DEMETALLATION OF BIOCRUDE FROM HYDROTHERMAL LIQUEFACTION
OF MICROALGAE

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by
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ABSTRACT

Hydrothermal liquefaction (HTL) converts wet biomass into a crude bio-oil that could be further treated to be acceptable for processing in petroleum refineries. HTL takes advantage of the properties of hot, compressed water near its critical point to break down biomacromolecules in the feedstock into smaller molecules. Algal biocrude oil is a complex matrix composed of tens of thousands of molecules including organic compounds containing inorganic elements such as S, P, and metals. Metals in crude oil can cause problems in refineries such as fouling, corrosion, and catalyst deactivation. Several issues presently prevent the substitution of HTL biocrude for petroleum and one is the presence of metals in the crude bio-oil. Iron is found to be the most abundant metal and can cause plugging the head of the catalyst beds that are used to hydrotreat algal biocrude oil. The scarcity of important information about the concentrations and distributions of different elements, including metals, in the product fractions from alga HTL motivates the present study.

We report herein on the effect of HTL time, temperature, biomass loading, water loading, and heating rate on the concentrations and distributions of 13 different elements in algal HTL biocrude and the aqueous phase co-product from both isothermal and fast (non-isothermal) HTL. We then use the results to inform the design of interventions to reduce the metal content in the biocrude and thus make it more amenable to processing in a conventional petroleum refinery. Those interventions include engineering the recovery solvents, altering product workup procedures, and applying heterogeneous catalysts during HTL. The Fe contents were reduced over 50% by use of methyl tert-butyl ether (MTBE) rather than dichloromethane as the organic solvent for biocrude recovery and they were reduced over 98% via additional application of a supported Ni catalyst during HTL. This work demonstrates that the hydrothermal treatment
conditions influence the metal content in biocrude and that judicious selection of solvent and catalyst can lead to significant reduction in the metal content in biocrude.

To better understand the pathways of iron removal and eliminate the interference of other macromolecules, metals, and ligands, we use hemin to represent the iron presence from microalgae. Using hemin as a model compound provides an unprecedented opportunity to examine the products formed from iron-containing molecules. Iron in hemin crude oil was reduced using the same solvent and catalysts combined that were employed during HTL of microalgae. The majority of the oil products are iron porphyrinic species after uncatalyzed hemin HTL, and those porphyrinic species are degraded into N₂ species when hemin is treated with demetallation catalysts.

Having proved that hemin is a robust model compound to predict iron behavior during HTL of microalgae, we further developed the first gravimetric and multiphase kinetic model for HTL of hemin. This model captures the empirical trends for both hemin product yields and iron distributions. Agreement is very good between calculated model values and observed values. The kinetic models of iron distributions for HTL of hemin and microalgae are compared against each other. Taking all the observations collectively, we also proposed optimal HTL conditions for a higher yield and crude oil quality in terms of iron presence. The conditions includes an 80 min of holding time, and a temperature range from 364 °C to 368 °C or from 380 °C to 400 °C. Finally, we used the same reaction network frame to model the iron data obtained in HTL of microalgae.

The investigation into iron behaviors by using a model compound and developing an empirical model can help enhance the overall process optimization. The elucidations on metal fates and the success in reducing metal contents in the algal crude oil will ultimately enable minimal capital by using the existing downstream refinery infrastructures for algal biocrude.
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Chapter 1

Introduction

1.1 Algae Feedstock for Biofuels

The growing economy worldwide has driven heavier reliance on energy consumption. According to U.S. Energy Information Administration (EIA), the fossil fuel sources—petroleum, natural gas, and coal—have made up at least 80% of total U.S. energy consumption over the past 100 years. Despite the rapid growth of global supply for fossil fuels, the EIA foresees half exhaustion of existing oil reserves and anticipated reserves by the year 2030. In recent years, production of nuclear, hydroelectric, and other renewable energies have slightly moderated the intensive uses of fossil fuels, but growing global demand for energy is expected to maintain the predominance of these three energy resources between now and year 2040.

One serious consequence of burning fossil fuels is Greenhouse Gas (GHG) emissions, which have led to climate change and several other environmental concerns. Human activity is most likely a driving factor contributing to global warming in the recent century. During the past 20 years, about three-quarters of human-made carbon dioxide emissions were from burning fossil fuels.

Biofuels are fuels produced from biomass—organic matters made up of plant materials and animal waste. With the ongoing demand for transportation fuels, along with growing concerns over global warming caused by carbon dioxide emissions, biofuels have been gaining popularity in recent decades. Compared to fossil fuels, biofuels are advantageous in terms of long term availability and GHG remission. Unlike fossil fuels, biomass can be replenished quickly and easily. Biomass can sequester carbon dioxide in the atmosphere or emitted from power plants, so there is no net increase in atmospheric CO₂ levels when biofuels are utilized. Thus it has the potential to be carbon neutral for biofuel production.
Production of fuels from microalgae has received attention over recent years for several reasons. Compared to terrestrial feedstocks, algae grow rapidly due to its potentially high photosynthetic efficiency, as well as achieve high biomass yields per acre of land. Algae can have high lipid content (up to 70%), which prompted the potential for achieving favorably high fuel yields from algal biomass. Algae can be cultivated on low-value land and water, such as brackish coastal water and seawater, so that it has no competition with crops or other biomass resources. During the cultivation process, algae’s nutrient uptake includes nitrogen, phosphorus, and sulfate nutrients, which can come from wastewater.

### 1.2 Hydrothermal liquefaction (HTL)

HTL is the process of obtaining low molecular weight crude oils from high molecular weight organic compounds by converting biomass in water at high temperature and pressure. HTL mostly occurs between about 200 and 400 °C, with a pressure between 10 and 40 MPa. These pressures are high enough to keep the water in liquid phase or supercritical phase. At temperatures up to around 500 °C, a catalyst is usually required for effective reforming and gasification. At higher temperatures above 500 °C, homogeneous gasification and thermolysis often take place. HTL biocrude oils tend to be viscous and tar-like, and 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. Biocrude oils often have a significant amount of heteroatom contents, primarily in the form of nitrogenous and oxygenated compounds. Bio-oil yields typically range from 25 to 60 wt %, and the heating value of the bio-oil is usually around 35 MJ/kg, which is close to that of petroleum-derived crudes (~42 MJ/kg). In addition to heteroatoms such as N, O, and S in algal biocrude oil, there is also the presence of metals. The most common metals in algal crude oil are Na, Fe, Ca, Cu, K, Mg, Ni, Zn, Mn, Cr, Al, with concentration ranging from a few ppm to a few thousand ppm. Conversely, the most abundant and detrimental metals in petroleum crude oil are Ni and V.
Biomass is mainly composed of varying ratios of macromolecules including carbohydrates, protein, lipids and lignin. During HTL, the reaction pathway could be described as: 1) Depolymerization of macromolecules into monomer units. 2) Decomposition of monomers by cleavage, dehydration, decarboxylation and deamination. 3) Recombination of reactive fragments.

The advantage of this process is that it eliminates the needs for feedstock drying. Since microalgae usually have a high moisture content and water possesses a high latent heat, HTL is more energy efficient than traditional thermochemical methods such as pyrolysis, which requires feedstock drying. The overall energy requirement of fast pyrolysis is theoretically 1.6 times more than that of HTL. Recent techno-economic analyses and life cycle assessments have shown that HTL has lower GHG emissions with a better energy return on investment as compared to conventional lipid extraction and transterification methods.

Compared to isothermal HTL which requires long (10-120 min) holding time, fast HTL examines non-isothermal HTL with rapid heating. Faeth et al. has the first published work on HTL of microalgae over much shorter time scale and obtained comparable or higher biocrude yields than from isothermal conditions. Others have studied fast HTL in batch reactors of corn stover, bacteria and yeast, and macroalgae.

**Properties of water at sub- and supercritical temperatures**

High-temperature water (HTW) is used as a medium for one of the primary processes to hydrothermally convert biomass into bio-crude oil and co-products. HTW refers to both pressurized hot water above 200 °C and supercritical water (T > 374 °C, P > 22.1MPa).

Properties of liquid water at elevated temperature exhibit great variability when compared to those of ambient liquid water. As the temperature increases, there is an increase in the diffusion rate and a decrease in the viscosity and surface tension. The dielectric constant decreases from 78 at 25 °C and 0.1 MPa to 14.07 at 350 °C and 20 MPa, which is similar to the range of polar organic solvents. This increases the solubility of organic compounds, such as fatty acids. Moreover, the ion product (Kw) for
HTW is about 3 orders of magnitude higher than that for ambient liquid water. Consequently, the H⁺ and OH⁻ ions from self-ionization of HTW are at such a high level that some acid- or base-dependent reactions could proceed in HTW even without catalysts, thus HTW could act as an acid- or base-catalyst.

1.3 Metals and Other Elements in Microalgae

The cultivation system of a large-scale microalgae production can be coupled with industrial waste resources, such as carbon dioxide from flue gas and nutrients from wastewater. Considerable studies have been conducted on exploitation of the metal binding ability of biomass for the treatment of metal enriched wastewaters. In this way, the cooperated system is able to minimize biofuel production costs and at the same time provide environmental remediation. This environmental technology is named as phytoremediation, which uses plants to stabilize various organic and inorganic pollutants present in solids, muds, or wastewater. However, one of the problems associated with phytoremediation is to handle and dispose of the biomass product. Thus, it is both economically valuable and environmentally friendly to utilize microalgae as an energy source while removing the heavy metals during the biomass conversion process.

Algae can accumulate high concentrations of inorganics depending on the supply in the external environment. The most common metals determined in algal crude oil are iron and sodium. Sodium is notorious for forming low-temperature melting compounds which cause the formed ash to become sticky, and prone to depositing on surfaces inside the reactor. Iron could affect the activity of catalysts, which results in an increase of gas and coke formation and reducing yields of crude oil. Iron also works its way into the hydrotreater feed as rust and scale from corrosion of upstream equipment and piping, as well as from unfiltered particulates present in the feed. Fe particulates also fill the interstitial spaces in the catalyst bed which will result in a higher than expected pressure drop. While sodium can often be
removed by a desalter in the refinery, iron has been gaining special interest because of its difficulty to be removed as an organic complex by a desalter.

![Figure 1-1: Example of heme, 2Fe-2S cluster and ferritin.](http://www.chemistry.wustl.edu/~edudev/LabTutorials/Ferritin/Ferritin.html)

It is important to have a preliminary knowledge of the intracellular molecular form of iron and its binding states on the probable sites of an algal cell. Iron supplied from the culture media is mostly in the form of dissolved chelated complexes, primarily bound to ethylenediaminetetraacetic acid (EDTA). These compounds are much more stable than inorganic iron salts, and are able to serve as trace metal buffers. Iron in microalgae could also be present in harvested microalgae slurry as a result of flocculation processes employed. Iron can be present in the form of heme, a ferrous containing porphyrin, or Fe-sulfur clusters in a variety of metalloproteins that are involved in countless metabolic pathways. Iron plays a particularly important role in oxygenic photosynthesis, as 50-90% of the metabolic Fe within phytoplankton resides in the photosynthetic apparatus. Fe is also involved in other cellular processes such as assimilation of NO₃, cellular respiration and vitamin synthesis in the form of Fe-containing enzymes. Some microalgae contain ferritins for Fe storage that are located in plastid.

**1.4 Issues Associated with Metals in Algal Biocrude**

Metal complexes may poison and foul catalysts, contribute to furnace coking, and cause undesirable side reactions in refinery operations, such as fluid cracking and hydrodesulfurization. Iron in
reﬁnery is harmful to the process, as it can react with hydrogen sulﬁdes from the petroleum hydrogenation process and produce iron sulﬁde, which adheres to the catalyst surface and degrades the activity and selectivity of the catalyst. Dunn et al.\textsuperscript{35} concluded that the iron deposition during catalytic cracking can cause 10% loss for feedstock conversion.

Iron content in petroleum crude oil can vary from different sources and time, but the general range falls within 0.1 to tens of ppm and some can contain 100 ppm or more of the iron content,\textsuperscript{36–40} which is orders of magnitude lower than iron content in algal crude oil.\textsuperscript{1} Iron at low concentration has mild poisoning effect during catalytic cracking. However, with a high iron content, reﬁneries have found this iron deposition phenomena deteriorating.\textsuperscript{41–45} With an iron concentration above 1000 ppm in the algal crude oil, the catalyst bed was found to be clogged after only tens of hours of continuous operation.\textsuperscript{1}

1.5 Metals in Petroleum Crude Oil and Demetallation Methods

To the best of my knowledge, there are no studies on metal removal from algal crude oil to date. However, a step similar to that in petroleum reﬁneries regarding demetallation of residual fossil fuels might be prudent, as the most undesirable metals in petroleum crude oil, nickel and vanadium, are also generally present in the form of porphyrins.\textsuperscript{46} The removal of metals can be a complex problem as they are chelated or complexed with ligands that are completely compatible with crude oil. However, certain reagents and methods have been used successfully to demetallate samples of petroleum crude oil.

