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Promising Pathway for Algal Biofuels through Wastewater Cultivation and Hydrothermal Conversion

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ABSTRACT: The purpose of this study is to demonstrate feasibility of an integrated wastewater algae-to-biocrude process that can sustainably cultivate algal biomass for biofuel production. This process used pilot-scale algal cultivation ponds fed with municipal wastewater as the nutrient source. The open ponds were self-inoculated from the wastewater source, resulting in a mixed-culture microalgal community with distinct differences compared to laboratory-maintained and fertilized monocultures: 29.0% dry weight (dw) ash, 48.9% ash-free dry weight (afdw) carbon, 37.5% afdw oxygen, and 14.0% afdw lipid. The harvested algae was processed using hydrothermal liquefaction at 350 °C (autogenous pressures up to 2000 psig) for 1 h using 3 g of freeze-dried algae and 50 mL of water. The yield of biocrude was 44.5 ± 4.7% afdw, with an elemental weight percent composition of 78.7% carbon, 10.1% hydrogen, 4.4% nitrogen, and 5.5% oxygen and an energy content of 39 MJ/kg. Hydrothermal processing also resulted in the formation of 18.4 ± 4.6% afdw aqueous co-products (ACPs) and 45.0 ± 5.9% dw solid biochar. The ACPs contained 4550 ± 460 mg L^{-1} organic carbon, 1640 ± 250 mg L^{-1} total nitrogen, and 3.5 mg L^{-1} total phosphorus. The solid biochar product contained >20% dw carbon with an energy density between 8 and 10 MJ kg^{-1}. This study is the first hydrothermal liquefaction paper of wastewater-derived microalgae. The municipal wastewater matrix and resultant mixed-culture biomass significantly influenced liquefaction product distribution, yielding a higher proportion of biochar, which may be a valuable co-product. This paper explores the potential for wastewater-fed algal systems integrated with hydrothermal liquefaction, which together overcome challenges identified by the 2012 National Research Council's report on algal biofuel sustainability.

1. INTRODUCTION

The substitution of petroleum-derived fuels with renewable, affordable, and low-carbon-emitting fuels is necessary to reduce continued detrimental human impact to the environment, support future economic growth, and increase energy independence. To achieve these goals, the U.S. government passed The Energy Independence and Security Act (EISA) in 2007, mandating 36 billion gallons of annual biofuel production by 2022. Algae has been studied intermittently for the past 30−50 years as a possible energy source, and it has tremendous potential to support the EISA goals because of its fast growth rates, noncompetitive nature to food markets, and ability to grow using nutrient waste streams (CO_2 from the atmosphere or flue gases and macronutrients contained in wastewater). However, a recent report[1] by the National Research Council (NRC) of the National Academies has identified several concerns about the sustainability of algal biofuels: limited supplies of water, nutrients, and appropriate land; greenhouse gas emissions and fossil energy-use over the life cycle of algal biofuels; introduction of genetically modified algae to natural water; and waste products generated by algal biofuel production. Algal biofuel technologies that do not address these concerns are likely to have limited viability.

Traditional conversion technologies of algal biomass to biofuels have mainly included lipid extraction or pyrolysis for biodiesel, hydrotreated renewable diesel, or bio-oil production. These technology pathways have significant drawbacks when using algal feedstocks. Lipid extraction requires extensive dewatering and organic solvents, diminishing the economics of fuel production and increasing environmental concerns. Whole cell conversion pathways, such as pyrolysis, alleviate environmental concerns from solvent extraction but still require extensive dewatering prior to conversion. A wet whole cell conversion pathway, hydrothermal liquefaction (HTL), has gained popularity in the past few years. HTL uses subcritical water as the chemical driving force to convert biomass to a carbon-rich biocrude.[2,3] Using microalgal feedstocks, biocrude yields from HTL processing are 5−30% greater than the initial algae lipid content[4−13] because other cellular components (beyond only lipids) are converted to biocrude.[2,3,8,14] Economic analyses of producing fuel oil, synthetic gasoline, and diesel from wood chips with 50 wt % moisture have shown that atmospheric flash pyrolysis is less costly than high-pressure liquefaction.[15] However, for high water content biomass, such as algae, the energy costs of dewatering may offset the energy demand of liquefaction processes. Also, HTL of algae gives a biocrude with a composition and energy density that more closely resembles petroleum crude than bio-oil from pyrolysis.[16]

The economics and sustainability of a viable algae-based fuel and chemical platform will depend upon a close integration of cultivation and conversion technology. To date, HTL studies of algal biomass have focused on product formation and characterization using fresh monoculture or marine alga feedstocks.[4−6,8−15] The algal biomass has typically been

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purchased from existing algae producers in the nutraceutical industry, in which the high-value products support the high cost of monoculture cultivation. However, it is generally believed that the renewable fuel industry must use low-cost feedstocks to compete with petroleum-derived fuels.[17] Because fertilizer inputs represent a large monetary and environmental cost for cultivation, sustainable algal biomass production should rely on macronutrients provided in wastewater.[18,19] Wastewater-fed algal cultivation systems also have a dual benefit of performing biological nutrient removal from wastewater.[20] Excess nutrients in receiving water bodies cause problematic algal blooms. In the Gulf region, such algal blooms have caused hypoxic zones and a collapse of aquatic wildlife communities. It is expected that future United States Environmental Protection Agency (U.S. EPA) regulations will require wastewater treatment plants to meet stringent nutrient discharge limits. Wastewater treatment engineers are investigating many treatment options to achieve these limits economically, including algal scrubbers.[21,22]

While municipal wastewater is a readily available water and nutrient source that can support mass algal biomass cultivation,[23] wastewater effluent has dynamic nutrient concentrations that vary daily and seasonally. This variable nutrient source is entirely different from the nitrogen-limited growth conditions that are known to yield high-lipid content biomass, such as 40−70% lipid by weight.[24] Wastewater-fed algae typically have low lipid [10−29% dry weight (dw)][20,25] and low carbon (30−37% dw)[26] contents, and these characteristics can have substantial effects on the HTL product formation and biocrude composition. In this work, we report on the cultivation of mixed-culture microalgae grown in pilot-scale wastewater-fed open pond reactors for the production of biocrude through HTL. The biofuel pathway and characterization of feedstock and conversion products are discussed in detail.

