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Effect of algal recycling rate on the performance of *Pediastrum boryanum* dominated wastewater treatment high rate algal pond

J. B. K. Park and R. J. Craggs

ABSTRACT

Recycling a portion of gravity harvested algae promoted the dominance of a rapidly settling colonial alga, *Pediastrum boryanum* (*P. boryanum*) and improved both biomass productivity and settleability in High Rate Algal Pond (HRAP) treating domestic wastewater. The effect of algal recycling rate on HRAP performance was investigated using 12 replicate mesocosms (18 L) that were operated semi-continuously under ambient conditions. Three experiments were conducted during different seasons with each experiment lasting up to 36 days. Recycling 10%, 25%, and 50% of the 'mass' of daily algal production all increased total biomass concentration in the mesocosms. However, recycling >10% reduced the organic content (volatile suspended solids (VSS)) of the mesocosm biomass from 83% to 68% and did not further increase biomass productivity (based on VSS). This indicates that if a HRAP is operated with a low algal concentration and does not utilise all the available sunlight, algal recycling increases the algal concentration up to an optimum level, resulting in higher algal biomass productivity. Recycling 10% of the daily algal production not only increased biomass productivity by ~40%, but increased biomass settleability by ~25%, which was probably a consequence of the ~30% increase in *P. boryanum* dominance in the mesocosms compared with controls without recycling.

Key words | algal biofuel, algal productivity, algal recycling, High Rate Algal Ponds (HRAPs), *Pediastrum boryanum*

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INTRODUCTION

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 (Benemann 2008; Craggs *et al.* 2011, 2014). Furthermore, the algal biomass produced and economically harvested from HRAP effluent could be converted to biofuels such as biogas, biodiesel, bioethanol, and bio-crude oil (Sukias & Craggs 2010; Vasudevan & Fu 2010; Craggs *et al.* 2011). To achieve efficient wastewater treatment and economic algal biofuel production both high algal biomass production and rapid and cost-effective harvest of algal biomass from HRAP effluent are required (Benemann 2008; Brennan & Owende 2010; Park *et al.* 2011b). Therefore, methods to improve algal biomass productivity and harvest efficiency would be of great benefit.

Gravity sedimentation is the most common and cost-effective method of algal biomass harvest (removal) from wastewater treatment HRAP effluent, because of the large volumes of wastewater that need to be treated and the low value of harvested algal biomass (Nurdogan & 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 algal biomass (Nurdogan & Oswald 1996; Brennan & Owende 2010; Park *et al.* 2011b). While various harvesting options including chemical and mechanical methods have been investigated previously (Brennan & Owende 2010; Mata *et al.* 2010), most technologies have high operation costs (Benemann 2008; Craggs *et al.* 2011). For example, chemical flocculation can be reliably used to remove small algae (<5 μm) 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

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used for the removal of algal biomass, but the high energy requirement makes it economically viable for only secondary thickening of primary harvested algae (with 1–2% solids) up to 20–30% solids for use in biofuel production (Benemann 2008; Alabi *et al.* 2009). Algae commonly found in wastewater treatment HRAPs (including *Scenedesmus* sp., *Micractinium* sp., *Actinastrum* sp., *Pediastrum* sp., and *Coelastrum* sp.) often form large-diameter (50–200 μm) settleable colonies, which enable cost effective and simple biomass harvest by gravity sedimentation (García *et al.* 2000; Benemann 2008; Park *et al.* 2011b). Park and Craggs (2010) reported that CO_2 addition to wastewater treatment HRAPs promoted the formation of large bioflocs of algal colonies associated with wastewater bacteria (diameter: >500 μm), which settled rapidly in a simple gravity harvester with a retention time of 12 hours or less. Therefore, operating wastewater treatment HRAPs to promote both the growth of particular settleable algal species (i.e. colonial species) and the formation of algal–bacterial bioflocs could greatly enhance the efficiency of algal harvest from the effluent.

