Effect of low temperature hydrothermal liquefaction on catalytic hydrodenitrogenation of algae biocrude and model macromolecules

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Abstract

Staged, low and high-temperature subcritical liquefaction were used to pretreat algae and generate a biocrude primarily characterized by free fatty acids and unsaturated hydrocarbons with reduced nitrogen heteroatom levels. Subsequently, catalytic hydrodenitrogenation and deoxygenation (HDN/HDO) was conducted using ruthenium (5% Ru/carbon) and cobalt molybdenum catalysts. Ru on carbon was the most effective HDO catalyst and generated the lowest level of nitrogen and heteroatoms in the resulting oil. A CoMo-S catalyst generated higher nitrogen and heteroatom levels, and resulted in a higher TAN value and lower heating value, probably due to the low sulfur levels in the biocrude. The highest quality oil was generated from a raceway strain using Ru/C and HTL pretreatment (225°C, 15 min) with a repeated batch HDN/HDO step (3.24% N, 8.2% O, TAN of 12, 1.25% water, HHV 40 MJ/kg) and had a boiling point range of ~20% kerosene, ~30% distillate fuel oil, and ~50% gas oil. A 57% reduction in nitrogen content of the oil was realized when repeated HDN/HDO was coupled with HTL pretreatment using Ru/C (4.3 wt.% N), relative to 7.5% N for single stage HTL/HDO. Final yields for the highest quality oil generated via the coupled HTL/HDO process ranged from 15-22%. Recovery and reuse of the catalyst (Ru/C) resulted in a significant decline in activity potentially caused by coking and metals deposition.

Keywords: Algae; Fuels; Heteroatoms; Hydrothermal Liquefaction; Catalyst;
Hydrodenitrogenation; Hydrodeoxygenation

1. Introduction

Algae have proven to be a viable biomass feedstock for conversion to fuel intermediates due to their high energy content and ability to grow autotrophically using carbon dioxide and sunlight. Hydrothermal liquefaction (HTL) of wet algae results in 29% higher bio-oil yield and 32% more energy recovery relative to other methods [1, 2]. Past studies indicate that HTL bio-oil is more energy dense and shows higher thermal and oxidative stability than bio-oil produced from biomass pyrolysis [1, 2]. HTL is performed using hot compressed water, since it is a highly reactive medium as it approaches its critical point (374 °C, 22.1 MPa) due to changes in properties such as acidity, solubility, density, dielectric constant and reactivity. The resultant bio-oil is a dark viscous liquid with an energy value 70-95% of that of petroleum fuel oil [3-5]. Under subcritical conditions, the hydrothermal liquefaction of algae yields multiple products from the hydrolysis/depolymerization of the algae macromolecules. These products include long-chain fatty acids (e.g., oleic acid), nitrogenated and oxygenated heterocyclics (e.g., pyrrolidine, phenol), and some long-chain hydrocarbons (e.g., pentadecane) [6]. Thus, HTL converts organic constituents of algae into a liquid bio-oil that in theory can be refined to diesel-like fuels via heteroatom removal mechanisms such as decarboxylation, decarbonylation, denitrogenation, and hydrodeoxygenation. The upgrading of HTL products such as amino acids, fatty acids, and carbohydrates has been investigated, yet there has been little research on catalytic conversion on mixtures of these molecules and the impact of nitrogen heteroatoms on catalytic upgrading [4, 7-9].

However, due to the large amount of protein present in algae biomass, bio-oil or biocrude has a large abundance of nitrogen heterocyclic compounds, which can cause problems in catalytic upgrading. Nitrogen has been shown to poison and deactivate biocrude upgrading catalysts [10, 11]. The nitrogenated compounds present in the bio-oil include nitrogen heterocyclics (pyridine, pyrrole, pyrrolidine, piperidine, and indole) and non-heterocyclics, such as, open-chain amines and amides (hexadecanenitrile, and hexadecanamides) [2, 3, 6, 12, 13]. Also, the presence of polypeptides and proteins lead to the high molecular weight compounds in the bio-oil in the range of 2,000-10,000 g mol⁻¹ [14]. These nitrogenated compounds can negatively affect catalytic activity and coupled with an abundance of oxygen in the biocrude, makes it unsuitable for co-processing with petroleum oil. Thus, it would be beneficial to create a biocrude low in nitrogen content and heteroatoms.

The catalytic processing units associated with petroleum refining have been tailored to low total acid number (TAN) and heteroatom levels in crude oil – typically, 0.3-6.6 mg of KOH/g or TAN, 2-8000 ppm nitrogen, and 0.4-4.5% sulfur [15, 16, 17]. Thus, given the high levels of free fatty acids and nitrogen in algae biocrude, fuel production or co-processing in a petroleum refinery requires heterogeneous catalytic hydrodeoxygenation (HDO) and hydrodenitrogenation (HDN). Bai et al. [18] performed a catalytic screening study in which algae oil generated from the liquefaction of *Chlorella* (350 °C, 60 min) was subjected to a two-stage upgrading process - a high-temperature upgrading stage using hydrogen gas without catalyst (350 °C, 4 hours) and supercritical upgrading stage using hydrogen gas with varying types of heterogeneous catalysts (400 °C, 4 hours; 10 wt%). Their work demonstrated that ruthenium on carbon (5 wt.%) and Raney nickel produced the best results. The two-stage process reduced the nitrogen content of the bio-oil from 8.0 to 2.0 wt%. Duan and Savage [19] conducted

a study on the effect of temperature (430 to 530 °C), time (2 to 6 hours), and catalyst (Mo₂C, HZSM-5, or Pt/C) loading (5 to 20 wt.%) on algae bio-oil quality. This work indicated that high reaction temperatures (530 °C), long contact times (6 hours) and high catalyst loading (10-20%) correlated with reduced nitrogen level (~5% to 1.5-2.5% N). The HDO temperature of 530 °C generated the lowest nitrogen content oil, yet higher amount of gas and coke phases.

This study was designed to determine if low temperature HTL (LT-HTL), followed by a higher temperature HTL, could be used to reduce nitrogen heteroatoms and then coupled with catalytic HDO/HDN to generate a refinery quality bio-oil. Performing a two-stage HTL process is a novel approach to minimize N and nitrogen heteroatoms in the HTL bio-oil. In theory it is possible to extract protein fractions from the biomass by a first stage low temperature HTL process, and separate the nitrogen rich aqueous fraction and then liquefy the remaining fractions to generate a bio-oil product via a second stage HTL step [20, 21, 22]. The nitrogen rich fraction in the aqueous phase from the pretreatment step can be recycled for algae cultivation, as indicated in recent studies [22, 23, 24]. Additional advantages of integrating nitrogen recovery with LT-HTL include a potential reduction in inhibitory compounds (e.g., phenolics, pyridines, carboxylic acids) and the resultant aqueous phase is sterile (contaminating microorganisms and viruses are eliminated). Moreover, LCA and process simulations of algae production indicate that scale-up of algae to liquid fuel processes is not sustainable without nitrogen and water recycling as part of an integrated design [25]. To the best of our knowledge the impact of nitrogen heteroatom reduction via a low temperature HTL step on catalytic HDO/HDN of algae bio-oil has not been studied.

2. Experimental

2.1. Materials

Freeze-dried *Spirulina platensis* was obtained from Earthrise Nutritionals LLC (Calipatria, CA), and *Nannochloropsis* sp. was obtained from Reed Mariculture ("Nanno 3600", strain CCMP525). A consortium of three algal strains (UGA Consortium henceforth), namely *Chlorella sorokiniana*, *Chlorella minutissima*, and *Scenedesmus bijuga*, were grown and harvested for use in this study as well. Ruthenium (5 wt% on carbon, 20 μm particle size, Ru/C) was obtained from Sigma-Aldrich (MO, USA). A cobalt oxide (3.4-4.5%) molybdenum oxide (11.5-14.5%) on alumina (Al_2O_3) catalyst (2.5 mm trilobe, CoMo) was obtained from Alfa Aesar (MS, USA). The CoMo/ Al_2O_3 catalyst was reduced in the presence of flowing hydrogen (100%) at 400 °C in a tubular packed bed reactor (1 in. ID); 100 mL min⁻¹ of hydrogen gas was passed over the catalyst for 2 hours (CoMo-H₂). In addition, pre-sulfided CoMo/ Al_2O_3 catalyst (2-7% CoS and 5-25% MoS, trilobe 2.5 mm, CoMo-S) was obtained from EureCat (EU). A mixed metal oxide catalyst (Red Mud) was obtained from Rio Tinto (Alcan, Canada). This catalyst was dried (105 °C), crushed, and sieved ($0.5 < d_p < 2$, mm). The red mud particles were reduced in the presence of flowing hydrogen (100%) at 300 °C in a tubular packed bed reactor (1 in. ID); 90 mL min⁻¹ of hydrogen gas was passed over the catalyst for 20 hours (RRM).

The Ru/C catalyst was selected due to its high activity for hydrodenitrogenation and deoxygenation activity of algal oil relative to a range of other catalysts [18]. CoMo metal catalyst was used given its reported HDO activity and hydrogenation of fast pyrolysis oil, vegetable oils, and free fatty acids [26, 27] (Parapati et al., 2014; Harnos et al., 2012) and for bi-metallic catalyst HDO activity in the presence of sulfur for algal oil [28] (Guo et al., 2015). It has been reported in the literature that CoMo sulfidation via sulfur in the feed can activate the material (i.e., form CoMo-S) leading to HDO activity, although the activity is lower than if pre-sulfided

catalyst is used [29] (Viljava et al., 2000). Thus, it was of interest to determine if sulfur in algal HTL feedstock would activate the catalyst and thus eliminate the need to supplement the feed with a source of sulfur. Red mud and iron catalysts have been demonstrated to hydrotreat vacuum residue [30], hydrocrack algae [31], and deoxygenate fatty acids [3]. We anticipated lower activity due to the presence of other minerals in red mud, but were interested to determine if its activity was high enough to consider using it as pretreatment step (partial reduction in nitrogen and some cracking of triglycerides) before co-processing. Also, it has been reported that red mud self-activates for hydrogenation activity, via thermal cracking, release of sulfur, and sulfidation [30] (Nguyen-Huy et al., 2012).