2. EXPERIMENTAL SECTION

2.1. Algal Growth and Characterization. Four 2500 gallon (height, 1.2 m; diameter, 3.17 m) open pond reactors were operated as continuous flow stirred tank reactors at the Lawrence Wastewater Treatment Plant (WWTP) in Lawrence, KS, from April to October 2011. The effluent from the secondary clarifier of the treatment plant was pumped into each reactor at a rate of 680 mL min^{-1}, corresponding to a hydraulic residence time (HRT) of 10 days. The Lawrence WWTP does not perform biological nutrient removal but does perform nitrification, resulting in an effluent with nitrate and phosphate. Aeration from fine-bubble air stones provided mixing with no addition of carbon dioxide gas. Top-down ecological control was implemented with *Gambusia* fish, which prey on zooplankton, such as *Daphnia*. Operation of these pond reactors are described in further detail by Sturm and Lamer[20] and Sturm et al.[27] Effluent from the four reactors continuously flowed to four separate gravity sedimentation tanks, each with a surface area of 1.56 ft^2 and an operating volume of 42.9 gallons. At the operational flow rate, each system had an overflow velocity of 6.7 m day^{-1}. The concentrated microalgae samples (1−1.5% solids) were collected from the bottom of each sedimentation tank daily and were immediately processed. Average reactor conditions were determined by weekly water quality measurements of wastewater feed and samples collected in each reactor. The nutrient concentrations of the municipal wastewater effluent and the growth reactors were determined through total and dissolved chemical analyses. Samples were filtered through pre-rinsed Whatman GF/F glass-fiber filters to obtain dissolved fractions. Digestion for total nitrogen (TN) and total dissolved nitrogen (TDN) was performed according to standard method 4500-N C, and the resulting nitrate was measured using standard method 4500-NO_3^- B. Total phosphorus (TP) and total dissolved phosphorus (TDP) measurements were obtained with standard method 4500-P E.[28]

Algae harvested from the settling tanks at the Lawrence WWTP were mixed together and centrifuged at 3220 relative centrifugal force (rcf) for 10 min, and the pellet was freeze-dried and ground with a conventional coffee grinder. Algae were then sent to Micro Analysis, Inc. (Wilmington, DE) for CHN and O elemental analysis, reporting an instrumental error of ±0.3 wt %. The higher heating value (HHV) of prepared (ground and freeze-dried) algae was measured using a Parr 6200 calorimeter using decane (99+%; Fisher) as a combustion agent. Moisture and ash were determined by ASTM E1755 using a Thermolyne 46100 high-temperature furnace.[29] Inorganic composition (non-CHNO elements) of algae was analyzed and quantified using proton-induced X-ray emission (PIXE) analysis performed by Elemental Analysis, Inc. (Lexington, KY). The gravimetric lipid content was measured using a modified Bligh−Dyer method.[30]

2.2. Reaction, Recovery, and Yield Determination. Hydrothermal processing was conducted in a 450 mL 4560 series mini benchtop reactor (Parr) attached to a 4848 model controller (Parr). The reactor was charged with 3 g of freeze-dried algae and 50 mL of Milli-Q water. Prior to heating, the reactor was purged with nitrogen. The reaction was conducted for 1 h at 350 °C with pressures up to 2000 psig and constant stirring at 150 rpm. Post-reaction, the reactor was allowed to cool overnight.

The product recovery method implemented is outlined in Figure 1a. After overnight equilibration, 50 mL of decane (99+%; Fisher) was introduced to the reactor and the temperature was increased to 70 °C for 30 min while stirring at 150 rpm to allow for clumps of biochar to be loosened, maximizing solvent exposure and biocrude recovery. The reactor contents were collected in preweighed centrifuge tubes and were centrifuged at 1730 rcf for several minutes, resulting in four layers [from top to bottom: solvent containing biocrude, solid biochar, aqueous co-product (ACP), and solid biochar] shown in Figure 1b.

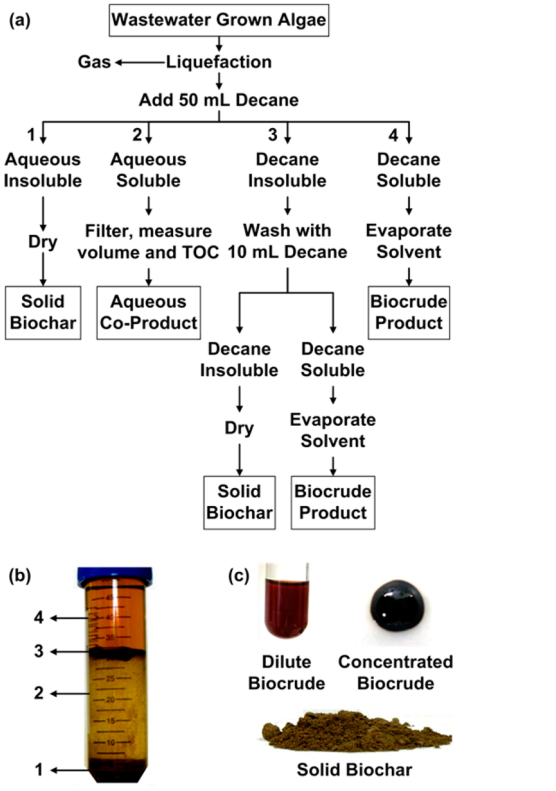

Figure 1. (a) Flow diagram of the solvent extraction and product recovery method used, (b) four layers after centrifugation of HTL products, and (c) photograph of (dilute and concentrated) biocrude and biochar.

