Implications of nutrient removal and biomass production by native and augmented algal populations at a municipal wastewater treatment plant

Ivy L. C. Drexler, Sascha Bekaan, Yasmin Eskandari and Daniel H. Yeh

ABSTRACT

Algal monocultures (*Chlorella sorokiniana* and *Botryococcus braunii*) and algal communities native to clarifiers of a wastewater treatment plant were batch cultivated in (1) clarified effluent following a biochemical oxygen demand (BOD) removal reactor post-BOD removal clarified effluent (PBCE), (2) clarified effluent following a nitrification reactor post-nitrification clarified effluent (PNCE), and (3) a reference media (RM). After 12 days, all algal species achieved nitrogen removal between 68 and 82% in PBCE and 37 and 99% in PNCE, and phosphorus removal between 91 and 100% in PBCE and 60 and 100% in PNCE. The pH of the wastewater samples increased above 9.8 after cultivation of each species, which likely aided ammonia volatilization and phosphorus adsorption. Both monocultures grew readily with wastewater as a feedstock, but *B. braunii* experienced significant crowding from endemic fauna. In most cases, native algal species' nutrient removal efficiency was competitive with augmented algal monocultures, and in some cases achieved a higher biomass yield, demonstrating the potential to utilize native species for nutrient polishing and algal biomass production.

Key words | *Botryococcus braunii*, *Chlorella sorokiniana*, microalgae, nutrient removal, phycoremediation

Ivy L. C. Drexler
Yasmin Eskandari
Daniel H. Yeh (corresponding author)
Department of Civil & Environmental Engineering,
University of South Florida,
4202 E. Fowler Ave, ENB118,
Tampa,
FL 33620,
USA
E-mail: *dhyeh@usf.edu*

Sascha Bekaan
Department of Chemical Engineering,
Saxion University of Applied Sciences,
P.O. Box 70.000,
7500 KB,
Enschede,
The Netherlands

INTRODUCTION

Although algae have most commonly been used in wastewater treatment lagoons for nutrient polishing prior to discharge, microalgae cultivation could be sustainably integrated into advanced wastewater treatment plants (WWTP), expanding infrastructure that serves dual purposes and effectively uses wastes as resources. Not only may integration decrease the environmental footprint and increase the energy return on investment of the two systems (Clarens *et al.* 2011; Beal *et al.* 2012), but without it, the economic feasibility of current algal cultivation technology is somewhat dubious (Pittman *et al.* 2011). Advanced WWTP not only contain adequate nutrients to sustain algae growth, but integrating the two processes can contribute economic benefits to plant operation as well (Drexler *et al.* 2014).

Many algal species can grow in municipal, industrial and agricultural wastewater and have been used in numerous wastewater treatment applications (Hoffman 1998). Aside from reducing loading in wastewater, algal biomass can be harvested for many bioproducts, including biofuels, fertilizers or fish feed (Mata *et al.* 2010).

This study involved the cultivation of two native algal communities derived from wastewater clarifiers (attached biofilm) and two monocultures (*Chlorella sorokiniana* and *Botryococcus braunii*) augmented with endemic cultures in unsterilized wastewater effluents. *Chlorella* spp. were chosen because of their relatively fast growth rate, resiliency and nutritional value (de-Bashan *et al.* 2008). *B. braunii*, on the other hand, grows much slower but produces large quantities of lipids, making it a desirable species for biofuel production (Metzger & Largeau 2005). If lipids were released into the wastewater stream, the resulting chemical oxygen demand (COD) could be treated by activated sludge processes. Both species can also be digested for biogas (Frigon *et al.* 2013). Because native species already thrive in the desired environment and pose a smaller threat of becoming an invasive species, phycoprospecting may reduce risks in algae-wastewater integration and help identify appropriate native species to produce for biofuel (Wilkie *et al.* 2011). Phycoprospecting may be especially convenient if the end use of algal biomass (i.e., soil amendment)

doi: 10.2166/wst.2014.340

is less sensitive to algal composition. Therefore, native algal communities were also examined.

The aim of this study was to compare the biomass yield and nutrient removal efficiencies of these four algal populations at two stages of an advanced WWTP with the algal growth and nutrient removal efficiency in a reference media (RM).

MATERIALS AND METHODS

Algae stock cultivation

Algal monocultures were obtained from the University of Texas (UTEX) Culture Collection of Algae. *C. sorokiniana* (UTEX #246) and *B. braunii* (UTEX #572) stock cultures were acclimated in RM and Bold 1NV media (UTEX, web.biosci.utexas.edu/utex/media.aspx) prior to batch tests. During the acclimation stage, both cultures were shaken at 150 rpm (Lab-Line Incubator-Shaker, Melrose Park, IL) at a 12/12 photoperiod (3000 lux) at room temperature (25 °C). RM contained the following components: $NaNO_3$ (1.47 mM); NH_4Cl (2.95 mM); K_2HPO_4 (0.43 mM); KH_2PO_4 (1.29 mM); $CaCl_2$ (0.25 mM); $MgSO_4$ (0.30 mM); NaCl (0.43 mM); $NaHCO_3$ (0.17 mM); Na_2SiO_3 (0.15 mM); citric acid (0.16 mM); ferric citrate (0.14 mM); $CuSO_4$ (0.08 μm); $ZnSO_4$ (0.15 μm); $CoCl_2$ (0.84 μm); $MnCl_2$ (0.061 μm); Na_2MoO_4 (0.052 μm); $FeCl_3$ (0.36 mM); $ZnCl_2$ (0.037 mM); and Vitamin B_{12}, thiamine, biotin per UTEX Bold 1NV recipe. Native algal communities (A and B) were harvested from the respective clarifier weirs, transported in cold storage and inoculated in batch experiments the same day.

