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Algal biofuels from urban wastewaters: Maximizing biomass yield using nutrients recycled from hydrothermal processing of biomass

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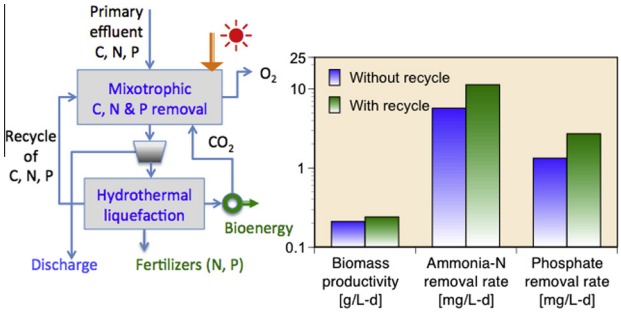
HIGHLIGHTS

- Demonstrated higher biomass growth in wastewater than in control.
- Demonstrated >90% nutrient removal from wastewater.
- Evaluated biomass growth in aqueous product obtained at six HTL-temperatures.
- Demonstrated higher biomass productivity in aqueous product of HTL.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent studies have proposed algal cultivation in urban wastewaters for the dual purpose of waste treatment and bioenergy production from the resulting biomass. This study proposes an enhancement to this approach that integrates cultivation of an acidophilic strain, *Galdieria sulphuraria* 5587.1, in a closed photobioreactor (PBR); hydrothermal liquefaction (HTL) of the wet algal biomass; and recirculation of the nutrient-rich aqueous product (AP) of HTL to the PBR to achieve higher biomass productivity than that could be achieved with raw wastewater. The premise is that recycling nutrients in the AP can maintain optimal C, N and P levels in the PBR to maximize biomass growth to increase energy returns. Growth studies on the test species validated growth on AP derived from HTL at temperatures from 180 to 300 °C. Doubling N and P concentrations over normal levels in wastewater resulted in biomass productivity gains of 20–25% while N and P removal rates also doubled.

1. Introduction

Urban wastewaters (UWWs) are laden with dissolved organic carbon (C) as well as nitrogen (N) and phosphorous (P) that must be removed to meet discharge standards. Typically, organic carbon, measured as biological oxygen demand (BOD), is effectively oxidized to CO_2 in the secondary treatment step by heterotrophic bacteria using oxygen as a terminal electron acceptor, while only ~15% of the dissolved nitrogen is incorporated into the resulting biomass (Ekenfelder and Grau, 1998). A fundamental limitation of this step is the C:N:P ratio in UWW relative to that in the biomass; there is not enough carbon in UWW for heterotrophic bacteria to simultaneously utilize all the N and P in UWW. As a result, tertiary treatment such as biological nutrient removal is required

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to meet the N and P discharge standards. Current technologies are thus energy intensive and operationally expensive, with only 25–50% of energy cost considered recoverable via anaerobic digestion of the resulting biomass (EPA Office of Water, 2006). In addition, an external carbon source, such as methanol (Tchobanoglous et al., 2003), has to be added to the tertiary process to complete nitrogen removal.

Several approaches to improve the energy efficiency of wastewater treatment (WWT) are in active development. One strategy substitutes anaerobic metabolism in various forms to overcome the need for providing dissolved oxygen (Ahn et al., 2004; McCarty et al., 2011). A second approach used in aquaculture is to supplement wastewater with additional organic carbon to balance the C:N:P stoichiometry (Ebeling et al., 2006); however, this is not a practical approach for the large volumes associated with urban wastewater. A third, more promising approach is the use of photosynthetic microorganisms to provide both oxygen from photosystem II and carbon via CO_2 fixation (Green et al., 1995; Oswald et al., 1953; Selvaratnam et al., 2014a). Photosynthetic oxygen evolution also supports BOD oxidation suggesting the potential for simultaneous removal of C, N and P in a single system that would also yield more energy-rich biomass than expected from a system dependent on heterotrophic activated sludge process.

