Major Research Project Thesis

The Impacts of Intermittent Mixing on High Rate Algal Pond Performance

Naomi Pirida Semi

Master of Environmental Health

College of Science and Engineering, June 2020

Contents

i. Summary

High rate algal ponds (HRAP) are a sustainable wastewater treatment system that is suitable for rural and remote communities. They use less land area, less energy for operation/solar voltaic with battery storage, have shorter retention times compared to conventional waste stabilisation ponds (WSPs) and most importantly achieve treatment results that are comparable to WSPs. The key features of a HRAP include a shallow raceway pond system, continuously yet gently mixed by a paddlewheel and in the presence of sunlight and algal photosynthesis wastewater is treated. However, there is the likelihood of the mixing being interrupted either due to failure/outages of the electricity supply or as part of managing the operational costs. Therefore, the aim of this study was to assess the performance of a HRAP when subjected to different intermittent mixing conditions.

The project was conducted at the Kingston on Murray HRAP facility studying two intermittent mixing regimes to assess treatment performance. In Regime 1, to illustrate an instance where there could be a power failure to operate the paddle wheel or if there was a mechanical failure in the system, the mixing was intermittently turned on than off for 5 days over a 15-day period to determine if treatment changed within the periods and or recovered to its initial stage when mixing restarted. In Regime 2, the second intermittent mixing condition, compared the operation of two HRAPs where the experimental HRAP was turned off for 12 hours daily in the evenings to depict a situation where this could be done to save electrical energy consumption and HRAP 2 was kept in continuous operation for comparison.

Parameters studied included nutrients, carbon content, suspended solids (SS) and chlorophyll *a*. The seasons in which the study was conducted were considered as well as they have an impact on the performance of HRAPs. In Regime 1 the results of SS, chlorophyll *a*, the carbon content and nutrients between the on and off phases were statistically insignificant to conclude that the performance between the on, off and on phases were different. In Regime 2, chlorophyll *a*, inorganic carbon content as well and $PO_4 – P$ were comparable. Particulate organic carbon (POC) content was over 60% in both seasons and HRAP conditions studied in this regime indicated an ample organic carbon pool and the algal biomass was maintained. Despite some differences, the HRAP performances were comparable and maintained overall.

These results can contribute towards seeking amendments for the operational guidelines for HRAP systems in South Australia.

ii. Declaration

I certify that this master's research project thesis does not incorporate without acknowledgment any material previously published or written by another person except where due reference is made within the text.

Naomi Pirida Semi

iii. Acknowledgements

To attempt a research project, let alone a major research project was something that I never envisioned I would do. To understand why, one has to know my background. I graduated from my undergrad in my home country, Papua New Guinea, in 2009. Throughout my undergrad, the notion of doing research was not encouraged but taught in a way that I for one felt that it was something that was unattainable or scary to do, for lack of better terms. And it was been something that had left a mark on me.

However, when I stated Master's degree in 2018, the teaching and learning environment in Flinders University changed my perspective on research and therefore I decided that I would do a major project as the component of my degree, might I add, trepidatiously.

And I am so happy with the decision that I made. I have gained so much through the process of the project. I have a growing interest in the field of my project and its possible application in my country, the opportunity to work in such excellent laboratory facilities I've ever been in, the opportunity to make friends with other students and staff and learn from each other within the research environment. Overall, I have fallen in love with research and hope to do more in the future.

On that note, I would like to acknowledge a number of who people have been with me through this journey. Firstly, my supervisor, Professor Howard Fallowfield, with his wealth of knowledge in the field, he has really guided me through this process despite the added challenges of Covid 19. His passion for wastewater treatment systems has really inspired me in developing a growing passion for the field and shaping the future of my profession. Dr. Ngai Ning (Ryan) Cheng, who guided me through the use of the laboratory equipment, the different analysis and for guidance throughout the process. You two have made me reach a place where I would have never imagined I would be at. Thank you so much!

The staff and other research students at the Environmental Health Department, for the conversations, for checking how I was going and for the friendship. Appreciation for Mr. Raj Indela and Jody Walker for help with the TOC and Nutrient analysis. I also would like to acknowledge the work of past researchers who through their work have guided my research. Thank You!

To my dear family and friends, who gave me the encouragement to go on during the days when I felt that I could not do it, when I had a lot of self-doubt, you uplifted me with positive words and prayers that boosted me on. Thank You!

Above all, I thank God for the Wisdom and knowledge that He so lavishly gives

~ Naomi Pirida Semi, 2194633 ~
June 2020

~Page intentionally left blank ~

The Impacts of Intermittent Mixing on High Rate Algal Pond Performance

1.0 Introduction

High rate algal ponds (HRAPs) are an alternative to conventional wastewater treatments systems. HRAPs use less land, simple operational equipment and require low energy but are very efficient in the treatment of wastewater within shorter timeframes in comparison to traditional waste stabilisation ponds (WSP) (Fallowfield and Garrett, 1985, Buchanan et al., 2018b). Secondarily, HRAPS are beneficial for the cultivation of algal biomass and the production of value-added products (Craggs et al., 2011, Park et al., 2011, Kumar et al., 2015).

The effectiveness of high rate algal ponds and their advantageous features in South Australia have been demonstrated (Buchanan et al., 2018b, Buchanan et al., 2018a). These features identified include the efficient removial of BOD_5 and nitrogen and the log_{10} reduction values (LVRs) of *E. coli*, the feacal indicator organisim, comparable to that of WSP, however, were achieved within a 5 days hydrolic retention time (HRT) at pond depth less then 0.4 m. Additionally, the evapourative water loss in the HRAP were lower than a WSP, a feature that is benefital for rural areas where treated wastewater reuse is part of water resource management plans. Moreover, HRPs use 66% less serface area therefore less captial cost. It is therefore applicable in remote communities as part of the Community Wastewater Management Systems (CWMS) that the Local Government Association (LGA) administers and who is also responsible for the design criteria for HRAPs in the state (LGA SA, 2019).

The HRAP system consists of a circular raceway pond, with depths less than 1 meter (Park et al., 2011) and a paddlewheel for mixing to encourage uniformity in the wastewater for maximum treatment. The growth of algae and its photosynthetic activity results in the assimilation of nutrients such as nitrates and phosphates in the wastewater and inactivation of bacterial pathogens (Evans et al., 2005, Buchanan et al., 2018a).

The paddlewheel that is required for the intentional mixing of the wastewater can be operated by alternate energy sources however, the reliability of such sources in remote communities can impact on the pond operation. Studies have looked at aspects of mixing for maximizing the production of algal biomass and the economics of operating commercial HRAPs however there are not many studies in literature looking at the impact of intermittent mixing on the performance of HRAPs in the treatment of wastewater.

This research paper begins with the literature review discussing the HRAPs components and functions of an HRAP, with key reference to the South Australian experience and discussions with regards to intermittent mixing and the gaps that justify the need for future research. Then it will discuss the research project that was conducted to study intermittent mixing in HRAPs and the discussions and conclusions drawn from it.

1.1 Background of HRAPs

HRAPs were first developed in California in the 1960s and were shown to be efficient in the treatment of wastewater from both domestic and industry (Picot et al., 1993, Evans et al., 2003, Evans et al., 2005). It has been widely studied and applied for use around the world due to the simplicity of the system, design, short retention times and effectiveness in the treatment of standard wastewater parameters. Many studies have reported reduction of BOD_5 and nutrients as well as with LRV for *E.coli* equal to or better than WSP (Buchanan et al., 2018b, Buchanan et al., 2018a, Young et al., 2017). HRAPs have been constructed and found to be effective in arid to semi – arid (Mediterranean) climates, studied for application in the subarctic climate (Grönlund et al., 2010) and in tropical climate (Young et al., 2017, García et al., 2006, Picot et al., 1991). One study found that localities where there is high rainfall are considered to be undesirable (Kumar et al., 2015) as there can be flooding due to the low depth of the system.

HRAPs are suitable for application in remote rural communities due to their low energy requirement, smaller land area use which then equates to low cost for construction and the shorter retention time for the wastewater while producing treatment results comparable or better than WSPs (Shelef and Azov, 1987, Fallowfield et al., 1992, Picot et al., 1993, García et al., 2006, Buchanan et al., 2018a). Other advantageous features include no accumulation of sludge and less odour (Cromar et al., 1996).

An HRAP constructed in the rural community of Kingston on Murray (KOM) in South Australia in 2008 caters for a population of approximately 300 people (Young et al., 2016). The wastewater is from the septic tanks and pre-treated onsite, gently mixed into the pond by a paddlewheel (Young et al., 2016) and according to design specifications set by the LGA has 4 to 10 days hydraulic retention time (HRT) (LGA SA, 2016) and depth between 0.3 m to 0.5 m (Young et al., 2017). In a comparative study, Buchanan et al. (2018a) found that HRAPs produced wastewater treatment results equal to or better than in traditional WSP.

1.2 HRAP design and process

The feasibility of the application of HRAPs depends on the local environment, solar radiation, temperature and matters such as land, water and cost (Kumar et al., 2015).

HRAP systems consist of a circular raceway pond for the wastewater flow, a paddlewheel for gentle mixing and where the actual treatment depends on algal biomass, solar radiance, bacteria and the hydraulic retention time (HRT). The wastewater influent source can be from a settling tank or pond (Picot et al, 1993), or septic tank effluent (Buchanan et al., 2018b). The effluent from the system can be used for irrigation of farmlands and recreational areas. The size of the HRAP depends on the estimate incoming flow of the locality of the system (Bahlaoui et al., 1997).

The different components of HRAPs and their roles in the treatment of wastewater water are further discussed.

Circular raceway pond, paddlewheel, HRT

The raceway ponds have depths between 0.2 to 1 meter (Park et al., 2011, Buchanan et al., 2018b). According to Picot et al. (1993) shallow depths between 0.3 m and 0.6m combined with the mixing and shorter HRT contribute to the reduced surface areas of HRAPs. This also contributes to reduced excavation costs (Buchanan et al., 2018a). The shallow depths allow for light penetration into the pond for the exposure of algal cells to light (Kumar et al., 2015). HRAPs can be operated at a constant depth and retention time however can impact level of treatment for some parameters between seasons as Buchanan et al., (2018) found in the treatment of nitorgen where in the warmer seasons treatment was not dependent on depth however inversly dependent on it in the cooler seasons. A secondary potential for HRAPs is algal biomass production especially for biofuel production (Young et al., 2017) however there are limitations. The limitation includes reliable production and the cost of harvest and low quantity of production (Craggs et al., 2011).

A paddlewheel is used to mix the wastewater to maintain a homogenous solution and this is also encouraged by baffles in the HRAP system. The blades of the paddlewheel can be made of materials such as galvanised steel (Craggs et al., 2004) or stainless steel (Evans et al., 2005) and require energy for the operation which can be from the electrical grid or alternate energy supply such as solar energy with battery storage. Mechanically they are simple with low maintenance requirements (Kumar et al., 2015). The paddlewheel gently circulates the wastewater and the baffles form a raceway to channel the flow (Craggs et al., 2004). The environment that the mixing creates is favourable for the growth of the algal biomass. The efficiency depends on the number of blades, speed and hydraulics (Kumar et al., 2015).

The hydraulic retention times (HRTs) can vary between 4 to 10 days (Picot et al., 1993) for an HRAP. The shorter HRT are beneficial as they reduce the loss of water through evaporations (Buchanan et al., 2018b). These water savings are important to rural communities where the reuse of treated wastewater is important for their agricultural practices. Conventional wastewater treatment lagoons, on the other hand, have a residence time of 66 to 70 days (Bahlaoui et al., 1997, (Buchanan et al., 2018b). More effective treatment is between spring and summer due to high solar radiation, however, with longer retention times in the winter when solar radiation is lower, treatment of wastewater is comparable to the warmer months (Bahlaoui et al., 1997). Therefore, temporal variations are to be expected but can be managed.

The shallow depth of a HRAP, the mixing by the paddlewheel ensure exposure of the volume of wastewater that enters to radiation and thereby the efficient treatment of wastewater parameters including E. coli, BOD_5 and the various nutrients (Young et al., 2017, Craggs et al., 2004)).

1.3 Treatment of Wastewater

HRAPs have been shown to provide better disinfection of wastewater (Fallowfield et al., 1996, Bahlaoui et al., 1997) or comparable results to WSP however where results are achieved in shorter retention times (Buchanan et al., 2018a). The algae absorb the nutrients and release oxygen through the process of photosynthesis which creates an aerobic condition for the treatment of wastewater where organic matter is broken down and assimilated by the algae (Craggs et al., 2004). There is also nitrification and BOD removal that occurs that will also be discussed.