Algal biomass production in wastewater treatment HRAPs could be enhanced if the algae were able to utilize more of the available incident light energy (Weissman & Benemann 1979; de la Noüe & Ní Eidhin 1988). 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 hydraulic retention time (HRT) (e.g. an 8 d HRT in winter and a 4 d HRT in summer) (Park *et al.* 2011a, 2013a) but MCRT and algal concentration are not optimized. 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 (de la Noüe & Ní Eidhin 1988; Park *et al.* 2011a, 2013a). Our previous work has shown that recycling a portion of gravity harvested algae ('algal recycling') from a pilot-scale wastewater treatment HRAP improved 'in-pond' algal biomass productivity and harvest efficiency by gravity sedimentation while maintaining the dominance of a readily settleable colonial alga, *Pediastrum boryanum* (*P. boryanum*) (Park *et al.* 2011a, 2013a, b). In particular, *P. boryanum* was maintained at >85% dominance in a pilot-scale HRAP for 2 years by algal recycling (Park *et al.* 2011a, 2013a). This result confirmed, for the first time in the literature, that species control was possible for similarly sized co-occurring algal colonies in an outdoor wastewater treatment HRAP (Park *et al.* 2011a, 2013a). Increased dominance of *P. boryanum* improved the biomass harvest efficiency by gravity sedimentation from less than 60% (in the control HRAP without recycling) to over 85% (Park *et al.* 2011a). Furthermore, algal recycling improved annual average biomass productivity by 18% to 10.9 g/m^2/d compared with the control HRAP without recycling (9.2 g/m^2/d) (Park *et al.* 2013a). During this pilot-scale HRAP study algal recycling was conducted on a volumetric basis with 1 L of gravity harvested algae recycled back to the HRAP each day (Park *et al.* 2011a, 2013a). Since HRAP algal biomass concentration varied seasonally between 100 and 220 g VSS/m^3 but the harvested algal solids concentration was less variable (28–35 g VSS/L), actual algal recycling rates varied widely from 35 to 470 g VSS/kg algal biomass produced/d (Park *et al.* 2011a). Therefore, the outdoor HRAP mesocosm experiments described in this paper were conducted to investigate how different algal recycling rates influenced the dominance of *P. boryanum*, algal biomass productivity and settleability and to determine an optimal algal recycling rate to enhance HRAP performance.

MATERIALS AND METHODS

A description of the two 8 m^3 pilot-scale HRAP systems located at the Ruakura Research Centre, Hamilton, New Zealand (37°47′S, 175°19′E) and annual operation parameters (July 2010–June 2011) is given in Park *et al.* (2013a). Twelve replicate HRAP mesocosms (plastic containers with a water depth of 0.3 m; volume of 18 L; surface area of 0.07 m^2; mixing with a magnetic stirrer; and 1% CO_2 added) were set up and operated under outdoor ambient conditions next to the two pilot-scale HRAPs. The containers were foil-wrapped so that sunlight only entered the culture through the water surface. All 12 mesocosms were initially filled with water from a pilot-scale HRAP dominated by *P. boryanum*, which is a readily settleable colonial alga. The HRAP water was pre-filtered using a 200 μm mesh to remove large invertebrates (e.g. *Daphnia* sp. or *Moina* sp.) to avoid potential algal grazing. The schematic diagram is presented in Figure 1 and the operational parameters are summarized in Table 1.

In Experiment 1, the effect of the relatively high algal recycling rates of 10%, 25%, and 50% on the 'mass' of algal biomass that was produced daily (M_{10}, M_{25}, and M_{50}) was investigated in triplicate mesocosms. In Experiment 2, triplicate mesocosms were operated with recycling of 1%, 2.5%, and 5% of the 'volume' of biomass that was produced daily (M_1, $M_{2.5}$, and M_5). Finally, in Experiment 3, the effect of a lower recycling rate was further investigated in triplicate

