CONSIDERATIONS ON THE OPERATION OF HIGH RATE ALGAL PONDS FOR WASTEWATER TREATMENT AND MICROALGAL BIOMASS PRODUCTION

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SUMMARY

High rate algal ponds (HRAP) are novel natural wastewater treatment systems which have been shown to have many advantages over other similar systems. In recent years they have also received considerable interest as the most promising system for coupling microalgae cultivation for biofuel feed and wastewater treatment. However, despite their advantages and potential, their application has been sluggish. The overall aim of this thesis was to investigate key factors limiting HRAPs application as wastewater treatment systems and microalgae bioreactors using large-scale, operational systems.

One major factor limiting HRAPs application as wastewater treatment systems is their absence as a treatment option in any official regulatory guidelines. Chapter 3 recounts the only independent validation of a HRAP system for inclusion as a wastewater treatment system option in official regulatory guidelines – the South Australian Community Wastewater Management Scheme (CWMS). Validation involved assessing the HRAP system's ability to inactivate three indicator organisms under winter conditions. The system met the national guideline validation objectives, resulting in two HRAP system designs being included in the CWMS. It is hoped this result will lead to the wider application of HRAPs, with a system based on one of these designs having already been constructed in Peterborough, Australia.

With the inclusion of a HRAP system as a wastewater treatment option in the CWMS likely resulting in their wider application, a well-designed pathogen inactivation model would help guide the design and operation of new systems. Unfortunately, there has been little research in this area with the only such model published 16 years ago. Chapter 4 describes the development and validation of a mechanistic pathogen inactivation model for HRAPs. The model attributes pathogen inactivation in HRAPs to solar radiation and uses laboratory measured inactivation values. This design is unique for pathogen inactivation models and allows for greater utility and flexibility when compared to traditional models based on system measurements. The model was successfully validated for two indicator organisms using a large-scale, operational HRAP. These results support the model design and encourage its

further development. The model also provided valuable insight into HRAPs operation that will guide the design of future systems.

After investigating the factors limiting HRAPs application as wastewater treatment systems, it was decided to investigate the key factors limiting their other main application as combination wastewater treatment systems and microalgae bioreactors. Biomass productivities below economically viable levels is one of the most significant limitations to this application. This is presumed to be caused by insufficient carbon in the wastewater, with the addition of CO_2 the most cited solution. Chapter 5 outlines a case study on the effect continuous CO_2 enrichment of wastewater has on HRAP wastewater treatment and biomass productivity. A HRAP was retrofitted into a major wastewater treatment plant and received secondary treated wastewater enriched with CO_2 by industrial biogas scrubbers. An identical HRAP receiving identical wastewater that had foregone enrichment was used as a control. CO_2 enrichment had no significant effect on biomass productivity and had a slightly negative effect on wastewater treatment – suggesting the microalgae were not carbon limited. This study is believed to be the closest representation in the literature to how such a design would perform in the real-world and the only study at such a scale to employ an adequate control.

Another major limitation of using HRAPs treating wastewater to cultivate microalgae for biofuel production is the lack of a cost-effective harvesting method. Autoflocculation, via magnesium hydroxide precipitation, is considered a potential method; however, it has not yet been demonstrated in wastewater treating HRAPs at a large-scale. Chapter 6 details the assessment of autoflocculation, via magnesium hydroxide precipitation, as a harvesting method for microalgae in HRAPs treating wastewater. Autoflocculation was induced in a large-scale, operational HRAP containing 33 m^3 of HRAP treated wastewater populated by a heterogenic mix of wild strain microalgae. A high level of flocculation efficiency, solids removal and nutrient removal was observed, suggesting this is a viable method for harvesting microalgae and treating wastewater in HRAPs. However, limitations with the

method were identified. This is believed to be the largest assessment of microalgae autoflocculation in HRAP treated wastewater and the only assessment conducted in-pond.

Overall, this thesis presents a unique collection of work on large-scale, operational HRAPs that not only supports the application of HRAPs as wastewater treatment but also provides essential information regarding their real-world application. It is also clear from this work that further research is required before HRAPs treating wastewater can be considered viable microalgae bioreactors for biofuel production, with previously overlooked complications to their real-world application elucidated. Nevertheless, this should not be considered an impediment to their application as wastewater treatment systems.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for the 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.

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CHAPTER 1. GENERAL INTRODUCTION

This chapter describes the current understanding in the scientific literature of high rate algal ponds (HRAP) as wastewater treatment systems and microalgae bioreactors. It begins by providing a foundation on the hazards posed by wastewater and how these hazards are typically managed. It then considers the use of natural wastewater treatment systems to manage these hazards focusing on waste stabilisation ponds (WSP). This is followed by a published mini-review on HRAPs which details their advantages over similar systems and key areas of research need. Next, HRAPs ability to remove pathogens is discussed in more detail, and the use of indicator organisms to assess the performance of wastewater treatment systems is detailed. Finally, the aims and outline of this thesis are stated.

1.1. Hazards and public health impacts of wastewater

Wastewater can be defined as water, which through human activity, has been contaminated with liquid and/or solid waste making it unsuitable for release into the environment or recycling (Tchobanoglous et al., 1991, Bani, 2011, Varela and Manaia, 2013). Its production is inherently linked with human activity and consequently poses a constant risk to human and environmental health (Henze and Comeau, 2008, Abdel-Raouf et al., 2012, Jain et al., 2013). The greatest sources of global wastewater production are domestic/commercial, industrial and agricultural activities (Akpor and Muchie, 2011, Deb and Dutta, 2017, Raouf et al., 2019). The hazards present in wastewater depend on the source but usually include some or all of the constituents listed in Table 1.1. (Kretschmer et al., 2002, Von Sperling, 2007b, Henze and Comeau, 2008, Petrie et al., 2015, Tran et al., 2018, Raouf et al., 2019).

Table 1.1. Common hazards found in wastewater and the harm they cause (Kretschmer et al., 2002, Von Sperling, 2007b, Henze and Comeau, 2008, Petrie et al., 2015, Tran et al., 2018, Raouf et al., 2019).

Hazards	Constituents	Harm caused
Biodegradable organic materials	Suspended solids Soluble organic materials	Eutrophication Sludge accumulation Aesthetic problems Pollutants adsorption Protection of pathogens
Nutrients	Nitrogen Ammonia Phosphorus	Eutrophication Toxicity
Other organic material	Detergents Pesticides Fats Oils Grease Solvents	Toxicity Non-biodegradability Bioaccumulation Aesthetic problems Foam Odour
Pathogens	Bacteria Fungi Helminths Protozoa Viruses	Infectious diseases of humans, other animals and plants
Metals	Mercury Lead Copper Zinc	Toxicity Bioaccumulation Accumulation in soil
Other inorganic material	Acids Bases Chloride Sodium Boron	Toxicity Corrosion Damage to soils Salinity
Emerging contaminants of concern	Antibiotics and other medications Hormones Illicit drugs Caffeine Nicotine	Toxicity Bioaccumulation

The harm caused by these hazards is significant, with poor sanitation and the subsequent contamination of drinking water with wastewater resulting in 432 000 diarrhoeal deaths annually worldwide (WHO, 2019). Wastewater exposure also has a negative effect on the

environment, which in turn has a negative effect on the economy as it reduces the natural capital of ecosystems and the services they can provide (Okoh et al., 2007, Gutterer et al., 2009, Akpor and Muchie, 2011, Saravanan et al., 2011). The World Bank estimates an annual lost of USD $260 billion to the global economy due to the effect poor sanitation has on human and environmental health (IWA, 2019).

The risk posed by these hazards is exacerbated by the recycling of wastewater, particularly if inadequately treated or not treated at all (Tanik, 2010, Mizyed, 2013, Varela and Manaia, 2013, Walls, 2015). Recycling wastewater is a common practice around the world, especially in water-scarce regions (Abdel-Raouf et al., 2012). It can be employed for many purposes with irrigation being by far the biggest consumer of recycled wastewater, with an estimated 20% of global food crops relying on the practice (Jimenez, 2007, Drechsel et al., 2015, Walls, 2015). At the beginning of the twenty-first century, it was estimated 20% of the global population was suffering from water scarcity with this predicted to increase to >60% by 2030 (Sato et al., 2013, Valipour and Singh, 2016). As water becomes scarcer due to population growth and climate change the practice of wastewater recycling will undoubtedly increase, subsequently increasing the risk of exposure to the hazards present in wastewater (Akpor and Muchie, 2011, Drechsel et al., 2015, Walls, 2015, Dickin et al., 2016).

1.1.1. Types of pathogens present in wastewater and the health hazards they pose

Of all the hazards present in wastewater, pathogens present probably the greatest risk to human and environmental health, particularly in low- and middle-income countries (Mara, 2004, Davies-Colley, 2005, Akpor and Muchie, 2011, Scheierling et al., 2011). Pathogenic organisms commonly found in wastewater generally fit into five categories: bacteria, fungi, helminths, protozoa and viruses (Keraita et al., 2008, Orlofsky et al., 2011, Chen et al., 2013b, Kokkinos et al., 2015, Walls, 2015, Gerardi and Zimmerman, 2016). Although not exhaustive, Table 1.2 lists some of the most important and common pathogens of concern found in wastewater.

Table 1.2. The concentration (organism L^{-1}), survival (days), infectious dose (organisms L^{-1}) and disease(s) caused by pathogens commonly found in wastewater (Keraita et al., 2008, Orlofsky et al., 2011, Chen et al., 2013b, Kokkinos et al., 2015, Walls, 2015, Gerardi and Zimmerman, 2016).

Group	Organism(s)	Concentration (orgs L^{-1})	Survival (d)	Infectious dose	Disease
Bacteria	*Salmonella* spp.	1-10^5	<30	10^4-10^7	Salmonellosis, typhoid
	Shigella spp.	10-10^4	<10	10-10^2	Bacillary dysentery
	Vibrio cholerae	10^2-10^5	ND[b]	10^3	Cholera
	Campylobacter jejuni	10-10^4	ND[b]	~500, 10^6[e]	Gastroenteritis
	Escherichia coli O157:H7	ND[b]	ND[b]	<100	Gastroenteritis
Fungi	*Aspergillus fumigatus*	ND[b]	ND[b]	ND[b]	Farmer's lung
	Candida spp.	ND[b]	ND[b]	ND[b]	Candidiasis
	Penicillium spp.	ND[b]	ND[b]	ND[b]	Penicilliosis
	Pseudallescheria boydii	ND[b]	ND[b]	ND[b]	Hyalohyphomycosis
Helminths	*Ascaris lumbricoides*	1-10^3	Years[c]	1-10	Ascariasis
	Ancylostoma duodenale/ Necator americanus	1-10^3	ND[b]	Low	Hook-worm/roundworm
	Trichuris trichiura	1-10^2	ND[b]	1	Whipworm
Protozoa	*Cryptosporidium parvum*	1-10^4	<70[d]	1-10	Gastroenteritis, cryptosporidiosis
	Entamoeba histolytica	1-10^2	<15	10-10^2	Amoebic dysentery
	Giardia intestinalis	10^2-10^5	ND[b]	25-100	Giardiasis
Viruses	Enteric viruses[a]	10^5-10^6	<50	1-10	Poliomyelitis, gastroenteritis, heart anomalies and hepatitis
	Rotavirus	10^2-10^5	ND[b]	1-10	Gastroenteritis

[a]Coxsackievirus, echovirus, hepatitis A, poliovirus
[b]No data
[c]As *Ascaris* spp. eggs
[d]As *Cryptosporidium* oocysts
[e]~500 (Orlofsky et al., 2011), 10^6 (Chen et al., 2013)

As can be seen in Table 1.2, many of the common pathogens found in wastewater have a low infectious dose and can still be a public and environmental health risk at very low concentrations (Araki et al., 2000, Varela and Manaia, 2013). These low infectious doses put pressure on wastewater treatment systems to achieve complete removal of pathogens, as even a single organism in treated effluent can pose a risk to public health. Additionally, many of the pathogens, particularly protozoa and helminths, are capable of surviving in the environment for extended periods (Araki et al., 2000, Campos, 2008, Keraita et al., 2008, Tanik, 2010). Thus, the release of pathogens can pose a long-term problem for public and environmental health.

In the future, as with the other hazards, the expected increase in wastewater recycling is expected to increase the risk of pathogens present in wastewater (Reinoso et al., 2011, Baum et al., 2013, Makkaew et al., 2016). Grangier et al. (2012) support this expectation reporting significantly higher rates of water-borne diseases in children living in areas of Aleppo, Syria, that employ wastewater irrigation, 75%, compared to children living in areas employing freshwater irrigation only, 13%.

1.2. Wastewater treatment

Given the significant hazard posed by wastewater to public and environmental health, it is deeply concerning the amount which undergoes no or only partial treatment. In less affluent regions it is estimated between 80-90% of wastewater is released untreated into the environment (Drechsel and Evans, 2010, Bhaduri et al., 2016, Cossio et al., 2018). Even in affluent regions, 30% of wastewater is released, untreated, into the environment (Orner and Mihelcic, 2018). Thus, on a global-scale, >50% of rivers, oceans, and lakes are contaminated with untreated wastewater (Baum et al., 2013). Furthermore, it is estimated 2.9×10^7 km^2 or 14% of all croplands globally are dependent on surface waters for irrigation which are contaminated with inadequately treated wastewater (Hong et al., 2018).

While the widespread lack of wastewater treatment has long been a concern, in the last ten years it has been brought to the forefront. In 2010, the United Nations (U.N.) General Assembly and the U.N. Human Rights Council formally recognised access to safe and clean drinking water and sanitation as an essential human right (Murthy, 2013, McGranahan, 2015, Sultana and Loftus, 2015, Adeel, 2017). The U.N. added to this commitment by addressing wastewater treatment directly in Goal 6 of their Sustainable Development Goals to '*Ensure availability and sustainable management of water and sanitation for all*' (Bhaduri et al., 2016, Adeel, 2017, Stafford-Smith et al., 2017). Within Goal 6, target 6.2 aims to achieve access to adequate and equitable sanitation for all and target 6.3 aims to improve water quality by reducing pollution, halving the proportion of untreated wastewater, and increasing recycling and safe reuse globally. Both aims are to be achieved by 2030 (Adeel, 2017, Orner and Mihelcic, 2018).

The main aim of wastewater treatment is to treat wastewater to a suitable quality where its release into the environment or reuse does not cause harm to public or environmental health (Mara, 2004, Muga and Mihelcic, 2008, Bani, 2011, Capodaglio, 2017). To achieve this aim, physical, biological and chemical processes are often used in succession (Asano and Levine, 1996, Okoh et al., 2007, Eladel et al., 2019). These successive processes are usually referred to as preliminary, primary, secondary, tertiary/advanced and disinfection stages (Metcalf & Eddy INC, 1991, Pescod, 1992, Maliva and Missimer, 2012, Eladel et al., 2019). It should also be noted that how these stages are named and described vary throughout the world.

1.2.1. Stages of wastewater treatment and conventional treatment systems

1.2.1.1. Preliminary treatment

Preliminary treatment involves the removal of material that would be detrimental to the operation and maintenance of wastewater treatment systems in later treatment stages (Metcalf & Eddy INC, 1991, Maliva and Missimer, 2012, Spellman, 2013). It is not always required with its application depending on the constituents expected to be present in the wastewater and whether it is affordable. Conventional preliminary treatment systems employed include screens, grit chambers, comminutors, pre-aeration and chemical addition (Maliva and Missimer, 2012, Spellman, 2013).

1.2.1.2. Primary treatment

The objective of primary treatment is to remove heavy organic and inorganic solids (Pescod, 1992, Sonune and Ghate, 2004).To achieve this objective primary treatment uses the physical treatment processes of sedimentation and floatation (Sonune and Ghate, 2004, Maliva and Missimer, 2012). Conventional systems used to perform this treatment include sedimentation tanks and anaerobic digesters (Pescod, 1992, Okoh et al., 2007, Abdel-Raouf et al., 2012).

1.2.1.3. Secondary treatment

Secondary treatment involves the removal of the remaining dissolved and suspended organic material from the primary treatment effluent (Gilbride et al., 2006, Bani, 2011). To remove this waste, this stage of treatment employs aerobic biological processes (Pescod, 1992, Okoh et al., 2007). Essentially this process involves the biodegradation of organic matter present in the wastewater by microorganisms that utilise it for growth and

reproduction, with carbon dioxide and water produced as by-products (Von Sperling, 2007b, Abdel-Raouf et al., 2012, Asthana et al., 2017). The systems typically used during this stage of treatment are trickling filters, rotating biological contactors and activated sludge plants (Okoh et al., 2007, Bani, 2011, Bodik and Kubaska, 2013).

1.2.1.4. Tertiary/advance and disinfection treatment

Tertiary treatment and advance treatment are often grouped because, while they do not have the same meaning, they have the same aim of removing pollutants that primary and secondary treatments cannot (Okoh et al., 2007, Amenu, 2013, Spellman, 2013, Nasser, 2016). Traditionally the main pollutants tertiary/advanced treatment focuses on removing are ammonium, phosphorus and heavy metals (Von Sperling, 2007b, Bodik and Kubaska, 2013, Asthana et al., 2017).

Tertiary treatment systems typically employed include sand, dual media and membrane filters and land-based or wetland processes (EPA SA, 2003, Okoh et al., 2007, Nasser, 2016). Advanced treatments generally employ sophisticated techniques, such as chemical precipitation, ozonation, nanofiltration, reverse osmosis and carbon adsorption (Milne et al., 2007, Abdel-Raouf et al., 2012).

While the previously listed stages of treatment do perform some inactivation of pathogens, there is no guarantee that they can completely remove all pathogens (Abdel-Raouf et al., 2012, Asthana et al., 2017). To achieve an effluent free of pathogens a disinfection treatment stage is employed, generally using chlorination but ozonation, ultraviolet radiation and peracetic acid can also be used (Abdel-Raouf et al., 2012, Amenu, 2013, Spellman, 2013, Asthana et al., 2017, Collivignarelli et al., 2017).

Tertiary/advanced and disinfection treatment processes are usually only applied when high effluent standards that cannot be reached through primary and secondary treatment are required, such as when the treated effluent is to be recycled in a way that brings it in close

proximity to people (Pescod, 1992, Petala et al., 2006, Toze, 2006, Menegaki et al., 2007, Chen et al., 2013a).

1.2.1.5. Limitations of conventional wastewater treatment systems

While providing satisfactory treatment and protection of human and environmental health, the majority of the conventional wastewater treatment systems mentioned previously are not suitable in all situations. This is due to expensive construction and operational costs, high energy demands, the requirement for specific materials and chemicals, and the requirement of expert knowledge to operate (Pescod, 1992, Garcia and Bécares, 1997, Jhansi and Mishra, 2013, Gu et al., 2016, Reymond et al., 2018). Additionally, many of these conventional systems are not environmentally friendly: requiring large amount of resources, emitting high quantities of greenhouse gases, and potentially releasing residuals in the treated effluent that are toxic to aquatic life (Oswald, 2003, Collivignarelli et al., 2017, Enesca et al., 2017, Gu et al., 2017, Reymond et al., 2018). A significant concern as operators move towards more sustainable practices.

Such factors make these systems infeasible for less affluent regions as well as those undergoing rapid development and as a consequence are often abandoned resulting in wasted resources and wastewater going untreated (Jhansi and Mishra, 2013, Starkl et al., 2013). In these regions, which are mainly peri-urban and rural, natural wastewater treatment systems offer a more affordable, environmentally friendly and simpler alternative (Pescod, 1992, Jhansi and Mishra, 2013, Reymond et al., 2018, Philip et al., 2019).

1.2.2. Natural wastewater treatment systems

Natural wastewater treatment systems are so-called because they rely almost entirely on natural processes, with little or none of the electrical or chemical input typically used by conventional treatment systems (Muga and Mihelcic, 2008, Verbyla et al., 2015). Natural

processes used for treatment include solar radiation, gravitational forces, wind action, soil, microorganisms, plants and animals (Sundaravadivel and Vigneswaran, 2001, Kaur et al., 2012, Al-Hashimi and Hussain, 2013, Garfí et al., 2017). Common natural systems include duckweed pond systems, WSPs, constructed wetlands, subsurface flow constructed wetlands, soil aquifer treatments, planted filters and HRAPs (Metcalf & Eddy INC, 1991, Starkl et al., 2013, Matamoros et al., 2015, Singh et al., 2015). There has been extensive use of these natural systems in regions unsuitable for more conventional systems, particularly in less affluent regions and remote communities (Reinoso et al., 2008, Singh et al., 2015, Garfí et al., 2017).

Globally, the most popular natural wastewater treatment systems are WSPs with >8000 systems in the USA, ~3000 in Germany, >2500 in France and ~100 in Columbia (Shilton and Walmsley, 2005, Abbas et al., 2006, Jiménez et al., 2010, Verbyla and Mihelcic, 2015, Ho et al., 2017). Additionally, they are the most popular treatment system used in Mexico, the Dominican Republic and Brazil, as well as being used in more than half of the wastewater treatment systems in the United States and New Zealand (Verbyla and Mihelcic, 2015, Ho et al., 2017)

1.2.2.1. Waste stabilisation ponds

WSPs, also called lagoons, are large, shallow constructed basins (Gloyna, 1971, Kayombo et al., 2004, Bani, 2011, Verbyla and Mihelcic, 2015). They are passive systems with mixing only performed by convection, wind action and influent flow (Shilton and Sweeney, 2005, Von Sperling, 2007a, Shoener et al., 2014). These systems are considered to be one of the best wastewater sanitation techniques available (Picot et al., 1992, Jimenez, 2007). Consequently, they are not only used for rural/remote communities but have been employed in major wastewater treatment systems that serve large cities.

There are three types of WSPs categorised by the biological activity occurring in the pond, namely anaerobic, facultative and maturation (Fallowfield and Garrett, 1985, Edberg et al., 2000, Bani, 2011, Hayati et al., 2013). In order to achieve adequate wastewater treatment, it is usually required to operate them in series (Kayombo et al., 2004, Mara, 2008). A typical series is comprised of an anaerobic pond followed by a facultative pond and, if a higher quality of effluent is required, one or more maturation ponds (Kayombo et al., 2004, Birchall et al., 2008, Mara, 2008). Multiple ponds of the same type can also be operated in parallel to allow flexibility in operation and maintenance, and accommodate the high seasonal variation of loading rates (Fallowfield and Garrett, 1985, Mara, 2005).

1.2.2.1.1. Anaerobic waste stabilisation ponds

Anaerobic ponds perform primary treatment of wastewater (Kayombo et al., 2004, Bani, 2011). They are the deepest ponds, being 2-5 m deep to accommodate the accumulation of sludge at their base and to maintain anaerobic conditions via a low volume to surface area ratio (Mara et al., 1992). Anaerobic conditions are also maintained through the receival of high organic loads between 100-350 g BOD m^{-3} d^{-1} equivalent to ≥3000 kg ha^{-1} d^{-1} (Mara and Horan, 2003, Mara, 2004, Bani, 2011, Abdel-Raouf et al., 2012). A theoretical hydraulic retention time (THRT) between 1-6 d is required for effective operation (Mara et al., 1992, Papadopoulos et al., 2003, Von Sperling, 2007a). Their primary function is to remove bulk suspended solids and organic/inorganic matter through settlement and biodegradation via acidogenesis, acetogenesis and methanogenesis (Fallowfield and Garrett, 1985, Pescod, 1992, Rajbhandari and Annachhatre, 2004). Their treatment performance is highly dependent on temperature with negligible biodegradation and subsequent poor performance occurring below 10°C (Mara et al., 1992, Abdel-Raouf et al., 2012). When designed and operated correctly they can remove 60% of BOD_5 at 20°C and up to 75% at 25°C (Okoh et al., 2007, Bani, 2011). They also perform some removal of pathogens through sedimentation (Mara and Horan, 2003, Stott et al., 2003).

1.2.2.1.2. Facultative waste stabilisation ponds

Facultative ponds usually perform secondary treatment on anaerobic effluent but can be used to perform primary treatment on raw wastewater (Plate 1.1) (Fallowfield and Garrett, 1985, Bani, 2011). Their primary function is to remove organic matter; however, they do provide some disinfection of pathogens (Abdel-Raouf et al., 2012, Al-Hashimi and Hussain, 2013). As their name suggests the upper layer of facultative ponds is aerobic due to algal photosynthesis while their bottom layer is composed of anaerobic sludge (Shilton and Walmsley, 2005, Larsdotter, 2006, Al-Hashimi and Hussain, 2013). Both layers contribute to the typically high organic matter removal rates achieved by the system, between 75-85% (Shilton and Walmsley, 2005, Von Sperling, 2007a, Al-Hashimi and Hussain, 2013). The upper layer removes organic matter through algal-bacterial interactions while the bottom layer biodegrades settled organic matter through the same processes occurring in anaerobic ponds (Shilton and Walmsley, 2005, Von Sperling, 2007a, Henze and Comeau, 2008). To promote and maintain a healthy algal population in the upper layer they are designed to receive lower organic loads, 100-400 kg ha^{-1} d^{-1}, than anaerobic ponds (Mara, 2004, Bani, 2011, Abdel-Raouf et al., 2012). They are also operated at shallower depths (1.2-1.8 m) and longer THRTs (20-50 d) (Fallowfield and Garrett, 1985, Mara et al., 1992, Pescod, 1992).

Plate 1.1. The facultative waste stabilisation pond at Kingston on Murray wastewater treatment plant, Australia.

1.2.2.1.3. Maturation waste stabilisation ponds

Maturation ponds perform tertiary treatment and disinfection of the facultative pond effluent (Mara, 2008, Bani, 2011). The primary function of maturation ponds is to reduce pathogen load before discharge or recycling of the effluent (Mara et al., 1992). To achieve this aim maturation ponds are shallower (1.0-1.5 m) than anaerobic and facultative ponds, supposedly to maximise sunlight penetration into the water column increasing solar disinfection (Bolton et al., 2010). Mara et al. (1992) states that maturation WSPs have been reported to remove 4-6 log units of faecal coliforms, 2-4 log units of faecal viruses and 100% of parasites. While their primary function is to remove pathogens, they are also able to achieve significant nutrient removal via aerobic processes (Kayombo et al., 2004, Shilton and Walmsley, 2005). Their shallowness and low organic loading rates result in an aerobic environment throughout their water depth (Mara et al., 1992, Martínez et al., 2016). A series

of smaller maturation ponds with shorter THRTs (5-15 d) are typically used instead of a single larger pond to ensure good hydraulic efficacy and reduce the concentration of algae in the final effluent (Mara et al., 1992, Pescod, 1992, Craggs, 2005, Shilton and Walmsley, 2005).

1.2.2.1.4. Limitations of waste stabilisation ponds

Even with the many positives of WSPs, there are problems with the systems. These disadvantages include sludge accumulation and odour release that needs to be managed (Singh et al., 2015); thermal/dissolved oxygen (DO)/pH stratification and hydraulic short-circuiting adversely affecting treatment performance (Sweeney et al., 2005, Sweeney et al., 2007); long THRT that reduces the volume and quality of effluent available for beneficial reuse (Jimenez, 2007, Mara, 2008, Jiménez et al., 2010); and large land requirements that limit their application in certain regions (Jimenez, 2007, Jhansi and Mishra, 2013, Martínez et al., 2014, Verbyla et al., 2016). In comparison, HRAPs provide an alternative to facultative and maturation WSPs without these disadvantages and with many additional advantages (Young et al., 2017).

1.3. Mini-review: High rate algal ponds, flexible systems for sustainable wastewater treatment

The following section of this chapter is a published journal article authored by Paul Young, Dr Michael Taylor and Professor Howard Fallowfield in *World Journal of Microbiology and Biotechnology*, published 10 May 2017, Volume *33*:6, Page 117 (Appendix A.1). Reproduced by permission of Springer Nature. The published version of the article can be found at https://link.springer.com/article/10.1007%2Fs11274-017-2282-x.

This was a jointly authored publication requested by Professor Emeritus Ian Maddox, Review-commissioning Editor for the *World Journal of Microbiology and Biotechnology*. The

publication was based on a literature search conducted by Paul Young and extensive discussions with all authors. All authors contributed to all sections of the publication.

The publication reviews the current understanding of HRAPs operation and performance. It begins by detailing the development of HRAPs, the science behind their design, the different ways they have been used and their reported wastewater treatment performance. This is followed by a review of studies comparing the wastewater treatment performance, cost-effectiveness and environmental impact of HRAPs compared to WSPs, the most prevalent natural wastewater treatment system. The article then discusses the use of HRAPs to produce value-added products, mainly microalgae biofuels. It reviews the current literature on the topic identifying the gaps in knowledge. Lastly, the article highlights other areas requiring further research, specifically pathogen disinfection and the removal of emerging contaminants.

1.3.1. Abstract

Over the last 20 years, there has been a growing requirement by governments around the world for organisations to adopt more sustainable practices. Wastewater treatment is no exception, with many currently used systems requiring large capital investment, land area and power consumption. High rate algal ponds offer a sustainable, efficient and lower cost option to the systems currently in use. They are shallow, mixed lagoon-based systems, which aim to maximise wastewater treatment by creating optimal conditions for algal growth and oxygen production — the key processes which remove nitrogen and organic waste in HRAP systems. This design means they can treat wastewater to an acceptable quality within a fifth of the time of other lagoon systems while using 50% less surface area. This smaller land requirement decreases both the construction costs and evaporative water losses, making larger volumes of treated water available for beneficial reuse. They are ideal for rural, peri-urban and remote communities as they require minimum power and little on-site management. This review will address the history of and current trends in high rate algal

pond development and application; a comparison of their performance with other systems when treating various wastewaters; and discuss their potential for production of value-added products. Finally, the review will consider areas requiring further research.

1.3.2. Keywords

Algae, High rate algal ponds, Wastewater, Wastewater treatment

1.3.3. Introduction

In recent years, there has been an increase in interest and research regarding high rate algal ponds (HRAP). This has largely been driven by their potential to grow large amounts of algae from which value-added products may be derived, rather than by their potential application to more sustainable wastewater treatment. The mini-review specifically focusses on the application of HRAPs for wastewater treatment and considers the secondary benefit of biomass production and utilisation, while also identifying knowledge gaps and the need for future research.

1.3.4. High rate algal ponds, past, present and future

HRAPs were developed at the University of California in the middle of the twentieth century while investigating the use of algal biomass for wastewater treatment (Oswald et al. 1957; Oswald and Golueke 1960). The term ‘high-rate pond’ was first used by Oswald (1963) to describe open raceway ponds that differ from other pond systems in that they aim to maximise their algal biomass concentration to increase their wastewater treatment efficiency (Plate 1.2) (Bahlaoui et al. 1997). Since their initial development in the USA, HRAPs have been operated in many countries including Israel (Shelef and Azov 1987), France (Picot et al. 1991), Morocco (El Hamouri 2009), the United Kingdom (Fallowfield and Garrett 1985b),

Spain (García et al. 2008), Australia (Young et al. 2016), China and New Zealand (Craggs et al. 2003a). Due to their reliance on algal photosynthesis, they are better suited and more easily operated in arid, semi-arid and tropical climates (Garcia et al. 2006; Sahoo and Seckbach 2015). They have been used to treat a variety of wastes including domestic (Chen et al. 2003), tannery (Rose et al. 1996), dairy (Craggs et al. 2003b) and piggery (Fallowfield and Garrett 1985a).

Plate 1.2. Two high rate algal ponds at Melbourne Water Western Treatment Plant, Werribee, Australia, fed anaerobic and facultative lagoon treated domestic wastewater.

HRAPs are considered a low-cost wastewater treatment system compared to conventional electromechanical systems with construction costs typically ~70% less than activated sludge systems, the major wastewater treatment system in the USA (DOE 2016). Operation cost is also reduced for HRAP as they require substantially less energy than activated sludge systems (Shilton et al. 2008; Woertz et al. 2009; Craggs et al. 2011). This reduction in energy not only reduces cost but also reduces greenhouse gas emissions, making them an

option to improve the sustainability of wastewater treatment trains (Acién et al. 2016). Due to their low-cost and simple operation HRAPs are ideal for operation in rural, peri-urban and remote communities when land availability is not constrained (Garcia et al. 2006; Acién et al. 2016; DOE 2016). Currently, these communities largely employ waste stabilisation ponds (WSP) as low-cost wastewater treatment systems. Using the descriptions in Buchanan (2014), an infrastructure and associated cost comparison between an in series facultative-maturation pond HRAP and a five cell WSP system, commonly used in rural South Australia, was undertaken (Table 1.3). The scenario assumed a population served of 700 persons with a flow of 140 L per person per day equaling a total wastewater flow of 100 kL d^{-1}. Given these assumptions, the cost of constructing the HRAP system was 39.2% of the WSP when operated at a depth of 0.32 and 47.5% when operated at a depth of 0.43 m.

Table 1.3. Comparison of the estimated infrastructure and associated costs of an in series, 5-cell facultative-maturation waste stabilisation pond system and an HRAP based on the descriptions by Buchanan (2014). The scenario assumed a population served of 700 persons with a flow of 140 L per person per day equalling a total wastewater flow of 100 m^{-3} d^{-1}. Assumptions made for HRAP pond design were external earth walls; 1:3 internal batter; internal plastic curtain walls; square shape – made for calculation simplicity; and a high-density polyethylene liner buried 1.5 m all sides. Pricing used for earthworks was \$12 m^{-3} and for installed plastic was \$15 m^{-2} based on 2011 estimates with all prices in Australian dollars (AUD). The evaporation rate used was based on the pan evaporation rates typically experienced in South Australia, 1.8-2 m.

Design parameters	**High rate algal pond**		**Waste stabilisation pond**
Pond Depth (m)	0.32	0.43	1.2
Freeboard (m)	0.2	0.25	0.8
Surface Area (m^2)	2,500	3,100	6,000
Surface area as percentage of WSP (%)	41.6	47	100
Annual evaporative loss (m^3)	4,500	5,580	10,800
Evaporative loss as percentage of treated water (%)	12.3	15.3	29.6
Top dimensions (m)	51.7	57.9	81.1
Bottom dimensions (m)	50.0	55.7	77.5
Internal Volume (m^3)	1,348	2,197	12,169
Liner Area (m^2)	2,831	3,525	6,816
Curtain Area (m^2)	104	151	504
Earthworks as percentage of WSP (%)	11.1	18.1	100
Estimated construction costs	**AUD**	**AUD**	**AUD**
High-density polyethylene liner	44,030	55,139	109,801
Earthworks	16,82	26,362	146,023
Paddlewheel assembly	20,000	20,000	
Buffer tank	20,000	20,000	
Total Construction	100,211	121,501	255,825
HRAP costs as a percentage of those for the WSP	39.2%	47.5%	100.0%

There has been extensive research into the ability of HRAPs to treat standard wastewater parameters (Table 1.4). Reported 5-day biological oxygen demand (BOD_5) removal rates range between 22-93.4% with a median of 59% (Banat et al. 1990; El Hamouri et al. 1995; Craggs et al. 2003a; El Hafiane and El Hamouri 2005; Buchanan 2014; Young et al. 2016). The removal of nitrogen and ammonium is considered to be mainly through incorporation into algal biomass and pH-dependent ammonia volatilisation with limited nitrification having been reported as well (Cromar et al. 1996; Garcia et al. 2000; Craggs et al. 2003a). The reported removal of total nitrogen ranges between 26.6-75.7% with a median of 61.23%

(Shelef et al. 1982; Banat et al. 1990; Picot et al. 1991, 1992; Chen et al. 2003; Craggs et al. 2003a; El Hafiane and El Hamouri 2005; Park and Craggs 2011) and ammonium removal ranges between 21.89-94% with a median 77% (Wood et al. 1989; Banat et al. 1990; Picot et al. 1991, 1992; El Hamouri et al. 1995; Craggs et al. 2003a; El Hafiane and El Hamouri 2005; Park and Craggs 2011; Buchanan 2014; Sutherland et al. 2014a). The two main mechanisms of phosphorus removal are thought to be through incorporation into the algal biomass and pH-dependent precipitation. Reports of total phosphorus removal ranges between 10.48-97.2% with a median of 42.73% (Shelef et al. 1982; Picot et al. 1991, 1992; Chen et al. 2003; El Hafiane and El Hamouri 2005; El Hamouri 2009; Sutherland et al. 2014a) and orthophosphate removals range between -3.75-71% with a median of 21.2% (Wood et al. 1989; Picot et al. 1991, 1992; El Hamouri et al. 1995; Chen et al. 2003; Craggs et al. 2003a; Buchanan 2014; Sutherland et al. 2014a). Disinfection in HRAPs is believed to be mainly dependent on solar irradiance (Craggs et al. 2004), pond depth and pH (Buchanan et al. 2011b) or all three (Fallowfield et al. 1996). Considering that depth influences the exposure of pond volume to solar radiation, and pH is influenced by algal photosynthesis, which in turn is influenced by solar radiation exposure, it could be theorised that overall these studies suggest depth is the main factor influencing disinfection in HRAPs. Reported $\log_{10}$ reduction values for *Escherichia coli* in HRAPs range between 1 and 3.01 $\log_{10}$ *E. coli* MPN 100 mL^{-1} with a median of 1.4 $\log_{10}$ *E. coli* MPN 100 mL^{-1} (Craggs et al. 2003a; Davies-Colley et al. 2003, 2005; El Hafiane and El Hamouri 2005; Buchanan 2014; Young et al. 2016). There is limited information on the removal of heavy metals by HRAPs, but the few existing studies point towards effective removal mainly through adsorption in algal and microbial biomass (Rose et al. 1998; Toumi et al. 2000).

Table 1.4. Dimensions, location and operating conditions of high rate algal ponds treating various wastewaters and their reported removals of standard wastewater parameters.

Author	Wastewater	Length (m)	Width (m)	Surface area (m^2)	Depth (m)	THRT (d)	Coordinates	Removal					
								BOD_5 (%)	Total nitrogen (%)	Ammonia (%)	Total phosphorous (%)	Orthophosphate (%)	*E. coli* LRV (*E. coli* MPN 100 mL^{-1})
Banat et al 1990	Facultative treated domestic wastewater	10	5		0.45	5	29.37, 47.97	90.37	59.65	90			
El Hamouri et al 1995	Grease/sand trap and anaerobic pond treated domestic wastewater			3023	0.4	4.2	latitude 30.55	32		78.92		53.05	
El Hamouri et al 1995	Grease/sand trap and anaerobic pond treated domestic wastewater			3023	0.4	4.2	latitude 30.56	45.13		21.89		30.51	
Buchanan 2014	Septic tank treated domestic wastewater			192	0.32	4.5	-34.14, 140.14	93.4		69.8		18.9	1.741
Buchanan 2014	Septic tank treated domestic wastewater			208	0.43	6.4	-34.14, 140.14	92.5		73.5		21.2	2.079
Buchanan 2014	Septic tank treated domestic wastewater			226	0.55	9.1	-34.14, 140.14	90.2		61.1		6.5	1.977
Buchanan 2014	Facultative treated domestic wastewater			192	0.32	4.5	-34.14, 140.14	72		72		0.1	2.52
Buchanan 2014	Facultative treated domestic wastewater			208	0.43	6.4	-34.14, 140.14	59		83		0.1	2.12
Buchanan 2014	Facultative treated domestic wastewater			226	0.55	9.1	-34.14, 140.14	51		35		0.02	3.01
Young et al 2016	Septic tank treated domestic wastewater			200	0.32	5	-34.14, 140.14	91.76					2.13

Table 1.4. (continued)

Author	Wastewater	Length (m)	Width (m)	Surface area (m^2)	Depth (m)	THRT (d)	Coordinates	Removal					
								BOD_5 (%)	Total nitrogen (%)	Ammonia (%)	Total phosphorous (%)	Orthophosphate (%)	*E. coli* LRV (*E. coli* MPN 100 mL^{-1})
Shelef et al 1982	Bar-screened domestic wastewater			120	0.4	3.4			75.2		95.7		

Shelef et al 1982	Bar-screened domestic wastewater			120	0.5	4.25		62.8		93.6		
Shelef et al 1982	Bar-screened domestic wastewater			120	0.35	2.9		72.7		95.2		
Shelef et al 1982	Bar-screened domestic wastewater			120	0.25	2		83.3		97.2		
Picot et al 1992	Facultative treated domestic wastewater	13.4	3.6	48	0.35	8	43.42, 3.59	34.33[g]	66.16	24.49	8.28	
Chen et al 2003	Settling tank treated wastewater				0.3	8	31.70, 122.37	75.2[g]	80.4	47.5	43.5	
Chen et al 2003	Settling tank treated wastewater				0.3	4	31.70, 122.38	75.7[g]	93.6	40.7	38.2	
Park and Craggs 2011	Anaerobic digested domestic wastewater			31.8	0.3	8	-37.78, 175.32	26.6	74.29			
Wood et al 1989	Settling tank treated wastewater	22	11		0.4		-25.75, 28.19		73.76		32.7	
Picot et al 1991	Primary pond treated domestic wastewater	12.4	3.8		0.35	8	43.42, 3.59	30.54[g]	92	31.58	71	
Picot et al 1991	Primary pond treated domestic wastewater	12.4	3.8		0.35	4	43.42, 3.59	47.81[g]	94	44.76	71	
Sutherland et al 2014a	Primary treated domestic wastewater			12500	0.35	7	-43.53, 172.68		47	37		

Table 1.4. (continued)

Author	Wastewater	Length (m)	Width (m)	Surface area (m^2)	Depth (m)	THRT (d)	Coordinates	Removal					
								BOD_5 (%)	Total nitrogen (%)	Ammonia (%)	Total phosphorous (%)	Orthophosphate (%)	*E. coli* LRV (*E. coli* MPN 100 mL^{-1})
Sutherland et al 2014a	Primary treated domestic wastewater			12500	0.35	9	-43.53, 172.69			53	22		
Sutherland et al 2014a	Primary treated domestic wastewater			12500	0.35	7	-43.53, 172.70			79	49		
Sutherland et al 2014a	Primary treated domestic wastewater			12500	0.35	5.5	-43.53, 172.71			77	20		
El Hafiane and El Hamouri 2005	Step up-flow anaerobic reactor and gravel filter treated domestic			790	0.35	3	33.98, -6.87	22	86[g]	86	66	59	1.23

	wastewater												
Craggs et al 2003a	Primary pond treated domestic wastewater	20.3	4.2	85	0.45	7.5	-37.30, 175.50	54.55	51.95[g]	91	15.32	-3.75	1.42
Craggs et al 2003a	Primary pond treated domestic wastewater	30.5	4.2	128.1	0.3	7.5	-37.30, 175.50	54.55	57.96[g]	85	10.48	13.75	1.49
Davies-Colley et al 2003	Domestic wastewater				0.3	7.5	-37.30, 175.50						1
Davies-Colley et al 2005	Anaerobic digester treated domestic and laboratory wastewater					8	-37.78, 175.32						1
Davies-Colley et al 2005	Anaerobic digester treated domestic and laboratory wastewater					8	-37.78, 175.32						1

Algal biomass concentration is maximised by creating an environment conducive to photosynthesis through maximising the pond volume's exposure to solar radiation by shallow ponding and mixing (Rawat et al. 2011). Operational depths of HRAPs range between 0.2 and 0.8 m with the most common being ~0.3 m (Craggs et al. 2003a; Park and Craggs 2011). Gentle mixing is predominantly carried out by a paddlewheel at surface water velocities between 0.15 and 0.3 m s^{-1} (Sutherland et al. 2015). Increasing algal biomass concentration increases wastewater treatment efficiency as it increases the mutual breakdown of organic waste by algae and bacteria (Craggs et al. 2004; El Hamouri 2009). This results in HRAP providing faster treatment than non-mixed pond systems and as such HRAP systems can operate at shorter theoretical hydraulic retention times (THRT) or have higher organic loading rates (Green et al. 1996; Buchanan 2014) with typical THRT ranging between 4 and 10 d (Picot et al. 1992). The high rates of algal photosynthesis also produce high concentrations of dissolved oxygen and high pH levels which both fluctuate diurnally (Craggs et al. 2004). During peak solar radiation, dissolved oxygen concentrations can reach supersaturation, and pH levels can reach as high as 11 (Norvill et al. 2016).