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The top organic layer (solvent containing biocrude) was removed by pipet, and the volume was recorded. A total of 30 mL of this fraction was separated and dried under N_2 flow at 30 °C until the mass was constant to determine the biocrude concentration and, thus, overall mass of biocrude. A second solvent recovery step was employed to remove any other contents still residing in the reactor, mainly biocrude and solid biochar adhering to the reactor walls. The second recovery step involved adding 50 mL of decane to the reactor and stirring at 600+ rpm with no heat for 30 min. The contents were collected, measured, and processed in the same manner as the previous recovery step. Significant biocrude and biochar were collected during the second recovery. The reactor was then cleaned with 50 mL of acetone at 200 °C for 30 min, where significant biocrude could be collected from fittings of the reactor head, but this mass was not added to the overall yield of the process. Lastly, the reactor was conditioned with 50 mL of water at 350 °C for 1 h to expose the reactor walls to subcritical water before the next experiment was performed. Three HTL experiments were performed to obtain a standard deviation of product yields.

Decane was selected as a solvent for biocrude recovery because it is a representative solvent that could be created from HTL conversion. If solvent extraction is deemed necessary during a commercial-scale liquefaction process, it is important to use a solvent that is cost-effective and will not disrupt downstream chemistry. Furthermore, a study by Valdez et al.[6] has indicated that decane can result in a higher oil yield compared to other solvents, such as dichloromethane.

The solid biochar residing between the solvent-containing biocrude and the ACP, layer 3 in Figure 1b, was carefully scraped out and collected. A total of 10 mL of decane was then added to this fraction of biochar, vortexed, and centrifuged for 5 min at 3220 rcf. After centrifugation, the solvent was completely pipetted from the biochar pellet and dried in the same manner. A significant biocrude mass was extracted from this biochar fraction and added to the overall biocrude yield. The ACP was filtered through pre-rinsed Whatman GF/F glass-fiber filters, and the volume was recorded. A portion of ACP was diluted with Milli-Q water and analyzed for total organic carbon (TOC) and TN using a Torch Combustion TOC/TN Analyzer. All solids were dried under N_2 flow at 30 °C, and the weights of each were combined for a total biochar mass. The energy content (HHV) and elemental analysis of the biochar and biocrude were determined in the same manner as the starting algae. A photograph of biocrude (dilute and concentrated) and biochar is shown in Figure 1c.

A control experiment was performed using the same type and amount of algae and water. Using the same reactor, the algae/water suspension was agitated at 150 rpm for 1 h at room temperature. The solvent recovery method described above was employed without the addition of heat at any point. Both recovery steps were implemented, and oil mass determination methods were consistent for both HTL and control experiments.

Gas chromatography/mass spectrometry (GC/MS) analysis of biocrude was performed using an Agilent 6890 Series GC−FID system with a 5973 network mass selective detector equipped with a HP-5MS column (5% phenyl methyl siloxane mobile phase; 30.0 m length; 250 μm diameter; and 0.25 μm thickness). The method used an injection volume of 1.0 μL with a split ratio of 20:1, an initial temperature of 40 °C, ramp of 8 °C/min to 250 °C, holding for 5 min, followed by a 20 °C/min ramp to 300 °C, and holding for 1 min. Samples were prepared for the GC/MS analysis in two ways: (1) a derivatized sample of concentrated biocrude and (2) a non-derivatized sample of dilute biocrude in the decane solvent. The derivatized sample was prepared using 10.0 mg of concentrated biocrude, weighed in a GC vial, with 100 μL of *n*-methyl-*n*-(trimethylsilyl)trifluoroacetamide (MSTFA; derivatizing agent supplied by Agilent) and 100 μL of pyridine [high-performance liquid chromatography (HPLC) grade, 99.5+%, Alfa Aesar] added, and diluted with 800 μL of *n*-heptane (99+ %, Sigma). The GC/MS analysis of the biocrude in the derivatized sample revealed that residual decane was present in the concentrated biocrude, even though the oil had been dried until a consistent weight was reached. A five-point (in triplicate) calibration curve was produced using known masses of decane in chloroform (HPLC grade; Fisher) to quantify residual decane in the concentrated biocrude and control experiment. The calibration curve had a R^2 value of 0.98. The amount of decane was quantified to be 1.0 wt % for biocrude and 0.65 wt % for the control. The mass of decane found was then subtracted from biocrude and control yields prior to reporting. The amount of residual decane contributes to less than 0.01 wt % of the C and H levels measured in the concentrated biocrude. The dilute, underivatized sample was 1.0 mL of solvent containing biocrude (fraction 4 in Figure 1b).

Yields are reported on an ash-free dry weight (afdw) basis to allow for direct comparison of this work to the majority of the literature and were calculated as follows:

$$\text{biocrude yield} = \frac{\sum m_{\text{biocrude}}}{m_{\text{algae}_{\text{organics}}}} \times 100 \quad (1)$$

$$\text{ACP yield} = \frac{[\text{TOC}] \times \text{aq}_{\text{vol}}}{m_{\text{C}_{\text{algae}}}} \times 100 \quad (2)$$

$$\text{biochar yield} = \frac{m_{\text{solids}} - m_{\text{ash}_{\text{algae}}}}{m_{\text{algae}_{\text{organics}}}} \times 100 \quad (3)$$

$$\text{gas yield} = 100 - \text{biocrude} - \text{ACP} - \text{biochar} \quad (4)$$

where $\sum m_{\text{biocrude}}$ is the summation of all calculated masses of biocrude from each fraction, $m_{\text{algae}_{\text{organics}}}$ is the mass of freeze-dried algae × (100 − ash % − moisture %), [TOC] is the concentration of measured total organic carbon, aq_{vol} is the measured filtered ACP volume, $m_{\text{C}_{\text{algae}}}$ is the mass of carbon (%, afdw) in algae, m_{solids} is the mass of dried solid co-product, and $m_{\text{ash}_{\text{algae}}}$ is the mass of ash in starting algae. The dry weight (%, dw) solid biochar yield was calculated by relating the total dry weight of product to the dry weight of algae used in the reaction.

