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Hydrothermal Liquefaction of Galdieria sulphuraria Grown on Municipal Wastewater

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Abstract. Subcritical hydrothermal liquefaction uses high temperatures (270-350°C) and high pressures (80-173 bar) to produce bio-crude oils that can be upgraded to liquid transportation fuels. In this study, two strains of *Galdieria sulphuraria*, an acidophilic, mixotrophic red microalgae, were cultivated on effluent from primary settling tanks at a municipal wastewater treatment plant. Samples were concentrated to 5 and 10 wt.% slurries after harvest and converted by hydrothermal liquefaction in a 1.8 L batch reactor. Reaction conditions included temperatures of 310, 330 and 350°C, and hold times of 5, 30 and 60 minutes. Yields and product properties were compared to those of hydrothermal liquefaction of *Galdieria sulphuraria* grown on media. Total oil yields were low (11-18 wt.%) and char yields were high (28-36 wt.%) compared to those from HTL of the algae grown on media (27-35 wt.% oil and 10-13 wt.% char), likely due to the higher ash content and lower lipid content of the algae grown on wastewater. The bio-crude oil, char, and aqueous phase samples were characterized to complete mass, energy and nutrient balances to characterize the tradeoffs in the algae growth and conversion systems for energy and nutrient recovery.

Keywords. algae, biofuels, energy recovery, hydrothermal process, waste treatment

Introduction

Algae in Wastewater Treatment

Greater demand for clean water in arid regions has led to a growing surge of research on improving wastewater treatment [1, 2]. To protect the environment and public health, dissolved organic carbon, nitrogen and phosphorous in the wastewater must be removed. Organic carbon, biochemical oxygen demand (BOD), is usually oxidized into gaseous carbon dioxide by heterotrophic bacteria and discharged into the atmosphere. Ammonium nitrogen is converted into nitrogen by nitrification/de-nitrification and discharged into the atmosphere. A tertiary treatment is used to eliminate remaining biological nutrients containing nitrogen and phosphorous. Current wastewater treatment technologies are subject to several problems: high energy consumption, expensive operation, the need for external carbon to complete nitrogen removal, and the under-utilization of carbon, nitrogen, and other nutrients in the wastewater [3]. To solve these problems, Oswald proposed a mixed algal/bacterial system to enhance the energy efficiency of wastewater treatment [4]. Oxygen provided by algae is used by the heterotrophic bacteria to oxidize the BOD into carbon dioxide, which is then captured via photosynthesis within the mixotrophic system. With the aid of solar energy, ammonium nitrogen and phosphates in the wastewater can be incorporated into energy-rich algal biomass without any additional external carbon input [5]. The stoichiometric ratio of C:N:P in wastewater is closer to that of algal biomass than bacterial biomass, indicating that an energy-intensive tertiary treatment for nitrogen removal might not be needed [3]. Finally, the produced algal biomass represents a conversion of the organic compounds in wastewater into a renewable bio-energy resource [6].

Energy Considerations for Algae Wastewater Treatment Systems

The feasibility of low-cost wastewater treatment has been demonstrated through optimization of algae production and coupling of energy generation and wastewater treatment [7-12]. High nutrient removal efficiencies, including of nitrogen and phosphorous, have been achieved from various wastewater sources using algae [8, 13-15]. One common design for low-cost algal production systems, the open raceway, has several issues that need to be addressed [16]. First, the shallow culture depth requires larger surface areas which results in considerable water evaporation and shorter bubble retention times for CO_2-enriched air; limited contact of CO_2 with algal biomass lowers algae productivity [17]. Second, low cell density in open raceways lowers harvesting efficiency and increases operating costs. Third, the open raceway design creates opportunities for contamination by undesired invaders. To address these challenges, a low-cost, enclosed photobioreactor has been developed which inhibits water evaporation, increases CO_2 recovery by the mixotrophic system for energy-rich biomass production, and avoids external contamination [3].

