Chemical properties of biocrude oil from the hydrothermal liquefaction of *Spirulina* algae, swine manure, and digested anaerobic sludge

Derek R. Vardon [a,*], B.K. Sharma [b], John Scott [b], Guo Yu [c], Zhichao Wang [c], Lance Schideman [c], Yuanhui Zhang [c], Timothy J. Strathmann [a]

[a] Dept. of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, Newmark Lab, 205 N. Mathews Ave., Urbana, IL 61801, USA
[b] Illinois Sustainable Technology Center, University of Illinois at Urbana-Champaign, 1 Hazelwood Dr., Champaign, IL 61820, USA
[c] Dept. of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Agricultural and Engineering Science Building, 1304 West Penn. Ave., Urbana, IL 61801, USA

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ABSTRACT

This study explores the influence of wastewater feedstock composition on hydrothermal liquefaction (HTL) biocrude oil properties and physico-chemical characteristics. *Spirulina* algae, swine manure, and digested sludge were converted under HTL conditions (300 °C, 10–12 MPa, and 30 min reaction time). Biocrude yields ranged from 9.4% (digested sludge) to 32.6% (*Spirulina*). Although similar higher heating values (32.0–34.7 MJ/kg) were estimated for all product oils, more detailed characterization revealed significant differences in biocrude chemistry. Feedstock composition influenced the individual compounds identified as well as the biocrude functional group chemistry. Molecular weights tracked with obdurate carbohydrate content and followed the order of *Spirulina* < swine manure < digested sludge. A similar trend was observed in boiling point distributions and the long branched aliphatic contents. These findings show the importance of HTL feedstock composition and highlight the need for better understanding of biocrude chemistries when considering bio-oil uses and upgrading requirements.

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1. Introduction

Hydrothermal liquefaction (HTL) is a promising technology for converting wastewater biomass into a liquid fuel (Cantrell et al., 2007). HTL has been applied to a wide range of wastewater feedstocks, including swine manure, cattle manure, microalgae, macroalgae, and sludge (Suzuki et al., 1988; Dote et al., 1994; He et al., 2000; Brown et al., 2010; Xiu et al., 2010a; Yin et al., 2010; Biller and Ross, 2011). During HTL, water serves as the reaction medium, alleviating the need to dewater biomass which can be a major energy input for biofuel production. Elevated temperature (200–350 °C) and pressure (5–15 MPa) are used to breakdown and re-form biomass macromolecules into biofuel (Peterson et al., 2008), subsequently referred to as biocrude oil. Self-separation of the biocrude oil from water is then facilitated as the reaction solution returns to standard conditions (Peterson et al., 2008). The recovered biocrude oil can be directly combusted or upgraded to approach petroleum oils (Dote et al., 1991; Elliott, 2007; Duan and Savage, 2011a). While the ability of HTL to convert a wide-range of wastewater feedstocks provides a significant waste-disposal benefit, it also presents a major challenge for optimization and downstream processes due to the diverse biocrude oil chemistry that can result.

HTL biocrude oils contain a diverse range of chemical compounds which can include straight and branched aliphatic compounds, aromatics and phenolic derivatives, carboxylic acids, esters, and nitrogenous ring structures (Brown et al., 2010; Xiu et al., 2010a; Yin et al., 2010; Zhou et al., 2010; Biller and Ross, 2011). The class of compounds identified in HTL biocrude oils has been shown to be influenced by the ratio of protein, lipid, and carbohydrate fractions in the initial biomass feedstock (Biller and Ross, 2011). Biocrude oils are often characterized by high heteroatom contents, primarily in the form of oxygenated and nitrogenous compounds. The high heteroatom content is the main distinguishing factor separating bio-oils from petroleum crude oils (Huber et al., 2006; Peterson et al., 2008; Demirbas, 2009) and results in undesirable biofuel qualities such as oil acidity, polymerization, high viscosity, and high-boiling distribution (Adjaye et al., 1992; Speight, 2001). Furthermore, the diverse chemical composition of biocrude oil affects the combustion performance, storage stability, upgrading response, and economic value (Huber et al., 2006). Therefore, the objective of this study was to examine and compare the influence of wastewater feedstock composition for three feedstocks (*Spirulina* algae, swine manure, and anaerobic digested sludge) on the bulk properties and physico-chemical characteristics of HTL biocrude oil.

* Corresponding author. Tel.: +1 217 766 3916.
  E-mail address: dvardon2@illinois.edu (D.R. Vardon).

The algal species *Spirulina* was selected for HTL conversion due to increased interest in integrating algal cultivation into wastewater treatment from both a water resource and renewable energy perspective (Pittman et al., 2010). *Spirulina* can thrive in municipal and agricultural wastewater effluents and the filamentous cell structure facilitates harvesting (Kosaric et al., 1974). The high protein content can also be converted into HTL biocrude oil (Biller and Ross, 2011) and the ability to capture CO_2 can be used to reduce a treatment facility's environmental footprint (Packer, 2009). Additionally, HTL of low-lipid algae such as *Spirulina* can provide a comparison with contributions focused on HTL of high-lipid species (Dote et al., 1994; Brown et al., 2010), which tend to exhibit relatively low total lipid content when cultured in wastewaters (Pittman et al., 2010). Swine manure was chosen as a representative solid waste generated by agricultural livestock facilities. In the United States alone, livestock manure accounts for ∼250 million tons of dry solids annually (Xiu et al., 2010a) and land application of these wastes has been linked to the spread of hormones, pathogens, and nutrient runoff (Cantrell et al., 2007). Swine manure also contains a moderate lipid and high carbohydrate content to provide a distinct biochemical comparison to *Spirulina* algae. Anaerobically digested sludge was selected as the final feedstock to compare the effect of high obdurate carbohydrate content on biocrude oil yields and chemistry and evaluate HTL as an alternative to current predominant disposal practices: land application and landfilling. Currently, over 7 million tons of dry anaerobically digested sludge is produced annually with only 60% going towards beneficial use (US Environmental Protection Agency, 1999).