Demetallation of petroleum crude oil has been studied with two stages wherein crude oil is ﬁrst obtained and then upgraded. The most common methods applied are physical methods, chemical methods and catalytic hydrosprocessing.\textsuperscript{46} Physical methods include distillation, solvent extraction and ﬁltration. Chemical methods include thermal processes such as visbreaking and coking. Catalytic hydrosprocessing is accomplished by the use of hydrometallization (HDM) catalysts. The fundamental reaction was
proposed as such: porphyrins are initially hydrogenated, form precursors which subsequently undergo ring cleavage reactions, and then deposit metals on the catalyst surface as depicted below:

\[
M-P \rightleftharpoons M-PH_2 \rightarrow M_{\text{deposition}} + \text{hydrocarbon}
\]

In this depiction, P represents the initial porphyrin complex, M represents metal, and M–PH₂ represents the hydrogenated metalloporphyrin intermediate. Investigations of non-porphyrin complexes have not been very extensive, and the transformation pathways during HDM are not known.

Microwave radiation was also used to remove metals from crude oil treated with acid or alkali, and has shown high efficiency. Microwave heating involves agitation of polar molecules or ions that oscillate under the effect of an oscillating electric or magnetic field.

Electrochemical removal of metals was also applied by using protonating agents to enhance the degradation of vanadium porphyrins. The percentage of demetallation was highly dependent on the electrodes, protonating agents, and electrolysis conditions.

There are also methods for resolving iron issues in petroleum crude oil including magnetic separation, iron passivation, and iron adsorption. As intuitive as it might be, magnetic separation is used for iron and its compounds that have a strong magnetism. However, the average deferric efficiency for crude oil using a magnetic separator is about 43%. Iron passivation is used to inhibit iron deposition onto the catalyst surfaces. However, about 15% - 20% of the installed capacity must be guaranteed to restore the activity of the catalyst. Iron adsorption uses sulfur in a fiber material to react with iron in crude oil, resulting in iron sulfide dispositions on the fiber. However, this technology requires sulfur-containing feedstocks and long-hour operations. The deferric efficiency is >75% only when the reaction is run continuously for 100 h.
1.6 Research Motivation and Summary

Following the study of hydrothermal liquefaction of microalgae, there has been no report of metal removal specific to algal biocrude oil, although metals are undesirable for the refinery process. There are very limited studies on metal contents and fates after HTL of microalgae.50–52 There are also several studies on the hydrotreating of algal biocrudes that aim to remove other undesirable components such as oxygen, sulfur, nitrogen, and aromatics using heterogeneous catalyst at relatively high temperature and pressure.53–58 Given the large gaps in the research field, it is of vital importance to study the metal fates, demetallation methods, and the kinetics in detail.

Chapter 2 provides an overview of the metals and elements presenting in microalgae and its HTL products. We investigated the effects of processing conditions on those element distributions into post HTL products. In Chapter 3, we introduced interventions to reduce metal contents, especially iron contents, in the resulting crude oil. The methods included engineering recovery solvents and applying heterogeneous catalysts. Chapter 4 uses hemin as an iron model compound in microalgae and mimics the iron fates during HTL applying the same interventions as in microalgae. Chapter 5 extended the study following Chapter 4, describing the development of kinetic models to calculate both the product yields and iron distributions from HTL of hemin. Chapter 6 contains the conclusions of work in this dissertation and recommendations for future research.
Chapter 2

Effects of Processing Conditions on Metal Contents and Distributions from Hydrothermal Liquefaction of Microalgae

2.1 Introduction

HTL biocrude, like petroleum, needs to be upgraded and refined to produce transportation fuels. One proposed approach for processing algal biocrude is to blend it with petroleum feedstocks and use existing refinery technology. However, the metals present in algal biocrude from HTL can have negative effects in a refinery. Iron could deposit on and reduce the activity of catalysts. Fe particulates can also fill the interstitial spaces in a catalyst bed, which will result in a higher than expected pressure drop. Jarvis et al. reported that the catalyst bed used to hydrotreat algal-derived HTL biocrudes (Fe > 700 ppm) plugged after only tens of hours of continuous processing. They reported the presence of about 100 unique iron-containing porphyrin molecules in algal biocrude from HTL. The most prevalent were \( \text{Ni}_4\text{Fe}_1 \) species. In addition, both the carbon number and double bond equivalent distribution of those iron porphyrins are similar to those of Ni- and V-porphyrins in petroleum crude oil. Other metals, such as sodium, can form low-temperature melting compounds that lead to deposition and buildup on surfaces inside the reactor. While sodium can often be removed by different demineralization methods, iron is more difficult to remove.

There have been some prior reports on the concentrations of metals and other biomass elements in the solid, aqueous-phase, and biocrude HTL product fractions. These prior reports for biocrude are limited to either just one or two HTL process conditions or one or two metals at different conditions for isothermal HTL for long residence times. There has been no published work on the fates of elements
other than Fe and Na from fast HTL,\textsuperscript{18} (HTL at short reaction times and fast heating rates\textsuperscript{25}). We agree with Patel et al.\textsuperscript{20} that there is a pressing need to examine short HTL residence times.

However, there have been no reports on how the HTL processing conditions influence the metal content in the biocrude. That the conditions might have an influence is suggested by prior work where supercritical water (SCW) treatment has been shown to remove metals, such as nickel from porphyrin structures, even in the absence of catalyst and added hydrogen.\textsuperscript{59} Additionally, there have been no reports on the metal content in algae biocrude produced via fast HTL\textsuperscript{14}, an emerging and more rapid approach for converting microalgae to biocrude.

Of course, manipulating HTL processing conditions may not alone be sufficient to produce biocrude that can be processed via existing refinery technology. The literature on both petroleum demetallation and on algae treatment provides some guidance, however, regarding process options and interventions that might be profitably explored to reduce metal content in biocrude even further. The most abundant metals in petroleum crude oil, nickel and vanadium, are also generally present in the form of porphyrins.\textsuperscript{46} Physical methods (e.g., distillation, solvent extraction), chemical methods (e.g., visbreaking, delayed coking), and catalytic methods (e.g., hydrotreatment) can be applied for demetallation.\textsuperscript{46}

Wang et al.\textsuperscript{60} showed that catalytic hydrotreatment of HTL algae biocrude (separated from the HTL mixture with isoparaffin) led to the accumulation of Fe on the carbon-supported metal (Pt, Ru, Ni, Co) catalysts. The goal of this study was not metal removal, however, so this result was largely mentioned in passing and noted simply as an observation. Wang et al.\textsuperscript{61} showed that washing algal biomass with water and extracting oils from algae with different organic solvents can have a very large impact on the metal content in the algal oil.

The scarcity of important information about the concentrations and distributions of different elements, including metals, in the product fractions from alga HTL motivates the present study. We report herein on the effect of HTL time, temperature, biomass loading, and water loading on the concentrations and distributions of 13 different elements in algal HTL biocrude and the aqueous phase co-product from both isothermal and fast (non-isothermal) HTL. The results can inform the design of interventions to
reduce the metal content in the biocrude and thus make it more amenable to processing in a conventional petroleum refinery.

2.2 Materials and Methods

2.2.1 Materials

*Nannochloropsis sp.* algae was provided by Sapphire Energy Inc., as dried powder with roughly 17.6 wt% of ash content and also purchased from Reed Mariculture Inc. with 5.9 wt% of ash content. Both were used as received. The biochemical composition information is analyzed by Cumberland Valley Analytical Service and is listed on Table 2-1. Dichloromethane (DCM), n-pentane, ethylene glycol butyl ether (EGBE) were obtained from Sigma-Aldrich with over 99% purity. A standard for quantifying metals (S-21+K oil) and Elemental Blank Oil were purchased from Conostan Division, Continental Oil Company. The standard comprises 22 elements (at 500 ppm each), which include all the metals of interest in algal biocrude. 1000 µg/ml iron and sodium standards in 2% HCl were purchased from High-Purity Standards.

Swagelok stainless steel caps and port connectors (nominal ½ in) were used to make 4.1 mL batch reactors. An Omega UWBT series wireless Bluetooth thermocouple was used to monitor the temperature inside the reactors.

<table>
<thead>
<tr>
<th></th>
<th>Sapphire</th>
<th>Reed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids</td>
<td>6</td>
<td>14.5</td>
</tr>
<tr>
<td>Proteins</td>
<td>43.1</td>
<td>58.6</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>33.3</td>
<td>20</td>
</tr>
<tr>
<td>Ash</td>
<td>17.6</td>
<td>5.9</td>
</tr>
</tbody>
</table>
2.2.2 Procedure

We loaded carefully measured amounts of algae biomass and deionized (DI) water into the reactors. The precise amounts of each were determined by the desired biomass loading (5, 10, 15 wt% algae) and system pressure. The reactors were placed in a sand bath preheated to the desired set point temperature (350, 400, 450 °C) for the desired holding time (0.5 – 60 min). A proxy reactor loaded with DI water and equipped with a thermocouple was also placed in the sand bath to record the reactor temperature as a function of holding time. The reactors were then removed from the sand bath and quickly quenched in ice water. We then let the reactors equilibrate for at least one hour prior to opening them.

We placed the loaded reactors in a fluidized sand bath (model IFB-51 from Techne) equipped with a temperature controller. The sand bath was preheated to the desired set point temperature. Figure B-1 shows the reactor temperature profiles for the sand bath set point temperatures used in this chapter. After conducting the reaction for the allotted time, we removed the reactors from the sand bath and quenched the reactions by placing them in an ice-water bath for 5 min. The reactors were then held at room temperature for at least 60 min before opening.

After opening the reactor, we first removed the aqueous phase (and some accompanying solids) and placed it in a conical tube. Next, we used aliquots of DCM to dissolve the organic material remaining in the reactor as well as in the cap, until all the contents were recovered. These aliquots were then transferred into the same conical tube. The total volume of DCM applied in each reactor was about 10 ml. The multiphase mixture was then separated via centrifugation. Centrifugation provided clear separation of an aqueous phase (on top), an organic phase (at the bottom), and a solid phase (in between). A glass pipet was then used to recover the organic phase. We were careful not to collect any of the aqueous or solid phases with the pipet. This approach did leave behind a very small amount of the organic layer. We flowed N₂ over the separated organic phase to evaporate the solvent and isolate the biocrude. The conditions used for evaporation of DCM were 38 °C for 2 hours. We separated the aqueous phase from
the solids by filtration. The rest of the product work-up procedure was the same as that described previously.62

The concentrations of different elements in the algal biomass, the biocrude, and the aqueous phase co-product were determined by Inductively Coupled Plasma Optical Emission Spectrometry (Agilent, ICP-OES 715). The injection system used a concentric glass K-style nebulizer and a double glass spray chamber for sample aspiration. The peristaltic pump was fitted with organic resistant tubings. The optimal parameters for sample introduction are: Power (1.35kW), Plasma flow (15.0 L/min), Nebulizer flow (0.5 L/min), Auxiliary gas flow (2.25 L/min), Pump rate (10 rpm). Biocrude oil samples were dissolved in EGBE on a 1:50 to 1:80 weight basis. A blank solution was created by adding a given volume of element blank oil so as to match the solution viscosity to that of the samples was analyzed as a control. Yttrium was used as an internal standard. The metals content in the algal biomass and selected aqueous phase product samples were determined using ICP-AES (Thermo ICAP 6500) by staff at Laboratory for Isotopes and Metals in the Environment (LIME) on campus.

We report product yields as the mass of the recovered product (e.g., biocrude) divided by the mass of algae (dry, ash free) loaded into the reactor. We typically ran three reactors at each experimental condition. Values reported are the means and reported uncertainties are standard deviations. For some experiments, a single reactor gave insufficient biocrude for analysis, so we combined the biocrude from the three reactors prior to analysis for these cases. As a result, some runs show no measured uncertainties for the concentrations of the different elements.

