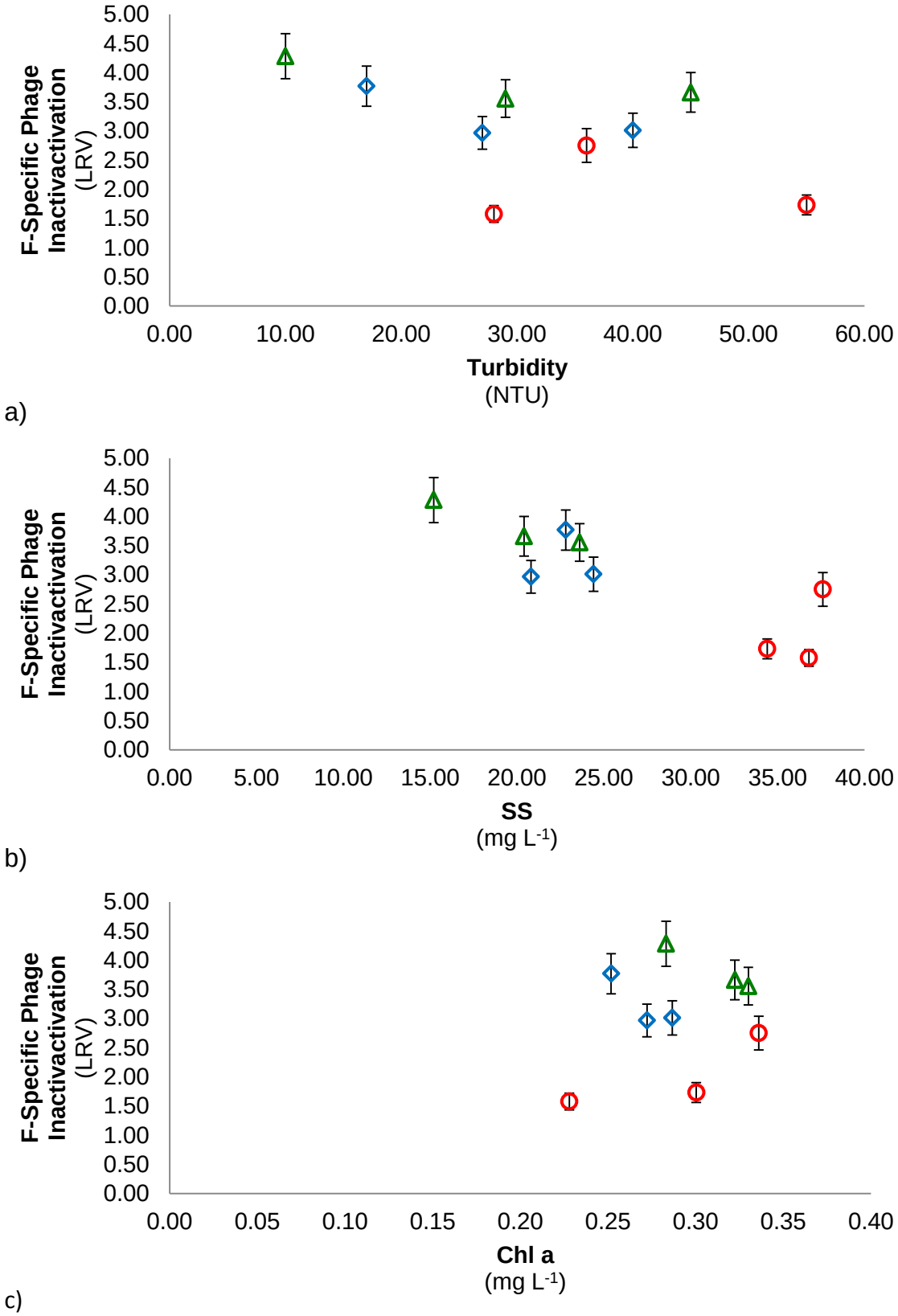


Figure 5.4: Influence of a) turbidity, b) SS and c) chl *a* on F-Specific phage log reduction values (LRV, log_{10} PFU 100 mL^{-1}, mean±SE) in solar exposed model HRAPs; $HRAP_L$ (○), $HRAP_L$ + SIP (◇) and $HRAP_L$ + LIP (△). Exposure time was 49.5 with solar irradiance ranging from 7.3-15.5 MJ m^{-2}

Table 5.7: Correlation matrix of F-Specific phage inactivation, sunlight exposure and water composition in the $HRAP_L$, $HRAP_L$ + SIP and $HRAP_L$ + LIP after 49.5 h exposed.

	BOD	Chl a	DO	K_L	LRV	pH	Solar Exposure	SS	Temperature	Turbidity
BOD ($mg\ L^{-1}$)	1									
Chl *a* ($mg\ L^{-1}$)	0.289[c]	1								
DO ($mg\ L^{-1}$)	0.278[c]	0.185[c]	1							
K_L ($log_{10}\ h^{-1}$)	0.193[c]	0.1365[c]	-0.664[b]	1						
LRV (log_{10} PFU $100mL^{-1}$)	0.534[b]	0.263[c]	-0.473[c]	0.891[a]	1					
pH	-0.641[b]	-0.458[c]	-0.193[c]	-0.067[d]	-0.321[c]	1				
Solar Exposure ($MJ\ m^{-2}$)	-0.386[c]	0.131[c]	-0.680[b]	0.697[b]	0.446[c]	0.387[c]	1			
SS ($mg\ L^{-1}$)	-0.751[b]	-0.014[d]	0.182	-0.564[b]	-0.856[a]	0.377[c]	-0.102[c]	1		
Temperature (°C)	-0.331[c]	-0.105[c]	-0.967[a]	0.687[b]	0.480[c]	0.254[c]	0.810[b]	-0.150[c]	1	
Turbidity (NTU)	-0.093[d]	0.436[c]	0.801[b]	-0.507[b]	-0.563[b]	-0.053[d]	-0.306[c]	0.483[c]	-0.739[b]	1

[a] strong correlation; if |r| is between 0.85 and 1.00

[b] moderate correlation; if |r| is between 0.50 and 0.85

[c] weak correlation; if |r| is between 0.10 and 0.50

[d] no correlation; if |r| is between 0.10 and 0.00

5.3.3 F-Specific phage inactivation: sunlight vs. dark incubation

Figure 5.5 shows the variation in removal when the model systems were solar exposed (pond uncovered) or dark incubated (pond covered). Increased inactivation occurred when the pond or IP was exposed to sunlight. Only the SIP was used for comparison since only a second SIP available. Table 5.8 presents the achieved inactivation rates (K_L and K_D; $\log_{10}$ h^{-1}) for both exposure conditions following 24 and 49.5 h incubations. LRV after 6.0 and 49.5 h are also presented in Table 5.8. LRV after 24 h was also calculated and reported in Appendix 6.1.

In general, the highest removal was observed for $HRAP_L$ + SIP ($LRV_{6.0}$ 0.80±0.04, $LRV_{49.5}$ 2.13±0.36) and lowest in the dark incubated $HRAP_D$ ($LRV_{6.0}$ 0.28±0.06, $LRV_{49.5}$ 1.08±0.30). The $HRAP_D$ + SIP and $HRAP_L$ exhibited removals that were similar (Table 5.8).

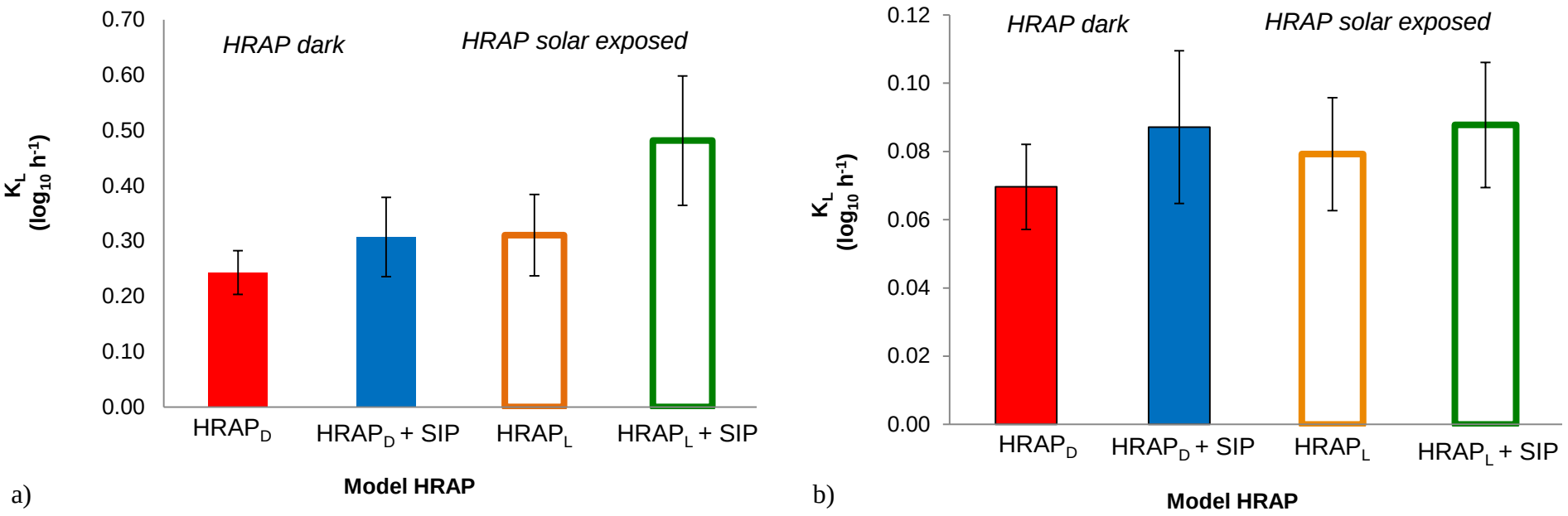


Figure 5.5: F-Specific phage inactivation rates (K_L, log_{10} h^{-1}; mean ± 1 SE) for the same model systems solar exposed and dark incubated after a) 24 and b) 49.5 h. Mean solar irradiations ranged were 9.70±1.15 and 8.83±1.19 MJ m^{-2}. Mean water temperatures ranged from 7.17-21.4°C ($HRAP_D$), 10.7-25.7°C ($HRAP_D$ + SIP), 10.7-22.0°C ($HRAP_L$) and 8.33-25.9°C ($HRAP_L$ + SIP).

Table 5.8: Comparison of F-Specific phage LRV_t (log_{10} PFU 100 mL^{-1}, mean±SE) inactivation rates (K_L, log_{10} h^{-1}, mean±SE) in model HRAPs when solar exposed (pond uncovered) and dark incubated (pond covered). Incubation period was 24 and 49.5 h.

Pond condition	Model HRAP	F-Specific phage Inactivation Rates			
		24 h		49.5 h	
		$LRV_{6.0}$	K_L	$LRV_{49.5}$	K_L
Dark incubated	$HRAP_D$	0.28±0.06	0.23±0.04	1.08±0.30	0.07±0.01
	$HRAP_D$ + SIP	0.50±013	0.31±0.07	1.50±0.36	0.09±0.02
Sunlight exposed	$HRAP_L$	0.44±0.07	0.31±0.07	1.65±0.44	0.08±0.02
	$HRAP_L$+ SIP	0.80±0.04	0.48±0.12	2.13±0.36	0.09±0.02

* LRV were calculated after 6.0 h

Tukey HSD post hoc test comparison was performed to distinguish significant difference in mean LRV between the analysed model systems. Results are presented in Table 5.9. After 6.0 h, a statistical difference was detected between the $HRAP_L$ + SIP and the other HRAPs ($p<0.05$). All other comparisons were not statistically significant ($p>0.05$) (Table 5.9). Prolonging exposure to 49.5 h confirmed the benefit of exposing the HRAP to light ($HRAP_D$ v $HRAP_L$, $p<0.05$, actual $p=0.023$; 95% CI: 0.03 to 0.37), with the difference in inactivation further increased when compared with the dark incubation of the HRAP by inclusion of the SIP with the $HRAP_L$ ($p<0.001$, actual $p=0.0002$; 95% CI; 0.32 to 0.66) (Table 5.9). Furthermore, sunlight inactivation increased with inclusion of the SIP to the solar exposed HRAP ($p<0.01$, actual $p= 0.004$; 95% CI: 0.12 to 0.46) (Table 5.9).

Statistical comparison was also performed on the obtained K_L values. Unfortunately, a significant difference was not detected between any of the systems after 24 and 49.5 h incubations (Tukey's post hoc comparison: Appendix 6.3).

When both temperature and solar exposure were controlled, model HRAP was shown to have a significant impact on inactivation ($p<0.05$; Appendix 6.2: Tables A6.5-A6.7). However, this was only observed for the 24 h K_L values and only between the $HRAP_L$ + SIP and $HRAP_D$ (both covariates) and the $HRAP_L$ (temperature) ($p<0.05$; Appendix 6.2: Tables A6.5-A6.7).

Table 5.9: Comparison of mean F-Specific phage log reduction (LRV; log_{10} PFU 100 mL^{-1}) in dark incubated ($HRAP_D$) and solar exposed ($HRAP_L$) model HRAPs after 6.0 h and 49.5 h. Statistical significance was at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level									
Model Systems		6 h				49.5 h			
		Difference	Lower	Upper	P-Value	Difference	Lower	Upper	P-Value
$HRAP_D$ + SIP	$HRAP_D$	0.09	-0.05	0.25	0.193	0.16	0.00	0.33	0.056
$HRAP_L$	$HRAP_D$	0.08	-0.05	0.22	0.250	0.20	0.03	0.37	0.023*
$HRAP_L$ + SIP	$HRAP_D$	0.29	0.15	0.43	0.002**	0.49	0.32	0.66	2.0E-04***
$HRAP_L$	$HRAP_D$ + SIP	-0.01	-0.15	0.13	0.996	0.04	-0.13	0.20	0.854
$HRAP_L$ + SIP	$HRAP_D$ + SIP	0.20	0.06	0.33	0.011*	0.33	0.16	0.50	0.002**
$HRAP_L$ + SIP	$HRAP_L$	0.20	0.07	0.34	0.009**	0.29	0.12	0.46	0.004**

Strength of statistical significance, * = p<0.05, ** = p<0.01

In situ water measurements for the different test conditions are summarised in Table 5.10. These conditions are representative of the 49.5 h incubation. Turbidity and SS in the model HRAPs ranged between 33.0-53.0 NTU and 12.0-32.4 mg SS L^{-1}.

Table 5.10: In situ pH, DO and water temperature for model systems operated in tap and wastewater under dark incubation.

Parameter	$HRAP_D$				$HRAP_D$+ SIP				$HRAP_L$				$HRAP_L$+ SIP			
	n	Mean±SE	Min	Max	n	Mean± SE	Min	max	n	Mean± SE	Min	max	n	Mean± SE	Min	max
pH	34	7.99±0.05	7.17	8.53	34	8.22±0.06	7.52	8.75	34	8.42±0.09	7.52	9.24	34	8.41±0.07	7.66	9.11
DO (mg L^{-1})	22	6.23±0.44	3.27	9.36	22	8.91±0.63	3.72	13.34	22	13.77±1.45	3.83	26.0	22	10.24±0.72	5.18	16.5
Temperature (°C)	34	15.84±0.58	7.17	21.4	34	15.90±0.66	10.7	25.7	34	15.01±0.48	10.7	22.0	34	15.89±0.70	8.33	25.9
Turbidity (NTU)	2	43.00±10.00	33.0	53.0	2	29.00±3.00	26.0	32.0	2	38.00±10.00	28.0	48.0	2	25.20±0.50	25.0	26.0
SS (mg L^{-1})	2	26.40±6.00	20.4	32.4	2	13.00±0.60	12.4	13.6	2	22.20±10.20	12.0	32.4	2	17.00±1.80	15.2	18.8
Chl *a* (mg L^{-1})	2	0.43±0.19	0.24	0.62	2	0.29±0.08	0.21	0.37	2	0.29±0.06	0.16	0.28	2	0.24±0.09	0.15	0.33

5.4 Discussion

To finalise the work with the model systems, an examination of the IP performance was carried out when both pond and IP were exposed to direct sunlight. Different sized IPs were again used with die-off compared against a solar exposed $HRAP_L$; no IP. The results could be used as an indication of the likely performance and contribution of the IP towards inactivation in the field.

5.4.1 F-Specific phage inactivation in the solar exposed model HRAPs

From the previous chapters, inclusion of a solar exposed IP to dark incubated (covered) model HRAPs was shown to have a positive influence on F-Specific phage (MS2) inactivation rates in both optically clear (tap, Chapter 3) and turbid waters (wastewater, Chapter 4). The contribution of the IP towards inactivation was again found to be positive when the model systems were operated with both pond and IP exposed to direct sunlight.

The results indicated that an improvement in F-Specific phage could be achieved from the addition of the IP. $HRAP_L$ + LIP ($LRV_{49.5}$ 3.83±0.23) and $HRAP_L$ + SIP ($LRV_{49.5}$ 3.25±0.26) were both shown to be significantly higher than $HRAP_L$ ($LRV_{49.5}$ 2.02±0.37) after 49.5 h (Tables 5.2 and 5.5). The identification of a significant different between $HRAP_L$ and $HRAP_L$ + IP meant the null hypothesis (H_0) that F-Specific phage die-off could not be improved in turbid wastewater when a solar exposed IP was included into an open pond was rejected. The alternate hypothesis (H_1) that the die-off can be improved with the inclusion of an IP was therefore accepted.

Results also indicated that on average when the IP was present inactivation was found to be between 1.6 and 1.4 times faster than when the IP was absent ($HRAP_L$: K_L 0.10±0.03) (Table 5.2). However, it was not until the effects of solar exposure were taken into account that inactivation with IP ($HRAP_L$ + LIP) was found to be significant (Table 5.4), adding further support to the importance of solar exposure on pathogen inactivation.

Under the conditions tested, increasing the surface area of the IP (LIP) made a significant impact on removal with the obtained LRV found to be statistically different from the SIP ($p<0.05$). In all cases inactivation achieved with the LIP was found to be always higher that the SIP. This therefore

supports the theory that greater removal can be achieved when the surface area of exposure is increased.

The obtained inactivation rates presented in Table 5.2 were found comparable to the F-RNA phage removals reported by Yu (2015) and Sinton *et al.* (2002) for wastewater effluents under simulated sunlight (i.e. UVB) and Young *et al.* (2016) under natural sunlight. The similarity in LRV between the model HRAPs presented and Young et al (2016) is a good indication that the findings obtained in these small scale systems could be replicated into the field. This would of course need to be examined, with examination carried out in Chapter 6.

Environmental factors can have both a positive or negative influence on disinfection in pond systems (Davies-Colley *et al.*, 1999). Increasing exposure to solar irradiance was predicted to have a greater impact on F-Specific phage removal rates, with this relationship well documented (Sinton *et al.*, 2002; Fisher *et al.*, 2012). Results from this research, provide evidence in support of this increase (Figure 5.2), with a covariate analysis suggesting 74% of the variability in F-Specific phage die-off observed in the model HRAPs could be accounted for by solar exposure (Appendix 6.2).

The addition of complexity to the water matrix; i.e. turbidity, algal growth, nutrients, solids etc, can have a strong impact on the effectiveness of solar inactivation (Davies-Colley *et al.*, 1997; Dias and von Sperling, 2017). DO, pH, temperature and presence of solids in the water can have a significant impact on sunlight inactivation of phage, with inactivation enhanced or inhibited (Curtis *et al.*, 1992; Davies-Colley *et al.*, 1997; Davies-Colley *et al.*, 1999). Evidence of this influence was evident in the model systems with $LRV_{49.5}$ higher when pH and temperature were increased. The inactivation was inversely related to SS concentration (Figures 5.3 and 5.4). Table 5.7 provides data which supports this influence with the correlation between LRV and these parameters found to be at least moderate. Overall, pH and DO were shown to be higher when the IP was present compared to its absence. Increased inactivation is common with elevated pH and DO with photo-oxidation (a solar inactivation mechanism) reliant on these parameters to provide the oxygen

required for inactivation (Davies-Colley *et al.*, 1997; Davies-Colley *et al.*, 1999; Paterson and Curtis, 2005).

5.4.2 F-Specific phage inactivation: dark incubation vs. solar exposure

Concurrent operation of the model systems under dark incubation (covered) and solar exposure (uncovered) yielded results consistent with the findings previously reported in this thesis and the reviewed literature; increased F-Specific phage die-off was observed in all systems exposed to direct sunlight. This observation was unsurprising, with sunlight having a strong influence on inactivation (Sinton *et al.*, 2002; Kohn and Nelson, 2007).

The work by Hawley (2012) performed a similar analysis, comparing light and dark inactivation of model systems operated with and without an IP. However, the Hawley (2012) study was conducted using optically clear tap water rather than the turbid wastewater considered here. Results from both studies identified the fastest inactivation following 24 h incubations were achieved in the solar exposed pond with the IP (i.e. $HRAP_L$ + SIP), and the slowest removal in the dark incubated pond without an IP (i.e. $HRAP_D$).

Furthermore, results indicated that for all the incubation times examined the HRAP + IP systems achieved higher LRV than the corresponding HRAP no-IP for both exposure conditions. This further supports the use of the IP as a versatile addition to treatment.

As noted above, inactivation was shown highest when systems were exposed to sunlight. It was interesting, however, that when the systems were exposed for 49.5h the die-off achieved in the $HRAP_D$ + SIP ($LRV_{49.5}$ 1.50±0.36) was found to be comparable statistically to the $HRAP_L$ ($LRV_{49.5}$ 1.65±0.44), only 0.15 logs separated the two systems (Table 5.8). From this it could be perceived that under the same operating conditions, operation with only the IP exposed to sunlight could achieve a removal that is just as effective as have a fully open system. Of course more work would still be required if this avenue was to be examined in detail, with the associated impacts on other processes vital to removal and water quality needing to be considered.

Increased inactivation of F-Specific phage in the solar exposed model systems provides a good indication that the system will be effective if applied to the field. The results thereby provide enough evidence to support an investigation of the IP in a field scale system. This will be examined in the proceeding chapter with a large scale IP applied to a fully functioning HRAP at Kingston on Murray, South Australia.

5.5 Conclusion

To complete investigation with the model systems, this chapter carried out an examination of the impact the IP had on pathogen removal when both pond and IP were exposed to sunlight. Experiments were therefore performed in open (uncovered) model systems with and without the IP addition. This also provided a closer representation to field conditions. The results from the study continued to show elevated inactivation whenever the IP was present, with removal observed to be higher than the solar exposed HRAP without the IP. This had been observed across all experiments and conditions analysed. Overall, the following conclusions could be made;

- Whenever IP was present elevated inactivation was achieved
- Removal was found to be improved with surface area was increased, but only with prolonged exposure
- Impact of sunlight on inactivation rates was further confirmed, with elevated removals achieved
- Removal was shown to be always higher when systems were exposed to sunlight compared to the dark, however a dark incubated with a solar exposed IP fitted can achieve equal removal to a solar exposed without an IP

Based on the findings presented in this chapter and the previous chapters the author is confident that the IP would produce results equally as effective if applied to the field. This will be addressed in the next chapter when the IP is up scaled and fitted into a large scale HRAP.

6. LARGE-SCALE IP AT KINGSTON ON MURRAY (KOM)

In this Chapter, some of the work and results presented have been presented as a part of an international conference preceding (see Appendix 7.1) which is uploaded on research gate,
citation: *Hawley & Fallowfield (2016). Inclusion of pond walls to enhance solar exposure and pathogen removal. In: 11th IWA Specialist Group Conference on Wastewater Pond Technology, University of Leeds.*

6.1 Introduction

Community wastewater management schemes (CWMS) have been adopted for rural townships as a means of collecting, treating and disposing of domestic effluent (Davies-Colley *et al.*, 2005; Fallowfield *et al.*, 2012; Young *et al.*, 2016). In these schemes effluent is collected via on-site septic tanks before being pumped out to nearby treatment ponds (Buchanan *et al.*, 2011b). Settling of the solid material occurs within these septic tanks, much the same as an anaerobic pond used in larger treatment systems (Davies-Colley *et al.*, 2005). Commonly in CWMS a waste stabilisation ponds (WSP) are used, however, a movement towards the use of high rate algal ponds (HRAPs) has been proposed for their associated advantages, including increased solar exposure essential for disinfection processes (Fallowfield *et al.*, 2012).

The HRAP provides increased exposure to sunlight via a large surface area to volume (S/V) ratio, raceway configuration and paddlewheel rotation, with the paddlewheel surfacing the deeper pond water (Fallowfield and Garrett, 1985; Hu *et al.*, 1996; Craggs *et al.*, 2004b; Park *et al.*, 2011). However, limited light penetration is still a concern with at least one third of the pond left unexposed due to attenuation and non-pathogen absorption with high algal concentrations the primary contributor (80%) (Sutherland *et al.*, 2014).

In the previous chapters inclusion of an inclined plane (IP) was shown to enhance pathogen removal (F-Specific phage: MS2) when implemented into model pond systems, under an array of different test conditions, which included optically clear water, wastewaters and both solar and dark incubation. However, as the overall aim of this research was to improve pathogen removal in HRAPs. Understanding the behaviour of the IP in the field on a larger scale was necessary.

An IP naturally occurs within WSPs and HRAPs in the form of the pond wall. The pond wall is generated with construction and serves no other purpose but for water containment. To complete this investigation, an IP was implemented into a large scale HRAP with the freeboard on the pond wall used as the exposed surface. Specifically this chapter aims to

- Assess the effectiveness of the large scale IP when implemented in the field
- Examine which parameters in the field are essential for F-Specific phage (MS2) removal with the IP
- Establish whether the IP had a significant impact on the resultant effluent quality
- Establish the practicality and efficiency of the concept in the field

6.2 Methods

6.2.1 Kingston on Murray (KoM) HRAP

The large scale IP was applied to the pre-existing HRAP at KoM (E 140°20' S34°14). The pond was constructed in 2008 and services the domestic and local wastewater from the township pre-treated in septic tanks. KoM has a population of approximately 140-300 people, but fluctuates depending on seasonal activities; school (particularly summer) holidays, fruit-picking and back packing influx. The treated effluent is used for woodlot irrigation.

The HRAP is divided into two channels (30 m x 5 m) with a single raceway. The surface area of the pond is 200 m^2 at a depth of approximately 0.32 m. The pond, operated at a theoretical hydraulic retention time of 5 d, has a volume of 60,000 L with a daily inflow volume of 12,000 L delivered in six pumping operations. Water is circulated and mixed in the pond via an 8 bladed paddlewheel at a velocity of 0.2 m s^{-1}.

A freeboard of 52 m^2 existed along the side of the pond wall (Plate 6.1). This freeboard spanned a width of 13 m between causeway and pond bend, with the measurements taken 30 cm from the top of the HDPE lining. Length of freeboard was determined from a pond depth of 0.32 m.

Plate 6.1: Example of the available freeboard for exposure on the HRAP at KoM. Surface area of the freeboard between the causeway and the start of the pond bend in the foreground was 52 m^2. Estimate was generated with a 0.32 m depth pond.

3.1.2 Large Scale IP (Pond Wall)

The large scale IP was positioned along the length of the pond, downstream from the paddlewheel. Details regarding the construction and design of the large scale IP are outlined in Chapter 2.1.2. Two IP manifold lengths were used, 5 m resulting in a 20 m^2 IP (HRAP + IP_{20}) and 9 m yielding a 36 m^2 IP (HRAP + IP_{36}). Plate 6.2 and 6.3 illustrates the two IP lengths used.

A pumping regime was employed to initiate or deactivate IP operation. The regime used at KoM is outlined in Table 6.1. Included are the details for the IDEC GT3A timer set-up. Additional details regarding the timer can be found in Appendix 8. Corresponding flow rates and HLR for both IP widths are also presented in Table 6.1.

Plate 6.2: 20 m^2 IP (white rectangle) retrofitted to the Kingston on Murray (KoM) HRAP pond wall. IP was situated downstream of the paddlewheel and approximately 30 cm from the top of the pond lining.

Plate 6.3: 36 m^2 IP (white rectangle) on the HRAP pond wall at Kingston on Murray (KoM), South Australia.

Table 6.1: IDEC GT3A timer configuration and IP operating regime. Regime was devised to switch the pumps attached to the IP on and off at specified time intervals.

IP		Pump Cycle	Timer details		Pump(s)	Flow Rate(s) ($L\ h^{-1}$)	HLR ($L\ m^{-2}\ h^{-1}$)
Dimension	Surface Area						
5 m x 4 m	20 m^2	5 days On 5 days Off 4 days On	Mode: Range: Dial: Hours:	B 10 h 12 120	Aqua Pro AP7500	3329.1	166.5
9 m x 4 m	36 m^2	7 days On 7 days Off 3 days On	Mode: Range: Dial: Hours:	B 10 h 16.8 168	Aqua Pro AP7500 Submersible Pump	8861.7	246.2

6.2.2 Sample Collection

Composite samples (800 mL) were collected twice daily, with 400 mL collected at 3:00 am and 3:00 pm using a refrigerated (~1°C) auto sampler (Avalanche® Sampler, Teledyne ISCO Lincoln, NE). The samples were retrieved after 14 or 24 days.

Inlet water (1.0 L) was collected from an onsite splitter box at the beginning of each experimental run.

6.2.3 Microbial Analysis

Determination of F-Specific phage presence in both inlet and pond water samples was performed using the quantitative double layer agar plaque assay described in Chapter 2. Pond samples were plated as neat (undiluted) and the inlet samples were diluted 10 fold (10^{-1}) in tryptone water (Oxoid).

6.2.4 Environmental and Water Analysis

Environmental conditions were monitored as described in Chapter 2. When available water temperature, pH and DO were logged onsite using T-Tech loggers.

6.2.5 Statistical Analysis

F-Specific phage inactivation was determined as log removal values (LRV_t; $\log_{10}$ PFU 100 mL^{-1}) with t representative of the number of days IP was in operation (i.e. 5 or 7 d). LRVs were determined from $\log_{10} N_0 - \log_{10} N_t$, where N_o is the initial concentration of F-Specific phage and N_t the concentration at time t (d). Independent samples t-tests were performed to establish the difference in mean removals between inactivation in the $HRAP_{KoM}$ with and without the inclusion of the IP. This test was also performed to establish the statistical significance for the monitored pond parameters between the treated effluent and the inlet wastewater.

6.3 Results

Application of the pond wall to the KoM HRAP was examined over two consecutive winter-spring periods (July – September) during 2015 (Trial 1; IP_{20}) and 2016 (Trial 2; IP_{36}) Data from this chapter has been adapted and incorporated as part of an international conference proceeding (Appendix 7.1).

6.3.1 KoM Inlet Wastewater

A summary of the physicochemical properties of the KoM inlet wastewater is presented in Appendix 9 (Table A9.1). There was little variation between the consecutive winter-spring sampling in Trail 1 and 2. Detectable F-Specific phage ranged from 2.60 to 4.37 log_{10} PFU 100 mL^{-1} with the mean recorded over both trials 3.18 $\pm$ 0.24 log_{10} PFU 100 mL^{-1}. During Trial 2 (04/08 - 23/09/2016) a 21% reduction in detectable F-Specific phage was observed with HRAP + IP_{36}; (2.72±0.10 log_{10} PFU 100 mL^{-1}) compared to Trial 1 (22/07 – 21/09/2015) with HRAP + IP_{20} (3.46±0.33 log_{10} PFU 100 mL^{-1}).

6.3.2 Inactivation of F-Specific phage by the KoM HRAP, performance under normal pond operation

Under normal pond operation, i.e. IP_{OFF}, the mean difference in phage concentrations between inlet wastewater and treated wastewater over the two Trials was 1.26 log_{10} PFU 100 mL^{-1}. The overall mean LRV_5 for the KoM HRAP + IP_{OFF} between June – September 2015-2016 was 1.27±0.13. A decrease in removal efficiency, however, was observed between the two Trials (Figure 6.1); with F-Specific phage LRV_5 of 1.89±0.12 in Trial 1 (2015) and a LRV_5 of 0.64±0.10 recorded in Trial 2 (2016).

A summary of the pond conditions is presented in Appendix 9 (Table A9.2). The variability is demonstrated in Figure 6.1. A statistically significant increase ($p<0.05$) in suspended solids, NTU and chlorophyll *a* was observed in Trial 2 in 2016 compared with Trial 1 in 2015 (Appendix 10: Table A10.1). These increases in 2016 were likely associated with the higher solar irradiance (13.92±0.85 MJ m^{-2}) in 2016 (Appendix 9: Table A9.2) leading to increased algal growth. Water temperature might have also been a factor however, no data was available for 2015, and thus a

comparison could not be performed. Although light attenuation was not measured these increases would significantly increase attenuation in pond water in 2016 compared with 2015. The lower LRV recorded in 2016 was likely a consequence of this increase in light attenuation.

