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Short communication

Optimizing energy yields from nutrient recycling using sequential hydrothermal liquefaction with *Galdieria sulphuraria*

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ABSTRACT

Hydrothermal liquefaction (HTL) provides a promising option for extracting bio-crude oil from wet algal biomass. One of the byproducts of HTL is an aqueous phase (AP) rich in organic carbon and nutrients. This study evaluated the hypothesis that recycling the AP to the cultivation step could enhance biomass productivity and net energy yield. Since the yields of bio-crude and nutrients post-HTL are functions of HTL reaction temperature, this study evaluated the impact of reaction temperature on net energy yield. Nutrient recycle experiments were conducted with a low-lipid, acidophilic strain, *Galdieria sulphuraria,* being developed for single-step removal of organic carbon, nitrogen and phosphorus from urban wastewater. *G. sulphuraria* was cultivated in different dilutions of the AP generated by HTL performed between 180 and 300 °C. Biomass productivity recorded in this study with recycled AP was greater than that in the control by a factor as much as 1.94. Estimates of net energy yields indicate the optimum temperature for the second-stage HTL bio-crude oil extraction from *G. sulphuraria* to be 280 to 300 °C.

1. Introduction

Microalgal feedstocks are being widely investigated as a third generation source for renewable chemicals and biofuel production. Currently, a potential bottleneck in this approach is the excessive energy requirement of drying harvested biomass prior to downstream processing. The U.S. Department of Energy has outlined two wet extraction options to overcome this bottleneck. The first one is an Algal Lipid Upgrading (ALU) option employing a wet acid pre-treatment to liberate carbohydrate for ethanol fermentation followed by lipid extraction of the remaining algal biomass and upgrading to bio-diesel [1]. The second option, known as AHTL, is based on hydrothermal liquefaction (HTL) of wet algal biomass [2].

HTL has been proposed in recent studies as an energy-efficient alternative to the current practice as it can eliminate the biomass drying step [3]. Further, HTL enables lipid-, protein-, and carbohydrate-contents of the biomass to be converted to energy-dense bio-crude oil through hydrolysis, re-polymerization, dehydration, decarboxylation, and deamination [3,4]. In the full AHTL option, wet biomass is converted to bio-crude oil via HTL while, the residual carbon in the aqueous fraction of HTL is converted to biogas via catalytic gasification. A portion of the resulting biogas is used to produce hydrogen gas for upgrading the bio-crude oil to renewable diesel fuel; and the nutrients solubilized from the aqueous and solid fraction are recycled to cultivation [2].

A two-step approach has been proposed recently where fermentable carbohydrate and organic nitrogen are extracted in the first HTL step under low-temperature, followed by the second higher-temperature HTL step to extract bio-crude oil. Key advantages claimed for this sequential HTL (SEQHTL) process are lower fraction of bio-char formation and a lower nitrogen content in the resulting HTL bio-oil fraction [5,6]. A more recent study by Jazrawi et al. [7] on high-protein algal biomass confirmed that nitrogen levels in HTL bio-oil are lower in a two-stage HTL operation but at the expense of lower yields of bio-oil. As a result, the techno-economic trade-offs between one- and two-stage HTL are thus likely to be complex. The low-temperature step of SEQHTL pathway is similar to the acid-pretreatment/carbohydrate recovery step in the ALU pathway; both afford the opportunity for using the resulting nutrient-rich aqueous fraction as a substrate for ethanol production or for increasing the algal biomass yield through recycling carbohydrate to heterotrophic or mixotrophic algal cultivation. Thus, the present study was motivated by the need for better data on the chemical characteristics and biological compatibility of HTL water extractives as a function of HTL-temperature coupled with an energetic analysis of the recycle pathway: using the extracted carbon and nitrogen to boost algal biomass productivity.