Advanced characterization methods were used to assess the molecular properties of biocrude oil and the influence of feedstock composition. Molecular-level characterization has been critical for designing engineering approaches to catalytically upgrade low-quality "heavy" petroleum oils (Speight, 2001) and will be necessary for transitioning to a biomass-based fuel infrastructure (Huber et al., 2006). Previous examples of molecular characterization used to better understand HTL chemistry include, but are not limited to, observing the conversion of carbohydrates into biocrude oil (Duan and Savage, 2011b), verifying the presence of aromatic hydrocarbons tied to increased bio-oil viscosity and density (Yin et al., 2010), determining the effects of reaction parameters (Xiu et al., 2010a; Yin et al., 2010) and catalyst addition (Duan and Savage, 2011b), and monitoring the deoxygenation of fatty acids through catalytic upgrading (Duan and Savage, 2011a). However, further understanding of the chemistry involved during HTL biomass conversion and upgrading process is needed to develop more efficient and sustainable biofuel and biochemical production methods (Huber et al., 2006).

In this study, both bulk properties (e.g., oil yield, elemental analysis, and heating value) and physico-chemical characteristics (e.g., molecular constituents, functional group allocation, proton and carbon speciation, molecular weight distribution, and boiling point distribution) of biocrude oils produced by liquefaction of different wastewater feedstocks were compared with each other as well as published results for petroleum crudes and tar sand bitumens. Low-boiling compounds were identified by gas chromatography–mass spectroscopy (GC–MS), functional group compositions were examined with Fourier Transform infrared (FTIR) and nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopies, and the molecular weight and boiling point distributions were analyzed with size exclusion chromatography (SEC) and simulated distillation (Sim-Dist), respectively. To our knowledge, this is the first study to compare the chemical characteristics of biocrudes generated from disparate wastewater feedstocks under identical HTL conditions.

2. Methods

2.1. Feedstock sources and characterization

Spirulina algae (solids content of 95%) were obtained in dry-powder form from Cyanotech (Kailua-Kona, Hawaii). Swine manure (solids content of 27%) was sampled from grower-finisher pen floors at the Swine Research Center at the University of Illinois at Urbana-Champaign. The pens contained a partially slotted floor for manure collection. Both the swine manure and algal samples were stored at 4 °C prior to processing. Digested anaerobic sludge (solids content of 26%) was collected from the outlet of an anaerobic digester at the Urbana-Champaign Sanitary District wastewater treatment plant (Urbana, IL) and was processed immediately to avoid compositional decay.

Forage analysis of feedstock samples (Midwest Laboratories; Omaha, NE) was conducted to determine crude protein, crude lipid, neutral detergent fiber (cellulose, lignin, and hemicellulose), acid detergent fiber (cellulose and lignin), lignin and ash content. Crude protein was determined by combustion method (AOAC 990.03). Crude lipids were measured gravimetrically after ether solvent extraction (AOAC 945.16). Ash content, representing the inorganic fraction of the feedstock, was determined by heating the sample at 600 °C to remove all organic carbon (AOAC 942.05). Total carbohydrates were calculated by subtraction. Carbohydrates were also subdivided into several categories that were measured separately and included: neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin fractions by measuring the organic residue remaining in an ANKOM Technology filtration apparatus after digestion. However, due to differences in the sample preparation and analysis procedures for individual component assays, results varied for each fraction (e.g., swine manure NDF > total carbohydrates).

2.2. HTL conversion

Samples were converted into biocrude oil under hydrothermal conditions (300 °C, 10–12 MPa, and 30 min retention time) in single runs for each feedstock using a Parr 4500 2-L reactor. Approximately 800 g of feedstock slurry adjusted to 20% solid content (w/w) was loaded into the reactor vessel, which was then sealed and purged with nitrogen three times to displace headspace gases and charged to an initial pressure of 0.65 MPa. Electric resistance heating was then used to raise the temperature to 300 °C and the feedstock was continuously mixed by a magnetic drive agitator. After a 30-min reaction time, the reactor was cooled rapidly to room temperature by circulating water through a cooling coil located inside the reactor. Reactor headspace gases were then carefully released into a gas collection assembly and the liquid and solid phases were removed from the reactor. The non-aqueous liquid fraction, which also contained visible solid residue, was extracted into dichloromethane (DCM) solvent using a separatory funnel. Deionized water (>18 MΩ) was added to the funnel to form a bi-layer, and the DCM-soluble portion was vacuum filtered (Whatman No. 44 ashless filter paper) to remove particulate matter. The DCM was then removed under a nitrogen stream at 40 °C. The focus of this study was on characterization of the DCM-soluble biocrude phase, so the gas, aqueous, and solid phases were not further characterized.

2.3. Biocrude oil characterization

2.3.1. Bulk characterization

Biocrude oil yield was determined gravimetrically and calculated as the ratio of the DCM-soluble biocrude mass to dry

feedstock organic mass (i.e., total dry mass minus ash content) (Suzuki et al., 1988; Dote et al., 1994; Yin et al., 2010; Biller and Ross, 2011). Elemental analysis was conducted at the University of Illinois Microanalysis Laboratory (Urbana, IL). Samples were processed for total carbon/hydrogen/nitrogen using an Exeter Analytical CE-440 Elemental Analyzer. Sulfur was measured by ICP-OES in axial mode (PerkinElmer Optima 2000DV) after digesting samples (PerkinElmer Multiwave 3000 Digester). Oxygen was assumed to account for the bulk of the remaining sample mass balance. Higher heating value (HHV; MJ/kg) was estimated using Dulong's formula (Brown et al., 2010; Zhou et al., 2010; Biller and Ross, 2011; Duan and Savage, 2011a, 2011b):

$$HHV = 0.3383C + 1.422\left(H - \frac{O}{8}\right) \tag{1}$$

where C, H, and O are the mass percentages of carbon, hydrogen and oxygen, respectively.

2.3.2. GC–MS

Separation was achieved with a Varian VF-5 ms phenyl–methyl GC column (30 m × 0.25 mm id, 1.0-μm film) using He carrier gas with flow rate of 1 mL/min. One microlitre of DCM extracts (2 wt.% biocrude) were injected at 270 °C with a split ratio of 30:1. The oven temperature was initially set to 60 °C with a hold time of 4 min, then increased at 5 °C/min until 280 °C and held constant for 15 min. The source temperature was 210 °C, electron ionization was set at 70 eV, and spectra were scanned from 35–550 m/z (0.2 s per scan and interscan delay of 0.1 s). Individual low molecular weight products were identified by matching fragmentation patterns against a NIST database. Before peak integration, the total ion chromatogram was baseline corrected and signal noise was filtered using Matlab software.