A change in water composition was observed between the pond water and the inlet (Appendix 10: Table A10.2). Concentrations of Chl *a*, turbidity and SS as expected increased. Table A10.2 in Appendix 10 presents the results of a statistical analysis (independent samples t-test) of the different water compositions, with most comparisons indicating the difference in means between the $HRAP_{KoM}$ + IP_{OFF} and the inlet were significant ($p<0.05$).

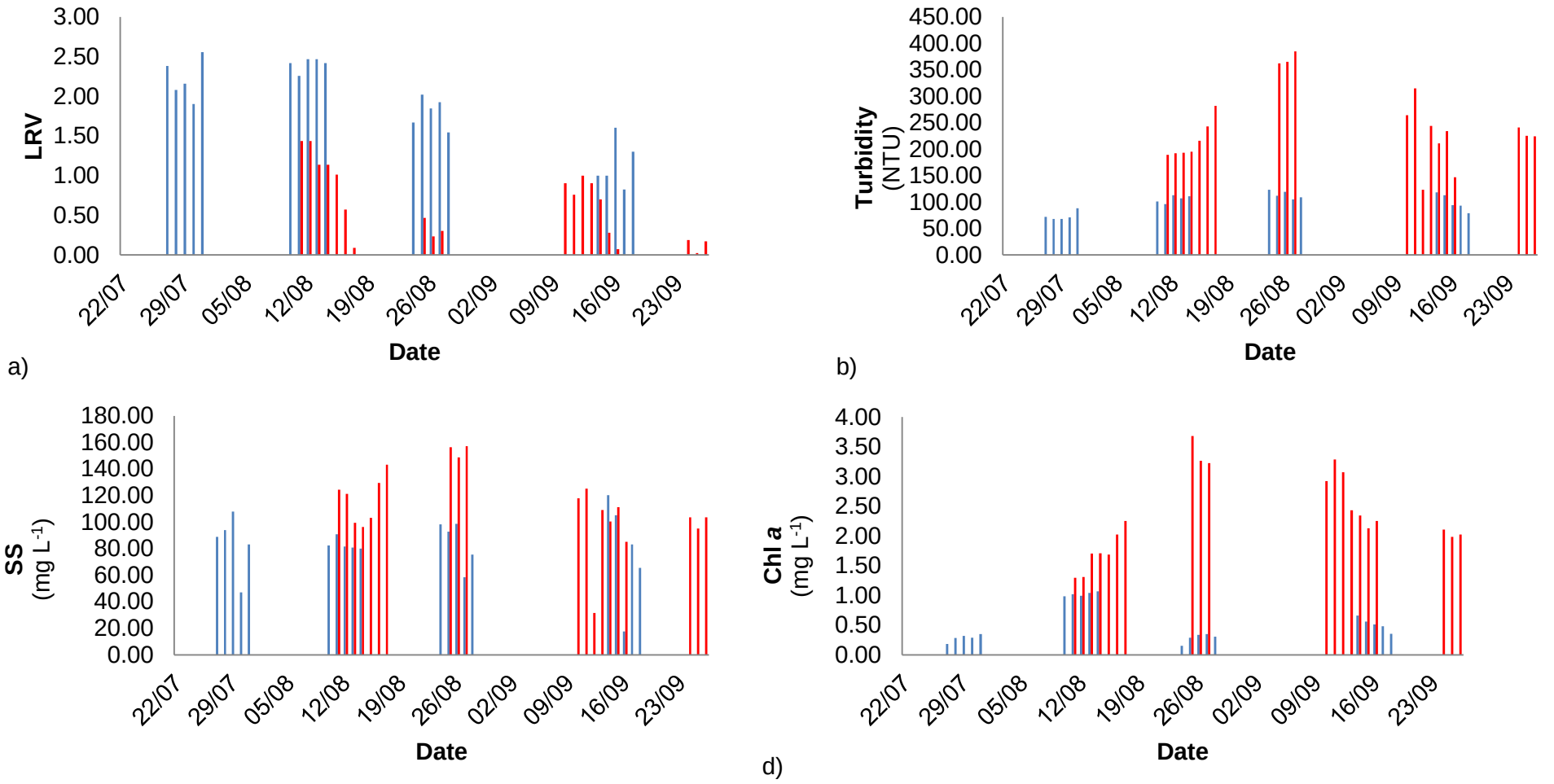


Figure 6.1: The variability in pond conditions observed in the $HRAP_{KoM}$ + IP_{OFF} throughout the sampling period: a) F-Specific phage LRV (log_{10} PFU 100 mL^{-1}) turbidity (NTU), b) suspended solids, SS (mg L^{-1}) and c) chlorophyll *a*, (mg Chl *a* L^{-1}). Sampling occurred over two consecutive winter-spring seasons; Trial 1; July-September 2015 (■) and Trial 2; August-September 2016 (■). Mean solar irradiances for the two seasons were 12.41±0.77 MJ m^{-2} (2015) and 13.92±0.85 MJ m^{-2} (2016).

6.3.3 F-Specific phage inactivation in the KoM HRAP with the pond wall

Initial examination of the inclusion of pond wall as an inclined plane to HRAP treatment in the field was performed using an area of 20 m^2 with the plane operated continuously for 5d. The pumping regime was devised to match the 5 d HRT of the HRAP with the whole pond volume passed over the IP within 5 d. A flow rate and HLR of 3329.1±91.4 L h^{-1} and 166.5 L m^{-2} h^{-1} was used (Table 6.1). The solar irradiance recorded for this period ranged between 3.2 and 20.8 MJ m^{-2}, the mean 12.65±0.70 MJ m^{-2}. The IP was operated for a total of 35 d. The mean LRV when the IP was operating was 2.04±0.11 (Appendix 9: Table A9.3). In contrast, over the same trial period (Trial 1) when the IP was not in operation the LRV_5 achieved in the $HRAP_{KoM}$ + IP_{OFF} was 1.89±0.12 (Appendix 9: Table A9.2), This was 0.15 LRVs lower than that observed in the $HRAP_{KoM}$ + IP_{20}. An independent samples t-test, however, identified the difference in means between the $HRAP_{KoM}$ + IP_{20} and $HRAP_{KoM}$ + IP_{OFF} not to be significant ($p>0.05$, actual $p=0.376$; 95% CI: -0.19 to 0.59).

Similar to the model systems, modification to the IP design and operation was performed in an attempt to significantly improve the removal performance with inclusion of the pond wall. This was achieved by altering the size of the exposed area on the pond wall as well as increasing the number of days each test condition was in operation (i.e. pumping regime). IP width was therefore extended to 9 m (36 m^2) and the days when the IP was on (or off) to 7 d. Solar irradiation and water temperatures during this period ranged from 7.50 to 21.50 MJ m^{-2} and 12.48 to 18.12 °C, respectively. The corresponding means were 14.29±0.59 MJ m^{-2} and 15.23±0.30°C. It should be noted that whilst the bleed valve was incorporated into the large scale IP it was never activated.

F-Specific phage inactivation in the $HRAP_{KoM}$ + IP_{36} was 1.41±0.16 (Appendix 9: Table A9.3), an LRV 0.77 higher than the corresponding $HRAP_{KoM}$ + IP_{OFF} (LRV_7 0.64±0.10) for the same sampling season (Trial 2, Appendix 9: Table A9.2). The difference between inactivation with the IP operational and inactivation under normal pond operation was significant with the resulting p value <0.001 (actual $p=0.0005$; 95% CI: 0.35 to 1.18). The LRV for $HRAP_{KoM}$ + IP_{36} was, however, 0.64 LRV lower than the recorded mean for the $HRAP_{KoM}$ + IP_{20} (Trial 1: Appendix 9: Table A9.3). This

is likely due to higher chl $_a$, SS and consequently turbidity resulting in greater UV light attenuation reducing phage inactivation.

Overall, considering combined data for HRAP + IP_{20} and HRAP + IP_{36}, the mean LRV was significantly higher ($p<0.05$, actual $p=0.028$; 95% CI: 0.04 to 0.84) when the IPs were operating ($HRAP_{KoM}$ + IP_{ON}, 1.76±0.10 LRV) compared to when the $HRAP_{KoM}$ was operating in their absence (LRV 1.27±0.13)

A summary of the F-Specific phage inactivation rates when either the 20 m^2 or the 36 m^2 IP was operating is shown in Table A10.3 of Appendix 10. The LRV recorded when operating the IP_{36} was less than that recorded for the IP_{20}. Turbidity, SS and chl *a* concentrations were significantly higher statistically ($p<0.05$, Appendix 10: Table A10.3) when the IP_{36} was operating, likely resulting in greater light attenuation and the consequent decrease in LRV, which was statistically significant, compared to when the IP_{20} was operated.

Figure 6.2 shows the trend in LRV, turbidity, SS and Chl a concentration for the collected samples in the $HRAP_{KoM}$ + IP_{ON} throughout the entire investigation.

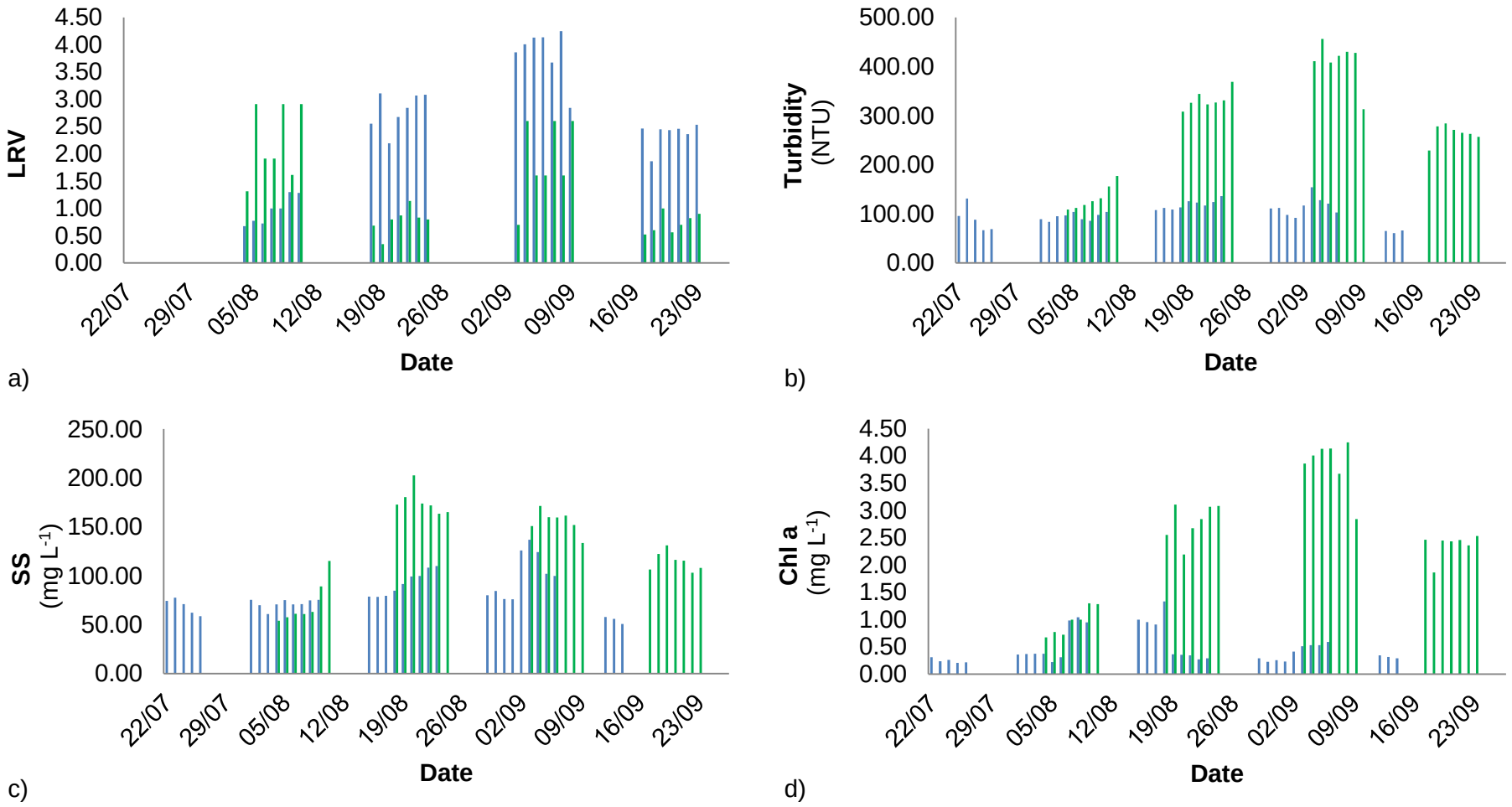


Figure 6.2: The variability in pond conditions observed in the $HRAP_{KoM}$ + IP_{ON} during Trial 1 (2015, ■) with 20 m^2 IP and Trial 2 (2016, ■) with 36 m^2 IP. Conditions included; a) F-Specific phage LRV (log_{10} PFU 100 mL^{-1}) turbidity (NTU), b) suspended solids, SS (mg L^{-1}) and c) chlorophyll *a*, (mg Chl *a* L^{-1}). Mean solar irradiances for 2015 and 2016 were 12.65 ± 0.70 MJ m^{-2} and 1 4.29 ± 0.59 MJ m^{-2} respectively.

The comparison between pond conditions in the HRAP when the IPs were operating and the inlet is presented in Table A10.4 (Appendix 10). As anticipated the changes in concentrations between the inlet and pond water were found to be significant statistically ($p<0.05$) except for BOD_5 ($p>0.05$) (Appendix 10: Table A10.4).

A similar comparison was performed between the normal HRAP operation and operation with the IP (Table 6.2). Higher concentrations were recorded for BOD_5, turbidity, SS and nutrients with the IP. However, as shown in Table 6.2 none of the differences between means were significant except the F-Specific phage concentration which was significantly lower ($p<0.001$, actual $p=1.22x10^{-04}$: 95% CI: 0.22 to 0.65) and the LRV was significantly higher ($p<0.01$, actual $p=0.003$; 95% CI: 0.17 to 0.82) when the IPs were operating. TN was also found to be significantly higher ($p<0.01$, actual $p=0.008$; 95% CI: 2.85 to 18.86) when the IPs were operating.

Table 6.3 shows that the operation of the IP_{20} for 5d period in July – September 2015 had no effect on wastewater composition or F-Specific phage inactivation when compared with data for the HRAP operated alone. The IP_{36} operated over the same period in 2016 resulted in significantly higher mean F-Specific phage LRV compared to that for the HRAP operated alone (Table 6.4). All the wastewater parameters which could influence light attenuation (NTU, SS & Chl *a*) were significantly higher ($p<0.05$) in 2016 when the IP_{36} was operating compared to 2015 when the IP_{20} was operated (Appendix 10; Table A10.4). This could be interpreted, given that the solar irradiance was not statistically different between years, that the inclusion of the IP increased the F-Specific phage LRV in turbid wastewaters.

Table 6.2: Independent samples T-Test comparison of water composition between water treated in the KoM HRAP under normal pond operation ($HRAP_{KoM}$ + IP_{OFF}) and water treated with the pond wall inclusion ($HRAP_{KoM}$ + IP_{ON}). Significance was at p<0.05.

Parameter	$HRAP_{KoM}$ + IP_{OFF}				$HRAP_{KoM}$ + IP_{ON}				T-Test results						
	n	Mean ± SE	Min	Max	n	Mean ±SE	Min	Max	Diff.	t	df	95% CI (lower and upper)		P value	sig
F-Specific phage	40	1.92±0.07	1.00	2.83	63	1.49±0.08	0.00	2.57	0.43	4.32	101	0.22	0.65	$1.22x10^{-04}$	***
LRV	40	1.27±0.13	0.02	2.56	63	1.76±0.10	0.35	2.19	-0.49	3.06	101	-0.82	-0.17	0.003	**
Turbidity	40	170.3±14.05	68.00	385.0	63	183.6±14.68	61.00	456.0	-13.34	-0.62	101	-56.13	29.46	0.538	-
SS	40	97.91±4.70	17.60	157.2	63	103.41±5.02	50.80	202.8	-5.51	-0.75	101	-20.07	9.05	0.455	-
BOD_5	16	15.66±3.32	1.40	42.30	26	18.60±2.37	1.40	42.30	-2.94	-0.74	40	-10.99	18.60	0.465	-
Chl *a*	40	1.43±0.17	0.15	3.60	63	1.37±0.16	0.21	4.25	0.06	0.25	101	-0.42	0.54	0.803	-
TOC	30	40.29±4.54	0.07	90.50	49	45.41±2.81	0.00	89.24	-5.12	1.02	77	-15.16	4.93	0.314	-
TC	30	54.23±6.01	0.14	114.1	49	61.82±3.92	0.16	120.1	-7.59	1.11	77	-21.26	6.07	0.272	-
IC	30	14.81±2.31	0.07	52.34	49	16.42±2.19	0.07	57.03	-1.61	0.48	77	-8.26	5.04	0.277	-
TN	30	65.81±4.91	0.00	88.07	49	76.67±0.96	61.49	88.65	-10.85	2.70	77	-18.86	-2.85	0.008	**
Solar Exposure	40	13.17±0.58	3.60	19.40	63	13.38±0.48	3.20	21.50	-0.22	-0.29	101	-1.72	1.28	0.774	-
Water Temperature	20	15.40±0.80	13.30	17.50	28	15.23±0.30	12.48	18.12	0.42	0.40	46	-0.68	1.01	0.688	-

Strength of statistical significance: * p<0.05 (significant), ** p<0.01 (highly significant), *** p<0.001 (extremely significant), - p>0.05 (not significant)

Table 6.3: Operation with a 20 m^2 IP ($HRAP_{KoM}$ + IP_{20}): Statistical comparison of F-Specific phage inactivation and mean pond conditions between the normal HRAP operation ($HRAP_{KoM}$ + IP_{OFF}; 5d) and operation with a 20 m^2 IP ($HRAP_{KoM}$ + IP_{20}; 5d). Values were collated from four collection periods throughout winter-spring 2015. Independent samples t-test was used with statistical significance determined for p<0.05.

Parameter	$HRAP_{KoM}$ + IP_{OFF}				$HRAP_{KoM}$ + IP_{20}				T-Test results						
	n	Mean ±SE	Min	Max	n	Mean ± SE	Min	Max	Difference	t	df	95% CI (lower and upper)		P value	Sig.
F-Specific phage	20	1.77±0.06	1.00	2.11	35	1.60±0.04	1.00	2.11	0.12	1.62	53	-0.03	0.28	0.110	-
LRV	20	1.89±0.12	0.82	2.56	35	2.04±0.11	0.76	3.37	-0.15	0.89	53	-0.50	0.19	0.376	-
Turbidity	20	98.00±4.06	68.00	123.00	35	102.66±3.69	61.00	154.0	-4.66	0.81	53	-16.23	6.91	0.423	-
SS	20	76.88±5.09	17.60	120.40	35	82.59±3.46	50.80	136.8	0.06	0.01	53	-11.93	12.04	0.993	-
BOD_5	10	20.78±4.47	1.40	42.30	21	20.52±2.60	1.40	42.30	0.27	0.06	28	-9.64	10.17	0.957	-
Chl *a*	20	0.53±0.07	0.151	1.07	35	0.47±0.05	0.209	1.335	0.05	0.61	53	-0.12	0.22	0.546	-
TOC	20	38.97±2.04	22.72	50.53	35	41.37±1.01	22.97	50.97	-2.41	1.18	53	-6.50	1.68	0.243	-
TC	20	49.97±2.64	29.44	68.00	35	51.41±1.52	33.29	71.32	-1.44	0.51	53	-7.11	4.24	0.614	-
IC	20	12.30±1.61	2.90	28.40	35	10.04±1.04	3.77	27.89	2.26	1.24	53	-7.11	4.24	0.222	-
TN	20	73.43±1.06	62.81	79.49	35	73.38±0.80	61.49	81.58	0.05	0.04	53	-2.60	2.71	0.968	-
Solar Exposure	20	12.41±0.77	7.10	19.40	35	12.65±0.70	3.20	20.80	-0.24	0.22	53	-2.44	1.95	0.824	-

Strength of statistical significance: * p<0.05 (significant), ** p<0.01 (highly significant), *** p<0.001 (extremely significant), - p>0.05 (not significant)

Table 6.4: Operation with a 36 m^2 IP ($HRAP_{KoM}$ + IP_{36}): Statistical comparison of F-Specific phage inactivation and mean pond conditions between the normal HRAP operation ($HRAP_{KoM}$ + IP_{OFF}; 7d) and operation with a 20 m^2 IP ($HRAP_{KoM}$ + IP_{36}; 7d). Values were collated throughout winter-spring 2016. Independent samples t-test was used with statistical significance determined for $p<0.05$.

Parameter	$HRAP_{KoM}$ + IP_{OFF}				$HRAP_{KoM}$ + IP_{36}				T-Test results						
	n	Mean ± SE	Min	Max	n	Mean ± SE	Min	Max	Diff.	t	df	95% CI (lower and upper)		P value	Sig.
F-Specific phage	20	2.12±0.10	1.49	2.83	28	1.35±0.16	0.00	2.57	0.77	3.76	46	0.36	1.18	0.001	***
LRV	20	0.64±0.10	0.02	1.44	28	1.41±0.16	0.35	2.91	-0.77	3.72	46	-1.18	-0.35	0.001	***
Turbidity	20	242.5±15.63	123.00	385.00	28	284.8±20.26	109.0	456.0	-42.25	1.54	46	-97.41	12.91	0.130	-
SS	20	113.2±6.34	31.60	157.20	28	129.44±8.15	54.00	202.8	-16.28	1.47	46	-38.53	5.97	0.165	-
BOD_5	6	7.13±2.27	2.80	14.10	6	12.22±5.02	1.40	31.00	-5.08	0.92	10	-17.35	7.18	0.378	-
Chl *a*	20	2.34±0.15	1.30	3.68	28	2.49±0.21	0.67	4.25	-0.16	0.56	46	-0.72	0.40	0.579	-
TOC	10	42.93±13.44	0.07	90.50	14	55.48±9.17	0.01	89.24	-12.55	0.80	22	-71.01	-39.96	0.432	-
TC	10	62.73±17.54	0.14	114.10	14	87.84±10.49	0.16	120.1	-25.11	1.30	22	-105.60	-70.08	0.206	-
IC	10	19.82±6.05	0.07	52.34	14	32.37±5.27	0.07	57.03	-29.27	1.56	22	-105.60	-70.08	0.134	-
TN	10	50.57±13.78	0.00	88.07	14	84.88±0.72	81.12	88.65	-34.31	2.97	22	-86.09	-83.67	0.007	**
Solar Exposure	20	13.92±0.85	3.60	18.90	28	14.29±0.59	7.50	21.50	-0.37	0.37	46	-2.39	1.65	0.712	-
Water Temperature	20	15.40±0.27	13.30	17.50	28	15.23±0.30	12.48	18.12	0.42	0.40	46	-0.68	1.01	0.688	-

6.3.4 Environmental and in situ pond conditions

Monitoring of effluent quality, and both pond and environmental conditions was performed to establish whether the measured parameters had a significant impact on pond and IP performance.

Solar Exposure

A plot of LRV against solar exposure is presented in Figure 6.3. In general the correlation between LRV in the HRAP (both IP_{ON} and IP_{OFF}) and daily solar exposure was negative (r = -0.672). A linear regression confirmed the connection between inactivation and solar irradiance, with the relationship significant for both pond conditions ($p<0.01$) despite low R^2 values.

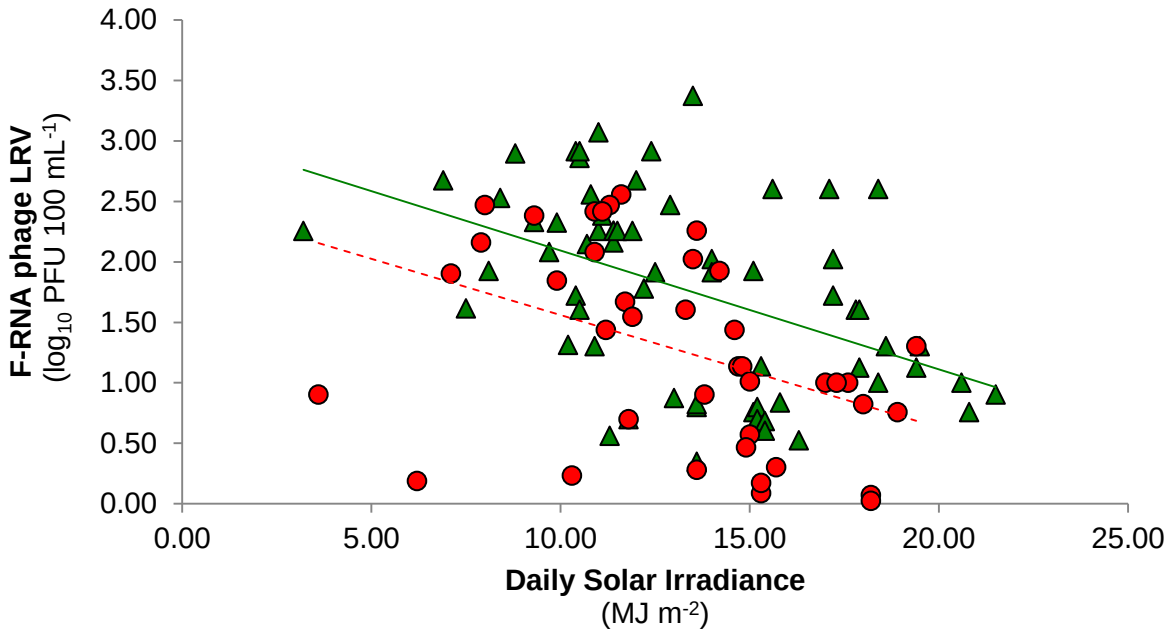


Figure 6.3: Influence of daily solar irradiation (MJ m^{-2}) on F-Specific phage removal (LRV) in the $HRAP_{KoM}$ + IP_{OFF} (●) and $HRAP_{KoM}$ + IP_{ON} (▲). Linear trend lines have been applied. Relationship identified significant for both IP_{OFF}: R^2 = 0.180, p<0.01 (actual p=0.001), and IP_{ON}; R^2 = 0.216, p<0.001 (actual p=0.001).

Similar plots of Chl *a*, turbidity and SS against solar irradiance are presented in Figures 6.4, 6.5 and 6.6 respectively. The relationship was identified to be positive for all three parameters, with concentrations increased with higher irradiance. However, linear regression identified this influence to be significant for the IP_{ON} only ($p<0.05$). R^2 values were again low. The increase observed with

these parameters could explain the lower LRV observed under higher irradiance, with Chl *a*, turbidity and SS contributing to light loss in the water column.

For both IP_{ON} and $IP_{OFF,}$ no significant relationships were detected between Chl *a*, SS and turbidity against solar irradiance for 2015 or 2016.

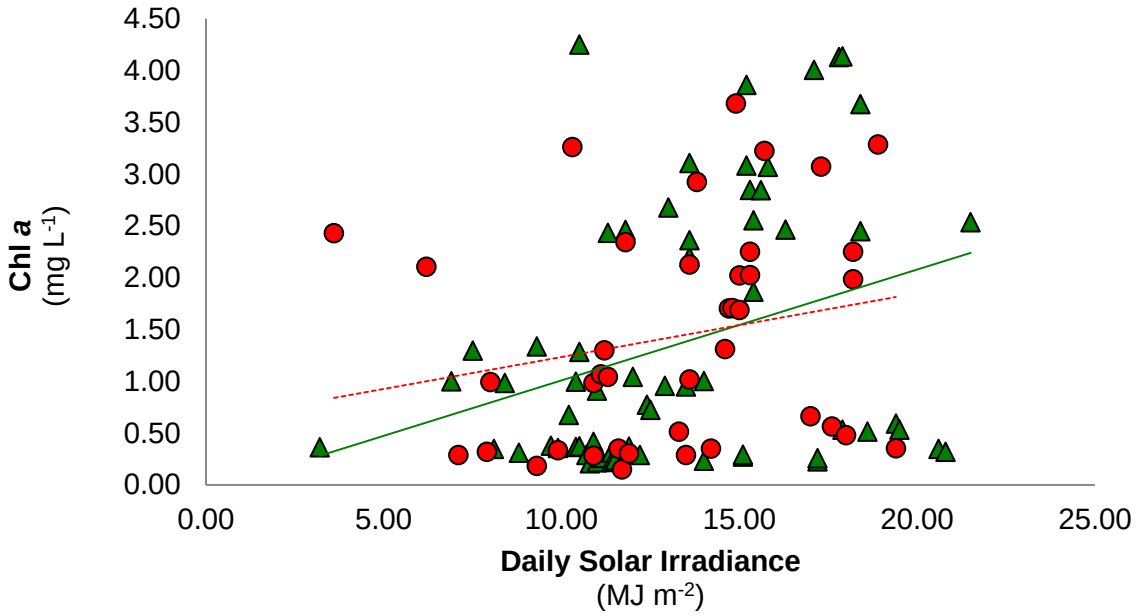


Figure 6.4: Influence of daily solar irradiation (MJ m^{-2}) on Chloropyll *a* (Chl *a*, mg L^{-1}) in the $HRAP_{KoM}$ + IP_{OFF} (●) and $HRAP_{KoM}$ + IP_{ON} (▲). Relationship significant for IP_{ON} (R^2 = 0.102, p<0.05, actual p=0.015), not for IP_{OFF} (R^2 = 0.046, p>0.05, actual p=0.184)

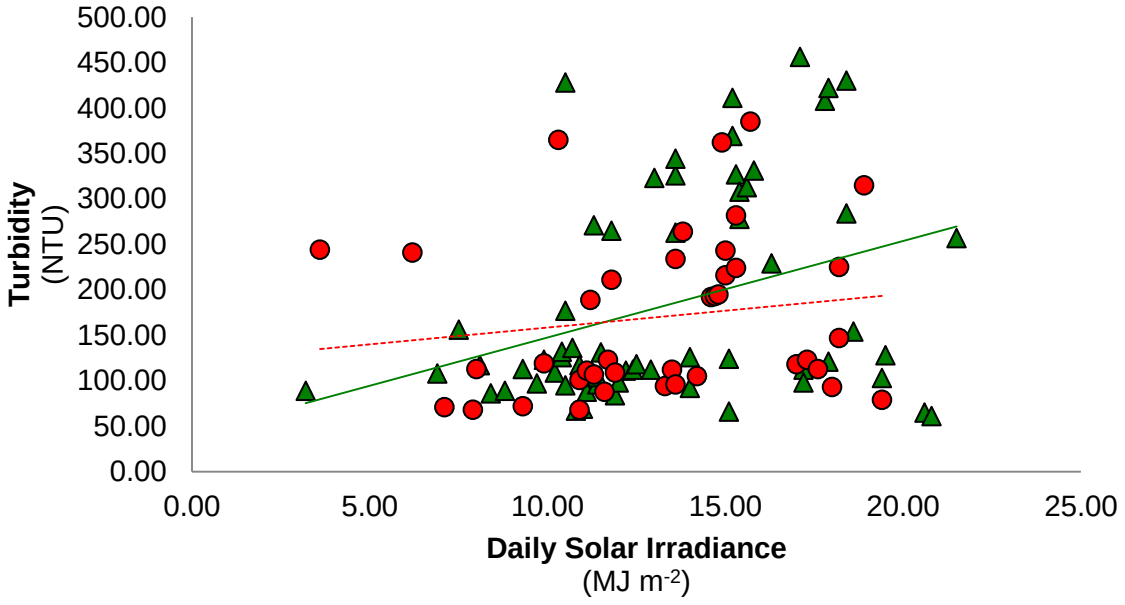


Figure 6.5: Influence of daily solar irradiation (MJ m^{-2}) on turbidity (NTU) in the $HRAP_{KoM}$ + IP_{OFF} (●) and $HRAP_{KoM}$ + IP_{ON} (▲). Relationship significant for IP_{ON} (R^2 = 0.119, p<0.01, actual p=0.006), not for IP_{OFF} (R^2 = 0.023, p>0.05, actual p=0.346)

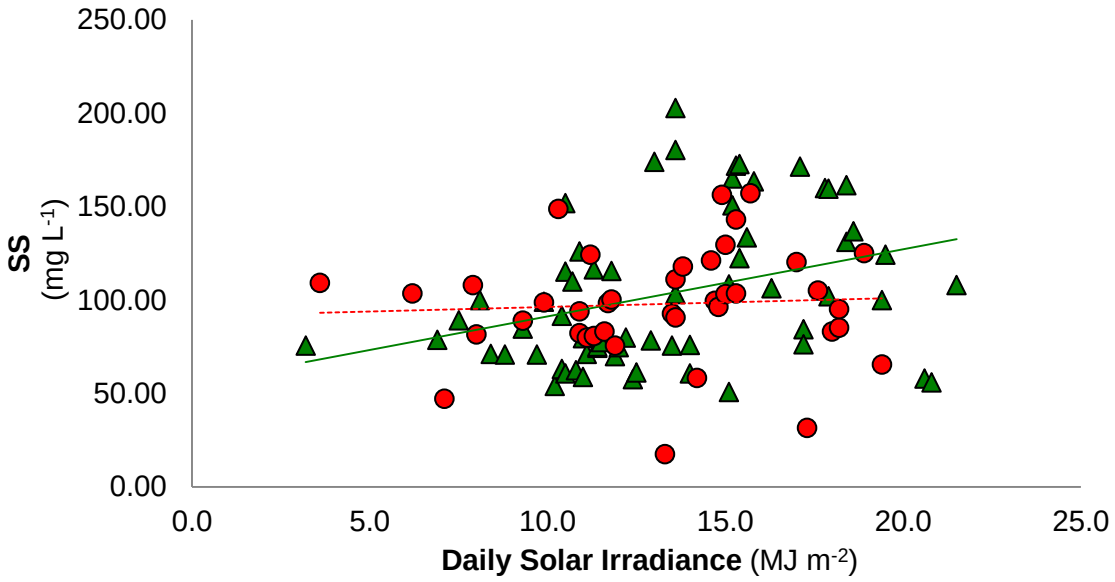


Figure 6.6: Influence of daily solar irradiation (MJ m^{-2}) on SS (mg L^{-1}) in the $HRAP_{KoM}$ + IP_{OFF} (●) and $HRAP_{KoM}$ + IP_{ON} (▲). Relationship significant for IP_{ON} (R^2 = 0.116, p<0.01, actual p=0.001), not for IP_{OFF} (R^2 = 0.004, p>0.05, actual p=0.709)

Pond Temperature

When possible the water temperature in the HRAP was logged and the daily means calculated. The means were calculated from the data recorded between 3:00 am and 2:59 am. Temperature was logged in 30 minute intervals.