The recent development of hydrothermal treatment processes for wet biomass conversion to bio-crude oil (Peterson et al., 2008) or bio-gas (Elliott, 2008) affords efficient ways for extracting energy from biomass produced at WWT plants. Hydrothermal liquefaction (HTL) is an emerging technology for wet biomass processing under moderate temperatures (150–350 °C) and pressures (15–20 MPa) (Biller et al., 2012). In HTL, bio-chemical compounds (e.g. lipids, proteins and carbohydrates) present in algal biomass undergo hydrolysis, repolymerization, dehydration, decarboxylation, and deamination (Peterson et al., 2008) to form energy-dense biocrude oil; bio-char; an aqueous product (AP) rich in organic C (~45% of initial feed), N and P; and gaseous products. Biocrude yields of up to 50% (Toor et al., 2013; Zhou et al., 2010) and energy recoveries up to 71% (Alba et al., 2012) have been reported with HTL of wet algal biomass. Other embodiments include sequential HTL, a two-stage process employing a lower temperature extraction of polysaccharides prior to bio-oil production (Chakraborty et al., 2012).

While the chemical composition of AP from HTL processes will vary depending on the process parameters such as temperature, pressure, duration the solids loading, and the species, typical compounds observed include polysaccharides (Chakraborty et al., 2012), soluble organic compounds, and basic nutrients (C, N, and P) (Alba et al., 2013; Biller et al., 2012). Recycling the nutrient-rich AP of HTL of biomass to the cultivation step can bridge the stoichiometric imbalance discussed earlier and boost biomass productivity, a critical design objective towards energy-positive and discharge compliant WWT. When algal WWT is coupled with HTL, of the resulting wet biomass, a secondary benefit is that excess sterile concentrated nutrients can be recovered. Hydrolysis of proteins to amino acids and further deamination of the amino acids release nitrogen in the form of NH_4^+ while phosphate PO_4^{3-} is released by hydrolysis of nucleotide backbones (Alba et al., 2012).

We propose a three-prong approach integrating algal cultivation in wastewater followed by HTL processing of the biomass, and recycling of the AP of HTL to boost biomass productivity and hence, maximize energy recovery. The integrated process is called POWER for Photosynthetically Oxygenated Waste to Energy Recovery. A simplified schematic of the POWER WWT approach is compared to the conventional method in Fig. 1. Oxygen, sterile concentrated forms of fertilizer, and bioenergy products are the outputs from

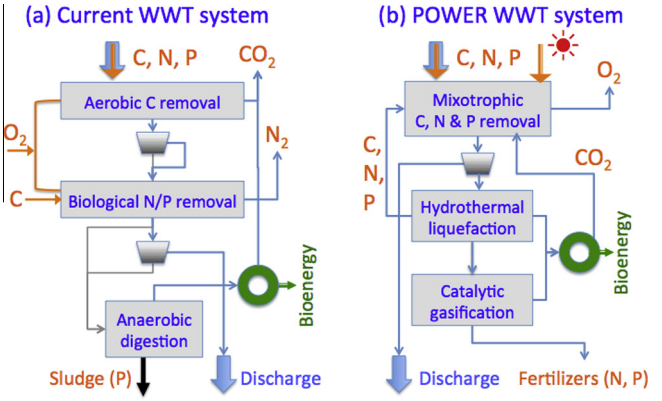

Fig. 1. Schematic of the inputs and outputs of the conventional wastewater treatment system (a) vs. proposed integrated system driven by the POWER algal photosynthesis process (b). Maximizing biomass productivity in POWER while eliminating the energy demand for aerobic carbon removal and the external carbon supply for nitrogen removal increases the net bioenergy output of the POWER system and eliminates sludge as an output stream.

such an integrated system compared to carbon dioxide, N_2 and sludge outputs from traditional treatment methods. Co-location of this system with anthropogenic sources of CO_2 affords an additional potential benefit.

A limited number of literature reports have proposed/evaluated a similar three-prong approach. Roberts et al. (2013) were the first ones to report on a pilot scale study to demonstrate that municipal wastewater could be used to cultivate algal biomass, which upon HTL treatment yielded 44.5% biocrude with an energy content of 39 MJ kg^{-1}. Based on the analysis of the AP of HTL, they had proposed that recycling of the AP could be an option to improve overall sustainability. Biller et al. (2012) have evaluated regrowth on AP of HTL process and demonstrated regrowth of algal biomass at different dilutions, achieving near-equal growths compared to standard media for *Chlorella* sp. (1%), *Chlorogloeopsis* sp. (0.25%), and *Spirulina* sp. (0.25%). Du et al. (2012) have demonstrated regrowth of *Chlorella vulgaris* on AP of HTL conducted at 200 °C, and diluted with distilled water at 2%, 1% and 0.5%. Regrowth rates with AP of HTL diluted with distilled water were greater than those observed with the standard growth medium; biomass productivity and final biomass concentrations were higher in the following order: 2% > 1% > 0.5%, negating the notion that high concentrations of carbon and nutrients in AP of HTL could be inhibitory to algal growth. They reported 45.5–59.9% removal of total nitrogen and 85.8–94.6% removal of total phosphorus under regrowth conditions.