To further enhance energy utilization in the algal wastewater treatment, a downstream biomass-to-biofuel conversion is needed, such as hydrothermal liquefaction [18, 19], anaerobic digestion [20], fast pyrolysis [21], or catalytic hydrothermal gasification [22]. Algal conversion to biofuel must accommodate the tradeoff between lipid content (as the primary energy-rich component) in algae and algae productivity [23]. Hydrothermal liquefaction (HTL) converts the lipid, carbohydrate and protein fractions of the algal biomass into bio-crude oil [24], and avoids the feedstock dewatering process [25]. Recently, novel algal wastewater treatment has been coupled with HTL to recover more energy [16, 26, 27]. The aqueous phase product from HTL process can be reused as nutrient media by WWT algae to increase algal productivity and the bioenergy potential of wastewater [27-29]. These concepts are implemented as the Environment-Enhancing Energy (E^2-Energy) system [26] and the Photosynthetically Oxygenated Waste-to-Energy Recovery (POWER) system [16, 27, 30] to improve the overall energy efficiency in the algal wastewater system via increasing biomass densities and optimizing C:N:P ratios in the wastewater. In some cases, surplus nutrients from algal wastewater treatment system can be recovered and used as fertilizers [30]. The key feature of these algal wastewater systems is the coupling with HTL processes; optimizing the HTL of algal biomass plays an essential role in achieving positive energy yields from algal wastewater treatment systems.

Hydrothermal Liquefaction of Algae

HTL has been demonstrated as an energetically favorable thermochemical conversion over the other biomass upgrading technologies [31]. The various HTL reactions are catalyzed by H^+ or OH^- ions derived from water at a subcritical state (180-370°C and 5-21 MPa) [24, 25, 32]. The lower dielectric constant of water at high temperatures is conducive to dissolving more organic molecules derived from algal biomass. Those reactions involve hydrolysis, dehydration, decarboxylation, repolymerization, and deamination [25, 33], which break larger biopolymers into bio-crude oil aqueous, char and gaseous phases [37], [38]. Bio-crude oil yields typically range from 30 to 60 wt.% (dry, ash-free), with energy recoveries from 50 to 70%. Greenhouse gas emissions are less and the energy return on investment is better for HTL [34], especially for bio-jet fuel production [35]. Since HTL can convert most biopolymers into bio-crude oil, even low-lipid and high-protein WWT algae could give a high yield of bio-crude oil [26, 29]. To date, bio-crude oil yields from HTL of WWT algae have ranged from 30 to 50 wt.% (dry, ash-free), with higher heating values of 35-39 MJ kg^{-1} [29, 36]—comparable to those from HTL of media-cultured algae. Recent work has focused on the optimization of the HTL process for WWT algal species.

Selvaratnam et a. [25] recently found that 180°C was the most suitable for producing a nutrient-rich aqueous phase, while 300°C and solid algal contents >10 wt.% were favor bio-crude oil production. In addition, different operating conditions have been shown to extract compounds from the different biopolymers (e.g. lipids [37], carbohydrates [19], and proteins [38]) in algae. At low temperatures, nutrient-containing compounds (derived from proteins and carbohydrates) could be transferred into the aqueous phase, while, at high temperatures, those compounds could be broken into smaller organic molecules and dissolved into the bio-crude oil phase. Jazrawi et al. [38] and Chakraborty et al. [19] have proposed two-step methods to extract proteins and carbohydrates, respectively, from algal biomass at temperatures lower than 200 °C, followed by HTL at higher temperatures to convert the organic residues into bio-crude oil, giving an aqueous phase with higher nutrient content, a bio-crude oil with higher yield and lower nitrogen content, and less biochar [39].