From the data retrieved the HRAP pond temperature ranged between 12.48 and 18.12°C, the mean 15.30±0.21°C. For the different test conditions the pond temperature ranged from 12.48 to 18.12°C (mean: 15.23±0.30°C) with the pond wall and 13.30 to 17.50°C (mean: 15.40±0.27°C) without the pond wall. A significant difference was not detected between the test conditions (t (46) = 0.40, p = 0.688, p>0.05; 95% CI: -0.68 to 1.01) (Table 6.4).

Figure 6.7 shows a scatter plot of LRV against mean daily water temperature. No distinct relationship was observed between removal and water temperature and either pond conditions. However, a negative relationship was suspected with LRV appearing to be lower for the warmer temperatures, particularly for the $HRAP_{KoM}$ + IP_{OFF}.

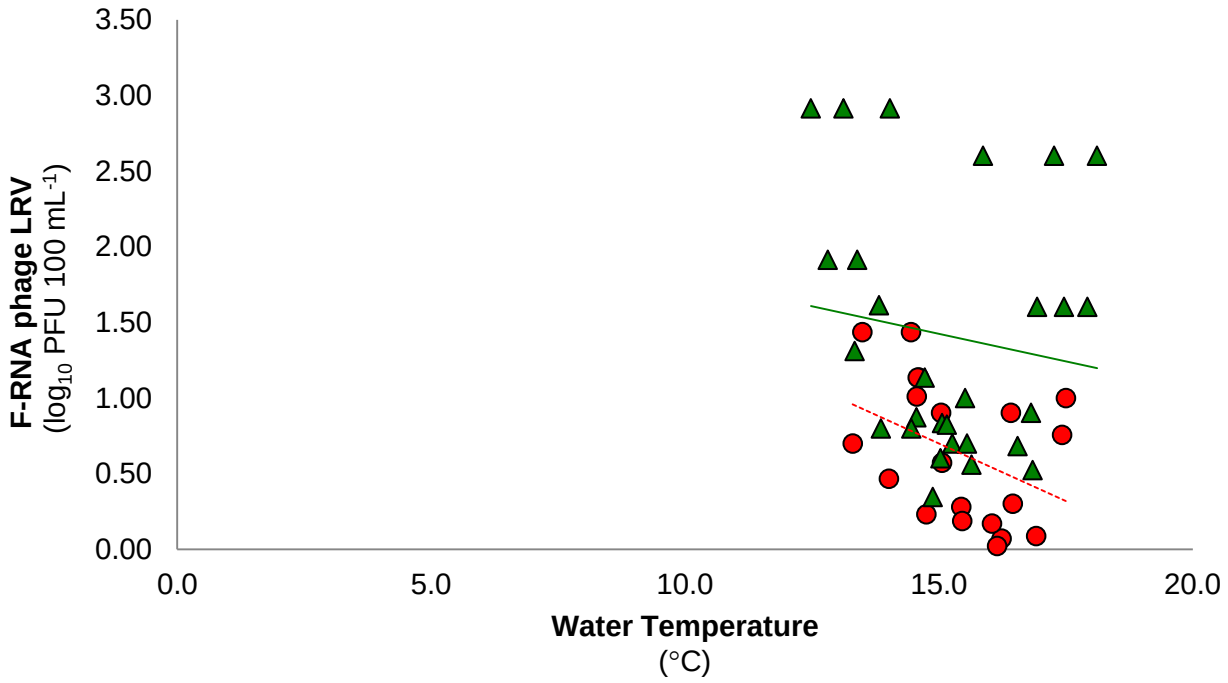


Figure 6.7: LRV against pond temperature for both $HRAP_{KoM}$ + IP_{OFF} (●) and $HRAP_{KoM}$ + IP_{ON} (▲). Reported values represent the daily mean temperatures logged in the pond between 3:00 am and 2:59 am. Resulting R^2 and p-values were; $HRAP_{KoM}$ + IP_{OFF}; R^2 0.1608, p=0.0835 (p>0.05) and $HRAP_{KoM}$ + IP_{ON}: R^2 0.020, p= 0.473 (p>0.05). Solar irradiances ranged between 3.6 and 21.5 MJ m^{-2}.

*Chlorophyll a (*Chl *a)*

Chl *a* was used as a surrogate for algal growth. In the $HRAP_{KoM}$ Chl *a* concentrations ranged between 0.15 and 4.25 mg L^{-1}. The highest was recorded for the $HRAP_{KoM}$ + IP_{ON}. The Mean Chl *a* concentrations for the $HRAP_{KoM}$ + IP_{OFF} and $HRAP_{KoM}$ + IP_{ON} as presented in Table A9.2 (Appendix 9) and 9.3 (Appendix 9) were 1.43±0.17 mg L^{-1} and 1.37±0.16 mg L^{-1}, respectively.

Figure 6.8 shows the influence Chl *a* had on F-Specific phage removal in the HRAP. For both pond conditions this influence was found to be negative with inactivation shown to be lower when the chl *a* concentration was high.

A linear regression confirmed the connection between inactivation and Chl *a* production, with the relationship significant for both pond conditions ($p<0.05$) despite low R^2 values.

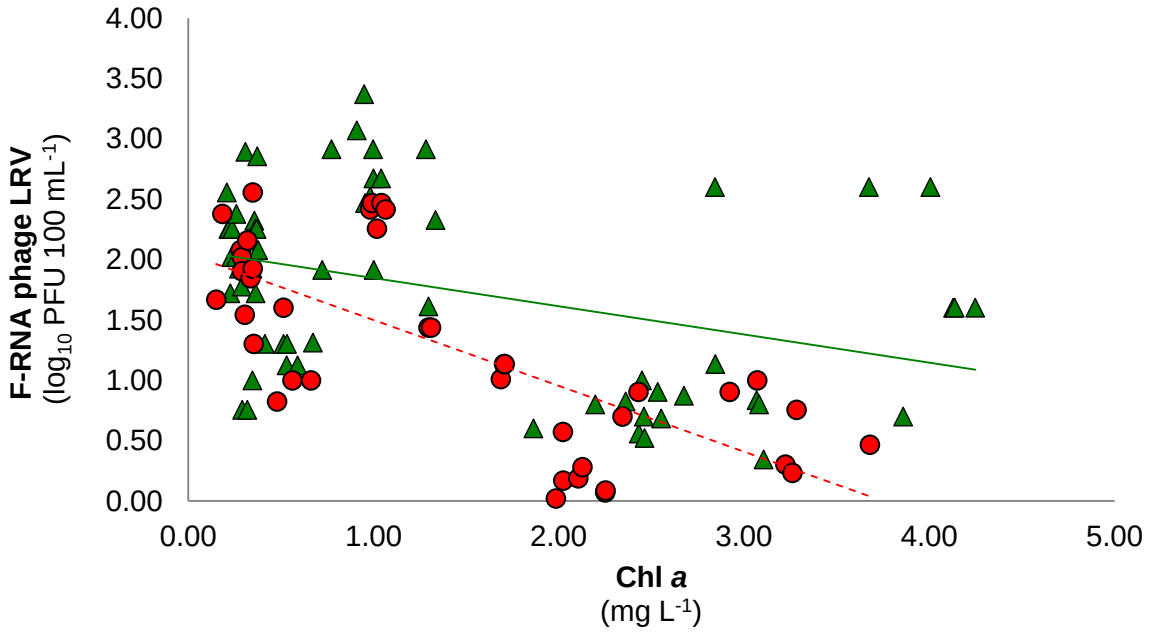


Figure 6.8: The relationship between F-Specific phage inactivation (LRV: log_{10} PFU 100 mL^{-1}) and Chlorophyll a (Chl *a*, mg L^{-1}) in the KoM HRAP when the pond was operated with ($HRAP_{KoM}$ + IP_{ON}, ▲) and without ($HRAP_{KoM}$ + IP_{OFF}, ●) the inclusion of the pond wall. Linear trendline has been included. Corresponding R^2 and p-values were; $HRAP_{KoM}$ + IP_{OFF}: R^2 = 0.516, p= 1.81 x 10^{-07} (p<0.001) and $HRAP_{KoM}$ + IP_{ON}: R^2 0.137, p= 0.003 (p<0.01). Daily solar irradiation ranged between 3.2 and 21.5 MJ m^{-2}

Turbidity and Suspended Solids (SS)

The mean concentrations for turbidity (NTU) and SS (mg L^{-1}) in the $HRAP_{KoM}$ + IP_{OFF} and $HRAP_{KoM}$ + IP_{ON} are outlined in Appendix 9 (Tables A9.2 and A9.3). In general, the $HRAP_{KoM}$ reported concentrations between 61–456 NTU and 17.6–202.8 mg SS L^{-1}. Again, the higher concentrations were exhibited when the IP was operated.

Figure 6.9 and 6.10 show the influence of turbidity and SS on F-Specific phage LRV. The impact of SS and turbidity on removal was identified negative, with reduction lower with increased concentrations. For both parameters and pond conditions this relationship was identified to be highly significant (p<0.001).

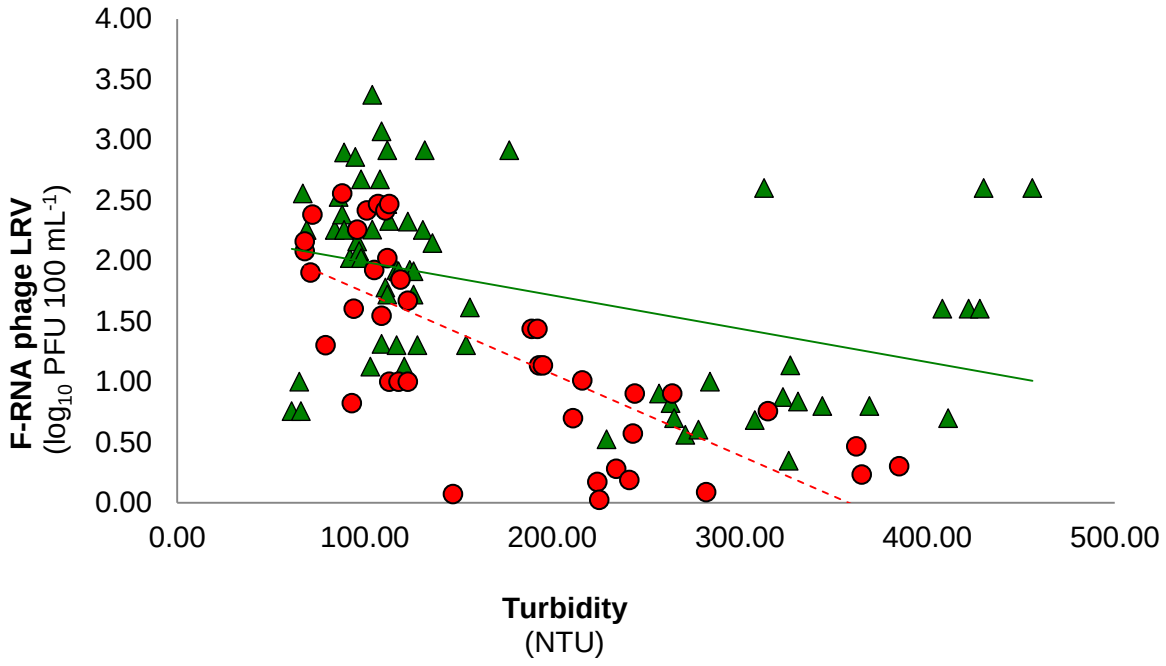


Figure 6.9: F-Specific phage inactivation (LRV: log_{10} PFU 100 mL^{-1}) against Turbidity (NTU) for the $HRAP_{KoM}$ + IP_{ON},(▲) and $HRAP_{KoM}$ + IP_{OFF} (●). Linear trendline has been included. Corresponding R^2 and p-values were; $HRAP_{KoM}$ + IP_{OFF}; R^2 0.555, p= 3.49 x 10^{-08} (p<0.001) and $HRAP_{KoM}$ + IP_{ON}: R^2 0.164, p= 0.001 (p<0.01). Daily solar irradiation ranged between 3.2 and 21.5 MJ m^{-2}.

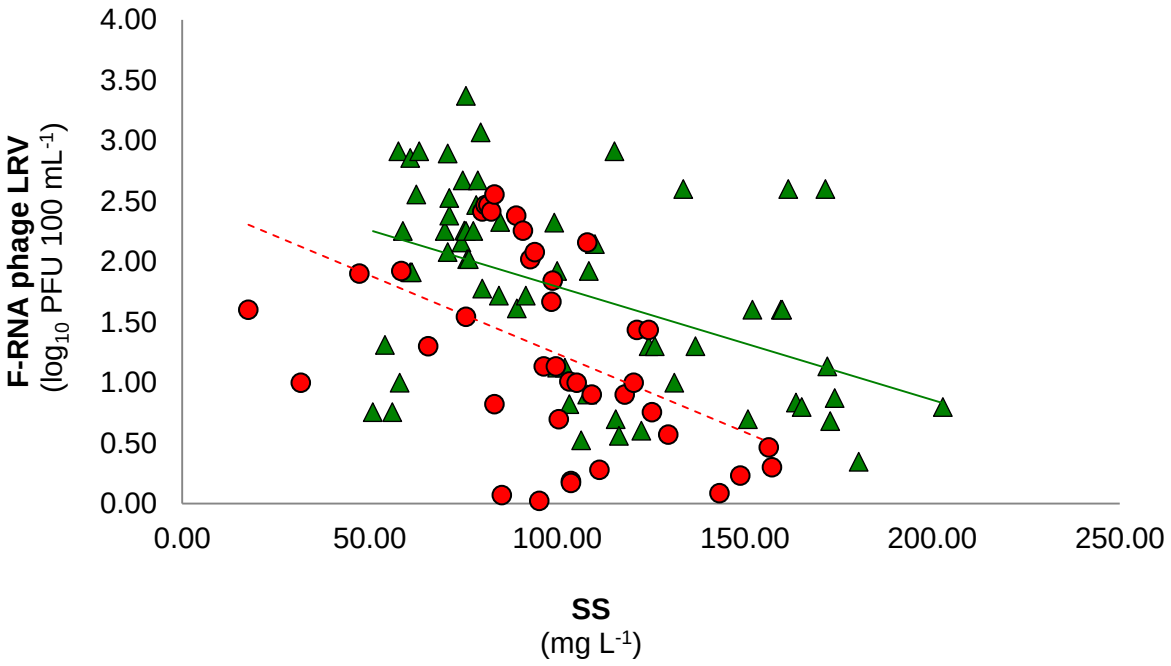


Figure 6.10: F-Specific phage inactivation (LRV: log_{10} PFU 100 mL^{-1}) against suspended solids (SS, mg L^{-1}) for the $HRAP_{KoM}$ + IP_{ON},(▲) and $HRAP_{KoM}$ + IP_{OFF} (●). Linear trendline has been included. Corresponding R^2 and p-values were; $HRAP_{KoM}$ + IP_{OFF}; R^2 0.230, p= 0.0002 (p<0.001) and $HRAP_{KoM}$ + IP_{ON}: R^2 0.219, p= 0.000108 (p<0.001). Daily solar irradiation ranged between 3.2 and 21.5 MJ m^{-2}.

6.3.5 Construction costs

Construction costs for the KoM IP were estimated and summarised in Table 6.5. Similarly the costs specific to the individual components are presented in Table 6.6. A more detailed account is presented in Appendix 11. The final estimate at the completion of the investigation for the large scale IP was approximately $718.87. Costs presented in this work are reported as Australian dollars (A$)

Operating costs were also estimated. Costs were predicted at approximately $1.08 per day, $394.2 per annum. This estimation was determined using a power rating of 150 W (as provided by Aqua Pro AP7500 multi-use pump manufacturer, Appendix 2) and an electrical cost of $0.30 kWh^{-1}. The overheads doubled to approximately $2.16 per day and $788.40 per annum when the two pumps were used.

Table 6.5: Associated construction costs for both IP systems used at the Kingston on Murray, KoM HRAP Costs are summarised in accordance to the IP itself and the additional costs associated from the modifications to the systems performed. Costs are also reported in Australian dollars (A$).

Estimated Construction Costs		Cost (A$)
IP_{20}	IP	$430.92
	IP + Filter	$477.24
IP_{36}	IP	$672.55
	IP + Filter	$718.87
	IP Extension	$241.63
Other	Filtration system	$ 46.32

Table 6.6: Break down of the costs associated with the individual components of the IP system. Costs are reported in Australian dollars (A$).

Component	Cost (A$)
$IP_{(total\ size)}$	$ 126.14
Pumps	$ 472.00
Timer	$ 45.68
Filtration	$ 46.32
Electrical	$ 28.73
TOTAL	**$ 718.87**

6.4 Discussion

The importance of sunlight for microbial inactivation in pond systems is emphasised throughout the literature, with its rapid dissipation due to attenuation and absorption also discussed (Kirk, 1994; Davies-Colley *et al.*, 1997; Sinton *et al.*, 2002; Craggs *et al.*, 2004b; Bolton *et al.*, 2010; Sutherland *et al.*, 2014; Sutherland *et al.*, 2015). Development of strategies to improve solar exposure in these pond systems is sought. In this study, the concept of running water down an inclined plane (IP) to improve disinfection was up scaled into the field where it was retrofitted into a fully functioning HRAP in the South Australian Riverland (Kingston on Murray, KoM). The wall surrounding the pond water had provided a suitable area for disinfection, with the HDPE pond lining used as the exposed surface. By up scaling the system the potential and practicality of the IP under more realistic conditions can be assessed.

Experiments performed in this chapter were operated throughout July-September of consecutive winter-spring sampling seasons (2015 and 2016). Selection of the sampling periods was based on what is known regarding pathogen removal in pond systems, solar irradiation on inclined planes and pond validation (Davies-Colley *et al.*, 2005; Navntoft *et al.*, 2012; Fallowfield *et al.*, 2018). Consequently, examination of the IP performance in the field had been performed under conditions considered to be worst case

6.4.1 KoM inlet wastewater

Minimal variation in the composition of the KoM inlet water had been observed throughout the investigation. Buchanan (2014) described the limited variation in HRAP inlet wastewater as a consequence of the two to three day retention time in household septic tanks prior to delivery onsite. During this time the wastewater is exposed to anaerobic conditions and allowed to settle resulting in the water composition becoming more uniform (Buchanan, 2014).

A slight drop in detectable F-Specific phage was observed in the inlet wastewater between the different sampling periods. However, this difference between concentrations was shown to be small and unlikely to of had a major bearing on the pond performance. Furthermore, it was identified that the overall mean F-Specific phage (3.18 ± 0.24 $\log_{10}$ PFU 100 mL^{-1}) in the inlet water

was similar but slightly lower than the 4.05±0.73 log_{10} PFU 100 mL^{-1} reported by Young *et al.* (2016) for the same system over a larger sampling period (ten months).

When the composition of the KoM inlet wastewater was compared to the current literature, the resulting concentrations were shown to equivalent or lower than those previously reported. For instance; the turbidity (NTU) and SS (mg L^{-1}) concentrations reported here were found to be similar to those reported for KoM inlet wastewater by Fallowfield *et al.* (2018) but lower than those reported elsewhere in the literature (Picot *et al.*, 1991; Picot *et al.*, 1992; El Hamouri *et al.*, 1994; Chen *et al.*, 2003; Craggs *et al.*, 2012a). The low BOD_5 (mg L^{-1}) presented here was the biggest deviation from the literature (Banat *et al.*, 1990; El Hamouri *et al.*, 1994; Craggs *et al.*, 2012a; Young *et al.*, 2016; Fallowfield *et al.*, 2018). It is uncertain as to why the concentration presented here was so low.

6.4.2 F-Specific phage inactivation in the large scale HRAP with and without the IP

An advantage of HRAPs is their ability to yield high microbial inactivation rates (Fallowfield *et al.*, 1996). This proficiency had been observed under both normal pond operation ($HRAP_{KoM}$ + IP_{OFF}) and with the inclusion of the IP ($HRAP_{KoM}$ + IP_{ON}). Comparison of the obtained LRVs with the current literature was difficult, with only a few studies having reported phage and virus removal in HRAPs, and no other study (to the author's knowledge) reporting removals by an IP. Nonetheless, the LRVs presented here were found to be comparable to the F-RNA phage inactivation reported by Fallowfield *et al.* (2018) and Young *et al.* (2016) in the same HRAP. Campos *et al.* (2002) observed a removal of 1.3 LRV of F-RNA phage in a facultative WSP with a theoretical hydraulic retention time (THRT) of 18 d; 13 d longer than the 5 d HRT used in the $HRAP_{KoM}$. Consequently, the results presented in this work further supports the findings by Buchanan *et al.* (2011a) that equal or better pathogen removal could be achieved in HRAPs in a fraction of the time needed by WSPs.

Addition of the IP into the large scale HRAP had been shown to produced results much like those observed with the model systems (Chapters 3, 4 and 5), with F-Specific phage inactivation

elevated. This elevation was apparent whenever the IP had been in operation, an observation that was consistent throughout the thesis.

Inclusion of the 20m^2 IP had resulted with a LRV that was found to be higher than the inactivation achieved when the pond was operated as normal. Corresponding LRV were 2.04±0.11 ($HRAP_{KoM}$ + IP_{20}) and 1.89±0.12 ($HRAP_{KoM}$ + IP_{OFF}), respectively. This improvement was, however, not statistically significant. Disruption to the water flow down the pond wall was believed to be a contributing factor, with reduced water restricting disinfection performance. Strategies to resolve this issue were devised and will be addressed below in Section 6.4.5. The LRV_5 for the $HRAP_{KoM}$ + IP_{20} was also found to be 1.2-1.8 times higher than the LRVs presented above for Campos *et al.* (2002), Young *et al.* (2016) and Fallowfield *et al.* (2018).

Modifying the surface area of the IP and the time in which the IP was in operation was shown to have a positive impact on the removal performance by the IP, with the difference in removal between the $HRAP_{KoM}$ + IP_{36} and the $HRAP_{KoM}$ + IP_{OFF}, statistically significant. What is more, the difference between the corresponding LRVs (i.e. LRV_7 1.41±0.16 and LRV_7 0.64±0.44) was approximately 2.0 times higher than achieved with the 5 m IP, despite the lower reduction values observed.

Overall, the reduction in F-Specific phage exhibited in the $HRAP_{KoM}$ were 1.27±0.13 (IP_{OFF}) and 1.76±0.10(IP_{ON}). These were found to be consistent with 1.0 – 4.0 LRV deemed to be acceptable for bacteriophage and virus removal by the Australian reuse guidelines for WSPs (NRMMC-EPHC-AHMC, 2006). Results from the statistical analysis presented in Table 6.2 indicates that the null the null hypothesis (H_0) that incorporating the pond wall as part of the treatment process will have no greater impact on the inactivation of F-Specific phage could be rejected, with the resulting p value ($p=0.003$) <0.05. The alternative hypothesis (H_1) that the pond will have a significant impact on inactivation rates in the field was therefore accepted.

The characteristics of the HRAP effluents for both IP_{ON} and IP_{OFF}, were typical for HRAPs (Picot *et al.*, 1991; Picot *et al.*, 1992; El Hamouri *et al.*, 1994; Chen *et al.*, 2003; Craggs *et al.*, 2012a; Young

et al., 2016; Fallowfield *et al.*, 2018). A reduction in BOD_5 (mg L^{-1}) was evident. However, the 19.4 and 4.25% removal observed in the $HRAP_{KoM}$ + IP_{OFF} and $HRAP_{KoM}$ + IP_{ON} was found to be significantly lower than previously reported. For instance Fallowfield *et al.* (2018), Craggs *et al.* (2012a), and Banat *et al.* (1990) reported BOD_5 removals of 90.6%, 46.8% and 90-95%, respectively. The low inlet concentration may have been a contributing factor. Nonetheless, the low BOD_5 concentrations exhibited are within the required range for the resultant effluents to be considered safe for reuse (NRMMC-EPHC-AHMC, 2006; WHO, 2006b),

The increase in Chl *a* (mg L^{-1}), SS (mg L^{-1}) and turbidity (NTU) is typical in these systems, linked with algal growth. Although the water composition was shown to be varied in the $HRAP_{KoM}$ between the sampling seasons, a similarity between the ponds was apparent when the HRAP was operated with and without the IP. This consistency indicates that running the pond water down the IP had no overall bearing on the composition of the water quality, pathogen removal the exception. This means that the integrity of the pond system can be maintained with the addition of the pond wall. However, as only one pond system was available for this research and the IP was switched on and off at set intervals, a more in depth analysis would be required to ensure this is the case.

In comparison to other HRAPs, the size of the freeboard presented at KoM (i.e. 56 m^2, total length 13 m) was considered to be quite large. Nonetheless, from the work carried out in the model systems and the results presented in this work, the author is confident the concept can be easily adapted and successful in HRAPs with smaller freeboards or more likely in other systems e.g. WSP.

6.4.3 Environmental factors

In most cases disinfection in pond systems can be explained by the environmental and in situ characteristics of the pond (Davies-Colley *et al.*, 1999). Typically inactivation will increase with pond temperature and solar irradiance (Sinton *et al.*, 2002; Davies-Colley, 2005). However, for both operating conditions this increase was not observed with F-Specific phage LRVs. Young et al. (2016), observed a similar result, suggesting the irradiances observed for the South Australian Riverland (study site) were higher than those required for maximum disinfection resulting with

disinfection becoming independent of irradiance. However, it was predicted that the high algal growth exhibited in the pond was a more likely explanation.

Algal growth plays a major role in the inactivation process in HRAPs by increasing pH and the concentration of reactive oxygen species (ROS) needed for inactivation (i.e. photo-oxidation) (Curtis *et al.*, 1992). However, this was not the case in the $HRAP_{KoM}$ for either operating conditions with the reduction in F-Specific phage decreased with chl *a* production. This was unsurprising, with Van der Steen *et al.* (2000) and Ansa *et al.* (2012) claiming algal to have a detrimental effect on inactivation when it reaches a specific concentration (i.e. optimum). Also algae have been classified as a main contributor to light loss in pond systems with algae contributing to attenuation and absorption. Sutherland *et al.* (2013) claimed up to 80% of the light absorbed in ponds could be attributed to light absorption by algae; the light used for photosynthetic activities. This connection between solar irradiance, Chl *a* (algae) and LRV is evident in Figures 6.3 and 6.4, respectively with algae believed responsible for the poor removal performance under increased irradiation.

A correlation has also been identified between algae growth (Chl *a*) and pond depth within the literature with increased algal production associated with shallower depths (Sutherland *et al.*, 2014). Shorter light paths in shallower pond depths are responsible with the algae receiving greater solar exposure (Sutherland *et al.*, 2014). On the pond wall, the water depth had been reduced to a thin layer; the surface area to volume ratio was therefore increased (S/V). It was therefore unsurprising that the highest recorded chl *a* concentration observed when the pond wall was in operation (i.e. 4.25 mg L^{-1} $HRAP_{KoM}$ + IP_{36}) (Appendix 9: Table A9.3). Increased algal production has also been reported when the exposed surfaces of photobioreactors (PBR) were tilted towards the sun (Tredici and Materassi, 1992; Hu *et al.*, 1996; Hu *et al.*, 1998; Tredici, 2004). Tredici (2004) outlined several advantages of using inclined slopes to amplify solar exposure for higher algal production yields. For instance; inclined planes have the capability of increasing both S/V volumes and turbulent flow, with high S/V resulting with higher cell production; a consequence of the greater solar exposure received over the surface (Tredici, 2004). These advantages can also be applied to improving pathogen removal.

SS and turbidity concentrations were also shown to be increased in the pond with the rise in concentrations linked to algae growth (Fallowfield *et al.*, 2018). For both operating conditions (i.e. IP_{ON} and IP_{OFF}) SS and turbidity were shown to have a similar impact on F-Specific phage removal with LRV decreased when concentrations were high. Furthermore, these parameters are also commonly associated with reduced light penetration in pond systems by contributing to light attenuation (Kirk, 1994; Bolton, 2012). Evidence of this is displayed in Figures 6.9 and 6.10 with LRV under higher solar irradiances heavily impacted.

6.4.4 Cost efficiency of the system

Significantly less energy is required to operate HRAPs compared to other treatment systems, such as activated sludge systems (Shilton *et al.*, 2008; Woertz *et al.*, 2009; Craggs *et al.*, 2011) making them desirable in resource limited areas and regions. Modification to the operation or construction of these ponds would therefore need to be considered this before application in the field to ensure the low cost status and practicality of these ponds is maintained.

As a guideline, Buchanan (2014) and Young *et al.* (2017) outlined the construction costs of the KoM HRAP and compared the associated costs with those reported for a South Australian WSP at Lyndoch (34.601179°S, 138.897011°E). They reported for a pond of 0.32 m depth (as used in this work), the total construction cost of the pond was approximately A$100,211 (Table 6.7), a cost 60.8% lower than the estimated cost for a WSP (A$255,825) (Buchanan, 2014; Young *et al.*, 2017). Addition of the IP was estimated to increase costs by 0.7%, raising the capital to A$100,931 approximately (Table 6.7).

Table 6.7: Comparison of construction costs between a South Australian High Rate Algal Pond (HRAP) and Waste Stabilisation Pond (WSP). Table is adapted from the work by (Buchanan, 2014) and Young *et al.* (2017). Costs are reported in Australian dollars, A$.