2.2. Algae Growth

A consortium of three algal strains (UGA Consortium henceforth), namely *Chlorella sorokiniana*, *Chlorella minutissima*, and *Scenedesmus bijuga*, were grown and harvested at The University of Georgia (USA). A monoculture inocula of the constituent strains were first grown in 20 L carboys under controlled conditions in a growth chamber at 25 ± 1 °C for 12 h with alternating light-dark cycles; the light intensity was $100 \mu\text{moles m}^{-2} \text{s}^{-1}$ with continuous air bubbling. The final consortium was prepared by mixing equal proportions (v/v) of each individual strain and then using it as inoculum at 10% v/v for outdoor cultivation in raceway ponds under green house facilities at an algae bioenergy lab at the University of Georgia (UGA). The raceway ponds are constructed of HDPE plastic and are 1.32 m wide, 2.18 m long, and 0.61 m deep with a working volume of approximately 500 L at 0.17 m water depth. Standard algae growth medium BG 11 was used in fresh water for cultivation [33 Supplemental CO₂ was derived from a commercial 10% CO₂ storage cylinder and used as a carbon source and for pH

control. The UGA designed carbonation column [33] was used for CO₂ mass transfer. Once the cell density in raceways reached 500 mg/L, the biomass was harvested using a continuous centrifuge and dried at 55 °C until constant weight was observed after multiple weighing. The dried biomass was packed in zip-lock bags and stored at 4 °C until further use. Subsequently, the dried microalgae was ground to a fine powder using a heavy-duty laboratory knife mill (Retsch SM 2000, Germany) with a screen size of 0.5 mm. The knife mills cutting blade rotor (1690 rpm, 60 Hz) was powered by a 1.5 kW electric motor.

Algal biomass, solid residues, HTL bio-oil, and HDO samples were analyzed for elemental C, H, N, S, O (ultimate analysis) using a Flash 2000 organic elemental analyzer (Thermo Scientific) following methods outlined in ASTM D 5291. The analyzer was calibrated using BBOT or 2,5-Bis (5-tert-butyl-2-benzo-oxazol-2-yl) thiophene (C–72.59%, H–6.06%, N–6.54%, O–7.42%, and S–7.43%) as the standard material. Atomic ratio and empirical formula of raw feedstock and the biocrudes were derived from the elemental results. Higher heating values (HHV) of solid and biocrude samples were measured using an isoperibol bomb calorimeter Model 1351 (Parr Instruments Co., Moline, Illinois) following ASTM D 5865 and D 4809 standard methods.

Carbohydrate content of the algal biomass was measured using the DuBois method [34] (DuBois et al., 1956), protein content was estimated by multiplying the elemental N content by a factor of 4.58 [35], and lipid content was measured by gravimetric method using an ANKOMXT10 automated extraction system (ANKOM Technology, Macedon, NY) where hexane was used as the extraction solvent. Compositional analysis of the three sources of algal biomass used in this work are presented in Table 1.

2.3. Liquefaction Apparatus and Experimental Procedure

2.3.1. Reactor Operation

The process flow and analytical methods used in the 4-stage conversion process are presented in Figure 1. Low temperature liquefaction pretreatment (PT, 125 to 225 °C) was performed in a batch reactor (Parr 5000 Multi Reactor System, 75 mL vessel). Vessels were charged with 7 g of algae and 32 g of deionized water, and were sealed using a PTFE gasket. The system contains six separate vessels, each with its own PTFE magnetic stir bar (300 rpm) and band heater in an aluminum block (250 W) generated a heating rate of $\sim 10\text{ }^{\circ}\text{C min}^{-1}$. The heat up times were 9, 14, and 21 minutes for 125, 175, and 225 °C, respectively and the residence time was taken as the point the reactor reached the temperature set-point. Vessels were pressurized with 300 psi (20.7 bar) helium prior to heating to ensure liquid water throughout the reaction. Once the reaction was complete vessels were removed from the heating block and the bottom sections of the vessels (below the screw cap) submerged in a water bath. At ambient temperatures, the vessels were depressurized and weighed to determine the mass of gas produced from the reaction.

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Table 1: Composition of algae biomass used in the HTL and HDO studies.

Parameter	Composition (% w/w, dry basis)		
	<i>Spirulina</i>	<i>UGA</i> (Raceway)	<i>Nannochloropsis</i> (Reed Mariculture)
C	43.1 ± 1.0	40.8 ± 1.0	51 ± 0.1
N	10.8 ± 0.2	7.6 ± 0.5	10.1 ± 0.3
S	ND	ND	0.8 ± 0.8
Protein	49.2 ± 0.78	34.6 ± 2.3	46.3 ± 1.4
Lipids	5.8 ± 10.5 (0.5-11)	6 ± 1.7	13.5 ± 9 (9 ^a -18 ^b)
Carbohydrates	16 ± 2	17 ± 8.7	14 ^b
Moisture	6.6 ± 0.06	5.2 ± 0.8	1.3 ^b
Ash	8.0 ± 0.04	13.4 ± 5.1	7.4 ^b

^a, Analysis performed at UGA, ^b, Reported by Reed Mariculture, ND: not detected

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The whole product from the low temperature liquefaction stage was transferred without additional solvent into a 50 mL centrifuge tube and centrifuged in a Sorvall Super T21 centrifuge at 10000 RPM for 20 minutes. The aqueous phase was decanted off and weighed, and the remaining solids were re-suspended in a fresh aliquot of deionized water (to generate a slurry of 47 g total). Solids were dried after centrifugation and decanting at 105°C for 4 hours for analysis.

High temperature liquefaction (HTL, 350 °C) was performed in a different batch reactor (Parr 4598 Micro-Stirred Reactor, 100 mL vessel) and were charged with either 7 g of algae and 40 g of deionized water, or with the re-suspended solid slurry, and sealed using a consumable Parr Grafoil gasket (flat flexible graphite). The mixture was stirred using a 4-blade impeller powered by a magnetic stirrer (model no. A1120HC6, 100 W variable speed) at 300 rpm. Vessels were heated using a ceramic fiber external jacket (700 W) to generate a heating rate of 14 °C min⁻¹. For this reactor system, the heat-up time ranged between 27 and 35 minutes with an average of 29 minutes and high temperature liquefaction was performed at 350 °C for 60 minutes and pressurized with 500 psi (34.5 bar) helium prior to heating. Once the reaction was complete, the external jacket was removed and the vessel cooled to ambient temperatures, then depressurized and weighed to determine the mass of gas produced from the reaction. The whole product was transferred without additional solvent into a 50 mL centrifuge tube for storage. Catalytic hydrodeoxygenation and repeated batch catalysis (HDO and RB, 350 °C) were performed in the Parr 4598 Micro-Stirred Reactor following the procedure listed above. The vessels were charged with catalyst while being charged with the reaction slurry, and 750 psi (51.7 bar) of hydrogen gas was used to fill the headspace and reactions were conducted for 240 minutes, stirring at either 300 or 500 RPM. The algal oil to hydrogen ratio for the HDO

experiments was ~11 g oil/ mol H₂ (based on room temperature conditions, 51.7 bar H₂, 60 ml of headspace, and a PT-HTL biocrude yield of 20%).

2.3.2. Hydrothermal Liquefaction and Catalytic Hydrodeoxygenation of Model Molecules

To better understand the interaction of protein, carbohydrate, and lipid hydrolysis products and their effect on catalytic hydrodeoxygenation, a series of experiments was performed using macromolecular model compounds at defined ratios. Model compounds were selected to best represent a range of algal strains – a lipid, protein, and carbohydrate model was selected based on free fatty acid composition, amino acid composition, and on simple sugar composition, respectively.

For lipid selection, a survey of *Spirulina platensis* was performed and palm oil selected as model a lipid. Literature indicates that lipids generated by this species are primarily composed of the C16 free fatty acid, palmitic acid [nearly 50%, 36]. Monosaccharide composition of 16 different algal strains indicates a significant amount of the simple sugars are in the form of glucose (21 to 87.5%, 37). The model carbohydrate selected was starch (potato starch), since most algae surveyed follow this pattern and it contains glucose as its primary monosaccharide [37]. Algal proteins were initially surveyed for amino acid composition and a model protein was to be selected based up similarities between that protein, and the average amino acid make-up of algal strains as a whole. However, literature analysis indicated no clear representable amino acid composition for algae that can be accurately modeled; each algae species surveyed had a very different amino acid compositions [37]. This criteria was thus eliminated and instead a model protein was selected based on more generalized characteristics and prior research performed on the model protein. The protein of highest frequency found in algae are part of a water-soluble

group of photosynthetic proteins called *Biliproteins* [38] (Simo et al., 2005). This protein group, comprised of allophycocyanin and c-phyococyanin, was found to have properties that can be modeled by most high-production proteins. Albumin was thus selected because of the knowledge already amassed in the literature and because of its macro-qualities (such as solubility) that make it similar to algae *Biliproteins* [39] (Stein, 1949).

