Investigation and modelling of high rate algal ponds utilising secondary effluent at Western Water, Bacchus Marsh Recycled Water Plant

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Abstract

There is growing interest in the ability of high rate algal ponds to treat wastewater. This method reduces the costs of algal production while treating the wastewater quicker and more efficiently than standard lagoon practices. Two parallel HRAPs were used in this study to treat secondary effluent. Nitrogen levels were significantly reduced with a mean reduction of 71% for ammonia and 64% of total nitrogen. The use of the HRAPs significantly increased the algal biomass levels compared to the algal growth in the storage lagoons, with a mean increase of 274%. Beneficial use of algae can be used to reduce treatment costs, so being able to predict and optimise the amount of algal biomass produced in HRAPs is vital. However, most models are complicated and require specific, detailed information. In this study, a predictive microalgal growth model was developed for high rate algal ponds by adapting two previously established models: the Steele and Monod models. The model could predict algal growth based on temperatures and solar radiation and account for limiting ammonia concentrations in an elevated pH environment with natural variations in the algal community. This model used experimental data that would be readily available to any established HRAP study.

Keywords: Algae, Algal biomass model, High rate algal ponds, Secondary effluent, Wastewater treatment

Introduction

High rate algal ponds (HRAP) are open shallow raceway ponds that are utilised for wastewater treatment and the production of microalgae. HRAPs have been used to treat wastewater for over half a century, and there have been many studies investigating HRAPs for wastewater treatment and biofuel production (Oswald et al. 1957, Craggs et al. 2012, Chisti 2016). For large-scale algal production, the use of HRAPs is one of the cheapest methods as they have a relatively low operating and construction cost. The cost of using microalgae to treat wastewater could potentially be offset by making biofuels with the algal biomass produced (Christenson and Sims 2011, Craggs et al. 2012). This method treats the wastewater and provides microalgae with nutrients, substantially improving the economics of the algal production (Rawat et al. 2013). These nutrients include; nitrogen, phosphorus, carbon and other micronutrients that are required for growth (Abdel-Raouf et al. 2012, Craggs et al. 2012).

Microalgae are simple, acellular or colonial organisms which require nutrients to grow. These nutrients can become limiting quite quickly especially in wastewater, where the carbon to nitrogen ratio is generally 3:1 instead of the required 6:1 (Benemann 2003, Sutherland et al. 2016). Modelling algal growth and being able to predict how well the microalgae will grow can help determine if and when the water may require extra nutrients or if other factors are affecting the microalgal growth. A predictive model can also be used to optimise processes and ensure harvesting is done at the ideal time to maximise biomass production. Furthermore, prediction of algal growth in wastewater will also help determine the discharge times or retention times required.

Due to substantial variation in different types of microalgae such as red microalgae, green microalgae and diatoms, prediction of algal growth in open HRAPs can be complex. It is known, however, that green algae are the most abundant algae found in wastewater treatment facilities, and that the majority of microalgae require a few essential elements for growth: nutrients, a specific temperature and a particular solar radiation range (Abdel-Raouf et al. 2012). The nutrients, most commonly needed are carbon, nitrogen and phosphorus. Ammonia is algae's preferred form of nitrogen and is assimilated into the biomass first (Glibert et al. 2016).

Temperature has a significant impact on the production of algae, and different species of algae prefer different temperature regimes. A large number of microalgae species prefer temperatures in the range of 20-30℃ (Undurraga et al. 2016). Solar radiation is the primary form of energy for microalgae, as they are photosynthetic organisms . Most algae have an optimal solar radiation range in which they prefer to grow, and the shallow design of HRAPs maximises the amount of light for algal growth (Young et al. 2017). Regular harvesting and mixing can help minimise self-shading that occurs at high concentrations. The effect of temperature and solar radiation on algal growth are strongly linked given their impact on photosynthesis and cellular respiration (Béchet et al. 2015).Nutrient availability in wastewater if not adequately controlled can severely diminish algal growth. Carbon is the primary nutrient which is limited in the secondary effluent. Algal cells contain 50% carbon by weight and, therefore, carbon drives the growth of the cell (Putt et al. 2011). Being able to incorporate nutrient concentrations, temperature and solar radiation into a growth model, and determine how fluctuations of each of them can affect the algal growth is essential for the design of HRAPs and optimisation of their operation.

