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Enhancing harvestable algal biomass production in wastewater treatment high rate algal ponds by recycling

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy
in
Environmental Engineering

at Massey University, Palmerston North,
New Zealand.

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2013

Abstract

High Rate Algal Ponds (HRAPs) are an efficient and cost-effective system for wastewater treatment and produce algal biomass which could be converted to biofuels. However, little research has been conducted to improve harvestable biomass production from these ponds. Laboratory and small-scale outdoor research reported in the literature indicates that selective biomass recycling is partially effective at controlling algal species in HRAP. This, therefore, offers the potential to select and maintain a rapidly settleable algal species. To date, algal species control of similarly sized, co-occurring algae has not been demonstrated in wastewater treatment HRAPs. Furthermore, the influence of algal recycling on biomass harvest efficiency, harvestable biomass productivity, net biomass energy yield and the growth of the dominant algal species in the HRAPs have never previously been investigated. The main hypothesis of this Ph.D. was: ‘Recycling a portion of gravity harvested biomass (‘recycling’) back into the HRAP improves harvestable biomass production’. To test this, a series of experiments was conducted using pilot-scale wastewater treatment HRAPs, outdoor mesocosms and laboratory microcosms. Firstly, the influence of recycling on species dominance and biomass harvest efficiency was investigated using two identical pilot-scale HRAPs over two years. This pilot-scale study showed that recycling promoted the dominance of a rapidly settling colonial alga, *Pediastrum boryanum*, and maintained its dominance over the two year experimental period. Moreover, *P. boryanum* dominance was relatively fast to establish and was then stable and sustainable between seasons. The higher dominance of *P. boryanum* in the HRAP with recycling improved biomass harvest efficiency by gravity sedimentation from ~60% in the control HRAP without recycling to 85%. Unexpectedly, recycling also improved the ‘in-pond’ biomass productivity by 20%. The combination of the increased biomass productivity of the HRAP and the increased biomass harvestability with recycling improved the ‘harvestable biomass productivity’ by 58%. Overall, recycling increased the net biomass energy yield by 66% through the combined improvements in biomass productivity, harvest efficiency and a small increase in algal biomass energy content. To determine the reproducibility of these findings and investigate the mechanisms responsible, twelve outdoor mesocosms were studied. This mesocosm research repeatedly confirmed that recycling can establish *P. boryanum* dominance, and improve biomass productivity and settleability. Settleability was not only found to be improved by recycling the ‘solid’ fraction of the harvested

biomass but also by recycling of the ‘liquid’ fraction, potentially indicating the presence of extracellular polymeric substances. Several possible mechanisms to explain the increase in biomass productivity were identified. However, after review all but two were discounted: (i) the mean cell residence time (MCRT) was extended thereby increasing the algal concentration and thus allowing better utilization of incident sunlight; and (ii) the relative proportion of algal growth stages (which may have different net growth rates) was shifted, resulting in an increase in the net growth rate of the algal culture. To investigate these mechanisms further, the life-cycle of *P. boryanum* was studied in detail and showed, for the first time in the literature, that its net growth rate does indeed vary between the three life-cycle stages (‘growth’ > ‘juvenile’ > ‘reproductive’). Given that the mesocosm studies in Chapter 4 showed that recycling increased the number of growth colonies by ~2-fold and juvenile colonies by ~4-fold then it is proposed that mechanism (ii) does appear to be viable. This Ph.D. work has demonstrated that recycling a portion of gravity harvested biomass could be a simple and practical method to enhance biomass productivity, harvest efficiency and energy content, which contribute to achieve higher ‘harvestable biomass productivity’ and ‘energy yield’ in wastewater treatment high rate algal ponds.

Acknowledgement

First to my supervisors Professor Andy Shilton at Massey University and Dr Rupert Craggs at NIWA (National Institute of Water and Atmospheric Research, Ltd) for their help, guidance and motivation throughout my research. Their support during my Ph.D. and all the valuable and critical comments on the research work particularly on a number of journal papers is greatly appreciated.

This Ph.D. research was funded by NIWA's MBIE funded project, "Wastewater Algal Biofuel Program". I am grateful for the financial support from Top Achiever Doctoral Scholarship commissioned by New Zealand Tertiary Education Commission (TEC). I wish to thank my employer, NIWA during my Ph.D. Without the financial support from MBIE, TEC and NIWA completion of this thesis would have been extremely difficult.

I would like to acknowledge Mr Karl Safi and Ms Karen Thompson at NIWA for their technical advice while developing the microcosm experiment set-up in the laboratory. I also wish to acknowledge Miss Donna Sutherland, Mr James Sukias and Dr Niall Broekhuizen at NIWA for their critical peer review of my various papers, which have been greatly helpful for the completion of this thesis.

Thanks must also go to the students from Europe for their valuable help during my outdoor experimental work conducted in the Ruakura HRAP research facility. In particular I wish to thank Mr Valerio Montemezzani from Italy, Mr Andy McDonald from U.S.A, Mr Tim Walles from Netherland, Mr TayFun Tastic and Mr Lukas Seibold both from Germany.

Lastly I would like to think my family for their patience, love and support throughout my years as a Ph.D. student.

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Structure of the thesis

The chapters of this Ph.D. thesis are presented as a series of scientific journal papers. These papers have either been accepted for publication or submitted for review. Consequently there was some repetition in the paper introductions and methods sections. In order to reduce this repetition in the thesis, the introduction of the chapters has been shortened (particularly Chapters 3, 4 and 5). The chapters have also had some minor editing to improve clarity and consistency. A preface is included for each of these chapters to help link the chapters together and illustrate how each of these chapters contributes to investigate the objectives of this thesis. Some formatting changes have been made to ensure consistent style within the thesis. For example, the labels for Figures and Tables have been modified to include the chapter number (e.g. Figure 2 in the third paper was changed to Figure 3.2 in Chapter 3). Where the published papers refer to other papers within the thesis, these references have been changed to the relevant chapter within the thesis.

The structure of this thesis complies with Massey University guidelines given in the Doctoral Handbook, 2011.

List of papers and contribution

A list of the chapters and relevant publications is given below.

Chapter 1

Park, J.B.K., Craggs, R.J., Shilton, A.N. (2011). Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology* 102 (1):35-42. (This publication have been cited 170 times according to Scopus by December 19th, 2013).

Chapter 2

Park, J.B.K., Craggs, R.J., Shilton, A.N. (2011). Recycling algae to improve species control and harvest efficiency from a high rate algal pond. *Water Research* 45 (20):6637-6649. (This publication have been cited 13 times according to Scopus by December 19th, 2013).

Chapter 3

Park, J.B.K., Craggs, R.J., Shilton, A.N. (2013). Enhancing biomass energy yield from pilot-scale high rate algal ponds with recycling. *Water Research*. 47 (13): 4422-4432.

Chapter 4

Park, J.B.K., Craggs, R.J., Shilton, A.N. (2013). Investigating why recycling gravity harvested algae increases harvestability and productivity in high rate algal ponds. *Water Research*. *Water Research* 47 (14): 4904-4917.

Chapter 5

Park, J.B.K., Craggs, R.J., Shilton, A.N. Growth, reproduction and life-cycle of the wastewater treatment High Rate Algal Pond alga, *Pediastrum boryanum*. Submitted to *Water Research*.

All the research that these papers are based on was conducted during my Ph.D. period. While the papers were completed with advice and editing from my supervisors, Professor Andy Shilton and Dr Rupert Craggs, I designed the experiments, conducted all experimental work,

analysed the results and led all the papers as the first corresponding author. My contribution to jointly authored chapters was clearly documented the end of each thesis chapter (DRC 16) signed by both the principal supervisor (Professor Andy Shilton) and myself.

Chapter 1

Introduction

Chapter preface

In this chapter the current literature on the application of high rate algal ponds (HRAP) for wastewater treatment and algal production is reviewed, and the benefits and opportunities of algal biomass production from wastewater treatment HRAP for biofuel production are explored. Moreover, the critical parameters limiting algal biomass production and harvest ('harvestable biomass production'), and practical options to enhance them in wastewater treatment HRAPs were investigated. There was a lack of published information available on practical methods to enhance harvestable biomass production in HRAP, even for simple techniques such as recycling a portion of gravity harvested biomass ('recycling') back to the pond. In particular, the influence of recycling on the species dominance of similarly sized, co-occurring algae has not been studied. Furthermore, the influence of recycling on biomass harvestability, productivity, and biomass energy yield has never been investigated.

This chapter is based on the following publication;

Park, J.B.K., Craggs, R.J., Shilton, A.N. (2011) Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology* 102 (1):35-42.

1.1. Introduction

Algae grown under controlled conditions could potentially produce substantially more oil per hectare than terrestrial oilseed crops such as palm, soy and canola (Sheehan et al. 1998; Chisti 2007, 2008; Benemann 2008b; Darzins et al. 2010). Therefore, less land area is potentially required to produce oil from algae than from other types of biomass. However, the capital and operation costs of systems for algal biofuel production are presently prohibitive (Sheehan et al. 1998; Benemann 2008b; Tampier 2009; Craggs et al. 2011; Benemann 2013). For example, Chisti (2008) calculated that algal bio-diesel production costs must drop almost 10-fold to be competitive with crude oil at \$100/barrel.

A niche opportunity may, however, exist where algal biomass is grown as a by-product of wastewater treatment in high rate algal ponds (HRAPs). HRAPs are shallow, open raceway ponds that are used for treatment of municipal, industrial and agricultural wastewaters. Large-scale production of algal biofuels using wastewater treatment HRAPs was first proposed by Oswald and Golueke (1960). The algal biomass produced and harvested as a by-product of these wastewater treatment systems could be converted through various pathways to biofuels, for example anaerobic digestion to biogas, transesterification of lipids to biodiesel, fermentation of carbohydrate to bioethanol and high temperature conversion to bio-crude oil (Craggs et al. 2011).

Algal growth and photosynthetic activity under different environmental conditions have been extensively studied over the last few decades (e.g. Oswald and Golueke 1960; Weissman and Goebel 1987; Tillett 1988; Walker 2002; Melis 2009; Walker 2009). Moreover, the many critical environmental (light and temperature), operational (pH, CO₂ and nutrients) and biological (zooplankton grazers and algal pathogens) parameters that affect HRAP wastewater treatment have been studied (Weissman and Goebel 1985; Richmond 1986; Nurdogan and Oswald 1995; Pulz 2001; Torzillo et al. 2003; Richmond 2004; Grobbelaar 2009). Over the last 50 years, full-scale wastewater treatment HRAPs have been built in the USA and several other countries as a component of Advanced Pond Systems (Craggs 2005). In particular, the National Institute of Water and Atmospheric Research Ltd. (NIWA) has conducted pilot-scale and full-scale research on HRAPs over the last decade to calibrate design and operation to New Zealand climatic conditions, and has shown that HRAP not only provide improved and more consistent wastewater treatment than conventional oxidation ponds, but have much higher productivity (annual

average, $\sim 8 \text{ g/m}^2/\text{d}$ volatile suspended solids) (Craggs et al. 1998, 2003, 2011; Sutherland et al. 2013). However, fundamental and field-scale research is needed to further optimise algal biomass production and particularly subsequent harvest ('harvestable biomass production') from wastewater treatment HRAPs while maintaining high effluent water quality.

This chapter reviews the current literature on both the application of HRAP for wastewater treatment and for dedicated algal biomass production, and defines the benefits and opportunities of algal biomass production from wastewater treatment HRAP for biofuel production. Critical parameters limiting algal biomass production and harvest are discussed and practical options to enhance harvestable biomass production in wastewater treatment HRAPs are identified. The specific objectives of this Ph.D. research that were derived from this literature review are proposed in Section 1.7.

1.2. High Rate Algal Ponds

HRAPs are gently mixed raceway-type ponds and have depths between 0.2 and 1 m (Figure 1.1). Mixing is normally provided by a paddlewheel to give a mean horizontal water velocity of between 0.15 and 0.3 m/s (Craggs 2005). Raceway configuration may be as a single loop or multiple loops around central dividing walls. The pond bottom is typically earth or clay lined (depending on soil conditions and local regulations). CO_2 may be added into a counter current gas sparging sump ($\sim 1.5 \text{ m}$ depth) that increases CO_2 uptake efficiency into the pond water.

1.2.1. HRAPs for treating wastewater

Many small communities and farms use single or two stage oxidation pond systems for wastewater treatment (Craggs 2005). These systems have generally performed well in terms of wastewater organic solids removal; however, nutrient removal, algal solids removal and disinfection are highly inconsistent, and the discharge of poor-quality effluents with respect to these parameters may negatively impact the receiving waters (Davies-Colley et al. 1995; Craggs et al. 2003, 2012). HRAPs retain the advantages of conventional oxidation ponds (simplicity and economy) but overcome many of their drawbacks (including poor and highly variable effluent quality, limited nutrient and pathogen removal), and could have the added benefit of recovering wastewater nutrients as harvestable algal/bacterial biomass for beneficial use as fertiliser, feed or biofuels.

Algae in the HRAPs provide photosynthetic oxygenation which promotes aerobic breakdown of dissolved organic compounds (i.e. BOD₅) in the wastewater by heterotrophic bacteria (Oswald 1996; Craggs 2005). Moreover, algae assimilate nutrients during their growth, and thus subsequent harvesting of the algal biomass recovers the nutrients from the wastewater (Craggs 2005; García et al. 2006; Park and Craggs 2010).

HRAP is a component of wastewater treatment Advanced Pond System which typically comprises advanced facultative ponds (or more recently anaerobic ponds), HRAP, algal settling ponds and maturation ponds in series (Craggs 2005; Craggs et al. 2011). Compared to activated sludge systems, which are presently one of the most common wastewater treatment technologies, the area required for an Advanced Pond System is approximately 50 times greater (based on design for BOD₅ removal and not accounting for the area needed for handling the waste activated sludge). However, the capital costs for construction and operating costs are lower than mechanical activated sludge systems (Downing et al. 2002; Craggs et al. 2011; 2012). Moreover, APS systems could also provide the co-benefits of enhanced algal biomass production for beneficial use (feed or biofuels), and recovery of nutrients from wastewater. Therefore, where land is available and climate conditions are appropriate, these systems could be widely applicable for many of the smaller communities worldwide for near tertiary-level wastewater treatment (i.e. enhanced nutrient removal and disinfection) and biofuel production (Benemann 2003; Craggs et al 2012; Benemann 2013). The simplest and most cost effective option to convert algal biomass to biofuel would be via an ambient temperature covered anaerobic digester pond to produce methane-rich biogas (Craggs et al. 2011), although more expensive heated and mixed anaerobic digesters could also be used (Sialve 2009). Biogas production rates from laboratory-scale ambient temperature covered digester ponds have been shown to be similar to those of heated mixed digesters (0.21-0.28 m³ CH₄/kg algal volatile solids (VS) added) (Sukias and Craggs 2010). Use of methane biogas produced by digesting algal biomass for electricity generation can produce approximately 0.6-0.8 kWh_{electricity} / kg algal VS assuming a methane energy content of 9.38 kWh/m³; Elliot et al 2012) and a ~30% generator conversion efficiency.

1.2.2. Economic and environmental advantages of biofuel production from wastewater treatment HRAP

The comparisons between algal production HRAP and wastewater treatment HRAP are summarized in Table 1.1. The costs of algal biomass production and harvest using wastewater treatment HRAP are essentially covered by the wastewater treatment plant capital and operation costs, and these systems may have significantly less environmental impacts in terms of water footprint, energy and fertiliser use, and eutrophication from discharge of residual nutrients in effluent.

While the demand for biofuel production is in part driven by environmental concerns, there is no doubt that building and operating HRAP dedicated solely to produce algal biomass for biofuel has a significant environmental impact in its own right. For example, fresh water resources are consumed via evaporation thus creating a water footprint. Indeed, Clarens et al (2010) concluded that algal production using freshwater and fertilizers would consume more energy, have higher greenhouse gas emissions and use more water than biofuel production from land-based crops such as switchgrass, canola and corn.

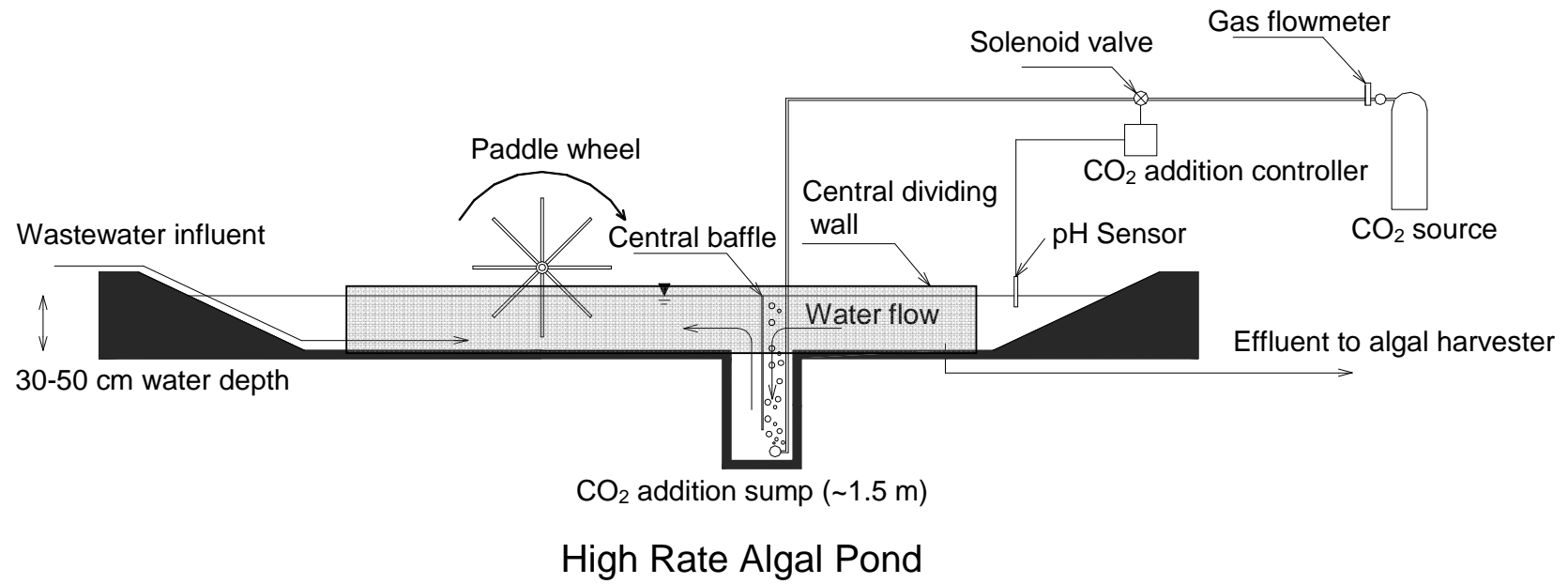


Figure 1.1: Side elevation of a high rate algal pond (HRAP) with CO₂ addition to enhance algal biomass production.

Table 1.1: Comparison of dedicated HRAP and wastewater treatment HRAP for biofuel production.

Factors	Dedicated HRAP	Wastewater HRAP	Reference
Capital costs	\$US 0.1 (unlined)– \$0.25 (lined) million/ha	Algae is free as a by-product	(Benemann, 2008a; Tampier, 2009)
Operation and maintenance costs	~ \$US 20k/ha	Algae is free as a by-product	(Benemann, 2003; van Harmelen and Oonk, 2006; Tampier, 2009; Craggs et al., 2010)
Land use	High	N/A	
Commercial availability	Already commercially applied for health products and pigments	Well established wastewater treatment	(Benemann, 2008a,b)
Most costly parameters	Water, Fertiliser, Harvesting, and Mixing	N/A because algae is free as a by-product	(Tampier, 2009)
Limiting factors for algal growth	Light, Temperature, Nutrients, CO ₂ (externally provided)	Light, Temperature, Nutrients (internally provided by wastewater), CO ₂ (partially provided by bacterial oxidation of wastewater organics and externally provided)	(Grobbelaar, 2009; Craggs et al., 2010)
Algal productivity	Could be >30 g/m ² /d but not reported in literature	High productivity may not be a main driver due to algal biomass as a by-product	(Benemann, 2002 & 2008a,b)
Water footprint	Significant (freshwater use and net evaporation loss)	Not applicable unless effluent is reused	(Carvalho et al., 2006)
Risk of contamination	High (growth medium re-use increases risk of contamination)	Contamination from incoming wastewater flow (grazers and fungal parasites)	(Schenk et al., 2008; Tampier, 2009)
Biomass harvesting	Expensive due to small size (<20 µm)	Could be expensive but gravity settling can be promoted by aggregation of colonial algae with wastewater bacteria	(Sheehan et al., 1998; Benemann, 2008a; Craggs et al., 2010)
Algal species control	Only limited success in high pH and high salinity	May be possible by selective biomass recirculation	(Schenk et al., 2008)

Algal biomass production from wastewater treatment HRAPs by contrast offers a far more attractive proposition from an environmental impact viewpoint. The impacts of its construction and operation are a necessity of providing wastewater treatment and thus the subsequent algal biomass yield represents a biofuel feedstock free of this environmental burden. Furthermore, the water and nutrients that are utilized in these systems are neutral in that they are otherwise wasted. The extraction of energy and subsequent application of the residual algal biomass to land represents a source of sustainable energy and fertilizer, thus offering net environmental benefit. Indeed, the use of HRAPs for wastewater treatment over other types of wastewater treatment can provide environment gains. For example, Shilton et al (2008) gave an example for a town of 25,000 people in the English countryside where using a pond treatment option instead of an electromechanical wastewater treatment system (e.g. activated sludge system) could save 35 million kWh over a 30-year design life. They went on to note that for the UK, where an average of 0.43 kg CO₂ is emitted per kWh of electricity produced, this amounts to 500 tonnes of CO₂ emitted per year which would require over 160 hectares (~400 acres) of pine forest to soak up (Shilton et al. 2008).

Pond systems are one of the most common types of wastewater treatment technology used by small to medium sized communities around the world. However, with increasing regulatory pressure to upgrade treatment for nutrient removal and subsequent algal harvesting and with greater recognition of the renewable energy production and potentially improved greenhouse gas management that HRAPs offer, it is likely that they will become increasingly widespread in communities of this size in the future.

1.3. Algal production

Many theoretical approaches to determine the maximum photosynthetic solar energy conversion efficiency have been described in the literature (Weissman and Goebel 1987; Tillett 1988; Walker 2002; Falkowski and Raven 2007; Melis 2009; Walker 2009), and these are summarized in Table 1.2. For example, photosynthesis requires four photons for each of two photosystems in order to produce a molecule of O₂ ($2\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{CH}_2\text{O} + \text{O}_2 + \text{H}_2\text{O}$). 9.7 photons in the average visible light are required for complete photosynthesis (Falkowski and Raven 2007). The light energy absorbed by algae is first stored as intermediate biochemical reductants (NADPH₂ and ATP) which are then used by the algal cells to produce new biomass (CH₂O) (Tillett 1988). Since, the energy content

of one mole of 'CH₂O' (E_{CH_2O}) is ~468 kJ (see Table 1.2) (Walker 2009), and the energy content of 9.7 photons of red (680 nm) light (E_P) is ~1408 kJ the arithmetic photosynthetic solar conversion efficiency (η_{pho}) is ~33% (see Table 1.2). However, because only ~48% of solar energy is photo-synthetically active radiation (PAR) and because 10–20% of the solar energy is lost by surface reflection, only 12.8–14.4% of solar energy (η_{theo}) can theoretically be converted into algal biomass.

Algae are susceptible to becoming light saturated and inhibited (Weissman and Benemann 1978; Tillett 1988; Walker 2009). While the light saturation level (L_{sat}) is dependent on algal strain and culture density (concentration), the growth of most algal species is inhibited at light levels $>200 \mu\text{Mol/m}^2/\text{s}$, which is only about 10–17% of maximum summer and winter solar PAR radiation (~2000 and ~1200 $\mu\text{Mol/m}^2/\text{s}$ respectively) (Ogbonna and Tanaka 2000; Torzillo et al. 2003). Therefore, the maximum algal photosynthetic conversion efficiency (η_{max}) is only 1.3–2.4% of total solar radiation (Benemann 2008a; Walker 2009).

Table 1.2. Maximum theoretical algal photosynthetic solar energy conversion efficiency (η_{max}).

Symbol	Parameter	Value	Description	References
E_{CH_2O}	Energy required to produce algal biomass (CH ₂ O)	468 kJ/mol	One gram of glucose (C ₆ H ₁₂ O ₆) releases 2813 kJ as heat. As glucose is made up of six molecules of CH ₂ O the energy content of CH ₂ O is at least 468 kJ $E_{CH_2O} = \frac{2813 \text{ kJ (C}_6\text{H}_{12}\text{O}_6 \text{ as heat)}}{6 \text{ mol CH}_2\text{O}} = 468 \text{ kJ/mol}$	(Walker, 2009)
E_p	Energy value of a photon	~176 kJ/photon	One photon of red light (680 nm) has an energy value of ~176 kJ. Eight photons (1408 kJ) are required for complete photosynthesis (PSI and PSII)	(Walker, 2000)
η_{pho}	Photosynthetic solar conversion efficiency	33.2%	$\eta_{pho} = \frac{E_{CH_2O} (468 \text{ kJ/mol})}{E_p \times 8 \text{ photons (1408 kJ)}} \times 100 = 33.2\%$	
η_{par}	The fraction of photosynthetically available solar radiation (PAR)	~48%	The visible light spectrum (light wavelength of 400 – 700 nm) is only available for algal growth	(Zhu et al., 2008; Melis, 2009; Walker, 2009)
L_r	Reflection loss	10 – 20%	Reflection loss at the pond water surface depending on solar angle and mixing conditions of the ponds	(Benemann et al., 1977; Weissman and Goebel, 1987; Tillett, 1988; Zhu et al., 2008)
η_{theo}	Theoretical efficiency of photosynthetic solar energy conversion	12.8 – 14.4%	Solar energy 12.8 – 14.4% can be theoretically fixed by algae as chemical energy $\eta_{theo} = \eta_{par} \times L_r \times \eta_{pho}$	
L_{sat}	Light saturation of algal photosystem	10-17%	Photosynthesis of most algal species is saturated at a solar radiation level of ~200 $\mu\text{mol/m}^2\text{/sec}$, which is about 10-17% of summer/winter maximum outdoor light intensity	(Tredici and Zittelli, 1998; Ogbonna and Tanaka, 2000; Torzillo et al., 2003)
η_{max}	Maximum efficiency of photosynthetic solar energy conversion into biomass	1.3 – 2.4%	Maximum solar energy of 1.3 – 2.4% can be fixed by algae $\eta_{max} = \eta_{theo} \times L_{sat}$	

The maximum algal photosynthetic conversion efficiency can be used to estimate the algal biomass productivity (P_{max}) from incident solar radiation (I_o). For example, taking our HRAP facility in Hamilton, New Zealand (37°47'S, 175°19'E) the summer algal biomass productivity (New Zealand summer months from December to February) could be determined from the average solar radiation as shown in Equation 1.1:

$$P_{max} = \frac{23.5 \text{ MJ/m}^2/\text{d}^{(1)} \times 2.4\%^{(2)}}{\sim 21 \text{ kJ/g}^{(3)}} \times 1000 \approx 27 \text{ g/m}^2/\text{d} \quad \text{Equation 1.1}$$

Where

- (1) Average solar radiation (I_o) from December 2008 to February 2009.
- (2) Maximum efficiency of photosynthetic solar energy conversion (η_{max}).
- (3) Energy content of algal biomass.

Because a proportion of the algal biomass will be lost by decay of the biomass (e.g. dark respiration), this loss of productivity needs to be accounted for and can be estimated as ~10% (Zhu et al. 2008). Therefore, the summer productivity at our HRAP facility in Hamilton, New Zealand is ~24 g/m²/d.

The summer productivity (~24 g/m²/d) estimated by the calculation is very similar to that measured at our experimental pilot-scale HRAP facility in Hamilton, New Zealand 25 g/m²/d, presented in Table 1.3). However, our experimental biomass productivity includes bacterial as well as algal biomass (accounting for both autotrophic and potentially heterotrophic algal growth). More detailed research over a prolonged period (> 1 year) is required to confirm the relationship between algal biomass productivity and solar radiation.

As shown in Table 1.3, experimental algal biomass productivities in HRAPs fed with either growth medium or wastewater range widely from 12-40 g/m²/d, depending on algal species, pond operation, climate and season.

Table 1.3: Algal biomass productivities measured in experimental fresh water and wastewater treatment HRAPs.

HRAP	Location	Species	Areal Productivity (g/m ² /d)		Surface area (m ²)	Total volume (m ³)	References	
			Total	Harvestable				
Algal production	Hawaii	<i>Tetraselmis suecica</i>	40	-			(Laws et al., 1988)	
	Hawaii	<i>Cyclotella cryptica</i>	29.7	-			(Laws et al., 1988)	
	Hawaii	<i>Platymonas sp</i>	26	-	48	5.8	(Sheehan et al., 1998)	
		<i>Cyclotella cryptica</i>	30	-	9.2	1.1	(Sheehan et al., 1998)	
	Hawaii	<i>T. suecica</i>	37.5	-	9.2	1.1	(Sheehan et al., 1998)	
	New Mexico	<i>Scenedesmus quadricauda</i>	14	-	100	22.5	(Weissman and Goebel, 1988)	
	New Mexico	<i>Chlorella sp.</i>	21	-	100	22.5	(Weissman and Goebel, 1988)	
		<i>Anabena siamensis</i>	12.9	-	2	0.3	(Richmond et al., 1993)	
	Wastewater treatment	California		18.4	14.8	1000	-	(Benemann, 1986)
		Israel	Mixed algal culture	33	-	120	-	(Shelef, 1982)
Israel		(e.g. <i>Scenedesmus sp.</i>	35	-	150	-	(Shelef, 1982)	
New Zealand		<i>Micractinium sp.</i>	25*	16.8	32	8	(Park and Craggs, 2010)	
		<i>Actinastrum sp.</i>	4.4 – 11.5	-	12500	4375	(Craggs et al. 2012)	
Philippine		<i>Pediastrum sp.</i>	15.3	11.9	100	-	(Oswald, 1987)	
Scotland		<i>Coelostrum sp.</i>	18*	-	13	-	(Cromar et al., 1996)	
Spain		<i>Chlorella sp.</i>	12.7 – 14.8	9.9-11.5	1.54	-	(García et al., 2006)	
Kuwait		<i>Ankistrodesmus sp.</i>)	15	-	12	-	(Banat et al., 1990)	

1.4. Parameters affecting algal biomass production

1.4.1. Light and Temperature

In the absence of nutrient limitation photosynthesis increases with increasing light intensity until the maximum algal growth rate is attained at the light saturation point (Boutterfas et al. 2002; Macedo et al. 2002; Torzillo et al. 2003; Richmond 2004). Increasing the light intensity beyond this point can lead to photo-oxidation (also known as photoinhibition), damaging the light receptors of the algae and decreasing the photosynthetic rate and productivity (Richmond et al. 2003; Richmond 2004). As algal concentration increases so does the shading effect this biomass creates. For example, an algal biomass concentration of 300 g TSS/m³ will absorb almost all of the available light (PAR) within the top 15 cm of the HRAP, leaving the rest of the pond depth in the dark. Typically HRAPs are designed with a depth of about 30 cm however turbulent eddies, resulting from water flow around the pond, and paddlewheel mixing provide a degree of vertical mixing through the pond depth thus ensuring that the algal biomass is intermittently exposed to light.

Algal biomass productivity increases with increasing pond temperature up to an optimum temperature above which increasing algal respiration and photorespiration reduce overall productivity (Tillett 1988; Sheehan et al. 1998; Pulz 2001). The optimal temperature measured under conditions of maximum algal growth rate (sufficient nutrient and light conditions) is 28–35°C for many algal species (Soeder et al. 1985). However, optimal temperature varies between algal species and when nutrient or light conditions are limiting. Growth often declines when algae are subjected to a sudden temperature change, for example, exposure of a high temperature adapted algal strain to 10°C resulted in a 50% reduction in chlorophyll-*a* in just 15 hours (Harris 1978).

Temperature can also alter the pond water ionic equilibria, pH, and gas (oxygen and CO₂) solubility, although different algal species are influenced to differing degrees by this effect (Boutterfas et al., 2002).

1.4.2. Pond water pH and CO₂ availability

The pH of the pond water affects many of the bio-chemical processes associated with algal growth and metabolism, including the bio-availability of CO₂ for photosynthesis and the availability and uptake of nutrient ions. Pond water pH is in turn a function of algal biomass productivity, algal/bacterial respiration, the alkalinity and ionic composition of culture medium, autotrophic and heterotrophic microbial activities (e.g. nitrification and denitrification) and the efficiency of a CO₂ addition system (García et al. 2000b; Craggs 2005; Heubeck et al. 2007; Park and Craggs 2010). Algal photosynthesis in HRAP raises daytime pH by consumption of CO₂ and HCO₃⁻, often exceeding pH 10 (Craggs 2005; Heubeck et al. 2007; Park and Craggs 2010). The elevated pH can enhance ammoniacal-N removal from the pond liquid via ammonia volatilization and phosphorus removal through phosphate precipitation with unchelated ferric iron, calcium and magnesium (García et al. 2000b; Craggs 2005). The equilibrium shift to higher free ammonia concentrations at high pH can significantly inhibit algal growth (Azov and Goldman 1982). For example, free ammonia concentrations of 34 and 51 g/m³ at pH 9.5 (20 – 25°C) reduced algal photosynthesis of the freshwater algae, *Scenedesmus obliquus*, by 50% and 90% respectively (Azov and Goldman 1982). Moreover, aerobic heterotrophic bacteria that oxidize organic matter in wastewater treatment HRAP have a pH range of 6.5 to 8.5, above which bacterial activity is increasingly inhibited (Craggs 2005). Therefore in wastewater treatment HRAPs, pH could not only influence algal growth but also dissolved organic matter oxidation and nitrogen removal efficiency.

The optimal pH of many freshwater algae is about 8 (Kong et al. 2010). A pH above or below 8 decreases productivity, for example Weissman and Goebel (1988) showed that the productivities of *Chaetoceros* sp. and *Chlorella* sp. were reduced by 22% when pH was raised from 8 to 9. Some algae are, however, capable of growing well above pH 8, such as *Amphora* sp. and *Ankistrodesmus* sp. which were not inhibited at pH 9 and 10 respectively (Weissman and Goebel 1988).

CO₂ availability within wastewater treatment HRAP predominantly depends upon heterotrophic oxidation of organic compounds by bacteria (Weissman and Goebel 1987; Oswald 1988; Craggs 2005). However typical domestic wastewater often contains

insufficient carbon to fully support optimal algal production (~4-7C:N ratio in domestic wastewater compared with ~15C:N in algal biomass) (Benemann 2003; van Harmelen and Oonk 2006). CO₂ addition has been shown to enhance algal biomass productivity in laboratory and pilot-scale wastewater treatment HRAPs (Azov et al. 1982; Benemann 2003; Park and Craggs 2011a) and is indeed standard practice at all commercial algal production HRAP systems (van Harmelen and Oonk 2006). By comparison, CO₂ addition is presently not used in wastewater treatment HRAPs except in a few small- to pilot-scale experimental trials and a 5 ha demonstration system in the Christchurch Wastewater Treatment Plant, New Zealand that was operated between 2009-2011 (Craggs et al. 2012).

1.4.3. Dissolved Oxygen

Intense daytime photosynthesis in HRAP can increase pond water dissolved oxygen levels to >200% saturation (García et al. 2000b; Molina et al. 2001; Park and Craggs 2010). High dissolved oxygen levels in excess of normal air saturation are believed to impact algal biomass productivity (Weissman and Goebel 1987; Molina et al. 2001). For example, Molina et al (2001) presented that photosynthetic activity, measured by oxygen generation under steady-state algal biomass concentrations, was reduced by 17-25% at 200-300% dissolved oxygen saturation. More research is required to demonstrate the effect of high oxygen levels on algal growth in outdoor HRAPs.

1.4.4. Nutrients

In commercial HRAP production systems fertiliser is usually added in excess to avoid nutrient limitation of algal growth (Acién Fernández et al. 2001). Assuming that algal biomass has the typical composition ($C_{106}H_{181}O_{45}N_{16}P$), a fertiliser with an N:P ratio of 16N:P (7.3 g N : 1 g P) would be required (Craggs 2005). However, this ratio of N:P can vary from about 4:1 to almost 40:1 depending on algal species and nutrient availability in algal culture (Craggs et al. 2011). Therefore, high productivity may be achieved even at relatively low N:P ratios in wastewater treatment HRAPs.

Nitrogen is a critical factor for regulating algal cell lipid content (Cooksey et al. 1987; Tillett 1988; Griffiths and Harrison 2008; Brennan and Owende 2010). While typically algae

contain approximately 20% lipid in their cell (Benemann 2008b and Chisti 2008), high lipid accumulation in algal cells of >40% occurs when nitrogen becomes the growth limiting factor (Cooksey et al. 1987; Tillett 1988). However, using nitrogen limitation to stimulate lipid accumulation in algal cells may in turn reduce algal growth (Coleman et al. 1987; Tillett 1988; Chelf 1990), suggesting that the two conditions of high lipid content and high algal biomass productivity may be mutually exclusive.

1.4.5. Zooplankton grazers and pathogens

HRAPs are susceptible to grazing by herbivorous protozoa and zooplankton (e.g. rotifers and cladocerans) which can reduce the algal biomass concentrations and production to low levels within just a few days (van Harmelen and Oonk 2006; Benemann 2008a). For example, rotifers and cladocerans at high densities (>105/L) were shown to reduce algal biomass concentrations by 90% within two days (Oswald 1980) and Cauchie et al (1995) measured a 99% reduction in algal chlorophyll-*a* due to *Daphnia* grazing.

Fungal parasitism and viral infection can also reduce the algal population in a conventional wastewater treatment pond within a few days. Moreover, the presence of viruses can trigger changes in algal cell structure, diversity and succession (Kagami et al. 2007).

1.5. Optimising harvestable algal biomass production in wastewater treatment HRAPs

Maximum algal biomass production in wastewater treatment HRAPs could be achieved by alleviating rate limiting conditions, overcoming inhibitory parameters and through control of algal grazers and pathogens. This section discusses some practical options to enhance net harvestable algal biomass production.

1.5.1. CO₂ addition

CO₂ addition to wastewater treatment HRAPs augments carbon availability for algal growth and also serves to mitigate pH inhibition. This can be simply achieved through control of the HRAP water daytime maximum pH to below 8.0 by CO₂ addition. While nutrient removal (which is often an important wastewater treatment objective) by physico-chemical processes such as ammonia volatilisation and phosphate precipitation may be reduced by CO₂ addition

to a wastewater treatment HRAP, it has been shown that this reduction in treatment can be offset by the increased algal biomass production and consequential nutrient assimilation into this biomass (Park and Craggs 2011b).