We use herein the reaction ordinate26,27 (R0) as a single metric to combine quantitatively the effects of both holding time (t) and temperature (T) and hence describe the severity of the reaction conditions.

\[
R_0 = \int_0^t e^{\frac{(T(t) - 100)}{14.75}} dt
\]  

(1)
2.3 Results and Discussion

Table 2-2 shows the conditions used in each fast (t ≤ 5 min) and isothermal (t ≥ 10 min) HTL experiment, the reactor pressure expected at the set-point temperatures, the corresponding values of the reaction ordinate (R₀), and the biocrude yields obtained. At the short holding times, the reactor contents did not reach the sand bath set point temperature and both temperature and pressure increased continually throughout the reaction. Figure B-1 shows the reactor temperature profile at each sand bath set point temperature. Mixing of the reactor contents likely occurred during heat up via convection currents induced by the radial temperature gradient that accompanied heating. Even so, physical processes such as algae cell rupture and transport of water and biopolymers in the reactor may play a role in this reacting system. Water loading (vol %) is the percentage of the reactor volume that is occupied by liquid water at room temperature. We typically used a high water loading, selected such that at the set point temperature the reactor would be 90% filled with liquid (for runs below the critical temperature of 374 °C) or the reactor pressure would be 40 MPa, which is a safe working pressure for the reactor (for runs at supercritical set point temperatures). We also considered two lower water loadings for experiments at 350 and 400 °C to explore the effect of this potential process variable. At the subcritical set point temperature of 350 °C, there will be both liquid water and a vapor phase present in the reactor at the water loadings used here. At the supercritical set point temperature, both liquid and vapor phases will co-exist until the critical temperature of water (374 °C) is reached and exceeded, at which point a single supercritical fluid phase will exist in the reactor. The algae loading is the wt% of the biomass in the algae slurry in the reactor. We typically used 15 wt% as the base case, and explored the effect of this variable by using two lower loadings (5 and 10 wt%) at 350 and 400 °C.

Table 2-3 provides the concentrations of ten metals (Ni, Ca, Mn, Zn, Cu, Al, K, Fe, Mg, Na) along with P, S, and Si in the algal HTL biocrude produced under the different conditions examined. The metal concentrations in the algae biomass was reported previously. The Run numbers correspond to those defined in Table 2-2. We omit Run 24 from Table 2-3 because there was insufficient biocrude for
analysis. Control experiments with just DI water and blank oil (no biomass) led to Fe and Ni levels of less than 1 ppm in the resultant organic phase.

Table 2-2: Summary of Reaction Conditions and Biocrude Yields for HTL

<table>
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<tr>
<th>run</th>
<th>set-point temperature (°C)</th>
<th>holding time (min)</th>
<th>algae loading (wt%)</th>
<th>water loading (vol%)</th>
<th>P* (MPa)</th>
<th>log (R_o)</th>
<th>biocrude yield (wt% daf)</th>
</tr>
</thead>
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<td>1</td>
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<td>54.6</td>
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<td>6.51</td>
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<td>11.78</td>
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* taken from steam tables for given set-point temperature and water density
### Table 2-3: Concentrations (ppm) of different elements in algal biocrude

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<tr>
<th>run</th>
<th>Ni</th>
<th>Ca</th>
<th>Mn</th>
<th>P</th>
<th>S</th>
<th>Si</th>
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<td>111±80</td>
<td>424±33</td>
<td>38±30</td>
<td>146±1.4</td>
<td>27±2</td>
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<td>1472±83</td>
<td>29±12</td>
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</tr>
<tr>
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<td>0.9±0.7</td>
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<td>7±10</td>
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n.d. – not determined
The most abundant elements at mild conditions were Ca, P, K, Fe, Mg, and Na. The Fe concentrations remained high (typically 1000 – 2500 ppm) throughout. The concentrations of Mn and Al were always among the lowest in the biocrude, and their levels exceeded 30 ppm in only a single run. The concentrations of Na and K showed considerable run-to-run variation, and the uncertainty, at times, exceeded the mean value. Even so, it is clear that the concentrations of alkali metals are relatively high at mild conditions.

2.3.1 Influence of Holding Time

Figure 2-1 displays the temporal variations of the concentrations of different elements in biocrude from HTL at a sandbath set-point temperature of 350 °C. The trends at 400 °C are very similar and hence not displayed. We plot only those species with concentrations exceeding 10 ppm. Note that the x-axis is a log scale.

The P, Na, Ca, and Mg, concentrations have their highest values at the shortest holding time and then decrease rapidly. Phosphorus, for example, decreases from 3800 ppm at one minute to about 100 ppm at a 3 min holding time. These short holding times correspond to the regime known as fast HTL, and it is clear that these large variations in concentrations would have been entirely missed if one examined solely conventional isothermal HTL, which employs holding times on the order of tens of minutes. Zn, Cu, and Ni do not show much variation with time, whereas the Fe concentration first increases and then decreases as holding time increases. Even with this decrease, however, after about 3 min, Fe is the most abundant of the elements depicted. These high levels of Fe in HTL biocrude are consistent with previous work on isothermal HTL.18

The temporal variation of the Fe concentration in the biocrude suggests that only a fraction of the Fe-containing structures (e.g., porphyrins) in the biomass are converted into DCM-soluble material (i.e., biocrude) at the mildest conditions explored. Applying increasingly severe conditions, however,
facilitates the degradation of these structures into ones that partition into the biocrude phase. This scenario of the mildest conditions being too mild to affect some of the structural changes that occurred under more severe conditions is consistent with our observation that the color of the biocrude solution was much lighter and semi-transparent at mild conditions and become darker and opaque as the holding time increased.

Figure 2-1: Concentrations of different elements in algal biocrude from HTL at 350 °C (15 wt% algae loading, 55 vol% water loading, Runs 1-7)

2.3.2 Influence of Reaction Severity

Figure 2-1 showed that Fe was the most abundant metal in biocrude from HTL at 350 °C, so we focus additional attention on iron. Figure 2-2 shows the effect of the reaction ordinate on the iron concentration in the biocrude and the biocrude yields from HTL with a 15 wt% biomass loading and the highest water vol% loading. Use of the reaction ordinate as a severity factor permits the plotting together
of data obtained from experiments at different set-point temperatures and thus gives a larger data set for examination.

![Biocrude Yield vs Iron Concentration](image)

Figure 2-2: Variation of the biocrude yield (wt. % d.a.f. basis) and iron concentration (ppm) in biocrude with the reaction ordinate (HTL with 15 wt% algae loading and highest water vol% loading, Runs 1-7, 12-19, and 25-29)

Figure 2-2 shows that at the least severe conditions examined, both the iron concentration in the biocrude and the biocrude yield are low. There appears to be little hydrothermal chemistry taking place at these mildest conditions and only a small fraction of the iron porphyrins in the biomass being transferred into the biocrude phase. As the reaction severity increases, both the biocrude yield and its iron concentration increase. The biocrude yield tends to be relatively steady at around 35-40 wt% from $R_o = 10^8$ to $10^{11}$. The Fe concentration, however, reaches its highest value at the low end of this reaction ordinate range and then decreases steadily such that its concentration at $R_o = 10^{11}$ is about half that at $R_o = 10^8$. The different effects of the reaction severity in this range on the biocrude yield (none) and the Fe concentration (decreases) points to an opportunity to select HTL conditions that could provide high biocrude yields and simultaneously provide reduced Fe concentrations. The region between $R_o = 10^9$ – $10^{11}$ appears to be promising for production of biocrude with reduced iron content from this particular
alga. This region corresponds to performing HTL at 400°C for 5 min or at 350 °C for 60 min, for example.

Although manipulating the HTL process variables can reduce the iron concentration in algal biocrude to some extent, Fe-free biocrude cannot be so produced from this alga. This outcome is perhaps due to the thermal stability of the Fe-containing porphyrin compounds in the biomass, which are subsequently transferred to the biocrude.

Previous work has reported on how yields of biocrude from HTL vary with the Reaction Ordinate.\textsuperscript{25,28,29} All of these reports show an initial increase in yield with increasing severity and then a subsequent decrease in yield as the Reaction Ordinate increases further to more severe processing conditions. This behavior is reasonable because the biomass macromolecules are not fully decomposed to biocrude at mild conditions and at severe conditions gasification of biocrude takes place.\textsuperscript{30} Though this general trend has been observed for all feedstocks to date, there are some differences in the shapes of these biocrude yield vs $R_o$ curves for the different feedstocks. For example, Faeth et al.\textsuperscript{25} reported the highest biocrude yields from algae at $R_o$ from $10^6 - 10^8$, whereas we observed the highest yields at about $R_o$ from $10^8 - 10^{11}$. Faeth et al. also reported a faster increase in biocrude yield with increasing severity factor than observed in the present study. Sheehan and Savage\textsuperscript{28} compared HTL biocrude yields for two model proteins, and observed a maximum yield around $R_o = 10^7 - 10^8$.

Phosphorus is an important element for HTL because it constitutes one of the elements needed to cultivate algal biomass. It is a mined resource and, ideally, an algal biorefinery would be designed to recover phosphorus after HTL and recycle it to the ponds or bioreactors used to grow the algal biomass. Consequently, it is desirable to have low levels of phosphorus in the biocrude from HTL. Phosphorus that resides in the aqueous phase or in the solids after HTL could be more readily recovered and reused.
Figure 2-3: Variation of phosphorus concentration in biocrude with reaction ordinate (HTL with 15 wt% algae loading and highest water vol% loading, Runs 1-7, 12-19, and 25-29)

Figure 2-3 shows that there is a sharp decrease in the P concentration in the biocrude during the earliest stages of HTL. The phosphorus concentration is a few thousand ppm when the reaction severity is very mild and it drops to tens of ppm by the time even moderately severe conditions are reached. It seems that some phosphorus-containing compounds partition into the biocrude during the early stages of the reaction, and that those compounds readily react in hot compressed water such that the phosphorus appears in compounds that are either water soluble or solids.

Figure 2-4 shows that the concentrations of Ca and Mg exhibit the same variation with reaction ordinate that was observed for P in Figure 2-3. This similarity is consistent with the hypothesis that some common chemistry connects the fates of phosphorus with those of magnesium and calcium.
Figure 2-4: Variation of calcium and magnesium concentration in biocrude with reaction ordinate (HTL with 15 wt% algae loading and highest water vol% loading, Runs 1-7, 12-19, and 25-29)

Magnesium in algae can be present in different forms including as Mg-centered porphyrins in chlorophyll. Figure 4 shows that the Mg concentration in biocrude decreases steadily with increasing reaction ordinate, which suggests that the biocrude Mg is not in the form of stable porphyrin structures. Rather, the Mg is in a form that can transfer into one of the other product fractions. One possible pathway is transfer to the aqueous phase and then combining with phosphate to form a precipitate. Alkali metals (e.g., K and Na) in the biocrude are also likely in the form of compounds that can support formation of aqueous-phase alkali metal ions during HTL. These results show that the initial biocrude, formed at mild conditions from fast HTL, is rich in alkali and alkaline earth metals that exist in structures permitting the ready transfer of the Na, K, Ca, or Mg atoms to either the aqueous or solid phases.
2.3.3 Influence of biomass and water loadings

Figure 2-5 shows the effect of the algae loading (5, 10, 15 wt%) on the concentrations of different elements in algal biocrude from HTL at 400 °C for 30 min. The data from HTL at 350 °C show the same trends.

![Figure 2-5: Effect of algae loading on concentrations of different elements in biocrude from HTL (400 °C, 30 min, highest water loading, Runs 18, 20, and 21)](image)

The biomass loadings have different effects on the various elements. The concentrations of S, Fe, Zn, and Cu are largely unaffected by variations in the biomass loading. There is some variation in the mean concentrations of Na and K with loading, but the uncertainties are large enough that these differences may lack statistical significance. All other elements in Figure 2-5, however, show the lowest concentrations at the 15 wt% biomass loading and, with the sole exception of Si, the highest concentrations at the 5 wt% loading. Indeed, the concentrations of Ca, P, and Mg are orders of magnitude
higher at the lowest algae loading. This result shows that the algae biomass loading might be a process variable that can be manipulated to influence the concentration of some elements in HTL biocrude. Of course, this being the first report on this topic, additional work with other species of algae and other HTL conditions is needed to test this hypothesis.

