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Algal-based, single-step treatment of urban wastewaters

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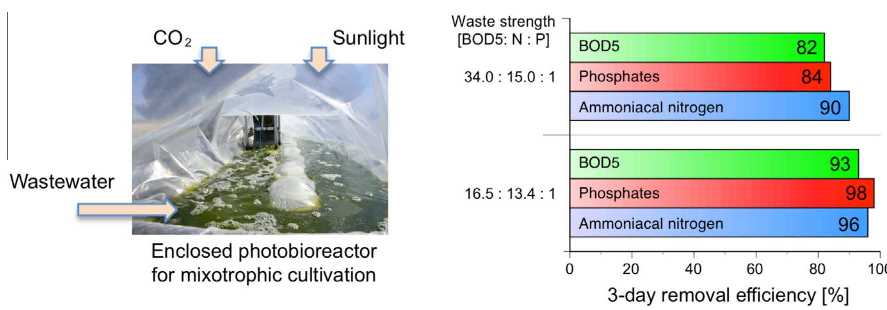
HIGHLIGHTS

- Demonstrated wastewater treatment by mixotrophic metabolism.
- Demonstrated single-step removal of BOD and nutrients.
- Demonstrated higher BOD removal by mixotrophic metabolism.
- Demonstrated comparable nutrient removal by mixotrophic metabolism.

GRAPHICAL ABSTRACT



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ABSTRACT

Currently, urban wastewaters (UWW) laden with organic carbon (BOD) and nutrients (ammoniacal nitrogen, N, and phosphates, P) are treated in multi-stage, energy-intensive process trains to meet the mandated discharge standards. This study presents a single-step process based on mixotrophic metabolism for simultaneous removal of carbon and nutrients from UWWs. The proposed system is designed specifically for hot, arid environments utilizing an acidophilic, thermotolerant algal species, *Galdieria sulphuraria*, and an enclosed photobioreactor to limit evaporation. Removal rates of BOD, N, and P recorded in this study (14.93, 7.23, and 1.38 mg L^{-1} d^{-1}, respectively) are comparable to literature reports. These results confirm that the mixotrophic system can reduce the energy costs associated with oxygen supply in current UWW treatment systems, and has the potential to generate more energy-rich biomass for net energy extraction from UWW.

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1. Introduction

Urban wastewater (UWW) treatment plants in current use have been designed and operated solely for the purpose of meeting the mandatory discharge regulations to protect receiving waters and public health. Technologies deployed in today's wastewater treatment plants to meet these regulations consume significant electrical energy and dissipate valuable carbon- and nutrient-content of the wastewater into the environment. For example, organic-content of UWW is aerobically mineralized to gaseous carbon dioxide and discharged into the atmosphere; ammonia-content is converted by nitrification/denitrification process to inert dinitrogen and discharged into the atmosphere. In recent years, there has been a shift in this paradigm where UWWs are being recognized as a renewable resource from which water, energy, nutrients, and useful chemicals could be reclaimed for beneficial use.

This study proposes an approach based on mixotrophic metabolism for energy-efficient and sustainable treatment of UWW. The

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premise of this approach is that, mixotrophic metabolism driven by sunlight and BOD oxidation can simultaneously remove BOD, N and P in UWWs to the required effluent standards. Results of this study demonstrate the feasibility of BOD, N and P removal in a single-step process to generate more energy-rich biomass than by current methods. The higher biomass yield enables energy extraction as gaseous or liquid biofuels via catalytic hydrothermal gasification (Elliott, 2008), anaerobic digestion (McCarty et al., 2011), or hydrothermal liquefaction (Biller and Ross, 2011; Chakraborty et al., 2012).

Critical to the success of the proposed approach is a low-cost, enclosed photobioreactor (PBR) developed by us, that minimizes evaporative water loss and retains metabolic gases (O_2 and CO_2) enabling mixotrophic oxidation of organic carbon for maximal conversion to biomass with fewer input requirements. Another embodiment in the proposed approach is hydrothermal liquefaction (HTL) of the biomass to extract its energy-content as biocrude with concomitant solubilization of its nutrient-content. Upon separation of the biocrude from the products of HTL, the nutrient-rich aqueous phase could be recycled to the cultivation step to increase biomass productivity as discussed later.