Numerous algal growth models have developed over the years, and currently, the models we use for predicting algal growth are complicated. These models tend to be species specific and less reliable for ambient open HRAPs that contain numerous species. In general, most models focus on one or two growth aspects, such as light and temperature, and the models are proven to be useful in a controlled laboratory setting (Jayaraman and Rhinehart 2015, Undurraga et al. 2016). However, they are less applicable to large-scale open ponds due to fluctuations in weather, nutrient concentrations and microalgal species. Most models require large amounts of pre-determined values such as optimal and minimal growth rates in certain conditions, and at specific nutrient concentrations. They are usually too specific to a particular set of conditions to be utilised outside of the controlled laboratory environment. Most of the models assume that either the nutrients are not limiting or that the temperature and light are kept constant. Some previous models are species-specific and would not be able to predict the biomass production in a naturally occurring culture (Wu et al. 2013, Béchet et al. 2015, Undurraga et al. 2016). A few studies have considered systems with numerous species, but tend to be very complicated and require data that is not commonly

available (Huesemann et al. 2016). A simple model with readily available data would be beneficial for design and operation of large-scale HRAPs.

The Steele model has been previously used to predict algal cell growth based on light intensity and is shown in equation (1)(Wu et al. 2013).

$$\mu = \mu_{max} \cdot \frac{I}{I_{opt}} \cdot e^{1\frac{I}{I_{opt}}} \quad (1)$$

Where μ (d^{-1}) is the specific growth rate under light intensity of I (lx); $\mu_{max(I)}$ (d^{-1}) is the maximum specific growth rate when light intensity is optimal; I (lx) is the light intensity, and I_{opt} (lx) is the optimal light intensity (Wu et al. 2013). Béchet et al. (2011) developed a universal temperature model for shallow algal ponds but it required vast amounts of information and site-specific data. A simple algal growth model which incorporates both temperature and solar radiation and their effects on each other would likely find broader application.

The Monod model has previously been used to describe the algal growth rate based on nutrient levels and is described in equation (2) (Wu et al. 2013, Béchet et al. 2015, Jayaraman and Rhinehart 2015).

$$\mu = \mu_{\max(N)} \cdot \frac{S_N}{K_N + S_N} \quad (2)$$

Where μ (d^{-1}) is the specific growth rate under the nutrient concentration of S_N (mg L^{-1}), $\mu_{max(N)}$ (d^{-1})is the maximal specific growth rate when nutrient concentrations are saturated, S_N (mg L^{-1}) is the nutrient concentration in the medium; and K_N is the half-saturation constant of the nutrient (Wu et al. 2013).

Here the use of microalgae to treat secondary effluent is examined, and the development of a simple model for biomass production in an open pond environment with elevated pH was investigated. The paper considered the following variables for prediction of algal growth; temperature, solar radiation and ammonia concentration.

Experimental Design/ Methods

Bacchus Marsh Recycled Water Plant (BMRWP)

The two HRAPs used in this experiment were located in Victoria, Australia at Western Waters' Bacchus Marsh Recycled Water Plant (BMRWP) (lat. 37°72'44.09'S, long. 144°47'61.20'E). The HRAPs received water from the third secondary treatment lagoon at the BMRWP that treats municipal wastewater in a series of lagoons, see Figure 1. Wastewater is first fed into an aeration lagoon (AL) which sparges air into the raw sewage to ensure an aerobic environment which enhances treatment and reduces odour. After three days, the aerobic effluent is fed into three primary settling lagoons operated in parallel (P1-P3), where the larger solids settle out and that operate as facultative lagoons. Subsequently, the primary effluent from all three primary ponds is combined and fed into a series of three secondary lagoons (S1-S3). The secondary lagoons further treat the water and remove nutrients mainly using algae. The effluent from the third secondary lagoon feeds into the winter storage lagoon (WS). The winter storage lagoon is a final polishing lagoon. The effluent from the winter storage lagoon is 'Class A' water, and is discharged onto the surrounding farmland. Typical effluent quality from secondary lagoon three is shown in Table 1.

Figure 1: Western Waters' Bacchus Marsh Recycled Water Plant. Aerobic lagoon (AL), Primary lagoons (P1-P3), Secondary Lagoons (S1-3) and Winter storage lagoon (WS) (Google 2017).

High Rate Algal Ponds (HRAPs)

The two HRAPs were single loop raceway ponds with a central baffle, had working depths of 0.3m, surface areas of 2.8m^2 and a total volume of 850L (see Figure 2). The HRAPs were continuously mixed with paddle wheels. Effluent from secondary lagoon three was pumped into the HRAPs via pipework and float valves. The HRAPs were cleaned and filled at the start of each run, and the water level was kept constant with the float valves. The HRAPs were operated in batch mode with a retention time for seven days during most of the year and a shorter retention time of four days for four summer runs.

Methods and investigation

Chemical methodology

Each HRAP had a YSI Quatro probe installed with sensors for temperature, conductivity, pH, ammonia and dissolved oxygen. The YSI Quatro probe recorded measurements on a half-hourly basis. Waters samples were taken at the start and end of each experiment, and additional samples were taken during each run to observe the changes in the nutrient concentrations, species diversity (not shown) and biomass concentrations. An initial sample was taken and passed through a 0.45µm syringe filter immediately, and the filtrate was tested for nitrite (NO_2-N) and orthophosphate (P-PO_4) concentrations using Hach kits and analysed on a handheld Hach DR890 colourimeter (Hach 2008). A second sample was taken and placed on ice and returned to the laboratory. It was filtered through a glass fibre grade C Whatman filter and tested for Ammonia (NH_3–N), nitrate (NO_3–N), total nitrogen (TN), and total phosphorus (TP) concentrations using Hach test kits and analysed on a bench top Hach spectrophotometer (Hach 2008).