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Previous algal HTL studies had focused on high-temperature HTL processing of biomass as a means of maximizing bio-crude yield. Yields of up to 50% [8,9] and energy recoveries up to 71% [10] have been reported. While the primary focus of early HTL studies had been on maximizing bio-crude yield, recent studies have begun to assess beneficial use of the byproducts of HTL, particularly, the aqueous phase. Biller et al. [11] demonstrated that *Chlorella* sp., *Spirulina*, and *Chlorogloeopsis* sp. could be cultivated in AP from HTL process diluted at 2%, 1%, and 0.5% respectively, at growth rates comparable to that with the standard media. Du et al. [12] have evaluated the growth of *Chlorella vulgaris* on AP of HTL conducted at 200 °C at dilutions of 2%, 1%, and 0.5%. Growth rates with AP diluted with distilled water showed higher regrowth than the standard growth medium; final biomass concentrations at the different dilutions were higher in the following order: 2% > 1% >0.5%. Total nitrogen removal in their study ranged 45.5–59.9% and phosphorous removal ranged 85.8–94.6%. Alba et al. [13] and Biller et al. [11] evaluated growth of *Desmodesmus sp.*, on AP of HTL and showed growth rates comparable to that with the control medium. However, they reported lower growth rates with AP of HTL process diluted with deionized water, which was attributed to lack of micronutrients. In a recent study, Zhou et al. [14] have showed bio-crude yields up to 50% with net positive energy yield with mixed algae-bacterial cultures growing in primary-settled wastewater mixed with AP of HTL of algal biomass and primary sludge conducted at 300 °C.

In this study, it is hypothesized that higher biomass productivity and, hence, higher net energy could be attained with the nutrient-rich AP recycled from HTL than with the standard growth medium. A theoretical analysis is presented first to justify this hypothesis and to simulate the effect of HTL temperature on net energy yield. The hypothesis is then validated with experimental growth data on an extremophile, *Galdieria sulphuraria* (hereafter *G. sulphuraria*), which is the most versatile known alga with respect to organic carbon utilization potential [15]. Furthermore, this strain grows at low pH where fewer heterotrophs survive to compete for carbohydrate and organic nitrogen sources. We have previously documented the utility of this strain for direct, one-step, energy-efficient municipal wastewater treatment defraying the cost of nutrients for algal feedstock production [16–19]. We and others have noted the synergy between algal WWT and HTL energy extraction [14]. Here we address the energetics of recycling low-temperature aqueous HTL extracts to boost biomass productivity.

2. Theoretical background

The premise of this study is that the net energy yield from HTL could be maximized by balancing the energy input to the process to raise the HTL temperature against the benefit of higher energy production achieved through higher bio-crude yield. The heat energy input to the HTL process as a function of the HTL temperature, E_{in} (T) [kJ d^{-1}], can be estimated from the following, assuming partial heat recovery from its effluent to preheat the influent:

$$E_{in}(T) = (1-\theta)\{c_{p,BM}BM + c_{p,w}Q\rho\}(T-T_f) \quad (1)$$

where, θ is the efficiency of heat recovery, $c_{p,BM}$ and $c_{p,w}$ are the specific heats [kJ kg^{-1} K^{-1}] of biomass and water respectively; BM is the biomass feed rate to HTL productivity [kg d^{-1}]; Q is the dewatered biomass flow rate to HTL [m^3 d^{-1}]; ρ is the density of broth [kg m^{-3}]; and T and T_f are the HTL temperature and feed temperature, respectively.

The energy equivalent of the bio-crude, E_{BCr} (T) [kJ d^{-1}], that can be extracted as a function of HTL temperature can be estimated from the following:

$$E_{BCr}(T) = \Delta G_{BCr}\{Y_{BCr}(T)BM\} \quad (2)$$

where, ΔG_{BCr} is the calorific value of bio-crude [kJ kg^{-1}]; and Y_{BCr} (T) is the gravimetric yield of bio-crude per unit biomass [%], hypothesized to be a function of the HTL temperature, T, for any given algal species.

Hence, the net energy yield from HTL process, E_{net} (T), can be estimated from Eqs. (1) and (2) as

$$\begin{aligned} E_{net}(T) &= [\Delta G_{BCr}\{Y_{BCr}(T)BM\}] - [(1-\theta)\{c_{p,BM}BM + c_{p,w}Q\rho\}(T-T_f)] \\ &= [\Delta G_{BCr}Y_{BCr}(T) - (1-\theta)c_{p,BM}(T-T_f)]BM - (1-\theta)c_{p,w}Q\rho(T-T_f). \end{aligned} \quad (3)$$

The above results support the premise that, at any given HTL reaction temperature, T, the net energy can be increased by increasing the biomass flow rate, BM. To discern the impact of the HTL reaction temperature on net energy yield, the variation of bio-crude yield Y_{BCr} as a function of the HTL reaction temperature needs to be established experimentally.