High ash content in WWT algal biomass is an important unresolved technical problem. Chen et al. [40] used physical pretreatments (e.g. centrifugation and ultrasonification) to reduce the ash content from 28.6 to 18.6 wt.% in the algal feedstock, leading to a higher yield of bio-crude oil from 30 to 55 wt.%. However, the energy and equipment costs need to be considered carefully prior to scaling up any such pretreatments.

Galdieria sulphuraria for Wastewater Treatment

Galdieria sulphuraria (hereafter *G. sulphuraria*) is a heterotrophic/photoautotrophic, acidophilic, and thermo-tolerant microalgae within the Cyanidiophyceae class, capable of growing at temperatures from 25 to 55°C [41]. This means that *G. sulphuraria* is able to survive in uncooled growth chambers where the temperatures reach almost 50°C in daylight during the warmer months (April to September in Las Cruces, NM). Not needing a cooling systems allows *G. sulphuraria* to be grown in a low-cost, closed photobioreactor (PBR) in a warm-arid environment using substantially less energy [42]. *G. sulphuraria* was originally found in acidic (pH = 0.5-4) geothermal hot springs within Yellowstone National Park (Wyoming, USA), meaning that the culture can be protected from pathogenic invaders and competitors by decreasing the pH of the wastewater. *G. sulphuraria* has moderately strong capabilities to remove BOD and other nutrients (nitrogen and phosphorous) from primary-settled wastewater [42, 43] at growth rates comparable to those in a control medium [3, 30]. *G. sulphuraria* is tolerant of high levels of salts and metals [44], and has the metabolic versatility to grow on a wide range of organic substrates [45]. Chen et al. [46] found that cultivation of low-lipid algae in wastewater can decrease algae production costs and mitigate eutrophication.

Study Objectives

The goal of this study is to compare the efficiency of HTL conversion on the same warm-weather strain of algae, *G. sulphuraria* 5587.1, grown on freshwater media and on municipal wastewater primary effluent, and a cool-weather strain, *G. sulphuraria* SOOS, grown on the same municipal wastewater. Efficiency is evaluated in terms of biomass composition, bio-crude oil yields, bio-crude oil chemistry, energy recovery in bio-crude oil and char fractions, and nutrient recovery from the aqueous phase product.

Materials and Methods

Algae Production and Harvest

Galdieria sulphuraria (CCMEE 5778.1) was identified by the Culture Collection of Microorganisms from Extreme Environments (University of Oregon). The freshwater media 5587.1 strain was grown in a modified cyanidium medium with the pH was adjusted to 2.5 with 10 N H_2SO_4, where the cyanidium medium contained 0.27 g L^{-1} KH_2PO_4, 1.32 g L^{-1} $(NH_4)_2SO_4$, 0.25 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.12 g L^{-1} NaCl, 0.07 g L^{-1} $CaCl_2 \cdot 2H_2O$, 1.0 mL of 0.29 g L^{-1} $FeCl_3$ solution, and 0.5 mL Nitch's trace element solution. The algae culture was expanded in 20 L carboys then transferred to an outdoor photobioreactor system (Solix Algredients, Fort Collins, CO), located at the NMSU Algal Growth Facility at the Fabian Garcia Plant Science Center in Las Cruces, NM. Growth conditions used natural photoperiod and light intensity, and the internal temperatures inside the growth bags was substantially warmer than the ambient air temperature. Algal cultures were harvested and concentrated by a custom-built high speed continuous centrifuge (AC26VHC, Type 265322CD, Pennwalt, India) at 15,000 rpm for 1-2 hours with a flow rate of 8 L/min. Samples were stored at -20°C prior to HTL conversion.