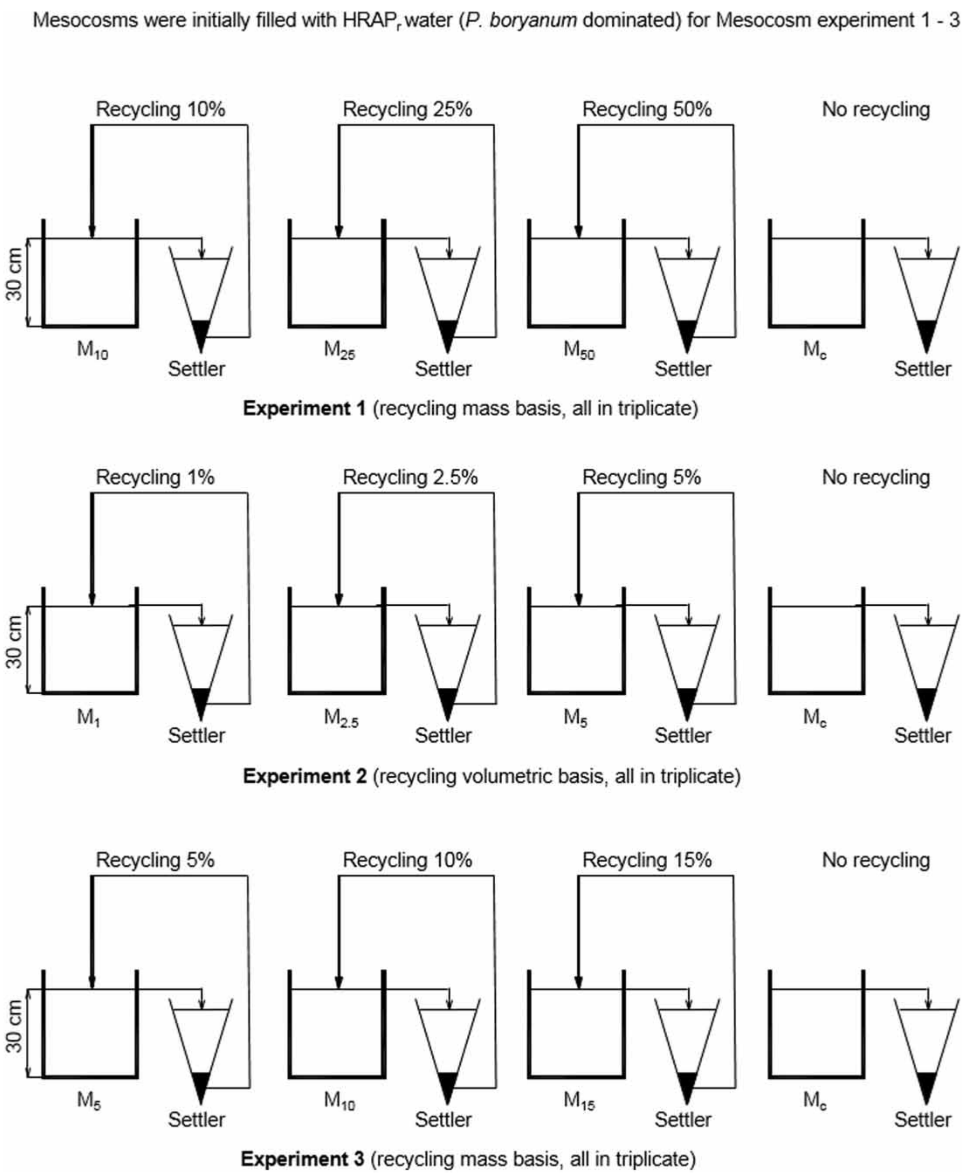

Figure 1 | Schematic diagram for the operation of the HRAP mesocosms investigating the effect of different algal recycling rates on biomass productivity, settleability and *P. boryanum* dominance.

mesocosms with recycling of 5%, 10%, and 15% of the 'mass' of algal biomass that was produced daily (M_5, M_{10}, and M_{15}). The mesocosms were operated as semi-continuous cultures with the same hydraulic retention time (HRT) as the pilot-scale HRAPs (for the season) by daily replacement of a portion of the mesocosm water with primary settled domestic wastewater. During Experiment 1 (conducted in the spring) the mesocosms were operated with a 6 d HRT by replacing 3 L of culture water each day. In Experiment 2 (conducted in the summer) the mesocosms were operated with a 4 d HRT by replacing 4.5 L of culture water each day. During Experiment 3 (conducted in the winter), the mesocosms were operated with an 8 d HRT by replacing 2.25 L of culture water each day. In all three experiments, the performance of the mesocosms with algal recycling was compared to triplicate control mesocosms (M_c) without recycling in terms of biomass productivity, settleability, and *P. boryanum* dominance.