Biller et al. (2012) and Alba et al. (2013) have evaluated regrowth of *Desmodesmus* sp. on AP of HTL process and showed growth rates with the extracts diluted with the control medium comparable to those with the control medium alone. However, regrowth was significantly lower with AP of HTL process diluted with deionized water, which was attributed to lack of micro nutrients rather than to any inhibitory effects of the high concentrations of C, N, and P in AP. Zhou et al. (2013) have recently demonstrated growth of mixed algae-bacteria in primary-settled wastewater fed with AP of HTL (done at a temperature of 300 °C) of algal biomass as well as primary sludge. They achieved biocrude yields up to 50% and energy contents up to 38 MJ kg^{-1}, with net positive energy yield.

1.1. Scope of this study

The proposed algal WWT system is uniquely different from previous studies in that, our medium is acidic (pH = 2.5 to 4) and

moderately hot (temperature = 35–45 °C), employing a heterotrophic/photoautotrophic microalga, *Galdieria sulphuraria* (hereafter *G. sulphuraria*), originating in geothermal springs adapted to pH of 1.0–4.0 and temperatures of 25–56 °C (Selvaratnam et al., 2014b). Developed specifically for warm-humid and warm-arid environments, the PBR design for this culture system is also uniquely different from those in previous studies; it is a closed design, fabricated out of plastic sheet, intended to trap solar light and heat, with CO_2-enriched headspace (2% vol/vol) (Fig. 2). Wet biomass harvested from the PBR is processed via HTL to recover its energy content as bio-crude and bio-char, while releasing the nutrients in concentrated dissolved form for partial recycling to the PBR to support higher biomass production. Excess nutrients from the HTL recycle stream is expected to be stockpiled for sale as fertilizer or used for cultivation of more algae with a different water supply for bio-energy production.

The motivation for the proposed cultivation system is to circumvent most of the limitations of current systems. The closed PBR design maximizes CO_2 utilization while reducing evaporation, odor emissions, and introduction of contaminates. The low pH contributes to efficient control of pathogens and invaders. The mixed trophic conditions that are not that light-dependent, enable higher biomass densities to be maintained. Recycling concentrated nutrients from the HTL process to the PBR enables higher biomass densities to be maintained in a self-sufficient manner. Photosynthetic capture of sunlight in the PBR amplifies the energy content of the wastewater, resulting in higher net energy yield than other pathways for recovering energy from UWWs.

Key components of the proposed system have been validated in our preliminary laboratory studies (Reddy, 2013; Selvaratnam et al., 2014a,b). Our previous reports on *G. sulphuraria* demonstrated its growth in sterilized primary-settled wastewater and its nutrient removal capabilities (Selvaratnam et al., 2014b). Ability of *G. sulphuraria* in removing BOD from primary-settled wastewater has also been demonstrated. This paper presents the growth of *G. sulphuraria* in primary-settled UWW, its nutrient removal capabilities, and, its regrowth rates in the AP of HTL *performed over a range of temperatures*. Even though the composition and energy content of the end products of HTL are known to be a function of process temperature, previous studies had not evaluated regrowth in AP generated at different temperatures; as such this study was motivated by the need to assess regrowth rates against the benefits of higher HTL temperatures. We evaluate the effect of HTL-temperature on the recovery of N and P and subsequent growth of *G. sulphuraria*. We also evaluate the net effect of doubling the N and P concentrations in primary settled wastewater on algal biomass productivity and N and P removal rates, and demonstrate that algal N and P removal rates do not change while biomass productivity increases by ~20%.

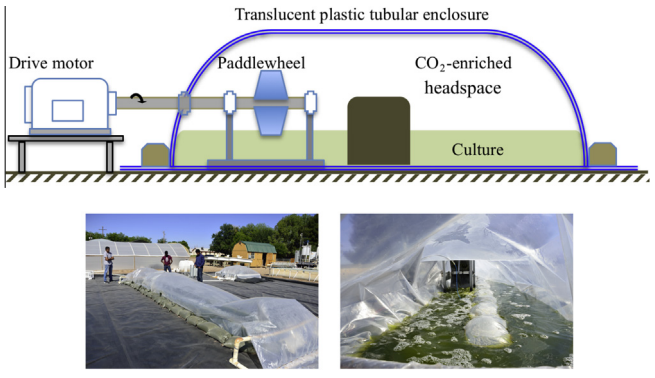

Fig. 2. Low-cost closed photobioreactor driven by paddlewheel for cultivating thermo-tolerant, acidophilic culture under CO_2-enriched headspace: schematic of sectional view and photographs of outdoor installation.