The macromolecular compounds were mixed proportionally in water (by weight) to generate defined ratios such that the final mixture contained 80 percent water and 20 percent macromolecules. The macromolecular ratios were systematically varied to achieve defined ratios of lipid:carbohydrate:protein and combinations according to Table 1SI. The hydrodeoxygenation (HDO) step (350°C, 30 min) was performed on the product of the HTL step (350°C, 240 min, 100% H₂, 3 g Ru/C) in its entirety; the only new additions to the vessel once the liquefaction has completed was hydrogen gas and catalyst. The headspace was pressurized with pure hydrogen to 750 psig after the vessel had been purged with hydrogen.

2.3.2. Phase Separation and Biocrude Extraction Procedure

Following high temperature liquefaction or catalytic upgrading, 40 mL of dichloromethane (DCM) was added to the whole product and mixed. This solution was filtered under vacuum through Whatman #4 filter paper (pore size 20-25 µm), and the cake was rinsed with 15 mL of additional DCM. The cake was weighed wet, dried at 105 °C for 4 hours, and weighed again to determine wet and dry solid yield, and estimated solid water content. The filtrate was then transferred into a separatory funnel (150 mL) and was allowed to phase separate for 15 minutes. The bottom phase (oil solubilized in DCM) was decanted and 1 g of anhydrous (magnesium perchlorate) was added to the decanted phase. This phase was filtered again under vacuum with

10 mL DCM used to rinse the anhydrous cake. The filtrate was transferred to a rotary evaporator (Rotovap) flask and distilled under vacuum pressure (40 mbar) in a 36 °C water bath for 30-45 minutes until all DCM was removed and the remaining product was weighed to determine the mass of bio-crude. Since the solvent-free bio-crude was very viscous, two different storage techniques were used, 1) a subsample was removed for analyses requiring a solvent-free product, and 2) the remaining bio-crude in the flask was re-suspended in DCM (approximately 6:1 DCM:oil by mass).

2.4. Analytical Methods

2.4.1. Oil Analysis

The elemental composition (C, H, N, and S) of the samples (solvent-free oils and dry solids) was measured using a Thermo Scientific Flash 2000 elemental analyzer according to the ASTM D5291 standard method. Issues with the biocrude being overly volatile during sample weighing were overcome by using two tin capsules (instead of one) and by loading and analyzing each replicate individually.

Boiling point distributions of the HTL algal oil and catalytically upgraded oil were estimated using two methods. First the GC/MS results were used to estimate a boiling point distribution. In this method the chromatograms (TIC) generated by the GC/MS were integrated using the same integration parameters for each chromatogram. The peak areas of the highest match factor compounds (i.e., the top hit using the NIST algorithm) were determined by integration and total peak area determined by summation of all peak (excluding any residual solvent). Then % peak area and cumulative peak area as a function of retention time were

determined. Normal boiling points for the identified compound were found from the NIST Web Book (all boiling points at P_{atm}). Subsequently, cumulative peak area versus boiling point plots were performed, and the boiling point distribution for petroleum cuts overlaid on the plots. In addition to the GC/MS technique, simulated distillation of the algal oils were estimated using thermogravimetric analysis (Mettler Toledo – Model TGA/SDTA851). In this method, 3-5 mg of oil was heated from 25 °C to 625 °C at 10°C/min in the presence of flowing N_2 (50 ml/min). During heating the change in mass was measured and the fraction of mass lost and the first derivative of the mass change with time was calculated. Subsequently, the mass, % mass lost, and first derivative (as an absolute value) were plotted as a function of temperature.

Gas Chromatography-Mass Spectrometry (GC/MS) was performed on an Agilent GC-MSD (Hewlett-Packard 5973 and 6890) with an HP5 MS Capillary Column (30 m x 0.25 μm x 0.25 μm). Separation was achieved using a temperature-programmed method of 40 °C, held for 4 minutes, with a 5 °C min⁻¹ ramp until 275 °C was reached, held for 5 minutes; the inlet was held at 260 °C, the split ratio was 50:1, the injection volume was 1 μL , the helium carrier gas flow was 0.8 mL min⁻¹, and the MS scan was 30-400 amu. In some cases the algal oil and catalytically upgraded oil were quantitatively analyzed using an external standard method and GC/MS analysis. Hexanol was used as the standard and added to the oil at 2.03 g/L and analyzed using the previously described methods. Neat compounds of 1-hydroxy-2-propanone, furfural, 2-methoxy phenol, 2-methoxy-4-methyl phenol, tetrahydrofuran, 1-butanol, and eugenol (99.9%, Sigma) were mixed with hexanol (2.03 g/L), acetone, and methanol to generate standard mixtures and analyzed on the GC/MS using identical methods. Standard curves were constructed using model compounds and 1-hexanol as an internal standard, in triplicate.

High Pressure Liquid Chromatography (HPLC) was performed on a Shimadzu LC-20 AT equipped with a RID-10A refractive index detector and a Coragel 64-H transgenomic analytical column (7.8 x 300 mm); the flow was 0.6 mL min⁻¹ for a 55 minute run time, the sample volume was 5 μ L, the mobile phase was 4 mN sulfuric acid, and the samples were analyzed at 6.89 MPa and 60 °C. About 2 mL of the oil was diluted with DI water at 1:1 ratio and centrifuged at 5000 rpm for 30 min, and then decanted. The supernatant was filtered through a 0.45 μ m filter into 2 mL auto-sampling vials. The sample was injected into the column using the LC-20 AT Shimadzu auto-injector. Samples were identified using standard curves constructed on isolated model compounds.

The higher heating value (HHV) of bio-crude was estimated using the elemental composition and the Dulong formula [Eq. 1, 40]. The nitrogen removal was calculated on a weight basis based on the initial amount of nitrogen in the dry/ash-free feedstock, according to Eq. 2. Total Acid Number (TAN) was determined according to the ASTM D974-12 standard method, in which sample dissolved in a solution of toluene, isopropanol, and water (500:495:5, by mass) is titrated with potassium hydroxide titrant (dissolved in isopropanol, molarity approx. 0.11 M) until the indicator solution (p-naphtholbenzein) drastically changes color. Total Acid Number (TAN) is typically reported as milligrams of KOH required to neutralize one gram sample (mg KOH (g oil)-1). pH was determined using potentiometry performed on an Orion 520A Digital pH meter (Orion Research Inc., USA). Water content was determined using a Mettler Toledo DL31 Karl Fischer Titrator. Water content was measured on a weight percentage basis using sample suspended in Hydranal, titrated with CombiTitrant 5 Keto.

$$\text{HHV} = 0.3383 * \%C + 1.422 * (\%H - (\%O/8)) \quad [\text{MJ kg}^{-1}] \quad (1)$$

$$\% \text{Nitrogen Removal} = \frac{(\% \text{N} * \text{DAF wt.})_{\text{feedstock}} - (\% \text{N} * \text{wt.})_{\text{product}}}{(\% \text{N} * \text{DAF wt.})_{\text{feedstock}}} \times 100 \quad (2)$$

2.4.1. Catalyst Characterization

Catalyst surface area was measured by N₂ adsorption over a relative pressure range (P/P₀) of 0.05-0.35 using a 7-point BET analysis equation (Quantachrome AUTOSORB-1C; Boynton Beach, FL, US). Pore size distribution, average pore radius, and total pore volume were estimated from N₂ desorption curves using BJH analysis. All samples were degassed ranging from 250 to 300 °C for 3-4 hours prior to analysis.

Recovered catalysts were washed with an equal volume mixture of toluene, acetone, and methanol to remove tar. Approximately 2 g of reacted catalyst was placed on a Whatman Filter (Qualitative #1 Filter Paper, 70 mm) and rinsed under vacuum with the solvent mixture. The rinsed catalyst was then dried at 105 °C for 1 hour, cooled to room temperature, and weighed to determine the mass of tar accumulated. Catalyst coke formation was determined by heating the washed catalyst (5-10 mg) in a thermogravimetric analyzer (Mettler Toledo TGA/SDTA 851e) at 10 °C/min to 600 or 800 °C under air flow. The change in the mass of catalyst from 100 to 380°C, relative to the original unreacted catalyst, was assumed to be due to the combustion of coke. Elemental concentrations in the recovered catalysts (Ca, Fe, K, Mg, Na, P, and S) were determined using inductively coupled plasma emission spectroscopy (ICP-OES) performed on a Spectro Arcos FHS16 AMETEK ICP-OES (Samples were digested using concentrated HNO₃ following EPA method 3051A).

2.4.2. Level of Replication and Statistical Analysis

The CHNSO analysis of the algae feedstocks and samples were performed in triplicate and reported as the mean and sample standard deviation (Tables 1 and 2). Lipid and carbohydrate analysis of the feedstocks were performed in duplicate and the mean and range reported (Table 1). Protein levels and HHV values were determined from the CHNS analysis, performed in triplicate, and sample standard deviation reported (Tables 1, 2, and 3). The total acid number (TAN) and % water were performed in triplicate and reported as the mean and sample standard deviation (Table 3). Percent heteroatom composition was determined from duplicate GC/MS analysis and reported as the mean and range (Table 3, Figures 4, 5, and 9). Surface area, pore size, and pore volume for fresh unreacted catalyst and coke levels for reacted catalysts are reported as the mean and range of duplicate analysis (Tables 4 and 5). The standard deviation between duplicates in the metals analysis of the recovered catalyst using ICP-OCP was less than 1 percent (Table 5SI, Figures 13SI-15SI). Fresh, unreacted catalyst was analyzed in duplicate for surface area, pore size, and pore volume and reported as the mean and range (Table 4). Catalyst coking analysis via TGA was performed in triplicate and reported as the mean and sample standard deviation (Table 5); however, due to limited supply of recovered catalyst (and problems with tar off-gassing during surface area analysis) we could not perform duplicate or triplicate analysis for the other properties. When analyzing differences between two means a small sample t-test was used assuming the null hypothesis, and the level of significance (α) is noted in the text.