Previous studies by the authors (Selvaratnam et al., 2014a,b) have documented the feasibility of a thermo-tolerant, acidophilic, heterotrophic/photoautotrophic alga, *Galdieria sulphuraria* (here after *G. sulphuraria*) as a successful and robust algal species for efficient N and P removal. The choice of *G. sulphuraria* in this study was motivated by its metabolic versatility that includes the ability to grow on the largest known range of organic substrates known among photosynthetic microorganism (Schonknecht et al., 2013). It is also an acidophile, growing between pH 1–4, conditions that rapidly inactivate plant and animal pathogens found in wastewater. The ability of *G. sulphuraria* to naturally acidify its growth medium from neutrality to optimum levels under heterotrophic conditions (Oesterhelt et al., 2007) makes it an ideal strain for mixotrophic treatment of UWW. This study demonstrates the ability of *G. sulphuraria* in removing BOD from UWW as well as nutrients to validate the premise that this species can be successfully cultivated in UWWs for energy-positive wastewater treatment.

A central design advantage of the mixotrophic system over traditional WWT systems stems from the fact that stoichiometric carbon-to-nitrogen (C:N) ratio in UWW is closer to that of algal biomass composition than to that of heterotrophic bacteria (Fig. 1). Even more important is that CO_2 capture via photosynthesis corrects the stoichiometric imbalance between C:N:P ratios in WW relative to either type of biomass to afford single-step biological treatment that can simultaneously achieve discharge standards for all three components. This offers a significant advantage over

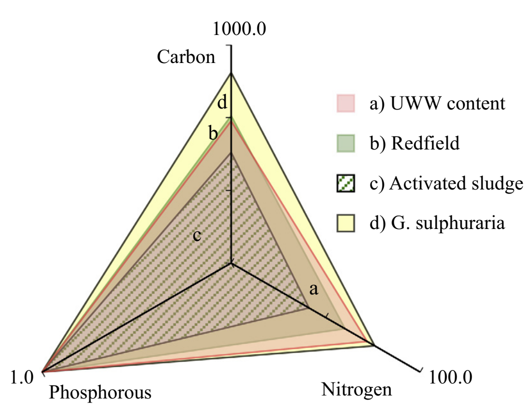

Fig. 1. C:N:P ratio in urban wastewater (UWW) (a) compared to composition of various biomasses cultivated with UWW: algal biomass according to Redfield formula (b); activated sludge (c); and *G. sulphuraria* (d).

the traditional practice that necessitates a two-step process including energy-intensive aeration:aerobic oxidation for BOD removal followed by nitrification/denitrification for N removal with external carbon supply to bridge the C:N imbalance.

The higher energy-efficiency of the mixotrophic system over the traditional system for wastewater treatment is due to the fact that the former is driven by photosynthesis, whereas the latter requires electrical energy to provide the necessary dissolved oxygen. Both processes are capable of generating biomass that can be converted to useable energy; for example, by anaerobic digestion to produce methane as energy carrier. An energetic comparison of the wastewater-to-biomass-to-methane conversion pathways has shown that the mixotrophic pathway can yield more than double the net electrical energy than the traditional pathway (Selvaratnam et al., 2014b). Sturm and Lamer (2011) have reported similar advantage of algal-based UWW treatment systems.

Several recent studies have built on the pioneering efforts of Oswald (1962, 1988), Oswald et al. (1953) to develop improved mixed algal/bacterial systems for UWW treatment with minimal energy input. While early studies had focused on using algal systems for polishing the secondary effluent to prevent eutrophication of receiving waters, later studies have demonstrated the feasibility of algal systems in treating the primary effluent as well as side-streams from various wastewater treatment process (Cho et al., 2011; Dalrymple et al., 2013; Wang et al., 2010). Wang et al. (2010) have demonstrated feasibility of algal treatment of four different side-streams at a wastewater treatment plant (wastewater before/after primary settling, wastewater after activated sludge tank, and centrate). More recent studies have extended the feasibility of algal systems to wastewater treatment and simultaneous energy generation (Lardon et al., 2009). This paper reports on the rates and efficiencies of removal of BOD, N, and P from primary-settled urban wastewater by *G. sulphuraria*.