G. sulphuraria SOOS was originally isolated from a diatomite shield site in the National Nature Reserve Soos, Czech Republic [47]. Wastewater treatment (WWT) *G. sulphuraria* 5587.1 and SOOS were cultivated using the same outdoor pilot-scale culture system [48] at the Jacob A. Hands Wastewater Treatment Facility (Las Cruces, NM) in raw primary effluent (primary settled wastewater) supplemented with the same cynanidium media components except for the ammonium sulfate and potassium phosphate since the wastewater was expected to contain the N and P needed for algae growth. The pH was again adjusted to 2.5 with 10 N H_2SO_4. The growth chamber headspace was filled with 2% CO_2-enriched air [48]. Temperature, pH, biological oxygen demand, and dissolved oxygen where recorded, but the temperature of the vessel was not controlled. *G. sulphuraria* 5587.1 was grown from approximately March through October, and *G. sulphuraria* SOOS was grown from November to February. A polyculture of the two strains was attempted during the three of the transition

periods but these polycultures crashed except for one in November-December 2015, so biomass from the polyculture was not included in the HTL conversion experiments. Algae cultures were harvested and concentrated by allowing cultures to settle overnight, removing supernatant water, and centrifuging in 6 L batches using a Avanti J-26 XP centrifuge (Beckman Coulter, Brea, CA) at 10,000 rpm for 5-10 min. After centrifuging, solid algal loading was approximately 14-15 wt.%. Samples were stored at -20°C prior to HTL conversion.

Hydrothermal Liquefaction

The HTL experiments were performed in a 1.8L model 4572 stainless steel batch reactor （Parr Instrument Co., Moline, IL) accompanied by a controller unit to set the desired temperature and detect the pressure. Algae slurries of 5 wt.% and 10 wt.% solid loading were reacted at 310, 330 and 350°C for 5, 30 and 60 min (holding time). In a typical experiment, 500 g of algae slurry was prepared by charging the reactor with concentrated algal slurry and deionized water. For the last run with the SOOS strain (350°C and 5 min) sample limitations required a reduction in total slurry mass to 378 g. Before running the reaction, the sealed reactor was purged with nitrogen for 5 min, and then pressurized with nitrogen up to approximately 200 psi (1.38 MPa). The reactor was stirred by an impeller type agitator continuously and heated at 2.8-3.3°C/min up the HTL temperature. After the reaction, the batch reactor was cooled to room temperature using a water jacket and the pressure released.

The original HTL products were agitated after adding 200 mL hexane into the reactor to extract volatile organics from aqueous phase into bio-crude phase. The aqueous, organic and solid phases were poured out into a 1L beaker, and the gaseous phase was vented into the fume hood. 150 mL of hexane was used to rinse the agitator and inner wall of the reactor in triplicate (50 mL each time), and the rinsed solution was also poured into that 1L beaker containing the original HTL solution. To enhance the efficiency of filtration, Whatman® No. 4 filter paper (pore size = 25 μm) and Whatman® No. 1 filter paper (pore size = 11 μm) were used to filtrate sequentially to collect the solid products (containing asphalt-like sticky black residue, biochar, and yellowish ash powders). The hexane-soluble product, defined as light bio-crude oil (LBO) was separated by a separatory funnel from the aqueous phase. The solid residue on the filter paper was rinsed by mixing 75 mL dichloromethane (DCM) with the solid residues, followed by filtrating the rinsed solid product from DCM-extracted organic phase, defined as heavy bio-crude oil (HBO). The solid residue was dried at room temperature overnight in a fume hood. The hexane-soluble and DCM-soluble fractions were vacuum evaporated at 47°C and 39ºC, respectively to remove the solvent. Aqueous and oil products were stored in the refrigerator and desiccator, respectively, prior to analysis.