2.3.3. FTIR and NMR

Additional functional group information was derived from FTIR and NMR spectroscopic data. FTIR spectra were collected using a Thermo Nicolet Nexus 670 Fourier Transform Infrared Spectrophotometer equipped with a single-bounce diamond attenuated total reflectance (ATR) accessory (Specac Golden Gate) and KBr beam splitter. Spectra were collected from 4000 to 525 cm^{-1} with 0.98-cm^{-1} resolution and averaged over 50 replicate scans using Omnic software. Background scans were conducted of the dry accessory at ambient temperature. Biocrudes were applied in thin films and allowed to dry to remove any trace solvent. The spectra were then collected after smearing ~30 mg of sample directly on the ATR crystal surface.

^{1}H NMR spectra were collected using a Varian Unity 400-MHz spectrometer outfitted with a 5-mm broadband probe. Samples (2.5% w/w) were prepared by dissolving 50–75 mg of biocrude oil in deuterated chloroform containing 0.03% tetramethylsilane (TMS) as an internal reference. Samples were then filtered (0.45-μm PTFE) to remove any suspended particulates before loading into 5 mm diameter NMR tubes. [1]H spectra were acquired with a 90° pulse angle, spinner frequency of 20 Hz, sweep width of 8000 Hz across 32 transients.

^{13}C NMR spectra were collected using a Varian Unity 600-MHz spectrometer outfitted with a 10-mm broadband probe. Samples were prepared at concentration of 7–10% (w/w) in deuterated chloroform with 0.03% TMS and filtered (0.45 μm PTFE) before loading into 10 mm diameter NMR tubes. Spectra were acquired with a 90° pulse angle at 25 °C with a relaxation time of 7 s and gated proton decoupling. Spectra were accumulated for >3600 transients to maximize the S/N ratio. Signal processing of NMR spectra was performed with NutsPro NMR Utility Transform Software Professional. Processed NMR spectra were exported to Matlab for integration

based on functional group designations for ^{1}H and ^{13}C NMR peaks according to Mullen et al. (2009). The signal area of residual dichloromethane solvent (5.32 ppm for ^{1}H NMR; 54 ppm for ^{13}C NMR) and trace protons from deuterated chloroform (7.26 ppm for ^{1}H NMR; 77 ppm for ^{13}C NMR) were excluded during spectrum integration.

2.3.4. SEC analysis

Molecular weight distributions were determined by SEC. Analysis was conducted using a Waters 2414 RI detector, Styragel HR1 SEC column (7.8 × 300 mm), Waters 600-MS System controller connected to a 600-mulitsolvent delivery system, and 717-plus autosampler connected to a Dionex U120 Universal Interface. Retention time calibration was obtained using six polystyrene standards with peak molecular weights of 250, 600, 1000, 1700, 2500, and 7000 Da (Polysciences Inc.). Biocrude samples (3% w/w) were dissolved in tetrahydrofuran and filtered (0.45-μm PTFE) to remove suspended particulates. The pump flow rate was 1.0 mL/min with THF as the mobile phase and injection volumes were set to 50 μL. The resulting chromatographic data was processed using Matlab according to the equations below. Cumulative signal intensities were normalized to unity between samples to allow for relative comparisons. The number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index were calculated based on component molecular weights (M_i) determined from the retention time calibration curve and signal intensities (N_i):

$$M_n = \frac{\sum M_i N_i}{\sum N_i} \tag{2}$$

$$M_w = \frac{\sum M_i^2 N_i}{\sum M_i N_i} \tag{3}$$

The polydispersity index (PDI), which is indicative of the spread in the molecular weight distribution of the biocrude oil constituents, was then calculated according to:

$$PDI = \frac{M_w}{M_n} \tag{4}$$

2.3.5. Sim-Dist analysis

Simulated distillations were modeled after ASTM-7169-05 method and performed using a HP 5890 Series II FID gas chromatograph and a Durabond DB-HT-SimDis GC column by Agilent-J&W Scientific (5 m × 0.53 mm id, 0.15 μm film). Helium (18 mL/min) was used as the carrier gas. The oven temperature was initially set to 0 °C, using CO_2 cryogenic cooling to improve solvent separation from low-boiling peaks, and raised to 420 °C at 10 °C/min and then held constant for 10 min. The injector volume was set to 1μL and the injector temperature was oven-tracked and followed the oven temperature program. Detector temperature was set to 450 °C, hydrogen gas set to 32 ml/min, airflow set to 400 ml/min, and nitrogen makeup set to 24 ml/min. Samples (1% w/w) and reference standards (0.5% w/w) were dissolved in DCM rather than the carbon disulfide outlined in the ASTM method due to improved biocrude oil solubility. Samples were filtered (0.45-μm PTFE) to remove any suspended particulates. Boiling points were determined by comparison against a series of reference standards purchased from Accustandard, including a DRH-2887 calibration mix, DRH-008S calibration mix, decane, hexadecane, PolyWax 850 and D-6352 Reference Gas Oil. Data were collected at a sampling rate of 1 Hz using Varian-STAR software and signal integration and distribution analysis was performed using MATLAB. For the Sim-Dist analysis, FID detector intensities were assumed to be directly proportional to the weight percent of sample eluted. The boiling

point–retention time relationship is known to be influenced by chemical structure and heteroatom content. Therefore, the Sim-Dist analysis of biocrude samples can only be considered a rough approximation since the heteroatom contents of the biocrude volatile components are significantly different from the mixture of *n*-alkane reference compounds used for calibration.