2. Methods

The algal culture used in this study, *G. sulphuraria* CCMEE 5587.1, was obtained from Culture Collection of Microorganisms from Extreme Environments (University of Oregon). Bacteria-free colonies were picked from glucose-containing agar plates to establish axenic stock cultures and cultivated in 16-mm borosilicate glass tubes (culture volume = 6 mL) with a parafilm wrap around the closure to minimize evaporative losses while permitting O_2 and CO_2 diffusion. In all the tests, initial pH level of the media was adjusted to 2.5 by adding 10 N sulfuric acid. The tubes were placed in a tissue-culture roller drum (New Brunswick Scientific Co., Edison, NJ, USA) housed in a CO_2-enriched (2–3% vol/vol) incubator (Percival, USA) and maintained at 40 °C in a 14-h/10-h light–dark cycle mimicking outdoor conditions. Biomass growth was analyzed daily, based on measurements of optical density at 750 nm (OD 750) using Beckman DU-530 UV/Vis spectrophotometer and converted to ash-free dry weight (AFDW, g L^{-1}) using the correlation established in the previous study (Selvaratnam et al., 2014b).

2.1. Test I – growth of G. sulphuraria in UWW

The goal of Test I was to demonstrate the ability of *G. sulphuraria* to grow in a medium representative of typical UWW (BOD:N:P ratio of 16.5:13.4:1) and assess its BOD, N, and P removal capabilities. In this test, the standard Cyanidium recipe for *G. sulphuraria* (Selvaratnam et al., 2014a) was modified as follows: instead of using deionized water to prepare the standard growth medium, filter-sterilized (0.45 μm filter unit, Thermo Scientific Inc.) primary effluent obtained from the Las Cruces Municipal Wastewater

Table 1
Summary of initial conditions in Tests I, II, and III.

	Test I	Test II	Test III		
			Medium 1	Medium 2	Medium 3
Biomass [g AFDW L^{-1}]	0.109	0.139	0.204	0.214	0.214
N–NH_3 [mg L^{-1}]	48.0	24.1	38.10	26.20	27.70
P–PO_4 [mg L^{-1}]	11.0	4.9	10.40	6.00	5.73
BOD_5 [mg L^{-1}]	59.4	54.4	–	–	–
BOD_5:N:P ratio	16.5:13.4:1	34.0:15.0:1	–	–	–

Table 2
Summary of results from Tests I, II, and III.

	Test I	Test II	Test III		
			Medium 1	Medium 2	Medium 3
N removal efficiency (3 day) [%]	96.0	90.0	96.00	94.00	94.00
P removal efficiency (3 day) [%]	98.0	84.0	78.00	90.00	98.00
BOD removal efficiency (3 day)	93.0	82.0	–	–	–
N removal rate (3 day) [mg L^{-1} d^{-1}]	15.5	7.2	12.18	8.21	8.65
P removal rate (3 day) [mg L^{-1} d^{-1}]	3.6	1.4	2.69	1.80	1.88

Treatment Plant was used; and, ammonium sulfate and mono-potassium phosphate from the standard recipe were excluded as the wastewater contained adequate N and P for biomass growth. Initial conditions in Test I (after filter-sterilization) are summarized in Table 1. Daily samples drawn over a period of 6 days were centrifuged and the filtered (0.2 micron) supernatant was analyzed for ammoniacal-nitrogen, phosphates, and BOD_5.

2.2. Test II – cultivation of G. sulphuraria in primary effluent

The goal of Test II was to validate the growth of *G. sulphuraria* in carbon-rich medium (BOD:N:P ratio of 34.0:15.0:1), and compare its BOD and nutrient removal capabilities with literature reports. Again, filter-sterilized primary effluent was used instead of deionized water to prepare modified Cyanidium medium excluding ammonium sulfate and mono-potassium phosphate dosage. Initial conditions in Test II (after filter-sterilization) are summarized in Table 1. Samples of inoculum were centrifuged and filtered (0.2 micron) supernatants were used to measure ammoniacal nitrogen, phosphates, and BOD_5 on a daily basis for 6 days.