As solar energy is the main energy source for HRAPs, the influence of depth and light attenuation on their wastewater treatment performance and biomass productivity has garnered research. Sutherland et al. (2014b) compared three pilot-scale HRAPs operated at different depths, 0.2, 0.3 and 0.4 m. There was no significant difference between the depths in the removal of ammonia and orthophosphate relative to inflow, but in regards to the total amount of ammonia removed and algal productivity, the 0.4 m outperformed the other depths. Buchanan (2014) studied the influence of depth on the wastewater treatment performance of a large-scale HRAP. The HRAP was operated at three different depths, 0.32, 0.43 and 0.55 m, while treating two different strengths of wastewater either septic tank treated domestic wastewater, or the same wastewater further treated by a facultative pond. When treating septic tank treated wastewater the 0.43 m depth slightly outperformed the 0.32 m depth, and both outperformed the 0.55 m depth. When treating the facultative pond

effluent, the 0.32 m depth had the best performance based on BOD_5 and *E. coli* removal while the 0.43 m depth had the best performance when removing ammonia. The results from both studies suggest that the optimal depth for a HRAP acting as a secondary wastewater treatment system is ~0.4 m and the results presented by Buchanan (2014) suggest the optimal depth for a HRAP acting as a tertiary wastewater treatment system is 0.32 m when removal of BOD_5 and *E. coli* are a priority and 0.43 m when ammonia removal is a priority. When interpreting these results, it should be considered that both these studies had limitations with Sutherland et al. (2014b) acknowledging that the light climate would be different in large-scale HRAPs and Buchanan (2014) only being able to run a single HRAP at a time meaning the different depths experienced different weather conditions. Ideally, to properly understand the effect of depth, two large-scale HRAPs should be operated concurrently at different depths while fed the same wastewater.

1.3.5. Comparison of high rate algal pond performance with other treatment systems

HRAPs have been considered as a replacement for other low-cost systems, mainly WSPs. However, before wide-scale replacement of WSP can occur, further comparisons of conventional wastewater treatment systems and HRAPs should be made under varied operational and geographic conditions. The comparison is made difficult as the performance of both systems can be affected by their specific location, meaning that compared systems must be geographically close. This can be difficult to arrange, and consequently, there are only a few studies comparing their performance in this way (Picot et al. 1992; Toumi et al. 2000; El Hamouri et al. 2003; Buchanan et al. 2011a; Buchanan 2014). These studies have shown that HRAPs have equal or better removal of standard wastewater parameters, with the one exception of orthophosphate removal in Buchanan (2014). The HRAPs also showed equal performance in the removal of pathogens and better performance in the removal of heavy metals (Picot et al. 1992; Toumi et al. 2000; El Hamouri et al. 2003; Buchanan et al.

2011a; Buchanan 2014). Toumi et al. (2000) demonstrated when compared to a facultative pond a HRAP was 1.3 times more efficient at removing zinc, ten times more efficient at removing copper and twice as efficient at removing lead.

This equivalence in treatment is significant because of the reduced time HRAPs take to achieve it — requiring at least 80% less THRT. This reduction in THRT means HRAPs have less standing volume than WSPs. Consequently, they are significantly smaller, with estimated reductions in size of 40% (El Hamouri et al. 2003) and 60% (Buchanan 2014).

This has two benefits, firstly construction costs, in particular, earthworks, are reduced with Buchanan (2014) estimating a reduction of 25-50%, and secondly, less treated effluent is lost by evaporation because of the reduction in surface area. This decrease in evaporative loss is of particular importance due to substantial reuse of wastewater for irrigation, particularly in less affluent areas which commonly experience high evaporation loss (Jimenez 2007). It has been estimated that the reduction in evaporative loss can be up to 90% (Buchanan et al. 2011a; Buchanan 2014). HRAPs have also been demonstrated to supersede WSPs in several further operational parameters: HRAPs do not require desludging; do not experience thermal stratification and hydraulic short-circuiting and produce higher concentrations of algal biomass which can be utilised (Fallowfield and Garrett 1985b; Cromar et al. 1996).

A notable disadvantage of HRAPs compared to WSPs is their requirement for a paddlewheel to mix the system, which can make it more difficult to operate the system where access to electricity is difficult. While there is no real solution to this problem, it is partially mitigated by the energy requirement being low so a small generator could be used (Shilton et al. 2008; Shoener et al. 2014). An ideal solution is to power the paddlewheel using solar panels, but the current cost would be prohibitive to the communities that would benefit the most, although it is predicted that in the near future there will be large drops in prices (Pinner and Rogers 2015).

Arbib et al. (2013) compared the wastewater treatment performance of an experimental HRAP to an experimental photobioreactor. The photobioreactor outperformed the HRAP in the removal of all standard wastewater parameters and produced a higher concentration of algal biomass (Arbib et al. 2013). Undermining this performance is the severe biofouling the photobioreactor experienced, causing cessation of the experiment: something a HRAP would not experience (Arbib et al. 2013). It should also be considered that photobioreactors cost substantially more to construct and operate as well as being more challenging to up-scale, all of which limit their application compared to HRAPs (Munoz and Guieysse 2006).

1.3.6. Potential for production of value-added products

The use of the wastewater-grown algal biomass for the production of value-added products has long been seen as a major attraction of HRAPs (Oswald and Golueke 1960; Shelef et al. 1982). Potential uses for the algal biomass include biofuel, animal feed, pigment production and fertiliser (Christenson and Sims 2011; Craggs et al. 2011). The low quality of the biomass, the potential contamination of the biomass by pathogens in the wastewater and the difficulty in maintaining monocultures in an open system mean that HRAP biomass is most suitable for biofuel production (Brennan and Owende 2010; Leu and Boussiba 2014; Shukla et al. 2017). For this reason, and the increasing interest in alternate renewable transport fuel options to replace fossil fuels the use of HRAP biomass has overwhelmingly focused on biofuel production (Pulz 2001; Brennan and Owende 2010; Leu and Boussiba 2014). This interest in using algal biomass as a source for creating biofuels has long been of interest, and this can be seen in the yearly publication and patents on algal biofuels following in-trend with the price of oil (US$) (Figure 1.1). Large-scale production of algal biofuels is hindered by the high cost of production, especially when compared to fossil fuel petroleum. It is thought that coupling biofuel production with wastewater treatment will reduce the cost (Driver et al. 2014). Essentially, the HRAP is used as a ready built low-cost reactor and wastewater as a low-cost feedstock for algae (Chen et al. 2015). While theoretically, this

coupling seems ideal where wastewater is transformed into biofuel and treated effluent for reuse, there are still many limitations to this application (Sutherland et al. 2015; Doma et al. 2016).

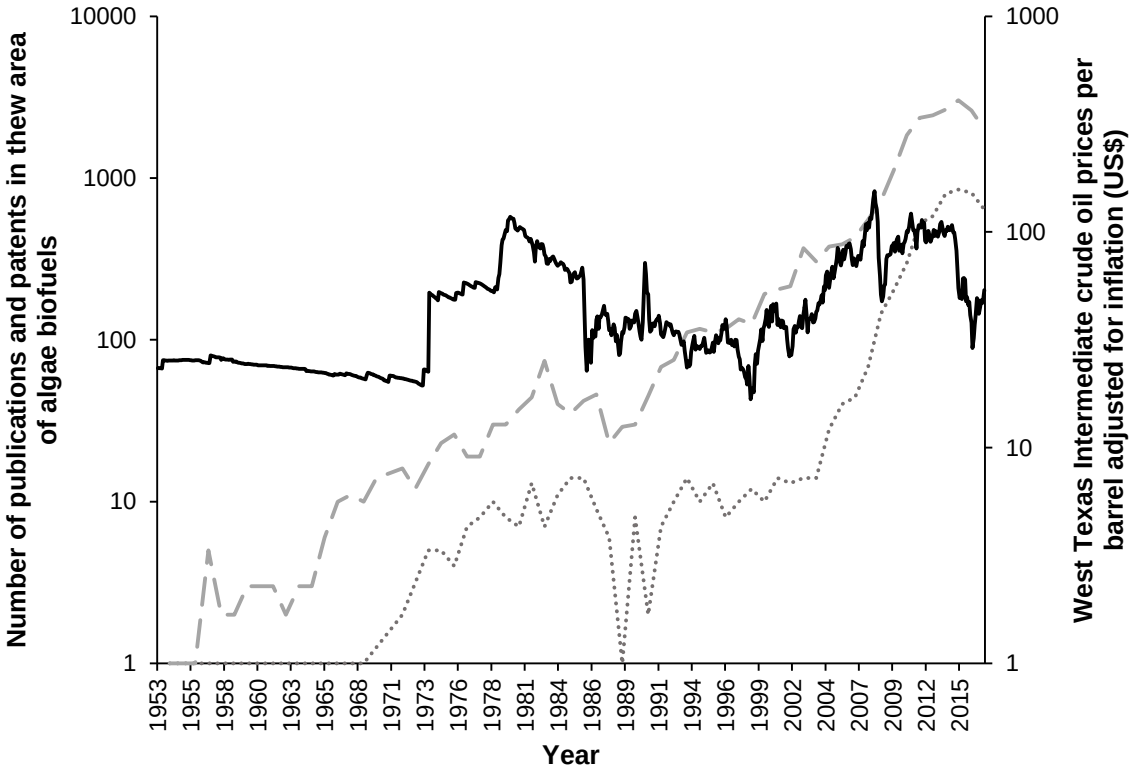

Figure 1.1. The number of patents (--) and publications (···) on algae biofuels and the West Texas Intermediate crude oil prices per barrel adjusted for inflation (US$) (Macrotrends 2017) (–) both on the logarithmic scale between 1953 and 2016.

Reliable and cheap harvesting is considered by many to be the most important limitation to the utilisation of the algal biomass to produce biofuels or any other value-added product with harvesting estimated to cost up 50% of the algal biomass (Greenwell et al. 2009; Hwang et al. 2016). The algal phyla that populate wastewater treating HRAPs, typically microalgae, are challenging to harvest due to their small cell size, <20 μm, similar density to water, 1.08-1.13 g mL^{-1}, and strong negative charge (Park et al. 2011). Out of the most well-known methods, sedimentation and flocculation are generally considered the most promising options as they are relatively cheap, simple to operate and easy to up-scale (Milledge and

Heaven 2013). Flocculation involves the addition of chemicals that triggers single-celled microalgae to aggregate into flocs that are more easily removed (Pahazri et al. 2016). Historically, the flocculants commonly used were metal salts, such as iron(III) chloride and alum, and cationic polymers such as chitosan and cationic starch (Pittman et al. 2011; Vandamme et al. 2013). There are difficulties with these flocculants the former with contamination of the biomass and the latter being influenced by pH and ionic conditions: cost can also be a limiting factor (Pittman et al. 2011; Vandamme et al. 2013). Flocculation involving the use of microorganisms and their products, bioflocculation, can involve the use of other algae, bacteria and fungi. Bioflocculation avoids chemical contamination to the biomass and has been promising but has yet to be proven outside of laboratory settings (Van Den Hende et al. 2011; Manheim and Nelson 2013; Wrede et al. 2014; Muradov et al. 2015). Sedimentation involves the use of gravitational forces to settle the algae from the liquid phase and is simple and relatively cheap method but has problems associated with reliability and speed (Milledge and Heaven 2013). Settling reliability can differ greatly between algae species, and it is thought selecting for more readably settable algal species may increase harvestability (Milledge and Heaven 2013). A novel way to do this has been recycling a portion of algal biomass harvested by sedimentation to increase yields in future harvests is another promising method that while demonstrated effectively in pilot-scale HRAPs has yet to be demonstrated in large-scale HRAPs (Park et al. 2013, 2015; Gutiérrez et al. 2016). Park and Craggs (2014) found recycling 10% of the daily algal biomass in a pilot HRAP dominated by the rapidly settling *Pediastrum boryanum* increased subsequent harvests settleability by 25% and biomass productivity by 40%.

Another major limitation to the utilisation of wastewater-grown algal biomass in HRAPs is the productivity achieved is well below the theoretical maximum of 50-60 g m^{-2} day^{-1} (Christenson and Sims 2011; Sutherland et al. 2015). Due to the high pH, it is believed that algal growth in wastewater is carbon limited and providing additional carbon would increase productivity (Craggs et al. 2012). The most popular solution to this problem has been adding

carbon to the HRAPs as carbon dioxide via flue gas, which has the bonus of reducing greenhouse gas emissions and as a consequence potentially earning tax and carbon credits (Munoz and Guieysse 2006; DOE 2016). There have been several studies on the effect carbon dioxide addition has on algal biomass productivity in HRAPs, and while some results have been promising, the interpretation is hampered by the experiments being laboratory based or using pilot-scale systems (Heubeck et al. 2007; de Godos et al. 2010; Van Den Hende et al. 2011), not having adequate controls (Park and Craggs 2010, 2011; Craggs et al. 2012) or using pure carbon dioxide which is lacking chemicals present in flue gas that may be toxic to algae (Chen et al. 2015; de Godos et al. 2016). Even if it were clear such addition substantially increased algal biomass, such systems would be limited in location to where suitable flue gas can be added, estimated to be <10% of flue gas emitting infrastructure in the USA, as transport of the gas is prohibitively expensive (Lundquist et al. 2010). Increasing productivity through the selection of high producing strains or genetic modification have also been considered, but there are problems in maintaining monocultures through predation/parasitism and more competitive wild strains (Christenson and Sims 2011; Sutherland et al. 2015).

1.3.7. Areas for further research

Increasing beneficial reuse of treated wastewater requires minimising the risk to the public of exposure to pathogenic microorganisms. Excluding *E. coli* and faecal indicators, there is a lack of information on the disinfection of many prominent pathogens and indicator organisms in large-scale, fully operational HRAPs. The only investigation into the removal of other bacteria by a HRAP was in a pilot-scale system which did show effective removal of the indicator organisms *Staphylococcus* spp. and *Clostridium perfrigens* (García et al. 2008). There is a notable absence of studies on the removal of pathogenic viruses. However, two studies on virus indicator organisms both showed effective removal (Davies-Colley et al. 2005; Young et al. 2016).

Research is also needed on the removal of pathogenic protozoa in full-scale HRAPs. Young et al. (2016) attempted using aerobic spore-forming bacteria as surrogate indicators of protozoa; the result was inconclusive and suggested they were unsuitable indicators for lagoon systems. Arkai et al. (2001) investigated the removal of *Cryptosporidium parvum* oocysts in a semi-permeable bag using a pilot-scale HRAP and showed removals of >98%. Studies on the removal of helminths have shown HRAPs perform removal, but primary treatment seems to be the main contributor (El Hamouri et al. 1994; El Hamouri et al. 1995; El Hamouri 2009). Given the extra treated effluent HRAPs produce for reuse, it is particularly important to determine their removal capabilities for the reference pathogens listed in *The World Health Organization Guidelines for the Safe Use of Wastewater Excreta and Greywater Volume II: Wastewater use in Agriculture* (2006). These are *Campylobacter* spp. for bacteria, rotavirus/norovirus for viruses, *Cryptosporidium* spp. for protozoa and *Ascaris lumbricoides* for helminths (WHO 2006; Mara et al. 2010).

Emerging contaminants are a wide-ranging group of primarily organic compounds that have recently been acknowledged as potentially posing a hazard to human and environmental health. As they are a recent problem, there have been few studies on the removal of emerging contaminants by HRAPs (de Godos et al. 2012; Matamoros et al. 2015). de Godos et al. (2012) measured the removal of the antibiotic tetracycline in a pilot-scale 24 L HRAP and found a removal of 69 ± 1%. Matamoros et al. (2015) measured the removal of 26 emerging contaminants including pesticides, pharmaceuticals, plasticisers and personal care products in a pilot-scale 470 L HRAP. They recorded removal efficiencies ranging from 0 to 99% depending on the chemical, season and THRT. They also performed an ecotoxicological risk assessment which showed following treatment the remaining concentration of chemicals had no acute toxicity risk (Matamoros et al. 2015). Both studies agreed that the major contributors to the HRAPs removal of emerging contaminants were photodegradation and biodegradation. Suggesting research on the removal of emerging contaminants in large-scale HRAPs is necessary as the light climate would be expected to

be different due to the difference in size of the pilot systems employed in previous studies and relative influence of the paddlewheel.

1.3.8. Conclusion

HRAPs present an alternative, or at least augmentative adjunct to current wastewater treatment systems which are costly to install, maintain and often unsuitable due to space and location constraints. HRAPs may provide a more flexible system with many of the advantages of a bioreactor, control over operational parameters, without the requirements of maintaining sterility and laboratory formulated feedstocks.

1.3.9. Reference

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1.4. Inactivation of pathogens in high rate algal ponds

Many of the factors that influence inactivation of pathogens in WSPs also influence inactivation in HRAPs (Gomez et al., 1995, Fallowfield et al., 1996, Araki et al., 2000, Mara

and Horan, 2003). These factors include solar radiation, pH, DO, temperature, predation, starvation and microcidal agents released by microorganisms (El Hamouri et al., 1994, Fallowfield et al., 1996, Bahlaoui et al., 1998, Stratton et al., 2015, Dar et al., 2019). Notably, settlement of organisms in HRAPs is reduced when compared to WSPs due to the constant mixing keeping them in suspension (Jupsin et al., 2003, Craggs, 2005, Sutherland et al., 2015, Young et al., 2016). It is therefore likely this factor plays a smaller role in pathogen inactivation in HRAPs than in WSPs. From the perspective of pathogens the HRAP environment also differs from the WSP environment by being more extreme, with greater exposure to solar radiation resulting in greater, yet highly fluctuating, pH values and DO concentrations (Nurdogan and Oswald, 1995, Fallowfield et al., 1996, Mara and Horan, 2003, Craggs et al., 2004, Munoz and Guieysse, 2006, El Hamouri, 2009, Park et al., 2011, Hawley and Fallowfield, 2016).

As with WSPs (Davies-Colley et al., 1999, Davies-Colley et al., 2005, Bolton et al., 2010, Reinoso et al., 2011), solar radiation is generally considered the most significant factor influencing pathogen inactivation either individually or in conjunction with other factors (El Hamouri et al., 1995, Fallowfield et al., 1996, Araki et al., 2001, Craggs et al., 2004). After reviewing the literature, Young et al. (2017) theorised depth was the primary influence on disinfection in HRAPs, yet this is probably due to its influence on solar radiation exposure in ponds. Fallowfield et al. (1996) supports this idea by suggesting that the influence depth has on pathogen inactivation in HRAPs may be mediated by solar radiation penetration and thus ultimately solar radiation itself.

1.4.1. Indicator organisms as tools to assess the performance of wastewater treatment systems

Given the significance of pathogens, it is crucial to assess the ability of wastewater treatment systems to remove them. Such assessments are made difficult by pathogens only being present in wastewater intermittently and their methods of enumeration being difficult, time-

consuming and expensive (Okoh et al., 2007, Akpor et al., 2014, Nguyen et al., 2014a). Also, given the wide and diverse range of pathogens, monitoring for each one is impractical (Okoh et al., 2007, Alcalde et al., 2012, Olaolu et al., 2014, Dias et al., 2017, Dar et al., 2019). Thus, to allow for the assessment of pathogen inactivation in wastewater treatment systems, organisms that represent the behaviour of the pathogens of interest in the system are measured instead – referred to as either indicator or surrogate organisms (Lucena et al., 2004, Mandilara et al., 2006, Lucena and Jofre, 2010, Dias et al., 2017).

Faecal coliforms, specifically *E. coli*, have long been used as the indicator organisms of choice for pathogen monitoring in wastewater treatment systems (Tree et al., 1997, Molleda et al., 2008, Alcalde et al., 2012, Carducci and Verani, 2013). *E. coli* has been a popular choice because they are ubiquitous in raw wastewater due to being found in great quantities in the human digestive tract; they are easy, rapid and cheap to culture; they are usually non-pathogenic, and they do not replicate in the environment – although recently this has been brought into question (Olaolu et al., 2014, Stratton et al., 2015, Verbyla et al., 2015). Consequently, *E. coli* is included in the *Australian Guidelines for Water Recycling: managing health and environmental risks (Phase 1)* (NRMMC, 2006) as a key parameter to assess wastewater treatment performance and water quality as well as overwhelmingly being the organism used to assess the inactivation of pathogens in HRAPs (Craggs et al., 2012, Sutherland et al., 2017, Young et al., 2017, Fallowfield et al., 2018).

While *E.coli* is still regarded as a useful indicator organism, there has been a movement away from it being used as an absolute indicator for pathogen inactivation in wastewater treatment systems (Salgot et al., 2006, Okoh et al., 2007, Verbyla et al., 2015, Dias et al., 2017). The reason for this is that it is considered a largely ineffective indicator for pathogenic viruses, protozoa and helminths in wastewater treatment systems (Lucena et al., 2004, Yates, 2007, Verbyla and Mihelcic, 2015, Dias et al., 2017). This is based upon the observation that these pathogens behave differently in wastewater treatment systems, in particular, being more resistant to inactivation mechanisms (Tree et al., 2005, Mandilara et

al., 2006, Carducci and Verani, 2013, Kokkinos et al., 2015, Dehghani et al., 2018). Such resistance is a serious issue as viruses are among the most important and potentially most hazardous contaminants in wastewater (Akpor and Muchie, 2011, Akpor et al., 2014, Olaolu et al., 2014, Dias et al., 2017) and protozoa are the parasites most commonly associated with waterborne disease outbreaks in developing countries (Dehghani et al., 2018). Consequently, it has been realised that there is a need for alternative indicators for these organisms (Lucena et al., 2004, Dias et al., 2017).

1.4.1.1. Bacteriophages as indicator organisms for pathogenic viruses in wastewater treatment systems

Bacteriophages have been strongly considered as alternate indicators for assessing the removal of pathogenic viruses by wastewater treatment systems (Costán-Longares et al., 2008, Akpor et al., 2014, Verbyla and Mihelcic, 2015, McMinn et al., 2017). Bacteriophages are viruses that infect bacteria (Campos, 2008, Olaolu et al., 2014, Xagoraraki et al., 2014). They are perceived as the best indicators for pathogenic viruses because they have a similar size, composition, morphology, structure, behaviour and survival characteristics to natural and anthropogenic stressors, particularly when compared to faecal coliforms and *E. coli* (Yahya et al., 2015, McMinn et al., 2017, Dias et al., 2018, Nappier et al., 2019). Additionally, they are easy to culture, usually non-pathogenic and are ubiquitous in wastewaters at reasonably high concentrations independent of origin or location (Mandilara et al., 2006, Yahya et al., 2015, Dias et al., 2018, Lee et al., 2018).

Despite these many positives of bacteriophages as indicators for pathogenic viruses during wastewater treatment, they are not perfect. The most commonly identified problem is that there is not a strong correlation between the removal values for bacteriophages and enteric pathogens (Jofre et al., 2016, Amarasiri et al., 2017, Dias et al., 2018). While this is not ideal, the significance of this has been questioned by both Jofre et al. (2016) and Amarasiri et al. (2017) who point out that the removal values for bacteriophages are almost always

lower than pathogenic viruses and the correlation between bacteriophages and pathogenic viruses is no worse than the correlation between different pathogenic viruses (Jofre et al., 2016, Amarasiri et al., 2017).

The three bacteriophages that are most commonly suggested as indicator organisms for the inactivation of pathogenic viruses in wastewater treatment systems are somatic coliphage, F-specific bacteriophage – including the subtype F-RNA bacteriophage and the strain MS2, and bacteriophage infecting *Bacteroides* spp. (Lucena and Jofre, 2010, Jebri et al., 2012, De Luca et al., 2013, Dias et al., 2018, Lee et al., 2018). Somatic coliphages were initially considered because of the three bacteriophages listed they appear in the highest concentrations in wastewater and are the easiest and cheapest to enumerate (Mandilara et al., 2006, Lucena and Jofre, 2010, Dias et al., 2018). However, they are suspected of replicating in the environment, while pathogenic viruses do not, undermining how well they represent pathogenic viruses (Lucena and Jofre, 2010, Ulbricht et al., 2014). Due to this F-specific bacteriophages and bacteriophages infecting *Bacteroides* spp. were considered because there is no evidence they replicate in the environment (Mandilara et al., 2006, Lucena and Jofre, 2010, De Luca et al., 2013) – although recently the MS2 strain of F-specific bacteriophage has been observed to replicate in synthetic secondary effluent wastewater under laboratory conditions (Voumard et al., 2019). F-specific bacteriophage was considered an ideal indicator organism candidate because of its physical resemblance to many viral pathogens, as well as still being in high concentrations and relatively cheap and easy to culture (Tree et al., 1997, Savichtcheva and Okabe, 2006, Lucena and Jofre, 2010, Lee et al., 2019). Bacteriophage infecting *Bacteroides* spp. were also considered because of their similar physical structure to pathogenic viruses (Verbyla and Mihelcic, 2015). Yet, compared to the other bacteriophage candidates considered those that infect *Bacteroides* spp. have received less attention as indicator organisms, as they appear in lower concentrations in wastewater and are more expensive and difficult to culture (Lucena and Jofre, 2010, Santiago-Rodriguez et al., 2013, Yahya et al., 2015). Additionally, there are

geographical limitations with the *Bacteroides* spp. infecting bacteriophages only being detected in specific locations (Santiago-Rodriguez et al., 2013).

Despite the idea that they potentially replicate in the environment, somatic coliphage and F-specific bacteriophages have still been extensively used – most likely because the significance of their replication has been brought under question (Jofre, 2009, Jofre et al., 2016). Specifically, somatic coliphages have been suggested as the best indicators for activated sludge plants and trickling filters (Dias et al., 2018). While it has been suggested that F-specific bacteriophages are better indicators for chlorination and UV irradiation as well as the MS2 strain being suggested as the best indicator organism for studying sunlight disinfection in WSPs (Verbyla and Mihelcic, 2015, Jofre et al., 2016). Both bacteriophage types have been recommended as the best indicators for membrane bioreactors by different studies (De Luca et al., 2013, Amarasiri et al., 2017). Importantly, the F-specific bacteriophage subtype F-RNA bacteriophage is the recommended indicator for pathogenic viruses in the *Australian Guidelines for Water Recycling: managing health and environmental risks (Phase 1)* (NRMMC, 2006). Considering the above, there currently seems to be no consensus on which of the two bacteriophages is the best indicator overall, with some even suggesting the best option is to use both concurrently (Jofre et al., 2016).

1.4.1.2. Choice of pathogen indicator organisms for the research presented in this thesis

Based on the evidence from previous research and feasibility of various pathogen indicator organisms, it was decided to use *E. coli* as indicators for bacterial pathogens and F-RNA bacteriophages as indicators for viral pathogens. The decision was based on these organisms being the ones recommended in the *Australian Guidelines for Water Recycling:* managing *health and environmental risks (Phase 1)* (NRMMC, 2006). There were two reasons that their inclusion in these guidelines were considered so important: firstly, one of the major advantages of HRAPs over other similar systems is the greater amount of treated

effluent available for reuse; and secondly, all of the research presented in this thesis was conducted in Australia.

1.5. Research aims and directions

It is clear from reviewing the literature that HRAPs are flexible and robust natural wastewater treatment systems that can provide many benefits to communities. Their potential as a system for the coupling of microalgae cultivation for biofuel feed and wastewater treatment has led to a considerable increase in research on the systems in recent years. However, this research has not translated into their wider application, particularly when compared to similar systems such as WSPs. This can partly be attributed to notable absences in the literature. Regarding their use as wastewater treatment systems, notable absences include HRAPs not being present as a wastewater treatment option in any official regulatory guidelines and not having a well-developed and validated pathogen inactivation model. While notable absences in the literature regarding their use as microalgae bioreactors include the assessment of CO_2 enrichment to improve biomass productivity under real-world conditions and the identification of a cost-effective method for harvesting microalgae. Specifically, there is an absence of research in these areas focusing on large-scale, operational HRAPs as well as those servicing communities. This is problematic as the results of laboratory-based experiments sometimes have difficulty translating to real-world systems. Due to this, a strong focus of this thesis was on research involving large-scale, operational HRAPs. While such practical studies as these presented in this thesis are difficult due to the high demand on time and resources, the likelihood of unforeseen complications, and often requiring collaboration across multiple institutions they are paramount if the wider application of HRAPs as wastewater treatment systems and microalgae bioreactors are to be realised.

The overall aim of this thesis was to investigate key factors limiting HRAPs application as wastewater treatment systems and microalgae bioreactors using large-scale, operational systems. More specifically, the thesis aimed to:

- Validate the wastewater treatment performance of a HRAP system for inclusion in the South Australian Community Wastewater Management Scheme (CWMS) as a wastewater treatment system option for rural communities in SA, Australia.

- Develop and validate an initial model for the inactivation of pathogens in HRAPs treating wastewater, which employs inactivation values obtained from independently measured laboratory experiments.

- Asses the effect CO_2 enrichment, via biogas scrubbing, has on the biomass production and wastewater treatment of a HRAP performing tertiary wastewater treatment as part of an existing major wastewater treatment plant.

- Assess the flocculation efficiency of autoflocculation, via magnesium hydroxide precipitation, in a large-scale, operational HRAP treating domestic wastewater.

To achieve these aims, this thesis was divided into four chapters, each addressing an individual aim (Chapter 3 to 6). The first two chapters focus on HRAPs as a wastewater treatment system, with Chapter 3 recounting the independent validation of a HRAP system for inclusion in the CWMS and Chapter 4 describing the development and validation of a mechanistic model for pathogen inactivation in HRAPs. The last two chapters focus on HRAPs as microalgae bioreactors, with Chapter 5 outlining a case study assessing the effect CO_2 enrichment has on the biomass productivity of a HRAP treating wastewater in a major wastewater treatment plant and Chapter 6 detailing the assessment of autoflocculation, via magnesium hydroxide precipitation, as an effective method to harvest microalgae from HRAPs.

CHAPTER 2. GENERAL MATERIALS AND METHODS

This chapter details the general materials and methods used throughout the research presented in this thesis. Specific materials and methods related to each experiment are presented in their corresponding chapter.

2.1. High rate algal ponds

2.1.1. The high rate algal pond system at Kingston on Murray, Australia.

Two identical high rate algal ponds (HRAP) were constructed at Kingston on Murray Wastewater Treatment Plant, Kingston on Murray, Australia (Plate 2.1). They were both single loop raceways 30 m long with flared channels 2.5 m wide at the base. In both HRAPs mixing was carried out by an 8 bladed paddlewheel at a speed to maintain a surface water velocity of 0.2 m s^{-1}. Depth of the HRAPs could be set by adjusting the height of the overflow pipe in the outlet standpipe. As inflow was relatively constant changing depth also changed theoretical hydraulic retention time (THRT) and surface area, with increased depth resulting in increased THRT and surface area.

The HRAPs received wastewater produced by the Kingston on Murray community after it had undergone prior treatment by on-site septic tanks. The community has an estimated population of 300 permanent residents and the typical commercial industries for an Australian rural community of that size. The influent into the ponds was approximately 12 kL d^{-1}, delivered by 6 pumping occurrences. After treatment, the effluent was transferred to an on-site storage pond and subsequently used to irrigate on-site native trees.

Plate 2.1. The high rate algal ponds at Kingston on Murray Wastewater Treatment Plant (Kingston on Murray, Australia)

2.1.1.1. Sampling method used for the high rate algal pond system at Kingston on Murray, Australia.

Inlet and HRAP samples were collected using refrigerated (1^0 C) auto-samplers: Avalanche® Sampler (Teledyne ISCO, Lincoln, USA) and 4700 Refrigerated Sampler (Teledyne ISCO, Lincoln, USA). When necessary grab samples were collected manually from the inlet. All collected samples were stored in an enclosed Esky at 1°C in the dark during transport.

2.1.2. The high rate algal pond system at Melbourne Water Western Treatment Plant, Werribee, Australia

Two identical HRAPs were constructed at the Melbourne Water Western Treatment Plant, Werribee, Australia (Plate 2.2). They were both single loop raceways 30 m long with flared channels 2.5 m wide at the base. In both HRAPs mixing was carried out by an 8 bladed paddlewheel at a speed to maintain a surface water velocity of 0.2 m s^{-1}. The HRAPs received wastewater from metropolitan Melbourne that had undergone prior treatment by the on-site covered anaerobic, aerated and facultative lagoons. Treated effluent from the HRAPs was returned to the raw wastewater inlet, at the head of the treatment plant, for processing through the normal treatment train. Operational depth of the HRAPs could be adjusted between 0.2 to 0.4 m using a height adjustable riser, while the operational THRT of each HRAP could be set between 2 to 8 d by adjusting gate valves on the inlet pipes.

The covered anaerobic lagoon produces biogas which is captured and used by an on-site AGL Power Plant. Before the biogas can be used it needs to undergo scrubbing to remove CO_2, which reduces energy efficiency, and H_2S, which is corrosive. The effluent from the facultative lagoon is used for scrubbing the biogas, and this is the same effluent that feeds into the HRAPs. One of the HRAPs was designed and built so that the influent feeding into it could be switched between just the facultative lagoon effluent or the same facultative lagoon effluent that had been used for scrubbing the biogas and was consequently enriched with CO_2.

Plate 2.2. The high rate algal ponds at the Melbourne Water Western Treatment Plant (Werribee, Australia)

2.1.2.1. Sampling method used for the high rate algal pond system at Melbourne Water Western Treatment Plant, Werribee, Australia

Sampling of the HRAPs was carried out using programmed auto-samplers (Teledyne ISCO, Lincoln, USA). Grab samples of the inlets were taken when personnel were on-site usually twice a week. Samples analysed by Flinders University were biologically inactivated by reducing their pH to 2 through the addition of 5 mL of sulphuric acid (1 M). When it was expected acid stabilisation would have a significant effect on the result, analyses were performed on non-acidified samples by the ALS Group Environmental Division, Scoresby, Australia – a National Association of Testing Authorities (NATA) accredited laboratory. These samples were collected manually from both inlets and HRAPs with analyses carried out within 24 h.

2.1.2.2. In-situ HRAP wastewater monitoring for the high rate algal pond system at Melbourne Water Western Treatment Plant, Werribee, Australia

The in-situ water temperature (°C) for the HRAPs was measured and recorded using T-TEC 6-3F: temperature data loggers with dual temperature sensors (Temperature Technology,

Adelaide, Australia). The thermistors were placed approximately 15 cm below the water surface.

pH was measured using ABB 4600 transmitter controllers with data collected using T-TEC 6-3F data loggers (Temperature Technology, Adelaide, Australia). All sensors were placed approximately 15 cm below the water surface.

2.2. Biomass measurements

2.2.1. Total suspended solids

The method used to measure total suspended solids (TSS; mg L^{-1}) was Test 2540 D (Total Suspended Solids Dried at 103-105°C) described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). Glass fibre filter papers (90 mm glass; 1.2 µm pore exclusion size) were dried overnight at 105°C and then weighed before filtration. Known volumes of the samples were filtered using a vacuum filtration apparatus. The filters were dried at 105°C for 24 h then weighed. Total suspended solids for the samples were equal to the sum of pre-filtration weight subtracted from the post-filtration weight, adjusted to 1 L volume.

2.2.2. Total suspended solids productivity

The equation used to calculate total suspended solids productivity (g m^{-2} d^{-1}) for each sample is presented in Equation 2.1.

$$Total\ suspend\ solids\ productivity\ (g\ m^{-2}\ d^{-1}) = \frac{(TSS\ x\ V_{pond}) \div THRT}{SA}$$

Where:

TSS = Total suspended solids (g L^{-1})

V_{pond} = Volume of pond (L)

THRT = Theoretical hydraulic retention time (d)

SA =Surface area (m^2)

Equation 2.1. Formula used to calculate total suspend solids productivity (g m^{-2} d^{-1}).

2.2.3. Total carbon, total organic carbon, inorganic carbon and total nitrogen

Total carbon (mg C L^{-1}), total organic carbon (mg C L^{-1}), inorganic carbon (mg C L^{-1}) and total nitrogen (mg N L^{-1}) were measured in each sample pre- and post-filtration using glass fibre filter papers (1.2 µm pore exclusion size). Analyses were performed using the Shimadzu TOC-LSCH analyser (Shimadzu, Kyoto, Japan).

2.2.4. Particulate organic carbon and particulate organic nitrogen

The particulate organic carbon concentration (POC; mg L^{-1}) for each sample was equal to the difference between the total organic carbon concentration (mg C L^{-1}) of the wastewater pre- and post-filtration. Likewise, the particulate organic nitrogen concentration (PON; mg L^{-1}) for each sample was equal to the difference between the total organic nitrogen (mg N L^{-1}) of the wastewater pre- and post-filtration.

2.2.5. Chlorophyll *a*

The method used to determine chlorophyll *a* was Test 10200 (Chlorophyll – trichromatic method) of *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). 25 mL of each sample was vacuumed filtered in replicate using glass microfiber filter papers (47 mm diameter; 1.2 µm pore exclusion size). The filter papers with the retained solids were folded inwards until they could be easily placed at the bottom of a McCartney bottle. 10 mL of 90% v/v acetone was added to the bottles which were then stored at 4°C in the dark for 24 h. After this period, 1.5 mL of the solution was centrifuged at 3000 g for 10 min. The absorbance of the supernatant was measured in triplicate at three different wavelengths: 664 nm, 647 nm and 630 nm, using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The acetone solution pre-extraction was used as a blank. Chlorophyll *a* absorbance for each sample was calculated using the mean absorbance at each wavelength and Equation 2.2.

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

Where:

$\mathrm{OD}_{664} = \mathrm{Absorbance\ at\ 664\ nm}$

$\mathrm{OD}_{664} = \mathrm{Absorbance\ at\ 647\ nm}$

$\mathrm{OD}_{664} = \mathrm{Absorbance\ at\ 630\ nm}$

Equation 2.2. Formula used to calculate chlorophyll *a* absorbance.

Chlorophyll *a* concentration was then calculated using Equation 2.3.

$$Chlorophyll\ a\ concentration\ (\mu\ L^{-1})\ =\ Chl.a\ abs.\ \times\ \frac{V_{acetone}}{V_{sample}}$$

Where:

Chl. *a* abs. = Chlorophyll *a* absorbance

$V_{acetone} = \text{Volume of acetone (mL)}$

$V_{sample} = \text{Volume of sample (L)}$

Equation 2.3. Formula used to calculate chlorophyll *a* concentration (µ L^{-1}).

2.2.6. Turbidity

The method used to measure turbidity (NTU) in the samples was Test 2130 B (Nephelometric Method) described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). Method 750 on a DR/2000 Spectrophotometer (Hach, Loveland, USA) was used for spectrophotometric readings with reverse osmosis filtered water used as a blank.

2.3. Nutrient analysis

2.3.1. 5-day biochemical oxygen demand

OxiTop® Biological Oxygen Demand Instrumentation (Xylem Analytics, Germany) was used to measure 5-day biochemical oxygen demand (BOD_5; mg BOD_5 L^{-1}). This method is based on the same principles as Test 5210 B (5-Day BOD Test) *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). The method involved filtering specified sample volumes based on expected BOD_5 concentration using glass fibre filter papers (90 mm diameter; 1.2 µm pore exclusion size). The filtrate was transferred into an amber sample bottle, a magnetic flea was added, then a sleeve containing two NaOH pellets was placed in the opening, followed by an OxiTop®-C being screwed on. The sample bottles were incubated in the dark in a Thermostatic cabinet (TS 606 G/2; WTW) for 5 d at 25°C. After incubation BOD_5 was measured using the OxiTop® OC 100 Controller.

2.3.2. Ammonium

The method used to measure ammonium (mg NH_4-N L^{-1}) in the samples was Test 4500-NH_3 H (Phenate Method) described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). Samples were filtered using glass fibre filter papers (90 mm diameter; 1.2 µm pore exclusion size) before measurements were undertaken using a Foss Fiastar 5000 Analysis System (Foss Pacific Pty Ltd, Mulgrave, Australia).

2.3.3 Nitrite/nitrate

The method used to measure nitrite/nitrate (mg NOx-N L^{-1}) in the samples was Test 4500-NO_3 F (Cadmium Reduction Method) described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). Samples were filtered using glass fibre filter papers (90 mm diameter; 1.2 µm pore exclusion size) before measurements were undertaken using a Foss Fiastar 5000 Analysis System (Foss Pacific Pty Ltd, Mulgrave, Australia).

2.3.4. Orthophosphate

The method used to measure orthophosphate (mg NH_4-N L^{-1}) in the samples was Test 4500-P D (Stannous Chloride Method) described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). Samples were filtered using glass fibre filter papers (90 mm diameter; 1.2 µm pore exclusion size) before measurements were undertaken using a Foss Fiastar 5000 Analysis System (Foss Pacific Pty Ltd, Mulgrave, Australia).

2.3.5. Calculation of nutrient removal

Nutrient removal for each parameter was calculated by subtracting the value measured in the HRAP from the value measured in the corresponding inlet collected at the time closest to the HRAP sample.

2.4. Microbiological analysis

2.4.1. *Escherichia coli* quantification

All measurements of *Escherichia coli* were performed using Colilert Quanti-Tray® (IDEXX Laboratories, Inc. Westbrook, USA). When required, dilutions were performed using sterile 0.1% buffered peptone water (Oxoid Ltd.). Results were reported as $\log_{10}$ *E. coli* Most Probable Number 100 mL^{-1} ($\log_{10}$ *E. coli* MPN 100 mL^{-1}).

2.4.2. F-RNA bacteriophage quantification

All measurements of F-RNA bacteriophage were performed using a double layer agar plaque assay method based on methods described by Debartolomeis and Cabelli (1991) and Noble et al. (2004). For assay preparation, 5 mL of each sample was added to 5 mL of 1.5% tryptone soya agar (Oxoid Ltd.) containing 1% of ampicillin/streptomycin stock and 10% host *E. coli* Famp (ATCC # 700891). The host *E. coli* had been grown for 24 h in 10 mL of tryptone soya broth (Oxoid Ltd.) at 37°C. The tubes were mixed by gentle inversion and poured onto a base agar layer of 1.5% TSA and ampicillin/streptomycin antibiotic stock (1%). Plates were gently swirled and allowed to set for 10 min before incubated for 24 h at 37°C. Plaques were enumerated and expressed as $\log_{10}$ plaque forming units per 100 mL ($\log_{10}$ PFU 100 mL^{-1}). When required, dilutions of the sample were performed before analysis using 0.5% sterile tryptone water (Oxoid Ltd.)

2.4.3. Calculation of log_{10} reduction values

HRAP disinfection performance was reported as log_{10} reduction values (LRV) for both *E.* coli and F-RNA bacteriophage. LRVs for both organisms were calculated using Equation 2.4.

$$Log_{10}\ reduction\ value\ of\ the\ organism = C_{final} - C_{intial}$$

Where:

$C_{final} = \log_{10}$ of the final concentration of the organism

$C_{inital} = \log_{10}$ of the initial concentration of the organism

Equation 2.4. Formula used to calculate log_{10} reduction values.

2.5. Statistical analysis

Statistical analyses were performed using Microsoft Office Excel 2016, IBM SPSS Statistics 23 (IBM Corp., 2015), Matlab and R Statistical Software (R Core Team, 2014) with the additional package Rcmdr (Fox, 2005). Unless stated otherwise, statistical significance for all analyses was accepted at $p<0.05$.

Graphs were created using Microsoft Office Excel 2016, IBM SPSS Statistics 23 (IBM Corp., 2015), Matlab, Graph Pad™ prism 5.0 (Graph Pad Software Inc. USA) and R Statistical Software (R Core Team, 2014) with the additional package ggplot2 (Wickham, 2009).

CHAPTER 3. INDEPENDENT VALIDATION AND REGULATORY AGENCY APPROVAL FOR HIGH RATE ALGAL PONDS TO TREAT WASTEWATER FROM RURAL COMMUNITIES

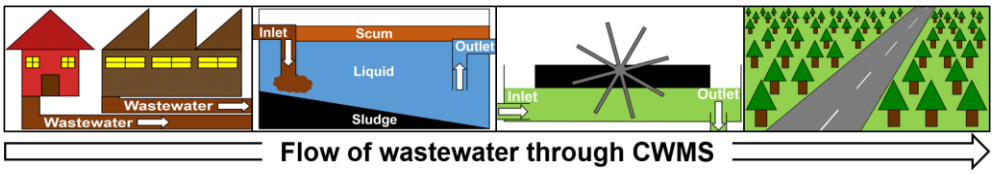
The following chapter is a published journal article authored by Professor Howard Fallowfield, Paul Young, Dr Michael J. Taylor, Dr Neil Buchanan, Professor Nancy Cromar, Dr Alex Keegan and Dr Paul Monis in *Environmental Science: Water Research & Technology,* published 8 November 2017, Volume 2018:4, Pages 195-205 (Appendix A.2). Reproduced by permission of The Royal Society of Chemistry. The published version of the article can be found at https://pubs.rsc.org/en/content/articlehtml/2018/ew/c7ew00228a.

