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Short Communication

Evaluation of a thermo-tolerant acidophilic alga, *Galdieria sulphuraria*, for nutrient removal from urban wastewaters

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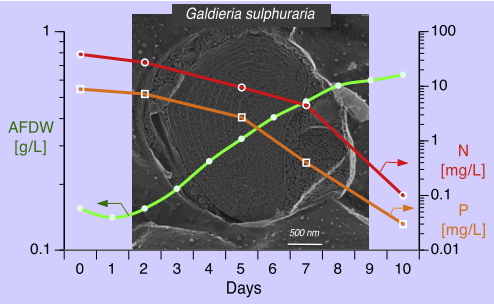
HIGHLIGHTS

- Cultivated *Galdieria sulphuraria* in acidified wastewater.
- Demonstrated ammoniacal nitrogen removal rate of 4.85 mg L^{-1} d^{-1}.
- Demonstrated phosphate removal rate of 1.21 mg L^{-1} d^{-1}.
- Closed reactor contained odors, minimized evaporation, and achieved cell density of 2.5 g AFDW L^{-1}.
- Achieved nutrient removal comparable to literature values from algae grown at neutral pH.

GRAPHICAL ABSTRACT



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ABSTRACT

Nutrient removal from primary wastewater effluent was tested using *Galdieria sulphuraria*, an acidophilic and moderately thermophilic alga. Biomass yield recorded in this study (27.42 g biomass per g nitrogen removed) is higher than the average reported in the literature (25.75 g g^{-1}) while, the theoretical yield estimated from the empirical molecular formula of algal biomass is 15.8 g g^{-1}. Seven-day removal efficiencies were 88.3% for ammoniacal-nitrogen and 95.5% for phosphates; corresponding removal rates were 4.85 and 1.21 mg L^{-1} d^{-1}. Although these rates are lower than the average literature values for other strains (6.36 and 1.34 mg L^{-1} d^{-1}, respectively), potential advantages of *G. sulphuraria* for accomplishing energy-positive nutrient removal are highlighted. Feasibility of growing *G. sulphuraria* outdoors at densities higher than in high-rate oxidation ponds is also demonstrated.

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1. Introduction

Urban wastewaters are laden with high levels of organic carbon and different forms of nitrogen (N) and phosphorous (P) that must be removed prior to discharge into receiving waters. Although traditional wastewater treatment plants (WWTPs) equipped with secondary treatment meet the discharge standards for organic carbon (BOD), they fall short of meeting the discharge standards for nutrients (Cabanelas et al., 2013). Many WWTPs are now required to add tertiary treatment of the secondary effluent to meet current discharge standards for nutrients.

The most common option for tertiary treatment, biological nutrient removal (BNR), converts NH_4–N into N_2 gas, eliminating its potential value as fertilizer, while entrapping P into biosolids for removal prior to discharge. Yet, BNR processes are energy intensive. Energy consumption in a 6-MGD urban wastewater treatment plant increased 41% following addition of BNR

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(Sturm and Lamer, 2011). Of the 656 major WWTPs (flows >10 MGD) that handle 70% of the wastewater flow in the US, 353 had to be retrofitted with tertiary processes to remove nutrients, incurring significant energy costs (Report on the Performance of Secondary Treatment Technology, 2013).

There is growing interest in developing energy-efficient and sustainable technologies that minimize or eliminate the energetic cost of managing urban wastewaters (McCarty et al., 2011). Urban wastewaters contain internal energy of 6.3–7.6 kJ L^{-1} (Heidrich et al., 2011), which is roughly 2–4 times the energy that is now being expended to treat them prior to discharge (Tyler et al., 2013). Recognizing that algal-based wastewater treatment systems use photosynthetic energy to drive nutrient removal, recent studies have sought to build on the early efforts of Oswalad and coworkers (Oswald et al., 1953) to develop improved algal systems for urban wastewater treatment. The premise of this approach is that, mixed algal/bacterial systems can simultaneously reduce BOD, N, and P in urban wastewaters. The energy-rich biomass produced would then serve as feedstock for producing gaseous or liquid biofuels via hydrothermal liquefaction (Chakraborty et al., 2012), catalytic hydrothermal gasification (Elliott, 2008), or anaerobic digestion (McCarty et al., 2011). This approach incorporates much of the internal energy of the wastewater into the biomass as well as solar energy captured via photosynthesis.