CO₂ addition has been shown to more than double algal biomass productivity in laboratory studies (Azov et al. 1982) and increase the productivity by ~30% in a New Zealand pilot-scale HRAP during summer (Park and Craggs 2011a). In this latter work, Park and Craggs (2010a;b) demonstrated that maintaining the pH below 8 using CO₂ addition reduced nitrogen loss mainly by reduced ammonia volatilization (approximately 5–9% N loss compared with 24% N loss in control HRAP without CO₂ addition) and this reduction enabled greater nitrogen recovery from the wastewater through assimilation into algal/bacterial biomass.

Many researchers (van Harmelen and Oonk 2006; Benemann 2008b; Chisti 2008; Lardon et al. 2009; Craggs et al. 2011) propose that waste gaseous emissions, such as flue gas from fossil fuel burning power plants, could be used as a CO₂ source to minimize operational costs in full scale applications. Alternatively at wastewater treatment facilities, if biogas is produced onsite from anaerobic digestion and burned for electricity/heat production, then this flue gas could be used as the CO₂ source for the HRAPs.

1.5.2. Control of grazers and parasites

Zooplankton grazers may be controlled through physical (heating, filtration, centrifugation, low DO concentration / high organic loading) and chemical treatments (application of chemicals / invertebrate hormone mimics, increase in pH and free ammonia concentration) (Schluter and Groeneweg 1981). As many zooplankton are able to survive extended periods of low DO (Schluter and Groeneweg 1981), pH adjustment up to a value of 11 is perhaps the most pragmatic method of control for most zooplankton (Benemann et al. 1978). Alternatively, since wastewaters generally contain high ammonia levels (~30 mg/L), the apparent toxic effects of elevated pH on zooplankton may actually be due to the increased free ammonia levels that results at higher pH (Oswald 1988).

Presently there are no general treatments to control fungal infections and, indeed, the presence and inhibitory effects of parasitic fungi on algal growth in the HRAPs have yet to be investigated.

1.5.3. Maintaining desirable algal species in wastewater treatment HRAPs

The ideal attributes of algal species for use in wastewater treatment HRAPs are: (1) high growth rate (high biomass productivity) when fed with wastewater nutrients which are predominantly ammoniacal-N and phosphate-P; (2) tolerance to seasonal and diurnal variation in outdoor growth conditions; (3) form large aggregates thereby enabling simple gravity harvest. High levels of valuable algal cell components (e.g. lipid for biodiesel) could also be desirable for biofuel conversion. Algal species dominance in the HRAP may be determined by many environmental (light and temperature), operational (pH, nutrient composition and concentration, hydraulic retention time) and biological parameters (algal pre-adaptation and seeding, grazers and parasites) (Sheehan et al. 1998; Benemann 2003). However, previous attempts to grow introduced algal species in the HRAP as monocultures for periods greater than 3 months have all failed due to contamination by native algae and/or zooplankton (Sheehan et al. 1998; Benemann 2008b).

Selective biomass recirculation based on algal size (or density) aimed at increasing the concentration of easily-harvestable algae (either float or settle), nutrient limitation and control of hydraulic retention time (culture dilution rate), has shown promise of being at least partially effective for algal species control (Benemann et al. 1977; Weissman and Benemann 1979; Tillett 1988). Benemann et al (1977) demonstrated algal species control in an outdoor wastewater treatment HRAP by selective recycling of microstrainer harvested algal biomass. The slow growing filamentous cyanobacterium, *Spirulina* sp. was maintained in dominant culture over the faster-growing unicellular contaminant, *Chlorella* sp. (Benemann et al. 1977; Weissman and Benemann 1978). However, practical methods of control of similar sized algal species have not yet been given in the literature (Weissman and Benemann 1979; Sheehan et al. 1998; Benemann 2003; Benemann 2008b) and research on the mechanisms of algal dominance is required.

1.5.4. Enhancing algal biomass harvest

Efficient algal biomass harvest (removal) is essential to achieve high quality wastewater treatment and cost-effective biofuel production (Benemann 2003; van Harmelen and Oonk 2006; Benemann 2008b). Algae are very difficult to remove due to their small cell size (<20 μm), similar density to water (1.08–1.13 g/ml) (Lavoie and de la Noue 1987), and strong negative surface charge particularly during exponential growth (Moraine et al. 1979). While various harvesting options have been investigated (Shen et al. 2009; Tampier 2009; Brennan and Owende 2010; Mata et al. 2010), most technologies including chemical and mechanical methods greatly increase the operational costs for algal biomass production and are only economically feasible for use during the production of high value products (Benemann 2008a; Craggs et al. 2011). For example, chemical flocculation can be reliably used to remove small algae (<5 μm) from pond water by forming large (1-5 mm) sized flocs (Sharma et al. 2006). However, the chemical reactions are highly sensitive to pH and the high doses of flocculants required produce large amounts of sludge and may leave a residue in the treated effluent. The high energy requirement of centrifugation makes it only economically viable for secondary thickening of harvested algae (1 – 2% solids) up to 30% solids (Tampier 2009).

Enhancing natural aggregation/bioflocculation of algae to encourage simple gravity settling would appear to be the most promising option to achieve both a high quality treated effluent in terms of total suspended solids removal and economically recovering algal biomass for biofuel use (García et al. 2000a; Benemann 2008a). Many of the algal species that dominate wastewater treatment HRAPs (*Scenedesmus* sp., *Micractinium* sp., *Actinastrum* sp., *Pediastrum* sp., *Dictyosphaerium* sp., *Coelastrum* sp.) often form large colonies (50-200 μm) (Benemann et al. 1978; Benemann et al. 1983; Benemann 1986; Park and Craggs 2010; Craggs et al. 2011). It is possible that enhancing aggregation can be achieved via nitrogen limitation and/or CO₂ addition (Weissman and Goebel 1985; Benemann and Oswald 1996), however more research is needed in this area and the exact mechanisms behind this phenomena have yet to even be investigated.

1.5.5. Recycling a portion of gravity harvested biomass

Algal biomass production in the wastewater treatment HRAPs could be enhanced if the algae were able to utilize more of the available incident light energy. This could be achieved by maintaining the optimal algal concentration (algal culture density) by adjusting the mean cell residence time (MCRT) for the ponds depending on season. Adjusting the MCRT can be accomplished by altering the hydraulic retention time (HRT) by changing either the inflow rate or the pond volume. The former is not practical for full-scale wastewater treatment HRAPs, which should treat all of the wastewater inflow. An alternative and practical strategy to optimize the MCRT (without changing the HRT) is to recycle a portion of gravity harvested algae back to the HRAP (Weissman and Benemann 1979; de la Noüe and Ní Eidhin 1988), which increases the algal concentration. Improvement of biomass productivity through recycling was shown in laboratory studies by Weissman and Benemann (1979) and de la Noüe and Ní Eidhin (1988). However, the influence of recycling on the growth of wastewater algae and particularly how critical environmental parameters (i.e. light and temperature) affect the growth of a dominant algal species in the HRAPs have not been studied previously.

1.6. Summary and research needs

High Rate Algal Pond (HRAP) could be a cost-effective and efficient wastewater treatment option to upgrade conventional oxidation ponds with minimal energy consumption compared with mechanical activated sludge systems. The algal biomass produced and harvested as a by-product from HRAPs may be converted to biofuels (e.g. biodiesel, biogas, bio-crude oil, and bioethanol). The costs of algal biomass production and harvest using wastewater treatment HRAPs are essentially covered by the wastewater treatment plant capital and operation costs, and the system will have considerably lower environmental impact than building and operating HRAP dedicated solely for algal biomass production for biofuels. Therefore, wastewater treatment HRAPs could serve as a ‘testing ground’ to develop large-scale algal biomass production, harvest and biofuel conversion technologies. However, both fundamental and field-scale research are required to promote algal biomass production and harvest (‘harvestable algal biomass production’) from wastewater treatment HRAPs.

Algal biomass production is limited by many parameters including: environmental (light and temperature); operational (pH, CO₂, DO and nutrients), and biological (zooplankton grazing, and pathogens such as fungal and viral infection). Previous research (Park and Craggs, 2011b) has already shown that CO₂ addition to HRAPs will enhance algal biomass production by augmenting daytime CO₂ availability and preventing free ammonia inhibition of algal and bacterial growth. However, further research is required in large-scale wastewater treatment HRAPs using low-cost CO₂ sources (biogas or generator exhaust gas) to minimize operational costs. The influence of zooplankton grazing and parasitism (fungal and viral) on HRAP wastewater treatment and algal biomass production also requires further research, since a greater understanding of how these organisms interact with HRAP algae may lead to the development of effective control methods.

Wastewater treatment and algal biofuel production both require rapid and cost-effective harvest of algal biomass from HRAP effluent, therefore, methods to improve biomass harvest efficiency would be of great benefit. While various harvesting options including chemical and mechanical methods have been extensively investigated, gravity sedimentation is the most common and cost-effective method to harvest algal biomass from HRAP effluent because of the large volumes of wastewater treated and the relatively low value of the algal biomass produced.

Algal species commonly found in the wastewater treatment HRAPs (including *Scenedesmus* sp., *Micractinium* sp., *Actinastrum* sp., *Pediastrum* sp., *Dictyosphaerium* sp. and *Coelastrum* sp.) often form large rapidly settleable colonies (diameter: 50-200 µm), which enable cost effective and simple biomass harvest by gravity sedimentation. Laboratory and small-scale outdoor research reported in the literature indicate that some improvement in algal species control may be achieved when selective biomass recycling (based on either algal size or density) is implemented. However, to date, algal species control of similarly sized, co-occurring algae has not been demonstrated in wastewater treatment HRAP.

‘In-pond’ biomass productivities in wastewater treatment HRAPs that have previously been reported in the literature are summarized in Table 1.3. In the context of biofuel production it is also necessary to consider both ‘harvestable biomass productivity’ and particularly

‘biomass energy yield’. However, limited information could be found in the literature on both of these subjects.

As addressed in Section 1.5.5, algal biomass production in HRAPs could be enhanced if the algae were able to utilize more of the available incident light energy. This could be achieved by maintaining optimal algal concentration (algal culture density) by adjusting the mean cell residence time (MCRT) of the ponds depending on season. Current operation of HRAP involves seasonal variation of HRT and consequently MCRT. A simple and practical strategy to optimize MCRT (without changing the HRT) is to recycle a portion of gravity harvested algae back to the HRAP. However, the influence of recycling on the growth of wastewater algae and particularly the dominance of similarly sized, co-occurring algae have not been previously studied. Furthermore, the influence of recycling on biomass harvestability, productivity, and biomass energy yield has never previously been investigated.

1.7. Ph.D. research scope

Wastewater treatment HRAPs often grow large rapidly settleable colonial algal species, which could be preferentially selected from the pond effluent by gravity settling. Therefore, recycling a portion of the gravity harvested biomass (‘recycling’) back to the HRAP could be a simple and practical method to enhance the dominance of rapidly settleable algae and consequently, harvestable biomass production.

The main hypothesis of this Ph.D. research was:

‘Recycling a portion of gravity harvested biomass back into the HRAP improves harvestable biomass production.’

To confirm this hypothesis, a series of experiments was conducted during the Ph.D. using pilot-scale wastewater treatment HRAPs, outdoor mesocosms and laboratory microcosms.

Firstly, the influence of recycling a portion of gravity harvested biomass on species dominance and biomass harvest efficiency was investigated using two identical pilot-scale HRAPs treating domestic wastewater, which were operated under outside ambient conditions over one year (Chapter 2).

To increase harvestable biomass production (i.e. net biomass production) in HRAPs it is necessary to consider both the ‘biomass productivity’ in the pond and subsequent ‘biomass harvest’ from the pond effluent. Moreover, in the context of biofuel production the energy content of the biomass needs to be measured to determine the actual ‘biomass energy yield’. Thus, the area of study in this Ph.D. research was to determine if recycling a portion of gravity harvested biomass back to the pilot-scale HRAP improves biomass productivity and how this contributes to an improved net biomass energy yield (Chapter 3).

To demonstrate the reproducibility of the pilot-scale research findings such as the increased biomass productivity, settleability and species control with recycling, outdoor mesocosms were operated with and without recycling adjacent to the pilot-scale HRAPs during two different seasons. A third mesocosm study compared recycling of the separated solid and liquid components of the harvested algal biomass with recycling of un-separated biomass to explore potential mechanisms that could account for the increased settleability and productivity (Chapter 4).

Finally, to further investigate the mechanisms behind the observed increase in biomass productivity, a laboratory microcosm study was conducted to determine the exact life-cycle of the dominant alga, *Pediastrum boryanum* s including the timing and net growth rates of each life-cycle stage (Chapter 5).

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GRADUATE RESEARCH SCHOOL

**STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING
PUBLICATIONS**

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Byung-Kwan Park

Name/Title of Principal Supervisor: Professor Andy Shilton

Name of Published Research Output and full reference: Park, J.B.K., Craggs, R.J., Shilton, A.N. (2011). Wastewater treatment high rate algal ponds for biofuel production. Bioresource Technology 102 (1):35-42.

In which Chapter is the Published Work: Chapter 1

• **Describe the contribution that the candidate has made to the Published Work:** While the paper was completed with advice and editing from my supervisors, Professor Andy Shilton and Dr Rupert Craggs, I searched all the literature, conducted critical review and led the paper as the first corresponding author.



Candidate's Signature

22nd August 2013

Date

22nd August 2013

Principal Supervisor's signature

Date

Chapter 2

Recycling algae to improve species control and harvest efficiency from a high rate algal pond

Chapter preface

The literature review in Chapter 1 identified that recycling a portion of gravity harvested biomass ('recycling') could be a simple and practical method to enhance 'harvestable biomass production' in wastewater treatment HRAPs. HRAPs often grow large, rapidly settleable colonial algal species which could be preferentially harvested from the pond effluent by gravity settling. Therefore recycling could increase the dominance of larger colonial species and enhance HRAP biomass harvest efficiency. In order to investigate the effect of recycling on species dominance and biomass harvest efficiency, two identical pilot-scale HRAPs treating domestic wastewater were operated over one year, with or without recycling.

This chapter is based on the following publication:

Park, J.B.K., Craggs, R.J., Shilton, A.N. (2011) Recycling algae to improve species control and harvest efficiency from a high rate algal pond. *Water Research* 45 (20):6637-6649.

Abstract

This chapter investigates the influence of recycling gravity harvested biomass ('recycling') on species dominance and harvest efficiency in wastewater treatment High Rate Algal Ponds (HRAPs). Two identical pilot-scale HRAPs treating domestic wastewater were operated over one year either with (HRAP_r) or without (HRAP_c) recycling. Biomass was harvested by gravity from the HRAP effluent using algal settling cones (ASC) and harvest efficiency was compared to settleability after 1 hour in Imhoff cones five times a week. A microscopic image analysis technique was developed to determine relative algal dominance based on biovolume and was conducted once a month. Recycling maintained the dominance of a rapidly settleable colonial alga, *Pediastrum boryanum* at >90% over one year in HRAP_r compared with only 53% in the control HRAP_c. Increased dominance of *P. boryanum* greatly improved biomass harvest efficiency (annual average of >85% for HRAP_r compared with ~60% for the control). Imhoff cone tests in the laboratory showed that biomass settleability was influenced by both the dominance of *P. boryanum* and the species composition of the remaining algae. Recycling increased the average biovolume of *P. boryanum* colonies by 50-80% by increasing mean cell residence time (by 0.5-3.4 d depending on season). These results indicate that recycling a portion of gravity harvested biomass could be a simple and effective operational method to maintain the dominance of rapidly settling algal species (e.g. *P. boryanum*), and enhance biomass harvest from the HRAP effluent by gravity sedimentation.

2.1. Introduction

Chapter 1 reported that High Rate Algal Ponds (HRAPs) could provide cost-effective and efficient wastewater treatment with minimal energy consumption and have considerable potential to upgrade conventional waste stabilization ponds. Furthermore, the algal biomass produced and harvested from these wastewater treatment systems could be converted through various pathways to biofuels, for example anaerobic digestion to biogas, transesterification of lipids to biodiesel, fermentation of carbohydrate to bioethanol and high temperature conversion to bio-crude oil (Sukias and Craggs, 2010; Vasudevan and Fu, 2010; Craggs et al., 2011). Wastewater treatment and algal biofuel production both require rapid and cost-effective harvest of algal biomass from HRAP effluent, therefore, methods to improve algal harvest efficiency would be of great benefit (Benemann, 2003; Chen and Yeh, 2005; van

Harmelen and Oonk, 2006; Brennan and Owende, 2010). However, algal cells are very costly to remove due to their small size ($<20\ \mu\text{m}$), similar density to water (1.08–1.13 g/ml) (Lavoie and de la Noue, 1986) and strong negative surface charge, particularly during exponential growth (Moraine et al., 1979; Chen and Yeh, 2005).

Gravity sedimentation is the most common and cost-effective method of algal biomass removal from wastewater treatment HRAP effluent because of the large volumes of wastewater treated and the low value of the algal biomass generated (Nurdogan and Oswald, 1996). However, the algal settling ponds which are typically used have relatively long retention times (1-2 days) and only remove 50-80% of the biomass (Nurdogan and Oswald, 1996; Brennan and Owende, 2010; Park and Craggs, 2010; Park et al., 2011). While various harvesting options including chemical and mechanical methods have been extensively investigated (Shen et al., 2009; Tampier, 2009; Brennan and Owende, 2010; Mata et al., 2010), most technologies greatly increase operational costs for algal production (Benemann, 2008a; Craggs et al., 2011). For example, chemical flocculation can be reliably used to remove small algae ($<5\ \mu\text{m}$) from pond effluent by forming large (1-5 mm) sized algal flocs (Sharma et al., 2006). However, the process is highly sensitive to pH and the high flocculent dose required produces large amounts of sludge. Mechanical centrifugation could be used for the removal of algal biomass, but the high energy requirement makes it only economically viable for secondary thickening of primary harvested algae (1-2% solids) up to 20-30% solids (Benemann, 2008b; Tampier, 2009).

Algal species commonly found in wastewater treatment HRAPs include: *Scenedesmus* sp., *Micractinium* sp., *Actinastrum* sp., *Pediastrum* sp., *Dictyosphaerium* sp. and *Coelastrum* sp.). These algae often form large settleable colonies (diameter: 50-200 μm), which enable cost effective and simple biomass removal (harvest) by gravity sedimentation (Lavoie and de la Noue 1986; García et al. 2000a; Benemann 2008b; Craggs et al. 2011; Park et al. 2011c). Park and Craggs (2010) reported that CO₂ addition to wastewater treatment HRAP promoted the formation of large bioflocs of algal colonies associated with wastewater bacteria (diameter: $>500\ \mu\text{m}$), which settle rapidly in simple gravity harvesters with a retention time of twelve hours or less. Therefore, operating wastewater treatment HRAPs to promote both the growth of particular settleable algal species (i.e. colonial species) and the aggregation of

algal-bacterial biomass could greatly enhance the efficiency of algal harvest from the effluent.

Recycling harvested algae based their size (or density) has previously been shown to increase the dominance of rapidly settleable algae in small-scale laboratory cultures (Benemann et al. 1977; Weissman and Benemann 1979; Tillett 1988). Another option to select for beneficial algal species is by adjustment of the HRAP hydraulic retention time to promote species based on their specific growth rate (Weissman and Benemann 1979). While there are several examples of successful species control (e.g. *Spirulina* sp. and *Dunaliella* sp.) in outdoor commercial algal production HRAPs, these algae grow under extreme conditions (e.g. high pH and salinity) that greatly reduce the potential for contamination (Weissman and Benemann, 1979; Sheehan et al., 1998; Benemann, 2003; Benemann, 2008b). The feasibility of algal species control in wastewater treatment HRAPs and the mechanisms involved are still poorly understood.

This chapter investigates the influence of recycling gravity harvested biomass ('recycling') on species dominance and harvest efficiency in a pilot-scale wastewater treatment HRAP over one year. Relative algal dominance was determined from algal biovolume using microscopic image analysis and correlated with gravity harvest and settling efficiency.

2.2. Materials and methods

2.2.1. Experimental pilot-scale HRAP systems

Experiments were conducted using two identical pilot-scale single-loop raceway HRAPs treating domestic wastewater at the Ruakura Research Centre, Hamilton, New Zealand (37°47'S, 175°19'E). The HRAPs were previously a part of wastewater treatment Advanced Pond System (APS) comprising an anaerobic digester, HRAP, two algal settling ponds and four maturation ponds in series. Each HRAP had a surface area of 32 m², a depth of 0.3 m and a total volume of 8 m³ with semi-circular end-walls; lined with high-density polyethylene (HDPE) plastic; and with a dividing wall (HDPE) separating the two raceway channels. A free standing, 1 m wide, galvanised steel paddlewheel circulated the pond water around the

HRAP raceway to give a mean surface velocity of 0.15 m/s. A schematic diagram of a pilot-scale HRAP is shown in Figure 2.1.

2.2.2. HRAP operation

The HRAPs were fed with primary settled sewage and were operated at different HRT depending on season to account for changes in environmental parameters such as light and temperature and their influence on wastewater treatment and algal growth (Chapter 1; Park and Craggs 2010). Hydraulic retention times (HRT) of 8 and 4 days in winter and summer were maintained respectively with total inflow of 1 and 2 m³/d to promote algal production. The influent sewage was diluted with 1:1 with tap water to simulate recycling of treated effluent after complete biomass and nutrient removal to reduce influent concentrations of organic and nutrients into the ponds. During the NZ spring (Sept 7–Nov 22, 2009) and autumn (Mar 17–May 25, 2010), the HRT of the HRAPs was maintained at 6 days with a total inflow of 1.3 m³/d (primary sewage diluted 1:1 with tap water). Separate 1 m³ water tanks were used to temporarily store the primary sewage and to store and dechlorinate tap water. Required volumes of the primary sewage and tap water were pumped into the HRAPs each hour using submersible pumps controlled by an electronic timer which was recalibrated at least twice a month.

The maximum pH of the HRAPs was maintained below 8 through pH controlled CO₂ addition to avoid free ammonia inhibition and to augment daytime carbon availability. The CO₂ addition system consisted of pure CO₂ (compressed in a gas cylinder), a gas regulator and flow meter (0-12 L/min range), a solenoid valve, and two gas diffusers placed on the pond bottom in turbulent zones (one just before the paddlewheel and the other before the downstream pond corner). Pond water pH was measured every five seconds using a pH probe and when the pH exceeded the pH 8 set point, the controller opened the solenoid valve and added CO₂ into the ponds (2 L/min) through the gas diffusers. When the pond water pH was reduced to 7.8, the controller closed the solenoid valve halting CO₂ addition. The pH probes were calibrated 1-2 times a week with standard pH solutions (pH 7 and 10). More details were previously reported in Park and Craggs (2010).

Pediastrum sp. was inoculated into both pilot-scale HRAPs using 500 L of pond water taken from three wastewater treatment mini-HRAPs (volume: 0.8 m³) that were operated next to the pilot-scale HRAPs. *Pediastrum* sp. naturally established in the mini-HRAPs in January 2009 and was dominant on March 13th 2009 when the inoculum was added. The pilot-scale HRAPs were completely drained and any solids which had accumulated on the pond bottom were removed. Once the inoculum had been added, each pilot-scale HRAP was then filled with primary settled sewage over 8 days (flow rate: 1 m³/d). Both pilot-scale HRAPs were operated without recycling until July 1st 2009.

2.2.3. Biomass removal and recycling gravity harvested biomass

Effluent from the HRAPs was taken from the pond bottom (upstream of the paddlewheel) and flowed by gravity into the first algal settling cone (ASC1, 250 L) at mid-depth. The effluent from the top of ASC1 then flowed into the second ASC (ASC2, 250 L) at mid-depth. The hydraulic retention time (HRT) of each ASC varied seasonally (3 h in summer and 6 h in winter) depending on the HRT in the HRAPs (4 d and 8 d respectively).

Algal biomass collected at the bottom of each ASC and was removed each day using a peristaltic pump (Masterflex, Cole-Palmer, HV-07523-60). One litre of the volume of the rapidly settling algal biomass that collected every 24 hours in the ASC1 following HRAP_r (shown in Figure 2.1) was added back to the HRAP_r each day. The algal recycling rate for HRAP_r was determined by multiplying the volume of biomass recycled per day (L/d) with the harvested biomass solids concentration (VSS g/L) and then dividing by the total mass of algal biomass harvested from the pilot-scale HRAP on that day (Table 2.1). The second HRAP was operated without recycling as a control (HRAP_c) with all other operational parameters the same as HRAP_r.

The operational parameters of the two HRAPs, one operated with recycling (HRAP_r) and the other without (HRAP_c) are also summarized in Table 2.1. Parameters include the design and weather compensated (actual) daily flow rate and hydraulic retention time (HRT), mean cell residence time (MCRT) and influent nutrient (NH₄⁺-N and PO₄³⁻-P) concentrations.

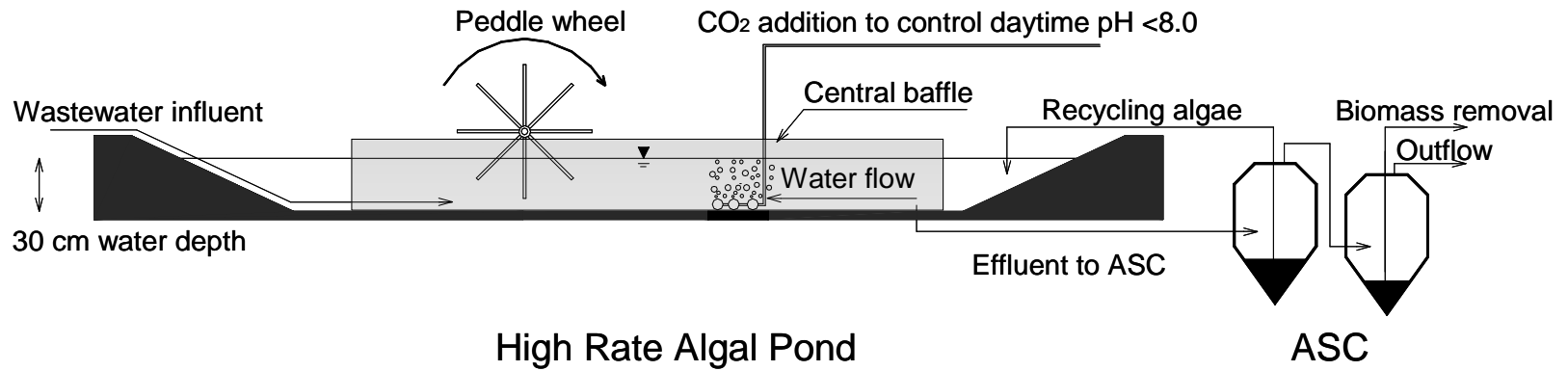


Figure 2.1: Schematic diagram of the pilot-scale high rate algal pond (HRAP) followed by two algal settling cones (ASCs) in series.

2.2.4. Measurement of algal harvest efficiency and Imhoff cone settling efficiency

Samples of HRAP water and ASC effluents were taken at least five times a week for the measurement of volatile suspended solids (VSS) according to Standard Methods (APHA 2008). Large inorganic particles and invertebrate grazers (e.g. *Moina* sp. or *Daphnia* sp.) were strained (1 mm mesh) from the pond water samples before analysis of VSS. Biomass harvest efficiency was determined as the percentage of the biomass in the HRAP water (measured as VSS) that was removed in each of the ASCs.

Biomass settling efficiency was measured five times a week using 1 litre Imhoff cones after 10, 30, and 60 minutes and 24 hours under laboratory conditions. Water samples (50 ml) were taken using a syringe from the mid depth of the Imhoff cone (~450 ml depth) and were used to measure VSS, which were then compared with the initial VSS to determine biomass settling efficiency.

Table 2.1: Operational parameters of wastewater treatment pilot-scale HRAPs with and without recycling in terms of design/ weather compensated inflow rate, influent nutrient concentrations (NH₄⁺-N and PO₄⁻-P), hydraulic retention time (HRT), mean cell residence time (MCRT) of algae and algal recycling rate to HRAP_r.

Parameters		Winter	Spring	Summer	Autumn	Winter
Days		67 days	76 days	113 days	70 days	34 days
Experimental period		(July 1-Sept 6, 09)	(Sept 7-Nov 22, 09)	(Nov 23, 09-Mar 16, 10)	(Mar 17-May 25, 10)	(May 26-Jun 30, 10)
Inflow to HRAPs (m ³ /d)	Designed	1.0	1.3	2.0	1.3	1.0
	Actual ⁽¹⁾	1.1±0.2	1.4±0.2	2.0±0.2	1.3±0.2	1.2±0.3
Influent (mg/L)	NH ₄ ⁺ -N	30.3±4.0	22.5±7.5	20.6±9.2	39.5±6.5	34.3±7.3
	PO ₄ ⁻ -P	5.5±1.1	4.0±1.0	4.0±0.7	5.9±1.0	4.8±1.2
HRAP HRT (d)	Designed	8.0	6.0	4.0	6.0	8.0
	Actual ⁽¹⁾	7.7±1.2	5.9±0.8	4.1±0.4	6.1±0.5	7.0±1.5
MCRT (d) ⁽²⁾	HRAP _r	8.9±1.4	6.5±0.9	4.6±0.4	7.2±0.6	10.4±2.2
	HRAP _c	7.7±1.2	5.9±0.8	4.1±0.4	6.1±0.5	7.0±1.5
Algal recycling rate to HRAP _r (g recycled/ kg production/ d) ⁽³⁾		105±32	66±25	76±22	170±89	212±8
ASC1 HRT (d)	Designed	6	4.5	3	4.5	6
	Actual	5.8±0.8	4.4±0.6	3.1±0.3	4.6±0.4	5.2±1.1
Harvested biomass conc. (VSS, g/L)		35±5.1	38±6.2	33±5.8	32±5.1	30±9.0
ASC2 HRT (h) ⁽⁴⁾	Designed	12	9	6	9	12
	Actual	11.6±1.7	8.9±1.1	6.2±0.5	9.2±0.8	10.5±2.2

Note:

- (1) Weather compensated (daily precipitation and evaporation) daily inflow and hydraulic retention time (HRT)
- (2) Calculated using Equation 1 below
- (3) Volume of algal biomass recycled (L/d) × harvested algal biomass concentration (g/L) ÷ the total mass of algae biomass harvested (kg)
- (4) Combined HRT of ASC1 and ASC2

Equation 1
$$MCRT = \frac{V_r X_r}{Q_c X_1 - Q_{re} X_h}$$

Where;

MCRT= Algal mean cell residence time (d)

V_r: HRAP volume (m³)

X_r: HRAP algal biomass concentration (g/m³)

Q_c: Compensated HRAP effluent flow rate (m³/d)

Q_{re}: Algal biomass recycled per day (L/d)

X_h: Harvested biomass concentration (g/L)

2.2.5. Microscopic image analysis

2.2.5.1. Processing of samples and equipment used

A Utermöhl chamber (25 mm diameter) was used to count pond water algal populations and for microscopic image analysis. A 1 ml sample of thoroughly mixed pond water was pipetted into the Utermöhl chamber, evenly distributed to cover the surface of the chamber and then settled for 30–60 minutes (depending on algal settleability). The Utermöhl chamber was then examined using an inverted light microscope with 160× magnification (Leica model) equipped with a Leica microscopic camera (DFS 420c). If the cell/colony density in the HRAP water sample was too high (>300 mg/L) to allow adjacent algal cells or colonies to be distinguished, a 1 ml sample of diluted HRAP water was used.

2.2.5.2. Taking microscopic images

Safi (2009) found that taking microscopic images of the whole chamber is neither necessary nor feasible, particularly when algal cell/colony numbers are high. If the algae have settled evenly across the base of the settling chamber (checked by scanning the whole chamber at low magnification), microscopic images can be taken of randomly selected fields of view (FOV). If the algae have not settled evenly, images may be taken of equally spaced FOV along transects that run perpendicular to any observed settling gradient (Safi 2009). The number of microscopic images required for the measurement of cell/colony dimensions and counts varied with algal population density (concentration) but were typically ~10. A stage micrometer was placed on the inverted light microscope and a picture was also taken on the same day to calibrate the scale setting of microscopic image analysis software (Leica Application Suite, LAS version 3.1.0).

2.2.5.3. Identification of algal species

The most abundant algal species in the microscopic images of the HRAP water were identified using an identification guide (Brook 2002). Dominant invertebrate grazers (e.g. *Moina*) were also identified and counted; and the occurrence of fungal hyphae in the algal/bacterial flocs was confirmed using the Calcofluor White protocol suggested by Kagami et al (2007) and Rasconi et al (2009).

2.2.5.4 Measurement of algal cell/colony dimensions and algal counts

Only viable algal cells/colonies were included (clear or collapsed cells and cell fragments were ignored). Due to the variable and sometimes high number of cells in the colonies of the algae that often dominate in the HRAPs (e.g. *Pediastrum* sp. can have 8, 16, 32, or 64 cells/colony and *Scenedesmus* sp. can have 2, 4, or 8 cells/colony), it was not practical to count every cell in a colony. Therefore, the number of colonies of each species was counted and the dimensions (length and width) of each colony were measured to determine the algal biovolume using microscopic image analysis software.

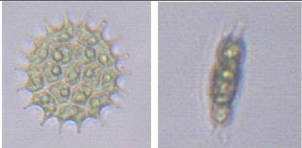




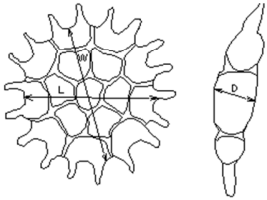
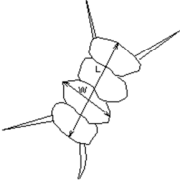
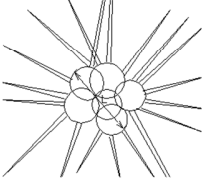
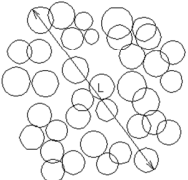
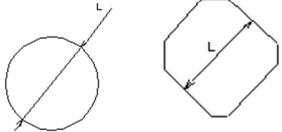
2.2.5.5. Total cell/colony number counts and measurement uncertainty

Lund et al. (1958) recommended that for a FOV count, a minimum of 100 cells/colonies should be enumerated to ensure that the count is representative of the sample ($\pm 20\%$ accuracy with 95% confidence limits). In this study, 200-500 algal cells or colonies were counted from ~10 microscopic images depending on the algal biomass concentration. Therefore counts for each pond water sample had an accuracy of $\pm 10\%$ with 95% confidence limits (Lund et al. 1958; Rott et al. 2007).

2.2.5.6. Calculation of algal biovolume

Algal biovolume is a more accurate measure of relative algal dominance (%) than cell counts because not all algal cells are the same size (Lyakh 2007; Rott et al. 2007; Vadrucci et al. 2007). Hillebrand et al (1999) and Vadrucci et al (2007) developed geometrical equations to calculate the biovolume of algal species of different shapes from microscopically measured linear dimensions. The equations for the five most dominant algae in the HRAPs (*Pediastrum* sp., *Scenedesmus* sp., *Micractinium* sp., *Dictyosphaerium* sp. and unicellular algae (including *Chlorella* sp., flagellates and *Thalassiosira* sp.)) are shown in Table 2.2 along with the number of cells per colony. Microscopic image analysis was conducted each month and the data used to calculate algal biovolume, which was in turn used to determine algal dominance in the HRAPs.

Table 2.2: Calculations of algal cell/colony biovolume using geometric measurement.

Dominant Algae	<i>Pediastrum</i> sp.	<i>Scenedesmus</i> sp.	<i>Micractinium</i> sp.	<i>Dictyosphaerium</i> sp.	Unicellular algae
Photo					
Shape of the cell/colony	Disc/star-shaped, flat and single-layered	Flat, straight or slightly curved	Cuboidal, Tetrahedral or Polyhedral (spherical cells)	Hollow, spherical	Spherical (<i>Chlorella</i> sp.), Cube (<i>Thalassiosira</i> sp.)
Number of cells/colony	4/8/16/32/64-celled	2/4/8-celled	>4 celled distinguishing into single cells	>4-celled distinguishing into single cells	Single cell
					
Calculation of single cell biovolume	$V = \frac{\pi}{4} \cdot L \cdot W \cdot D$ (Elliptic disc)	$V = \frac{\pi}{6} \cdot \left(\frac{L^2}{4}\right) \cdot W \times 4$ (Prolate spheroid)	$V = \frac{\pi}{6} \cdot L^3$ (Colonial sphere)	$V = \frac{\pi}{6} \cdot L^3$ (Colonial sphere)	$V = \frac{\pi}{6} \cdot d^3$ (Single sphere) $V = d^3$ (Cube)
	L: length (μm) W: width (μm) D: depth (μm)	L: length (μm) W: width (μm)	L: length (μm)	L: length (μm)	L: length (μm)

2.2.6. Water quality monitoring

Weekly samples of HRAP influent (primary settled sewage) and effluent were taken and then analysed using standard methods (APHA, 2008) for the following parameters: ammoniacal-N ($\text{NH}_4^+\text{-N}$), dissolved reactive phosphorus (DRP). During the period when a unicellular diatom (*Thalassiosira* sp.) occurred in the ponds (6th to 30th March 2010) reactive Silica (as SiO_2) was also measured (APHA, 2008) at weekly intervals.

2.3. Results and discussion

2.3.1. Algal species and algal biovolume

The types of algae found in the HRAPs over the one year experimental period are summarized in Table 2.3. These included 13 genera of green algae which are commonly found in eutrophic waters and four colonial algae including: two species of *Pediastrum* sp., *Scenedesmus* sp., *Micractinium pusillum*, and *Dictyosphaerium* sp., which typically dominate wastewater treatment HRAPs around the world (Benemann et al. 1978; Benemann et al. 1983; Benemann 1986; García et al. 2000a; Park and Craggs 2010; Craggs et al. 2011). The two species of *Pediastrum* (*P. boryanum* and *P. duplex*) were easily distinguished since *P. duplex* has intercellular spaces and *P. boryanum* does not.

The cell or colony biovolume of the five most abundant algae in the HRAPs (4 colonial species and unicellular algae (including the diatom, *Thalassiosira* sp.) were calculated by image analysis of microscopic photographs of pond water samples. A positive correlation ($r=0.82$) was found between algal biovolume and biomass concentration (Figure 2.2), indicating that biovolume is a particularly useful measure of algal biomass for wastewater treatment HRAPs. Because the HRAPs selected for colonial algae (such as *Micractinium* sp., *Dictyosphaerium* sp., *Scenedesmus* sp., and *Pediastrum* sp.) that have different shapes and varying numbers of cells. For example, the biovolume of a *Pediastrum* sp. colony can vary with both the number of cells (8, 16, 32 or 64 cells) and the size of the cells within the colony depending on life-cycle stage and culture conditions.

Table 2.3: Wastewater algae found in the wastewater treatment HRAPs over one year experimental period.