This was a jointly authored publication with the data collected by the FUSA and AWQC. Paul Young and Dr Neil Buchanan were responsible for the data collected by FUSA while the data collected by AWQC was attributed to Dr Paul Monis and Dr Alex Keegan. Paul Young performed data analysis in discussion with Professor Howard Fallowfield, Dr Paul Monis and Dr Michael Taylor. Manuscript writing and editing was performed by Paul Young with Professor Howard Fallowfield.

Given the demonstrated advantages high rate algal ponds (HRAP) have over similar systems, it is surprising they have been largely ignored when installing new wastewater treatment systems. This is likely due, in part, to their omission as a wastewater treatment system option in any official regulatory guidelines resulting in them being overlooked for systems already accepted in the guidelines. This chapter recounts the only independent validation of a HRAP system for inclusion as a wastewater treatment system option in official regulatory guidelines, the South Australian Community Wastewater Management Scheme. Additionally, the use of refrigerated auto-samplers as an alternative to traditional grab sampling during the validation of rural wastewater treatment systems was assessed.

3.1. Table of contents entry

This is the first validation of a HRAP accepted by a regulatory agency and resulted in the system being incorporated into the South Australian Community Wastewater Management Scheme – depicted below.



3.2. Abstract

Despite the many recognised benefits, the application of high rate algal ponds (HRAP) to manage wastewater treatment in small communities has been limited. To be incorporated into the South Australian Community Wastewater Management Scheme (CWMS), new wastewater treatment systems are required to undergo validation and obtain regulatory approval from the South Australian Department of Health, Wastewater Management Group. A HRAP system at Kingston on Murray, Australia, underwent validation to be incorporated into the CWMS. The process was consistent with the Australian National Guidelines, which requires the demonstration of the $\log_{10}$ reduction values (LRV) for indicator organisms achieved by the wastewater treatment system. These were required to be measured twice weekly, over a 10-week period in below average solar radiation and temperature conditions, by an independent National Association of Testing Authorities accredited laboratory. The Australian Water Quality Centre was commissioned to assess the removal of *Escherichia coli*, F-RNA bacteriophage and aerobic spore-forming bacteria (ASFB). Flinders University of South Australia concurrently monitored the removal of the same organisms and other standard wastewater parameters. While ASFB were shown to be unsuitable indicators of protozoa in natural pond systems, the system effectively removed *E. coli* and F-RNA bacteriophage with the treated effluent meeting the limits set by the guidelines for effluent

reuse for non-food crop irrigation: a 5th percentile LRV of >1.0 for F-RNA bacteriophage and a median *E. coli* concentration of <4.0 $\log_{10}$ *E. coli* MPN 100 mL^{-1}. Based on these results, two configurations of HRAP systems were approved to be incorporated into the CWMS.

3.3. Water impact statement

HRAPs occupy less surface area and have lower capital costs than other pond systems. Communities lacking centralised sewage systems are often in water-scarce regions – shorter HRAP retention times and consequently reduced evaporation increases effluent volume for reuse. The validation of these systems by a regulatory agency legitimises them as alternatives to other pond systems, facilitating more wide-scale application of HRAPs.

3.4. Introduction

In rural South Australian communities, treatment of wastewater is managed by Community Wastewater Management Schemes (CWMS) with the assistance of the Local Government Association of South Australia (LGA SA). As of 2016, 172 CWMS were operating in 45 district councils, treating wastewater from approximately 180 000 individuals or approximately 15% of the South Australian population. Ninety of these were waste stabilisation pond (WSP)-based systems, reflecting a preference for these systems. Drivers for this preference include the limited expertise available to manage, operate and maintain electro-mechanical wastewater treatment systems in these communities; and increasing awareness in rural communities of issues associated with energy supply, cost of operation and associated greenhouse gas emissions.

In CWMS, the first stage of treatment is performed in on-site septic tanks where the bulk solid portion of the waste is settled out and undergoes anaerobic digestion. The treated liquid phase is then reticulated to a centralised WSP system for further treatment before disposal or beneficial reuse. The recommended WSP system configuration comprises five

cells, each with a recommended depth of 1.2 m. The first WSP is a facultative pond, required to have a theoretical hydraulic retention time (THRT) of 36 d, while the remaining four are maturation ponds, operated in series, each having a THRT of 7.5 d. This equates to a recommended total THRT of 66 d for CWMS WSP systems.

In 2009, the Health and Environment Group at Flinders University of South Australia (FUSA) commissioned the construction of a high rate algal pond (HRAP) system for research on the treatment of wastewater at the Kingston on Murray CWMS. The initial aims of the project were: to compare the treatment performance of a CWMS WSP system with the HRAP at Kingston on Murray; determine the optimum operating conditions to maximise HRAP performance; and to provide criteria for HRAP design and operation in South Australia. This research showed that in comparison to a CWMS WSP operated at Lyndoch, Australia, the HRAP at Kingston on Murray achieved *Escherichia coli* die-off rates and 5-day biochemical oxygen demand (BOD_5) removal rates four to six times higher and ammonia removal rates eight to seventeen times higher with at least 50% less evaporative losses (Buchanan et al., 2011, Buchanan, 2014) This reduction in treatment time reduces area requirement and consequently construction costs, while the reduced evaporative loss means more water is available for beneficial reuse in water-scare regions, such as rural Australia (Young et al., 2017).

After establishing the many benefits HRAPs provide over WSPs, approval for HRAPs to be included as an alternative treatment option to WSPs in the CWMS design guidelines was sought from the South Australian Department of Health, Wastewater Management Group (DoHWMG). The validation process required for approval is consistent with the *Australian National Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1)* (NRMMC, 2006), which employ the concept of disability-adjusted life years (DALYs) with the tolerable risk accepted as 10^{-6} DALYs per capita per year, equivalent to an annual risk of diarrhoeal illness of 1 per 1000 people. The public health risk associated with exposure to waterborne pathogens in treated wastewaters intended for disposal or reuse are managed

by health-based performance targets derived from the guidelines to ensure the tolerable risk is not exceeded. The initial concentration of the organisms in the wastewater, data relating to their passage through components of the wastewater treatment train, the frequency of exposure and likely ingestion volume associated with the reuse water are considered in the derivation of the target $\log_{10}$ reduction values (LRV) of indicators for bacterial, viral and protozoan pathogens. The treated wastewater from CWMS is most commonly used to irrigate non-food crops, typically woodlots. The target LRVs for this reuse application for enteric organisms are 5.0 for viruses, 4.0 for bacteria and 3.5 for protozoa, with an additional treated wastewater quality objective of a median concentration of <4.0 $\log_{10}$ *E. coli* 100 mL^{-1} (NRMMC, 2006). A minimum 5th percentile of 1.0 $\log_{10}$ reduction of viruses is required following treatment since on-site controls can contribute further to exposure reduction. *E. coli* and F-RNA bacteriophage were used as indicators for pathogenic bacteria and viruses as recommended by the guidelines (NRMMC, 2006). Following consultation with DoHWMG, aerobic spore-forming bacteria (ASFB) were chosen as indicators for pathogenic protozoa.

The validation took place between 1 August and 10 October 2013. It was required to be carried out in below average solar radiation and temperature conditions with twenty inlet and twenty outlet samples taken over 10 weeks, with the 5th percentiles of the LRVs used as the performance values for the validation. This sampling strategy is employed to reflect the worst-case scenario when determining system performance. It was also a requirement for validation that sample collection and microbiological analysis be conducted by a National Association of Testing Authorities (NATA) accredited laboratory. Consequently, the Australian Water Quality Centre (AWQC), South Australian Water Corporation was engaged by the LGA SA to undertake this analysis. This involved the manual collection of inlet and outlet samples over the 10-week period, followed by laboratory analysis of the samples within 24 hours of collection. Concurrently during the validation, FUSA employed an auto-sampler to collect composite treated wastewater samples, which were stored at 1°C before retrieval and microbiological analysis comparable to that conducted by the AWQC. The

required validation of wastewater treatment systems in rural and remote communities is logistically difficult and expensive. Uniquely, this validation enabled comparison and evaluation of two different sampling strategies, daily ‘grab’ sampling versus composite daily sampling and refrigerated storage.

3.5. Material and methods

3.5.1. Wastewater treatment plant site

The HRAP system was operated by FUSA. It consisted of two HRAPs operated in series at the Kingston on Murray wastewater treatment site (34°14'34.1"S 140°19'48.7"E). The HRAPs were both single loop, HDPE sheet lined raceways, each 30 m long with a single channel width of 2.5 m. Within the HRAPs, wastewater was circulated at a mean surface velocity of 0.2 m s^{-1} by an 8 blade, stainless steel paddlewheel. Over the course of the validation, both HRAPs were operated at a depth of 0.30 m, with a surface area of 200 m^2 and a THRT of 5 d.

The first HRAP in the series (HRAP1) received septic tank-treated domestic wastewater produced by the South Australian rural town Kingston on Murray. The town had a population of approximately 300 permanent residents, with the usual variety of commercial activities associated with a small rural Australian town, as well as a school and a seasonal backpacker hostel. Wastewater depth within HRAP1 was controlled by a calibrated ultrasonic depth sensor (U-Gage, Banner Engineering Corp., Minneapolis, USA) activating a submersed pump which transferred the wastewater from HRAP1 into the second HRAP in the series (HRAP2). The treated effluent from HRAP2 was pumped, again under ultrasonic depth control, to the storage pond before discharge via an irrigation system.

Wastewater inflow into HRAP1 was monitored via Mag-Flow meters (ABB Ltd, Zurich, Switzerland) installed on both the HRAP inlet and outlet pipes. Over that period the average

daily inflow was 12.13 m^3 d^{-1}, with a minimum of 6.8 m^3 d^{-1} and a maximum of 18.9 m^3 d^{-1}. The observed variation in the daily flows was due to the fluctuations in the population of the township and was not subject to a regular, predictable pattern. The mean daily flow was 12 m^3, consistent with the long-term average. The daily inflow comes from a central pumping station in the township, which is activated and deactivated by float switches. The height between the activating and deactivating float switches was set so that each pumping consisted of approximately 2000 L delivered over 20 minutes (100 L min^{-1}). Theoretically, the pump was set to activate 6 times per day. In practice, the pump was activated in clusters, typically 2 pump activations in the morning, another in the early afternoon, 2 more activations in the evening and a final activation just after midnight.

3.5.2. Sampling strategy

Sampling was carried out between 1 August and 10 October 2013 during below average solar radiation and temperature conditions. Grab samples from both HRAPs and the inlet were collected on Monday and Thursday of each week at approximately 7 am and shipped immediately on ice by road freight to Adelaide for analysis. Samples were processed within 5-8 hours of collection by the AWQC laboratories. FUSA collected samples over the same period as the AWQC. Effluent samples from the two HRAPs were collected twice daily, at 3 am and 3 pm, by a refrigerated (1°C) auto-sampler, (Avalanche® Sampler, Teledyne ISCO, Lincoln, USA). The two samples collected each day formed a daily composite sample (1 L). The results for these samples were considered an average over the day. The median sample storage time in the auto-sampler at 1°C was 12.5 d (range 8-14 d). To obtain a fresh sample of wastewater entering the pond, during every visit to retrieve the samples taken by the auto-sampler, a single wastewater grab sample (1 L) was taken from the inlet when the septic tank effluent was pumped from the transfer station into the pond. After the samples had been retrieved, they were transported refrigerated at 1 °C in the dark and analysed

within 24 h.

3.5.3. Microbiological analysis

3.5.3.1. Enumeration of *E. coli*

100 mL of each sample were analysed for *E. coli* using AWQC NATA accredited methods. A defined substrate medium (Colilert, IDEXX Laboratories, Inc. Westbrook, USA) was used for the detection and enumeration of *E. coli*, following Australian Standard AS 4276.21-2005: *Water Microbiology – Examination for coliforms and Escherichia coli – Determination of most probable number (MPN) using enzyme hydrolysable substrates* (Standards Australia, 2005).

FUSA quantified *E. coli* for each sample using a single Colilert Quanti-Tray® (IDEXX Laboratories, Inc. Westbrook, USA) according to the manufacturer's instructions. The values were reported as *E. coli* Most Probable Number (MPN) 100 mL^{-1}.

3.5.3.2. F-RNA bacteriophage enumeration

F-RNA bacteriophage quantification was performed by AWQC using 1 mL of each sample employing a plaque assay, according to the methodology described in Appendix D of the *UV Disinfection Guidance Manual* (US EPA, 2006).

F-RNA bacteriophage quantification was carried out at FUSA using a double layer agar plaque assay method (Debartolomeis and Cabelli, 1991, Noble et al., 2004). Duplicate 5 mL aliquots were used for each HRAP sample. 1 mL of each inlet sample was diluted in 9 mL of tryptone water (Oxoid Ltd), which was divided into 5 mL aliquots, both of which were enumerated.

3.5.3.3. Aerobic spore-forming bacteria enumeration

AWQC enumerated ASFB using an in-house method. 100 mL of each sample was heat treated at 80°C for 12 min, followed by serial dilution and membrane filtration (0.45 µm pore size) of 100 mL of sample. The organisms retained on the filter were cultured on tryptone soy agar at 30°C for 42-50 hours. Confirmation of colonies as *Bacillus sp.* was by Gram staining.

To enumerate ASFB, FUSA used the filtration and pasteurisation method described in Young et al. (2016), which was adapted from Rice et al. (1996).

3.5.4. Wastewater analysis

3.5.4.1. Biochemical oxygen demand

BOD_5 was measured using an OxiTopControl OC 100 controller (Xylem Analytics, Germany) following incubation in the dark at 25°C using OxiTop-C measuring heads in accordance with the manufacturer's instructions (WTW, 2006). The BOD_5 concentration was expressed as mg BOD_5 L^{-1}.

3.5.4.2. Suspended solids

Suspended solids were determined for each sample as described in Test 2540 D of *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992).

3.5.4.3. Turbidity

All samples were tested using the nephelometric method described in Test 2130 B (Nephelometric Method) of *Standard Methods for the Examination of Water and Wastewater*

(Greenberg et al., 1992). A Hach DR/2000 (Hach, Loveland, USA) was used for spectrophotometric readings and reported in nephelometric turbidity units (NTU).

3.5.4.4. Chlorophyll *a*

All samples were tested using the spectrophotometric method described in Test 10200 (Chlorophyll – trichromatic method) of *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). A Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) was used for spectrophotometric readings.

3.5.5. Environmental parameters

Data on solar irradiance experienced by the HRAP system at Kingston on Murray over the validation period was collected as the mean daily global solar exposure (kWh m^{-2}) from the weather station at Kingston on Murray, Australia (34.22° S, 140.34° E; Bureau of Meteorology). This weather station was ~3 km away from the HRAP system.

Daily minimum air temperature (°C) and maximum air temperature (°C) over the validation period were collected from the weather station at Renmark Aerodrome, Australia (34.20° S, 140.68° E) (Bureau of Meteorology). This weather station was ~34 km away from the HRAP system.

3.5.6. Log_{10} reduction value calculations

The LRVs of the indicator organisms for each of the HRAPs were equal to the difference between the log_{10} concentration of the organisms entering each HRAP and the log_{10} concentration of the organisms leaving each HRAP. The LRVs of the indicator organisms for the combined HRAP treatment were equal to the difference between the log_{10} concentration

of the organisms entering HRAP1 and the $\log_{10}$ concentration of the organisms leaving HRAP2.

3.5.7. Statistical analysis

Statistical analysis and graphical preparation were carried out using Analyse-it for Microsoft Excel (version 2.20; Analyse-it Software, Ltd, http://www.analyse-it.com/, 2009); R statistical software (R Core Team, 2012) with the additional packages rcmdr (Fox, 2005) and ggplot2 (Wickham, 2009); and IBM SPSS Statistics 23 (IBM Corp., 2015)

Microbiological results from each laboratory were statistically compared where the sampling regimes aligned. All data sets were tested for normality using Shapiro-Wilk test for normality (Table 3.7). Data sets found to be normally distributed were analysed using independent-samples *t*-test for equality of means while those found to violate normality were compared using independent-samples Mann-Whitney U test. Significance was tested to the 0.05 level for all statistical comparisons.

3.6. Results and discussion

3.6.1. Prevailing weather conditions during the validation period

'Natural' wastewater treatment systems, which are largely dependent upon prevailing weather conditions for their effectiveness, are required to be validated when solar irradiance and temperature, the main contributors to pathogen inactivation and algal growth in HRAPs, are low (Goldman, 1979, Fallowfield and Garrett, 1985, Grobbelaar, 1991, Maynard et al., 1999, Benchokroun et al., 2003, Bolton et al., 2010). The validation of the HRAP at Kingston on Murray was conducted over 10 weeks in the winter and spring of 2013. During the 10 weeks of validation, the daily mean global solar exposure, 4.23 ± 1.29 kW h m^{-2}, was 15.4%

less than the 2013 daily annual mean of 5.00 kW h m^{-2}, although it increased towards the end of the validation period (Figure 3.1). The mean daily minimum air temperature during the validation was 7.61 ± 4.19°C, 23.13% lower than the annual mean minimum air temperature, 9.9°C, recorded for 2013 (Figure. 3.2). Similarly, the mean daily maximum air temperature, 23.11 ± 5.22°C, during the validation was 10.08% less than the annual mean daily maximum air temperature of 25.7 °C.

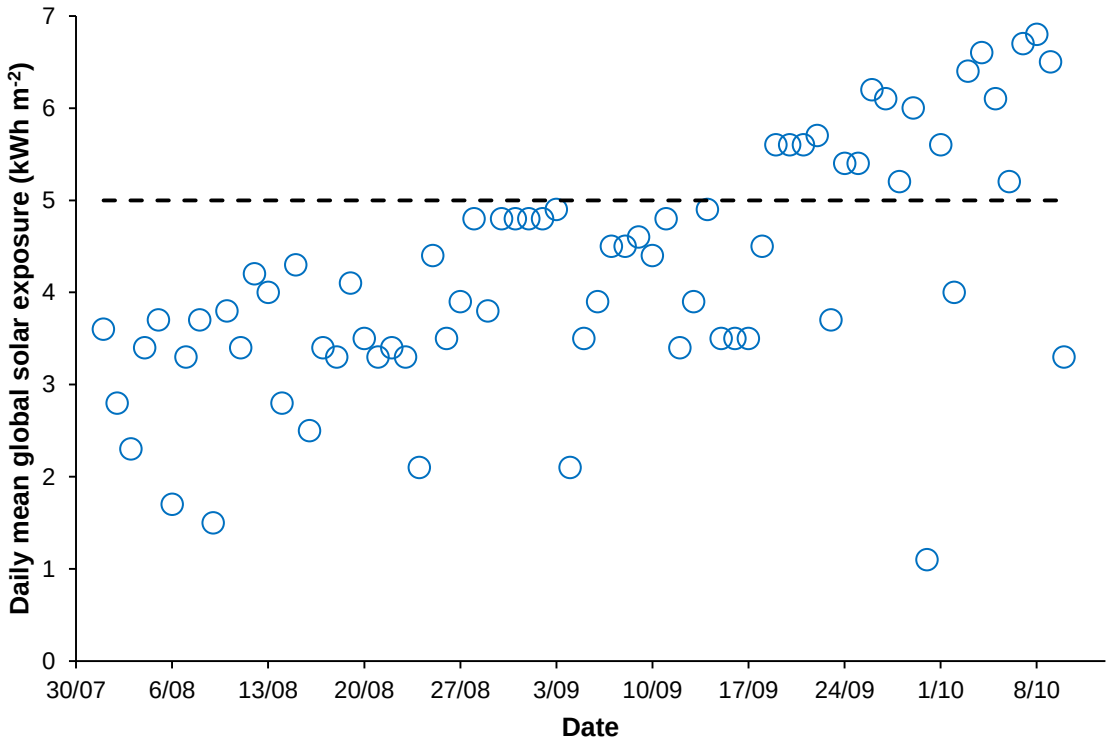


Figure 3.1. Scatterplot of the daily mean global solar exposure (kWh m^{-2}; O) measured by the weather station at Kingston on Murray, Australia, (34.22° S, 140.34° E) between the 1 August and 10 October 2013. Included is the yearly mean of the mean daily global solar exposure (kWh m^{-2}; dashed line) for 2013 measured by the weather station.

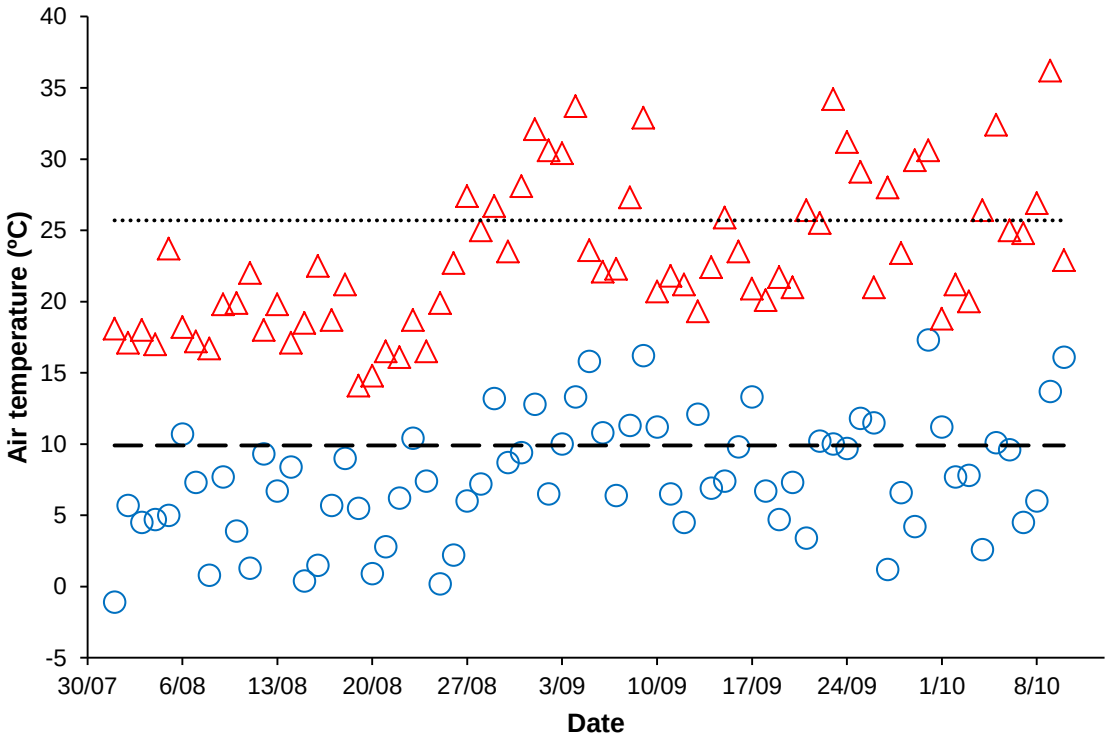


Figure 3.2. Scatterplot of the daily maximum air temperature (°C; Δ) and daily minimum air temperature (°C; O) measured by the weather station at the Renmark Aerodrome, Australia (34.20° S, 140.68° E) between the 1 August and 10 October 2013. Included is the yearly mean of the daily maximum air temperature (°C), 25.70°C, (dotted line) and daily minimum air temperature (°C), 9.90°C, (dashed line) for 2013 measured by the weather station.

3.6.2. Wastewater characteristics during the validation period

The BOD_5, suspended solids and turbidity of the wastewater within HRAPs 1 and 2 during the validation period was typical of that associated with HRAPs treating domestic wastewater. The mean inlet BOD_5 concentration to HRAP1 from the Kingston on Murray septic tanks was 180.83 ± 72.55 mg BOD_5 L^{-1} (Table 3.1). Following treatment in HRAP1, the mean BOD_5 concentration was reduced by 90.6% to 16.95 ± 14.06 mg BOD_5 L^{-1}, which was then the inlet concentration to HRAP2. The mean BOD_5 removal from the inlet wastewater following treatment in HRAP1 was consistent with that reported for longitudinal studies on the same pond, 91.76% and 93.4%, (Buchanan, 2014, Young et al., 2016) and similar to removal rates reported for other HRAPs treating domestic wastewater (Young et al., 2017). Following treatment in HRAP2, the mean BOD_5 concentration at the outlet of HRAP2 was 23.85 ± 10.92 mg BOD_5 L^{-1}, higher than the HRAP2 inlet water supplied from

HRAP1 and independent-samples Mann-Whitney U test showed that this difference was statistically significant ($p = 0.007$; $n = 40$). The most likely reason for the increased BOD_5 concentration in HRAP2 is that the ageing biomass in the pond was degrading and releasing extracellular material into suspension, increasing the organic matter concentration. The median filtered BOD_5 concentration over the 10-week period for HRAPs 1 and 2 compared favourably with the acceptable annual median guideline value of 20 mg BOD_5 L^{-1} (NRMMC, 2006).

The suspended solids concentrations in the HRAPs were slightly less than those reported for HRAPs treating domestic wastewater (Picot et al., 1991, Picot et al., 1992, Chen et al., 2003). The mean suspended solids (mg L^{-1}) concentration of the inlet wastewater to HRAP1 was 56.67 ± 14.17 mg L^{-1}, and biomass production in HRAP1 increased this three-fold to 141.65 ± 59.80 mg L^{-1} (Table 3.1). The suspended solids decreased slightly in HRAP2 to 119.58 ± 42.94 mg L^{-1}, providing supporting evidence that the ageing biomass was degrading.

The mean chlorophyll *a* concentrations of the HRAP wastewaters, a surrogate measure of algal biomass, were similar in the HRAPs: 1.99 ± 1.25 mg L^{-1} in HRAP1 and 1.56 ± 0.86 mg L^{-1} in HRAP2 (Table 3.1). The lower chlorophyll *a* concentration in HRAP2 adds additional supporting evidence that the ageing biomass was degrading. These chlorophyll *a* concentrations were comparable to those reported for other HRAPs treating domestic wastewater (Picot et al., 1991, Picot et al., 1992, Chen et al., 2003).

The mean turbidity of the wastewater in HRAP1, 185.28 ± 60.47 NTU, and HRAP2, 161.54 ± 53.08 NTU, was double that of the original inlet wastewater from septic tanks: 83.67 ± 22.37 NTU (Table 3.1). This increased turbidity from the inlet to the HRAPs was most likely caused by the algal biomass growing in the ponds.

Table 3.1. Characteristics of inlet, HRAP1 and HRAP2 wastewater: Mean, standard deviation, median and number of samples analysed (n) for 5-day biochemical oxygen demand (mg BOD_5 L^{-1}), suspended solids (mg L^{-1}), turbidity (NTU) and chlorophyll *a* (mg L^{-1}) at Kingston on Murray, Australia, between the 1 August and 10 October 2013.

	5-day biochemical oxygen demand (mg BOD_5 L^{-1})			Suspended solids (mg L^{-1})			Turbidity (NTU)			Chlorophyll *a* (mg L^{-1})	
	Inlet	**HRAP1**	**HRAP2**	**Inlet**	**HRAP1**	**HRAP2**	**Inlet**	**HRAP1**	**HRAP2**	**HRAP1**	**HRAP2**
Mean	180.83	16.95	23.85	56.67	141.65	119.58	83.67	185.28	161.54	1.99	1.56
Standard deviation	72.55	14.06	10.92	14.17	59.80	42.94	22.37	60.47	53.08	1.25	0.86
Median	205.5	11	19.5	61	134	125	94	165	163	1.35	1.47
n	6	20	20	6	69	69	3	69	69	69	69

3.6.3. Microbiological validation of HRAP performance

3.6.3.1. Log_{10} reduction values for indicator organisms following treatment in the HRAPs

The mean, median and 5th percentile LRVs for the faecal indicator organisms following treatment in the HRAPs measured by AWQC and FUSA are shown in Tables 3.2 and 3.3 respectively. The temporal variation in LRVs for *E. coli* measured for the HRAPs operated in series over the 10-week validation period is shown in Figure. 3.1. The *E. coli* LRV values ranged between 1.27-5.89 as determined by AWQC and 2.16-4.69 as determined by FUSA. The mean *E. coli* LRV for HRAP1 determined by AWQC was higher than that measured by FUSA, while the opposite was the case for HRAP2. Consequently, there was little difference between the two laboratories' mean LRV for the HRAPs operated in series. The mean LRV measured by AWQC was 3.30 ± 1.28, whereas the mean LRV measured by FUSA was 2.89 ± 0.75 (Figure. 3.3). The regulatory agency, DoHWMG, required determination of 5th percentile values for *E. coli* LRV, which for the HRAPs operated in series, at a combined retention time of 10 d, were 1.82 and 2.0 as determined by AWQC and FUSA respectively.

The mean *E. coli* LRVs for HRAP1 determined for both AWQC and FUSA were similar to those reported for other HRAPs (Davies-Colley et al., 2003, Davies-Colley et al., 2005) Notably, the mean *E. coli* LRVs for HRAP1 operated at a 5 d THRT were similar to the 2.02

± 0.65 LRV reported for the facultative WSP operated at a 27.5 d THRT at the CWMS at Lyndoch, Australia (Buchanan, 2014).

The median concentration of *E. coli* in the effluent following treatment in the HRAPs with a combined THRT of 10 d as measured at 3.13 $\log_{10}$ *E. coli* MPN 100 mL^{-1} by AWQC and 3.30 $\log_{10}$ *E. coli* MPN 100 mL^{-1} by FUSA (Table 3.6).

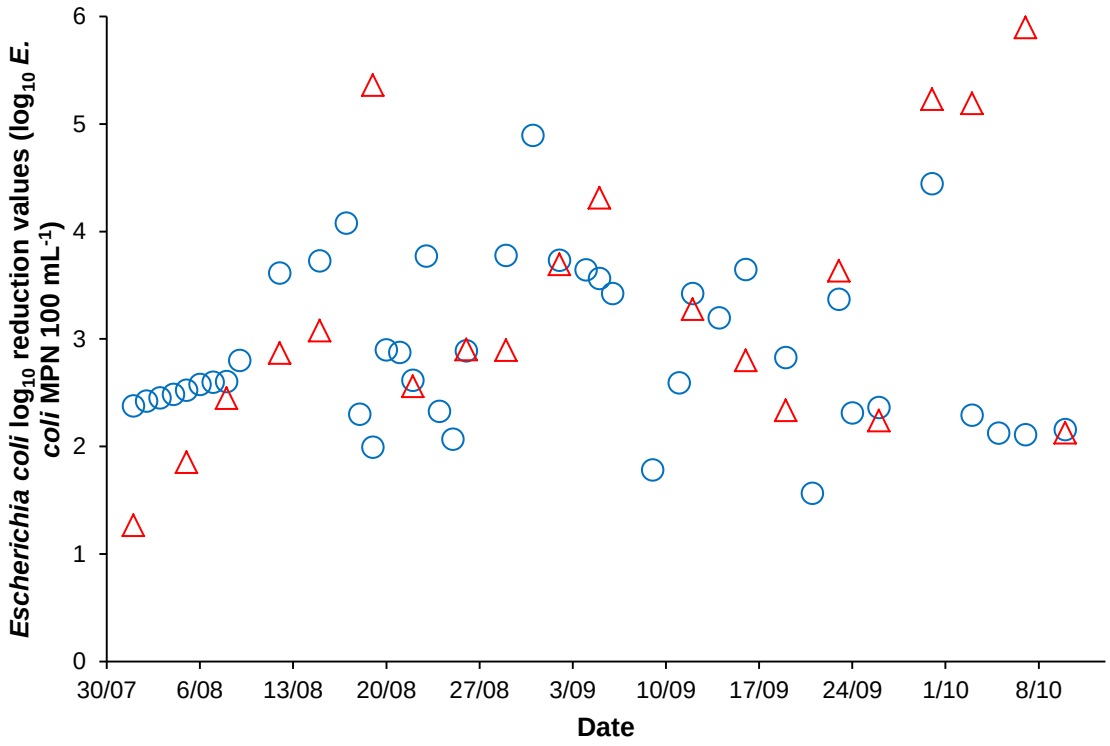


Figure 3.3. Scatterplot of the Australian Water Quality Centre's (Δ) and the Flinders University of South Australia's (O) $\log_{10}$ reduction values of *Escherichia coli* for the HRAPs operated in series at Kingston on Murray, Australia, between the 1 August and 10 October 2013.

The LRVs for F-RNA bacteriophage ranged between 1.61-4.76 as determined by AWQC and 1.13-5.04 as determined by FUSA (Figure 3.4). The F-RNA bacteriophage mean LRVs followed a similar pattern to those determined for *E. coli* with the AWQC derived values for the mean LRV higher for HRAP1 and lower for HRAP2 than those obtained by FUSA. The mean LRVs for F-RNA bacteriophage for the HRAPs operated in series measured by AWQC over the validation period was 2.32 ± 0.74 (Table 3.2) compared with 2.87 ± 0.89 determined

by FUSA (Table 3.3). The 5th percentile LRVs for F-RNA bacteriophage were 1.61 and 1.50 as determined by AWQC and FUSA respectively.

AWQC and FUSA data both showed that the HRAPs consistently inactivated F-RNA bacteriophage over the validation period. There are no data available in the literature for F-RNA bacteriophage inactivation by other HRAPs. Davies-Colley et al. (2005) reported approximately a 1 LRV for somatic phage by a HRAP treating domestic wastewater during summer. An F-RNA bacteriophage LRV of 1.3 has been reported for facultative WSPs with THRT of 18 d (Campos et al., 2002) which compares with the mean 1.17 and 2.25 LRVs determined by AWQC and FUSA for HRAP1 with a 5 d THRT. The mean F-RNA bacteriophage LRVs for HRAP2 were less than the annual mean LRV of 1.72 reported for a pilot maturation WSP (Alcalde et al., 2003); however, the LRV for the WSP reduced to 0.42 when considering only the winter data, less than the LRVs reported for the HRAP (Alcalde et al., 2003).

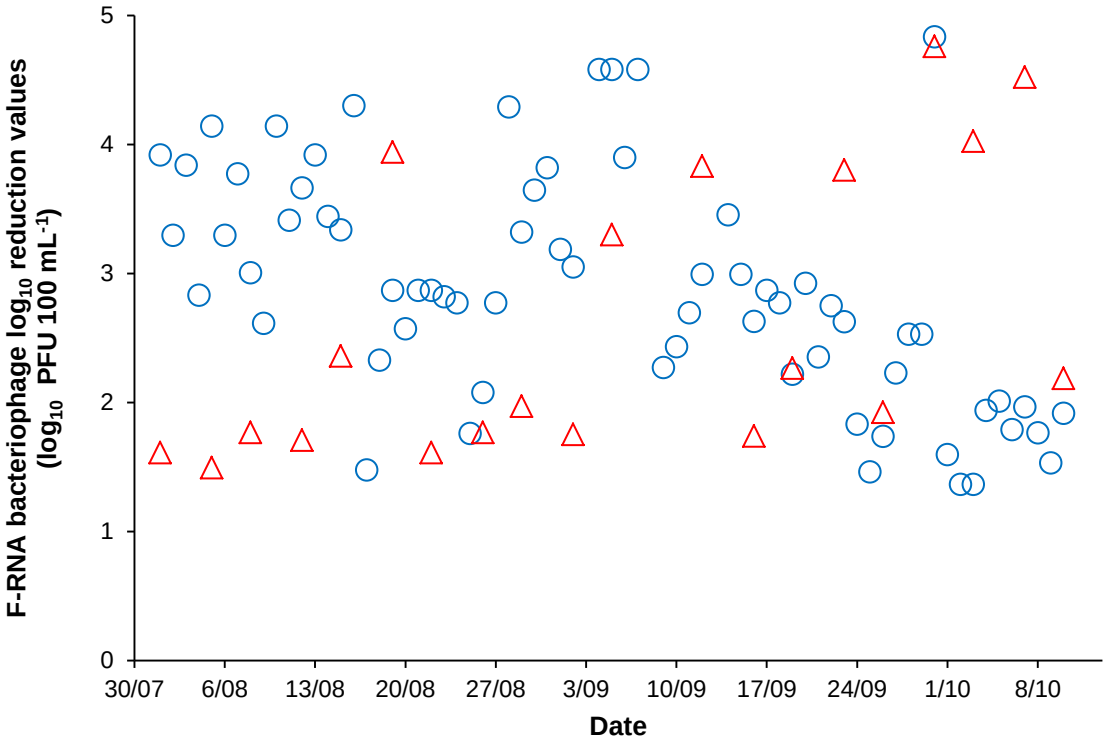


Figure 3.4. Scatterplot of the Australian Water Quality Centre's (Δ) and the Flinders University of South Australia's (O) $\log_{10}$ reduction values of F-RNA bacteriophage for the HRAPs operated in series at Kingston on Murray, Australia, between the 1 August and 10 October 2013.

Overall, the HRAPs showed inactivation of *E. coli* and F-RNA bacteriophage equivalent to those reported for WSPs. However, the inactivation rates were achieved using considerably shorter THRTs than those commonly employed for WSPs. The shorter THRTs reduce both the area requirement and the cost of construction for HRAPs compared to WSPs typically employed in CWMS in rural South Australia.

ASFB were shown to be unsuitable indicators for protozoa in open systems as analysis by both laboratories frequently showed higher concentrations of ASFB in the HRAP's treated effluent than was entering in the influent from septic tanks. Young et al. (2016) proposed the likely causes of increased ASFB in the HRAP effluent were ASFB being transported into the HRAPs by wind-blown soil and/or by propagation of influent spores in the HRAPs triggered by increases in temperature. It was concluded that ASFB were an unsuitable indicator for *Cryptosporidium* spp. and other protozoa in natural pond systems and *E. coli* should be used as an indicator in HRAPs (Young et al., 2016).

Table 3.2. Data collected by the Australian Water Quality Centre: Mean, standard deviation, median, 5th percentile and number of samples analysed (n) of the log_{10} reduction values for *Escherichia coli*, F-RNA bacteriophage and aerobic spore-forming bacteria for HRAP1, HRAP2 considered individually and in series at Kingston on Murray, Australia, between 1 August and 10 October 2013.

	***E. coli* log_{10} reduction values**			**F-RNA bacteriophage log_{10} reduction values**			**Aerobic spore-forming bacteria log_{10} reduction values**		
	HRAP1	**HRAP2**	**In series**	**HRAP1**	**HRAP2**	**In series**	**HRAP1**	**HRAP2**	**In series**
Mean	1.81	1.49	3.30	1.17	1.16	2.32	0.18	-0.24	-0.05
Standard Deviation	0.46	1.21	1.28	0.38	0.73	0.74	0.47	0.29	0.37
Median	1.76	0.93	2.90	1.30	0.88	2.08	0.04	-0.15	-0.20
5th percentile	1.24	0.37	1.82	0.62	0.35	1.61	-0.30	-0.52	-0.40
n	20	20	20	20	20	20	20	20	20

Table 3.3. Data collected by Flinders University of South Australia: Mean, standard deviation, median, 5th percentile and number of samples analysed (n) of the log_{10} reduction values for *Escherichia coli*, F-RNA bacteriophage and aerobic spore-forming bacteria for HRAP1, HRAP2 considered individually and in series at Kingston on Murray, Australia, between 1 August and 10 October 2013.

	***E. coli* log_{10} reduction values**			**F-RNA bacteriophage log_{10} reduction values**			**Aerobic spore-forming bacteria log_{10} reduction values**		
	HRAP1	**HRAP2**	**In series**	**HRAP1**	**HRAP2**	**In series**	**HRAP1**	**HRAP2**	**In series**
Mean	2.00	0.88	2.89	2.25	0.63	2.87	0.07	0.24	0.31
Standard Deviation	0.58	0.52	0.75	0.64	0.72	0.89	0.31	0.24	0.35
Median	1.91	0.86	2.61	2.15	0.42	2.83	0.02	0.24	0.32
5th percentile	1.22	0.13	2.00	1.37	-0.23	1.50	-0.34	-0.01	-0.27
n	42	42	42	67	67	68	57	57	57

The influence of environmental parameters on the LRVs achieved in HRAP1 is explored in more detail in *Inactivation of indicator organisms in wastewater treated by a high rate algal pond system* (Young et al., 2016). This publication details a longitudinal study on HRAP1 disinfection carried out by FUSA between July 2013 to May 2014, of which some of the data presented here is a component. Data presented in both publications includes *E. coli*, F-RNA bacteriophage, ASFB, BOD_5, chlorophyll *a* concentrations in the inlet and HRAP1 as well the LRVs achieved by HRAP1 for all indicator organisms.

AWQC's independent validation data for the HRAP system showed the treated effluent met the limits set by the NRMMC (2006) guidelines for effluent reuse for non-food crop irrigation with a winter 5th percentile LRV of >1.0 for F-RNA bacteriophage and a median *E. coli* concentration of <4.0 log_{10} *E. coli* MPN 100 mL^{-1}. Based on these disinfection results, in

2016, DoHWMG approved a HRAP based system comprising of a single HRAP receiving septic tank effluent operated at depths between 0.3-0.5 m at a 10 d THRT to be an alternative to installing the standard 5 cell 1.2 m deep WSP system with a 66 d THRT when new systems are required. Additionally, based on these results and those in Buchanan (2014), the DoHWMG approved a second configuration of a HRAP based system, one which would replace existing facultative WSPs in need of upgrade with a single HRAP operated at a depth between 0.3-0.5 m at a 5 d THRT, while retaining the traditional in series, 4 cell (30 d THRT) maturation WSPs. The removal of helminths was not considered in the validation since they are not endemic in most parts of Australia. In areas where helminths infections are prevalent, a minimum 25 d total treatment time is required based on the *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1)* (NRMMC, 2006). As such, the configuration approved by DoHWMG to ensure helminth die-off was a 10 d THRT in a HRAP with an additional 15 d THRT in a storage lagoon before discharge or reuse. These design guidelines were published in *Design Guideline for a High Rate Algal Pond (HRAP) – as an Element in Wastewater Treatment Trains*.

3.6.3.2. Comparison between grab and refrigerated auto-sampler sampling methods

This study also enabled comparison between two methods of sampling and subsequent analysis. The mean *E. coli* inlet concentrations measured by the two laboratories were similar with the AWQC reporting a value of 6.19 ± 0.31 $\log_{10}$ *E. coli* MPN 100 mL^{-1} and FUSA reporting a mean of 6.16 ± 0.39 $\log_{10}$ *E. coli* MPN 100 mL^{-1} (Table 3.4). The same value, 5.05 ± 0.50 $\log_{10}$ PFU 100 mL^{-1}, for mean inlet F-RNA bacteriophage concentration was obtained by both AWQC and FUSA, although the median values differed (Table 3.4). Independent samples *t*-test for equality of means was performed between the results obtained by each laboratory for both organisms found that for both *E. coli* ($p = 0.97$; $n = 12$) and F-RNA bacteriophage ($p=0.65$; $n=12$) there was no statistically significant difference between the results. As both laboratories employed grab sampling for the inlet, the results of

the analysis suggest that the analytical methods used by both laboratories for enumeration of these organisms in wastewater were equivalent.