Design Parameters	High Rate Algal Pond (HRAP)	Waste Stabilisation Pond (WSP)
Pond depth (m)	0.32	1.20
Freeboard (m)	0.20	0.80
Surface area (m^2)	2,500	6,000
Surface area as a percentage of WSP (%)	41.6	100
Annual evaporative loss (m^3)	4,500	10,800
Evaporative loss as percentage of treated water (%)	12.3	29.6
Top dimensions (m)	51.7	81.1
Bottom dimensions (m)	50.0	77.5
Internal volume (m^3)	1,348	12,169
Linear area (m^2)	2,831	6,816
Curtain area (m^2)	104	504
Earthworks as percentage of WSP (%)	11.1	100
Estimated construction costs	A$	A$
HDPE liner	44,030	109,801
Earthworks	16,820	146,023
Paddlewheel assembly	20,000	-
Buffer tank	20,000	-
Total Construction	**100,211**	**255,825**
HRAP costs as a percentage of those for the WSP	**39.2%**	**100%**

Operation of the IP in the field was considered to be fairly inexpensive with running costs estimated to be less than A$800 per year (Section 6.3.5). This value however is only a rough estimate with expenses expected to fluctuate due to the ever changing global and local electricity prices and variation in pump size, power rating and cost. Incorporation of a solar panel onsite will help to reduce operation costs and ensure operation is as economical as possible. Addition of a solar panel to run not only the IP but the HRAP itself will further the appeal of this pond system in resource limited areas.

6.4.5 Future Research

In the field, the addition of the IP was shown to be positive and the outcome promising. However, the author acknowledges the necessity of further research to ensure to the IP is used to its fullest, with its full potential achieved. Below outlines some of the potential areas where future research could be directed.

For instance, it would be beneficial if two independent HRAPs could be run simultaneously onsite, such that inactivation with and without the addition of the pond wall can be examined more accurately with inactivation achieved under the same environmental conditions.

For pond validation it is required that inactivation rates are determined under the worst case scenario; i.e. performed throughout winter where solar irradiances are low (Fallowfield *et al.*, 2018). However, it would be of advantage to monitor the performance of the systems throughout the course of a year to determine whether performance is impacted by seasonal variation and establish the longevity of the system; i.e. how it withstands the elements and continuous use.

Operation of the IP throughout summer could exhibit the highest removal rates overall within the systems, however it may lead to even greater production of algal biomass which could potentially have a greater impact on removal efficiency. In the work by (Navntoft *et al.*, 2012) retrieval of greater solar radiation could be achieved on a tilted surface throughout winter months. If this was true and depending on the results achieved throughout summer, it could be that the IP may not need to be operated for the whole year, just throughout the months with lower irradiances. If this was the case, the operational costs would be significantly reduced, maintain the economical status of the pond and further adding to the appeal of the system.

To further maximise the removal efficiency of the IP. Pumping the inlet water directly down the pond wall before entry into the pond could see even greater removal rates with all the incoming phage being exposed to sunlight.

6.5 Conclusion

In this chapter, the concept of using an inclined plane to enhance solar exposure and pathogen removal was up scaled into a fully functioning HRAP with the work conducted as a pilot scheme to assess the plausibility of the IP in the field. Mixed results were presented in this study with significantly improved removal of F-Specific phage observed only once the size and operation of the IP was increased. Overall, the following conclusions could be made;

- Increased F-Specific phage inactivation was observed whenever the IP was in operation
- Improved removal was observed with modification to the IP; i.e. increased exposure surface area
- Increased removal was not observed under high solar irradiances with algal growth impacting removal performance

Whilst the results were promising additional work is still required before the system can be fully integrated as part of the treatment strategy in these pond systems. The main areas that would be of interest for future research would be:

- Direct comparison between normal and pond wall operation with the two systems operated side by side.
- Having the pond wall continually in operation to observe the long term effects the IP has on removal and effluent quality
- Directly pumping water down the wall such that the whole volume is passed down the wall before entering the system

Furthermore the results indicate this concept is not restricted to HRAPs or wastewater and could be applied to any pond system where there is a large sloped area available, WSP in particular could benefit.

7. GENERAL CONCLUSION

This chapter summarises the key findings from the results and discussions presented in each experimental chapter. A more detailed discussion was presented at the end of each corresponding chapters. Presented is also an overview of the potential areas for future research.

Sunlight is crucial for F-Specific phage inactivation in pond systems used for wastewater treatment, however, it is rapidly attenuated with depth by suspended solids, algae and turbidity (Kirk, 1994; Bolton, 2012). As a way to combat the adverse effects of light attenuation on pathogen removal, this thesis presented the concept of applying inclined planes to natural treatment systems to increase pathogen inactivation. Wastewater flowing over a solar exposed, inclined plane as a thin layer enhances pathogen exposure to damaging UV light by reducing the negative impacts associated with light attenuation. Hawley (2012) first introduced the concept of using an IP for improved disinfection with the study focussed solely on assessing whether inactivation could be improved with an IP under sunny and overcast conditions. The work presented in this study builds on the initial work by Hawley (2012), focussing on expanding the concept across a range of different water types, operating conditions and system scales (i.e. model and field based scales), with the objective to determine IP effectiveness.

With limited information available regarding phage and virus inactivation in HRAPs this study took the opportunity to bridge the gap. F-Specific RNA bacteriophage MS2; a known and commonly used surrogate for enteric viruses (Havelaar and Melse, 2003) was therefore chosen to assess inactivation with the IP.

Below outlines the respective aims of this research as presented in Chapter 1, with the findings relevant to each summarised accordingly.

> *Aim 1: Examine pathogen inactivation in HRAPs with the addition of an inclined plane and establish 'proof of concept' in laboratory scaled model systems*

Chapters 3, 4 and 5 examined the inclusion of an inclined plane to model HRAPs under different exposure conditions and water types to establish whether enhanced inactivation was achieved by incorporation of an IP.

Chapter 3 reported on MS2 inactivation in dark model HRAPs operated using optically clear 'tap' water with and without a solar exposed IP. These conditions were chosen as they enabled the contribution of the IP towards inactivation to be determined without the added complexities associated with other water types, such as turbidity and the presence of solids. Complexities know to restrict light penetration (Curtis *et al.*, 1994; Tchobanoglous *et al.*, 2003; Bolton *et al.*, 2010). It was clear that in all instances where the IP was present MS2 inactivation was greater than without the IP. Log_{10} removal values, measured over 24h (LRV_{24}), were 1.2-1.6 higher when an IP was incorporated compared those determined for the dark incubated, $HRAP_D$. Results presented in Chapter 3 also confirmed the sensitivity of MS2 to sunlight outlined within the literature (Sinton *et al.*, 2002) and confirmed inactivation was increased at higher solar irradiances.

Chapter 4 continued the examination with dark incubated model HRAPs with and without IPs, only this time the tap water was replaced with wastewater. Selection of wastewater allowed the performance of the IP to be assessed in the presence of light attenuating suspended solids, algae and turbidity. Proof of concept was again identified with elevated inactivation observed whenever the IP was present. However, modifications to the system were required before statistically significant difference between $HRAP_D$ and $HRAP_D$ + IP were identified (addressed under Aim 2).

The influence of water type on IP efficiency was also reported in Chapter 4 with inactivation evidently faster in optically clear water opposed to wastewater, the differences in water composition were believed responsible. Irrespective of water source, however, the systems including an IP continued to record greater inactivation than the corresponding systems without an IP.

Chapter 5 concluded the examination in the model systems reporting IP induced inactivation rates when ponds and IPs were both solar exposed in wastewater. This provided an evaluation of the IP under more realistic operating conditions and enabled a more accurate assessment of the

effectiveness of the IP when compared with an HRAP without an IP also exposed to sunlight. The results continued to support the inclusion of the IP, with higher inactivation rates exhibited whenever the IP was in use.

Chapter 5 reported similar observations to Chapters 3 and 4 in the sense that elevated inactivation rates within the presence of the IP were observed in model systems where both IP and pond were exposed to sunlight. Comparable inactivation was identified between all model systems examined in this chapter and the LRV achieved in a HRAP in the field (Young *et al.*, 2016), with the LRV presented in this study tending to be higher.

Overall, proof of concept was established using model systems, indicating the IP to be an affective addition for enhancing solar exposure and pathogen inactivation. The findings primarily from Chapter 5 support the adoption of the IP concept into the field with results likely to be positive. This adaption was also investigated and addressed below under Aim 2.

> *Aim 2 Evaluate different design parameters of the inclined plane system to improve performance and establish system efficiency.*

Chapters 3, 4 and 5 reported on the influence surface area, incubation time and HLR had on the effectiveness of the IP. It was found that when surface area of the IP was increased faster inactivation rates (K_D) and higher LRV could be achieved in majority of the experiments performed. The LRV obtained by incorporation of the LIP was shown not to improve LRV compared to the SIP, except when both pond and IP were solar exposed.

Increasing incubation time was also assessed to determine whether there was a significant difference between the SIP and LIP with more prolonged exposure. The SIP and LIP both recorded significantly enhanced F-Specific phage inactivation in wastewater, when compared to the HRAP alone, following 49.5 h incubation (Chapters 4 and 5). In contrast, incubation between 7.5 – 24 h resulted in significant increases in inactivation in the optically clear water when either SIP or LIP was incorporated with the HRAP compared to those achieved solely by the HRAP (Chapter 3).

Chapter 4 reported on the influence HLR had on inactivation with the IP, identifying that a statistically similar LRV could be obtained when IPs of different length and surface area were operated at the same HLR under the same climatic conditions. This was true for all HLR examined. This information can be applied to scale or adapt the system into the field or other pond systems.

Aim 3: Establish the effectiveness of the inclined plane system when up-scaled into an already established pond system (i.e. Kingston on Murray).

With proof of concept identified in the model HRAPs the logical progression was to expand the system into the field as a pilot system. Chapter 6 reported the impact the IP had on F-Specific phage (MS2) inactivation in a fully functional HRAP. The slope of the HDPE lined pond wall was adapted to form an IP by addition of a distribution manifold. Since there was no control HRAP available (with no IP) the IP was operated for 7d and the data obtained compared with an adjacent 7d period when the IP was not operated. Whenever IP was on the LRVs were higher than the HRAP under normal operation, much like the model systems presented in Chapters 3, 4 and 5. However, increasing surface area from $20m^2$ to $36m^2$ was required before the increase in inactivation by IP incorporation was statistically significant.

Chapter 6 also reported the relationship between sunlight irradiance and F-Specific phage inactivation to be negative. An observation consistent with the other research carried out in the KoM HRAP (Young *et al.*, 2016) but not with the model systems. Increased algal growth was believed responsible with the algae inhibiting light exposure (Sutherland *et al.*, 2015).

HRAPs are considered to be low cost system with minimal energy required for operation (Shilton *et al.*, 2008; Craggs *et al.*, 2011; Buchanan, 2014; Young *et al.*, 2016). The estimated costs associated with the IP as outlined in Chapter 6, found the IP to be economical as well as beneficial with the potential for reduced costs when coupled with a solar PV panel.

Overall the thesis carried out a comprehensive, novel set of experiments both in field and on a laboratory model scale. From the results presented there was enough evidence to support the IP as a beneficial addition to pond systems and recommend additional work to be carried out to better

improve the system and assess the additional impacts and benefits that could be obtained from the use of the IP. The thesis also acknowledges the concept as being not restricted to HRAPs but could easily be adapted into any pond system where an inclined surface is available. The proposed future directions can be summarised as follows;

- Assess the impact of the IP when incorporated into other pond systems such as WSPs to establish whether inactivation will be equally or better improved.
- Assess the effectiveness of the IP at reducing other pathogen species to provide a more rounded overview of the impact the IP and the increased solar exposure associated with it has on microbial inactivation
- Further examine the IP under different seasonal and climatic conditions. It may also be an advantage to assess the concept in regions or areas where solar irradiances are lower
- Continue to assess the impact of the IP in the field by operating the IP continuously over an extended period
- Conduct a more direct comparison of the HRAP + IP against HRAP under normal operation by running identical systems concurrently in the field at the same site, i.e. side by side comparison
- Examine whether inactivation and IP performance can be further improved when the water is delivered directly down the pond wall such that the whole volume is exposed to sunlight before entering the pond (i.e. from inlet straight down the wall).
- Examine the impacts of the IP on water loss and evaporation rates in the field and under different environmental and operational conditions

8. APPENDICES

Appendix 1: Additional information for model HRAPs aquarium pumps

Appendix 1.1: Aqua One 102 Maxi Power Head Aquarium Pump

Table A 1.1: Aqua One power head range specification for 100 series Maxi Power Head aquarium pump. Information adapted from manufacturer's manual available from https://www.aquaone.co.uk/documents/PH100series_instructions_lowres.pdf

Specifications:	
Model	102
Flow	500 L h^{-1}
Head	1.05 m
Inlet/Outlet Size	9 +13 mm
Cable Length	10 m
Power	8 W
Voltage	12 V (50 Hz)
Size	23 x 4.5 x 8.5 cm

Appendix 1.2: AQUAPRO AP1050 Water feature Pump (02AS002B)

Table A 1.2: Specification for the Aqua Pro AP1050 water feature pump. Information adapted from manufacturer's manual available from https://system.na2.netsuite.com/core/media/media.nl?id=6743&c=4144647&h=3750e5a14a398729e6d0&_xt=.pdf

Specifications:	
Model	AP1050
Flow	1050 L
Head	1.80 m
Inlet/Outlet Size	12 mm
Cable Length	10 m
Power	18 W
Voltage	240 V

Table A 1.3: Maximum pumping head range for Aqua Pro AP1050 water feature pump. Information adapted from https://aquatecequipment.com/product/aquapro-ap1050c-compact-waterfeature-pump-2/

Head (m)	Volume (L)
200	0
1.50	250
1.00	540
0.50	760
0.00	1050

Appendix 2: Additional information for pumps used for large scale IP operation

Appendix 2.1: AQUARPO AP7500HM Multi-Use Pump (02AH550)

Table A 2.1: Specification for the Aqua Pro AP7500HM Multi-Use pump. Information adapted from manufacturer's manual available from https://system.na2.netsuite.com/core/media/media.nl?id=1829&c=4144647&h=d61145e039c028c46b45&_xt=.pdf

Specifications:	
Model	AP7500HM
Flow	7500 L
Max. Height	4.20 m
Cable Length	10 m
Power	150 W
Voltage	240 V
Size	385 x 150 x 181 mm

Table A 2.2: Maximum pumping head range for Aqua Pro AP7500HM Multi-Use pump. Information adapted from https://aquatecequipment.com/product/aquapro-ap1050c-compact-waterfeature-pump-2/

Head (m)	Volume (L)
5.00	-
4.50	0
4.00	900
3.50	2600
3.00	3700
2.50	4400
200	5200
1.50	6000
1.00	6500
0.50	7200
0.00	7500

Appendix 3: Estimated water loss in model HRAPs

Table A 3.1: Estimated water loss in the model HRAPs; $HRAP_D$ and $HRAP_D$ +SIP when bulk water was dark incubated and IP solar exposed. Water loss was calculated from the change in pond depth (m) and volume (L) after being operated for 24 h. Starting volume 86.93 L at a 0.30 m depth.

Exp.	Solar Exposure ($MJ\ m^{-2}$)	$HRAP_D$				$HRAP_D$ + SIP			
		Depth (m)	Final Volume (L)	Volume lost (L)	Pond volume lost (%)	Depth (m)	Final Volume (L)	Volume lost (L)	Pond volume lost (%)
1	7.8	0.01	86.64	0.29	0.33	0.01	86.64	0.29	0.33
2	17.3	0.00	86.93	0.00	1.00	0.03	86.06	0.87	1.00
3	28.1	0.01	86.64	0.29	0.33	0.05	85.48	1.45	1.67
Mean±SE	17.73±5.86	0.07±0.03	86.73±0.10	0.19±0.10	0.22±0.11	0.15±0.00	0.30±0.12	86.06±0.33	1.00±0.38

Appendix 4: Statistical comparisons for Chapter 3 – optically clear water Model HRAPs

Appendix 4.1: T-Test and Tukey HSD post hoc comparison

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Table A 4.1: Independent samples t-test (Welch) showing the comparison between MS2 inactivation rates ($LRV_{7.5}$; log_{10} PFU $100mL^{-1}$) in the $HRAP_D$ and $HRAP_D$ + SIP after 7.5 h when dark incubated in optically clear water. Statistical significance was at p<0.05.

	Model System	n	Mean±SE	SD	t	df	Difference	Lower	Upper	p-value	*d*
$LRV_{7.5}$	$HRAP_D$	6	0.57±0.07	0.15	3.22	5	0.78	0.14	1.41	0.026	2.04
	$HRAP_D$ + SIP	6	1.34±0.23	0.69							

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01, Cohen's *d effect size included*

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Table A 4.2: Tukey's post hoc comparison of variance between MS2 inactivation rates (K_D; log_{10} h^{-1}) in the model HRAPs after 24 h when an IP has been included. Statistical significance at p<0.05.

Parameter	Model HRAP		Tukey multiple comparison of means 95% family-wise confidence level					
			Difference	Lower	Upper	p-value	Sig	*d*
K_D	$HRAP_D$ + SIP	$HRAP_D$	0.63	0.20	1.05	0.008	**	1.90
	$HRAP_D$	$HRAP_D$ + SIP	-0.63	-1.05	-0.20	0.008	**	1.90

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01, Cohen's *d effect size included*

Appendix 4.2: Linear regression outputs of MS2 inactivation against environmental conditions in the model HRAPs

Table A 4.3: Linear relationship between K_D values in the $HRAP_D$ + SIP and various environmental conditions including a) water temperature, b) solar exposure, c) pH, d) DO, e) UVA, and f) UVB

a) K_D vs. Temperature

Call:
lm(formula = Kd ~ Temperature, data = c3.sip.insitu)
Residuals:

2	3	4	5	6
0.014	-0.048	0.200	-0.140	-0.025

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.491	0.458	-1.072	0.363
Temperature	0.06	0.021	2.819	0.067

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error:	0.144	on 3 DF		
Multiple R^2	0.726		Adjusted R^2	0.635
F-statistic:	7.945	on 1 and 3 DF	p-value:	0.067

(1 observation deleted due to missingness)
>Confidence intervals (Kd.vs.Temperature level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	-0.491	-1.950	0.967
Temperature	0.060	-0.008	0.127

b) K_D vs. Solar Exposure

Call:

lm(formula = Kd ~ Solar.Exposure, data = c3.sip.insitu)
Residuals:

1	2	3	4	5	6
0.273	0.347	-0.305	-0.058	-0.099	-0.159

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.227	0.269	0.842	0.447
Solar Exposure	0.060	0.020	3.006	0.040*

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error:	0.286	on 4 DF		
Multiple R^2	0.693		Adjusted R^2	0.616
F-statistic:	9.033	on 1 and 4 DF	p-value:	0.040*

>Confidence intervals (Kd.vs.Solar.Exposure.SIP level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	0.227	0.269	0.842
Solar Exposure	0.060	0.020	3.006

c) K_D vs. pH

Call:

lm(formula = Kd ~ pH, data = c3.sip.insitu)

Residuals:

2	3	4	5	6
-0.021	0.019	0.325	-0.002	-0.321

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-2.216	5.760	-0.385	0.726
pH	0.363	0.695	0.522	0.638

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error: 0.264 on 3 DF

Multiple R^2 0.083 Adjusted R^2 -0.223

F-statistic: 0.272 on 1 and 3 DF p-value: 0.638

(1 observation deleted due to missingness)

> Confint(SIP.kd.v.ph, level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	-2.216	-20.546	16.114
pH	0.363	-1.850	2.576

d) K_D vs. DO

Call:

lm(formula = Kd ~ DO, data = c3.sip.insitu)

Residuals:

2	3	4	5	6
0.197	-0.046	-0.014	0.051	-0.188

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.449	0.160	2.810	0.067
DO	0.056	0.024	2.377	0.098

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error: 0.162 on 3 DF

Multiple R^2 0.653 Adjusted R^2 0.538

F-statistic: 5.650 on 1 and 3 DF p-value: 0.098

(1 observation deleted due to missingness)

> Confint(SIP.kd.v.DO, level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	0.449	-0.060	0.958
DO	0.056	-0.019	0.131

e) K_D vs. UVA

Call:

lm(formula = Kd ~ UVA, data = c3.sip.insitu)

Residuals:

2	3	4	5	6
0.228	-0.088	0.160	-0.060	-0.240

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.515	0.233	2.216	0.113
UVA	0.023	0.018	1.294	0.286

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error: 0.221 on 3 DF

Multiple R^2: 0.358, Adjusted R^2: 0.144

F-statistic: 1.674 on 1 and 3 DF, p-value: 0.286

(1 observation deleted due to missingness)

> Confint(SIP.kd.v.UVA, level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	0.515	-0.225	1.255
UVA	0.023	-0.034	0.081

e) K_D vs. UVB

Call:

lm(formula = Kd ~ UVB, data = c3.sip.insitu)

Residuals:

2	3	4	5	6
0.162	-0.135	0.094	0.097	-0.217

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.536	0.164	3.258	0.047*
UVB	0.986	0.549	1.795	0.171

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error: 0.192 on 3 DF

Multiple R^2: 0.518, Adjusted R^2: 0.357

F-statistic: 3.222 on 1 and 3 DF, p-value: 0.171

(1 observation deleted due to missingness)

> Confint(SIP.kd.v.UVB, level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	0.536	0.012	1.059
UVB	0.986	-0.762	2.734

Appendix 4.3: ANCOVA outputs for MS2 inactivation in the model HRAPs

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Table A 4.4: ANCOVA results and descriptive statistics for MS2 inactivation by model HRAP when controlled for solar exposure (MJ m^{-2}) after 24 h. Model HRAPs were incubated in optically clear (tap) water. Data refers to configuration I; $HRAP_D$ vs. $HRAP_D$ + SIP. Statistical significance was at $p<0.05$.

F-Specific Phage Inactivation Rates (K_D)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	6	0.35	0.07	0.35[a]	0.11	0.10	0.60
$HRAP_D$ + SIP	6	0.95	0.46	0.95[a]	0.11	0.70	1.21

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	1	1.09	1.09	14.69	0.004	**	0.62
Solar Exposure	1	0.43	0.43	5.72	0.040	*	0.39
Residual	9	0.67	0.07				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + SIP	$HRAP_D$	0.60	0.25	0.96	0.004	**
$HRAP_D$	$HRAP_D$ + SIP	-0.60	-0.96	-0.25	0.004	**

a. Covariates appearing in the model are evaluated at the following values; Solar exposure = 12.20 MJ m^{-2}; Bonferroni CI adjustment applied

b. R^2 = 0.819, Adj. R^2 = 0.768

c. Homogeneity tested: Levene's test = F (1,10) =0.421, p>0.05, actual p=0.421

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 4.5: ANCOVA results and descriptive statistics for MS2 inactivation by model HRAP when controlled for water temperature (°C) after 24 h. Model HRAPs were incubated in optically clear (tap) water. Data refers to configuration I; $HRAP_D$ vs. $HRAP_D$ + SIP. Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_D)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	5	0.34	0.07	0.33[a]	0.06	0.18	0.48
$HRAP_D$ + SIP	5	0.79	0.24	0.80[a]	0.06	0.65	0.94

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	1	0.54	0.54	27.79	0.001	***	0.80
Temperature	1	0.11	0.11	5.75	0.048	*	0.45
Residual	7	0.14	0.02				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + SIP	$HRAP_D$	0.47	0.24	0.66	0.001	***
$HRAP_D$	$HRAP_D$ + SIP	-0.47	-0.66	-0.24	0.001	***

a. Covariates appearing in the model are evaluated at the following values; Temperature = 21.63°C; Bonferroni CI adjustment applied

b. R^2 = 0.819, Adj. R^2 = 0.768

c. Homogeneity tested: Levene's test = F (1,8) =0.181, p>0.05, actual p=0.682

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 4.6: ANCOVA results and descriptive statistics for MS2 inactivation by model HRAP when controlled for both solar exposure (MJ m^{-2}) and water temperature (°C) after 24 h. Model HRAPs were incubated in optically clear (tap) water. Data refers to configuration I; $HRAP_D$ vs. $HRAP_D$ + SIP. Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_D)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	5	0.34	0.07	0.33	0.07	0.13	0.99
$HRAP_D$ + SIP	5	0.79	0.24	0.79	0.07	0.60	0.53

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	1	0.54	0.54	24.43	0.003	**	0.80
Solar Exposure	1	0.01	0.01	0.21	0.681	-	0.03
Temperature	1	0.07	0.07	13.23	0.122	-	0.33
Residual	7	0.13	0.02				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + SIP	$HRAP_D$	0.47	0.22	0.68	0.003	**
$HRAP_D$	$HRAP_D$ + SIP	-0.47	-0.68	-0.22	0.003	**

a. Covariates appearing in the model are evaluated at the following values; Temperature = 21.63°C; Bonferroni CI adjustment applied

b. R^2 = 0.825, Adj. R^2 = 0.738

c. Homogeneity tested: Levene's test = F (1,10) =0.421, p>0.05, actual p=0.421

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 4.7: ANCOVA results and descriptive statistics for MS2 inactivation by model HRAP when controlled for solar exposure (MJ m^{-2}) after 24 h. Model HRAPs were incubated in optically clear (tap) water. Data refers to configuration II; $HRAP_D$, $HRAP_D$ + SIP and $HRAP_D$ + LIP. Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_D)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	4	0.93	1.10	0.93	0.32	0.94	2.89
$HRAP_D$+ SIP	4	1.49	0.92	1.49	0.32	0.51	2.46
$HRAP_D$ + LIP	4	1.91	0.71	1.91	0.32	-0.05	1.90

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	1.95	0.98	2.35	0.158	-	0.37
Solar Exposure	1	4.34	4.34	10.42	0.012	*	0.57
Residual	8	3.33	0.42				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + LIP	$HRAP_D$	0.99	-0.32	2.29	0.139	-
$HRAP_D$ + LIP	$HRAP_D$ + SIP	0.43	-0.88	1.73	0.634	-
$HRAP_D$ + SIP	$HRAP_D$	0.56	-0.75	1.86	0.474	-

a. Covariates appearing in the model are evaluated at the following values; Solar exposure = 25.25 MJ m^{-2}; Bonferroni CI adjustment applied

b. R^2 = 0.654, Adj. R^2 = 0.524

c. Homogeneity tested: Levene's test = F (2, 8) = 0.193, p>0.05, actual p=0.828

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 4.8: ANCOVA results and descriptive statistics for MS2 inactivation by model HRAP when controlled for temperature (°C) after 24 h. Model HRAPs were incubated in optically clear (tap) water. Data refers to configuration II; $HRAP_D$, $HRAP_D$ + SIP and $HRAP_D$ + LIP. Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_D)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	4	0.93	1.10	0.75	0.51	-0.79	2.28
$HRAP_D$ + SIP	4	1.49	0.92	1.56	0.47	0.13	2.99
$HRAP_D$ + LIP	4	1.91	0.71	2.02	0.48	0.57	3.47

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	3.03	1.33	3.94	0.064	-	0.28
Temperature	1	4.59	0.71	11.92	0.009	-	0.09
Error	8	3.0	0.87				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + LIP	$HRAP_D$	0.99	-0.27	2.23	0.123	-
$HRAP_D$ + LIP	$HRAP_D$ + SIP	0.56	-0.70	1.81	0.449	-
$HRAP_D$ + SIP	$HRAP_D$	-0.43	-1.68	0.83	0.612	-

a. Covariates appearing in the model are evaluated at the following values; Temperature = 23.63 °C; Bonferroni CI adjustment applied

b. R^2 = 0.68, Adj. R^2 = 0.56

c. Homogeneity tested: Levene's test = F (2, 9) = 1.65, p>0.05, actual p=0.246

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 4.9: ANCOVA results and descriptive statistics for MS2 inactivation by model HRAP when controlled for solar exposure (MJ m^{-2}) after 24 h. Model HRAPs were incubated in optically clear (tap) water. Data refers to configuration II; $HRAP_D$, $HRAP_D$ + SIP and $HRAP_D$ + LIP. Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_D)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	4	0.93	1.10	0.80	0.21	0.14	1.46
$HRAP_D$ + SIP	4	1.49	0.92	1.58	0.21	0.92	2.23
$HRAP_D$ + LIP	4	1.91	0.71	1.95	0.21	1.30	2.60

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	2.68	1.34	7.78	0.017	*	0.69
Solar Exposure	1	1.87	1.87	10.86	0.013	*	0.61
Temperature	1	2.12	2.12	12.32	0.010	*	0.64
Error	7	1.21	0.17				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + LIP	$HRAP_D$	1.16	0.12	1.85	0.015	*
$HRAP_D$ + LIP	$HRAP_D$ + SIP	0.37	-0.44	1.29	0.452	-
$HRAP_D$ + SIP	$HRAP_D$	0.78	-0.31	1.42	0.080	-

a. Covariates appearing in the model are evaluated at the following values; Solar exposure = 25.25 MJ m^{-2} and temperature = 23.63°C; Bonferroni CI adjustment applied

b. R^2 = 0.875, Adj. R^2 = 0.803

c. Homogeneity tested: Levene's test = F (2, 9) = 1.65, p>0.05, actual p=0.246

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Appendix 5: Statistical comparisons for Chapter 4 – dark incubated model HRAPs (wastewater)

Appendix 5.1: Summary of F-Specific phage removal after 24 h (LRV_{24})

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Table A 5.1: Summary of the total F-Specific phage removal (LRV_{24}; log_{10} PFU 100 mL^{-1}) in model systems; $HRAP_d$ and $HRAP_d$ + SIP after 24 h when dark incubated in turbid wastewater.

Time (t; h)	Model HRAP	LRV_{24} ± SE
24	$HRAP_d$	1.45±0.27
	$HRAP_d$ + SIP	2.35±0.26

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Table A 5.2: Summary of the total F-Specific phage removal (LRV_{24}; log_{10} PFU 100 mL^{-1}) in model systems; $HRAP_d$, $HRAP_d$ + SIP and $HRAP_d$ + LIP after 24 h. Systems were dark incubated in wastewater

Time (t; h)	Model HRAP	LRV_{24} ± SE
24	$HRAP_d$	0.73±0.40
	$HRAP_d$ + SIP	1.15±0.47
	$HRAP_d$ + LIP	1.40±0.54

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Table A 5.3: Summary of the total F-Specific phage removal (LRV_{24}; log_{10} PFU 100 mL^{-1}) achieved in the model HRAPs for optically clear (tap) and turbid (wastewater) waters. Incubation period was 24 h.