3. Materials and methods

3.1. Algae stock cultures and inoculum production

The unicellular red algae *G. sulphuraria* CCMEE 5587.1 [20] used in this study was obtained from the Culture Collection of Microorganisms from Extreme Environments (University of Oregon) now at Environmental Molecular Sciences Division at Pacific Northwest National Laboratory. The algal stock solution was grown in Standard Cyanidium medium [20], which was modified to contain twice the standard ammonium sulfate concentration [18] (Table 1). The inoculum for test algal cultures used in this study was derived from single colonies scaled up autotrophically in the modified Cyanidium medium recipe. The incubator (Percival, IA, USA) was set at 40 °C with a 14-h light/10-h dark cycle. The CO_2 level inside the incubator was maintained at 2–3% (vol/vol). The inocula for the dark growth experiments in this study were all pre-adapted in the different media to avoid adaptation-related effects. The *G. sulphuraria* biomass used to produce the HTL aqueous phases at different temperatures was grown outdoors in a 4000-L photobioreactor as described previously [18].

3.2. Hydrothermal liquefaction and aqueous phase recovery

50 g slurry of *G. sulphuraria* at a solids content of 10% was prepared by adding sufficient deionized water to the harvested biomass. The feed biomass was then transferred into a 100 mL Parr bench top reactor, and the reactor was purged with nitrogen to remove any air. The reactor was then pressurized with nitrogen gas to control rapid boiling of water and the reactor vessel was heated to the test temperature (180 °C, 200 °C, 225 °C, 250 °C, 275 °C, 300 °C) by a heating jacket. Upon reaching the desired test temperature, the reaction was continued for 30 min. Thereafter, the reactor was allowed to cool down to room temperature. A 30 mL aliquot of dichloromethane (DCM) was added to the product

Table 1
Media preparation for growth on diluted AP of HTL.

Dilution	Composition[a]		Final NH_3–N conc. in medium [mg L^{-1}]
	AP	CM[b]	
1%	1%	99%	40
2%	2%	98%	80
4%	4%	96%	160
8%	8%	92%	320

[a] Volumetric composition.
[b] Modified Cyanidium medium prepared without any $(NH_4)_2SO_4$. The complete recipe for modified CM medium is as follows: $(NH_4)2SO_4$, 0.188 g L^{-1}; KH_2PO_4, 0.0143 g L^{-1}; NaCl, 0.12 g L^{-1}; $MgSO_4.7H_2O$, 0.25 g L^{-1}; $CaCl_2.2H_2O$, 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 the pH adjusted to 2.5 with 10 N H_2SO_4. Includes vitamin component of f/2 algal medium (vitamins B1, B12 and biotin).

mixture and stirred in the reactor for 2 min to collect any residual bio-crude from the reactor walls. Subsequently the product mixture with DCM was transferred onto a filter paper placed on top of a separation funnel. The bio-char separated on top of the filter paper was washed with 5 mL of DCM again before collecting and drying it. The DCM phase with bio-crude oil was separated at the bottom and DCM was evaporated in a rotary evaporator. The weight of the bio-crude and bio-char were noted after the drying step. The remaining aqueous phase was collected into sample vials and stored at −5 °C along with bio-crude samples.

3.3. Chemical analysis of AP generated by HTL

Ammoniacal nitrogen (NH_3–N), total nitrogen (TN), and phosphate in HTL AP samples were measured using HACH DR6000 spectrophotometer (HACH, Colorado, USA) (Salicylate TNT Method 10031, TNTplus 828 Method 10208 and Phosver 3 Method 8048). Carbohydrate levels in the AP samples were measured using phenol sulphuric acid assay method [21].