The energy-advantage of the mixed algal/bacterial process can be illustrated by comparing two scenarios: (1) anaerobic digestion of algal biomass cultivated in wastewater to produce methane as an energy carrier; (2) activated sludge treatment of wastewater coupled with anaerobic digestion of the waste biomass to produce methane as an energy carrier. Considering the stoichiometric biomass yields per unit N-consumed in the two scenarios, and the electrical energy equivalence of methane, the mixed process is estimated to yield 175% more net electrical energy (Table 1). Likewise, another study has estimated that algal-based urban wastewater systems have the potential to recover $62{,}700 \times 10^6$ kW h yr^{-1} of energy from the Nation's wastewaters whereas anaerobic systems could extract only 5000×10^6 kW h yr^{-1} (Sturm and Lamer, 2011).

Although the above comparisons favoring the algal-based systems are based on theoretical estimates, only a few studies have experimentally quantified their ability to remove BOD and nutrients from urban and industrial wastewaters (for e.g. Park et al., 2011). This study proposes a potentially energy-positive WWTP process specifically intended for warm-to-hot, arid regions where water is precious. This paper presents nutrient removal ability of an algal extremophile, *Galdieria sulphuraria*, with a broad genetic capacity for organic carbon utilization (Schonknecht et al., 2013). *G. sulphuraria* can thrive at pH 0.5–4 and temperatures up to 56 °C, conditions that many competitors, predators, viruses, and pathogens will not tolerate. Both laboratory assessment of nutrient removal capability and outdoor cultivation results are presented.

Table 1
Potential for energy recovery per unit nitrogen consumed: activated sludge process vs. mixotrophic process.

	Process	
	Activated sludge	Mixotrophic
Biomass formula	$C_5H_7O_2N$[d]	$C_{106}H_{263}O_{110}N_{16}P$[e]
Electrical energy input for aeration[a]	32 kJ $(g\Delta N)^{-1}$	–
Stoichiometric biomass yield	8.1 g $(g\Delta N)^{-1}$	15.8 g $(g\Delta N)^{-1}$
Biological methane potential[b]	5.3 L $(g\Delta N)^{-1}$	6.3 L $(g\Delta N)^{-1}$
Electrical energy producible from methane[c]	56.9 kJ $(g\Delta N)^{-1}$	67.7 kJ $(g\Delta N)^{-1}$
Net electrical energy producible	24.9 kJ $(g\Delta N)^{-1}$	67.7 kJ $(g\Delta N)^{-1}$

[a] Assumptions: 0.45 g biomass/g ΔCOD; 0.5 g O_2/g ΔCOD; 1 W h/g O_2.
[b] Speece (1996).
[c] Lower heating value of methane = 35.8 kJ/L; energy conversion efficiency = 30%.
[d] Speece (1996).
[e] Redfield et al. (1963).

2. Methods

An independent isolate of the unicellular red algae *G. sulphuraria* CCMEE 5587.1 (Toplin et al., 2008) (hereafter *G. sulphuraria*) obtained from the Culture Collection of Microorganisms from Extreme Environments (University of Oregon) was assessed in this study. The test cultures were grown in 16 mm borosilicate glass tubes closed with plastic caps and sealed with parafilm to reduce evaporation. Each tube was inoculated with 6 mL of culture and placed in the outer rim of a Tissue Culture Roller Drum Apparatus (New Brunswick Scientific, Eppendorf, CT, USA) rotating at 16 rpm. The roller drum was housed inside an incubator (Percival, IA, USA) maintained at 40 °C with a 14 h/10 h light/dark cycle. The CO_2 level inside the incubator was kept constant at 2–3% (vol/vol).