Characterization of Feedstock and HTL Products Characterization

Lipid, carbohydrate, protein and ash contents of the algae biomass were measured by the standard methods developed by the National Renewable Energy Laboratory [49-52]. Weighed samples were placed into a programmable box furnace (Cole – Parmer, Vernon Hills, IL) at 575°C for 180 min with 3 replications. Moisture contents of algal biomass and aqueous samples were measured using a FreeZone 12 L Console Freeze Dry System with Stoppering Tray Dryer (Labconco, Kansas City, MO). Samples were analyzed in triplicate. Higher heating values of the feedstocks, light bio-crude oils, and chars were measured in duplicate using a model 6725 semi-micro bomb calorimeter (Parr Instrument Co., Moline, IL) to calculate the energy recovery. Elemental CHNS content was measured using a Series II 2400 elemental analyzer (Perkin Elmer, Waltham, MA). The analyzer was calibrated using cystine and acetanilide. Samples were analyzed in duplicate. Ammonium, total nitrogen, phosphate, and total phosphorus contents of the aqueous phase products were measured using DRB200 and DR 6000 (HACH Company, Loveland, CO) following the Salicylate 10031, Persulfate 10072, Ascorbic Acid 8048, and Acid Persulfate Digestion 8190 methods, respectively. Samples were analyzed in triplicate. Total carbon (TC) and total organic carbon (TOC) of the aqueous phase products were measured using a model TOC-VCPH analyzer (Shimadzu Corp., Kyoto, Japan). Algal biomass, bio-crude oil, and char samples (0.2 g) were digested with 6 ml of 30% HCl and 6 ml of 70% HNO3 in a Multiwave 3000 microwave digestion system (Anton Paar, Ashland, VA) to quantify total metal content. Digestates, and aqueous phase samples, were diluted to 100 mL with deionized (DI) water and the cation content measured using an Optima 4300 DV inductively coupled plasma optical emission spectrophotometer (ICP-OES) (PerkinElmer, Waltham, MA).

Results and Discussion

Feedstock Characteristics

The compositions of the algae feedstocks are shown in Table 1. The lipid content of *G. sulphuraria* is relatively low compared to other algae species that have been used for biofuels, such as *Nannochloropsis sp.,* and the protein content is relatively high. One concern that frequently needs to be addressed for wastewater water algae is a mineral content than algae grown on a controlled media. Total metal content analysis of *G. sulphuraria* 5587.1 grown on wastewater (Table 2) shows that it a substantial amount of the mineral matter is Si (~7 wt.% of the dry algal biomass), followed by lesser amounts of S, P, Fe, K, Na, Ca and Mg.

Table 1. Proximate, elemental and biochemical composition of algae biomass grown on wastewater compared to the composition of algae biomass grown on a controlled media (unpublished data, manuscript under review).

Algal Species	***G. sulphuraria* 5587.1**	***G. sulphuraria* SOOS**	***G. sulphuraria* 5587.1 (grown on media)**
Proximate Analysis			
Moisture Content wt.% (wet basis)		12.7	31.0 ± 0.3
Ash Content wt.% (dry basis)			10.4 ± 0.5
HHV MJ/kg (dry basis)	24.5	24.9	20.5 ± 1.0
Elemental Analysis wt.% (dry basis)			
Carbon			44.5 ± 0.2
Hydrogen			7.7 ± 1.3
Nitrogen			9.5 ± 0.2
Sulfur			3.0 ± 0.6
Oxygen [d]			25.4 ± 2.6
Biochemical Analysis wt.% (dry, ash free basis)			
Lipid			5.5 ± 0.7
Protein			45.3 ± 1.0
Carbohydrate			14.5 ± 1.0

Table 2. Total elemental content by microwave acid digestion and ICP of the algal biomass and HTL products from *G. sulphuraria* 5587.1 converted at 350°C, 60 min, and 10 wt.% solid algae content. The aqueous (AP) phase was analyzed without undergoing microwave digestion. A blank cell represents a non-detect.