Figure 2-6: Effect of water loading on concentrations of different elements in biocrude from HTL (400 °C, 30 min, 15 wt% biomass loading, Runs 18, 22, and 23)

Figure 2-6 shows that the water loading had no effect on the concentrations of S, Fe, Zn, Cu, and Ni in the biocrude from HTL at 400 °C for 30 min. The data from HTL at 350 °C show the same trends. The concentrations of Mn, Ca, P, Mg, and Si are each at least an order of magnitude lower at the highest water loading compared with the two lower water loadings. This result shows that the water loading might be another process variable that can be manipulated to influence the concentration of some metals in HTL biocrude.
It is difficult to discern from these few experiments alone how precisely the water loading is influencing the results. As Table 2-2 shows, the different water loadings correspond to different reactor pressures, so there might be a pressure effect. Additionally, the properties of supercritical water are density dependent, so the different values of the ion product (log $K_w = -20.1, -15.9, -12.8$, respectively) or dielectric constant (2.5, 5.0, 9.9, respectively) at the low, medium, and high water loadings ($\rho_w = 0.15, 0.30, 0.50$ g cm$^{-3}$), respectively, might be responsible.

### 2.3.4 Partitioning of elements into biocrude and aqueous phases

After HTL, the different elements in the biomass partition differently into the aqueous and biocrude phases. How those different amounts change with the HTL processing conditions is vital for knowing what conditions to select to facilitate recovery of different elements. Knowing the amounts of different elements loaded into the reactor (in the algal biomass), and recovered post-HTL in the biocrude and in the aqueous phase, permits an assessment of the effects of the HTL conditions on the partitioning between phases.

Figure 2-7 provides the results for HTL at 400 °C. Results for other HTL conditions appear in the Supporting Information. Given the small quantities of biomass used in the mini batch reactors and the unavoidable loss of some biocrude and aqueous phase product during sample transfers and handling, the recoveries presented in this section represent lower bounds.

Under all conditions examined in this work, less than 3% of the Mn and Al in the algal biomass was present in the aqueous and organic phases, so we do not show those elements in Figure 2-7. These metals appear to remain in the solids throughout the HTL process, regardless of the reaction severity. This observation is consistent with the results of Tian et al.$^{21}$, who reported that Al was the most abundant element in the solid residue after HTL of algae.
Generally, about 80 - 90% of the sodium and 60 - 80% of the potassium are in the aqueous phase, regardless of the HTL conditions. Although the sodium content in the biocrude is quite high compared to other metals, less than 1% of the Na in the biomass resided in the biocrude. Patel et al.\textsuperscript{20} studied the effect of reaction conditions (isothermal HTL with subcritical temperatures) on Na and K in the aqueous phase co-product and also reported that the concentrations of both metals vary little with temperature and reaction time.
Figure 2-7: Recoveries (%) of different biomass elements in the aqueous phase (blue) and biocrude phase (black) after HTL at 400 °C (15 wt% algae loading, highest water vol %, Runs 12-19)

Only traces of Ca were in the aqueous and biocrude phases for HTL holding times longer than 2 min. The results for Mg (not shown in Fig 2-7) were very similar to those for Ca. These elements appear...
to be present almost exclusively in the solid residue. The results above for Na, K, Ca, and Mg are consistent with previous work\textsuperscript{14,15} showing that after HTL, most of the sodium and potassium is distributed to the aqueous phase and most of the calcium and magnesium was in the solid residue.

Zinc, copper, and iron show similar trends with respect to HTL holding time. The fraction of these metals that partition into the biocrude increases with time and reaches a maximum (60 – 90%) at 3 min before slowly decreasing at longer times. Less than 3% of the Zn, Cu, and Fe partition into the aqueous phase.

About 5 – 10% of the biomass phosphorus is present in the aqueous phase after HTL, less than 5% is in the biocrude, and the largest portion is in the solid phase. We did not determine the phosphorus speciation in the aqueous phase, but previous reports\textsuperscript{16,31} indicate that most of the P in the aqueous phase co-product was present as free phosphate (orthophosphate). Of course, phosphate, if present in the post-HTL aqueous phase, could combine with elements such as calcium and magnesium to form phosphate precipitates. This scenario is consistent with the present results where Ca, Mg, and P all appear to reside primarily in the residual solids. Indeed, the literature indicates that the fate of phosphorus can be linked, in part, to the presence of elements such as Ca and Mg since these can combine with P to form phosphate precipitates.\textsuperscript{14,19}

Taken collectively, the results in Figure 2-7 show that each element tends to partition largely to either the aqueous, organic, or solid phases after HTL. For some elements, such as Fe, Zn, and S, the preferred phase is a function of the reaction severity. At both the milder and the more severe HTL conditions explored, these elements tend to partition primarily into the solid products. At moderate HTL severity, Fe and Zn partition primarily into the biocrude and S primarily into the aqueous phase. Thus, the HTL processing conditions can be used to manipulate the amounts of some elements in the different product fractions. Additional work is required to elucidate fully the reasons that the reaction severity has the effects observed. At present, however, we hypothesize that hydrothermal reactions of the different
biomass-derived compounds play a role, as does the pH of the aqueous phase becoming more basic as the reaction severity increases and more of the N atoms are converted to ammonia\textsuperscript{14,20}.

### 2.4 Conclusion

1) At mild HTL conditions, biocrude yields are low, and this nascent biocrude is much richer in P, Na, Ca, and Mg than is the biocrude produced in higher yields from HTL at moderate conditions.

2) Iron is always the most abundant metal in biocrude produced at moderate HTL conditions. There appears to be a region of reaction severity where high biocrude yields can be obtained along with comparatively lower iron concentrations. Thus, the HTL conditions can be used to alter the concentrations of some metals in biocrude.

3) The biomass and water loadings are potential process variables for altering the concentrations of some elements in algal biocrude. The concentrations of Ca, P, Si, and Mg were at least an order of magnitude lower at the highest biomass loading (15 wt\%) and the highest water loading. Other elements (S, Fe, Zn, Cu) were insensitive to the different biomass and water loadings.

4) At all HTL conditions examined, Al, Mn, Ca, Mg, and P were present primarily in the solid residue. Na and K were present primarily in the aqueous-phase co-product. At moderate HTL conditions, Fe, Zn, and Cu were primarily in the biocrude phase, but at mild and severe conditions, they were primarily in the solid residue.
Chapter 3

Using Solvents and Heterogeneous Catalysts to Reduce Metal Content in Crude Bio-oil from Hydrothermal Liquefaction of Microalga

3.1 Introduction

Hydrothermal liquefaction (HTL) converts algal biomass into crude bio-oil (biocrude) using hot (200-400 °C), compressed (5-40 MPa) water.\textsuperscript{64-66} HTL directly converts wet biomass feedstocks and thus can be more energy efficient than competing methods of producing biocrude that require dry feedstocks.

The identity and amounts of the metals and other elements in algal biocrude depend on the identity and types of elements in the microalgae being processed, which in turn depends on the nutrient elements provided in the growth media. Elements in algae macronutrients include nitrogen, phosphorus, sulfur, potassium and magnesium. Micronutrients (e.g., Fe, Mn, Co, Zn, Cu, Mo) are present in smaller amounts (30 - 2.5 ppm) in the growth media.\textsuperscript{67} The following inorganic elements were found to be present in microalgae: N, P, K, Mg, Ca, S, Fe, Cu, Mn, Zn, Mo, Na, Co, V, Si, Fe, Cr, Cd, Cl, B, and I.\textsuperscript{68} Many of these elements play important roles in microalgae such as biological redox reactions (e.g., Fe, Mn, Cu), acid-base catalysis (e.g., Zn, Ni), transmission and storage of information and energy (K, Ca), and in structural cross-links (e.g. S, Si).\textsuperscript{68}

A likely path for using algal biocrude is to blend it with petroleum feedstocks prior to refining. Blending infrastructure for petroleum crudes is already in place at refineries.\textsuperscript{57} Some components in algae biocrude, including metals, would pose problems in refineries, however. Iron, for example, could reduce the activity of catalysts and form particulates that fill interstitial spaces in catalyst beds,\textsuperscript{69} which will result in a higher than expected pressure drop.\textsuperscript{32} Indeed, Jarvis et al.\textsuperscript{1} observed that a catalyst bed used to hydrotreat algal-derived HTL biocrudes (Fe > 700 ppm) plugged after only tens of hours of continuous
processing. Other metals (e.g., sodium) form low-temperature melting compounds that cause any ash formed to become sticky and prone to depositing on surfaces inside the reactor. While sodium can often be removed by demineralization methods, such as desalting, iron is more difficult to remove.

Solvents might be useful for reducing the metal content of algae biocrude since solvents are used in petroleum demetallation (often via asphaltene precipitation) to remove nickel and vanadium. There are few studies on process interventions to reduce metals content in algal biocrude oil and none involving solvents other than that in our earlier preliminary work.

Laboratory-scale HTL typically uses an organic solvent (e.g., dichloromethane) to recover the biocrude formed, and the specific solvent used can affect the biocrude yield and elemental and molecular compositions. For example, a solvent could increase the biocrude yield if it also contacts the aqueous phase co-product and extracts polar organic molecules from that phase and transfers them to the biocrude phase. Though this extraction from the aqueous phase increases the biocrude yield, such molecules tend to contain more heteroatoms and have less heating value than do water-insoluble biocrude molecules.

To the best of our knowledge, there are no prior reports on how different solvents influence the metal contents in the biocrude from HTL, and no reports on whether contact between the solvent and the aqueous phase product increases or reduces the metal content in the biocrude.

Recognizing both the potential and the lack of information about how solvents could be used to reduce the metal content in algae biocrude, we aimed to determine how different organic solvents would affect the biocrude yield and its metal content. Two separation methods have been tested. In the first method, the organic solvent was in contact with all of the post-HTL product phases and then the biocrude phase was separated out. In the second method, the aqueous and solid products were filtered out first before an organic solvent was used to recover the biocrude product.
3.2 Materials and Methods

3.2.1 Materials

*Nannochloropsis* sp. algae was obtained from Sapphire Energy Inc. in dried powder form. It had 17.6 wt% ash content and was used as received. Deionized (DI) water was used throughout the experiments. All solvents (see Table 3-1) were HPLC grade with purity ≥ 99.5% (Sigma Aldrich, USA). An S-21+K oil-based metal calibration standard (Conostan), which included all the metals detected in microalgae, was used for metal analysis via ICP-OES (Agilent 710). The batch reactors comprised two nominal half-inch Swagelok 316 stainless steel caps and one port connector. The total internal volume of the assembled reactor was about 4.1 mL. High Pressure Valve in 3/8” O.D. tubing size is purchased from HiP High Pressure Equipment and is constructed with the reactor through a reducing union to pressurize hydrogen gas.

This study employed non-polar solvents (e.g., hexane and MTBE), polar protic solvents (e.g., ethanol, methanol), and polar aprotic solvents (e.g., dichloromethane). Table 1 lists the solvents and provides several physical properties (e.g., normal boiling point ($T_b$), density ($\rho$), dielectric constant ($\varepsilon$)) for each. $E_P^N$ is an empirical normalized measure of solvent polarity based on measured solvatochromic shifts. The Hansen Solubility Parameter (HSP) for each solvent includes the contributions shown from nonpolar interactions (dispersion), polar interactions (dipole-dipole and dipole-induced dipole), and hydrogen bonding or other specific interactions including Lewis acid-base interactions.

Table 3-1: Solvents used and selected physical properties
For the catalysts used in this study, γ-Alumina, activated charcoal, and ruthenium 5% on activated charcoal were obtained from Sigma-Aldrich as powders and used as received. Cobalt molybdenum oxide supported on alumina (3.4-4.5% cobalt oxide, 11.5-14.5% molybdenum oxide) was purchased from Alfa Aesar and ground into powder before use. Nickel on silica-alumina (66±5% nickel) and aluminum silicate were purchased as powders from Alfa Aesar and used as received.

### 3.2.2 Procedure

All reactors were conditioned with 2 mL of DI water at 350 °C for one hour prior to use to season and clean the interior of the reactors. All experiments were performed in triplicate. We loaded the reactors with dry microalgae powder and DI water such that the mixture was 15 wt% biomass on an ash-free basis. We added an amount of water to each reactor such that it would expand to 95% of the reactor volume at 350 °C and such that the pressure would be 22 MPa at 400 °C. We immersed the loaded and sealed reactors in a preheated sandbath at two different HTL conditions: 350 °C for 60 min or 400 °C for 5 min. These conditions were selected because earlier work showed they produced high biocrude yields but relatively low metal contents in the biocrude. Moreover, these conditions include both traditional isothermal HTL and fast HTL, a rapid-heating version of HTL that gives high biocrude yields in short times. After the desired reaction time, we took the reactors out of the sand bath and quickly quenched.
them in an ice bath to stop the reactions. The cooled reactors were then dried and kept at room temperature for at least two hours to equilibrate before opening.