Bacteriological parameters

The Australian Guidelines for Wastewater Recycling: Managing Health and Environmental Risk (Phase 1) (AGWR) (NRMMC, 2006) sets the indicative log removals of enteric pathogens and indicator organisms which include guidelines for *Escherichia coli* (*E.coli*), viruses and protozoa from wastewater treatment facilities. Usually, unlike bacterial pathogens, viruses and protoza are infectious at usually low doses therefore a cause for concern to public health. Indicator ogranisms and surrogates of the pathogens are used during the validation and in the countinuos monitoring of the treatment process.

In a HRAP, the interaction between the sunlight radiation, pH, pond depth and heterotopic bacteria with the action of photosynthesis disinfects wastewater by breaking down pathogenic bacteria (Fallowfield et al., 1996) especially *E. coli*. Sunlight radiation exposure to the wastewater is important and effective in the inactivation of the indicator bacteria (Craggs et al., 2004). The presence of *E. coli* is evaluated by the determination of the log_{10} reduction value (LRV). LRV is a parameter set in World Health Organization Guidelines (2006) in the enumeration of microbial pathogens in wastewater that will be used in agriculture or aquacultures for the risks to public health. LRV are reported to be between 1 and 3.01 log_{10} MPN 100 mL^{-1} for HRAPs (Young et al., 2016, Buchanan et al., 2018b, Buchanan et al., 2018a, Young et al., 2017). These are comparable to other studies but achieved in shorter retention times in comparison to WSP.

The treatement of pathogenic viruses from faeces in water and wastewaster treatment is monitored by the use of FRNA coliphages and somatic coliphages (Havelaar et al., 1985) . FRNA coliphages and its subgroup like MS2 are good indicators of pathogenic viruses (Young et al., 2016, Havelaar et al., 1985) as their size is closest to enentric viruses. Young et al. (2016) reported that a HRAP treated FRNA bacteriophage up to approximatley 1.6 LRV (log_{10} PFU 100 mL^{-1}) which complies to Australian Guidelines for Water Recycling (NRMMC, 2006)

There is limited information on the ability of the HRAP to treat protozoa. Young et al. (2016) used the surrogate Aerobic spore-forming bacteria (ASFB) to be the indicator for pathogenic protozoa in the same study, however, this proved to be unsuccessful as the LRV were often less than zero, indicating the volume of ASFB increased from the inlet to the outlet therefore creating a challenge in determining the treatment in natural wastewater treatment systems.

Physiochemical Parameters

HRAPs are efficient in the treatment of standard physicochemical parameters in wastewater such as biological oxygen demand (BOD), nutrients which include ammonia and phosphates as well as the carbon content dynamics within a system. *Figure 1.3.1 (*Removed due to copyright restriction) depicts the processes that are happening in this system that depends on algal biomass and bacteria in treating these parameters. There are two groups of microorganisms in a HRAP cycle which interact directly and indirectly with the organic matter. These are the aerobic bacteria and algae that keep the process in an equilibrium state through the algal oxygen production and the uptake of this by the bacteria. It is a symbiotic state achieved together with the composition of biomass controlled by the organic carbon loading rate (Cromar and Fallowfield, 1997, Fallowfield and Garrett, 1985). The bacterial biomass degradation releases the nutrients and carbon dioxide (CO_2) for algal photosynthesis. Atmospheric CO_2 is another source of inorganic carbon. Algae have the ability to use both CO_2 and bicarbonate (HCO_3^-) as their inorganic carbon source (Markou and Georgakakis, 2011).

It is important to note that wastewater cultures have an internal carbon pool which can be converted to a carbon source through changing CO_2 concentrating mechanisms (CCM) or accessible forms or particulate organic carbon (POCs).

BOD is reduced by the respiration of bacteria utilising oxygen produced from the photosynthesis of the algal biomass as the nutrients such a phosphate and nitrates are incorporated into the biomass (Cromar et al., 1996, Fallowfield and Garrett, 1985). Comparable removal of BOD in HRAP and WSP is reported however achieved at a shorted residence for the former (Buchanan et al., 2018a). BOD removal is over 90% (Buchanan et al., 2018b, Fallowfield and Garrett, 1985) however drops from 90% to just over 85% during winter months for 3 day residence times, therefore, Shelef and Azov (1987) recommended 4 days HRT for better removal.

Nutrient removal is regulated by factors that determine algal growth and activity such as retention time, solar radiation and temperature (Cromar et al., 1996, García et al., 2000). Algae prefer the assimilation of ammonium to obtain nitrogen instead of nitrates and or nitrite (Evans et al., 2005). Thus, the main mode of nutrients removal in HRAPs is through algae growth (Fallowfield and Garrett, 1985, Gracia et al., 2000). This is also referred to as the direct removal whereby the nutrient is assimilated in algal cells. The indirect process, on the other hand, is by the increase in pH of the wastewater and algae concoction due to algal photosynthesis which then results in the volatilisation of the ammonium ion ($NH_4 – N$) including the precipitation of phosphates ($PO_4 – P$) (Gracia et al., 2000 Fallowfield et al., 1996). The retention time plays an important role in the nitrification of ammonia, which is predominant form found in in HRAPs (Evans et al., 2005) where if the conditions are limited the NH_3 can reach toxic levels within a system.

Many studies both in experimental and pilot HRAP settings have achieved over 70% removal of phosphate, 50% to 70% removal of ammonia and or nitrate and over 90% removal of BOD (Shelef et al., 1982, Fallowfield and Garret, 1985, Picot et al., 1993, Cromar et al., 1996, Bahlaoui et al., 1997, García et al., 2000, Buchanan et al., 2018a, Buchanan et al., 2018b).

Changes in factors such as residence time, pH and seasonal changes have an influence on the reduction of nutrients in the wastewater.

There have been many studies to study optimum nutrient removal in a HRAP. A study carried out by García et al. (2000) reported removal rate of nitrogen being high in the spring and summer months and lower during winter and autumn. Picot et al. (1993) studied the diurnal variations where treatment was efficient during the daylight hours and dropped during the dark hours when the HRAP was subjected to sequential effluent flow. Garcia et al (2006) found that the diurnal variations of total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD), did not affect the performance of a HRAP in treating wastewater. Buchanan et al (2018a) in a study comparing the treatment of wastewater between a facultative WSP and HRAP found that BOD and NH_4 – N removal was comparable between the two and or better in the latter system however PO_4 – P removal although achieved by both systems, was better in the WSP.

1.4 Mixing in HRAPs

Mixing in the HRAP is the single largest factor distinguishing HRAPs from WSPs. Mixing in HRAPs by the paddlewheel is a key component to the success in the operation in the treatment of wastewater. The simple mixing mechanism along with the presence of baffles in the HRAP regulate the hydrodynamics of the pond, therefore, making the system efficient in its function (Fallowfield et al., 1996). The mixing is not for aeration, but purposely to ensure the even distribution of materials, avoid settling of sediments and flocculation of algal matter (Pham et al., 2018). For this project, there was not paper sighted which showed how HRAPs would perform when algal matter is resuspended should a system be "Off" in the night and restarted in the morning, additionally would there be sufficient energy to do this. Paddlewheels contribute to providing a conducive environment for the physicochemical process needed for wastewater treatment and encourages uniform radiance exposure which is beneficial for the growth of the algal biomass (Richmond, 2004, Sutherland et al., 2014, Pham et al., 2018).

The gentle mixing produces average velocities between 0.05 – 0.2 m/s (Fallowfield and Garrett, 1985). The eddies that are caused as a result of the mixing reduces the algal biomass residence in the darker layers of the pond (Rogers et al., 2014, Kumar et al., 2015). Energy is needed for the mixing and accounts to most of the operational cost for a HRAP (Kumar et al., 2015).

Intermittent Mixing

An important factor for consideration in the operation of an HRAP is the likelihood of events that can impact its operation especially with regards to interruption to the continuous mixing by the paddlewheel. This can be either a mechanical or electrical failure leading to the disruption of the continuous mixing or even intentional actions. There is very limited research on the performance of HRAPs in intermittent mixing conditions as alluded to also by Sutherland et al. (2014).

Intermittent mixing can be as a result to impacts on the energy supply to operate the paddlewheel, failure in the gearbox or impacts on components of alternate energy sources which can include continuous cloudy days impacting on solar radiation needed for solar – powered HRAP plants such as the KOM HRAP Facility. On the other hand, intermittent mixing could be intentional.

Low solar radiation impacts and causes a deficit in the amount of solar energy needed for operation. Daily solar exposure is from 1 to 35 MJ/m^2 where the lowest readings are for winter or cloudy days from longitudinal data from the Australian Bureau of Meteorology (BOM) (Bureau of Meteorology, 2019) in the KOM locale. Radiant exposure data from KOM for the past decade from 2008 to 2018 (***index i***) show that especially in autumn and winter there were 4 consecutive cloudy days and some data indicating more than 5 days. The lack of or minimal solar exposure means reduced supply of energy to solar panels to recharge the battery storage subsequently leading to a system shutdown. These will create an intermittent mixing state of the HRAP where can there can be possibly up to 5 days of no mixing.

Moreover, intermittent mixing could be an intentional action by HRAP operators who may decide to take such an action for the purpose of minimising operational and capital cost by turning off the energy source especially if using generators (or having one as a backup) in the night in the absence of sunlight.

There has been no publication sighted during the duration of this project that specifically explores the impact on the disinfection and treatment of wastewater by an HRAP in an intermittent mixing state, whether in a short term for up to 5 day (or longer) or how long can mixing be stopped before the performance is affected.

Sutherland et al. (2014) in a series of laboratory studies using various intermittent mixing regimes focused on the removal of nutrients to enhance the yield of different species of algal biomass. The laboratory studies mimicking HRAPs were 5L black buckets with 200mm of wastewater culture were subjected to different mixing regimes. The paddlewheel of an HRAP was mimicked by a magnetic stirrer. There were three mixing regimes; continuous, every 45 minutes, every 90 minutes and no mixing. The results showed that there were benefits from subjecting HRAPs to intermittent mixing.

Fallowfield and Garrett (1985) found that algal activity maintained when mixing was changed from a continuous mixing regime to 8-hour mixing regime at the same time energy use was decreased hence the likelihood that wastewater treatment was achieved. For diurnal efficiency in treatment, it was found that stopping the effluent flow in a HRAP from 11 pm to 11 am produced high levels of removal for ammonia and phosphates compared to a HRAP with continuous discharge (Picot et al., 1993).

The focus on the yield and production is also the focus of a study by Kumar et al (2015). They reported that mixing in the night can be ceased or reduced to avoid biomass loss. Rogers et al. (2014) proposed a number of approaches including decreasing the rate of the paddlewheel during the night, having variable operational rates, by operating at different rates for different blocks of time and having the operation ceased during winter but using the using of alternate mixing equipment such as airlift pumps and mixing boards to reduce the cost of operation and enhancing the algal biomass production. Both these studies were theoretical and focused on the application of a HRAP for the production of algal biomass for biofuel.

1.5 Research aims

High rate algal ponds are effective in the treatment of wastewater with results that are comparable or better to those of traditional systems but achieved in shorter retention times. The system depends on irradiance, algal photosynthesis and the gentle continuous mixing by the paddlewheel for wastewater treatment. The validation of the HRAP system in South Australia to be incorporated into the Community Wastewater Management Scheme (CWMC) was based on a continuous mixing system (Fallowfield et al., 2018).

However, as HRAPs are applicable in remote rural communities there is the likelihood of impacts to the continuous mixing resulting in an intermittent mixing state for shorter periods up to a few days or few hours. Such situations may be as a result of a failure of energy sources to operate the paddlewheel or intentional action taken to minimize capital and operational costs by turning 'off' mixing during the hours of darkness and the impacts on the treatment performance.

And there is very limited literature looking into the impacts on HRAP performance in such scenarios.

Therefore, the aim of this research project was to study the performance of a HRAP when subjected to intermittent mixing conditions. This was by studying the recovery within a HRAP after it had been turned off for a period of time and another was to compare between an experimental and control pond by subjecting one to an intermittent mixing state. The focus of this study was in the effectiveness of treatment of physicochemical parameters.

2.0 Materials and methods

The effectiveness of intermittent mixing on HRAP performance was studied by analysing biochemical parameters especially oxygen demand (BOD_5), total suspended solids (TSS), chlorophyll *a* and nutrients.

2.1 Sampling Location - HRAP Facility at Kingston on Murray

The project was carried out at the HRAP Facility located in Kingston - on – Murray (KoM; E 140°200 S 34°140). The township is over 214 km north – east of Adelaide within the District Council of Loxton Waikerie in South Australia. The HRAP was built in 2008 and serves a population of approximately 300 permanent residents. There is a school and a backpacker accommodation is also situated in the town. The two HRAPs are fed by the wastewater that is pre-treated in residential septic tanks then pumped to the facility. The two ponds have a dimension of 30 m X 5 m with a surface area of 200 m^2. The depth is maintained at 0.3 m and the mixing is by paddle wheel. The surface fluid velocity was 0.2 m s^{-1}.