In Experiment 1, mesocosm effluent samples (100 ml) were taken daily to measure total and volatile suspended solids (TSS/VSS) according to *Standard Methods* (APHA 2008) and used to determine algal biomass productivity. Algal biomass productivity was calculated using Equation (1) which includes subtracting the increase in VSS

Table 1 | HRAP mesocosm experiment operational parameters (including: algal recycling rate (as a % of either the 'mass' of daily algal production for Experiments 1 and 3 or 'volume' of daily algal production for Experiment 2); HRT and MCRT)) and the experimental results (including: biomass productivity; settleability; and *P. boryanum* dominance)

		Experiment 1				**Experiment 2**				**Experiment 3**			
Experimental period		Sept 7–18, 2010 (11 days in spring)				Feb 14–Mar 22, 2011 (36 days in summer)				Aug 29–Oct 4, 2011 (36 days in winter)			
Initial pond water (Inoculum)		*P. boryanum* dominated $HRAP_r$ water[1]											
Mesocosms		M_c[2]	M_{10}[3]	M_{25}	M_{50}	M_c	M_1[4]	$M_{2.5}$	M_5	M_c	M_5[5]	M_{10}	M_{15}
Algal recycling rate	% harvested volume	n/a	1.0–2.6	2.5–6.5	5.0–13	n/a	1	2.5	5	n/a	0.5–1.3	1.0–2.6	1.5–4.0
	% harvested mass	n/a	10	25	50	n/a	2.2–3.5	5.2–8.8	11.1–21.4	n/a	5	10	15
HRT (d)	Designed (d)	6				4				8			
MCRT (d)[6]		6.2 ± 0.3	6.82 ± 0.2	7.8 ± 0.3	9.3 ± 0.3	3.8 ± 0.2	4.1 ± 0.2	4.3 ± 0.5	4.7 ± 0.2	7.6 ± 0.2	8.0 ± 0.3	8.4 ± 0.3	8.7 ± 0.2
Mean biomass productivity ($g/m^2/d$)		11.5 ± 0.7	13.1 ± 0.8	13.5 ± 1.0	13.8 ± 1.0	11.0 ± 1.6	14.2 ± 2.1	15.2 ± 2.3	12.4 ± 1.6	7.9 ± 1.9	8.1 ± 0.5	8.6 ± 2.3	9.1 ± 2.4
1-h settleability (%)		–	–	–	–	65.3 ± 4.7	77.7 ± 8.5	82.2 ± 2.9	78.7 ± 5.1	54.1 ± 11.5	57.4 ± 10.4	72.9 ± 11.6	76.2 ± 12.7
% *P. boryanum* dominance on Day 36		–	–	–	–	67 ± 7.2	70 ± 5.5	90 ± 3.4	93 ± 5.5	50 ± 7.2	55 ± 5.5	87 ± 3.7	88 ± 5.7

Notes:

(1) *P. boryanum* dominance of >90%.

(2) M_c: Mesocosms without recycling as a control.

(3) $M_{10,\ 25,\ or\ 50}$: Mesocosms with recycling 10%, 25%, and 50% fixed mass of gravity harvested biomass (Experiment 1).

(4) $M_{1,\ 2.5,\ or\ 5}$: Mesocosms with recycling 1%, 2.5%, and 5% fixed volume of gravity harvested biomass (Experiment 2).

(5) $M_{5,\ 10,\ or\ 15}$: Mesocosms with recycling 5%, 10%, and 15% fixed mass of gravity harvested biomass (Experiment 3).

(6) MCRT: Equation is previously presented in Park *et al.* (2011a, b).

concentration due to algal 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.co.nz/).

$$P = \frac{(C \times Q_c) - R}{A}, \tag{1}$$

$Q_c = Q_{\text{inf}} + ((\text{rainfall–evaporation}) \times \text{mesocosm surface area})$, where P: algal biomass productivity (g/m^2/d), C: algal biomass concentration in the mesocosm (VSS, g/m^3), A: mesocosm surface area (0.07 m^2), R: biomass recycled per day (2.5 ml/d), Q_c: adjusted daily mesocosm outflow (m^3/d), Q_{inf}: daily inflow (Experiment 1: 3 L/d; Experiment 2: 4.5 L/d; and Experiment 3: 2.3 L/d).

In Experiments 2 and 3, algal biomass productivity and settleability were determined three times a week and relative algal dominance was also determined using microscopic analysis on three occasions (Days 0, 18, and 36) based on the technique developed in Park *et al.* (2011b). The settleability of the algal biomass in the effluent removed from each mesocosm was measured in the laboratory over 1 h using 1 L 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 initial VSS of the mesocosm effluent to give the biomass settleability.