3. Results and Discussion

3.1. Effect of Catalytic Upgrading Parameters on Bio-oil Quality

Biocrude quality is partly determined based on the nitrogen content and heteroatom levels in the oil. Performing catalytic hydrodeoxygenation (HDO) alone without liquefaction as a

373 pretreatment gave the least favorable results and generated a biocrude with a nitrogen content of
374 6.4% and 20.6% yield. High-temperature liquefaction (HTL) alone produced biocrude with a
375 nitrogen content of 5.8% and 26.7% yield (Table 2). Relative to HTL/HDO only, adding a low-
376 temperature pretreatment liquefaction (PT) step reduced the nitrogen and sulfur content across
377 any combination of stages with the most effective nitrogen reduction being obtained using all
378 three conversion stages (Table 2, $\alpha=0.05$, level of significance). However, implementing the
379 pretreatment stage reduced the biocrude yield an average of 14%

Table 2: Effect of catalytic hydrodeoxygenation (HDO) on ultimate analysis and HHV properties of biocrude and hydrogen consumption from a 3-stage conversion of *UGA Consortium* using Ru/C [PT- 225°C, 15 min → HTL-350°C, 60 min → HDO- 350°C, 240 min].

Stage Combination			Cat. Loading	Agitation	Ultimate Analysis (dry basis, %, w/w)					HHV (MJ/kg)	Yield (% DAF feed) ^b	H ₂ Consumption (mmol hr ⁻¹ g _{cat} ⁻¹)
PT	HTL	HDO	(%, w/w)	rpm	C	H	N	S	O ^a			
x	x		NA	300	71.4±0.4	8.8±0.1	5.9±0.1	ND	13.9±0.4	34.2±0.28	17.4 (11.5±4.4) ^c	NA
	x		NA	300	71.6 ± 2.1	8.7 ± 0.2	5.8 ± 0.2	1.17±0.72	13.4 ± 3.2	34.2±0.92	26.7 (27±2.8) ^c	NA
		x	10	300	74.3 ± 0.3	9.4 ± 0.1	6.4 ± 0.1	0.80±0.79	9.2 ± 1.1	36.8±0.16	20.6 (22.6±3.9) ^c	25.5(28.7±5.3) ^c
	x	x	10	300	74.6 ± 0.1	9.3 ± 0.2	5.8 ± 0.2	ND	10.4 ± 0.1	36.6±0.51	22.1 (20±2) ^c	22.1(24.3±6.4) ^c
x		x	10	300	74.4 ± 0.1	9.4 ± 0.1	5.0 ± 0.1	ND	11.2 ± 0.7	36.6±0.13	9.5 (9.5±1.2) ^c	25.9(22.6±14.1) ^c
x	x	x	10	300	74.5 ± 0.7	9.3 ± 0.1	4.8 ± 0.1	ND	12.2 ± 0.9	36.3±0.47	6.2 (7.3±1.5) ^c	25.4(31.4±5.7) ^c
	x	x	30	300	75.6 ± 0.3	10.6 ± 0.1	4.9 ± 0.1	ND	8.9 ± 0.4	39.0±0.33	24.3 (26.6±4.7) ^d	11.9(11.7±0.3) ^d
x		x	30	300	77.4 ± 0.4	11.7± 0.1	3.4 ± 0.1	ND	7.5 ± 0.5	41.5±0.40	13.8 (12.9±1.8) ^d	11.9 (11.8±0.12) ^d
x	x	x	30	300	79.0 ± 1.1	12.0 ± 0.2	3.0 ± 0.2	ND	6.1 ± 0.9	42.7±0.97	17.3 (14.8±5.0) ^d	12.0(11.75±0.5) ^d
x	x	x	30	500	66.8 ± 3.6	8.6 ± 0.6	2.4 ± 0.2	ND	20.2 ± 4.3	29.9±2.6	22.2 (22.5±0.53) ^d	13.8(13.9±0.26) ^d
x	x	x	30	700	72.6 ± 0.7	10.6 ± 0.2	3.1 ± 0.1	ND	13.8 ± 0.1	37.2±0.78	17.2 (19.9±5.4) ^d	13.6(13.7±0.24) ^d

^a Determined by difference. ^b % w/w of dry/ash-free feedstock fed initially. NA: Not applicable. ND: Not detected or below detection limit. ^c Numbers inside brackets indicate mean and standard deviation of Ru/C HDO experiments grouping UGA Consortium, *Nannochloropsis*, and *Spirulina*. ^d Numbers inside brackets indicates mean and range of Ru/C HDO experiments grouping UGA Consortium and *Nannochloropsis*.

(Table 2). This is likely due to the loss in hydrolyzed material that occurs after the pretreatment stage, since it is theorized that hydrolyzed proteins and carbohydrate partition to the aqueous phase. Total nitrogen removal was large (90-96%, relative to starting algae biomass) for all three strains tested [41] (Costanzo et al., 2015), if pretreatment was implemented before HTL. Yet, nitrogen removal relative to a single stage, batch HTL process was 0, 19, and 23% for the UGA Consortium (Table 2), *Spirulina*, and *Nannochloropsis*, respectively [41] (Costanzo et al., 2015). It's unclear why the UGA consortium acted in such a distinctly different manner. Using a similar two-stage HTL approach, Jazrawi et al., 2015 achieved a 54% reduction in nitrogen relative to a single-stage HTL, generating a bio-oil with 3.4% nitrogen [42]. As discussed later and presented in Costanzo et al., 2015, GC/MS analysis indicated a 30% reduction in nitrogen heteroatoms due to the low temperature pretreatment step. Up to this point in the work, catalytic HDO/HDN studies were conducted at a 10 wt% catalyst loading and 300 rpm, resulting in nitrogen contents ranging from 4.9 to 6.4 wt% (Table 2).

Subsequently, additional improvements in catalytic HDO/HDN of the algae were implemented to reduce the nitrogen content and improve the properties of the resultant oil including higher catalyst loadings and increased agitation rates. Increasing the catalyst loading from 10% to 30% decreased the nitrogen content by 38% when using Ru/carbon (Table 2). Similar trends were observed across all stage combinations. Next a series of experiments were performed in which the agitation rate was increased in the HDO step at the highest catalyst loading (30 wt.%). To limit the number of experiments only two algal oils (the *Nannochloropsis* and UGA Raceway generated strains) and one catalyst were tested (Ru/C catalyst). It was assumed that any effect on nitrogen content and deoxygenation would be similar with the other catalysts. Hydrogen consumption increased up to an agitation rate of 500 rpm and leveled; there

was no additional increase in H₂ consumption at an agitation rate of 700 rpm (Fig. 2). The most effective reduction in nitrogen occurred when using all three conversion stages with a higher agitation rate during HDO (2.4% nitrogen at a biocrude yield of 22.2%-Table 2).

Compositional analysis of the resulting oil using GC/MS clearly illustrated an improvement in the composition quality of the biocrude with the addition of the pretreatment (PT) and catalytic upgrading (HDO) stages, compared to HTL alone (Fig. 3). The improvements from the addition of the pretreatment stage (as compared to HTL alone) were due to a significant reduction in the nitrogen heteroatoms (Fig. 3). When comparing the 3-stage conversion process

Figure 2: Effect of agitation rate on H₂ consumption during HDO of algal oil using Ru/Carbon. First, an algae suspension was treated via a two stage HTL (225°C, 300 psig → 350°C, 500psig) with aqueous nitrogen removed between the stages, then a catalytic HDO step was conducted on the algal oil at 350°C for 4 h (100% H₂ in headspace at time zero, 750 psig). All experiments were conducted with 2.1 g-cat (Ru/C)/40 g algal oil. The average and range of oil yield (wt.%) and H₂ consumption for the two strains are reported (UGA Raceway and *Nannochloropsis*).

475 **Figure 3:** GC/MS analysis of *UGA Consortium* biocrude using Ru/C [PT: 225 °C, 15 min; HTL: 350 °C, 60 min; HDO and RB: 350 °C, 240 min] – HTL only (A), PT and HTL
 476 (B), PT, HTL, and HDO (C), and PT, HTL, RB/HDO (D). Identified compounds are (1) pyrazine, (2) methyl-pyrazine, (3) ethyl benzene, (4) 1-methyl-2-pyrrolidinone, (5) 1-ethyl-2-
 477 pyrrolidinone, (6) methyl-piperidine, (7) 1-pentadecene, (8) 3,7,11,15-tetramethyl-2-hexadecene, (9) hexadecanoic acid, (10) hexadecanamide, (11) toluene, (12) ethyl benzene, (13)
 478 decane, (14) undecane, (15) dodecane, (16) tridecane, (17) tetradecane, (18) pentadecane, (19) hexadecane, (20) heptadecane, (21) phytane, (22) nonadecane, (23) eicosane, (24)
 479 heneicosane, (25) docosane, (26) tricosane, (27) tetracosane, (28) pentacosane, (29) hexacosane, (30) heptacosane, (31) octacosane, (32) nonacosane, (33) triacontane. RB indicates
 480 repeated batch HDO.

involving PT, HTL, and HDO to the 2-stage process without HDO, it is clear that there are multiple catalytic mechanisms leading to primarily straight chain saturated hydrocarbons. Free fatty acids (hexadecanoic acid) were decarboxylated to form long chain hydrocarbons and unsaturated free fatty acids (oleic acid) were hydrogenated and decarboxylated so that the hydrocarbons formed were saturated. Any unsaturated hydrocarbons formed from the initial two steps (e.g., 1-pentadecene) were hydrogenated into hydrocarbons.