3. RESULTS AND DISCUSSION

3.1. Algal Growth and Characterization. With microscopic analysis, 18 different algal species (Table 1) were identified in the open ponds. One batch of harvested algae from the Lawrence WWTP was evaluated and used for this study to ensure that a consistent sample was used for all trials. The average water quality and nutrient concentrations in the wastewater effluent feed and algal ponds can be found in Table 2, producing an average biomass production rate[20] of 12 g m^{-2} day^{-1}. The average TN concentration of the wastewater effluent feed was 29.3 ± 4.8 mg L^{-1}, and the average TP concentration was 2.65 ± 0.10 mg L^{-1}. During operation of the pond reactors in the previous year (2010), the average TN of the wastewater effluent was 19.5 ± 4.5 mg L^{-1} and the average TP was 3.21 ± 0.93 mg L^{-1}.[20] The measured concentrations over these two periods of operation are similar to the textbook

Table 1. List of Identified Algal Species Cultivated Using Wastewater Effluent as Growth Media in Open Ponds

species identified	
Scenedesmus quadricauda	*Cladophora* sp.
Navicula sp.	*Golenkinia radiata*
Scenedesmus bijuga	*Selenastrum* sp.
Oscillatoria sp.	*Cosmarium* sp.
Micractinium pusillum	*Pediastrum boryanum*
Merismopedia sp.	*Microcystis* sp.
Chlorella sp.	*Oedogonium* sp.
Cryptomonas sp.	*Cosmarium* sp.
Cyclotella sp.	*Spirogyra* sp.

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Table 2. Average Water Quality of Pond Reactors at the Lawrence WWTP

	average measured value		
water quality parameter	wastewater effluent feed	growth reactors	analysis method
total nitrogen (TN; mg L^{-1})	29.3 ± 4.8	27.0 ± 4.9	standard method 4500-N C[a]
total dissolved nitrogen (TDN; mg L^{-1})	28.9 ± 5.2	22.4 ± 5.8	standard method 4500-N C[a]
total phosphorus (TP; mg L^{-1})	2.65 ± 0.10	3.41 ± 0.67	standard method 4500-P E[a]
total dissolved phosphorus (TDP; mg L^{-1})	2.52 ± 0.12	2.75 ± 1.1	standard method 4500-P E[a]
total suspended solids (TSS; mg L^{-1})	13.3 ± 7.5	85.5 ± 22	standard method 2540 D[a]
pH	6.89 ± 0.31	7.44 ± 0.87	field meter[b]
temperature (°C)	25.7 ± 0.65	28.1 ± 2.2	field meter[b]
dissolved oxygen (DO; mg L^{-1})	2.71 ± 2.0	7.45 ± 0.51	field meter[b]

[a]Standard methods can be found in the book by Eaton et al.[28] [b]YSI 556, Yellow Springs, OH.

TN concentration for wastewater effluent (20 mg L^{-1}), while the TP concentrations are slightly lower than the textbook TP concentration (10 mg L^{-1}).[31] Similar to the majority of WWTPs in the U.S., the Lawrence WWTP does not perform biological nutrient removal (no phosphorus removal or denitrification). Only 206 of the more than 12 000 municipal WWTPs in the country reported use of denitrification or phosphorus removal technologies in the U.S. EPA 2008 Clean Watersheds Needs Survey.[32] Thus, the Lawrence WWTP effluent is representative of average municipal wastewater effluent characteristics in the U.S.

The wastewater-cultured algae had a high intercellular ash content of 29.0% dw noncombustible material compared to other reports of monoculture microalgae, with ash contents as low as 5% and up to 25%.[8,12] The majority, ∼70%, of this noncombustible material is calcium and silica, which is directly related to the municipal wastewater effluent growth media. The high levels of silica can be ascribed to the presence of diatoms, as confirmed by the species listed in Table 1. The high calcium is attributed to the municipal water source having an average water hardness of 40−120 mg L^{-1} as $CaCO_3$, with calcium concentrations of 24−43 mg L^{-1}.[33] A high ash content is also normally found in marine macroalgae because of high levels of sodium and chloride.[10] Because the algae produced for this study had a high inorganic composition, the product yields were subsequently based on an afdw basis to understand the degree of organic conversion.

The organic and inorganic elemental analyses of the algae can be found in Table 3. The measured carbon content of the algae was 34.7% dw, similar to other wastewater-cultivated algae (30−37% dw),[26] or 48.9% afdw, which is lower than 51.3−57.8% afdw of commercially grown microalgae reported in the literature.[8] Because most of the HTL studies reported in the literature use algae provided by companies, it is difficult to directly compare algal growth conditions between studies. Adding CO_2 to the algal tanks may increase the carbon content of microalgae, but this was not performed in the wastewater pilot. Although the algae have a lower carbon content, they have a high oxygen content of 37.5% afdw. This oxygen content is 5−15% higher than what has been reported for microalgae used in previous HTL studies.[4−13] The combination of the low carbon and high oxygen contents directly affects the HHV of the algal biomass, which was measured at 15.1 MJ kg^{-1}. Ross et al.[12] reported HHV for *Spirulina* sp. and *Chlorella vulgaris* to be 21.2 and 23.2 MJ kg^{-1}, respectively. However, the carbon content for both species was near 54% afdw, while the oxygen content was closer to 27% afdw. These results indicate that microalgae grown using municipal wastewater effluent are unique compared to fertilized monoculture algae that are typically studied for HTL processing.