Time (t; h)	Water Type	Model HRAP	LRV_{24} ± SE
24	Tap Water	$HRAP_d$	2.59±0.02
		$HRAP_d$ + SIP	3.50±0.03
	Wastewater	$HRAP_d$	1.19±0.03
		$HRAP_d$ + SIP	1.66±0.003

Appendix 5.2: ANOVA and ANCOVA of F-Specific phage die-off in the model HRAPs

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Table A 5.4: ANCOVA of F-Specific Phage inactivation rates (K_L: log_{10} h^{-1}) in model HRAPs with and without an IP after 24 h when controlled for solar exposure (MJ m^{-2}). Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_D)							
Model HRAP	n	Observed Mean	SD	Adjusted Mean[a]	SE	95% CI	
						lower	upper
$HRAP_d$	5	0.24	0.07	0.24	0.01	0.20	0.28
$HRAP_d$ + SIP	5	0.30	0.08	0.30	0.01	0.26	0.34

ANOVA							
Source	df	SS	MS	F	p value	Sig.	Partial η^2
Model HRAP	1	0.01	0.01	8.77	0.021	*	0.56
Solar Exposure	1	0.04	0.04	36.92	5.02E-04	***	0.84
Residual	7	0.01	9.52E-04				

Tukey's Pairwise Comparison						
Comparison		Difference	lower	upper	p value	Sig.
$HRAP_d$ + SIP	$HRAP_d$	0.06	0.01	0.10	0.021	*
$HRAP_d$	$HRAP_d$ + SIP	-0.06	-0.10	-0.01	0.021	*

a. covariates appearing in the model are evaluated at the following values; Solar exposure = 19.44 MJ m^{-2}; Bonferroni CI adjustment applied

b. R^2 = 0.867, Adj. R^2 = 0.829

c. Homogeneity tested: Levene's test = F (1,8) = 0.108, p>0.05, actual p=0.751

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 5.5: ANCOVA of F-Specific Phage inactivation rates (K_d: log_{10} h^{-1}) in model HRAPs with and without an IP after 24 h when controlled for temperature (°C). Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_d)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_d$	5	0.24	0.07	0.24	0.02	0.17	0.30
$HRAP_d$ + SIP	5	0.30	0.08	0.30	0.02	0.23	0.36

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	1	0.01	0.01	3.48	0.104	-	0.33
Temperature	1	0.01	0.02	9.57	0.018	*	0.58
Residual	7	0.02	0.003				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_d$ + SIP	$HRAP_d$	0.06	-0.02	0.13	0.104	-
$HRAP_d$	$HRAP_d$ + SIP	-0.06	-0.13	0.02	0.104	-

a. Covariates appearing in the model are evaluated at the following values; Solar exposure = 25.43 MJ m^{-2}; Bonferroni CI adjustment applied

b. R^2 = 0.648, Adj. R^2 = 0.547

c. Homogeneity tested: Levene's test = F (1,8) = 0.004, p>0.05, actual p=0.951

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 5.6: ANCOVA of F-Specific Phage inactivation rates (K_d: log_{10} h^{-1}) in model HRAPs with and without an IP after 24 h when controlled for solar exposure (MJ m^{-2}) and temperature (°C). Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_d)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_d$	5	0.24	0.08	0.24	0.02	0.20	0.28
$HRAP_d$ + SIP	5	0.30	0.07	0.30	0.02	0.25	0.34

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	1	0.01	0.008	7.51	0.034	*	0.57
Solar Exposure	1	0.01	0.011	9.92	0.020	*	0.62
Temperature	1	6.95E-06	6.95E-06	0.01	0.939	-	0.001
Residual	6	0.01	0.001				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_d$ + SIP	$HRAP_d$	0.06	0.01	0.11	0.034	*
$HRAP_d$	$HRAP_d$ + SIP	-0.06	-0.11	-0.01	0.034	*

a. Covariates appearing in the model are evaluated at the following values; Solar exposure = 19.44 MJ m^{-2} and temperature = 23.09°C; Bonferroni CI adjustment applied

b. R^2 = 0.867, Adj. R^2 = 0.801

c. Homogeneity tested: Levene's test = F (1, 8) = 0.151, p>0.05, actual p=0.707

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 5.7: ANCOVA of F-Specific Phage inactivation rates (K_d: log_{10} h^{-1}) in model HRAPs with and without an IP after 24 h when controlled for solar exposure (MJ m^{-2}). Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_d)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_d$	6	0.23	0.06	0.23	0.02	0.16	0.29
$HRAP_d$ + LIP	6	0.38	0.09	0.38	0.02	0.31	0.44
$HRAP_d$ + SIP	6	0.29	0.06	0.29	0.02	0.22	0.35

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	0.02	0.03	9.69	0.002	**	0.58
Solar Exposure	1	0.02	0.02	6.36	0.03	*	0.31
Residual	14	0.003	0.003				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_d$ + LIP	$HRAP_d$	0.15	0.06	0.24	0.002	**
$HRAP_d$ + SIP	$HRAP_d$	0.06	-0.03	0.15	0.205	-
$HRAP_d$ + LIP	$HRAP_d$ + SIP	0.09	-0.001	0.18	0.054	-

a. Covariates appearing in the model are evaluated at the following values; temperature = 24.31°C; Bonferroni CI adjustment applied

b. R^2 = 0.646, Adj. R^2 = 0.570

c. Homogeneity tested: Levene's test = F (2, 15) = 2.38, p>0.05, actual p=0.126

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 5.8: ANCOVA of F-Specific Phage inactivation rates (K_d: log_{10} h^{-1}) in model HRAPs with and without an IP after 24 h when controlled for temperature (°C). Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_d)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_d$	6	0.23	0.06	0.23	0.02	0.17	0.30
$HRAP_d$ + LIP	6	0.38	0.09	0.37	0.02	0.30	0.43
$HRAP_d$ + SIP	6	0.29	0.06	0.29	0.02	0.22	0.35

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	0.05	0.027	7.82	0.003	**	0.53
Temperature	1	0.02	0.022	6.43	0.024	*	0.32
Residual	14	0.05	0.003				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_d$ + LIP	$HRAP_d$	-0.14	-0.28	-0.06	0.004	**
$HRAP_d$ + SIP	$HRAP_d$	-0.06	-0.15	0.03	0.286	-
$HRAP_d$ + LIP	$HRAP_d$ + SIP	0.08	-0.001	0.18	0.083	-

a. Covariates appearing in the model are evaluated at the following values; temperature = 24.31°C; Bonferroni CI adjustment applied

b. R^2 = 0.647, Adj. R^2 = 0.572

c. Homogeneity tested: Levene's test = F (2, 15) = 1.15, p>0.05, actual p=0.344

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 5.9: ANCOVA of F-Specific Phage inactivation rates (K_d: log_{10} h^{-1}) in model HRAPs with and without an IP after 24 h when controlled for solar exposure (MJ m^{-2}) and temperature (°C). Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_d)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_d$	6	0.23	0.06	0.23	0.02	0.17	0.30
$HRAP_d$ + LIP	6	0.38	0.09	0.37	0.02	0.31	0.44
$HRAP_d$ + SIP	6	0.29	0.06	0.29	0.02	0.22	0.35

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	0.06	0.029	8.46	0.004	**	0.57
Solar Exposure	1	0.004	0.004	1.29	0.277	-	0.09
Temperature	1	0.01	0.005	1.34	0.268	-	0.09
Residual	13	0.04	0.003				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_d$ + LIP	$HRAP_d$	-0.11	-0.24	-0.06	0.003	**
$HRAP_d$ + SIP	$HRAP_d$	-0.06	-0.15	0.03	0.238	-
$HRAP_d$ + LIP	$HRAP_d$ + SIP	0.08	-0.001	0.18	0.070	-

a. Covariates appearing in the model are evaluated at the following values; solar exposure = 25.43 MJ m^{-2} and temperature = 24.31°C; Bonferroni CI adjustment applied

b. R^2 = 0.679, Adj. R^2 = 0.580

c. Homogeneity tested: Levene's test = F (2, 15) = 1.76, p>0.05, actual p=0.207

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 5.10: ANCOVA of F-Specific Phage inactivation rates (K_d: log_{10} h^{-1}) in model HRAPs with and without an IP after 49.5 h when controlled for solar exposure (MJ m^{-2}). Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_d)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_d$	6	0.11	0.10	0.11	0.02	0.06	0.15
$HRAP_d$ + LIP	6	0.17	0.14	0.17	0.02	0.12	0.22
$HRAP_d$ + SIP	6	0.14	0.12	0.14	0.02	0.09	0.19

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	0.01	0.01	3.07	0.078	-	0.31
Solar Exposure	1	0.19	0.19	103.5	7.55E-08	***	0.88
Residual	14	0.03	0.002				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_d$ + LIP	$HRAP_d$	0.06	-0.004	0.13	0.066	-
$HRAP_d$ + SIP	$HRAP_d$	0.04	-0.03	0.10	0.337	-
$HRAP_d$ + LIP	$HRAP_d$ + SIP	0.03	-0.04	0.09	0.590	-

a. Covariates appearing in the model are evaluated at the following values; Solar exposure = 25.43 MJ m^{-2}; Bonferroni CI adjustment applied

b. R^2 = 0.888, Adj. R^2 =0.864

c. Homogeneity tested: Levene's test = F (2, 6) = 1.27, p>0.05, actual p=0.346

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 5.11: ANCOVA of F-Specific Phage inactivation rates (K_d: log_{10} h^{-1}) in model HRAPs with and without an IP after 49.5 h when controlled for temperature (°C). Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_d)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_d$	6	0.11	0.10	0.12	0.02	0.06	0.18
$HRAP_d$ + LIP	6	0.17	0.14	0.15	0.02	0.09	0.30
$HRAP_d$ + SIP	6	0.14	0.12	0.15	0.02	0.09	0.21

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	0.004	0.002	0.58	0.512	-	0.08
Temperature	1	0.18	0.18	58.77	2.24E-06	***	0.81
Residual	14	0.04	0.003				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_d$ + LIP	$HRAP_d$	0.03	-0.021	0.15	0.995	-
$HRAP_d$ + SIP	$HRAP_d$	0.03	-0.05	0.12	0.60	
$HRAP_d$ + LIP	$HRAP_d$ + SIP	-0.003	-0.06	0.11	0.66	

a. Covariates appearing in the model are evaluated at the following values; temperature = 24.31°C; Bonferroni CI adjustment applied

b. R^2 = 0.817, Adj. R^2 = 0.778

c. Homogeneity tested: Levene's test = F (2, 6) = 2.29, p>0.05, actual p=0.182

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 5.12: ANCOVA of F-Specific Phage inactivation rates (K_d: log_{10} h^{-1}) in model HRAPs with and without an IP after 49.5 h when controlled for solar exposure (MJ m^{-2}) and temperature (°C). Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_d)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_d$	6	0.11	0.10	0.11	0.02	0.07	0.15
$HRAP_d$ + LIP	6	0.17	0.14	0.16	0.02	0.12	0.20
$HRAP_d$ + SIP	6	0.14	0.12	0.15	0.02	0.10	0.19

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	0.01	0.003	2.54	0.117	-	0.28
Solar Exposure	1	0.03	0.03	17.86	9.91E-4	***	0.58
Temperature	1	0.01	0.01	6.11	0.028	**	0.32
Residual	13	0.02	0.001				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_d$ + LIP	$HRAP_d$	0.05	0.01	0.12	0.114	-
$HRAP_d$ + SIP	$HRAP_d$	0.03	-0.02	0.09	0.280	-
$HRAP_d$ + LIP	$HRAP_d$ + SIP	0.01	-0.03	0.08	0.810	-

a. Covariates appearing in the model are evaluated at the following values; solar exposure = 25.43 MJ m^{-2} and temperature = 24.31°C; Bonferroni CI adjustment applied

b. R^2 = 0.924, Adj. R^2 = 0.901

c. Homogeneity tested: Levene's test = F (2, 6) = 1.42, p>0.05, actual p=0.313

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 5.13: ANCOVA output for comparison between F-Specific phage inactivation rates (K_d) determined for solar exposed model HRAPs with and without an IP after 24 h when solar exposure (MJ m^{-2}) is controlled. Statistical significance was at p<0.05.

F-Specific Phage Inactivation Rates (K_d)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_d$ (TW)	3	0.39	0.05	0.39	0.03	0.31	0.48
$HRAP_d$ + SIP (TW)	3	0.17	0.02	0.17	0.03	0.09	0.26
$HRAP_d$ (WW)	3	0.70	0.11	0.70	0.03	0.61	0.78
$HRAP_d$ + SIP (WW)	3	0.20	0.06	0.20	0.03	0.12	0.28

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	3	0.35	0.12	85.59	0.0002	**	0.99
Solar Exposure	1	0.02	0.02	11.62	0.042	*	0.80
Error	3	0.00	0.00				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_d$ + SIP (TW)	$HRAP_d$ (TW)	0.30	0.07	0.53	0.023	*
$HRAP_d$ (WW)	$HRAP_d$ (TW)	-0.22	-0.45	0.01	0.056	-
$HRAP_d$ + SIP (WW)	$HRAP_d$ (TW)	-0.19	-0.42	0.04	0.081	-
$HRAP_d$ + SIP (TW)	$HRAP_d$ (WW)	0.52	0.29	0.75	0.005	**
$HRAP_d$ + SIP (WW)	$HRAP_d$ (WW)	0.03	-0.20		1.000	-
$HRAP_d$ + SIP (WW)	$HRAP_d$ + SIP (TW)	-0.50	-0.72	-0.27	0.005	**

a. Covariates appearing in the model are evaluated at the following values; Solar exposure=8.20 MJ m-2; Bonferroni CI adjustment applied

b. R2= 0.989, Adj. R2 = 0.974

c. Homogeneity tested: Levene's test = F (3, 4) = 0.19, p>0.05, actual p=0.902

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Appendix 5.3: Tukey Post hoc comparison

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Table A 5.14: Independent samples t-test showing the comparison between mean F-Specific phage inactivation ($LRV_{7.5}$ and K_d) in the $HRAP_d$ and $HRAP_d$ + SIP after 24 h incubated. Statistical significance was at p<0.05.

	Model System	n	Mean±SE	t	Df	Difference	Lower	Upper	p-value	*d*
$LRV_{7.5}$	$HRAP_d$	5	0.29±0.02	3.38	8	0.13	0.04	0.21	0.010**	3.41
	$HRAP_d$ + SIP	5	0.42±0.03							

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01; Effect size (*d) was calculated using Cohen's d.*

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Table A 5.15: Independent samples t-test showing the comparison between in situ pond conditions (pH, DO and temperature) in the $HRAP_d$ and $HRAP_d$ + SIP after 24 h incubated. Statistical significance was at p<0.05.

	Model System	n	Mean±SE	SD	t	df	Difference	Lower	Upper	p-value	*d*
pH	$HRAP_d$	5	7.83±0.07	0.15	1.86	8	-0.20	-0.46	0.05	0.103	1.23
	$HRAP_d$ + SIP	5	8.04±0.09	0.19							
DO (mg L^{-1})	$HRAP_d$	5	4.26±0.77	1.71	0.85	8	-1.38	-5.34	2.58	0.428	0.54
	$HRAP_d$ + SIP	5	5.64±1.43	3.20							
Temperature (°C)	$HRAP_d$	5	23.17±2.40	5.36	0.04	8	0.15	-7.67	7.96	0.967	-0.03
	$HRAP_d$ + SIP	5	23.02±2.40	5.36							

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01

Table A 5.16: Tukey's post hoc comparison of variance between in situ pH, DO (mg L^{-1}) and temperature (°C) concentrations in the model HRAPs after 49.5 h. Statistical significance at p<0.05

Parameter	Model HRAP		Tukey multiple comparison of means 95% family-wise confidence level				
			Difference	Lower	Upper	p-value	Sig.
pH	HRAPd+SIP	HRAPd	0.33	0.15	0.50	$1.32x10^{-04}$	***
	HRAPd+LIP	HRAPd	0.42	0.24	0.59	$2.42x10^{-06}$	***
	HRAPd+SIP	HRAPd+LIP	-0.09	-0.27	0.08	0.422	-
DO (mg L^{-1})	HRAPd+SIP	HRAPd	1.07	0.34	1.80	0.003	***
	HRAPd+LIP	HRAPd	0.57	-0.16	1.30	0.149	-
	HRAPd+SIP	HRAPd+LIP	0.50	-0.23	1.23	0.231	-
Water Temperature (°C)	HRAPd+SIP	HRAPd	0.25	-2.80	3.30	0.978	-
	HRAPd+LIP	HRAPd	1.47	-1.58	4.52	0.475	-
	HRAPd+SIP	HRAPd+LIP	-1.22	-4.27	1.83	0.598	-

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01

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Table A 5.17: Tukey's post hoc comparison of variance between in situ concentrations in the model HRAPs after 49.5 h. Statistical significance at p<0.05

Parameter	Model HRAP		Tukey multiple comparison of means 95% family-wise confidence level			
			Difference	Lower	Upper	p-value
Turbidity (NTU)	HRAPd+SIP	HRAPd	1.00	1.00	-41.14	43.14
	HRAPd+LIP	HRAPd	8.50	0.94	-33.64	50.64
	HRAPd+SIP	HRAPd+LIP	-7.50	0.96	-49.64	34.64
SS (mg L^{-1})	HRAPd+SIP	HRAPd	8.00	0.90	-24.21	40.21
	HRAPd+LIP	HRAPd	14.93	0.57	-17.27	47.14
	HRAPd+SIP	HRAPd+LIP	-6.93	0.93	-39.14	25.27
Chl a (mg L^{-1})	HRAPd+SIP	HRAPd	0.07	0.99	-0.70	0.84
	HRAPd+LIP	HRAPd	0.08	0.99	-0.68	0.85
	HRAPd+SIP	HRAPd+LIP	-0.02	1.00	-0.78	0.75
TOC (mg L^{-1})	HRAPd+SIP	HRAPd	1.62	1.00	-20.76	24.00
	HRAPd+LIP	HRAPd	6.07	0.87	-16.31	28.45
	HRAPd+SIP	HRAPd+LIP	-4.45	0.94	-26.83	17.93
TC (mg L^{-1})	HRAPd+SIP	HRAPd	5.17	0.96	-23.76	34.11
	HRAPd+LIP	HRAPd	7.41	0.89	-21.52	36.35
	HRAPd+SIP	HRAPd+LIP	-2.24	1.00	-31.17	26.69
IC (mg L^{-1})	HRAPd+SIP	HRAPd	5.22	0.85	-12.90	23.34
	HRAPd+LIP	HRAPd	1.34	1.00	-16.78	19.46
	HRAPd+SIP	HRAPd+LIP	3.88	0.93	-14.24	22.00
TN (mg L^{-1})	HRAPd+SIP	HRAPd	2.63	0.81	-5.72	10.98
	HRAPd+LIP	HRAPd	1.46	0.96	-6.89	9.81
	HRAPd+SIP	HRAPd+LIP	1.17	0.98	-7.18	9.52

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Table A 5.18: Tukey's post hoc comparison of variance between starting (Mt Barker Inlet) and final in situ concentrations in the model HRAPs after 49.5 h. Statistical significance at p<0.05

Parameter	Model HRAP		Tukey multiple comparison of means 95% family-wise confidence level			
			Difference	Lower	Upper	p-value
Turbidity	Inlet	$HRAP_d$	12.00	-30.14	54.14	0.85
		$HRAP_d$ + SIP	3.50	-38.64	45.64	1.00
		$HRAP_d$ + LIP	11.00	-31.14	53.14	0.88
SS	Inlet	$HRAP_d$	9.33	-22.87	41.54	0.85
		$HRAP_d$ + SIP	-5.60	-37.81	26.61	0.96
		$HRAP_d$ + LIP	1.33	-30.87	33.54	1.00
Chl a	Inlet	$HRAP_d$	0.16	-0.61	0.92	0.94
		$HRAP_d$ + SIP	0.07	-0.70	0.84	0.99
		$HRAP_d$ + LIP	0.09	-0.68	0.86	0.99
TOC	Inlet	$HRAP_d$	2.72	-19.66	25.09	0.99
		$HRAP_d$ + SIP	-3.36	-25.73	19.02	0.97
		$HRAP_d$ + LIP	1.09	-21.28	23.47	1.00
TC	Inlet	$HRAP_d$	11.06	-17.87	39.99	0.71
		$HRAP_d$ + SIP	3.65	-25.29	32.58	0.98
		$HRAP_d$ + LIP	5.89	-23.05	34.82	0.94
IC	Inlet	$HRAP_d$	8.35	-9.77	26.46	0.58
		$HRAP_d$ + SIP	7.01	-11.11	25.12	0.70
		$HRAP_d$ + LIP	3.13	-14.99	21.24	0.96
TN	Inlet	$HRAP_d$	-1.83	-10.18	6.52	0.93
		$HRAP_d$ + SIP	-3.29	-11.64	5.06	0.69
		$HRAP_d$ + LIP	-4.46	-12.81	3.89	0.46

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Table A 5.19: Statistical comparison between mean F-Specific phage log reduction values (LRV; log_{10} PFU 100 mL^{-1}) in the $HRAP_d$ + SIP and $HRAP_d$ + LIP when operated at similar hydraulic loading rates (HLR; L m^{-2} h^{-1}) and different flow rates (Q; L h^{-1}). Statistical significance at p<0.05

	HLR	Q	Tukey multiple comparison of means 95% family-wise confidence level					
			Model Systems	Difference	Lower	Upper	p-value	Sig.
HLR.1	167.8±2.4	62.1±0.9	$HRAP_L$+SIP	-0.15	-0.86	0.56	0.998	-
	174.4±1.4	130.8±1.2	$HRAP_L$+LIP					
HLR.2	232.7±4.9	86.1±1.8	$HRAP_L$+SIP	-0.19	-0.87	0.50	0.990	-
	232.8±0.8	174.6±0.6	$HRAP_L$+LIP					
HLR.3	300.0±0.8	110.0±0.3	$HRAP_L$+SIP	-0.06	-0.74	0.63	1.000	-
	300.0±0.8	225.0±0.6	$HRAP_L$+LIP					
HLR.4	350.3±8.4	129.6±3.1	$HRAP_L$+SIP	-0.10	-0.84	0.64	1.000	-
	350.3±1.2	262.7±0.9	$HRAP_L$+LIP					

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01,

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Table A 5.20: Tukey's post hoc comparison of variance between mean F-Specific phage log reduction values (LRV; log_{10} PFU 100 mL^{-1}) obtained in model systems operated in different water types over a 7.5 h incubation period. Model systems examined included both a $HRAP_d$ and $HRAP_d$ + SIP operated in optically clear (tap) water or wastewater. Statistical significance was at p<0.05.

Tukey multiple comparison of means 95% family-wise confidence level						
Model Systems		Difference	Lower	Upper	p-value	Sig.
$HRAP_d$ + SIP (TW)	$HRAP_d$ (TW)	0.28	-0.29	0.85	0.322	-
$HRAP_d$ (WW)	$HRAP_d$ (TW)	-0.40	-0.97	0.17	0.138	-
$HRAP_d$ +SIP (WW)	$HRAP_d$ (TW)	-0.26	-0.83	0.31	0.377	-
$HRAP_d$ +SIP (TW)	$HRAP_d$ (WW)	0.68	0.11	1.25	0.027	*
$HRAP_d$ +SIP (WW)	$HRAP_d$ +SIP (TW)	0.15	-0.42	0.72	0.732	-
$HRAP_d$ +SIP (WW)	$HRAP_d$ (WW)	-0.54	-1.11	0.03	0.060	-

Strength of statistical significance, - = p>0.05, * = p<0.05, ** = p<0.01

Appendix 5.4: Linear regression outputs of F-Specific phage inactivation against environmental conditions in the model HRAPs

Table A 5.21: Linear relationships between F-specific phage K_D values in the $HRAP_D$ + SIP and a) solar exposure (MJ m^{-2}) b) UVA (W m^{-2}), and c) UVB (W m^{-2})

a) K_D vs. solar exposure

Call:

lm (formula = Kd ~ solar exposure, data = C4.LvS.KdvEnviro.SIP)

Residuals:

7	8	9	10	11	12
0.039	-0.006	-0.038	-0.036	0.040	0.001

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.025	0.030	-0.84	0.448
Solar exposure	0.011	0.002	6.608	0.003**

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error: 0.039 on 4 DF

Multiple R^2	0.916	Adjusted R^2	0.895
F-statistic:	43.67 on 1 and 4 DF	p-value:	0.003

>Confidence intervals (Kd.vs.solar exposure.SIP level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	-0.025	-0.107	0.058
Solar Exposure	0.011	0.006	0.015

b) K_D vs. UVA

Call:

lm (formula = Kd ~ UVA, data = C4.LvS.KdvEnviro.SIP)

Residuals:

7	8	9	10	11	12
0.065	-0.057	0.032	-0.037	0.017	-0.019

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.033	0.042	-0.796	0.471
UVA	0.009	0.002	4.805	0.009**

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error: 0.051 on 4 DF

Multiple R^2	0.852	Adjusted R^2	0.815
F-statistic:	23.08 on 1 and 4 DF	p-value:	0.009

>Confidence intervals (Kd Vs. UVA, .SIP level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	-0.033	-0.150	0.083
UVA	0.009	0.004	0.014

c) K_D vs. UVB

Call:

lm (formula = Kd ~ UVB, data = C4.LvS.KdvEnviro.SIP)

Residuals:

7	8	9	10	11	12
0.166	-0.043	0.003	-0.078	-0.004	-0.044

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.045	0.065	0.689	0.529
UVB	0.216	0.114	1.889	0.132

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error: 0.097 on 4 DF

Multiple R^2	0.471	Adjusted R^2	0.339
F-statistic:	3.57 on 1 and 4 DF	p-value:	0.132

>Confidence intervals (Kd Vs. UVB, .SIP level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	0.045	-0.135	0.224
UVB	0.216	-0.102	0.534

Table A 5.22: Linear relationships between F-specific phage K_D values in the $HRAP_D$ + LIP and a) solar exposure (MJ m^{-2}) b) UVA (W m^{-2}), and c) UVB (W m^{-2})

a) K_D vs. Solar Exposure

Call:

lm (formula = Kd ~ solar exposure, data = C4.LvS.KdvEnviro.LIP)

Residuals:

13	14	15	16	17	18
0.051	-0.008	-0.054	-0.029	0.038	0.002

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.029	0.034	-0.845	0.445
Solar exposure	0.012	0.002	6.766	0.002**

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error:	0.044	on 4 DF		
Multiple R^2	0.920		Adjusted R^2	0.900
F-statistic:	45.78	on 1 and 4 DF	p-value:	0.002

>Confidence intervals (Kd.vs.solar exposure.LIP level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	-0.029	-0.123	0.066
Solar Exposure	0.012	0.007	0.017

b) K_D vs. UVA

Call:

lm (formula = Kd ~ UVA, data = C4.LvS.KdvEnviro.SIP)

Residuals:

13	14	15	16	17	18
0.080	-0.068	0.027	-0.029	0.011	-0.022

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.040	0.047	-0.841	0.448
UVA	0.010	0.002	5.038	0.007**

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error:	0.058	on 4 DF		
Multiple R^2	0.864		Adjusted R^2	0.830
F-statistic:	25.38	on 1 and 4 DF	p-value:	0.007

>Confidence intervals (Kd Vs. UVA, .SIP level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	-0.040	-0.171	0.091
UVA	0.010	0.005	0.016

c) K_D vs. UVB

Call:

lm (formula = Kd ~ UVB, data = C4.LvS.KdvEnviro.LIP)

Residuals:

13	14	15	16	17	18
0.199	-0.051	-0.006	-0.078	-0.014	-0.050

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.053	0.076	0.698	0.523
UVB	0.254	0.134	1.896	0.131

Signif. codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.'0.1 ' ' 1

Residual standard error:	0.113	on 4 DF		
Multiple R^2	0.473		Adjusted R^2	0.342
F-statistic:	3.594	on 1 and 4 DF	p-value:	0.131

>Confidence intervals (Kd Vs. UVB, .LIP level=0.95)

	Estimate	2.5% (lower)	97.5% (upper)
(Intercept)	0.053	-0.157	0.263
UVB	0.254	-0.118	0.625

Appendix 6: Statistical comparisons for Chapter 5 – solar exposed model HRAPs (wastewater)

Appendix 6.1: Summary of F-Specific phage removal after 24 h (LRV_{24})

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Table A 6.1: Summary of the total F-Specific phage removal (LRV_{24}; log_{10} PFU 100 mL^{-1}) achieved in the model HRAPs with and without an IP after 24 h when bulk water and IP are exposed to sunlight.

Time (t; h)	Model HRAP	LRV_{24} ± SE
24	$HRAP_L$	0.74±0.41
	$HRAP_L$ + SIP	1.20±0.42
	$HRAP_L$ + LIP	1.46±0.33

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Table A 6.2: Summary of the total F-Specific phage removal (LRV_{24}; log_{10} PFU 100 mL^{-1}) achieved in the model HRAPs with and without an IP after 24 h when exposed to sunlight or dark incubated.

Time (t; h)	Pond Condition	Model HRAP	LRV_{24} ± SE
24	Dark Incubation	$HRAP_D$	0.19±0.02
		$HRAP_D$ + SIP	0.64±0.13
	Sunlight Exposure	$HRAP_L$	0.58±0.04
		$HRAP_L$ + SIP	0.98±0.04

Appendix 6.2: ANOVA and ANCOVA of F-Specific phage die-off in the model HRAPs

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Table A 6.3: ANCOVA output for comparison between F-Specific phage inactivation rates (K_L) determined for solar exposed model HRAPs with and without an IP after 24 h.

F-Specific Phage Inactivation Rates (K_L)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_L$	3	0.26	0.08	0.26	0.06	0.06	0.46
$HRAP_L$ + SIP	3	0.46	0.26	0.46	0.06	0.25	0.66
$HRAP_L$ + LIP	3	0.46	0.26	0.46	0.06	0.26	0.66

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Solar Exposure	1	0.24	0.24	25.54	0.005	**	0.825
Model HRAP	2	0.08	0.04	3.93	0.094	-	0.611
Error	5	0.05	0.01				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_L$ + LIP	$HRAP_L$	0.197	0.07	0.46	0.129	-
$HRAP_L$ + SIP	$HRAP_L$	0.200	0.07	0.47	0.123	-
$HRAP_L$ + LIP	$HRAP_L$ + SIP	-0.003	-0.27	0.26	0.999	-

a. covariates appearing in the model are evaluated at the following values; Solar Exposure = 9.80 MJ m^{-2}, Bonferroni CI adjustment applied

b. R^2 = 0.863, Adj. R^2 = 0.780

c. Homogeneity tested: Levene's test = F (2, 6) = 0.989, p>0.05, actual p=0.425

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 6.4: ANCOVA output for comparison between F-Specific phage inactivation rates (K_L) determined for solar exposed model HRAPs with and without an IP after 49.5 h.

F-Specific Phage Inactivation Rates (K_L)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_L$	3	0.10	0.06	0.10	0.01	0.06	0.14
$HRAP_L$ + SIP	3	0.16	0.02	0.16	0.01	0.12	0.20
$HRAP_L$ + LIP	3	0.14	0.03	0.14	0.01	0.10	0.18

ANOVA							
Source	**df**	**SS**	**MS**	**F**	**p value**	**Sig.**	**Partial η^2**
Model HRAP	2	0.005	0.003	7.20	0.034	*	0.74
Solar Exposure	1	0.009	0.009	23.26	0.005	***	0.82
Error	5	0.002	3.66E-04				

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_L$ + LIP	$HRAP_L$	0.06	0.01	0.11	0.034	*
$HRAP_L$ + LIP	$HRAP_L$ + SIP	0.05	-0.04	0.06	0.689	-
$HRAP_L$ + SIP	$HRAP_L$	0.02	-0.01	0.09	0.085	-

a. Covariates appearing in the model are evaluated at the following values; Solar Exposure = 10.68 MJ m^{-2}, Bonferroni CI adjustment applied

b. R^2 = 0.883, Adj. R^2 = 0.812

c. Homogeneity tested: Levene's test = F (2, 6) = 3.38, p>0.05, actual p=0.104

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 6.5: ANCOVA output for comparison between F-Specific phage inactivation rates (K_L) determined for solar exposed ($HRAP_L$ and $HRAP_L$ + SIP) and dark incubated ($HRAP_D$ and $HRAP_D$ +SIP) model HRAPs with and without an IP when controlled for solar exposure (MJ m^{-2}) after 24 h.

F-Specific Phage Inactivation Rates (K_L)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	3	0.24	0.14	0.24	0.05	0.07	0.41
$HRAP_D$ + SIP	3	0.31	0.06	0.31	0.05	0.14	0.48
$HRAP_L$	3	0.31	0.07	0.31	0.05	0.14	0.48
$HRAP_L$ + SIP	3	0.48	0.16	0.48	0.05	0.31	0.65

ANOVA						
Source	**df**	**SS**	**MS**	**F**	**p value**	**Partial η^2**
Model HRAP	3	0.09	0.03	4.05	0.058	0.64
Solar Exposure	1	0.05	0.05	7.00	0.033	0.50
Error	7	0.05	0.01			

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + SIP	$HRAP_D$	0.06	-0.17	0.30	0.809	-
$HRAP_L$	$HRAP_D$	0.07	-0.17	0.31	0.786	-
$HRAP_L$ + SIP	$HRAP_D$	0.24	8.93E-04	0.48	0.049	*
$HRAP_L$	$HRAP_D$ + SIP	0.00	-0.23	0.24	1.000	-
$HRAP_L$ + SIP	$HRAP_D$ + SIP	0.17	-0.06	0.41	0.158	-
$HRAP_L$ + SIP	$HRAP_L$	0.17	-0.07	0.41	0.168	-

a. Covariates appearing in the model are evaluated at the following values; Solar Exposure = 9.70 MJ m^{-2}, Bonferroni CI adjustment applied

b. R^2 = 0.732, Adj. R^2 = 0.579

c. Homogeneity tested: Levene's test = F (3,8) = 0.37, p>0.05, actual p=0.778

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 6.6: ANCOVA output for comparison between F-Specific phage inactivation rates (K_L) determined for solar exposed ($HRAP_L$ and $HRAP_L$ + SIP) and dark incubated ($HRAP_D$ and $HRAP_D$ +SIP) model HRAPs with and without an IP when controlled for water temperature (°C) after 24 h.