3. Results and discussion

3.1. Feedstock composition

Forage analysis of the feedstocks indicated that *Spirulina* had the highest overall organic matter content on a dry weight basis (90%) compared to swine manure (84%) and digested sludge (69%) as shown in Table 1. The organic matter in *Spirulina* was comprised primarily of crude protein (64%) with relatively smaller amounts of crude lipids (5%) and fibrous carbohydrates (2%) accounted for in the neutral detergent fiber (NDF) fraction (e.g., lignin, cellulose, and hemicellulose). *Spirulina* had a much lower crude lipid content than other microalgal species examined for HTL biocrude oil production such as *Chlorella* (25%) (Biller and Ross, 2011), *Nannochloropsis* (28%) (Brown et al., 2010), and *Botryochoacus brauni* (50%) (Dote et al., 1994). In contrast, swine manure had a more uniform nutritional composition with a distribution of NDF (44%), crude protein (25%) and crude lipids (22%). Lastly, digested anaerobic sludge consisted primarily of NDF (51%) and crude protein (42%) with very low crude lipid content (<1%) and a much higher acid detergent fiber fraction (ADF) (36%), representative of lignin and cellulose that are poorly biodegradable during wastewater treatment processes (Jain et al., 1992).

3.2. HTL conversion

3.2.1. Biocrude oil yields

The varying feedstock compositions were reflected in the HTL biocrude oil yields calculated on an ash-free basis as shown in Table 2. *Spirulina* had the highest biocrude oil yield (32.6%), with swine manure having a moderately lower yield (30.2%), despite having much higher initial lipid content (22%) than *Spirulina* (5%). Previous research has identified the following general trend for conversion efficiency: lipids > protein > carbohydrates (Biller and Ross, 2011). The slightly lower biocrude oil yield of swine manure in comparison to *Spirulina* may be explained by the higher carbohydrate content of manure, which has a lower conversion efficiency compared to protein and offsets the higher lipid content of the manure feedstock. We suspect that low carbohydrate conversion efficiency is related to higher hemicellulose and lignin content, the latter of which has been shown to decrease HTL oil yields (Zhong and Wei, 2004). Digested anaerobic sludge provided much lower yield (9.4%), even after accounting for the higher ash content, and may be attributed to the larger carbohydrate content with low conversion efficiency due to higher hemicellulose and lignin.

Direct comparison of HTL biocrude yields can be difficult due to varying conversion conditions (e.g., reaction temperature,

Table 1
Forage analysis of wastewater biomass feedstocks for HTL.

Forage analysis (%)	*Spirulina* algae	Swine manure	Anaerobic sludge
Crude protein	64	25	15
Crude lipid	5	22	<1
Total carbohydrates	21	37	54
NDF	2	44	51
ADF	1	14	36
Lignin	<1	3	10
Ash	10	16	31

Table 2
Conversion yields and bulk properties of HTL biocrude oil obtained from different wastewater feedstocks.

Feedstock	Oil yield%	C%	H%	N%	O%	S%	HHV (MJ/kg)
Spirulina algae	32.6	68.9	8.9	6.5	14.9	0.86	33.2
Swine manure	30.2	71.2	9.5	3.7	15.6	0.12	34.7
Anaerobic sludge	9.4	66.6	9.2	4.3	18.9	0.97	32.0

retention time, headspace gas, agitation, solids loading) that can significantly impact yields (Yin et al., 2010). However, yields with *Spriulina* obtained in this study were comparable to those reported by Biller and Ross (2011) (29%) and lower than those of moderate-to-high lipid species such as *Chlorella* (36%) (Biller and Ross, 2011), *Nannochloropsis* (43%) (Brown et al., 2010), and *B. brauni* (~55%) (Dote et al., 1994). The swine manure biocrude oil yield was somewhat higher compared to a recent study by Xiu et al. (2010a) (25%) and considerably lower than results previously reported by members of our group (54%) (He et al., 2000), with differences likely due to varying manure compositions, conversion parameters, and bio crude oil recovery methods (e.g., solvent selection, heating, and recirculation). Lastly, biocrude yield with digested sludge was significantly lower compared to values reported by Suzuki et al. (1988) (25%), which may be due to disparate feed compositions or differing extents of digestion before disposal.

These results point to ability of HTL to convert high organic content feedstocks into biocrude oil, regardless of lipid content, through the partial utilization of both protein and carbohydrate fractions (Biller and Ross, 2011). This holds promise for utilizing low-lipid algae, such as *Spirulina*, that can be cultivated in wastewater environments and processed alongside raw sewage solids and bacterial sludges. Furthermore, low-lipid algal species tend to have faster growth rates in a wastewater environment compared to high-lipid algae (Becker, 1994). Swine manure biocrude oil yields were also significant, warranting further investigation into the potential environmental and economic benefits of HTL compared to typical land application practices. HTL of digested anaerobic sludge did not yield much oil, suggesting the need to continue current disposal methods. Alternatively, wastewater sludges collected earlier in the treatment system (e.g., undigested primary or secondary sludge) can produce higher HTL biocrude oil yields due to higher lipid and protein contents, but comes with the tradeoff of diverting organic substrate currently used for producing methane during anaerobic digestion.

3.2.2. Elemental analysis and higher heating value

Despite the varying biocrude oil yields, bulk elemental analysis (Table 2) resulted in only small differences in estimated higher heating values (HHV) of the product oils, ranging from 32.0 to 34.7 MJ/kg. Previous HTL reports with microalgae, manure, and sludge feedstocks show a somewhat wider range of HHVs (25–39 MJ/kg) (Suzuki et al., 1988; Brown et al., 2010; Xiu et al., 2010a; Yin et al., 2010; Biller and Ross, 2011), with the exception of the high-hydrocarbon algal species *B. brauni* (45.9 MJ/kg) (Dote et al., 1994). Petroleum crude oils, in contrast, can range from 41 to 48 MJ/kg (Speight, 2001). The lower HHV values of the wastewater biocrudes are a byproduct of the much higher nitrogen (3.7–6.5%) and oxygen (14.9–18.9%) contents in comparison with petroleum crudes (Speight, 2001) and North American tar sand bitumens (Bunger et al., 1979), which typically have less than 2.0% of each element. The high protein content of *Spirulina* carried over into the biocrude which had the highest nitrogen content (6.5%) of the three oils. Anaerobic sludge, with its high ADF content (lignin and cellulose), produced a biocrude with the greatest oxygen content (18.9%) and lowest HHV. In contrast to the oxygen and

nitrogen data, the sulfur content of the HTL biocrudes were relatively low (<1%) compared to many petroleum crudes, which can range from 0.05% to 6.0% (Speight, 2001). The high oxygen and nitrogen content is a primary factor that distinguishes biocrude oils from petroleum crudes, and upgrading processes for lowering heteroatom content represent a significant challenge to the biofuel industry.