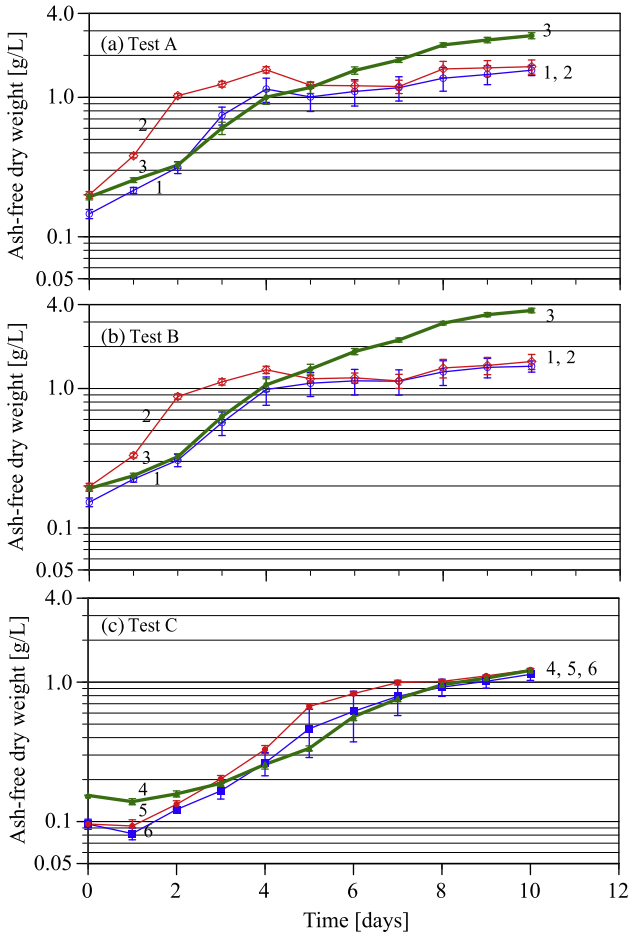

Fig. 1. Biomass growth profiles of *G. sulphuraria* in Tests A–C. Numbers correspond to media codes: Code 1 – Modified Cyanidium medium (MCM), prepared with DI water; Code 2 – MCM + 20 mM glucose, prepared with DI water; Code 3 – MCM, prepared with autoclaved primary effluent; Code 4 – MCM with no N & P + 40 ppm $(NH_4)_2SO_4$ + 10 ppm KH_2PO_4, prepared with DI water; Code 5 – MCM with no N & P + 40 ppm $(NH_4)_2SO_4$ + 10 ppm KH_2PO_4 + 20 mM glucose, prepared with DI water; Code 6 – MCM with no N & P, prepared with autoclaved primary effluent. Composition of modified Cyanidium medium (Andersen, 2005), CM: $(NH_4)_2SO_4$, 2.64 g/L; KH_2PO_4, 0.27 g/L; NaCl, 0.12 g/L; $MgSO_4{\cdot}7H_2O$, 0.25 g/L; $CaCl_2{\cdot}2H_2O$, 0.07 g/L; Nitch's trace element solution, 0.5 mL; $FeCl_3$ (0.29 g/L), 1.0 mL, and pH adjusted to 2.5 with 10 N H_2SO_4 . Includes vitamin component of f/2 algal medium (vitamins B1, B12 and biotin).

Various growth media tested in this study are detailed in Fig. 1. The performance of the strain was evaluated in Test A at a fixed temperature of 40 °C; and in Test B under a diurnal temperature regime (from 26 to 42 °C over a 10-h period) to mimic outdoor conditions. Test C was conducted at 40 °C. Since the N and P levels in the modified Cyanidium medium (560 ppm NH_3–N and 190 ppm P) are higher than those in the primary effluent, in Test C, the medium was prepared excluding the original amounts of N and P, and then, dosed with 40 ppm of N and 10 ppm of P to simulate the N and P levels in primary effluent. The initial pH of all cultures was set at 2.5 and which stayed within 0.3 pH units throughout the duration of the experiments.

Wastewater samples for testing were collected downstream of the primary settling tank at the municipal WWTP, Las Cruces, NM. Upon collection of the sample, large solid particles were removed by gravity settling, and then autoclaved at 121 °C and stored at 4 °C. The clear supernatant of the stored sample was used in the experiments to make up the growth medium. At the beginning of each test, the inoculum was centrifuged (Sorvall Biofuge primo., Thermo Scientific, USA) and the algae pellets were re-suspended in the control medium of the particular test and left for 24 h at 40 °C, 14 h/10 h light/dark photoperiod for preadaptation. Biomass growth thereafter was quantified daily, in terms of the optical density (OD) measured with Beckman DU530 spectrophotometer (Beckman Coulter Inc., USA) at a wavelength of 750 nm:

$$\text{AFDW } [\text{g L}^{-1}] = 0.54\ (\text{OD @ 750 nm}) + 0.023;$$
$$n = 12;\ r^2 = 0.997$$

For each test condition, 3 glass tubes were withdrawn from the drum on days 2, 5, 7 and 10 to serve as triplicates for measuring nutrient levels. Samples from each tube were first centrifuged at 4000 rpm for 10 min and the supernatant was diluted for analyses. Soluble ammonia-N and P were determined using HACH DR6000 spectrophotometer (HACH, Colorado, USA) (Salicylate TNT Method 10031 and Phosver 3 Method 8048). Outdoor cultivation was conducted in an enclosed polyethylene bag measuring 1 × 3 m, inflated to ~10% above ambient pressure with 1–2% CO_2 in air. Mixing was provided by a paddlewheel placed inside the bag. Culture depth was 10 cm.