Plate 2.1 Wastewater Inlet

Plate 2.2 HRAP Pond 1

There is an inlet (*Plate 2.1*) for the incoming wastewater, two HRAPs (*Plate 2.2*) and a storage pond for the treated wastewater. The facility also has a shed that houses the control panels for the pond and the solar power controls (*Plate 2.3*). The facility is powered by 5.3 kW h^{-1} solar voltaic cells (*figure 4*). The hydraulic retention time (HRT) for the HRAPs is 10 days.

Plate 2.3 Control panels in shed

Plate 2.4 Solar panels providing electricity to HRAP at KOM

2.2 HRAP Operation during the study period

For the first part of the study a HRAP (experimental) was subjected to an intermittent mixing regime of 5 days (half the retention time) on/off to assess the behaviour and recovery of the pond under this condition. This mixing condition was termed as Regime 1.

In the second study an HRAP 1 (control) was continuously mixed by the paddlewheel to achieve a mean surface velocity of 0.2m s^{-1} operation while HRAP 2 (experimental) was intermittently mixed 12 hours on/off at an equivalent surface velocity as described in Table 2.1

Paddlewheel operational condition Experimental	Period Operated	No. of Sample Collected
5 Days ON/OFF	03/08/19 – 17/08/19	5 ea
12 Hours ON/OFF (OFF: 6pm to 6am)	21/08/19 – 14/09/19 26/02/20 – 24/03/20	25 ea 28 ea

Table 2.1 HRAP 1 operational conditions over the study period. Control HRAP 2 was continuously mixed over the whole study period at a mean surface velocity of 0.2 m s-1; HRAP 1 was similarly mixed when the paddlewheel was operating.

The intermittent mixing was initiated by the automatic cessation of the paddlewheel by remote programming. This mixing regime was termed as Regime 2.

Samples were also taken from the inlet into the HRAP facility.

Wastewater sampling

Due to the distance of the HRAP site from the laboratory facilities, the samples were collected between August 2019 and April 2020 using ISCO refrigerated (<4°C) autosamplers (Avalanche® and ISCO4700). A daily composite sample was obtained by collecting wastewater (400 mL) at 3 am and 3 pm each day. The samples were retrieved after 14 days and transported to the laboratory in portable coolers for analysis.

Analysis of wastewater

2.2.1 Biological oxygen demand (BOD$_5$)

The 5-day BOD analysis was carried out as per APHA (1992) Test 5210 B (5 day BOD analysis) using the OxiTop® Control BOD measuring system as per the manufacturer's instructions. BOD$_5$ was analysed for all samples in Regime 1, for the second condition, every third sample from HRAP every third samples was analysed due to the sample volume. 250 mL of sample from the ponds and the inlet was measured into the OxiTop® bottles and a magnetic bar was added for the purpose of stirring. Three pellets of NaOH were place in a rubber quiver inside the neck of the bottle to absorb CO_2. The bottle was then closed with the OxiTop® head and the measurement was started by the controller, placed in the dark, temperature-controlled cabinet (20 or 25°C) on magnetic stirrers for 5 days after which BOD5 was recorded using the OxiTop controller.

Fig 2.1 OxiTop Controller reading BOD$_5$ from Measuring head

2.2.2 *Total Suspended Solids (TSS)*

The suspended solids within a HRAP is made up of algae, bacteria, zooplankton and detritus (ALBAZOD) (Buchanan et al., 2018). Wastewater TSS was analysed for the HRAPs and the inlet.

A known volume of a well-mixed sample was filtered through a 90 mm GF/C Whatman Filter which had been pre-dried (105°C / 24 h) and weighed. Following the filtration, the filters were then dried for a minimum of 24 hours at 105 °C. The final weight, rounded to three decimal places, was recorded and the final TSS in mg SS L^{-1} (APHA, 1992) was calculated using the following equation:

$$\text{TSS (mg L}^{-1}\text{)} = \frac{\text{(weight of filter and residue (mg) – weight of filter (mg)) x 1000}}{\text{Sample volume (ml)}}$$

The filtrate was froze for subsequent nutrient and total organic carbon analysis.

Additionally, unfiltered samples were also stored.

2.2.3 *Chlorophyll 'a' analysis*

The level of chlorophyll 'a' in a HRAP is a surrogate measure of the algal concentration, which is a vital component of the treatment process in this system.

The 90% acetone extraction method by Jeffrey and Humphrey (1975) was used in the analysis of chlorophyll *a*. 25 ml of sample was filtered through a 47 mm GF/C filter (Whatman Ltd; pore size 1.2 µm). The filter was then placed in a scintillation vial with 10 ml of 90 % (v/v) acetone/water and stored in the dark at 4 °C for 24 hours. Then 1 mL of acetone extract was placed into an Eppendorf micro - centrifuge tube and centrifuged for 5 minutes at 12,000 rpm to remove particulate matter. The clarified extracted was then transferred to a glass micro – cuvette (light path length 1 cm) and absorbance determined spectrophotometrically (UV – 1700 Spectrophotometer Shimadzu) of 664 nm, 647 nm and 630 nm.

The chlorophyll '*a*' concentration was calculated using the following equations:

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

where: OD_{664}, OD_{647} and OD_{630} are the absorbance at the respective wavelengths (nm)

Chl.*a* (µg L^{-1}) = Chl.*a* absorbance x (volume of acetone (ml) / sample volume (L))

2.2.4 Nutrients

Nutrients in wastewater can include ammonia (NH_4-N), nitrates (NO_3-N) and nitrite (NO_2-N) as well as phosphates (PO_4-P).

The San++ Automated Wet Chemistry Analyzer (Skalar Analytical BV, 2020) a continuous flow analyser was used for nutrients analysis of the GF/C filtrates (above) which applies APHA (1992) standard methods principles. 10 mL aliquot of samples were prepared and set into the automatic sampler. The parameters were pre-set into the computer interface of the San++ Automated Wet Chemistry Analyzer (Skalar Analytical BV, 2020) along with the standards that are required for the analysis.

2.2.5 Carbon analysis

A Shimadzu (2018) TOC-L series analyser was used for the analysis of total carbon which also comprises total organic carbon (TOC) and organic carbon (IC). GF/C filtrates (above) were analysed including homogenised unfiltered samples using APHA (1992) standard methods. Vials containing the samples were set into the analyser for analysis. The TOC- L analyser (Shimadzu,2018) applies the 680°C combustion catalytic oxidation method. Results from wastewater filtrates will be described as soluble IC, TC, TOC (in particular) using nomenclature sTC, sTOC, sTC.

Additionally, the particulate organic carbon (POC) was determined from the difference of the unfiltered TOC and filtered samples. POC is the undissolved organic carbon fraction of TOC.

Figure 2.2 Shimadzu (2018) TOC-L series analyser

2.2.6 Statistical analysis

Graphical analysis of data was done with MS Excel 2010. For statistical tests, the independent samples T – Test for the Equality of means and Levene's Test for the Equality of variances was completed using the SPSS Software (IBM Corp, 2018). Statistical significance was accepted at >95% confidence ($p \leq 0.05$). Results were presented as means ± SD.

3.0 Results

The samples analysed were collected between August 2019 to September 2019 (late winter to early spring) and from late February 2020 to March 2020 (late summer to early autumn) from the two HRAPs located at KOM. HRAP 1 was the Experimental and HRAP 2, the Control. There were 2 studies of intermittent mixing in this study. The first part of the study was to subject a HRAP to 5 days on and off regime, referred to as Regime 1, to assess the behaviour of the HRAP when it was turned off and how it recovered when it was turned on again. In the second part, the experimental HRAP was subjected to 12 hours on/off intermittent mixing regime (Regime 2) where mixing was off from 6pm to 6am. HRAP 2, was run continuously as the control.

The BOD_5, suspended solids, nutrients, TOC/IC/TC and chlorophyll *a* analysis of the wastewater were the focus of this project by evaluating their concentration in the respective intermittent mixing regimes for the two parts of this study.

3.1 HRAP 5 Days ON/OFF Results

The intermittent mixing in the HRAP was initiated by switching the electricity supply on or off for the operation of the paddlewheel the regimes. *Figure 3.1.1* shows the fluctuating electricity during the 5 days on and off regime beginning August 3 2019 to August 17 2029 as supplied by Dematec from logger located on site. The blue line is for dissolved oxygen and the yellow is for the turbidity.

Figure 3.1.1 Alternating electricity over the 5 days on/off to the HRAP indicated by the green lines beginning August 3 2019

The composition of the wastewater in the HRAP that was subject to 5 days with the mixing on followed by 5 days with mixing off and 5 days with mixing resumed are shown in *Table 3.1.1* which includes the mean, standard deviation (SD), median and the number of samples analysed for each parameter. Overall, the results did not show large differences in the composition between the phases of the study.

Parameters	5 Days ON				5 Days OFF				5 Days ON			
	Mean	SD	Median	n	Mean	SD	Median	n	Mean	SD	Median	n
BOD_5 (mg L^{-1})	155	9.9	155	2	75.35	88.6	75.35	2	95.5	91.22	95.5	2
SS (mg L^{-1})	287.4	40.89	300	5	247.86	106.85	201	5	184.29	49.07	169	5
Chl *a* (mg L^{-1})	2.75	1.38	2.62	5	2.99	1.33	2.56	15	3.05	2.06	2.60	5
NH_3 - N (mg L^{-1})	6.27	1.32	6.16	5	6.36	1.11	6.19	15	9.25	5.23	11.19	5
NO_2- NO_3 - N (mg L^{-1})	9.49	3.99	7.33	5	13.52	1.45	13.95	15	10.85	3.22	10.39	5
PO4 - P (mg L^{-1})	5.85	0.72	5.78	5	5.51	1.17	5.5	15	6.58	0.66	6.85	5
TC (mg L^{-1})	39.90	5.06	38.59	5	40.47	3.37	39.59	15	44.60	15.73	49.81	5
IC (mg L^{-1})	13.89	1.46	13.88	5	14.55	2.29	13.54	15	17.09	9.21	21.2	5
TOC (mg L^{-1})	26.01	3.77	24.58	5	25.92	1.85	27.06	15	27.51	6.60	29.33	5

Table 3.1.1 Summary of the physicochemical parameters and standard deviation during the 5 days ON/OFF intermittent HRAP Operation during winter 2019.

The organic load (BOD_5) is reduced by the action of bacterial respiration giving off CO_2 which the algal matter utilises in photosynthesis (Fallowfield and Garrett, 1985). In this study, over the paddlewheel on/off regime, the BOD_5 dropped during the off phase, when on again the BOD_5 increased but not to the level before cessation of mixing. (ON: 155 mg $L^{-1,}$ OFF: 75.35 mg L^{-1}, ON: 95.5 mg L^{-1}. Suspended solids behaved similarly, the mean values within the respective pond conditions gradually decreasing through the different periods in the regime (287.4 mg L^{-1}, 247.86 mg L^{-1} and 184.29 mg $L^{-1)}$ mixing on, off and on however high and overlapping standard deviations in the values suggest there was no significant difference amongst the values. The fall in concentration of SS from day 3 in the OFF phase *(Figure 3.1.2)* may be related to the settling of the suspended particulate matter due to the mixing turned off.

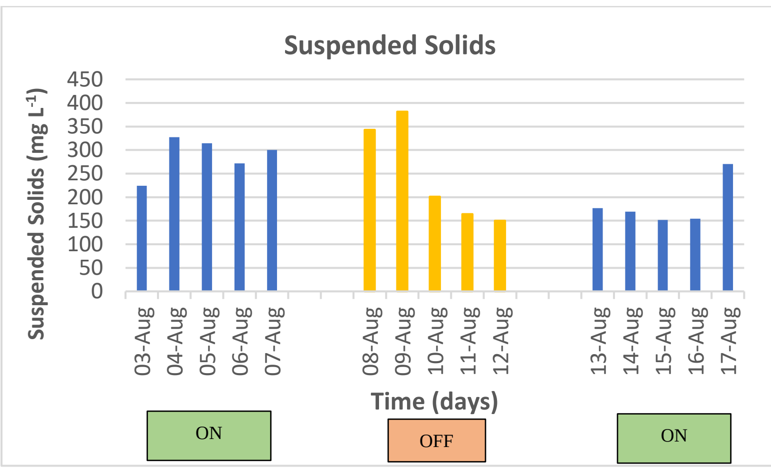

Figure 3.1.2 Suspended Solids concentration in the HRAP during the 5 days on/off mixing regime in winter 2019

From *Figure 3.1.3* the range of chlorophyll *a* was between 1 to 3 mg L^{-1} throughout the study. The mean chlorophyll *a* concentration over the study period slightly increased (mixing on>off>on) as shown in Table *3.1.1*, although the increase was unlikely significant.