2. Methods

2.1. Test strain and feedstock

An independent isolate of the unicellular red algae *G. sulphuraria* CCMEE 5587.1 (Toplin et al., 2008) obtained from the Culture Collection of Microorganisms from Extreme Environments (University of Oregon) was assessed in this study. The strain was grown in an incubator (Percival, IA, USA) at 40 °C with a 14-h light/10-h dark cycle. Standard cyanidium medium (Toplin et al., 2008) modified to contain twice the standard ammonium sulfate concentration and supplemented with vitamin component of f/2 algal medium (Andersen, 2005) was used to maintain the algae feedstock. Cultures were streaked onto agar plates and single colonies were then picked to start axenic cultures from culture plates to modified cyanidium medium (MCM) scaling up the volume to 1-L Erlenmeyer flasks. Constituents of the new standard cyanidium medium are as follows: $(NH_4)_2SO_4$, 2.64 g L^{-1}; KH_2PO_4, 0.27 g L^{-1}; NaCl, 0.12 g L^{-1}; $MgSO_4{\cdot}7H_2O$, 0.25 g L^{-1}; $CaCl_2{\cdot}2H_2O$, 0.07 g L^{-1}; Nitch's Trace Element Solution, 0.5 mL; $FeCl_3$ (solution = 0.29 g L^{-1}), 1.0 mL; pH adjusted to 2.5 with 10 N H_2SO_4 .

Wastewater used in this study was collected downstream of the primary settling tank at the municipal wastewater treatment plant, Las Cruces, NM. Upon collection of the sample, large solid particles were removed by gravity settling and stored at 4 °C. The clear supernatant 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 set medium of the particular test and left for 24 h at 40 °C, 14-h light/10-h dark photoperiod for preadaptation.

2.2. Experimental conditions

2.2.1. Growth experiment with primary-settled wastewater

One set of experiments (Test I) was designed to compare the growth and nutrient removal rates of *G. sulphuraria* in primary-settled UWW (sterilized and unsterilized) against that in the modified cyanidium medium (MCM) prepared in nanopure water. In this test, the three media compositions coded as follows were evaluated: (a) MCM prepared in nanopure water, serving as the control; (b) MCM without any N and P compounds, but prepared in sterilized primary-settled UWW; and (c) MCM without any N and P compounds, but prepared in unsterilized wastewater. In case (b), the sterilization was done with Nalgene Rapid-Flow filter units (0.45 μm) (Thermo Scientific, USA).

Since the primary-settled wastewater typically contains 40 ppm of NH_3-N and 10 ppm of phosphate, the media in all the three tests were adjusted to the same initial amount of N and P concentrations. The composition of MCM included: $(NH_4)_2SO_4$, 0.188 g L^{-1} (40 ppm NH_3-N); KH_2PO_4, 0.0143 g L^{-1} (10 ppm phosphate); NaCl, 0.12 g L^{-1}; $MgSO_4{\cdot}7H_2O$, 0.25 g L^{-1}; $CaCl_2{\cdot}2H_2O$, 0.07 g L^{-1}; Nitch's Trace Element Solution, 0.5 mL; $FeCl_3$ (solution = 0.29 g L^{-1}), 1.0 mL, and vitamin component of f/2 algal medium (vitamins B1, B12 and biotin). The pH of the medium was adjusted to 2.5 with 10 N H_2SO_4 .

2.2.2. Growth experiments with aqueous product of HTL

The composition of the AP of HTL of biomass is known to depend on the HTL-temperature. To assess the stimulatory or inhibitory effects, if any, of those constituents, one set of experiments (Test II) was designed to evaluate the growth of *G. sulphuraria* in the AP resulting from HTL conducted at six temperatures in the range of

180–300 °C against growth in the control medium with MCM. In this initial evaluation, the AP was diluted with MCM to lower its NH_3-N level to 40 mg L^{-1} to make direct comparison with MCM. The volumetric percent AP in regrowth experiments derived at the different HTL-temperatures were as follows: 180 °C, 3.0%; 200 °C, 4.7%; 225 °C, 3.4%; 250 °C, 2.2%; 275 °C, 1.7%; and 300 °C, 1.5%. Details of the HTL procedure can be found elsewhere (Reddy, 2013).