Table 1: Minimum, maximum, mean and standard deviation of the nutrient concentrations of HRAP influent measured during the 12 months of operation (May 2016 - May 2017).

	Annual					
	Nitrate and Nitrite (mg/L)	**Ammonia (mg/L)**	**Total N (mg/L)**	**Total P (mg/L)**	**Biomass (mg/L)**	**pH**
Min	0.45	0.41	2.89	4.28	5.00	8.03
Max	8.70	25.50	29.53	12.31	129.00	10.10
Mean	2.93	10.31	13.30	8.11	41.93	8.68
Std. Dev	2.21	9.13	9.76	2.23	2.77	0.61
	Autumn					
	Nitrate and Nitrite (mg/L)	**Ammonia (mg/L)**	**Total N (mg/L)**	**Total P (mg/L)**	**Biomass (mg/L)**	**pH**
Min	0.90	0.82	3.80	5.82	12.00	8.10
Max	2.90	19.70	23.64	7.97	92.00	9.63
Mean	1.52	6.56	9.70	6.76	50.40	8.74
Std. Dev	0.81	7.85	8.36	0.78	37.45	0.70
	Winter					
	Nitrate and Nitrite (mg/L)	**Ammonia (mg/L)**	**Total N (mg/L)**	**Total P (mg/L)**	**Biomass (mg/L)**	**pH**
Min	1.10	12.95	15.27	6.26	5.00	8.03
Max	8.70	25.50	29.53	10.76	29.00	8.57
Mean	3.99	20.29	23.87	8.26	15.50	8.23
Std. Dev	3.11	4.59	5.32	1.68	9.41	0.25
	Spring					
	Nitrate and Nitrite (mg/L)	**Ammonia (mg/L)**	**Total N (mg/L)**	**Total P (mg/L)**	**Biomass (mg/L)**	**pH**
Min	2.10	4.48	7.05	9.23	8.50	8.14
Max	6.80	23.20	26.67	12.31	50.00	8.89
Mean	4.34	13.10	15.53	10.93	30.70	8.47
Std. Dev	1.77	7.65	9.17	1.36	18.27	0.28
	Summer					
	Nitrate and Nitrite (mg/L)	**Ammonia (mg/L)**	**Total N (mg/L)**	**Total P (mg/L)**	**Biomass (mg/L)**	**pH**
Min	0.45	0.41	2.89	4.28	28.50	8.21
Max	4.00	2.64	6.93	8.70	129.00	10.10
Mean	1.85	1.28	4.10	6.49	71.10	9.18
Std. Dev	1.36	0.84	1.63	1.69	36.73	0.68

Biological Methods

Dry weight and absorbance were used to measured algal biomass. Dried biomass was determined using the method described in Standard Methods for the Examination of Water and Wastewater 20th edn (1998). Absorbance was measured at 440nm, 680nm and 750nm (results not shown) using a Biochrom Libra S22 spectrophotometer (Standard Methods for the Examination of Water and Wastewater 20th edn 1998, Wang et al. 2010, Huesemann et al. 2013). Growth rates were determined using the dried biomass results.

$$G = \frac{fb-ib}{t} \quad (3)$$

Where G is growth rate in (mg/L/D^{-1}), *fb* is the final biomass in (mg/L), *ib* is the initial biomass (mg/L), and *t* is time (D(days). Growth rates were taken from the growth phase of the algal growth, and this was assumed to be the case for all runs unless then ammonia concentration was less than 1.3mg/L. For ammonia concentrations <1.3mg/L the biomass production rates dropped, and the algae were in the stationary or death phase of their growth.