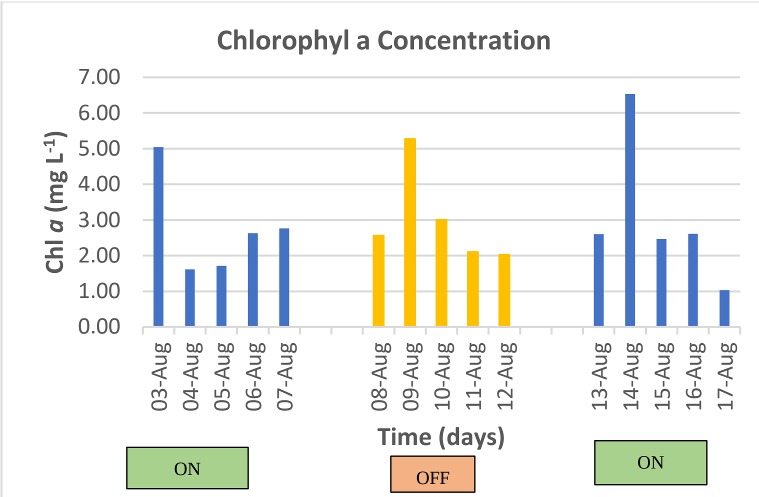

Figure 3.1.3 Chlorophyll concentration in the HRAP with a concentration between 1 mg L^{-1} and 3 mg L^{-1} throughout the during the 15 days ON/OF/ON mixing regime

There was slight increase in NH_3 through the study period where at the final ON period, the mean concentration was approximately 30% more than at the start of the study, however, the NH_3-N concentration changed little between the initial 5 days mixing period and the subsequent 5 days when mixing was halted. The NO_2 – N and NO_3 – N values were higher in the OFF phase (13.52 mg L^{-1}) compared to the ON phases (9.49 mg L^{-1} and 10.85 mg L^{-1}). Moreover, the mean concentrations for the ON phases were almost similar. From *Figure 3.1.4* it can be seen that there was an increase of NO_2 – N and NO_3 – N from the latter days of the initial ON phase and through the OFF days the possibly value due to reduced uptake and then concentration drops when the pond is mixed again. The PO_4-P concentration varied little throughout the study period of Regime 1 (*Figure 3.1.4*).

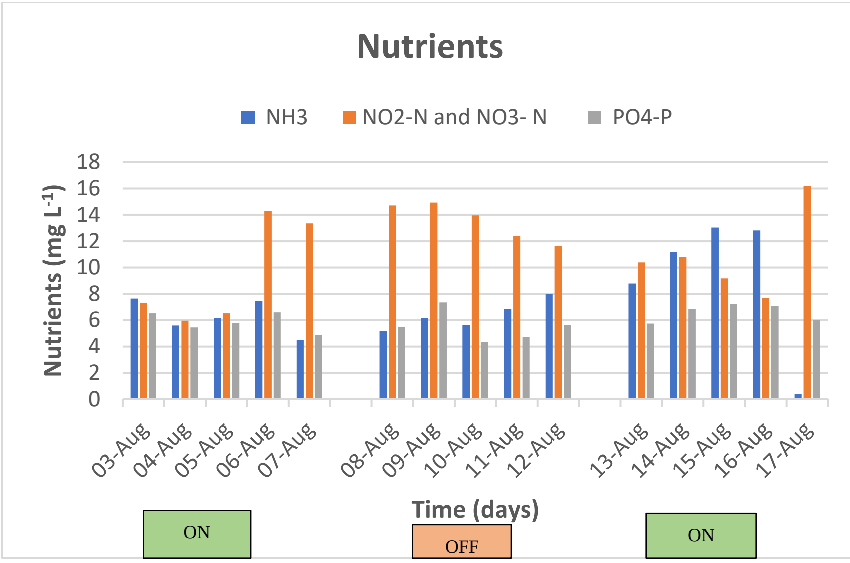

Figure 3.1.4 Nutrient concentration in the HRAP during the 15 days ON/OFF/ON regime in winter 2019.

Considering the analysis of GF/C filtrates of the HRAP wastewater, the mean sTC concentration differed little between the three operational conditions (*Table 3.1.1*), however, the mean sIC concentration slightly increased from its initial concentration when mixing was halted (13.89 mg L^{-1} to 14.55 mg L^{-1}) and then further increased on the recommencement of mixing to 17.08 mg L^{-1} (*Table 3.1.1; Figure 3.1.5*). The mean sTOC slightly decreased in the OFF phase from 26.01 mg L^{-1} to 25.92 mg L^{-1} and increased when restarted again (27.51 mg L^{-1}), a result close to the initial ON period. The concentration of sTOC through the study is between 15 mg L^{-1} to 33 mg L^{-1}. These results were indicative of the continuous respiration of organic matter within the system that continues despite the operational status.

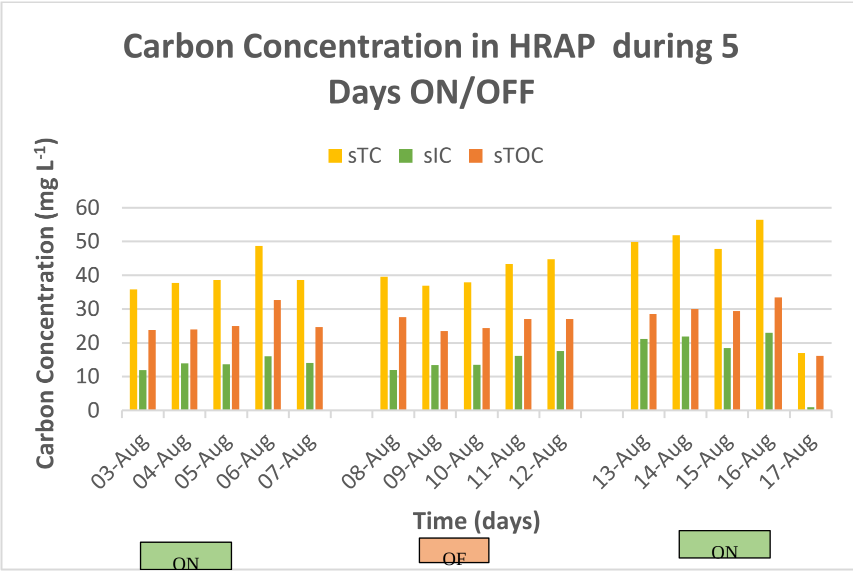

Figure 3.1.5 Carbon concentration in the HRAP during the 5 days ON/OFF regime in winter 2019 with almost uniform performance for $_{s}$IC and $_{s}$TOC

Statistical analysis of 5 Days ON/OFF data

Statistical analysis results are in *Table 3.1.2 a,b,c,d* confirmed that there was insignificant (p > 0.05) differences in all the variables concentrations between the different mixing periods (ON/OFF/ON). Furthermore, statistical analysis of the latter on (ON_2) period compared to the initial on (ON_1) period also show insignificant difference except for suspended solids.

(a) SS (mg L^{-1})	ON_1/OFF	OFF/ON_2	ON_2/ON_1
Are means significantly different (P < 0.05)?	No	No	Yes
P value	.462	.181	0.000
F	12.865	27.080	2.679
df	8	8	8
t	.773	1.596	-5.770
Mean Difference	39.54	77.57	-117.12

Table 3.1.2 (a) Significant differences in the suspended solids concentration between the ON/OFF phases (P < 0.05). A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

(b) Chl *a* (mg L^{-1})	ON_1/OFF	OFF/ON_2	ON_2/ON_1
Are means significantly different (P < 0.05)?	No	No	No
P value	.787	.959	.795
F	.000	.448	.421
df	8	8	8
t	-.280	-.053	.269
Mean Difference	-.2400	-.058	.298

Table 3.1.2 (b)Non - significant differences in the Chl a concentration between the ON/OFF phases (P < 0.05). A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

(c) $NO_2 - N$ and NO_3 - N (mg L^{-1})	ON_1/OFF	OFF/ON_2	ON_2/ON_1
Are means significantly different (P < 0.05)?	No	No	No
P value	0.87	.129	.571
F	20.503	.871	1.547
df	5.037	8	8
t	-2.125	1.693	.591
Mean Difference	-4.032	2.676	1.356

Table3.1.2 (c) Non - significant differences in the $NO_2 - N$ and NO_3 - N concentration between the ON/OFF phases (P < 0.05). A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

(d) sIC (mg L^{-1})	ON_1/OFF	OFF/ON_2	ON_2/ON_1
Are means significantly different (P < 0.05)?	No	No	No
P value	.599	.567	.465
F	2.462	3.193	4.635
df	8	8	8
t	-.547	-.597	.767
Mean Difference	-.664	-2.534	3.1981

Table 3.1.2 (d) Non - significant differences in the sIC concentration between the ON/OFF phases (P < 0.05). A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

(e) sTOC (mg L^{-1})	ON_1/OFF	OFF/ON_2	ON_2/ON_1
Are means significantly different (P < 0.05)?	No	No	No
P value	.961	.619	.672
F	1.064	2.378	.742
df	8	8	8
t	.050	-.518	.439
Mean Difference	.094	.619	1.494

Table 3.1.2 (e) Non - significant differences in the sTOC concentration between the ON/OFF phases (P < 0.05). A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

There were no inlet data for comparison in this regime. This study was over 15 winter days in 2019.

3.2 HRAP 12 hours ON/OFF mixing regime

In Regime 2, the experimental pond was subjected to intermittent mixing by switching of the electricity that ran the paddle wheel off for 12 hours daily between 6pm and 6am while the control pond was in continuous operation. *Figure 3.2.1 a,b* shows the change in electricity flow for the experimental pond in green when it was stopped for 12 hours and the grey graph is the continuous supply to the control pond for the two season during this regime supplied by Dematec from logger located on site.

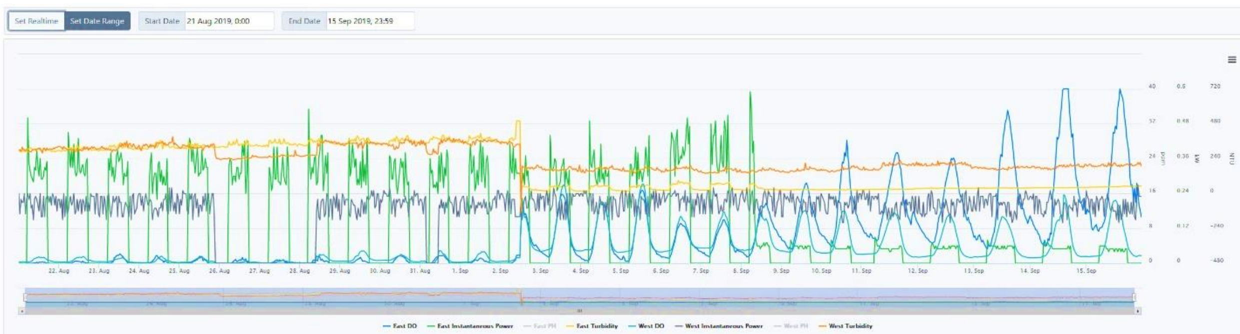

Figure 3.2.1 a Intermittent electricity supply for 12 hours for the experimental HRAP indicated by the green graph while the purple graph is the continuous power supply to the control HRAP during the Winter – Spring season

Figure 3.2.1 b Intermittent electricity supply for 12 hours for the experimental HRAP indicated by the green graph while the purple graph is the continuous power supply to the control HRAP during the Summer - Autumn season

The overall results of the 12 hours ON/OFF intermittent mixing regime showing number of samples analysed (n), the mean ± SD and median concentrations for the wastewater parameters are shown in *Table 3.2.1*. There were no significant differences in the mean concentration of the parameters between the control and the experimental HRAP overall, suggesting intermittent mixing had little effect upon HRAP process when compared with the continuously mixed HRAP.

Intermittent Mixing Regime 2	Continuously mixed								Mixing 12 Hours ON/OFF			
	Inlet				Control HRAP				Experimental HRAP			
	Mean	SD	Median	*n*	Mean	SD	Median	*n*	Mean	SD	Median	*n*
BOD_5 (mg L^{-1})	86.7	-	88.9	14	99.81	-	120	17	106.83	-	121.5	18
SS (mg L^{-1})	27.73	33.25	14.29	51	264.34	62.74	270	51	290.60	68.22	300	53
Chl *a* (mg L^{-1})	-		-	-	3.28		2.83	52	4.21		4.1	53
NH_3 (mg L^{-1})	44.06	28.51	34.39	38	8.56	4.27	10.02	51	7.56	5.79	7.43	53
NO_2^- NO_3^- (mg L^{-1})	7.49	7.11	6.24	38	7.86	4.74	7.68	51	12.35	6.72	9.38	53
PO_4 - P (mg L^{-1})	7.01	3.26	5.37	38	7.51	3.30	7.13	51	7.55	3.10	6.80	53
$_s$TC (mg L^{-1})	62.08		70.19	32	44.18		41.22	41	39.77		38.04	42
$_s$IC (mg L^{-1})	39.86		47.45	32	17.68		19.71	41	13.57		15.85	42
$_s$TOC (mg L^{-1})	22.22		22.34	32	26.50		20.4	41	26.21		20	42

*Table 3.2.1 HRAP 12 hours ON/OFF mixing regime mean results show no significant difference between the control and the experimental HRAP, although there is a high variability in the BOD_5 and SS. * Unfiltered BOD_5*

The average BOD_5 concentration was higher in the HRAPs than the inlet and was due to whole pond samples being used in the analysis of the HRAP wastewater. The analysis therefore included endogenous respiration of the algal biomass. The inlet values were low as the wastewater was pre-treated by septic tanks.