2.2.3. Growth experiments with supplemental nutrients

A third set of experiments (Test III) was conducted to validate the premise that the productivity of the test species could be improved at higher nutrient levels made possible with the recycling of the AP of HTL of the biomass. In this test, experiments were conducted at three different initial biomass densities (of 0.1, 0.2, and 0.4 g L^{-1}), each at two different initial N and P levels: one at typical UWW levels of 40 mg ammoniacal N L^{-1} and 10 mg phosphate L^{-1}; and the other at twice those levels (80 mg ammoniacal N L^{-1} and 20 mg phosphate L^{-1}) to simulate recycling of AP of HTL; the growth medium in this test was prepared in nanopure water with all other constituents as in the standard MCM medium.

The above growth studies were conducted in 16 mL borosilicate glass tubes, as detailed in Selvaratnam et al. (2014b). Each tube was inoculated with 6 mL of culture and placed in the outer rim of a roller drum (New Brunswick Scientific, Eppendorf., Connecticut, USA) rotating at 16 rpm. The roller drum was housed inside an incubator (Percival, IA, USA) where the CO_2 level was maintained at 2–3% (vol/vol) throughout the experiments.

2.3. Growth measurements and nutrient analyses

2.3.1. Optical density measurements

Biomass growth 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. Ash free dry weight (AFDW, g L^{-1}) for the corresponding OD750 values were calculated using the following correlation derived for *G. sulphuraria* (Selvaratnam et al., 2014b):

$$\text{AFDW} = 0.54 \times OD750 + 0.023$$

$n = 12$; $r^2 = 0.997$.

2.3.2. N and P measurements

During the growth experiments, 3 glass tubes were removed on days 1, 3, 5 and 7 to serve as triplicates for measuring the nutrient levels. Culture samples from each tube were first centrifuged at 4000 rpm for 10 min and the supernatant was diluted prior to analyses. Dissolved concentrations of ammonia nitrogen and phosphorus (phosphate) were determined using HACH DR 6000 (HACH, Colorado, USA) spectrophotometer (Salicylate TNT Method 10031 and Phosver 3 Method 8048).

3. Results and discussion

3.1. Growth of G. sulphuraria in primary-settled UWW

Results of Test I summarized in Fig. 3 show that primary-settled UWW is a suitable growth medium for *G. sulphuraria*. Exponential growth is observed from 1–7 days in the test. Based on the growth rates estimated from the growth profiles (day 1–7), both sterilized UWW (code b) and unsterilized UWW (code c) growth media showed slightly higher growth rates (0.186 ± 0.012, 0.175 ± 0.012 g L^{-1} d^{-1}) compared to that in the control medium, code a (0.134 ± 0.010). The lag phase was also shorter in cultures grown on sterilized or un-sterilized UWW. These observations in UWW-containing media indicate the presence of heterotrophic

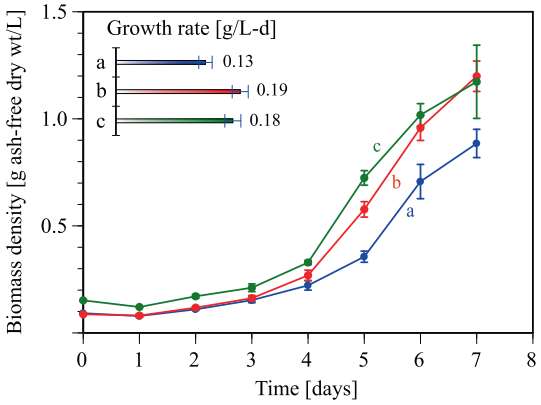

Fig. 3. Temporal growth profiles of *G. sulphuraria* in Test I in cyanidium medium prepared as follows: (a) in nanopure water; (b) in sterilized primary-settled wastewater; and (c) in unsterilized primary-settled wastewater. In all cases, initial N was adjusted to 40 mg L^{-1} and initial phosphate to 10 mg L^{-1}. Error bars indicate SD from triplicates. Inset shows growth rates estimated from the growth curves with std. dev.

metabolism by *G. sulphuraria* (code b) and surviving heterotrophic UWW microorganisms (code c). Furthermore, enhanced growth in the filter-sterilized wastewater medium relative to control medium without organic carbon is consistent with genomic (Schonknecht et al., 2013) and biochemical evidence (Gross and Schnarrenberger, 1995; Oesterhelt and Gross, 2002) for robust heterotrophic metabolism in *G. sulphuraria*. The pH levels used in this experiment dramatically reduce the viability of UWW bacteria, a topic under active study in our laboratories. Volumetric growth rates in these small-scale laboratory tests are in the same range as outdoor growth rates (0.165 g L^{-1} d^{-1}) recorded in enclosed, horizontal photobioreactors (Fig. 2) at 10 cm depth conducted at Las Cruces, NM (Selvaratnam et al., 2014b).