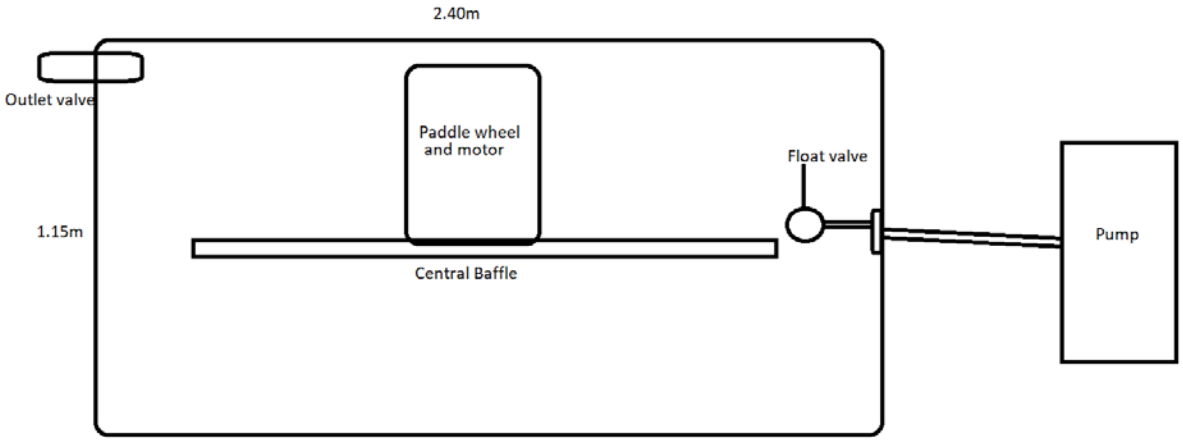

Figure 2: Schematic of HRAP and Pump.

Model

Development of a growth model for algal biomass focused on the main factors required for algal growth and utilised data that was easily accessible and available. The model has considered three factors; solar radiation, temperature and ammonia concentration. Temperature and solar radiation were the main drivers for algal growth with solar radiation providing energy and temperature regulating the kinetics of enzyme activation for growth (Singh and Singh 2015). Ammonia concentration was included to identify when the system was nitrogen-limited, and a concentration of 0.3mg/L was used as the minimum ammonia concentration below which the algal growth rate was in the stationary or death phase based on experimental data. Algal growth is believed to occur exponentially if there are no limiting factors. Limiting factors can include physical, chemical and biological elements; physical factors include; solar radiation, temperature, self-shading, the design of the HRAP and mixing. Chemical factors that can limit growth include nutrient concentrations, nutrient availability, toxicants and pH. Biological factors that can limit growth are predators, interspecies competition, parasites and microbiological contaminants such as bacteria, fungi or viruses (Collos and Harrison 2014, Carney et al. 2016, Chisti 2016, Mehrabadi et al. 2016).

Modified versions of Steele's and Monod's equations were utilised in this study. The modified Steele's equation was used to determine the effect of solar radiation and temperature on algal growth, and the modified Monod's equation was employed to investigate the impact limiting ammonia concentrations had on the algal growth. Steele's equation was modified to include both temperature and solar radiation. The optimum value used in the equation was determined from results observed in the studies experimental period. The temperature and solar radiation were

multiplied together and replaced the light intensity value used in the Steele's model (see equation 4).

$$\mu = \mu_{\max(tl)} \cdot \frac{tl}{tl_{opt}} \quad (4)$$

Where μ (mg/L/D) is the specific growth rate under conditions of *tl* (°C.W.m^{-2}); $\mu_{max(tl)}$ (g/L/D) is the maximum specific growth rate when temperature and solar radiation was optimal; *tl* (°C.W.m^{-2}) is the temperature (°C) multiplied by solar radiation (Wm^{-2}). The use of both temperature and solar radiation incorporated effects associated with the kinetics of enzyme activation as well as the amount of energy available for growth. Mean solar radiation and mean temperature results were utilised based on data over the trial period. The temperature was recorded on a half hourly basis from the YSI Quatro probe in the HRAPs. Solar radiation was recorded on a half hourly basis by Western Water's weather station.

A modified Monod equation (5) was used to incorporate the negative effect limited ammonia concentration would have on biomass productivity.

$$\mu N = \frac{N}{n+N} \cdot \mu \quad (5)$$

Where μN (mg/L/D) is the biomass growth rate under *N* at μ, *N* (mg/L/D) is the ammonia concentration, *n* is the ammonia half saturation constant, and μ is the biomass growth rate predicted by the Steele equation. The ammonia half saturation constant was determined using the ammonia concentration when the growth rate was optimal in the experimental data. The Monod equation was only utilised when the initial ammonia concentration was below 1.3mg/L. This allowed for less than 1mg/L of ammonia to be utilised per day, as 0.3mg/L ammonia was determined from the HRAP experiments to be the minimum ammonia concentration required for growth. Above the concentration of 1.3mg/L ammonia algal growth was believed not to be limited by ammonia concentrations.

Statistics

Mean, minimum and maximum values for the influent water quality results were analysed. Percentages values were used to show the changes in nutrient concentrations and biomass concentrations during HRAP trials, and the results were separated into annual and seasonal values to highlight the significant variations that occur throughout the year. R^2 values were used to signify the accuracy of the correlation between the recorded growth rates and predicted growth rates. R^2 values for exponential and linear growth rates for each HRAP trials were compared using the using the t-test assuming equal variances, and a p-value of 0.05 used. Root mean square error (RMSE) was used to determine the amount of error there was between the recorded and predicted daily biomass productivity data sets. Graphs and trendlines were compiled using Microsoft Excel 365.