3.2. Repeated Catalytic HDO Effect

In an attempt to further reduce the nitrogen content of the algal oil, a series of HTL/HDO experiments were performed in which the HDO step was repeated (Table 3). First, an algae suspension was treated via a two stage HTL (225°C, 300 psig→350°C, 500psig) with aqueous nitrogen removed between the stages. Next the catalytic HDO step was conducted two times on the algal oil at 350°C for 4 h (100% H₂ in headspace at time zero, 750 psig). Note, the same batch of catalyst was used for both HDO steps. These HDO experiments were conducted with Ru/carbon, H₂ reduced iron oxides, H₂ reduced CoMo, and sulfide CoMo using *Nannochloropsis* and the UGA raceway strain.

Table 3 contains the biocrude quality characteristics of oils generated via this repeated HDO process. Across all three strains, Ru/C catalyst achieved the most favorable results in all measured characteristics (total acid number [TAN], water content [%W], percent heteroatoms [%HA], nitrogen and oxygen content, and higher heating value [HHV]). Ru/C was the most effective at producing a low-nitrogen biocrude, with the best results being with a 4-stage conversion of UGA Consortium (3.2% nitrogen, $\alpha=0.05$). Comparatively, sulfided cobalt

503 molybdenum (CoMo-S) produced a biocrude with 4.1% nitrogen under the same conditions.

504 Reduced red mud

505 **Table 3:** Properties of oil generated by repeated batch catalysis [PT-225°C 15 min→HTL: 350°C-60 min →RB/HDO-350°C, 240 min].

Oil Properties After HTL/HDO Stage and Reaction Conditions										
Algae Strain	Catalyst	Stage 1-HTL		Acidity/Water		Heteroatoms			%O ^e	Physical Properties HHV (MJ/kg)
		T, °C	τ, min	TAN	%Water	%HA ^a	%N ^b	%O ^{c,d}		
UGA <i>Nannochloropsis</i> <i>Spirulina</i>	Ru/C	225	15	11.6 ± 1.4	1.25 ± 0.6	0.0±0.0	3.24 ± 0.2	9.31±0.66	8.2	39.81±0.73
	Ru/C	225	15	47.05 ± 4.7	3.6 ± 1.5	9.1±7.5	3.76 ± 0.5	9.57±1.66	6.4	39.66±1.54
	Ru/C	225	15	NP	0.33 ± 0.32	33.2±13.4	NP	NP	NP	NP
UGA	Ru/C	225	15	11.6 ± 1.4	1.25 ± 0.6	0.0±0.0	3.24 ± 0.2	9.31±0.66	8.2	39.81±0.73
	CoMo-S	225	15	63.1 ± 3.9	0.8 ± 0.08	46.1±13.6	4.1 ± 0.05	12.47±0.86	11.8	36.10±1.02
<i>Nannochloropsis</i> <i>Spirulina</i>	Ru/C	225	15	47.05 ± 4.7	3.6 ± 1.5	0.0±0.0	3.76 ± 0.5	9.57±1.66	6.4	39.66±1.54
	CoMo-H2	225	15	70.8 ± 1.8	1.24 ± 0.23	64±11.8	4.91 ± 0.04	7.42±0.46	6.3	39.0±1.4
	CoMo-S	225	15	65.8 ± 8.7	1.0 ± 0.2	23.6±13.0	3.82 ± 0.04	5.96±0.28	5.1	41.37±0.32
	RRM-300C	225	15	67.8 ± 1.2	1.4 ± 0.25	59±3.9	4.9 ± 0.11	9.85±0.61	8.6	37.64±0.62
	CoMo-S	225	15	74 ± 5.5	0.81 ± 0.04	47±5.8	4.81±0.39	14.23±0.20	13.5	34.96±1.02
UGA <i>Nannochloropsis</i> <i>Spirulina</i>	Ru/C	175	15	20.8	NP	14.1±3.5	3.15 ± 0.15	19.54±2.37	NP	33.1±3.7
		225	15	11.6 ± 1.4	1.25 ± 0.6	0.0±0.0	3.24 ± 0.20	9.31±0.66	8.2	39.81±0.73
	Ru/C	175	15	6.3 ± 0.35	0.25 ± 0.034	21.8±17.3	3.92 ± 0.03	15.12±0.52	14.9	35.50±0.56
		225	15	47.05 ± 4.7	3.6 ± 1.5	9.1±7.5	3.76 ± 0.50	9.57±1.66	6.4	39.66±1.54
	Ru/C	175	15	18.8 ± 0.6	0.17± 0.04	NP	NP	NP	NP	NP
		225	15	NP	0.33 ± 0.32	33.2±13.4	NP	NP	NP	NP
	Ru/C Reuse	225	15	171 ± 0.9	NP	20±4.8	3.85 ± 0.04	7.66±0.15	NP	40.24±0.19
		225	15	171 ± 0.9	NP	20±4.8	3.85 ± 0.04	7.66±0.15	NP	40.24±0.19

506 ^a, % Heteroatoms based on % peak area in GC/MS analysis – includes phenolics, ketones, nitrogenated aromatics, amides, fatty acids

507 NP – not performed, not enough oil for analysis; ^b, CHNS using two tin holders and single sample analysis, which solved volatility issue and

508 resulted in consistent results

509 ^c, Oxygen determined by difference, ^d, Sulfur was not detected in any of the samples, ^e, Oxygen content in water subtracted from %O

(RRM) and hydrogen reduced cobalt molybdenum (CoMo-H₂) were both less effective at nitrogen removal than Ru/C, producing a biocrude from *Nannochloropsis* with 4.9% nitrogen (compared to 3.8% with Ru/C under the same conditions, $\alpha=0.05$).

In addition to CHNS analysis of the repeated HDO samples, an alternative method to estimate heteroatom composition in the algae was performed. In this method, results from the GC/MS analysis of each sample were used to estimate the composition of key compounds based on % peak area. As outlined in the experimental methods section, each sample was analyzed under the same conditions (e.g., injection volume, GC conditions, MS conditions), compounds identified based on a NIST 2008 database and search algorithm, the peak areas determined via integration of the total ion chromatogram or TIC (all based on the same integration parameters), and % peak area calculated based on the total peak area. Typical output of this analysis is shown in Tables 2SI and 3SI. Subsequently, these data were analyzed to determine the % composition (based on peak area) for different classes of compounds, which included aromatics (e.g., toluene, ethyl benzene), long chain hydrocarbons including branched hydrocarbons (C₁₀-C₂₀ and >C₂₀), nitrogenated aromatics (pyridines), long chain amides and nitriles (octadecanamide), polycyclic aromatics, phenolics (phenol), and oxygenates (ketones). These results were then used to compare/contrast catalyst type effects, strain differences, and pretreatment effects. The Ru/C catalyst generated significantly higher levels of long chain hydrocarbons compared to other catalysts (Figs. 4 and 5); e.g., for the UGA strain, Ru/C generated an oil with ~95% long chain hydrocarbons compared to ~39% for CoMo-S (based on % peak area). Moreover, contrary to CoMo-S, the Ru/C treated oil had minimal heteroatom percentages. For example, no heteroatoms were present when using the UGA strain (Fig. 4), and phenolics and long chain amides were 5.9

532 ± 6.9 and 1.84 ± 1.99 % respectively for *Nannochloropsis* oil (Fig. 5). However, CoMo-S
533 treated oil resulted in 25 ± 3.2 % phenolics (e.g.,

Figure 4: Compositional analysis of algal oil, from UGA strains, after repeated catalytic HDO (Ru/C vs. CoMo-S), based on GC/MS analysis and % peak area. AH – aromatic hydrocarbons, LCH – long chain hydrocarbons, LCH>C20 – long chain hydrocarbons greater than C20, NA-nitrogenated aromatics, LCA-long chain amides, PAH – polycyclic aromatics.

538

539 **Figure 5:** Compositional analysis of algal oil, from *Nannochloropsis*, after catalytic HDO (Ru/C, CoMo-H2, CoMo-S, RRM), based
 540 on GC/MS analysis and % peak area. AH – aromatic hydrocarbons, LCH – long chain hydrocarbons, LCH>C20 – long chain
 541 hydrocarbons greater than C20, NA-nitrogenated aromatics, LCA-long chain amides, PAH – polycyclic aromatics.

phenol, 4-methyl phenol), 9.2 ± 1.4 % ketones, 12.3 ± 5.2 % nitrogenated aromatics (1-methyl-Indan), and 2.9 ± 1.2 % long chain amides (e.g., octadecanamide – Fig. 4). Similar trends were observed for the *Nannochloropsis* strain (Fig. 5). When comparing algae strains and Ru/C or CoMo-S as the catalyst, the most noticeable trend was the significantly higher percentages of heteroatoms ($\alpha=0.10$, Ru/C, UGA strain versus *Spirulina* or *Nannochloropsis*) and incomplete deoxygenation of the FFA's (based on higher TAN numbers, $\alpha=0.05$) when using *Spirulina* or *Nannochloropsis* (Table 3). We attribute this to the significantly higher protein level ($\alpha=0.05$) in *Spirulina* and *Nannochloropsis*, and potentially higher H₂ demand for heteroatom denitrogenation. It is possible that the nitrogenated aromatics are preferentially deoxygenated (or compete for active sites) relative to the FFA's leading to incomplete HDO of the FFA's. Finally, since previous work indicated that a lower pretreatment HTL temperature (175°C) generated higher protein levels we wanted to determine if this lower temperature would still result in significant HDO/HDN of the algal oil. As observed in Table 3, the lower HTL temperature resulted in higher heteroatom percentages and incomplete deoxygenation of FFA's, and lower HHV ($\alpha=0.05$), for both UGA and *Nannochloropsis* strains.