F-Specific Phage Inactivation Rates (K_L)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	3	0.24	0.14	0.25	0.04	0.10	0.40
$HRAP_D$ + SIP	3	0.31	0.06	0.30	0.04	0.15	0.44
$HRAP_L$	3	0.31	0.07	0.29	0.04	0.15	0.44
$HRAP_L$ + SIP	3	0.48	0.16	0.50	0.04	0.35	0.65
ANOVA							
Source	**df**		**SS**	**MS**	**F**	**p value**	**Partial η^2**
Model HRAP	3		0.11	0.04	6.53	0.019	0.74
Temperature	1		0.07	0.07	11.91	0.011	0.63
Error	7		0.04	0.01			
Tukey's Pairwise Comparison							
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**	
$HRAP_d$ + SIP	$HRAP_D$	0.05	-0.14	0.27	0.868	-	
$HRAP_L$	$HRAP_D$	0.04	-0.14	0.27	0.890	-	
$HRAP_L$ + SIP	$HRAP_D$	0.25	0.03	0.44	0.019	*	
$HRAP_L$	$HRAP_D$ + SIP	0.00	-0.20	0.21	1.000	-	
$HRAP_L$ + SIP	$HRAP_D$ + SIP	0.20	-0.03	0.38	0.053	-	
$HRAP_L$ + SIP	$HRAP_L$	0.21	0.03	0.38	0.050	*	

a. Covariates appearing in the model are evaluated at the following values; Temperature = 15.91 °C, Bonferroni CI adjustment applied

b. R^2 = 0.803, Adj. R^2 = 0.690

c. Homogeneity tested: Levene's test = F (3,8) = 2.27, p>0.05, actual p=0.157

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 6.7: ANCOVA output for comparison between F-Specific phage inactivation rates (K_L) determined for solar exposed ($HRAP_L$ and $HRAP_L$ + SIP) and dark incubated ($HRAP_D$ and $HRAP_D$ +SIP) model HRAPs with and without an IP when controlled for solar exposure (MJ m^{-2}) and water temperature (°C) after 24 h.

F-Specific Phage Inactivation Rates (K_L)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	3	0.24	0.14	0.25	0.04	0.10	0.39
$HRAP_D$ + SIP	3	0.31	0.06	0.30	0.04	0.16	0.44
$HRAP_L$	3	0.31	0.07	0.30	0.04	0.15	0.44
$HRAP_L$ + SIP	3	0.48	0.16	0.50	0.04	0.35	0.64

ANOVA						
Source	**df**	**SS**	**MS**	**F**	**p value**	**Partial η^2**
Model HRAP	3	0.11	0.04	7.02	0.022	0.78
Solar Exposure	1	0.01	0.01	1.98	0.209	0.25
Temperature	1	0.02	0.02	4.79	0.071	0.44
Error	6	0.03	0.01			

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + SIP	$HRAP_D$	0.05	-0.14	0.26	0.809	
$HRAP_L$	$HRAP_D$	0.05	0.13	0.27	0.823	-
$HRAP_L$ + SIP	$HRAP_D$	0.25	0.04	0.44	0.020	*
$HRAP_L$	$HRAP_D$ + SIP	0.00	-0.20	0.20	1.000	-
$HRAP_L$ + SIP	$HRAP_D$ + SIP	0.20	-0.03	0.37	0.057	-
$HRAP_L$ + SIP	$HRAP_L$	0.20	-0.03	0.37	0.057	-

a. Covariates appearing in the model are evaluated at the following values; Solar exposure = 9.70 MJ m^{-2}, Temperature = 15.91 °C, Bonferroni CI adjustment applied

b. R^2 = 0.852, Adj. R^2 = 0.728

c. Homogeneity tested: Levene's test = F (3,8) = 0.781, p>0.05, actual p=0.537

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 6.8: ANCOVA output for comparison between F-Specific phage inactivation rates (K_L) determined for solar exposed ($HRAP_L$ and $HRAP_L$ + SIP) and dark incubated ($HRAP_D$ and $HRAP_D$ +SIP) model HRAPs with and without an IP when controlled for solar exposure (MJ m^{-2}) after 49.5 h.

F-Specific Phage Inactivation Rates (K_L)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	3	0.07	0.03	0.07	0.02	0.02	0.12
$HRAP_D$ + SIP	3	0.09	0.03	0.09	0.02	0.04	0.14
$HRAP_L$	3	0.08	0.02	0.08	0.02	0.03	0.13
$HRAP_L$ + SIP	3	0.09	0.02	0.09	0.02	0.04	0.14

ANOVA						
Source	**df**	**SS**	**MS**	**F**	**p value**	**Partial η^2**
Model HRAP	3	6.58E-04	2.19E-04	0.32	0.813	0.12
Solar Exposure	1	8.91E-04	8.91E-04	1.29	0.294	0.16
Error	7	0.005	6.93E-04			

Tukey's Pairwise Comparison						
Comparison		**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + SIP	$HRAP_D$	0.02	-0.05	0.09	0.842	-
$HRAP_L$	$HRAP_D$	0.01	-0.06	0.08	0.964	-
$HRAP_L$ + SIP	$HRAP_D$	0.02	-0.05	0.09	0.828	-
$HRAP_L$	$HRAP_D$ + SIP	-0.01	-0.08	0.06	0.983	-
$HRAP_L$ + SIP	$HRAP_D$ + SIP	6.67E-04	-0.07	0.07	1.000	-
$HRAP_L$ + SIP	$HRAP_L$	0.01	-0.06	0.08	0.979	-

a. Covariates appearing in the model are evaluated at the following values; Solar exposure = 9.27 MJ m^{-2}, Bonferroni CI adjustment applied

b. R^2 = 0.240, Adj. R^2 = -0.195

c. Homogeneity tested: Levene's test = F (3,8) = 0.268, p>0.05, p = 0.268

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 6.9: ANCOVA output for comparison between F-Specific phage inactivation rates (K_L) determined for solar exposed ($HRAP_L$ and $HRAP_L$ + SIP) and dark incubated ($HRAP_D$ and $HRAP_D$ +SIP) model HRAPs with and without an IP when controlled for water temperature (°C) after 49.5 h.

F-Specific Phage Inactivation Rates (K_L)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	3	0.07	0.03	0.07	0.02	0.02	0.12
$HRAP_D$ + SIP	3	0.09	0.03	0.09	0.02	0.04	0.14
$HRAP_L$	3	0.08	0.02	0.08	0.02	0.03	0.13
$HRAP_L$ + SIP	3	0.09	0.02	0.09	0.02	0.04	0.14
ANOVA							
Source	**df**		**SS**	**MS**	**F**	**p value**	**Partial η^2**
Model HRAP	3		7.77E-04	2.59E-04	0.38	0.773	0.139
Temperature	1		9.29E-04	9.29E-04	1.35	0.283	0.162
Error	7		0.005	6.87E-04			
Tukey's Pairwise Comparison							
Comparison			**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + SIP	$HRAP_D$		0.02	-0.05	0.09	0.786	-
$HRAP_L$	$HRAP_D$		0.01	-0.06	0.08	0.911	-
$HRAP_L$ + SIP	$HRAP_D$		0.02	-0.05	0.09	0.806	-
$HRAP_L$	$HRAP_D$ + SIP		-0.01	-0.08	0.06	0.992	-
$HRAP_L$ + SIP	$HRAP_D$ + SIP		-9.38E-04	-0.07	0.07	1.000	-
$HRAP_L$ + SIP	$HRAP_L$		0.01	-0.06	0.08	0.995	-

a. Covariates appearing in the model are evaluated at the following values; temperature = 15.66 °C, Bonferroni CI adjustment applied

b. R^2 = 0.249, Adj. R^2 = -0.181

c. Homogeneity tested: Levene's test = F (3,8) = 0.127, p>0.05, p = 0.941

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Table A 6.10: ANCOVA output for comparison between F-Specific phage inactivation rates (K_L) determined for solar exposed ($HRAP_L$ and $HRAP_L$ + SIP) and dark incubated ($HRAP_D$ and $HRAP_D$ +SIP) model HRAPs with and without an IP when controlled for both solar exposure (MJ m^{-2}) and water temperature (°C) after 49.5 h.

F-Specific Phage Inactivation Rates (K_L)							
Model HRAP	**n**	**Observed Mean**	**SD**	**Adjusted Mean[a]**	**SE**	**95% CI**	
						lower	**upper**
$HRAP_D$	3	0.07	0.03	0.07	0.01	0.03	0.10
$HRAP_D$ + SIP	3	0.09	0.03	0.09	0.01	0.05	0.12
$HRAP_L$	3	0.08	0.02	0.08	0.01	0.05	0.12
$HRAP_L$ + SIP	3	0.09	0.02	0.09	0.01	0.05	0.12
ANOVA							
Source	**df**		**SS**	**MS**	**F**	**p value**	**Partial η^2**
Model HRAP	3		9.97E-04	3.32E-04	1.19	0.391	0.11
Solar Exposure	1		0.003	0.003	11.16	0.016	0.35
Temperature	1		0.003	0.003	11.3	0.015	0.35
Error	6		0.002	2.80E-04			
Tukey's Pairwise Comparison							
Comparison			**Difference**	**lower**	**upper**	**p value**	**Sig.**
$HRAP_D$ + SIP	$HRAP_D$		0.023	-0.03	0.07	0.403	-
$HRAP_L$	$HRAP_D$		0.019	-0.03	0.06	0.553	-
$HRAP_L$ + SIP	$HRAP_D$		0.020	-0.03	0.07	0.496	-
$HRAP_L$	$HRAP_D$ + SIP		-0.004	-0.05	0.04	0.990	-
$HRAP_L$ + SIP	$HRAP_D$ + SIP		-0.003	-0.05	0.05	0.996	-
$HRAP_L$ + SIP	$HRAP_L$		0.001	-0.04	0.05	1.000	-

a. Covariates appearing in the model are evaluated at the following values; solar exposure = 9.27 MJ m^{-2}, temperature = 15.66 °C, Bonferroni CI adjustment applied

b. R^2 = 0.738, Adj. R^2 = 0.520

c. Homogeneity tested: Levene's test = F (3,8) = 2.189, p>0.05, p = 0.167

d. Strength of significance: '-' p>0.05, '*' p<0.05, '**' p<0.01, '***' p<0.001

Appendix 6.3: Tukey's Post Hoc comparisons

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Table A 6.11: Tukey's post hoc comparison of variance for in situ water conditions in the model HRAPs ($HRAP_L$, $HRAP_L$ + SIP and $HRAP_L$ + LIP) after 49.5 h when fully exposed to sunlight. Statistical significance was at p<0.05.

Parameter	Model HRAP		Tukey multiple comparison of means 95% family-wise confidence level				
			Difference	Lower	Upper	p-value	Sig.
pH	$HRAP_L$ + SIP	$HRAP_L$	0.06	-0.14	0.25	0.776	-
	$HRAP_L$ + LIP	$HRAP_L$ + SIP	-0.01	-0.20	0.18	0.989	-
	$HRAP_L$ + SIP	$HRAP_L$ + LIP	0.01	-0.18	0.20	0.989	-
DO (mg L^{-1})	$HRAP_L$ + SIP	$HRAP_L$	0.37	-1.06	1.80	0.812	-
	$HRAP_L$ + LIP	$HRAP_L$ + SIP	1.13	-0.30	2.56	0.151	-
	$HRAP_L$ + SIP	$HRAP_L$ + LIP	-0.76	-2.19	0.67	0.421	-
Temperature (°C)	$HRAP_L$ + SIP	$HRAP_L$	-0.16	-2.90	2.59	0.990	-
	$HRAP_L$ + LIP	$HRAP_L$ + SIP	-0.17	-2.92	2.57	0.987	-
	$HRAP_L$ + SIP	$HRAP_L$ + LIP	0.02	-2.73	2.76	1.000	-
Turbidity (NTU)	$HRAP_L$ + SIP	$HRAP_L$	-11.67	-48.04	24.70	0.612	-
	$HRAP_L$ + LIP	$HRAP_L$ + SIP	-11.67	-48.04	24.70	0.612	-
	$HRAP_L$ + SIP	$HRAP_L$ + LIP	0.00	-36.37	36.37	1.000	-
SS (mg L^{-1})	$HRAP_L$ + SIP	$HRAP_L$	-13.60	-20.69	-6.51	0.003	**
	$HRAP_L$ + LIP	$HRAP_L$ + SIP	-16.53	-23.62	-9.45	0.001	***
	$HRAP_L$ + SIP	$HRAP_L$ + LIP	2.93	-4.15	10.02	0.460	-
Chl *a* (mg L^{-1})	$HRAP_L$ + SIP	$HRAP_L$	-0.02	-0.11	0.07	0.825	-
	$HRAP_L$ + LIP	$HRAP_L$ + SIP	-0.04	-0.13	0.05	0.398	-
	$HRAP_L$ + SIP	$HRAP_L$ + LIP	0.02	-0.07	0.11	0.717	-

Table A 6.12: Tukey's post hoc comparison of variance for in situ water conditions in the model HRAPs ($HRAP_L$, $HRAP_L$ + SIP and $HRAP_L$ + LIP) after 49.5 h when fully exposed to sunlight. Statistical significance was at p<0.05.

Time (t; h)	Model HRAP		Tukey multiple comparison of means 95% family-wise confidence level				
			Difference	Lower	Upper	p-value	Sig.
24	$HRAP_D$ + SIP	$HRAP_D$	0.06	-0.24	0.37	0.904	-
	$HRAP_L$	$HRAP_D$	0.07	-0.24	0.37	0.891	-
	$HRAP_L$ + SIP	$HRAP_D$	0.24	-0.07	0.54	0.132	-
	$HRAP_L$	$HRAP_D$+ SIP	0.00	0.30	0.31	1.000	-
	$HRAP_L$ + SIP	$HRAP_D$ + SIP	0.17	-0.13	0.48	0.325	-
	$HRAP_L$ + SIP	$HRAP_L$	0.17	-0.13	0.48	0.339	-
49.5	$HRAP_d$ + SIP	$HRAP_D$	0.02	-0.05	0.09	0.849	-
	$HRAP_L$	$HRAP_D$	0.01	-0.06	0.08	0.966	-
	$HRAP_L$ + SIP	$HRAP_D$	0.02	-0.05	0.09	0.835	-
	$HRAP_L$	$HRAP_D$ + SIP	-0.01	-0.08	0.06	0.984	-
	$HRAP_L$ + SIP	$HRAP_D$ + SIP	6.67E-04	-0.07	0.07	1.000	-
	$HRAP_L$ + SIP	$HRAP_L$	0.01	-0.06	0.08	0.980	-

Appendix 7: Publications
Appendix 7.1: Conference Proceedings

Inclusion of pond walls to enhance solar exposure and pathogen removal

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ABSTRACT
Pathogen removal in wastewater treatment ponds is limited by poor light penetration in the water column. Solar exposure is increased in high rate algal ponds through paddlewheel mixing and shallow pond depths however; insufficiently exposed areas remain. To address this inclusion of an inclined plane to these treatment ponds was considered to increase the area available for disinfection and improve reduction rates. The application of inclined planes was investigated in a laboratory based model system and a pre-existing high rate algal pond in the field. Corresponding controls were assessed for comparison under normal pond conditions. Reduction of F-RNA bacteriophage; MS2 was significantly higher in model systems where an inclined plane was present ($P>0.05$), with an increase of 1.0 Log_{10} reduction difference observed after 49.5 h exposure. Systems operated at similar hydraulic loading rates presented identical reduction rates regardless of slope length. Preliminary results from the field based system were not as conclusive, with no observable difference identified. Despite initial results from the field proof of concept had been presented in this paper.

KEYWORDS
High rate algal ponds; inclined planes; pathogen removal; solar exposure; thin films.

INTRODUCTION
Water scarcity has led to increased wastewater reuse. To ensure wastewater quality is suitable pathogen removal must be achieved adequately. Wastewater treatment ponds provide effective treatment without the need for chemical additives. Germicidal properties of sunlight are effective at decreasing microbial populations in these ponds (Clancy *et al.*, 2000, Davies-Colley *et al.*, 1999). Reduction occurs through the direct (photo-inactivation) or indirect (photo-oxidation) inactivation of pathogens where the organisms genetic material becomes damaged through the absorption of ultraviolet (UV) and visible (Vis) light (Muela *et al.*, 2002, Sinton *et al.*, 2002). Poor light penetration within the water column however, can restrict disinfection through the decay of light intensity (Bolton *et al.*, 2010). This reduction is a result of attenuation (scattering of light particles), rapid absorption and short light wavelengths (Acher *et al.*, 1997, Caslake *et al.*, 2004, Curtis *et al.*, 1994). Pond depth, exposure time and water composition are also contributing factors (Al-Juboori *et al.*, 2010,

Fallowfield *et al.*, 1996, Kirk, 1994). Light availability in highly turbid pond waters was shown to decrease as pond depth increased (Fallowfield *et al.*, 1996, Kirk, 1994). The maximum penetration depths of UVA (~315-400 nm), UVB (~280-315 nm) and Vis (~400-700 nm) wavelengths in 1 m turbid waste stabilisation pond (WSP) water was identified as 0.03 m, 0.07 m and 0.14 m respectively (Bolton, 2012). This supports both Haag & Hoigne (1986) who claimed that majority of the pathogen inactivating light is absorbed in the first 1.0 m of water and Kohn & Nelson (2007) who indicated 99% of UVB absorption is within the first 0.03 m of water. For improved disinfection it is therefore essential that the amount of light available for microbial reduction is increased and these factors lessened.

Increased light exposure is exhibited in high rate algal ponds (HRAPs) through continual water mixing and a large surface area to volume ratio (Craggs *et al.*, 2004a, Mara, 2012). A shallow pond depth (~0.2-1.0 m) and raceway configuration is used in these ponds (Park *et al.*, 2011) with a theoretical hydraulic retention time (THRT) between 2-8 days (Shilton, 2005). Buchanan *et al.* (2011) identified disinfection in these ponds could be achieved six times faster than other treatment ponds, with less water lost through evaporation. A paddlewheel is unique design feature incorporated into HRAPs to improve solar exposure and ensure systems are well mixed and circulated throughout the pond (Craggs *et al.*, 2004b, Fallowfield and Garrett, 1985). Rotation of the wheel surfaces water deep within the column and exposes it to sunlight (Hu *et al.*, 1996). Pathogen removal is increased in HRAPs through this elevated exposure. Removal rates have been largely reported within HRAPs for bacteria (i.e. *Escherichia coli* and other faecal coliforms) (El Hamouri *et al.*, 1994, Fallowfield *et al.*, 1996, Garcia and Becares, 1997, Wells, 2005), helminth (El Hamouri *et al.*, 1994), and protozoa (i.e. *Cryptosporidium*) (Araki *et al.*, 2001). However the removal of enteric viruses and their indicator organism surrogates; bacteriophage is less documented. As a result the removal of F-RNA bacteriophage in particular MS2 will be explored throughout.

High algal concentrations within the system will reduce the quantity of light available in the column by 80% in HRAPs leaving at least one third of the pond unexposed (Sutherland *et al.*, 2014). As there are areas that remain unexposed even with the paddlewheel mixing complete removal of pathogens from within these systems may not be sufficiently achieved and must be addressed. Running water over an increased surface area in a thin film is hypothesised to improve both reduction rates and solar irradiation exposure and increase inactivation.

An inclined plane already exists in WSPs and an HRAP in the form of the pond walls. These walls are formed during construction and present an angle of approximately 45°. They have no role in treatment except for pond containment, but are considered suitable for increasing exposure to solar irradiance. The quantity of solar irradiance (UV energy) on an inclined plane has been reported. For a 37° incline exposure was increased by 3-4% (Navntoft *et al.*, 2012) and by 10% when angle of the incline was equal to the latitude and in the direction of the sun (Duffie and Beckman, 2013, Iqbal, 1983). During winter where irradiance is lower, Navontoft et *al.* (2012) identified a 50 and 76% improvement in both global spectra and solar UV radiation. A study by Hawley (2012) had briefly explored the use of the inclined plane (IP) in a model HRAP system to improve solar irradiation and pathogen removal with positive results identified under overcast and sunny conditions, further investigation however is necessary for the system to be considered plausible. This paper therefore aims to investigate microbial inactivation in HRAPs with the inclusion of an IP to both model and large scaled HRAPs.

METHODS

Experiments were conducted in model HRAPs at Flinders University, South Australia and at a pre-existing HRAP at Kingston on Murray (KOM), South Australia (34.242641°S, 140.329529°E). The KOM HRAP had a 60,000 L capacity with a depth of 0.32 m, a surface area of 200 m^2 and a theoretical hydraulic retention time (THRT) of 5 d. Pre-treated domestic wastewater from the local township was pumped into the pond at a daily inflow of 12,000 L d^{-1}.

HRAP Systems and Inclined Planes

Model HRAP Systems. The first part of the experiment was conducted using outdoor model systems. Model HRAPs were constructed from 100 L plastic vessels lined with plastic bin liners. Vessels were filled to a volume of 87.0 L at 0.30 m depth with domestic wastewater collected from local wastewater treatment plant (Mt. Barker). Wastewater was taken from the inlet to DAF (dissolved air flotation). Model systems were dark incubated with IP solely exposed to sunlight. A non-IP control was used for comparative purposes of normal HRAP conditions. For water circulation and IP operation aquarium pumps (Aqua One 102 maxi power head and Aqua PRO AP950 submersible) were used. A valve system was incorporated for flow adjustments. Operating conditions for model systems are outlined in Table 1.

Large Scale HRAP. The second part of the experiment looked at a larger in-field pilot system attached to the KOM HRAP. Aqua Pro AP7500 HM multi-use pump was used for wastewater circulation through IP system. No control pond was available for comparison thus, a timer system (IDEEC GT3A) was incorporated to turn IP on or off at set intervals (i.e. every 5 days), inactivation in the HRAP (control) was compared with inactivation determined when the IP was also operating. IP flow rate was set at approximately 3310.92 L h^{-1} giving a hydraulic loading rate (HLR) of 165.5 L h^{-1} m^{-2} over a 5 d hydraulic retention time (HRT).
Over the 5 d HRT the entire pond volume (60,000L) was estimated to pass over the manifold 1.65 times (0.33 cycles d^{-1}).

Inclined Planes (IP). Two types of inclined planes were used successfully in this study; a model IP and a naturally occurring HRAP wall. The Model IP was constructed from 5.0 and 6.0 mm black Perspex connected to a steel base. Two sizes, short (0.55 m) and long (1.10 m) were used with a surface area of 0.37 m^2 and 0.75 m^2, respectively. To generate a thin film 2 mm holes were evenly distributed along a piece of plastic tubing attached to the top of each plane. The second IP was constructed along the length of the pre-existing pond wall of the HRAP, situated downstream of the paddlewheel. IP had a surface area of 20 m^2. The distribution manifold utilised 3 mm holes.

Table 1. Model HRAP operating characteristics for different slope lengths; Short IP (0.55 m) and Long IP (1.10 m)

	Area	Initial	1		2		3		4	
	(m^2)	Q	HLR	Q	HLR	Q	HLR	Q	HLR	Q
Short IP	0.37	129.6	350.3	129.6	167.8	62.1	232.7	87.7	300.0	111.0
Long IP	0.75	131.1	350.3	262.7	174.4	130.8	232.8	174.6	300.0	225.0

Q: flow rate ($L.h^{-1}$), HLR: hydraulic loading rate (L h^{-1} m^{-2})

Sample Collection, environmental conditions, water quality analysis

Model HRAP Systems. Triplicate (10 mL) samples were collected every 1.5 h between 9:00 am and 5:30 pm. 120 mL samples collected every 3 h for water analysis including 1 L samples before and after each run. In situ water measurements were recorded at each collection interval, including dissolved oxygen (DO; YSI model 55, Xylem), pH (370 pH meter, Jenway), and water temperature (YSI model 55, Xylem). UVA and UVB radiation were also monitored using a Solar Light data logging radiometer-PMA2100 with sensors for both UVA (PMA 2110-WP, Solar Light) and UVB (PMA 2106-WP, Solar Light). Daily solar exposure (MJ m-2) and hours of sunshine were monitored from the Australian Bureau of Meteorology (BOM) with data obtained from Adelaide airport (9.58 km away); station number 23034. Other parameters included daily rainfall and minimum and maximum temperature.

Large Scale HRAP. Systems were run four times during early winter – late spring 2015 (i.e. July, August, and September). Composite samples (2 x 400 mL) were collected twice daily at 3:00 pm and 3:00 am over a 14 d sampling period. Due to the remoteness of the large HRAP samples were collected and stored at ~1°C via a refrigerated auto-sampler (Avalanche®, Teledyne ISCO). During transport samples were stored under dark incubation in an esky. Analysis was conducted within 24 – 48 h after being brought back to the laboratory.

Water Quality Analysis. Water quality was analysed using APHA (1992) standard wastewater methods, for BOD_5, chlorophyll a (chl a), nutrient concentrations, suspended solids (SS), and turbidity

Microbial Quantification

Enteric virus indicator organisms; F-RNA bacteriophage were used to determine microbial inactivation within the systems. Model systems were spiked with F-RNA bacteriophage MS2 (ATTC#15597-B1). KOM samples were reported as F-RNA phage due to uncertainty phage detected was MS2. Quantification of MS2 and FRNA bacteriophage present in the systems was carried out using quantitative double layer agar plaque assay modified from Debarolomeis & Cabelli (1991) and Noble *et al.* (2004). *Escherichia coli* Famp (ATTC# 700891) was used as the bacterial host. Both host and MS2 stock (model HRAPs) were grown with ampicillin sodium salt (Sigma) and streptomycin sulphate (Sigma) antibiotics. Samples were serially diluted (10x) with 0.5% tryptone water (Oxoid) for analysis when required. Counts were reported as plaque forming units (PFU) 100 mL for both systems.

Statistical Analysis

Log_{10} reduction was calculated using Equation 1; where N_0 was the initial microbial concentration and N_t was the microbial concentration at time t.

$$Log_{10}\, N_0 - Log_{10}\, N_t$$

Equation 1.

Inactivation rates (K) were determined from the slope of linear regression of log_{10} removal against hours exposed. Statistical analyses were carried out using statistical software packages; SPSS (IBM Corp., 2011) and R (R Core Team., 2012). Tests included linear regression, independent t-tests, and one-way analysis of variance (ANOVA) with Tukey's post hoc comparison. Statistical significance was inferred at $P<0.05$.

RESULTS AND DISCUSSION

Model HRAP Systems + IP

MS2 numbers were shown to decrease with time for all model HRAP systems. Reduction was visibly greater with the inclusion of the IP (Figure 1) and was increased further when exposure time was extended from 24.0 h (Figure 1a) to 49.5 h (Figure 1b).

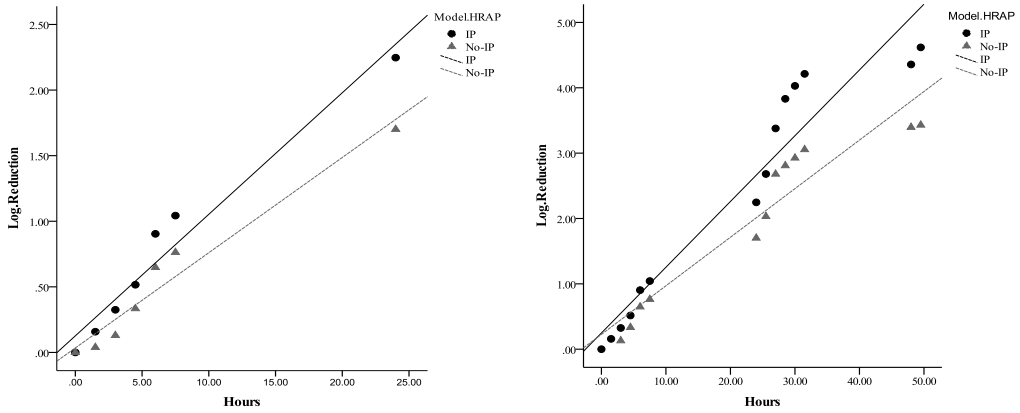

Figure 1. Semi-Log plot of Log_{10} reduction of MS2 against time (h) after **a**) 24.0 h and **b**) 49.5 h exposure : for the HRAP + IP system (●) and the non-IP HRAP control (▲)

As the model systems were primarily exposed under dark incubation and only the IP solar exposed this increased reduction from the IP supports Sinton *et al.* (2002) that F-RNA phage (i.e. MS2) exhibit a higher sensitivity to sunlight and inactivation is significantly greater when operated in the presence of sunlight compared to the absence. Inactivation rates are presented in Table 2. A significant difference (P>0.05) was observed between the inactivation rates after 24.0 h exposure with the mean Log_{10} MS2 removal in the HRAP+IP system (0.093±0.008 h^{-1}) higher compared to the Non-IP HRAP control (0.073±0.007 h^{-1}). A 24.1% increase was observed after 24.0 h exposure. Al Juboori *et al.* (1994) and Kirk (1994) suggested the amount of time pathogens are exposed to sunlight can restrict the disinfection effectiveness of solar irradiation and therefore increasing the time available for exposure should result in an elevated removal rate. This was evident when the systems were operated over 49.5 h (Figure 1b) with a difference of 1.0 log_{10} reduction achieved between both the non-IP control after 49.5 h exposure and the HRAP+IP system after 24.0 h. A significant difference (P>0.05) was also identified in the inactivation rates achieved after 49.5 h for the HRAP+IP (0.101± 0.009 h^{-1}) and the Non-IP HRAP control (0.077±0.006 h^{-1}).

A direct comparison of the inactivation rates with previous research could not be made as this is a novel concept. Comparison with inactivation rates obtained under sunlight exposure suggested that inactivation rates for the Non-IP HRAP and the HRAP+IP at 24.0 h and 49.5 h were higher than those exhibited by Sinton *et al.* (2002) for F-RNA phage in WSP effluent over winter when exposed to sunlight (0.050 h-1) and in the absence of light (0.014 h-1). However higher removal rates were reported Kohn & Nelson (2007) in the presence of UVB; the main disinfecting wavelength (0.56 h^{-1}) and lower in the absence of UVB (0.39 h^{-1})

Table 2. MS2 inactivation rates for Model HRAP systems; Non-IP HRAP and HRAP+IP after 24.0 and 49.5 h exposure.

Time (h)	Model HRAP	Inactivation Rates						
		Mean	SD	LRV	n	R^2	P-Value	Inactivation

								(K)
24.0	No-IP	0.516	0.599	0.145	7	0.954	0.0001554	0.073±0.007
	IP	0.743	0.763	0.586	7	0.960	0.0001074	0.093±0.008
49.5	No-IP	1.710	1.344	0.594	14	0.932	2.394e-08	0.077±0.006
	IP	2.307	1.763	1.521	14	0.921	5.631e-08	0.101±0.009

Note n=mean of all reps at time for system, total n; No-IP 24.0h n=21, 49.5 = 36; IP 24.0h =42, 49.5h = 72, p-value, *=0.001

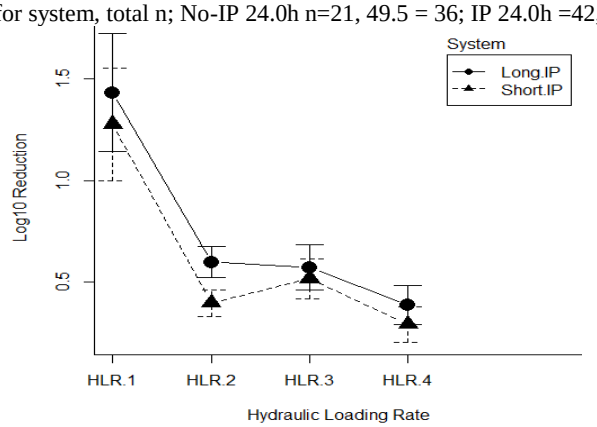

Figure 1: Mean values for log_{10} MS2 reduction (Log_{10} N_0-N_t) for both Short IP (▲) (0.55 m, 0.37m^2) and Long IP (•) (1.10 m, 0.75m^2) at different hydraulic loading rates (HLR; L h^{-1} m^{-2}); HLR 1**;** 350.3 L h^{-1} m^{-2}, HLR 2;167.8-174.4 L h^{-1} m^{-2}, HLR 3; 323.7-232.8 L h^{-1} m^{-2} and HLR 4; 300 L h^{-1} m^{-2})

No further improvement in inactivation was recorded when IP length was increased from 0.55 m (Short IP) to 1.10 m (Long IP). Figure 2 shows mean log_{10} MS2 removal for both short IP and long IP at varied flow rates and similar HLR. The difference between means inactivation was not significant (P<0.05). From this it can be perceived that regardless of IP length, if HLR is kept similar, a comparable or predicted reduction rate is likely to be observed. This relationship assisted up scaling from model systems into a larger field scale.