2.3. Test III – heterotrophic growth of G. sulphuraria

The goal of Test III was to assess the growth capability of *G. sulphuraria* in urban wastewaters in comparison to growth in modified Cyanidium growth medium. In this test, the following three culture media were compared: Medium 1: modified Cyanidium medium prepared in deionized water, but with the N and P levels adjusted to match those in UWW; Medium 2: Cyanidium medium prepared with filter-sterilized primary effluent excluding ammonium sulfate and mono-potassium phosphate; and Medium 3: raw filter-sterilized primary-settled wastewater. Initial conditions in Test III are summarized in Table 1. Dissolved concentrations of ammoniacal-nitrogen and phosphate were analyzed on days 0, 3, 6 and 10, in triplicate.

Dissolved ammoniacal-nitrogen and phosphates were measured using HACH DR 6000 spectrophotometer with salicylate TNT method 10031 and Phosver 3 method 8048. Dissolved BOD_5 without nitrification inhibition was measured following the dilution method 8043 (Hach Company, USA, 2012) in transparent 300-mL Wheaton BOD bottles (Wheaton, USA).

3. Results and discussion

Results from Tests I, II, and III are summarized in Table 2.

3.1. Results for Test I

Temporal profiles of biomass, BOD_5 and nutrients recorded in Test I conducted at a typical BOD:N:P ratio (16.5:13.4:1) are shown in Fig. 2a–d. The cultures grew with minimal lag phase, and reached a maximum density of 1.5 g L^{-1} in 6 days, by which time, both N and P had been reduced to negligible levels. On day 3, *G. sulphuraria* had achieved maximum growth rate of 0.5 g L^{-1} d^{-1} at a density of 1 g L^{-1}, by which time, BOD had been reduced from 60 to 3.7 mg L^{-1} (>93%), N from 48 to 1.5 mg L^{-1} (>96%), and P from 11 to 0.2 mg L^{-1} (>98%). The BOD removal rate in this test can be