3.3. Physico-chemical characterization

3.3.1. Gas chromatography–mass spectroscopy

In contrast to bulk elemental analysis, chemical characterization of HTL biocrude oils from different feedstocks exhibited a marked degree of variation. With GC–MS, >40 compounds were identified for each biocrude sample that comprised >50% of the total ion chromatogram (TIC) peak area. Compounds that represented >1% of the total peak area of the chromatograms are listed in Table 3 with spectra and identified peaks provided in the Supplemental Information (Fig. S1). It should be noted that only a fraction of the products formed by HTL are identifiable by GC–MS due to the high molecular weights and boiling point distributions of the biocrude (see sections below) and the temperature limit of the instrument (maximum boiling point detected ~350 °C). Furthermore, some low boiling point compounds may have been masked by the solvent peak or lost when evaporating the DCM used to recover the biocrude oil.

The high protein content of *Spirulina* produced biocrude oil that contained a high percentage of nitrogenous compounds, similar to findings by Biller and Ross (2011). The most prevalent peak in the spectrum was O-decylhydroxylamine (11.8% of the total ion chromatogram), which may result from the decomposition of proteins, followed by tridecene (4.7%), dimethyl-dioxaspiro-undecanone, (4.6%), and trimethyl-dodecanol (4.0%). Strikingly, no straight-chain hydrocarbons were identified among the major compounds, though several minor hydrocarbons were identified, including pentadecene (0.5%), heptadecene (0.9%), and docecane (0.4%). The most prevalent compound, O-decylhydroxylamine, contained a 10-carbon chain backbone with an amine moiety, which may be derived from the decarboxylation and rearrangement of amino acids that can occur under HTL conditions (Peterson et al., 2008). Catalytic upgrading can be used to remove nitrogen through hydrodenitrogenation (HDN) and limit regulated NO_x emissions during biofuel combustion. However, due to their basicity, nitrogen compounds are highly problematic for conventional hydro-treatment catalysts since they strongly adsorb to active acidic sites and require more severe treatment conditions (Furimsky and Massoth, 1999).

The swine manure feedstock, with a more balanced distribution of bulk carbohydrates and crude lipids, produced biocrude oil that contained a mix of phenolic- and lipid-derived compounds, similar to findings by Xiu et al. (2010b). The major GC–MS peaks in the swine biocrude were identified as pentanoic acid (11.1%), ethyl-phenol (10.1%), hexanoic acid (5.2%), methylphenol, (3.9%) and cholesterol compounds (2.5%). The phenol compounds are likely derived from the carbohydrate and protein fraction, which have been identified during the HTL degradation of lignin (Demirbas, 2000) and model protein compounds (Biller and Ross, 2011). Similarly, the fatty acid and cholesterol compounds (2.5%) are likely derived from the crude lipid fraction, with fatty acids being identified during the decomposition of triglycerides under HTL conditions (Peterson et al., 2008; Biller and Ross, 2011). The crude protein in the manure also led to nitrogenous compounds, including succinimide, indole, and pyrazine, however to a lesser extent (<2% peak area) than for *Spirulina* biocrude. The oxygen-rich compounds are of particular concern since they can polymerize, leading to storage instability (Adjaye et al., 1992). Profiling of the

oxygenated compounds prior to application of hydrodeoxygenation (HDO) upgrading processes is beneficial, since hydrogen consumption and operation severity are highly dependent on the concentration and structure of oxygenated compounds present in oil (Furimsky, 2000).

The digested anaerobic sludge feedstock, which was mainly comprised of ADF (cellulose and lignin) and crude protein, resulted in biocrude oil containing a high percentage of ester, phenolic and nitrogenous compounds. To our knowledge, no GC–MS information has previously been reported on digested sludge biocrude oil. The major compounds identified were dodecyl acrylate (3.8%), benzoylformic acid (3.5%), phenol (2.9%), and O-decylhydroxylamine (2.7%). Interestingly, no compounds were identified with a peak area >4% in the sludge biocrude. Cholesterol derivatives were also observed despite a very low crude lipid content (<1%) measured in the initial feedstock.

The high heteroatom compounds that comprised the major classes for all HTL biocrudes are in stark contrast to the aliphatic and aromatic compounds that predominate in GC–MS analysis of petroleum crude oil (Speight, 2001). As mentioned previously, the high heteroatom content of HTL compounds is a major concern, particularly in the low boiling fraction that is traditionally used for the transportation-grade fuel production.

3.3.2. FTIR spectroscopy

FTIR analysis of the biocrude oils (spectra provided in Fig. S2, Supplemental Information) allowed for a more comprehensive comparison of "whole" oil functional group characteristics when compared to GC–MS, with spectral band assignments and interpretation based on previous studies (Xiu et al., 2010a,b; Yin et al., 2010; Zhou et al., 2010; Duan and Savage, 2011b). Similar to elemental analysis, the high carbon and hydrogen content of HTL biocrude oil produced prominent C—H stretch (3000–2840 cm^{-1}), CH$_2$ bending (1465 cm^{-1}), and CH$_3$ bending (1375 cm^{-1}). Significant heteroatom functionality (1730–1150 cm^{-1}) was also observed for all biocrude samples as expected from the elemental analysis and GC–MS characterization. The high protein content of *Spirulina* algae produced biocrude oil with prominent N—H bending peaks (1680–1600 cm^{-1} and 1575–1525 cm^{-1}), consistent with nitrogenous compounds identified by GC–MS. In contrast, the moderate lipid and fibrous carbohydrate content of swine manure produced biocrude oil with strong C=O stretch (1730–1700 cm^{-1}), C—O stretch (1320–1210 cm^{-1}), and C—O alcohol stretch peaks (1260–1000 cm^{-1}), consistent with the fatty acids/esters and carbohydrate derivatives identified by GC–MS and previous FTIR peaks identified in HTL biocrude derived from swine manure (Xiu et al., 2010a,b). Lastly, digested sludge had the highest ADF (lignin and cellulose) and produced biocrude oil with C—O stretch peaks (1320–1210 cm^{-1}). The moderate protein of sludge was also reflected in the biocrude with weaker N—H bending peaks (1680–1600 cm^{-1}) compared to those found in oil produced from *Spirulina*. The biocrude oil spectra are distinct from spectra reported for conventional petroleum crudes (Wilt et al., 1998) and heavy asphaltic crudes (Akrami et al., 1997), which display stronger alkane peaks (3000–2800 cm^{-1}) and exhibit weaker heteroatom associated peaks congruent with their higher hydrocarbon and lower heteroatom contents.