Phylum	Dominance	HRAP _r		HRAP _c	
		Genus	Species	Genus	Species
Green algae (Chlorophyta)	Dominant algae	<i>Pediastrum</i>	<i>boryanum</i>	<i>Pediastrum</i>	<i>boryanum</i>
		<i>Pediastrum</i>	<i>duplex</i>	<i>Pediastrum</i>	<i>duplex</i>
		<i>Scenedesmus</i>	sp.	<i>Scenedesmus</i>	sp.
		<i>Micractinium</i>	<i>pusillum</i>	<i>Micractinium</i>	<i>pusillum</i>
		<i>Dictyosphaerium</i>	sp.	<i>Dictyosphaeriu</i>	sp.
	Occasionally found algae	<i>Gonium</i>	sp.	<i>Gonium</i>	sp.
		<i>Ankistrodesmus</i>	<i>falcatus</i>	<i>Ankistrodesmu</i>	<i>falcatus</i>
		<i>Monoraphidium</i>	sp.	<i>Monoraphidium</i>	sp.
		<i>Pandorina</i>	sp.	<i>Dictyosphaeriu</i>	sp.
		<i>Radiococcus</i>	sp.	<i>Kirchneriella</i>	sp.
		<i>Kirchneriella</i>	sp.	<i>Actinastrum</i>	<i>hantzschii</i>
		<i>Actinastrum</i>	<i>hantzschii</i>	<i>Coelastrum</i>	sp.
		<i>Coelastrum</i>	sp.	<i>Chlamydomon</i>	sp.
		Diatoms (Bacillariophyceae)	-	<i>Thalassiosira</i>	sp.

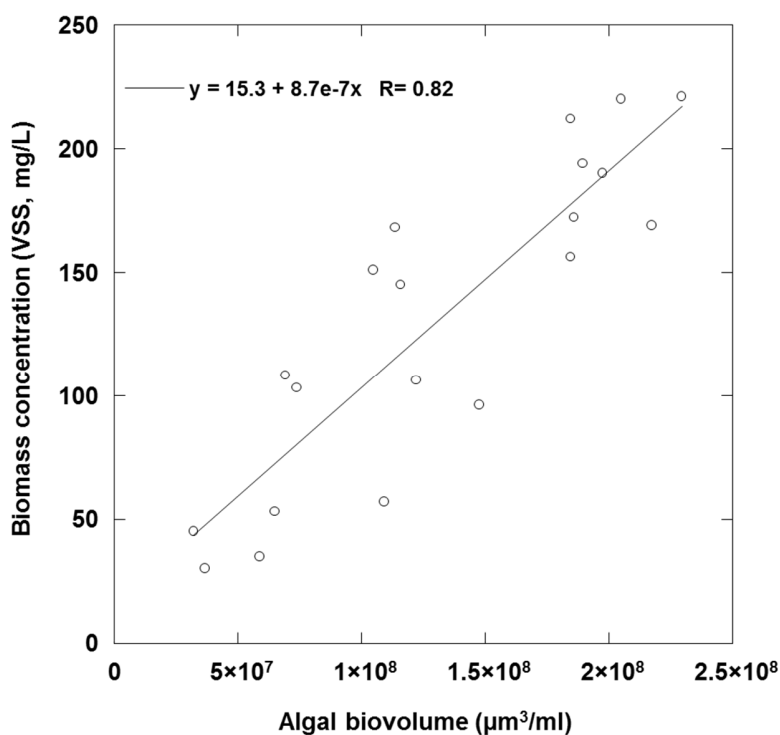


Figure 2.2: The relationship between the algal biovolume and biomass concentration (as VSS) in the HRAPs (R=0.82).

2.3.2. *Influence of recycling on algal dominance*

The relative dominance of the five most abundant algae in the HRAPs was determined at monthly intervals based on biovolume (Figure 2.3). The colonial algae *Pediastrum* sp., *Micractinium* sp., and *Dictyosphaerium* sp. were each dominant in the control pond (HRAP_c) for periods of two months or more (Figure 2.3a). Changes in dominance between these algae quickly occurred within a few weeks. For example, *Pediastrum* sp. was replaced by *Micractinium* sp. in October 2009, which was replaced by *Pediastrum* sp. in December 2009, which was replaced by the unicellular diatom *Thalassiosira* sp. in March 2010, which was then replaced by *Dictyosphaerium* sp. in April 2010. These shifts in algal dominance were probably caused by changes in environmental conditions (notably seasonal variation of solar radiation and pond water temperature which are known to affect species selection, succession and co-existence) and HRAP operational parameters such as hydraulic retention time (Benemann et al. 1977; Harris 1978; Sommers 1984; Oswald 1988). García et al (2000) also reported similar changes in relation to environmental parameters by the dominant algae (including *Dictyosphaerium* sp., *Chlorella* sp., *Micractinium* sp., and *Scenedesmus* sp.) of a small-scale (0.5 m³) wastewater treatment HRAP without CO₂ addition in Spain over a one year experimental period.

As can be seen in Figure 2.3b, recycling increased the dominance of *Pediastrum* sp. (76-99% dominance) in HRAP_r compared to the control HRAP_c (0-98% dominance) during the one year experimental period. Other colonial algae including *Micractinium* sp., *Scenedesmus* sp., *Dictyosphaerium* sp. and the unicellular diatom *Thalassiosira* sp. were temporarily present and co-existed with *Pediastrum* sp. in HRAP_r but at much lower populations than in HRAP_c. Maintaining dominance of a single algal species (*Pediastrum* sp., >90% dominance) over similarly sized algal species in wastewater treatment HRAP for over one year has not been previously reported in the literature. This suggests that recycling of gravity harvested biomass could provide a simple method to promote the dominance of rapidly settleable colonial algal species such as *Pediastrum* sp. in the HRAPs.

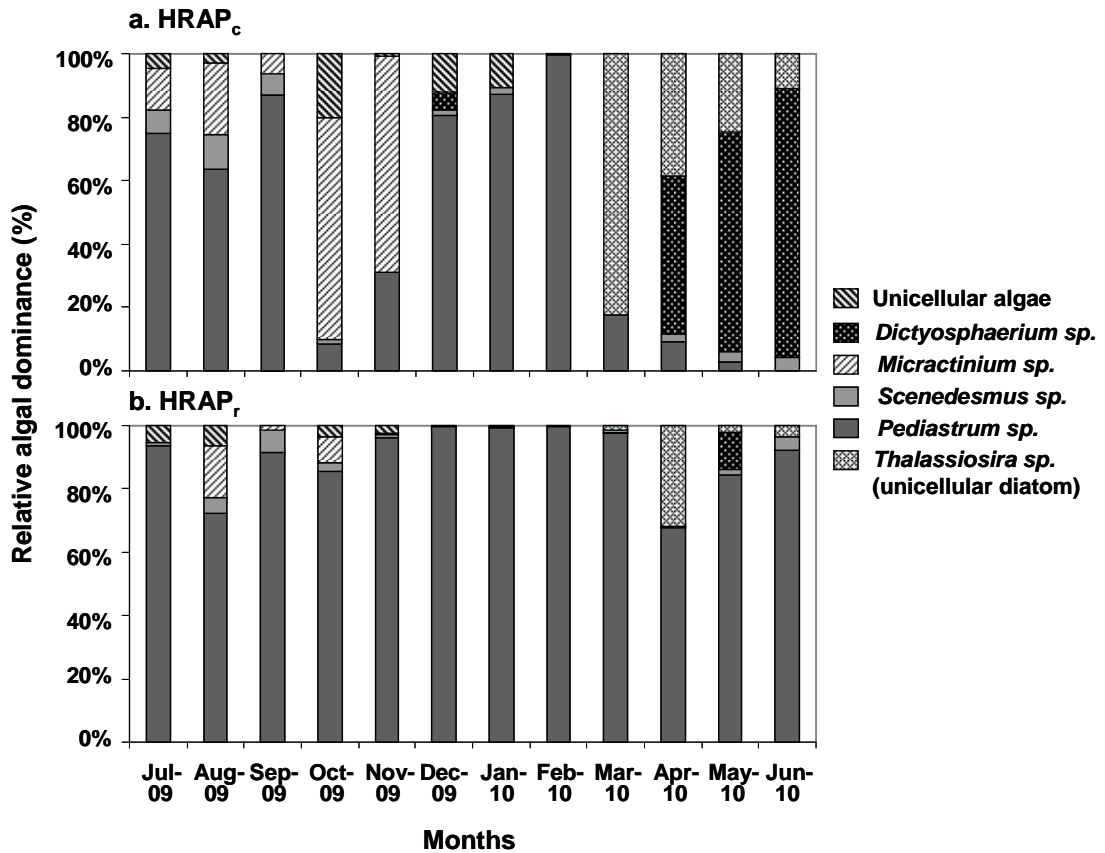


Figure 2.3: Algal dominance based on calculated biovolume in the pilot-scale wastewater treatment HRAPs over a one year experimental period (from July 2009 to June 2010), a: control HRAP_c without recycling and; b: HRAP_r with recycling.

2.3.3. Settling characteristics of the dominant algal species

Recycling preferentially selected for algae that settle rapidly. Since all algae in the HRAP effluent are exposed to the same settling conditions (e.g. water viscosity (η) and temperature) in the algae settling cones, differences in settleability between species, therefore, depend on their physiological state, cell or colony size, and morphology (Smith 1982; Alldredge and Gotschalk 1989; Chen and Yeh 2005; Choi et al. 2006). While the size of algal cells or colonies affects the settling velocity according to the Stoke's law (see equation in Table 2.4), the morphology (i.e. size and irregularity) of the algal cell or colony also influences settling velocity according to the frictional drag force exerted as it falls through the fluid under the pull of gravity (Smith 1982; Padisák et al. 2003; Chen and Yeh 2005; Choi et al. 2006).

Therefore, algae that have a lower surface area to volume ratio (thus therefore reduced drag) settle faster than algae that have the same density but a larger surface area (Padisák et al. 2003; Choi et al. 2006).

In order to investigate the influence of algal cell or colony size alone on the settling velocity (assuming for simplification that form resistance (Φ) of different algae are the same), the average biovolume, the calculated nominal radius (r_s) and the approximate theoretical settling velocity (V_{theo}) of the five dominant algae were determined using the one year experimental data and are summarized in Table 2.4. The nominal radius of the *Pediastrum* sp. colonies (the most dominant algae in both HRAPs) ranged from 5.1 to 13.5 μm and depended on colony age and the number of cells per colony (8, 16, 32, or 64). The nominal radius of *Pediastrum* sp. colonies were 1.4-3.8 times larger than that of the unicellular algae (3.6 μm) and nearly 2 times larger than those of the other colonial algal species present (4.8-5.3 μm for 2-4 celled *Scenedesmus* sp., 5.5 μm for *Micractinium* sp., and 7.9 μm for *Dictyosphaerium* sp.). The theoretical settling velocity, V_{theo} , of each alga was calculated from its biovolume (Table 2.4). The larger biovolume and nominal radius of *Pediastrum* sp. colonies indicates that they could have better settling characteristics (calculated theoretical settling velocity, V_{theo} was 2-14 times greater than that of any other algae present in the HRAPs). Therefore, when the biomass was dominated by *Pediastrum* sp., it was easily harvested by gravity sedimentation.

Table 2.4: Average algal cell or colony biovolume of the four dominant algal species / type over the one year experimental period, the number of cells, relative abundance with genera (%), nominal radius (ϕ , μm) of a sphere of equivalent biovolume to the algal cell or colony, and the approximate relative settling velocity (r^2).

Algal species	Cell numbers within a colony	Relative abundance within genera (%)	Total cell counts (counts/ml)	Average biovolume of cell/colony ($\mu\text{m}^3 \pm \text{s.d.}$)	Total biovolume ($\mu\text{m}^3 \pm \text{s.d.}$)	Nominal radius (r_s , $\mu\text{m} \pm \text{s.d.}$)	Relative radius (r_r) ⁽¹⁾	V_{theo} ⁽²⁾
<i>Pediastrum</i> sp.	8-celled	7.2	9.59E+06	561±308	9.59E+06	5.1±4.2	1.4	2.0
	16-celled	40.2	3.37E+04	1834±670	5.33E+07	7.6±5.4	2.1	4.5
	32-celled	43.2	1.78E+04	4115±2009	5.73E+07	9.9±7.8	2.8	7.7
	64-celled	9.3	1.10E+03	10219±3217	1.23E+07	13.5±9.2	3.8	14.1
<i>Scenedesmus</i> sp.	2-celled	20.4	8.20E+02	458±265	4.91E+05	4.8±4.0	1.3	1.8
	4-celled	79.6	2.87E+03	617±292	1.92E+06	5.3±4.1	1.5	2.2
<i>Micractinium</i> sp.	-	-	7.96E+03	679±446	2.13E+06	5.5±4.7	1.5	2.3
<i>Dictyosphaerium</i> sp.	-	-	5.45E+03	2073±118	1.20E+06	7.9±3.0	2.2	4.9
Unicellular algae	-	-	4.04E+04	193±82	7.53E+06	3.6±2.7	1.0	1.0

Note;

(1) Radius relative to a unicellular algae

(2) V_{theo} (theoretical relative settling velocity) is proportional to r_r^2 according to Stoke's law ($V_s = \frac{2 r_s^2 g (\rho_p - \rho_f)}{9 \eta \Phi_r}$), assuming all other parameters are same

Where, g Gravitational acceleration, ($\rho_p - \rho_f$) excess density between particles and fluid, η Viscosity of the medium

r_s nominal radius of the sphere of equivalent biovolume to the algae, Φ_r Form Resistance (the effects of algal shape upon settling)

2.3.4. Biomass harvest depending on dominant algal species

The biomass concentrations in the HRAP, ASC1 and ASC2 effluents and the biomass harvest efficiency after ASC2 are shown in Figure 2.4. Biomass concentrations in the HRAP effluent varied from ~30 to 340 g VSS /m³ during the one year experimental period depending on seasonal algal growth and invertebrate grazing. However, biomass concentrations of the ASC effluents and harvest efficiency were highly dependent on the algae that were dominant in the HRAP at that time. Increased dominance (annual average of >90%, Figure 2.3) of *Pediastrum* sp. in HRAP_r with recycling greatly improved biomass harvest efficiency by gravity sedimentation (>75% and >85% harvest efficiency after ASC1 and ASC2 respectively). Low final effluent biomass concentrations (<20 g/m³) were consistently achieved for HRAP_r during the one year experimental period (Figure 2.4b). In contrast, the control pond (HRAP_c) had inconsistent and rather poor biomass harvest efficiency (48% and 64% harvest efficiency after ASC1 and 2 respectively), mainly because less settleable algae were dominant in this pond (Figure 2.3a). For example, increased dominance of *Pediastrum* sp. in the control HRAP_c (80-98% dominance over 3 months from December to February 2010) greatly enhanced the biomass harvest efficiency to 90% (which was nearly comparable to that in HRAP_r with >90% *Pediastrum* dominance). However, by June 2010 the dominance of *Pediastrum* sp. in HRAP_c had declined to less than 20%, as *Pediastrum* sp. was replaced by poorly-settleable algae (*Thalassiosira* sp. (~85% dominance) by March 2010, then *Dictyosphaerium* sp. (dominance increased from 40% to 85% by June 2010). These results demonstrate that maintaining rapidly settleable algae such as *Pediastrum* sp. as the dominant species could provide a way of promoting efficient biomass harvest by simple gravity sedimentation.

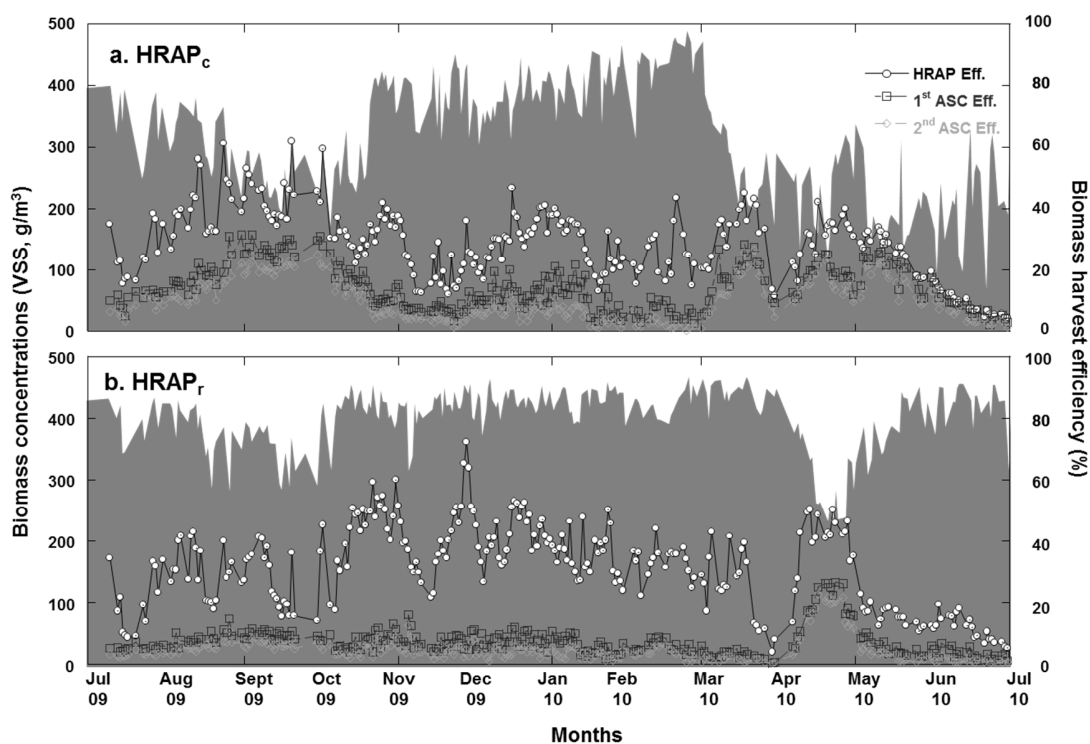


Figure 2.4: Biomass concentrations in the effluents from two pilot-scale HRAPs and primary and secondary algal settling cones (ASCs); and calculated total removal efficiency measured over one year: a.: HRAP_c operated without recycling; b.: HRAP_r operated with recycling.

During a four week period from 6th to 30th March 2010 a unicellular diatom (*Thalassiosira* sp.) temporarily established in both HRAPs (>82% dominance in HRAP_c and 32% dominance in HRAP_r; Figure 2.3). This was an interesting observation, since it is very unusual for diatoms to grow at high concentration in wastewater treatment HRAPs and has not been previously observed at our wastewater treatment HRAP research facility, or reported in the literature (Benemann et al., 1978; Benemann et al., 1983; Benemann, 1986; García et al., 2000; Craggs et al., 2010; Park and Craggs, 2010). The occurrence of *Thalassiosira* sp. may be explained by a temporary increase in the dissolved silica concentration (SiO₂) of the influent wastewater, since silica is an essential element for diatom growth and the typical low concentration in wastewater limits the growth of diatoms. Analysis of the influent wastewater to the HRAPs for SiO₂ during the period of *Thalassiosira* sp. occurrence showed a high dissolved silica level (~30 mg/L as SiO₂), which declined to less than 5 mg/L by May 2010

when the diatom was no longer present in the HRAPs. *Thalassiosira* sp. has very low settling efficiency and consequently its occurrence greatly reduced harvest efficiency of the ASCs for both HRAPs (HRAP_c: 48% harvest efficiency; HRAP_r: 64% harvest efficiency).

Micractinium sp. and *Dictyosphaerium* sp. were dominant in the control HRAP_c for periods of two months (*Micractinium* sp.: ~70% dominance from October to November 2009; *Dictyosphaerium* sp.: 70-85% dominance from May to June 2010; Figure 2.3a). These algae can form colonies of typically >100 spherical single cells and have a nominal radius of 5.5-7.9 µm. When these two species were dominant in the ponds biomass harvest efficiency was particularly poor (63-77% for *Micractinium* sp. and only 16-38% for *Dictyosphaerium* sp., Figure 2.4a). In contrast, during the period from December to February 2010 when *Pediastrum* sp. was dominant very high biomass harvest efficiency (>85% after the ASC2 with 6 h HRT) was achieved. The lower settling efficiency of *Micractinium* sp. and *Dictyosphaerium* sp. compared with *Pediastrum* sp. might be due to lower density and higher drag resulting from the dispersed structure of the colonies (i.e. large spaces between groups of cells) compared with *Pediastrum* sp. colonies in which the cells are tightly packed together.

Over the one year experimental period, nutrient removal efficiency (86-98% NH₄⁺-N and 50-75% PO₄⁻-P) of HRAP_r (in which *Pediastrum* sp. was maintained at >90% dominance) was similar to that (90-96% NH₄⁺-N and 52-68% PO₄³⁻-P) of the control HRAP_c (which had a mixed population of algae). Further detailed wastewater treatment performance of the HRAPs in terms of organic compounds (total suspended solids, volatile suspended solids and BOD₅) and nutrient (N and P) removal and a nitrogen mass balance with CO₂ addition have been described previously (Park and Craggs, 2010; 2011a; 2011b).

2.3.5. Enhancing biomass harvest in wastewater treatment HRAPs

The Imhoff cone settling efficiency of algae in the HRAP effluents after 10, 30 and 60 minutes and 24 hours was measured throughout the year and was found to be greatly improved when *Pediastrum* sp. was dominant (Figure 2.5a). Average biomass settling efficiencies of 75.3±13.7%, 86.0±9.1%, 93.6±2.8%, and 99.2±1.6% were achieved after 10, 30 and 60 minutes, and 24 hours of settling respectively, when *Pediastrum* sp. was present

at over 80% dominance in the HRAPs. However, as *Pediastrum* sp. dominance declined to less than 40%, Imhoff cone settling efficiencies reduced to $19.8\pm 8.6\%$, $25.6\pm 10.2\%$, $35.2\pm 10.1\%$ and $76.0\pm 10.9\%$ for the respective settling periods. These settling efficiency test results confirmed that the dominance of *Pediastrum* sp. in the HRAP promoted settling efficiency particularly for settling periods of less than 1 hour.

Overall Imhoff cone settling efficiency was also influenced by settling characteristics of the algae that co-existed with *Pediastrum* sp. (Figure 2.5a). For example, when *Pediastrum* sp. was present at 70% dominance and co-existed with other colonial algae such as *Micractinium* sp., and *Scenedesmus* sp. (shown in circle “i”) the 10 minute settling efficiency was high (58%). However, when the co-existing algae were mainly poorly-settleable unicellular algae such as *Thalassiosira* sp. (average cell size $3.6\pm 2.7\ \mu\text{m}$) (shown in circle “ii”), the 10 minute settling efficiency was only 38%.

Analysis of the data on the population density of unicellular algae (counts/ml) including *Thalassiosira* sp. and settling efficiency during the one year experimental period indicates that settling efficiency at all settling times declined if the unicellular algal population was greater than 1×10^5 cells/ml (Figure 2.5b). Therefore, controlling the population density of the poorly-settleable unicellular algae to below this level could be necessary to achieve efficient biomass harvest.

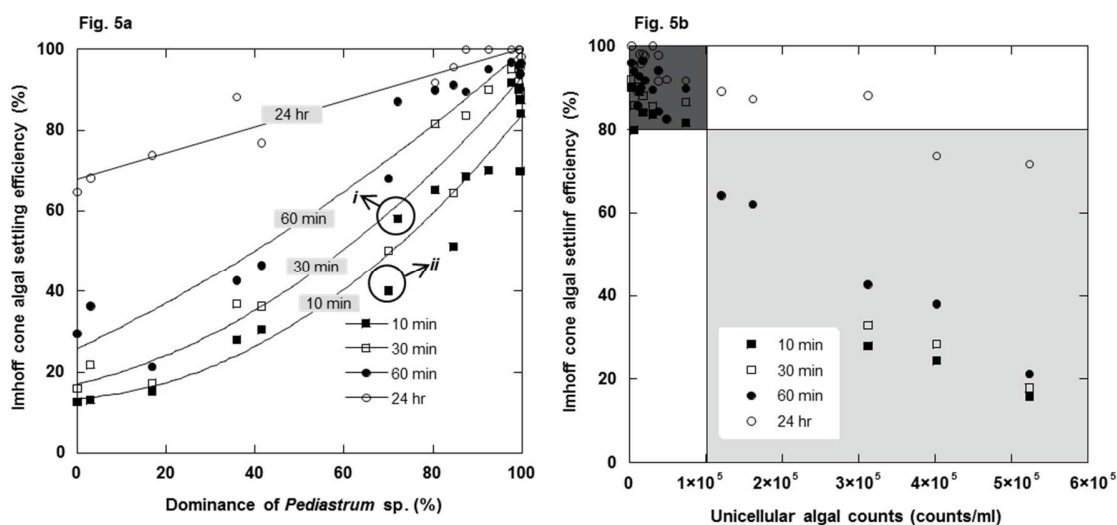


Figure 2.5: Relationship between a. *Pediastrum* sp. dominance and biomass settling efficiency after 10, 30, and 60 minute and 24 hour settling in an Imhoff cone (a.) (i, Note: within circle a: 72% *Pediastrum* sp. + 16% *Micractinium* sp. + 12% *Scenedesmus* sp. ii: 70% *Pediastrum* sp. + 30% Unicellular algae) and, b. relationship between unicellular algal counts (counts/ml) and biomass settling efficiency in an Imhoff cone.

The percentage solids (as % total solids) of the biomass collected at the bottom of an Imhoff cone after a 24 hour settling period were also influenced by the settling characteristics of the dominant algal species and other biological factors (e.g. zooplankton grazing and fungal infection) in the HRAPs (Figure 2.6). *Pediastrum* sp. dominant biomass (>90% dominance) was harvested as 2.5-3.0% solids by 24 hour gravity sedimentation (December 2009 for HRAP_c: Figure 2.6a, November-December 2009 and May-June 2010 for HRAP_i: Figure 2.6b). However, when less settleable colonial (*Micractinium* sp., and *Dictyosphaerium* sp.) and poorly settleable unicellular algae (*Thalassiosira* sp.) were dominant in the control HRAP_c, the % solids of harvested biomass was only about 1.5-2.0% (Figure 2.6a). Moreover, fungal infection in both HRAPs during the summer period of *Pediastrum* dominance (January – February 2010) reduced the settled biomass solids concentrations from 2.5-3.0% to less than 2.0%, which may have been due to the lower density of algal/fungal flocs.

Two species of *Pediastrum* (*P. duplex* and *P. boryanum*) were present in the HRAPs, of which, *P. boryanum* (intercellular space absent) was the most prevalent species throughout

the one year experimental period. In particular, during the three month summer period from December 2009 to February 2010, *P. boryanum* accounted for 80% to 98% of total biomass in both HRAPs. This period of *P. boryanum* prevalence coincided with very high biomass harvest efficiency (80 to 95%) in the two ASCs (combined HRT of 6 hours) (Figure 2.4) of both HRAPs. Imhoff cone settling experiments in the laboratory conducted during the same period showed that recycling in HRAP_r enhanced the biomass settling efficiency by 8-15% ($76.0 \pm 6.4\%$, $89.3 \pm 3.5\%$, and $97.2 \pm 1.9\%$ removal after 10, 30, and 60 minutes respectively compared with $59.3 \pm 13.5\%$, $76.5 \pm 8.9\%$, and $88.2 \pm 9.9\%$ for the control, *P-value*: <0.005 , one-way ANOVA, Figure 2.7). The improved biomass settling efficiency in HRAP_r may be explained by the presence of larger colonies of *P. boryanum* as recycling of gravity harvested biomass at a recycling rate of 66-212 g /kg produced /d (depending on season, Table 2.1) extended the mean cell residence time (MCRT) or period for growth in the pond by ~0.5 d in summer and ~3.4 d in winter. Extending the MCRT increased the average size and biovolume of *P. boryanum* colonies in HRAP_r by 13-30% and 50-80% respectively compared to those in the control HRAP_c measured over the three month period when *P. boryanum* was prevalent in both HRAPs (December 2009 to February 2010) (Figure 2.8).

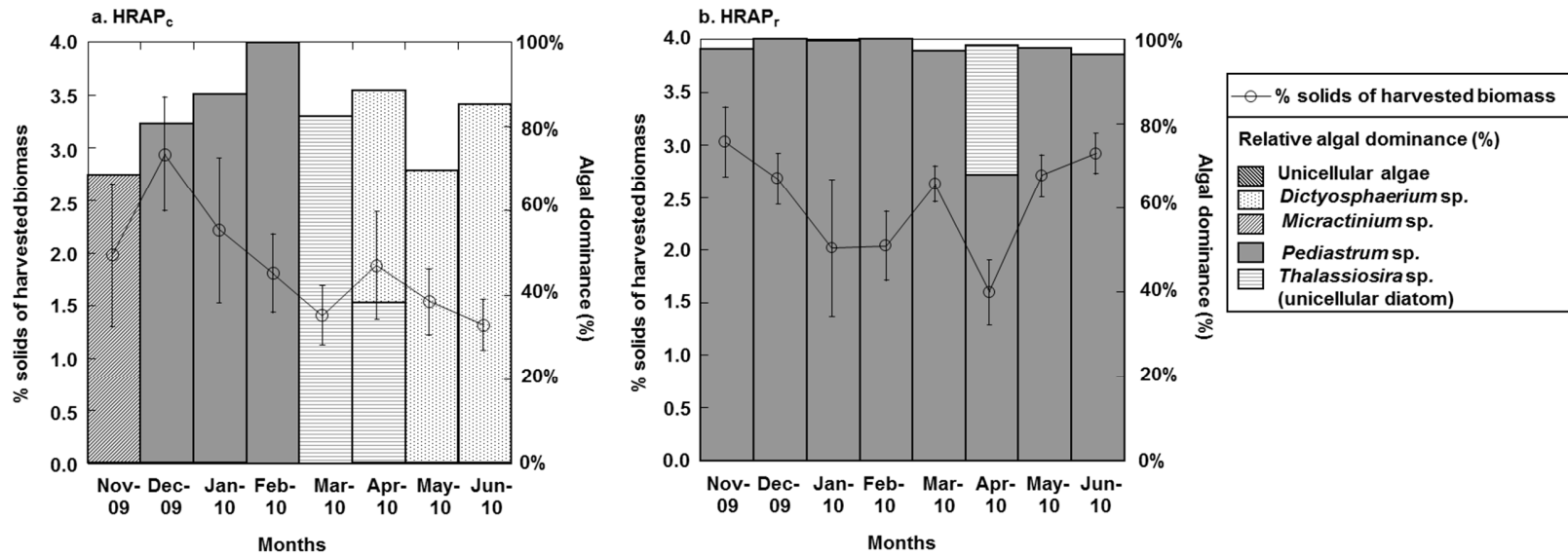


Figure 2.6: The relationship between the percentage solids of the biomass (as VSS) collected from the bottom of the Imhoff cone after 24 hours and dominant algae in HRAP_r with recycling (Fig. 5a) and HRAP_c without recycling (Fig. 5b) (Note: possible fungal infection was observed in both HRAPs in summer from Jan to Feb 2010).

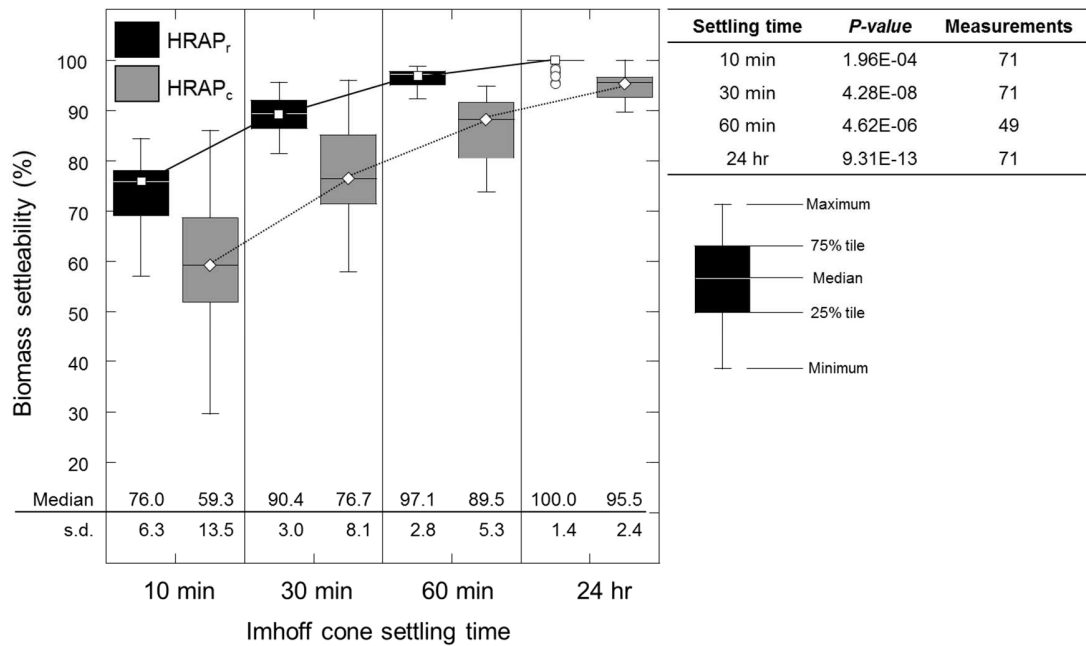


Figure 2.7: Imhoff cone settling efficiency after 10, 30, and 60 minutes and 24 hours in laboratory measured from December 2009 to February 2010 when *P. boryanum* dominance was similar (>80%) in both HRAPs with and without recycling (The data for each settling were compared to investigate the effect of recycling using one-way ANOVA analysis).

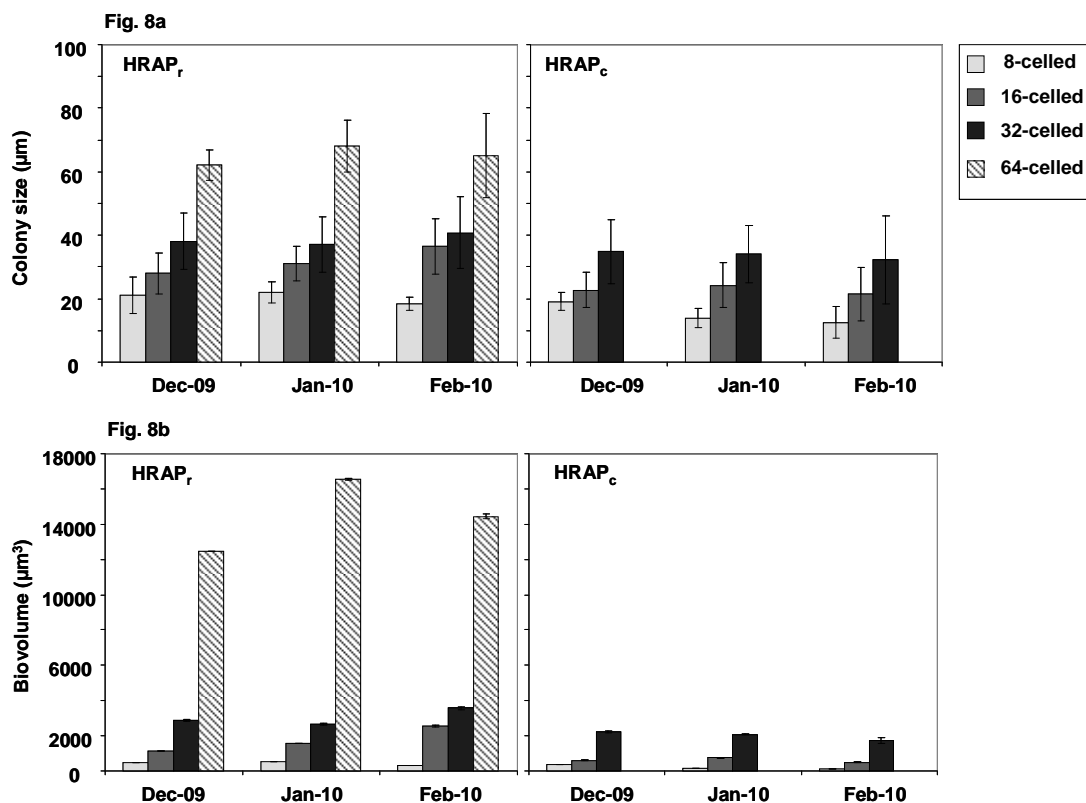


Figure 2.8: Average size (a) and biovolume (b) of *P. boryanum* colonies with different numbers of cells (8-64) in HRAP_r with recycling and HRAP_c without recycling during the period of *P. boryanum* dominance (>80%).

2.4. Conclusions

Recycling gravity harvested biomass to HRAP_r increased the dominance of a rapidly settleable colonial alga, *Pediastrum boryanum* to >90% which was maintained for the majority of the one year study compared to the control HRAP_c (53% dominance). The long term (one year) maintenance of algal species dominance over similarly sized co-occurring algae has not been previously reported for a wastewater treatment HRAP. Increased dominance of *P. boryanum* greatly improved biomass harvest efficiency (90% from HRAP_r effluent compared to 60% from the control). Imhoff cone tests measuring biomass settleability demonstrated that the dominance of *P. boryanum* and the species composition of remaining algae both influenced biomass settleability. Moreover the settling characteristics of the dominant algae in the HRAPs also influenced the concentration of 24

hour settled biomass. Recycling increased the average biovolume of *P. boryanum* colonies in HRAP_T effluent by 50-80%, possibly as a result of increasing the mean cell residence time, which enables algae to grow for longer. These results show that recycling gravity harvested biomass is a simple and effective operational method to promote and maintain the dominance of a rapidly settleable algal species such as *P. boryanum* to enhance biomass harvest by gravity sedimentation.

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MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

**STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING
PUBLICATIONS**

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Byung-Kwan Park

Name/Title of Principal Supervisor: Professor Andy Shilton

Name of Published Research Output and full reference: Park, J.B.K., Craggs, R.J., Shilton, A.N. (2011). Recycling algae to improve species control and harvest efficiency from a high rate algal pond. Water Research 45 (20):6637-6649.

In which Chapter is the Published Work: Chapter 2

• **Describe the contribution that the candidate has made to the Published Work:** While the paper was completed with advice and editing from my supervisors, Professor Andy Shilton and Dr Rupert Craggs, I designed the experiments, conducted all experimental work, analysed the results and led the paper as the first corresponding author.

Candidate's Signature

22nd August 2013

Date

Principal Supervisor's signature

22nd August 2013

Date

Chapter 3

Enhancing biomass energy yield from pilot-scale high rate algal ponds with recycling

Chapter preface

The first year of the pilot-scale HRAP study reported in Chapter 2 showed that recycling a portion of gravity harvested biomass (“recycling”) promoted the dominance of a large rapidly settling colonial alga, *Pediastrum boryanum* resulting in an improvement of biomass harvest efficiency. To improve ‘harvestable biomass production’ from HRAPs, it would be advantageous to not only improve biomass harvestability (i.e. settleability) as demonstrated in Chapter 2, but also to increase biomass productivity. Moreover, in the context of algal biomass production for biofuels, the energy content of the biomass needs to be measured to determine the actual ‘biomass energy yield’. Thus, the main objective of the research presented in this chapter was to determine if recycling also improves biomass productivity in a pilot-scale HRAP and how this contributes to an improved net biomass energy yield. This chapter presents two years of data from the pilot-scale HRAPs. In Year 1 biomass productivity and energy yield were measured for the HRAPs operated with and without recycling. In Year 2 the study was extended to investigate whether seeding *P. boryanum* dominant biomass into a HRAP initially dominated by a poorly-settleable alga, *Dictyosphaerium* sp., would promote a change in species dominance to *P. boryanum*.