3.3. Simulated Distillation Analysis

As described in the experimental methods section, simulated distillation was performed by GC/MS and TGA analysis of the HDO treated algal oil. The boiling point range for petroleum products applied to the analysis is shown in Table 4SI. TGA analysis was performed to capture boiling point distributions for fractions that the GC/MS could not analyze; i.e., boiling points greater than 400-500 °C. It should be noted that there were several data points that did not follow the expected trend (cumulative % peak area versus boiling point) based on the standard curve

generated from the Gas Oil reference. It is possible that the column used and the GC/MS method did not allow for complete recovery and integration of some compounds. These differences were primarily in samples with high levels of heteroatoms. The most notable errors (differences in assigned boiling points based on the gas oil reference and identified compounds) occurred with samples containing nitrogenated and oxygenated compounds – e.g., 4-methyl phenol, 3-ethylpyridine, indole, hexadecanoic acid, hexadecanamide, octadecanamide.

Simulated distillation via GC/MS and TGA analysis indicated that the repeated HDO runs with Ru/C generated a mixture of compounds (~70-80 %) with boiling points between 200 and 360 °C when using UGA and *Nannochloropsis* HTL generated oil. Using the petroleum cuts presented in Table 4SI this suggests an oil similar to a mixture of kerosene, distillate fuel oil, and gas oil. These results were corroborated by using a Gas Oil reference (Table 4SI) and applying the same GC/MS methods for simulated distillation analysis of this standard and then overlaying against our algae HDO results. The algal oil generated via HDO using Ru/C from either the UGA strains or *Nannochloropsis* had boiling point distributions similar to Gas Oil, but with a larger fraction of higher boiling point components ~ 95% between 190 and 500 °C versus 25% for the Gas Oil reference (Fig. 6 and 7; data for other conditions are shown in the supplemental material).

Using CoMo-S catalyst generated an oil with a lower boiling point fraction than Ru/C for the same algae strains; ~10-20% between 80-180°C (Fig. 2SI, 3SI, and 5SI). This was primarily due to the formation of ketones, phenolics, and pyridines when using CoMo-S catalyst. Significantly lower boiling point fractions (~20-30% between 80-180°C) were also observed for the other catalysts including CoMoH₂ and RRM (Fig. 4SI). TGA analysis of the HDO treated

588 algae also suggested a higher boiling point fraction when using CoMo catalyst (H₂ reduced and
589 sulfide, Fig. 2SI and 3SI). This may have been due to the presence of hexadecanamide and
590 octadecanamide in these oils, which have higher boiling points than their denitrogenated forms
591 (e.g., 317°C for octadecane vs. 408 °C for octadecanamide). Finally, the effect of starting with
592 high protein concentration in the algae strain on the boiling point range was most noticeable
593 when using *Spirulina*. When comparing the same catalyst (Ru/C) and HTL/HDO conditions one
594 can see that a much lower boiling fraction (80-180°C) is generated in the oil; ~ 15% for
595 *Spirulina* versus ~ 6-7% for *Nannochloropsis*, compared to 0% for the UGA strains (Fig. 8). One
596 can't rule out the possibility that cell wall structure differences between the three strains and not

597 **Figure 6:** Simulated distillation analysis of a 3 stage HTL/HDO algal oil generated from UGA strains using Ru/C catalyst for HDO (2.1 g cat, 2
598 HDO runs). MN is medium naphtha, HN is heavy naphtha, DFO is distillate fuel oil. BP is boiling point. Bottom right figure is for TGA analysis.

Figure 7: Comparison of simulated distillation analysis between a Gas Oil reference (shown in Fig. 6SI) and 3 stage HTL/HDO algal oil generated from different algae strains using Ru/C. MN is medium naphtha, HN is heavy naphtha, DFO is distillate fuel oil (according to Table 4SI).

Figure 8: Simulated distillation analysis of 3 stage HTL/HDO algal oil generated from different algae strains using Ru/C. MN is medium naphtha, HN is heavy naphtha, DFO is distillate fuel oil.

just initial protein content, may have also played a role in protein separation in the HTL step thus effecting the catalytic HDO step.

Finally, the Gas Oil reference (ASTM D2887, Supelco) was analyzed using our GC/MS method to verify the simulated distillation method. This Gas Oil reference has a boiling point (BP) range from 121 to 454 °C. Our GC/MS method confirmed this BP range and is shown in Fig. 6SI. Except for the presence of BTEX at the low BP range (compounds with retention time < 10 min, Fig. 6SI), a majority of the compounds were long chain straight chain alkanes – octane (C8) to heptacosane (C27). The simulated distillation curves for the Gas Oil reference were then plotted against the catalytic HDO results (Fig. 7). As shown in Fig. 7, the upgraded algal oil had higher a boiling point range then the Gas Oil reference. This is due to the absence or low levels of benzene, xylene, and C8-C12 alkanes. This difference is also noticeable when comparing chromatograms of the Gas Oil reference with the HDO treated algae.

Oil yields ranged from 15-22% for conditions that resulted in the highest quality oil (lowest level of nitrogenated and oxygenated compounds). For example, an oil yield of 15-23% was measured using Ru/C for UGA and *Nannochloropsis* strains (Fig. 7SI) and these oils had heteroatoms percentages (based on GC/MS) ranging from 0 to 9% (Table 3). Oil yields using CoMo-S were in the same range as Ru/C (Fig. 7SI), yet the oil quality based on heteroatom percentage was poor; e.g., heteroatom percentages ranged from 24-46% for UGA and *Nannochloropsis* strains when using CoMo-S (Table 3). Oil yields were similar when using the other catalysts (CoMo-H₂ and RRM), yet there was incomplete hydrodeoxygenation and hydrodenitrogenation. Oil yields were also higher ($\alpha=0.05$, Ru/C-225°C vs. 175°C) when using a lower temperature pretreatment HTL temperature (Fig. 7SI), yet the quality of the oil was poor

(higher heteroatom levels) due to incomplete protein hydrolysis and limited nitrogen reduction in the algal oil before HDO (Table 3).

3.3. Catalyst Reuse and Characterization

In one experiment the Ru/C catalyst was recycled and reused in a subsequent catalytic HDO step. First, a 4 stage HTL/HDO sequence was initiated. The whole product at the end of this run was filtered and then re-used (catalyst and solids) as catalyst for another 4 stage HTL/HDO sequence. Qualitatively, the two different oil products look very similar in composition based on GC/MS analysis (Fig. 8SI). However, review of the chromatograms indicated peaks of nitrogenated compounds (e.g., indoles) that were not apparent in the first treatment. More detailed analysis of the chromatograms indicates higher levels of heteroatoms in the upgraded oil using the recycled catalyst, suggesting catalyst poisoning of active sites or coking, resulting in blockage of active sites (Fig. 9). Oils yields were similar for fresh and reused catalyst – 20.9 wt.% (g oil/g dry algae) for the fresh Ru/C and 23.0 wt.% (g/g) for the reused Ru/C catalyst.

Recovery of the reused Ru-C catalyst and analysis of its physical properties indicated significant tar accumulation and subsequent reduction in surface area and pore volume. As noted in Figure 9, the amount of tar accumulated was similar between a single use and second use of Ru-C catalyst (3.92% for a single run and 3.7% for a second use). However, after the Ru-C catalyst was washed with solvent and degassed, surface area and pore volume were reduced significantly – i.e., an 85% reduction in surface area and 75% reduction in pore volume resulted. One reuse step of the catalyst caused further reduction in these values to a 92% and 86% reduction in surface area and pore volume, respectively (Fig. 9, Fig. 9SI). Catalysts used in the HDO step were recovered and a significant reduction in surface area and pore volume was

Figure 9: Compositional analysis of algal oil, from UGA strains, after catalytic HDO (Ru/C) using recycled catalyst, based on GC/MS analysis and % peak area (A, AH – aromatic hydrocarbons, LCH – long chain hydrocarbons, LCH>C20 – long chain hydrocarbons greater than C20, NA-nitrogenated aromatics, LCA-long chain amides, PAH – polycyclic aromatics) and Ru on carbon catalyst properties (tar, surface area and pore volume via BET) after repeated batch HDO and one reuse for HDO of UGA Raceway oil generated from a two-step HTL (B, UGA\225\350\350\Ru-C\RU is the reused catalyst result).

716 **Table 4:** Catalyst properties after repeated batch HDO of HTL-generated algal oil [PT-225°C, 15 min→HTL-350 °C, 60 min→RB/HDO-350
717 °C, 240 min].