We used two different methods to recover the reaction products. The first method, used with all of the solvents in Table 3-1, was to remove the aqueous and solid product phases from the reactor and separate them by using a syringe filter. The biocrude remained in the reactor because it was a sticky, tar-like material that did not flow freely at room temperature. We then added multiple aliquots of one of the solvents (about 10 mL in total) to the reactors to dissolve and recover the biocrude. We stopped when the solvent being removed from the reactor became transparent and light in color. The organic phase was passed through the same syringe filter so that the solvent could dissolve any biocrude that adhered to the solids initially removed from the reactor. In this method, the aqueous phase product from HTL and the organic solvent applied for biocrude recovery never came into contact.

The second method was applied only with the first five solvents in Table 3-1, which are not miscible with water. After treating the reactors with one of the solvents, we combined and mixed the aqueous, solids, and organic phases together in a centrifuge tube. We then separated the organic phase after centrifuging, using the product work-up procedure described previously.62 This method differs from the first method in that it provided contact between the aqueous-phase products and the organic solvent used to recover biocrude.

For both product recovery methods, the organic solvent was evaporated in flowing N₂ at 30 psi using a Labconco evaporator (Model: 73200). The evaporation temperature was set to 40°C for all samples, and evaporation time could vary from 2 hours to 10 hours depending on the solvents that were used. We then recorded the mass of the algal biocrude obtained. Biocrude yields were calculated as the mass of biocrude divided by the mass of microalgae (ash-free basis) loaded into the reactors. The biocrude samples and standard solutions were diluted about 50 – 100 fold by ethylene glycol butyl ether prior to determination of the metals and inorganic element content by Inductively Coupled Plasma (ICP-OES) as described previously.75
Atlantic Microlab, Inc. (Norcross, GA) determined the C, H, and N composition of the biocrude.

### 3.3 Results and Discussion on Solvent Extraction

#### 3.3.1 Biocrude yields

Using different solvents and different methods to recover the biocrude resulted in different biocrude yields from HTL of the same feedstock at the same nominal HTL conditions. The biocrude yields were as low as 20% and as high as 45%. The lowest biocrude yield was obtained when using hexane for extracting all the product phases. The highest yield was obtained when treating microalgae at 400 °C and 5 min, and using ethyl acetate to recover biocrude after removing the aqueous phase co-product and filtering out the solid products.

#### 3.3.1.1 Effect of Solvent

We first consider the results obtained wherein the solvent had no contact with the aqueous phase, as this allows comparison using all eight solvents employed. For HTL at 350 °C and 60 min, Figure 3-1 shows that MTBE, ethyl acetate, chloroform and DCM gave the highest biocrude yields (Runs 6, 10, 14, 18) and hexane (Run 2) gave the lowest. For HTL at 400 °C and 5 min (Figure C-1), MTBE, ethyl acetate, and chloroform again gave the highest yields (Runs 8, 12, 16) and DCM (Run 20) gave the lowest. When there was contact between the solvent and the aqueous phase products, again, hexane gave the lowest biocrude yields (Runs 1 and 3) and the chlorinated solvents (Runs 13, 15, 17, 19) and MTBE (Runs 5 and 7) gave the highest. This result held for both HTL conditions explored and Figures C-2 and C-3 provide the details.
Valdez et al. also observed that the yield of a given product fraction from HTL of microalgae was influenced by the solvent used to recover the biocrude. For example, the biocrude yield ranged from 30 to 39 wt%, with hexadecane and decane giving the highest yields and DCM giving the lowest. Yang et al. also used a range of different solvents to recover biocrude after HTL and observed the highest biocrude yield (~ 40%) when using THF and toluene as solvents. Ethyl acetate, acetone, DCM, ether, and methanol gave nearly 30% biocrude yields. Hexane gave a biocrude yield of only 8.3%. Qian et al. observed highest biocrude yields from HTL of sewage sludge, when using chlorinated solvents such as DCM and chloroform, and the lowest biocrude yields were with use of the nonpolar solvent (hexane).

Valdez et al. found a higher biocrude yield when using hexane compared to other studies noted probably because of the different work-up procedures applied. We did see a big difference in biocrude yield when applying hexane as solvent using different product work-up procedures. As in the present study, the yield of biocrude did not vary monotonically with the polarity of the solvent \( (E^N_T) \), but instead yields are higher with medium polarity solvents and lower with polar or nonpolar solvents, suggesting that the biocrude contained both high and low polarity compounds. This is expected as polar solvents will likely cause the precipitation of less-polar compounds formed from HTL, vice versa for nonpolar solvents.

Although the bio-oil yield is most likely related with solvent polarity, there is a marginal relationship between yield and solvent properties when using polarity as a sole predictor. Multiple linear regression (MLR) is used to model the relationship between two or more explanatory variables and a response variable by fitting a linear equation to observed data. Therefore, we proposed a MLR model to predict bio-oil yield with three solvent properties: dielectric constant \( (\varepsilon) \), dipole \( (\delta_D) \) and \( E^N_T \), after considering the properties in Table 3-1. Those three predictors are chosen in order to get a correctly specified model following stepwise regression and best subsets regression. With p-value less than 0.0005, bio-oil yield is significantly associated with those three variables. The model equation is obtained as Bio-oil yield (%) = 23.90 - 1.820 \( \varepsilon \) + 3.084 \( \delta_D \) + 31.5 \( E^N_T \). The adjusted R-square in this model is 58.12%. Admittedly, there is a big multicollinearity in this model because dielectric constant, dipole, and polarity
are highly correlated. However, using this model does a good job explaining the variation in the bio-oil yields with different solvents used in this study. Figure 3-1 compares bio-oil yield data from experimental and correlated by MLR at 350 °C and 60 min condition.

![Graph showing bio-oil yield comparison](image)

Figure 3-1: Biocrude yield from algae HTL at 350 °C and 60 min with no contact between solvent and aqueous phase co-product (experimental and predicted using MLR)

### 3.3.1.2 Effect of Solvent Contact with Aqueous Phase

The results from all of the solvents except DCM show that the biocrude yield from HTL runs where the solvent contacted the aqueous phase was less than that from the analogous runs where there was no contact. For these solvents, it appears that the aqueous phase was able to extract some presumably polar components from the organic phase that it had not extracted while in the reactor prior to addition of the solvent. This potential extraction might have been frustrated by the small water-biocrude interfacial area available in the reactor and the biocrude phase at room temperature being a very viscous, tar-like material. Adding organic solvent to the reactor and then mixing the reactor contents (both aqueous and
organic phases) increases the area for mass transfer, makes polar compounds more accessible, and facilitates extraction. The differences in yields with and without contact between solvent and the aqueous phase co-product exceeded the sum of the experimental uncertainties in biocrude yields for hexane and ethyl acetate.

To test the hypothesis that water could extract components from the original biocrude, we did a control experiment (Run 31). After using the conditions in Run 8 to generate the organic (MTBE) phase, we added to it an amount of DI water equal to the amount of the HTL aqueous phase co-product. After vortexing and sonicating to ensure adequate mixing, the sample was centrifuged. The top layer with MTBE was separated, dried, and weighed. The bio-oil yield in this experiment was 20.9 ± 1.5 wt%, much lower than the 37 ± 10 wt% yield obtained in the original experiment. This control experiment confirmed that material originally in the biocrude fraction can partition into an aqueous phase when it is provided the opportunity.

DCM was the solvent that behaved differently than the others. Here, the biocrude yield was higher when the solvent contacted the aqueous phase. This outcome for DCM is consistent with previous studies. Xu et al. found that about 8.4% of the total biocrude from algae HTL was extracted from the aqueous phase by DCM. Similarly, Lopez et al. found that the biocrude yield was a few percent higher when allowing DCM to contact and mix with all of the post-HTL product phases. The present results and these literature studies show that mixing DCM with the aqueous product would transfer some of the less polar organic molecules to the biocrude phase from the aqueous phase.
Table 3-2: Experimental HTL conditions and extraction methods and biocrude yields

<table>
<thead>
<tr>
<th>Run</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Holding time (min)</th>
<th>Aqueous and solvent contact</th>
<th>Yield (%)</th>
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<td></td>
<td>350</td>
<td>60</td>
<td>N</td>
<td>20.3±2.0</td>
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<tr>
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<td></td>
<td>400</td>
<td>5</td>
<td>Y</td>
<td>13.0±1.5</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>400</td>
<td>5</td>
<td>N</td>
<td>28.8±3.2</td>
</tr>
<tr>
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<td>60</td>
<td>Y</td>
<td>29.9±1.2</td>
</tr>
<tr>
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<td></td>
<td>350</td>
<td>60</td>
<td>N</td>
<td>36.8±6.4</td>
</tr>
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<td></td>
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<td>29.4±2.1</td>
</tr>
<tr>
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<td></td>
<td>400</td>
<td>5</td>
<td>N</td>
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</tr>
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<td>N</td>
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<td>Y</td>
<td>NA</td>
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</table>

* Same as Run 8, but MTBE solution with biocrude was extracted with DI water prior to evaporating the MTBE solvent
Taken collectively, these results provide the insight that there is a competition for HTL product molecules between the organic and aqueous phases when both are in contact and have sufficient interfacial area. Polar molecules that might have been in the biocrude initially (perhaps trapped physically) can be extracted from the organic solution by the aqueous phase whereas some water-soluble molecules in the aqueous phase can be extracted by the organic solvent. The net direction of transfer of molecules will determine whether the contact between organic solvent and aqueous phase increases or decreases the biocrude yield.
<table>
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<th>K</th>
<th>Mg</th>
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<th>P</th>
<th>S</th>
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Odd numbered runs from 1 – 19 included contact between the organic solvent with biocrude and the aqueous phase co-product. All other runs had no such contact between the organic and aqueous phases.

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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*a run numbers correspond to those in Table 3-2

*Run 32-34 are control experiments with organic solvents being added to aqueous phase co-product.

**Odd number runs from 1 – 19 included contact between the organic solvent with biocrude and the aqueous phase co-product. All other runs had no such contact between the organic and aqueous phases.**
3.3.2 Metal content in biocrude

The effects of solvent choice and product recovery method on biocrude metal concentrations are discussed in this section. Table 3-3 provides the concentrations of different elements in the algae biocrudes. Regardless of the organic solvent used, when there was no contact between the solvent and the aqueous phase product, the most abundant metals in the biocrude oil are Na and K, typically at levels of several thousand ppm. Fe was also abundant in the biocrude and typically at the thousand ppm level. When the organic and aqueous phases had been in contact with each other, Fe was the most abundant metal in the biocrude and the Na and K concentrations were, at most, a few hundred ppm. Depending on the solvents used, there could be hundreds of ppm of Ca, Mg, P, S, and Zn. The concentrations of Ni, Mn, Cu, and Al are usually under 100 ppm.

3.3.2.1 Iron in biocrude

Figure 3-2 shows the iron concentration in the biocrude recovered using different solvents at the two HTL conditions and with no contact between the solvent and the aqueous phase. The solvent used to recover the biocrude can influence its iron content. The Fe concentration was as low as ~800 ppm when using MTBE and >2000 ppm when using chlorinated solvents. Interestingly, the biocrude solution was also much darker when using the chlorinated solvents. In general, the less polar solvents (hexane and MTBE) resulted in the lowest iron content in the biocrude, and it was about half of the iron concentration in the biocrude recovered using the other solvents. This outcome is consistent with our earlier preliminary report,\textsuperscript{75} which showed that using the less polar MTBE (instead of DCM) can reduce by about 50% the iron content in biocrude from HTL of algae at 400 °C for 60 min. It is also consistent with the use of
alkanes such as hexane to precipitate asphaltenes and thereby reduce the metal content of heavy petroleum crude oils.

The data in Table 3-3 show that contact between the organic and aqueous phases can also influence the Fe content. Under the isothermal HTL condition (350 °C and 60 min), the iron content was lower when the organic solvent was mixed with and contacted the aqueous phase co-product. However, under the fast HTL condition (400 °C and 5 min), mixing the organic and aqueous phases had the opposite effect.

![Figure 3-2: Iron concentration in biocrude from algae HTL at two conditions. The solvent did not contact the aqueous phase](image-url)
The results demonstrate that solvents with low dielectric constants and $E^N_f$ values, such as hexane and MTBE produce biocrude with less iron. As is shown in the next paragraph, the solution properties that seem to correlate best with the biocrude higher iron content are the solvent viscosity and the dispersion force component of the solubility parameter. Although the precise reasons for the effect of different solvents on the iron content in biocrude are not clear, it is clear that the effects are not due to a single solvent property such as polarity, dielectric constant, or Hansen Solubility Parameter.

