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Algal recycling enhances algal productivity and settleability in *Pediastrum boryanum* pure cultures

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ABSTRACT

Recycling a portion of gravity harvested algae (i.e. algae and associated bacteria biomass) has been shown to improve both algal biomass productivity and harvest efficiency by maintaining the dominance of a rapidly-settleable colonial alga, *Pediastrum boryanum* in both pilot-scale wastewater treatment High Rate Algal Ponds (HRAP) and outdoor mesocosms. While algal recycling did not change the relative proportions of algae and bacteria in the HRAP culture, the contribution of the wastewater bacteria to the improved algal biomass productivity and settleability with the recycling was not certain and still required investigation. *P. boryanum* was therefore isolated from the HRAP and grown in pure culture on synthetic wastewater growth media under laboratory conditions. The influence of recycling on the productivity and settleability of the pure *P. boryanum* culture was then determined without wastewater bacteria present. 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 of 10% of gravity harvested algae. The cultures with recycling (P_r) had higher algal productivity than the controls (P_c) when the cultures were operated at both 4 and 3 d hydraulic retention times by 11% and 38% respectively. Furthermore, algal recycling also improved 1 h settleability from ~60% to ~85% by increasing the average *P. boryanum* colony size due to the extended mean cell residence time and promoted formation of large algal bio-flocs (>500 μm diameter). These results demonstrate that the presence of wastewater bacteria was not necessary to improve algal productivity and settleability with algal recycling.

1. Introduction

Microalgae grown under controlled conditions could produce >20 times more oil per hectare than terrestrial oilseed crops such as soy and canola (Sheehan et al., 1998; Chisti, 2007; Benemann, 2008; Park et al., 2011b). However, the capital and operational costs of systems for algal biofuel production are presently prohibitive (Sheehan et al., 1998; Schenk et al., 2008; Benemann, 2008; Craggs et al., 2009; Tampier, 2009; Park et al., 2011b). A niche opportunity may exist where algal biomass produced and harvested as a by-product of wastewater treatment in High Rate Algal Ponds (HRAPs) could be used as a substrate for biofuel conversion such as biogas, bio-ethanol, bio-diesel and bio-crude oil through various biological and chemical pathways (Vasudevan and Fu, 2010; Sukias and Craggs, 2010; Craggs et al., 2011; Campbell et al., 2011; Craggs et al., 2014; Park and Craggs, 2014; Sutherland et al., 2014). However, both wastewater treatment and algal biofuel production essentially require high algal biomass production followed by rapid and cost-effective algal harvest from HRAP effluent. Therefore, improvements in algal biomass production and its subsequent harvest efficiency (i.e. 'harvestable algal biomass production') are crucial to achieve both efficient wastewater treatment and economic algal biofuel production (Benemann, 2003; Chen and Yeh, 2005; van Harmelen and Oonk, 2006; Brennan and Owende, 2010; Pittman et al., 2011; Craggs et al., 2014).

The colonial, non-motile green microalga, *Pediastrum boryanum* has been shown to be a beneficial algal species for wastewater treatment High Rate Algal Ponds (HRAP) (Park et al., 2011a, 2013a; Mehrabadi et al., 2015). This was mainly because the outer layer of the cell wall contains large quantities of silica (Johnson et al., 2011) making the colony dense compared to the other algae. Moreover, the colonies have a large diameter (50–200 μm) compared with other co-occurring algal species (e.g. ~2–5 μm for unicellular

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algae), resulting in greater settleability (Park et al., 2011a). Our previous study (Park et al., 2011a) showed that recycling a portion of gravity harvested algae ('algal recycling') in a pilot-scale wastewater treatment HRAP was found to promote the dominance of *P. boryanum* from 53% to greater than 90% over the one year experimental period in New Zealand climatic conditions. Moreover, a second one year study showed that seeding the original control HRAP (that had no recycling) with gravity harvested *P. boryanum* dominated biomass taken from the HRAP with recycling shifted the algal dominance from 89% *Dictyosphaerium* sp. (a poorly-settleable alga) to over 90% *P. boryanum* within just 5 months (Park et al., 2013a). The results from the two year pilot-scale HRAP studies confirmed, for the first time in the literature, that recycling a portion of gravity harvested algae could enable algal species control of similarly sized co-occurring algal colonies in an outdoor wastewater treatment HRAP (Park et al., 2011a, 2013a).