3. Results and discussion

Biomass growth profiles recorded in Tests A–C are presented in Fig. 1. Exponential growth is observed from 0–4 days in Tests A and B; and from 2–8 days in Test C. Based on the growth rates estimated from the above growth profiles (compared in Fig. 2), and the final biomass densities attained (Code 1: 1.146 ± 0.226 vs. 0.984 ± 0.167 g L^{-1}; and) in Tests A and B under the temperature regimes tested, the effect of temperature was deemed insignificant. As such, Test C was conducted at 40 °C.

In Tests A and B, where the exponential phase lasted 4 days, growth rate was highest in the case with CM supplemented with glucose (Code 2), while that with primary effluent (Code 3) was lowest. However, following the exponential phase, growth with primary effluent (Code 3) overtook the other two (Test A), and attained a density of 2.7 g L^{-1}, while the other two saturated at ~1.6 g L^{-1}. The initial stimulation of growth with glucose (Code 2) in the two temperature regimes (Tests A and B) is attributed to the heterotrophic nature of *G. sulphuraria* and confirmed its ability to grown on primary effluent.

Test C was designed to assess the growth of *G. sulphuraria* at N and P levels similar to those in primary effluent. Growth rates in Test C with the three media were comparable (Fig. 2): Code 4: 0.133 ± 0.021 g L^{-1} d^{-1}; Code 5: 0.146 ± 0.002 g L^{-1} d^{-1}; and Code 6: 0.133 ± 0.007 g L^{-1} d^{-1}. In this test, growth with glucose (Code 5) was stimulated initially, but reached saturation by day six. However, at the end of the exponential phase, all three media reached the same density of 1.2 g L^{-1}. Overall, Tests A–C showed that *G. sulphuraria* can be grown in primary effluent at growth rates comparable to that with the baseline CM medium.

3.1. Nutrient removal by G. sulphuraria

Temporal NH_4–N profiles recorded in Test C are shown in Fig. 3a. Removal efficiencies of NH_4–N over 7 days with the different test media were as follows: Code 4: 90.5%; Code 5: 89.0%; and Code 6: 88.3%. At the end of 10 days, N levels were below the detection limit; removal rate of ammoniacal-N with primary effluent (Code 6) was 4.85 mg L^{-1} d^{-1}. Biomass yield with primary effluent (Code 6) was found to be 27.42 g biomass per g nitrogen removed, whereas the theoretical yield estimated from the empirical molecular formula of biomass is 15.8 g g^{-1} (Table 1).

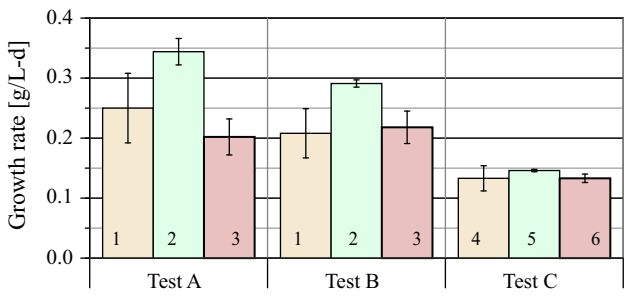

Fig. 2. Growth rate in Tests A–C determined from the exponential growth period in each test. Numbers correspond to medium codes: see Fig. 1 for description.