MLR is applied here to assess iron content in bio-oil with various solvent properties using the same statistical method in section 3.3.3.1. The best model was obtained with viscosity and dispersion force as two predictors. The adjusted R-square is 66.10%. The equation is expressed as Iron content (ppm) = -1939+1163*viscosity (cP)+159.8* $\delta_D$ (MPa$^{1/2}$). As is shown in figure 3-3, the MLR model can...
fairly correlate iron content data obtained at 350 °C and 60 min. As this model correlated, both viscosity and dispersion force have positive effects on iron content in bio-oil.

3.3.2.2 Other transition metals in biocrude

Mn and Al are always in low concentrations (< 40 ppm) in biocrude regardless of the HTL conditions, solvent choices, and biocrude recovery method. The Ni concentration was always < 200 ppm, and it was highest (100-200 ppm) when biocrude was recovered by chloroform and the solvent was mixed with the aqueous phase co-product. The Zn concentration was typically 100 – 200 ppm and the Cu concentration was typically 25 – 75 ppm. The results showed little variation and no clear trends for the different experimental runs.

3.3.2.3 Alkali and alkaline earth metals in biocrude

Six of the seven highest calcium concentrations in Table 3-3 (a few hundred ppm) are from the use of hexane and chloroform as extraction solvents. The Ca concentration was lower in other cases. The Mg concentration was higher when using hexane, ethanol, and methanol as the solvents for recovering biocrude.
Figure 3-4: Na and K concentrations in biocrude from algae HTL at different conditions. No contact between the solvent and aqueous phase co-product.

As shown in Figure 3-4, the concentrations of both Na and K in the biocrude are lower when chloroform, DCM, and acetone are used. Although hexane and MTBE have low dielectric constants and $E_{lN}$ values, the Na and K concentration in these biocrudes are comparable to those obtained with polar solvents like ethanol and methanol.

The concentrations of sodium and potassium in biocrude are orders of magnitude higher when the aqueous phase co-product and organic solvent never came into contact during product recovery. We believe these high Na and K levels in the biocrude are due to compounds trapped in the biocrude that could have been transferred to the aqueous phase had there been sufficient opportunity. The biocrude is tar-like at ambient conditions and adheres to the reactor walls. At ambient conditions, the aqueous phase co-product can contact only the external surface of the biocrude, so much of Na and K remains in the biocrude. This Na and K can be transferred to the aqueous phase when it is liberated via dissolution of the
biocrude in an organic solvent. Thus, the biocrude samples obtained when there is contact between the solvent and the aqueous phase have much less Na and K than does the biocrude without contact.

The solubility of Na+ in solvents increases in the order of acetone < ethanol < methanol.\textsuperscript{53} This ordering also agrees with the experimental results in Figure 3-4 for the biocrude sodium concentrations.

### 3.3.2.4 Sulfur and Phosphorus

Phosphorus also has a higher concentration when extracting solvent didn’t contact with the aqueous phase, but less significant than alkali metals. This is because the concentration of phosphorus is largely affected by the presence of metals such as magnesium and calcium, which can form precipitates and transfer to the solid phase. This is also why we did not observe the very same phenomenon in magnesium and calcium contents when using two extraction methods.

### 3.3.2.5 Control experiments

Run 27 is a control experiment where dry algae biomass was extracted by DCM at ambient conditions. The material obtained after filtering the solids and evaporating DCM contained high amounts of K, Mg, Na, P, and Ca but much less Fe and Zn than did the biocrudes obtained through hydrothermal treatment. This outcome is reasonable because the heavier metals such as Fe and Zn reside in molecules such as porphyrins that likely do not become accessible until the cellular material and the biomolecules are degraded.

Run 28 is a control experiment where both algae and DI water were added in the same amounts used for HTL, but the reactor was kept at room temperature and then subjected to the same product work up used for the HTL reactions. With DI water being present, there were higher concentrations of K, Na, Mg, Ca, and P in the “biocrude” than in run 27 with just dry algae.
Previous control experiments showed that Fe and Ni levels were less than 1 ppm when no biomass was in the reactor under 400°C processing condition, indicating the metal corrosion from the reactor walls did not meaningfully contribute to metal concentrations reported from HTL under those conditions. However, Ross et al. has reported a nickel concentration of 41 ppm from the leaching of the 316 stainless steel reactors under 300-350 °C. Another study found 0-4 ppm of nickel using different 316 stainless steel reactors.

Comparing results from Run 31 and Run 8, there are no variations in the concentrations of the transition metals such as Zn, Cu, and Fe. On the other hand, P, K, and Na concentrations in Run 31 were significantly reduced from Run 8, indicating that compounds containing these elements were extracted by DI water from the Run 8 biocrude after its dissolution in MTBE.

Run 32 is simply the original aqueous phase product from HTL. In Run 33 and 34, eight mL of DCM and MTBE were added separately to two mL of the aqueous phase products, adequately stirred, and separated. The aqueous phases after contacting with the solvents were then analyzed. After the extraction, both the DCM and MTBE phases had a light yellow color, suggesting that some material was extracted from the aqueous phase product into the organic phase.

3.3.3 Solvent mixtures

The results presented herein show that no single solvent will provide both a high biocrude yield and low concentrations of metals in the biocrude. This outcome led us to explore solvent mixtures. A mixture containing one solvent that gave high biocrude yields and a second solvent that gave low metal concentrations, for example, might be able to recover more biocrude than would a pure solvent but do so with low metal concentrations. We used two binary solvent mixtures in a 1:1 volume ratio. The total amount of solvent used was the same as was used with a single solvent to recover the biocrude. There was no contact between the aqueous phase co-product and the solvents used.
The first mixture contained MTBE and chloroform (Run 29), because using chloroform alone led to a high biocrude oil yield, while using MTBE resulted in lower metal content in the biocrude. The second mixture was hexane and ethanol (Run 30), which combined a strongly polar and a nonpolar solvent. The biocrude yield for Run 29 is as high as that from using chloroform alone (both ~ 40 wt%), and is the highest biocrude yield achieved in this study. The biocrude yield for Run 30 is about 36 wt%, which is higher than using either ethanol (30 wt%) or hexane (29 wt%) alone. Therefore, using these binary solvent systems can either increase the biocrude yield or maintain a high biocrude yield.

Figure 3-5: Concentrations of metals and other elements in biocrude from HTL at 400 °C, 5 min using MTBE (Run 8), chloroform (Run 16), and their mixture (Run 29, 1:1 v/v). No contact between the aqueous-phase product and solvent.

The metal contents in biocrude when using binary solvent mixtures in comparison to a single solvent are shown in Figure 3-3 and 3-4. Using MTBE and chloroform as a binary solvent led to biocrude metal contents that were often higher than those from using chloroform alone. Thus, using MTBE and
chloroform in combination provided the desired high biocrude yield obtainable with chloroform, but it did not provide the desired lower metal concentrations obtainable with using MTBE. There appears to be no advantage to using this solvent mixture.

The case is different, however, when using hexane and ethanol as a binary solvent. In this case, the concentrations of Fe, Ni, and Cu are no different than those from using a single solvent, but the concentrations of S, Al, K, and Na are the lowest when using the binary solvent. The concentrations of the remaining elements fall between those from using one of the pure solvents. This hexane-ethanol solvent mixture appears to show some promise. It provides an increase (> 6%) in biocrude yield relative to either pure solvent, and a comparable or lower metal content in the biocrude oil for many of the elements.

Figure 3-6: Concentrations of metals and other elements in biocrude from HTL at 400 °C, 5 min using hexane (Run 4), ethanol (Run 24), and their mixture (Run 30, 1:1 v/v). No contact between aqueous-phase product and solvent.
The ratio of the co-solvents used in this study is 1:1. Different ratios could have an effect on the algal biocrude oil yield and on its metal contents. Additionally factors such as extraction temperature and pH could also affect the metal distributions in the product phases. These topics are all worthy of consideration for future research.

3.3.4 Discussion

3.3.4.1 Solvation of metal electrolytes

When the aqueous phase product and extraction solvent never came into contact, the solvation of electrolytes into organic phases can be simplified using Born equation:

\[ \Delta_{el}G = -A_{el} \frac{z^2}{r} (1 - \frac{1}{\varepsilon}) \]

where \( \Delta_{el}G \) is the solvation energy, \( A_{el} = \frac{N \cdot A \cdot e^2}{8 \pi \varepsilon_0} = 69.5 \text{ kJ/mol} \), \( z \) is the nuclear charge of the ion, \( r \) is the radius of the ion in nm, and \( \varepsilon \) is the dielectric constant (permittivity) of the solvent. The Born equation estimates the Gibbs energy of the process, using a model which simplifies the ion solvation process as a large molecule in a heterogeneous dielectric medium. The Born equation shows that highly charged, small ions are more stabilized in solvents. For same ions, they are more stable in solvents with higher dielectric constant. In addition, if the dielectric constant of the solvent is \( \varepsilon > 40 \), electrolytes are almost completely dissociated into ions. On the other hand, only slight or practically no ionic dissociation takes place when the dielectric constant is small enough. This is understandable because the high dielectric constant allows complete ionic dissociation. The possible reason for metal not varying monotonically with dielectric constant is that the crude oil is a complexed mixture containing different electrolytes and non-electrolytes. There are solute and solvent interactions that could change the solvation. The presence of other ions will cause non-ideal electrical fields. There is also a complex
formation between metal cations and anionic ligands. The nonelectrolyte may be a co-solvent that can also affect the solution properties (e.g., lower the relative dielectric constant, $\varepsilon$, or increase the solubility of other nonelectrolytes).\textsuperscript{86} In addition, there are also solute-solute interactions. For example, dipole-dipole interactions can occur between polar solute particles in organic solvents. When the interactions are attractive (instead of repulsive), the dipole interactions will cause solute aggregation. The second kind of solute-solute interaction is hydrogen bonding, when a hydrogen atom bonds to a strongly electronegative atom existing in the vicinity of another electronegative atom that can accept hydrogen bond. Biocrude contains a lot of molecules that could form hydrogen bonding, such as carboxylic acids and acidic phosphate esters.

### 3.3.4.2 Solvent extraction of metal nonelectrolytes

Most metallic organic solutes are expected to be soluble in the organic solvents. When the extraction solvent is in contact with the aqueous phase, a nonelectrolyte solute is present in both phases. The distribution ratio $D$ of a metal species $M$ is denoted as,

$$D = \frac{\text{concentrations of all species containing } Fe \text{ in organic phase}}{\text{concentrations of all species containing } Fe \text{ in aqueous phase}} = \frac{[M]_{t,org}}{[M]_{t,aq}}$$

The distribution ratio is also affected by the addition of electrolytes. When the solubility of $M$ in both phases are low enough, the distribution coefficient can be approximated as

$$D = \frac{[M]_{t,org}}{[M]_{t,aq}} = \frac{[S]_{org}}{[S]_{aq}}$$

Here, $[S]$ is the solubility of $M$ is the solution. The effect (usually a salting-out effect but sometimes a salting-in effect) is complicated.\textsuperscript{86} Also, another example of the deviation from the distribution law is the distribution of halogens. For example, chloride could come from the algae biomass. However, the general rule is found in the molecular interaction of the solute with water and with the usual
organic solvents: 1) Because the forces of hydrogen bonds in water phase are stronger than the van der Waals forces in nonpolar organic phases, the energy required for destruction of solvent-solvent interaction is larger in water than in organic phases. 2) Because the smaller molecular sizes and polar nature of water, the van der Waal forces between the solute and solvent molecules are also stronger in water phase than in organic phase. 3) Thus the distribution into water is unfavorable from the first point but favorable from the second. 87

### 3.4 Results and Discussion on Catalyst Intervention

Table 3-4 shows the effects of different metal catalysts and catalyst supports on the Fe content in HTL biocrude. CoMo/Al₂O₃ (Run 5) and Ni/Al₂O₃-SiO₂ (Run 11) reduced the Fe content in the biocrude by more than an order of magnitude relative to HTL with no solid additive (Run 1). To determine whether the reduced Fe content was simply due to adsorption on the catalyst support, we performed control experiments with γ-alumina (Run 2) and aluminum silicate alone (Run 8). Comparing these results with Run 1 (no solid added) shows that the metal concentrations in the biocrude remain unchanged when using the catalyst supports. This outcome supports the hypothesis that adsorption onto catalyst supports is not the demetallation mechanism in Runs 5 and 11 where CoMo and Ni were also present. Run 7 shows that activated charcoal, which is a common adsorbent and catalyst support, provided a modest reduction in the Fe content of the biocrude. Run 12 shows the addition of ruthenium on activated charcoal as a catalyst and it has the similar reduction on iron as using activated charcoal alone.