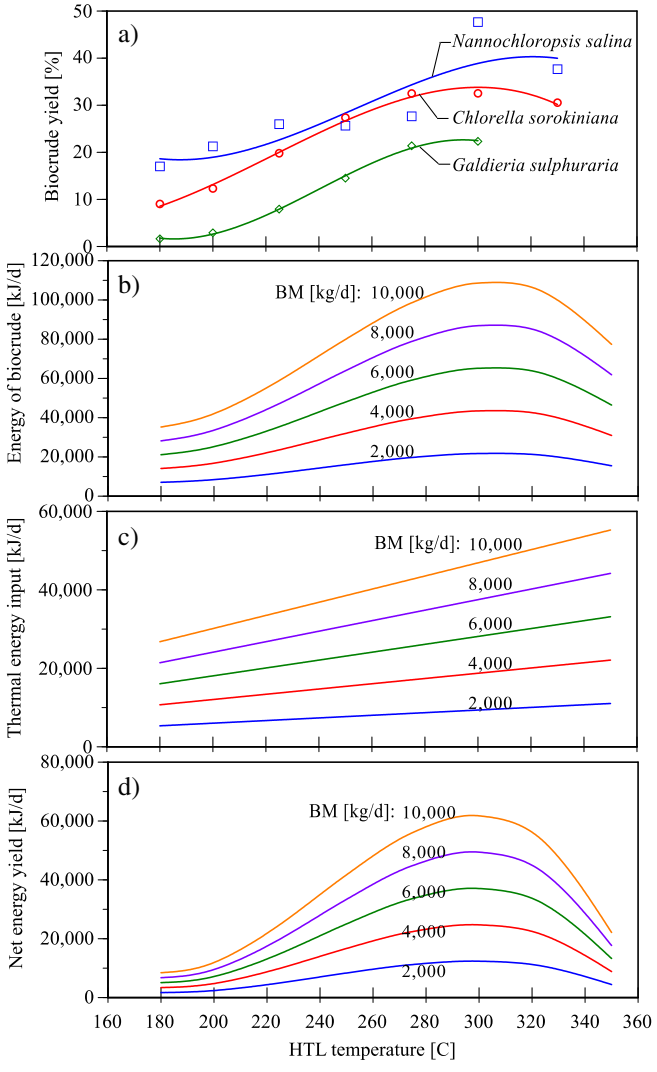

Fig. 1. Measured bio-crude yield as a function of HTL-temperature (a); simulated energy equivalent of bio-crude as a function of biomass productivity and HTL-temperature (b); simulated thermal energy input as a function of HTL-temperature (c); and, simulated net energy yield as a function of biomass productivity and HTL-temperature (d). Symbols represent measured data; lines represent fitted or simulated curves.

3.4. Growth experiment conditions

Heterotrophic (dark) growth of *G. sulphuraria* was evaluated in four different dilutions of AP (1%–8%) from HTL treatment of *G. sulphuraria* biomass at six different temperatures (180 to 300 °C) as summarized in Table 1. (For example, at 1% dilution, the medium was prepared by mixing 1% of AP from HTL at the test temperature with 99% Modified Cyanidium medium without any $(NH_4)_2SO_4$) see Table 1. The control treatment group (Modified Cyanidium medium) did not have a reduced carbon source, providing a direct test of carbon source utilization from the AP derived at each temperature.

Growth experiments were conducted using microplate assays (culture volume of 250 μL), where the plates were housed in an incubator maintained at 40 °C with 2–3% CO_2 level in the headspace, without any illumination. Perimeter wells of the microplates were filled with deionized water to avoid edge effects [22]. Algal growth was followed daily by measuring the optical density at 750 nm (OD750) with a SpectraMax M2 microplate reader (Molecular Devices, CA, USA). Ash free dry weight (AFDW, g L^{-1}) for the corresponding OD750 values were calculated using the following correlation derived for *G. sulphuraria* in our previous study [18]:

$$AFDW = 0.54 \times 0D_{750} + 0.023 \quad (4)$$

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

4. Results & discussion

Bio-crude yields of three different algal species as a function of HTL temperature (measured in our laboratory over HTL temperature range of 180 to 330 °C, [23]) are shown in Fig. 1-a. For the purpose of simulation, a generic polynomial relationship between bio-crude yield and