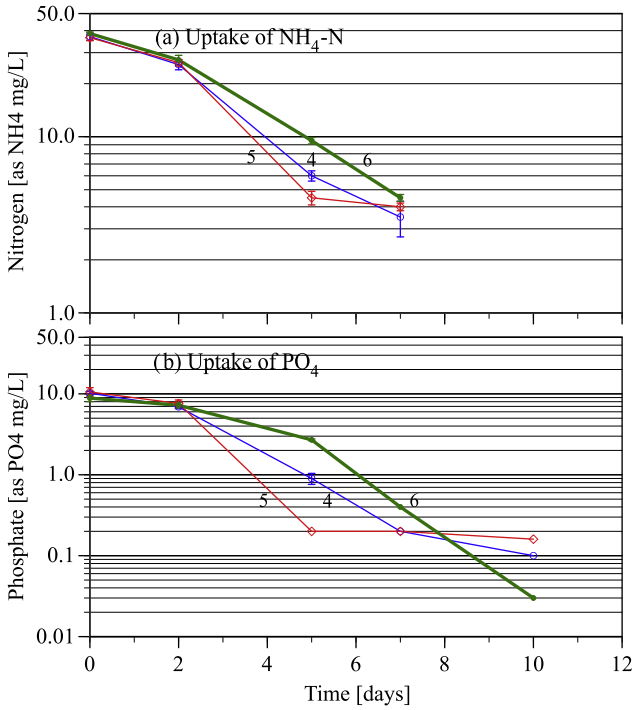

Fig. 3. Uptake of nutrients by *G. sulphuraria* in Test C numbers correspond to media codes: see Fig. 1 for description.

Temporal P profiles recorded in Test C are shown in Fig. 3b. Removal efficiencies of P over 7 days with the different test media were: Code 4: 98%; Code 5: 98.1%; and Code 6: 95.5%. At the end of 10 days, phosphate levels were below the detection limit; removal rate of phosphate with primary effluent (Code 6) was 1.21 mg L^{-1} d^{-1}.

3.2. This study vs. literature reports

Results of this study are compared with available literature studies for variety of species grown with different streams of urban wastewaters at different initial concentrations of N and P (Table 2). Biomass yield in this study with primary effluent (27.42 g biomass per g nitrogen removed) is slightly higher than the average reported in the literature (25.75 g g^{-1}). Higher biomass yields from WWTP are preferable to maximize net energy production via anaerobic digestion or hydrothermal processing (Chakraborty et al., 2012).

Removal rates of nitrogen and phosphates from primary effluent (Test C, Code 6) found in this study (4.85 and 1.21 mg L^{-1} d^{-1}, respectively), are somewhat lower than the corresponding rates reported previously with other algal strains that averaged 6.36 and 1.34 mg L^{-1} d^{-1} (Table 2). Most of the higher removals reported in the literature are associated with higher initial concentrations of N and P in the growth medium (55–96 mg N/L vs. 40 mg N/L in this study).

3.3. Outdoor cultivation of G. sulphuraria

To further demonstrate the utility of *G. sulphuraria*, cultures were grown outdoors in an enclosed PBR at 100 L of culture per m^2, supplemented with 1–2% CO_2/air sparged at gas-to-culture volume ratio of 0.02 min^{-1} (VVM). The mean areal productivity from three successive batch cultures in the period May 18 to July 13 (2013) was 10.6 g m^{-2} d^{-1} (S.D. = 5.1). The growth curve for the peak culture is shown in Fig. 4, with exponential growth during days 1–5 followed by linear growth at 0.165 g L^{-1} d^{-1} (16 g m^{-2} d^{-1}).

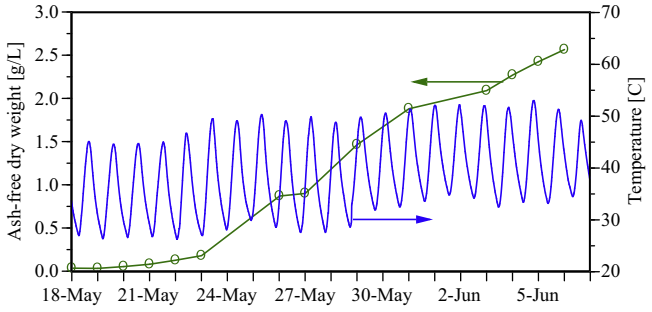

Fig. 4. Temperature and biomass profiles under outdoor cultivation of *G. sulphuraria* in enclosed photobioreactor.

These results compare favorably with volumetric and areal productivities observed in a more expensive, temperature-regulated vertical PBR system previously tested at the same location (Quinn et al., 2012). The final biomass concentration in this study was three times higher than the maximum achieved with secondary wastewater treatment in a tubular PBR and nine-times that achieved with the same water source in a high-rate algal pond (HRAP) system (for e.g. Park et al., 2011). Significantly, cell densities achieved in full sunlight exceed the highest level observed in the laboratory (Test B, Code 3) by a factor of 1.32. Diurnal temperature profile superimposed in Fig. 4 demonstrates the magnitude of passive solar heat gain as well as nighttime heat losses in the horizontal PBRs.