Results & Discussion

Water Quality

Influent water quality to the HRAPs varied throughout the year with seasonal fluctuations. The most substantial variations were between summer and winter. Table 1 indicates that in the warmer summer months the ammonia concentrations were reduced and the biomass concentrations were elevated, the reverse of which was found in the colder winter months with high ammonia and low

biomass concentrations. The concentration of ammonia, microalga's preferred nitrogen source, was highest in winter with a mean ammonia concentration of 20.29mg/L, and lowest in summer with a mean concentration of 1.28mg/L (Glibert et al. 2016). This indicates that in the lagoons before the final secondary lagoon, large amounts of ammonia were removed during summer, however during winter ammonia removal was not as effective. Total nitrogen followed the same pattern with the highest mean concentration recorded in winter and the lowest in summer with 23.87mg/L and 4.10mg/L respectively. This was inversely reflected in the influents starting concentration of biomass when summer had the highest biomass concentration, and winter had the lowest with 129mg/L and 5mg/L respectively.

High Rate Algal Ponds

The two HRAPs were operated under the same conditions during the 12 months of operation and acted as duplicate experiments. Similar nutrient removal and biomass concentrations were recorded in both HRAPs during the operating period (May 2016- May 2017). The HRAPs had an annual mean removal of 71% of ammonia and 64% of total nitrogen, and an annual mean increase in biomass concentrations by 274% (see Table 2). The highest seasonal percentage removal of ammonia and total nitrogen were recorded in spring with a mean removal of 97% and 84% respectively. Spring also had the highest seasonal increase in biomass, with a mean increase of 399%. Winter had the lowest seasonal ammonia and total nitrogen removal and smallest biomass increase with means of 59%, 34 % and 160% respectively. Based on this research and previous research (Wu et al. 2013, Mehrabadi et al. 2016), it is clear there is a relationship between nitrogen removal and biomass production. This relationship is displayed in Figure 3, which shows the ammonia concentration versus biomass concentrations from a spring HRAP run with a 7-day retention time (R^2 value of 0.99). As the biomass increased, the ammonia decreased in a linear fashion. The low biomass production rate and nutrient removal in winter were due to the lower temperatures and is reflected in the low biomass concentrations and high ammonia concentrations in the colder winter and high biomass concentrations and low ammonia concentrations in the warmer summer (see Table 2). Low temperatures hinder microalgal growth due to slower enzyme reactions and less available energy (Singh and Singh 2015).

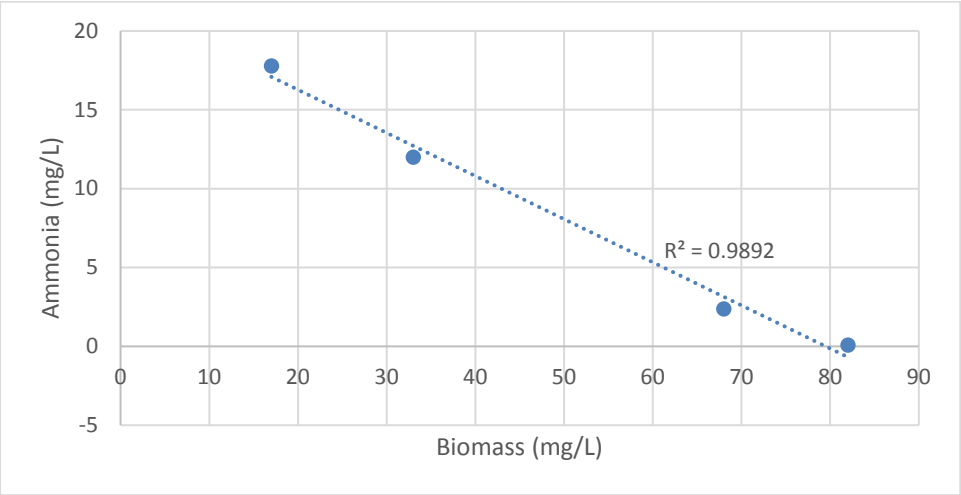

Figure 3: Ammonia (mg/L) versus Biomass (mg/L) for an HRAP run commencing on the 5th of October 2016 with a retention time of 7 days (sampling on days 0, 2, 5 and 7).

Table 2: Mean percentage nutrient removal results and mean percentage algal biomass increases during HRAP trials throughout the experimental period.