Experimental design

Algae were cultivated using clarified process streams from two sequential stages of the Howard F. Curren Advanced Wastewater Treatment Plant in Tampa, FL, USA, described previously in Drexler *et al.* (2014). Wastewater used as growth media was (1) clarified effluent following a high purity oxygen carbonaceous biochemical oxygen demand (BOD) removal reactor (post-BOD removal clarified effluent (PBCE)) and (2) clarified effluent following a nitrification reactor (post-nitrification clarified effluent (PNCE)). These sampling locations were chosen because of their differing dominant nitrogen sources (i.e., ammonia in the PBCE and nitrate in the PNCE), and because clarified effluent would allow better light penetration than activated sludge. For comparison, algae were also cultivated in (3) RM containing both nitrogen sources.

Cultivation bioassays were conducted in 12-day batch tests in the laboratory, utilizing four algal populations: *C. sorokiniana, B. braunii,* an algal biofilm community native to the post-BOD removal clarifier (community A), and an algal biofilm community native to the post-nitrification clarifier (community B). Each population was cultivated in three types of growth media (PBCE, PNCE, and RM), resulting in a 4 × 3 experimental matrix. Initial water quality conditions of the media are shown in Table 1, where the difference in nitrogen speciation between PBCE and PNCE is evident. Although increasing the pH of the RM was considered, it was not adjusted since the pH remained within the range preferred by *C. sorokiniana* and typical of activated sludge.

Wastewater was collected at the beginning of each species' batch test, which, due to variations at the wastewater plant, resulted in slightly different characteristics among the series. However, ammonia and nitrate concentrations among the series were fairly stable (RSD of 6.5% and 5.4%, respectively), and, after inoculation, phosphorus concentrations were not limiting in any case. Because the intention of the study was to test the feasibility of growing the algal species in unaltered wastewater to mimic field conditions, wastewater was not autoclaved or augmented with nutrients prior to experimental use for the bioassays.

Statistical comparisons of the initial and final conditions (pH, total and soluble nitrogen, ammonia, nitrate, phosphate, COD, total suspended solids (TSS), and optical density (OD)) were made using the Student's *t*-test. Comparisons with *p*-values less than 0.05 ($p < 0.05$) were considered 'significant' and are referred to accordingly.

Triplicate Erlenmeyer flasks (500 mL for *C. sorokiniana*, communities A and B; 125 mL for *B. braunii* due to limited available inoculant) were filled with growth media (400 mL for *C. sorokiniana*, communities A and B; 100 mL for

Table 1 | Average initial conditions for post-BOD removal clarified effluent (PBCE), post-nitrification clarified effluent (PNCE), and reference media (RM) between four batch studies. Standard deviation is shown in parentheses. All units are in mg/L, except for pH

Parameter	PBCE	PNCE	RM
Soluble nitrogen	34.3(4.0)	34.5(1.9)	46.3(4.3)
Ammonia-N	29.1(1.9)	0.0(0.0)	20.8(0.3)
Nitrate-N	1.1(0.5)	30.9(1.7)	22.2(0.5)
Phosphate-P	7.3(3.1)	7.8(4.5)	151.2(12.4)
Soluble COD	58.8(11.8)	35.8(9.5)	92.0(3.1)
pH	7.4(0.1)	7.6(0.2)	6.5(0.3)

B. braunii) and inoculated with *C. sorokiniana* or *B. braunii* until the biomass density was approximately 0.09 g/L. Native species were dewatered (but not dried) to remove remnant free liquid (Whatman glass fibre filters 934-AH), and a known amount of biomass (approximately 0.1 g) were added to each flask. All flasks were cultivated in the conditions described above.

Analytical methods

Water quality tests were conducted using the following HACH methods: COD (method no. 8000), total nitrogen (TN) (method no. 10072), nitrate-nitrogen (method no. 10020), ammonia-nitrogen (method no. 10031), and phosphate (method no. 10127). Phenol-sulfuric acid carbohydrate assays and Lowry protein assays were conducted to measure soluble carbohydrate and protein (Ferlita 2011). OD was measured at 680 nm (HACH DR/4000 spectrophotometer), and pH was measured with Oakton pH probes. TSS were measured according to Standard Methods 2540D.

RESULTS AND DISCUSSION

pH changes

In wastewater treatment, pH regulates not only biological health but also physical–chemical processes. All algal communities significantly increased the pH in both wastewater samples from a range of 7.4–7.6 to a range of 9.8–11.0, most likely due to the consumption of alkaline compounds (Uusitalo 1996) and the photosynthetic removal of carbon dioxide, as the cultures were not artificially aerated. The largest pH increase of the RM occurred when cultivating *Chlorella* spp. (from 6.0 to 6.8) and algal community A (from 6.5 to 7.8), but increased just slightly when cultivating *B. braunii* (from 6.6 to 6.8) and decreased when cultivating algal community B (from 6.8 to 6.3). The pH change in the RM was not significant due to the addition of the buffering agent $NaHCO_3$.

Although activated sludge processes generally operate within a pH range of 6–8, other physical chemical processes may require an alkaline pH. Therefore, strategically placing an algal reactor ahead of a treatment step requiring an alkaline pH could be advantageous for reducing chemical inputs. On the other hand, drastic pH increases may be harmful to downstream pH-sensitive processes. The extent of pH elevation may be lower in continuous growth at a WWTP than the batch bioassays due to the constant supply of carbon dioxide rich wastewater following aerobic respiration. The net impact of algae-induced pH changes on biological processes in a full-scale integration of algal cultivation, and wastewater treatment deserves further study.