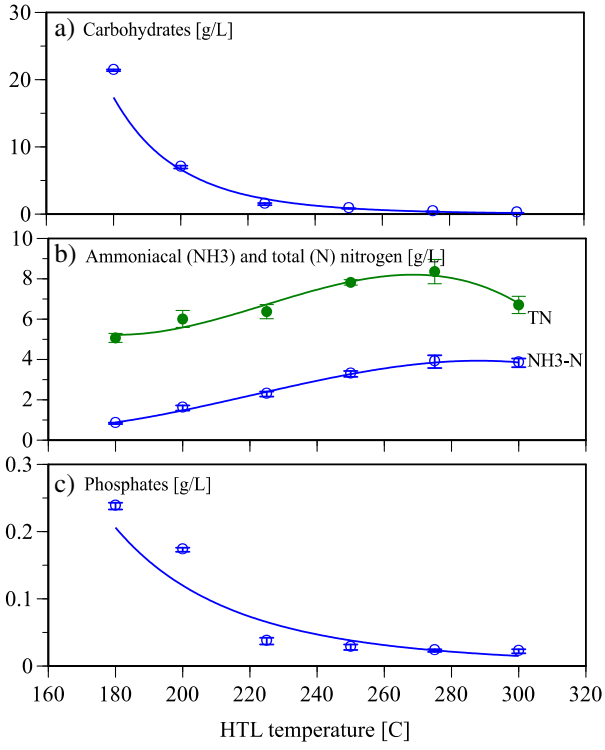

Fig. 2. Composition of aqueous product of HTL as a function of HTL reaction temperature: carbohydrates (a); ammoniacal and total nitrogen (b); and phosphates (c). Symbols represent measured data; error bars indicate std. dev. of triplicate measurements; lines represent fitted curves.

HTL temperature was formulated as follows by combining the above results over the tested temperature range ($r^2 = 0.94$):

$$Y_{BCr} = -2.22 \times 10^{-5} T_{THL}^3 + 1.60 \times 10^{-2} T_{THL}^2 - 3.56\ T_{THL} + 263.61. \quad (5)$$

Similar 3rd order polynomial relationship can be derived from data from the literature (for example [10] and [24]). From Fig. 1-a, it can be deduced that, as the yield increases with T_{THL}, more of the carbon in the biomass will be channeled towards bio-crude while the resulting AP would become leaner in carbon; and, at lower T_{THL}, AP would be richer in carbon. The decline in crude oil yield at higher temperature may be attributed, in part, to loss of carbon via the gas phase; as reported by Christensen et al. [24] gas yield increased with T_{THL}, and its methane content increased from 3 to 7% as T_{THL} increased from 400 to 420 °C.

Even though the bio-crude yield increases with HTL temperature to a certain point, performing HTL at such higher temperatures demand higher thermal energy input to the process and may result in reduced net energy yield. Simulations based on Eqs. (1) to (3) with the empirical relationship given by Eq. (5) are presented in Fig. 1-b to 1-d to illustrate the opposing impacts of HTL temperature and the occurrence of a local optimal HTL temperature as a function of biomass production, BM; this optimal temperature will be a function of the algal species.

The simulation results also show that, increasing biomass production is an option to maximize the net energy yield. As the AP of HTL is known to contain high concentrations of solubilized organics [5] and nutrients [11,13], it has been proposed that diluted AP could serve as a valuable source of carbon and nutrients for boosting biomass production under heterotrophic conditions. Concentrations of carbohydrates and nutrients in the AP samples generated at the six HTL temperatures tested in this study are summarized in Fig. 2.

Total nitrogen levels in the AP generally increased with HTL temperature (from 5066 mg L^{-1} at 180 °C to 8300 mg L^{-1} at 300 °C, Fig. 2-b) as more biomass is solubilized and converted to bio-crude. Based on the N-content of *G. sulphuraria* estimated in this study (=4–8%), the above recovery of nitrogen translates to 50–75% over this temperature range. Jena et al. [25] reported that total Kjeldahl-N levels of a fresh water alga *Chlorella minutissima* were greater than 16,000 mg L^{-1} with 78.4% of the N accounted for in the form of ammonia and nitrates. Yu et al. [26] reported a positive relationship of nitrogen recovery with process temperatures and theoretical yields of 75% recovery of N in the aqueous fraction from a low lipid algae *Chlorella pyrenoidosa*. The temperature-dependent decrease in AP phosphate levels shown in Fig. 2 c is interpreted as hydrolysis of organic phosphate and subsequent precipitation with calcium, iron and other metal ions.