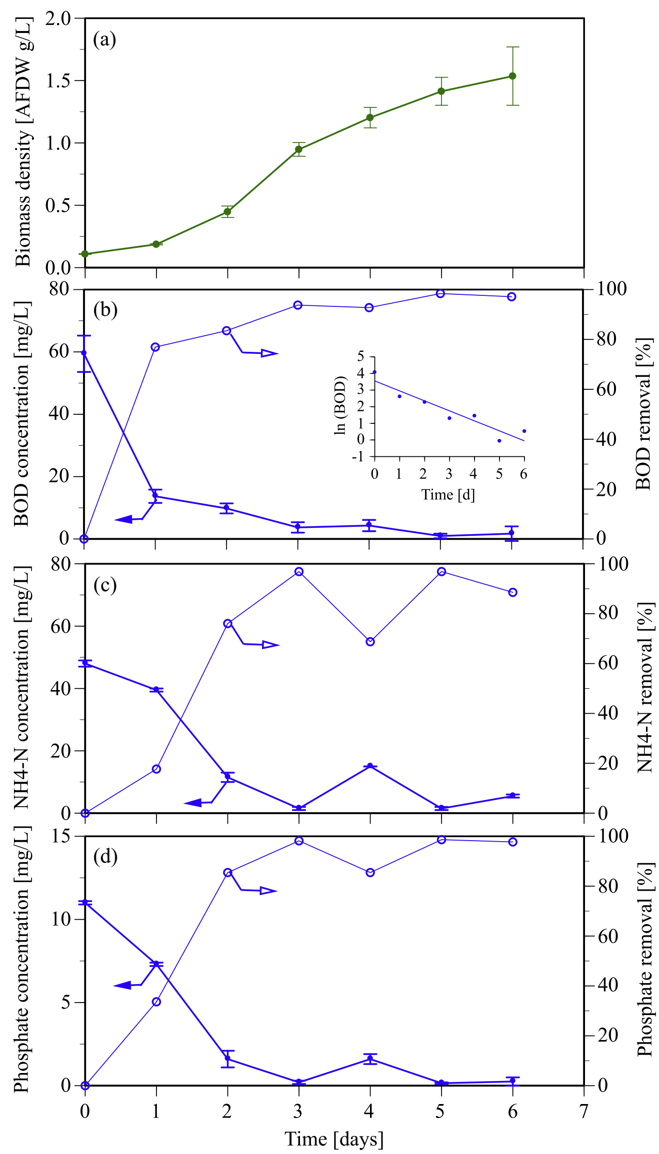

Fig. 2. Results of Test I at BOD:N:P ratio in medium of 16.5:13.4:1: (a) biomass growth; (b) BOD removal; (c) nitrogen removal; and (d) phosphate removal.

approximated by first order reaction of rate constant = 0.7 d^{-1} (r^2 = 0.825, Fig. 2, inset), which is about 50% higher than that of typical activated sludge process (Metcalf et al., 2004). The volumetric removals of BOD_5, N, and P over the first three days were 18.57, 15.5, and 3.6 mg L^{-1} d^{-1}, respectively.

During Test I, nutrient uptake preceded the period of maximum growth. More than 90% nutrient removal was achieved by the end of day 3 (Table 2), when only two thirds of the final biomass density was achieved. This offset between residence time requirements to meet nutrient recovery requirements and maximum biomass density creates a trade-off with respect to operational timing. Maximum energy recovery from UWW requires maximum biomass density but plant capital costs will scale directly with land area requirement, a function of the hydraulic retention time. Detailed techno-economic modeling using the removal rates recorded in this study will be required to achieve the desired outcome.

3.2. Results of Test II

Temporal profiles of biomass, BOD_5, and nutrients recorded in Test II conducted with carbon rich medium (BOD:N:P of 34.0:15.0:1) are shown in Fig. 3a–d. As shown in Fig. 3a, the cultures reached the maximum density of 1.3 g L^{-1} by day 6 with maximum growth rate of 0.4 g L^{-1} d^{-1} on day 2. By 3rd day, BOD had dropped from 54.4 to 9.6 mg L^{-1} (82%), N from 24.1 to 2.4 mg L^{-1} (90%) and P from 4.9 to 0.8 mg L^{-1} (84%). Volumetric removals of BOD_5, N, and P were 14.93, 7.23, and 1.38 mg L^{-1} d^{-1}, respectively. The first order reaction rate constant in Test II was 0.47 d^{-1}. The lower removal rates of BOD_5, N and P in Test II compared to Test I are attributed to the lower initial concentrations of BOD_5 and nutrients in the media. Comparing Figs. 2 and 3, these results show that BOD, N and P levels in wastewater can be limiting for growth of *G. sulphuraria* and support the notion that higher levels of BOD, N and P will support accelerated growth rates, faster nutrient removal rates, higher final biomass yields, and hence, higher energy yields.

Test II reveals the sensitivity of nutrient removal rates to changes in BOD:N:P ratios (Table 1). Optimizing these ratios is critical to minimizing hydraulic residence times and plant costs. Recovery of N and P from the aqueous and solid fractions after hydrothermal liquefaction of the biomass and recycling to the cultivation step as proposed would enable optimization of these ratios in a coupled WWT/HTL system (Biller et al., 2012; Nelson et al., 2013).