Total and soluble nitrogen

The TN removal efficiency of communities A and B was not significantly different than that of *C. sorokiniana* in PBCE, and outperformed *C. sorokiniana* in PNCE (Figure 1). The removal efficiency of *B. braunii* was higher than the native species communities in all but one batch, but all nitrogen removal may not be solely attributable to *B. braunii* due to the presence of endemic fauna in unsterilized wastewater.

The final soluble nitrogen concentrations were significantly lower than the initial values in all samples (except community A in RM). Results agree with previous batch *Chlorella* sp. studies that achieved 61–86% removal (Li *et al.* 2011; Zhu *et al.* 2013). With the exception of *B. braunii*, all species achieved greater removal of soluble nitrogen in the PBCE than the PNCE. Soluble nitrogen removal in the RM was considerably less than that in wastewater effluents. As native species' removal was comparable to augmented cultures, phycoprospecting may be as effective and less operationally intense than maintaining augmented cultures.

Ammonia-nitrogen

All species achieved significantly higher ammonia removal in PBCE than the RM (Figure 1). The final ammonia concentrations were significantly lower than the initial concentrations in all bioassays, and ammonia removal in the PBCE agreed with previous studies of *Chlorella* sp. that achieved 60–100% removal (Ruiz-Marin *et al.* 2010; Li *et al.* 2011). Ammonia removal by the monocultures was not significantly different from that of the native species in PBCE, except *B. braunii* batch cultures achieved significantly higher removal than community A.

The location-specific nitrogen balance profiles for each algal population are presented in Figure 2. Ammonia removal in batch cultures of *C. sorokiniana, B. braunii,* and community B cultivated in PBCE all appear to be aided by volatilization due to elevated pH in these cultures. Volatilization has previously been found to be highly correlated with free ammonia concentration in algae ponds (Zimmo *et al.* 2003). In previous studies (Gustin & Marinsek-Logar 2011), when pH rose above 10, aeration-aided ammonia volatilization reached above 80%; although flasks were not

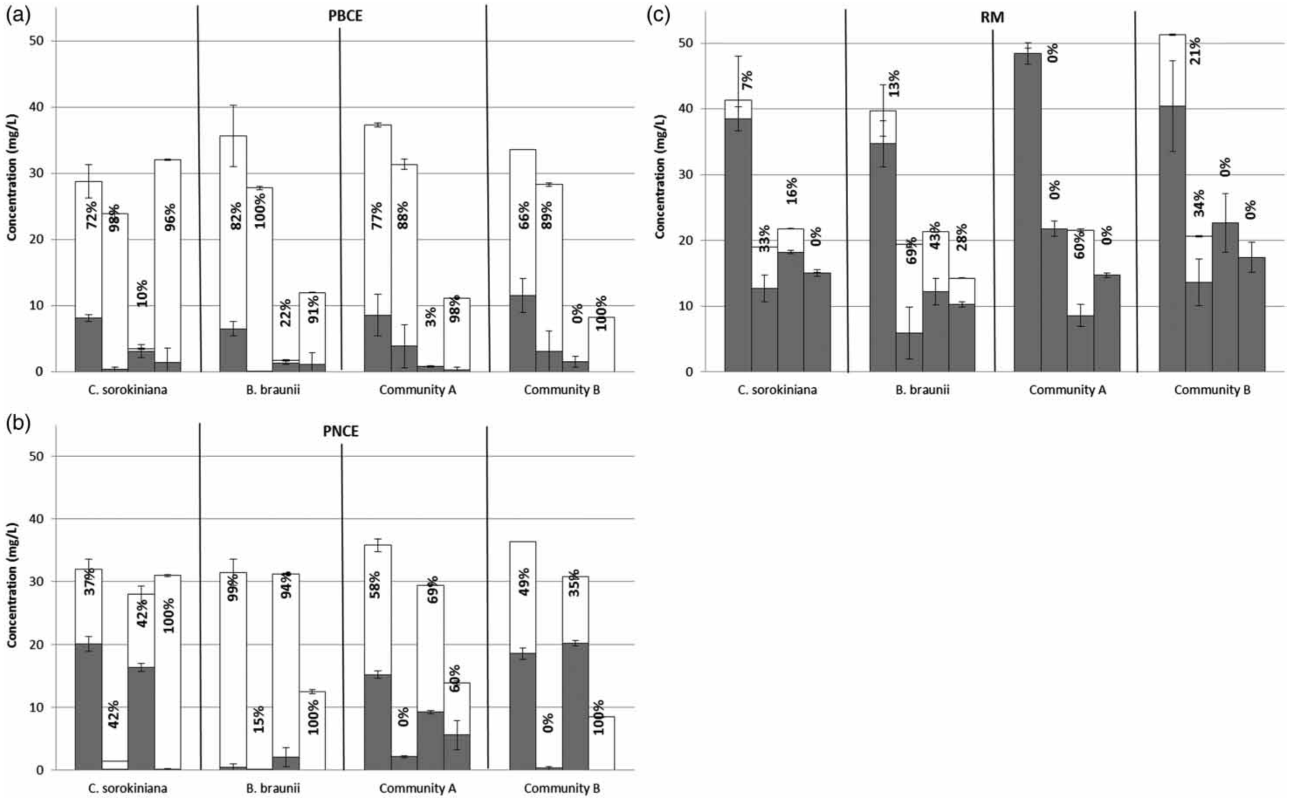

Figure 1 | Change in soluble nitrogen, ammonia-N, nitrate-N, and phosphate-P after 12-day cultivation of four algal populations in three growth media. Bars of each group represent the following from left to right: soluble nitrogen, ammonia-nitrogen, nitrate-nitrogen and phosphate-phosphorus. Initial concentration is shown as white bars; final concentration is shown as black bars. Error bars depict one standard deviation. PBCE: post-BOD removal clarified effluent; PNCE: post-nitrification clarified effluent; RM: reference media.