	Autumn	Winter	Spring	Summer	Annual
Nitrate and Nitrite (mg/L)	4%	-83%	3%	-11%	-6%
Orthophosphate (mg/L)	15%	3%	53%	57%	32%
Ammonia (mg/L)	68%	56%	97%	65%	71%
Total Nitrogen (mg/L)	45%	34%	84%	26%	64%
Total Phosphorus (mg/L)	21%	5%	60%	61%	37%
Dried Biomass (mg/L)	215%	160%	399%	299%	274%
750nm	269%	237%	513%	267%	330%
680nm	245%	198%	483%	256%	302%
440nm	216%	149%	397%	223%	252%

According to Redfield (1958) the ratio of carbon: nitrogen: phosphorus in algae is approximately 106:16:1. A mean HRAP influent N: P ratio of 1.64:1 and a maximum N: P ratio of 4.16:1 were recorded during this study. This confirms that there was no phosphorus limitation during the experiments and microalgae were believed to have taken up extra phosphorus as a form of luxury uptake (Wang et al. 2010, Beuckels et al. 2015). Nitrogen was thought to be a limiting factor in some runs, other than carbon, and carbon limitation was highlighted by the elevated pH values.

Table 3 shows an annual mean pH value of 9.35, a maximum of 11.02 and a minimum of 7.93 during the algal growth runs. The highest mean pH results were recorded in summer with a mean of 9.98, the lowest in winter with a mean of 8.71, and autumn and spring means were 9.15 and 9.30 respectively. These elevated pH levels negatively affect the growth of the microalgae. When pH exceeds 8.3, the amount of free/ dissolved CO_2 reduces to virtually zero. Algae are still able to grow in elevated pH conditions, but the growth rates are diminished by the algae's need to break down bicarbonate to access the carbon. High pH values cause ammonia volatilization, and this would further hinder the growth of algae due to a lack of nitrogen (Cai et al. 2013).

Table 3: Mean, standard deviation, maximum and minimum pH results in each season and throughout the experimental period (May 2016 - May 2017)

	Autumn	Winter	Spring	Summer	Annual
Mean	9.15	8.71	9.30	9.98	9.35
Max	9.63	8.82	10.18	10.49	11.02
Min	8.69	8.41	8.29	9.10	7.93
Mean Std. Dev	0.22	0.11	0.43	0.31	0.27

A large variety of microalgae inhabited the lagoons and the HRAPs. These included *Scenedesmus sp., Dictyosphaerium sp., Chlamydomonas sp., Euglena sp., Micractinium sp., Golenkinia sp.* and *Oocystis* sp. The species diversity and population density varied throughout the year responding to fluctuations in temperature and nutrients. Large amounts of zooplankton were observed in the third secondary lagoon. These included; rotifers (*Brachionus* sp.), cladocerans, (*Daphnia* sp.) and copepods, (C*yclops* sp.) but these zooplankton were not found in significant numbers in the HRAPs. Montemezzani et al. (2015) stated that zooplankton might be killed by the

generation of shear forces exerted by a water pump. It is believed that during the process of transferring the water from the lagoon to the HRAPs the shear forces exerted on the zooplankton in the pipework and pump, killed large amounts of zooplankton due to powerful impacts with the pipe walls causing the zooplankton to be damaged. Elevate pH levels, and free ammonia toxicity may also have a lethal effect on the zooplankton population (Montemezzani et al. 2015).

Environmental Factors

Previous studies showed the significant impact solar radiation and temperature have on algal growth (Singh and Singh 2015, Huesemann et al. 2016). The concentration of algal biomass recorded during each run was plotted against the number of days passed in that run, linear and exponential trendlines were plotted, and the R^2 values recorded in Table 4. ANOVA and t-test analysis on the two sets of R^2 values showed no difference. Algae are expected to grow exponentially, and a linear growth relationship indicates that the algal growth was limited. The use of linear relationship in the predictive growth rate modelling was determined to be more accurate than an exponential relationship for this data due to the slightly higher mean R^2 value: the linear and exponential mean R^2 values were 0.80 and 0.77 respectively.

Table 4: R^2 values based on time versus biomass concentration for each separate HRAP run.

	Linear R^2	**Exponential R^2**
25/05/2016	0.9767	0.9757
3/06/2016	0.9483	0.9934
18/07/2016	0.9786	0.99
31/08/2016	0.0112	0.0206
19/09/2016	0.5595	0.1317
5/10/2016	0.9917	0.9629
19/10/2016	0.9713	0.9713
16/11/2016	0.7068	0.6712
2/12/2016	0.9924	0.9676
12/12/2016	0.8846	0.7394
6/01/2017	0.5967	0.6318
16/01/2017	0.8957	0.8581
13/02/2017	0.8935	0.8563
6/03/2017	0.882	0.9083
13/03/2017	0.7923	0.7835
10/04/2017	0.8313	0.8193
1/05/2017	0.982	0.9875
29/05/2017	0.5578	0.5152
Mean	0.8029	0.7657

The growth rate was correlated to solar radiation, ammonia concentration and temperature. Linear regression of the mean temperature and growth rate resulted in an R^2 value of 0.52, while linear regression of mean solar radiation and growth rate achieved an R^2 value of 0.65. The effect of differing photoperiods was investigated and gave the same R^2 value as solar radiation. While these R^2 values are low, the results do indicate a relationship between daily production and mean temperature and solar radiation during the growth period. A simple combination of mean

temperature and mean solar radiation was tested, and when measured against growth rate an R^2 value of 0.66 was achieved as shown in Figure 4.