3.2. Nutrient removal by G. sulphuraria

Temporal NH_3-N profiles recorded in Test I over 7 days are shown in Fig. 4a. Removal efficiencies estimated from these profiles are as follows: 99.7% for code a; 95.2% for code b; and 99.4% in code c. The removal rates of NH_3-N were 4.71 mg L^{-1} d^{-1} for code b and 4.97 mg L^{-1} d^{-1} for code c; the respective biomass yields were 33.92 and 30.23 g biomass per g nitrogen removed. These values are twice the theoretical yield of 15.83 g g^{-1} estimated from the "Redfield Ratio" often attributed to algal biomass, $C_{106}H_{263}O_{110}N_{16}P$ (Redfield et al., 1963) and consistent with emerging evidence that C:N:P ratios are highly variable in algal biomass in response to genetic and environmental constraints (Geider and Roche, 2002).

Temporal phosphate profiles recorded in Test I over 7 days are shown in Fig. 4.b. Removal efficiencies estimated from these profiles are as follows:>99% for code a; 96.2% for code b; and 97.8% for code c. The removal rates of phosphate were 1.68 mg L^{-1} d^{-1} for code b and 1.47 mg L^{-1} d^{-1} for code c. Results of Test I show that *G. sulphuraria* can be used to remove both NH_3-N and Phosphate from the primary effluent to discharge standards mandated by the regulatory agencies.

Comparison of Figs. 3 and 4 also demonstrates that approximately 50% of the initial N and P had been removed from the medium by the end of the lag phase on day 4. Nearly complete nutrient removal preceded the time to maximum cell density cell density (data not shown).

3.3. Growth of G. sulphuraria in AP of HTL

Growth profiles of *G. sulphuraria* as a function of HTL-temperature recorded in Test II are presented in Fig. 5, along with the growth

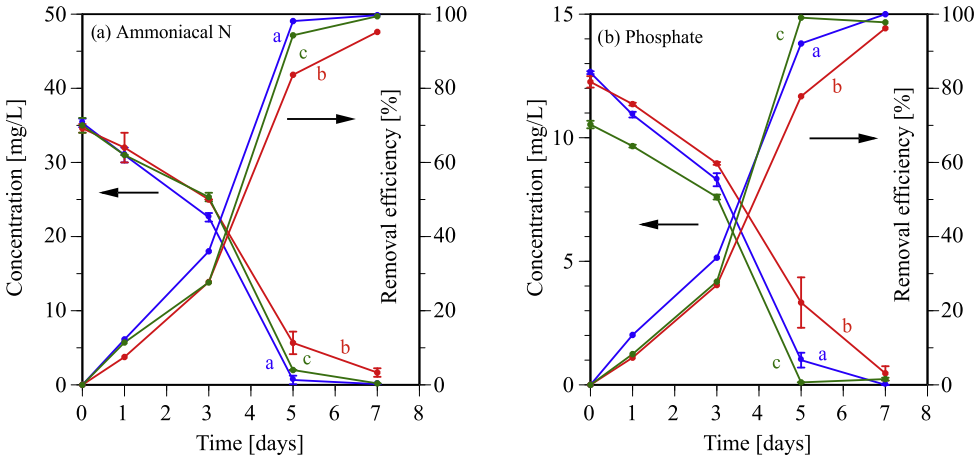

Fig. 4. (a) Ammoniacal nitrogen and (b) phosphate reduction by G. *sulphuraria* in the three media in Test I. See Fig. 3 for media codes. Error bars indicate SD from triplicates.

rates during the exponential phase (days 2–10). The media with AP from HTL at 200 °C showed minimal growth (0.015 ± 0.001 g L^{-1} d^{-1}) over 10 days. A gradual increase in growth rate was noted as the HTL-temperature increased from 225 °C to 300 °C. Growth rate in the control media (0.114 ± 0.014 g L^{-1} d^{-1}) was matched by growth rates in the HTL-temperature range of 250–300 °C. Growth in AP from HTL-temperatures higher than 225 °C and AP from 180 °C were comparable to that in the control medium with MCM.