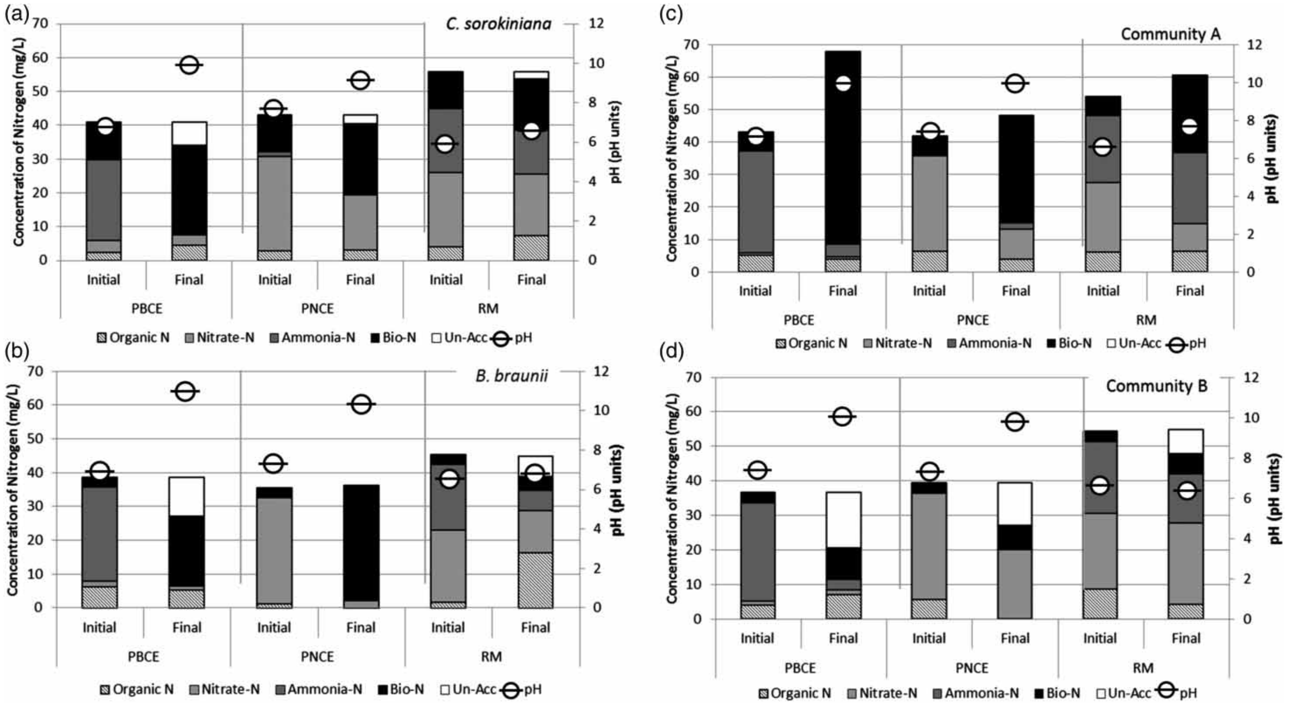

Figure 2 | Nitrogen profiles of four algal populations cultivated in three growth media. Bio-N is the mass of nitrogen bound in biomass. Un-Acc is nitrogen that is not accounted for in measured tests or calculations; it could represent volatized or denitrified nitrogen. PBCE: post-BOD removal clarified effluent; PNCE: post-nitrification clarified effluent; RM: reference media.

aerated in this study, unaccounted for ammonia in PBCE batch tests were between 32 and 45%, which is within a reasonable range of unaided volatilization for the pH range of this study. Ammonia removal could also have been aided by struvite precipitation. Furthermore, pH remained below 8.0 in the RM (due to the buffering agent added) for all batch tests, and overall ammonia removal was significantly less in the RM than both wastewater effluents.

Nitrate-nitrogen

Nitrate removal was significant for all algal populations in the PNCE, as shown in Figure 1. *B. braunii* achieved a considerably higher nitrate removal efficiency (94%) than all other species (35–69%), which agrees with previous work (Sawayama *et al.* 1992), where *B. braunii* achieved near complete removal of nitrate in batch tests. However, due to background fauna, not all of the nitrate removal may be attributable to *B. braunii* alone.

Gaps in the nitrogen balance profiles (Figure 2) observed in the PNCE and RM may be explained (aside from experimental error) by denitrification. Previous work (Zimmo *et al.* 2004) has shown that denitrification contributes significantly (approximately 25% of TN loss) to nitrogen removal in algae-based ponds, and bacteria genetically similar to denitrifying bacteria have previously been found in wastewater process streams immediately following nitrification (Ghosh & Love 2011). Therefore, denitrification by coexisting bacteria (in background wastewater or introduced with inoculant) was considered a possible cause of unaccounted for nitrogen removal, even though conditions in this study may be different than field conditions. Unfortunately, a direct measure of denitrification was not possible, and genetic testing of the algal bacteria communities was not conducted to verify the presence of denitrifying organisms. Denitrification is, therefore, merely a potential explanation.