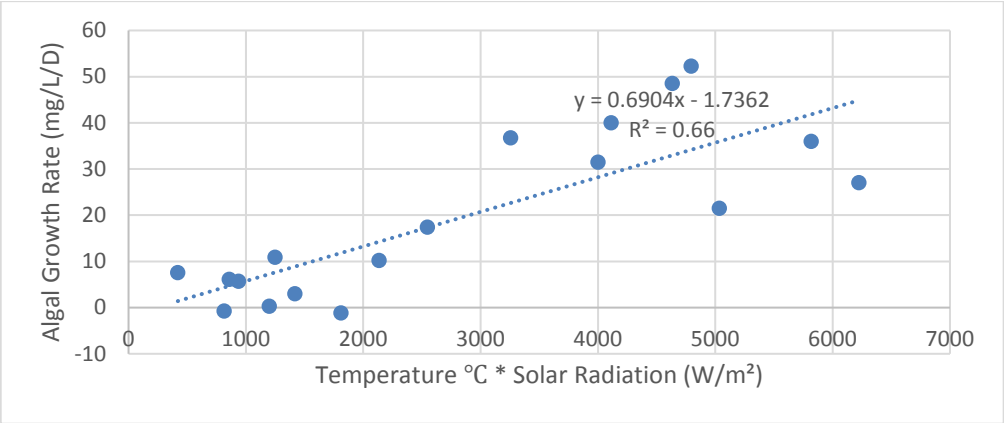

Figure 4: Algal Growth rate (mg/L/D) versus mean temperature (°C) x mean solar radiation (W/m²).

Model Validation

The use of modified versions of the Steele and Monod equations was shown to enhance the predictive capabilities of this work. Steele equation was modified by removing the exponential function from the equations as it was determined that the algal growth rates in the HRAP followed a linear trend rather than the expected exponential trend. This result is caused by the various ecological and chemical limitations the HRAPs contain. For example, most models developed for use in a laboratory do not incorporate light limitation factors such as diurnal variation and self-shading which are common in an HRAP. Inter-species competition between algae can also affect the rate of growth. The modified Steele equation (4) was used to model the effect of light and temperature. When comparing the predicted growth rate using the modified Steele equation against the recorded growth rate an R^2 value of 0.66 resulted. The main outliers were HRAPs trials in which high temperatures and solar radiations were observed, such as during the summer months. In these instances, the model overpredicted the observed biomass growth, and this was believed to be caused by an ammonia limitation during these trials. Removing the results in which the initial ammonia concentrations were less than 1.3 mg/L increased the R^2 value to 0.83. Overprediction of algal biomass was also observed under cold temperatures conditions where growth measurements were inaccurate due to the low algal biomass concentrations.

To correct for the ammonia limitation, the modified Monod model was used to predict the biomass production based on the amount of ammonia that was available. 2mg/L of ammonia was determined to be sufficient for normal growth in these experiments, and that below 1.3 mg/L of available ammonia in the system was limiting. The model typically requires a maximum growth rate based on the optimal growth rate determined by saturated nitrogen conditions. However, the maximum growth rate used was the predicted value calculated using the modified Steele equation using solar radiation and temperature.

A combination of the two equations achieved an R^2 value of 0.82 when comparing the predicted growth rates and the recorded growth rates (See Figure 5). This result is promising, and while more complex models could predict the algae growth more accurately, this would require more detailed characterisation of the growth conditions. Wu et al. (2013) used an integrated growth model on nine different wastewater samples and obtained R^2 values ranging from 0.64 to 0.99,

however, each wastewater sample had different model parameters, thus adding to the complexity of the modelling. An RMSE value of 8.82mg/L/D was obtained when comparing all the predicted daily biomass productivity to the recorded daily biomass productivity. The daily biomass productivity data was separated into two sets, above and below the average temperature, to highlight the differences in RMSE. The RMSE of the above average temperature (15°C) data is 5.47mg/L/D, and the RMSE of the below average temperature data is 10.77mg/L/D. These results are displayed in Figure 5, which outlines which data corresponds to which RMSE set. This highlights that there is a more significant error for model predictions at colder temperatures than at warmer temperatures. Therefore, estimating growth times and lagoon areas for operation of HRAP during cold weather is prone to greater risk than for higher temperatures, while better estimates can be made for higher temperatures (>15C) that correspond to the usual conditions for industrial use of HRAPs. The discrepancies in the current model could be attributed to a few factors experienced during the study. Firstly, elevated pH levels reduce the amount of available free carbon in the system, and this negatively affects growth. Secondly, the difference in algal species and cyanobacteria composition throughout the year would affect the growth rate. Thirdly, there were two results obtained in winter 2016 in which the biomass productivity rates were slightly negative, and while these results were expected to be low, negative values were not expected. The negative values could have been produced by higher respiration rates compared to their photosynthetic rates or experimental error. Fourthly, evaporation could have contributed to the higher than predicted biomass concentrations in summer. The water in the HRAPs was kept constant via the addition of effluent from the secondary lagoon three; this additional water would have contained unaccounted for ammonia and consequently enhanced biomass growth rates. Lastly, zooplankton may have consumed algae and skewed the results, although zooplankton were killed by the pump when the lagoons were filled with secondary effluent.