4.1. Growth in recycled AP generated by HTL

Since the composition of AP generated by HTL, the energy input to the HTL process, and the bio-crude yield are all functions of the

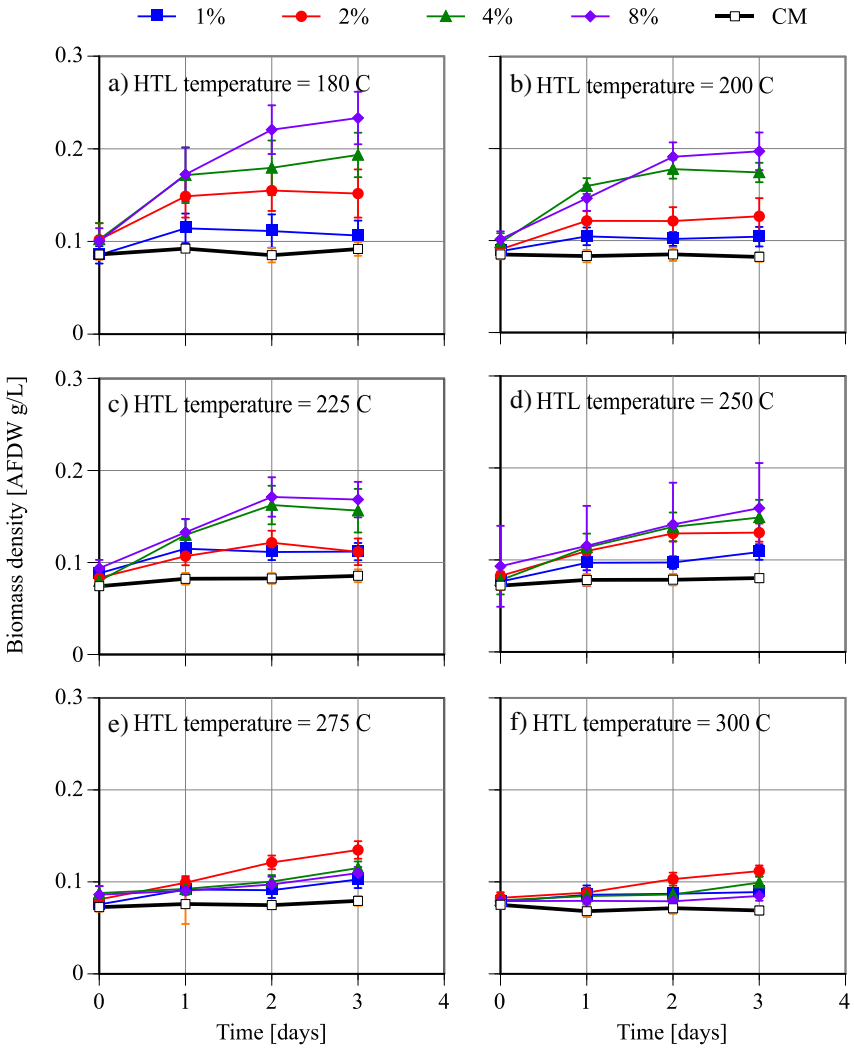

Fig. 3. Growth profiles of *Galdieria sulphuraria* in aqueous product of HTL as a function of dilution and HTL-temperature. Soild symbols represent measured biomass densities at indicated dilutions; hollow symbol represents biomass density with control medium; error bars represent std. dev. from biological triplicates.

HTL-temperature, this study attempted to identify the optimal HTL-temperature and the appropriate dilution of the AP for recycling to the cultivation step so that eventually the net energy yield of the integrated process could be maximized. Because the ammoniacal nitrogen levels varied from 833–3833 mg L^{-1} across the six AP samples, it was decided to normalize the all nitrogen levels to 4000 mg L^{-1} (by adding $(NH_4)_2SO_4$ to the samples) prior to initiating the growth studies. This eliminated nitrogen availability as a variable in the growth experiments intended to evaluate biomass growth on carbohydrates in the recycled aqueous products of HTL.

Growth curves of *G. sulphuraria* cultivated in recycled aqueous products of HTL as a function of HTL temperature and dilution are summarized in Fig. 3 a)–f) relative to that in the standard growth medium without any carbon source. Two generalizations can be made from these growth curves. First, at any given dilution, biomass growth *decreased* with *increase* of HTL temperature. At the dilution of 1%, for example, the 3-day biomass density decreased 16.5% as the HTL temperature increased from 180 to 300 °C; while at the dilution of 4% the corresponding decrease was 48.7%. Second, at any given HTL temperature, *growth increased* with increasing AP concentration. For instance, at the HTL temperature of 180 °C, 3-day biomass density increased 81.8% as the AP concentration increased from 1% to 4%; while at 300 °C, the corresponding increase was 11.7%. Given the negligible amount of carbohydrate detected in the AP produced at 275 and 300 °C (Fig. 2 a), we interpret the growth observed with AP from these processing temperatures as likely due to catabolism of lipid molecules. Free fatty acids have been identified as major products in both the bio-crude and aqueous product fractions from algal HTL processing [27].