Nitrogen preference of algae species

In the presence of both ammonia and nitrate nitrogen, many algal species, including *Chlorella* sp., will preferentially utilize ammonia (Ruiz *et al.* 2011). *B. braunii*, on the other hand, has previously been grown successfully in nitrate dominated secondary wastewater (Sawayama *et al.* 1992; An *et al.* 2003). Accordingly, it was hypothesized that the native species would prefer the nitrogen species dominant in their respective clarifier.

To investigate nitrogen preferences, the RM included both nitrogen species. Although *C. sorokiniana* and *B. braunii* achieved significant removal of both ammonia and nitrate in the RM, the removal efficiency of ammonia was significantly higher than that of nitrate (Figure 1(c)). The native species appeared to prefer the opposite nitrogen species as was expected, as community A had significant removal of nitrate and community B had significant removal of ammonia. Differences in the environmental conditions in batch tests compared to the native environment (i.e., attached versus suspended growth, light intensity) may have selected for different dominant species (with potentially different nitrogen preferences) than would be dominant in the native environment.

The amount of nitrogen measured in the final biomass of community A greatly exceeded the initial soluble nitrogen available (Figure 2(c)), suggesting either analytical error and/or nitrogen fixation. Previous studies identifying algal species in secondary treated wastewater (Ghosh & Love 2011) noted the presence of cyanobacteria, which have been shown to fix nitrogen. Nitrogen fixed by community A cultivated in the PBCE, PNCE and RM amounted to approximately 25, 6.5 and 6.5 mg/L, respectively (corresponding to approximately 57, 15 and 12%, respectively, of the initial available nitrogen). Although it is possible that laboratory conditions favored nitrogen fixing bacteria, it is unlikely that nitrogen fixation occurred because ammonia and nitrate remained at the end of the experiments. It is more likely that the difference is explained by experimental error or a yet unidentified factor.

Phosphate-phosphorus

Due to algal assimilation and/or adsorption, phosphate-phosphorus removal was significant for all algal communities in both wastewater effluents (Figure 1). An abiotic trial, where the pH of the RM was artificially raised, confirmed that phosphate did not precipitate significantly until pH values were higher than 8. Increased pH, such as that seen in the wastewater effluents, has been shown to encourage phosphate precipitation and adsorption to algal cells (Zhu *et al.* 2013) and may have aided phosphorus removal in this study. Phosphate removal was somewhat higher than previously reported for *Chlorella* sp. in domestic sewage treatment in batch cultivation (12–92% (Ruiz-Marin *et al.* 2010; Li *et al.* 2011)), but similar to that found previously by *B. braunii* in secondary wastewater (Sawayama *et al.* 1992). In the PBCE, the native communities provided equal removal efficiency as the augmented cultures; although community A had significantly less phosphorus removal in the PNCE than other species, the community still achieved an average of 60% removal. The phosphorus removal in RM was not significant (except in the *B. braunii* batch tests), where pH did not exceed 8 in any case due to the presence of a buffering agent. Although the RM had a much higher phosphorus concentration than the clarified effluent, phosphorus is not considered toxic to algae (Carpenter *et al.* 1998) and therefore would not have a detrimental effect on growth. Similarly, the elevated phosphorus concentration would not offer an advantage, since all cultures began with adequate N:P ratio (Redfield 1958) and excess phosphorus concentrations.

Communities A and B achieved comparable phosphate removal to the augmented cultures. If an algal-integrated WWTP did not require a monoculture with specific traits, cultivating phycoprospected populations that could achieve the same nutrient removal may be more economical and less difficult than maintaining an augmented culture, while still producing value-added algal products.

Organic carbon

The final soluble COD concentration increased significantly from an initial (33–82 mg/L) to final (76–141 mg/L) concentration range when cultivating *C. sorokiniana* in all media. Soluble COD also increased when cultivating *B. braunii* (from 84 mg/L to 227 mg/L) and community A (from 60 mg/L to 107 mg/L) in the PBCE. Similarly, soluble COD increased in the PNCE (from 25 to 49 mg/L) after cultivating community B.

The findings are contrary to two similar batch test studies (Li *et al.* 2011; Zhu *et al.* 2013) which achieved a minimum of 67% soluble COD removal depending on environmental factors. Both studies, however, were run on high strength wastewater with COD concentrations at least four times higher than those in this study. Hence, heterotrophic processes were likely dominant. Although COD removal was rapid in the first 2–3 days of the experiments, it eventually reached steady state above 100 mg/L. However, a batch study cultivating *B. braunii* in secondarily treated domestic sewage with lower nutrient loading also observed an increase in soluble organic carbon (Sawayama *et al.* 1992).

An increase in soluble COD could be due to an increased production of extracellular polymeric substances (EPS), which can be induced via environmental stresses (Babel *et al.* 2002) and should be accompanied by increased soluble carbohydrate, protein and/or organic nitrogen (Her *et al.* 2004). Slight increases of these components did occur, though not significantly, in the batch cultures in this study (Table 2). Organic nitrogen concentration increased when *C. sorokiniana* was cultivated in PBCE and PNCE (from 2.5 to 4.4 mg/L and 2.8 to 3.0 mg/L, respectively). Soluble carbohydrate and protein increased when cultivating *B. braunii* (from 20.1 to 26.8 mg/L and 16.3 to 16.7 mg/L, respectively) and community A (from 8.9 to 41.0 mg/L and 10.2 to 17.6 mg/L, respectively) in PBCE. Similar increases in carbohydrate were observed when cultivating community B in the PNCE and PBCE (from 6.9 to 12.9 mg/L and 7.7 to 11.8 mg/L, respectively).