3.3.3. NMR spectroscopy

NMR spectra (Figs. S3 and S4, Supplemental Information) provided complementary functional group information to FTIR spectra and the ability to quantify and compare integration areas between spectra. Similar to FTIR, ^{1}H NMR spectra showed a high percentage of aliphatic functional groups for all HTL biocrude oils; a summary of integrated peak area regions assigned to different functional group classes is provided Fig. 1a. The swine manure

Table 3
Major compounds representing >1% of the GC–MS total ion chromatogram areas from HTL biocrude oils generated from different wastewater feedstocks.

No. of ID	Spirulina algae			Swine manure			Anaerobic sludge		
	RT (min)	Compound	Area (%)[a]	RT (min)	Compound	Area (%)[a]	RT (min)	Compound	Area (%)[a]
1	6.9	Phenylglyoxal	2.7	7.3	Pentanoic acid	11.1	6.9	Benzoylformic acid	3.5
2	7.3	Phenol	3.0	7.3	Hexanoic acid	5.2	7.3	Phenol	2.0
3	10.3	Phenol, 4-methyl	2.5	8.0	Pyrazine	1.3	10.3	Phenol, 4-methyl	2.9
4	13.1	Phenol, 4-ethyl	1.1	10.3	Phenol-4 methyl	3.9	13.1	Phenol, 4-ethyl	2.0
5	16.8	Indole	1.8	10.6	Mequinol	2.8	19.3	4-Methylindole	1.3
6	18.5	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	1.6	13.1	Phenol, 4-ethyl	2.9	21.5	1-Tridecanol	2.2
7	19.1	2,5-Pyrrolidinedione, 1-propyl	3.1	16.2	Phenol, 4-ethyl-2-methoxy-	10.1	22.1	Hydroxylamine, O-decyl-	2.7
8	19.4	1,5-Dioxaspiro[5.5]undecan-9-one, 3,3-dimethyl-	4.6	19.1	N-2-Hydroxyethyl-succinimide	1.4	23.4	7-Tetradecene	1.7
9	21.6	Benzonitrile, 2,4,6-trimethyl-	1.0	19.3	[1]H-Indole, 4-methyl	1.1	26.6	Dodecyl acrylate	3.8
10	26.7	Hydroxylamine, O-decyl-	11.8	21.9	1-Hexadecanol	1.3	45.7	Cholest-4-ene	2.2
11	29.2	1-Dodecanol, 3,7,11-trimethyl-	1.0	26.6	2-Propenoic acid, tridecyl ester	3.2	46.1	Cholest-2-ene	1.5
12	29.5	1-Dodecanol, 3,7,11-trimethyl-	4.0	45.7	Cholest-4-ene	1.3	46.3	Cholest-7-ene, (5.alpha.)-	1.3
13	29.7	5-Tridecene, (Z)-	4.7	48.3	5.alpha.-Ergost-8(14)-ene	1.2			

[a] Area represents percent of total ion chromatogram.

and sludge-derived biocrude contained the highest percentage (68–69%) of alkane functional groups (0.5–1.5 ppm). The high alkane content may be attributed to the fatty acids and alkanes in swine manure biocrude, derived from decomposition of feedstock triglycerides under HTL conditions (Peterson et al., 2008; Biller and Ross, 2011), and the high percentage of aliphatic esters identified in the sludge biocrude GC–MS spectra. *Spirulina* biocrude exhibited the lowest alkane functionality but the highest percentage (53%) of α-to-heteroatom/unsaturated functionality (1.5–3.0 ppm), possibly due to the large number of nitrogenous and oxygenated compounds derived from the feedstock's high protein that have been shown to resonate in this area (Mullen et al., 2009; Zhou et al., 2010). All biocrudes displayed a low percentage (<2%) of methoxy/carbohydrate functionality (4.4–6.0 ppm), which is consistent with carbohydrates converting into biocrude oil (Duan and Savage, 2011b) or partitioning into the aqueous phase (Anastasakis and Ross, 2011). In general, the range of alkane percentages for all feedstocks (53–69%) was similar to prior ^{1}H NMR analyses of HTL biocrudes produced from the microalgae *Nannochloropsis* (Duan and Savage, 2011b) and the macroalgae *Enteromorpha prolifera* (Zhou et al., 2010). Aromatic/hetero-aromatic functionality was also observed in all biocrude oils (6.0–8.5 ppm) in agreement with findings from FTIR. Aldehyde functionality (9.5–10.0 ppm) was absent from all samples despite the observed C=O functional groups (1730–1700 cm^{−1}) by FTIR. The appearance of such FTIR bands can also be due to other carbonyl-bearing groups like protonated carboxylic acids, carboxylic acid esters, amides, and ketones.

^{13}C NMR spectra provided greater detail due to their large chemical shift regions and pointed to a high aliphatic content (0–55 ppm) for all biocrude oils as shown in the integrated peak area regions in Fig. 1b. All samples displayed greater than 65% total aliphatic carbon with sludge biocrude containing the highest proportion (76%), followed by swine manure (71%) and algae (66%) biocrude. Aliphatics were further subdivided into short (0–28 ppm) and long-branched aliphatics (28–55 ppm), with *Spirulina* biocrude containing the highest proportion of short aliphatics (27% of total spectrum area) and sludge biocrude containing the highest proportion of long-branched aliphatics (54%). All spectra also contained a high proportion of aromatic and olefin carbon assignments (95–165 ppm) which was evident in the FTIR spectra (1600 and 1475 cm^{−1}). The aromatic-olefin ranking between biocrudes was