Traditional wastewater treatment with algal-bacterial systems utilizes high-rate ponds that typically achieve 0.5–0.8 g L^{-1} final cell densities (Oswald et al., 1953). Open pond systems are subject to high evaporation rates when operated in arid environments. The wastewater treatment system described here avoids evaporative water losses, opening the possibility of algal wastewater treatment in arid environments where sunlight is rarely limiting but where freshwater supplies are scarce. Furthermore, this enclosed cultivation system achieved 3–5-fold higher final cell densities than high-rate pond systems. High cell density at harvest lowers energy costs, a significant component of life-cycle carbon allocations in algal production systems.

Table 2
Nutrient removal rates from urban wastewater: this study vs. literature results.

Source[a]	Strain[b]	Culture medium[c]	Initial conc. [mg L^{-1}]		Growth rate [mg L^{-1} d^{-1}]	Removal rate [mg L^{-1} d^{-1}]		Yield[d] [g g^{-1}]
			NH_4–N	PO_4^{3-}		NH_4–N	PO_4^{3-}	
A	1	APE	40.0	10.0	133.1	4.85	1.21	27.42
B	2	PCE	47.4	11.5	224.8	2.63	0.40	85.48
	3	PCE	43.8	7.1	57.7	3.03	0.51	19.04
C	4	SCE	14.6	4.9	43.8	1.76	0.41	24.89
		SCE	18.4	3.9	46.3	2.26	0.36	20.49
		SCE	17.0	4.7	37.5	2.09	0.33	17.94
		SCE	18.9	3.8	51.3	2.34	0.34	21.92
D	5	CEN	95.6	125.8	210.0	6.02	2.90	34.86
		CEN	95.6	125.8	164.5	4.52	3.08	36.37
		CEN	95.6	125.8	70.5	6.90	8.58	10.21
E	6	PCE	33.6	1.5	138.0	4.80	1.54	28.75
		PCE	55.9	1.7	177.0	7.99	0.57	22.15
		PCE	84.6	1.7	227.0	7.69	0.58	29.52
	7	PCE	23.3	1.7	78.0	3.33	0.24	23.42
F	8	PCE	39.0	2.1	212.2	9.75	0.52	21.77
		PCE	39.0	2.1	271.1	12.9	0.70	20.86
		PCE	39.0	2.1	104.3	10.9	0.70	9.51
		PCE	39.0	2.1	204.4	19.2	0.98	10.65

[a] A, This study; B, Cabanelas et al. (2013); C, Su et al. (2011); D, Hu et al. (2012); E, Samori et al. (2013); F, Woertz et al. (2009).
[b] 1, *Galdieria sulphuraria* 5587.1; 2, *Botryococcus terribilis*; 3, *Chlorella vulgaris*; 4, Algal + bacterial culture; 5, *A. protothecoides*; 6, *Desmodesmus communis*; 7, Algal consortia; 8, Green algae + diatoms.
[c] PCE, primary clarifier effluent; APE, autoclaved primary effluent; CEN, centrate; SCE, secondary clarifier effluent.
[d] Biomass yield per unit ammoniacal-nitrogen consumed.

The diurnal temperature variations in enclosed cultivation systems impose a requirement for thermophilic algae. *G. sulphuraria* was chosen for this reason and also because it is an obligate acidophile able to grow well only below pH 4 (Schonknecht et al., 2013). The low pH of acidified wastewater could be beneficial in rapid destabilization of enteric pathogens present in primary settled wastewater. These extremes of temperature and pH are expected to drastically reduce the number of microorganisms present in the wastewater treatment system and afford a level of biochemical control not possible in open pond systems. This bodes well for further optimization of overall system design to leverage biological and chemical engineering approaches to reduce the required footprint of an algae-based wastewater treatment systems and minimize it's hydraulic residence time.

4. Conclusion

Based on the results of this study, it can be concluded that *G. sulphuraria* can be cultivated in primary effluent to achieve high nutrient removal efficiencies and at removal rates comparable to other strains. The high biomass yield recorded under laboratory conditions as well as the high areal productivity achieved under outdoor conditions in the closed PBR configuration that minimizes evaporative losses and contamination, hold promise for *G. sulphuraria* as a preferred strain for energy-efficient nutrient removal from urban wastewaters.

Acknowledgements

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