In situ water measurements identified water temperature (18.21±4.02°C), pH (7.50±0.39) and DO (3.87±4.04 mg L^{-1}) to be marginally higher but not significantly in the HRAP + IP system compared to the Non-IP HRAP control (16.48±3.19°C, 7.08±0.32 and 3.69±3.79 mg respectively mg L^{-1}). Alternatively chl. a and turbidity were higher in the Non-IP HRAP control (0.34±0.37 mg chl a L^{-1} and 46.03±48.90 NTU) than in the HRAP+ IP (0.23±0.26 mg chl a L^{-1} and 38.89±41.34 NTU).

Table 3. Analysis of the KOM HRAP treated wastewater during July-September 2015 for normal pond conditions (IP Off) and inclusion of the pond wall operating as an inclined plane (IP On).

Pump	Parameter	Mean	SD	n	Min.	Max.
IP-off	F-RNA Phage (Log_{10}PFU 100 mL^{-1})	1.72	0.29	20	1.00	2.11
	Log Removal (Log_{10}PFU 100 mL^{-1})	1.78	0.57	15	0.82	2.47
	Chl. a (mg L^{-1})	0.53	0.32	20	0.15	1.07
	SS (mg L^{-1})	76.88	23.13	20	17.60	120.40
	Turbidity (NTU)	98.00	18.14	20	68.00	123.00
	BOD_5 (mg L^{-1})	10.39	14.43	20	0.00	42.30
	Solar exposure (MJ m^{-2})	12.41	3.46	20	7.10	19.40
IP-On						
	F-RNA Phage (Log_{10}PFU 100 mL^{-1})	1.60	0.26	35	1.00	2.11

Log Removal (Log_{10}PFU 100 mL^{-1})	1.94	0.71	26	0.76	3.37
Chl. a (mg L^{-1})	0.47	0.29	35	0.21	1.34
SS (mg L^{-1})	82.59	20.46	35	50.80	136.80
Turbidity (NTU)	102.66	21.82	35	61.00	154.00
BOD5 (mg L^{-1})	11.72	13.48	35	0.00	42.30
Solar exposure (MJ m^{-2})	12.65	4.12	35	3.20	20.80

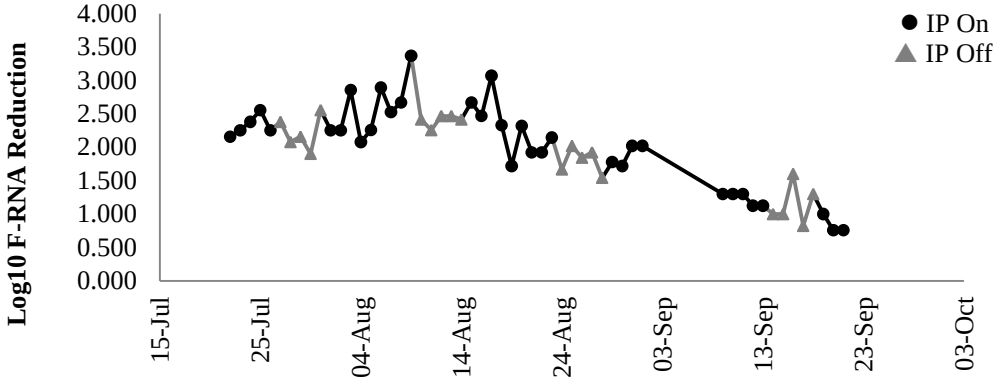

Figure 3. Kingston on Murray (KOM) HRAP F-RNA phage reduction (log_{10}PFU 100 mL^{-1}) for normal pond conditions (IP Off,▲) and pond wall IP inclusion (IP On,●) during July-September 2015. Operating cycle was IP on for 5 d, off for 5 d, on for 4 d.

Large Scale HRAP + IP

KOM IP was run four times over three months during winter-spring (2015). Table 3 shows the wastewater composition and operating conditions over the three month trial period. Mean inlet F-RNA phage concentration was 3.86±0.80 log_{10}PFU 100 mL^{-1}. Figure 3 shows the F-RNA phage log_{10} removal from the HRAP over the trial periods. No significant difference ($P>0.05$) was observed in the mean F-RNA phage removal rates when pond was operated under normal conditions (No-IP) and when operated with the IP addition (IP). Inclusion of the IP (pond wall) had yielded a higher mean F-RNA bacteriophage log_{10} reduction value (LRV) of 1.94±0.71 Log_{10}PFU 100 mL^{-1}. The mean LRV achieved throughout this study was reported after a three month period, thus a true comparison cannot be made between the two systems and the LRV reported by Young *et al.* (2015). However, they were shown to achieve those reported in the Australian reuse guidelines for WSPs (1.0-4.0 Log10 removal for viruses and bacteriophage) (NRMMC-EPHC-AHMC, 2006), suggesting treated water with the IP is suitable for reuse.

This was largely caused by hole clogging with algae and other particulate matter, preventing water flow. The poor flow over the manifold would help to explain the poor reduction performance exhibited with the increase in solar exposure shown in Figure 4. The clogging can be easily rectified by enlarging hole diameter to 5 mm for example. Placing a filter around the pump without inhibiting pump performance may restrict large particles from entering the system thus reducing blocking. Absence of a control pond is another limiting factor. As no pond was available the IP was forced to be switched off after 5 d. During this "off" period it is likely incomplete siphoning of water out of the tubing may have occurred which facilitated algal growth. In an ideal situation the IP would not need to be switched off so this build up may not occur. Tilting one end of the piping slightly may help to ensure all water is drained. It was possible that the operating regime was insufficient to service a pond of KOM's calibre (i.e. 60,000L) or for a significant difference to be observed. Extending the IP to allow more of the manifold to be used should also increase exposure further, if a different HLR is used.

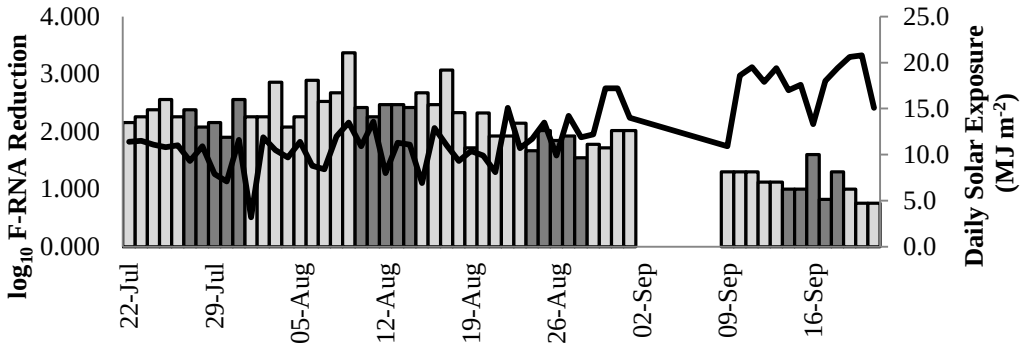

Figure 4. Plot of daily solar exposure (MJ m-2) and obtained Log_{10} F-RNA bacteriophage reduction (log_{10}PFU 100 mL^{-1}) obtained in the KOM HRAP when IP was operated (lighter bars) and normal pond conditions were run (darker bars) during July-September 2015.

CONCLUSION

Applying pond walls and thin films to HRAPs to improve solar exposure and pathogen removal had exhibited mixed results. A clear improvement obtained from the model systems indicated the concept is effective and feasible. Incorporation into the field however was not as positive with no distinguishable difference observed. This concept should not be dismissed based on these initial findings as it is likely with slight modifications a significant improvement can be detected. Further analysis is therefore required. For instance running both model and large scale IP under higher solar exposure (as these were conducted under the worst case scenario) should see reduction increased further. Increasing both IP length in the field to enable exposure over an even larger surface area and extending the length of each operating cycle, i.e. from five days to seven. A successful outcome of this system would be beneficial to both the water industry and consumer by providing a higher quality effluent safer for reuse leading to reduced disease spread and other health associated effects, with operating conditions optimised. Also this system provides a cost efficient extension where construction and operating cost are minimal and additional land space is unnecessary. This concept is not restricted to HRAPs or wastewater and could be applied to any pond system where there is a large sloped area available.

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Appendix 7.2: Publications

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Pond walls: Inclined planes to improve pathogen removal in pond systems for wastewater treatment?

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ABSTRACT

Attenuation of sunlight in wastewater treatment ponds reduces the depth of the water exposed to disinfecting irradiances. Shallow pond depths and paddlewheel rotation increases exposure of pathogens to sunlight in high rate algal ponds. Generation of thin films, using pond walls as inclined planes may increase inactivation of pathogens by increasing sunlight exposure. The performance of a laboratory based model system incorporating an inclined plane (IP) was evaluated. F-RNA bacteriophage, in tap water or wastewater, was exposed to sunlight only on the IP with the bulk water incubated in the dark. MS2 inactivation was significantly higher when the inclined plane was present ($P<0.05$) with a 63% increase observed. Prolonged exposure increased MS2 die-off irrespective of IP presence. Versatility of the IP was also demonstrated with faster inactivation observed in both optically clear 'tap' and wastewaters. IPs of different surface areas produced similar inactivation rates when operated at similar hydraulic loading rates regardless of slope length.

KEYWORDS

High rate algal ponds, inclined plane, dark inactivation, pathogen removal, solar exposure

INTRODUCTION

Wastewater treatment ponds utilise the germicidal properties of sunlight for the disinfection of pathogens (Clancy *et al.* 2000). Ultraviolet (UV) and visible (Vis) light when absorbed; directly (photo-inactivation) or indirectly (photo-oxidation), cause the genetic material or membranes of the organism to be damaged (Muela *et al.* 2002). In wastewater, however, attenuation reduces the depth

of light penetration through the water column, particularly of the more germicidal, shorter wavelength UVB spectrum, (Caslake *et al.* 2004). This light decay is more prominent in turbid waters, and increases with pond depth (Kirk 1994; Fallowfield *et al.* 1996). In turbid water, the majority of the light involved in inactivation is absorbed in the first 1 m of water (Haag & Hoigne 1986) and UVB in the first 0.03 m (Kohn & Nelson 2007). More specifically, Bolton (2012) identified the extinction depths of 0.03 m (UVB; 280-315 nm), 0.07 m (UVA; 315-400 nm) and 0.14 m (Vis; 400-700 nm), waste stabilisation pond (WSP) effluent, respectively. For improved disinfection, it is essential that the availability of light within the water column is increased and the effects of attenuation reduced.

High rate algal ponds (HRAPs) are, intentionally mixed, shallow treatment ponds (0.2- 0.5 m) arranged in a raceway configuration (Park *et al.* 2011). The hydraulic retention time (HRT) of these ponds is between 2-8 days (Shilton 2005). Increased exposure to sunlight is achieved in these ponds through large surface area to volume ratios and continual mixing, most commonly by paddlewheel (Fallowfield & Garrett 1985). Elevated removal rates are achieved in these ponds with disinfection up to six times faster than other waste stabilisation pond (WSP) systems (Buchanan *et al.* 2011). The removal of helminth (El Hamouri *et al.* 1994), protozoa (Araki *et al.* 2001) and bacteria (El Hamouri *et al.* 1994; Fallowfield *et al.* 1996) have been reported with a focus on the reduction of faecal coliforms and *E. coli*. The removal of enteric viruses is a priority to protect human health; however, limited information exists regarding the removal of these viruses and their bacteriophage surrogates. To help bridge the gap this study will focus on the removal of MS2 an F-RNA bacteriophage.

The existing pond walls surrounding both HRAPs and WSPs provide a natural inclined plane (45°), formed during construction. At present, these embankments serve no purpose other than to contain the pond water. However, they may provide an opportunity for a cost effective means of increasing

solar exposure, and consequently virus inactivation within the systems. Generation of a thin film of wastewater flowing down the pond wall will increase exposure to disinfecting wavelengths of light. The solar exposure (UV energy) experienced by an inclined plane has been characterised. An increase of 3-4 % was reported when the incline was 37° compared to a horizontal surface (Navntoft *et al.* 2012). A 10% increase was reported when the plane was in the direction of the sun and the incline equal to the degree of latitude (Iqbal 1983; Duffie & Beckman 2013).

The objective of this research was to establish if increasing the area available for solar exposure, through the addition of an IP and the generation of a thin film improved the inactivation of the F-RNA coliphage MS2. To achieve this, the study examined and compared removal rates achieved in model HRAPs in the presence and absence of an IP.

METHODS

Inclined Planes (IP)

IPs were constructed from black Perspex sheet (width 0.67m) fixed to a steel frame base. Two sizes were used, a small-IP (SIP; length 0.55 m, 0.37 m^2) and a large -IP (LIP; length 1.10 m, 0.75 m^2). A valved manifold was attached to the top of the plane through which water was pumped (Aqua PRO AP950) and the flow rates controlled to generate the thin film on the plane. Figure 1 provides a schematic diagram of the IPs used.

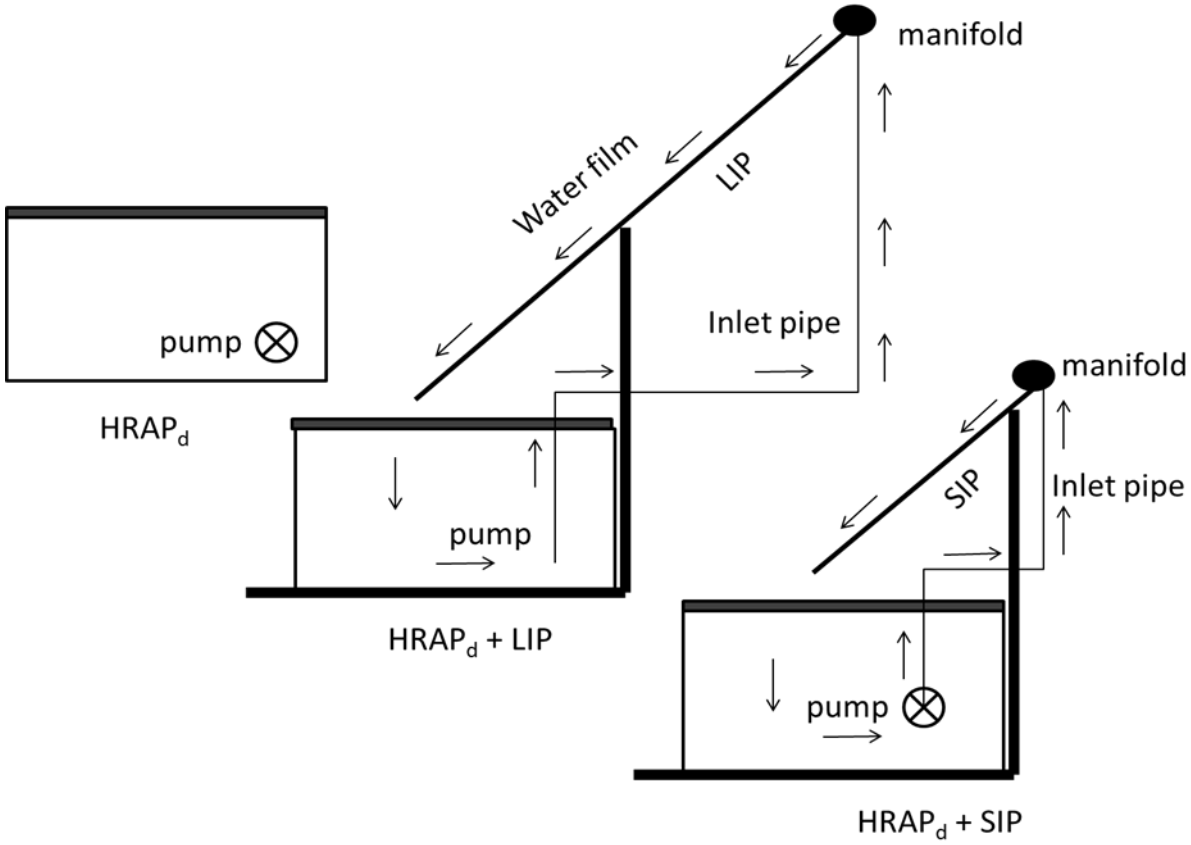

Figure 1: Schematic diagram of the model HRAP$_d$ and HRAP$_d$ + IP used. Two size IPs were used; a short (SIP, 0.37 m^2, 0.55 m) and a long (LIP, 0.75m^2, 1.10 m) IP. Diagram also demonstrates the function of the IP where pond water is pumped to the top of the IP via an inlet pipe connected to both pump and manifold. The water is converted into a thin film by holes in the manifold and exposed to sunlight as it travels down the IP back to the pond. Systems were operated concurrently.

Model HRAP systems

Model HRAPs were constructed from 100 L plastic vessels, filled to a depth of 0.30 m (87.0 L) with either optically clear (tap) water or wastewater collected from a treatment plant comprising an aerated lagoon and maturation pond (Mt Barker, South Australia). The bulk water in the plastic vessels was continuously mixed using aquarium pumps (Aqua One 102). In order to determine the inactivation potential of the IP the bulk water in the vessels was covered so that only the IP was exposed to sunlight. A similarly mixed and covered HRAP without an IP (HRAP$_d$) was used for comparison (Figure 1). Operating conditions are presented in Table 1.

Table 1. Model HRAP operating characteristics for two slopes of differing lengths and surface area; Small-IP (SIP; 0.37 m^2) and Large-IP (LIP; 0.75 m^2), where Q: flow rate ($L.h^{-1}$), HLR: hydraulic loading rate ($L\ h^{-1}\ m^{-2}$).

Model	**Area**	**Initial**	**1**		**2**		**3**		**4**	
HRAP	**(m^2)**	**Q**	HLR	Q	HLR	Q	HLR	Q	HLR	Q
SIP	0.37	129.6	350.3	129.6	167.8	62.1	232.7	87.7	300.0	111.0
LIP	0.75	131.1	350.3	262.7	174.4	130.8	232.8	174.6	300.0	225.0

Sample collection

Triplicate 10 mL samples were collected in 1.5 h intervals over a 24 h period. For water analysis, 120 mL samples were collected 3 hourly with an additional 1 L collected prior to and at completion of each experimental run.

Environmental conditions

In situ water measurements were recorded at each collection interval. Parameters monitored included; water temperature (YSI model 55, Xylem), pH (370 pH meter, Jenway) and dissolved oxygen (DO, YSI model 55, Xylem). Onsite UVA and UVB radiation was recorded using a Solar Light data logging radiometer-PMA2100 with UVA (PMA 2110-WP, Solar Light) and UVB (PMA 2106-WP, Solar Light) sensors. Daily solar exposure ($MJ\ m^{-2}$) and sunshine duration (h) were monitored from the Australian Bureau of Meteorology (BOM). Data was obtained from Adelaide airport (Station number 23034; 9.58 km from site).

Water Quality Analysis

Water quality was assessed, using Greenberg *et al.* (1992) standard methods for the analysis of wastewater methods, for turbidity (NTU), suspended solid (SS, $mg\ L^{-1}$), and chlorophyll *a* (chl *a*, $mg\ L^{-1}$).

Microbial Quantification

F-RNA bacteriophage MS2 (ATTC#15597-B1) was spiked into all model systems ($3.09x10^{10}$ PFU 100 mL^{-1}) and inactivation determined using the quantitative double layer agar plaque assay adapted from Debartolomeis and Cabelli (1991) and Noble *et al.* (2004). Briefly, *E. coli* Famp (ATTC# 700891) was used as the bacterial host. Both host and MS2 stock for HRAP inoculation were grown with ampicillin sodium salt (Sigma) and streptomycin sulphate (Sigma) antibiotics. Samples were serially diluted (10x) with 0.5% tryptone water (Oxoid) for analysis when required. Counts were reported as plaque forming units (PFU) 100 mL for both systems.

Statistical Analysis

Log_{10} reduction values (LRV) were calculated using Equation 1; where N_0 represents initial concentration, N_t was concentration at time t and LRV_t is the log_{10} reduction obtained after t hours.

$$LRV_t = \log_{10} N_0 - \log_{10} \underline{N_t}$$

Equation 1.

Inactivation rate constants (K) were determined using GInaFiT; a Microsoft Excel add-in (Geeraerd *et al.* 2005). Statistical analyses were carried out using statistical software packages; R version 3.1.2 (Vienna, Austria) and SPSS version 20.0 (Armonk, NY). Normal distribution of data was determined from Quantile-Comparison (Q-Q) plots and Shapiro-Wilk normality tests. Statistical analyses s included linear regression (multiple with stepwise regression), independent samples t-tests, one-way analysis of variance (ANOVA) with Tukey's post hoc comparison and Pearson's product correlation. Statistical significance was inferred at $p < 0.05$.

RESULTS AND DISCUSSION

MS2 inactivation with IP inclusion

The global solar irradiation received during this experiment ranged from 26.0 to 28.2 MJ m^{-1} with a mean of 26.9±1.1 MJ m^{-1}. Water temperatures ranged from 17.4 to 32.6°C in the model systems with means of 26.0±3.7°C ($HRAP_d$) and 25.9±5.2°C ($HRAP_d$+SIP). Elevated MS2 inactivation was achieved by the incorporation of the SIP (Table 2). The inclusion of the SIP resulted in an inactivation 1.6 times higher than the $HRAP_d$ operated in the absence of the IP. The difference between the mean LRVs was statistically significant ($P<0.05$).

Table 2 shows the inactivation rates obtained after 24 h incubation. The log_{10} linear + tail model described by Geeraerd *et al.* (2000) was identified as the best representation of the data. Tailing of the data suggests a lag in die-off for the presence of a mixed or sub culture where one of the populations exhibits a greater resistance to disinfection (Bevilacqua *et al.* 2015). In Table 2, the K_{max} values are derived from the log_{10} linear + tail inactivation curves.

Table 2. MS2 inactivation in model systems; log_{10} reduction values (LRV) and inactivation rate constant (K_{max}, mean ± standard deviation for $HRAP_d$ and $HRAP_d$ + SIP, after 24.0 h incubation. R^2 and p-values relate to the strength of the statistics associated with the determination K_{max}.

Time (h)	**System**	**Inactivation**				
		n	LRV_t	K_{max}	R^2	P-value
24.0	$HRAP_d$	21	1.445	0.240±0.067	0.7795	2.2×10^{-12}
	$HRAP_d$+SIP	21	2.354	0.297±0.078	0.9110	2.0×10^{-12}

Since the bulk water of both HRAP was in the dark, the elevated MS2 inactivation seen in the $HRAP_d$+SIP can be attributed to the exposure of water to sunlight received whilst on the slope. The inclusion of the IP increased the LRV_{24} by 63% when compared with the $HRAP_d$.

A direct comparison could not be made with the current literature due to the novel nature of this research. Comparison was made with studies examining F-RNA phage inactivation in other pond systems, for example WSPs, under both dark and solar exposures. The values reported here were

higher than the inactivation rates reported by Sinton *et al.* (2002), who examined the inactivation of F-RNA phage in WSP effluent exposed to sunlight (summer; 0.070 h^{-1}, winter; 0.050 h^{-1}) and in the absence of light (0.014 h^{-1}).

The role of hydraulic loading rate

Further experiments comparing the performance of SIP and LIP at different flow rates which maintained different but constant HLR on the two IPs within each experiment resulted in MS2 $LRV_{49.5}$ that were statistically similar ($P \geq 0.05$), an observation consistent across the four HLRs examined (Figure 2). The similar inactivation rates for slopes of different lengths and surface areas operated over a range of HLRs suggests that HLR is the factor which influences inactivation by the IP operated under the same climatic conditions. The higher inactivation exhibited for HLR 1 (Figure 2) was attributed to the higher solar irradiance received throughout the experimental period. Corresponding solar irradiances for the HLR experiments were 18.5±4.3 (HLR1), 8.9±10.7 (HLR2), 4.4±3.5 (HLR3) and 5.5±6.2 MJ m^{-2} (HLR4).

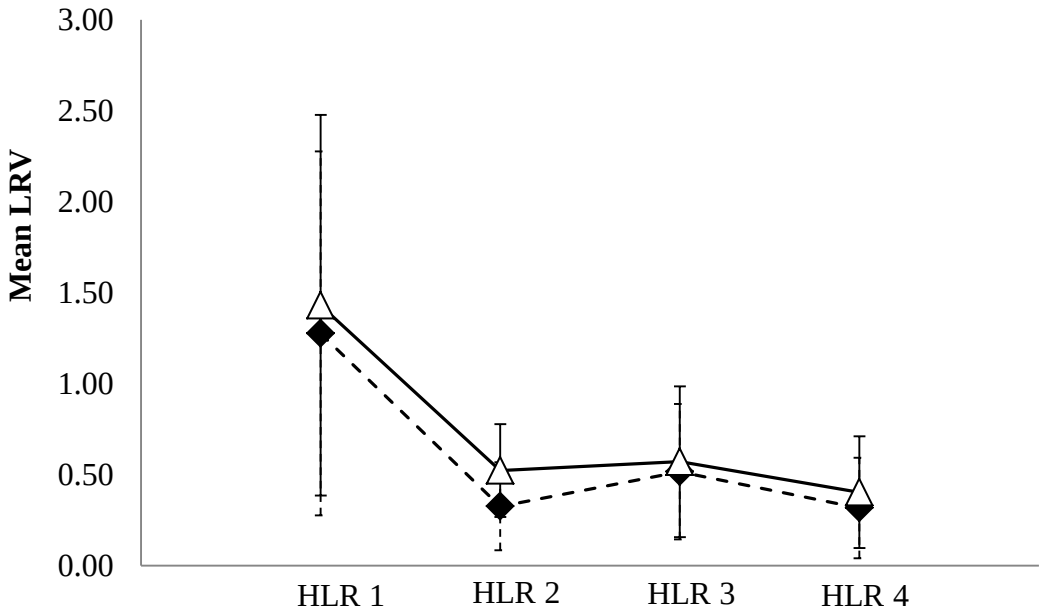

Figure 2. MS2 $\log_{10}$ reduction values (mean ± standard deviation) obtained after an incubation time of 49.5 h for small (◆) and large (▲) IP operated at different hydraulic loading rates (HLR: L m^{-2} h^{-1}). Mean hydraulic loading rates were HLR1, 350.3 L m^{-2} h^{-1}, HLR 2, 171.1 L m^{-2} h^{-1}, HLR 3, 232.75 L m^{-2} h^{-1} and HLR 4, 300 L m^{-2} h^{-1}.

Incubation Time

Solar and dark disinfection are affected by the duration of incubation and exposure to sunlight (Kirk 1994). Figure 3 shows that inactivation was related to incubation time both in the dark incubated $HRAP_d$ and in the $HRAP_d$ + IP exposed to sunlight. The LRV_{5d} were 1.882±0.282, 2.852±0.627 and 3.046±0.322 for the HRAP, HRAP+SIP and HRAP+LIP, respectively.

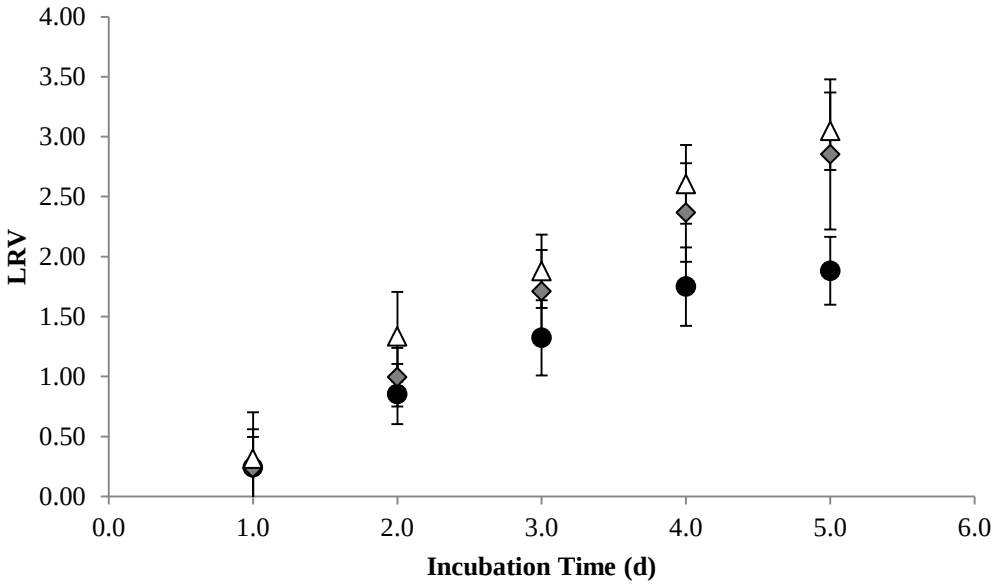

Figure 3. The relationship between LRV (mean ± 1 standard deviation) and the incubation time for the dark incubated $HRAP_d$ (●), $HRAP_d$ +SIP (◆) and $HRAP_d$ + LIP (▲). The inclined planes (IP) were operated at an HLR of 300.0 L m^{-2} h^{-1}

In situ water conditions

Inactivation can vary depending on different environmental parameters. Higher pH, DO and water temperature were identified in the $HRAP_d$+LIP (pH 7.73±0.42, 6.08±2.01 mg DO L^{-1}, 21.9±6.0°C) and HRAP+SIP (pH 7.64±0.41, 6.58±2.11 mg DO L^{-1}, 20.7±5.9°C) compared to the $HRAP_d$ (pH7.31±0.43, 5.51±2.26 mg DO L^{-1}, 20.4±6.2°C). However, pH was the only parameter identified as being significantly higher in the $HRAP_d$ +IP. This is likely a response to the elevated chlorophyll

a levels identified in the systems incorporating IPs. The chl *a* levels were 0.76±0.53 ($HRAP_d$+LIP), 0.71±0.38 ($HRAP_d$+SIP) and 0.49±0.21 mg L^{-1} ($HRAP_d$), respectively.

Summer incubations exhibited greater MS2 inactivation (Figure 4) indicative of the effect of higher solar irradiances received throughout summer. During the experimental period the mean summer irradiance; 27.9±1.1 MJ m^{-2}, was 2.6 and 5.5 times higher than the mean irradiance received during autumn (11.2±8.1 MJ m^{-2}) and winter (9.9±0.0 MJ m^{-2}), respectively.

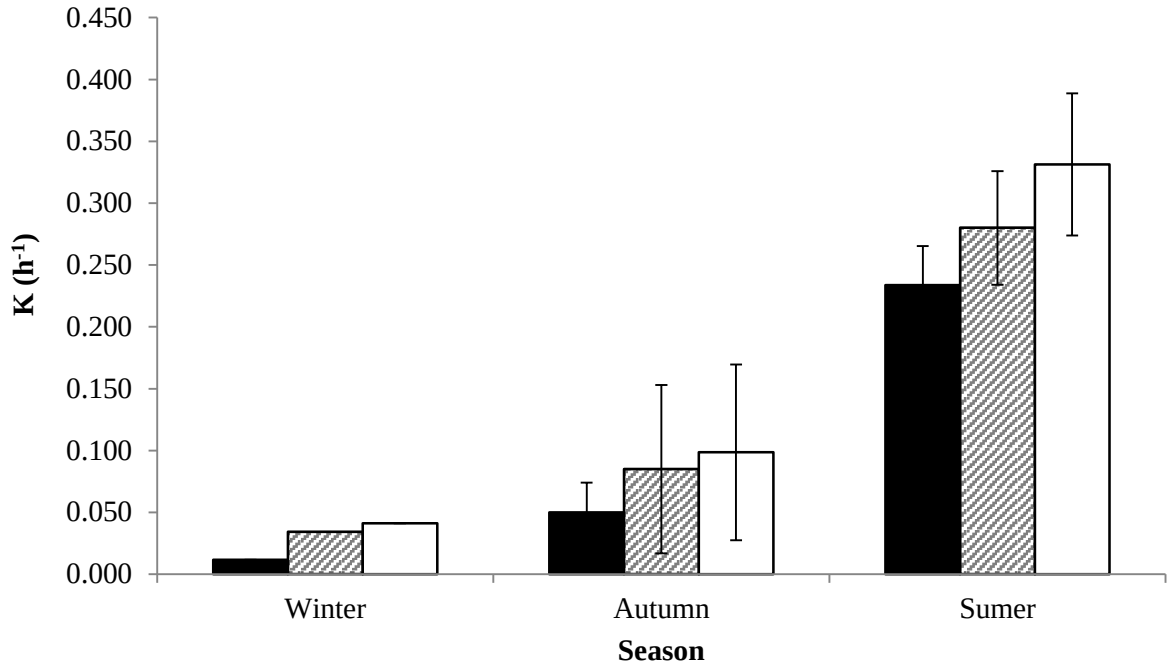

Figure 4. Seasonal MS2 inactivation rates (K h^{-1}) recorded in the model systems; $HRAP_d$ (■), $HRAP_d$+SIP (▨) and $HRAP_d$+LIP (□). Solar radiation ranged from 9.9±0.0 MJ m^{-2} (winter), 11.2±8.1 MJ m^{-2} (autumn) and 27.9±1.1 MJ m^{-2} (summer).