This chapter is based on the following publication:

Park, J.B.K., Craggs, R.J., Shilton, A.N. (2013). Enhancing biomass energy yield from pilot-scale high rate algal ponds with recycling. *Water Research*. 47, (13): 4422-4432.

Abstract

This chapter investigates the effect of recycling gravity harvested biomass ('recycling') on 'harvestable biomass production' and 'energy yield' in wastewater treatment High Rate Algal Ponds (HRAPs). Two 8 m³ pilot-scale HRAPs treating domestic wastewater were operated in parallel and monitored over a 2-year period. Volatile suspended solids were measured in the effluents from both HRAPs and their algal settling cones (ASCs) to determine biomass productivity and harvest efficiency. The energy content of the biomass was also measured. Multiplying biomass productivity and harvest efficiency gives the 'harvestable biomass productivity' and multiplying this by the energy content defines the net 'biomass energy yield'. In Year 1, recycling was implemented in one of the ponds (HRAP_r) and improved harvestable biomass productivity by 58% compared with the control (HRAP_c) without recycling (HRAP_r: 9.2 g/m²/d; HRAP_c: 5.8 g/m²/d). The energy content of the biomass grown in HRAP_r, which was dominated by *Pediastrum boryanum*, was 15% higher than that from the control pond which contained a mixed culture of 4-5 different algae (HRAP_r: 21.5 kJ/g; HRAP_c: 18.6 kJ/g). In Year 2, HRAP_c was then seeded with the biomass harvested from the *P. boryanum* dominated HRAP_r. This had the effect of shifting algal dominance from 89% *Dictyosphaerium* sp. (which is poorly-settleable) to over 90% *P. boryanum* in just 5 months. Operation of HRAP_c was then switched to recycling its own harvested biomass, which maintained *P. boryanum* dominance for the rest of Year 2. Importantly this work also showed that *P. boryanum* dominance was relatively fast to establish and was then stable and sustainable between seasons. With regard to the overall improvement in biomass energy yield, which is a critical parameter in the context of algal production for biofuels, the combined improvements that recycling caused in biomass productivity, harvest efficiency and energy content enhanced the net biomass energy yield by 66% (HRAP_r: 195 kJ/m²/day; HRAP_c: 118 kJ/m²/day).

3.1. Introduction

Chapter 2 showed that the dominance (based on algal biovolume) of a rapidly settling colonial alga, *Pediastrum boryanum* increased to more than 90% in HRAP_r with recycling compared with 53% dominance in the control HRAP_c that had no recycling. The higher dominance of *P. boryanum* coincided with an improved biomass harvest efficiency by 25%

compared with the control (annual average in HRAP_r: 85%; HRAP_c: 60%) with algal recycling rate of 66-212 g/ kg produced/ d depending on season.

Any method to improve the net biomass yield from wastewater treatment HRAPs could benefit energy production potential. However, this is not only a function of biomass productivity in the pond, but also biomass harvest efficiency from the pond effluent. Therefore it is necessary to consider the ‘harvestable biomass productivity’ which reflects the biomass productivity multiplied by the harvest efficiency.

While the methods of enhancing both the biomass productivity and harvest efficiency to improve harvestable biomass productivity were previously discussed in Chapter 1 and then demonstrated in Chapter 2, in the context of biofuel production it is also necessary to consider the energy content of the biomass to determine the actual ‘biomass energy yield’. For example, if algae are to be used for as a feedstock for biodiesel production, high algal lipid content is important (Wang et al. 2008; Patil et al. 2011; Pittman et al. 2011; Singh et al. 2011).

Biomass productivities in wastewater treatment HRAPs have been reported in the literature (summarized in Table 1.3, Chapter 1), however, neither the ‘harvestable biomass productivity’ nor the actual ‘biomass energy yield’ have been considered previously. Furthermore, the effect of recycling on these parameters has never previously been studied. This chapter presents two years of data from the pilot-scale HRAPs. In Year 1 harvestable biomass productivity and energy yield were determined for the HRAPs operated with and without recycling. In Year 2 the study was extended to investigate the influence of seeding *P. boryanum* biomass into a HRAP dominated by a poorly-settleable alga, *Dictyosphaerium* sp.

3.2. Materials and methods

3.2.1. Operation of pilot-scale high rate algal pond

A description of the two pilot-scale HRAP systems (HRAP_r with recycling; HRAP_c without recycling) and general operational parameters in Year 1 is given in Table 2.1 (Chapter 2). In Year 2, operational parameters are summarized in Table 3.1 and a schematic diagram of the experimental set-up is also illustrated in Figure 3.1.

Table 3.1: Year 2 (July 2010 to June 2011) pilot-scale wastewater treatment HRAP and ASC operational parameters including design/actual inflow rate, hydraulic retention time (HRT), solar radiation, pond water temperature, mean cell residence time (MCRT), and algal recycling rate.

Parameters		Winter in 2010	Spring	Summer	Autumn	Winter in 2011
Days		61	90	110	71	30
Experimental period		(July 1-Aug 31, 10)	(Sept 1-Nov 30, 10)	(Dec 1, 10-Mar 21, 11)	(Mar 22- May 31, 11)	(Jun 1-Jun 30, 11)
Solar radiation (MJ/m ² /d)		8.4±3.5	18.7±7.3	21.1±7.5	10.6±4.9	6.0±2.5
Temperature (°C)		9.2±2.4	14.2±2.9	19.6±2.5	13.3±2.8	10.3±2.0
Inflow (m ³ /d)	Design	1.0	1.3	2.0	1.3	1.0
	Actual ⁽¹⁾	1.1±0.2	1.3±0.2	2.0±0.4	1.4±0.3	1.1±0.3
HRAP HRT (d)	Design	8.0	6.0	4.0	6.0	8.0
	Actual ⁽¹⁾	7.6±1.0	6.2±0.6	4.1±0.5	5.9±0.8	7.3±1.3
MCRT (d) ⁽²⁾	HRAP _r	9.3±1.8	6.2±1.0	4.6±0.2	8.0±2.6	9.4±1.7
	HRAP _c	13.1±6.6	6.9±1.3	4.7±0.3	8.0±2.3	9.0±2.0
Algal recycling rate (g recycled/ kg produced/ d) ⁽³⁾	HRAP _r	60-375	49-174	35-121	64-470	117-313
	HRAP _c	89-513	81-363	41-184	113-393	92-246
ASC HRT (d)	Design	6	4.5	3	4.5	6
	Actual	5.7±0.8	4.6±0.5	3.1±0.4	4.4±0.6	5.5±1.0

Note:

(1) Weather compensated (daily precipitation and evaporation) daily inflow and hydraulic retention time (HRT)

(2) Calculated using Equation 1 below

(3) Determined based on the volume of biomass recycled (1 L/d), harvested biomass concentration (g/L) and HRAP volume (8 m³)

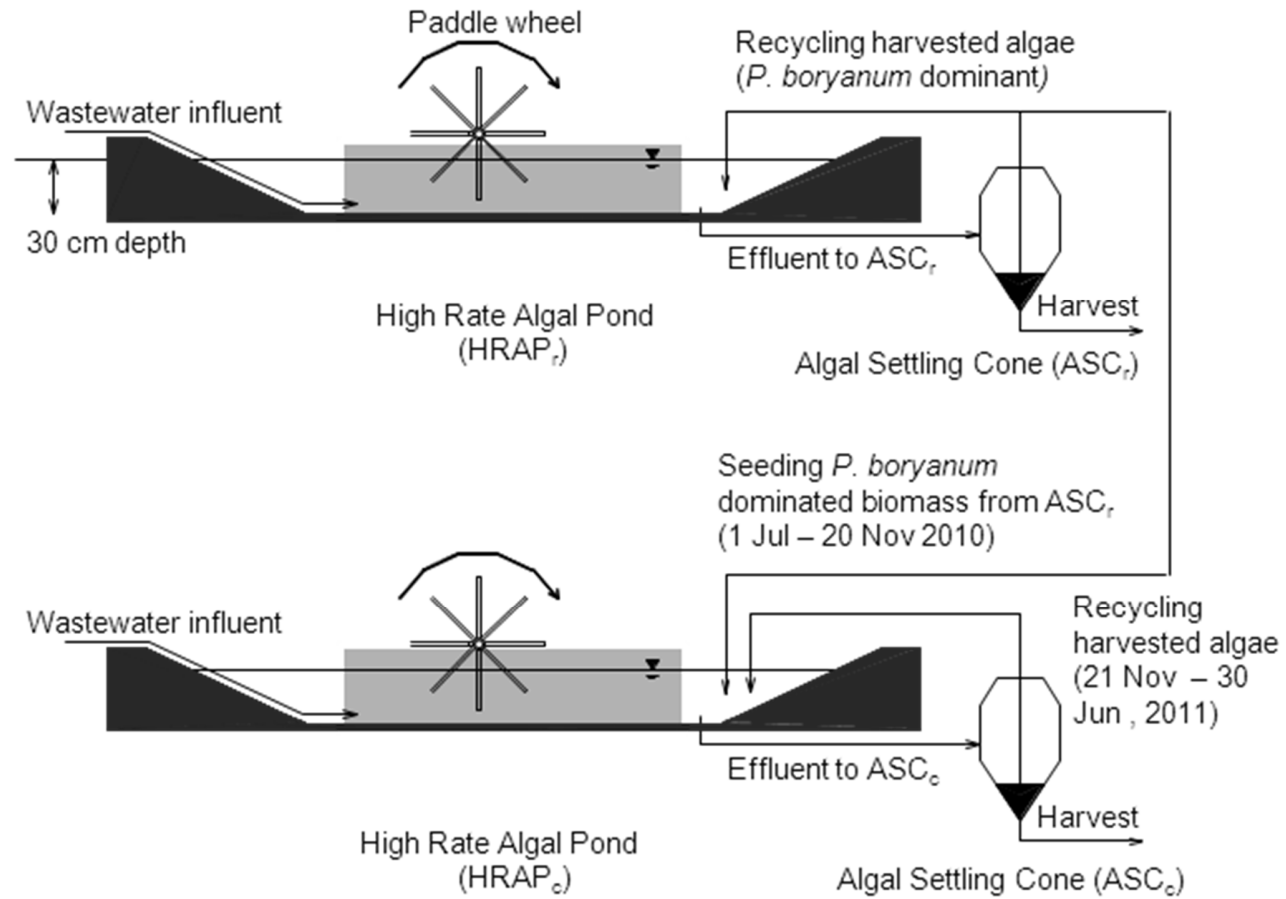


Figure 3.1: Schematic diagram for the operation of the pilot-scale HRAPs with recycling in Year 2 (July 2010-June 2011).

As described in Chapter 2, in Year 1 settled biomass was removed from ASC_r each day and one litre of the gravity harvested algal biomass was recycled back to HRAP_r (Figure 3.1). The second HRAP_c was operated without recycling as a control with all other operational parameters the same as HRAP_r. At the end of Year 1, HRAP_r was dominated by a rapidly settleable alga, *Pediastrum boryanum* (92% dominance) and HRAP_c was dominated by a poorly settleable alga, *Dictyosphaerium* sp. (90% dominance). The excess amount of algal biomass collected from both ASCs each day was added to an anaerobic digester for biogas production.

In Year 2, HRAP_c was then seeded with the biomass harvested from the *P. boryanum* dominated HRAP_r (one litre of the biomass from ASC_r, as shown in Figure 3.1). After the dominance of *P. boryanum* reached 85% in HRAP_c (November 2010), the ‘seeding’ of harvested biomass from HRAP_r was replaced by ‘recycling’ back from its own settler, ASC_c, for the rest of Year 2.

Recycling extended the mean cell residence time (MCRT) of algae in the HRAPs. Thus, the MCRT was calculated using Equation 3.1 and presented in Table 3.1.

$$MCRT = \frac{V X}{Q_c X - Q_{re} X_h} \quad \text{Equation 3.1}$$

Where;

$MCRT$ = Algal mean cell residence time (d)

V : HRAP volume (m³)

X : HRAP biomass concentration (VSS, g/m³)

Q_c : Net evaporation compensated HRAP effluent flow rate (m³/d)

Q_{re} : Biomass recycled per day (l/d)

X_h : Harvested biomass concentration (VSS g/L)

3.2.2. *Measurement of biomass productivity*

Samples of HRAP effluents were taken 2-3 times a week for the measurement of volatile suspended solids (VSS) and analysed according to Standard Methods (APHA 2008). Biomass productivity of the HRAPs was calculated based on the VSS concentration (Equation 2). The measured VSS concentration was adjusted by subtracting the increase due to the recycled biomass and compensating for any increase or decrease in daily flow due to rainfall or evaporation. Daily rainfall and evaporation data (calculated using the Penman–Monteith method from daily mean temperature, wind speed, relative humidity and solar radiation) for the experimental site were downloaded from NIWA National Climate Database (<http://cliflo-niwa.niwa.co.nz/>).

$$P = \frac{(C \times Q_c) - R}{A} \quad \text{Equation 3.2}$$

$$Q_c = Q_{inf} + ((\text{rainfall} - \text{evaporation}) \times \text{pond surface area}) \quad \text{Equation 3.3}$$

$$R = \text{Harvested biomass concentration (VSS, g/L)} \times 1 \text{ L/d (fixed volume)} \quad \text{Equation 3.4}$$

Where;

Q_c : Net evaporation compensated HRAP effluent flow rate (m³/d)

Q_{inf} : Daily inflow (m³/d)

P : Biomass productivity (g/m²/d)

C : Biomass concentration in HRAPs (VSS, g/m³)

A : HRAP surface area (32 m²)

R : Recycled biomass per day (g/d)

Harvestable biomass productivity (which reflects the actual capture of biomass by the ASC) was calculated by multiplying the measured biomass productivity with harvest efficiency of the ASCs.

3.2.3. Measurement of the biomass energy content

For nine months in Year 1 (October 2009 - July 2010), the energy content of the HRAP biomass (kJ/g) was measured monthly using a bomb calorimeter (Leco, AC 350, Leco Corporation, St Joseph, MI, USA) to determine biomass energy yield (as kJ/m²/d) in the HRAPs, which was calculated by multiplying the harvestable biomass productivity (g/m²/d) with the energy content of the biomass (kJ/g).

3.2.4. Measurement of biomass harvest efficiency

Samples of HRAP water and ASC effluents (i.e. final discharge) were taken 2-3 times a week for the measurement of volatile suspended solids (VSS) according to standard methods (APHA 2008). Large non-algal particles (e.g. leaves) and invertebrate grazers (e.g. *Moina* sp. or *Daphnia* sp.) were strained (1 mm mesh) from the pond water samples before VSS analysis. Biomass harvest efficiency from the HRAP effluents was determined as the percentage of the biomass (VSS) in the HRAP water that was removed in the ASC. Samples of harvested biomass collected in the respective ASC's were also analysed 2-3 times a week for volatile suspended solids (VSS) to calculate the algal recycling rate.

3.2.5. Microscopic image analysis

Relative algal dominance based on algal biovolume ($\mu\text{m}^3/\text{ml}$) in the pilot-scale HRAPs was determined monthly using the microscopic image analysis technique developed previously in Chapter 2. The most abundant algal species in the microscopic images of the HRAP water were also identified using an identification guide (Brook 2002).

3.3. Results and discussion

3.3.1. *Recycling gravity harvested biomass improves the dominance of P. boryanum*

In Year 1, recycling a portion of gravity harvested biomass ('recycling') in HRAP_r increased, and maintained the dominance of *P. boryanum* (annual average of >90%) compared with the control HRAP_c without recycling (53% dominance) (reported in Chapter 2).

In Year 2, this research was extended to investigate the effect of adding gravity harvested biomass into the HRAPs and the relative algal dominance is presented in Figure 3.2. Seeding *P. boryanum* dominated biomass (collected from HRAP_r) into the *Dictyosphaerium* sp. dominated HRAP_c was able to shift the algal dominance in HRAP_c, to 85% *P. boryanum* in five months (Figure 3.2a). For the remaining seven months in Year 2, recycling a portion of gravity harvested biomass from HRAP_c (instead of HRAP_r) maintained *P. boryanum* dominance at about 90% (Figure 3.2a).

Recycling in HRAP_r consistently maintained the dominance of *P. boryanum* at 90% throughout the second year of the study (Figure 3.2b), replicating the results of the first year of the study (Chapter 2). These results confirm that recycling (or seeding) a small portion of *P. boryanum* dominated gravity harvested biomass provides a simple and effective way to improve (or maintain) the dominance of rapidly settleable alga, such as *P. boryanum*, in wastewater treatment HRAPs.

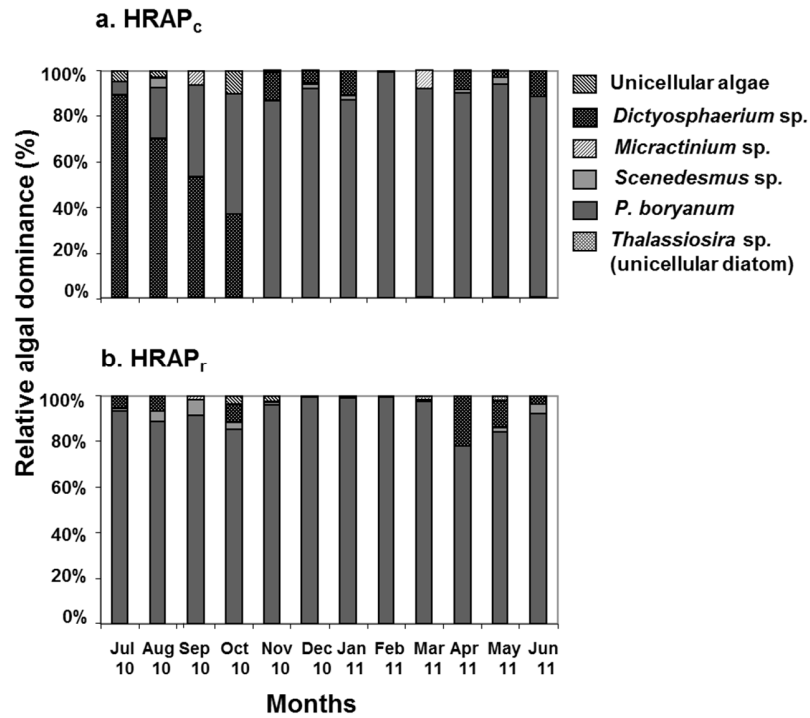


Figure 3.2: Algal dominance based on calculated algal biovolume in Year 2 (from July 2010 to June 2011), a: HRAP_c initially dominated by *Dictyosphaerium* sp.; b: HRAP_r dominated by *P. boryanum*.

3.3.2. Recycling improves biomass harvest efficiency

In Year 1, biomass harvest efficiency was highly dependent on the algae that were dominant in the HRAPs (Chapter 2). For example, as shown in Figure 3.3a, during the initial five month period (Period 1) when *Dictyosphaerium* sp. (poorly-settleable) was prevalent in HRAP_c, it had lower and less consistent biomass harvest efficiency (data summarized in Table 3.2). However, in Period 2 when *P. boryanum* dominance was well established in HRAP_c as a result of recycling, biomass harvest efficiency increased to 87% (Table 3.2). HRAP_r had similar performance with >80% biomass harvest efficiency.

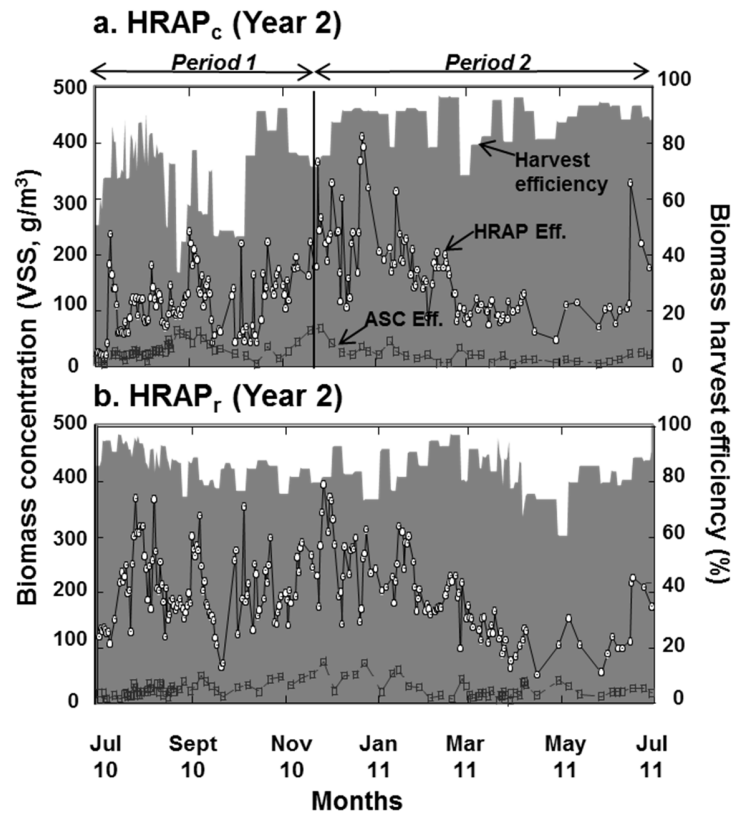


Figure 3.3: Biomass concentration (VSS) in the HRAP effluents and algal settling cones (ASCs) (Primary Y-axis) and biomass harvest efficiency in the ASCs (Shaded area; Secondary Y-axis) in Year 2. a: HRAP_c initially operated with seeding *P. boryanum* dominated biomass (Period 1) and then recycling harvested biomass (Period 2) when *P. boryanum* dominance was improved; b: HRAP_r with recycling.

Table 3.2: Year 2 (July 2010 to June 2011) HRAP average TSS/VSS concentrations (and the proportion of VSS in TSS), biomass and harvestable biomass productivities (based on VSS), biomass harvest efficiency and harvested biomass concentration.

Parameters		Winter in 2010	Spring	Summer	Autumn	Winter in 2011
Days		61	90	110	71	30
Experimental period		(July 1-Aug 31, 10)	(Sept 1-Nov 30, 10)	(Dec 1, 10-Mar 21, 11)	(Mar 21- May 31, 11)	(Jun 1-Jun 30, 11)
HRAP _r	TSS (g/m ³)	250.7±76.2	266.7±90.5	235.0±75.8	119.5±33.5	186.2±64.1
	VSS (g/m ³)	208.6±64.1	220.5±75.0	198.3±58.9	99.9±30.8	163.3±57.9
	% VSS	83.1±2.3	84.2±2.4	83.9±2.5	81.1±2.9	85.2±2.4
HRAP _c	TSS (g/m ³)	121.7±59.0	176.4±88.7	201.2±97.5	122.6±30.0	218.0±60.1
	VSS (g/m ³)	99.3±47.1	148.4±71.9	164.5±75.4	98.5±25.8	160.4±49.5
	% VSS	81.5±2.8	82.4±2.7	80.4±2.7	81.6±2.2	80.5±2.8
Biomass productivity (g VSS/m ² /d)	HRAP _r	6.7±3.2	8.7±2.6	12.7±4.0	4.3±1.5	5.1±2.4
	HRAP _c	3.3±1.5	5.8±2.6	12.1±4.7	4.1±1.1	5.4±2.4
% harvest efficiency (based on VSS)	HRAP _r	89.3±4.8	82.5±6.0	86.7±6.3	80.1±22.6	83.1±4.2
	HRAP _c	69.9±13.1	59.0±15.5	87.6±7.9	89.0±5.9	90.5±2.5
Harvestable biomass productivity (g VSS/m ² /d)	HRAP _r	6.1±2.9	7.2±2.2	10.6±3.5	3.2±1.0	4.6±2.7
	HRAP _c	2.3±1.2	4.3±2.4	10.4±4.5	3.6±1.1	4.5±2.6
Harvested biomass concentration (VSS, g/L)	HRAP _r	31±9.6	28±7.7	29.9±6.5	27.0±10.8	35.2±5.3
	HRAP _c	18±7.1	22±6.9	30.2±5.1	26.2±5.3	28.7±11.0

3.3.3. Biomass productivity

This section presents biomass productivity (and harvestable biomass productivity) data from 2 years of the pilot-scale HRAP studies.

3.3.3.1. Biomass productivity in Year 1 (from Jul 09-Jun 10)

In Year 1 (Figure 3.4 and data summarized in Table 3.3), recycling in HRAP_r improved biomass productivity by 20% compared with HRAP_c without recycling (HRAP_c: 9.2 g/m²/d; HRAP_r: 10.9 g/m²/d). However, as previously discussed, harvestable biomass productivity is a more important parameter since it takes into account both biomass productivity and harvest efficiency. As reported in Chapter 2, since recycling also improved biomass harvest efficiency from 63% to 85%, the harvestable biomass productivity increased from 5.8 g/m²/d (HRAP_c) to 9.2 g/m²/d (HRAP_r) as noted in Figure 3.4.

The benefits of recycling on harvestable biomass production are illustrated by comparison of our results with a previous study (García et al 2006) in which an outdoor experimental HRAP received domestic wastewater in Barcelona, Spain for one year (pond surface area: 1.54 m²; water depth: 0.3 m; HRT of the HRAP: 7 - 10 days; no CO₂ addition). They reported annual average biomass productivity (as TSS) of 12 g/m²/d in the pond and TSS removal efficiency of 77% in a subsequent gravity settler (HRT of the settler: 1-3 d). Assuming a VSS to TSS ratio of 80% (as we typically measured), approximately 10 g VSS/m²/d of biomass productivity was achieved. This value is similar to the biomass productivity measured in our HRAPs (HRAP_r: 10.9 g VSS/m²/d; HRAP_c: 9.2 g VSS/m²/d) with similar solar radiation (Barcelona: 15 MJ/m²/d (García et al. 2006); Hamilton: 14.5 MJ/m²/d). However, we had 16% higher harvestable biomass productivity (HRAP_r: 9.2 g/m²/d) than García et al (2006) reported (7.7 g VSS/m²/d) as a result of the improved harvestability of HRAP_r effluent (annual average of 85% in ASC_r compared with 77% of García et al (2006)), despite our ASC_r having a 4-6 times shorter HRT (6-12 h depending on season) than their gravity harvester (HRT: 1-3 d).

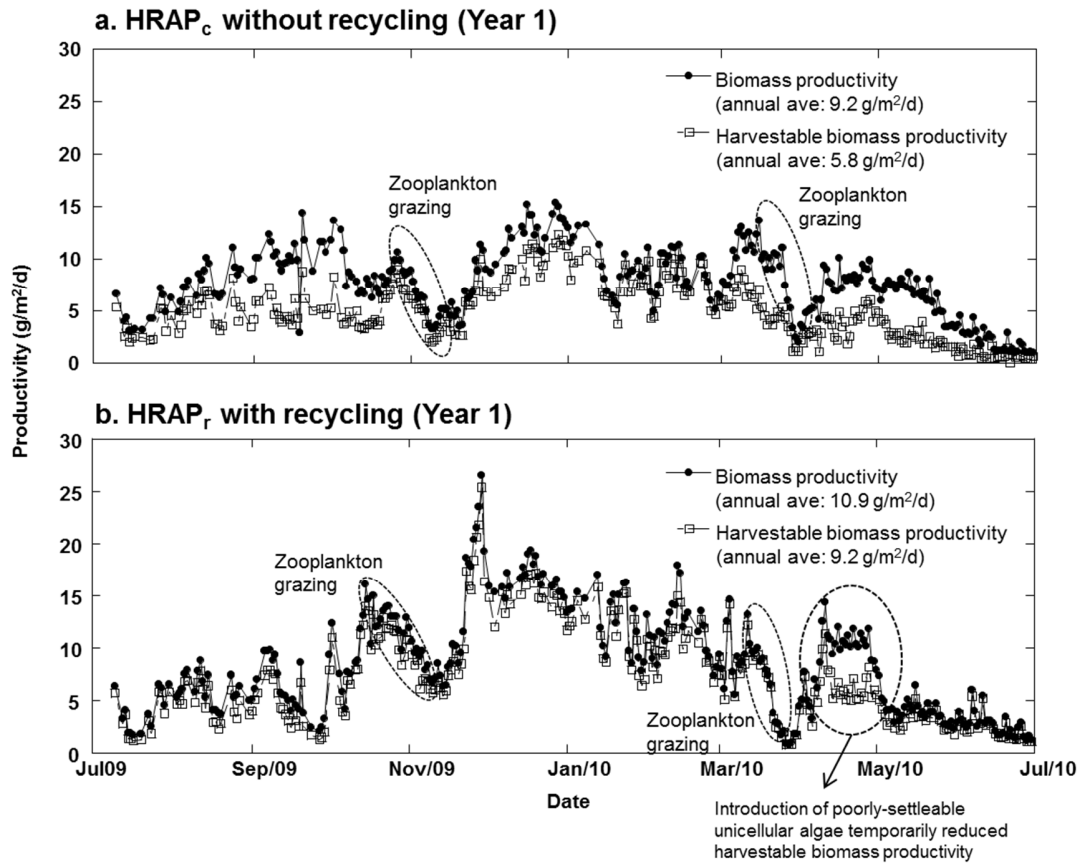


Figure 3.4: Biomass productivity in the HRAPs (HRAP_r with recycling; HRAP_c: without recycling) and harvestable biomass productivity in ASCs in Year 1 (July 2009 to June 2010) (note: Both HRAPs were susceptible to grazing by zooplankton (e.g. rotifers, *Moina* sp. or *Daphnia* sp.), which reduced biomass concentrations when a population of *Moina* sp. increased to ~560 individuals/L).

Table 3.3: Year 1 (July 2009 – June 2010) biomass and harvestable biomass productivities in the pilot-scale HRAPs (HRAP_r: with recycling; HRAP_c: without recycling).

Parameter		Winter in 2009	Spring	Summer	Autumn	Winter in 2010
Days		67 days	76 days	113 days	70 days	34 days
Experimental period		(July 1-Sept 6, 09)	(Sept 7-Nov 22, 09)	(Nov 23, 09-Mar 16, 10)	(Mar 17-May 25, 10)	(May 26-Jun 30, 10)
Dominant algae ⁽¹⁾	HRAP _r	<i>P. boryanum</i> (~90%)	<i>P. boryanum</i> (~92%)	<i>P. boryanum</i> (~98%)	<i>P. boryanum</i> (~80%)	<i>P. boryanum</i> (~90%)
	HRAP _c	<i>P. boryanum</i> (~70%)	<i>Micractinium</i> sp. (>70%)	<i>P. boryanum</i> (~90%)	Unicellular algae (~45%) <i>Dictyosphaerium</i> sp. (~40%)	<i>Dictyosphaerium</i> sp. (~80%)
Biomass productivity (g VSS/m ² /d) ⁽²⁾	HRAP _r	6.0±1.8	8.9±3.3	13.5±3.9	6.4±3.4	2.6±1.1
	HRAP _c	7.2±2.8	8.4±4.5	10.6±2.6	7.3±1.9	2.3±1.1
Harvestable biomass productivity (g VSS/m ² /d)	HRAP _r	4.9±1.4	7.3±2.7	12.2±3.5	4.8±2.6	2.2±0.9
	HRAP _c	4.9±1.9	5.3±2.8	8.1±2.0	3.3±0.8	1.3±0.6

Note: (1) Algal dominance in the HRAPs was previously shown in Chapter 2

(2) Weather compensated (daily precipitation and evaporation) productivity calculated using Equation 3.2.

If the HRAP is being operated at a low hydraulic retention time (HRT), the algal population (concentration) may have been insufficient to fully utilize the incident light energy and available nutrients, resulting in sub-optimal biomass production. Implementing biomass recycling separates the mean cell retention time (MCRT) from the HRT. In Year 1, recycling in HRAP_r extended the MCRT so that it was longer than the HRT (by 0.5 d in summer and 3.4 d in winter, Chapter 2). Therefore, it is feasible that the increases in the MCRT and thus concentration contributed to improved biomass production in HRAP_r by enabling more light and nutrients to be utilized.

Gravity settling in ASC_r selected for larger *P. boryanum* colonies from the HRAP_r effluent (Figure 3.5), which were then recycled back to the pond. Approximately 55% of the colonies in the harvested biomass had a diameter of >35 µm compared with only 25% in the mixed pond water (Figure 3.5). Not only does this have clear benefits in terms of improved harvest efficiency (and thus the overall harvestable biomass productivity) as discussed above, but, it may also explain the improvement in the biomass productivity in the pond itself. Research undertaken by Tukaj et al. (2003) on *Scenedesmus armatus*, noted that the maximum algal growth rate occurred at when the alga had reached about 80% of their full size (i.e. reproductive colonies). This indicates that there is a variation in the efficiency at which the alga can convert light energy to biomass throughout different stages of their life-cycle. If *P. boryanum* exhibits similar behaviour it therefore offers a possible explanation as to why recycling was observed to improve biomass productivity, because recycling increases the proportion of large colonies with higher net growth rates.

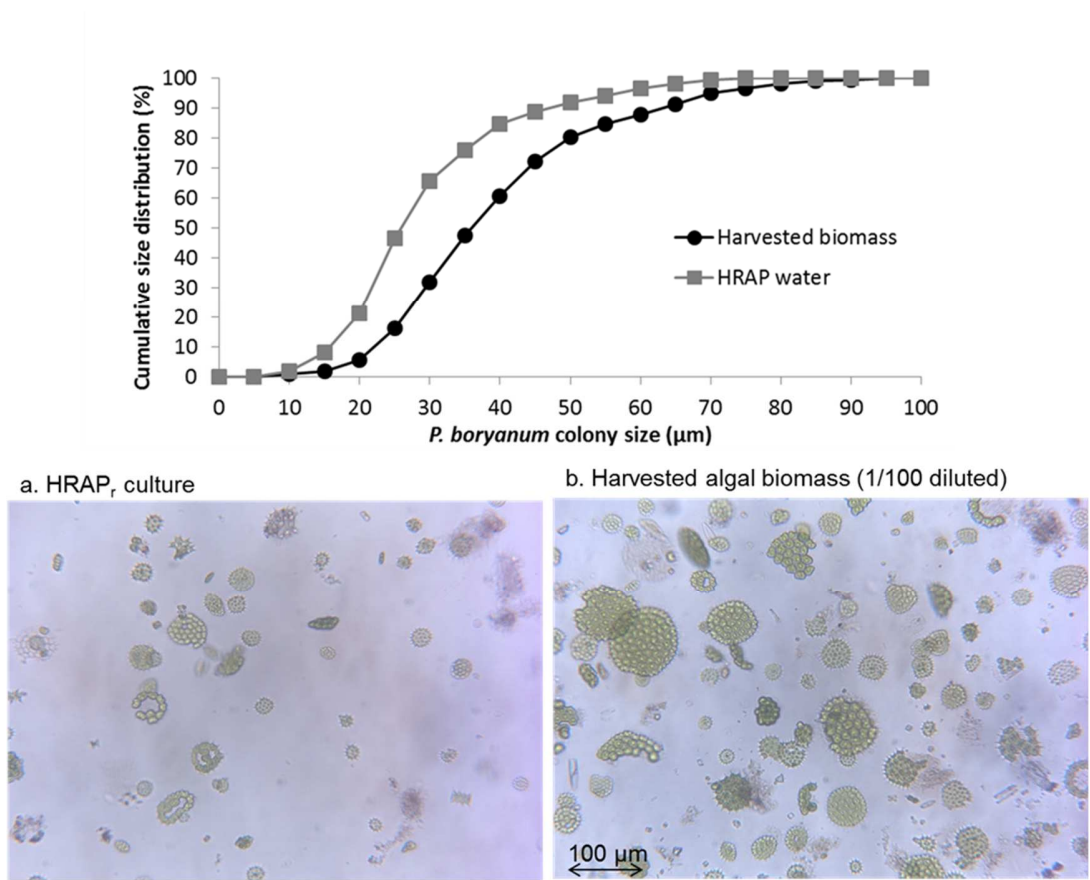


Figure 3.5: Size distribution of *P. boryanum* colonies in gravity settled biomass that collected in ASC_r and the HRAP_r water and microscopic photos of a. the HRAP_r water and b. the settled biomass (1/100 diluted) which were recycled back to HRAP_r.

3.3.3.2. Biomass productivity in Year 2 (from Jul 10 - Jun 11)

Over the seasons, biomass productivity in the HRAPs varied from 3.3 to 12.1 g/m²/d in HRAP_c and from 4.3 to 12.7 g/m²/d in HRAP_r, as shown in Table 3.2.

For the five month period (Period 1) before *P. boryanum* was established as the dominant species in HRAP_c, this pond had significantly lower biomass productivity than that in HRAP_r where *P. boryanum* had been established by recycling (Figure 3.6; Table 3.2). However, when both HRAPs had similar *P. boryanum* dominance (Period 2), similar biomass productivities (summer: 12 g/m²/d; autumn: 4 g/m²/d; winter in 2011: 5 g/m²/d, Table 3.2) and harvestable biomass productivities (summer: 11 g/m²/d; autumn: 4 g/m²/d; winter in 2011: 4.5 g/m²/d, Table 3.2) were obtained in both ponds.

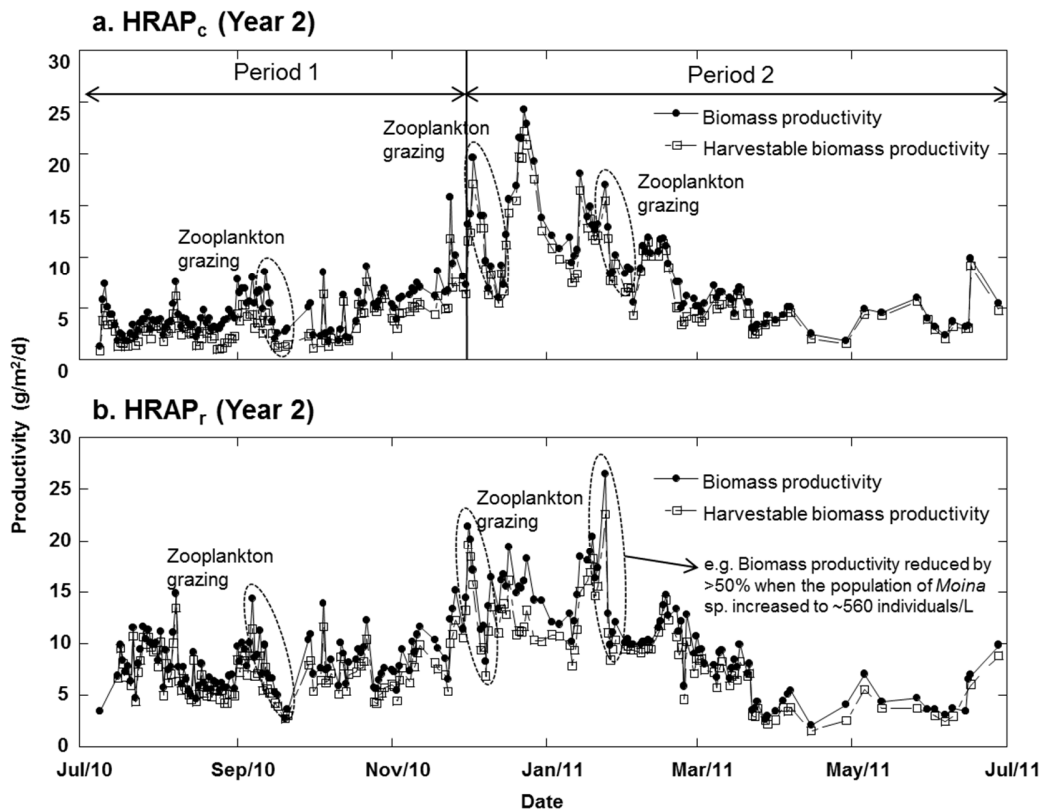


Figure 3.6: Biomass productivity in the HRAPs (both with recycling) and harvestable biomass productivity in ASCs in Year 2 (July 2010 to June 2011).