Additional analyses of the AP need to be carried out to resolve the inhibitory effect noted at the HTL-temperature of 200 °C. The N concentration in the re-growth experiments was normalized to the measured ammoniacal N in the AP extracts at each HTL temperature. As such, the volume of AP in the cultures from 200 and 225 °C HTL reactions was higher, 4.7% and 3.4% respectively, than all the others which were at 3% AP or less (see Section 2.2.2). Inhibition of growth of a consortium of algae by HTL-AP has been reported previously at values above 0.5% (Zhou et al., 2013). Our results suggest that the inhibitor primarily increases the lag time rather than the slope of the post-lag growth curve (Fig. 5). Furthermore, *G. sulphuraria* cultures appear to be less sensitive to HTL-derived inhibition than the mixed algal cultures reported by Zhou et al. (2013). In general, AP samples from higher HTL process temperatures demonstrated nutrient bioavailability and proof of principle acceptability for reuse in WWT photobioreactors to boost biomass productivity.

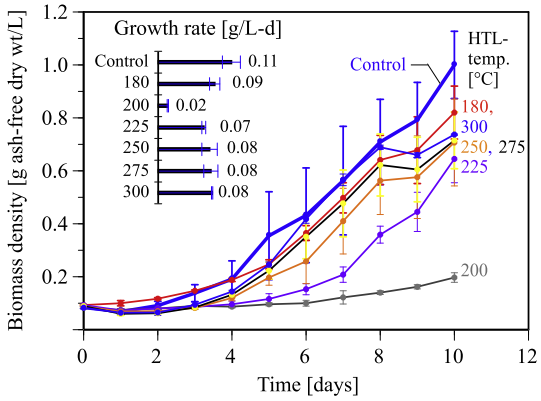

Fig. 5. Growth profiles of G. *sulphuraria* in Test II in control medium vs. aqueous product of HTL performed at various temperatures, all adjusted for initial N of 40 mg L^{-1} and initial phosphate of 10 mg L^{-1}. Error bars indicate SD from triplicates. Inset shows growth rates estimated from the growth curves with std. dev.

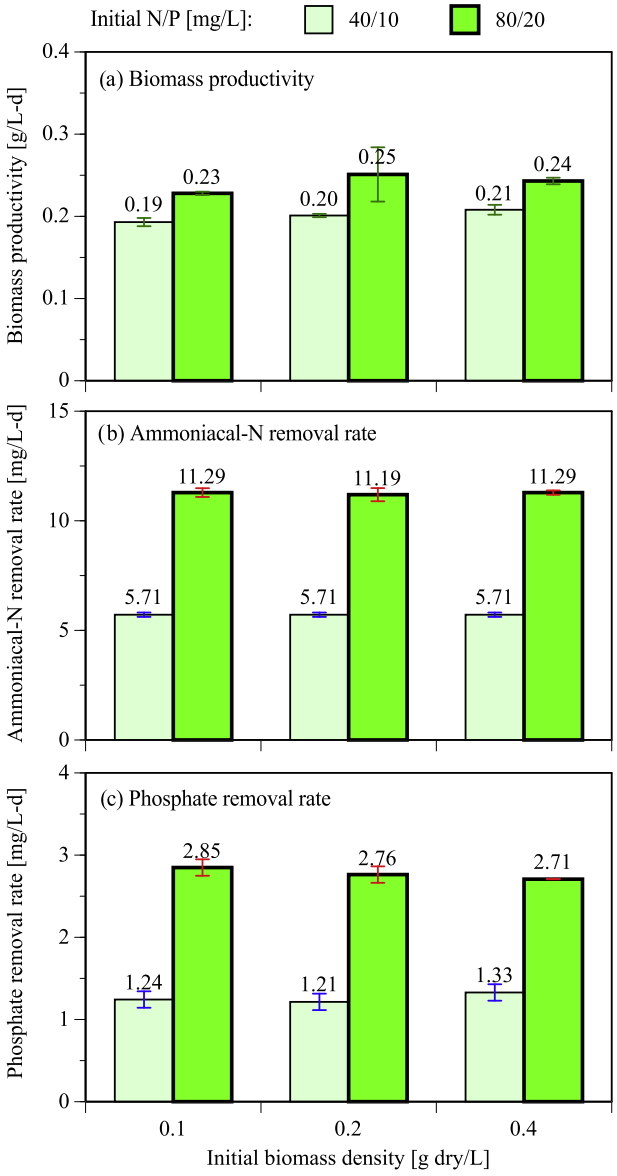

Fig. 6. Biomass productivity (a); nitrogen removal rate (b); and phosphate removal rate (c), as a function of initial biomass density and initial nutrient N/P ratio.