Table 3. (a) Cultivated Algal Composition (Ultimate and Proximate) and HHV and (b) Total Inorganic Composition of Algae, Measured by PIXE Normalized to Inorganic Material

(a) cultured microalgae (%, afdw)					
C	H	N	O	HHV (MJ kg^{-1})	ash = 29.0%
48.9	7.1	8.4	37.5	15.1	moisture = 4.7%

(b) algae inorganic composition (wt %)								
Na	Mg	Al	Si	P	S	Cl	K	Ca
Ti	Mn	Fe	Co	Ni	Cu	Zn	Br	Sr
0.7	1.7	0.8	35.6	16.4	5.0	0.7	2.7	34.0
0.10	0.3	1.7	0.03	0.01	0.02	0.3	0.1	0.1

3.2. Product Yields and Characterization. Results comparing the initial lipid content, control, and biocrude produced through HTL are found in Table 4. Good agreement was found between the Bligh−Dyer method and the solvent extraction/oil recovery method used for the control experiment at 14.0 and 13.95% afdw, respectively. HTL of cultivated algae yielded 44.5% afdw biocrude, 31.5% more than the initial lipid content. The biocrude yield in this work is on the high end compared to HTL studies on fertilized monoculture algae, typically reported from 19 to 46.5%, corresponding to yields 6−30% above the initial lipid content.[4−6,8−14,34]

Table 4. Oil Yields from Starting Algae, Control, and HTL Using 3 g of Algae/50 mL of Water at 350 °C for 1 h

oil yields (%, afdw)		
lipid content[a]	control	biocrude
14.0	13.95	44.5

[a]Measured by the Bligh−Dyer method.[30]

HTL yielded other co-products besides biocrude, and these are shown in Table 5 on an afdw basis. The main product was the biocrude at 44.5 ± 4.7%, followed by 21.0 ± 8.6% biochar, 18.4 ± 4.6% ACP, and 16.1 ± 8.0% gas. The gas is reported by difference; however, it has been verified using an ideal gas law calculation, assuming that all of the gas is CO_2 with equilibrated temperature (18 °C), pressure (4 psig), and a reactor head space volume of 400 mL.

Table 5. Bulk Yields from the HTL Conversion Method

HTL bulk yields (%, afdw)			
biocrude	biochar	ACP	gas[a]
44.5 ± 4.7	21.0 ± 8.6	18.4 ± 4.6	16.1 ± 8.0

[a]Measured by difference; the ideal gas law calculates 10.1% afdw.

Detailed chromatogram(s) of the biocrude obtained are shown in Figure 2. Peaks identified with the National Institute of Standards and Technology (NIST) library at a 90%

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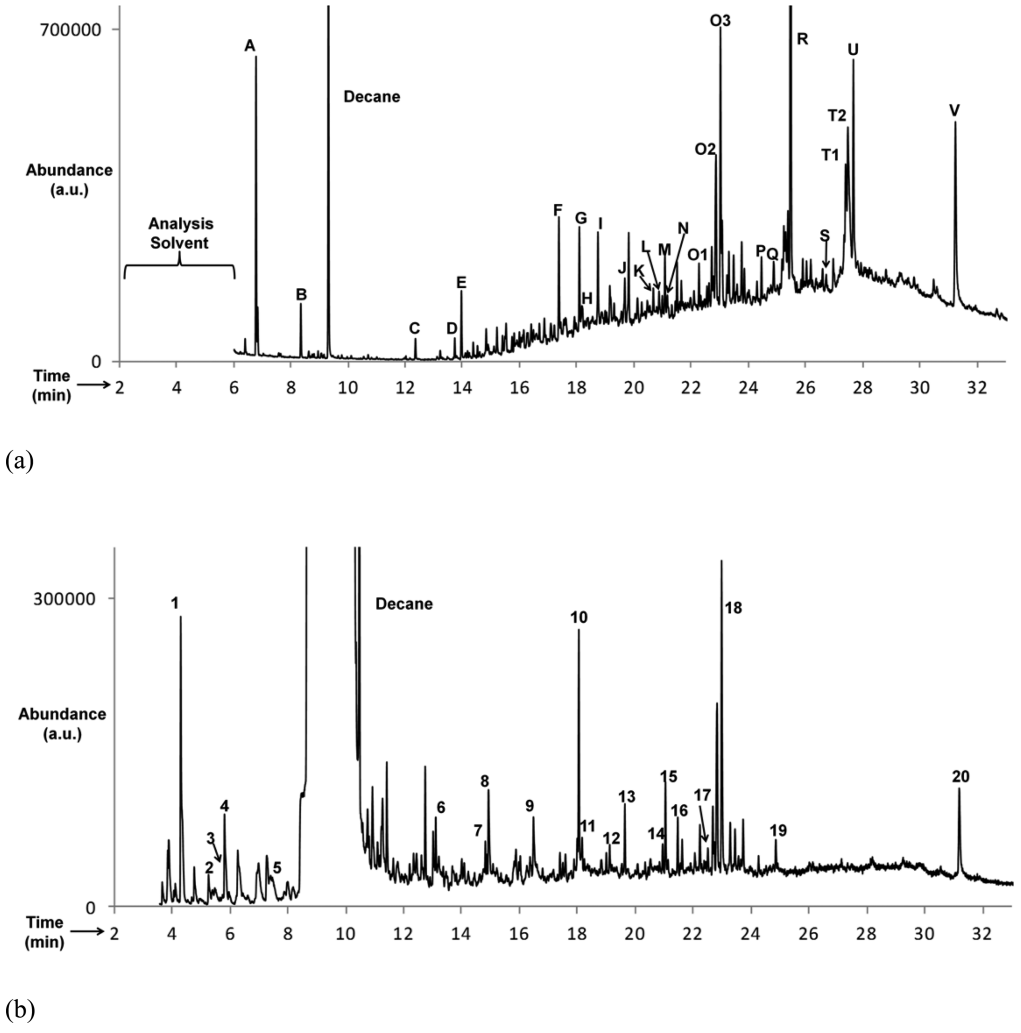

Figure 2. Chromatogram of biocrude produced using samples of (a) concentrated derivitized sample and (b) non-derivitized biocrude in decane from the reactor.

confidence level and above are labeled and reported. These compounds included aliphatics, phenols, fatty acids, ketones, and indoles, with the presence of hydrogen sulfide and at least one amine, pyrrole, pyridine, and phthalate. A complete list of identified peaks, retention times, and structures are shown in Table 6. Many of these compounds are consistent with previous studies reporting the components of biocrude from HTL on monoculture algae.[6,12] Two different sample preparations were employed for GC/MS analysis to allow for more complete compound identification. The first sample preparation was a derivatized sample of concentrated biocrude providing better separation and identification of functional groups (Figure 2a). Derivatizing with MSTFA alters compounds containing −OH, −NH, and −SH by replacing the hydrogen atoms with $Si(CH_3)$ to give $-X-Si(CH_3)$, where X is either an oxygen, nitrogen, or sulfur atom. Hydrogen sulfide, methyl amine, phenols, and free fatty acids are clearly identified using the derivatization. The second sample preparation, dilute biocrude in decane, was used to determine if any compounds were being lost during drying/concentration (Figure 2b).