in the opposite order of the total aliphatics, with the *Spirulina* biocrude containing the highest percentage (32%), followed by swine manure (19%) and sludge biocrude (18%). This ranking was reflected in α-to-heteroatom/unsaturated functionality determined by ^{1}H NMR (1.5–3.0 ppm) and followed a similar order (*Spirulina* algae > swine manure ∼ digested sludge). Low percentages of alcohol/ethers/carbohydrates (55–95 ppm) were also observed in all ^{13}C NMR spectra, prompting further inquiry into the extent that carbohydrates in the feedstock are converted into biocrude oil (Duan and Savage, 2011b) or partition into the aqueous phase (Anastasakis and Ross, 2011). Ketones/aldehydes were also present in low amounts, similar to findings with HTL algal biocrude oil (Duan and Savage, 2011b). Although minor, swine manure biocrude contained the highest percentage of esters/carboxylic acids (165–180 ppm), consistent with the fatty acids identified by GC–MS and similar to findings with the moderate lipid algal species *Nannochloropsis* (Duan and Savage, 2011b).

Direct comparison of HTL biocrude with conventional petroleum sources is rather difficult due to differing reported spectra integration ranges; however, the work conducted by Gupta et al. (1986) with Bombay petroleum crude and synthetic coal-derived oil is rather revealing. These authors reported that Bombay petroleum crude ^{13}C NMR spectra area contained 87% total aliphatic content (9–60 ppm) while the synthetic coal-derived oil only displayed 56% of the spectra area, placing HTL biocrudes in between the two sources.

3.3.4. SEC analysis of molecular weight

The molecular weight (MW) distributions measured by SEC (Fig. 2) further emphasize the distinctive properties of HTL biocrudes produced from various feedstocks. The weight-average molecular weights (M_w), which are more heavily biased by high MW molecules in the oil mixtures, were similar for biocrudes generated from *Spirulina* (1870 Da) and swine manure (1890 Da), but significantly higher for the digested sludge biocrude (3470 Da) (Table 4). The number-average molecular weights (M_n), which are more biased by low MW molecules, followed a similar trend with *Spirulina* (890 Da) and swine manure biocrude (780 Da) exhibiting similar values, and sludge biocrude having a much higher value (1340 Da). The HTL biocrude MWs were significantly higher than that of conventional petroleum crudes ($\sim M_n$ 250) (Speight, 2001) while *Spirulina* and swine manure biocrude fell within the
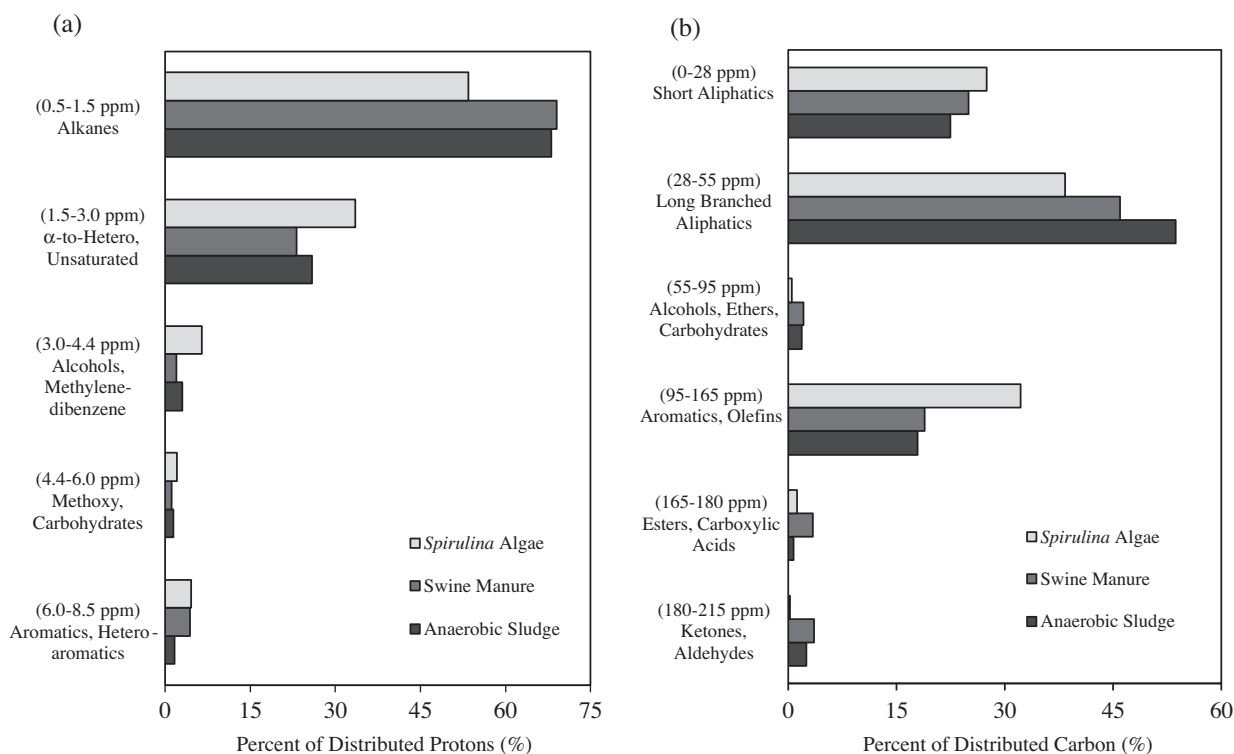
Fig. 1. ^{1}H NMR (a) and ^{13}C NMR (b) spectral distribution of functional groups present in biocrude oils produced from different wastewater feedstocks based on integrated peak areas assigned to characteristic spectral regions.
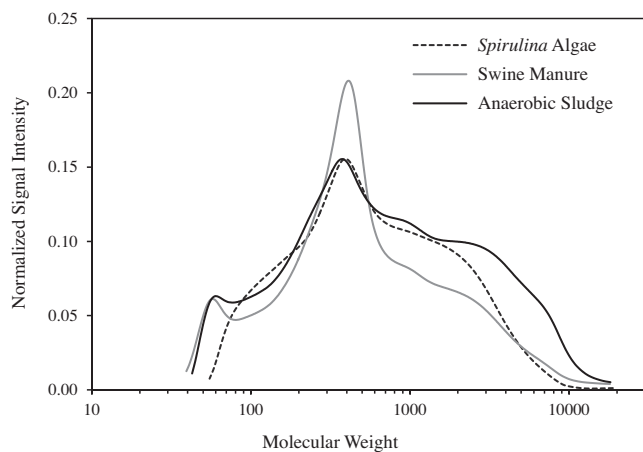

Fig. 2. SEC molecular weight distributions of HTL biocrude oils produced from different wastewater feedstocks.

upper-range of North American tar sand bitumens (M_n 538–820) (Bunger et al., 1979).

