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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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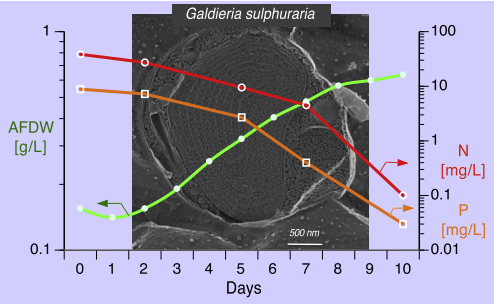
HIGHLIGHTS

- 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).

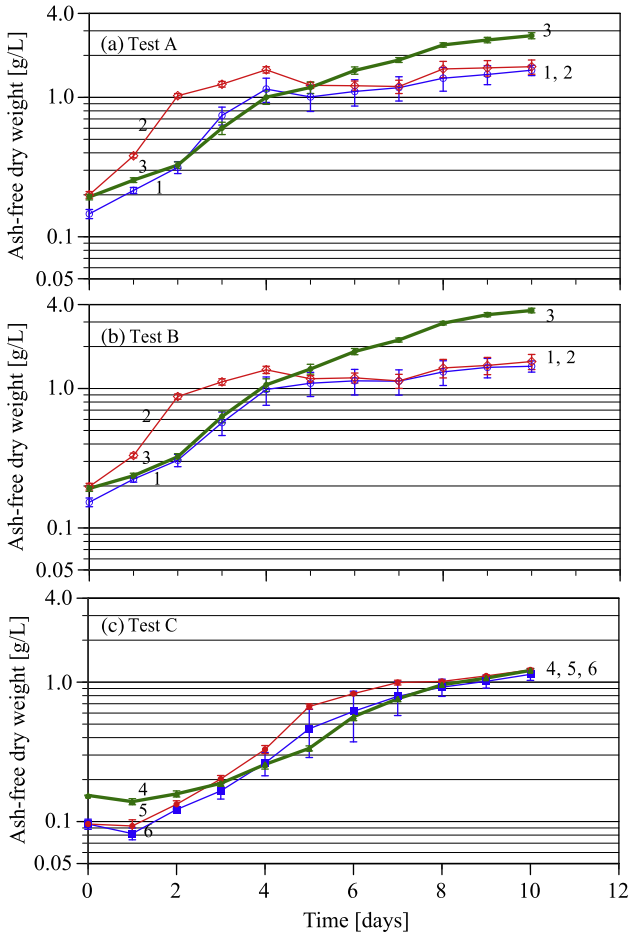

Fig. 1. Biomass growth profiles of *G. sulphuraria* in Tests A–C. Numbers correspond to media codes: Code 1 – Modified Cyanidium medium (MCM), prepared with DI water; Code 2 – MCM + 20 mM glucose, prepared with DI water; Code 3 – MCM, prepared with autoclaved primary effluent; Code 4 – MCM with no N & 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).

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