There was little difference between the BOD_5 of the control and experimental pond in the winter – spring season (*Table 3.2.2 a*.) The HRAP mean BOD_5 concentration in the summer – autumn season, were both higher than the inlet (161.7 mg $L^{-1}$and 142.74 mg L^{-1}), reflecting the increased algal biomass production and its endogenous respiration. During winter – spring, the BOD_5 in the inlet was higher (123.75 mg L^{-1}) than in the summer – autumn period (71.93 mg L^{-1}; *Table 3.2.2 a & b*) which can be also seen on *Figure 3.2.2*. Care should be exercised in the interpretation of these results since they are confounded by the use of unfiltered wastewater samples to determine the BOD_5 in the HRAPs.

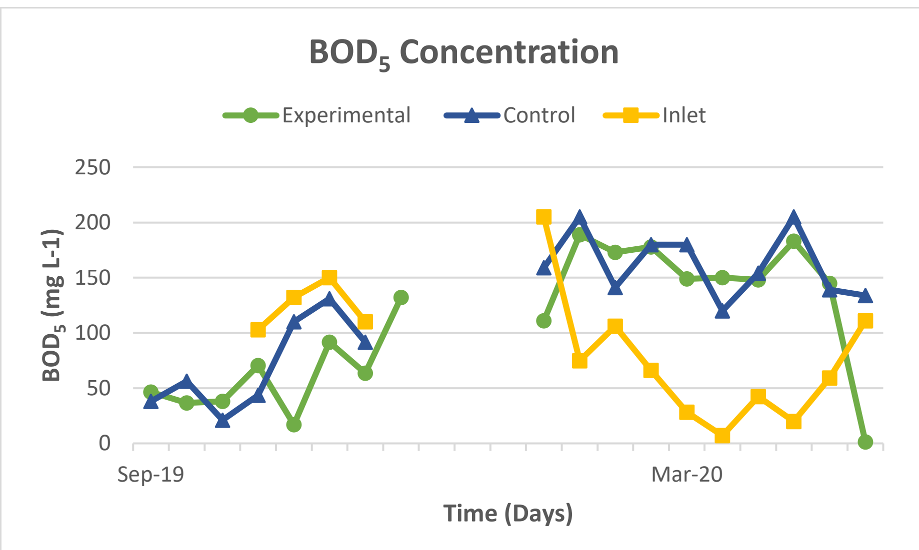

Figure 3.2.2 Experimental and Control HRAP BOD_5 concentration lower in Winter – Spring however readings are reflective of the whole pond sample utilised in the analysis

The average SS concentration was higher by 9% in the experimental HRAP (*Table 3.1.1*). The mean chlorophyll *a* concentration was also higher in the experimental HRAP, 4.21 mg L^{-1} compared with 3.28 mg L^{-1} in the control HRAP, however there was little variance between the two ponds as can be seen in *Figure 3.2.4*.

The mean SS concentration was approximately the same in both seasons but were very variable. This variation in the control and experimental HRAP concentration can be seen in *Figure 3.2.3*.

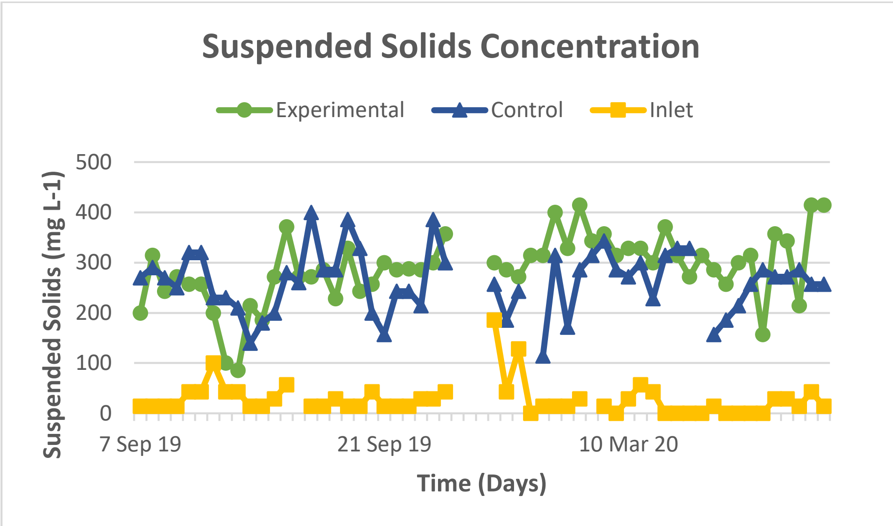

Figure 3.2.3 Suspended solids concentration of Inlet, Control and Experimental HRAP during the 12 hours ON/OFF Regime over the two different seasons

The chlorophyll *a*, the surrogate for algae, overall, there was minimal difference in the amount present in the two seasons and between the ponds. However, the experimental HRAPs had higher means. In the control, the mean amount was higher in the winter – spring season with 3.78 mg L^{-1} compared to 2.83 mg L^{-1}(Table *3.2.2 a and b). Figure 3.2.4* shows the chlorophyll *a* concentration was variable through the two seasons with the experimental HRAP a lot higher.

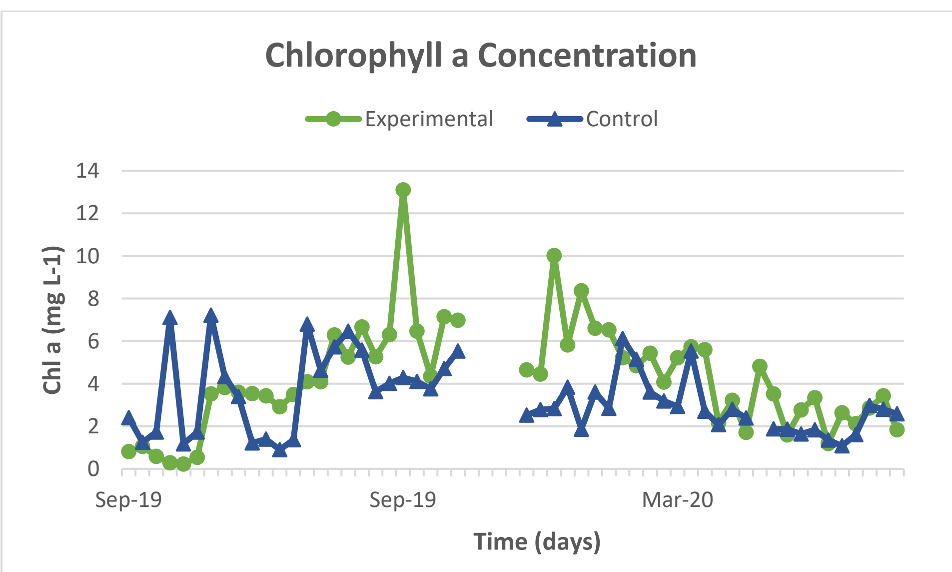

Figure 3.2.4 Chlorophyll a concentration of Inlet, Control and Experimental HRAP during the 12 hours ON/OFF Regime over the two different seasons showing high variations

Soluble inorganic carbon (sIC) concentration was higher in the summer – autumn season and the mean concentration in the control was slightly higher than that of the experimental HRAP (*Figure 3.2.5*). The sIC concentration is indicative of the carbon available for algal photosynthesis and other chemoautotrophic processes such as nitrification. The amount of sIC in the inlet was 39.86 mg L^{-1} and in the control 17.68 mg L^{-1} and 13.57 mg L^{-1} in the experimental HRAP. The lower concentrations are possibly indicative of the uptake of sIC in the photosynthesis process that is occurring within the HRAP system (Fallowfield and Garrett, 1985). sTOC mean concentration was also higher in the summer – autumn study period was 39.19 mg L^{-1} and 39.14 mg L^{-1} for the control and experimental HRAPs respectively, a very lower difference in their operation. In *Figure 3.2.56*, the sTOC concentration of the inlet, control and experimental HRAPs filtrates had concentrations between 10mg L^{-1} and 30 mg L^{-1}. Organic carbon is an important source of inorganic carbon for the algal photosynthesis following mineralisation by bacteria.

The soluble TC concentration of the wastewater filtrates is shown in *Figure 3.2.7* where the concentration for in, control and experimental were similar in the Winter – Spring season. In the summer – autumn season, the inlet concentration remained same with the previous but there was a slight increase in the concentration in the control and experimental however between these there were no significant differences.

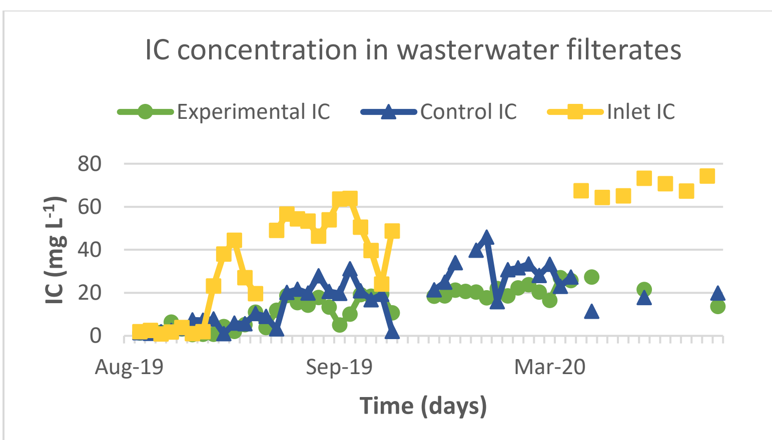

Figure 3.2.5 Soluble IC concentration over the two seasons during Regime 2 during the intermittent mixing Regime 2 for the inlet, control and experimental HRAP

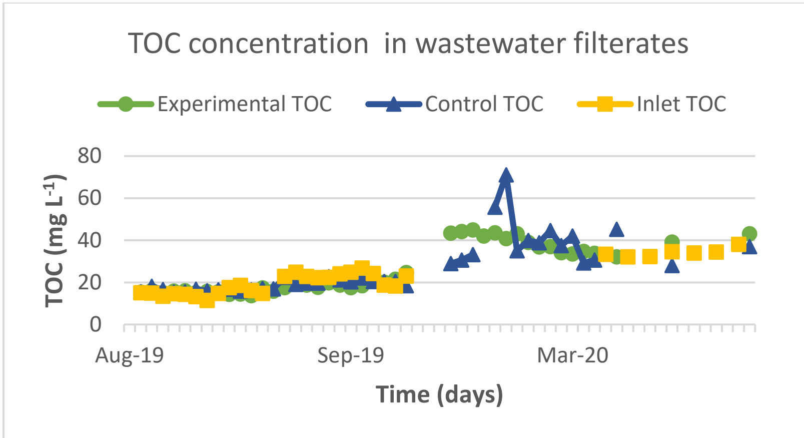

Figure 3.2.6 Comparable soluble TOC concentration over the two seasons during Regime 2 during the intermittent mixing Regime 2 for the inlet, control and experimental HRAP

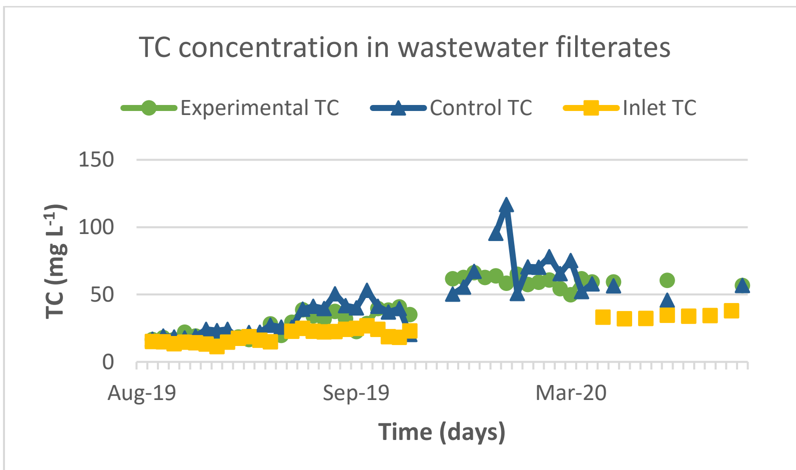

Figure 3.2.7 Soluble TC concentration over the two seasons during intermittent mixing Regime 2 for the inlet, control and experimental HRAP

POC was calculated from the difference of unfiltered organic carbon and filtered organic carbon.