Table 3.4. Mean, standard deviation, median and number of samples analysed (n) of the Australian Water Quality Centre's and Flinders University of South Australia's concentration for *Escherichia coli* (log_{10} *E. coli* MPN 100 mL^{-1}) and F-RNA bacteriophage (log_{10} PFU 100 mL^{-1}) in the inlet wastewater at Kingston on Murray, Australia, between 1 August and 10 October 2013

	***Escherichia coli* concentration (log_{10} *E. coli* MPN 100 mL^{-1})**		**F-RNA bacteriophage concentration (log_{10} PFU 100 mL^{-1})**	
	AWQC	**FUSA**	**AWQC**	**FUSA**
Mean	6.19	6.16	5.05	5.05
Standard deviation	0.31	0.39	0.50	0.50
Median	6.11	6.07	4.95	4.81
n	20	6	20	6

Independent-samples *t*-test for equality of means analysis also showed there was no statistically significant difference between the mean HRAP2 concentrations of *E. coli* as determined by AWQC using grab sampling and FUSA using composite sampling (Table 3.6). The AWQC mean concentration value for *E. coli* was 2.89 ± 1.19 log_{10} *E. coli* MPN 100 $mL^{-1,}$ and the FUSA mean was 3.17 ± 0.72 log_{10} *E. coli* MPN 100 mL^{-1} (p=0.51; n=40). Independent-samples Mann-Whitney U test indicated there was no statistically significant difference between the mean F-RNA bacteriophage concentration in HRAP2 determined by the each of the laboratories (Table 3.6). The F-RNA mean concentration for HRAP2 determined by AWQC was 2.43 ± 1.06 log_{10} PFU 100 mL^{-1} and the mean concentration determined by FUSA was 2.11 ± 0.92 log_{10} PFU 100 mL^{-1} (p=0.19; n=40). Considering the result of the statistical analysis for the inlet samples, the result of the statistical analysis of the HRAP2 samples, which only differed in methodology by FUSA collecting samples by refrigerated auto-sampler, suggests that the different sampling strategies employed did not produce results for the enumeration of either organisms which were statistically significantly different.

Contrasting with the previous results, an independent-samples Mann-Whitney U test indicated there was a statistically significant difference between the results obtained by each laboratory for mean concentration F-RNA bacteriophage in HRAP1 determined by AWQC using grab sampling and FUSA using refrigerated, composite sampling (Table 3.5). The mean F-RNA bacteriophage concentration determined by AWQC was 3.88 ± 0.50 $\log_{10}$ PFU 100 mL^{-1}, and the mean determined by FUSA was 2.74 ± 0.63 $\log_{10}$ PFU 100 mL^{-1} ($p<0.001$; n=40). It is unclear why the result from this statistical analysis differs from the previous results given that all sampling was carried out at the same time, the same sampling strategies were employed, and the same enumeration methods were used for both HRAP1 and HRAP2. Without understanding the cause for this difference, it is difficult to construe the significance, if any, of this result. Independent-samples *t*-test for equality of means analysis suggested there was no statistically significant difference between the *E. coli* concentrations measured by both laboratories in HRAP2. The *E. coli* mean concentration determined by AWQC was 4.38 ± 0.41 $\log_{10}$ *E. coli* MPN 100 $mL^{-1,}$ and the FUSA mean was 4.05 ± 0.54 $\log_{10}$ *E. coli* MPN 100 mL^{-1} ($p=0.07$; n=40). This result provides additional support that the different sampling strategies employed by each laboratory did not affect the microbiological analysis.

Table 3.5. Mean, standard deviation, median and number of samples analysed (n) of the Australian Water Quality Centre's and Flinders University of South Australia's concentration for *Escherichia coli* ($\log_{10}$ *E. coli* MPN 100 mL^{-1}) and F-RNA bacteriophage ($\log_{10}$ PFU 100 mL^{-1}) in the HRAP1 wastewater at Kingston on Murray, Australia, between 1 August and 10 October 2013.

	***Escherichia coli* concentration ($\log_{10}$ *E. coli* MPN 100 mL^{-1})**		**F-RNA bacteriophage concentration ($\log_{10}$ PFU 100 mL^{-1})**	
	AWQC	**FUSA**	**AWQC**	**FUSA**
Mean	4.38	4.05	3.88	2.74
Standard deviation	0.41	0.54	0.5	0.63
Median	4.25	3.95	3.91	2.78
n	20	42	20	67

Table 3.6. Mean, standard deviation, median and number of samples analysed (n) of the Australian

Water Quality Centre's and Flinders University of South Australia's concentration for *Escherichia coli* (log_{10} *E. coli* MPN 100 mL^{-1}) and F-RNA bacteriophage (log_{10} PFU 100 mL^{-1}) in the HRAP2 wastewater at Kingston on Murray, Australia, between 1 August and 10 October 2013.

	***Escherichia coli* concentration (log_{10} *E. coli* MPN 100 mL^{-1})**		**F-RNA bacteriophage concentration (log_{10} PFU 100 mL^{-1})**	
	AWQC	**FUSA**	**AWQC**	**FUSA**
Mean	2.89	3.17	2.43	2.11
Standard deviation	1.19	0.72	1.06	0.92
Median	3.13	3.30	2.75	2.04
n	20	42	20	68

There have been few studies on the dark die-off of *E. coli* in wastewater stored for the length of time utilised during this validation. Mayer et al. (2015) measured dark die-off of *E. coli* in wastewater stored in a refrigerated auto-sampler at 5°C. They reported a dark die-off of approximately 0.8 log_{10} *E. coli* MPN 100 mL^{-1} over 11 d: similar to the mean time, 11.83 d, the samples were left in the auto-sampler before collection during the validation (Mayer et al., 2015). This result is supported by Buchanan (2014) who measured the dark die-off of *E. coli* in wastewater stored at 2.5°C in the laboratory to be approximately 0.8 log_{10} *E. coli* MPN 100 mL^{-1} at 11 d. The significance of these results to what was happening to the organisms in the refrigerated auto-samplers during the validation is unclear, particularly when considering the lower storage temperature used in the validation, 1°C, and the values for the dark die-off of *E. coli* being similar to the standard deviation of the mean concentrations of *E. coli* measured in both HRAPs by each laboratory (Tables 3.5 & Table 3.6).

As the regulator validates new wastewater treatment systems based on final LRVs, the most important result from the statistical analyses was that there was no statistically significant difference between the final LRVs determined by each laboratory for *E. coli* using independent-samples *t*-test for equality of means (p=0.37; n=40) and F-RNA bacteriophage using independent-samples Mann-Whitney U test (p=0.20; n=40).

Validation of wastewater treatment systems in rural and remote communities is a challenging and expensive process. The Kingston on Murray HRAP system was a 500 km round trip from Adelaide, the location of both analytical laboratories. Personnel were required on-site to

conduct manual ‘grab’ sampling twice per week over a 10-week period and to arrange transport on ice to AWQC to enable analysis to be conducted within 24 h of sampling. The use of refrigerated (1°C) auto-samplers to collect and store the samples before retrieval was an alternate approach which may significantly reduce both the cost and logistical complexity associated with the validation of treatment systems in remote locations. Furthermore, application of refrigerated auto-samplers enables samples to be taken more frequently, resulting in a larger dataset for the validation. Further research is required to elucidate the behaviour of organisms stored in dark refrigerated auto-samplers for extended periods, but considering the results of this study, the employment of refrigerated, portable auto-samplers should be considered an economical option for validation of rural wastewater treatment systems.

3.7. Conclusions

To the authors' knowledge, this is the first time the independent validation of a HRAP has been accepted by a regulatory agency. The results from the validation provide robust evidence that HRAPs are an effective alternate treatment option to other conventional natural pond systems, such as WSPs. The results also demonstrated the HRAP treated effluent met the Australian reuse guideline requirements for irrigation of non-food crops. Consequently, HRAPs were approved to be incorporated into the South Australian CWMS as an alternative option to the conventional WSP systems currently used. The comparison between the AWQC and FUSA methodology suggests that refrigerated auto-samplers may present a simpler and cheaper method for monitoring remote wastewater treatment systems.

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3.9. Supplementary material

Table 3.7. Results of Shapiro-Wilk test for normality on concentration and log_{10} reduction values for *Escherichia coli*, F-RNA bacteriophage and aerobic spore-forming bacteria in the inlet, HRAP1, HRAP2 and in series measured by either Australian Water Quality Centre or Flinders University of South Australia at the HRAP system in Kingston on Murray, Australia, between 1 August and 10 October 2013. Results of Shapiro-Wilk test for normality on inlet, HRAP1 and HRAP2 BOD_5 conentration measusered by Flinders University of South Australia concurrently is also included.

Samples	Statistic	Df	Significance
FUSA inlet *Escherichia coli* concentration	.938	6	.644
FUSA inlet F-RNA bacteriophage concentration	.820	6	.089
AWQC inlet *Escherichia coli* concentration	.875	6	.246
AWQC inlet F-RNA bacteriophage concentration	.886	6	.297
AWQC inlet aerobic spore-forming bacteria concentration	.956	6	.789
FUSA HRAP1 *Escherichia coli* concentration	.929	20	.150
FUSA HRAP2 *Escherichia coli* concentration	.980	20	.939
FUSA HRAP1 F-RNA bacteriophage concentration	.977	20	.885
FUSA HRAP2 F-RNA bacteriophage concentration	.965	20	.654
FUSA HRAP1 *Escherichia coli* log_{10} reduction values	.927	20	.138
FUSA HRAP2 *Escherichia coli* log_{10} reduction values	.941	20	.254
FUSA in series *Escherichia coli* log_{10} reduction values	.926	20	.130
FUSA HRAP1 F-RNA bacteriophage log_{10} reduction values	.938	20	.218
FUSA HRAP2 F-RNA bacteriophage log_{10} reduction values	.933	20	.178
FUSA in series F-RNA bacteriophage log_{10} reduction values	.973	20	.823
AWQC HRAP1 *Escherichia coli* concentration	.942	20	.258
AWQC HRAP2 *Escherichia coli* concentration	.914	20	.076
AWQC HRAP1 F-RNA bacteriophage concentration	.950	20	.362
AWQC HRAP2 F-RNA bacteriophage concentration	.899	20	.040
AWQC HRAP1 *Escherichia coli* log_{10} reduction values	.935	20	.193
AWQC HRAP2 *Escherichia coli* log_{10} reduction values	.826	20	.002
AWQC in series *Escherichia coli* log_{10} reduction values	.924	20	.119
AWQC HRAP1 F-RNA bacteriophage log_{10} reduction values	.941	20	.253
AWQC HRAP2 F-RNA bacteriophage log_{10} reduction values	.835	20	.003
AWQC in series F-RNA bacteriophage log_{10} reduction values	.821	20	.002
FUSA inlet BOD_5 concentration	.924	6	.534
FUSA HRAP1 BOD_5 concentration	.755	20	.000
FUSA HRAP2 BOD_5 concentration	.842	20	.004

CHAPTER 4. DEVELOPMENT AND VALIDATION OF A MODEL FOR THE INACTIVATION OF PATHOGENS IN HIGH RATE ALGAL PONDS

The following chapter is written as a journal article to be submitted to *Water Science Technology*. It was authored by Paul Young, Dr Simon Williams, Dr Neil Buchanan, Dr Natalie F. Bolton and Professor Howard Fallowfield.

This was a jointly authored publication with the data collected by Paul Young and Dr Neil Buchanan. Data analysis was performed by Paul Young and Dr Simon Williams. Manuscript writing and editing was performed by Paul Young with Dr Simon Williams and Professor Howard Fallowfield.

With HRAPs having been around since the middle of the 20th century, it is surprising there has only been one study in the literature that modelled pathogen inactivation by them. It is also surprising that the study did not encourage further research into the area with no other studies on modelling HRAP pathogen inactivation being published in the 16 years since. The absence of such models has likely contributed to HRAPs limited application as officials often use models as tools to guide the design and operation of wastewater treatment systems. Considering HRAPs recent acceptance as a wastewater treatment system option in the South Australian Community Wastewater Management Scheme will likely result in their wider application, the development of a new HRAP pathogen inactivation model seems timely. This chapter describes the development and validation of a pathogen inactivation model for HRAPs. It is believed to be the only study in the literature that developed a mechanistic model for pathogen inactivation in HRAPs using laboratory measured solar radiation inactivation values and then validated it using a large-scale, operational HRAP. Additionally, the developed model also provided insight into optimum HRAP operation when intermittent influent feeding is employed, common in rural communities.

4.1. Abstract

Despite being smaller, cheaper to construct and providing more effluent for reuse than waste stabilisation ponds, high rate algal ponds (HRAP) have not been widely applied. This is believed to be due to the lack of models on pathogen inactivation in HRAPs guiding design and operation criteria. A HRAP pathogen inactivation model was developed with inactivation attributed to solar radiation and light-independent processes. The inactivation values used for each mechanism in the model were independently measured in the laboratory. In this study, the inactivation of two indicator organisms, *Escherichia coli* and F-RNA bacteriophage, were modelled. The results of the model were validated using a large-scale, operational HRAP. The model predicted concentrations and measured concentrations for all comparisons were well fitted. Paired t-test comparisons supported these fits, reporting that for all but one comparison the model predicted concentrations and measured concentrations did not differ significantly. These results confirm the model was well designed and further development is warranted. The model also provided guidance on HRAP operation, showing that when intermittent feeding of the influent is employed no more than 4% of the pond volume should be introduced over a period no longer than 4% of the theoretical hydraulic retention time.

4.2. Keywords

Escherichia coli; F-RNA bacteriophage; high rate algal ponds; indicator organisms; pathogen inactivation; wastewater treatment

4.3. Introduction

High rate algal ponds (HRAP) are natural wastewater treatment systems ideal for low-income rural and peri-urban regions where the more complex and expensive wastewater treatment systems used in urban regions are unsuitable (Garcia and Bécares, 1997, Palmer

et al., 2001). They differ from the most prominent natural wastewater treatment systems, waste stabilisation ponds (WSP), by being comparatively shallow, raceway systems mixed by a paddlewheel (Picot et al., 1992, Buchanan et al., 2018a). The shallow ponding and mixing maximises the solar exposure experienced by the pond volume, improving treatment efficiency by promoting the microalgal-bacterial assimilation and degradation of nutrients and the solar disinfection of pathogens (Craggs et al., 2004, Young et al., 2016). The improved treatment efficiency means HRAPs can perform equal wastewater treatment to WSPs in significantly shorter theoretical hydraulic retention times (THRT) (Picot et al., 1992, Buchanan et al., 2018b). This shortened THRT reduces the standing pond volume, which subsequently reduces the size of HRAPs by making them viable in areas where land availability prevents the use of WSPs (Fallowfield et al., 1996, Buchanan et al., 2018b). The reduced volume of HRAPs also results in a smaller surface area which, in combination with their shorter THRT, results in them experiencing reduced evaporative losses compared to WSPs (Young et al., 2017). This leads to more effluent being available for reuse which is significant in many arid and semi-arid regions that rely heavily on wastewater reuse to meet water needs (Qadir and Mwachiro, 2017, Young et al., 2017). Additionally, the reduced size of HRAPs means less earthworks are required for construction compared to WSPs, thus reducing construction costs and subsequent green-house gas emissions produced via earthworks (Young et al., 2017).

With these advantages over WSPs, it is surprising that the uptake of HRAPs as a wastewater treatment system has been limited (Fallowfield et al., 2018). A likely hindrance to their use is the relative lack of models on their wastewater treatment performance, particularly when compared to the number of models available for WSPs (Sah et al., 2012). Models are a useful tool to describe and understand wastewater treatment systems at a minimal cost (Fallowfield et al., 1992, Alvarado et al., 2012, Sah et al., 2012). They can provide guidance for developing design and operational criteria, particularly when considering new environmental conditions (Sah et al., 2012, Butler et al., 2017). They are

also able to elucidate previously overlooked or unknown research areas (Fallowfield et al., 1992). For these reasons well developed, flexible models that have undergone validation are strong drivers for the implementation and use of wastewater treatment systems.

The majority of models on wastewater treating HRAPs have mainly focused on algae cultivation (Buhr and Miller, 1983, Fallowfield et al., 1992, Bello et al., 2017) with relatively few considering wastewater treatment performance (Craggs et al., 2004, Yang, 2011). This predilection is most likely caused by the considerable interest in using HRAPs as a bioreactor to grow microalgae for biofuel production (Sutherland et al., 2017, Young et al., 2017). While this application of wastewater treating HRAPs is important and should be explored it should not come at the expense of research into their wastewater treatment performance, particularly as coupling microalgae cultivation with wastewater treatment is currently considered the most economically viable way to produce microalgae for biofuel production using HRAPs (Lundquist et al., 2010). Another reason there should be more of a focus on modelling HRAP wastewater treatment performance is it can help predict and ensure effluent quality, which is vital as one of the major advantages HRAPs have over other systems is the increased effluent available for reuse (Garcia and Bécares, 1997, Young et al., 2017). Ensuring effluent quality is especially important if it is to be used for irrigation of food crops or will be in close proximity to people, common both in water-scarce and low-income regions, as residual pathogens pose a significant risk to human and environmental health (Fallowfield et al., 1996, Qadir and Mwachiro, 2017).

Despite its apparent importance, there has only been one published study modelling disinfection in HRAPs (Craggs et al., 2004). The study developed a simple model for *Escherichia coli* disinfection based on results measured in a pilot-scale HRAP which was part of a trial Advanced Pond System on a dairy farm at Anchor Products Hautapu, New Zealand. The HRAP was operated at a depth of 0.2 m and a surface area of 37.5 m^2. It received dairy farm effluent that had already undergone treatment in an anaerobic pond. To allow for easier monitoring of *E. coli,* the HRAP was operated in batch mode at a THRT of 2

d. The model developed treats the HRAP as a complete-mix reactor with a dark die-off rate derived from the measured night-time inactivation rates, and a solar radiation inactivation rate derived from the measured daylight inactivation rates which had been corrected for dark die-off. The model was well-fitted to the measured data with removal occurring rapidly during daylight hours and slowly overnight. Through developing and analysing this model, Craggs et al. (2004) elucidated valuable insights into HRAP disinfection. Probably the most important were that solar radiation was responsible for approximately 75% of *E. coli* disinfection in the HRAP and that pH and dissolved oxygen (DO), traditionally considered contributors to disinfection in natural wastewater treatment systems (Fallowfield et al., 1996, Sah et al., 2012), have little influence on disinfection within the ranges used in the study, 8.0-9.2 pH and 0-22 g m^{-3} DO. Despite these valuable insights, some improvements could be made to the model. Firstly, the model developed treats the HRAP as a batch system which is not practical when considering the continuous stream of wastewater experienced in most systems. Secondly, the model only considers the disinfection of *E. coli*, which while being the most common indicator organism for water quality and pathogenic bacteria (Campos, 2008, Lucena and Jofre, 2010), is generally not considered a suitable indicator for other pathogenic organisms, particularly pathogenic viruses and protozoa (Campos, 2008, Lucena and Jofre, 2010).

The aim of this study was to develop and validate an initial model for the inactivation of pathogens in HRAPs treating wastewater, which employed inactivation values obtained from independently measured laboratory experiments. It was believed that developing an inactivation model in such a way would make it more flexible and broadly applicable. The authors are unaware of any other study in the literature that developed such a model and then validated it using a large-scale, operational HRAP. It is also to the authors' knowledge the first study to model the inactivation of a viral indicator organism in a HRAP.

4.4. Methods

4.4.1. Model development

The model treats the passage of the indicator organisms through the HRAP as a mass balance. Initially, the organisms enter the systems at a set concentration in a set influent volume and rate of addition. The concentration of the measured organisms is then adjusted hourly accounting for decreases caused by inactivation. The concentration of the organisms in the influent, as well as the influent volume and rate of addition, are all able to be adjusted to match the system being modelled.

Like other models developed for HRAPs, the model presented in this publication treats HRAPs as a complete-mix reactor (Craggs et al., 2004) with negligible settling (Jupsin et al., 2003). Consequently, the model considers the HRAP environment to be homogenous with all the pond volume experiencing the same level of solar radiation exposure and all organisms spending equal time at all depths.

The inactivation mechanisms used in this model are solar radiation and light-independent processes, commonly referred to as dark die-off. Solar radiation was chosen as a mechanism as it is widely considered the major contributor to pathogen inactivation in natural wastewater treatment systems (Craggs et al., 2004, Davies-Colley, 2005, Young et al., 2016). Light-independent processes were included in the model because they are considered to have a significant influence on overall pathogen inactivation in natural wastewater treatment systems (Craggs et al., 2004, Davies-Colley, 2005). To model the inactivation effects of solar radiation and light-independent processes, inactivation values measured independently in the laboratory were used. The solar radiation mechanism in the model was comprised of three environmentally relative wavelengths, ultraviolet B (UVB), ultraviolet A (UVA) and visible light (Vis) (Lian et al., 2018), with inactivation values independently measured for each wavelength. Light-independent processes were assigned

a singular inactivation value that was also measured independently. Ideally, measurements across all inactivation mechanisms were made in identical water matrices. The total inactivation of the model was equal to the sum of all the solar radiation mechanisms and the light-independent process.

The organisms modelled in this study were *E. coli*, the traditional indicator organism for pathogens in wastewater (Davies-Colley, 2005, Campos, 2008), and F-RNA bacteriophage, a common indicator for the viral pathogens in wastewater (Tree et al., 2005, Campos, 2008, Young et al., 2016, Hawley and Fallowfield, 2018). The inactivation values used in the model for these organisms are presented in Table 4.1.

Table 4.1. Ultraviolet B (UVB), ultraviolet A (UVA) visible light (Vis) and light-independent inactivation rates (k (h^{-1})) for *Escherichia coli* and MS2. The *E. coli* inactivation rates were measured (Bolton et al 2012) in buffered reverse osmosis water while the MS2 inactivation rates were measured in buffered, filtered (0.2 µm) wastewater that had already undergone treatment at Mount Barker wastewater treatment plant.

Organism and water matrix	Wavelength	Inactivation rate (k h^{-1})	Water temperature (°C)	pH	Dissolved oxygen (mg L^{-1})	n
E. coli in RO water	UVB	2.78	20	9.5	8.5	8
	UVA	0.188	20	9.5	8.5	12
	Vis	0.125	20	9.5	8.5	8
	Light-independent	0.001	20	9.5	8.5	12
MS2 in wastewater	UVB	0.289	20	9.5	8.5	14
	UVA	0.119	20	9.5	8.5	14
	Vis	0.047	20	9.5	8.5	8
	Light-independent	0.03	20	9.5	8.5	14

Attenuation affects light penetration and consequently the depth of influence the solar radiation mechanisms have in the system (Curtis et al., 1994, Hawley and Fallowfield, 2018). The depth of influence each wavelength had in the model was determined by premeasured attenuation values. The attenuation values used in this model run are presented in Table 4.2. The mechanism of light-independent processes was treated as if it influenced the entire depth of the model.

Table 4.2. Mean attenuation (m) for each of the wavelengths, Ultraviolet B, Ultraviolet A and Visible

light, comprising solar inactivation in the model. Measurements were made in the high rate algal at Kingston on Murray, Australia (Bolton, 2012).

	Attenuation (m)			Turbidity (NTU)	Chlorophyll *a* (μg L^{-1})	Total suspended solids (mg L^{-1})
	UVB	UVA	Vis			
n	6	6	6	4	5	5
Mean	0.1	0.13	0.53	115.4	1169.2	73.0
Standard deviation	0.03	0.04	0.18	121.2	2366.3	66.8

The length of time the model experiences solar radiation was set at a predetermined value. For the model runs presented in this study the period of solar exposure was set at 12 h. The effect of light-independent processes in the model was treated as a constant in the model. The model was created using MATLAB statistical software (The MathWorks, Inc.).

4.4.2. Model validation

4.4.2.1. High rate algal pond

The model was validated using a high-density polyethylene lined, single loop HRAP, 30 m long with a single channel width of 2.5m, located in rural South Australia (34°14′34.1″S 140°19′48.7″E). During model validation, the HRAP was operated at a depth of 0.3 m and hydraulic retention time (THRT) of 5 d. Wastewater within the HRAP was circulated at a mean surface velocity of 0.2 m s^{-1} by an 8 blade, stainless steel paddlewheel. The HRAP was intermittently fed septic tank treated wastewater produced by the rural community Kingston on Murray, Australia. The community has an approximate population of 300 permanent residents with a school, a seasonal backpacker hostel and other common commercial activities associated with a rural Australian town. Over a 24 h period, there were typically six inlet pumping events each delivering approximately 2 m^3 of wastewater to the HRAP, resulting in an approximate average daily inflow of 12 m^3 d^{-1}.

4.4.2.2. Sampling strategy

HRAP samples were collected automatically using a 4700 Refrigerated Sampler (1^0 C; Teledyne ISCO, Lincoln, USA) while inlet samples were also collected automatically using a refrigerated Avalanche® Sampler (1^0 C; Teledyne ISCO, Lincoln, USA). The sample volume collected for both the HRAP and inlet was 800 mL.

F-RNA bacteriophage samples for both the HRAP and inlet wastewater were collected at two-hour intervals to observe fluctuations in concentration throughout a day. *E. coli* samples for the inlet were also collected every two hours while HRAP samples were collected at two, four and six hours. Sampling programs were carried out numerous times and at different months throughout the year. This was done to determine a mean concentration for both the organisms in the HRAP and inlet.

4.4.2.3. *Escherichia coli* enumeration

E. coli quantification was carried out using Colilert Quanti-Tray® (IDEXX Laboratories, Inc. Westbrook, USA) according to the manufacturer's instructions. Inlet samples were diluted $10^{-3,}$ and HRAP samples were undiluted. The values were reported as *E. coli* Most Probable Number (MPN) 100 mL^{-1}.

4.4.2.4. F-RNA bacteriophage enumeration

F-RNA bacteriophage quantification was carried out using the double layer agar plaque assay method described in Fallowfield et al. (2018). Duplicate 5 mL aliquots were used for each HRAP sample. One millilitre of each inlet sample was diluted in 9 mL of tryptone water (Oxoid Ltd), which was divided into 5 mL aliquots, both of which were enumerated.

4.4.2.5. Total suspended solids

Total suspended solids (mg L^{-1}) were measured for all HRAP samples using Test 2540 D (Total Suspended Solids Dried at 103-105°C) described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992).

4.4.2.6. Prevailing weather conditions

Data on the solar radiation and ambient temperature experienced by the HRAP system at Kingston on Murray during the sampling periods were collected from an Australian Bureau of Meteorology operated weather station ~3 km away (34.22° S, 140.34° E). Solar radiation was reported as daily global solar exposure (kWh m^{-2}), and ambient temperature was reported as daily maximum and minimum air temperature (ºC).

4.4.2.7. Statistical analysis

Statistical analysis was carried out using MATLAB statistical software (The MathWorks, Inc.) and Microsoft Excel. The model predicted and HRAP measured organism concentrations were compared using running paired t-tests with the level of significance set at 0.05. All graphs were made using MATLAB statistical software (The MathWorks, Inc.).

4.5. Results and discussion

4.5.1. Model development

From the discussion earlier, it can be seen that each of the inactivation mechanisms have a different rate (Table 4.1) and operate over differing depth scales (Table 4.2). They also have a time-dependent boundary condition because of the light activated nature of the inactivation mechanisms.

It is assumed a pond of depth, d, with total volume, V. The decay rate for the light-independent processes is k_d. An initial concentration of organisms in the pond of ρ_0. In darkness, the model for the concentration of the organisms in the pond $\rho(t)$ is:

$$\frac{d\rho}{dt} = -k_d\rho, \qquad \rho(0) = \rho_0$$

which has the solution

$$\rho(t) = \rho_0 \exp\{-k_d t\},$$

the standard exponential decay.

Now light actuation is introduced. It is assumed light is available for l hours in a 24 h period starting at time t_0. Assuming S inactivation modes operating at depths d_j with rates k_j, then the total solar radiation inactivation rate k is

$$k = \sum_{i=0}^{S} \frac{\min(d, d_i)}{d} k_i$$

now the day-night concentration formula, $\rho_{DN}(t)$ is given by

$$\rho_{DN}(t) = \rho_0 e^{-k_d t} \times \left(1 + e^{-kt} \sum_{j=0}^{\infty} \big(u(t - t_0 - 24j) - u(t - t_0 - 24j - l)\big)\right)$$

where *u* is the step function. In reality, this simply increases the decay rate from k_d to $k_d + k$ during daylight hours.

To model different inflow regimes, we denote I the total inflow at each of the N pumpings in 24 h. Then the final model $\rho_F(t)$ is

$$\rho_F(t) = \rho_{DN}(t) + \sum_{p=1}^{\infty} \frac{I}{V} e^{-k_d(t-pN)} \left(1 + e^{-k(t-pN)} \sum_{n=0}^{\infty} u(t - t_0 - 24\text{j}) - u(t - t_0 - 24j - l\right)$$

Figure 4.1 shows an example for *E. coli* with the three solar radiation inactivation vectors UVB, UVA and Vis operating to depths of 0.1 m, 0.13 m and 0.53 m with rates of 2.78 h^{-1},

0.188 h^{-1} and 0.125 h^{-1}, respectively. The inactivation rate for light-independent processes was 0.001 h^{-1}. The theoretical HRAP was 0.3 m deep with a THRT of 5 d and a total volume of 60 m^3. It received 12 $m^3 d^{-1}$ of wastewater in 6 pumping events of 2 m^3.

From Figure 4.1, it can be seen the unusual dependence of the inactivation. Instead of a steady decay over the nominal residence time, we see a steep decay, driven by the high daytime inactivation rate, to a steady state because of the restocking of the pond with organisms by pumping. The initial time is after pumping at night-time when the decay rate is slowest. In order for the pond to remain stable, we need

$$\rho_f(t_n) - \rho_f(t_n - 24) < 0$$

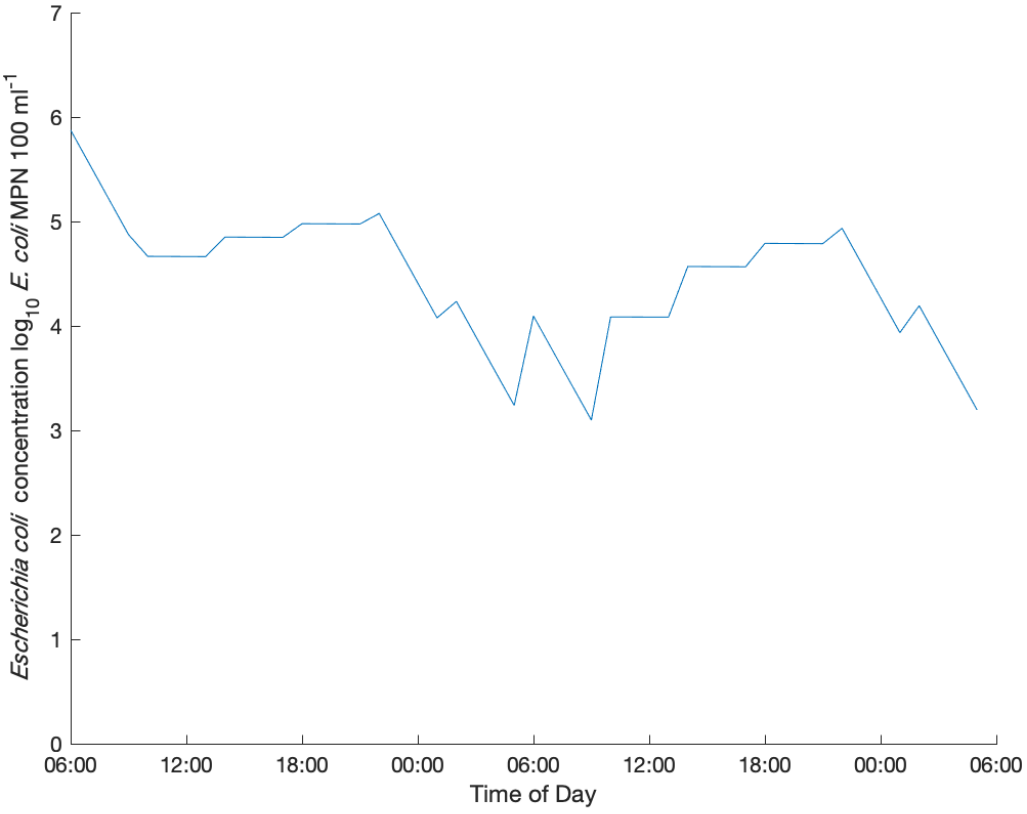

Figure 4.1. Example of model predicted *Escherichia coli* concentrations (log_{10} *E. coli* MPN 100 mL^{-1}) in a theoretical high rate algal pond over a 48 h.

4.5.2. Model validation

Validation of the model was carried out by comparing the model predicted concentrations for the organisms with the concentrations of the organisms measured at the Kingston on Murray HRAP.

Intensive HRAP *E. coli* concentration data presented in the figures was collected by Dr Neil Buchanan.

4.5.3. *Escherichia coli*

The model predicted inactivation for *E. coli* was produced using inactivation rates measured in RO water (Table 4.1) and attenuation values measured in a HRAP (Table 4.2). The reason these attenuation values were used despite being measured in a different water matrix is so the model can provide the most accurate representation of what is occurring in the real-world HRAP. While there was not perfect symmetry, there was a good fit between the model predicted concentrations and the measured concentrations of *E. coli* for all sampling periods (Figure 4.2-4.4).

Running paired sample t-tests showed there was no statistically significant difference between the model predicted concentration and the actual measured concentration for all data sets with Figure 4.2-4.4 having p-values of 0.0623, 0.6011 and 0.2159, respectively. This is despite differences in mean daily global solar exposure and, maximum and minimum air temperatures.

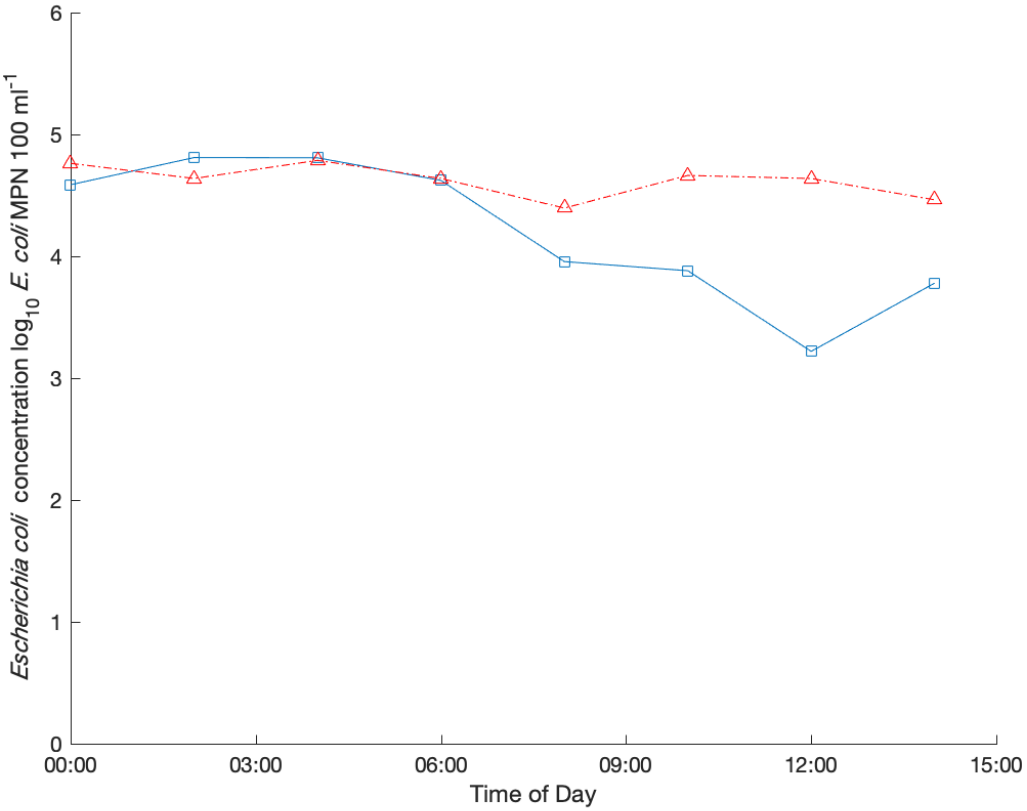

Figure 4.2. Comparison between the *Escherichia coli* (log_{10} *E. coli* MPN 100 mL^{-1}) model predicted concentrations (□) and the concentrations measured in the high rate algal pond at Kingston on Murray, Australia, over a 14 h period on 6 May 2010 (△).

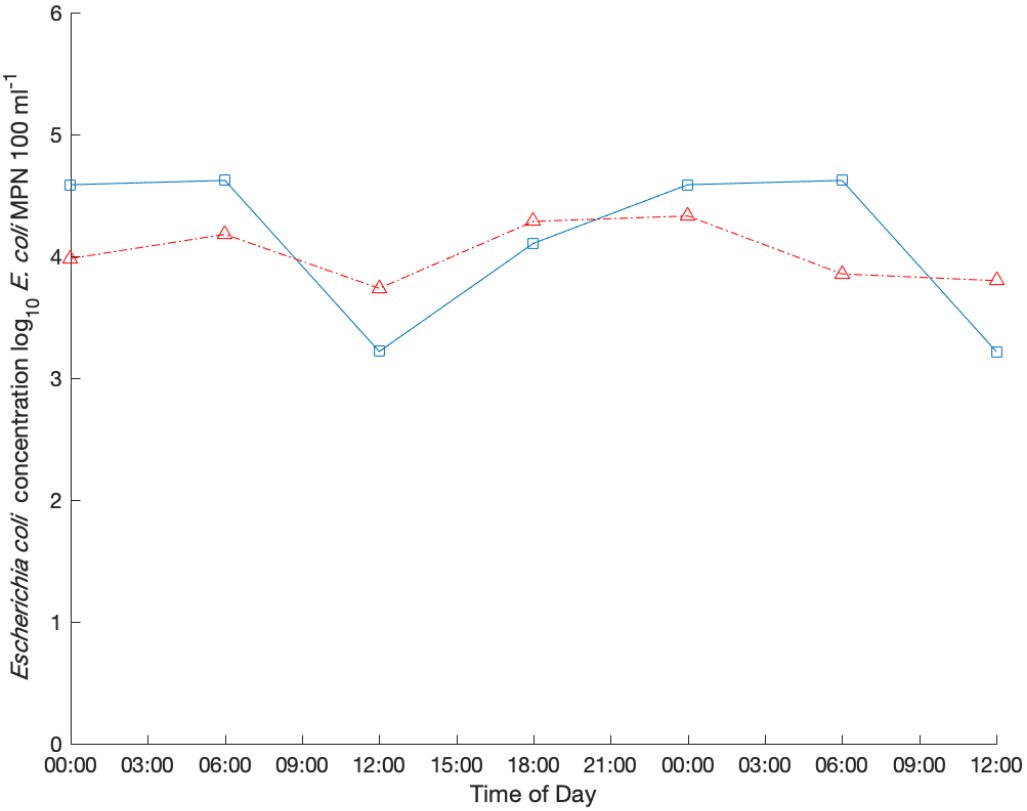

Figure 4.3. Comparison between the *Escherichia coli* (log_{10} *E. coli* MPN 100 mL^{-1}) model predicted concentrations (□) and the concentrations measured in the high rate algal pond at Kingston on Murray, Australia, over a 36 h period between 4-5 June 2010 (△).

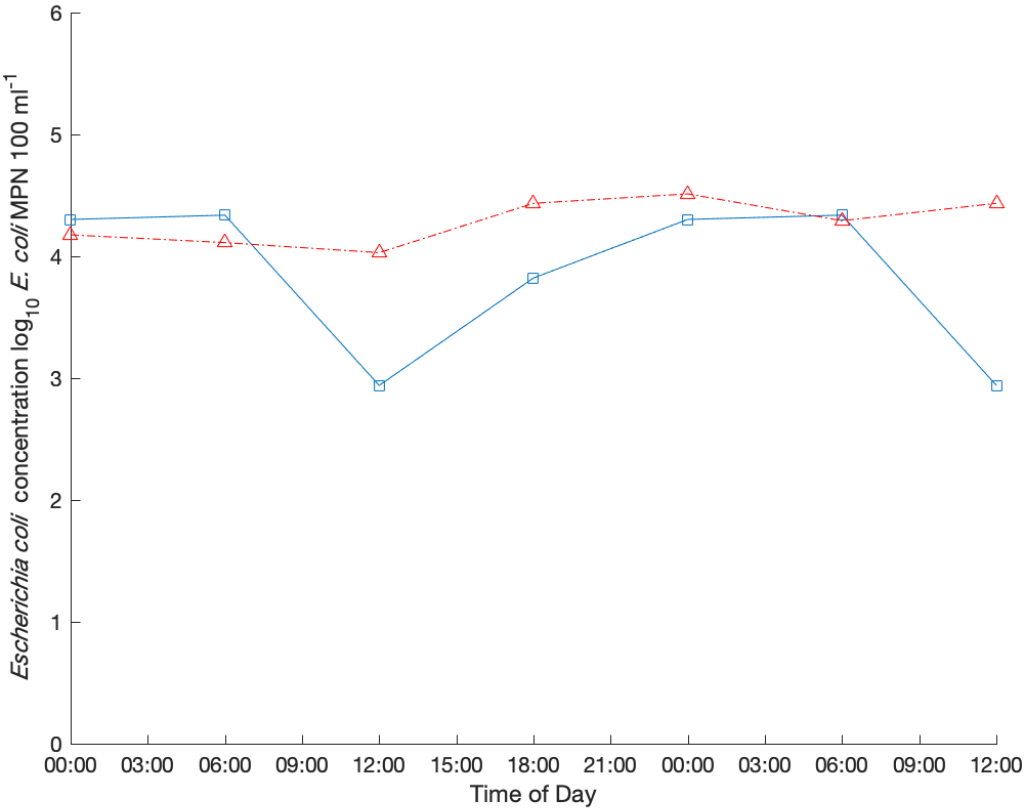

Figure 4.4. Comparison between the *Escherichia coli* (log_{10} *E. coli* MPN 100 mL^{-1}) model predicted concentrations (□) and the concentrations measured in the high rate algal pond at Kingston on Murray, Australia, over a 36 h period between 6-7 June 2010 (△).

4.5.4. F-RNA bacteriophage

Additional complexity was added when modelling F-RNA bacteriophage inactivation. The model predicted inactivation for F-RNA bacteriophage was produced using inactivation rates for MS2, an F-RNA bacteriophage, measured in treated wastewater that had been buffered and filtered (0.2 µm). This model run used attenuation values previously measured in a HRAP (Table 2). This is believed to be a more accurate representation of what is occurring in a real-world HRAP.

Running paired sample t-tests showed that the model predicted concentrations and the measured concentrations for F-RNA bacteriophage were not significantly different for the 11 August 2015 ($p=0.8473$; Figure 4.5) and the 15 September 2015 ($p=0.6527$; Figure 4.6) data sets, but were significantly different for the 25 August 2015 data set ($p<0.001$; Figure 4.7).

There was no apparent reason for this data set to be different from the others as there was little difference between weather conditions and total suspended solids.

The fit between the model predicted concentrations and the actual measured concentrations for F-RNA bacteriophage were once again not identical but followed a similar trend. This was even true for the data set where the difference was statistically significant. Interestingly, the measured concentrations of the organism experienced less variation than the model predicted data. This perhaps indicates that HRAPs are more resilient to 'shock' loading than commonly believed.