3.3. Results for Test III

Temporal profiles of biomass, BOD_5 and nutrients recorded in Test III with the three media are shown in Fig. 4a–c. As with the

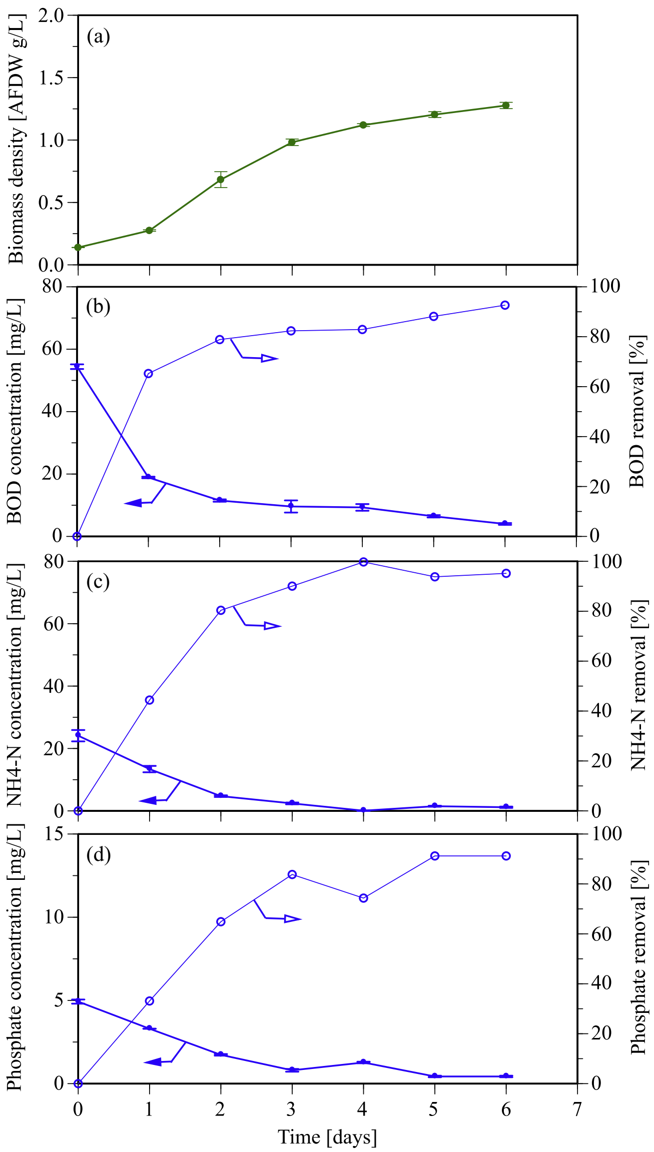

Fig. 3. Results of Test II at BOD:N:P ratio in medium of 34.0:15.0:1: (a) biomass growth; (b) BOD removal; (c) nitrogen removal; and (d) phosphate removal.

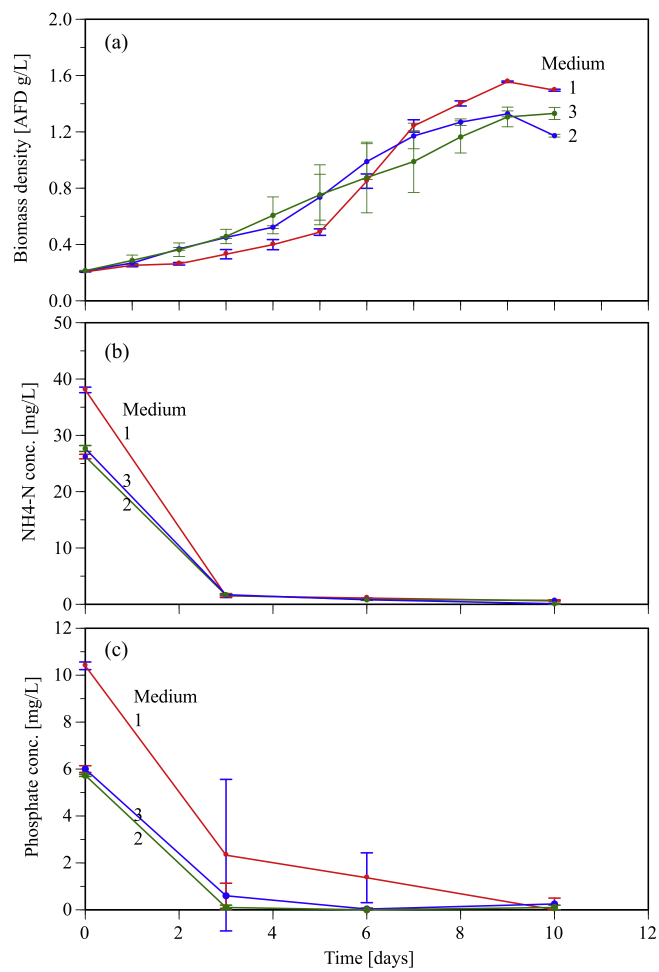

Fig. 4. Results of Test III in media 1, 2, and 3: (a) biomass growth; (b) nitrogen removal; and (c) phosphate removal.