Particulate organic carbon (POC) was also considered and calculated using the equation;

Unfiltered TOC – filtered TOC = Particulate Organic Carbon (POC)

The inlet wastewater contained low concentration of POC (*Figure 3.2.8*). In both seasons POC concentration was between 64 – 75% (*Table 3.2.2 a,b*) in the control and experimental ponds in both the cooler season and warmer season. POC is representative of algal organic carbon (AOC) and bacterial organic carbon (BOC), providing an understanding into both primary and secondary productivity. Whereas chlorophyll *a* concentration only represents the increase or decrease of

AOC, POC also provides insight to the bacterial productivity and an overall biomass increase (*Figure 3.2.8*).

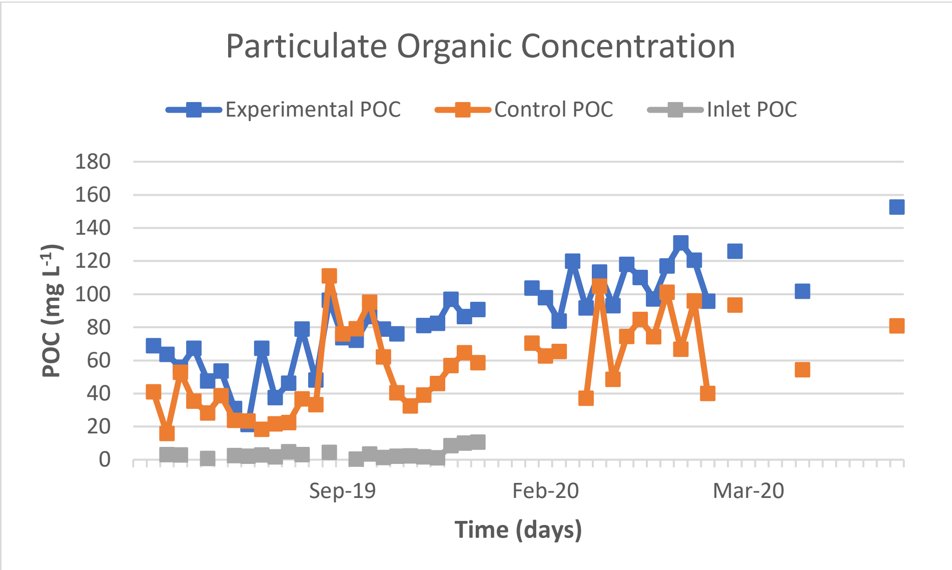

Figure 3.2.8 POC Concentration in the Control and Experimental HRAP and Inlet during Regime 2

The POC concentration was higher in the experimental pond over both seasons in regime 2 (*figure 3.2.7*) and the inlet POC was low in concentration.

	Inlet	Control	Experimental
Mean POC (mg L-1)	3.4	46.1	66.98
Mean Unfiltered TOC (mg L-1)	22.8	64.5	84.39
% POC in HRAP	14.91%	71.47%	79.37%

Table 3.2.2 (a) Intermittent mixing 12 hours ON/OFF POC results for the Inlet, Control HRAP and Experimental HRAP for late winter, early spring 2020. There is minimal difference between the HRAPs

	Inlet	Control	Experimental
Mean POC (mg L-1)	N/A	72.1	110.13
Mean Unfiltered TOC (mg L-1)	N/A	111.7	151.1
% POC in HRAP	N/A	64.58%	72.90%

*Table 3.2.2 (b) Intermittent mixing 12 hours ON/OFF POC results for the Control HRAP and Experimental HRAP for late summer, early autumn 2020 with no significant differences *no inlet samples in this period*

An additional calculation of the whole pond IC and TOC were calculated (*Table 3.2.3*). In both seasons, the amount of TOC was higher than the IC, which it should be and since the POC were over 60%, this indicated that more organic matter was in the particulate form.

		Whole Pond IC			Whole pond TOC		
		mean	SD	n	mean	SD	n
Winter - Spring	Control	11.42	8.99	25	64.5	26.01	25
	Experimental	9.42	6.90	24	84.39	22.48	24
Summer - Autumn	Control	30.33	6.54	25	111.7	24.5	25
	Experimental	26.64	4.618	28	151.1	19.8	28

Table 3.2.3 Whole pond IC and TOC for both the winter – spring and summer to autumn season

Weather and sunlight are an essential part for the effectiveness of the HRAP treatment process therefore variation to them can impact on the performance (Shelef and Azov, 1987). *Table 3.2.4 (a) (b)* show the seasonal variations in performance during the 12 hours on/off regime. The first season of the study was from later winter to early spring 2019 (winter – spring) and the second season was late summer to early autumn 2020 (summer – autumn). There were distinctions between the performance of the two HRAPs in the different seasons for a number of the parameters.

12 Hours ON/OFF: Winter – Spring												
	Inlet				**Control HRAP**				**Experimental HRAP**			
	Mean	SD	Median	*n*	Mean	SD	Median	*n*	Mean	SD	Median	*n*
BOD_5 (mg L^{-1})	123.75	21.42	121	4	70.26	40.99	56.4	7	61.94	36.54	54.95	8
SS (mg L^{-1})	29.17	20.41	21.43	24	264.34	68.50	260	25	258.93	66.06	271.43	25
Chl *a* (mg L^{-1})	-	-	-	-	3.78	2.06	4.01	25	4.15	2.90	3.81	25
NH_3 (mg L^{-1})	25.03	11.46	29.03	25	7.33	4.63	5.97	25	2.82	2.84	1.69	25
NO_2^- & NO_3^- (mg L^{-1})	11.12	6.19	9.21	25	11.56	3.31	11.41	25	9.51	3.63	8.83	25
PO_4 - P (mg L^{-1})	4.75	0.86	4.94	25	4.94	1.70	5.41	25	5.11	1.26	5.36	25
sTC (mg L^{-1})	50.92	27.29	56.90	24	29.88	11.15	25.66	25	26.03	9.00	22.42	25
sIC (mg L^{-1})	32.01	22.89	38.73	24	11.50	9.27	7.76	25	8.62	6.82	6.25	25
sTOC (mg L^{-1})	18.90	4.61	18.43	24	18.38	2.35	18.07	25	17.41	2.61	17.28	25
TN (mg L^{-1})	82.30	14.60	83.73	24	55.92	5.66	57.65	25	37.56	7.99	36.11	25

*Table 3.2.4 (a) Late winter and early spring 2019 Intermittent mixing 12 hours ON/OFF concentration for variables for the control and experimental HRAP and inlet. * Unfiltered BOD_5*

12 Hours ON/OFF: Summer - Autumn												
	Inlet				Control HRAP				Experimental HRAP			
	Mean	SD	Median	*n*	Mean	SD	Median	*n*	Mean	SD	Median	n
BOD_5 (mg L^{-1})	71.93	58.05	62.7	10	161.7	29.69	156.5	10	142.74	54.80	149.5	10
SS (mg L^{-1})	26.46	41.88	14.29	27	258.79	57.90	271.43	26	318.88	57.68	314.29	28
Chl *a* (µg L^{-1})	-	-	-	-	2.83	1.22	2.78	27	4.28	2.10	4.26	28
NH_3 (mg L-1)	80.01	6.83	79.09	14	9.74	3.61	10.68	26	11.80	4.25	11.47	28
NO_2^- & NO_3^- (mg L^{-1})	0.57	0.44	0.4	14	4.30	2.73	3.71	26	14.88	7.73	12.06	28
PO_4 - P (mg L^{-1})	11.27	0.61	11.38	14	9.98	2.46	9.92	26	9.72	2.59	11.05	28
sTC (mg L^{-1})	102.92	5.75	101.7	7	66.54	18.54	61.67	16	59.98	3.99	60.53	17
sIC (mg L^{-1})	68.85	3.93	67.39	7	27.35	9.07	27.63	16	20.84	3.67	20.57	17
sTOC (mg L^{-1})	34.07	2.01	33.89	7	39.19	11.27	37.18	16	39.14	4.37	39.07	17
TN (mg L^{-1})	101.72	5.48	101.1	7	26.71	9.45	25.45	16	37.11	6.03	36.15	17

*Table 3.2.4 (b) Mean concentration of the variables are higher for late summer, early autumn 2020 Intermittent mixing 12 hours ON/OFF concentration for variables for the control and experimental HRAP and inlet. * Unfiltered BOD_5*

The concentration of ammonia at inlet was 44.06 mg NH_3-N mgL^{-1}. The concentration decreased in the HRAPs treated wastewater by inclusion into algal biomass and nitrification oxidising the ammonium to NO_2-N and NO_3-N (Evans et al., 2005). The mean concentration of NO_2^- and NO_3^- was higher by over 35% in the experimental pond than in the control reflecting higher rates of nitrification. Towards the end, as can be seen in *Figure 3.2.9*, the NO_2^- and NO_3^- increased drastically in the experimental pond, between 20 – 25 mg L^{-1} this correlated with concentration changes in NH_3 depicted in *Figure 3.2.10* where the inlet was about 80mg L^{-1} and the experimental pond was below 20 mg L^{-1}.

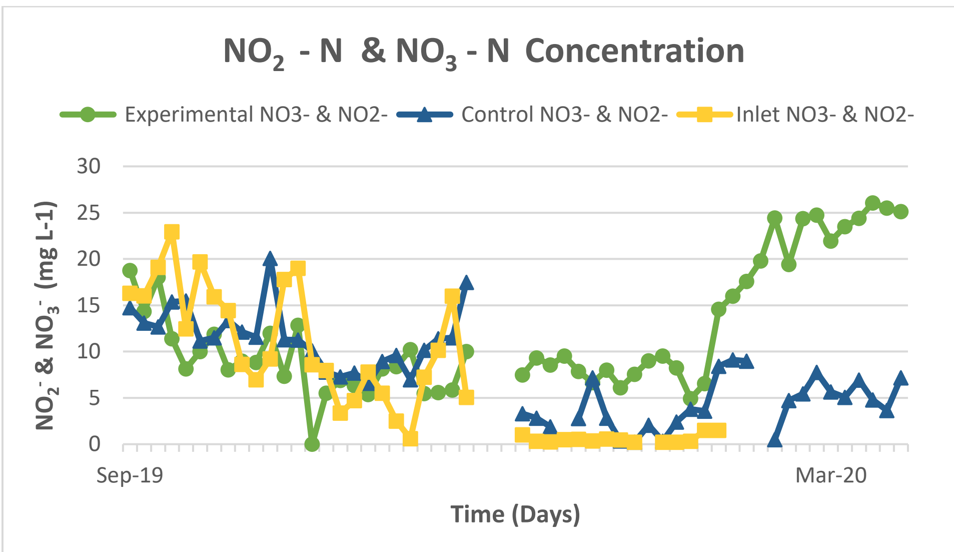

Figure 3.2.9 NO_2^- and NO_3^- concentration of Inlet, Control and Experimental HRAP during the 12 hours ON/OFF Regime over the two different seasons. There was higher concentration in the experimental HRAP in the Summer – Autumn season

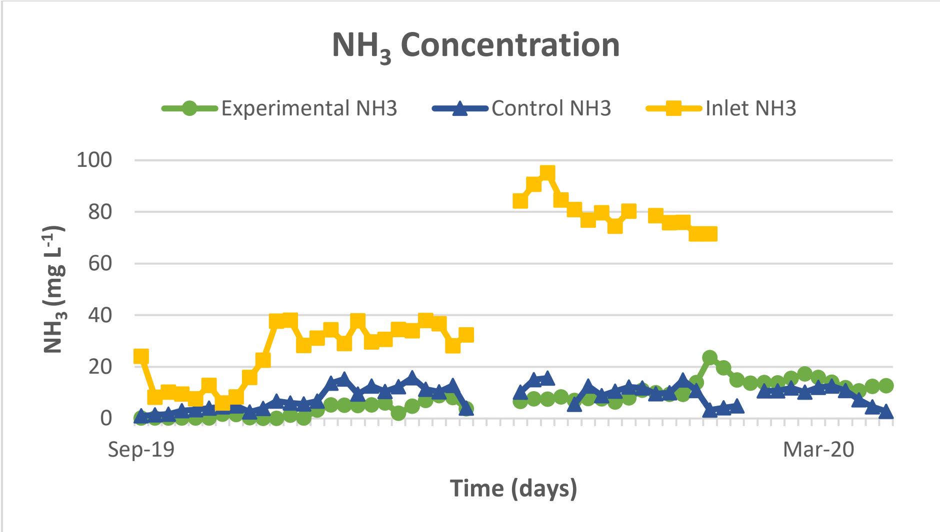

Figure 3.2.10 Comparable NH_3 concentrations in the control and experimental HRAPs during the 12 hours ON/OFF regime

Nutrient removal from a HRAP is via assimilation by the algal biomass or ammonia volatilisation as well as precipitation especially for phosphate (Picot et al., 1991). The mean concentration of ammonia entering the HRAPs in the summer – autumn season was 80.01 mg L^{-1} compared 25.03 mg L^{-1} in winter – spring. These values however decreased in the HRAPs as NH_3 was being utilised. Mean concentration was slightly higher in the control HRAP during winter – spring but it was higher in the experimental pond during summer – autumn. The NO_2^- and NO_3^- mean concentrations were highest in the summer – autumn (14.88mg L^{-1}) which can be seen also on *Figure 3.2.9*.