Table 1
Comparison of biological nitrogen removal technologies vs. algal approach.

Factors	Nitrification/denitrification[a]	CANON[b]	Aerobic deammonification[c]	DEAMOX[d]	Algal approach[e]
Discharge-liquid Discharge-gas	NO_3^-, NO_2^-, N_2O, N_2, CO_2	NO_3^-, N_2	NO_3^-, N_2, CO_2	NO_3^-, N_2	O_2
Oxygen demand (g O_2 g^{-1} $NH4^+$-N)	4.18	3.66	3.07	–	–
Biomass produced (g cells g^{-1} NH_4^+-N)	0.61	0.11	0.25	0.76	15.8
Energy consumed (kWh kg^{-1} NH_4^+-N)	3.43	3.00	2.52	–	–
Energy produced (kWh kg^{-1} NH_4^+-N)	0.9	0.17	0.38	1.13	15
Net energy (kWh kg^{-1} NH_4^+-N)	−2.53	−2.83	−2.14	1.13	15

[a,b] Ahn (2006).
[c] Musabyimana (2008).
[d] Kalyuzhnyi and Gladchenko (2009).
[e] Ebeling et al. (2006).

3.4. Increased productivity at higher nutrient levels

Results of Test III evaluating the effects of doubling the N and P levles on biomass productivity and nutrient removal rates are summarized in Fig. 6. Productivity increased at the higher nutrient level regardless of the initial inoculation cell density. Importantly, N and P removal rates at twice normal N and P levels (80 and 20 mg L^{-1} respectively) also doubled such that no increase in residence time is required to achieve the increase in biomass productivity (Fig. 6). These results add credence to the premise that recycling of AP of HTL can boost biomass productivity by 20–25% without reliance on external nutrient supplementation. Maintaining higher biomass densities and higher productivities can translate to higher net bioenergy yields relative to conventional WWT.

3.5. Proposed vs. current approaches for nutrient removal

To demonstrate the energy-advantage of the proposed approach in wastewater treatment, currently available biological nutrient removal (BNR) processes are evaluated against the proposed approach in terms of the net energy associated with the process per unit mass of nitrogen removed, as an example. For this evaluation, the following BNR processes were chosen: nitrification/denitrification, CANON (Completely autotrophic nitrogen removal over nitrite), aerobic deammonification, and DEAMOX (Denitrifying Ammonium Oxidation) (Ahn, 2006; Mosquera-Corral et al., 2005; Mulder, 2003). The energy input to each process and the energy that can be harvested from the biomass generated by each process are estimated from their respective stoichiometric equations. The energy input is assumed to be that equivalent to provide the stoichiometric oxygen demands of the processes; the energy output is assumed to be that equivalent to the methane potentials of the biomass generated by the processes; hence, the net energy associated with each process. Details of these estimations are included in the SI.

The nitrification–denitrification, CANON, and the aerobic deammonification processes require oxygen, while the DEAMOX and the algal processes do not. The energy equivalent of the oxygen supply is estimated as 1.22 kWhr kg^{-1} O_2 (Yerachmiel et al., 1991). Biomass productions and methane potentials of the biomass generated are estimated assuming typical empirical formulae (details in SI). Key process metrics estimated from the stoichiometry for the four current BNR process and the algal system are summarized in Table 1 to illustrate the energy-advantage of the proposed approach. Typically, the BNR processes follow the activated sludge process where additional energy is input to meet the oxygen demand for BOD removal. In contrast, BOD removal in the algal system is accomplished without any external oxygen input. Clearly, the advantage of the algal system will be still higher if BOD removal is included in the comparison.

4. Conclusions

This study confirmed that *G. sulphuraria* can be cultivated in UWW achieving comparable growth rates and higher nutrient removals than with the artificial growth medium. Tests with the AP of HTL conducted over 180–300 °C confirmed that *G. sulphuraria* could be grown at rates comparable to that in the control medium. Biomass productivity recorded with initial level of 80 mg NH_3-N L^{-1} and 20 mg phosphate L^{-1} was higher than that with typical primary settled wastewater, validating our premise that recycling the nutrient-rich aqueous product of hydrothermal liquefaction of algal biomass has the potential to boost biomass productivity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.01.134.

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