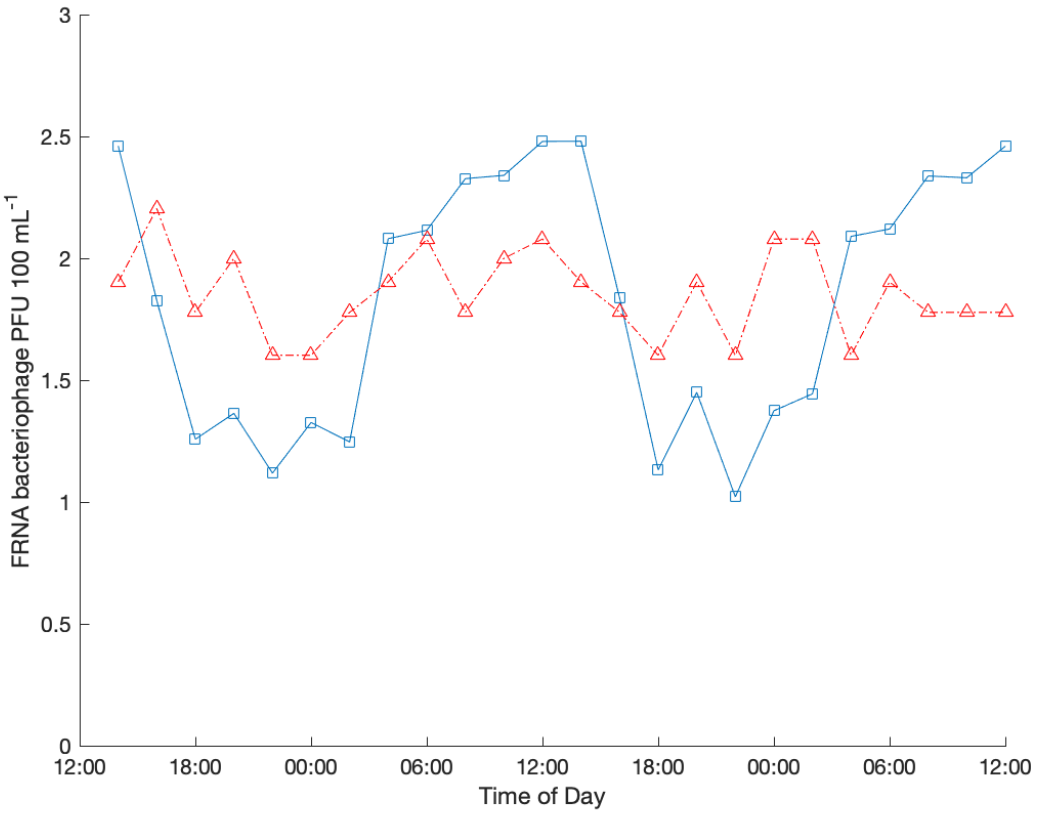

Figure 4.5. Comparison between the F-RNA bacteriophage (log_{10} PFU 100 mL^{-1}) model predicted concentrations (□) and the concentrations measured in the high rate algal pond at Kingston on Murray, Australia, over a 48 h period between 11-13 August 2015 (△).

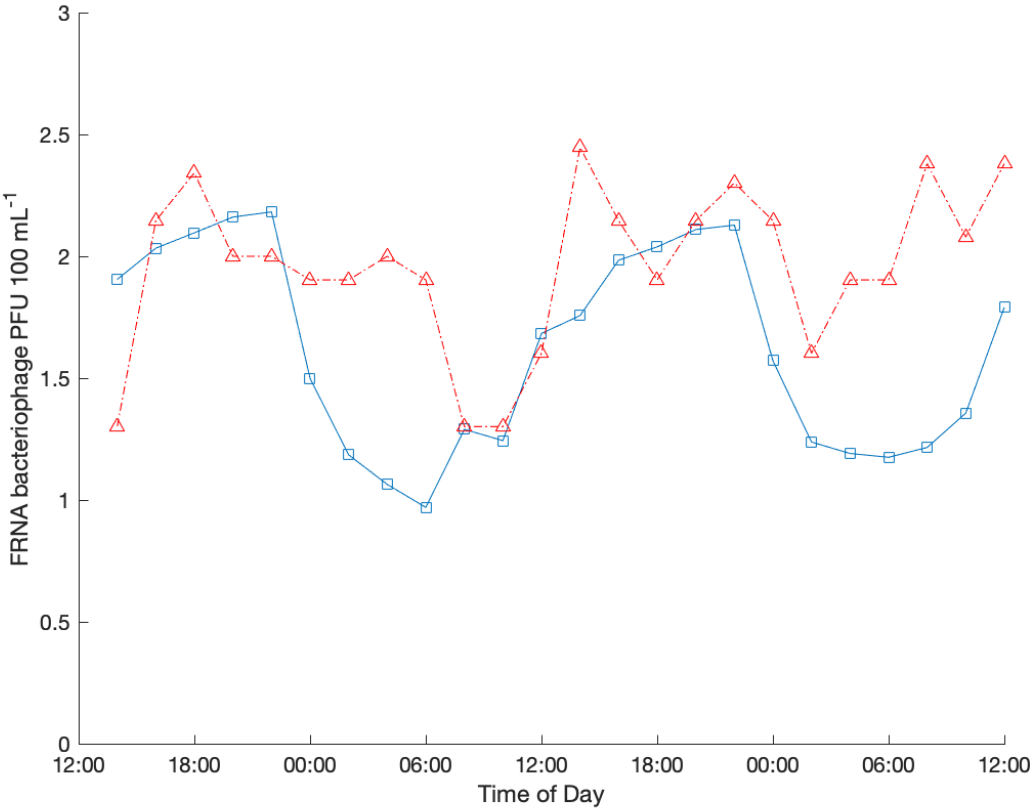

Figure 4.6. Comparison between the F-RNA bacteriophage (log_{10} PFU 100 mL^{-1}) model predicted concentrations (□) and the concentrations measured in the high rate algal pond at Kingston on Murray, Australia, over a 48h period between 25-27 August 2015 (△).

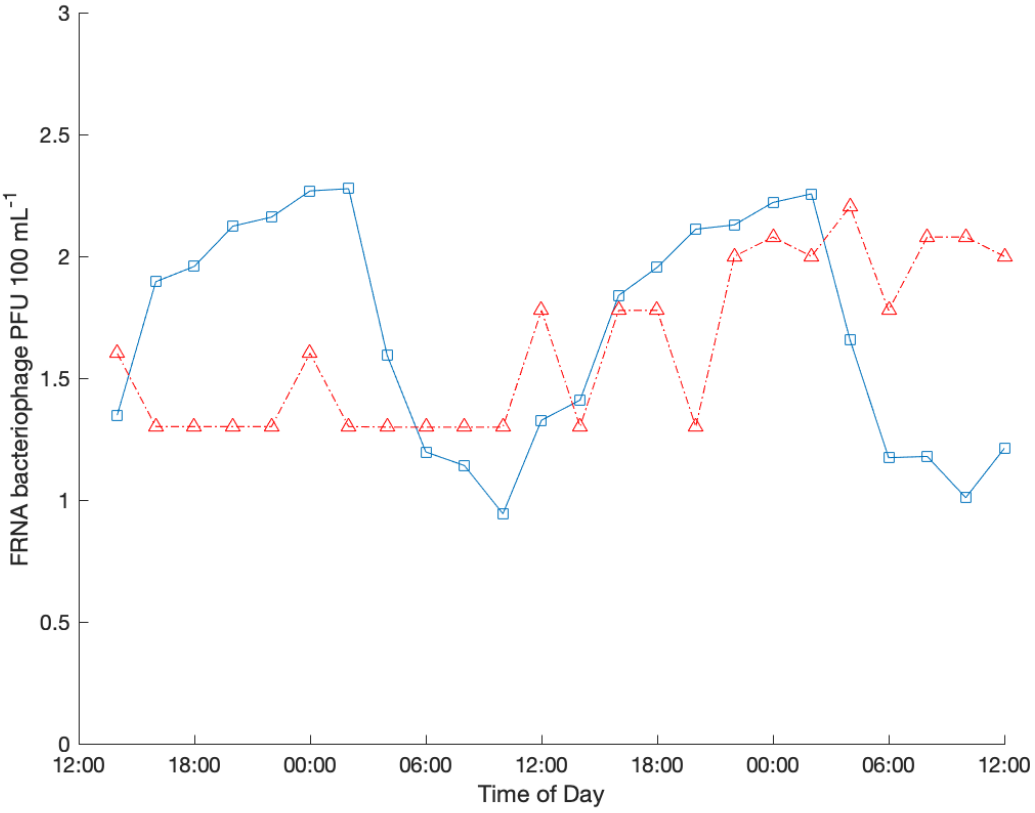

Figure 4.7. Comparison between the F-RNA bacteriophage (log_{10} PFU 100 mL^{-1}) model predicted concentrations (□) and the concentrations measured in the high rate algal pond at Kingston on Murray, Australia, over a 48 h period between 15-17 September 2015 (△).

4.5.5. Model overview and future directions

The results of the model validation suggest the model has been well designed with a good fit between the model predicted concentrations and the measured concentrations for both organisms – falling within a single order of magnitude. This is also supported by the majority of the paired t-test comparisons, which reported no statistically significant difference between the model concentrations and the measured concentrations. Overall, these results suggest the concept of an inactivation model for pathogens and indicator organisms in HRAPs based on independently measured laboratory inactivation values for solar radiation and light-independent processes is sound and should be further developed.

The version of the model presented here was used as a proof of concept, and for this reason, there was a conscious effort made to keep it simple as possible. With this in mind,

there is room to add future complexity and make the model a more comprehensive representation of a real-world system. A possible future development for the model is changing how solar radiation exposure and inactivation behave. In the model presented here, the length of the day was kept at a fixed 12 h period with solar radiation exposure beginning or ending instantaneously. This schedule could be adjusted to suit variance in daylight hours caused by both location and season. Additionally, how the model transitions between light and dark could also be changed in future versions. In this current version of the model, the inactivation values for the solar radiation mechanisms are a constant value throughout the day. This transition could be adjusted to reflect better how solar radiation intensity changes over a day.

Despite pH and DO being considered contributors to pathogen inactivation in natural wastewater treatment systems (Curtis et al., 1992, Benchokroun et al., 2003, Craggs et al., 2004), it was decided to omit them as individual inactivation factors in this version of the model. As mentioned previously, maintaining model simplicity was a focus during model development, and the inclusion of these factors would add considerable complexity. The decision to exclude pH and DO as individual factors was supported by Craggs et al. (2004) reporting a similar observation and subsequently also choosing to exclude these factors from their model – describing them as 'secondary order factors'. It should be noted due to the methods used to measure the inactivation values used in this model, the influence pH and DO had on inactivation is to some degree included in the model as part of the solar radiation and light-independent processes inactivation values (Table 4.1). This is probably more appropriate than including them as individual factors considering the relationships pH and DO have been reported to have with solar radiation and light-independent processes in the disinfection of pathogens in HRAPs and other natural wastewater treatment systems. (Curtis et al., 1992, Davies-Colley et al., 1999, Benchokroun et al., 2003). Additionally, encompassing these factors into a single value like this could help maintain model simplicity.

Unlike most other models, this model used inactivation rates measured in the laboratory independent of HRAP monitoring. In theory, this independence of inactivation rates means the resulting model is less of a model on a specific system and more a model that represents the behaviour of HRAP systems more broadly. Potentially this makes the model presented here more flexible and widely applicable.

An advantage of this model is it can be easily used for any organism for which solar radiation and light-independent processes inactivation rates are already available. This is pertinent as it allows for the potential modelling of other indicator organisms and pathogens in HRAPs. It is particularly relevant when considering that one of the key advantages of HRAPs over other similar systems is the additional effluent available for reuse with pathogens being a major hazard of the practice.

The model also provided guidance on HRAP operation criteria and subsequently assisted in the development of the South Australian Community Wastewater Management Scheme (CWMS) HRAP guidelines. Recently, HRAPs were approved by a regulatory agency, the South Australian Department of Health Wastewater Management Group, as an alternative to conventional wastewater treatment systems in the CWMS – mainly WSPs (Fallowfield et al., 2018). The CWMS manages the wastewater collection, removal and disposal for rural communities in South Australia (Fallowfield et al., 2018). The model showed that the inactivation of pathogens in HRAPs was improved if the addition of influent was continuous at flow rates equivalent to those required to achieve the THRT. This is not always possible in many communities serviced by the CWMS, as often community septic tank effluent is delivered to a collection sump where it is then pumped intermittently to the next stage of wastewater treatment system – in this scenario a HRAP. This leads to ‘pulse’ loading of the HRAPs, adversely affecting the inactivation of pathogens. If continuous loading is not possible, the model indicated that to maintain HRAP pathogen inactivation performance no more than 4% of the pond volume should be introduced over a period no longer than 4% of the THRT – the ‘4% rule’. This operational guidance was included in the new CWMS

guidelines, *Design Guideline for a High Rate Algal Pond (HRAP) – as an Element in Wastewater Treatment Trains.*

4.6. Conclusion

A HRAP pathogen inactivation model employing inactivation values independently measured in the laboratory was successfully developed. The inactivation of the indicator organisms *E. coli* and F-RNA bacteriophage were modelled with the models subsequently validated using a large-scale, operational HRAP. The results of the validation confirm that this type of design for an inactivation model is satisfactory and that the modelling of other organisms and further development should be pursued. The model also provided valuable insight into HRAP operation subsequently assisting in the development of South Australian CWMS operational guidelines. It is hoped that the model developed in this study and the information gained from its development and validation will lead to wider application of HRAPs.

4.7. Acknowledgements

The authors acknowledge the support provided by the Local Government Association of South Australia, the Loxton Waikerie District Council and Richard Gayler. The authors would also like to acknowledge the support provided by Raj Indela, Technical Assistant, Health and Environment Group, College of Science and Engineering, Flinders University.

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CHAPTER 5. CASE STUDY ON THE EFFECT CONTINUOUS CO_2 ENRICHMENT, VIA BIOGAS SCRUBBING, HAS ON BIOMASS PRODUCTION AND WASTEWATER TREATMENT IN A HIGH RATE ALGAL POND

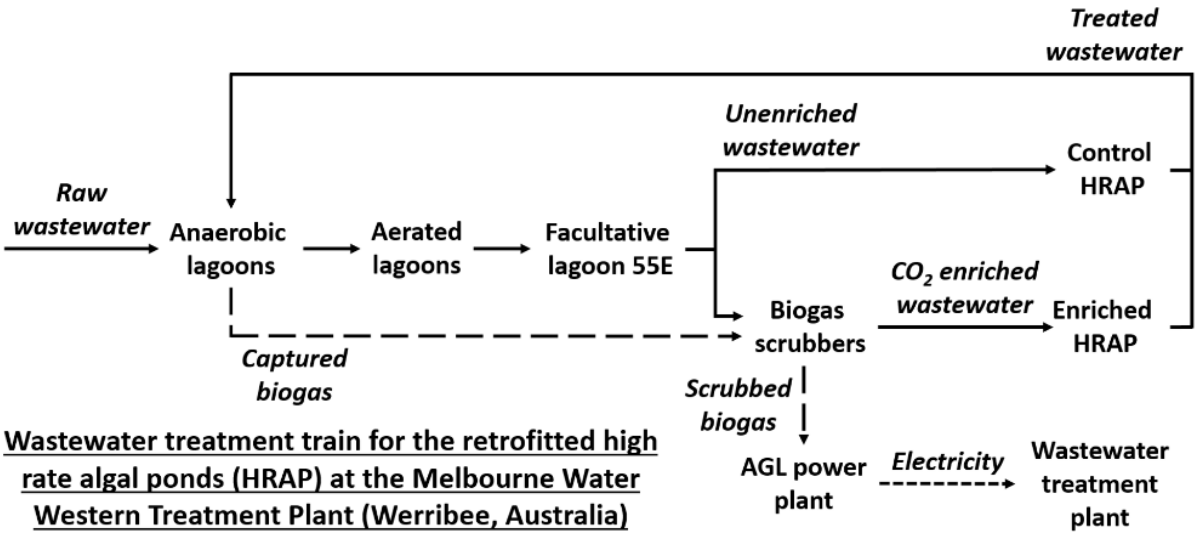
The following chapter is a published journal article authored by Paul Young, Dr Michael J. Taylor, Dr Neil Buchanan, Justin Lewis and Professor Howard Fallowfield in the Journal of Environmental Management, published 1 December 2019, Volume 251, Page 109614 (Appendix A.3). Reproduced by permission of Elsevier. The published version of the article can be found at https://www.sciencedirect.com/science/article/pii/S0301479719313325.

This was a jointly authored publication with the data collected by Paul Young, Dr Michael Taylor and Justin Lewis. Data analysis was performed by Paul Young in discussion with Dr Michael Taylor and Professor Howard Fallowfield. Manuscript writing and editing was performed by Paul Young with the assistance of Professor Howard Fallowfield.

As previously mentioned, the most significant driver for research into high rate algal ponds (HRAP) treating wastewater has been their use as bioreactors to cultivate microalgae for the production of biofuels. It was therefore seen as necessary to investigate the major limitations to HRAPs application for this purpose. One of the two main factors considered to be limiting wastewater treating HRAPs application for this purpose is biomass productivities below economically viable concentrations for the cost competitive production of biofuels. This has mainly been attributed to the growth of microalgae in wastewater treating HRAPs being carbon limited. The enrichment of wastewater with carbon via CO_2 is widely considered the best way to overcome this limitation with many laboratory and pilot-scale studies reporting CO_2 addition to have a positive effect on microalgae productivity in wastewater. However, there have been relatively few studies on large-scale, operational HRAPs and none with adequate controls, raising doubts about the method's real-world application. This chapter outlines a case study on the effect continuous CO_2 enrichment of wastewater, via an

industry standard biogas scrubber, has on the biomass production and wastewater treatment of a HRAP retrofitted into a major wastewater treatment plant. It is believed to be the closest representation in the literature to a real-world scenario where a wastewater treating HRAP is enriched with CO_2 to increase biomass productivity. It is also believed to be the only study at such a scale to use an adequate control.

5.1. Graphical abstract



Wastewater treatment train for the retrofitted high rate algal ponds (HRAP) at the Melbourne Water Western Treatment Plant (Werribee, Australia)

5.2. Highlights

- The most representative study on CO_2 enrichment in high rate algal ponds (HRAP)
- The only large-scale HRAP wastewater CO_2 enrichment study with an adequate control
- Chlorophyll *a* was the only biomass measure that was increased in the Enriched HRAP
- The Control HRAP had slightly increased mean removals for total N and NH_4-N
- The Enriched HRAP had slightly increased mean nitrate production

5.3. Abstract

Microalgae grown in high rate algal ponds (HRAP) treating wastewater are considered a promising feed for biofuel production. Biomass productivity is often considered to be limited by carbon availability, with the addition of CO_2 being the proposed solution. Biogas from anaerobic wastewater treatment potentially provides a cheap, co-located CO_2 source. Two identical 223 m^2 HRAPs were constructed at Melbourne Water's Western Treatment Plant, where biogas from an anaerobic lagoon is used to generate electricity. One HRAP was fed secondary treated wastewater that had been enriched with CO_2 recovered from the biogas using industry standard biogas scrubbers, the Enriched HRAP, while the other HRAP was fed the same wastewater except it had bypassed the biogas scrubbers, the Control HRAP. The biomass production and wastewater treatment performance of the two HRAPs was compared over 12 months. The inlet to the Enriched HRAP had significantly higher free CO_2 and inorganic carbon, 175.00 ± 49.30 mg L^{-1} and 110.00 ± 10.2 mg L^{-1}, than the inlet to the Control HRAP, 9.30 ± 7.08 mg L^{-1} and 89.62 ± 5.12 mg L^{-1}. There were no significant differences in biomass production between the HRAPs as measured by dry matter, particulate organic carbon or nitrogen. Chlorophyll *a* was statistically higher in the Enriched HRAP; however, this measurement is potentially unreliable. Regarding wastewater treatment, only total nitrogen and ammonium removal differed significantly between the HRAPs, with the Control HRAP, 59.13 ± 21.13% and 76.46 ± 32.33%, slightly outperforming the Enriched HRAP, 53.52 ± 17.41% and 68.76 ± 31.17%. Overall, neither biomass production nor wastewater treatment was meaningfully improved by CO_2 enrichment; however, wastewater treatment was still effective in both HRAPs.

5.4. Keywords

High rate algal ponds; Wastewater treatment; CO_2 addition; Biogas scrubbing; Microalgal biomass

5.5. Introduction

A desire to move towards more sustainable, carbon neutral practices has led to a significant increase of interest in biofuels (Rawat et al., 2013, Posadas et al., 2016). Microalgae-derived biofuels are of particular interest, as algal culture does not need to compete with food crops for arable land or fresh water, and they have a higher growth rate and lipid content than other candidate crops (Rawat et al., 2013, Posadas et al., 2016). Despite these advantages, there are still many limitations to the economic production of microalgae-derived biofuels (Chen et al., 2015). One major limitation is the prohibitively high cost of nutrients sourced from fertiliser (Chen et al., 2015). The coupling of microalgae-derived biofuels and wastewater treatment is widely considered a cost-effective and sustainable solution to this problem (Rawat et al., 2013, Chen et al., 2015).

The high concentration of nutrients in wastewater make it an excellent medium for microalgae and associated microbial growth, which removes these nutrients by assimilation and degradation, effectively treating the wastewater (Rawat et al., 2013, Chen et al., 2015). An economic analysis of microalgal biofuel production by Lundquist et al. (2010) concluded that, for the near future, this coupling was the only economically viable way to produce biofuels from microalgae, and only if wastewater treatment was the primary objective. High rate algal ponds (HRAP) are widely considered to be the most suitable reactors for this coupling due to their relatively low construction cost, low energy requirement, ease of operation and ability to be scaled up (de Godos et al., 2014, Posadas et al., 2016, Lundquist et al., 2010).

HRAPs are shallow, open raceway ponds mixed by a paddlewheel (Fallowfield et al., 2018, Buchanan et al., 2018). The design aims to maximise the ponds' exposure to solar radiation, which acts both as a pathogen disinfectant and driver, through microalgal photosynthesis, of microalgal-bacterial assimilation and degradation of nutrients (Young et al., 2016). The extensive solar exposure microalgae experience in these systems results in microalgal

productivities considerably higher than other natural, pond-based wastewater treatment systems (Craggs et al., 2012). The concept of utilising the microalgae grown in HRAPs as part of wastewater treatment for biofuel production has been considered for nearly as long as these systems have existed (Young et al., 2017). It should be recognised that the biomass produced in HRAPs treating wastewater, while predominantly a mixed population of microalgae, also comprises bacteria, zooplankton and detritus (Cromar and Fallowfield, 1992).

Despite the many advantages of using wastewater-grown biomass in HRAPs for biofuel production, there are still limitations (Young et al., 2017, Sutherland et al., 2017). One major limitation is that the biomass productivity achieved is currently below the level where it would be economically competitive with fossil fuels and well below the theoretical maximum for microalgae, 40-60 g m^{-2} d^{-1} (de Godos et al., 2014, Young et al., 2017). This low-level of production is thought to be caused mainly by the microalgae being carbon limited due to wastewater having a significantly lower carbon to nitrogen ratio compared to microalgal biomass (Craggs et al., 2014, Park and Craggs, 2011, de Godos et al., 2016). While there appears to be considerable confusion in the literature regarding C to N ratios, it is still widely accepted that the microalgal biomass in HRAPs treating wastewater is carbon limited. It has been suggested that the addition of inorganic carbon, as gaseous carbon dioxide, could be used to solve this problem (Heubeck et al., 2007, Craggs et al., 2012). The rationale behind this is that the CO_2 dissolved in the pond volume would provide a readily available source of additional carbon for the microalgae to use for growth during times of carbon limitation (Craggs et al., 2014, Chen et al., 2015)

CO_2 can be obtained from several sources including commercially manufactured pure CO_2, flue gas or biogas (Heubeck et al., 2007, Craggs et al., 2014, Park and Craggs, 2011). Commercially manufactured pure CO_2 is prohibitively expensive for the economical production of biomass while flue gas contains contaminants that are potentially problematic, and few sources are in locations appropriate for HRAP construction (Chen et al., 2015, de

Godos et al., 2014, Young et al., 2017). Consequently, biogas produced via anaerobic wastewater treatment seems to provide the most promising source of CO_2 to improve microalgal biomass production in HRAPs.

Biogas produced via anaerobic wastewater treatment is rich in methane, 35−75%, and, consequently, is often used as a renewable fuel (Osorio and Torres, 2009, Heubeck et al., 2007, Serejo et al., 2015, Muñoz et al., 2015). Before it can be used, CO_2 and H_2S need to be removed ('scrubbing') from the biogas to improve engine efficiency and prevent corrosion (Heubeck et al., 2007, Osorio and Torres, 2009). Wastewater is commonly used for this application, and it is here that biogas presents itself as a promising source of CO_2 (Muñoz et al., 2015). The waste CO_2 enriches the wastewater with inorganic carbon that the microalgae in HRAPs can use for growth. This coupling is attractive as both wastewater and CO_2 can be found on-site at the same wastewater treatment plant as 'free' waste streams. This results in biogas being more economical than other potential CO_2 sources due to reduced sourcing, transport and infrastructure costs. Additionally, the HRAP may easily be incorporated into the wastewater treatment plant even without CO_2 enrichment. While this coupling has shown promise, the number of studies, particularly those looking at the real-world application, are scarce (Heubeck et al., 2007, Muñoz et al., 2015, Serejo et al., 2015).

Melbourne Water is one of the top 150 energy users and greenhouse gas emitters in Australia. As part of their Climate Resilience Plan, they set a target for zero net greenhouse gas emissions. One of the action areas of Melbourne Water's Climate Resilience Plan was to investigate cost-effective on-site renewable energy generation options. Consistent with this strategy, biogas captured from a covered anaerobic lagoon is used to generate on-site electricity in a 10 MWh rated plant operated by Australian Gas and Light (AGL). Prior to combustion, counter-current biogas scrubbers continuously remove H_2S and CO_2 using wastewater from a facultative lagoon, Lagoon 55E, resulting in CO_2 enriched wastewater. In July 2012, the Smart Water Fund, Victoria, supported by Melbourne Water, financed construction and research on two parallel HRAPs at Melbourne Water's Western Treatment

Plant. This enabled a study comparing the wastewater treatment and biomass production of a HRAP fed wastewater continuously enriched with CO_2 via industry standard, biogas scrubbers and a HRAP fed the same wastewater that had bypassed the biogas scrubbers. To the authors' knowledge, this comparative study is the closest representation of a real-world scenario, with the HRAPs retrofitted to a major wastewater treatment plant that already had the existing industrial-scale infrastructure in place for biogas capture and scrubbing. The study was conducted in two parts, firstly between 19 June and 2 December 2014 (winter and spring) and secondly between 4 January and 25 July 2016 (summer, autumn and winter), to encompass all weather conditions experienced throughout a year.

5.5. Material and methods

5.5.1. Design and operation of the high rate algal ponds

Two HRAPs, designed by Flinders University, were constructed at the Melbourne Water Western Treatment Plant (37°58'29.8"S 144°38'27.1"E). Each HRAP was a single pass, lined (high-density polyethylene), 223 m^2 raceway, mixed by a paddlewheel, which provided a linear surface velocity of ~0.4 m s^{-1}.

The Western Treatment Plant receives approximately 450 ML d^{-1} of wastewater from metropolitan Melbourne. Primary treatment is carried out in a covered anaerobic lagoon, followed by an aerated lagoon and subsequent facultative lagoon treatment. Wastewater from the facultative lagoon, Lagoon 55E, the first in the series after the aerated lagoon, was supplied to the AGL on-site power station to be used for biogas 'scrubbing'. The wastewater was transported to the top of the industry standard biogas scrubbers, with biogas from the covered anaerobic lagoon being delivered to the base of the scrubbers. The gas scrubbing towers were packed with cascading plastic pall rings to increase both the turbulence and the effective surface area of the liquid phase to aid gas diffusion into the wastewater liquid

phase. The biogas is scrubbed to remove H_2S and CO_2, after which it is used in the on-site power station to generate electricity. This electricity is mostly used to power the wastewater treatment plant.

The wastewater, enriched with CO_2 recovered from the biogas, was fed to one of the identical HRAPs (Enriched HRAP). The other HRAP was fed the same wastewater supplied to the AGL facility but which had bypassed the biogas scrubbers (Control HRAP). The inlet wastewater was introduced below the surface of each HRAP to minimise potential outgassing of CO_2 to the atmosphere. The HRAPs were both operated at a depth of 0.3 m at a hydraulic retention time of 4 d. Treated effluent from the HRAPs was returned to the raw wastewater inlet at the head of the works for processing through the normal treatment train.

5.5.2. Meteorological monitoring

Climate and meteorological data were collected from the closest weather stations to the HRAPs, via the Australian Bureau of Meteorology databases (Australian Bureau of Meteorology, 2018). The closest weather station that measured daily global solar exposure (kWh m^{-2}) was located at the Werribee Racecourse (37.90° S, 44.64° E), approximately 8 km from the HRAPs. Daily maximum and minimum temperature (°C) were measured by the weather station at the RAAF Laverton Williams Base (37.86° S, 144.76° E) approximately 16 km from the HRAPs at the Melbourne Water Western Treatment Plant (Werribee, Australia).

5.5.3. In-situ HRAP wastewater monitoring

The pH of wastewater in the HRAPs was continuously monitored (Model 4600 transmitter controller; ABB, Ltd., Thebarton, Australia), with the mean of the data logged continuously at 60-minute intervals and presented as daily averages. The sensors were located next to the paddlewheel on the water return side, approximately 15 cm below the water's surface.

5.5.4. Analysis of wastewater by Flinders University.

5.5.4.1. Wastewater sample collection

Programmed auto-samplers (Teledyne ISCO, Lincoln, USA) were used to collect wastewater from each HRAP at 03:00 and 15:00 on two consecutive days, representing a two-day composite sample. The auto-samplers sampled from the water return side of the paddlewheel. Grab samples of inlet wastewater to the respective HRAPs were collected twice weekly. Samples were biologically inactivated by adjustment to pH 2 by addition of 5 mL sulphuric acid (1 M). These acid stabilised samples were retrieved and conveyed to Flinders University for further analysis.

5.5.4.2 Total carbon, total organic carbon and total nitrogen

The total carbon (mg L^{-1}), organic carbon (mg L^{-1}) and total nitrogen (mg L^{-1}) content of the wastewater pre- and post-filtration through a glass fibre filter (GF/C, 1.2 µm; Whatman Ltd) were determined using a Shimadzu TOC-LSCH analyser with auto-sampler (Shimadzu, Kyoto, Japan). The particulate organic carbon (POC; mg L^{-1}) was calculated as the difference between the total organic carbon of the whole and filtered wastewater. Similarly, particulate organic nitrogen (PON; mg L^{-1}) was calculated as the difference between the total organic nitrogen of the whole and filtered wastewater.

5.5.4.3. Total suspended solids and productivity

Total suspended solids (mg L^{-1}) for all samples were determined using Test 2540 D (Total Suspended Solids Dried at 103-105°C) described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). Total suspended solids productivity (g m^{-}

^{2} d^{-1}) was derived from the product of total suspended solids and daily effluent outflow divided by the effective surface area of the HRAP.

5.5.4.4. Chlorophyll *a*

Chlorophyll *a* (µg L^{-1}) was determined for each sample using Test 10200 (Chlorophyll – trichromatic method) in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992).

5.5.4.5. Ammonium (NH_4-N), nitrite/nitrate (NOx-N) and orthophosphate (PO_4-P)

Ammonium (NH_4-N; mg L^{-1}), nitrite/nitrate (NO_X-N; mg L^{-1}) and orthophosphate (PO_4-P; mg L^{-1}) were analysed for each sample after filtration through a glass fibre filter (GF/C, 1.2 µm; Whatman Ltd) using the Foss Fiastar 5000 Analysis System (Foss Pacific Pty Ltd, Mulgrave, Australia). This system uses techniques that are automated forms of systems described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al., 1992). The method used for ammonium analysis was the Phenate Method described in Test 4500-NH_3 H (Greenberg et al., 1992). Nitrite/nitrate analysis was undertaken using the Cadmium Reduction Method described in Test 4500-NO_3 F (Greenberg et al., 1992). The technique used for orthophosphate analysis was the Stannous Chloride Method described in Test 4500-P D (Greenberg et al., 1992).

5.5.5. Analysis of wastewater by ALS Group

Analyses that were likely to be adversely affected by acid stabilisation were carried out by ALS Group Environmental Division, Scoresby, Adelaide; a National Association of Testing Authorities (NATA) accredited laboratory. Non-acidified samples from both inlets and HRAPs were collected intermittently and analysed within 24 h. Analyses carried out by ALS were

post-filtration 5-day biochemical oxygen demand (BOD_5) (mg BOD_5 L^{-1}) (method EP030F), free CO_2 (mg L^{-1}) (method 4500-CO2D; APHA 1992) and inorganic carbon (mg C L^{-1}) (method EP006). *E. coli* (MPN 100 mL^{-1}) was determined by ALS using the Colilert™ chromogenic substrate technique (IDEXX Laboratories, Westbrook, USA). *E. coli* removal values were reported as log_{10} reduction values (LRV) and were calculated by subtracting the *E. coli* (log_{10} MPN 100 mL^{-1}) concentration in the HRAP outlet wastewater from the respective *E. coli* (log_{10} MPN 100 mL^{-1}) concentration in the inlet wastewater. Identification and enumeration of microalgae and cyanobacteria was also undertaken by ALS Group using standard counting chambers and light microscopy. The identification was to genus level; cell numbers were expressed as log_{10} cells mL^{-1}.

5.5.6. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics, version 23 (Armonk, NY). Data was checked for normality using the Kolmogorov-Smirnov Test and the Shapiro-Wilk Test. Means were compared using independent-samples Mann-Whitney U test. Significant differences in means (p-value<0.05) were reported. Graphs were made using either IBM SPSS Statistics, version 23 (Armonk, NY) or Graph Pad™ prism 5.0 (Graph PAD Software Inc. USA).

HRAP removals for parameters measured by ALS were calculated by subtracting the value measured in the HRAP from the value measured in the corresponding inlet on the same date. The majority of HRAP parameters measured by Flinders University did not have an inlet measurement taken on the same day, so a different approach had to be taken: where the inlet sample that was collected on the date closest to the HRAP sample was used.

5.6. Results

5.6.1. Prevailing weather conditions

The weather data collected shows typical trends for the temperate oceanic climate experienced in southern Victoria, Australia, with temperature and solar minima during June-July and maxima during December-February. The mean daily global exposure (kWh m^{-2}) during the study, 3.67 ± 2.03 kWh m^{-2}, was slightly lower than the long-term mean, 4.2 kWh m^{-2} (Figure 5.5). The mean maximum, 19.73 ± 6.10ºC, and minimum, 10.17 ± 4.75ºC, air temperatures were in line with the long-term means for the area (Figure 5.6).

5.6.2. Inlet wastewater composition

As anticipated, following passage through the biogas scrubbers, the Enriched Inlet had statistically significant elevated levels of total inorganic carbon (mg C L^{-1}) ($p<0.000$) and free CO_2 (mg L^{-1}) ($p=0.002$) compared to the Control Inlet. There were, however, no other statistically significant differences in the composition of the inlet wastewaters (Table 5.1).

Table 5.1. Statistical comparison (Mann-Whitney U test) of the composition of the wastewater from Lagoon 55E post-biogas scrubbers and fed to the Enriched high rate algal pond (HRAP) and wastewater from Lagoon 55E which had bypassed the scrubbers and fed to the Control HRAP. Significant statistical difference accepted at $p < 0.05$.

Parameter	Sample location	n	Mean	Standard deviation	Standard error of mean	p value
Free CO_2 (mg L^{-1})	Enriched Inlet	6	175.0	49.30	20.12	0.002
	Control Inlet	6	9.30	7.08	3.26	
Total inorganic carbon (mg C L^{-1})	Enriched Inlet	13	110.0	10.21	2.83	<0.000
	Control Inlet	13	89.62	5.12	1.42	
Total suspended solids (mg L^{-1})	Enriched Inlet	55	64.50	106.0	14.30	0.518
	Control Inlet	54	54.06	80.28	10.93	
Chlorophyll *a* (µg L^{-1})	Enriched Inlet	55	405.3	705.9	95.19	0.608
	Control Inlet	54	273.8	423.6	57.65	
BOD_5* (mg BOD_5 L^{-1})	Enriched Inlet	25	35.0	43.35	8.67	0.55
	Control Inlet	25	30.8	47.69	9.54	
Total nitrogen* (mg N L^{-1})	Enriched Inlet	55	76.20	10.03	1.35	0.340
	Control Inlet	54	77.65	10.58	1.44	
Ammonium* (mg NH_4-N L^{-1})	Enriched Inlet	55	55.84	19.11	2.58	0.328
	Control Inlet	54	57.64	19.90	2.71	
Nitrite/Nitrate* (mg NO_x-N L^{-1})	Enriched Inlet	55	0.21	1.02	0.14	0.506
	Control Inlet	54	0.11	0.66	0.09	
Orthophosphate* (mg PO_4-P L^{-1})	Enriched Inlet	55	9.55	3.87	0.52	0.082
	Control Inlet	54	8.61	3.05	0.41	
***E. coli* (log_{10} *E. coli* MPN 100 mL^{-1})**	Enriched Inlet	21	4.48	0.70	0.11	0.777
	Control Inlet	20	4.42	0.77	0.17	

*Measured in filtrate (GF/C, 1.2 µm)

5.6.3. In-situ monitored HRAP pH

Although following a similar trend over the study, the daily mean pH for the HRAPs were found to differ significantly ($p=0.001$). The Enriched HRAP had a mean pH of 7.41 ± 0.74 with a range of pH 5.00 to 8.89 while the Control HRAP had a higher mean pH of 8.03 ± 0.58 with a range of pH of 5.37 to 9.30.

5.6.4. Biomass production

Of the four biomass measurements used in this study, only mean chlorophyll *a* concentration was found to be significantly different between the HRAPs, with the Enriched HRAP having a

higher concentration than the Control HRAP (Table 5.2). These results were reflected in the statistical analysis of the biomass measurements by Spearman's rank correlation coefficient (Table 5.4), which showed a strong correlation between all the biomass measurements except for chlorophyll *a*.

Table 5.2. Statistical comparison (Mann-Whitney U test) between the biomass productivity of the Enriched high rate algal pond (HRAP) fed wastewater from Lagoon 55E enriched with CO_2 via biogas scrubbers and the Control HRAP fed wastewater from the same lagoon but which had bypassed the biogas scrubbers. Significant difference between means accepted at $p < 0.05$.

Parameter	Sample location	n	Mean	Standard deviation	Standard error mean	p value
Total suspended solids (mg L^{-1})	Enriched HRAP	176	223.9	161.9	12.20	0.592
	Control HRAP	185	251.9	232.40	17.09	
Total suspended solids productivity (g m^{-2} d^{-1})	Enriched HRAP	176	14.06	10.61	0.77	0.592
	Control HRAP	185	15.81	14.59	1.07	
Particulate organic carbon (mg C L^{-1})	Enriched HRAP	176	85.76	69.09	5.21	0.670
	Control HRAP	185	83.70	73.70	5.42	
Particulate organic nitrogen (mg N L^{-1})	Enriched HRAP	176	12.72	10.14	0.76	0.966
	Control HRAP	185	12.54	9.65	0.71	
Chlorophyll *a* (µg L^{-1})	Enriched HRAP	176	937.2	705.9	59.36	0.004
	Control HRAP	185	835.7	1311	96.40	

5.6.4.1. Total suspended solids and productivity

There was no statistically significant difference ($p=0.592$) in total suspended solids (mg L^{-1}) between the Enriched HRAP and the Control HRAP and consequently no significant difference ($p=0.592$) in total suspended solids productivity (g m^{-2} d^{-1}). The mean concentration of total suspended solids was slightly elevated in the Control HRAP (Table 5.2). Figure 5.1 shows total suspended solids productivity was higher in both HRAPs between late winter and early summer, August-November. Notable peaks in total suspended

solids productivity occurred in August and April in the Control HRAP and in May in the Enriched HRAP.

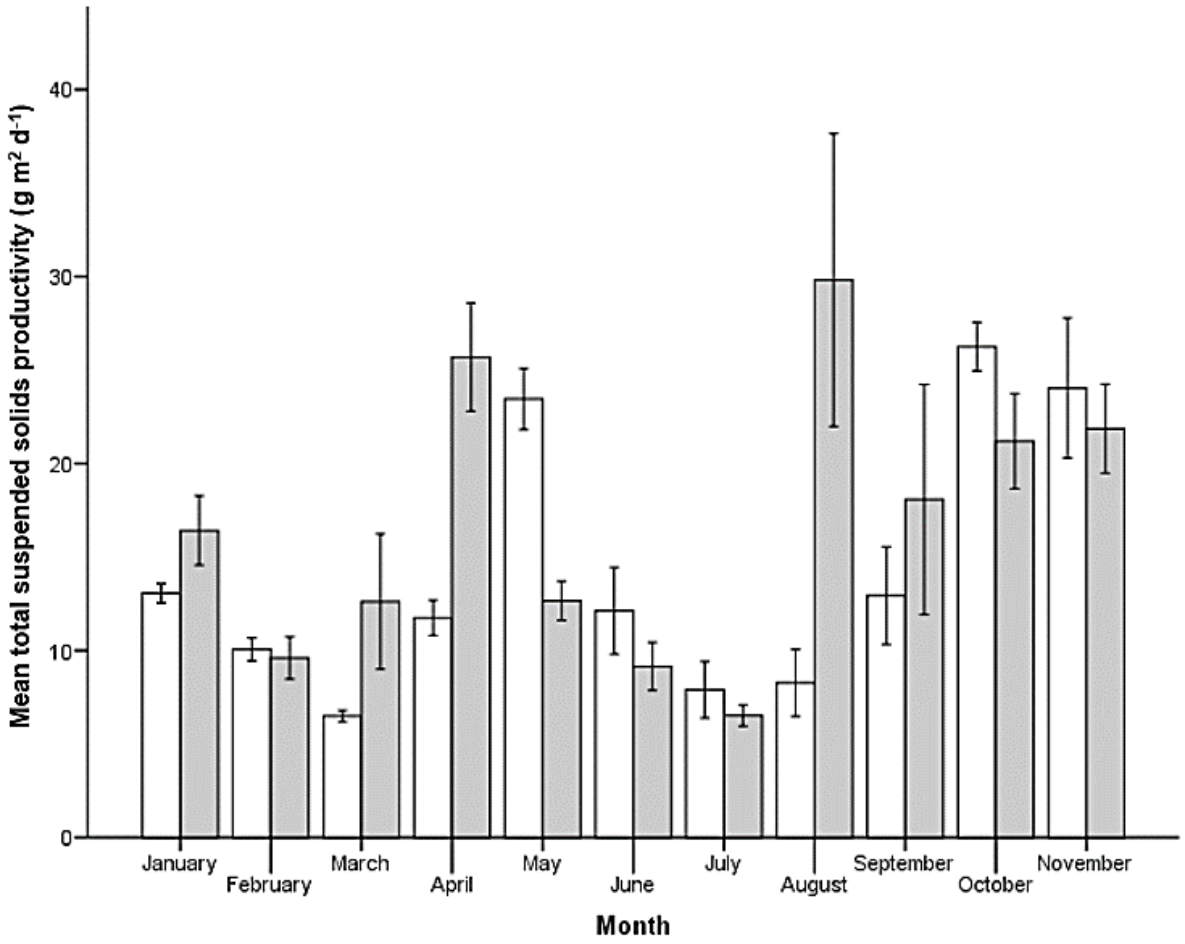


Figure 5.1. Monthly mean total suspended solids productivity (g m^{-2} d^{-1}) of Enriched high rate algal pond (□) and Control high rate algal pond (■) at the Melbourne Water Western Treatment Plant (Werribee, Australia), firstly between 19 June and 2 December 2014 and secondly between 4 January and 24 July 2016. Data shown as the mean ± 1 standard error.

5.6.4.2. Particulate organic carbon and particulate organic nitrogen

The changes in mean monthly values of POC in both HRAPs over an annual cycle is shown in Figure 5.2. Generally, the monthly means for both HRAPs were similar, although there were exceptions in May and October when the POC concentration was higher in the Enriched HRAP. In contrast, the POC in the Control HRAP was higher in August and

January. Overall there was no statistical difference in the concentration of POC or PON between the HRAPs (Table 5.2).