previous tests, N and P were removed to negligible levels by day three. *G. sulphuraria* cultures began to grow without any lag period in Media 2 and 3. The productivity in Medium 3 was not statistically different from that in Medium 2 (p value = 0.8503), but was higher than that recoded with the modified Cyanidium medium (p value = 0.034). The volumetric removals of N in the Media 1, 2 and 3 over the first 3 days were 12.18, 8.21 and 8.65 mg L^{-1} d^{-1} respectively; corresponding removals of P were 2.69, 1.80, and 1.88 mg L^{-1} d^{-1}, respectively. Removal efficiencies (3 days) of N in this test ranged 94–96%, and of P ranged 77–98%. These results support the utility value of this strain in large-scale cost-effective wastewater treatment.

Higher cell densities by day 3 in carbon-containing Media 2 and 3 than that in non-carbon containing Medium 1 show the important contribution of mixotrophic growth on the overall growth pattern for this organism. Previous work with *G. sulphuraria* 074G has shown that external glucose uptake suppressed photosynthesis suggesting this organism may prioritize heterotrophic metabolism over photosynthesis (Oesterhelt et al., 2007). The rapid removal of over 60% of the BOD observed in this study within the first day is consistent with this idea. Indeed, *G. sulphuraria* is the most versatile alga known with respect to growth on organic carbon sources (Schonknecht et al., 2013). Since microscopic evidence of bacterial growth in the acidic conditions used in these experiments was not observed, it is concluded that *G. sulphuraria* is directly responsible for the BOD uptake shown in Fig. 4.

The maximum growth rates of *G. sulphuraria* demonstrated in Fig. 4 in carbon containing media are 2–5 times higher than the maximum growth rates for strict phototrophic cultures of *G. sulphuraria* grown in outdoor PBRs (Selvaratnam et al., 2014b), cultures of *Nannochloropsis salina* grown in outdoor PBRs (Quinn et al., 2012) and cultures of *Chlorella sorokiniana* (Huesemann et al., 2013). The faster growth rates observed here are likely the direct effect of oxidation of organic carbon in wastewater by *G. sulphuraria* during the 10-h dark period used in these experiments preventing respiratory losses of internal carbon that would otherwise occur under phototrophic conditions. Furthermore, as noted by Huesemann et al. (2013), light attenuation in algal cultures leads to a very shallow photic zone at the top of a culture such that the average cell will spend 90% of its time in the dark zone during daylight hours depending on mixing energy.

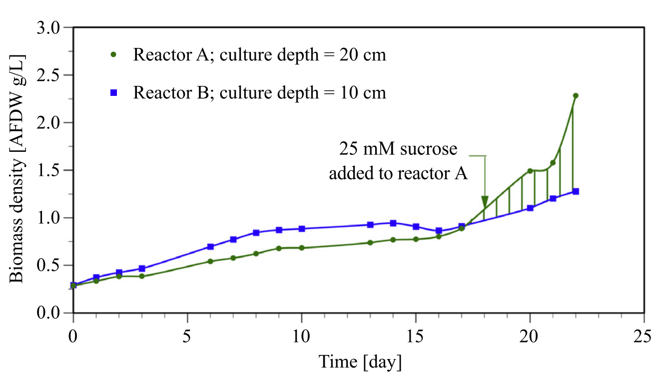

Fig. 5. Results of outdoor test in two enclosed photobioreactors: reactor A with culture depth = 20 cm; and reactor B with culture depth = 10 cm. Shaded area represents increased growth due to mixotrophy following the addition of 25 mM sucrose to reactor A.