Gravity settled algal biomass containing rapidly settleable *P. boryanum* colonies was collected from each Imhoff cone daily (using a tap in the bottom of the Imhoff cone, Figure 1) and then a portion of the collected algal biomass was recycled back to the mesocosm depending on the algal recycling rate needed. The volume of the algal biomass that was recycled was determined each day using Equations (2) and (3), and varied depending on the previous days' average algal biomass concentration in the mesocosm effluents and the average algal settleability. For example, to maintain a 10% recycling rate in M_{10} during Experiments 1 and 3 (Figure 1), the volume of algal biomass recycled each day varied from 6.7 to 7.5 ml or from 5 to 5.6 ml for the 6 d and 8 d HRT, respectively. Later comparison with the average algal biomass concentration in the mesocosm effluents on the day of recycling showed that the actual mass recycling rates were maintained at ~10% in both experiments (Experiment 1: 9.5%–11.0%; and Experiment 3: 9.4%–10.8%).

$$X_m = C_a \times V_e, \tag{2}$$

where X_m: total algal mass produced (mg), C_a: algal biomass concentration in mesocosm effluents, V_e: the mesocosm water replaced (4.5 L for a 4 d HRT; 3 L for a 6 d HRT; and 2.25 L for an 8 d HRT).

$$V_r = \frac{V_a \times X_r}{X_T}, \tag{3}$$

where V_r: algae volume to be recycled (ml), V_a: total algae volume collected from the Imhoff cone (a 20 ml fixed volume was collected), X_r: algae mass to be recycled based on an algal recycling rate ($X_r = X_m \times$ % algal recycling rate), X_T: total algae mass collected in the 1 L Imhoff cone ($X_T = C_a \times$ % algal settling efficiency $\times$1 L Imhoff cone volume).

RESULTS AND DISCUSSION

Experiment 1 investigated the influence of recycling 10%, 25%, or 50% of daily algal production on biomass productivity in the mesocosms and was conducted during a NZ spring. The total biomass (TSS) concentration, organic content of the biomass (%VSS of the TSS), and biomass productivity (based on VSS) are presented in Figure 2 and the data are summarized in Table 1. The TSS concentration increased in all of the mesocosms with a greater increase found in the mesocosms that had a higher amount of recycling (Figure 2(a)). However, the organic content of the biomass (%VSS of the TSS) in the mesocosms with a high level of algal recycling (M_{25} and M_{50}) declined from 83% to 68%, while it was maintained at ~80% in the mesocosms that had low level (M_{10}) or no (M_0) algal recycling. These results suggest that if the algal biomass concentration in the mesocosm algal cultures had already reached the optimum level (assuming for a 6 d HRT ~380 mg VSS/L, or 410 mg TSS/L on Day 7, Figure 2(a)), recycling a higher mass of the daily algal production (i.e. 25% or 50%) increased the concentration of inorganic compounds (e.g. non-volatile or inert material). This might have reduced sunlight penetration through the mesocosm algal culture, potentially causing light limitation in M_{25} and M_{50}. Recycling 10% of the daily algal production increased algal biomass productivity by ~20% to ~13 g/m^2/d compared with that of the control mesocosms that had no recycling (11.5 g/m^2/d) (Table 1; Figure 1(c)). However, recycling greater than 10% (i.e. 25% and 50%) did not increase biomass productivity further. These results indicate that if the algal concentration (i.e. algal population density) in the