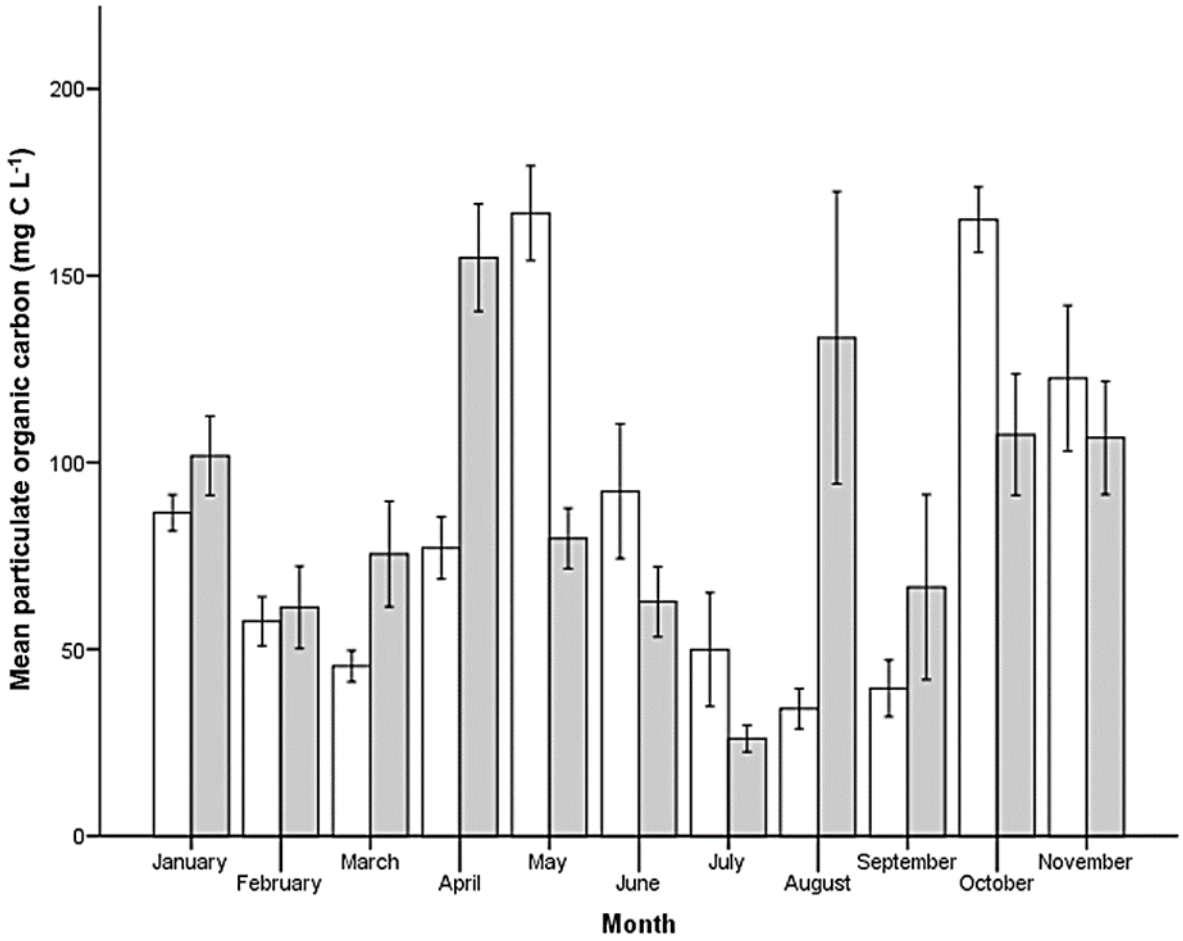


Figure 5.2. Monthly mean particulate organic carbon concentration (mg C L^{-1}) of Enriched high rate algal pond (□) and Control high rate algal pond (■) at the Melbourne Water Western Treatment Plant (Werribee, Australia), firstly between 19 June and 2 December 2014 and secondly between 4 January and 24 July 2016. Data shown as the mean ± 1 standard error.

There was a statistically significant, linear relationship (Figure 5.3) between molar POC and PON of the biomass for both the Enriched HRAP and Control HRAP. Consequently, the molar C:N ratios for the HRAPs were also similar 7.78:1 and 8.20:1 for the Enriched HRAP and the Control HRAP respectively.

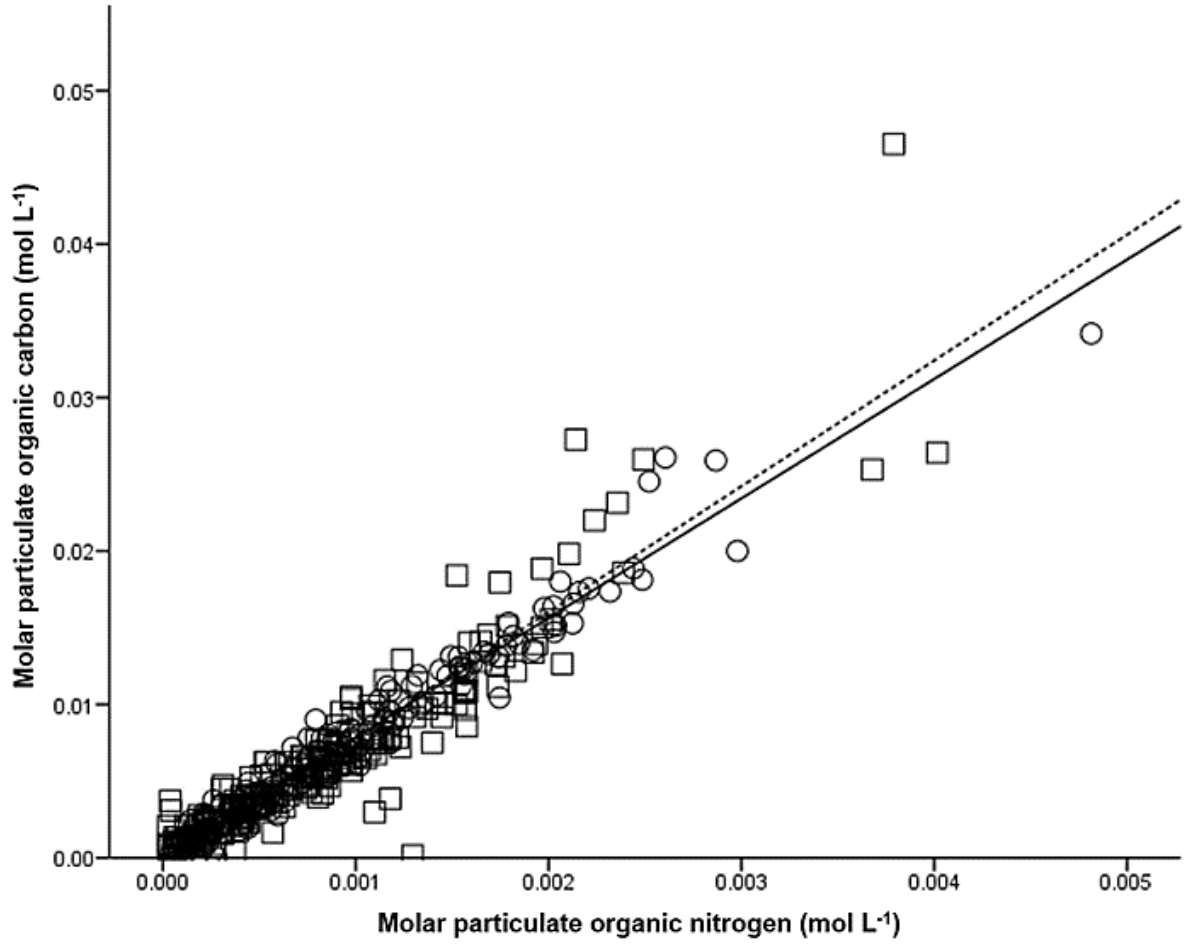


Figure 5.3. Ratio of molar particulate organic carbon:nitrogen in the suspended solid material harvested from the Enriched high rate algal pond (□) and Control high rate algal pond (o) at Melbourne Water Western Treatment Plant (Werribee, Australia), firstly between 19 June and 2 December 2014 and secondly between 4 January and 25 July 2016. Linear regressions were fitted to both the Enriched high rate algal pond (R_2=0.96, p<0.000, n=176) and the Control high rate algal pond (R_2=0.84, p<0.000, n=185).

5.6.4.3. Chlorophyll *a* in the HRAPs

The mean chlorophyll *a* concentration for the Enriched HRAP, 937.2 ± 705.9 µg L^{-1}, was 10.9% higher than the Control HRAP, 835.7 ± 1311 µg L^{-1}. A Mann-Whitney U test showed that this difference between the HRAPs was statistically significant (p=0.004). Both the HRAPs' chlorophyll *a* concentration fluctuated greatly between samples and months, with significant elevations in both ponds occurring independently of each other at different times during the experimental period (Figure 5.4). These fluctuations may be due to seasonal effects but showed little consistency between HRAPs.

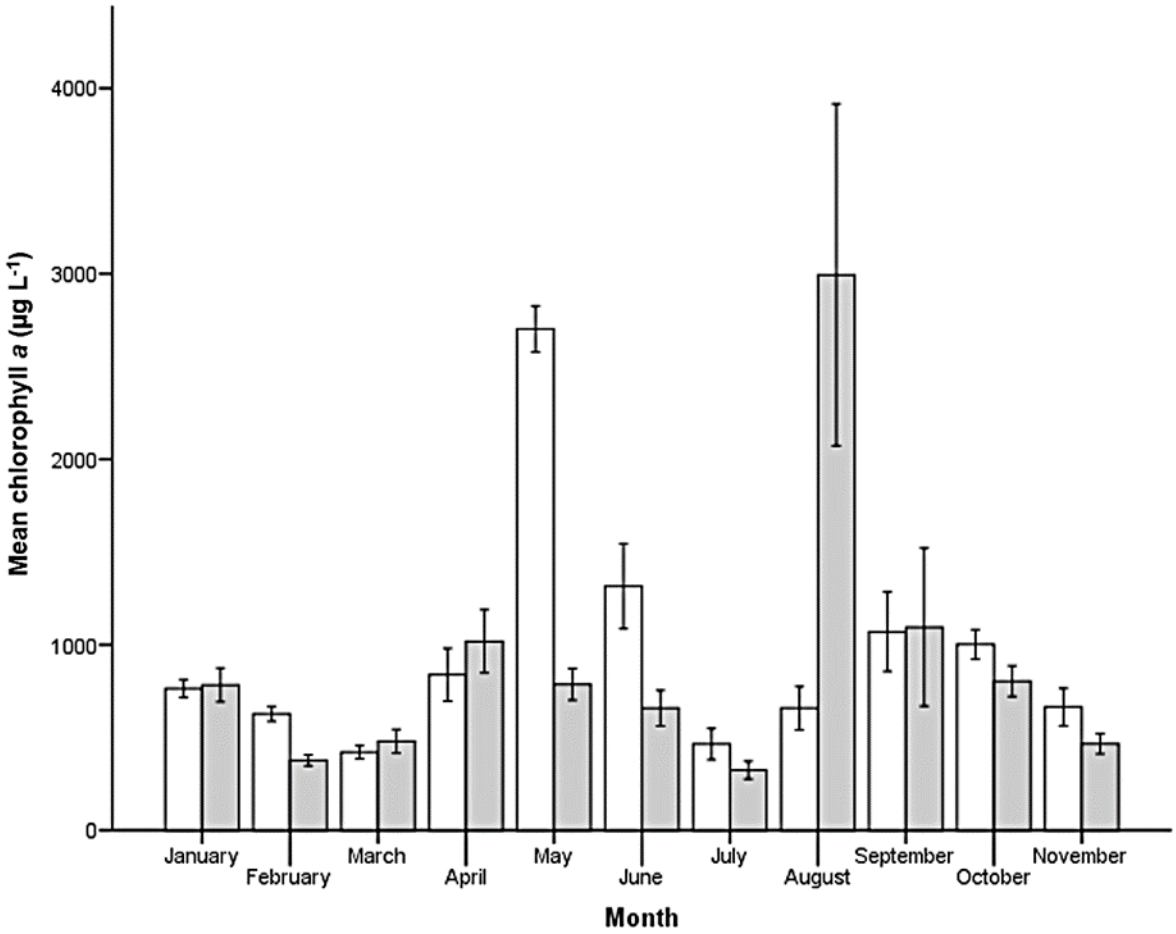


Figure 5.4. Monthly mean chlorophyll *a* (µg L^{-1}) of Enriched high rate algal pond (□) and Control high rate algal pond (▨) at the Melbourne Water Western Treatment Plant (Werribee, Australia), firstly between 19 June and 2 December 2014 and secondly between 4 January and 24 July 2016. Data shown as the mean ± 1 standard error.

5.6.4.4. Algal species composition

Genera of the orders Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cryptophyceae, Cyanophyceae and Euglenophyceae, were identified and enumerated in wastewater samples taken from Lagoon 55E, the Enriched and the Control HRAP (Table 5.5). Cyanophyceae, (*Aphanocapsa* sp., *Leptolyngbya* sp. *Planktolyngbya* sp. *Pseudanabaena* sp.) predominated in the wastewater collected from Lagoon 55E and comprised 88% and 93% of the total phytoplankton population in September and December respectively. Chlorophyceae, (*Chlamydomonas* spp. *Chlorococcoid* spp.) comprised only 11% and 7% of the total phytoplankton population in Lagoon 55E in September and December respectively. Interestingly, the phytoplankton population of the Lagoon 55E wastewater was significantly

changed following passage through the HRAPs. In the Enriched HRAP, the percentage of Cyanophyceae decreased to 55% and 1.9% in September and December respectively, with Chlorophyceae increasing to 55% and 98% of the phytoplankton in the same months. The shift following passage through a HRAP from a phytoplankton community dominated by Cyanophyceae in Lagoon 55E wastewater to a Chlorophycean population was arguably more pronounced in the Control HRAP where they comprised 95% and 99% of the population in September and December respectively. The Cyanophyceae were replaced in both ponds by *Micractinium* sp., *Pediastrum* sp., *Oocystis* sp., *Scenedesmus* sp. (Table 5.5).

5.6.5. Wastewater treatment performance

Overall, there was little difference between the wastewater treatment performance of the HRAPs with only the nitrogen parameters differing significantly (Table 5.3).

Table 5.3. Statistical comparison (Mann-Whitney U test) between the wastewater treatment performance of the Enriched high rate algal pond (HRAP) fed wastewater from Lagoon 55E enriched with CO_2 via biogas scrubbers and the Control HRAP fed wastewater from the same lagoon but which had bypassed the biogas scrubbers. Significant difference between means accepted at $p < 0.05$.

Parameter	HRAP	n	Mean	Standard deviation	Standard error mean	p value
BOD_5^* (mg BOD_5 L^{-1})	Enriched HRAP	31	16.74	17.50	3.14	0.821
	Control HRAP	31	20.65	33.21	5.96	
BOD_5^* removal (%)	Enriched HRAP	25	48.89	32.50	6.50	0.138
	Control HRAP	25	36.63	27.78	5.56	
Total nitrogen* (mg N L^{-1})	Enriched HRAP	176	31.75	9.53	0.72	<0.000
	Control HRAP	185	27.50	10.39	0.76	
Total nitrogen removal* (%)	Enriched HRAP	176	53.52	17.41	1.31	<0.000
	Control HRAP	185	59.13	21.13	1.55	
Ammonium* (mg NH_4-N L^{-1})	Enriched HRAP	176	12.25	13.28	1.00	<0.000
	Control HRAP	185	9.02	13.70	1.00	
Ammonium* removal (%)	Enriched HRAP	176	68.76	31.17	2.35	<0.000
	Control HRAP	185	76.46	32.33	2.38	
Nitrite/Nitrate* (mg NO_x-N L^{-1})	Enriched HRAP	176	12.16	8.28	0.62	0.011
	Control HRAP	185	10.25	9.22	0.68	
Orthophosphate* (mg PO_4-P L^{-1})	Enriched HRAP	176	9.76	8.10	0.61	0.942
	Control HRAP	185	10.41	8.83	0.65	
Orthophosphate* removal (%)	Enriched HRAP	176	16.67	21.57	1.63	0.513
	Control HRAP	185	17.17	22.88	1.68	
E. coli (log_{10} *E. coli* MPN 100 mL^{-1})	Enriched HRAP	22	3.38	0.52	0.11	0.712
	Control HRAP	21	3.31	0.77	0.17	
E. coli log_{10} reduction values (log_{10} *E. coli* MPN)	Enriched HRAP	21	1.15	0.52	0.11	0.662
	Control HRAP	20	1.22	0.49	0.11	

*Measured in filtrate (GF/C, 1.2 µm)

5.6.5.1. BOD_5

The mean BOD_5 concentrations in the HRAPS were similar, with the Enriched HRAP having a mean of 16.74 ± 17.50 mg BOD_5 L^{-1} and the Control HRAP having a mean of 20.65 ± 33.21 mg BOD_5 L^{-1} (Table 5.3). The mean BOD_5 removal for both the HRAPS was also similar, with the Enriched HRAP removing 48.89 ± 32.50% and the Control HRAP removing 36.63 ± 27.78%. The difference in BOD_5 concentration or removal between the HRAPs was not statistically significant (p=0.821 and p=0.138 respectively).

5.6.5.2. Total nitrogen, ammonium and nitrite/nitrate

Mean total nitrogen concentration was higher in the treated wastewater leaving the Enriched HRAP, 31.75 ± 9.53 mg N L^{-1}, than that leaving the Control HRAP, 27.50 ± 10.39 mg N L^{-1}, while for mean total nitrogen removal the inverse was true with the Control HRAP having a higher mean removal, 59.13 ± 21.13%, than the Enriched HRAP 53.52 ± 17.41% (Table 5.3). The difference between the HRAPs for both mean total nitrogen concentration and removal were found to be statistically significant (p<0.000).

The treated effluent leaving the Enriched HRAP had a higher mean ammonium concentration, 12.25 ± 13.28 mg NH_4-N L^{-1}, than that leaving the Control HRAP, 9.02 ± 13.70 mg NH_4-N L^{-1} (Table 5.3). This difference in mean ammonium concentration between the HRAPs was found to be statistically significant (p<0.000). The Control HRAP had a mean ammonium removal of 76.46 ± 32.33%, which was higher than the mean ammonium removal in the Enriched HRAP, 68.76 ± 31.17% (Table 5.3). This difference in mean ammonium removal between the HRAPs was found to be statistically significant (p<0.000).

The nitrite/nitrate concentration for both HRAPs increased over the study. The nitrite/nitrate concentration was significantly higher, statistically (p=0.011), in the Enriched HRAP (12.16 ± 8.28 mg NO_X-N L^{-1}) compared to the Control HRAP (10.55 ± 9.22 mg NO_X-N L^{-1}).

5.6.5.3. Orthophosphate

During the experimental period, neither HRAP effectively removed orthophosphate, with the Enriched HRAP and Control HRAP having mean removals of 16.67 ± 21.57% and 17.17 ± 22.88% respectively (Table 5.3). This difference in removal between the HRAPs was found not to be statistically significant (p=0.513). The mean orthophosphate removal values for the HRAPs were not reflected in the differences seen between the mean orthophosphate concentrations of the inlets and their corresponding HRAPs: 9.55 ± 3.87 mg PO_4-P L^{-1} for the Enriched Inlet compared to 9.76 ± 8.10 mg PO_4-P L^{-1} for the Enriched HRAP and 9.55 ± 3.87 mg PO_4-P L^{-1} for the Control Inlet compared to 10.41 ± 8.83 mg PO_4-P L^{-1} for the Control HRAP.

5.6.5.4. *Escherichia coli*

Both the mean *E. coli* concentration and LRVs were near identical between the HRAPs, and neither values were statistically significantly different (p=0.712; p=0.662 respectively). The Enriched HRAP had a mean *E. coli* concentration and LRV of 3.38 ± 0.52 $\log_{10}$ *E. coli* MPN 100 mL^{-1} and 1.15 ± 0.52, while the Control HRAP had a mean *E. coli* concentration of 3.31 ± 0.77 $\log_{10}$ *E. coli* MPN 100 mL^{-1} and mean LRV of 1.22 ± 0.49 (Table 5.3).

5.7. Discussion

5.7.1. Influent composition

The lack of statistically significant differences between the inlet wastewater composition of the two HRAPs for all the measured parameters, excluding CO_2 and inorganic carbon, suggest the Control HRAP was effective for determining the influence of CO_2 enrichment on biomass production and wastewater treatment performance.

The large, statistically significant, difference between the Enriched and Control Inlet wastewaters, in both free CO_2 and total inorganic carbon concentrations, provides strong evidence of CO_2 enrichment in the Enriched HRAP by the scrubbed biogas captured from the anaerobic ponds. The lower daily mean pH in the Enriched HRAP compared to the Control HRAP, which was found to be statistically significant, also supports a condition of comparative CO_2 enrichment. The high pH, <8.5, typically experienced in unenriched HRAPs during peaks in solar radiation, are considered symptomatic of carbon limitation. Consequently, a decrease in pH is often used to demonstrate effective CO_2 enrichment (Craggs et al., 2014).

The influent to both the HRAPs had <300 mg BOD_5 L^{-1}, a consequence of the substantial prior treatment the wastewater fed to the HRAPs had undergone. This condition was identified by Azov et al. (1982) for likely carbon limitation of microalgae growth in wastewater. This suggests that the biomass production of the HRAPs in this study might respond to CO_2 enrichment.

5.7.2. Biomass production

The addition of CO_2 scrubbed from biogas had no statistically significant effect on microalgal productivity as indicated by all measurements used except for chlorophyll *a*, which was significantly higher in the Enriched HRAP. However, caution is required when interpreting this result since chlorophyll *a* had a poor correlation, using Spearman's rank correlation coefficient, with the other biomass measures employed in the study. Furthermore, the standard deviation on the estimation of the mean chlorophyll *a* concentration was high for both HRAPs. Ramaraj et al. (2013) similarly compared chlorophyll *a* concentration to algal biomass concentration, measured by total suspended solids. They reported that chlorophyll *a* had no discernible relationship with algal biomass and should, therefore, be considered an unreliable measure. The utility of chlorophyll *a* as a surrogate biomass measure is further confounded by the observed variation in the chlorophyll *a* content of dry algal biomass,

which ranges from 0.25 to 10%, depending on environmental conditions and species (da Silva Ferreira and Sant'Anna, 2017, Bowie et al., 1985).

The use of POC as a measure of primary and secondary biomass production in this study is unique for research investigating the effect of CO_2 enrichment on HRAP performance, and it is recommended for use in future studies. While other studies on HRAPs have measured POC, it has been used in conjunction with differential filtration as a way to distinguish between algae and bacteria in the biomass (Broekhuizen et al., 2012).

The molar C:N ratio of the biomass in both the HRAPs were similar to the molar C:N ratio reported for algal biomass, 6.6:1 Redfield (1934) inferring that the bulk of the particulate material present in the HRAPs was likely microalgae. The shift from Cyanophyceae to Chlorophyceae predominance following passage through a HRAP is most likely due to the imposed mixing adversely affecting cyanobacterial growth and competition; however, this observation requires further investigation. The species of Chlorophyceae observed in the HRAPs are those commonly reported in wastewater HRAPs (Sutherland et al., 2017, Canovas et al., 1996, Craggs et al., 2014). In agreement with Sutherland et al. (2017) the wastewater treatment performance and biomass productivity of the HRAPs in our study did not seem to be significantly affected by changes in the microalgae populations.

The results of this study contradict the results of other similar studies which reported CO_2 enrichment increased biomass productivity in HRAPs treating wastewater (Craggs et al., 2012, de Godos et al., 2016, Park and Craggs, 2011). This can be explained by differences in experimental design between this and other studies. Park and Craggs (2011) reported that enrichment of a 31.8 m^2 HRAP at Hamilton, New Zealand with pure CO_2 increased median areal algal/bacterial biomass productivity (15.3 g m^{-2} d^{-1}) by approximately 30% when compared to an identical control HRAP (10.6 g m^{-2} d^{-1}). Both HRAPs were fed a 1:1 mixture of anaerobic digester effluent and tap water and the enriched HRAP intermittently received industrial, pure CO_2 via two gas diffusers placed on the pond bottom. CO_2 was added in such a way as to maintain wastewater pH at 8. Four 1.25 ha HRAPs constructed at the

Christchurch wastewater treatment plant, New Zealand, were enriched with CO_2 via on-site generator exhaust while treating primary effluent from the wastewater treatment plant (Craggs et al., 2012). As with the previous study, CO_2 was added in such a way as to maintain wastewater pH between 7.5-8.5 via sumps situated at the bottom of the HRAPs. Algal/bacterial biomass concentration, calculated as volatile suspended solids, for the four HRAPs ranged between 4.4 and 11.5 g m^{-2} day^{-1}. As the study did not include a control HRAP that was not enriched with CO_2, it is difficult to interpret the influence CO_2 enrichment had on biomass production. de Godos et al. (2016) investigated the effect THRT, and CO_2 enrichment had on the biomass production of four 32 m^2 HRAPs fed effluent pre-treated by an Upflow Anaerobic Sludge Blanket reactor in Chiclana de la Frontera, Spain. Two HRAPs were enriched with pure manufactured CO_2 sparged to the bottom of the pond to maintain the pH between 7.9 and 8.1 while the remaining two HRAPs were operated as controls. For each treatment, one HRAP was operated at a short THRT while the other was operated at a long THRT. They reported that, while CO_2 increased biomass productivity, expressed as volatile suspended solids, the THRT had a greater effect.

The most significant difference between this and other similar studies was the source of CO_2 and how it was added. The other studies used either pure bottled CO_2 (Park and Craggs, 2011, de Godos et al., 2016) or combusted exhaust gas (Craggs et al., 2012) and all added CO_2 under pH control. Biomass production may be adversely influenced by high concentrations of CO_2 and by contaminants associated with the source of that CO_2, e.g. SO_X, NO_X in flue gases (Heubeck et al., 2007, Kumar et al., 2015, Bhola et al., 2014). It has been reported that to maximise biomass production CO_2 should be added in such a way as to maintain pH within a constant range ideal for algal growth (Kumar et al., 2015). However, in this study, such control over the quality and addition of the CO_2 was not practicable since the HRAPs were retrofitted to an existing wastewater treatment plant, which was already using secondary treated wastewater and industrial scrubbers to condition biogas prior to combustion. Consequently, the quality of the CO_2 and the schedule of its addition were

subservient to the requirements of the power plant. Management of CO_2 concentrations in the wastewater specifically for algal growth would likely increase the complexity and cost of biogas scrubbing. This study presents a practical assessment of the performance of an HRAP retrofitted to existing industry standard biogas scrubbers.

5.7.3. Wastewater treatment

CO_2 addition seemed only to have a significant effect on nitrogen parameters in the wastewaters. The Control HRAP outperformed the Enriched HRAP in the removal of total nitrogen and ammonium, and while these differences were found to be statistically significant, the actual difference in values was small. Inorganic nitrogen is removed by uptake into the biomass and, additionally, for ammonia by volatilisation from the wastewater at high pH generated by algal photosynthesis (Young et al., 2017). The lower total nitrogen and ammonium removal in the Enriched HRAP is probably best explained by the lower pH resulting in decreased ammonium volatilisation (Heubeck et al., 2007, Park and Craggs, 2010).

The higher nitrite/nitrate production in the Enriched HRAP may be due to the higher inorganic carbon concentration increasing the availability of inorganic carbon for autotrophic nitrifying bacteria, or the lower pH helping establish a larger competent nitrifying population (de Godos et al., 2010, Park and Craggs, 2011, Posadas et al., 2016). Increased nitrification has been observed in other HRAPs enriched with CO_2 (de Godos et al., 2010, Park and Craggs, 2011).

There were no significant differences between the HRAPs in regards to the other wastewater treatment performance parameters. Overall, the wastewater treatment performances of both the HRAPs, while at the lower end, were still within the range of values reported for other HRAPs (Sutherland et al., 2017, Young et al., 2017, Fallowfield et al., 2018). The lower values for removal seen in this study could perhaps be explained by the wastewater already

having undergone significant treatment – with this phenomenon being observed for other similar systems that were also fed wastewater that had undergone significant prior treatment (Buchanan et al., 2018, Fallowfield et al., 2018). Even so, these results confirm that CO_2 enrichment is not a prerequisite for HRAPs to perform competent wastewater treatment and production of biomass for utilisation. Additionally, they also demonstrate that HRAPs can be easily integrated into major wastewater treatment plants.

5.7.4. Integration of CO_2 enrichment, wastewater treatment and biomass production

The integration of CO_2 recovered from power generation with combined wastewater treatment and biomass production is potentially limited by the frequency of geographic co-location of both processes. Anaerobic digestion coupled with electricity generation, commonly practised in the wastewater industry, provides a co-located source of CO_2. Commercial gas scrubbers are, however, required to operate continuously to remove H_2S and associated CO_2. This provides little opportunity to regulate CO_2 concentrations in the liquid phase. Similarly, the flow of wastewater to treatment plants is also relatively constant and requires continuous treatment. The wastewater from the AGL gas scrubbers used in our study was continuously enriched with CO_2 and supplied at a constant rate for treatment in the HRAP, as opposed to being enriched intermittently under pH control as in many other studies (Craggs et al., 2012, de Godos et al., 2016, Park and Craggs, 2011). Consequently, this study better reflects the likely industrial-scale integration of CO_2 enriched wastewater treatment with biomass production. This unregulated CO_2 enrichment, however, most likely contributed to the comparatively low pH seen in the Enriched HRAP and could have influenced the effect of CO_2 enrichment on biomass production (Kumar et al., 2015).

Keeping construction and operational costs to a minimum is imperative if biomass-derived biofuel is to be competitive with fossil fuels. Consequently, this was a priority during the design, construction and operation of the system used in this investigation. The site was

chosen because it was a major wastewater treatment plant that captured and processed biogas on-site. This meant both wastewater and CO_2 were readily available as serendipitous resources, reducing sourcing and transportation costs. The HRAPs were of sufficient size to be integrated into the already existing wastewater treatment chain and had an adequate wastewater treatment performance fulfilling the criteria set out by Lundquist et al. (2010). As the biogas is used by an on-site commercial power plant operated by AGL industry standard scrubbers were required to remove the excess CO_2 into the wastewater. Given these details, to the authors' knowledge, this experiment is the closest representation in the literature to a real-world scenario of a CO_2 enriched HRAP being operated for wastewater treatment and biomass production.

5.8. Conclusions

CO_2 enrichment of wastewater had no meaningful effect on biomass production nor wastewater treatment performance of a HRAP. This result differed from the majority of other similar studies which used alternate CO_2 sources and enrichment technologies; however, to the authors' knowledge, this is the closest representation to a real-life scenario reported in the literature and the only large-scale study to have an adequate control. This study also demonstrated that HRAPs provided adequate wastewater treatment, without CO_2 enrichment, and could be integrated successfully into a large wastewater treatment train.

5.9. Acknowledgements

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Manager, Craig Mayhew, Asset Manager, AGL and Raj Indela, Technical Assistant, Health and Environment Group, College of Science and Engineering, Flinders University.

5.10. Conflict of interest statement

The authors have no real or perceived conflicts of interest, which could have influenced the outcome of the research.

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5.12. Supplementary material

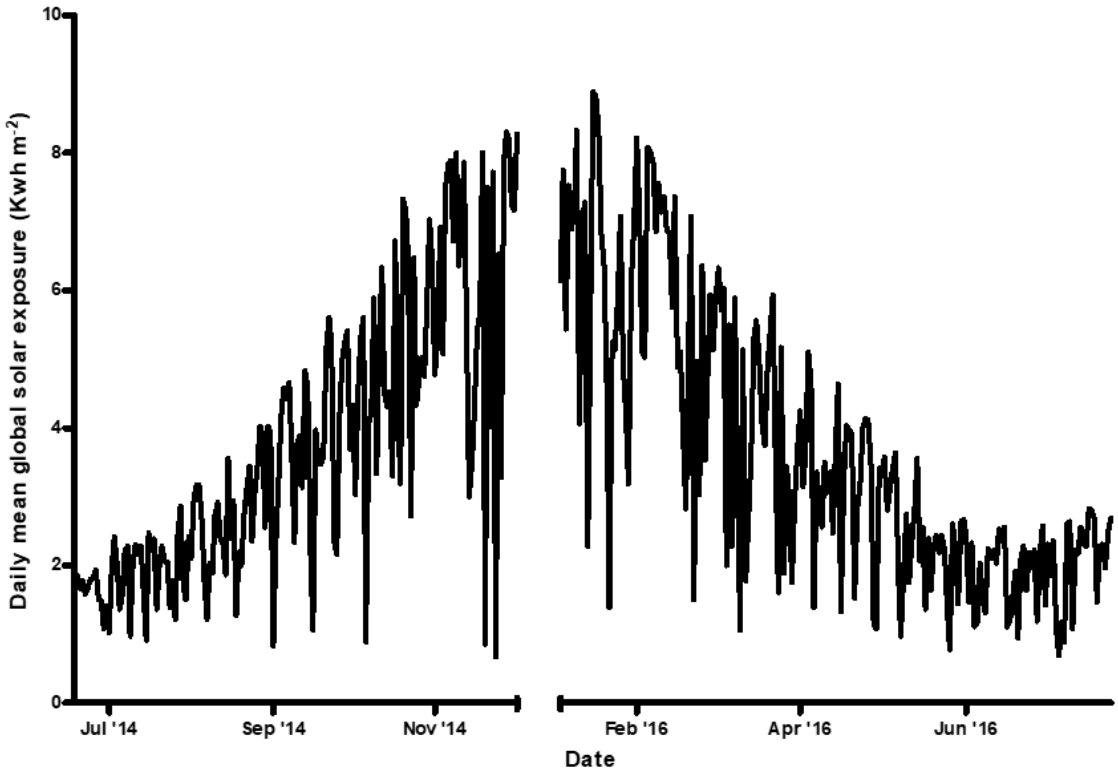


Figure 5.5. Daily mean global exposure (kWh m^{-2}) measured at the Werribee Racecourse (37.90° S, 44.64° E) approximately 8 km from the high rate algal ponds at the Melbourne Water Western Treatment Plant (Werribee, Australia) between 19 June 2014 and 24 July 2016.

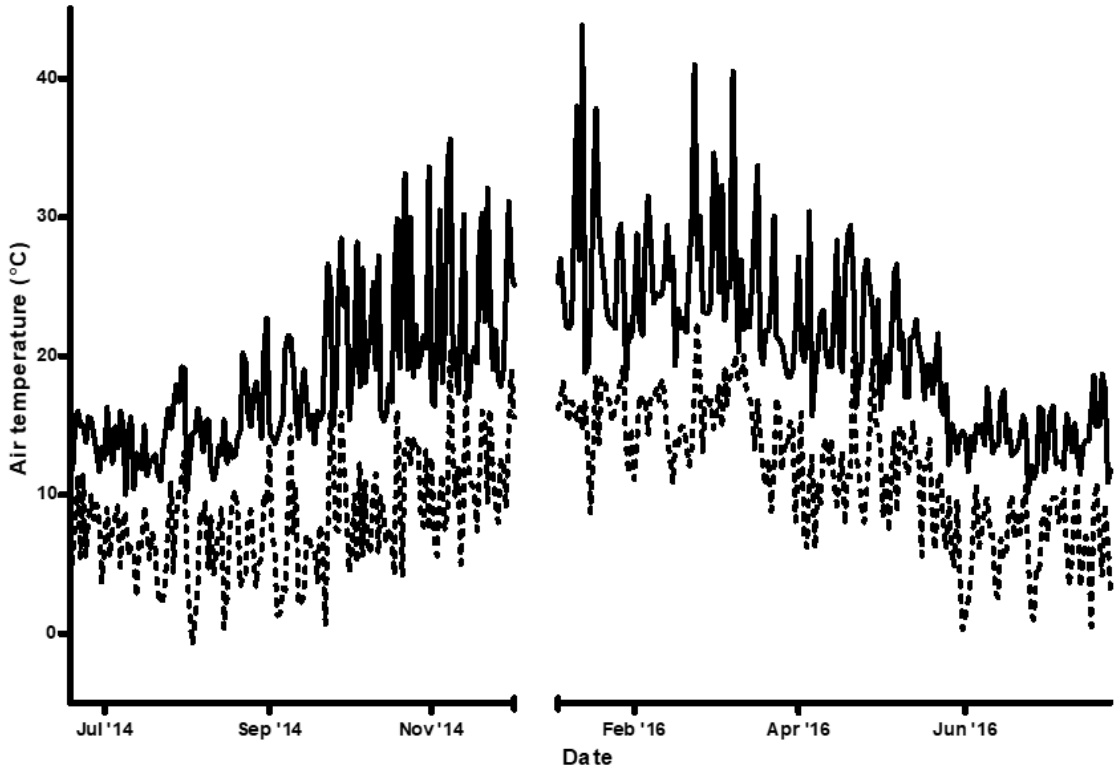


Figure 5.6. Daily maximum (-) and minimum (··) air temperature (°C) measured at the RAAF Base Williams (37.86° S, 144.76° E) approximately 16 km from the high rate algal ponds at the Melbourne Water Western Treatment Plant (Werribee, Australia) between 19 June 2014 and 24 July 2016.

Table 5.4. Spearman's rank correlation coefficient showing significant correlations and fit of correlations between multiple measures of biomass within the Enriched and Control high rate algal ponds. Correlation is significant at the 0.01 level (2-tailed).

		Total suspended solids (mg L^{-1})	Total suspended solids productivity (g m^{-2} d^{-1})	Particulate organic carbon (mg C L^{-1})	Particulate organic nitrogen (mg N L^{-1})
Total suspended solids (mg L^{-1})	**Correlation Coefficient**				
	Sig. (2-tailed)				
	n				
Total suspended solids productivity (g m^{-2} d^{-1})	**Correlation Coefficient**	1.000			
	Sig. (2-tailed)	0.000			
	n	361			
Particulate organic carbon (mg C L^{-1})	**Correlation Coefficient**	0.872	0.872		
	Sig. (2-tailed)	0.000	0.000		
	n	361	361		
Particulate organic nitrogen (mg N L^{-1})	**Correlation Coefficient**	0.836	0.836	0.948	
	Sig. (2-tailed)	0.000	0.000	0.000	
	n	361	361	361	
Chlorophyll *a* (ugL^{-1})	**Correlation Coefficient**	0.692	0.692	0.698	0.619
	Sig. (2-tailed)	0.000	0.000	0.000	0.000
	n	361	361	361	361

Table 5.5. Microalgal cell counts (log_{10} cells mL^{-1}) enumerated in Lagoon 55E wastewater, the Enriched high rate algal pond (HRAP) fed wastewater from Lagoon 55E enriched with free CO_2 and inorganic carbon and in wastewater in the Control high rate algal pond (HRAP) fed wastewater, which had bypassed the biogas scrubbers, from the same lagoon. Microalgal were identified to the genus level and only genera that constituted ≥1% of the total microalgal cell count shown of the samples were presented.

	18 September 2014			16 October 2014		29 December 2014			5 January 2016	
	Lagoon 55E	**Enriched HRAP**	**Control HRAP**	**Enriched HRAP**	**Control HRAP**	**Lagoon 55E**	**Enriched HRAP**	**Control HRAP**	**Enriched HRAP**	**Control HRAP**
BACILLARIOPHYCEAE										
Pennales		4.36					4.24	3.90	3.65	3.99
CHLOROPHYCEAE										
Chlamydomonads	4.22	4.42	4.59		5.75	3.89			3.52	3.71
Chlorococcoids	4.22	4.76	5.83			3.63	4.81	4.15	3.60	3.91
Coelastrum								4.36		
Dictyosphaerium							3.93			
Micractinium		5.48	6.15		6.02	3.23			3.58	5.14
Pediastrum							4.61	4.75	4.81	5.22
Oocystis		5.41		6.07				3.90		
Scenedesmus		5.01		4.66			4.95	5.43	3.58	
Selenastrum			4.77							
CHRYSOPHYCEAE										
Other *Chrysophytes*						3.91				
CRYPTOPHYCEAE										
Cryptomonads						3.23				
CYANOPHYCEAE										
***Aphanocapsa* (small cells)**	5.36					3.91				
***Aphanothece* (small cells)**		5.62				5.01				
Leptolyngbya	4.29					3.23				3.77
***Merismopedia* (small cells)**						3.71			3.58	
Cyanodictyon				4.25						
Cyanothamnos		5.07								

Table 5.5. (continued)

	18 September 2014			16 October 2014		29 December 2014			5 January 2016	
	Lagoon 55E	**Enriched HRAP**	**Control HRAP**	**Enriched HRAP**	**Control HRAP**	**Lagoon 55E**	**Enriched HRAP**	**Control HRAP**	**Enriched HRAP**	**Control HRAP**
***Phormidium* (small cells)**							4.72	4.49		4.17
***Phormidium* (medium cells)**										
***Planktolyngbya* (short filaments)**	4.09		4.53						4.35	
***Planktolyngbya* (long filaments)**	4.17		4.64						3.85	3.77
Pseudanabaena		4.42				3.97	5.23	4.76	4.98	4.83
Synechocystis						3.33				
Total count	5.49	6.15	6.36	6.11	6.22	5.18	5.67	5.68	5.35	5.64

CHAPTER 6. AUTOFLOCCULATION OF MICROALGAE, VIA MAGNESIUM HYDROXIDE PRECIPITATION, IN A HIGH RATE ALGAL POND TREATING DOMESTIC WASTEWATER IN THE SOUTH AUSTRALIAN RIVERLAND

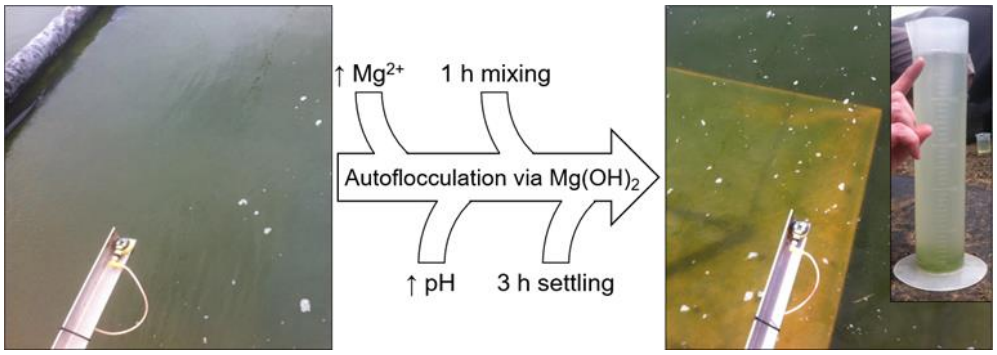
The following chapter is written as a journal article for submission to *Environmental Science: Water Research & Technology*. It was authored by Paul Young, Jordan Phasey, Dr Ilka Wallis, Professor Dries Vandamme and Professor Howard Fallowfield.

This was a jointly authored publication with the data collected by Paul Young and Jordan Phasey. Data analysis was performed by Paul Young. Modelling was performed by Dr Ilka Wallis and Paul Young. Manuscript writing and editing was performed by Paul Young with the assistance of Professor Dries Vandamme and Professor Howard Fallowfield.

The absence of a cost-effective harvesting method is widely considered the other most significant limitation to wastewater treating high rate algal ponds (HRAP) application as microalgae bioreactors for biofuel production. A method that has shown potential in laboratory studies using growth media and microalgae monocultures is autoflocculation via magnesium hydroxide precipitation. However, there has only been one assessment of this method using HRAP wastewater populated by a heterogenic mix of wild microalgae and this was only at a limited scale in a harvesting tank. Such studies are necessary to assess the suitability of a method for real-world application properly. This chapter details the assessment of microalgae autoflocculation in a large-scale, operational HRAP treating wastewater via magnesium hydroxide precipitation. It is believed to be the largest assessment of microalgae autoflocculation, via magnesium hydroxide precipitation, in HRAP treated wastewater in the literature and the only assessment conducted in-pond. It is also believed to be the closest representation to the method's real-world application in the literature.

6.1. Table of contents entry

Visual confirmation of microalgae flocculation in a high rate algal pond containing wastewater by autoflocculation, via magnesium hydroxide precipitation – the closest representation to the method's real-world application in the literature.



6.2. Abstract

High rate algal ponds (HRAP) are considered a promising system for coupling wastewater treatment with the growth of microalgae biomass for biofuel production. However, the absence of a cost-effective harvesting method limits their application for this purpose. Autoflocculation, via magnesium hydroxide precipitation, has been proposed as a harvesting method but requires further assessment in large-scale operational HRAPs treating wastewater. In this study, autoflocculation, via magnesium hydroxide precipitation, was assessed in a HRAP containing 33 m^3 of HRAP treated wastewater populated by a heterogenic mix of wild strain microalgae. Autoflocculation was induced by increasing the magnesium concentration in the HRAP to 82.63 ± 0.09 mg L^{-1} and raising the pH to 10.91 with slaked lime. The HRAP was then paddlewheel-mixed for 1 h and left to settle for 3 h. A high flocculation efficiency of 91.52 ± 0.47% was achieved with good removal of suspended solids. Excellent nutrient removal was also observed, particularly for total phosphorus, 91.10 ± 0.11%. The total chemical cost for the method was higher than those used for other flocculation methods; however, post-field study reaction modelling clearly showed the method could be optimised which would result in a substantial reduction in cost. Overall,

autoflocculation, via magnesium hydroxide precipitation, was shown to be a promising method for harvesting microalgae and nutrients in large-scale operational HRAPs treating wastewater. To the authors' knowledge, this is the largest assessment of this method in the literature and the closest representation to its real-world application. It is also the only in-pond assessment.