Although EPS may contribute to internal organic loading at a wastewater plant, it could help reduce the external chemical demand required for denitrification if it is readily biodegradable and was integrated ahead of denitrification. The effect of algal cultivation on soluble COD concentration in continuous culture and subsequent processes is deserving of further study.

Table 2 | Data summary of the initial and final soluble organic nitrogen, chemical oxygen demand, carbohydrate and protein concentration of each experimental series

		Org-N		**COD**		**Carbohydrate**		**Protein**	
Species media		**Initial**	**End**	**Initial**	**End**	**Initial**	**End**	**Initial**	**End**
B. braunii	PBCE	6.2	5.1	84	227	20.1	26.8	16.3	16.7
	PNCE	1.3	0.1	69	66	21.9	10.6	18.8	7.7
	RM	1.6	16.5	120	86	26.8	11.5	10.5	8.8
C. sorokiniana	PBCE	2.5	4.4	43	115	8.6	5.8	13.1	10.2
	PNCE	2.8	3.0	33	76	8.3	3.9	11.1	9.1
	RM	11.9	7.6	82	141	8.6	7.7	10.3	13.7
Comm. A	PBCE	5.2	3.9	60	107	8.9	41.0	10.2	17.6
	PNCE	6.4	3.9	45	74	6.8	54.0	9.9	11.6
	RM	6.1	18.0	90	90	8.0	26.8	10.3	3.0
Comm. B	PBCE	4.1	7.0	73	73	7.7	11.8	16.9	10.6
	PNCE	5.6	0.0	25	49	6.9	12.9	13.2	10.0
	RM	8.6	4.2	92	81	19.1	5.9	7.1	8.2

Final yield

Both monocultures grew readily in the wastewater effluents, confirming the feasibility of municipal wastewater as a feedstock for algae cultivation. The TSS yield of *C. sorokiniana* and *B. braunii* was significantly higher in both wastewater effluents than the yield in RM (Figure 3). The TSS yield of communities A and B were significantly higher in the PBCE than the yield in PNCE. Because batch cultivation occurred in unsterilized wastewater, some biomass yield could be attributed to growth of endemic species.

The final concentration of *C. sorokiniana* and *B. braunii* cultivated in wastewater effluents was lower than that reported in other studies (An *et al.* 2003; Ruiz *et al.* 2011). Because the N:P ratio in all batch tests was lower than 16:1 (Redfield 1958), phosphorus was not considered to be limiting in any case. Instead, the lower yield is most likely a result of not aerating the bioassays, or a suboptimal light regime, as carbon dioxide and light are important factors for algal growth.

When final yield was normalized to initial concentration, *B. braunii* had a significantly higher final TSS than *C. sorokiniana* and community B when grown in both wastewater effluents. However, other endemic species had colonized the *B. braunii* batch tests, as other faster growing endemic species in the unsterilized wastewater overtook *B. braunii*. *C. sorokiniana* had a significantly higher final TSS than community B in both wastewater effluents. Community A,