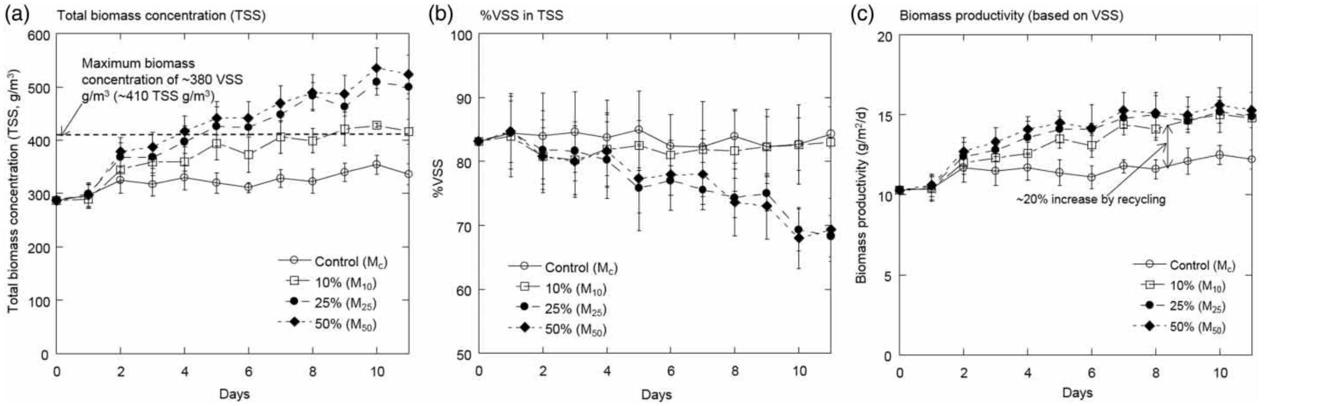

Figure 2 | Experiment 1: Mean ± s.d. of triplicate mesocosm biomass productivity with recycling of 10%, 25%, and 50% of the mass of the daily algal production in spring (September 7–18, 2011).

algal culture is below that which can be supported by the available environmental conditions (such as light, temperature, and nutrients), algal recycling (at ~10% of the mass of daily algal production) could increase the algal concentration to an optimal level, resulting in a higher net conversion of incident light energy to biomass in the algal culture (i.e. higher biomass productivity).

Potential mechanisms to explain the increase in biomass productivity with algal recycling were identified in our previous studies (Park *et al*. 2013a, b, 2014) including: (i) the MCRT was extended thereby enabling algae to grow for longer, thus increasing the algal concentration, so that incident sunlight was more fully utilized, and (ii) the relative proportions of algal growth stages (which have different specific growth rates) was shifted, resulting in an increase in the net growth rate of the algal culture. Furthermore, a microcosm study of the life-cycle of *P. boryanum* (i.e. the dominant alga in the current study) (Park *et al*. 2014) confirmed that the net growth rate varies between the life-cycle stages ('growth' > 'juvenile' > 'reproductive'). This

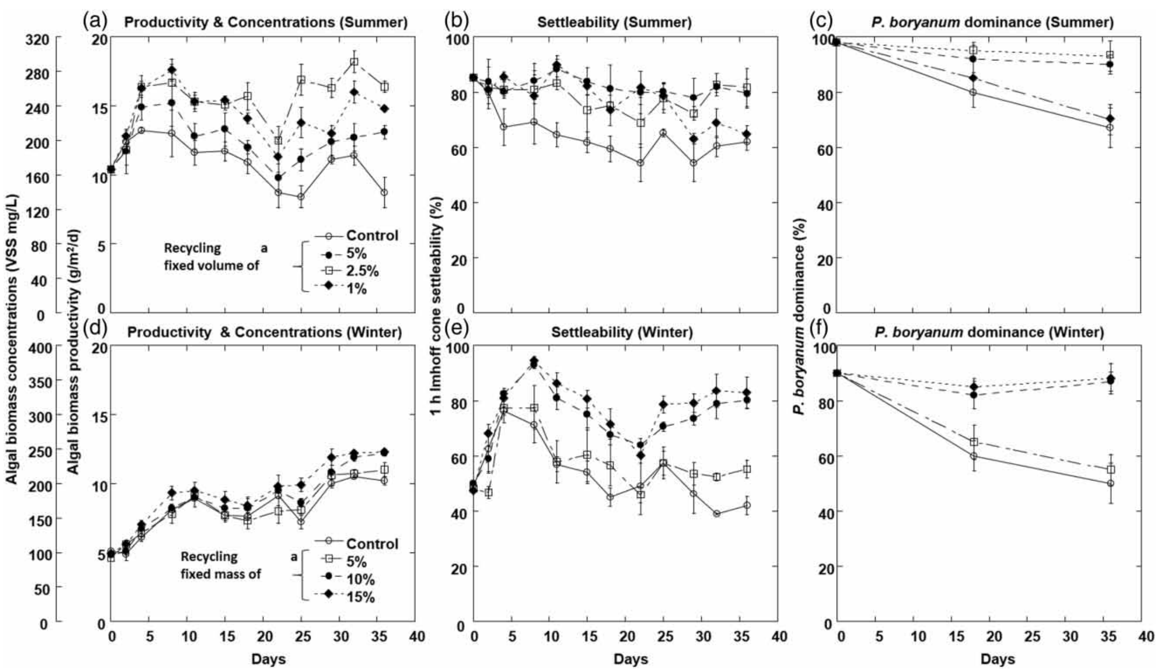

Figure 3 | Experiments 2 and 3: Mean ± s.d. of triplicate mesocosm biomass productivity ((a) and (d)); 1-h settleability ((b) and (e)); *P. boryanum* %-dominance ((c) and (f)) with recycling of 1%, 2.5%, and 5% of the daily volume of gravity harvested algae during the summer (February 14–March 22, 2011) or recycling 5%, 10%, and 15% of the daily mass of gravity harvested algae during the winter (August 29–October 4, 2011).

suggests that as well as improving algal biomass productivity by extending the MCRT, it is likely that algal recycling also increased the net growth rate of the algal culture by 'seeding' the pond with faster growing colonies (i.e. gravity harvesting selected for large 'growth' colonies and 'juvenile' colonies that were soon released from large 'reproduction' colonies).