A comparison of the chromatograms indicates that compounds evaporated with the decane solvent. There are fewer peaks eluting under 15 min in the concentrated biocrude than the dilute biocrude. Specifically, 2-methyl-2-cyclopenten-1-one (7.29 min), 2-decanone (13.13 min), 1-tridecene (14.84 min), and 5*H*-1-pyridine (14.96 min) are absent in Figure 2a. These compounds would not be altered from derivatization and, if present, would elute at the same time in Figure 2a. Other compounds were identified in the dilute biocrude but not the concentrated biocrude. These are listed as followed with their corresponding identification confidence level: cyclopentanone (65%), ethylbenzene (87%), *p*-xylene (76%), styrene (64%), dimethyl pyrazine (86%), dimethyl pyridine (68%), and dodecane (86%). It is likely that these compounds have been lost during solvent evaporation as previously suggested by Brown et al.[5]

Using the wastewater cultivation strategy and HTL conversion, we obtained biocrude with similar properties to petroleum crude and significant benefits to biocrude produced from fertilized, monoculture microalgae. The elemental analysis for the biocrude is shown in Table 7. The carbon content of the biocrude was 78.7 wt % compared to others reported in the literature ranging from 68 to 76 wt % carbon.[5,6,8,12,14,34] The high carbon content is especially significant given the relatively low carbon content of the feedstock algae. The hydrogen content of biocrude was measured at 10.1 wt %, which is comparable to previous studies.[17] The heteroatom (N and O) content of biocrude is the major difference compared to

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Table 6. Identified Compounds Using GC/MS of (a) Concentrated Derivatized Biocrude and (b) Biocrude Diluted in Decane Straight from the Reactor

(a)

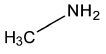
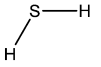
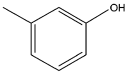
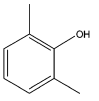
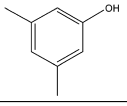
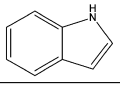
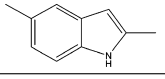
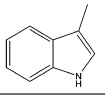
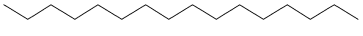
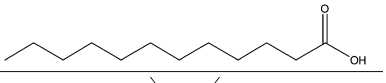
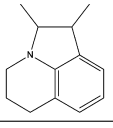
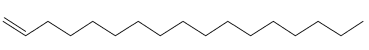
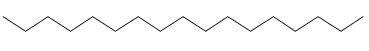
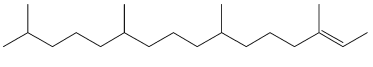
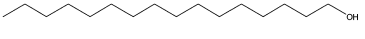
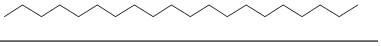
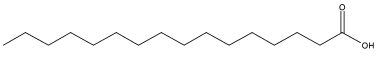
Peaks as Labeled in Fig. 2a	Retention Time (min)	Compound	Structure
A	6.77	Methyl amine	
B	8.33	Hydrogen sulfide	
C	12.34	Methyl phenol	
D	13.72	2,6-Dimethyl phenol	
E	13.95	3,5-Dimethyl phenol	
F	17.36	Indole	
G	18.07	1-Pentadecene	
H	18.17	1H-Indole, 2,5-dimethyl-	
I	18.72	1H-Indole, 3-methyl-	
J	19.66	Hexadecane	
K	20.45	Dodecanoic acid	
L	20.66	1,7-Trimethylene-2,3-dimethylindole	
M	20.97	1-Heptadecene	
N	21.07	Heptadecane	
O1	22.26	2-Phytene Isomer	
O2	22.85		
O3	23.01		
P	24.44	Hexadecanol	
Q	24.87	Eicosane	
R	25.46	Palmitic acid	

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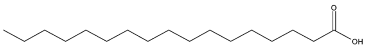
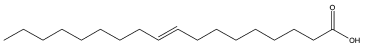
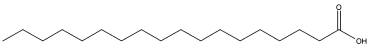
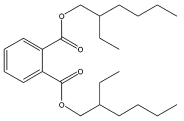
Table 6. continued

Peaks as Labeled in Fig. 2a	Retention Time (min)	Compound	Structure
S	26.58	Heptadecanoic acid	
T1	27.38	Oleic acid	
T2	27.46		
U	27.65	Stearic acid	
V	31.22	Bis(2-ethylhexyl) phthalate	

(b)

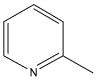
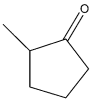
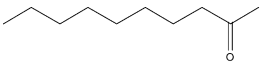
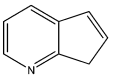
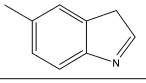
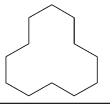
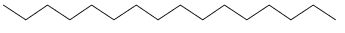
Peaks as Labeled in Fig. 2b	Retention Time (min)	Compound	Structure
1	4.32	Toluene	
2	5.28	1-Ethyl-1H-Pyrrole	
3	5.41	2-Methyl pyridine	
4	5.83	2-Methyl cyclopentanone	
5	7.29	2-Methyl-2-cyclopenten-1-one	
6	13.13	2-Decanone	
7	14.84	1-Tridecene	
8	14.96	5H-1-pyridine	
9	16.50	5-Methyl-1H-Indole	
10	18.07	1-Pentadecene	
11	18.18	Pentadecane	
12	19.02	Cyclododecane	
13	19.66	Hexadecane	