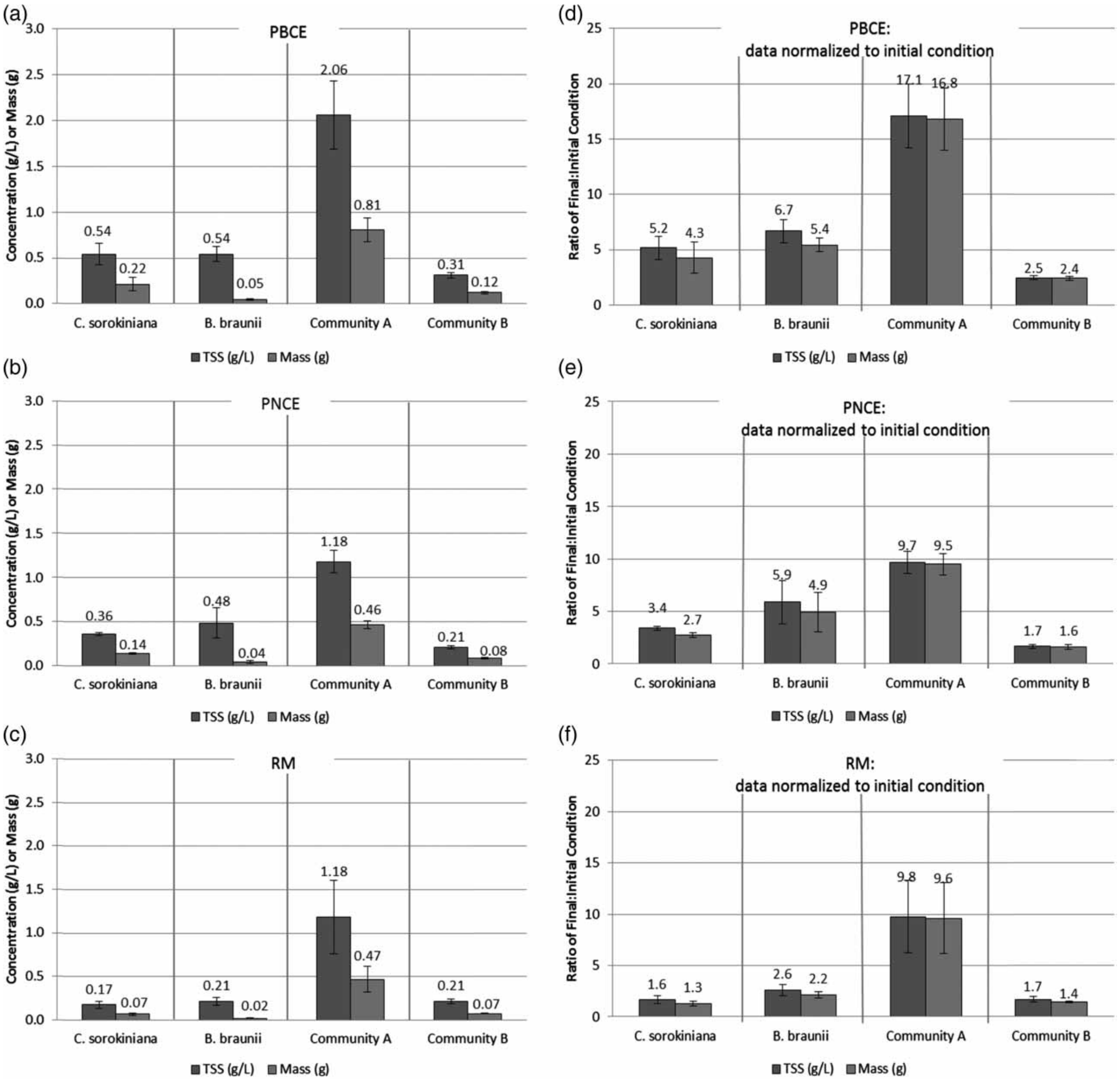

Figure 3 | Final yield as TSS and mass as dry weight of four algal populations cultivated in three growth media ((a)–(c)). Final yield normalized to initial TSS and mass as dry weight ((d)–(f)). PBCE: post-BOD removal clarified effluent; PNCE: post-nitrification clarified effluent; RM: reference media.

however, had the highest final TSS of all species in both wastewater effluents. Similar trends were seen in mass yield as dry weight for each species and growth media. Since native species produced as much or more biomass than augmented cultures in many cases, if biomass volume is the goal of integration (i.e., for biogas production or fertilizers), native species may be an acceptable alternative to augmented monocultures.

Specific growth rate was only determined for the *C. sorokiniana* series, due to the other cultures' heterogeneity inhibiting the ability to take a representative sample without significant culture disruption. The specific growth rate of *C. sorokiniana* was significantly higher in both wastewater samples than the RM ($0.11\,d^{-1}$), and the growth rate in the PBCE ($0.52\,d^{-1}$) was significantly higher than the PNCE ($0.40\,d^{-1}$). The growth rates were within the range of those previously reported for *Chlorella* sp. in wastewater (Ruiz *et al.* 2011; Zhu *et al.* 2013). The lower growth rate in the RM may have been due to the lack of algae–bacteria interactions in the sterilized media.

Community interactions

Because wastewater effluents were not sterilized prior to batch tests to better simulate scale-up conditions, diverse endemic algae and bacteria, which have been shown to be quite diverse (Ghosh & Love 2011), were cultivated along with the target species. Although community profiling tests were not conducted on the final algal communities, qualitative inspection suggested that *B. braunii* was overcrowded by endemic species in the wastewater effluents (to a lesser extent in the PNCE than the PBCE), while remaining dominant in the RM. Microscopic inspection of communities A and B also indicated that the dominant groups shifted when grown in different growth media. On the other hand, the fast growing *C. sorokiniana* remained dominant in all series. Due to species competitiveness, it may be difficult to maintain the dominance of a slow-growing monoculture with wastewater as a feedstock, unless a means of segregation or isolation is implemented, such as a membrane bioreactor (Sawayama *et al.* 1992; Prieto 2011), that may reduce competition by removing native species while allowing passage of nutrients.

CONCLUSION

This study shows that algal biofilm communities native to the clarifiers at two stages in an advanced wastewater treatment plant have had similar nutrient removal and biomass production capability as other extensively studied monocultures (*C. sorokiniana* and *B. braunii*.). After 12 days, all algal species achieved nitrogen removal between 68 and 99% and phosphorus removal between 60 and 100% in clarified effluents. All species investigated achieved higher yield in the ammonia-dominated PBCE, which may be where future algal photobioreactors should be incorporated into a wastewater treatment train. If a treatment plant integrates algae cultivation into its process train and is less concerned with specific biomass traits, cultivating native species can provide similar nutrient removal and biomass production benefits compared to augmented algal monocultures. Elevated pH in the batch series was significant and could be advantageous in continuous operation if an algal reactor was placed ahead of a treatment process (i.e., coagulation) that required an elevated pH. Similarly, increased soluble COD concentrations could be used to decrease external carbon inputs for denitrification. Both topics (and the degradability of the soluble COD produced) deserve further study. Not surprisingly, significant endemic species growth was apparent in unsterilized wastewater; if clean monocultures are required or predation is a concern, an isolation mechanism in the algal reactor may be recommended.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge funding from the US Department of Education Graduate Assistants in Areas of National Need (GAANN) Fellowship, the National Science Foundation (Awards 1236746 and 1200682), Florida Energy Systems Consortium, and the Dutch 'Clean Tech Community' student exchange program from Saxion University. The authors also acknowledge laboratory support from Alexandra Parish, Robert Bair, Maria Delpilar, Ana Lucia Prieto and Melanie Pickett, and logistical support from the staff at the Howard F. Curren Advanced Wastewater Treatment Plant in Tampa, FL, especially plant manager Tim Ware.

REFERENCES

An, J. Y., Sim, S. J., Lee, J. S. & Kim, B. W. 2003 Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. *J. Appl. Phys.* **15** (2–3), 185–191.

Babel, S., Takizama, S. & Ozaki, H. 2002 Factors affecting seasonal variation of membrane filtration resistance caused by *Chlorella* algae. *Water Res.* **36** (5), 1193–1202.