3.3.4. Biomass energy yield in the pilot-scale HRAPs

The effect of recycling on biomass energy yield (as kJ/m²/d), which is a critical parameter in the context of algal production for biofuels, was investigated during the nine months monitoring period (Oct 09 - Jun 10) in Year 1.

The energy content of the biomass grown in the HRAPs varied from 19 to 22 kJ/g depending on the dominant algal species and seasonal climatic conditions (Figure 3.7). These values are in line with literature values for algae grown in the laboratory (~18-34 kJ/g) (Tillett 1988; Lugar 2012). The energy content of the biomass varied with season (higher in summer from Dec 09-Feb 10 than in winter from Mar-Jun 10, Figure 3.7a) and with dominant algal species (21.2±0.3 kJ/g in HRAP_r with 85% *P. boryanum* dominance; 20.3±0.8 kJ/g in HRAP_c with 80% *Dictyosphaerium* sp. dominance). Thus, not only did the dominance of *P. boryanum*

increase the biomass productivity and harvest efficiency, but it also improved the energy content of the biomass produced.

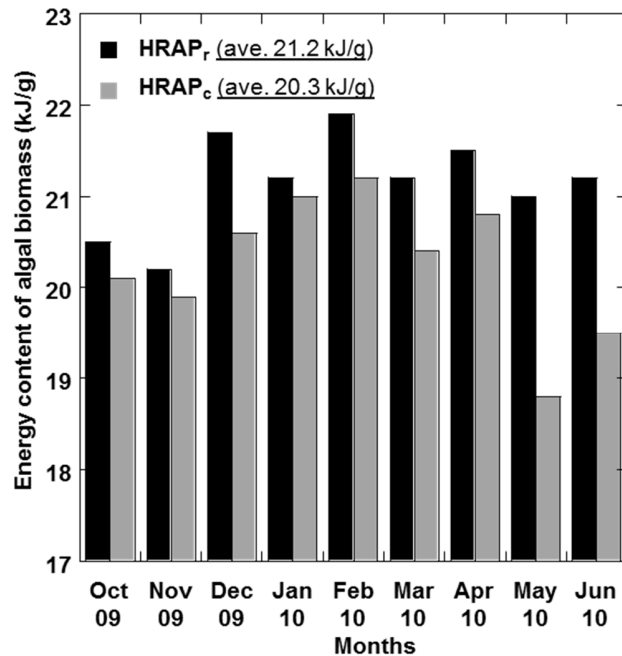


Figure 3.7: Energy content of biomass (KJ/g) in the pilot-scale HRAPs (HRAP_r with recycling; HRAP_c: without recycling) over the nine months in Year 1 (October 2009 to June 2010).

As shown in Figure 3.8a, the improvements that were triggered by recycling amounted to a total increase in biomass energy yield in HRAP_r by 66% compared with the control HRAP_c (HRAP_r: 195 kJ/m²/d; HRAP_c: 117 kJ/m²/d). The increase in biomass harvest efficiency had the greatest influence (60%) on the improvement of biomass energy yield compared with the increase in biomass productivity contributing 32% and the increase in biomass energy content at 8% (Figure 3.8b).

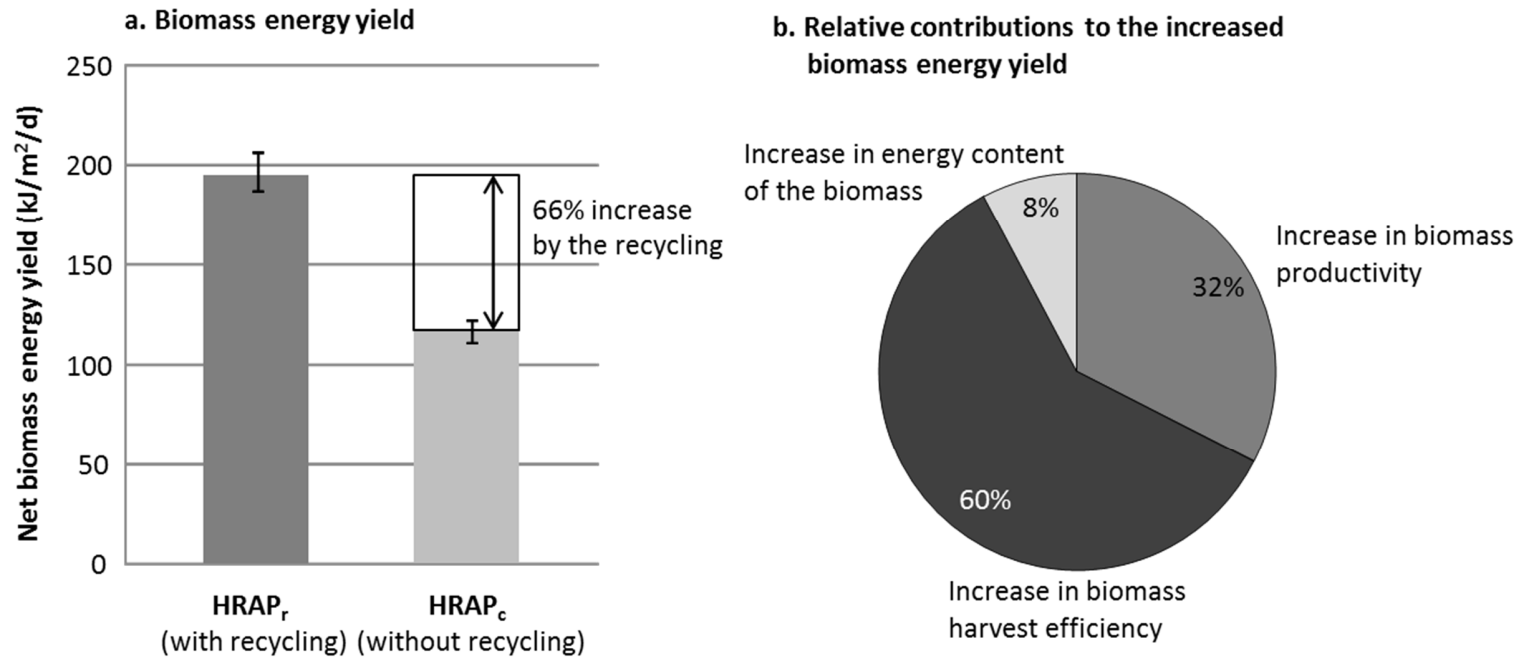


Figure 3.8: a: Biomass energy yield (as kJ/m²/d) including the increased biomass productivity, harvest efficiency and energy content of biomass in the wastewater treatment pilot-scale HRAPs (HRAP_r with recycling; HRAP_c: without recycling) from October 2009-June 2010; b: Relative contributions of increased biomass productivity, harvest efficiency and energy content of biomass to the increased biomass energy yield (note: the energy used for recycling was not accounted for, because the energy consumption was less than 0.5% compared with the energy produced in HRAP_r).

3.4. Conclusions

This work showed that seeding *P. boryanum* dominated biomass into a *Dictyosphaerium* sp. dominated pond (HRAP_c) changed the dominant alga to *P. boryanum*. This result demonstrates, for the first time in the literature, that species control is possible for similarly sized co-occurring algal colonies in outdoor wastewater treatment HRAP for 2 years by recycling a portion of gravity harvested biomass (“recycling”). Recycling could be a simple and practical way to improve ‘harvestable biomass productivity’ in wastewater treatment HRAPs (HRAP_r with recycling: 9.2 g/m²/d; HRAP_c without recycling: 5.8 g/m²/d). Furthermore the biomass energy yield was increased by 66% (HRAP_r: 195 kJ/m²/day; HRAP_c: 118 kJ/m²/day) through the combined improvements in biomass productivity, harvest efficiency and energy content with the recycling.

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GRADUATE RESEARCH SCHOOL

**STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING
PUBLICATIONS**

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Byung-Kwan Park

Name/Title of Principal Supervisor: Professor Andy Shilton

Name of Published Research Output and full reference: Park, J.B.K., Craggs, R.J., Shilton, A.N. (2013). Enhancing biomass energy yield from pilot-scale high rate algal ponds with recycling. Water Research. 47, (13): 4422-4432.

In which Chapter is the Published Work: Chapter 3

• **Describe the contribution that the candidate has made to the Published Work:** While the paper was completed with advice and editing from my supervisors, Professor Andy Shilton and Dr Rupert Craggs, I designed the experiments, conducted all experimental work, analysed the results and led the paper as the first corresponding author.

Candidate's Signature

22nd August 2013

Date

Principal Supervisor's signature

22nd August 2013

Date

Chapter 4

Investigating why recycling gravity harvested algae increases harvestability and productivity

Chapter preface

The pilot-scale HRAP studies conducted over two years (reported in Chapters 2 and 3) showed that recycling a portion of gravity harvested biomass (“recycling”) promoted the dominance of a rapidly settling colonial alga, *Pediastrum boryanum* and improved both ‘harvestable biomass productivity’ and ‘energy yield’. In order to demonstrate the reproducibility of these findings, twelve outdoor HRAP mesocosms were operated with and without recycling adjacent to the pilot-scale HRAPs over two different seasons. A further mesocosm study compared recycling of the separated solid and liquid components of the harvested algal biomass with recycling of un-separated biomass to explore potential mechanisms that could account for the increased settleability and productivity of the dominant alga, *Pediastrum boryanum*.

This chapter is based on the following publication:

Park, J.B.K., Craggs, R.J., Shilton, A.N. (2013). Investigating why recycling gravity harvested algae increases harvestability and productivity in high rate algal ponds. *Water Research*. 47(14), 4904-4917.

Abstract

Chapters 2 and 3 reported that recycling gravity harvested biomass ('recycling') promoted the dominance of a rapidly settling colonial alga, *Pediastrum boryanum* and improved harvestability and biomass productivity in pilot-scale wastewater treatment High Rate Algal Ponds (HRAPs). In order to demonstrate the reproducibility of these findings, twelve 18 L outdoor HRAP mesocosms were operated with and without recycling adjacent to the pilot-scale HRAPs over two different seasons. A further mesocosm study compared recycling of the separated solid and liquid components of the harvested algal biomass with recycling un-separated biomass to explore potential mechanisms that could account for the increased settleability and productivity. These mesocosm studies confirmed that recycling promoted *P. boryanum* dominance, improved 1h-settleability by >20% and increased biomass productivity by >25% compared with controls that had no recycling. Settleability was improved by both the separated solid containing large and fast settling colonies and liquid fraction, which is possibly due to the presence of extracellular polymeric substances that improve settleability. While there are many possible mechanisms that could account for the increased productivity with recycling, all but two were systematically eliminated: (i) the mean cell residence time was extended thereby increasing the algal concentration and so allowing better utilization of incident sunlight and, (ii) the relative proportions of algal growth stages (which have different net growth rates) was shifted, possibly resulting in an increase in the net growth rate of the culture.

4.1. Introduction

The previous two year pilot-scale HRAP studies (Chapters 2 and 3) demonstrated that recycling a portion of gravity harvested biomass ('recycling') improved the dominance of the rapidly settleable colonial alga, *Pediastrum boryanum* and resulted in an improvement in both biomass productivity and harvest efficiency (i.e. 'harvestable biomass productivity'). Biomass energy yield (a critical parameter in the context of algal production for biofuels) was improved by 66% with recycling (195 kJ/m²/day compared to 118 kJ/m²/day for the HRAP without recycling).

This chapter attempted to replicate the previous pilot-scale findings in mesocosm experiments conducted under different seasonal conditions. In addition to comparing species dominance and settleability, the research also included biomass productivity (Experiments 1 and 2). A further mesocosm study compared recycling of the separated solid and liquid components of the harvested algal biomass with recycling un-separated biomass to explore potential mechanisms that could account for the increased settleability and productivity of *P. boryanum*.

4.2. Materials and methods

In order to undertake multiple replicates, mesocosms were used (twelve including controls). The experiments were conducted next to the pilot-scale HRAPs that were also monitored, so that the validity of using the mesocosms to represent HRAP's could be verified.

4.2.1. Operation of the pilot-scale HRAPs with recycling

Full details of the operation of the two pilot-scale HRAPs (surface area: 31.8 m², depth: 0.30 m, volume: 8 m³, wastewater: primary sewage (0.5-1 m³/d)) one with recycling (HRAP_r) and one control without recycling (HRAP_c) are described in Chapter 2.

4.2.2. HRAP mesocosm experiments

Twelve replicate mesocosms (plastic containers with a water depth of 0.3 m; filled volume of 18 L; surface area: 0.07 m²) were set-up and operated next to the two pilot-scale HRAPs. The containers were foil-wrapped to ensure that sunlight only entered through the mesocosm water surface. The experiments were sequentially conducted over three seasons (Experiment 1: autumn; Experiment 2: winter; Experiment 3: spring) with each experiment lasting 36-39 days. The mesocosm operational parameters including the pond water used (either HRAP_r or HRAP_c), initial algal dominance, algal recycling rate, and the hydraulic retention time (HRT) are summarized in Table 4.1 for each experiment. A schematic diagram for the mesocosm experimental set-up is given in Figure 4.1.

Table 4.1: The mesocosm operational parameters including the pond water used (either HRAP_r or HRAP_c); initial algal dominance; algal recycling rate; and the hydraulic retention time (HRT) in each experiment.

Period	Experiment 1				Experiment 2				Experiment 3			
	16 April - 25 May 2010 (Autumn)				26 July - 31 August 2010 (winter)				4 October - 12 November 2010 (Spring)			
Pond water used	HRAP _r ⁽¹⁾		HRAP _c ⁽²⁾		HRAP _r		HRAP _c		HRAP _r			
Initial algal dominance (%)	67.5% <i>Pediastrum</i> sp. 32.5% Unicellular diatom		50% <i>Dictyosphaerium</i> sp. 38% Unicellular diatom 10% <i>Pediastrum</i> sp. 2% <i>Scenedesmus</i> sp.		98.5% <i>Pediastrum</i> sp. 1.2% Unicellular diatom 0.3% <i>Scenedesmus</i> sp.		73.6% <i>Dictyosphaerium</i> sp. 10.4% <i>Scenedesmus</i> sp. 9.5% Unicellular diatom 6.5% <i>Pediastrum</i> sp.		90.4% <i>Pediastrum</i> sp. 9.2% <i>Dictyosphaerium</i> sp. 0.4% <i>Scenedesmus</i> sp.			
Mesocosm (abbreviations)	M _c ⁽³⁾	M _r ⁽⁴⁾	M _c	M _r	M _c	M _r	M _c	M _r	M _c	M _L ⁽⁵⁾	M _S ⁽⁶⁾	M _r
Recycling : Total biomass	-	√	-	√	-	√	-	√	-	-	-	√
: Solid component only ⁽⁷⁾	-	-	-	-	-	-	-	-	-	-	√	-
: Liquid component only ⁽⁷⁾	-	-	-	-	-	-	-	-	-	√	-	-
Algal recycling rate (g recycled/ kg produced/ d)	-	61-193	-	51-186	-	65-119	-	57-192	-	-	48-161	50-161
HRT (d)	Designed				8				6			
MCRT (d) ⁽⁸⁾	6.0±0.6	6.9±0.3	6.0±0.6	6.8±0.3	7.8±1.0	9.4±0.3	7.8±1.0	10.2±0.3	6.0±0.8	6.0±0.8	6.7±0.2	6.7±0.2

Note: (1) Pilot-scale HRAP with recycling (HRAP_r)

(2) Pilot-scale HRAP without recycling (HRAP_c)

(3) M_c: Control mesocosms without recycling

(4) M_r: Mesocosms with recycling total biomass (the solid + liquid)

(5) M_L: Mesocosms with recycling the liquid component only

(6) M_S: Mesocosms with recycling the solid component only

(7) Harvested biomass was centrifuged and the filtered supernatant (the liquid component) was then recycled to M_L. The remaining solid component was recycled to M_S

(8) Mean cell residence time (MCRT): calculated using Equation 4.1

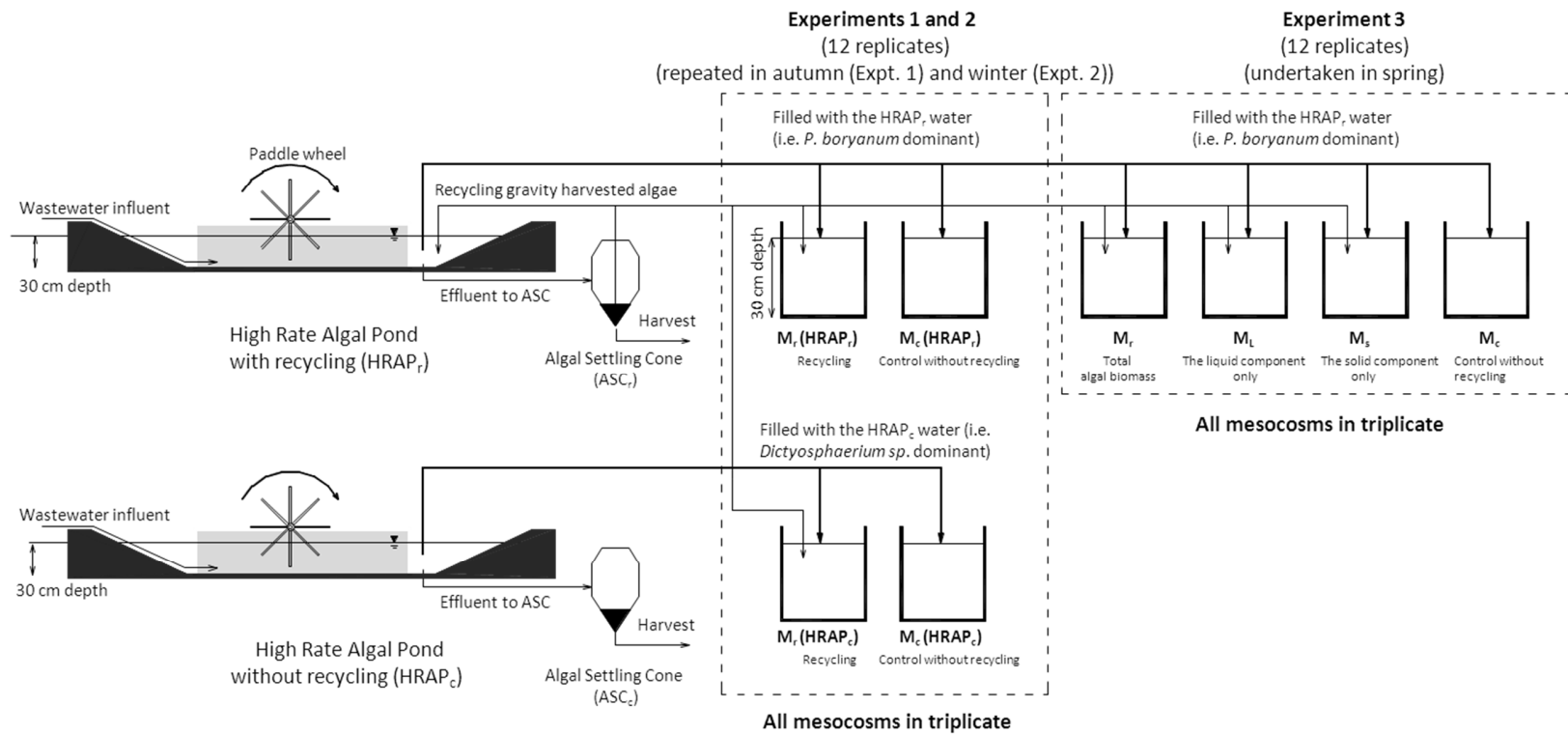


Figure 4.1: Schematic diagram for the operation of mesocosms adjacent to the pilot-scale wastewater treatment HRAPs in Experiments 1-3.

In Experiments 1 and 2, six of the twelve mesocosms were initially filled with water from HRAP_r dominated by *P. boryanum* (a rapidly settleable alga) and the other six with water from HRAP_c dominated by *Dictyosphaerium* sp. (a poorly-settleable alga). The HRAP waters used for the mesocosms were pre-filtered using a 200 µm mesh to remove large invertebrates (e.g. *Daphnia* sp. or *Moina* sp.) to avoid potential algal grazing. Of the six, three were operated with recycling (M_r) and three without recycling (as controls, M_c). For example, the triplicate mesocosms denoted as M_r(HRAP_r) were initially filled with HRAP_r water and operated with recycling.

Each day settled biomass was removed from the bottom of the algal settling cone (ASC_r) (*P. boryanum* dominated biomass) and 2.5 ml was added to the mesocosms (M_r), which was the same algal recycling rate as that used in the pilot-scale HRAP_r. Ultimately, because we wished to determine the net biomass productivity, the mass of solids that was recycled was subtracted from the total biomass yield from the mesocosms.

Recycling extended the mean cell residence time (MCRT) in the mesocosms (M_r) and was calculated using Equation 4.1.

$$MCRT = \frac{V X}{Q_c X - Q_{re} X_h} \quad \text{Equation 4.1}$$

Where;

$MCRT$ = Algal mean cell residence time (d)

V : Mesocosm volume (m³)

X : Mesocosm biomass concentration (VSS, g/m³)

Q_c : Net evaporation compensated HRAP effluent flow rate (m³/d)

Q_{re} : Biomass recycled per day (1 L/d)

X_h : Harvested biomass concentration (VSS g/L)

The mesocosms were operated as semi-continuous cultures with the same HRT as the pilot-scale HRAPs (for the time of year) by daily replacement (at ~9 am) of a portion of the mesocosm water with primary settled sewage. During Experiments 1 and 3 (conducted in the New Zealand autumn and spring respectively) a 6 d HRT was maintained by replacing 3 L of mesocosm water each day with 3 L primary sewage. Due to rainfall and/or evaporation the volume removed each day varied slightly, but this was easily managed by removing the water down to a 15 L volume (marked by a line around the side of the bucket) and then 3 L of primary sewage was added to make 18 L total water volume. During Experiment 2 (conducted in the New Zealand winter), an 8 d HRT was maintained by replacing 2.3 L of mesocosm water each day.

To further investigate why recycling triggered the effects observed in Experiments 1 and 2, Experiment 3 was conducted to compare the effect of recycling the separated solid and liquid components of the recycled harvested biomass. All twelve mesocosms were filled with water from the pilot-scale HRAP_r (*P. boryanum* dominance of ~90%): three in which the separated solid component was recycled (M_S); three in which the separated liquid component was recycled (M_L); and three in which the total harvested biomass (both solid and liquid components, M_r) was recycled; and three control mesocosms without recycling (M_c).

The solid and liquid components of the harvested biomass collected from ASC_r were separated by centrifugation (3000 rpm for 5 minutes), with the supernatant (liquid component) recycled to M_L and the solid component re-suspended with de-chlorinated water and then recycled back to M_S at the same mass recycling rate as used for M_r . All other operational conditions and measurements were the same as in Experiments 1 and 2.

4.2.3. Measurement of biomass productivity and settleability

Mesocosm effluent samples (100 ml) were taken 2-3 times a week during each experiment to measure volatile suspended solids (VSS) according to Standard Methods (APHA 2008) and used to determine biomass productivity. Biomass productivity was calculated using Equation 4.2 which includes subtracting the increase in VSS concentration of M_r and M_S due to the recycling from the measured VSS concentration and adjusting for any increase or decrease in daily outflow from the mesocosms due to rainfall or evaporation. Daily rainfall

and evaporation data for the experimental site were downloaded from NIWA's National Climate Database (<http://cliflo-niwa.niwa.co.nz/>).

$$P = \frac{(C \times Q_c) - R}{A} \quad \text{Equation 4.2}$$

$$Q_c = Q_{inf} + ((\text{rainfall} - \text{evaporation}) \times \text{mesocosm surface area})$$

Where;

P: Biomass productivity (g/m²/d)

C: Biomass concentration in the mesocosm (VSS, g/m³)

A: Mesocosm surface area (0.07 m²)

R: Biomass recycled per day (2.5 ml/d)

Q_c: Adjusted daily mesocosm outflow (m³/d)

Q_{inf}: Daily inflow (3 L/d for Experiment 1 and 3, and 2.3 L/d in Experiment 2)

The settleability of the biomass in the effluent removed from each mesocosm was measured in the laboratory over 1 hour using 1 litre Imhoff cones. A 50 ml water sample was then taken from the mid-depth of the Imhoff cone and used to measure VSS, which was compared with the VSS of the mesocosm effluent to give the 1h-settleability.

4.2.4. Microscopic analysis

Microscopic image analysis was conducted three times during each experiment (Experiment 1: Day 0, 18 and 39; Experiment 2: Day 0, 18 and 36) to measure algal dominance based on the biovolume (µm³/ml) of each species present. This was calculated by multiplying the number of colonies of a species (counts/ml) by the average colony biovolume (µm³/colony). Full details of the methods and equipment used to identify algal species, count and measure

the dimensions of algal cells/colonies, and calculate algal biovolume were described in Chapter 2.

Microscopic image analysis was extended in Experiment 3 (measured three times on Day 0, 18 and 39) to investigate the influence of recycling the solid or liquid components of harvested biomass on *P. boryanum* colony size and the relative proportions (the number of colonies, biovolume) of different life-cycle stages (juvenile, growth, and reproductive) of *P. boryanum* in the mesocosms. ‘Juvenile’ colonies have a diameter of typically less than 20 µm and are still enclosed within a vesicle; ‘growth’ colonies are actively growing and; ‘reproductive’ colonies contain at least one cell that has released a new juvenile colony (Davis 1967; Millington 1981; Millington et al. 1981). Since the number of cells per *P. boryanum* colony varies (colonies predominantly have 8, 16 or 32 cells but some have 4 or 64 cells), the influence of recycling on the diameter and biovolume of 8-, 16- and 32-celled *P. boryanum* colonies was also determined. *P. boryanum* colonies of each life-cycle stage are shown in Figure 4.2 and the complete life-cycle of *P. boryanum* is described in Chapter 5.

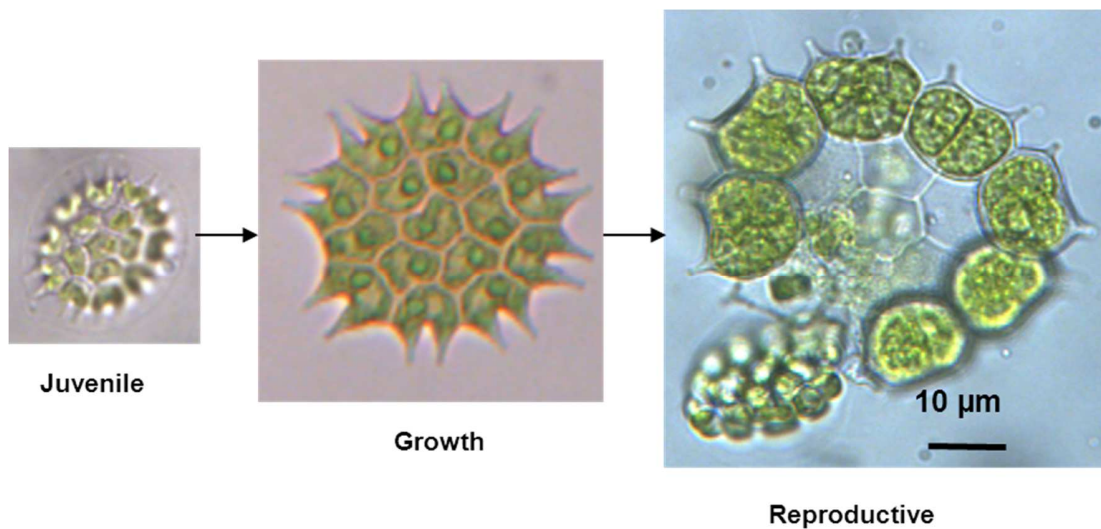


Figure 4.2: Life-cycle stages (juvenile, growth and reproductive) of *P. boryanum*.

4.2.5. Particle size distribution of algal cells, colonies and algal/bacterial aggregates

On the final day (Day 39) of Experiment 3, mesocosm effluent samples (100 ml) were taken to the University of Auckland for analysis of the size distribution of algal cells, colonies and algal/bacterial aggregates. A Malvern Mastersizer 2000[®] (Malvern Instruments Ltd., Malvern, UK, using laser diffraction with stirrer speed: 250 rpm; pump: 900 rpm; ultrasonic: off) was used following the protocol developed by Houghton et al (2002) and Ehlers et al (2011). Three consecutive measurements were made of each sample and averaged to produce a particle size distribution curve (recorded as percent particle volume in 70 discrete sizes ranging between 0.45 and 2000 μm).

4.3. Results and Discussion

4.3.1. Effect of recycling on species dominance

Given previous results that recycling can significantly change algal dominance in pilot-scale HRAP (Chapter 2 and 3), Experiments 1 and 2 attempted to replicate this effect in triplicate mesocosms studied over two different seasons. In Experiment 1, recycling was confirmed to increase *P. boryanum* dominance in M_r (Figure 4.3-1b&d) compared with the controls (M_c) (Figure 4.3-1a&c) in all cases. For example, recycling to the *P. boryanum* dominated mesocosms ($M_r(\text{HRAP}_r)$) increased the dominance of *P. boryanum* from 67 to greater than 90% by Day 39 (Figure 4.3-1d). In particular, recycling to the *Dictyosphaerium* sp. dominated mesocosms ($M_r(\text{HRAP}_c)$) increased the dominance of *P. boryanum* from less than 10% to 67% by Day 39 (Figure 4.3-1b), while the dominance of *Dictyosphaerium* sp. was reduced from 50% to less than 10% during the same period. In contrast, the dominance of *P. boryanum* in the *P. boryanum* dominated control mesocosms without recycling ($M_c(\text{HRAP}_r)$) declined from 68% to less than 40% (Figure 4.3-1c), while the dominance of *Dictyosphaerium* sp. in the *Dictyosphaerium* sp. dominated control mesocosms ($M_c(\text{HRAP}_c)$) increased from 50 to ~70% by Day 39 (Figure 4.3-1a).

The ability of the mesocosms to replicate the change in algal dominance in the pilot-scale HRAPs was confirmed by comparing the mesocosm results with data collected from the pilot-scale HRAPs during the experimental period (previously reported in Chapter 2). It was

found that the dominance of *P. boryanum* in the mesocosms ($M_r(\text{HRAP}_r)$) increased to 90% (Figure 4.3-1d), which was similar to the pilot-scale HRAP_r with 85%, and the dominance of *Dictyosphaerium* sp. in the mesocosms ($M_c(\text{HRAP}_c)$) increased to 68%, which was almost the same as the pilot-scale HRAP_c with 70% (Figure 2.3 in Chapter 2).

This increase in *P. boryanum* dominance in the mesocosms with recycling (i.e. recycling *P. boryanum* dominated biomass) was further confirmed using the same experimental set-up in Experiment 2. Recycling increased the dominance of *P. boryanum* in the *Dictyosphaerium* sp. dominated mesocosms ($M_r(\text{HRAP}_c)$) from 6.5 to 42% (Figure 4.3-2b) or maintained the dominance of *P. boryanum* in the *P. boryanum* dominated mesocosms ($M_r(\text{HRAP}_r)$) at greater than 90% (Figure 4.3-2d) compared with the controls (Figure 4.3-2a&c).

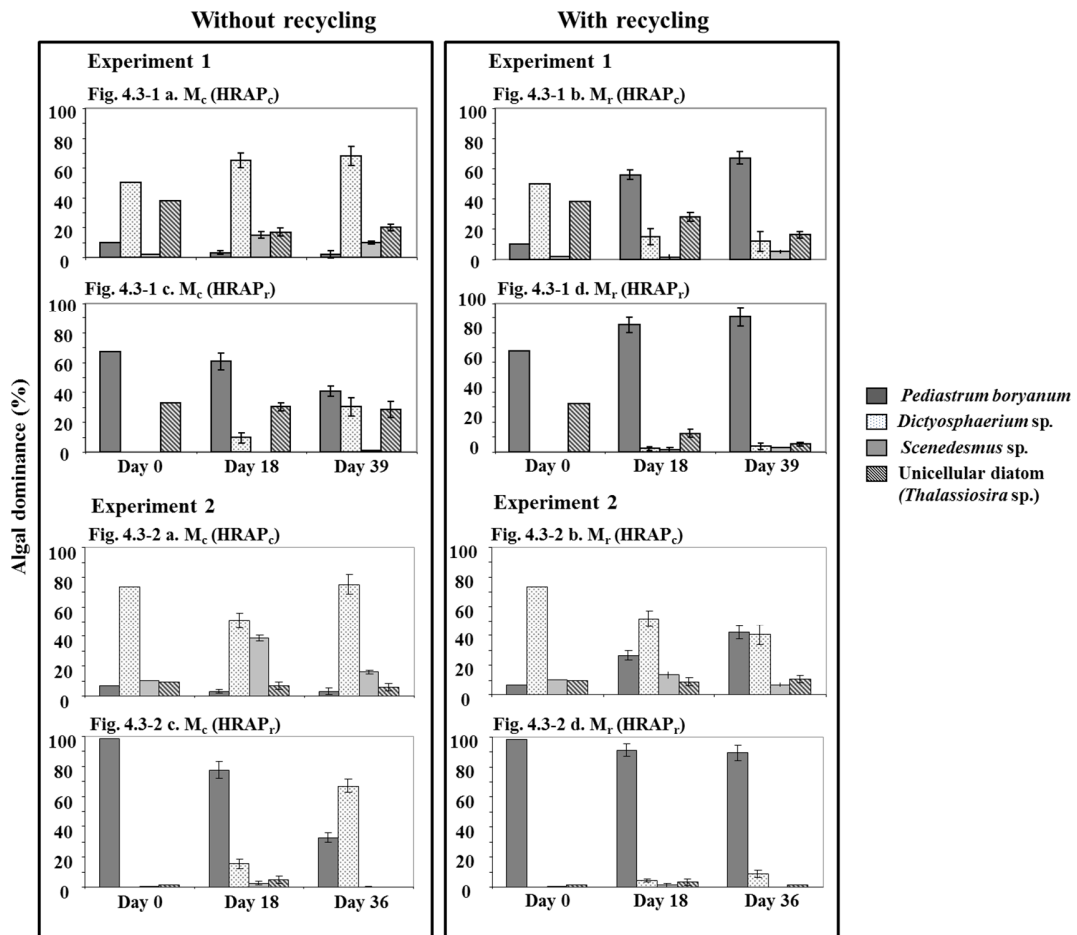


Figure 4.3: Algal dominance (based on biovolume; mean of triplicate and \pm s.d.) in the mesocosms with (M_r) and without recycling (M_c) in Experiments 1 and 2.

Weissman and Benemann (1979) conducted algal competition experiments in the laboratory with mixed cultures of *Chlorella* sp. (a unicellular green alga, cell diameter of 2-8 μm) and *Spirulina* *geitleri* (a filamentous blue-green alga, $\sim 6 \mu\text{m}$ in diameter and 100-400 μm long). They also found that recycling selectively harvested *Spirulina* biomass (using a 26 μm mesh microstrainer) maintained the dominance of *Spirulina* in the mixed culture, compared with cultures without recycling in which *Chlorella* sp. always out-competed *Spirulina*. The work of Weissman and Benemann (1979) showed that species control was possible for algae with substantial size differences. However, the results from these mesocosm studies combined with the previous pilot-scale HRAP studies (reported in Chapter 2 and 3) have shown for the first time in the literature that species control is also possible for similarly sized co-occurring algal colonies.

In order to further investigate why recycling caused the algal dominance to change, Experiment 3 determined the effect of recycling the separated solid and liquid components of the harvested algal biomass back to *P. boryanum* dominated mesocosms (a schematic is shown in Figure 4.1).

Recycling the solid component (M_S) consistently maintained the dominance of *P. boryanum* at about 90% over the 39 day experimental period in the similar way to that shown in the mesocosms with total biomass recycling (M_T) (Figure 4.4c&d). However, recycling the liquid component (M_L) reduced the dominance of *P. boryanum* from 90% to less than 70%, which was a similar decline to that shown in the control mesocosms (M_C) without recycling (from 90 to 67%) (Figure 4.4a&b). Furthermore, both M_C and M_L had a similar increase in the dominance of *Dictyosphaerium* sp. (from 5% to $\sim 30\%$ by Day 39). These results indicate that recycling the solid component of the harvested biomass (i.e. the *P. boryanum* colonies) was the key to promote the dominance of *P. boryanum* in the mesocosms.

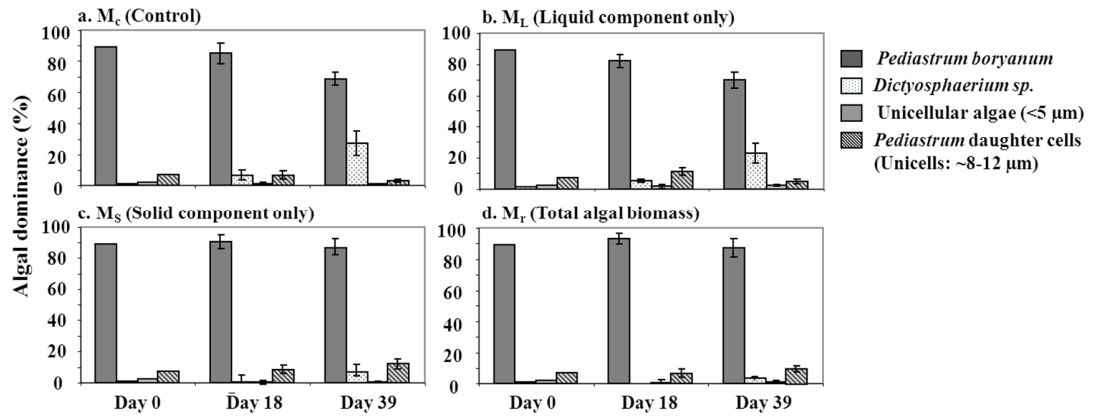


Figure 4.4: Algal dominance (based on algal biovolume; mean of triplicate and \pm s.d.) in the mesocosms with recycling the liquid component (M_L), the solid component (M_S), and total biomass (M_T) and the control (M_c) without recycling in Experiment 3 (Note: All mesocosms were initially filled with *P. boryanum* dominated pilot-scale HRAP_r water).