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Table 6. continued

Peaks as Labeled in Fig. 2a	Retention Time (min)	Compound	Structure
14	20.97	1-Heptadecene	
15	21.07	Heptadecane	
16	21.49	1-Nonadecene	
17	22.46	4-Methyl-dodec-3-en-1-ol	
18	23.00	2-Phytene Isomer	
19	24.88	Eicosane	
20	31.22	Bis(2-ethylhexyl) phthalate	

Table 7. Ultimate Analysis and HHV of Biocrude and Biochar Produced through HTL of Cultivated Algae

	elemental composition				
	C	H	N	O	HHV (MJ kg^{-1})
biocrude (wt %)	78.7	10.1	4.4	5.5	39.0
biochar (%, dw)	20.4	2.0	1.8	10.8	8–10

petroleum crude (typically 0.1–2.0 wt % N and 0.1–1.5 wt % O)[4,5,35] and is important to analyze for upgrading purposes. The biocrude produced in this work had a nitrogen content of 4.4 wt % and an oxygen content of 5.5 wt %. The oxygen content is typically 10–30[36,37] and 10–20 wt %[4,8] for pyrolysis-produced algal oils and HTL biocrude from fertilized, monoculture microalgae, respectively. When the heteroatom content of biocrude produced in this study is compared to most HTL studies, nitrogen is comparable while oxygen is lower, better resembling heavy petroleum crude.[14] One study has reported an oxygen content of HTL-produced biocrude near 5 wt %[17] for *Nannochloropsis* sp., but a reaction temperature of 500 °C was used. This increase in temperature decreased oxygen but sacrificed hydrogen (7.1 wt %) and the overall biocrude yield (16 wt %). The biocrude produced in this study had a low oxygen content, did not sacrifice hydrogen, and maintained high organic conversion to biocrude. To the authors' knowledge, only one other study shows a high carbon, low oxygen, and high hydrogen biocrude. Sapphire Energy reported HTL-produced biocrude (reaction temperature of 260 °C) from *Nannochloropsis* sp. with 77.7, 11.7, and 5.7 wt % for carbon, hydrogen, and oxygen, respectively.[38] However, a very different procedure was used for oil recovery (variable heating after reducing pH with H_3PO_4), and no information was provided on algal growth conditions or the elemental analysis of the feedstock algae; therefore, a direct comparison is not possible.

The relationship between the H/C ratio and the O/C ratio and the trade-off between maximizing H/C while minimizing O/C can be seen in a van Krevelen diagram. Higher H/C atomic ratios and lower O/C atomic ratios indicate that the energy content of the substance (feedstock, crude, or fuel) is high with less need for extensive upgrading. A van Krevelen diagram, adapted from Ross et al.,[12] comparing our biocrude to other reported HTL biocrude, coal, and petroleum crude is shown in Figure 3. Light and heavy petroleum crudes, points

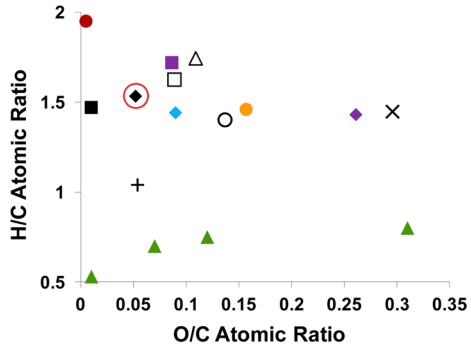

Figure 3. Van Krevelen diagram comparing (black diamond circled in red) biocrude-produced wastewater algae, (red circle) light and (black square) heavy petroleum crude,[12] (green triangle) coal,[12] and HTL biocrude from (white square) *Nannochloropsis* sp.,[8] (white triangle and orange circle) *Chlorella* sp.,[8,12] (white circle) *Porphyridium* sp.,[8] (purple diamond) *Enteromorpha prolifera*,[10] (purple square) *Spirulina* sp.,[8] (times sign) *Dunaliella tertiolecta*,[11] (plus sign) *Laminaria saccharina*,[9] and (blue diamond) *Desmodesmus* sp.[14]

closest to the *y* axis, are provided for comparison. Different coals are found closest to the *x* axis, and different HTL-produced biocrudes (from algae) are located throughout the chart. The circled data point is the biocrude produced in this work. As seen, the O/C ratio for this biocrude is the lowest (farthest left on the *x* axis) while still maintaining a high H/C ratio, which suggests that the least amount of heteroatom upgrading would be needed.

The measured HHV of the biocrude was 39 MJ kg^{-1} compared to the petroleum crude HHV of 41–43 MJ kg^{-1}. The large HHV is attributed to the high C and H and low O contents of the biocrude. Previous studies on *Spirulina* sp. and *Chorella vulgaris* have shown that adding alkali catalysts to HTL reactors results in lower oxygen content and larger HHV when compared to adding organic acids.[12] Furthermore, studies have shown that alkali catalysts are detrimental to oil formation when algae has a high starting initial lipid content but are

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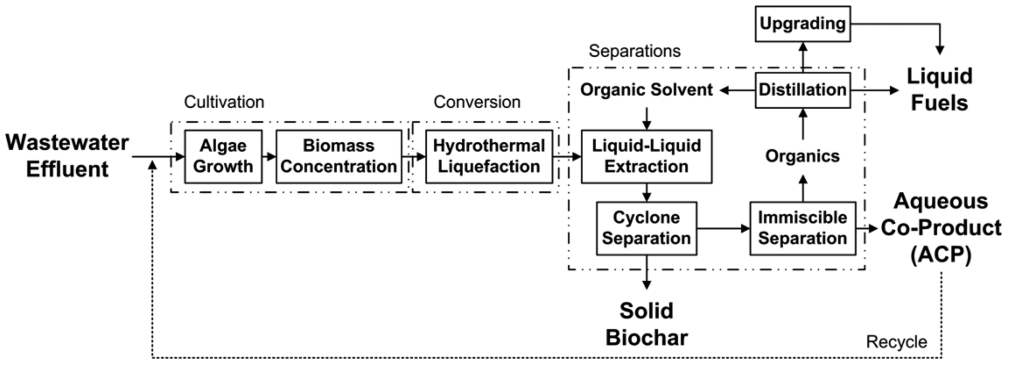

Figure 4. Potential block flow diagram of the continuous algal biofuel pathway.

advantageous when algae is high in carbohydrates.[8] On the basis of the elemental distribution of the inorganic composition of the cultivated algae, alkali species are present during the HTL reaction. While the exact role of the alkali species is unclear, it is possible that they are catalyzing reactions to reduce the final biocrude oxygen content. Thus, the higher ash of wastewater-fed algae could be advantageous for producing a biocrude with lower oxygen and larger HHV.