Beal, C. M., Stillwell, A. S., King, C. W., Cohen, S. M., Berberoglu, H., Bhattarai, R. P., Connelly, R. L., Webber, M. E. & Hebner, R. E. 2012 Energy return on investment for algal biofuel production coupled with wastewater treatment. *Water Environ. Res.* **84** (9), 692–710.

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N. & Smith, V. H. 1998 Non-point pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* **8** (3), 559–568.

Clarens, A. F., Nassau, H., Resurreccion, E. P., White, M. A. & Colosi, L. M. 2011 Environmental impacts of algae-derived biodiesel and bioelectricity for transportation. *Environ. Sci. Technol.* **45** (17), 7554–7560.

de-Bashan, L. E., Trejo, A., Huss, V. A. R., Hernandez, J. P. & Bashan, Y. 2008 *Chlorella sorokiniana* UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. *Bioresour. Technol.* **99** (11), 4980–4989.

Drexler, I. L. C., Joustra, C., Prieto, A. L., Bair, R. & Yeh, D. H. 2014 AlgaeSim: a model for integrated algal biofuel production and wastewater treatment. *Water Environ. Res.* **86** (2), 164–176.

Ferlita, R. R. 2011 In situ biofiltration of dissolved organic carbon in reverse osmosis membrane filtration. PhD Thesis, Civil & Environmental Engineering, University of South Florida, Tampa, FL.

Frigon, J. C., Abdou, R. H., McGinn, P. J., O'Leary, S. J. B. & Guiot, S. R. 2013 Screening microalgae strains for their productivity in methane following anaerobic digestion. *Appl. Energ.* **108** (August 2013), 100–107.

Ghosh, S. & Love, N. 2011 Application of *rbcL* based molecular diversity analysis to algae in wastewater treatment plants. *Bioresour. Technol.* **102** (3), 3619–3622.

Gustin, S. & Marinsek-Logar, R. 2011 Effect of pH, temperature and air flow rate on the continuous ammonia stripping of the anaerobic digestion effluent. *Process Saf. Environ.* **89** (1), 61–66.

Her, N., Amy, G., Park, H. R. & Song, M. 2004 Characterizing algogenic organic matter (AOM) and evaluating associated NF membrane fouling. *Water Res.* **38** (6), 1427–1438.

Hoffman, J. P. 1998 Minireview: wastewater treatment with suspended and nonsuspended algae. *J. Phycol.* **34**, 757–763.

Li, Y., Zhou, W., Hu, B., Min, M., Chen, P. & Ruan, R. 2011 Integration of algae cultivation as biodiesel production feedstock with municipal wastewater treatment: strains screening and significance evaluation of environmental factors. *Bioresour. Technol.* **102** (23), 10861–10867.

Mata, T. M., Martins, A. A. & Caetano, N. S. 2010 Microalgae for biodiesel production and other applications: a review. *Renew. Sustain. Energy Rev.* **14** (1), 217–232.

Metzger, P. & Largeau, C. 2005 *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl. Microbiol. Biotechnol.* 66 (5), 486–496.

Pittman, J. K., Dean, A. P. & Osundeko, O. 2011 The potential of sustainable algal biofuel production using wastewater resources. *Bioresour. Technol.* **102** (1), 17–25.

Prieto, A. L. 2011 *Sequential anaerobic and algal membrane bioreactor (A2MBR) system for sustainable sanitation and resource recovery from domestic wastewater*. PhD Thesis, Civil & Environmental Engineering, University of South Florida, Tampa, FL.

Redfield, A. C. 1958 The biological control of chemical factors in the environment. *Am Sci.* **46** (3) 205–221.

Ruiz, J., Alvarez, P., Arbib, Z., Garrido, C., Barragan, J. & Perales, J. A. 2011 Effect of nitrogen and phosphorus concentration on their removal kinetics in treated urban wastewater by *Chlorella vulgaris*. *Int. J. Phytorem.* **13** (9), 884–896.

Ruiz-Marin, A., Mendoza-Espinosa, L. G. & Stephenson, T. 2010 Growth and nutrient removal in free and immobilized green alga in batch and semi-continuous cultures treating real wastewater. *Bioresour. Technol.* **101** (1), 58–64.

Sawayama, S., Minowa, T., Dote, Y. & Yokoyama, S. 1992 Growth of the hydrocarbon-rich microalga *Botryococcus braunii* in secondarily treated sewage. *Appl. Microbiol. Biotechnol.* **38** (1), 135–138.

Uusitalo, J. 1996 Algal carbon uptake and the difference between alkalinity and high pH ('alkalization'), exemplified with a pH drift experiment. *Sci. Mar* **60** (Suppl. 1), 129–134.

Wilkie, A. C., Edmundson, S. J. & Duncan, J. G. 2011 Indigenous algae for local bioresource production: phycoprospecting. *Energy Sustain. Dev.* **15** (4), 366–371.

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P. & Yuen, Z. 2013 Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Res.* **47** (13), 4294–4302.

Zimmo, O. R., van der Steen, N. P. & Gijzen, H. J. 2003 Comparison of ammonia volatilization rates in algae and duckweed-based waste stabilization ponds treating domestic wastewater. *Water Res.* **37** (19), 4587–4594.

Zimmo, O. R., van der Steen, N. P. & Gijzen, H. J. 2004 Nitrogen mass balance across pilot-scale algae and duckweed-based wastewater stabilization ponds. *Water Res.* **38** (4), 913–920.

First received 19 March 2014; accepted in revised form 21 July 2014. Available online 4 August 2014