Biomass densities attained after 3-days of growth in the AP as a function of HTL temperature and dilution are illustrated in Fig. 4-a and summarized in Fig. 4-b confirming the hypothesis that higher biomass productivity could, in fact, be attained by recycling the AP of HTL than with the standard growth medium. These findings also confirm that *G. sulphuraria* can be successfully grown heterotrophically on recycled AP.

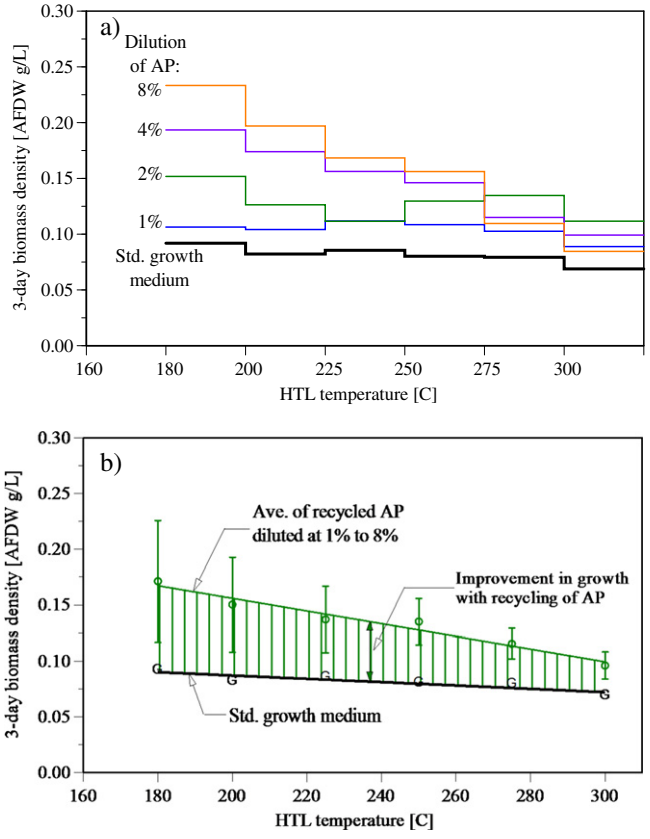

Fig. 4. 3-day biomass density of *Galdieria sulphuraria* cultivated in aqueous products of HTL as a function of HTL-temperature and dilution (a); average of 3-day biomass density with AP at different dilutions vs. with standard growth medium (b).

The decrease in biomass growth with increase of HTL temperature is attributed to decreasing levels of carbohydrates in the AP at higher HTL temperatures; as HTL temperature is increased more of the carbon in the biomass feed is converted to bio-crude, bio-char and off-gases while less of it remains in the AP that is recycled [5]. Carbohydrate level in the AP as a function of HTL temperature shown in Fig. 2-a confirms this supposition. A previous study of the carbon and nitrogen products from hydrothermal liquefaction of low-lipid microalgae at reaction temperatures greater than 220 °C resulted in 35–40% of the carbon being converted to compounds found in the aqueous product [26]. Broch et al. [28] also studied hydrothermal carbonization of *Spirulina* and reported that total organic carbon accounted for less than 50% of the dissolved mass.

Overall, these results validate the rationale for the two-stage sequential HTL process to provide fermentable carbohydrate in low-temperature AP to enhance biomass yield and improve net energy yield from algal biomass. This approach could be optimized to produce ethanol, for example; or to recycle carbon along with the nitrogen and phosphorus released at low HTL temperatures to maximize growth of *G. sulphuraria* [22]. Minimizing bio-char production and diverting nitrogen from incorporation into bio-crude compounds produced at higher temperatures are additional advantages of this approach.

5. Conclusions

Theoretical analysis and the experimental results presented here indicate that by integrating biomass cultivation with HTL of the biomass at the appropriate HTL temperature and recycling the AP of HTL can enhance biomass productivity and net energy yield. This approach can be engineered to maintain the optimal C:N:P ratio in the cultivation reactor [16]. Nutrients in excess of that is recycled could be harvested for beneficial use as fertilizers. Further optimization of the process may be possible by engineering the sequential HTL configuration to minimize energy input, maximize energy output, and diversify product yield.

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