Run 3-6 and Run 9-11 show the effect of processing conditions on catalytic demetallation. We conclude from there that a more severe reaction condition (higher temperature with longer holding time) will promote demetalltion by using the CoMo/Al₂O₃ and Ni/Al₂O₃-SiO₂ catalyst. Run 13 and Run 14 are experiments where 10 MPa of hydrogen gas was used to purge and pressurize the reactors before they are put in to the sand bath. The addition of hydrogen gas facilitates further reduction of the iron content in
crude oil when using Ru/C catalyst (Run 13) but has no effect when using only the catalyst support (Run 14). These results are the first for catalytic demetallation of algal biocrude, so the mechanism is not yet clear. If the chemistry here is similar to that in petroleum crude oil HDM using the same type of catalysts, one expects that the porphyrins are first hydrogenated, and then undergo ring cleavage reactions that deposit the metal on the catalyst surface. 46

In short, the presence of the Ni catalyst reduced the iron content to just 3% of its value from noncatalytic HTL and the sodium concentration was reduced to below the ICP detection limit. These materials are promising catalysts for in situ demetallation of algae biocrude during its production via HTL.

Table 3-4: Fe concentration (ppm) and yields (wt %) for biocrude produced from HTL with different catalysts, all using MTBE as a recovery solvent

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Set point Temp (°C)</th>
<th>Holding Time (min)</th>
<th>Catalyst loading (wt%)</th>
<th>Solid material added to reactor</th>
<th>Fe conc. (ppm)</th>
<th>Biocrude Yield (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400</td>
<td>60</td>
<td>N/A</td>
<td>N/A</td>
<td>468±108</td>
<td>26.5±0.47</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>60</td>
<td>25</td>
<td>yAl2O3</td>
<td>600±88</td>
<td>26.5±1.7</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>10</td>
<td>12.5</td>
<td>CoMo/Al2O3</td>
<td>242±99</td>
<td>27.4±2.0</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>30</td>
<td>12.5</td>
<td>CoMo/Al2O3</td>
<td>168±91</td>
<td>26.6±1.1</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>60</td>
<td>12.5</td>
<td>CoMo/Al2O3</td>
<td>35±4</td>
<td>22.5±1.9</td>
</tr>
<tr>
<td>6</td>
<td>350</td>
<td>60</td>
<td>12.5</td>
<td>CoMo/Al2O3</td>
<td>642±69</td>
<td>27.3±0.6</td>
</tr>
<tr>
<td>7</td>
<td>400</td>
<td>60</td>
<td>25</td>
<td>Activated Charcoal</td>
<td>130±68</td>
<td>22.9±1.2</td>
</tr>
<tr>
<td>8</td>
<td>400</td>
<td>60</td>
<td>25</td>
<td>Aluminum Silicate</td>
<td>590±76</td>
<td>25.9±2.7</td>
</tr>
<tr>
<td>9</td>
<td>400</td>
<td>10</td>
<td>25</td>
<td>Ni/Aluminum Silicate</td>
<td>166±12</td>
<td>29.5±0.2</td>
</tr>
<tr>
<td>10</td>
<td>400</td>
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<td>45±20</td>
<td>26.5±2.1</td>
</tr>
<tr>
<td>11</td>
<td>400</td>
<td>60</td>
<td>25</td>
<td>Ni/Aluminum Silicate</td>
<td>16±10</td>
<td>24.9±1.4</td>
</tr>
<tr>
<td>12</td>
<td>400</td>
<td>60</td>
<td>25</td>
<td>Ru/C</td>
<td>128±73</td>
<td>27.5±2.6</td>
</tr>
<tr>
<td>13*</td>
<td>400</td>
<td>60</td>
<td>25</td>
<td>Ru/C</td>
<td>30±3</td>
<td>26.1±1.8</td>
</tr>
<tr>
<td>14*</td>
<td>400</td>
<td>60</td>
<td>25</td>
<td>yAl2O3</td>
<td>870±192</td>
<td>25.9±2.2</td>
</tr>
</tbody>
</table>

Run 13 and 14 were pressurized with 10 MPa of H₂
The elemental composition of some of the biocrude samples are shown in table 3-5. The higher heating value (HHV) was calculated by using Dulong’s formula,\(^8\) where C, H, and O represent the weight percentage of each element.

\[ \text{HHV (MJ/kg)} = 0.338C + 1.428 \left( \frac{H - O}{8} \right) \]

The compositions of the biocrude fractions are about the same for catalytic and noncatalytic reactions. After using CoMo/Alumina during the demetallation process, there was an increase in the carbon and hydrogen contents in biocrude oil, and a decrease in oxygen content. Thus, the presence of catalyst will lead to some extent of deoxygenation, which helps improve the quality of biocrude oil.

Table 3-5: Elemental Compositions (wt%) of algal crude oil. Holding time is 60 min. Biomass loading is 15 wt%.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>O</th>
<th>H/C</th>
<th>N/C</th>
<th>HHV</th>
</tr>
</thead>
<tbody>
<tr>
<td>350°C</td>
<td>N/A</td>
<td>MTBE</td>
<td>77.7</td>
<td>10.45</td>
<td>4.61</td>
<td>7.24</td>
<td>1.61</td>
<td>0.83</td>
<td>39.89</td>
</tr>
<tr>
<td>350°C</td>
<td>Activated Carbon</td>
<td>MTBE</td>
<td>77.69</td>
<td>10.73</td>
<td>4.25</td>
<td>7.33</td>
<td>1.66</td>
<td>0.77</td>
<td>40.27</td>
</tr>
<tr>
<td>350°C</td>
<td>CoMo/Alumina</td>
<td>MTBE</td>
<td>78.36</td>
<td>10.47</td>
<td>4.34</td>
<td>6.83</td>
<td>1.60</td>
<td>0.78</td>
<td>40.22</td>
</tr>
<tr>
<td>400°C</td>
<td>N/A</td>
<td>DCM</td>
<td>78.94</td>
<td>10.24</td>
<td>4.18</td>
<td>6.64</td>
<td>1.56</td>
<td>0.74</td>
<td>40.12</td>
</tr>
<tr>
<td>400°C</td>
<td>N/A</td>
<td>MTBE</td>
<td>78.92</td>
<td>10.41</td>
<td>3.74</td>
<td>6.93</td>
<td>1.58</td>
<td>0.66</td>
<td>40.30</td>
</tr>
<tr>
<td>400°C</td>
<td>Activated Carbon</td>
<td>MTBE</td>
<td>78.62</td>
<td>10.92</td>
<td>3.24</td>
<td>7.22</td>
<td>1.67</td>
<td>0.58</td>
<td>40.88</td>
</tr>
<tr>
<td>400°C</td>
<td>CoMo/Alumina</td>
<td>MTBE</td>
<td>79.27</td>
<td>10.68</td>
<td>3.85</td>
<td>6.2</td>
<td>1.62</td>
<td>0.68</td>
<td>40.94</td>
</tr>
</tbody>
</table>

3.5 Conclusion

In this study, we used eight different organic solvents and two different extraction methods (with the extraction solvent contacting the HTL aqueous products or not) under two HTL conditions that would result in a higher biocrude yield and lower iron contents. Both single and a preliminary test of binary solvents were used in this study. The results showed that using an organic solvent for recovering biocrude after HTL from laboratory-scale operations can have significant effects on oil yields and metal contents. The different extraction methods can also have an effect on biocrude oil yields, with DCM having a higher yield when extracting all the phases together while other less polar solvents having higher yields.
when filtering out the aqueous and solid products. The influence of different extraction methods on metal contents in biocrude oil is the most obvious with alkali metals. Sodium and potassium contents tended to be orders of magnitude higher when extraction solvents and aqueous phase never came into contact. The effect of extraction methods on other metals is less influential and consistent. Using MTBE as a recovery solvent without contacting the aqueous phase product when extracting biocrude can have the highest biocrude oil yield and lowest iron contents in crude oil, despite the relatively higher cost of the solvent. Choosing the right binary solvents is a viable choice for a higher bio-oil yield and comparable or lower metal contents in the crude oil. However, changing the processing condition and using the solvent recovery method alone would not reduce the metal contents to a desirable level. Other interventions such as applying heterogeneous catalysts should be considered if looking for a higher quality biocrude oil. These results are valuable for choosing the appropriate solvents in industrial applications, considering the cost, convenience for operations, and environmental impacts etc. The results highlighted the need of developing solvent-free separation methods for the HTL products to have a desirable yield as well as lower metal contents. In practice, the application of organic solvents with or without contact of the HTL aqueous product must consider simplicity in operation, relative safety, and potential for scaling up to industrial plant level.

The Fe and Na content in biocrude from algae HTL is sensitive to the HTL conditions employed, the solvent employed to recover biocrude, and the presence of metal catalysts and activated charcoal. Having demonstrated the efficacy of these process interventions in reducing the metal content of biocrude, additional work is needed with other solvents, catalysts, process conditions, and algae species to advance understanding of the applicability of these effects. Additionally, catalytic studies with Fe-containing model compounds could permit post-reaction catalyst characterization, and thereby shed light on some of the chemical details in this process.
Chapter 4

Hydrothermal Demetallation of Hemin and Its Product Characterization

4.1 Introduction

In the previous chapters, iron is found to be the only metal present in *Nannochloropsis* sp. algal biocrude oil in a concentration larger than 1000 ppm. Similar conclusion was drawn by Jarvis et al., where *Tetraselmis* sp. algae biocrude was derived from HTL. Iron was found to be the only metal that is over 1000 ppm in their crude oil (1289 ppm). The presence of high concentrations of iron could cause detrimental effects such as catalyst deactivation in the hydrotreatment unit. Therefore, in our previous studies, we investigated the effects of different interventions to reduce iron contents in the resulting crude oil, which included altering processing conditions, engineering recovery solvents, and applying heterogeneous catalysts. However, since microalgae is a complex mixture, we do not know if those interventions would only be effective for microalgae system because of the presence of other molecules. That is to say, we do not know if the presence of other molecules, metals, and ligands would affect the interventions on iron contents. Furthermore, it is also difficult to explore the demetallation mechanisms and identify iron-related molecular products using the microalgae system. Thus, we need a simplified model compound that could well represent iron fates during HTL of microalgae to further develop the demetallation studies.

Iron in microalgae can be present in the form of heme, a ferrous containing porphyrin, or Fe-sulfur clusters in a variety of metalloproteins that are involved in countless metabolic pathways. The majority of iron is in the form of heme. Heme is involved with many biological
functions such as oxygen sensing, electron transport, signal transduction, and antioxidant defense enzymes. After HTL, porphyrinic iron can still stay in the resulting algal biocrude as Jarvis et al. identified more than 100 unique iron porphyrin compounds in biocrude oils derived from two algae species: *Tetraselmis* sp. and cyanobacteria. They discovered that those iron porphyrin compounds have similar carbon number and double bond equivalent (DBE) distributions to the metal porphyrins (e.g. Ni and V) reported for petroleum. While Ni and V porphyrin compounds were studied extensively in petroleum fields, we find very scarce information with respect to the fates of iron porphyrinic compounds under hydrothermal treatment. Therefore, given the similar iron containing molecular structures presenting in both microalgae and the algal biocrude oil, and the knowledge gap in iron porphyrin reactions during HTL, in this chapter, we use hemin as a model compound to specifically examine iron fates during HTL. The structures of hemin and heme are compared in figure 5-1. Heme and hemin are very similar in structures, except that the iron ion in heme is ferrous (Fe$^{2+}$), whereas it is ferric (Fe$^{3+}$) attached with chloride in hemin. The nucleus of all porphyrin molecules is a large cyclic structure composed of four pyrrole rings linked at their alpha positons by –CH- groups, referred to as methene bridges. These four bridge positions are known as meso positions, which are also the most reactive sites on porphyrins. A variety of reactions including nitration, halogenation, and oxidative cleavage can occur at meso carbons. The other eight peripheral positions that are available for substitution are called beta positions.
Figure 4-1: Molecular structures of heme (left) and hemin (right)

4.2 Materials and Methods

4.2.1 Materials

Hemin (porcine) was purchased from Alfa Aesar with 98+% purity. DCM, MTBE, and EGBE were HPLC grade with purity ≥ 99.5% purchased from Sigma Aldrich. NaOH was purchased from Sigma Aldrich. Elemental Blank Oil was purchased from Conostan Division, Continental Oil Company. Iron standard in oil phase (FEOMS 1000 µg/mL iron in mineral oil) was purchased from High-Purity Standards. Swagelok stainless steel caps and port connectors (nominal ½ in) were used to make 4.1 mL batch reactors.