Algae Strain/Catalyst	PT Temperature (°C)	Surface Area (m ² g ⁻¹)	Avg. Pore Size (diameter, Å)	Pore Volume (cm ³ g ⁻¹)	H ₂ Consumption (mmol hr ⁻¹ g ⁻¹)	
					HDO	RB
Fresh Catalyst						
Ru/C	NA	714±14.2	14.4±0.49	0.52±0.03	NA	NA
CoMo	NA	246±14	47.4±2.6	0.29±.0015	NA	NA
CoMo-H ₂	NA	244±12	49.2±6.01	0.30±0.0004	NA	NA
CoMo-S	NA	180±29	52±6.3	0.23±0.008	NA	NA
RRM	NA	28±3.7	42.2±34.7	0.04±0.002	NA	NA
UGA Consortium						
Ru/C	225	110	47.2	0.13	12.2	12.2
CoMo-S	225	73	56.3	0.10	12.1	12.1
Ru/C	175	122	44.0	0.13	12.0	12.0
Ru/C Reuse ^b	225	56	53.0	0.08	6.1	6.1
Nannochloropsis						
Ru/C	225	181	41.2	0.19	12.5	12.5
CoMo-H ₂	225	224	46.8	0.26	12.3	12.3
CoMo-S	225	125	47.1	0.15	12.3	12.3
RRM	225	27	6.0	0.0041	12.3	12.3
Ru/C	175	200	40.2	0.20	12.8	12.8
Ru/C	225	181	41.2	0.19	12.5	12.5
Spirulina						
Ru/C	225	347	33.7	0.29	12.2	12.2
CoMo-S	225	102	56.3	0.10	12.1	12.1
Ru/C	175	405	112	0.32	12.4	12.4

718 ^a Catalysts analyzed prior to use. ^b Catalyst from "reuse" study. NA: Not applicable

measured for all used catalysts ($\alpha=0.05$, analysis was performed by pooling results for all strains for a given catalyst; sample standard deviation for the recovered catalysts was calculated using R/d_2 , where R is the range and $d_2=1.128$). The catalysts with the lowest reduction in surface area and pore volume were the least effective HDO/HDN catalysts – i.e., CoMoH₂, CoMo-S, and RRM (Fig. 9SI and Table 4).

Although the low temperature pretreatment significantly reduced the nitrogen levels in the HTL oil (relative to the amount of nitrogen in the feedstock) and reduced heteroatom levels (Fig. 3), the final % N and TAN levels after catalytic upgrading were still too high for the product to be considered acceptable as a fuel or for co-processing (Table 3). These results are consistent with the recent work reported by Biller et al., 2015 [43] and Bai et al., 2014 [18] for the catalytic hydroprocessing of algal oil [CoMo-S and NiMoS; 350 and 405°C; 2 h and a range of catalysts, respectively] generated by continuous (350°C, 206 bar, 6 min) and batch hydrothermal liquefaction [350°C, 1h]. Using batch hydroprocessing the nitrogen content was reduced to 4.6-4.7% at 350°C and decreased to 2.4-2.6% at 405°C [Biller et al., 2015]. Bai et al., 2014, pretreated algal oil by hydrothermal reaction at 350°C for 4 hours in the presence of hydrogen (6 MPa) without a catalyst and subsequently performed catalytic HDO at 400°C to generate an oil with low nitrogen and oxygen levels (2.6% N, 1.1% O, 30 TAN, Ru/C). When using CoMo-S, Bai et al., 2014 did report a higher % O and TAN level in the oil of 4.8% and 61.5, compared to Ru/C. Our two-stage process coupled with HDO using Ru/C did result in lower nitrogen levels compared to Biller et al., 2015 (3.2-3.8 vs. 4.7%) and sulfur levels below CHNS detection, yet our % O levels were higher. Similar to Bai et al., 2014 our % O, heteroatom levels, and TAN levels in the oils were higher when using the sulfided CoMo catalyst for the UGA consortium (Table 3). The oxygen levels in the oils generated using our two-stage process ranged from 5-

15%, when subtracting the oxygen contribution due to water (Table 3), significantly higher than the 1-4.7 % O reported by Biller et al., 2015 (the lowest % O level occurred at 405°C) and 1-5% in Bai et al., 2004. Our final oxygen levels in the upgraded oils were process and strain dependent. In general, lower pretreatment temperatures resulted in higher oxygen levels, HTL/HDO using *Nannochloropsis* produced lower oxygen levels than the other strains, and Ru/C generated lower oxygen and TAN levels compared to CoMo-S (Table 3). The higher % O levels in our oils generated from the two-stage HTL process coupled with HDO may be due to our lower reaction temperature used during HDO (350°C versus 400-405°C in Biller et al., 2015 and Bai et al., 2014).

Recently, Elliott et al., 2013 [44] reported a continuous hydroprocessing step capable of upgrading algal oil generated from continuous HTL to form a final oil with significantly improved properties compared to our results and other batch HDO processing reports – i.e., oil with <0.05-0.25 %N, 3.7-<50 ppm S, <0.1-0.2 TAN [using a CoMo-S catalyst]. The most noticeable differences were the use of a trickle-bed with continuous H₂ addition (reportedly in great excess, yet not clearly defined), solids separation during HTL (e.g., precipitation of inorganics salts), and oil separation after HTL via spontaneous phase oil/aqueous phase formation (i.e., a solvent was not used to extract the biocrude or oil). The continuous HTL process did result in an algal oil feed to the HDO step that was lower in nitrogen compared to our feed (mean of 4.3±0.30 versus 5.9±0.1 %N, $\alpha=0.05$, level of significance). As discussed later, removal of the inorganic salts (e.g., calcium phosphate) may have minimized catalytic deactivation [Elliott et al., 2013], compared to our process where alkali metals were only potentially removed in the first step of the low temperature pretreatment. However, both Biller et al., 2015 and Bai et al., 2014 did remove solids generated during HTL before catalytic upgrading,

yet did not achieve the oil properties reported by Elliott et al., 2013. As in Elliott et al., 2013, hydrogen was added in excess of the requirement for complete reduction in our work. Taking the typical oil yield from the two-stage HTL process (~20%), the amount of algae (7 g) and hydrogen (0.13 moles-room temperature, 51.7 bar H₂, 60 ml of headspace) charged, and assuming the resultant biocrude is palmitic acid, one calculates eight times the molar requirement of hydrogen for complete reduction (3 mol H₂ per mole of palmitic with 2 moles H₂O formed). If the feed is assumed to be 94% palmitic acid and 6% pyridine (a possible nitrogen heteroatom model compound) then one estimates hydrogen was in excess by a factor six. However, the low H₂ volume to oil ratio, coupled with lack of continuous H₂ addition in the batch reactors may have reduced the rate of H₂ mass transfer and thus inhibited complete HDN and HDO of the algal biocrude.

Given the incomplete HDN and HDO of our algal biocrude, significant reduction in surface area and pore volume in the recovered catalysts (Table 4), and increase in heteroatom levels upon catalyst reuse (Fig. 9), additional analysis of the recovered catalysts was performed. Relative to unreacted catalysts, we observed an increase in sulfur on the Ru/C catalyst (645 ppm to 5080±112 ppm), but Ca and P levels increased significantly as well, and to a much higher level than sulfur (e.g., 114 ppm to 30,000 ppm P; see Table 5SI). It was also observed that sulfur deposition did not increase when the Ru/C was reused, yet Ca, Fe, Mg, and P levels increased significantly (see Table 5SI and Figure 13SI). This suggests that deposition of alkali metals and phosphorous may have contributed to catalyst deactivation, more so than sulfur poisoning. This trend in metal accumulation was not as significant for CoMo or CoMo-S, but a decline in sulfur for the CoMo-S catalyst was observed (Figure 14SI). To the best of our knowledge, there have been limited time on stream studies (TOS) or multiple reuse studies for HDO/HDN/HDS of

algae oil reported in the literature. However, TOS for HDO/HDS of crude vegetable oil sources have been reported and may be used to understand deactivation causes. Kubicka and Horacek 2011 [45], performed prolonged TOS studies (100-150 h) with crude rapeseed oil using CoMo-S/Al₂O₃. Results indicated the presence of phospholipids, when hydrolyzed under reaction conditions, release phosphorous and in the presence alkali metals form deposits on the catalyst bed. Significant reduction in HDO activity also occurred due to deposition of alkali metals and phosphorous catalyzed oligmerization products leading to coke formation [45]. Finally, we did analyze the effect of the low temperature pretreatment on the metal levels in the algal biocrude. For two out of three algae strains, pretreatment reduced metals levels in the biocrude (e.g., Ca was reduced by 0-40%, P was reduced by 17-35% and sulfur by 60-80% Figure 14SI). Yet for the UGA consortium, although P was reduced, metal and sulfur levels increased slightly (Fig. 14SI). However, in all cases alkali metal and phosphorous levels were high enough to have potentially contributed to coke formation and catalyst deactivation (Fig. 14SI).

3.4. Catalytic HDO of Model Macromolecules

3.4.1. Individual Macromolecules (Ru/C HDO/HDN)

Coupled HTL/ HDO experimentation were conducted on isolated palm oil (rich in C16:0 and C18:1D.9 fatty acids) using Ru/C catalyst and the biocrude generated revealed an abundance of free fatty acids (mainly C16:0 and C18:1D.9 FFA's), indicating triglyceride hydrolysis. The biocrude yield from this experiment was 100%, indicating that nearly all lipid mass charged to the reactor was converted to free fatty acids via HTL hydrolysis (Table 6SI). Further, HDO on this biocrude fraction demonstrated decarboxylation of the fatty acids to

hydrocarbons. The primary peaks in the HDO biocrude identified by GC/MS were saturated straight-chain hydrocarbons, primarily in the 15-17 carbon range (data not shown). Hydrocarbons as short as methane (identified in the gas phase by GC-TCD) and as long as 20+ carbons were identified, signifying cracking and reforming of hydrocarbon chains occurs under these reaction conditions; similarly, branched straight-chain hydrocarbons were observed.