Element	Feedstock (mg/kg)	LBO (mg/kg)	HBO (mg/kg)	Char (mg/kg)	AP (mg/L)
Al	76,170	612	597	28,3400	1.1
S	27,480	27,830	16,990	11,990	1,432
P	8,157			19,031	5.4
Fe	6,107	58	1226	14,241	0.05
K	1,915			933	214
Na	896			174	112
Mg	689			976	40
Ca	569			339	22
Cr	255	2.5	20	740	
Cu	132	2.3	30	257	0.25
Pb	51				
Zn	21		1.3	35	0.06
V	17		2.9	37	0.03
Mn	15		2.7	34	
Mo	14		1.3	41	
Li	13	0.8	0.8	1.7	0.03
Bi	12			12	
Ni	11		39	51	0.02
Tl	8.8			10	0.10
Ba	7.2			12	0.08
Sr	6.5				0.47
Cd	1.6	0.5	0.4	0.8	0.01

HTL Yields

Hydrothermal liquefaction conditions and yields are summarized in Table 3, with yields representing the average of duplicate reaction runs. For *G. sulphuraria* 5587.1, light (hexane-soluble) bio-crude oil (LBO) yields ranged from 5-11 wt.% on a dry feedstock basis, while heavy (hexane-insoluble, DCM-soluble) bio-crude oil (HBO) oil and char yields ranged from 6-7 wt.% and 26-26 wt.%, respectively. The HTL conditions giving the highest oil yield was 350°C for 30 min. HTL of *G. sulphuraria* SOOS gave substantially higher LBO yields (15-22 wt.%) and substantially lower (0.3-1 wt.%) HBO yields than HTL of *G. sulphuraria* 5587.1 under the same conditions; as with the 5587.1 strain, the higher temperature and shorter reaction time favored oil yields. HBO + Char yields are reported due to the very low amount and high viscosity of HBO recoverable from the solid for the SOOS strain, which made accurate weighing difficult. Oil yields observed in this study were comparable to slightly higher than those from previous HTL of wastewater-cultivated *G. sulphuraria*, where no distinction was made between light and heavy bio-crude oils [16].

Table 3. Product yields from HTL under different operating conditions. All yields are reported on a dry feedstock basis.

Temperature (°C)	Time (min)	LBO (wt.%)	HBO + Char (wt.%)	HBO (wt.%)	Char (wt.%)	Aqueous Phase (wt.%)	Gases/Losses (wt.%)
G. sulphuraria 5587.1							
310	60	8.6	36.0	6.0	30.1	25.4	30.0
330	60	9.0	42.8	6.9	35.9	17.5	30.7
350	5	9.8	35.9	7.1	28.8	13.2	41.1
350	30	10.8	37.0	7.3	29.7	12.0	40.2
350	60	7.6	42.9	7.3	35.6	14.2	35.2
350 (10 wt.%)	60	5.3	42.1	5.8	36.3	9.8	42.8
G. sulphuraria SOOS							
310	60	14.7	26.5	0.3	26.2	28.9	29.9
350	5	22.0	24.3	1.0	23.3	28.8	24.9
350	60	19.3	23.6	1.0	22.6	13.4	43.7

Bio-crude Oil and Char Characteristics

Table 4 shows the energy contents of the light bio-crude oils and the chars, as well as their energy contents relative to the energy contained in the algal biomass. Small sample recoveries and high viscosity of the heavy bio-crude oils limited the characterizations that could be done. The energy content was slightly higher for the SOOS strain LBO (38-41 MJ/kg) than the 5587.1 strain LBO (34-39 MJ/kg), with the higher HTL temperature slightly favoring LBO energy content. The SOOS stain chars also had higher energy contents (16-23 MJ/kg) than the 5587.1 strain chars (9-12 MJ/kg), indicating that the SOOS strain may provide better overall energy recovery than the 5587.1 strain.

Table 4. Higher heating values of light bio-crude oils (LBO) and chars under different operating conditions. Relative energy is compared to the energy available in the original biomass accounting for product yield on a dry basis.