Polydispersity values (PDI) are indicative of the spread in the molecular weight distribution, with higher PDI values correlating to greater differences in the minimum and maximum molecular weights from the average molecular weight. Digested sludge biocrude exhibited the largest spread in molecular weights (PDI = 2.59) followed by swine manure (PDI = 2.42) and *Spirulina* biocrude (2.10). The high MW and PDI of sludge biocrude are consistent with the high oxygen content derived from polymer-linking functional groups (e.g., esters, ethers) (Adjaye et al., 1992). These parameters also tracked with the percentage of long-chain branched aliphatics identified by ^{13}C NMR and were closely tied

to the boiling point distributions discussed in the following section.

3.3.5. Simulated distillation (Sim-Dist)

Sim-Dist was used to estimate the boiling point distribution for HTL biocrudes (Fig. 3), which fell predominantly within the range of heavy vacuum gas oil (343–538 °C). *Spirulina* biocrude had the greatest percentage (30%) of low boiling point compounds (bp < 343 °C), corresponding to heavy naphtha, kerosene, and gas oil fractions. In contrast, sludge-derived biocrude had the highest percentage (21%) of high boiling point compounds (bp > 538 °C), corresponding to vacuum residua. Swine manure biocrude fell in between, with the greatest percentage (69%) of mid-boiling point compounds (bp 343–538 °C) and the lowest percentage of the other two categories. The order of bulk boiling fraction was similar to the order of weight-average molecular weights (MW_w) and the percentage of long-branched aliphatics identified by ^{13}C NMR (*Spirulina* algae < swine manure < digested sludge).

The compound boiling point-retention time relationship for Sim-Dist is a function of structure and heteroatom content, so only qualitative comparisons can be made since the Sim-Dist data was calibrated using *n*-alkane standards. It is notable that similar trends were observed for SEC and Sim-Dist data. For example, the vacuum residua fraction (bp > 538 °C), corresponding to *n*-alkane compounds greater than or equal to *n*-$C_{43}H_{88}$ (604 Da), follows the order of biocrudes derived from swine manure < *Spirulina* algae < anaerobic sludge. Analogously, the MW distribution percentage between 604 and 11,000 Da follows the same trend. The distillable fraction of HTL biocrude oils (bp < 538 °C) ranged from 77% to 83% and is comparable to conventional petroleum crude oil sources that vary by region (e.g., 66% distillable for Venezuelan crude, 98% for Pennsylvania crude) (Speight, 2001). In addition, it is greater than the distillable fraction of North American tar sand bitumens (44–65%) (Bunger et al., 1979).

Table 4
Molecular weight distribution parameters of HTL biocrude oil from different wastewater feedstocks.

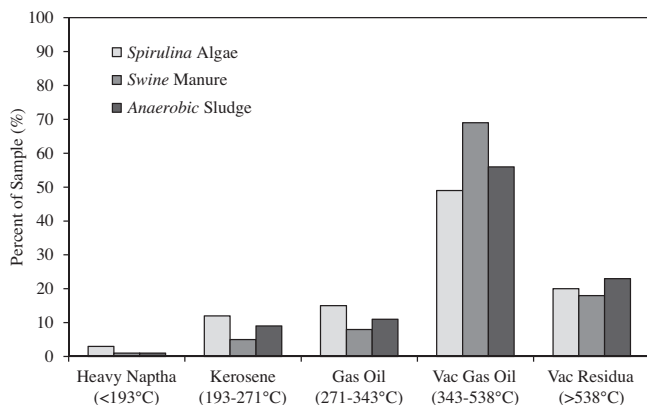| MW dist	*Spirulina* algae	Swine manure	Anaerobic sludge
M_w	1870	1890	3470
M_n	890	780	1340
PDI	2.10	2.42	2.59

Fig. 3. Sim-Dist boiling point fractions of HTL biocrude oils derived from different wastewater feedstocks.

Based on these results, raw HTL biocrude oil produced from the studied wastewater feedstocks may be best suited for bunker crude, boiler, or asphalt applications, similar to vacuum gas oil and vacuum residua fractions produced from petroleum crude. The high heteroatom content (oxygenated and nitrogenous compounds), molecular weight, and boiling point distribution of the biocrudes emphasize the need for bio-oil upgrading before HTL biocrudes can serve as a transportation fuel substitute for conventional petroleum. Several bio-oil upgrading routes have been pursued, including hydrodeoxygenation (HDO), hydrodenitrogenation (HDN), and hydrocracking with catalysts in conventional hydrotreatment operations and supercritical water (Huber et al., 2006; Elliott, 2007; Peterson et al., 2008; Demirbas, 2009; Duan and Savage, 2011a). The use of molecular-level characterization techniques described in this work will be beneficial for tracking and better understanding improvements in biocrude oil chemistry that occur during the upgrading process. However, further research is needed to improve the control of biomass conversion chemistry, enhance upgrading catalyst activity, selectivity, and longevity, and scale-up of both process technologies to demonstrable scales (Huber et al., 2006; Elliott, 2007; Demirbas, 2009).

4. Conclusion

Detailed multi-method characterization demonstrates that feedstock organic content and nutritional composition greatly affect HTL biocrude oil yields and chemistry, despite having similar bulk elemental distributions. The feedstock nutritional profile was reflected in the heteroatom content, type of compounds, and functionality observed in the resulting HTL biocrude oils. The tie between feedstock nutritional profile and HTL biocrude molecular makeup emphasizes the need for feedstock characterization and selection based on the intended downstream application. The molecular-level information gained from complementary methods can also help researchers design functional group-specific chemical strategies and processes to further reduce heteroatom content and improve HTL biocrude properties.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.06.041.

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