The HTL conversion of the cultivated algae resulted in an ACP containing ~50% of the cellular nitrogen of the algae, with a fraction of the cellular phosphorus. The concentrations of carbon (TOC), nitrogen (TN), and phosphorus (TP) in the ACP were measured at 4550 ± 460, 1640 ± 250, and 3.5 mg L^{-1}, respectively. It is speculated that the bulk of the algal phosphorus resides in the biochar. The macronutrient concentrations in the ACP compared to the wastewater effluent feed were 56:1 for TN and 1:1 for TP. The NRC report[1] has specifically listed the availability of N and P as a "high importance" concern for algal biofuel sustainability. Fortier and Sturm[23] have shown that wastewater treatment facilities alone would not supply adequate levels of nutrients to meet high demands of fuel consumption. The ability to capture N and P from HTL co-products and recycle them for algal cultivation would increase the potential of algal fuel production. Few studies to date have supplemented algal culture media with ACP from HTL processing. Jena et al.[39] reported an optimal recycle fraction of 0.2 vol % ACP diluted with deionized water achieving an algal growth rate of 0.035 g L^{-1} day^{-1} compared to a rate of 0.07 g L^{-1} day^{-1} for algae cultivated in BG 11 growth media. Zhou et al.[40] showed that recycling higher amounts of ACP from two HTL feedstocks (swine manure and *Spirulina* sp.) had inhibitory effects on algal growth. When the ACP was diluted with F/2 medium, they determined that algal growth was inhibited when supplemented with greater than 5 vol % swine manure ACP and 1 vol % *Spirulina* sp. ACP. Previous studies on *Spirulina* sp. and *C. vulgaris* used TOC analysis to show that a significant amount of the organic products are water-soluble.[12] Acetic acid, glycerol, and 3-pyridinol have been identified as the primary water-soluble organics in the ACP produced during HTL of *Enteromorpha prolifera* macroalgae.[10] Dependent upon the organics identified, it may be possible through separation to produce value-added organics and allow for higher levels of the ACP to be recycled for algae growth. Overall, further research is necessary to determine the recycle potential and value of the ACP produced from HTL.

Another major co-product of HTL conversion is biochar. On a dry weight basis, 45.0 ± 5.9% dw of biomass is retained in the form of solid biochar because of high amounts of non-combustible intercellular material. It may be assumed that the majority of biochar is comprised of the inorganic elements found in the feedstock algae, listed in Table 3b. The biochar is also 35 wt % organic, containing 20.4% dw carbon, as indicated in Table 7. This is reported as a weighted average of the two fractions of biochar produced, shown in Figure 1b, where the ultimate analysis differed by <2 wt % between fractions. The measured HHV of the biochar was 8−10 MJ kg^{-1}, depending upon which fraction of biochar was evaluated. Beyond burning the biochar for energy recovery, it could be used for soil amendments,[41] absorbents,[42] and catalysts.[43] Further research is ongoing to determine properties for the suitability of various applications.

3.3. Potential Algal Biofuel Production Pathway. A potential block flow diagram of a continuous algal biofuel operation is shown in Figure 4. Wastewater effluent provides water and nutrients for algal cultivation followed by biomass concentration to levels suitable for HTL. Following HTL conversion, if extraction of the biocrude is necessary, the separation from the ACP can be achieved using solvents that are generated during the HTL process, as demonstrated in this work. The biochar can be recovered using a cyclone and further processed for specific applications. The ACP and biocrude will separate because of immiscibility, allowing for the ACP to be recovered and recycled for algal cultivation. Finally, the biocrude produced can be distilled and upgraded to fuels and chemicals. This potential pathway begins to address some of the main concerns for economic and environmental sustainability of algal biofuels. The data collected in this study show a theoretical production rate of 6−9 barrels day^{-1} biocrude and >1500 kg day^{-1} biochar from available water and nutrients provided by the Lawrence, KS, municipal wastewater treatment plant. Although this scale of production would not be sufficient to meet the majority of the fuel demand for the city without the use of supplemental sources of water and nutrients, this proposed system allows for the wastewater treatment plant to both reduce nutrient loads in the previously discharged effluent while increasing revenue through sale of conversion products. Once algal cultivation is optimized with municipal wastewater feeds and ACP nutrient recycling, the production potential should increase.

4. CONCLUSION

The described algal cultivation strategy (open pond, wastewater-fed, naturally inoculated mixed culture) and HTL conversion (whole, wet biomass to produce a drop-in crude replacement and co-products) were successfully demonstrated. High yields of biocrude, 44.5% afdw, were obtained with

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superior quality compared to the biocrude from HTL of fertilized, monoculture microalgae. The biocrude approached a similar quality to petroleum crude oil in terms of ultimate analysis and energy content. The biocrude oil had over 10 wt % hydrogen, less than 6 wt % oxygen, and 80 wt % carbon, yielding a HHV of 39 MJ kg^{-1}. GC/MS analysis of the biocrude contained a significant number of straight-chain and branched hydrocarbons and mono- and polyaromatics in addition to fatty acids. Significant co-product formation was also observed with 45% dw biochar and >18% afdw ACP, with only 16% afdw converted to gas. The co-products could greatly enhance sustainability and the value chain for algal biofuels, adding markets in carbon sequestration, soil amendments, absorbents, and fertilizers. This promising demonstration requires further work upon optimizing the energy balance of the conversion method in conjunction with the cultivation strategy and determining the efficacy of the identified co-product markets.

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Notes

The authors declare no competing financial interest.

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