Temperature (°C)	Time (min)	LBO HHV (MJ/kg)	LBO Relative Energy (%)	Char HHV (MJ/kg)	Char Relative Energy (%)
G. sulphuraria 5587.1					
310	60	37.6 ± 1.4	13	13.7 ± 3.5	17
330	60	34.5 ± 1.4	13	12.4 ± 10	18
350	5	38.9 ± 1.4	16	10.2 ± 3.1	12
350	30	39.4 ± 2.8	17	9.3 ± 0.9	11
350	60	36.8 ± 1.5	11	12.0 ± 0.8	17
350 (10 wt.%)	60	38.7 ± 1.7	8	10.6 ± 0.9	16
G. sulphuraria SOOS					
310	60	38.6 ± 3.1	23	23.4 ± 5.4	25
350	5	37.8 ± 0.2	33	19.4 ± 2.0	18
350	60	41.4 ± 0.1	32	15.6 ± 0.3	14

Aqueous Phase Characteristics

The nutrient composition and water chemistry characteristics of the HTL aqueous phase products are shown in Table 5. In general, the aqueous phase products were slightly basic with pH values ranging between 8 and 9, and saline, with electrical

conductivity values ranging from 11-28 mS/cm. The SOOS strain aqueous phase products were more saline than those of the 5587.1 strain made under the same conditions. Notably, increasing the solid algal content of the HTL reaction from 5 to 10 wt.%, approximately doubled the electrical conductivity and the nutrient content of the aqueous phase product. While total metal analysis (Table 2) shows that the vast majority of minerals partition into the char product, a substantial amount of salt remains in the aqueous phase. The amount of carbon partitioning into aqueous phase ranged from 5-10 g/L. As with the mineral content, doubling the solid algal content approximately doubled the concentration of total and organic carbon in the aqueous phase. Most (72-94%) of the carbon in the aqueous phase product was organic.

Table 5. Aqueous phase composition for total N and P, ammonium, phosphate, total carbon (TC), total organic carbon (TOC), pH, and electrical conductivity (EC). All data are the average of two HTL conversion runs.

Temperature (°C)	Time (min)	NH_4^+ (g/L)	Total N (g/L)	PO_4^{3-} (mg/L)	Total P (mg/L)	TOC (g/L)	TC (g/L)	pH	EC (mS/cm)
G. sulphuraria 5587.1									
310	60	1.6	2.8	62	565	5.4	6.0	8.22	13.1
330	60	1.6	2.9	57	697	6.3	6.7	7.96	11.3
350	5	1.4	2.1	66	779	4.3	5.0	8.28	12.7
350	30	1.7	2.6	105	581	4.4	5.3	8.36	12.5
350	60	2.0	2.8	33	214	3.7	4.8	8.71	14.0
350 (10 wt.%)	60	4.0	5.6	2	895	9.6	10.7	8.64	28.2
G. sulphuraria SOOS									
310	60	2.3	3.2	37	100	6.7	8.2	8.39	17.3
350	5	2.6	4.3	29	3,567	6.1	7.8	8.79	17.0
350	60	2.7	3.6	20	1,547	5.3	7.3	8.52	20.2

Conclusions

G. sulphuraria SOOS gave higher bio-crude oil yields, higher bio-crude oil energy content, higher char energy content, and overall better energy recovery. Higher temperatures and shorter reaction times favored light bio-crude oil production. *G. sulphuraria* 5587.1 produced more heavy bio-crude oils while the bio-crude oil produced from *G. sulphuraria* SOOS was almost completely hexane soluble, which can indicate a composition more favorable for upgrading into hydrocarbon fuels. For both strains of algae, the amount of total phosphorus recovered in the aqueous phase was substantial. HTL conditions that favored bio-crude oil yield, also favored increased recovery of nutrients in the aqueous phase, therefore, temperatures around 350°C and reaction times between 5 and 30 minutes are recommended for HTL conversion of *G. sulphuraria* grown on wastewater. This first data on HTL conversion of *G. sulphuraria* SOOS suggests that the strain is promising as a wastewater-grown algae feedstock and more research is warranted.

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