4.3.2. Effect of recycling on settleability

Since species type (e.g. either colonial or unicellular algae) can significantly influence settleability (as described in Chapter 2), the effect of the shift in algal dominance found in Section 4.3.1 was quantified in Figure 4.5 and summarized with statistical analysis in Table 4.2.

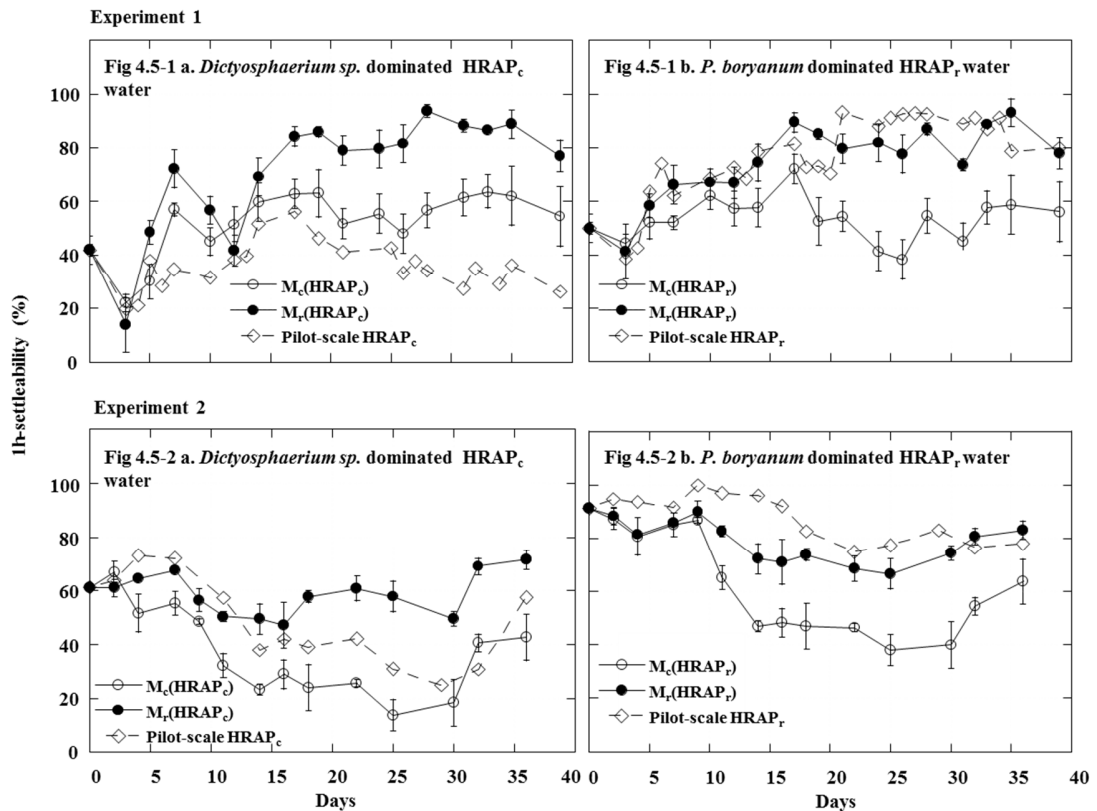


Figure 4.5: 1h-settleability (mean of triplicate and \pm s.d.) of the mesocosms effluents with (M_r) and without recycling (M_c), and the pilot-scale HRAP effluents during Experiments 1 and 2.

As shown in Figures 4.5-1a and 4.5-2a, when the mesocosms were initially filled with water from the pilot-scale HRAP_c (which had no recycling) and then recycling was implemented in the mesocosms (M_r (HRAP_c)), 1h-settleability increased. Furthermore, this effect was reversible, as shown in Figures 4.5-1b and 4.5-2b, when mesocosms were filled with water from the pilot-scale HRAP_r (which had recycling), but no recycling was then provided in the mesocosms (M_c (HRAP_r)), 1h-settleability decreased. The change in all cases was very similar at approximately 20% (p -value: <0.005 , one-way ANOVA; Table 4.2).

As previously addressed, Experiment 3 investigated the relative significance of recycling either the separated solid or liquid components of harvested biomass. It was found that recycling both the solid (M_s) and liquid (M_L) components maintained similar 1h-settleability (78% and 79% respectively) compared to 79% when the total biomass (the combined solid

and liquid components) was recycled (M_r) (Figure 4.6). In contrast, the control mesocosms (M_c) which had no recycling decreased 1h-settleability to less than 60% (as had been observed in Experiments 1 and 2).

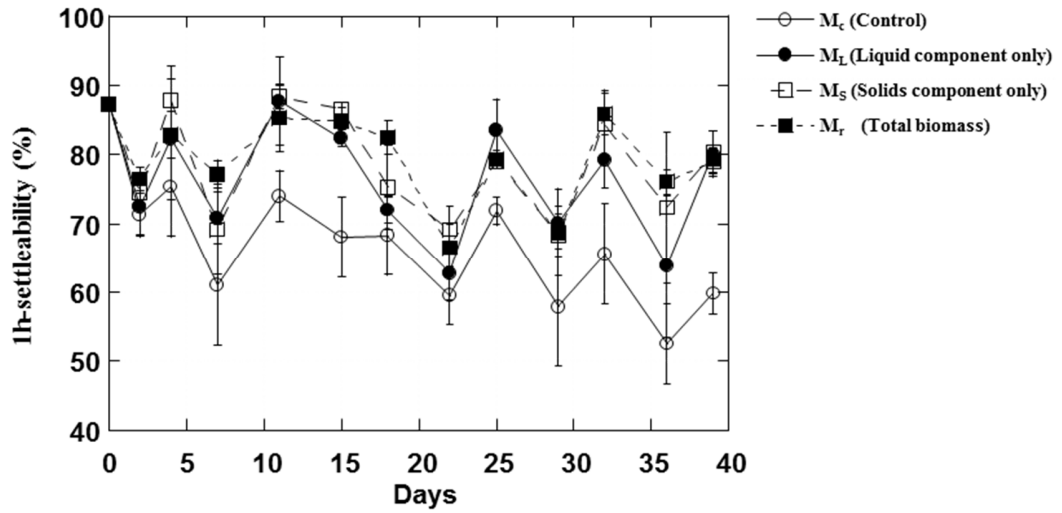


Figure 4.6: 1h-settleability (mean of triplicate and \pm s.d.) of the mesocosm effluents with recycling liquid components (M_L), the solid component (M_S), and total biomass (M_r) and the control without recycling (M_c) in Experiments 3.

Table 4.2: Biomass productivity, 1h-settleability, and statistical analysis for the effect of recycling on biomass productivity and 1h-settleability during Experiment 1-3.

Inoculum	Experiment 1				Experiment 2				Experiment 3			
	HRAP _r		HRAP _c		HRAP _r		HRAP _c		HRAP _r			
Mesocosm	M _c (HRAP _r)	M _r (HRAP _r)	M _c (HRAP _c)	M _r (HRAP _c)	M _c (HRAP _r)	M _r (HRAP _r)	M _c (HRAP _c)	M _r (HRAP _c)	M _c	M _L	M _S	M _r
Ave. algal biomass productivity (g/m ² /d) ⁽¹⁾	7.0±0.8	9.6±0.8	7.8±0.6	10.6±0.5	5.4±0.5	7.3±0.3	4.6±0.2	6.4±0.2	9.5±0.7	10.1±0.4	13.9±0.5	14.1±0.6
% increase compared with the control (M _c)	-	37.1±0.8	-	35.9±0.5	-	35.2±0.4	-	39.1±0.2	-	-	37.6±0.5	39.6±0.6
1 h settleability (%)	54.6±6.4	77.6±5.3	55.5±5.6	79.0±3.9	59.1±5.4	81.1±4.1	36.4±5.2	59.4±4.4	68.3±5.7	78.3±4.0	79.1±3.2	79.3±2.3
% increase compared with the control (M _c)	-	23.0±5.5	-	23.5±4.2	-	22.0±3.2	-	23.0±3.3	-	10.0±4.0	10.8±3.2	11.0±2.1
Variables ⁽²⁾	<i>p-value</i>											
Effect of recycling on Total biomass	0.005		0.005		0.008		0.000		-	n/a	n/a	0.001
1 h settleability Solid fraction only	n/a		n/a		n/a		n/a		-	n/a	0.003	n/a
Liquid fraction only	n/a		n/a		n/a		n/a		-	0.018	n/a	n/a
Significance	>99.5% confidence		>99.5% confidence		>95% confidence		>99.5% confidence		-	>95% confidence	>99.5% confidence	>99.5% confidence
Effect (all positive)	Very strong		Very strong		Strong		Very strong		-	Strong	Very strong	Very strong
Effect of recycling on productivity Total biomass	0.028		0.069		0.099		0.000		-	n/a	n/a	0.005
Solid fraction only	n/a		n/a		n/a		n/a		-	n/a	0.008	n/a
Liquid fraction only	n/a		n/a		n/a		n/a		-	0.591	n/a	n/a
Significance	>95% confidence		>90% confidence		>90% confidence		>99.5% confidence		-	>90% confidence	>95% confidence	>99.5% confidence
Effect (all positive)	Strong		Moderate		Moderate		Very strong		-	Moderate	Strong	Very strong
Note: (1) Recycled algal biomass was subtracted from the algal concentration of M _r and M _s (compensated algal productivity) (2) Significance was compared between M _c and M _r for Experiments 1-2 and between M _c and M _S , M _L , M _r for Experiment 3 to investigate the effect of algal recycling on productivity and 1 h settleability (One-way ANOVA analysis)												

The increased 1h-settleability achieved by recycling compared to the control is most probably attributable to the formation of larger sized algal colonies (i.e. *P. boryanum* colonies) and/or algal/bacterial aggregates in the culture. These would have a lower surface area to volume ratio (and therefore reduced drag) resulting in a higher settling velocity.

As shown in Figure 4.4a, when recycling was ceased in the control mesocosms (M_c), there was a subsequent reduction in the dominance of *P. boryanum* and an increase in *Dictyosphaerium* sp. dominance (which is poorly settleable). Since *P. boryanum* colonies are significantly larger (and thus have greater settling velocity) than *Dictyosphaerium* sp. colonies (previously addressed in Chapter 2), the shift in algal dominance to the smaller colonies would explain the decrease in the 1h-settleability observed in the control mesocosms. However, a similar loss of *P. boryanum* dominance was also observed in M_L (Figure 4.4b), and thus it appears that there is another mechanism that may contribute to the changes in biomass settleability.

Extracellular polymeric substances (EPS): The formation of algal/bacterial aggregates has been observed for species such *Scenedesmus* sp. (a common wastewater pond alga) due to the presence of EPS excreted either by algae themselves or by bacteria (e.g. *Paenibacillus polymyxa*) (Laspidou and Rittmann 2002; Lee et al., 2009; Ehlers et al., 2011; Kim et al., 2011). Laspidou and Rittmann (2002) reported that most bacteria produce extracellular polymeric substances (EPS), which promote the formation of microbial aggregates. Environmental stresses such as extreme pH, temperature or nutrient depletion may induce bioflocculation by promoting EPS production (Benemann and Oswald 1996; Higgins and Novak 1997; Lee et al. 2009). This suggests that in the compacted and dark conditions at the bottom of the algal settling cone (ASC_r), environmental stressors such as low oxygen concentrations due to respiration may have triggered an increase in the release of EPS by the algae and/or bacteria into the surrounding liquid. Recycling of this liquid component containing a high EPS content could have promoted formation of larger algal/bacterial aggregates in the M_L mesocosms compared with the controls (M_c).

In order to assess the effect of recycling each component of harvested biomass on the formation of large aggregates, the particle size distribution of the mesocosm effluents was determined in Experiment 3. As shown in Figure 4.7, recycling the solid and/or liquid

components similarly increased the upper size of the particle distribution from 400 μm (for M_c) to 1500 μm (for M_r , M_S and M_L) which included a second peak of particle size of 500-800 μm . These results further imply that EPS in the recycled liquid component contributed to the formation of large aggregates of biomass.

Recycling the solid component extended the mean cell residence time (MCRT) of *P. boryanum* colonies, enabling the colonies to grow larger. The previous pilot-scale HRAP study (Chapter 2) presented that recycling extended the mean cell residence time (MCRT) in the pond by 0.5 d in summer and 3.4 d in winter. This resulted in an increase in the average size (biovolume) of *P. boryanum* colonies of 50-80% in HRAP_r compared to that in the control HRAP_c. This previous finding was further confirmed in the replicate mesocosms in Experiment 3. As shown in Figure 4.8, recycling the solid component (M_S) or total algal biomass (M_r) similarly increased the average colony biovolume (by 2-5 times) compared with recycling the liquid component (M_L) or the control (M_c) without recycling.

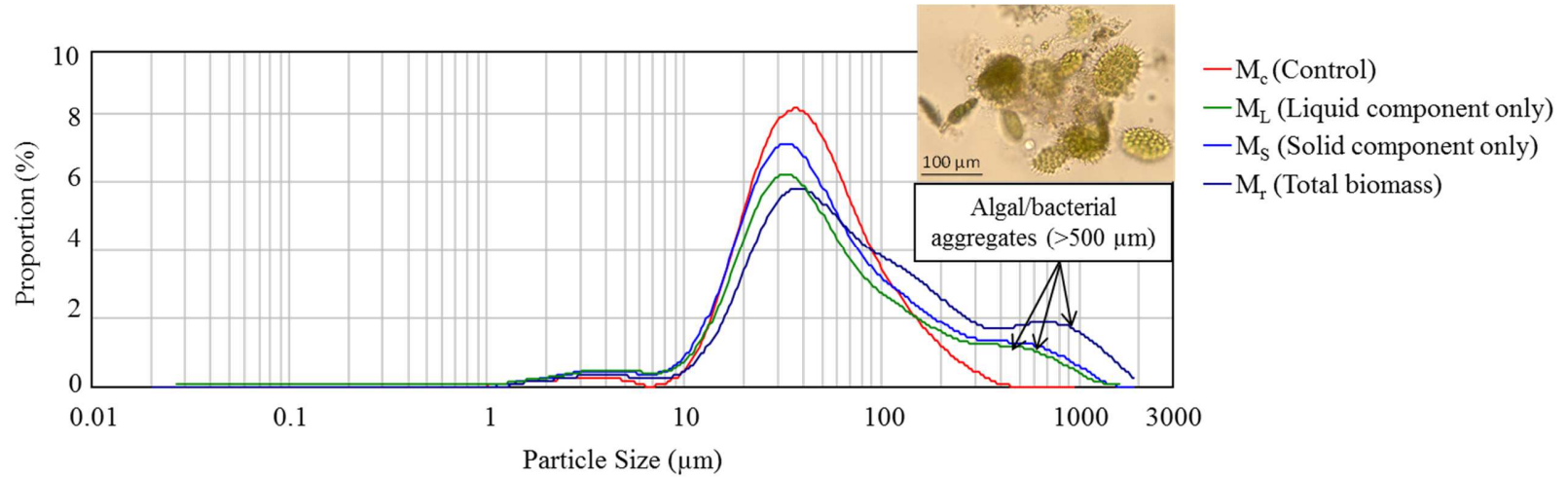


Figure 4.7: Average particle size distribution of algal cells, colonies and algal/bacterial aggregates in the mesocosms with recycling the liquid component (M_L), the solid component (M_S), and total biomass (M_r) and the control (M_c) without recycling in Experiment 3 (on Day 39).

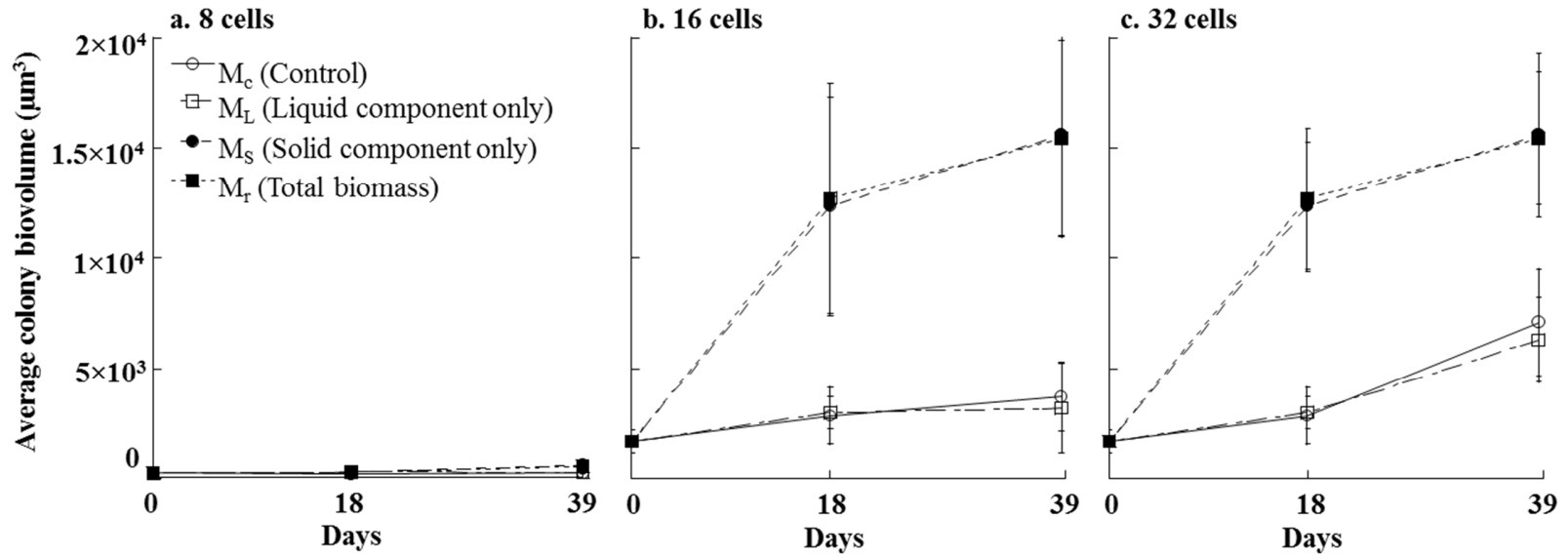


Figure 4.8: Average biovolume of 8-, 16- and 32-celled *P. boryanum* colonies in the mesocosms (mean of triplicate and \pm s.d.) with recycling of the liquid component (M_L), the solid component (M_S), the total biomass (M_T) and the control (M_c) without recycling in Experiment 3.

4.3.3. Enhancing biomass productivity with recycling

In addition to the settleability as discussed above, biomass productivity is also of crucial significance in optimizing HRAPs for wastewater treatment and biofuel production. In this section, the effect of recycling on net biomass productivity is presented in Figure 4.9 and summarized with statistical analysis in Table 4.2.

The biomass productivity in all the mesocosms declined during Experiment 1 (Figure 4.9-1). This is due to the autumn seasonal decrease in both average solar radiation (from 11.9 ± 2.5 to 6.8 ± 3.1 MJ/m²/d) and average water temperature (from 16.9 ± 1.8 to 11.0 ± 2.1 °C). However, the mesocosms with recycling (M_r) had greater (approximately 36% higher) biomass productivity than the controls (M_c) without recycling (Figure 4.9-1a&b; summarized in Table 4.2). The results of Experiment 2 (conducted in winter) further confirmed that recycling improved biomass productivity (by greater than 20%) in M_r compared with the controls (M_c) (Figure 4.9-2a&b; summarized in Table 4.2).

The biomass productivities (Figure 4.9) and 1h-settleabilities (Figure 4.5) of the pilot-scale HRAPs at the time when Experiments 1 and 2 were conducted were similar to those of the mesocosms that were operated in the same way (i.e. $HRAP_r$ and $M_r(HRAP_r)$; $HRAP_c$ and $M_c(HRAP_c)$). These results indicate that the experiments conducted using 18 L, 0.3 m deep mixed mesocosms were a good representation of the performance of pilot-scale HRAPs in terms of biomass productivity, settleability and algal dominance.

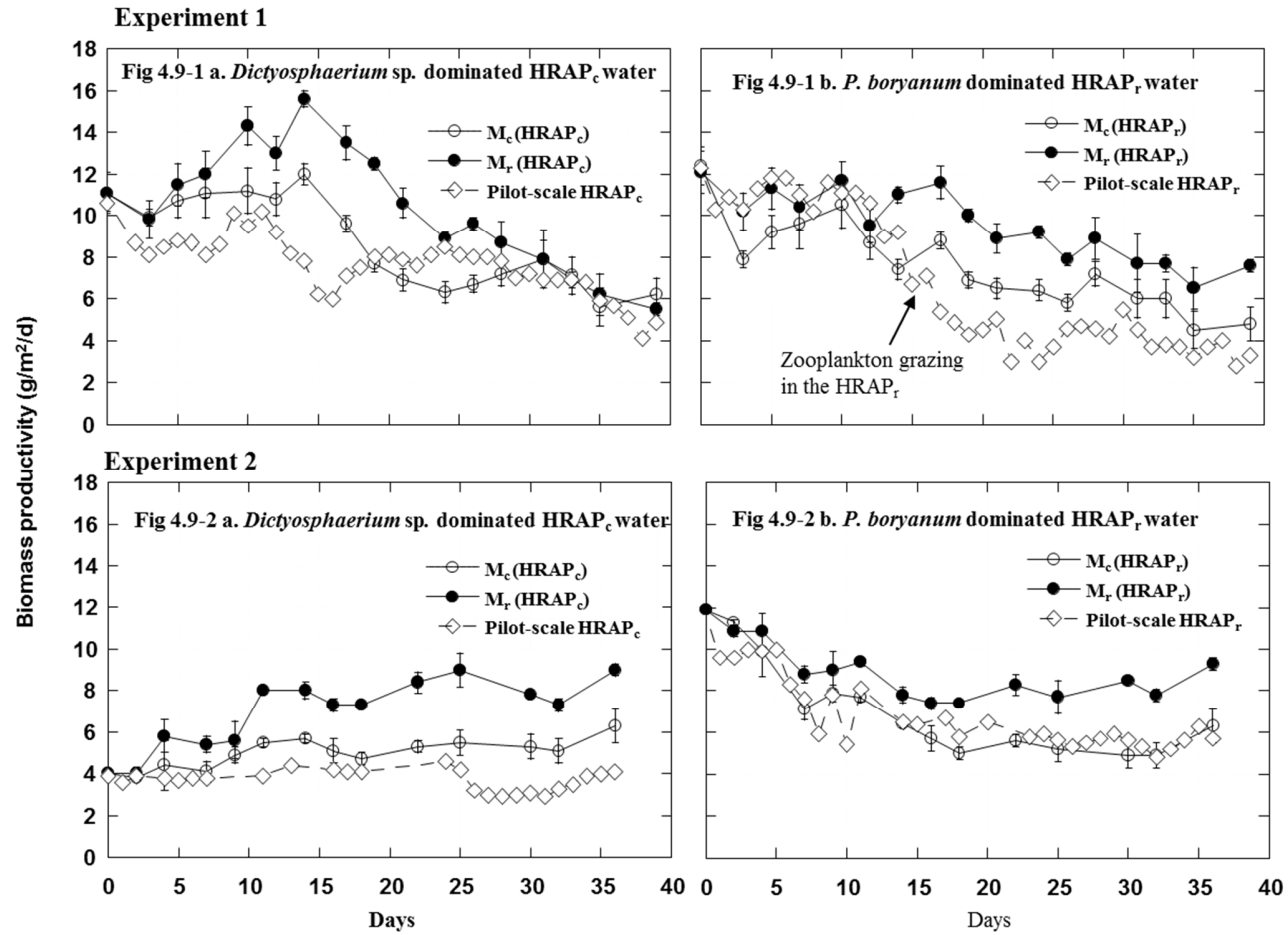


Figure 4.9: Biomass productivity in the mesocosms (mean of triplicate and \pm s.d.) with (M_r) and without recycling (M_c) in Experiments 1 and 2.

In Experiment 3, recycling the solid component (M_S) and total biomass (M_r) both increased biomass productivity by about 40% (M_S : 13.9 ± 0.5 g/m²/d; M_r : 14.1 ± 0.6 g/m²/d) compared with the control (M_c) or recycling the liquid component (M_L) (M_c : 9.5 ± 0.7 g/m²/d; M_L : 10.1 ± 0.4 g/m²/d) (Figure 4.10; *p*-value: <0.01, Table 4.2). This result indicated a link between recycling the solid component (i.e. *P. boryanum* colonies) and the improvement of biomass productivity.

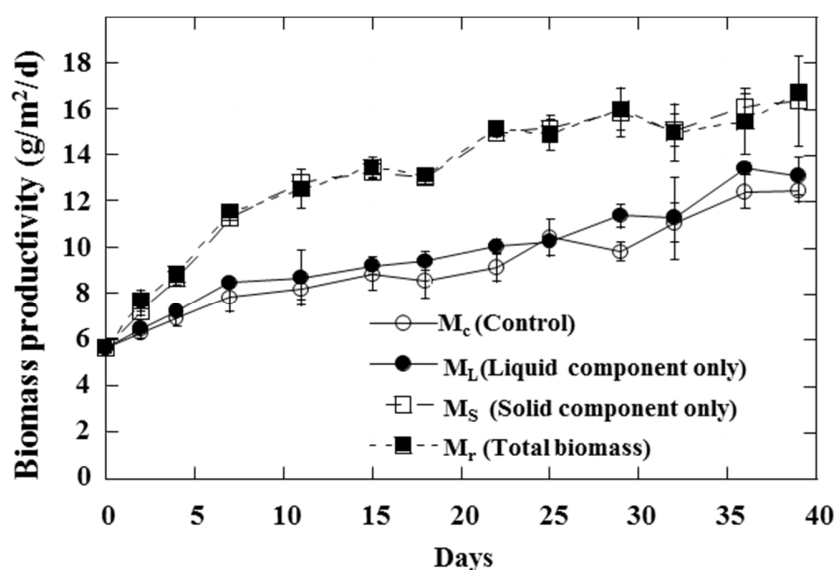


Figure 4.10: Biomass productivity in the mesocosms (mean of triplicate and \pm s.d.) with recycling the liquid component (M_L), the solid component (M_S), total biomass (M_r) and the control (M_c) without recycling in Experiment 3.

4.3.3.1. Potential mechanisms behind the improved biomass productivity with recycling

There are several potential mechanisms that may explain why recycling resulted in (or would appear to result in) an increase in biomass production. These are discussed below:

1. Could recycling have increased the production of bacterial biomass as opposed to algal biomass?

The relative ratio of algae (*P. boryanum*), algal/bacterial aggregates, picophytoplankton, and bacterial-cells was previously estimated by measuring both the size and particulate organic carbon (POC) of each component in the pilot-scale HRAP_r in May 2009 (Broekhuizen et al.

2012). This study determined that more than 60% of the total biomass in the mixed pond water culture was algae as opposed to only about 5% as bacteria, with the remaining 35% of algae and bacterial aggregates. Moreover Experiment 3 of the current study found a strong positive correlation ($r^2=0.941$) between the mesocosm biomass concentration (VSS) and total algal biovolume (as $\mu\text{m}^3/\text{ml}$) (Figure 4.11). This indicates that recycling did not change the relative proportions of algae and bacteria, and thus the increase in biomass productivity was due to a similar increase in both algal and bacterial biomass.

To investigate the influence of the wastewater bacteria on the increase of biomass productivity with recycling, *P. boryanum* was grown in pure culture on synthetic growth media under laboratory conditions (Appendix A). *P. boryanum* pure cultures with recycling of harvested algae were shown to have higher algal productivity (by 11% at a 4 d HRT and 38% at 3 d HRT) than the controls that had no recycling, confirming that the presence of wastewater bacteria was not necessary to enhance algal productivity with recycling.

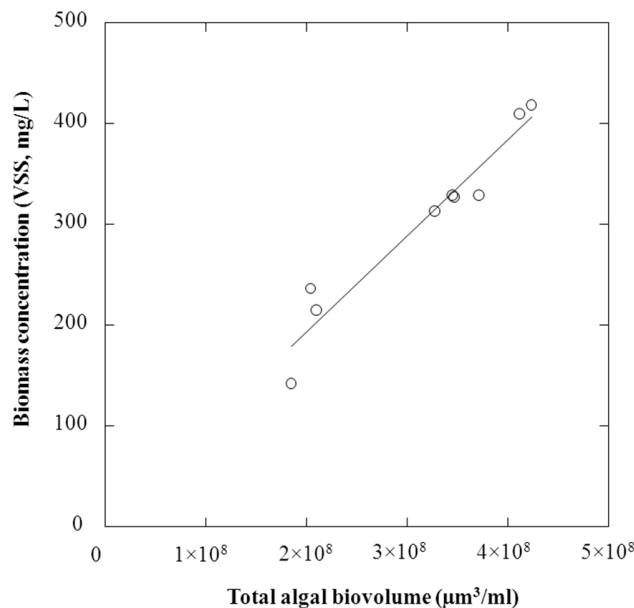


Figure 4.11: The correlation between the total algal biovolume ($\mu\text{m}^3/\text{ml}$; integrating biovolume of three algal species including *P. boryanum*, *Dictyosphaerium* sp. and unicellular algae) and biomass concentration (VSS) in all the mesocosms in Experiment 3 ($r^2=0.941$).

2. Is higher biomass production in the mesocosms with recycling due to the presence of larger algal colonies and aggregates because they were less susceptible to grazing by invertebrates, as has been suggested by Schluter et al (1987), Kagami et al (2005) and Hambright et al (2007)?

This was not a factor that caused the increased biomass production measured in these mesocosm studies, because the inoculums used for the mesocosms (i.e. HRAP culture) were initially filtered through a 200 μm mesh to remove large invertebrates (e.g. *Daphnia* sp. or *Moina* sp.). Moreover, no invertebrates were found during routine microscopic inspection of the mesocosms throughout the experimental periods.

3. Could the increase in biomass production have resulted from a shift in cell composition?

The biomass productivity of the recycled pond (as $\text{g}/\text{m}^2/\text{d}$) could have increased while the overall biomass energy yield (as $\text{kJ}/\text{m}^2/\text{d}$) remained unchanged due to a decrease in energy content of the biomass. However, the pilot-scale HRAP study (Chapter 3) showed that as well as having a higher biomass productivity in HRAP_r with recycling, the energy content of *P. boryanum* dominated biomass (21.2 ± 0.3 kJ/g) in the HRAP_r was also slightly higher than that of the *Dictyosphaerium* sp. dominated biomass (20.3 ± 0.8 kJ/g) in the control HRAP_c without recycling.

4. Does *P. boryanum* have a higher specific growth rate than other co-occurring species?

Specific growth rates of three algal species, *P. boryanum*, *Scenedesmus* sp. and *Micractinium* sp. isolated from the HRAP were determined in the laboratory under constant temperature and light conditions (20°C; 250 $\mu\text{Mol}/\text{m}^2/\text{s}$; 12/12 light and dark cycle). *P. boryanum* was found to have a slightly lower specific rate than the other two algae, which commonly occurred in the pond (Table 4.3). Furthermore, in the outdoor mesocosm Experiments 1 and 2 which were conducted in two different seasons, *P. boryanum* did not outgrow the other algal species. Indeed, the dominance of *P. boryanum* in the initially *P. boryanum* dominated control mesocosms without recycling ($M_c(\text{HRAP}_r)$) actually declined from 68% to less than 40% in Experiment 1 (Figure 4.3-1c) and from more than 90% to ~40%

in Experiment 2 (Figure 4.3-2c). The dominance of *Dictyosphaerium* sp., by comparison, increased in the control mesocosms in both experiments.

Table 4.3: Specific growth rates of three algal species (*Scenedesmus* sp. *Micractinium* sp., and *P. boryanum*) isolated from a wastewater treatment pilot-scale HRAP under constant laboratory conditions (Temperature: 20°C; light intensity: 250 $\mu\text{Mol}/\text{m}^2/\text{s}$; 12/12 light and dark cycle).

Algae genus/species	Specific growth rate (d^{-1})
<i>P. boryanum</i>	0.22 \pm 0.03
<i>Scenedesmus</i> sp.	0.24 \pm 0.05
<i>Micractinium</i> sp.	0.27 \pm 0.04

- Are algae unable to fully utilize available light at the short hydraulic retention time (HRT)?

If the pond was being operated with a short HRT, the algal population (concentration) may have been insufficient to fully utilize the incident light energy, resulting in sub-optimal algal biomass production. Implementing recycling increases the mean cell retention time (MCRT) without changing the HRT. In this mesocosm study recycling extended the MCRT by 0.5-1.4 d depending on season (Table 4.1). It is, therefore, feasible that the increased MCRT by recycling potentially contributed to the improved biomass production in the mesocosms (M_s and M_r) by enabling more light to be utilized.

- Could the higher proportion of larger algal colonies by recycling increase the net growth rate of the algae, and thus increase biomass production?

The previous pilot-scale HRAP study (Chapter 3) showed that gravity settling selected larger *P. boryanum* colonies from the HRAP effluent, which were then recycled back to the pond. This implies that recycling increases the relative proportion of large *P. boryanum* colonies compared with the pond that had no recycling. An increase in the proportion of large colonies may have contributed to the increased biomass production in two different ways:

6a. Do larger *P. boryanum* colonies grow faster relative to other life-cycle colonies?

Increasing the proportion of larger colonies by recycling may increase the net growth rate of the algal culture. Tukaj et al. (2003) found that the maximum growth of *Scenedesmus armatus* (as $\mu\text{m}^3/\text{h}$) occurred at when the cells reached about 80% of their full size. If larger *P. boryanum* colonies also have a higher growth rate than other life-cycle stages, then preferentially recycling large *P. boryanum* colonies would therefore have contributed to the observed increase in biomass production. The three different life-cycle stages ('juvenile', 'growth' and 'reproductive') of *P. boryanum* are shown in Figure 4.2.

6b. Does the higher number (and proportion) of large reproductive colonies that results from recycling increase the net growth rate of the algal culture?

Recycling during Experiment 3 not only increased the number, but also the proportion of reproductive colonies in M_S and M_R compared to the control (Figure 4.12a&b). The higher number (and proportion) of reproductive colonies may have contributed to the observed ~4-fold higher juvenile numbers in the mesocosms with recycling (M_S and M_R) compared to those without recycling (M_C and M_L , Figure 4.12c) after 39 days although the controls also had higher contamination (~70% *P. boryanum* dominance in the controls compared with ~90% in the mesocosms with recycling, Figure 4.4). This result implies that if the new juvenile colonies had a higher growth rate than the parent reproductive colony, then the net growth rate of the algal culture would be higher by recycling, which may have contributed to the observed increase in biomass production.

In summary we considered six potential mechanisms to explain the observed increase in biomass productivity achieved from recycling of which only Mechanisms 5 and 6 appear to be plausible.

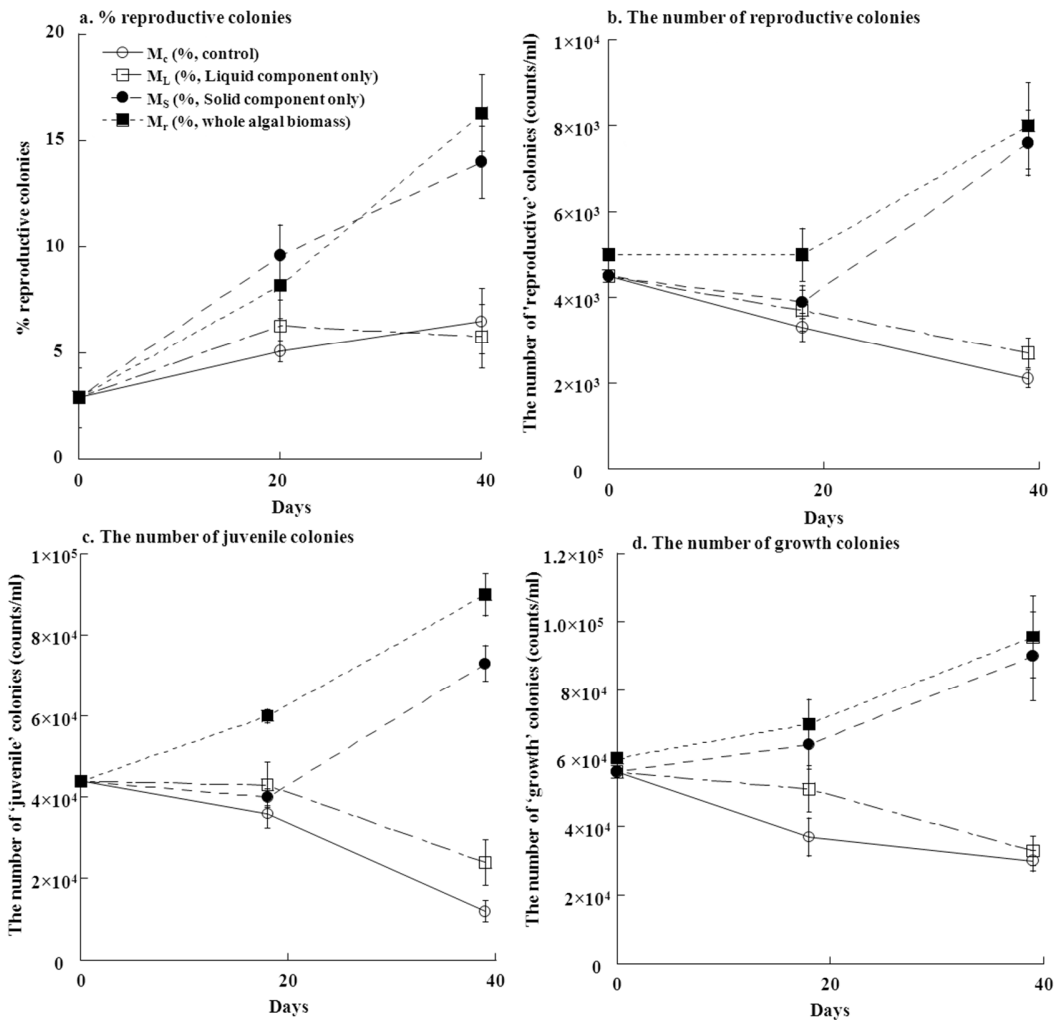


Figure 4.12: a. Proportion of reproductively matured *P. boryanum* colonies; b. the number of reproductive colonies; c. the number of juvenile colonies; d. the number of growth colony in the mesocosms (mean of triplicate and \pm s.d.) with recycling the liquid component (M_L), the solid component (M_S), total algal biomass (M_r) and the control (M_c) without recycling in Experiment 3.