As a result of higher dominance of the readily settleable alga (*P. boryanum*) in the HRAP with recycling ($HRAP_r$), biomass harvest from the HRAP effluent using a simple gravity harvester (settling time of only ~3–6 h depending on the seasons) was greatly improved with annual average harvest efficiency of 85% in the $HRAP_r$ compared with only ~60% in the control $HRAP_c$ (Park et al., 2011a). Furthermore, interestingly algal recycling also improved biomass productivity by 20% compared with the control $HRAP_c$ without recycling ($HRAP_r$: 11 $g/m^2/d$; $HRAP_c$: 9 $g/m^2/d$) (Park et al., 2013a). Therefore, the combination of the increased algal biomass productivity and increased settleability with recycling improved the 'harvestable biomass productivity' (i.e. net biomass yield) by 58% compared with the control pond ($HRAP_r$: 9.2 $g/m^2/d$; $HRAP_c$: 5.8 $g/m^2/d$) (Park et al., 2013a). This is particularly important because improvement of the harvestable biomass productivity from wastewater treatment HRAPs could benefit both the energy production potential and wastewater treatment performance in terms of nutrient recovery and final HRAP effluent quality. Overall, algal recycling increased the biomass energy yield by 66% ($HRAP_r$: 195 $kJ/m^2/day$; $HRAP_c$: 118 $kJ/m^2/day$) through the combined improvements in biomass productivity, harvest efficiency and a slight increase in algal biomass energy content (Park et al., 2013a).

The increased biomass settleability achieved by recycling in the pilot-scale HRAP and mesocosm studies (Park et al., 2011a, 2013b) was most probably attributable to the formation of larger sized algal colonies (i.e. *P. boryanum* colonies) and/or large bio-floccs of algae and bacteria that co-exist in the HRAP. Many researchers (Benemann et al., 1980; Choi et al., 1998; Lee et al., 2009, 2013) reported that most bacteria produce extracellular polymeric substances (EPS), which promote the formation of microbial aggregates (i.e. bio-flocculation), which probably contributed to the increase in biomass settleability in the HRAP cultures. Recycling experiments using the separated liquid and solid components of the biomass were conducted in the HRAP mesocosms to further assess the effect of algal recycling on the formation of large bio-floccs and the particle size distribution (Park et al., 2013b). Recycling the solid and/or liquid components all increased the upper size of the particle distribution from 400 μm (for the control without recycling) to 1500 μm, which included a second peak of particle size of 500–800 μm due to the formation of large bio-floccs. These results imply that EPS in the recycled liquid component (excreted from either bacteria or algae, or both) may have contributed to the formation of large aggregates of biomass in the mesocosms (Park et al., 2013b). However, the contribution of the wastewater bacteria (or algae) to the improved settleability with algal recycling was uncertain and required further detailed investigation. Therefore, *P. boryanum*, which was the most dominant algal species in both pilot-scale wastewater treatment HRAPs and mesocosms, was isolated from one of the pilot-scale wastewater treatment HRAP and grown in pure culture on inorganic growth media (with similar nutrient concentrations to wastewater) under laboratory conditions. The influence of algal recycling on the productivity and settleability of the pure *P. boryanum* culture was then determined without presence of wastewater bacteria.

2. Materials and methods

2.1. Isolation of P. boryanum and composition of synthetic growth media

P. boryanum colonies were isolated in summer 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, sterilized equipment and sterile liquid growth medium. The composition of the medium was based on Bold 3N medium (containing inorganic carbon and adjusting initial pH to ~7.0) (Shi et al., 2007), but adjusted to contain 5 mg/L of PO_4^{3-}-P (K_2HPO_4) and 20 mg/L NH_4^+-N ($(NH_4)_2SO_4$). The N and P concentrations mimicked the concentrations in the diluted domestic wastewater that was fed into the pilot-scale HRAPs (Park and Craggs, 2011).

Cultures of colonies that were isolated during the summer were grown in algal growth chambers (Contherm Scientific Ltd 6150CP–6400CP) that simulated summer light and temperature conditions respectively (Table 1).

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

The temperatures of the growth chambers simulating summer conditions were determined using water temperature data from the pilot-scale HRAP that was collected using a multi-probe Data-Sonde® at 15 min intervals during the New Zealand summer (December to February) 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) (Table 1).

The light intensity for the summer growth chamber was determined based on the average irradiation over the 30 cm depth of the pilot-scale HRAP using the equation suggested by Morowitz (1950).

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

I_{ave}: Average light irradiation over the depth ($\mu Mol/m^2/s$)
I_0: Light irradiation on the pond surface ($\mu Mol/m^2/s$)
L: Pond depth (cm)

Table 1
Light/dark cycle, temperature, light intensity of the algal growth chamber simulating New Zealand summer conditions.

		Simulated summer conditions
Day	Hours	14
	Light intensity ($\mu Mol/m^2/s$)	250
	Temperature (°C)	25
Night	Hours	10
	Light intensity ($\mu Mol/m^2/s$)	0
	Temperature (°C)	18