During the second mesocosm experiment (conducted in summer with a 4 d HRT), the mesocosms ($M_{2.5}$) with 2.5% volume recycling (equivalent to recycling 5.2%–8.8% mass of daily algal production) had both the highest algal biomass productivity (15.2 g/m^2/d) and settleability (82.2% after 1 h settling in a Imhoff cone) compared with the controls that had no recycling (M_c: 11.0 g/m^2/d and 65.3%), or mesocosms with 1% recycling (M_1: 14.2 g/m^2/d and 77.7%) or 5% recycling (M_5: 12 g/m^2/d and 78.7%) (Figures 3(a) and 3(b)). Moreover, the high *P. boryanum* dominance (~90%) in mesocosms $M_{2.5}$ and M_5 was maintained throughout the 36-day experimental period, whereas that of M_1 and M_c both declined to less than 75% (Figure 3(c)). As was reported previously in Park *et al*. (2013a), the dominance of *P. boryanum* declined in the control mesocosms without algal recycling (M_c) and in mesocosms with too low a recycling rate (M_1), resulting in an increase in the dominance of poorly settleable algae (i.e. *Dictyosphaerium* sp.). Since *P. boryanum* colonies are significantly larger (and thus have greater settling velocity) than *Dictyosphaerium* sp. colonies, the shift in algal dominance to the poorly settleable algae explains the decrease in the 1-h settleability observed in M_c and M_1. Moreover, Park *et al.* (2013a) found two potential mechanisms including: (i) recycling the 'solid' fraction of the harvested algae selected for larger and faster settling colonies and (ii) the recycled 'liquid' fraction of harvested algae may contain extracellular polymeric substances. Both would result in an increase in average settling velocity due to the formation of large bioflocs, which then improve biomass settleability. Overall, Experiment 2 indicated that recycling ~2.5% volume of the daily algal production (equivalent to 5.2%–8.8% mass of daily algal production) was sufficient to promote algal biomass productivity and settleability by maintaining the *P. boryanum* dominance in wastewater treatment HRAPs during NZ summer conditions. Finally, the third mesocosm experiment (conducted in winter with an 8 d HRT) recycled either 5%, 10%, or 15% of the mass of daily algal production back to the respective mesocosms (M_5, M_{10}, and M_{15}). All of the mesocosms had low algal biomass productivities compared with those measured in Experiments 1 and 2 due to the seasonal decrease in both average solar radiation (summer: 20.8 ± 7.2 MJ/m^2; and winter: 7.6 ± 2.18 MJ/m^2) and average water temperature (summer: 21.0 ± 2.5°C; and winter: 10.6 ± 2.8°C). However, recycling either 10% (M_{10}) or 15% (M_{15}) of the mass of daily algal production increased biomass productivity by ~10% (Figure 3(d)) and settleability by ~35% (Figure 3(e)) compared with recycling 5% (M_5) or the control without recycling (M_c). Furthermore, recycling 10% (M_{10}) or 15% (M_{15}) also maintained the dominance of *P. boryanum* (at >85%) throughout the 36-day experimental period, while the dominance of *P. boryanum* declined to <60% by Day 36 with recycling at 5% (M_5) or in the control (M_c) without recycling (Figure 3(f)).

CONCLUSIONS

This work investigated how different algal recycling rates affect the performance of the HRAPs and showed that recycling ~10% of the 'mass' of daily algal production in HRAP mesocosms improved biomass productivity (by ~40%), 1-h settleability (by ~25%) and *P. boryanum* dominance (by ~30%) compared with controls that had no recycling. These results indicate that if the algal concentration in the algal culture is below that which can be supported by the available environmental conditions (such as light, temperature, and nutrients), algal recycling could increase the algal concentration up to an optimal level, resulting in higher biomass productivity.

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