The removal efficiencies of nutrients were calculated using the following equation (Buchanan et al., 2018b).

$$\frac{C_0 - C_e}{C_0}$$

where, C_0=inlet concentration (mg L^{-1}) and C_e=outlet concentration (mg L^{-1}).

Table 3.2.5 shows that NH_3 decreased by 80% because 65% was being converted into oxidized nitrogen in the experimental HRAP. The rate was lower in the control.

	NO_2^- & NO_3^-		PO_4-P		NH_3	
	Experimental	Control	Experimental	Control	Experimental	Control
% Removal	-65%	-5%	-7.7%	-7%	83%	81%

Table 3.2.5 Removal efficiencies of nutrients in the 12 hours ON/OFF regime

The PO_4-P (*Figure 3.1.11*) concentration was higher during Summer – Autumn, 9.98 mg L^{-1} and 9.72 mg L^{-1}, in the control and experimental HRAPs respectively. There was a lower mean concentration in winter – spring (4.04 mg L^{-1} and 5.11 mg L^{-1}). Overall, there was minimal difference in the performance of the two HRAPs in both seasons

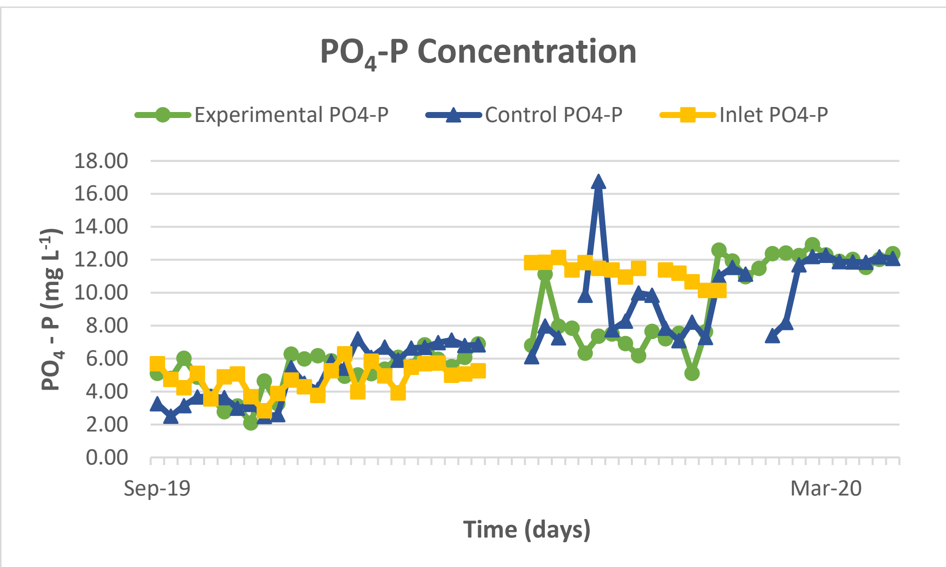

Figure 3.2.11 Similar PO_4-P concentration in the winter – spring season but increased in the Summer – Autumn season

4.0 Discussion

The advantageous features of HRAPs make them conducive for application in remote and rural communities. However, there is the likelihood of interruptions to the operation of the paddle wheel if there is a failure to the power supply, malfunction or an intentional action to stop the mixing at night to save on energy consumption therefore the cost of operation. Therefore, this project studied the impacts of intermittent mixing on performance of HRAPs. The HRAP facility at KOM was subjected to two different mixing regimes to depict the mentioned scenarios and HRAP performance were studied. In Regime 1, the mixing of a HRAP was run for 5 days, stopped for 5 days and then restarted. This assessment was to ascertain how the pond performed within the phases and how it changed between the phases to maintain treatment integrity. In the second intermittent mixing regime an experimental HRAP was turned off for 12 hours from 6pm to 6am daily from August 2019 to March 2020 and its performance was compared with a HRAP in continuous operation. The treatment variables that were assessed included BOD_5, suspended solids, chlorophyll *a* and the nutrient and carbon content. However, for both regimes, the whole ponds samples were used in the BOD analysis therefore the results indicated the endogenous respiration of the algal biomass. Regime 1 was conducted in winter and Regime 2 was over two seasonal periods and these have an impact on the results that were obtained for this study.

The hypothesis for this study was that intermittent mixing within a HRAP does not impact on the performance of a HRAP.

5 days ON/OFF

In Regime 1, even though there were some changes in concentration to the studied variables within the 5 days on, off and on again, the results were not statistically significant between the periods and from the initial and final results.

Biological oxygen demand is the indicator of the amount of oxygen required by organic matter in wastewater system. HRAPs have high BOD_5 removal rates which is also affected by the seasons. Azov and Shelef (1982) reported that the BOD_5 removal in HRAPs were lower in the winter and Buchanan et al. (2018b) also described generally lower BOD_5 in the colder periods of operation thus suggesting that the oxidation of organic carbon to CO_2 for algal photosynthesis was also lower. In this study, the BOD_5 concentration was lower in the off period compared to when the mixing was on indicating that despite the paddlewheel being off there continued to be organic respiration but at a lower rate, however, as mixing supports algal photosynthesis activity, there was less oxygen for the demand consequently low BOD_5 results. However, as whole pond samples were used in the BOD analysis the results indicated the included endogenous respiration of the algal biomass. The analysis of filtered samples would give the concentration of the oxygen that is utilised by the organic matter but another issue with this was the need to consider the particulate BOD in the sample.

There are a number of environmental parameters that influence the growth of algal growth rates including light intensity, temperature, nutrients and pH. Ratchford and Fallowfield (2003), studied two species of algae on the effect of light/dark cycles times on their recovery from photoinhibition which is where there is supersaturation of light on the surface layer of the HRAP. In that study, photoinhibition occurred at irradiances >300 µmol/m^2 /s at temperatures >15°C. Oxygen generation decreased rapidly when cells were continuously irradiated. They suggested from their study that the adverse effects of photoinhibition could be improved by algae staying for periods of time in the dark. Exposure time rather than the total light dose appeared to determine the effect of light: dark cycle times on photosynthesis (Ratchford & Fallowfield, 2003). Mixing in a HRAP is essential because it promotes algal growth (García et al., 2006) by exposing the algal cells to solar radiation and prevents thermal stratification (Fallowfield and Garrett, 1985). Ratchford and Fallowfield (2003) state that the turbulent flow from the mixing moves the algae in and out of the light and dark zones therefore influencing its productivity. The amount of light available for algal biomass is controlled by the amount of attenuation within a HRAP and the internal – self shading in the algae cell (Sutherland et al., 2015). Light passing through the water column declines with depth and it is being absorbed or scattered by the biomass (Sutherland et al., 2015). In relation to the current study, stopping of the paddlewheel for 5 days may have decreased the oxygen generation and therefore affected the population of the algae due to the unfavourable conditions for HRAP operation. However, it can be seen that the change was gradual over the 5 days that the pond was off. And when the mixing restarted, the HRAP was able to recover over steadily as the algal matter was cycled through the light and dark (L/D) phase again. The changes between the two phases were statistically insignificant to conclude that the off phase had a detrimental impact on the algal biomass which is an important component in a HRAP wastewater treatment system.

The decrease in chlorophyll *a* in the off period could also be seen in the decline of the suspended solids. Suspended solids consist of ALBAZOD which include algae, which makes up a larger portion, bacteria, zooplankton and detritus and mixing of the HRAP is important to prevent settling (Mihalyfalvy et al., 1998). Algal biomass can spontaneously settle or form flocs (Garcia and Hernandez-Marine, 2000) in mixed solution and the lack of mixing may promote the sedimentation and flocculation of suspended solids which can be seen with the identical decline of suspended solids and Chlorophyll *a* when the mixing was turned off. Seasonal variation can be a determinant factor in a HRAP operation and performance. Regime 1 was during the winter and several studies found that yields of algal biomass are lower compared to warmer seasons (Azov and Shelef, 1982, Buchanan et al., 2018b). During winter there were low levels of sunlight hence low algal concentration (Fallowfield and Garrett, 1985). When the mixing resumed, there were gradual changes noticed in the HRAP as the system recovered from the off phase.

Nitrogen removal via nitrification can only occur under conditions of adequate DO. Additionally, the extent of nitrification in a HRAP can be influenced by the carbon in the influent, the concentration of ammonia and the retention time (Evans et al., 2005). Buchanan et al. (2018b) found that nitrification was higher in the cold months where they stated that such results were due to the impacts on the nitrifying bacterial population in the warmer months by the irradiance and temperature or the increased competition for substrates by the algal biomass and autotrophs and others in the warmer months. Moreover, that the pond depth determined the extent of nitrification. There can be a likelihood that with no mixing the wastewater remained undisturbed therefore nitrification was much higher in the off phase of the current study.

According to Azov et al. (1982), about 48% of the influent carbon is inorganic and 52% in organic. Unionised, dissolved CO_2 are the forms of carbon preferred by most algal species for photosynthesis. In the HRAP this will mostly come from daytime bacterial respiration. The main nutrients NH_3 and CO_2 for algal photosynthesis are released by the degradation of bacterial biomass (Azov et al., 1982) which is a slow process. This can be the possible reason that there was no significant change in the sTC/sIC/sTOC concentration in the on and off periods. Soluble total carbon concentration increased by 10% to 15% when mixing was turned on again and may be a consequence of the increase in the bacterial respiration indicated by the high BOD in that phase or as a results resuspension of suspended matter within the HRAP.

In a mixed HRAP system, the diurnal pattern of solar irradiance (including temperature) produces great variations in the parameters that are influenced by algal photosynthesis such as DO and pH. As a result of these variations the physio – chemical reactions that are impacted by these parameters are also impacted and the performance changes during the diurnal cycle (García et al., 2006). In spite of this, the results from this investigation showed no significant changes to the HRAP performance in a 5 days intermittent mixing on and off period. The statistical analysis showed there was no significant difference between the latter on phase and the initial pond stage therefore suggesting that the HRAP has recovered to its original state.

12 hours ON/OFF

During Regime 2 in the current study the mixing was stopped between 6pm and 6am to determine whether the performance of the HRAP would be affected if subjected to such a condition. The time can be validated where García et al. (2006) found that HRAP performance slowed down in the dark hours where the rate of ammonia volatilisation and nitrification decreased, and the same with PO_4 – P and suspended solids. The outcome of their study showed that the diurnal variations of the treatment of SS, nutrients and carbon did not have a serious impact on the reliability in the treatment of the wastewater. These results had also been obtained by Picot et al. (1993) who additionally stated that even with continual mixing in the night there was negligible change to the performance of the HRAP in the removal of NH_4 – N, PO_4 – P and suspended solids. Fallowfield and Garrett (1985) who studied a HRAP operated on an 8-hour intermittent mixing during day light found that algal productivity was maintained during this time as energy use was reduced.

García et al. (2006) in their study of diurnal changes in temperature during HRAP operation concluded that there were no changes to the treatment performance of a HRAP. They found that during the night till dawn, the lack of algal photosynthetic activity together with the continuous respiration of algae and other microorganisms resulted in low dissolved oxygen concentration at dawn however at sunrise, the photosynthetic activity increased. For the current study, despite the experimental ponds being turned off for 12 hours during the non-active period of the HRAP, there were no significant differences in the performances of the two ponds.

Regime 2 was conducted over two seasonal periods, later winter to spring 2019 and late summer to early autumn 2020 and seasonal differences were evident. And from the comparison of the results, the concentrations from the first seasonal period were higher than the latter which were warmer months. According to literature, warmer months are more favourable to HRAP performance compared to colder seasons in the year additionally diurnal temperature changes also impact the rate of treatment (Picot et al., 1991, García et al., 2006, Shelef and Azov, 1987, Cromar et al., 1996).

Nutrients were assimilated in the ponds as seen by the decrease in ammonia concentration in the HRAPs. Nitrification rate depends on oxygen concentration greater than 1 g per m^{-3}, temperatures greater than 8°C and pH between 6 and 9. Picot et al. (1993) and García et al. (2006) reported that ammonia volatilisation decreased at night and phosphorous precipitation also declined. Additionally, they added that nitrification occurs at a very low rate in the dark due to the lower DO reducing the ammonia oxidation and nitrite and nitrate concentrations decrease at night. Nitrogen removal in this study was within what is reported in literature between 54% and 96% (Cromar et al., 1996). Picot et al. (1993) reported that even with continuous mixing in the night, the HRAP performance for the removal of nutrients was low therefore if there was not mixing such as in this current study, treatment efficiencies will thus be low.