6.3. Water impact statement

Using microalgae grown in wastewater in high rate algal ponds (HRAP) for biofuel production has been hindered by the absence of a cost-effective harvesting method. Autoflocculation, via magnesium hydroxide precipitation, was confirmed as a promising method in large-scale operational HRAPs. This result is a step towards the successful integration of wastewater treatment and microalgae derived biofuels in HRAPs.

6.4. Introduction

The idea of using high rate algal ponds (HRAP) as a combined wastewater treatment system and microalgae bioreactor for biofuel production feed has been considered for decades (Craggs et al., 2012, Young et al., 2017). Interest in this idea has increased over recent years, as it is widely believed that, for the foreseeable future, this combination is the only economically viable way to produce microalgae derived biofuels (Lundquist et al., 2010, Young et al., 2017, Sutherland et al., 2018). In this arrangement, wastewater would be a cheap growth medium for microalgae which, as they grow, treat the wastewater by removing nutrients (Rawat et al., 2013, Chen et al., 2015, Cuellar-Bermudez et al., 2017). HRAPs are currently considered the best candidates for this combination due to their relatively high biomass productivities, inexpensive construction and operation, and ease of scale-up (Lundquist et al., 2010, De Godos et al., 2014, Kumar et al., 2015, Mendoza Martin, 2016, Arbib et al., 2017).

HRAPs are natural wastewater treatment systems that employ the symbiotic relationship between algae and bacteria to remove organic matter and nutrients from wastewater via assimilation and degradation (Young et al., 2016, Buchanan et al., 2018a). As algae are essential to HRAPs performance, these ponds are designed to maximise algal growth through maximising solar radiation exposure via shallow ponding and continuous mixing (Young et al., 2016, Buchanan et al., 2018a). Paddlewheels principally perform mixing, typically circulating the wastewater around the raceways at mean surface water velocities between 0.15 and 0.3 m s^{-1} (Young et al., 2017). HRAPs have been widely demonstrated to be an effective wastewater treatment system (Young et al., 2017, Buchanan et al., 2018a).

Cost-effective harvesting is considered one of the biggest hurdles to the economic production of microalgae derived biofuels via HRAPs treating wastewater (Vandamme et al., 2013, Hwang et al., 2016, Roselet et al., 2019). This is because microalgae are difficult to harvest due to their small cell size, 5-50 µm, negative surface charge, similar density to water, 1.08-1.13 g mL^{-1}, and relatively low concentrations in the medium, 0.5-5 g L^{-1} (Park et al., 2011, Vandamme et al., 2012, Kim et al., 2013, Drira et al., 2016). It is estimated that the harvesting process represents 20-30% of the total costs of microalgae derived biofuel production (Kim et al., 2013, Drira et al., 2016). Therefore, the development of a cost-effective harvesting method would provide a significant step towards the economic production of microalgae derived biofuels via HRAPs treating wastewater. To gain a comprehensive understanding of all the challenges facing microalgae production of biofuels see Lyon et al. (2015), Barry et al. (2016), and Salama et al. (2017).

Centrifugation is commonly used to harvest microalgae for the production of high-value products, >\$10,000 T^{-1}; however, it is too expensive and energy-intensive for the production of low-value products, <\$1000 T^{-1} (Vandamme et al., 2012, Vandamme et al., 2013, Roselet et al., 2019). This is especially so for microalgae derived biofuel as its production needs to be competitive with fossil fuel prices (Lundquist et al., 2010, Arbib et al., 2017, Roselet et al., 2019). Flocculation has been suggested as a potential low-cost method of harvesting

microalgae for this purpose (Vandamme et al., 2013, Wan et al., 2015, Lama et al., 2016, Vandamme et al., 2018).

Flocculation involves the aggregation of single microalgae cells into larger, 0.5-1.0 mm, and heavier particles called ‘flocs’ that can be easily separated from the medium via gravity sedimentation (Park et al., 2011, Vandamme et al., 2013, Muylaert et al., 2017, Sutherland et al., 2018). It can be employed as a sole harvesting technique, but it is typically suggested it be used in conjunction with another harvesting method as part of a two-step process (García-Pérez et al., 2014, Branyikova et al., 2018). In this process flocculation is used as a first step to remove the bulk of the water, concentrating the dilute suspension of microalgae by 20-100 times and resulting in slurry with a microalgae concentration of 1-5% w/v (Park et al., 2011, Vandamme et al., 2013, García-Pérez et al., 2014, Muylaert et al., 2017). The second step involves the removal of the remaining water via mechanical processes, most commonly centrifugation, resulting in a paste with a microalgae solids concentration of 15-30% w/v (Park et al., 2011, Vandamme et al., 2013, Muylaert et al., 2015a, Muylaert et al., 2017, Branyikova et al., 2018). It is widely believed this two-step process would reduce the cost of harvesting via centrifugation by one order of magnitude due to the substantially decreased volumes needing to be processed (Vandamme et al., 2012, Vandamme et al., 2015b, Branyikova et al., 2018, Vandamme et al., 2018).

Of all flocculation methods, autoflocculation, also known as alkaline flocculation, has perhaps the greatest potential for use in this scenario (Kim et al., 2013, Vandamme et al., 2013, García-Pérez et al., 2014, Vandamme et al., 2015b, Branyikova et al., 2018, Matter et al., 2019). It involves the flocculation of microalgal cells brought about by the precipitation of calcium phosphate, calcium carbonate and/or magnesium hydroxide at pH>9 (Vandamme et al., 2015b, Phasey et al., 2017, Vandamme et al., 2018, Roselet et al., 2019). While this phenomenon has been observed to occur spontaneously for microalgae in wastewater, often the addition of precipitating ions and a base to raise pH are required (Kim et al., 2013, Nguyen et al., 2014, Muylaert et al., 2015a, Vandamme et al., 2015b, Drira et al., 2016,

Zhang et al., 2016). Even though it is not as ideal as 'free' spontaneous flocculation, the low cost of the additives required to induce autoflocculation suggest the method is still economically competitive with other harvesting methods (Vandamme et al., 2013, Vandamme et al., 2015b, Drira et al., 2016, Phasey et al., 2017, Branyikova et al., 2018). These additives also have the advantage of being low in toxicity, environmentally friendly, recoverable and reusable (Vandamme et al., 2013, Zhao et al., 2014, Vandamme et al., 2015a, Vandamme et al., 2015b, Zhang et al., 2016, Muylaert et al., 2017). Additionally, the process to induce autoflocculation is relatively simple requiring only the addition of the chemicals and mixing (Vandamme et al., 2015b, Phasey et al., 2017). As HRAPs already have a means of mixing wastewater, they are particularly well-suited for this flocculation process.

Autoflocculation, via magnesium hydroxide precipitation, currently seems to be the most encouraging autoflocculation method (Smith and Davis, 2012, Brady et al., 2014). It has been shown to achieve effective flocculation, often >90%, for a variety of different microalgae species in a variety of different media under laboratory conditions (Vandamme et al., 2012, García-Pérez et al., 2014, Nguyen et al., 2014, Lama et al., 2016, Aléman-Nava et al., 2017, Vandamme et al., 2018). However, none of these studies were conducted in wastewater which undermines how representative these results would be of autoflocculation in a HRAP treating wastewater; and they all used monocultures which are impractical to maintain for long periods in HRAPs treating wastewater (Park et al., 2011, Vandamme et al., 2013, Cuellar-Bermudez et al., 2017, Young et al., 2017). Furthermore, as they were all laboratory studies, it is possible 'real-world' complications due to up-scaling and using different systems may have been overlooked.

To the authors' knowledge, there is only one study in the literature that assessed the efficiency of autoflocculation, via magnesium hydroxide precipitation, on a wild algae culture from an operational-scale HRAP treating domestic wastewater (Drira et al., 2016). They installed a small-scale, conical shaped, high-density polyethylene harvesting tank alongside

the HRAP. The tank collected 1 m^3 of *Chlorella* sp. dominated HRAP wastewater. As the wastewater already had a high concentration of magnesium, ~80 mg L^{-1}, Drira et al. (2016) opted only to add a base, sodium hydroxide at 1 g L^{-1}, to increase the pH of the collected wastewater to 12 and induce flocculation. After chemical addition, the wastewater was left to flocculate and settle for 24 h. Microalgae flocculation and sedimentation >96% was achieved after 20 min with slight improvements after 24 h. While the results of the study did provide valuable insight into the autoflocculation process in HRAPs treating wastewater, the relatively small-scale of the system might limit their applicability to larger systems.

The aim of this study was to assess the flocculation efficiency of autoflocculation, via magnesium hydroxide precipitation, in a large-scale, operational HRAP treating domestic wastewater. This was achieved by using a HRAP system consisting of two identical HRAPs operated in series. The first HRAP received and treated domestic wastewater while the second HRAP received the treated effluent and was used as a flocculation basin. The authors are unaware of any similar study reported in the literature conducted at such a scale to date and believe it to be the closest representation to the method's real-world application.

6.5. Materials and methods

6.5.1. Kingston on Murray domestic wastewater treatment system

The Community Wastewater Management Scheme (CWMS) wastewater treatment system at Kingston on Murray in the South Australian Riverland was selected for this study. The system services the small rural community of Kingston on Murray which has a population of approximately 300 permanent residents and undertakes the typical commercial activities for an Australian rural community of its size. It consists of two identical HRAPs which were operated in series. The first HRAP in the series received domestic wastewater from Kingston on Murray after it had undergone treatment via on-site septic tanks, while the second HRAP

received the treated effluent from the first. The treated effluent from the second HRAP is pumped to a storage pond and reused for on-site irrigation. During the trial, the first HRAP was operated continuously (Wastewater Treatment HRAP; Plate 6.5) and the second HRAP was used as a flocculation basin (Flocculation HRAP). The physical dimensions of the HRAPs as used in this trial are described in Table 6.1.

Table 6.1. Physical dimensions of the Wastewater Treatment high rate algal pond (HRAP) and Flocculation HRAP at the Kingston on Murray wastewater treatment system, Australia

	Wastewater Treatment HRAP	**Flocculation HRAP**
Length (m)	30	30
Width (m)	5	5
Volume (m^3)	64	33
Theoretical hydraulic residence time (d)	5	N/A
Depth (m)	0.32	0.20
Circulation speed ($m\ s^{-1}$)	0.2	0.1
Circulate rate ($L\ s^{-1}$)	160	50

6.5.2. Jar test experiments on the autoflocculation of microalgae in high rate algal pond treated domestic wastewater via magnesium hydroxide precipitation

Before the in-pond trial was conducted, jar tests were performed on the Wastewater Treatment HRAP effluent to determine the minimum concentration of magnesium required to achieve a flocculation efficiency of >99%. These were performed by firstly adding known amounts of dissolved magnesium chloride, as $MgCl_2.6H_2O$, to 1 L test jars (Scientific Equipment Manufacturers Pty. Ltd., Australia), and then raising the pH to 11 using slaked lime. After chemical addition, rapid mixing was performed at 100 rpm for 5 min, followed by slow mixing at 15 rpm for 15 min and settling for 30 min. The supernatant was decanted from the settled sludge and stored at 1°C for chemical and nutrient analysis.

6.5.3. In-pond trial on the autoflocculation of microalgae in high rate algal pond treated domestic wastewater via magnesium hydroxide precipitation

To perform the in-pond trial 33 m^3 of the Wastewater Treatment HRAP effluent was pumped to the recently emptied and cleaned Flocculation HRAP. Then, based upon the jar-test results, 20 kg of magnesium chloride, as $MgCl_2.6H_2O$, was added to the effluent in the Flocculation HRAP to increase the concentration of magnesium to 80 mg L^{-1}.

After magnesium chloride addition, the addition of a base was required to artificially raise the pH of the wastewater to 11 to induce autoflocculation via magnesium hydroxide precipitation. Slaked lime was selected as the base to use in this study because it is considered the most cost-efficient base available and it poses a low health risk (Vandamme et al., 2012, Lama et al., 2016). The amount of slaked lime necessary to raise the pH of the Flocculation HRAP to 11 was determined through titration. A 10 g L^{-1} suspension of slaked lime was prepared by rapid mixing and was added in 1 mL increments to 1 L of Flocculation HRAP wastewater post-magnesium chloride addition. Based on the titration results, 6 kg of slaked lime, equivalent to 182 mg L^{-1}, was added to the Flocculation HRAP to raise the pH of the wastewater to 11 (Figure 6.3). The addition took approximately 2 h via a dosing apparatus (Plate 6.1) and was performed directly above the paddlewheel with considerable splashing. This method of addition was deliberate to help facilitate even mixing of the slaked lime within the HRAP. It is possible the splashing led to the absorption of atmospheric CO_2, which reduced the basicity of the water. Additionally, the long time for addition may also have contributed to atmospheric CO_2 absorption.

Plate 6.1. The dosage apparatus used to add magnesium chloride and slaked lime to the Flocculation high rate algal pond (HRAP) at Kingston on Murray wastewater treatment system, Australia. Also pictured is the paddlewheel in the Flocculation HRAP that was used for mixing after chemical addition.

Following chemical addition, the Flocculation HRAP was mixed by the paddlewheel at 0.1 m s^{-1} for 1 h. After mixing, samples of the effluent were collected in 1000 mL measuring cylinders from 3 locations in the Flocculation HRAP to observe settling (Plate 6.2). A further 3 h of settling took place prior to sample collection from the three locations in the HRAP for chemical, nutrient and microbiological analysis.

Plate 6.2. The emptied and cleaned Flocculation high rate algal pond (HRAP) at the Kingston on Murray wastewater treatment system, Australia, prior to the transfer of the Wastewater Treatment HRAP effluent. The location of the three sampling points (1-3) used to determine the efficacy of flocculation are also shown.

6.5.4. Sample analysis

Chemical and nutrient analyses of samples, as well as microalgae identification and enumeration, were performed by the Australian Water Quality Centre, Adelaide, Australia – a National Association of Testing Authorities accredited laboratory. Analyses performed were determination of pH (method T0010-01; APHA 4500-H B), determination of conductivity (method T0016-01; APHA 2510 B), ammonia/ammonium - automated flow colorimetry (method T0100-01; APHA 4500-NH3 G), alkalinity - automated acidimetric titration (method T0101-01; APHA 2320 B), chloride - discrete analyser (method T0104-02; APHA 4500-Cl- E), filterable reactive phosphorus - automated flow colorimetry (method T0108-01; APHA 4500-P G), phosphorus - total by discrete analyser (method T0109-01; APHA 4500-P F), nitrogen - total Kjeldahl by discrete analyser (method T0112-01; APHA 4500-N org A),

biochemical oxygen demand (method T0153-01; APHA 5210 B), chemical oxygen demand (method T0155-01; APHA 5220 B), total and dissolved organic carbon (Shimadzu TOC VCSH; method T0158-09; SM5310B), suspended solids (method T0160-01; APHA 4500), chlorophyll *a* & *b* and phaeophytin (method T0380-02; ISO 10260, 1992), microalgae & cyanobacteria - scan & identification (method T0393; Hötzel and Croome, 1999), preparation of samples for metal analysis (method W-052; APHA 3030A to 3030D) and determination of metals - ICP spectrometry by ICP2 (method TIC-004; APHA 3120).

Escherichia coli enumeration was performed by the Health and Environment Group at Flinders University using Colilert Quanti-Tray® (IDEXX Laboratories, Inc. Westbrook, USA) most probable number (MPN) chromogenic substrate assay.

As is the convention, flocculation efficiency was calculated as the percentage reduction in turbidity before and after flocculation (Equation 6.1).

$$Flocculation\ effciency = 100 - (100 \times \left(\frac{T_1}{T_0}\right))$$

Where,

T_0 = Turbidity pre-flocculation (NTU)

T_1 = Turbidity post-flocculation (NTU)

Equation 6.1. Formula used to calculate flocculation efficiency (%).

6.5.5. Statistical analysis

Data handling and analysis was performed using Microsoft Office Excel 2016 and SPSS Statistics, version 23 (Armonk, NY). Graphs were made using Prism 8 (GraphPad Software, CA USA).

Descriptive statistics were calculated for all eligible data. A linear quadratic survival regression was used to assess the relationship between *E. coli* and pH data. The level of

significance was set at $p<0.05$. Bivariate Pearson correlation coefficient was used to assess the relationship between flocculation efficiency, total suspended solids, volatile suspended solids and phosphorus with the level of significance set at $p<0.01$

6.5.6. Geochemical model of the Flocculation high rate algal pond during the autoflocculation process

Based on the wastewater characterisation and the observed hydrochemical changes following slaked lime addition (Table 6.3), a geochemical model was formulated, which provided a quantitative assessment of the predominant geochemical reactions within the Flocculation HRAP during the autoflocculation process. Modelling was undertaken using the code PHREEQC-2 (Parkhurst and Appelo, 1999) in conjunction with the standard PHREEQC database, extended by the phases brucite ($Mg(OH)_2$; magnesium hydroxide) and calcium hydroxide ($Ca(OH)_2$; slaked lime) (Ball and Nordstrom, 1991).

The geochemical model was based on a reaction network of mixed equilibrium and kinetic reactions. Slaked lime was included as an equilibrium phase in the model to account for the observed dissolution of slaked lime and the subsequent rise in pH and calcium. Hydroxyapatite ($Ca_5(PO_4)_3OH$) was allowed to precipitate under the equilibrium assumption based on calculated saturation indices of the wastewater and the observed reduction in phosphate. Magnesium hydroxide was modelled as a kinetic reaction on the basis of the standard formulation of dissolution and precipitation of minerals (Equation 6.2).

$$R_k = k_k \left(1 - \left(\frac{IAP}{K_{sp}}\right)\right)$$

Where,

k_k = empirical constant

IAP/K_{sp} = saturation ratio

Equation 6.2. Standard formulation of dissolution and precipitation of minerals

K_k was adjusted during model calibration until simulated precipitation rates of magnesium hydroxide were consistent with observed reaction rates by Pokrovsky and Schott (2004), who quantified magnesium hydroxide precipitation rates as a function of pH and magnesium concentration.

The model input concentrations of the wastewater were based on the chemical analysis post-magnesium chloride addition (Table 6.3). Slaked lime addition was based on the measured added quantity of 6 kg; however, calibration of the geochemical model to the observed data suggests that approximately 80% of the added slaked lime was dissolved after 4 h, at which point samples were taken. This is consistent with the reported incomplete mixing of the added slaked lime into the 33 m^3 of wastewater due to reduced paddlewheel circulation speeds resulting in reduced contact time between effluent and paddlewheel. Water compositions were charge-balanced with PHREEQC-2 by adjusting the chloride concentration.

The implemented reaction network allowed for the close replication of the observed hydrochemical changes within the wastewater. The developed model was used to explore how operational conditions can be improved for cost-effectiveness, including the reduction in added slaked lime and magnesium chloride and its effect on magnesium hydroxide precipitation and therefore autoflocculation (Figure 6.4).

6.6. Results and discussion

6.6.1. Algal species composition in the high rate algal pond treated domestic wastewater

The effluent from the Wastewater Treatment HRAP was found to be dominated by microalgae species of the genus *Micractinium* sp., with relativity minor abundances of species from the genera *Scenedesmus* sp. and *Monoraphidium* sp. (Table 6.2). Microalgae species from these genera have been regularly observed in HRAPs treating wastewater, particularly *Micractinium* sp. and *Scenedesmus* sp. (Canovas et al., 1996, Craggs et al., 2014, Sutherland et al., 2017, Young et al., 2019). As *Micractinium* sp. and *Scenedesmus* sp. are colonial algae, they readily settle by gravity upon cessation of HRAP mixing (Craggs et al., 2014). Possibly due to the dominance of these colonial microalgae in the Wastewater Treatment HRAP effluent, the biomass present in the effluent settled readily when HRAP mixing ceased, achieving a 25% decrease in turbidity in 30 min in the absence of chemical additives. This result suggests that the biomass present in this study was receptive towards flocculation.

Table 6.2. Microalgae and diatom cell concentration (cells mL^{-1}) in the effluent from the Wastewater Treatment high rate algal at the Kingston on Murray wastewater treatment system, Australia. Also included is chlorophyll *a* (µg L^{-1}) and pheophytin a (µg L^{-1}) concentration.

	Cell count (cells mL^{-1})
Algae	
Micractinium sp.	882,000
Scenedesmus sp.	10,000-100,000
Monoraphidium sp.	1,000-10,000
Diatoms	
Nitzschia sp.	1,000-10,000
Chlorophyll *a* (µg L^{-1})	1,590
Pheophytin a (µg L^{-1})	226

6.6.2. Jar test experiments on the autoflocculation of microalgae in high rate algal pond treated domestic wastewater via magnesium hydroxide precipitation

Based on the observed reduction in turbidity, an extremely high flocculation efficiency of 99.15% was achieved for microalgae in Wastewater Treatment HRAP effluent at a magnesium concentration of 80.1 mg L^{-1} and a pH of 11. Similar levels of removal were also observed for total suspended solids, 97.39%, and volatile suspended solids, 98.18%, at the same magnesium concentration (Figure 6.1a).

Flocculation of microalgae can also play a significant role in HRAP wastewater treatment. Significant removal of nutrients was observed at a magnesium concentration of 80.1 mg L^{-1}, with 29.45% of ammonia, 47.11% of total Kjeldahl nitrogen and 97.03% of total phosphorus removed (Figure 6.1b). Total phosphorus removal was well described by flocculation efficiency, total suspended solids removal and volatile suspended solids removal while the removals of the nitrogen parameters were not. It is likely the same factors that influenced flocculation efficiency and solids removal via magnesium hydroxide precipitation also heavily influenced phosphorus removal but had a negligible influence on the removals of inorganic nitrogen. This difference in the removal of the two nutrients was due to their different mechanisms of removal in HRAPs. Aside from assimilation into the microalgae biomass, nitrogen species are mainly removed in HRAPs by volatilisation at high pH and phosphorus removal is mainly caused by precipitation (Young et al., 2017, Park et al., 2011). As flocculation and solids removal were brought about by the precipitation of magnesium hydroxide, the relationship between these parameters and the removal of phosphorus was likely reflecting the relationship between magnesium hydroxide and phosphorus precipitation. The relationship observed between phosphorus, flocculation efficiency, total suspended solids and volatile suspended solids was supported by a bivariate Pearson correlation coefficient reporting statistically significant, $p=0.000$, strong correlations, >0.98, between all parameters (Table 6.4). Overall, these results for the removal of nutrients support the proposition that in addition to harvesting the biomass, autoflocculation can also

assist in wastewater treatment and nutrient recovery in HRAPs (Park et al., 2011, Kim et al., 2013, Vandamme et al., 2013, Phasey et al., 2017).

Considering these high removal rates, and the little improvement seen in them at higher magnesium concentrations, it was decided to increase the concentration of magnesium in the Flocculation HRAP to 80 mg L^{-1}. This decision was based on the desire to use the minimum concentration of magnesium required to achieve a flocculation efficiency of >99% This was in order to keep costs to a minimum while still having a high a very flocculation efficiency, which would be imperative in real-world applications.

When compared to the result of other studies conducted in the laboratory the magnesium concentration used in these jar tests to achieve a similar level of flocculation efficiency was exceedingly high (Vandamme et al., 2012, García-Pérez et al., 2014, Nguyen et al., 2014, Lama et al., 2016, Aléman-Nava et al., 2017, Vandamme et al., 2018). This was probably due to differences in medium and microalgae culture. In this study, the medium used was treated wastewater from the Wastewater Treatment HRAP and the microalgae culture used was the heterogenic mix of wild strains that had colonised the HRAP, in comparison, the other studies were performed using growth media and monoculture laboratory strains. Consequently, although the results presented here disagree with the other studies, they are arguably more representative of how the process would operate in the real world. When investigating autoflocculation via calcium phosphate precipitation, Phasey et al. (2017) found a similar inconsistency between a study using a laboratory culture and growth media and their study using wastewater and the microalgae naturally present. The concentration of calcium phosphate needed to induce autoflocculation in their wastewater samples was at least ten times higher than the values reported in the other study. They attributed this to the wastewater used in their study having a charge density 20-50 times higher than that of the media used in the laboratory study caused by the higher concentration of organic matter present in the wastewater. They correctly concluded that caution should be taken when

extrapolating the results of laboratory studies to microalgae in wastewater, particularly when determining chemical doses to induce flocculation.

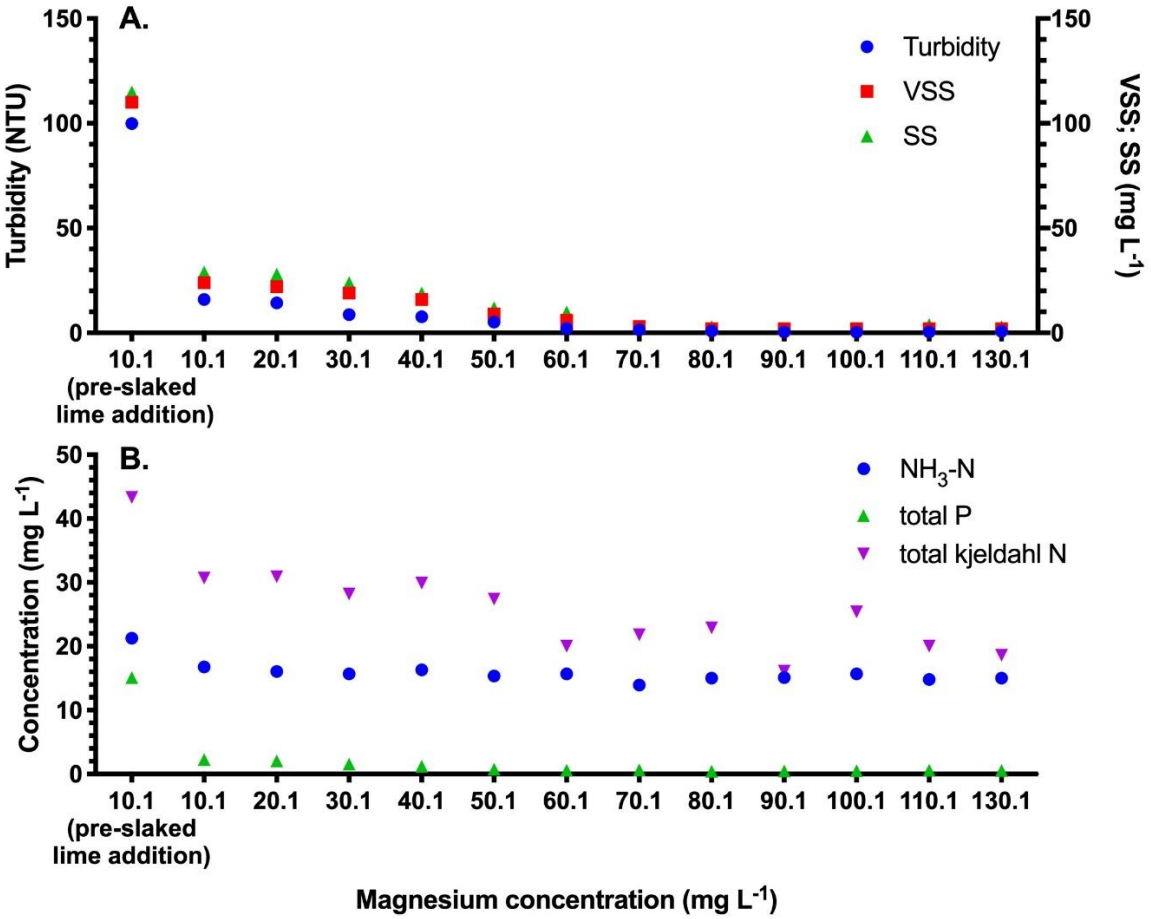


Figure 6.1. Reduction in **(A)** turbidity (NTU), volatile suspended solids (mg L^{-1}) and suspended solids (mg L^{-1}) concentration; and **(B)** ammonia (mg L^{-1}), organic nitrogen (mg L^{-1}) and total phosphorus (mg L^{-1}) concentration in Wastewater Treatment High Rate Algal Pond (HRAP) effluent at different magnesium concentrations (mg L^{-1}). Slaked lime was used to raise the pH of the effluent to 11. Wastewater Treatment HRAP effluent pre-slaked lime addition was use as a control.

6.6.3. In-pond trial on the autoflocculation of microalgae in high rate algal pond treated domestic wastewater via magnesium hydroxide precipitation

A high flocculation efficiency of 91.96 ± 8.47% was achieved in the Flocculation HRAP at a magnesium concentration of 82.63 ± 0.09 mg L^{-1} and a maximum pH of 10.91, after 1 h of mixing and 3 h of settling (Table 6.3). Lower but substantial removals were also seen for

total suspended solids, 84.93 ± 0.82%, and volatile suspended solids, 86.05 ± 1.71% (Table 6.3).

All these removal values were less than those achieved in the corresponding jar test. This was considered to be primarily due to differences in the completeness of mixing between the jar test and the Flocculation HRAP, which presumedly reduced chemical interaction and resulted in reduced magnesium hydroxide precipitation. The significantly larger volume of the effluent in the Flocculation HRAP, 33 m^3, compared to the jar-test, 1 L, makes it inherently more difficult to mix (Table 6.1). When operating correctly, paddlewheels are widely believed to provide proper mixing in HRAPs even at large sizes. (Sutherland et al., 2015, Hawley and Fallowfield, 2016, Young et al., 2017). Unfortunately, during the mixing step, the circulation speed of the paddlewheel in the Flocculation HRAP was slower, 0.1 m s^{-1}, than its designed speed, 0.2 m s^{-1}. This was likely due to the effluent being at a lower depth, 0.2 m, than the designed depth, 0.3 m, resulting in reduced contact between the effluent and paddlewheel (Table 6.1). Therefore, these results should be viewed in the light that more complete mixing is certainly possible and would likely result in increased flocculation efficiency.

There was visual confirmation of flocculation and sedimentation in the Flocculation HRAP. Precipitation of magnesium hydroxide was observed upon addition of slaked lime (Table 6.3), with evident floc formation occurring (Plate 6.6 & Plate 6.7). The three measuring cylinder samples had significant floc formation and underwent rapid sedimentation (Plate 6.3). Another visual confirming floc formation was the improved visibility of the concrete plinth upon which the paddlewheel is mounted. Prior to slaked lime addition, the plinth, which was ~0.05 m below the surface, was not visible through the effluent. After slaked lime addition, the plinth was visible, and very clearly visible after cessation of paddlewheel mixing and being left to settle for 3 h (Plate 6.4). This level of clarity was observed throughout the Flocculation HRAP with the bottom of the pond clearly visible in all locations.

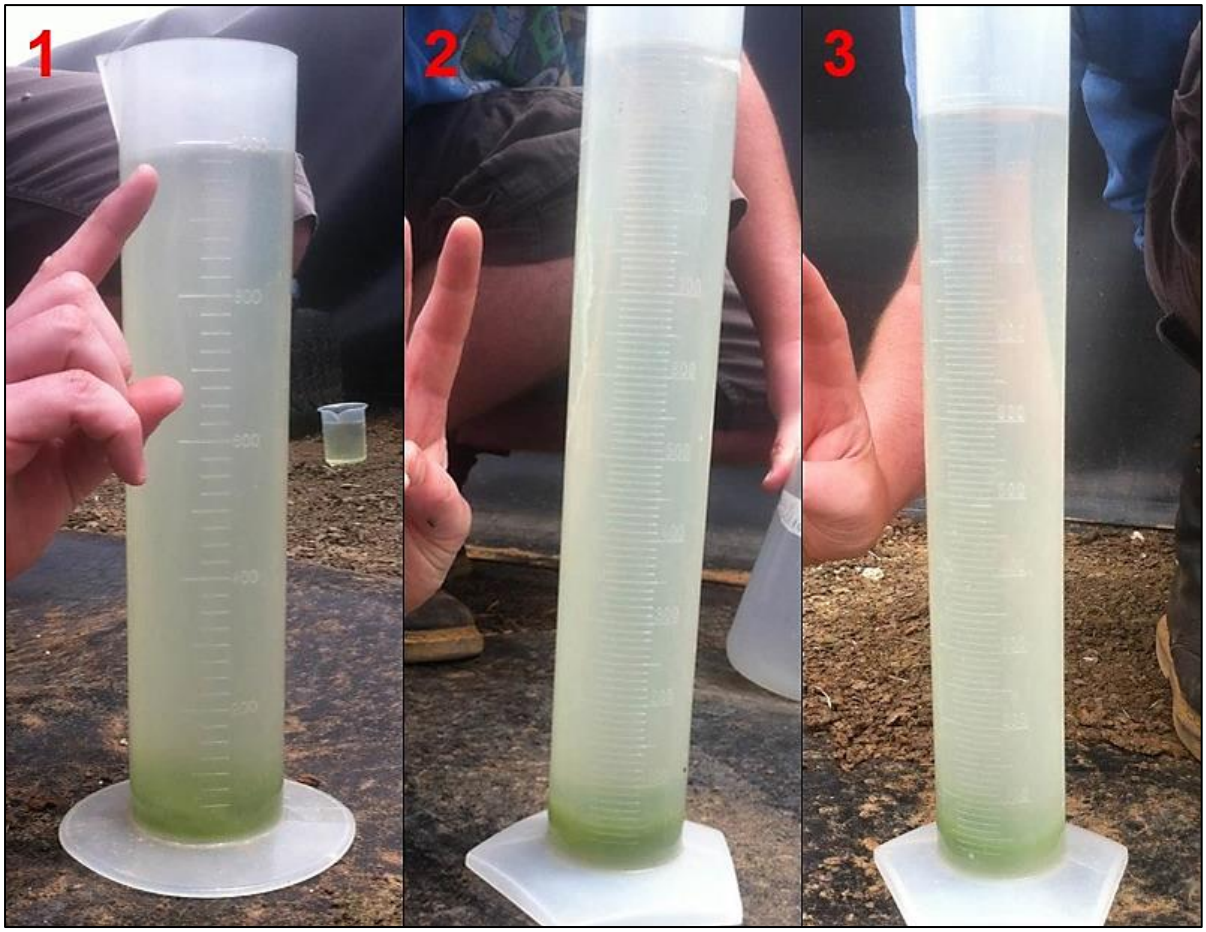


Plate 6.3. Visible flocculation and sedimentation in 1 L samples of wastewater taken from the 3 sampling points (see Plate 6.2) in the Flocculation high rate algal pond at the Kingston on Murray wastewater treatment system, Australia, after magnesium chloride and slaked lime addition followed by 1 h of mixing.

Plate 6.4. Visible flocculation and sedimentation of the microalgae biomass in the Flocculation high rate algal pond at the Kingston on Murray wastewater treatment system, Australia, post-autoflocculation via magnesium hydroxide. The plinth the paddlewheel is mounted on was clearly visible.

The flocculation efficiency achieved in this study was slightly less than the result reported by Drira et al. (2016) for the HRAP wastewater in a 1 m^3 the harvesting tank. This discrepancy was likely caused by the difference in the amount of magnesium hydroxide that precipitated in the two studies. The magnesium concentrations prior to flocculation were similar in both studies with a concentration of 82.63 ± 0.09 mg L^{-1} reported in this study and Drira et al. (2016) reporting a concentration of ~80 mg L^{-1}. However, after flocculation, the magnesium concentration between the studies was substantially different with the Flocculation HRAP having a significantly higher concentration, 71.17 ± 0.05 mg L^{-1}, than the harvesting tank, ~15 mg L^{-1}. This difference is likely to be the result of the higher pH achieved in the harvesting tank, pH 12 (Drira et al. 2016), compared to the Flocculation HRAP, pH 10.9. This explanation was supported by PHREEQC modelling. When the Flocculation HRAP was

simulated, attaining a similarly high pH of 11.7 by more than doubling the amount of slaked lime added to the wastewater, the magnesium hydroxide precipitation was considerably enhanced, and the magnesium concentration decreased from the initial 82.63 mg L^{-1} to 15.7 mg L^{-1}, an almost identical value to that reported by Drira et al. (2016) This result illustrates the strong influence of pH on magnesium precipitation (Figure 6.5). It is noteworthy, that despite the difference in observed magnesium concentrations between the harvesting tank (Drira et al. 2016) and the Flocculation HRAP, only a comparatively small difference in flocculation efficiency, ~5%, was observed between the two studies This suggests the influence increasing magnesium hydroxide precipitation has on microalgae flocculation efficiency decreases as the amount of magnesium hydroxide precipitating increases. This relationship was also observed during the jar tests (Figure 6.1a).

After flocculation, the supernatant in the Flocculation HRAP had 31.28 ± 2.89% less ammonia, 49.78 ± 3.18% less total Kjeldahl nitrogen and 91.10 ± 0.11% less total phosphorus (Table 6.3). Phosphorus removal in the Flocculation HRAP was less than observed in the corresponding jar test, which experienced a similar relative percentage decrease to those observed for flocculation efficiency, and total and volatile suspended solids removal. This was likely due to the presumed reduction in precipitation experienced in the Flocculation HRAP compared to the jar test, as this is one of the main processes for phosphorus removal in HRAPs (Park et al., 2011, Young et al., 2017). In comparison, the level of nitrogen removal observed for the Flocculation HRAP was very similar to the level observed in the jar test. This was likely due to the pH of the Flocculation HRAP and jar test being close, as volatilisation of ammonia at high pH is one of the main mechanisms of nitrogen removal in HRAPs (Park et al., 2011, Young et al., 2017).

The high removal of phosphorus is probably the most important wastewater treatment result. This is because phosphorus removal by HRAPs is variable or poor and therefore a simple method to increase phosphorus removal while also harvesting microalgae biomass was of great interest (Nurdogan and Oswald, 1995, Young et al., 2017). Additionally, due to the

increasing demand on finite global phosphorus reserves, there has been a strong push towards more sustainable practices of phosphorus use (Vandamme et al., 2013, Egle et al., 2016, Phasey et al., 2017). Recovering phosphorus from waste streams, especially wastewater, has been considered one such practice (Cornel et al., 2009, Egle et al., 2016). Unfortunately, the flocculants traditionally used by wastewater treatment plants, iron and aluminium salts, are toxic and contaminate the recovered phosphorus (Phasey et al., 2017). The high phosphorus removal results from this study in conjunction with PHREEQC modelling predicting the precipitation of 0.07 g L^{-1} hydroxyapatite post-autoflocculation, suggest that autoflocculation, via magnesium hydroxide precipitation, is a potential low toxicity method of recovering phosphorus from wastewater and should be further investigated.

It has been suggested that the high pH needed to induce autoflocculation can also play a significant role in pathogen disinfection (Semerjian and Ayoub, 2003, Vandamme et al., 2012). It is well-known exposure to extreme pH levels for sustained periods can have a deleterious effect on pathogens (Semerjian and Ayoub, 2003). This phenomenon was observed here study with *E coli* concentration being negatively correlated to the length of time the organisms were exposed to the Flocculation HRAP wastewater post-slaked lime addition to raise the pH to a maximum of 10.91 (Figure 6.2). This negative correlation was found to be statistically significant ($p<0.01$) and resulted in a final *E. coli* concentration of 0 $\log_{10}$ *E. coli* MPN 100 mL^{-1} after 6 h of exposure.

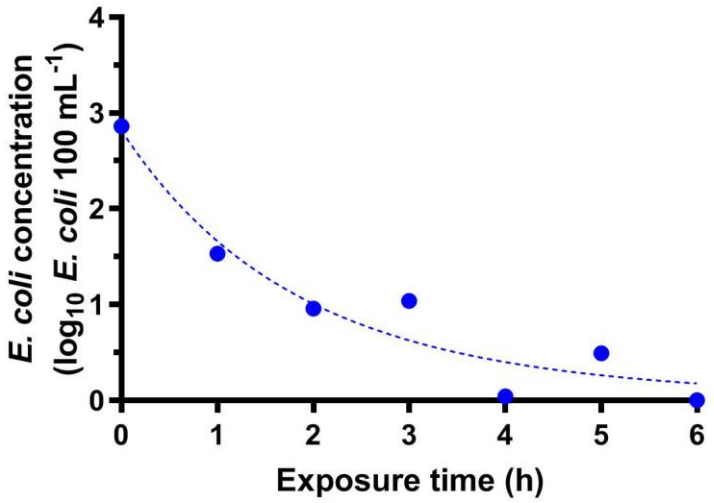


Figure 6.2. Effect of exposure time (h) to the wastewater from the Flocculation high rate algal pond (HRAP) at the Kingston on Murray wastewater treatment system, Australia, post-slaked lime addition which raised the pH to a maximum of 10.91 on *Escherichia coli* concentration (●; $\log_{10}$ *E.coli* 100 mL^{-1}). An exponential decay function was fitted to the data (R^2=0.9322).

The results of the physical and chemical analysis of the wastewater from the Flocculation HRAP post-flocculation demonstrate that autoflocculation, via magnesium hydroxide precipitation, can be used as a method to improve the quality of HRAP effluent (Table 6.3). Recently a HRAP system underwent independent validation and subsequently was approved to be included in the CWMS as a wastewater treatment system (Fallowfield et al., 2018). As part of this, the effluent from HRAP systems was accepted to meet the requirements of the *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1)* (NRMMC, 2006) for non-food crop irrigation – ordinarily woodlots. The improved effluent quality achieved by flocculation and sedimentation in this study met the requirements of the guidelines for suspended solids concentration, <30 mg L^{-1}, and *E. coli* concentration, <1000 *E. coli* MPN 100 mL^{-1}, for irrigation of commercial crops that undergo processing and have no contact with the ground (NRMMC, 2006). A filtered BOD_5 concentration of <20 mg L^{-1} is also a requirement of the guidelines, but unfortunately, this was not measured in this study. However, there has been previously published data on the filtered BOD_5 concentration of the effluent from the HRAP system used in this study that consistently meets this requirement even without flocculation (Young et al., 2016, Buchanan

et al., 2018a, Buchanan et al., 2018b). In combination, these results suggest that autoflocculation, via magnesium hydroxide precipitation, could be used to improve HRAP effluent, thereby increasing its application as a recycled resource for irrigation.

Table 6.3. Physical and chemical analysis of the wastewater in the Flocculation high rate algal (HRAP) at the Kingston on Murray wastewater treatment system, Australia, pre-autoflocculation via magnesium hydroxide precipitation, post-magnesium chloride addition, and post-autoflocculation via magnesium hydroxide precipitation. Also included is the removal of the parameters from the Flocculation HRAP wastewater post-autoflocculation via magnesium hydroxide precipitation (%).

	Flocculation HRAP wastewater	Post-magnesium chloride addition	Post-autoflocculation[a]	Removal (%)[a]
Turbidity (NTU)	99.90		8.47 ± 0.57	91.52 ± 0.57
Total suspended solids (mg L^{-1})	115.00	104.00	17.33 ± 1.15	84.93 ± 1.00
Volatile suspended solids (mg L^{-1})	110.00	100.00	15.33 ± 2.31	86.06 ± 2.10
Unfiltered 5-day biochemical oxygen demand (mg L^{-1})	141.00	75.00	49.67 ± 45.32	64.78 ± 32.15
Chemical oxygen demand (mg L^{-1})	244.00	234.00	110.67 ± 39.26	54.64 ± 16.09
Dissolved organic carbon (mg L^{-1})	22.80	25.80		
Ammonia (mg L^{-1})	21.29	20.76	14.63 ± 0.75	31.28 ± 3.54
Total Kjeldahl nitrogen (mg L^{-1})	43.30	41.90	21.77 ± 1.69	49.78 ± 3.89
Organic nitrogen (mg L^{-1})	22.00	21.10	7.14 ± 2.18	67.56 ± 9.93
Filtered reactive phosphorous (mg L^{-1})	12.20	12.50	0.10 ± 0.02	99.22 ± 0.13
Total phosphorus (mg L^{-1})	15.10	14.80	1.34 ± 0.02	91.10 ± 0.14
Calcium (mg L^{-1})	23.20	25.20	68.10 ± 0.10	
Magnesium (mg L^{-1})	10.10	82.63 ± 0.09[a]	71.17 ± 0.06	13.84 ± 0.00
Sulfate (mg L^{-1})	41.4	42.3	41.00 ± 0.17	
Potassium (mg L^{-1})	33.9	35.4	35.17 ± 0.12	
Sodium (mg L^{-1})	89.2	92.3	90.43 ± 0.35	
Chloride (mg L^{-1})	99	264	283 ± 29.46	
Electrical conductivity (µS cm^{-1})	949.00	1550.00	1546.67 ± 5.77	
pH	7.4	7.9	10.3	
Alkalinity (mg L^{-1})	51.00	48.00	115.67 ± 1.15	
Bicarbonate (mg L^{-1})	62.00	58.00	0.00 ± 0.00	
Carbonate (mg L^{-1})	0.00	0.00	63.00 ± 1.73	
Hydroxide (mg L^{-1})	0.00	0.00	3.67 ± 1.53	

[a]Results are the mean ± standard deviation of samples collected in triplicate.