4.4. Conclusions

The mesocosm experiments in this chapter confirmed that recycling gravity harvested biomass promoted *P. boryanum* dominance and improved 1h-settleability by >20% compared with controls that had no recycling. Recycling of the separated solid and liquid components both maintained 1h-settleability at >80% compared with the controls (~68%),

increased the distribution of particle size from 10-400 μm (in the control) to 10-1500 μm , and promoted formation of larger algal/bacterial aggregates ($>500 \mu\text{m}$). While settleability was improved by recycling of the separated 'solid' fraction of harvested biomass (selecting for larger and faster settling colonies), the similar results obtained from recycling the separated 'liquid only' fraction of harvested biomass implies a second mechanism. This may be attributable to the increased production of extracellular polymeric substances that improve settleability. Recycling also improved biomass productivity by $>25\%$ in all Experiments (1-3). There are range of possible mechanisms that could explain the increase in biomass productivity. However, after review all but two were discounted: (i) the mean cell residence time was extended thereby increasing the algal concentration and thus allowing better utilization of incident sunlight; and (ii) the relative proportion of algal growth stages (which may have different net growth rates) was shifted, potentially resulting in an increase in the overall growth rate of the algal culture.

4.5. Acknowledgement

The authors would like to acknowledge Dr Clark Ehlers for the measurement of particle size distribution using Malvern Mastersizer 2000[®].

4.6. References

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**STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING
PUBLICATIONS**

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Byung-Kwan Park

Name/Title of Principal Supervisor: Professor Andy Shilton

Name of Published Research Output and full reference: Park, J.B.K., Craggs, R.J., Shilton, A.N. (2013). Investigating why recycling gravity harvested algae increases harvestability and productivity in high rate algal ponds. Water Research. 47(14), 4904-4917.

In which Chapter is the Published Work: Chapter 4

• **Describe the contribution that the candidate has made to the Published Work:** While the paper was completed with advice and editing from my supervisors, Professor Andy Shilton and Dr Rupert Craggs, I designed the experiments, conducted all experimental work, analysed the results and led the paper as the first corresponding author.



Candidate's Signature

22nd August 2013

Date

Principal Supervisor's signature

22nd August 2013

Date

Chapter 5

Growth, reproduction and life-cycle of *Pediastrum boryanum*

Chapter preface

The pilot-scale HRAP studies (Chapters 2 and 3) and mesocosm studies (Chapter 4) showed that recycling a portion of gravity harvested biomass (“recycling”) promoted the dominance of a rapidly settling colonial alga, *Pediastrum boryanum* and resulted in increased biomass productivity and harvest efficiency (‘harvestable biomass productivity’). Two main mechanisms behind the improved productivity by recycling were suggested in Chapter 4: (i) the mean cell residence time (MCRT) was extended thereby increasing the algal concentration enabling better utilization of the incident sunlight and (ii) the relative proportion of algal growth stages (which may have different net growth rates) was shifted, possibly resulting in an increase in the net growth rate of the algal culture. In order to investigate these mechanisms, the complete life-cycle of *P. boryanum* as well as the timing and net growth rate of each life-cycle stage were determined in laboratory microcosm experiments.

This chapter is based on the following publication:

Park, J.B.K., Craggs, R.J., Shilton, A.N. Growth, reproduction and life-cycle of the wastewater treatment High Rate Algal Pond alga, *Pediastrum boryanum*: Implications for HRAP operation. Prepared for submission to Environmental Science and Technology.

Abstract

This chapter describes for the first time the detailed life-cycle of *Pediastrum boryanum*, a dominant alga in wastewater treatment High Rate Algal Ponds (HRAPs). Experiments determined the exact timing and net growth rate of each *P. boryanum* life-cycle stage ('juvenile', 'growth' and 'reproductive'). Juvenile 16-celled colonies of *P. boryanum* were grown in microcosms under four combinations of light (250 or 120 $\mu\text{Mol/m}^2/\text{s}$) and temperature (20 or 10°C) conditions to simulate summer and winter ambient conditions. The microcosms were cultured on an inverted microscope and a single colony photographed at 15 minute intervals until reproduction was complete. Two asexual life-cycles and a rarely occurring sexual life-cycle were observed. The time required to achieve asexual reproductive maturity increased with lower light or temperature (e.g. high light and high temperature: 52 h; low light and low temperature: 307 h). This indicates that the minimum hydraulic retention time (HRT) or mean cell residence time (MCRT) needs to be higher than these values to enable *P. boryanum* to grow in HRAP under ambient conditions. Recycling a portion of gravity harvested algal biomass is a simple means of extending the MCRT enabling algae to grow for longer and increasing the algal concentration. This could contribute to better conversion of available light into biomass, resulting in higher biomass productivity. This microcosm study showed, for the first time in the literature, that the net growth rate of *P. boryanum* colonies varied between the three life-cycle stages ('growth' > 'juvenile' > 'reproductive'). The previous mesocosm studies in Chapter 4 showed that recycling increased the number of growth colonies by ~2-fold and juvenile colonies by ~4-fold. Therefore, as well as improving productivity by extending the MCRT, it is likely that recycling also increased the net growth rate of the algal culture by 'seeding' the pond with faster growing colonies (i.e. both 'growth' and 'juveniles').

5.1. Introduction

Chapters 2, 3, and 4 reported that the algal genus *Pediastrum* and particularly the species *Pediastrum boryanum* has beneficial attributes for wastewater treatment in High Rate Algal Ponds (HRAP), particularly its higher productivity and efficient removal by simple gravity sedimentation. *P. boryanum* colonies in a pilot-scale HRAP were shown to have 6-60 times greater biovolume (560-12000 μm^3 depending on colony age and cell numbers) than co-occurring colonial algae such as *Desmodesmus* sp. (~530 μm^3), *Micractinium* sp.

(~680 μm^3) or *Dictyosphaerium* sp. (~2070 μm^3), and unicellular algae (~200 μm^3 such as *Chlorella* sp.).

P. boryanum was maintained at greater than 85% dominance in the pilot-scale HRAP_r for two years by recycling a portion of gravity harvested algal biomass ('recycling') (Chapters 2 and 3). Increased dominance of *P. boryanum* improved biomass harvest efficiency from less than 60% (in the control HRAP_c without recycling) to over 85% (Chapter 2). Furthermore, recycling improved annual average biomass productivity by ~20% in the HRAP_r compared with the control HRAP_c over one year (Chapter 3). Subsequent mesocosm studies conducted adjacent to the pilot-scale HRAPs further confirmed that recycling improved *P. boryanum* dominance by 20%, 1h-settleability by 20%, and biomass productivity by 25% compared with control mesocosms which had no recycling (Chapter 4).

The increased productivity and settleability that were achieved by maintaining *P. boryanum* dominance in HRAP could improve the economic viability of HRAP for both wastewater treatment and algal bio-energy production. Two main mechanisms behind the improved productivity by recycling were identified following the HRAP mesocosm studies (Chapter 4): (i) when the HRAP is operated with too short a HRT so that not all available light is utilized, recycling extends the mean cell residence time (MCRT) thereby increasing the algal concentration enabling better utilization of the incident sunlight and, (ii) the relative proportion of algal growth stages (which may have different net growth rates) was shifted, resulting in an increase in the net growth rate of the algal culture. To investigate these mechanisms further, the life-cycle of *P. boryanum* was required, including the exact timing and net growth rate of each life-cycle stage ('juvenile', 'growth' and 'reproductive').

Many environmental (light and temperature), operational (pH, CO₂ and nutrients) and biological (zooplankton grazers and algal pathogens) parameters can influence algal productivity in HRAPs. In particular, light, which provides the energy source, and temperature, which influences the rates of biochemical reactions are key environmental parameters for the growth of photoautotrophic algal cultures that are not nutrient limited (Sandnes et al. 2005; Carvalho et al. 2006). The life-cycle and intracellular structure of *Pediastrum* sp. (*P. boryanum*, *P. duplex*, *P. simplex*, and *P. tetras*) and their morphological response to environmental conditions have only been studied to a limited

extent and the exact details of the life-cycle of *P. boryanum* has not been described previously (Davis 1967; Millington and Gawlik 1967; Neustupa and Hodac 2005; Rojo et al. 2009). To investigate these mechanisms further, the research in this chapter, has observed for the first time, the complete life-cycle of *P. boryanum* including determination of the timing and net growth rate of each life-cycle stage ('juvenile', 'growth' and 'reproductive') in response to the key parameters of light and temperature.

5.2. Materials and methods

P. boryanum was grown in microcosms to determine the timing and net growth rate of each life-cycle stage and how they were affected by summer and winter light and temperature conditions. Batch cultures of *P. boryanum* were then grown under similar summer and winter conditions, to relate the colony life-cycle data to culture growth and production.

5.2.1. Isolation of *Pediastrum boryanum* from a wastewater treatment HRAP

P. boryanum colonies were isolated in two separate seasons (summer and winter) from a pilot-scale HRAP treating domestic wastewater at the Ruakura Research Station, Hamilton, New Zealand. A technique combining serial dilution and isolation of single-cells was conducted using an inverted microscope and sterilized equipment and sterile liquid growth medium. The composition of the medium was based on Bold 3N medium (Shi et al. 2007), but adjusted to contain 5 mg/L of $\text{PO}_4^{3-}\text{-P}$ (K_2HPO_4) and 20 mg/L $\text{NH}_4^+\text{-N}$ ($(\text{NH}_4)_2\text{SO}_4$). The N and P concentrations was to mimic the concentrations of the diluted domestic wastewater that was fed into the pilot-scale HRAPs, which were simulating operation with recirculation of treated effluent (Chapters 2 and 3).

Cultures of colonies that were isolated during the winter and summer were grown in algal growth chambers (Contherm Scientific Ltd 6150CP-6400CP) that simulated winter and summer light and temperature conditions respectively (Table 5.1).

Table 5.1: Culture conditions of the summer and winter algal growth chambers.

	Parameters	Summer growth chamber	Winter growth chamber
Day	Hours	14	10
	Light intensity ($\mu\text{Mol}/\text{m}^2/\text{s}$)	250	120
	Temperature ($^{\circ}\text{C}$)	25	13
Night	Hours	10	14
	Light intensity ($\mu\text{Mol}/\text{m}^2/\text{s}$)	0	0
	Temperature ($^{\circ}\text{C}$)	19	9

5.2.2. Microcosm experiments to determine the influence of light and temperature on the life-cycle of *P. boryanum*

A microcosm was formed in a Utermöhl chamber containing 10 ml of the growth medium which was then placed on an inverted light microscope (Leica Microsystems, 400 \times magnification) equipped with a microscope camera (Leica DFS 420c) with an external light source (Leica CIS 150; programmed for a 12:12h light:dark cycle) (Figure 5.1). The microscope light was also programmed to turn on 5 seconds before and off 2 seconds after taking a photo to minimize the effects of its very high light intensity ($\sim 5000 \mu\text{Mol}/\text{m}^2/\text{s}$ which is ~ 2.5 fold greater than maximum summer day light intensity) on the colony growth.

P. boryanum colonies can have either 4, 8, 16, 32, 64, or 128 cells per colony that are arranged as a single layer disc (Moner 1953; Davis 1963; Slavinski and Hillson 1984). In Chapter 2, monthly monitoring of *P. boryanum* dominance in a pilot-scale HRAP over one year showed that 16-celled colonies accounted for more than 50% of the *P. boryanum* biomass. Therefore, 16-celled colonies were used in this study. For each experiment a juvenile 16-celled colony (still enclosed within a vesicle) was isolated from a pure stock culture and placed into the microcosm.

The experiments were replicated three times and were conducted under four combinations of conditions; high and low light intensity (250 and 120 $\mu\text{Mol}/\text{m}^2/\text{s}$) and high and low temperature (20 and 10 $^{\circ}\text{C}$) in a culture room. The light intensity of the inverted microscope light source was measured using a Licor LI-190 Quantum Sensor placed beneath the base of the microcosm containing 10 ml of growth medium.

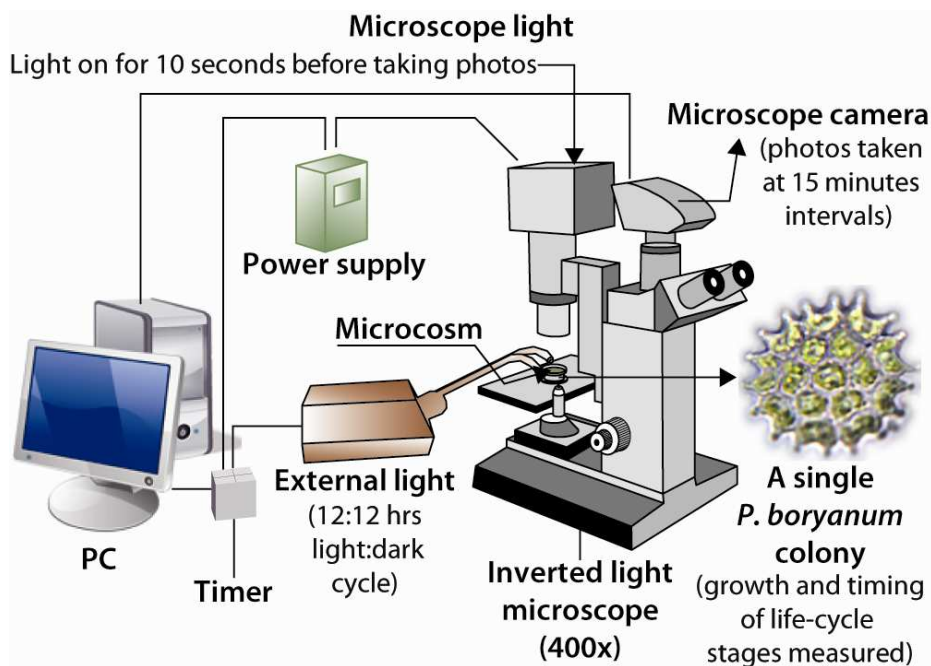
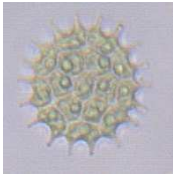

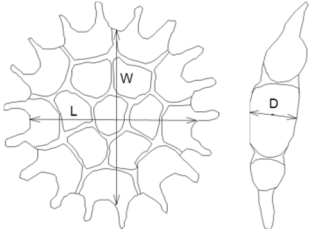


Figure 5.1: Microcosm experimental set-up on and inverted microscope to monitor and measure the growth of a single 16-celled *Pediastrum boryanum* colony.

5.2.2.1. Microscopic image analysis

Photographs were taken through the microscope at 15 minute intervals during the light period and at 60 minute intervals during the dark period (to minimize the effect of the very high intensity of the microscope light) until reproduction was complete. Growth of the *P. boryanum* colony (measured as an increase in biovolume), and timing of the different life-cycle stages were determined by microscopic image analysis (Leica Application Suite, LAS version 3.1.0) at the end of each experiment. Biovolume was calculated from colony length, width and thickness using Equation 5.1 (Table 5.2).

Table 5.2: Measurement of the diameter and biovolume of *P. boryanum* using microscopic image analysis (note: as the colony was placed flat on the surface of the microcosm, depth of the colony was not directly measured. Colony depth (D) was calculated by multiplying the colony width (W) 0.067 (a ratio based on >500 measurements of *P. boryanum* colony width and depth, Chapter 2).

Microscope photo	Top view	Side view
		
Geometric measurement		
Calculation of colony biovolume	Equation 5.1: $V = \frac{\pi}{4} \cdot L \cdot W \cdot D$	
	L : length (μm) W : width (μm) D : depth (μm)	

5.2.3. *P. boryanum* batch culture experiments

Batch cultures of *P. boryanum* were then grown in 1 L sterile culture flasks under similar summer and winter conditions, which was to enable the colony life-cycle data to be related to culture growth and production. Pure *P. boryanum* cultures (800 ml) were made up from 200 ml of *P. boryanum* inoculum (taken from an exponential phase stock culture) and 600 ml of fresh growth media. The cultures were grown for 20 days in triplicate under simulated summer or winter conditions using inocula that had been isolated and cultured under the same conditions (Table 5.1). Each culture was sampled twice a week to determine the number and biovolume of *P. boryanum* colonies in each life-cycle stage (juvenile, growth or reproductive). Bacterial contamination in the cultures was also examined at least twice a week by microscopic analysis.

5.2.3.1. Determination of light and temperature levels for the summer and winter growth chambers

The temperatures of the growth chambers that simulated winter and summer conditions were determined using water temperature data from pilot-scale HRAP that was collected using a multi-probe DataSonde® at 15 minute intervals during the New Zealand summer (December to February) and winter (June to August) during one year (2008-2009) (Park and Craggs 2010). Since there was a large diurnal variation in pond water temperature, the median of the daily daytime medians and the median of the daily night-time median temperatures were calculated and used as the temperature settings for the simulated summer (light, 25 °C: dark, 19°C) and winter (light, 13°C: dark, 9°C) conditions (Table 5.1).

Previous studies in the literature have used a very wide range of light intensities (~20-500 $\mu\text{Mol}/\text{m}^2/\text{s}$) in laboratory studies (Sandnes et al. 2005; Zhu et al. 2008; Rojo et al. 2009). No exact method has been given previously to determine the light intensity to simulate the light environment in an outdoor HRAP (i.e. ~30 cm deep, mixed algal culture with an average algal biomass concentration of ~220 mg/L in summer and ~180 mg/L in winter, Chapter 3). The average irradiation over the 30 cm depth of the pilot-scale HRAP can be determined using Equation 5.2 suggested by Morowitz (1950).

$$I_{ave} = \frac{1}{L} \int_0^L I_0 e^{-kx} dx = I_0 \left(\frac{1 - e^{-kL}}{kL} \right) \quad \text{Equation 5.2}$$

Where;

I_{ave} : Average light irradiation over the depth ($\mu\text{Mol}/\text{m}^2/\text{s}$)

I_0 : Light irradiation on the pond surface ($\mu\text{Mol}/\text{m}^2/\text{s}$)

L : Pond depth (cm)

k : Attenuation coefficient depending on algal biomass concentration in pond

Light profiles within the 30 cm deep pond (measured at a 1 cm interval from 0-10 cm and a 5 cm interval from 10-25 cm, using a LI-190 Quantum Sensor) were measured three times on a fine clear day (~12 pm) when the algal biomass concentration in the pond was approximately at the average level (winter: 180 mg/L; summer: 220 mg/L) (Chapter 3)

and plotted as shown in Figure 5.2. The attenuation coefficient (k) was determined using an exponential curve fit in Microsoft Excel (Figure 5.2). Average light irradiances of 250 and 120 $\mu\text{Mol}/\text{m}^2/\text{s}$ were calculated and used as the light settings for the simulated summer and winter conditions respectively.

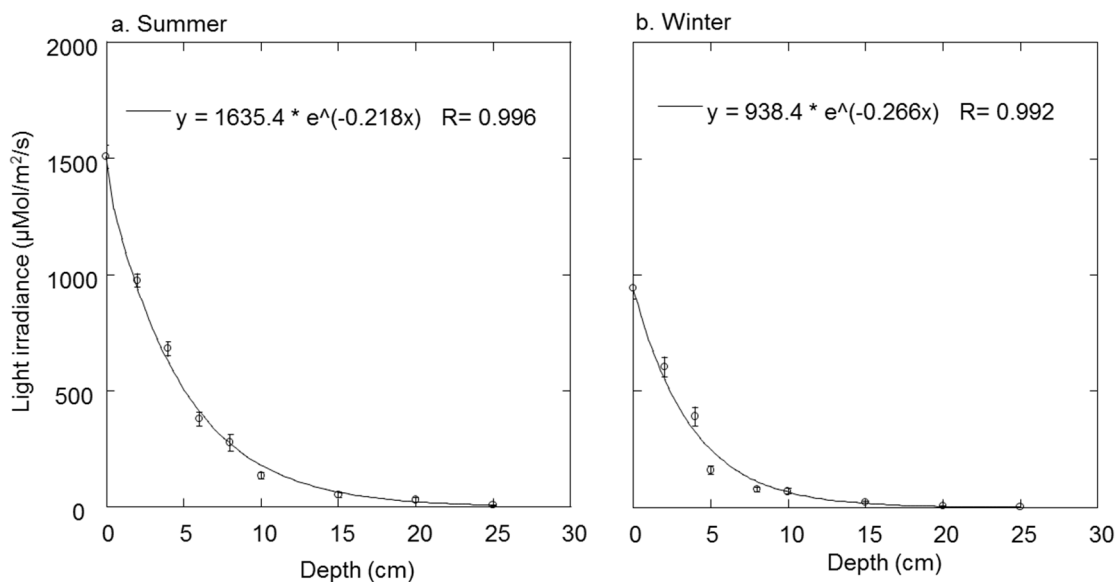


Figure 5.2: The light profiles (mean of triplicate and \pm s.d.) within the 30 cm deep pilot-scale HRAP in summer (a) and winter (b) to determine the average light irradiances (Note: the light profiles were measured on a fine clear day at ~ 12 pm when the algal biomass concentration in the pond was approximately at the average level for summer: 220 mg/L (a) and winter: 180 mg/L (b)).

5.3. Results and Discussion

5.3.1. The life-cycle of *Pediastrum boryanum*

Microcosm experiments were conducted to determine the life-cycle of *P. boryanum* by monitoring the growth of single 16-celled juvenile colonies until reproduction was complete. The influence of light and temperature on the growth of each life-cycle stage ('juvenile', 'growth' and 'reproductive') is summarized in Table 5.3 and the exact life-cycles of *Pediastrum boryanum* including asexual and sexual life-cycles is illustrated in Figure 5.3. A common asexual life-cycle (LC 1) and a rarely occurring asexual life-cycle (LC 2) were identified (Figure 5.3). Both asexual life-cycles began with the reproducing cell of a colony dividing and forming motile zoospores (typically 16 zoospores), which were released from the cell within a vesicle.

In LC 1, the zoospores swarmed for approximately 4 minutes within the vesicle, then became non-motile and aggregated to form a disc, which became a new juvenile colony (with 16 cells). This colony consisted of a central cell surrounded by concentric rings of 6 and then 9 cells. The juvenile colony grew slowly within the vesicle for approximately 4 h (high temperature and high light) to 25 h (low temperature and low light) until the vesicle disintegrated. A growth stage then followed in which two spines (prongs) grew out from the external wall of each of the 9 peripheral cells. Pyrenoids (the site of carbon dioxide fixation within algae chloroplasts; (Moner and Chapman 1960)) developed within all cells of the colony. The colony continued to grow for up to 52 h (2.2 d, high temperature and high light) or 307 h (12.8 d, low temperature and low light) when it reached reproductive maturity (Table 5.3) at which time the pyrenoids disappeared.

In LC 2, the vesicle ruptured soon (~4 minutes) after zoospore disc formation, releasing individual cells into the culture medium (Figure 5.3). The single cells grew to reproductive maturity, and divided forming motile zoospores which were released from the cell within a vesicle (after approx. 72 h). The timing of the stages of LC 2 (e.g. formation of the pyrenoid, reproductive maturity, and zoospore formation) were similar to those of LC 1. The juvenile colony then either grew through LC 1 or if the vesicle ruptured and released single cells, underwent another cycle of LC 2. Some aspects of *Pediastrum* sp. intracellular development have been previously described in the literature (Hawkins and Leedale 1971; Millington et al. 1981; Gawlik and Millington 1989) and much of asexual LC 1 has been described for *Pediastrum simplex* (Davis 1967). However, determination of the precise timing of each life-cycle stage, by observing the growth of a single juvenile colony microscopically has not been reported previously. Moreover, this is the first time that another asexual life-cycle (LC 2) has been described.

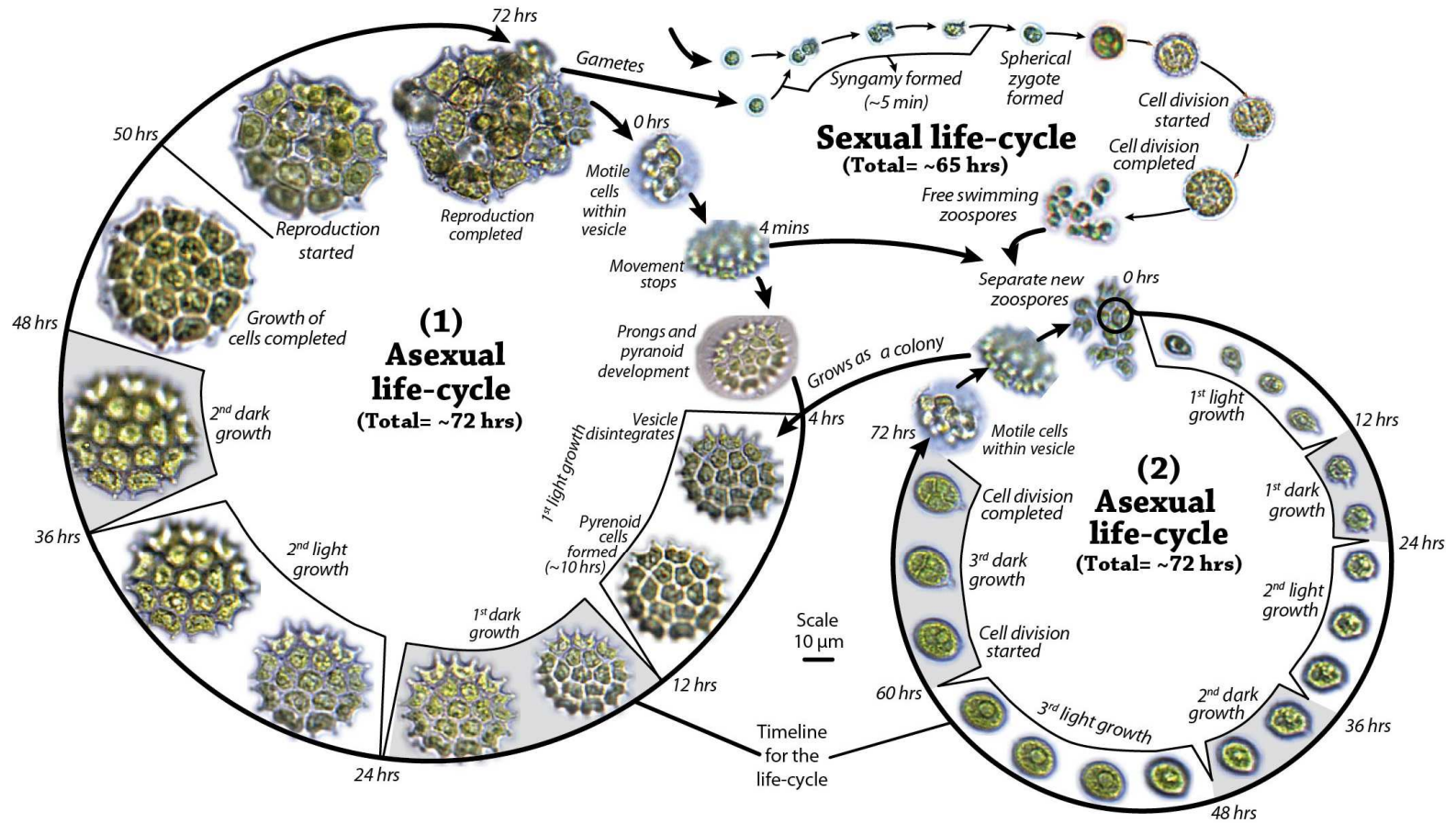


Figure 5.3: The asexual and sexual life-cycles of *Pediastrum boryanum* determined by observation of the growth of single cells/colonies grown in a microcosm under 250 µMol/m²/s (12:12 h light and dark cycle) at 20°C.

Table 5.3: The influence of light and temperature on the growth of 16-celled *P. boryanum* colonies.

Temperature		High (20°C)		Low (10°C)	
Light intensity ($\mu\text{Mol/m}^2/\text{s}$)		High 250	Low 120	High 250	Low 120
Juvenile stage	Ave. length of time (h)	3.7 \pm 1.3	7.2 \pm 1.1	6.6 \pm 1.2	25 \pm 5.5
	Ave. diameter (μm)	20.9 \pm 0.2	20.0 \pm 0.6	20.9 \pm 0.6	20.2 \pm 0.3
	Ave. biovolume (μm^3)	480 \pm 4.2	418 \pm 12.2	480 \pm 3.2	432 \pm 6.2
Growth stage	Ave. length of time (h)	49.1 \pm 5.1	124.8 \pm 10.2	108 \pm 9.6	280.8 \pm 16
	Ave. diameter (μm)	28.3 \pm 0.9	39.7 \pm 1.3	34.8 \pm 1.3	36.6 \pm 0.6
	Ave. biovolume (μm^3)	1334 \pm 42	3680 \pm 62	3030 \pm 31	3352 \pm 45
Reproductive stage	Ave. time to reach reproductive maturity (h)	52.8 \pm 4.0 (~2.2 d)	132.5 \pm 10.5 (~5.5 d)	114.6 \pm 6.5 (~4.7 d)	307 \pm 10.5 (~12.8 d)
	Ave. diameter (μm)	38 \pm 1.0	56.1 \pm 0.6	55 \pm 0.5	61.7 \pm 1.2
	Ave. biovolume (μm^3)	2887 \pm 234	9291 \pm 301	8755 \pm 241	12360 \pm 735

Sexual reproduction of *P. boryanum* was only observed once in all of the experiments, and then only under high light and high temperature conditions (Figure 5.3). The sexual life-cycle of other *Pediastrum* sp. such as *P. duplex*, *P. tetra*, and *P. simplex* have been described in the literature and are also reported to occur very rarely (Davis 1967; Millington 1981; Millington et al. 1981; Rojo et al. 2009). As illustrated in Figure 5.3, sexually reproductive cells were observed to divide into two gametes (biflagellate motile haploid cells, $<2 \mu\text{m}$) that were released into the culture medium. When two gametes from different mother cells came into contact, they fused together and within about 5 minutes formed a spherical zygote. The spherical zygote grew for about 65 hours (~3 days) and then released a vesicle containing zoospores (in a similar way to the asexual life-cycles). The zoospores swarmed for approximately 4 minutes within the vesicle and once released from the vesicle each zoospore grew and formed a new colony (in 3 or more days) as in the asexual LC 2.

5.3.2. Influence of light and temperature on the life-cycle of P. boryanum

The effect of the light intensity and temperature on the occurrence of LC 1 and 2 is summarized with statistical analysis in Table 5.4. The laboratory microcosm investigations found that most (80-98%) cells of mature *P. boryanum* colonies reproduced by LC 1 under the experimental conditions tested (Figure 5.4), although the proportion of cells reproducing by LC 2 did increase at the higher temperature (20°C compared with 10°C) (Figure 5.4a and c). Occurrence of LC 2 was influenced by temperature particularly under higher light conditions (*p-value*: <0.001 , one-way ANOVA; Table 5.4) and by light under both high and low temperature conditions (*p-value*: <0.05 , one-way ANOVA; Table 5.4). The increased occurrence of early vesicle rupture and LC 2 at higher temperature might be due to higher production or activity of enzymes such as autolysin that dissolve the vesicle membrane and release the zoospores before they have fused into a new colony (Millington and Labavitch 1986).

As addressed in Chapter 2, the size of algal cells or colonies affects the settling velocity. Therefore an increase in the occurrence of the LC 2 releasing small individual cells may reduce algal settleability from the HRAP effluent. As shown in Figure 5.4a and c, the occurrence of LC 2 was reduced when the light intensity was low in the simulated summer conditions (i.e. high temperature). This result may suggest that operating the HRAP with somewhat higher algal concentration (and thus increasing light attenuation through the

pond depth) may reduce the number of small, poorly-settleable individual cells reproducing by LC 2, which could possibly increase algal settleability from the HRAP effluent.

Table 5.4: One-way ANOVA analysis of the effect of the light intensity and temperature on the occurrence of LC 1 and 2.

Parameters		LC 1	LC 2
Effect of Temp. under High Light	<i>P-value</i>	<0.001	<0.001
	Significance	99.9%	99.9%
	Effect	Very strong	Very strong
Effect of Temp under Low Light	<i>P-value</i>	<0.01	<0.01
	Significance	99.0%	99.0%
	Effect	Strong	Strong
Effect of Light under High Temp.	<i>P-value</i>	<0.005	<0.05
	Significance	99.5%	95.0%
	Effect	Very strong	Strong
Effect of Light under Low Temp.	<i>P-value</i>	<0.005	<0.05
	Significance	99.5%	95.0%
	Effect	Very strong	Strong

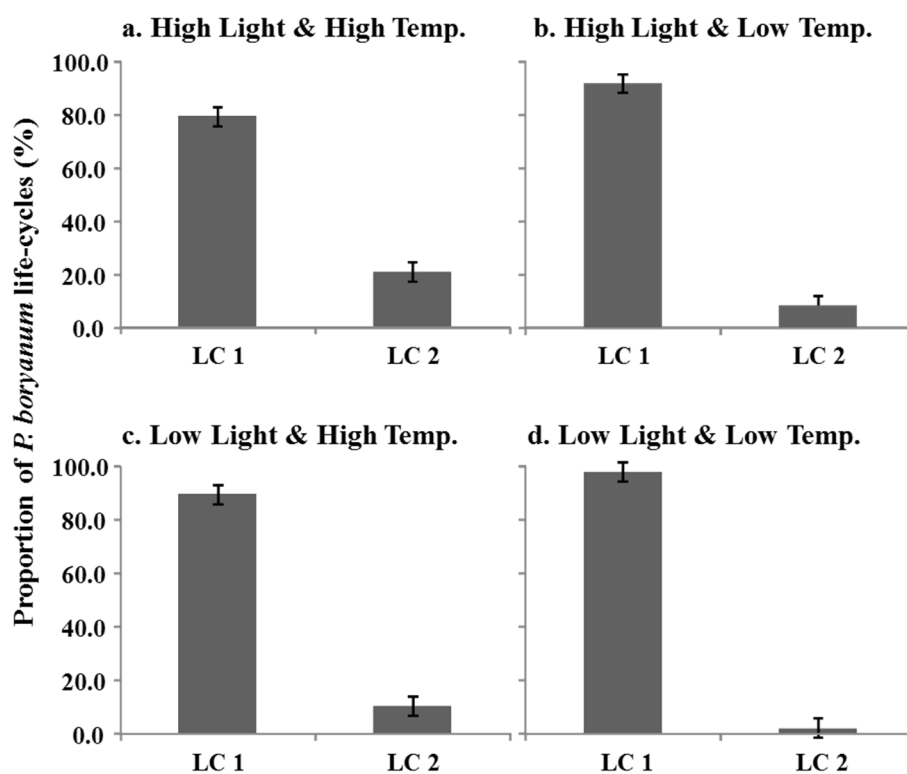


Figure 5.4: Relative proportion of two asexual reproduction cycles (LC 1 and LC 2) in *P. boryanum* microcosm experiments under high or low light (250 or 120 $\mu\text{Mol/m}^2/\text{s}$) and high or low temperature (20 or 10⁰C) conditions.

The growth of *P. boryanum* colonies (in terms of the average length of time and biovolume to reach the next life-cycle stage) in the microcosm experiments varies with light and temperature conditions (Figure 5.5 and summarized in Table 5.3). After a short 'juvenile' stage, colony biovolume rapidly increased in the 'growth' stage until the 'reproductive' stage when growth declined (Figure 5.5). The number of cells within a *Pediastrum* colony remained constant after juvenile colony formation, and thus colony growth was only by an increase in the biovolume of individual cells. This process of colony biovolume growth differs substantially from that of co-occurring colonial algal species in a wastewater treatment HRAP. *Dictyosphaerium* sp. (i.e. commonly found in the pilot-scale HRAP) (Chapters 2 and 3), mainly increases colony biovolume by increasing the number of cells per colony. These colonies then disintegrate into single cells, which divide to form new colonies (Irfanullah and Moss 2006).

The time necessary for colonies to reach reproductive maturity under simulated high light summer conditions (high temperature, 20°C) was 52 h (2.2 d) (Figure 5.5a; Table 5.3). However under low light summer conditions (i.e. high temperature but low light intensity, simulating an extended period of cloudy weather or where HRAP algal concentrations are high) this increased to 132 h (5.5 d). Similarly, the time taken to achieve reproductive maturity in simulated winter conditions (low temperature, 10°C) increased from 114 h (4.7 d) under high light, to 307 h (12.8 d) under low light (Figure 5.5b; Table 5.3).

The size of reproductively mature colonies also increased from 2880 μm^3 under the high light summer optimal conditions to 12360 μm^3 under the low light winter conditions (low temperature and low light) (Table 5.3). These results were further confirmed by the pure batch culture study of *P. boryanum*, showing that under the simulated winter conditions colonies need to grow larger before reproduction commenced (Figure 5.6). Previous researchers have found that when algae were grown under sub-optimal conditions, the rates of metabolic processes (e.g. enzyme activity, photosynthesis, respiration, and cell division) decrease, so that more time is required to reach reproductive maturity (Mihara and Hase 1971; Laws 1982; Falkowski 1984; Geider et al. 1986; Agustí 1991). Geider et al (1986) and Agustí (1991) also reported that under sub-optimal conditions, algae require larger intracellular energy reserves (such as carbohydrate or lipid vacuoles) to initiate cell division to form zoospores. Therefore, the increase in colony biovolume under low light and temperature conditions may have been due to the accumulation of sufficient energy reserves to initiate cell division.

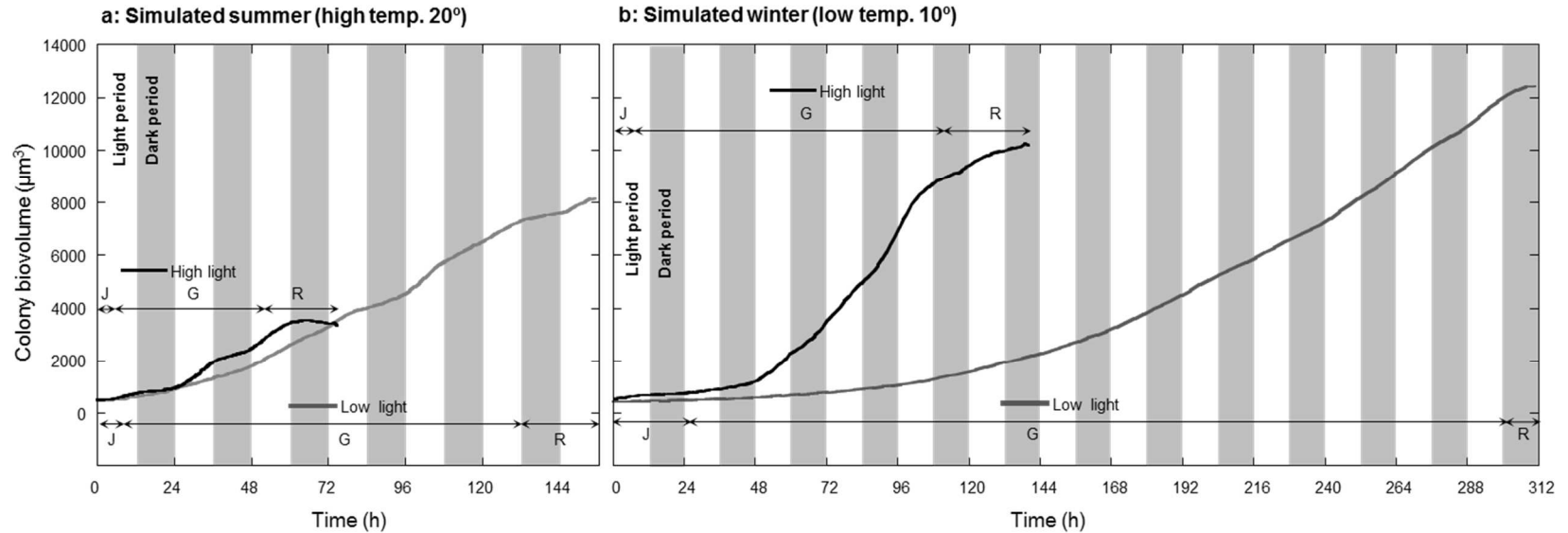


Figure 5.5: Increase in colony biovolume of a single 16-celled *P. boryanum* colony cultured in a microcosm under simulated summer (a: high temperature, 20°C) and winter (b: low temperature, 10°C) conditions at two light intensities (high: 250 $\mu\text{Mol}/\text{m}^2/\text{s}$; low: 120 $\mu\text{Mol}/\text{m}^2/\text{s}$). (Note: three consecutive experiments were conducted for each set of conditions and the results averaged to produce a growth curve. Life-cycle stages: 'J': juvenile; 'G': growth; 'R': reproductive).