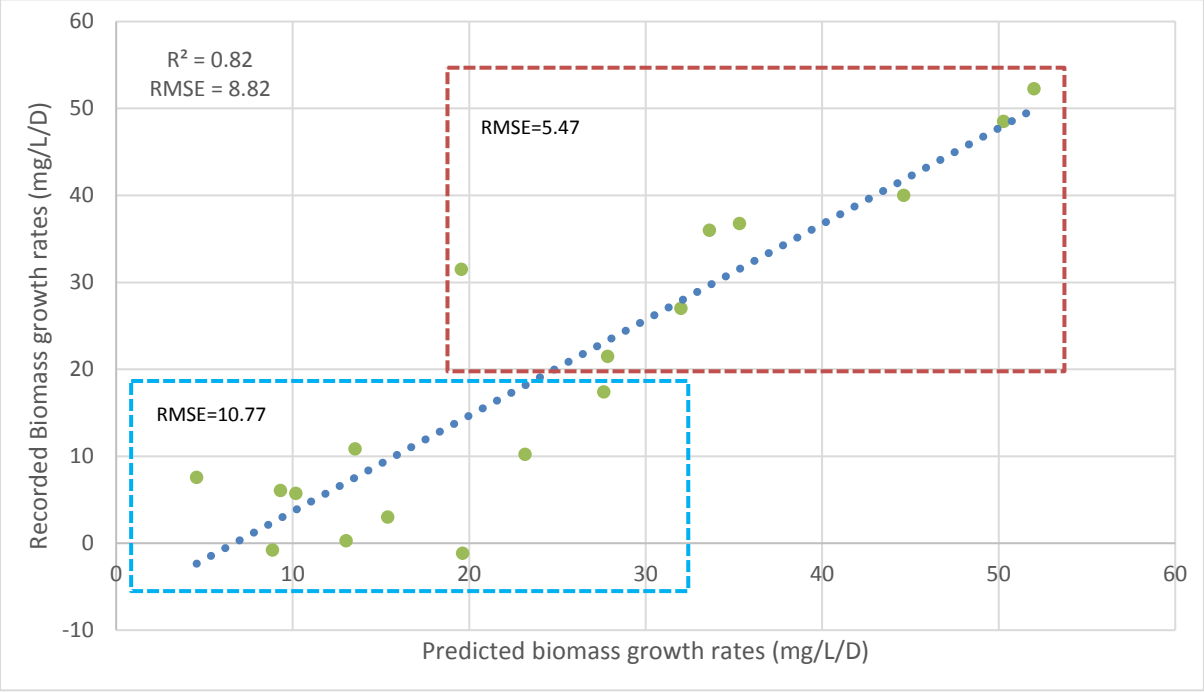

Figure 5: The values from the combined modified Steele and Monod models. Predicted algal growth rates (mg/L/D) versus recorded algal growth rates (mg/L/D).

From the proposed relationship and initial ammonia concentration, we can estimate how long it would take for microalgae to reach a stationary growth phase. The relationship will predict the growth rate and consumption of ammonia during growth. This will assist with identifying the optimum harvesting period and retention times for HRAPs. Additionally, a significant deviation of growth rates from this relationship might also identify if there are limiting conditions for growth or if its growth can be enhanced by the addition of nutrients or a change in pH.

Conclusion

This study investigated the use of batch HRAPs to remove nutrients from secondary effluent by means of growing algae. The two HRAPs were operated in parallel for 12 months with no control of pH, nutrient concentration or the algal community. The HRAPs significantly reduced the nutrient concentrations by means of incorporation of nutrients into algal biomass. This further reduced the nutrient level from the secondary effluent before its discharge for future use. A simple predictive algal growth model was developed from modified versions of the Steele and Monod models. The model incorporated temperature, solar radiation and ammonia concentrations which varied significantly throughout the year, and an R^2 correlation of 0.82 was obtained with an RMSE value of 8.82 mg/L/D. A predictive algal biomass model for HRAPs that requires readily available data is of key importance for the development of more extensive wastewater treatment HRAPs. This model can help predict if effluent quality and environmental conditions are suitable for a large scale HRAP from limited data and if HRAPs would be viable for biomass cultivation.

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