These results support those of Sinton *et al.* (1999) who identified slower inactivation of F-RNA phage throughout winter (both in sunlight and the dark).

Effect of water type

Figure 5 shows the variation of MS2 inactivation in clear (tap) waters and turbid wastewater. Inactivation was found to be 0.358 $\log_{10}$ h^{-1} slower in wastewater (0.186±0.019 $\log_{10}$ h^{-1}) than in tap water (0.544±0.214 $\log_{10}$ h^{-1}), suggesting inactivation efficiency was affected by water type. High turbidity, the presence of algae and particulate matter were likely responsible for the slower inactivation rates, with all capable of affecting light dispersion in the water column (Curtis *et al.* 1994). Davies-Colley *et al.* (1999) and Kohn and Nelson (2007) also reported lower F-RNA phage inactivation in waste stabilisation pond (WSP) effluent than in reverse osmosis (RO) water. Inclusion of the IP continued to produce elevated inactivation in wastewater and tap water, with the $HRAP_d$+SIP yielding an inactivation 1.5 and 1.2 times higher than the tap water and wastewater $HRAP_d$, respectively.

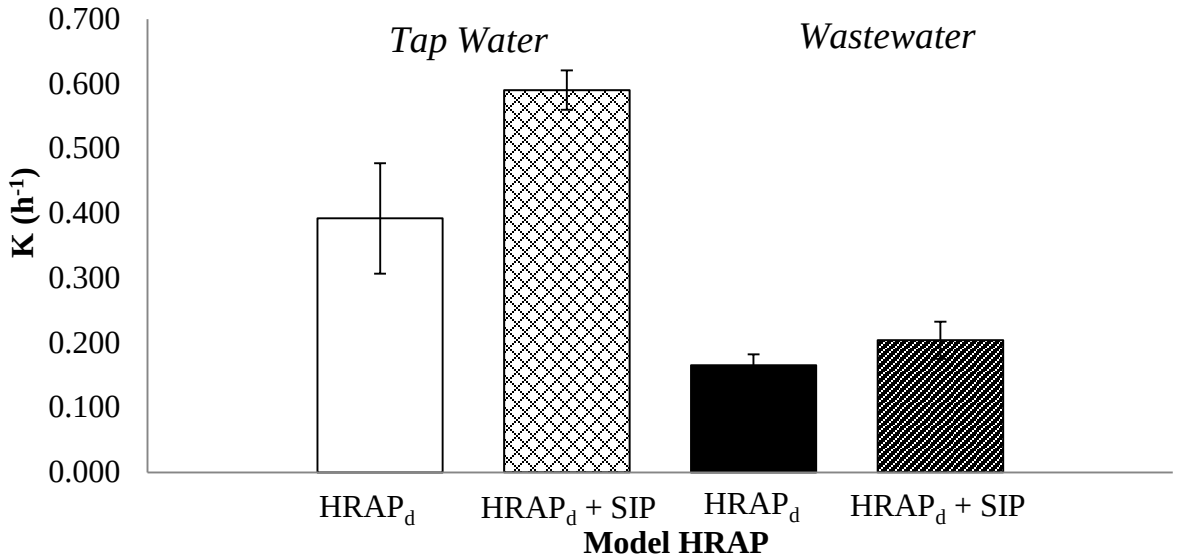

Figure 5. MS2 inactivation rates (K, h^{-1}; mean ± 1 standard deviation) for systems operated with either tap water or wastewater over 24 h. Solar irradiation ranged from 5.6 MJ m^{-2} to 10.8 MJ m^{-2}. Mean water temperatures ranged from 10.8 to 28.1°C in the tap water and 10.3 to 27.3°C in the wastewater.

CONCLUSION

It is clear from the results that pathogen inactivation can be improved by the inclusion of an inclined plane to a model HRAP. Inactivation increased by 63% following inclusion of the inclined plane compared to the dark incubated pond. Hydraulic loading rate was identified as an important factor influencing inactivation rates. The improvement in inactivation rates following incorporation of an inclined plane was less for turbid wastewaters compared to operation with optically clear tap waters. Additional work is still required with the aim of transferring the concept to a fully functioning field system. It would be beneficial to gain an understanding of the inactivation efficiency when both pond and IP are solar exposed and exposed to different seasonal variations. Multiple disciplines within the water industry would benefit from this research with higher quality effluent produced, safer for reuse via the management of the risk associated with exposure to pathogens.

ACKNOWLEDGEMENTS

We would like to acknowledge; Flinders Medical Centre biomedical engineers for the construction of the model HRAPs, the CWMS operators at Mt Barker Wastewater Treatment Plant for their assistance and permission to collect wastewater samples when required.

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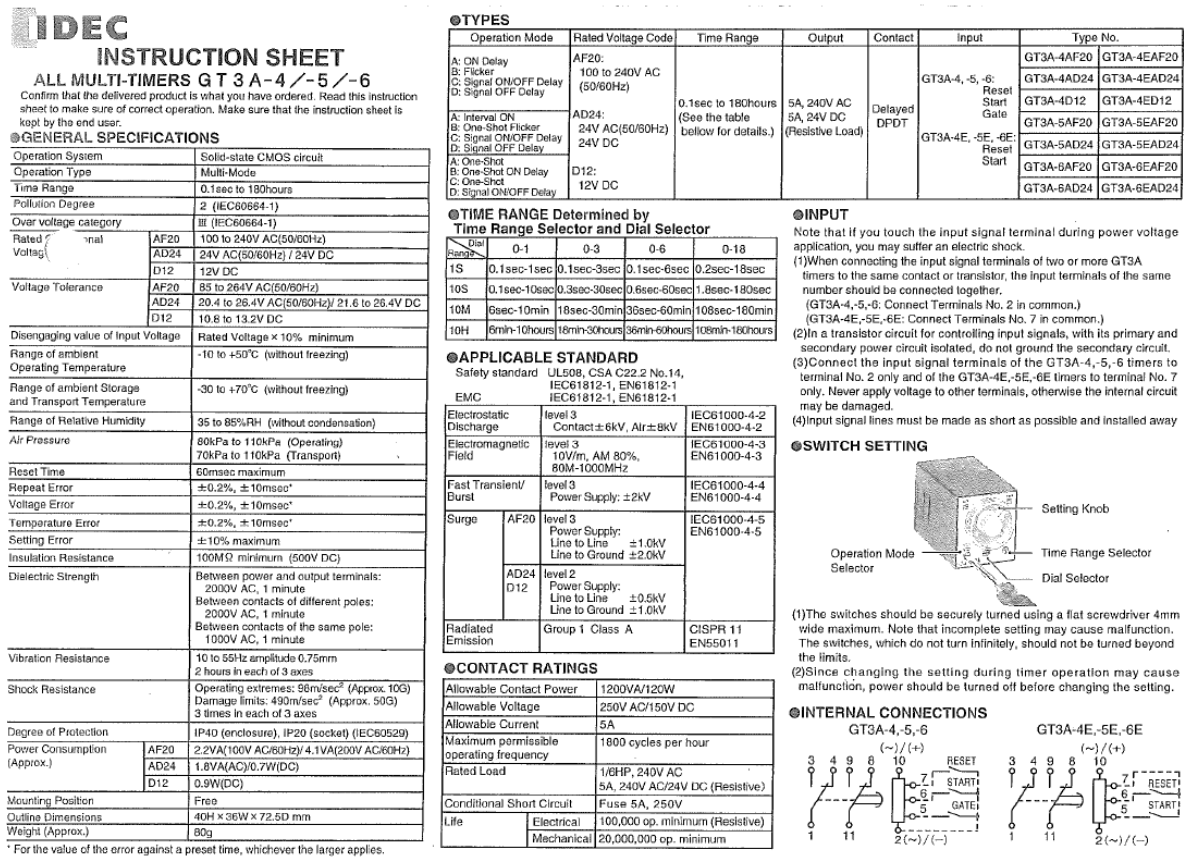
Appendix 8: IDEC GT3A Timer



IDEC

INSTRUCTION SHEET

ALL MULTI-TIMERS G T 3 A-4／-5／-6

Confirm that the delivered product is what you have ordered. Read this instruction sheet to make sure of correct operation. Make sure that the instruction sheet is kept by the end user.

●GENERAL SPECIFICATIONS

Operation System		Solid-state CMOS circuit
Operation Type		Multi-Mode
Time Range		0.1sec to 180hours
Pollution Degree		2 (IEC60664-1)
Over voltage category		III (IEC60664-1)
Rated Voltage	AF20	100 to 240V AC(50/60Hz)
	AD24	24V AC(50/60Hz) / 24V DC
	D12	12V DC
Voltage Tolerance	AF20	85 to 264V AC(50/60Hz)
	AD24	20.4 to 26.4V AC(50/60Hz)/ 21.6 to 26.4V DC
	D12	10.8 to 13.2V DC
Disengaging value of Input Voltage		Rated Voltage × 10% minimum
Range of ambient Operating Temperature		-10 to +50°C (without freezing)
Range of ambient Storage and Transport Temperature		-30 to +70°C (without freezing)
Range of Relative Humidity		35 to 85%RH (without condensation)
Air Pressure		80kPa to 110kPa (Operating) 70kPa to 110kPa (Transport)
Reset Time		60msec maximum
Repeat Error		±0.2%, ±10msec*
Voltage Error		±0.2%, ±10msec*
Temperature Error		±0.2%, ±10msec*
Setting Error		±10% maximum
Insulation Resistance		100MΩ minimum (500V DC)
Dielectric Strength		Between power and output terminals: 2000V AC, 1 minute Between contacts of different poles: 2000V AC, 1 minute Between contacts of the same pole: 1000V AC, 1 minute
Vibration Resistance		10 to 55Hz amplitude 0.75mm 2 hours in each of 3 axes
Shock Resistance		Operating extremes: 98m/sec² (Approx. 10G) Damage limits: 490m/sec² (Approx. 50G) 3 times in each of 3 axes
Degree of Protection		IP40 (enclosure), IP20 (socket) (IEC60529)
Power Consumption (Approx.)	AF20	2.2VA(100V AC/60Hz)/ 4.1VA(200V AC/60Hz)
	AD24	1.8VA(AC)/0.7W(DC)
	D12	0.9W(DC)
Mounting Position		Free
Outline Dimensions		40H × 36W × 72.5D mm
Weight (Approx.)		80g

* For the value of the error against a preset time, whichever the larger applies.

●TYPES

Operation Mode	Rated Voltage Code	Time Range	Output	Contact	Input	Type No.	
A: ON Delay B: Flicker C: Signal ON/OFF Delay D: Signal OFF Delay	AF20: 100 to 240V AC (50/60Hz)	0.1sec to 180hours (See the table bellow for details.)	5A, 240V AC 5A, 24V DC (Resistive Load)	Delayed DPDT	GT3A-4, -5, -6: Reset Start Gate	GT3A-4AF20	GT3A-4EAF20
						GT3A-4AD24	GT3A-4EAD24
A: Interval ON B: One-Shot Flicker C: Signal ON/OFF Delay D: Signal OFF Delay	AD24: 24V AC(50/60Hz) 24V DC					GT3A-4D12	GT3A-4ED12
						GT3A-5AF20	GT3A-5EAF20
A: One-Shot B: One-Shot ON Delay C: One-Shot D: Signal ON/OFF Delay	D12: 12V DC				GT3A-4E, -5E, -6E: Reset Start	GT3A-5AD24	GT3A-5EAD24
						GT3A-6AF20	GT3A-6EAF20
						GT3A-6AD24	GT3A-6EAD24

●TIME RANGE Determined by Time Range Selector and Dial Selector

Dial / Range	0-1	0-3	0-6	0-18
1S	0.1sec-1sec	0.1sec-3sec	0.1sec-6sec	0.2sec-18sec
10S	0.1sec-10sec	0.3sec-30sec	0.6sec-60sec	1.8sec-180sec
10M	6sec-10min	18sec-30min	36sec-60min	108sec-180min
10H	6min-10hours	18min-30hours	36min-60hours	108min-180hours

●APPLICABLE STANDARD

Safety standard UL508, CSA C22.2 No.14, IEC61812-1, EN61812-1

EMC IEC61812-1, EN61812-1

Electrostatic Discharge		level 3 Contact±6kV, Air±8kV	IEC61000-4-2 EN61000-4-2
Electromagnetic Field		level 3 10V/m, AM 80%, 80M-1000MHz	IEC61000-4-3 EN61000-4-3
Fast Transient/ Burst		level 3 Power Supply: ±2kV	IEC61000-4-4 EN61000-4-4
Surge	AF20	level 3 Power Supply: Line to Line ±1.0kV Line to Ground ±2.0kV	IEC61000-4-5 EN61000-4-5
	AD24 D12	level 2 Power Supply: Line to Line ±0.5kV Line to Ground ±1.0kV	
Radiated Emission		Group 1 Class A	CISPR 11 EN55011

●CONTACT RATINGS

Allowable Contact Power		1200VA/120W
Allowable Voltage		250V AC/150V DC
Allowable Current		5A
Maximum permissible operating frequency		1800 cycles per hour
Rated Load		1/6HP, 240V AC 5A, 240V AC/24V DC (Resistive)
Conditional Short Circuit		Fuse 5A, 250V
Life	Electrical	100,000 op. minimum (Resistive)
	Mechanical	20,000,000 op. minimum

●INPUT

Note that if you touch the input signal terminal during power voltage application, you may suffer an electric shock.

(1)When connecting the input signal terminals of two or more GT3A timers to the same contact or transistor, the input terminals of the same number should be connected together.
(GT3A-4,-5,-6: Connect Terminals No. 2 in common.)
(GT3A-4E,-5E,-6E: Connect Terminals No. 7 in common.)

(2)In a transistor circuit for controlling input signals, with its primary and secondary power circuit isolated, do not ground the secondary circuit.

(3)Connect the input signal terminals of the GT3A-4,-5,-6 timers to terminal No. 2 only and of the GT3A-4E,-5E,-6E timers to terminal No. 7 only. Never apply voltage to other terminals, otherwise the internal circuit may be damaged.

(4)Input signal lines must be made as short as possible and installed away

●SWITCH SETTING

Setting Knob
Operation Mode Selector
Time Range Selector
Dial Selector

(1)The switches should be securely turned using a flat screwdriver 4mm wide maximum. Note that incomplete setting may cause malfunction. The switches, which do not turn infinitely, should not be turned beyond the limits.

(2)Since changing the setting during timer operation may cause malfunction, power should be turned off before changing the setting.

●INTERNAL CONNECTIONS

GT3A-4,-5,-6

GT3A-4E,-5E,-6E

Figure A 8.1: Specifications and set-up guide for the IDEC GT3A timer used to initiate large scale IP on and off

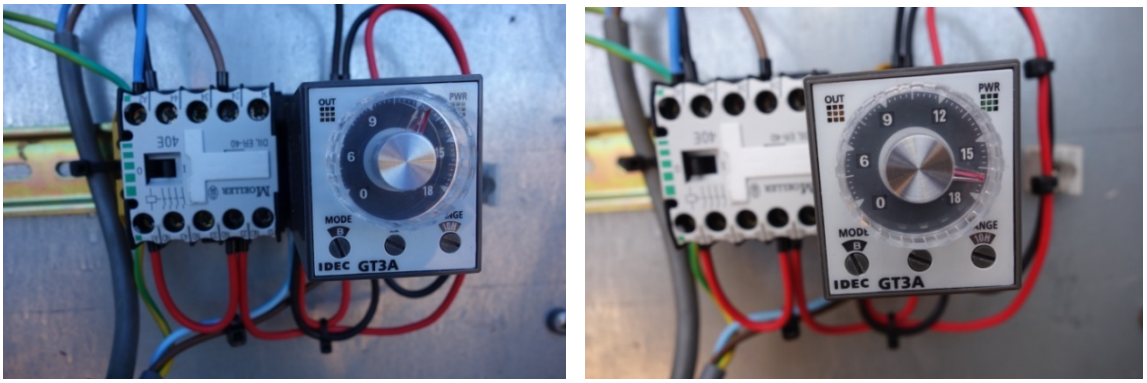


Plate A 8.1: IDEC GT3A timer set-up at Kingston on Murray (KoM) to operate large scale IP for a) 5 and b) 7 d.

Appendix 9: KoM pond summaries

Section 6.3.1 Page 176

Table A 9.1: KoM Inlet wastewater characterisation. A summary of the mean, standard error (SE), median and number of samples analysed for the inlet wastewater collected from the onsite splitter box at KoM. Samples were collected over a two year period between 2015 and 2016 with collections taken throughout winter and spring.

Parameter	Units	Trial 1 (2015)			Trial 2 (2016)			Combined data Mean		
		n	mean±SE	Median	n	mean±SE	Median	n	mean±SE	Median
F-Specific phage	log_{10} PFU 100 mL^{-1}	5	3.46±0.33	3.62	3	2.72±0.10	2.65	8	3.18±0.24	2.879
Turbidity	NTU	5	81.50±6.70	81.50	3	97.00±5.69	94.00	8	87.31±5.25	86.50
SS	mg L^{-1}	5	52.40±8.99	52.40	3	6.93±5.07	64.00	8	54.10±5.91	54.00
BOD_5	mg L^{-1}	5	19.87±3.91	18.55	3	24.87±6.85	31.00	8	22.20±3.46	20.20
TOC	mg L^{-1}	5	30.51±4.63	30.51	3	32.11±5.07	28.23	8	31.11±3.24	29.37
TC	mg L^{-1}	5	122.58±3.12	122.58	3	129.25±11.84	118.40	8	125.08±4.47	120.49
IC	mg L^{-1}	5	92.05±4.76	90.84	3	93.67±3.33	90.51	8	92.66±3.06	90.68
TN	mg L^{-1}	5	100.91±2.37	101.1	3	93.87±9.49	102.6	8	98.27±3.65	101.1

Table A 9.2: Mean ± SE and n for the KoM HRAP when operated without the inclusion of the pond wall. Monitoring occurred over 2015 and 2016 with the average values included.

Parameter	Units	Trial 1 (2015)			Trial 2 (2016)			Combined		
		n	Mean ± SE[1]	Median	n	Mean ± SE[2]	Median	n	Mean ± SE	Median
F-Specific phage	log_{10} PFU 100 mL^{-1}	20	1.77±0.06	1.78	20	2.12±0.10	2.11	40	1.92±0.07	1.87
LRV_5	log_{10} PFU 100 mL^{-1}	20	1.89±0.12	1.97	20	0.64±0.10	0.64	40	1.27±0.13	1.14
Turbidity	NTU	20	98.00±4.06	103.0	20	242.50±15.63	229.5	40	170.25±14.05	123.0
SS	mg L^{-1}	20	82.65±5.09	83.20	20	113.16±6.34	110.20	40	97.91±4.07	98.60
BOD_5	mg L^{-1}	10	20.78±4.47	21.80	6	7.13±2.27	4.50	16	15.66±3.32	14.10
Chl *a*	mg L^{-1}	20	0.53±0.07	0.35	20	2.33±0.15	2.19	40	1.43±0.17	1.18
TOC	mg L^{-1}	20	38.97±2.04	38.68	10	42.93±13.44	42.85	30	40.29±4.54	38.68
TC	mg L^{-1}	20	49.97±2.64	51.02	10	62.73±17.54	88.71	30	54.23±6.01	52.21
IC	mg L^{-1}	20	12.30±1.61	14.32	10	19.82±6.05	23.33	30	14.81±2.31	14.82
TN	mg L^{-1}	20	73.43±1.06	75.11	10	50.57±13.78	81.49	30	65.81±4.91	75.48
Solar Exposure	MJ m^{-2}	20	12.41±0.77	11.65	20	13.92±0.85	14.85	40	13.17±0.58	13.60
Water Temperature	°C	NA	NA	NA	20	15.40±0.27	15.25	20	15.40±0.27	15.25

Table A 9.3: Mean±SE and n for the KoM HRAP when operated with the inclusion of the pond wall. Two IP surface areas were used; IP_{20} (20 m^2) and IP_{36} (36 m^2) with systems operated in intervals of five and seven days. Monitoring occurred during winter-spring of 2015 (Trial 1; IP_{20}) and 2016 (Trial 2; IP_{36}) with the average values included.

Parameter	Units	IP_{20}			IP_{36}			Combined		
		n	Mean ± SE[1]	Median	n	Mean ±SE	Median	n	Mean ± SE	Median
F-Specific Phage	log_{10} PFU 100 mL^{-1}	35	1.60±0.04	1.60	28	1.35±0.16	1.65	63	1.49±0.08	1.60
LRV	log_{10} PFU 100 mL^{-1}	35	2.04±0.11	2.16	28	1.41±0.16	1.07	63	1.76±0.10	1.91
Turbidity	NTU	35	102.66±3.69	104.0	28	284.75±20.26	296.0	63	183.59±14.68	123.00
SS	mg L^{-1}	35	82.59±3.46	76.40	28	129.4±8.15	132.4	63	103.41±5.02	91.60
BOD_5	mg L^{-1}	21	20.52±2.60	19.70	6	12.22±5.02	7.05	27	18.60±2.37	19.00
Chl a	mg L^{-1}	35	0.47±0.05	0.358	28	2.49±0.21	2.50	63	1.37±0.16	0.909
TOC	mg L^{-1}	35	41.37±1.01	42.62	14	55.48±9.17	62.45	49	45.41±2.81	42.62
TC	mg L^{-1}	35	51.41±1.52	52.34	14	87.84±10.49	101.1	49	61.82±3.92	54.57
IC	mg L^{-1}	35	10.04±1.04	8.42	14	32.37±5.27	27.18	49	16.42±2.19	10.63
TN	mg L^{-1}	35	73.38±0.80	74.28	14	84.88±0.72	85.74	49	76.67±0.96	75.40
Solar Exposure	MJ m^{-2}	35	12.65±0.70	11.40	28	14.29±0.59	14.60	63	13.38±0.48	12.90
Water Temperature	°C	NA	NA	NA	28	15.23±0.30	15.11	28	15.23±0.30	15.11

[1] Values were achieved under the following operating conditions: Pumping intervals; 5 d, Flow rate (Q); 3329.1 L h^{-1}, hydraulic loading rate (HLR); 166.5 L m^{-2} h^{-1},
[2] Values were achieved under the following operating conditions: Pumping intervals; 7 d, Flow rate (Q); 8861.7 L h^{-1}, hydraulic loading rate (HLR); 246.2 L m^{-2} h^{-1},

Appendix 10: Comparisons between KoM inlet, $HRAP_{KoM}$ + IP_{OFF} and $HRAP_{KoM}$ + IP_{ON}

Section 6.3.2 Page 176

Table A 10.1: Statistical comparison of F-Specific phage inactivation and mean pond conditions between the normal HRAP operation during Trial 1 (2015) and Trial 2 (2016). Independent samples t-test was used with statistical significance determined for p<0.05.

Parameter	Trial 1 (2015)		Trial 2 (2016)		T-Test results						
	n	Mean ± SE	n	Mean ± SE	Difference	t	df	95% CI Lower	95% CI Upper	p value	Sig.
F-Specific phage	20	1.77±0.06	20	2.11±0.10	0.40	-3.38	33	-0.63	-0.16	0.0021	**
LRV	20	1.89±0.12	20	0.64±0.10	-1.25	7.89	37	0.93	1.57	1.84×10^{-09}	***
Turbidity	20	98.00±4.06	20	242.50±15.63	144.5	-8.95	22	-178.03	-110.97	1.05×10^{-08}	***
SS	20	82.65±5.09	20	113.16±6.34	30.51	-3.75	36	-47.00	-14.02	0.0006	***
BOD_5	10	20.78±4.47	6	7.13±2.27	-13.65	2.72	13	2.10	24.82	0.018	*
Chl *a*	20	0.53±0.07	20	2.35±0.15	1.81	-10.67	27	-2.16	-1.46	4.02×10^{-11}	***
TOC	20	38.97±2.04	10	42.93±13.44	3.96	-0.29	9	-34.51	26.59	0.777	-
TC	20	49.97±2.64	10	62.73±17.54	12.76	-0.72	9	-52.61	27.10	0.489	-
IC	20	12.30±1.61	10	19.82±6.05	7.52	-1.2	10	-21.41	6.38	0.257	-
TN	20	73.43±1.06	10	50.57±13.78	22.87	1.65	9	-8.34	54.08	0.132	-
Solar Exposure	20	12.41±0.77	20	13.92±0.85	1.51	-1.31	38	-3.84	0.822	0.198	-

Table A 10.2: Independent samples T-Test comparison of water composition between water treated in the KoM HRAP under normal pond operation and the inlet wastewater composition. Significance was at P <0.05.

Parameter	Inlet		$HRAP_{KoM}$ + IP_{OFF}		T-Test results							
	n	Mean ± SE	n	Mean ± SE	Difference	% change	t	df	95% CI Lower	95% CI Upper	p value	
F-Specific phage	8	3.18±0.24	40	1.92±0.07	1.26	39.70 ↓	7.002	46	0.90	1.63	0.0001	***
Turbidity	8	87.31±5.25	40	170.25±14.05	-82.94	94.99 ↑	2.611	46	-146.87	-19.00	0.0121	*
SS	8	54.10±5.91	40	97.91±4.07	-43.81	80.97 ↑	4.02	46	-65.74	-21.87	0.0002	***
BOD_5	8	22.20±3.46	16	15.66±3.32	6.54	19.37 ↓	1.18	21	-5.00	18.08	0.252	-
Chl a	8	31.11±3.24	40	1.43±0.17	-1.43	143.1 ↑	3.79	46	-2.19	-0.67	0.0004	***
TOC	8	125.08±4.47	30	40.29±4.54	-9.18	29.51 ↑	1.02	36	-27.49	9.14	0.316	-
TC	8	92.66±3.06	30	54.23±6.01	70.85	56.66 ↓	5.92	36	46.59	95.11	0.0001	***
IC	8	98.27±3.65	30	14.81±2.31	77.85	84.02 ↓	16.31	36	68.17	87.53	0.0001	***
TN	8	3.18±0.24	30	65.81±4.91	32.46	33.03 ↓	3.32	36	12.62	52.29	0.002	**

Strength of statistical significance: * p<0.05 (significant), ** p<0.01 (highly significant), *** p<0.001 (extremely significant), - p>0.05 (not significant)

Table A 10.3: Independent samples T-Test comparison of HRAP wastewater composition when either the 20 m^2 or the 36 m^2 IP was operating. Statistical significance was at p<0.05.

Parameter	IP_{20}				IP_{36}				T-Test results				
	n	Mean ± SE	Min	Max	n	Mean ± SE	Min	Max	Difference	t	df	P value	
F-Specific phage	35	1.60±0.04	1.00	2.11	28	1.35±0.16	0.00	2.57	0.25	1.66	61	0.103	-
LRV	35	2.04±0.11	0.76	3.37	28	1.41±0.16	0.35	2.91	0.64	3.42	61	0.001	**
Turbidity	35	102.66±3.69	61.00	154.0	28	284.8±20.26	109.0	456.0	-182.09	-9.82	61	3.58×10^{-14}	***
SS	35	82.59±3.46	50.80	136.8	28	129.44±8.15	54.00	202.8	-46.85	-5.68	61	3.97×10^{-7}	***
BOD_5	21	20.52±2.60	1.40	42.30	6	12.22±0.21	1.40	31.00	8.30	1.52	24	0.143	-
Chl *a*	35	0.47±0.05	0.21	1.34	28	2.49±0.21	0.67	4.25	-2.02	-10.40	61	3.93×10^{-15}	***
TOC	35	41.37±1.01	22.97	50.97	14	55.48±9.17	0.01	89.24	-14.11	-2.38	47	0.021	*
TC	35	51.41±1.52	33.29	71.32	14	87.84±10.49	0.16	120.1	-36.43	-5.23	47	3.83×10^{-6}	***
IC	35	10.04±1.04	3.77	27.89	14	32.37±5.27	0.07	57.03	-22.33	-6.09	47	1.98×10^{-7}	***
TN	35	73.38±0.80	61.49	81.58	14	84.88±0.72	81.12	88.65	-11.50	-8.545	47	3.96×10^{-11}	***
Solar Exposure	35	12.65±0.70	3.20	20.80	28	14.29±0.59	7.50	21.50	-1.64	-1.740	61	0.087	-

Strength of statistical significance: * p<0.05, ** p<0.01, *** p<0.001, - p>0.05 (not significant)

Table A 10.4: Independent samples T-Test comparison of water composition between water treated in the KoM HRAP when the IPs were operating and the inlet wastewater composition. Statistical significance was at p<0.05.

Parameter	Inlet				$HRAP_{KoM}$ + IP_{ON}				T-Test results					
	n	Mean ± SE	Min	Max	n	Mean ± SE	Min	Max	Difference	% change	t	df	P value	
F-Specific phage	8	3.18±0.24	2.60	4.37	63	1.49±0.08	0.00	2.57	1.70	-53.25 ↓	7.48	69	0.0001	***
Turbidity	8	87.3±5.25	63.00	108.0	63	183.6±14.68	61.00	456.0	-96.28	110.3 ↑	2.40	69	0.023	*
SS	8	54.1±5.91	34.40	84.00	63	103.41±5.02	50.80	202.8	-49.31	91.15 ↑	3.44	69	0.001	***
BOD_5	7	22.20±3.46	11.20	32.40	26	18.60±2.37	1.40	42.30	3.600	-4.25 ↓	0.73	69	0.470	-
Chl *a*	8	0.00±0.000	0.000	0.000	63	1.37±0.16	0.21	4.25	-1.37	137.0 ↑	3.05	69	0.003	**
TOC	8	31.11±3.24	17.34	42.35	49	45.41±2.81	0.00	89.24	-14.29	45.94 ↑	2.01	55	0.049	*
TC	8	125.1±4.47	114.5	152.9	49	61.82±3.92	0.16	120.1	63.26	-50.27 ↓	6.38	55	0.0001	***
IC	8	92.66±3.06	78.88	108.6	49	16.42±2.19	0.07	57.03	76.24	-82.28 ↓	13.63	55	0.0001	***
TN	8	98.27±3.65	74.92	108.70	49	76.67±0.96	61.49	88.65	21.60	-21.98 ↓	7.78	55	0.0001	***

Strength of statistical significance: * p<0.05 (significant), ** p<0.01 (highly significant), *** p<0.001 (extremely significant), - p>0.05 (not significant)

Appendix 11: Detailed summary of the associated costs with the large scale IP at KoM

Table A 11.1: Full summary of the total associated costs with the large scale IP construction. Costs are reported in Australian Dollars (A$) and are representative of both IP sizes used (i.e. 5 m and 9 m).

	Item	Qty	Price per Item	Total Cost
IP				
	AquaPro Multi-Use Pump	1	$ 236.00	$ 236.00
	Conduit - 25 mm Grey Electrical	3	$ 4.35	$ 13.05
	Conduit - 25 mm Elbow	2	$ 1.30	$ 2.60
	Conduit - 25 mm End Cap	2	$ 0.50	$ 1.00
	Conduit - Inspection T-Piece	1	$ 2.00	$ 2.00
	Conduit - Cement Solvent PVC Blue	1	$ 5.72	$ 5.72
	Irrigation - Pipe 25 mm x 50 m	1	$ 59.00	$ 59.00
	Irrigation - Elbow Poly Barbed 25 mm	2	$ 1.59	$ 3.18
	Irrigation - Barbed Tee	1	$ 1.27	$ 1.27
	Hose Clamp - 20-32 mm Stainless Steel	12	$ 1.28	$ 15.36
	Hose Clamp - 20-32 mm Plastic	10	$ 0.64	$ 6.40
	Valve - 25mm In line Barbed Tap	2	$ 7.78	$ 15.56
Other				
	Power - Extension lead 10 m	1	$ 19.73	$ 19.73
	Power - Lead Safety Box	1	$ 9.00	$ 9.00
	Insect Screen PE Cyclone 910mm x 2.05 m	1	$ 21.50	$ 21.50
	Black Storage Container 60 L	1	$ 10.00	$ 10.00
	Cable Ties - 100 x 2.5 mm 100 pk	1	$ 2.37	$ 2.37
	Cable Ties - 300 x 4.8 mm 100	1	$ 12.45	$ 12.45
	TOTAL			**A$ 436.19**

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