GC/MS analysis of biocrude generated from HTL of albumin indicated the formation of nitrogenated compounds such as pyrroles, pyrrolidinones, piperdines, and short chain amines, and aromatics (primarily styrene-data not shown). HDO/HDN of this biocrude generated long chain hydrocarbons (5,8-diethyl-dodecane), amides (hexadecanamide, octadecanamide) and nitriles (hexadecanenitrile), and the aromatics, toluene and ethyl benzene (styrene was not present). Hydrothermal liquefaction of starch formed a range of cyclic ketones (e.g., cyclopentanone, 2 and 3-methyl-cyclopentene-1-one) and 2,5-hexadione. Hydrodeoxygenation of this liquid generated a range of aromatic hydrocarbons (toluene, ethyl benzene, xylene, and methylated benzenes), long chain hydrocarbons (tetradecane, pentadecane, and heptadecane), and naphthalenes.

3.4.2. Catalytic HDO of Model Mixtures

More interesting and insightful results were observed when studying the effect of protein addition to lipid and carbohydrate (starch) HDO. Catalytic HTL/HDO of a lipid/carbohydrate mixture (no protein) gave a similar suite of products and lower oil yield, when compared to treatment of the individual macromolecules (Fig. 10SI, Table 6SI), suggesting the catalytic pathways acted independently of each other. Addition of protein to the lipid resulted in the formation of long chain amides and nitriles, as well as pyridines, pyrroles, and indoles from HTL

only, and upon HDO many of these compounds persisted. The formation of the amides and nitriles suggests that the fatty acids generated during HTL undergo condensation reactions with ammonia generated from protein hydrolysis and deamination of the amino acids. Their persistence suggests incomplete HDO due to increased hydrogen demand and/or catalyst deactivation due to the presence of nitrogen heteroatoms (e.g., indole). Catalytic HDO/HDN of the carbohydrate/protein mixture primarily generated aromatic hydrocarbons (toluene, ethyl benzene, 3-methyl phenol) and nitrogenated compounds (pyridines, pyrroles, pyrrolidines, indoles) – based on qualitative GC/MS analysis nitrogen removal was clearly incomplete. Catalytic HDO/HDN of model compound mixtures simulating the lipid:carbohydrate:protein ratio typical of *Spirulina* (1:2.2:8) and *Nannochloropsis* (2:1:4) generated a bio-oil similar in composition to HDO/HDN of the algae strains (Fig. 10). However, HDO/HDN treatment of the 1:1:1 mixture resulted in significant levels of solids and no clear formation of an oil phase. After filtering the solids, GC/MS analysis of the remaining liquid phase could not detect the presence any fatty acids or hydrocarbons. The formation of dark brown solids and inactivation of HDO/HDN for the 1:1:1 mixture suggests a network of Maillard reactions occurred, potentially between the amino acids and carbohydrates formed during HTL (Fig. 11 and reference 46). The fact that this event occurred at a 1:1:1 ratio and not the others (at least not significant enough to inactivate HDO/HDN) suggests the carbohydrate to protein ratio is critically important in thermal processing of algae. A speculative reaction pathway highlighting the sequential and cross reactions based on these results is presented in Figure 11.

3.4.3. Catalyst Characterization

857 The Ru/C catalyst used in these experiments was recovered and analyzed for surface area, pore
858 volume, tar, and coke. The highest tar percentages were found with pure protein, in
859 mixtures with proteins, or in highest protein content algae (the one exception being a mixture
860 without lipid, Table 5). F o r e x a m p l e , mixtures involving equal parts of each
861 macromolecule had a tar content of 26%, followed by the *Nannochloropsis sp.* model mixture at
862 18%. The lowest tar content was measured in the mixtures without protein (lipids and
863 carbohydrate, 4.1%) and without lipids (proteins and carbohydrate, 3.6%), compared to 6.8 and
864 9.6% in lipid only and protein only, respectively. The highest coke levels were observed for the
865 lipid/protein and starch/lipid mixtures (28% and 17%), catalytic HDO of starch (21%), and in
866 highest protein content algae (Table 5). TGA analysis indicated the formation of a coke
867 component

HTL Only

HTL/HDO

Figure 10: GC/MS chromatograms for biocrude generated from conversion of lipids, carbohydrates, and proteins (1:2.2:8). Identified compounds are (1) toluene, (2) 2-methylpyrazine, (3) ethyl benzene, (4) styrene, (5) 1-propyl-2-pyrrolidinone, (6) methylpiperidine, (7) pentadecane, (8) pentadecanenitrile, (9) 1,2-Dipalmitin (C16:0), (10) oleic acid (C18:1D.9), (11) hexadecanamide, (12) octadecanamide, (13) 1,2-Dipalmitin, (14) 9-Octadecenamide, n-butyl-, (15) oleic diethanolamide, (16) branched pyridine, (17) branched pyrrolidinone, (18) methylphenol, (19) ethylphenol, (20) tetradecane, (21) hexadecane, (22) heptadecane, (23) octadecane, (24) octadecanenitrile.

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907 **Figure 11:** Potential reaction pathway for hydrothermal liquefaction (HTL) and HDO/HDN of lipids, protein, and carbohydrates in algae. FFA's indicate free
908 fatty acids; AA's indicate amino acids. Dotted lines indicate cross reactions.

Table 5: Catalyst coke, tar, and characterization data for two-stage conversion (HTL and HDO) using model compound mixtures compared to treatment of algae strains [HTL-350 °C, 30 min→HDO-350 °C for model compounds, 240 min PT-225°C, 15 min→HTL-350 °C, 60 min→RB/HDO-350 °C, 240 min for algae].

Representation	Coking ^a (%, w/w)	Tar (%, w/w)	Average Pore Size (Radius, Å)	Total Pore Volume (cm ³ g ⁻¹)	Surface Area (m ² g ⁻¹)
Untreated Ru/C Control	NA	NA	11.37	0.52	721.4
Only Lipid	8.3±6.7	6.80	15.27	0.2410	631.6
Only Carb.	21.0±4.2	8.87	18.50	0.0625	135.3
Only Protein	0.0±0.0	9.57	13.46	0.0807	239.8
No Protein	17.2±5.9	4.12	13.44	0.0694	206.6
No Carb.	28.5±1.8	11.94	ND	ND	ND
No Lipid	0.0±0.0	3.57	ND	ND	ND
Equal Parts	10.8±2.7	25.95	ND	ND	ND
<i>Spirulina platensis</i> Model	0.0±0.1	6.91	11.98	0.0514	171.6
<i>Nannochloropsis</i> Model	7.4±1.6	17.71	ND	ND	ND
UGA Consortium	8.8±5.3	3.92	47.2	0.13	110
UGA Consortium Reuse	11.7±6.6	3.70	53	0.08	56
<i>Nannochloropsis</i>	2.0±3.5	5.97	41.2	0.19	181
<i>Spirulina platensis</i>	25.8±40	8.82	33.7	0.29	347

^aDetermined by comparing to the mass loss of the control catalyst. NA: Not applicable, ND: Not determined.

Note, for samples listed as ND we could not successfully degas these recovered catalysts

peak from starch that combusts from 280-400°C. When protein was added to lipid or starch, and in the high protein algae (*Spirulina*), the coke peak at 280-400 °C increased and a new combustion peak appeared from 150-280°C (Fig. 12SI). These results suggest that protein and carbohydrate hydrolysis products contribute to catalyst deactivation leading to a decrease in deoxygenation and denitrogenation, and lower quality biocrude. Interestingly, HDO/HDN of the protein only, starch/protein and lipid/starch/protein mixture (1:1:1) showed lower levels of coking, indicating any deposited tar was removed by solvent rinsing. This does not preclude the possibility that nitrogen heteroatoms generated during HTL poisoned the catalyst by chemisorption to active sites (for mixtures with protein). Unsurprisingly, all used catalysts showed a decrease in surface area compared to the unused control (Table 5). HTL/HDO of lipid only indicated a decrease of only 12.4%, compared to 67-78% for the other conditions (Table 5). HTL/HDO of starch resulted in the most significant decrease in surface area (81.2%).

4. Conclusions

A low temperature hydrothermal liquefaction step (PT, 225 °C, 15 min) before high-temperature (HTL) subcritical liquefaction stages (350 °C, 60 min) and subsequent catalytic hydrodeoxygenation (HDO) reduced nitrogen heteroatoms in biocrude from algae. This pretreatment step generated a biocrude primarily characterized by free fatty acids, unsaturated hydrocarbons, and long chain heteroatoms with reduced nitrogen heteroatom levels. Both steps are critical, since PT/HDO generated low oil yields. Catalytic HDO of this oil using Ru/carbon catalyst (5% Ru) generated the lowest nitrogen (2.4-3.1%) and heteroatom level. Due to low sulfur levels in the biocrude and loss of sulfur, a CoMo-S catalyst generated higher nitrogen and

heteroatom levels, and resulted in a higher TAN value and lower heating value, compared to Ru/C. The highest quality oil (based on %N and heteroatom levels) was similar to gas oil with a higher boiling point range of ~20%:200-280°C, ~30%:280-350°C, and ~50%:350-460°C. Final yields for the highest quality oil generated via the coupled HTL/HDO using Ru/C ranged from 15-22% and indicate the severe penalty in nitrogen removal from high protein algal feedstocks. Analysis of recovered catalyst and reuse studies indicate that catalyst deactivation may have occurred due to alkali metal and phosphorous deposition contributing to coking, and as a result of Maillard reactions between sugars and amino acids generated during HTL and HDO steps. Although nitrogen heteroatoms levels exceeded fuel or petroleum refining requirements and catalyst deactivation occurred, probably due to Maillard reaction products and metals deposition, the two stage HTL process holds promise for high lipid/low protein algae biomass, due to the significant reduction in nitrogen heteroatoms in the resulting biocrude.

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