In the winter – spring season, the concentrations of the variables were lower compared to the summer - spring season. According to Fallowfield and Garrett (1985) the lower biomass concentration allows for greater light penetration and in the warmer seasons the algal concentration is higher and the sunlight penetration is reduced. They also stated that when there are high irradiances, the algal growth depends on the temperature and when there is low irradiance the rate depends in the photosynthetic rate in a well-mixed system. The algal biomass, studied using chlorophyll *a* as surrogate, has an important function in stripping nutrients in a HRAP (Fallowfield and Garrett, 1985) and at the same time the process increases the productivity of the biomass. During Regime 2, the mean concentration for the experimental HRAP was 4.21 mg L^{-1} and 3.28 mg L^{-1} for the control. The Chl *a* productivity was higher in the experimental HRAP than the control HRAP. However, these values are within the rage, 2 – 5mg L^{-1}, that Cromar et al. (1996) found to be the optimum concentration of algal matter for efficient nutrient removal.

Unlike Regime 1, there was no apparent indication of sedimentation or flocculation of suspended solids as the result charts shows near consistent correlation between the control and the experimental HRAP.

Algal biomass prefer unionised dissolved carbon which is mostly available during daytime bacterial respiration which release CO_2 into the pond and during the night in lower layers, both algae and bacteria demand oxygen for respiration and will produce CO_2 (Azov and Shelef, 1982). The carbon concentration in the control and experimental ponds had minimal difference in both seasons. POC is a measure of particulate organic matter in the wastewater, it was defined as suspended organic matter following the filtration. Hence, POC comprises of algae and zooplankton cells, detritus and bacteria. Particulate organic carbon (POC) was over 64 to 71 % in the former during the cooler and the warmer seasons and between 79 to 72 % for the experimental pond. This was the available carbon pool if there was limited carbon availability.

Even through there was lower efficiency of treatment in cooler months as also discovered by Picot et al. (1991) combined with intermittent mixing, the treatment within the experimental pond did not show any statistical difference in comparison to the control pond.

The is no decrease in the BOD but instead an increase due to the use of whole pond samples in this study.

5.0 Conclusion

Higher rate algal ponds are an effective wastewater treatment system that requires less land compared to traditional WSP and is economical. Consisting of a meandering shallow pond, constantly mixed by a paddle wheel, the HRAP treats the wastewater with in the presence of sunlight and with algal biomass. Due to its applicability in remote and rural communities, there are challenges that may rise including impact or outage to the power supply that runs the paddle wheel or intentional stopping of the mixing intermittently as part of managing operational costs. As the validation of the system was given on a basis of a continuous mixed system, this study was carried out to determine if intermittent mixing impacted on the HRAP performance and to seek adjustments to guidelines if possible. Therefore, two intermittent conditions were studied. Mixing Regime 1 subjected a HRAP to 5 days on and off mixing and Regime 2 subjected on HRAP to 12 hours on and off regime. The first regime was to study the reaction of the pond over the study period and the second was to compare the performance between a control and experimental HRAP. In spite of the intermittent mixing, for both regimes studied, the results within the respective studies generally showed no statistically significant changes in the performance. The seasonal and diurnal variations made little difference as well. Thus, premeditated intermittent mixing conditions in a HRAP does not impact the treatment performance. In this type of HRAP system, tested with two realistic intermittent mixing conditions, there was no significant impact on treatment performance. The results from this project can contribute towards changing HRAP operational guidelines should amendments be sought.

6.0 References

APHA 1992. *Standard methods for the examination of water and wastewater,* Washington, D.C., Washington, D.C. : APHA-AWWA-WPCF.

Azov, Y. & Shelef, G. 1982. Operation of high-rate oxidation ponds: theory and experiments. *Water Research,* 16**,** 1153-1160.

Buchanan, N., Young, P., Cromar, N. J. & Fallowfield, H. J. 2018a. Comparison of the treatment performance of a high rate algal pond and a facultative waste stabilisation pond operating in rural South Australia. *Water science and technology : a journal of the International Association on Water Pollution Research,* 78**,** 3-11.

Buchanan, N. A., Young, P., Cromar, N. J. & Fallowfield, H. J. 2018b. Performance of a high rate algal pond treating septic tank effluent from a community wastewater management scheme in rural South Australia. *Algal Research,* 35**,** 325-332.

Bureau Of Meteorology. 2019. *Climate Data Online* [Online]. Australia: Commonwealth of Australia Available: http://www.bom.gov.au/climate/data/ [Accessed 5/11/2019 2019].

Bureau Of Meteorology. 2019. *Climate Data Online* [Online]. Australia: Commonwealth of Australia Available: http://www.bom.gov.au/climate/data/ [Accessed 9/04/2020 2019].

Craggs, R., Heubeck, S., Lundquist, T. & Benemann, Jr. 2011. Algal biofuels from wastewater treatment high rate algal ponds. *Water Sci. Technol.,* 63**,** 660-665.

Craggs, R. J., Zwart, A., Nagels, J. W. & Davies-Colley, R. J. 2004. Modelling sunlight disinfection in a high rate pond. *Ecological Engineering,* 22**,** 113-122.

Cromar, N. & Fallowfield, H. 1997. Effect of nutrient loading and retention time on performance of high rate algal ponds. *Journal of Applied Phycology,* 9**,** 301-309.

Cromar, N., Fallowfield, H. & Martin, N. 1996. Influence of environmental parameters on biomass production and nutrient removal in a high rate algal pond operated by continuous culture. 34.

Evans, R., Fallowfield, H. & Cromar, N. 2003. Characterisation of oxygen dynamics within a high-rate algal pond system used to treat abattoir wastewater. 48**,** 61-68.

Evans, R., Cromar, N. & Fallowfield, H. 2005. Performance of a pilot-scale high rate algal pond system treating abattoir wastewater in rural South Australia: nitrification and denitrification. *Water Science & Technology,* 51**,** 117-124.

Fallowfield, H., Mesple, F., Martin, N., Casellas, C. & Bontoux, J. 1992. Validation of computer-models for high-rate algal pond operation for waste-water treatment using data from Mediterranean and Scottish pilot scale systems - implications for management in coastal regions. *Water Sci. Technol.,* 25**,** 215-224.

Fallowfield, H., Cromar, N. & Evison, L. 1996. Coliform die-off rate constants in a high rate algal pond and the effect of operational and environmental variables. 34.

Fallowfield, H. J. & Garrett, M. K. 1985. The treatment of wastes by algal culture. *Journal of Applied Bacteriology,* 59**,** 187S-205S.

Fallowfield, H. J., Young, P., Taylor, M. J., Buchanan, N., Cromar, N., Keegan, A. & Monis, P. 2018. Independent validation and regulatory agency approval for high rate algal ponds to treat wastewater from rural communities. *Environmental Science: Water Research & Technology,* 4**,** 195-205.

García, J., Green, B. F., Lundquist, T., Mujeriego, R., Hernández-Mariné, M. & Oswald, W. J. 2006. Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. *Bioresource Technology,* 97**,** 1709-1715.

Garcia, J. & Hernandez-Marine, M. 2000. Influence of phytoplankton composition on biomass removal from high-rate oxidation lagoons by means of sedimentation and spontaneous flocculation. *Water Environment Research,* 72**,** 230.

García, J., Mujeriego, R. & Hernández-Mariné, M. 2000. High rate algal pond operating strategies for urban wastewater nitrogen removal. *Journal of Applied Phycology,* 12**,** 331-339.

Grönlund, E., Hanaeus, J., Johansson, E. & Falk, S. 2010. Performance of an experimental wastewater treatment high-rate algal pond in subarctic climate. *Water environment research : a research publication of the Water Environment Federation,* 82**,** 830-839.

Kumar, K., Mishra, S. K., Shrivastav, A., Park, M. S. & Yang, J.-W. 2015. Recent trends in the mass cultivation of algae in raceway ponds. *Renewable and Sustainable Energy Reviews,* 51**,** 875-885.

Havelaar, A., Hogeboom, W. & Pot, R. 1985. F specific RNA bacteriophages in sewage: methodology and occurrence. *Water Science and Technology,* 17**,** 645-655.

IBM CORP 2018. IBM SPSS Statistics for Windows *In:* CORP., I. (ed.) 25 ed. Armonk, NY.

Jeffrey, S. W. & Humphrey, G. F. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen,* 167**,** 191-194.

LGA SA 2019. South Australian Community Wastewater Management System (CWMS) Design Criteria

In: Local Government Association Of South Australia & South Australia Department Of Health And Wellbeing (eds.). Adelaide Local Government Association of South Australia

Markou, G. & Georgakakis, D. 2011. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Applied Energy,* 88**,** 3389-3401.

Mihalyfalvy, E., Johnston, H., Garrett, M., Fallowfield, H. & Cromar, N. 1998. Improved mixing of high rate algal ponds. *Water Res.,* 32**,** 1334-1337.

NRMMC, E. A. 2006. Australian Guidelines for Wastewater Recycling: Managing Health and Environmental Risks (Phase 1) *Environment Protection and Heritage Council, Australian Health Ministers' Conference*

Australia: Natural Resource Management Ministerial Council.

Park, J. B. K., Craggs, R. J. & Shilton, A. N. 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology,* 102**,** 35-42.

Pham, L. A., Laurent, J., Bois, P. & Wanko, A. 2018. Impacts of operational conditions on oxygen transfer rate, mixing characteristics and residence time distribution in a pilot scale high rate algal pond. *Water Science and Technology,* 78**,** 1782-1791.

Picot, B., Moersidik, S., Casellas, C. & Bontoux, J. 1993. Using diurnal variations in a high rate algal pond for management pattern. *Water Science & Technology,* 28**,** 169-175.

Picot, B., El Halouani, H., Casellas, C., Moersidik, S. & Bontoux, J. 1991. Nutrient removal by high rate pond system in a Mediterranean climate (France). 23**,** 1535-1541.

Picot, B., Moersidik, S., Casellas, C. & Bontoux, J. 1993. Using diurnal variations in a high rate algal pond for management pattern. *Water Science & Technology,* 28**,** 169-175.

Ratchford, I. A. J. & Fallowfield, H. J. 2003. The effect of light:dark cycles of medium frequency on photosynthesis by Chlorella vulgaris and the implications for waste stabilisation pond design and performance. *Water Science and Technology,* 48**,** 69-74.

Rogers, J. N., Rosenberg, J. N., Guzman, B. J., Oh, V. H., Mimbela, L. E., Ghassemi, A., Betenbaugh, M. J., Oyler, G. A. & Donohue, M. D. 2014. A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. *Algal Research,* 4**,** 76-88.

Sebastian, S. & Nair, K. V. K. 1984. Total removal of coliforms and E. coli from domestic sewage by high-rate pond mass culture of Scenedesmus obliquus. *Environmental Pollution. Series A, Ecological and Biological,* 34**,** 197-206.

Shelef, G., Azov, Y. & Moraine, R. 1982. Nutrients removal and recovery in a two-stage high-rate algal wastewater treatment system. *Water Science & Technology,* 14**,** 87-100.

Shelef, G. & Azov, Y. 1987. High-rate oxidation ponds: The Israeli experience. *Water Science and Technology,* 19**,** 249-255.

Shimadzu Corporation (2018). *TOC-L*. [online] Shimadzu. Available at: https://www.shimadzu.com/an/toc/lab/toc-l.html [Accessed 16 Apr. 2020].

Skalar Analytical BV. 2020. *San++ Automated Wet Chemistry Analyzer - Continuous Flow Analyzer (CFA)* [Online]. The Netherlands: Skalar Analytical B.V. Available: https://www.skalar.com/analyzers/automated-wet-chemistry-analyzers/ [Accessed 22/03/2020 2020].

Sutherland, D. L., Turnbull, M. H., Broady, P. A. & Craggs, R. J. 2014. Wastewater microalgal production, nutrient removal and physiological adaptation in response to changes in mixing frequency. *Water Research,* 61**,** 130-140.

Sutherland, D. L., Montemezzani, V., Howard-Williams, C., Turnbull, M. H., Broady, P. A. & Craggs, R. J. 2015. Modifying the high rate algal pond light environment and its effects on light absorption and photosynthesis. *Water Research,* 70**,** 86-96.

WHO. 2006. *Guidelines for the safe use of wastewater, excreta and greywater*, Geneva, Geneva : World Health Organization.

Young, P., Buchanan, N. & Fallowfield, H. J. 2016. Inactivation of indicator organisms in wastewater treated by a high rate algal pond system. *Journal of Applied Microbiology,* 121**,** 577-586.

Young, P., Taylor, M. & Fallowfield, H. J. 2017. Mini-review: high rate algal ponds, flexible systems for sustainable wastewater treatment. *World Journal of Microbiology and Biotechnology,* 33**,** 117.