After flocculation, the residual magnesium concentration in the supernatant of the Flocculation HRAP was 71.2 mg L^{-1} (Table 6.3). This was remarkably high and indicated that

only a small quantity, 11.46 mg L^{-1}, of the magnesium present, 82.63 ± 0.09 mg L^{-1}, precipitated with the flocs. The PHREEQC simulations confirm this moderate reduction in aqueous magnesium, reporting a concentration of 70.2 mg L^{-1} in the supernatant due to magnesium hydroxide precipitation under the induced pH conditions. The PHREEQC simulations reported 0.99 kg of magnesium hydroxide would have precipitated after 4 h in the Flocculation HRAP, the time of sampling post-autoflocculation (Table 6.3). Precipitation is thereby governed by the solubility product of $(Mg^{2+})(OH^{-})^2$, and a maximum amount of 1.52 kg of magnesium hydroxide is able to precipitate once equilibrium in regards to magnesium hydroxide ($SI_{magnesium\ hydroxide}$=0) is established after ~1 d (Figure 6.4). This translates into a maximum possible reduction in dissolved magnesium of 19.2 mg L^{-1} under the invoked wastewater pH.

The limited observed reduction in dissolved magnesium concentrations could be explained in two ways. Firstly, the required magnesium to induce flocculation was overestimated and was thus in excess in the Flocculation HRAP. Secondly, the maximum pH reached in the Flocculation HRAP, 10.91, might not have been high enough to induce precipitation of most of the magnesium present. Scenario modelling on the basis of the PHREEQC model confirms that the attained wastewater pH contributes to the limited magnitude of reduction. Through variation of the amount of slaked lime added to the wastewater, saturation in respect to magnesium hydroxide ($SI_{magnesium\ hydroxide}$) can be simulated over a wide range of pH conditions (Figure 6.5). Magnesium precipitation commences at about pH 9.9 when $SI_{magnesium\ hydroxide}$>0. The maximum pH of 10.91 reached in the Flocculation HRAP was therefore sufficient to induce magnesium precipitation; however, further increases in pH see a rapid decrease in dissolved magnesium concentrations, until, at about pH 11.9 when all dissolved magnesium is removed from the solution. PHREEQC simulations also confirm that lower initial magnesium concentrations could have been chosen without considerable impact on the amount of magnesium precipitation. Reducing the initial magnesium concentration by half, 41.35 mg L^{-1}, leads only to a moderate drop of 15% in the precipitated amount of

magnesium hydroxide; however, once the initial magnesium concentration is below, 30 mg L^{-1}, the amount of magnesium hydroxide which is able to precipitate diminishes rapidly (Figure 6.6). Based on these results, it is clear that the method used in this study to induce magnesium hydroxide precipitation could be optimised, potentially reducing resources and costs. Muylaert et al. (2015b) recommended that the operation of flocculation should be controlled by the mass of magnesium hydroxide precipitate required to induce flocculation and the buffering capacity of the medium instead of trying to reach a final pH. Considering the outcomes of this study this method should be further investigated.

While the results of the scenario modelling strongly suggest the method used in this study to induce magnesium hydroxide precipitation could be optimised, it is unclear what effect it would have on microalgae flocculation. The relationship between magnesium hydroxide precipitation and flocculation efficiency in wastewater is not present in the literature, making it difficult to estimate how microalgae flocculation would alter behaviour under these scenarios. However, when considering both the significant difference in magnesium hydroxide precipitation and the small difference in flocculation efficiency between this study and Drira et al. (2016) it seems that any increase in magnesium hydroxide precipitation would have a modest effect on flocculation efficiency. Ultimately, understanding the relationship between magnesium hydroxide precipitation and microalgae flocculation efficiency in wastewater is necessary to optimise the method of autoflocculation, via magnesium hydroxide precipitation, at scale and as such requires further investigation.

6.6.4. Cost estimation and design considerations for the in-pond trial on the autoflocculation of microalgae in high rate algal pond treated domestic wastewater via magnesium hydroxide precipitation

Cost is arguably the most important factor in selecting a harvesting method. In this study, 6 kg of slaked lime was used to raise the pH of the effluent in the Flocculation HRAP. Communication with an Australian water provider indicated the cost of bulk slaked lime to be \$232.65 USD t^{-1} or \$0.23 USD kg^{-1}, excluding transport costs. Having tested 33 m^{-3} of wastewater, this results in an alkali cost of \$0.042 USD m^{-3} for the autoflocculation process used in this study. In wastewater that already has a sufficient magnesium concentration to induce autoflocculation, such as in Drira et al. (2016), this would be the only cost for autoflocculation via magnesium hydroxide precipitation. At this total cost autoflocculation, via magnesium hydroxide precipitation, would be one of the cheapest harvesting methods available (Phasey et al., 2017).

In this study, magnesium addition was required with 20 kg of magnesium chloride used. Bulk magnesium chloride was quoted as \$500.94 USD t^{-1} or \$0.50 USD kg^{-1}, excluding transport costs, by an Australian chemical wholesaler. This equates to a magnesium chloride cost of \$0.30 USD m^{-3} for the autoflocculation process used in this study. A novel way to reduce the cost of magnesium could be by sourcing it from wastewater or brackish water containing high concentrations of magnesium rather than purchasing it (Sharma et al., 2013, Barros et al., 2015). Currently, it is unknown if this environmentally sourced magnesium would behave the same and achieve the same flocculation efficiency as the purchased chemical; however, considering the potential savings, this should be explored further. Further cost reductions could also be gained by recovering the magnesium from the flocculated biomass and reusing it (Zhao et al., 2014). Vandamme et al. (2015a) recovered 95% of precipitated magnesium hydroxide from flocculated and pre-concentrated microalgae biomass by mild acidification, pH 8 for 30 min, with no significant effect on the biomass.

Based on these estimates for slaked lime and magnesium chloride, the total cost for autoflocculation, via magnesium hydroxide precipitation, in this study was \$0.346 USD m^{-3}. This cost is higher than the total chemical costs reported for all flocculation methods by Phasey et al. (2017), suggesting the method might be too costly for commercial application. However, as discussed previously, the results of the wastewater analysis post-flocculation and sedimentation suggest only a small fraction of magnesium acted in flocculation (Table 6.3). PHREEQC modelling supports this suggestion, reporting that excessive magnesium was used in this study, and a similar level of magnesium precipitation could be achieved at half the magnesium concentration. This reduction in magnesium would result in the total cost of the process being reduced to \$0.17 USD m^{-3}, which is competitive with other flocculants (Phasey et al., 2017). It is important to note that as the relationship between magnesium hydroxide precipitation and microalgae flocculation is unclear, it is unknown to what degree the effect any alteration in magnesium hydroxide precipitation would have on flocculation efficiency.

Operationally, autoflocculation, via magnesium hydroxide precipitation, was simple to achieve in the HRAP system, requiring no sophisticated chemical dosing infrastructure. This study demonstrated that HRAPs are a suitable system to use as a flocculation tank for this method when operated as a batch process. They have the capacity to hold large volumes of wastewater, and while paddlewheel mixing was not optimal in this study, it seems that with minimal changes the paddlewheel can provide cheap and adequate mixing of the wastewater to induce a high level of flocculation. In this design HRAPs would offer a simple to operate system that could be used to treat wastewater and harvest algal biomass, potentially resulting in reduced capital costs by negating the need to install external harvesting equipment

Unfortunately, the way the HRAPs were operated in this study does have limitations. One major limitation is that the process of operation for wastewater treatment systems is subservient to wastewater production. At a particular population size, and likely not a high

one, wastewater production becomes continuous, making it impossible to operate the HRAP as a batch process while also maintaining adequate wastewater treatment. A potential design to navigate this limitation would be to operate two HRAPs in parallel as sequencing batch reactors each alternating as a wastewater treatment system and a biomass harvesting basin. This design would mean wastewater treatment was unobstructed during flocculation and has the added advantage of during times of high influent flow, such as holiday seasons or heavy rain events, both HRAPs could be operated as wastewater treatment systems to accommodate the increased volume. However, this design also has problems as it would double the size and cost of the system, reducing any potential savings in construction and raising whether external harvesting equipment would be more suitable. Another limitation is the absence of a cost-effective process to remove the settled microalgal biomass from the HRAPs after it has flocculated and settled. It is likely that such a process would require the redesigning of HRAPs. Potential alterations to the HRAP design include the incorporation of a channel in the floor of the pond or reworking of the pond floor into a v-notch.

6.7. Conclusion

Autoflocculation, via magnesium hydroxide precipitation, achieved a high level of flocculation efficiency and solids removal in jar test experiments on HRAP treated wastewater. It also played a role in wastewater treatment achieving good nutrient removal, particularly for phosphorus. The in-pond trial of the method had lower but still high levels of flocculation efficiency, solids removal and nutrient removal. Based on these results, autoflocculation, via magnesium hydroxide precipitation, is a promising method for microalgae biomass recovery, wastewater treatment and nutrient recovery in HRAPs treating wastewater. However, there is potential for optimisation of the process in regards to mixing and chemical addition.

6.8. Acknowledgements

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6.10. Supplementary material

Plate 6.5. The Wastewater Treatment high rate algal pond at the Kingston on Murray wastewater treatment system, Australia.

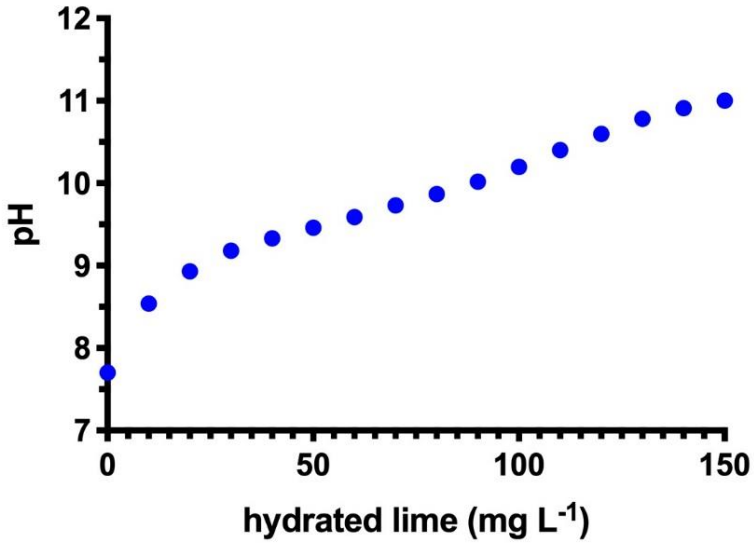

Figure 6.3. Concentration of slaked lime (mg L^{-1}) necessary to achieve pH 11 in wastewater from the Flocculation high rate algal pond at the Kingston on Murray wastewater treatment system, Australia, post-magnesium chloride addition.

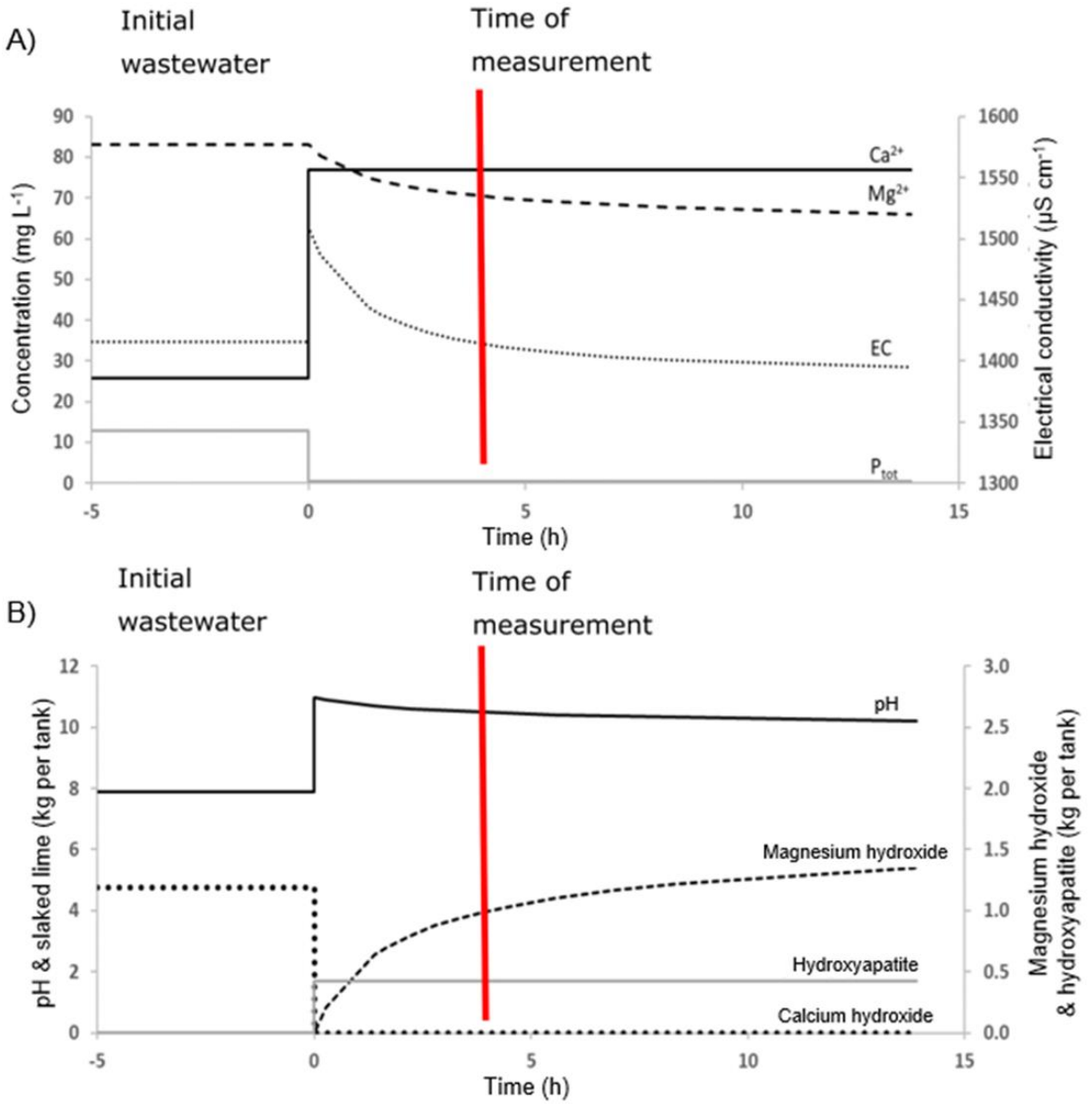

Figure 6.4. PHREEQC simulation results of the changes in: **(A)** calcium (mg L^{-1}), magnesium (mg L^{-1}), phosphorus (mg L^{-1}) and electrical conductivity (µS cm^{-1}); and **(B)** magnesium hydroxide (kg per tank), hydroxyapatite(kg per tank), slaked lime (kg per tank) and pH in the Flocculation high rate algal pond wastewater post-slaked lime addition. Parameter values measured post-magnesium chloride addition (Table 2) were used for initial wastewater values in the simulation.

Table 6.4. Bivariate Pearson correlation coefficient showing significant correlations and fit of correlations between the flocculation efficiency (%), total suspended solids (mg L^{-1}), volatile suspended solids (mg L^{-1}) and total phosphorus (mg L^{-1}) in the test jars on high rate algal pond effluent. Correlation is significant at the 0.01 level (2-tailed).

		Flocculation efficiency (%)	Total suspended solids (mg L^{-1})	Volatile suspended solids (mg L^{-1})
Total suspended solids (mg L^{-1})	**Pearson Correlation**	-0.988		
	Sig. (2-tailed)	0.000		
	N	13		
Volatile suspended solids (mg L^{-1})	**Pearson Correlation**	-0.995	0.998	
	Sig. (2-tailed)	0.000	0.000	
	N	13	13	
Total phosphorus (mg L^{-1})	**Pearson Correlation**	-0.999	0.981	0.990
	Sig. (2-tailed)	0.000	0.000	0.000
	N	13	13	13

Plate 6.6. Visible precipitation of magnesium hydroxide and microalgae flocculation in the wastewater contained within the dosing apparatus during slaked lime addition. The wastewater was from the Flocculation high rate algal pond at the Kingston on Murray wastewater treatment system, Australia, post-magnesium addition.

Plate 6.7. Visible floc formation in the Flocculation high rate algal pond at the Kingston on Murray wastewater treatment system, Australia, post-magnesium and slaked lime addition.

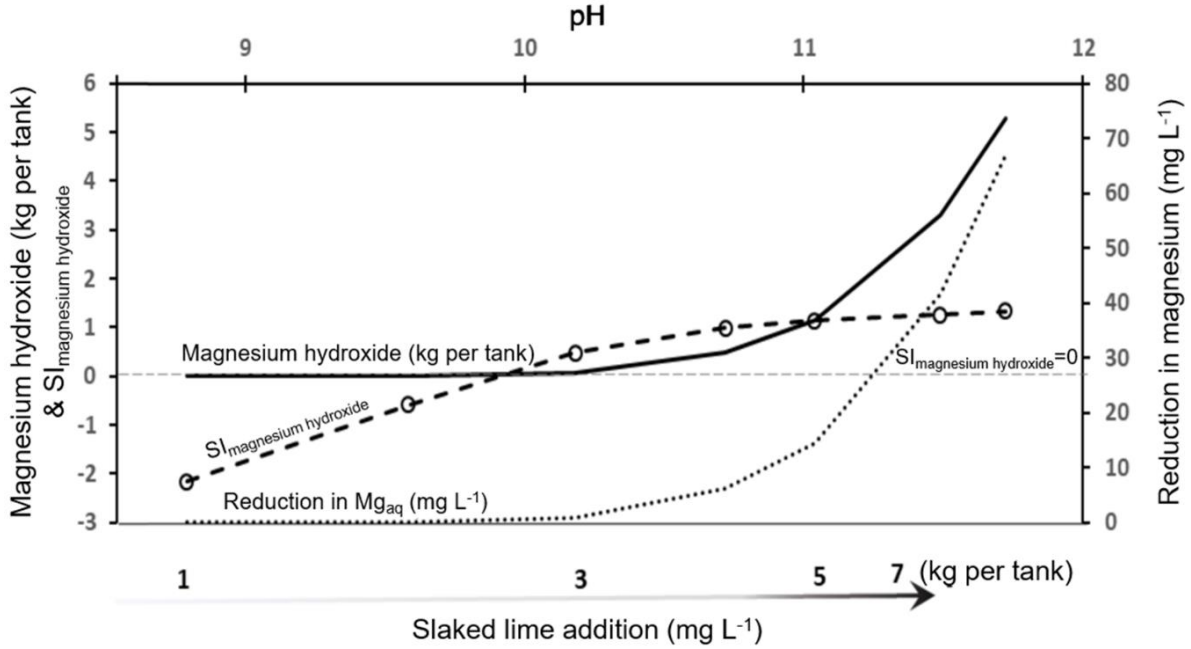

Figure 6.5. Simulated impact of changes in slaked lime (kg per tank) addition and therefore pH on dissolved magnesium concentrations (mg L^{-1}), the saturation in respect to magnesium hydroxide ($SI_{magnesium\ hydroxide}$) and magnesium hydroxide precipitation (kg per tank).

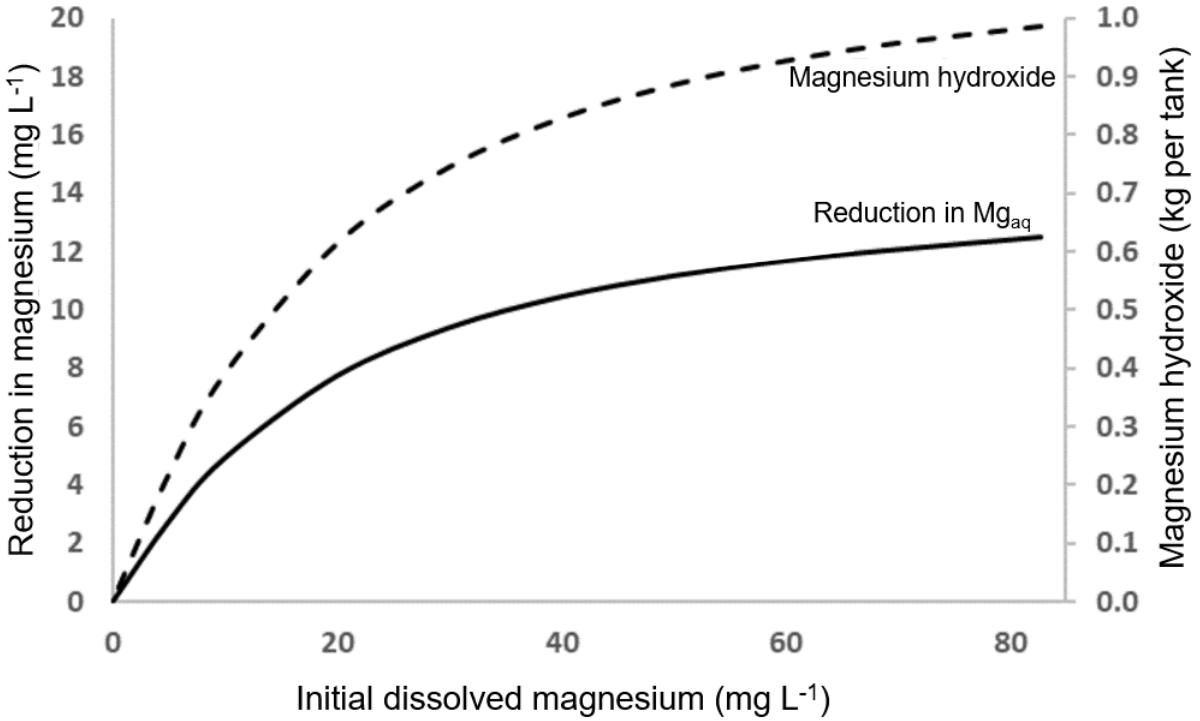

Figure 6.6. Simulated reduction in dissolved magnesium concentration (mg L-1) and increase in precipitation of magnesium hydroxide (kg per tank) for varying initial magnesium concentrations in the wastewater. Parameter values measured post-magnesium chloride addition (Table 6.3) were used for initial wastewater values in the simulation.

CHAPTER 7. GENERAL CONCLUSIONS AND FUTURE RESEARCH

This chapter summarises the results and conclusions for each study presented in Chapters 3 to 6. Also, presented is an overview of the potential areas for future research on high rate algal ponds (HRAP). More detailed discussions of the results for each study can be found in its corresponding chapter.

7.1. General conclusions

High rate algal ponds (HRAP) are flexible and robust natural wastewater treatment systems. They provide more efficient wastewater treatment than waste stabilisation ponds (WSP) while using less land, being cheaper to construct and providing more effluent for reuse (Young et al., 2017). They have also been shown to be a more sustainable, environmentally friendly alternative to electro-mechanical systems, as well as being cheaper and simpler to operate (Young et al., 2017). Additionally, as a side effect of their treatment, they provide relatively high concentrations of microalgae biomass to be used for the production of value-added products, particularly biofuels (Young et al., 2017). For these reasons, HRAPs have received considerable interest as systems for coupling microalgae cultivation for biofuel feed and wastewater treatment. Unfortunately, this has not resulted in their wide-spread application. This is probably because of notable absences in the literature, especially concerning large-scale, operational HRAPs. Research on such systems is rarely performed due to the high demand it has on time and resources and the rarity of such systems; however, such studies are a necessary step for the implementation of systems. Therefore, research on these systems was the focus of the studies contained within this thesis.

The overall aim of this thesis was to investigate the factors limiting HRAPs application as wastewater treatment systems and microalgae bioreactors using large-scale, operational systems. To achieve this overall aim, this thesis focused on four key research areas that would be required for the wider application of HRAPs, two of which focused on HRAPs use

as wastewater treatment systems, while the other two focused on the use of HRAPs as combination wastewater treatment systems and microalgae bioreactors for biofuel production.

Outlined below are the specific aims of the thesis as presented in Chapter 1, with the findings relevant to each chapter summarised accordingly.

Aim: Validate the wastewater treatment performance of a HRAP system for inclusion in the South Australian Community Wastewater Management Scheme (CWMS) as a wastewater treatment system option for rural communities in SA, Australia.

When installing new wastewater treatment systems, regulators refer to official guidelines to guide their choice of system. The absence of HRAPs in any official regulatory guidelines results in them being overlooked for traditional systems already present in the guidelines, such as WSPs. It is believed the inclusion of HRAPs in official regulatory guidelines will lead to their wider application. In this thesis, Chapter 3 recounted the only independent validation of a HRAP system for inclusion as a wastewater treatment system option in official regulatory guidelines, the SA CWMS. The HRAP system at Kingston on Murray, Australia, successfully met the removal objectives of the validation for the bacterial indicator *Escherichia coli* and the viral indicator F-RNA bacteriophage, achieving $\log_{10}$ reduction values (LRV) of 3.30 ± 1.28 and 2.32 ± 0.74 respectively. Aerobic spore-forming bacteria were found to be unsuitable indicators for natural treatment systems, and consequently, their results were not considered. The HRAP system also met the wastewater quality objectives set out by the *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1)* for effluent reuse in non-food crop irrigation, with a median concentration of <4.0 $\log_{10}$ *E. coli* 100 mL^{-1} and a minimum 5th percentile of 1.0 $\log_{10}$ reduction of viruses (NRMMC, 2006).

Based on the results of the validation, a HRAP system design comprising a single HRAP receiving septic tank effluent operated at depths between 0.3-0.5 m and a theoretical hydraulic retention time (THRT) of 10 d was approved by the South Australia Department of Health, Wastewater Management Group (DoHWMG) for inclusion as a wastewater treatment option in the CWMS. Additionally, based on these results and those reported in Buchanan (2014), a second design for a HRAP system was also approved by DoHWMG for inclusion as a wastewater treatment option in the CWMS. This second design comprised a single HRAP receiving septic tank effluent operated at depths between 0.3-0.5 m and a 5 d THRT, followed by the traditional in series, 4 cell maturation WSPs. Both HRAP system designs were published in *Design Guideline for a High Rate Algal Pond (HRAP) – as an Element in Wastewater Treatment Trains*. It is believed this is the first time HRAPs have been included in official regulatory guidelines as a wastewater treatment option for communities. The inclusion of these designs in the CWMS should lead to the wider application of HRAPs with a system based on one of the designs, comprised of two 0.5 ha HRAPs, having already been installed in Peterborough, Australia. This newly installed wastewater treatment system at Peterborough, Australia, won the Australian Water Association's (AWA) 2019 SA Infrastructure Innovation Project Award and subsequently went into the running for the AWA's national award.

This study also provided support for the use of refrigerated auto-samplers during validation of rural wastewater treatment systems. Statistical analysis revealed no significant difference between the LRVs measured by the Australian Water Quality Centre, Adelaide, Australia using grab sampling and Flinders University, Adelaide, Australia, using refrigerated auto-samplers. This suggests that the different sampling methods employed had no significant effect on the outcome of the validation. Due to remoteness, validation of rural wastewater treatment systems can be a challenging and expensive process with on-site personnel required for grab sampling. Considering the results of this analysis, refrigerated auto-

samplers may present a simpler and cheaper method for sampling remote wastewater treatment systems during validation, which can also potentially provide larger datasets.

Aim: Develop and validate an initial model for the inactivation of pathogens in HRAPs treating wastewater, which employs inactivation values obtained from independently measured laboratory experiments.

Well-designed pathogen inactivation models provide essential information on how to best design and operate a wastewater treatment system, especially where final disposal of treated effluent is to irrigation. A pathogen inactivation model for HRAPs could be a valuable tool for CWMS officials when installing new systems based on the recently approved designs. In this thesis, Chapter 4 described the development and validation of a pathogen inactivation model for HRAPs; only the second ever and the first in 16 years (Craggs et al., 2004). Solar radiation is generally considered to have the most significant influence on pathogen survival in HRAPs and as such, was treated as the primary contributor to pathogen inactivation in the model (Craggs et al., 2004). Uniquely, the model used solar inactivation values for the organisms measured in the laboratory compared to traditional models that use inactivation values measured in the systems of interest. This novel design approach makes the model more widely applicable than traditional models as it is less a model on a specific system and more a model that represents the behaviour of HRAP systems generally. The model was validated for the bacterial indicator organisms *E coli* and the viral indicator organism F-RNA bacteriophage using a large-scale, operational HRAP located at Kingston on Murray, Australia. The model predicted concentrations and the measured concentrations for all comparisons were similar and well fitted. Paired t-test comparisons supported these fits, reporting that for all *E. coli* comparisons and two of the three F-RNA bacteriophage comparisons, the model predicted concentrations and measured concentrations did not differ significantly. The results of the validation confirm the model was well designed and should

be the focus of more research. This is believed to be the only study in the literature that developed a mechanistic model for pathogen inactivation using laboratory measured solar radiation inactivation values and then validated it using a large-scale, operational HRAP.

The model also provided insight into the most effective method for HRAP operation when influent feeding is intermittent, which is common in rural communities. It showed that to maintain effluent quality when intermittent feeding of the influent is employed, no more than 4% of the pond volume should be introduced over a period no longer than 4% of the THRT. This insight was incorporated into the new CWMS guidelines, *Design Guideline for a High Rate Algal Pond (HRAP) – as an Element in Wastewater Treatment Trains*, and as such will help guide the implementation of new HRAP systems.

Aim: Asses the effect CO_2 enrichment, via biogas scrubbing, has on the biomass production and wastewater treatment of a HRAP performing tertiary wastewater treatment as part of an existing major wastewater treatment plant.

Having addressed some of the key factors limiting HRAPs application as wastewater treatment systems, it was pertinent to explore the key factors limiting their other main application as combination wastewater treatment systems and bioreactors for cultivating microalgae to be used as biofuel feed. Biomass productivities below economically viable concentrations for cost-competitive biofuel production is considered one of the two main factors limiting HRAPs use for this application. It is widely believed this is caused by the microalgae in the wastewater being carbon limited with CO_2 enrichment of the wastewater considered the best solution. In this thesis, Chapter 5 outlines a case study on the effect continuous CO_2 enrichment of wastewater, via an industry standard biogas scrubber, has on the biomass production and wastewater treatment of a HRAP retrofitted into a major wastewater treatment plant, Melbourne Water's Western Treatment Plant, Werribee,

Australia. During this assessment, as a control, another identical HRAP was operated in parallel and fed identical wastewater that had foregone CO_2 enrichment. This is believed to be the only such assessment at this scale to use an adequate control, providing a better assessment of the influence of CO_2 enrichment by negating confounding factors. There were no significant differences in biomass production between the HRAPs as measured by dry matter, particulate organic carbon or nitrogen. Conversely, mean chlorophyll *a* concentration was found to be significantly higher in the CO_2 enriched HRAP; however, this result was considered potentially unreliable. Regarding wastewater treatment, CO_2 had a similarly small effect with only total nitrogen and ammonium removal differing significantly between the HRAPs, being slightly decreased in the CO_2 enriched HRAP. Overall, neither biomass production nor wastewater treatment were meaningfully improved by CO_2 enrichment in this assessment; however, both HRAPs still provided effective wastewater treatment. These results suggest that in at least this scenario, microalgae growth in a wastewater treating HRAP is was not limited by carbon. The results also provide strong evidence refuting the widely held belief that HRAPs require CO_2 enrichment of wastewater to be effective wastewater treatment systems. This is significant as this belief potentially limited HRAPs application in the absence of cheap CO_2 and increased their capital costs due to additional CO_2 enrichment infrastructure.

A key focus of this study was to provide as close to a real-life scenario assessment of the effect of CO_2 enrichment, via biogas scrubbing, on the biomass productivity and wastewater treatment. To achieve this, the HRAPs were treated as if they were vital components of the treatment train. They were fed domestic wastewater produced by the greater Melbourne area after it had undergone secondary treatment in the plant. Biogas was collected from on-site anaerobic ponds and the CO_2 used for enrichment scrubbed from the biogas using industrial standard scrubbers. Both wastewater and biogas were fed continuously to the HRAPs as this would most likely be the case if they were integrated into the treatment train. Due to these design choices, this study is believed to be the closest representation in the

literature to a real-world scenario of a CO_2 enriched HRAP being operated for wastewater treatment and biomass production.

Aim: Assess the flocculation efficiency of autoflocculation, via magnesium hydroxide precipitation, in a large-scale, operational HRAP treating domestic wastewater.

The other main factor limiting HRAPs use as combination wastewater treatment systems and bioreactors for cultivating microalgae to be used as biofuel feed is the lack of a cost-effective harvesting method. Autoflocculation, via magnesium hydroxide precipitation, is considered a potential method for this application; however, most of the studies investigating this method have been in the laboratory using growth media and microalgae monocultures, undermining their real-world applicability. In this thesis, Chapter 6 focused on assessing the flocculation efficiency of autoflocculation, via magnesium hydroxide precipitation, in a large-scale, operational HRAP treating domestic wastewater, the first study in the literature to do this. Overwhelmingly, studies on autoflocculation, via magnesium hydroxide precipitation, have focused on laboratory assessments using microalgae monocultures and growth media (Vandamme et al., 2012, García-Pérez et al., 2014, Nguyen et al., 2014b, Lama et al., 2016, Aléman-Nava et al., 2017, Vandamme et al., 2018). To better represent how the method would behave in a real-life scenario, this study used 33 m^3 of HRAP treated domestic wastewater from a rural community, Kingston on Murray, Australia, naturally populated with a heterogenic mix of wild strain microalgae. Autoflocculation, via magnesium hydroxide precipitation, achieved a high level of flocculation efficiency and total and volatile solids removal. A good level of nutrient removal was also achieved by autoflocculation, particularly for phosphorus. Cost analysis estimated the total chemical cost for the method to be higher than those reported for other flocculation methods. However, chemical analysis and geochemical modelling indicated the concentrations of the chemicals added were excessive, with the recommended corrections likely resulting in a substantial cost reduction. Overall, the

results of this study suggest autoflocculation, via magnesium hydroxide precipitation, is a promising method for harvesting microalgae biomass, wastewater treatment and nutrient recovery in HRAPs treating wastewater. This is believed to be the largest assessment of autoflocculation in HRAP treated wastewater in the literature, and the closest representation to the method's real-world application. It is also the only reported study to perform this method in-pond, a potentially simpler and cheaper option to standard harvesting tanks.

Despite the positive results, this study also highlighted key limitations to the real-world application of this method. Firstly, while HRAPs provide a cheap and simple basin for flocculation an effective method to remove the settled biomass from the HRAP is still elusive. This is beyond the scope of this study but is an issue that will need to be addressed if this method is going to see real-world application. Secondly, the HRAP was operated as a batch system which is infeasible when, as it typically is, the influent is fed semi-continuously or continuously. This could be overcome with the use of multiple HRAPs, but it is unclear what would be the optimal configuration, and the additional infrastructure would reduce any savings gained by using this method, bringing into question the efficacy of its use.

In conclusion, this thesis achieved the overall aim of investigating the factors limiting HRAPs application as wastewater treatment systems and microalgae bioreactors using large-scale, operational systems. Regarding their use as wastewater treatment systems, key research areas were addressed by the validation and inclusion of a HRAP system into official regulatory guidelines and the development and validation of a unique pathogen inactivation model. Also, all studies undertaken in this thesis clearly demonstrated HRAPs ability to perform effective wastewater treatment. These results strongly support the application of HRAPs as wastewater treatment systems and will hopefully lead to their wider application, allowing consumers access to their many benefits. Regarding the use of HRAPs as systems for coupling wastewater treatment with microalgae cultivation for biofuel feed, the results presented in this thesis indicate that considerably more research is necessary before this application can be realised in an economically viable way. CO_2 enrichment was clearly

shown to have no significant effect on biomass productivity and a slight negative effect on the wastewater treatment performance of the HRAP in the examined scenario. This suggests the suboptimal biomass productivities experienced in HRAPs treating wastewater are not caused by the microalgae being carbon limited and, therefore, other causes should be investigated. While even though autoflocculation, via magnesium hydroxide precipitation, was shown to be a promising harvesting method for use in large-scale, operational HRAPs treating domestic wastewater, key limitations that will need to be addressed before its real-world application were identified. Nevertheless, the realisation that considerably more research is required before HRAPs are realised as economic systems for coupling wastewater treatment with microalgae cultivation for biofuel feed should not be seen as an impediment to their application as wastewater treatment systems.

7.2. Areas for future research

Having established HRAPs as a wastewater treatment for rural communities, a logical next step would be to assess how they would operate as part of a treatment train in a major wastewater treatment plant. Both WSPs and electro-mechanical system are commonly employed in major wastewater treatment systems. HRAPs offer the same advantages over these systems in major wastewater treatment systems as they as do for rural communities. The use of HRAPs as low greenhouse gas emitting alternatives to electro-mechanical systems for aeration and nitrogen removal is of particular interest due to the desire of the water industry to become more sustainable. It has been estimated HRAPs require 20-110 kWh ML^{-1} of wastewater treated which is substantially less than the estimated 230-1000 kWh ML^{-1} of wastewater treated required by the most commonly employed electro-mechanical systems, activated sludge systems (Craggs et al., 2013, Arashiro et al., 2018, Lopes et al., 2018, Daverey et al., 2019). Preliminary research into this area was conducted during the CO_2 enrichment study with the results indicating HRAPs can perform adequate tertiary wastewater treatment in the treatment train of a major wastewater treatment plant.

Given the potential reduction in costs and complexity, and the potential larger datasets collected further research into the application of refrigerated, auto-samplers seems warranted. Key research areas include determination of the ideal temperature for storage, determination of the maximum storage time before organism concentrations and LRVs are significantly affected, and the assessment of other organisms' behaviour when stored in dark refrigerated auto-samplers for extended periods. Also, while not directly relevant to the validation of wastewater treatment systems, it would be of considerable interest to assess the effect extended periods of storage in dark refrigerated auto-samplers have on other non-microbiological parameters.

Further development of the HRAP pathogen inactivation model presented in this thesis is supported by it being the first such model in 16 years, its successful validation and the guidance it provided on optimum HRAP design and operation. Modifying the behaviour of solar radiation in the model to reflect real-world behaviour better is perhaps the best candidate for further development. Modification of the solar radiation exposure time could be introduced to match better the location and season of the system being modelled. Diurnal variance to solar radiation intensity could also be introduced to reflect better the changes in intensity seen over a day. Other areas for further development include the modelling and validation of other organisms, particularly pathogens, and the modelling of other systems particularly those that receive a continuous influent feed and those operating in different climatic conditions.

With the results of the CO_2 enrichment study suggesting the growth of microalgae in wastewater treating HRAPs is not carbon limited other impediments to HRAPs reaching economically viable biomass productivities should be investigated. Predation/infection is considered a major impediment to HRAP biomass productivity, limiting growth and causing population crashes (Cho et al., 2017, Day et al., 2017, Poorey, 2017). While predation by zooplankton has received considerable research, the influence of other predators/pathogens has mostly been ignored, namely algae viruses (Kraft et al., 2020). This is a problem as

viruses have been shown to play an important role in algal ecosystems in natural environments and are therefore considered to have a significant influence on algal ecosystems in HRAPs (Coy et al., 2018, Kraft et al., 2020). Considering this and the dearth of research in this area, investigating the effect algae viruses have on algal ecosystems in HRAPs is a recommended area for future research.

Based on the literature review and results of the CO_2 enrichment study, it seems unlikely that the economic production of biofuel from microalgae grown in HRAPs treating wastewater will be realised in the near future. For this reason, it is recommended that other uses for the biomass produced by wastewater treating HRAPs be considered. One such use that should receive more research is the harvesting of nutrients essential to agriculture namely nitrogen and phosphorus. The demand for both these nutrients is ever increasing driven by the need to feed the increasing global population (Wágner, 2016). Unfortunately, the primary method to form nitrogen, the Haber-Bosch process, is extremely energy intensive and phosphorous is a finite resource with the global reserves expected to be depleted within one-hundred years (Wágner, 2016, Perin et al., 2019). Harvesting these nutrients from wastewater has been suggested as a sustainable process to help meet the demand for these nutrients (Solovchenko et al., 2016, Melia et al., 2017). In HRAPs, these nutrients can be harvested in two main ways, both of which require more research. Firstly, the nutrients can be harvested as part of microalgae which have accumulated these nutrients during their growth (Renuka et al., 2018, Perera et al., 2019). The nutrients can either be extracted from the microalgae or the microalgae can be used as a biofertiliser. Secondly, in the case of phosphorus, the nutrient can be removed from wastewater directly generally via precipitation (Solovchenko et al., 2016, Melia et al., 2017). The idea of directly removing phosphorus from HRAPs is supported by the autoflocculation study which showed phosphorus can be precipitated at a high efficiency from wastewater using a relatively simple method.

While autoflocculation, via magnesium hydroxide precipitation, was shown to be an effective method for harvesting microalgae in wastewater treating HRAPs, it was clear that the process used in this study could be improved. Chemical analysis and geochemical modelling showed that excessive magnesium chloride was used in this study and resulted in an inflated cost estimate. Consequently, determining the optimal magnesium chloride concentration to achieve high flocculation efficiency at the minimum cost is an important research need, especially for the real-world application of this method. Another area that requires research is determining the best way to remove the settled biomass from the pond. HRAPs were shown to be suitable basins for the autoflocculation of microalgae in large quantities of wastewater but a cost-effective method to remove the settled biomass from the HRAPs has yet to be discovered. Perhaps the most promising approach is the redesigning HRAPs. New designs could involve the inclusion of a channel in the floor of the pond or the reworking of the floor of the pond into a v-notch.

7.3. Closing statement

Considered as a whole, this thesis presents a collection of unique work, which not only addresses key areas of research regarding the application of HRAPs as wastewater treatment systems and microalgae bioreactors but does so at rarely used real-world scales. While such work is difficult due to the high demand on time and resources, the potential for unforeseen complications, and often requiring collaboration across multiple institutions, it is paramount if the wider application of HRAPs as wastewater treatment systems and microalgae bioreactors are to be realised. The key insights into the design and operation of large-scale, operational HRAPs presented in this thesis could not have been gained by studies in the laboratory or on small-scale systems. Due to this, it is believed the work presented in this thesis provides one of the most significant steps towards the wider application of HRAPs as wastewater treatment systems.

CHAPTER 8. REFERENCES

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APPENDIX A. PUBLICATIONS PRESENTED IN THIS THESIS

A.1. Mini-review: high rate algal ponds, flexible systems for sustainable wastewater treatment

Removed due to copyright. The published article can be found at https://link.springer.com/article/10.1007%2Fs11274-017-2282-x.

A.2. Independent validation and regulatory agency approval for high rate algal ponds to treat wastewater from rural communities

Removed due to copyright. The published article can be found at https://pubs.rsc.org/en/content/articlehtml/2018/ew/c7ew00228a.

A.3. Case study on the effect continuous CO_2 enrichment, via biogas scrubbing, has on biomass production and wastewater treatment in a high rate algal pond

Removed due to copyright. The published article can be found at https://www.sciencedirect.com/science/article/pii/S0301479719313325.