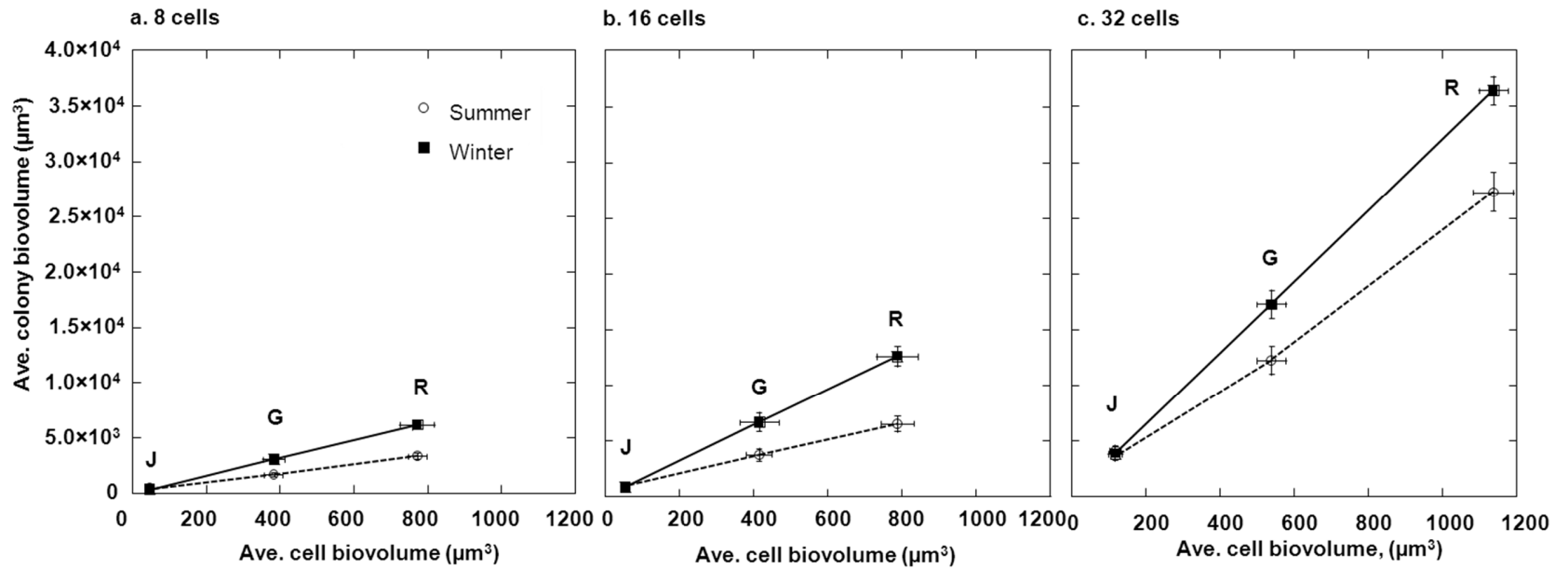


Figure 5.6: Average colony biovolumes for three different *P. boryanum* life-cycle stages ('J': juvenile stage; 'G': growth stage; 'R': reproductive stage) under simulated winter and summer growth conditions in laboratory batch cultures (a: 8-celled colony, b: 16-celled colony and c: 32-celled colony).

5.3.3. Implications for wastewater treatment HRAP

The time required to complete the life-cycle of *P. boryanum* gives an indication of the absolute minimum mean cell residence time (MCRT) for *P. boryanum* dominant HRAP operation, i.e. the minimum MCRT to enable *P. boryanum* to grow in the pond. The results presented in Table 5.3 indicate that minimum MCRTs of 52 h (~2.2 d) and 114 h (~4.7 d) would be required to maintain a viable population of *P. boryanum* under the simulated high light summer and winter conditions respectively. Pragmatically, MCRTs in full scale HRAPs need to be somewhat higher than these minimum values to provide resilience against variation of inflow rate which changes the hydraulic retention time (HRT) and fluctuations in environmental parameters such as light and temperature which impact on the algal growth rate.

In commercial algal production systems the MCRT is easily controlled by adjustment of the inflow rate and thus the HRT (which is the same as the MCRT in systems that have no recycling). However, increasing the MCRT of a full-scale wastewater treatment HRAP by decreasing the inflow rate is not practical because the system must treat all of the wastewater inflow. One option is to increase the HRT (and thus the MCRT) by increasing the pond depth (and so increasing the pond volume). However, another simple and practical means for increasing the MCRT without changing the HRT is to recycle a small portion of the harvested algal biomass back into the HRAP (Chapter 1).

The pilot-scale HRAP study in Chapter 2 showed that recycling only ~10% of daily algal biomass production increased the MCRT by ~0.5 day in summer and ~1.2 days in winter. This increased the algal concentration and resulted in a 20% higher 'in-pond' biomass productivity measured consistently over the one year experimental period compared with the control without recycling (Chapter 3). This benefit of recycling was further replicated in the HRAP mesocosm studies in Chapter 4 where recycling similarly improved biomass productivity by >25%.

Six mechanisms were reviewed in Chapter 4 as possible explanations for this increase in biomass productivity. For example, the possibility of the increase in biomass productivity being due to an increase in bacterial biomass production was considered but refuted by

replicating the effect using pure *P. boryanum* cultures in the laboratory (Appendix A). At the conclusion of Chapter 4 the two remaining plausible mechanisms were:

- (i) the HRAP mean cell residence time (MCRT) was extended thereby enabling algae to grow for longer and increasing the algal concentration, so that incident sunlight was more fully utilized, and
- (ii) the relative proportion of algal growth stages (which may have different net growth rates) was shifted, resulting in an increase in the net growth rate of the algal culture.

Mechanism (i) would certainly be expected if the HRAP was operating at an algal concentration that was sub-optimal for utilizing the incident sunlight. However, mechanism (ii) is relatively novel because limited information could be found in the literature, reporting that differential growth rates can exist across the different stages of an algal life-cycle (Tukaj et al, 2003) and no prior publications have identified this as a potential mechanism (or an operation technique) to improve biomass productivity in HRAP. Indeed, to date, detailed observation of the life-cycle of *P. boryanum* has not previously been reported to the extent that net growth rate data for the different life-cycle stages was available.

In the following discussion, aided by the improved understanding of the life-cycle of *P. boryanum*, we seek to examine the likelihood of mechanism (ii) being viable.

1. Was there any time when the increased biomass productivity could not be explained by mechanism (i) alone?

As shown in Figure 5.7, light profiles measured in the HRAP showed that at certain periods the incident light reached the bottom of the pond when biomass concentration was lower than ~200 mg/L in both summer and winter. During such periods biomass productivity could be improved when the algal concentration was increased by recycling thus making more efficient utilization of the incident light. However, there were also periods during when the control HRAP_c had biomass concentrations well in excess of 200 mg/L and thus a portion of the pond depth was in the dark due to light attenuation, but recycling still improved biomass productivity (e.g. December 2009; *P. boryanum* was dominant in both HRAPs; HRAP_r with

recycling: productivity of 17.6 g/m²/d; HRAP_c without recycling: productivity of 13.6 g/m²/d, Chapter 3). This further increase in productivity may be supported by vertical mixing within the 30 cm deep HRAP enabling sufficient light availability for algal growth despite the overall decrease in light in the pond caused by the increased biomass (Grobbeelaar 1994; Grobbeelaar et al 1996; Chapter 1). Although, the second mechanism may be needed to fully explain the increase in biomass productivity achieved with recycling:

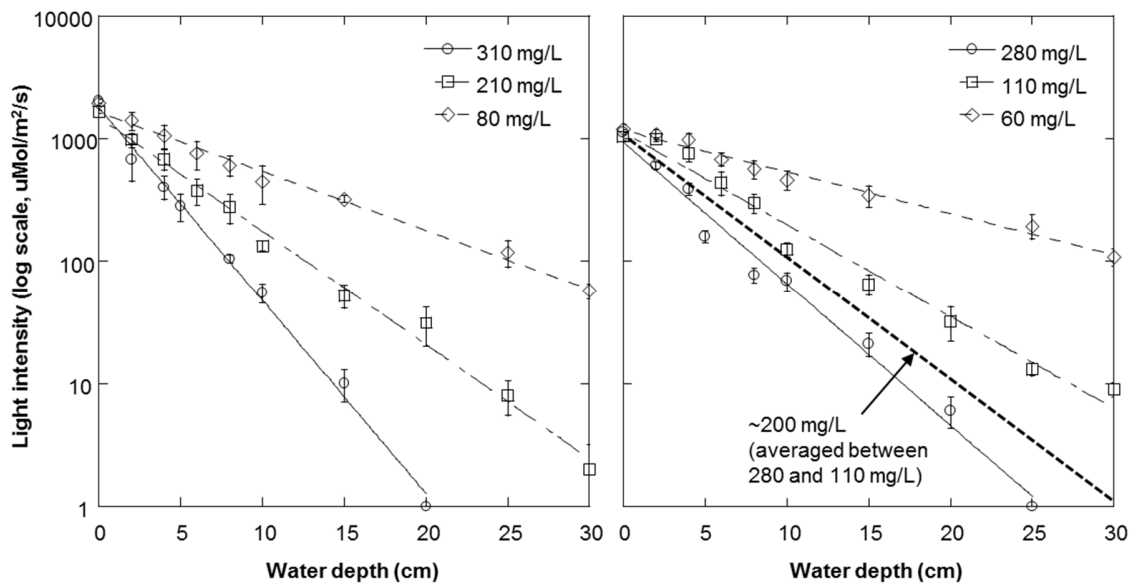


Figure 5.7: Light profiles within the 30 cm deep pilot-scale HRAP depending on culture biomass density (measured as TSS) in summer (a) and winter (b).

2. Do *P. boryanum* life-cycle stages have different growth rates and does recycling alter the proportion of life-cycle stages, resulting in an increase in the net growth rate of the pond culture?

Microcosm experiments with single *P. boryanum* colonies showed, for the first time in the literature, that the net growth rate of *P. boryanum* does vary with life-cycle stage ('growth' > 'juvenile' > 'reproductive') under all the experimental conditions tested (Figure 5.8 and Table 5.5).

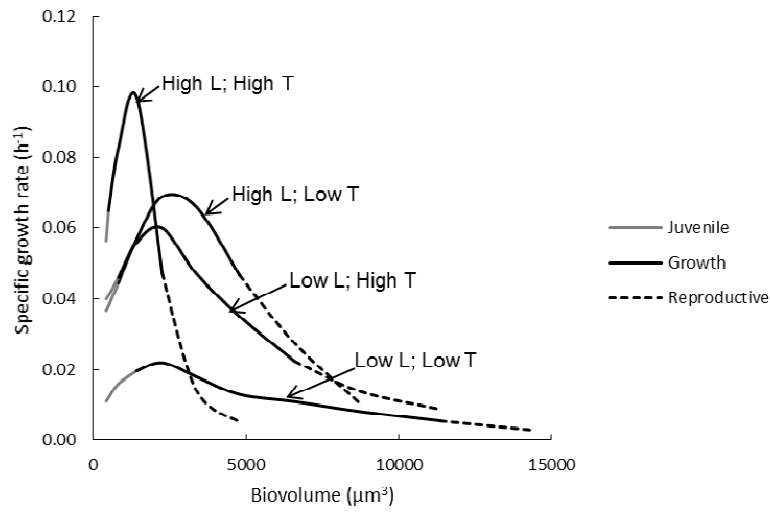


Figure 5.8: Net growth rate (h^{-1}) plotted against biovolume (μm^3) under high and low light conditions (250 and 120 $\mu\text{Mol}/\text{m}^2/\text{s}$ respectively) and at two temperatures (high at 20°C and low at 10°C) ('L': Light; 'T': Temperature).

Table 5.5: Net growth rate (h^{-1}) of different life-cycle stages ('juvenile', 'growth' and 'reproductive') of *Pediastrum boryanum* colonies.

Temperature		High (20°C)		Low (10°C)	
Light intensity ($\mu\text{Mol}/\text{m}^2/\text{s}$)		High 250	Low 120	High 250	Low 120
Juvenile	Ave. growth rate (h^{-1})	0.061±0.006	0.040±0.004	0.042±0.003	0.012±0.004
Growth	Ave. growth rate (h^{-1})	0.073±0.022	0.057±0.011	0.044±0.014	0.015±0.006
Reproductive	Ave. growth rate (h^{-1})	0.022±0.020	0.028±0.018	0.015±0.007	0.004±0.001
Average growth rate of colony (h^{-1})		0.051±0.036	0.044±0.020	0.037±0.018	0.013±0.006
Maximum growth rate of colony (h^{-1})		0.097±0.023	0.069±0.012	0.060±0.002	0.022±0.008

Note: Net growth rate was calculated using Equation 5.3 below;

$$\text{Equation 5.3: Net growth rate } (\mu) = \frac{\ln(m_{t_2}/m_{t_1})}{t_2 - t_1}; t_2 > t_1$$

where m_t are biovolumes at the different time points (t_1 and t_2) respectively

The previous pilot-scale HRAP study (Chapter 3) showed that gravity settling selected for larger *P. boryanum* colonies from the HRAP effluent, which were then recycled back to the pond. Since the largest ‘reproductive’ colonies actually have the slowest growth rate (Figure 5.8 and Table 5.5), recycling reproductive colonies back to the HRAP would not further improve biomass productivity. However the mesocosm study in Chapter 4 showed that recycling increased the number of growth colonies by ~2-fold (Figure 4.12d) and juvenile colonies by ~4-fold (Figure 4.12c) compared to the controls without recycling, indicating that recycling of larger colonies appears to effectively work to ‘seed’ the HRAP with the faster growing ‘growth’ and ‘juvenile’ colonies.

Further research is required to carefully determine the change of size distribution and proportion of each life-cycle stage in a *P. boryanum* culture with recycling. However, given that this study has shown that net growth rate varies between life-cycle stages (‘growth’ > ‘juvenile’ > ‘reproductive’) and mesocosm studies showed that there were times when recycling increased the proportion of algae with higher growth rates (‘juveniles’ and ‘growth’ stage colonies) then mechanism (ii) does appear to be viable.

5.4. Conclusions

A common and a rarely occurring asexual life-cycle of *Pediastrum boryanum* (LC 1 and LC 2) and a very rare sexual life-cycle were observed in detail for the first time. Study of the common asexual life-cycle (LC1) under simulated summer and winter conditions indicates that minimum mean cell residence times (MCRTs) of 2.2 and 4.7 days respectively are required to promote reproduction and maintain culture productivity. This study confirmed that recycling a small portion of harvested biomass could be a simple and practical method to extend the MCRT (without changing hydraulic retention time) to longer than the minimum values, increasing the algal concentration. This probably contributed to better conversion of available light into biomass, resulting in the higher biomass productivity observed in the HRAP. The life-cycle study showed for the first time, that the net growth rate varies between *P. boryanum* life-cycle stages (‘growth’ > ‘juvenile’ > ‘reproductive’). The previous mesocosm studies in Chapter 4 showed that recycling increased the number of growth colonies by ~2-fold and juvenile colonies by ~4-fold. Therefore, as well as improving productivity by extending the MCRT, it is likely that recycling also increased the net growth

rate of the algal culture by ‘seeding’ the pond with faster growing colonies (i.e. both ‘growth’ and ‘juveniles’).

5.5. Acknowledgement

Authors would like to acknowledge Mr Karl Safi (Algal Ecologist) and Ms Karen Thompson for their technical advice while developing the microcosm experiment set-up.

5.6. References

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MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

**STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING
PUBLICATIONS**

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Byung-Kwan Park

Name/Title of Principal Supervisor: Professor Andy Shilton

Name of Published Research Output and full reference: Submitted to Water Research.

In which Chapter is the Published Work: Chapter 5

• **Describe the contribution that the candidate has made to the Published Work:** While the paper was completed with advice and editing from my supervisors, Professor Andy Shilton and Dr Rupert Craggs, I designed the experiments, conducted all experimental work, analysed the results and led the paper as the first corresponding author.

Candidate's Signature

22nd August 2013

Date

Principal Supervisor's signature

22nd August 2013

Date

Chapter 6

Conclusions

High Rate Algal Ponds (HRAPs) are an efficient and cost-effective system for wastewater treatment and produce algal biomass which could be economically converted to biofuels. However, little fundamental research has been conducted to optimise biomass production and harvest ('harvestable biomass production') from these ponds. The main hypothesis of this Ph.D. was: 'Recycling a portion of gravity harvested biomass back into the HRAP improves harvestable biomass production'. To confirm this hypothesis, a series of experiments was conducted using pilot-scale wastewater treatment HRAPs, outdoor mesocosms and laboratory microcosms.

Firstly, to investigate the influence of recycling on species dominance and biomass harvest efficiency, two identical pilot-scale HRAPs treating domestic wastewater were operated under ambient conditions over two years either with or without recycling. In Year 1, recycling promoted the dominance of a rapidly settling colonial alga, *Pediastrum boryanum* from 53% to greater than 90% (Chapter 2). In Year 2, seeding the original control HRAP with *P. boryanum* dominated biomass harvested from the HRAP with recycling shifted the algal dominance from 89% *Dictyosphaerium* sp. (a poorly-settleable alga) to over 90% *P. boryanum* in just 5 months (Chapter 3). These results over two years showed for the first time in the literature, that recycling a portion of gravity harvested biomass could enable species control of similarly sized co-occurring algal colonies in an outdoor wastewater treatment HRAP.

The higher dominance of the rapidly settleable alga, *P. boryanum* in the HRAP with recycling improved biomass harvest from the HRAP effluent by gravity sedimentation (annual average harvest efficiency was 85% compared with only ~60% in the control without recycling Chapter 2). Unexpectedly, recycling also improved the in-pond biomass productivity of the HRAP by 20% compared with the control HRAP without recycling (HRAP_r: 11 g/m²/d; HRAP_c: 9 g/m²/d) (Chapter 3). The combination of the increased biomass productivity of the HRAP and the increased biomass harvestability with recycling improved the 'harvestable biomass productivity' by 58% compared with the control (HRAP_r: 9.2 g/m²/d; HRAP_c: 5.8 g/m²/d). This is particularly important because improvement of the harvestable biomass productivity (i.e. net biomass yield) from wastewater treatment HRAPs could benefit the energy production potential. Overall, recycling increased the net biomass energy yield by

66% (HRAP_r: 195 kJ/m²/day; HRAP_c: 118 kJ/m²/day) through the combined improvements in biomass productivity, harvest efficiency and a small increase in algal biomass energy content (Chapter 3).

To confirm the reproducibility of these findings and investigate the mechanisms responsible, twelve outdoor 18 L mesocosms were studied over two different seasons. This research confirmed that recycling established *P. boryanum* dominance, and improved biomass productivity (by >25%) and settleability (by >20%) compared with controls that had no recycling. Another mesocosm study compared recycling of the separated solid and liquid components of harvested biomass to that of un-separated biomass. Settleability was improved by recycling of the ‘solid’ fraction, selecting for larger and faster settling colonies. Surprisingly recycling the ‘liquid’ fraction caused a similar improvement in settleability as recycling the solid fraction, potentially indicating that recycling the liquid fraction of harvested biomass may contain extracellular polymeric substances that improve settleability.

Several possible mechanisms to explain the increase in biomass productivity with recycling were identified, including an increase in bacterial biomass production (although this was refuted by laboratory pure culture experiments, Appendix A). However, the two most plausible mechanisms were: (i) the HRAP mean cell residence time (MCRT) was extended thereby enabling algae to grow for longer and increasing the algal concentration, so that incident sunlight was more fully utilized, and (ii) the relative proportions of algal growth stages (which have different net growth rates) was shifted, resulting in an increase in the net growth rate of the algal culture (Chapter 4).

To investigate the mechanisms behind the improved productivity, a microcosm study of the life-cycle of *P. boryanum* was conducted under four combinations of light (250 or 120 μMol/m²/s) and temperature (20 or 10°C) conditions (Bold 3N medium used), including determination of the timing and the net growth rates of each of the life-cycle stages (‘juvenile’, ‘growth’ and ‘reproductive’) (Chapter 5). This study showed for the first time that the net growth rate varies between *P. boryanum* life-cycle stages (‘growth’ > ‘juvenile’ > ‘reproductive’). The previous mesocosm studies in Chapter 4 showed that recycling increased the number of growth colonies by ~2-fold and juvenile colonies by ~4-fold. Therefore, as well as improving productivity by extending the MCRT, it is likely that

recycling also increased the net growth rate of the algal culture by ‘seeding’ the pond with faster growing colonies (i.e. both ‘growth’ and ‘juveniles’).

This Ph.D. work has demonstrated that recycling a portion of gravity harvested biomass could be a simple and practical method to enhance biomass productivity, harvest efficiency and energy content, which contribute to achieve higher ‘harvestable biomass productivity’ and ‘energy yield’ in wastewater treatment high rate algal ponds.

Appendix A

Algal recycling enhances biomass productivity in *Pediastrum boryanum* pure cultures

Preface

Chapters 2, 3 and 4 showed that recycling a portion of gravity harvested biomass (i.e. algae and associated wastewater bacteria biomass) improved biomass productivity in both the pilot-scale wastewater treatment HRAPs and mesocosms. While recycling did not change the relative proportions of algae and bacteria in the HRAP culture (Chapter 4), the contribution of the wastewater bacteria to the improved biomass productivity with recycling was not certain and still required investigation. Therefore *Pediastrum boryanum* was grown in pure culture on synthetic growth media (containing inorganic carbon) under laboratory conditions to determine the influence of recycling on the productivity of *P. boryanum* without the presence of wastewater bacteria.

Abstract

Recycling a portion of gravity harvested biomass (i.e. algae and associated wastewater bacterial biomass) has been shown to improve biomass productivity by maintaining the dominance of a rapidly-settleable colonial alga, *Pediastrum boryanum* in both pilot-scale wastewater treatment High Rate Algal Ponds (HRAP) and mesocosms. While recycling did not change the relative proportions of algae and bacteria in the HRAP culture, the contribution of the wastewater bacteria to the improved biomass productivity with recycling was not certain and required further investigation. *P. boryanum* was therefore grown in pure culture on synthetic growth media (containing inorganic carbon) under laboratory conditions to determine the influence of recycling on the productivity of *P. boryanum* without the presence of wastewater bacteria. Six 1 L *P. boryanum* cultures were grown over 30 days in a laboratory growth chamber simulating New Zealand summer conditions either with (P_r) or without (P_c) recycling (100 mg harvested biomass recycled / g produced / day). The productivity of cultures with recycling (P_r) was higher than that of the controls without recycling (P_c) when the cultures were operated at both 4 and 3 d HRTs (by 11% and 38% respectively). This result confirmed that the presence of wastewater bacteria was not necessary to improve algal productivity with recycling.

1. Materials and methods

1.1. Isolation of *P. boryanum* and composition of synthetic growth media

Details of the method of *P. boryanum* isolation from a wastewater treatment pilot-scale HRAP and the composition of the sterilized synthetic wastewater growth media (based on Bold 3N medium, (Shi et al. 2007)) were described previously in Chapter 5.

1.2. Operation of pure *P. boryanum* semi-continuous cultures

A schematic diagram of the laboratory experiment is shown in Figure 1, and the simulated summer growth conditions summarized in Table 1. Justification for the light and temperature used in the simulated summer culture conditions was given in Chapter 5. All glassware and micropipette were sterilized using an Autoclave (TOMY, High Pressure Steam Sterilizer ES-315) to minimize contamination of bacteria and fungi during the experiment.

Table 1: Light/dark cycle, temperature, light intensity of the simulated summer algal growth chamber.

		Simulated Summer Conditions
Day	Hours	14
	Light intensity ($\mu\text{Mol}/\text{m}^2/\text{s}$)	250
	Temperature ($^{\circ}\text{C}$)	25
Night	Hours	10
	Light intensity ($\mu\text{Mol}/\text{m}^2/\text{s}$)	0
	Temperature ($^{\circ}\text{C}$)	18

Six 1 L pure cultures of *P. boryanum* (200 ml of *P. boryanum* stock culture and 600 ml of Bold 3 growth medium) were grown over 30 days either with recycling (P_r) or without (P_c). The cultures were mixed with a magnetic stirrer bar and 1% CO_2 in air was added continuously. The hydraulic retention time (HRT) was maintained at 4 days for the first 20 days (D0-D19) and then reduced to 3 days for the remaining 10 days of the experiment (D20-D30). The cultures were grown in semi-continuous culture by replacing a portion of the algal culture with new growth media daily (200 ml during the 4 d HRT and 265 ml during the 3 d HRT).

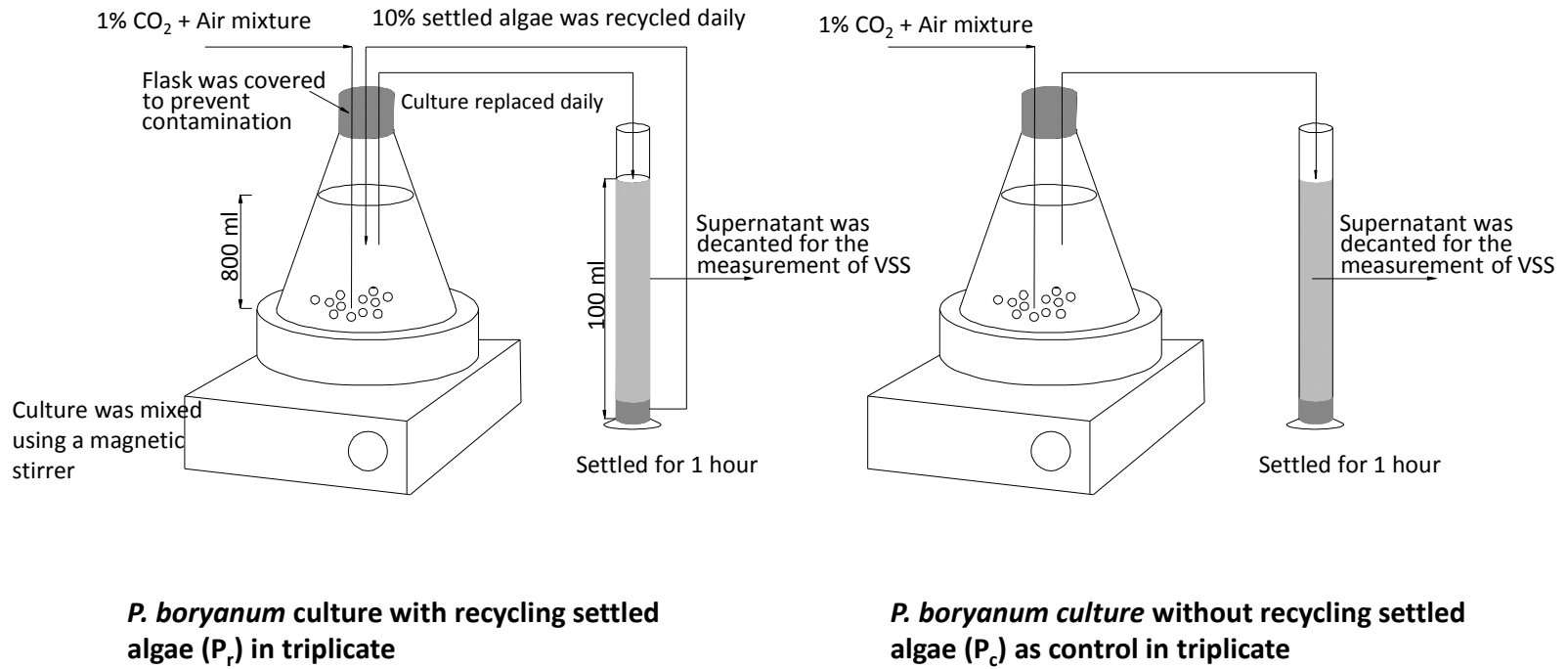


Figure 1: A schematic diagram for the *Pediastrum boryanum* cultures with (P_r) recycling and without (P_c).

Algal settleability (results not presented) was determined by settling 100 ml of the culture effluent in a settling chamber for 1 hour, after which a 90 ml volume of supernatant was gently decanted and used to measure VSS and compared with that of the culture effluent. The remaining 10 ml containing rapidly-settleable *P. boryanum* colonies was used for recycling back to the P_r cultures. A 10% mass recycling rate (100 mg harvested biomass recycled / g produced / day) was previously shown to give the highest increase in algal biomass productivity in outdoor mesocosm studies (Park et al. Submitted). Based on the 10% by mass recycling rate, the volume that was recycled each day (2.2-2.6 ml or 2.8-3.3 ml for the 4 d and 3 d HRT respectively) was determined using the previous days' average algal concentration in the P_r culture effluents and the average settleability. Later comparison with the average algal concentration in the P_r culture effluents on the day of recycling showed that the actual mass recycling rates during the 4 d and 3 d HRT culture periods were 10.3-11.0% and 9.9-10.3% respectively.

1.3. Analysis

The volatile suspended solids (VSS) of the culture effluent samples was measured at least three times a week according to Standard Methods (APHA, 2008) using 100 ml of the sample of culture effluent. Algal productivity (mg/L/d), was calculated based on the VSS values using Equation 1. The initial increase in VSS concentration in the P_r cultures due to the recycling was subtracted from the measured VSS concentration before calculating the productivity.

$$P = \frac{(C \times Q) - R}{V} \quad \text{Equation 1}$$

Where;

P: Volumetric algal productivity (mg/L/d)

C: Algal concentration of the culture effluent (VSS, mg/L)

Q: Culture volume replaced daily (4 d HRT: 0.2 L/d; 3 d HRT: 0.265 L/d)

R: Recycled algal mass per day (mg/d)

V: Total culture volume (0.8 L)

Microscopic image analysis was conducted to determine the colony size distribution in the cultures on Day 19. Full details of the methods and equipment used to count and measure the dimensions of colonies were described in Chapter 2 and 5. Bacterial contamination in the cultures was also examined at least twice a week by microscopic analysis.

2. Results and Discussion

2.1. Improving algal productivity by recycling

The influence of recycling on productivity (measured as mg/L/d) is presented in Figure 2 and the results are summarized with statistical analysis in Table 2. When initially operated with a 4 d hydraulic retention time (HRT), both cultures (P_r and P_c) had an initial fast growth phase (D0-D8) followed by slower growth phase (D9-D19) (Figure 2). During the fast growth phase over the first 8 days, the algal concentration of both cultures rapidly increased from 35 mg/L to 196 mg/L (P_r) and 185 mg/L (P_c). Thus, the P_r cultures had slightly higher productivity than the controls (P_c) (P_r : 54.1 ± 2.7 g/m³/d; P_c : 51.3 ± 1.9 g/m³/d on Day 8). When both cultures were in the slower growth phase (D9-D19), the P_r cultures had 11% higher average productivity than the controls (P_r : 62.4 ± 2.6 g/m³/d; P_c : 56.1 ± 1.3 g/m³/d; *P-value*: <0.05, Table 2). From days 20 to 30 when the HRT was reduced to 3 days, the algal concentration in both cultures declined (Figure 2). However, the average productivity in the P_r cultures (50.6 ± 11.2 mg/L/d) was 38% higher (*P-value*: <0.001, Table 2) than that of the controls (33.5 ± 2.5 mg/L/d).

The previous studies in both pilot-scale HRAPs and mesocosms showed that recycling a portion of gravity harvested biomass, containing algae and associated wastewater bacterial biomass, improved biomass productivity by >20% (Chapter 3 and 4). This study using pure *P. boryanum* cultures has showed that recycling algae alone (i.e. gravity harvested *P. boryanum* colonies) also increased algal productivity, confirming that the presence of wastewater bacteria was not necessary to achieve the increased productivity with recycling in the pilot-scale HRAPs and mesocosms.

Table 2: Average algal concentration, productivity, and one-way ANOVA analysis of the effect of recycling on productivity.

Hydraulic Retention Time (HRT, d)	4		3	
Culture periods	Day 0 - 19		Day 20 - 30	
	P _c (control)	P _r (recycling)	P _c (control)	P _r (recycling)
Ave. algal conc. (mg VSS/L)	218.2±13.7 ⁽¹⁾	242.0±16.0 ⁽¹⁾	114.2±68.2	177.7±61.2
Ave. productivity (g VSS/m ³ /d)	56.1±1.3	62.4±2.6	36.5±2.5	50.6±11.2
Effect of recycling on productivity				
% increase	n/a	11.2±2.2	n/a	38.6±9.7
<i>P</i> -value ⁽²⁾	n/a	<0.05	n/a	<0.001
Significance	n/a	>99.5% confidence	n/a	>99.99% confidence
Effect	n/a	Strong	n/a	Very strong

Note:

(1) Calculated when the growth of *P. boryanum* reached steady state growth phase on Day 8

(2) Compared productivity between P_c and P_r using One-way ANOVA analysis

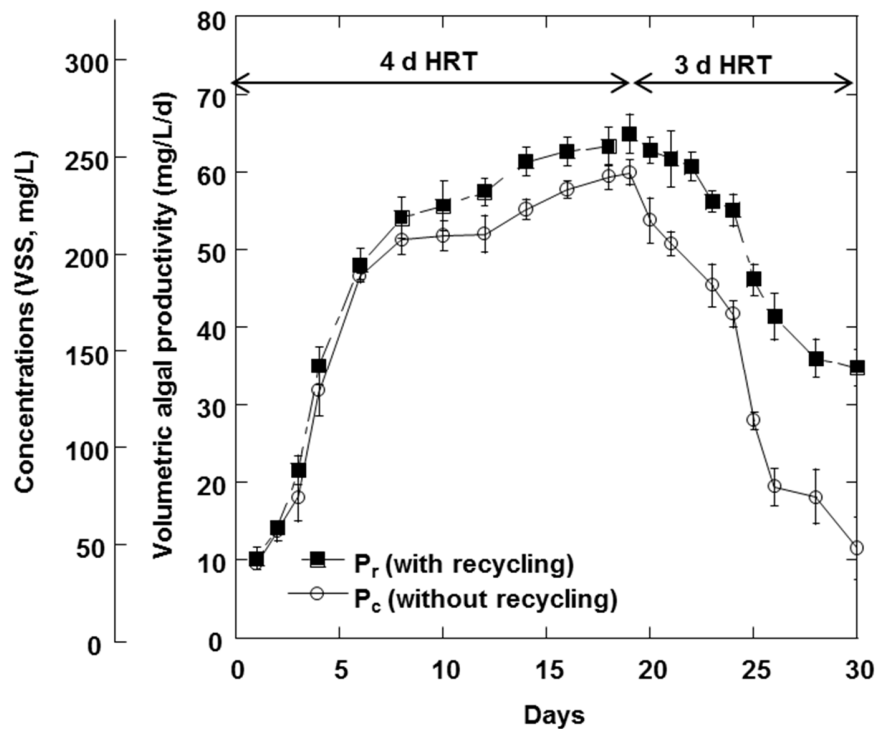


Figure 2: Algal concentration and productivity in pure cultures of *P. boryanum* with recycling (P_r) and without recycling (P_c) under simulated summer culture conditions over 30 days at two HRTs (4 d: D0 - D19 and 3 d: D20 - D30).

3. Conclusions

This laboratory study confirmed that wastewater bacteria are not necessary for the improvement of biomass productivity achieved by recycling in the pilot-scale HRAPs and mesocosms. Recycling algae alone (i.e. large gravity harvested *P. boryanum* colonies) increased the average algal productivity by 11% at a 4 d HRT and 38% in a 3 d HRT in the pure *P. boryanum* cultures (P_r) compared with the controls (P_c).

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Appendix B

Measurement uncertainty for biomass productivity

Preface

This appendix is to determine measurement uncertainty for the biomass productivity during the period of the pilot-scale HRAP studies over two years (Chapters 2 and 3)

The measurement of the VSS concentration (C) and flow rate (Q_{inf}) could affect the calculated biomass productivity of the HRAPs (P). Thus, the uncertainty (% error) for the biomass productivity was calculated by adding together the uncertainty in flow measurement ($u(f)$: Equation 1) and VSS analysis ($u(C)$: Equation 2).

1. Uncertainty for flow measurement was due to the error associated with the flow rate of the submersible pump (DOC3/A, Lowara). To determine the uncertainty, the variation of flow rate of the pumps was measured at least three times during each 1 year experiment when the pumps were recalibrated and then uncertainty was calculated using Equation 1 (Huang et al. 2010). The uncertainty ($u(f)$) was estimated at $\pm 0.8\%$ based on the mean flow rate (\bar{f}) of 1.31 L/sec and standard deviation ($\overline{\Delta f}$) of the flow rate of 0.01 L/sec.

$$u(f) = \frac{\overline{\Delta f}}{\bar{f}} \times 100$$

Equation 1

$u(f)$: uncertainty for flow measurement

Δf : The standard deviation of the measured flow rate

$\overline{\Delta f}$: The mean value of Δf

\bar{f} : The mean value of the measured flow rate

2. Uncertainty for VSS analysis included the errors associated with: the weighing of the pre-washed and oven dried GF/C filters using a 4 digit analytical balance (Denver, SI 234); the accuracy of measuring the sample volume using a 50 ml measuring cylinder); drying (at 100°C) and incinerating (at 450°C). To determine the uncertainty for VSS analysis, a pond water sample was taken from the each HRAP and six replicates of VSS were measured and then uncertainty were calculated using Equation 5 (Huang et al. 2010). VSS of 265.7 ± 6.5 mg/L ($u(C)$: 2.4%) for the HRAP_r and VSS

Appendix B

of 210.3 ± 5.4 mg/L ($u(C)$: 2.6%) for the HRAP_c was determined in October 25, 2009. Thus, the uncertainty for VSS analysis can be estimated at $\pm 2.5\%$.

$$u(C) = \frac{\overline{\Delta X}}{\bar{X}} \times 100$$

Equation 2

$u(C)$: Uncertainty for VSS analysis

ΔX : The standard deviation of the measured VSS concentration

$\overline{\Delta X}$: The mean value of ΔX

\bar{X} : The mean value of the measured VSS concentration

Overall, the uncertainty in the biomass productivity based on VSS measurement was identified at $\pm 3.3\%$ (adding $u(C)$ of $\pm 2.5\%$ and $u(f)$ of $\pm 0.8\%$).