Based on the above results, a further test was conducted to assess the growth of *G. sulphuraria* under outdoor conditions. In this test, two enclosed photobioreactors (A and B) were initiated with the standard Cyanidium medium; A with a culture depth of 20 cm; and B, with a culture depth of 10 cm. Both reactors were operated under strict phototrophic conditions for the first two weeks; thereafter, reactor A was dosed with 25 mM sucrose to simulate wastewater growth conditions with organic carbon present in outdoor PBRs like those envisioned for WWT.

Results of this test are illustrated in Fig. 5. Growth rates in the two reactors under phototrophic conditions were comparable, averaging 0.035 g L^{-1} d^{-1}; as expected, that in reactor B with the shallower depth was slightly higher. Upon addition of 25 mM of sucrose, growth rate in reactor A increased eightfold to 0.282 g L^{-1} d^{-1} translating to an aerial productivity of 56.6 g m^{-2} d^{-1} at a culture depth of 20 cm. This lends credibility to the premise of this study that *G. sulphuraria* could be of value in removing organic carbon from UWW. It is suggested that the availability of an external carbon source to support cellular energy needs via respiration both at night and in shaded zones in the daytime supports the high productivities observed under laboratory conditions with wastewater (Fig. 2) and in the outdoor, sucrose-supplemented PBR cultures in reactor A (Fig. 5). Otherwise, respiratory energy generation will consume internal carbon reserves, decreasing biomass productivity. Hydrothermal liquefaction can be manipulated to recover fermentable carbon at lower temperatures in a two-stage system (Chakraborty et al., 2012). This is another example of the potential synergy between algal WWT and hydrothermal liquefaction of the resulting algal sludge material (Zhou et al., 2013).

3.4. Comparison with literature studies

Results of this study are compared in Table 3 with those reported in the literature. While the reported studies have been conducted with different algal species grown in different wastewater sources, the comparison is made on the basis of BOD_5 and

Table 3
Comparison of literature results on primary effluent with this study.

Algal species	Initial concentration [mg L^{-1}]		Removal rate [mg L^{-1} d^{-1}]			Source
	N–NH_3	P–PO_4	Organics	N–NH_3	P–PO_4	
Wild type *Chlorella* sp.	32.2	6.86 (TP)	41.33 (COD)	5.73	1.89	Wang et al. (2010)
Chlorella vulgaris	43.9	7.11	75.56 (COD)	3.03	0.51	Cabanelas et al. (2013)
Botryococus terribilis	47.4	11.5	53.71 (COD)	2.63	0.4	Cabanelas et al. (2013)
Green algae and diatoms	39	2.1	–	9.75	0.52	Woertz et al. (2009)
	39	2.1	–	12.99	0.69	
	39	2.1	–	10.97	0.69	
	39	2.1	–	19.2	0.98	
Desmodesmus communis	33.6	1.54	–	4.8	1.54 (TP)	Samori et al. (2013)
	55.9	1.72	–	7.99	0.57 (TP)	
	84.6	1.73	–	7.69	0.58 (TP)	
G. sulphuraria	48.0	11.0	18.57 (BOD)	15.5	3.6	This study, Test I
G. sulphuraria	24.1	4.9	14.93 (BOD)	7.23	1.38	This study, Test II

nutrient removal rates. The ammonical nitrogen and phosphate removal rates (15.5 and 3.6 mg L^{-1} d^{-1} respectively) found in this study are comparable to those reported in the literature. This comparison further supports the selection of *G. sulphuraria* as a beneficial strain for urban wastewater treatment.

4. Conclusion

G. sulphuraria was shown to be capable of higher growth rate in the primary effluent than in the control medium. BOD removal rate by *G. sulphuraria* was shown to be greater than that of the activated sludge process; nutrient removals by this strain are comparable to those reported in the literature. Since mixotrophic metabolism does not require energy for oxygenation, it can conserve the energy currently consumed for aerobic BOD removal. By converting most of the carbon in the wastewater to biomass, it enables higher energy recovery than by current practice. Mixotrophic approach has the potential for energy-positive wastewater treatment.

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