4.2.2 Procedure

3 wt% of hemin, 12 wt% of elemental blank oil, selected weight percent (12.5% -100% relative to hemin loaded) of heterogeneous catalyst, and DI water were loaded into 4.1 mL batch
reactors. The elemental blank oil does not contain any metals. The purpose of the blank oil is to allow minimal loadings of hemin and to ensure a dilution ratio of less than 100 when preparing samples for ICP analysis. Adding the blank oil can be cost effective and the oil product obtained can be in a consistent mass.

The product workup follows the same procedure as described in Chapter 2. In summary, DCM and MTBE were used as recovering solvents. Solvent in small aliquots was used to wash the reactors until the washing aliquots containing products became clear. For a few of the HTL product samples, we extracted them with DCM first and then rinsed the reactors with 10 mL 0.1 M NaOH solution. The purpose of introducing NaOH solution is to dissolve unreacted hemin into the aqueous phase. After centrifuging, aqueous phase was separated out first and solid phase was then filtered out. Oil phase solutions containing either DCM or MTBE solvent were dried at 40 °C and 45 °C separately. Dried oil phase was then collected and subjected to metal analysis using ICP-OES.

Spent catalyst with the HTL solid products were characterized by SEM-EDS on campus using NanoSEM 630 (FEI, Hillsboro, OR) with the X-max detector (Oxford Instruments, Concord, MA) at 10 kV.

Hemin oil products without adding the elemental blank oil in the reactor was collected and analyzed with a Shimadzu GC-MS QP-2010 Ultra with a 0.25 mm inner diameter Agilent DB-SMS column (30 m x 0.25 μm). Inlet temp was 200°C, flow rate was 2mL/min, and starting oven temp was 35 °C holding for 1 min, ramp was 5.6 °C /min to 315 °C (final oven temp).

The same samples were also analyzed by Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometry at UC Berkeley. The procedures for sample preparation and FT-ICR MS operation were adapted from Jarvis et al.1
4.3 Results and Discussion

Table 4-1 summarizes the reaction conditions and the resulting iron content in the oil phase. Different intervention variables such as catalyst type, catalyst loading, temperature, time, and recovery solvent are included and explored. The oil produced was mostly from the elemental blank oil and only a very small fraction of the product is from hemin reaction. The iron content solely comes from the product formed from hemin since the blank oil is free of iron.

Table 4-1: HTL conditions and the iron content (ppm) in the resulting hemin oil. Catalyst loading is relative to hemin loaded.

<table>
<thead>
<tr>
<th>Run</th>
<th>Catalyst</th>
<th>Catalyst loading (%</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Iron in oil (ppm)</th>
<th>Special condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N/A</td>
<td>N/A</td>
<td>DCM</td>
<td>200</td>
<td>60</td>
<td>29±21</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>N/A</td>
<td>N/A</td>
<td>DCM</td>
<td>250</td>
<td>60</td>
<td>14±10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>N/A</td>
<td>N/A</td>
<td>DCM</td>
<td>300</td>
<td>60</td>
<td>1625</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>N/A</td>
<td>N/A</td>
<td>DCM</td>
<td>350</td>
<td>60</td>
<td>4974</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>N/A</td>
<td>N/A</td>
<td>DCM</td>
<td>400</td>
<td>3</td>
<td>3182±510</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>N/A</td>
<td>N/A</td>
<td>DCM</td>
<td>400</td>
<td>60</td>
<td>4262±240</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>N/A</td>
<td>N/A</td>
<td>DCM</td>
<td>400</td>
<td>80</td>
<td>2560</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>N/A</td>
<td>N/A</td>
<td>DCM</td>
<td>400</td>
<td>100</td>
<td>2760±360</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>N/A</td>
<td>N/A</td>
<td>MTBE</td>
<td>400</td>
<td>60</td>
<td>3301±512</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>AC</td>
<td>25</td>
<td>DCM</td>
<td>400</td>
<td>10</td>
<td>5696±1343</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>AC</td>
<td>25</td>
<td>DCM</td>
<td>400</td>
<td>30</td>
<td>4879±1006</td>
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</tr>
<tr>
<td>12</td>
<td>AC</td>
<td>25</td>
<td>DCM</td>
<td>400</td>
<td>60</td>
<td>3080±690</td>
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</tr>
<tr>
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<td>DCM</td>
<td>400</td>
<td>30</td>
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</tr>
<tr>
<td>14</td>
<td>AC</td>
<td>45</td>
<td>DCM</td>
<td>400</td>
<td>60</td>
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<td></td>
</tr>
<tr>
<td>15</td>
<td>AC</td>
<td>65</td>
<td>DCM</td>
<td>400</td>
<td>60</td>
<td>2727±372</td>
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</tr>
<tr>
<td>16</td>
<td>AC</td>
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<td>MTBE</td>
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<td>60</td>
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<tr>
<td>17</td>
<td>AC</td>
<td>100</td>
<td>MTBE</td>
<td>400</td>
<td>60</td>
<td>73±24</td>
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<td>DCM</td>
<td>400</td>
<td>60</td>
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<td>DCM</td>
<td>400</td>
<td>30</td>
<td>3782±230</td>
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<tr>
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<td>25</td>
<td>DCM</td>
<td>400</td>
<td>60</td>
<td>3215±274</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>CoMo/Al2O3</td>
<td>25</td>
<td>MTBE</td>
<td>400</td>
<td>60</td>
<td>2308±158</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>CoMo/Al2O3</td>
<td>50</td>
<td>MTBE</td>
<td>400</td>
<td>60</td>
<td>1440±127</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>CoMo/Al2O3</td>
<td>100</td>
<td>MTBE</td>
<td>400</td>
<td>60</td>
<td>740±68</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Ni/Al2O3-SiO2</td>
<td>25</td>
<td>DCM</td>
<td>300</td>
<td>60</td>
<td>453±131</td>
<td></td>
</tr>
</tbody>
</table>
4.3.1 The effect of reaction conditions

4.3.3.1 The effect of reaction temperature

Figure 4-2 shows the effect of reaction temperature on the iron content in hemin crude oil. There is minimal iron (14-29 ppm) in crude oil when hemin is processed at either 200 °C or 250 °C. Hemin starts decomposing at temperatures 300 °C and above, which results in an oil phase containing about 1600 ppm of iron. At 350 °C with the same holding time of 60 min, iron contents in crude oil is about 3 times as that processed at 300 °C, indicating a more extensive decomposition of hemin under subcritical water environment. At 400 °C (Run 6), the iron content is lower than that at 350 °C but still much higher than that at 300 °C, indicating a temperature as high as 400 °C could cause the iron molecules in oil to break down and deposit into solids phases. As a conclusion, hemin does not decompose at mild temperatures (temperatures below 300 °C).
Hemin degrades as temperature increases. The porphyrin fragments in oil will not stay stable and will further break down at higher temperatures (e.g. 400 °C).

![Bar chart showing temperature effect on iron content (ppm) in hemin crude oil.](chart.png)

Figure 4-2: Temperature effect on iron content (ppm) in hemin crude oil. Holding times are at 60 min. Recovery solvent is DCM.

Run 34 is a control experiment where the reactor is placed under room temperature. Since hemin does not dissolve in water, the purpose of this control experiment is to test if hemin will partly dissolve in the blank oil used. The iron in hemin oil is comparable to that in Run 1, which indicates that hemin does not react under 200 °C. Run 33 is another control experiment where hemin was not added into the reactor heated at 400 °C and 60 min. This is to test if the blank oil and DI water together will cause the leaching of iron from the reactor walls. The result demonstrates that there is very minimal iron leached from the reactor itself. Run 36 is a control experiment where post-HTL products were further washed with 0.1 M NaOH solution. The colour of the subsequent aqueous phase is much darker than the aqueous phase product directly obtained from HTL, indicating there is possible unreacted hemin. However, the iron content in
the oil phase is not affected comparing Run 36 with Run 8, which means that the solutes extracted in NaOH are mostly from the solid phase.

4.3.3.2 The effect of holding time

Figure 4-3 illustrates briefly on the effect of holding time on iron contents in the oil at 400 °C. At 3 min (Run 5), iron content in crude oil is already as high as about 3180 ppm. The reactant temperature at 3 min reaches 394 °C (Figure B-2). As the holding time increases, iron concentration increases to about 4300 ppm at 60 min and then decreases to the range of 2560-2760 ppm. This variation demonstrates that when the reaction temperature is high enough, hemin reactants will decompose quickly as temperature goes up, reaching a maximum of iron content in the oil phase, and then iron porphyrins will slowly decompose as time increases.

Furthermore, holding time can be crucial in terms of catalytic effects. The effect of holding time on Ni/Al₂O₃-SiO₂ catalytic demetallation is shown from Run 27 to Run 31. Despite the high standard deviation from Run 40, we can still conclude that up to 40 min, although iron content is reduced to a fraction of its original value, there is still hundreds of ppm of iron in the oil phase. However, when the holding time is above 50 min, almost all the iron is removed from the oil. Iron content reduces almost 50% when holding time increases from 10 min to 60 min.
Figure 4-3: Holding time effect on iron content (ppm) in hemin crude oil. Set temperature is at 400 °C. Recovery solvent is DCM.

4.3.2 The effect of recovery solvents and heterogeneous catalysts

Comparing Run 6 and Run 9 one sees that using MTBE reduces by about 25% the iron content in the crude oil compared with using DCM.

In this study, we examined the same catalysts that are proven to be effective in algal biocrude oil demetallation. The effect of using activated carbon on iron concentration is shown from Run 10 to Run 17. Activated carbon can reduce iron content to 3080 ppm (Run 12) compared to the original value 4260 ppm (Run 6). An increasing catalyst loading (Run 12, 14, 15) exerts no appreciable effect on further reducing the iron content. However, with the introduction of MTBE as a recovery solvent, increasing catalyst loading can have a tremendous
effect on iron content, as all of the iron in the oil phase is reduced when the catalyst loading is the same as hemin loading (Run 17). The addition Ru support on activated carbon does not help reduce iron contents in the oil product (Run 32). The effect of CoMo/Al₂O₃ catalyst on iron content is shown from Run 18 to Run 23. The ability of CoMo/Al₂O₃ to reduce metal content is comparable to activated carbon. Similarly, increasing CoMo/Al₂O₃ loading with DCM as a recovery solvent does not have an effect on demetallation in the oil phase. However, using MTBE as a recovery solvent and the same catalyst loading as the initial hemin reactant, iron can be reduced to 740 ppm in the crude oil (Run23). Future studies can be conducted on higher loadings of CoMo/Al₂O₃ using MTBE as a recovery solvent to examine if the iron can be totally removed.

The effect of Ni/Al₂O₃-SiO₂ catalyst on iron concentration is shown from Run 24 to Run 31. Ni/Al₂O₃-SiO₂ is the only catalyst that is able to remove nearly all the iron in crude oil under the selected operation condition (Run 31). Both reaction temperature and holding time have significant effects on catalytic demetallation using Ni/Al₂O₃-SiO₂. For the same holding time of 60 min and the same catalyst loading, iron content is reduced to 28%-38% of its original value under reaction temperature of 300 °C (Run 24) and 350 °C (Run 25). However, at 400 °C, almost all of the iron in the crude oil is removed.

Although the Ni/Al₂O₃-SiO₂ catalyst can be effective in demetallation on its own, we detected a high content of Ni (thousands of ppm) in the resulting crude oil. Jocz et al.⁹⁵ tested the stability of Ni catalyst in batch reactors at 400 °C for 60 min, and found the oxidation and dissolution of the catalyst in the supercritical water. The trade-off between iron and nickel in crude oil might still be worthwhile, since the existing infrastructure has already targeted on nickel removal from petroleum crude hydprocessing. However, from the conditions we examined, using MTBE and a high loading of activated carbon would be the selected combination condition examined to reduce iron content in crude oil without introducing other metals.
The SEM images of the spent activated carbon and Ni/Al₂O₃-SiO₂ together with the solid products from HTL recovered by DCM are shown in Figure 4-4. This result can give just a rough guide of catalyst behavior since it is not possible to separate the spent catalysts from the solid products from HTL. Thus the spent catalyst contains a fraction of the solid residues. Also, the catalyst surface is not flat and polished in the map. Nevertheless, we do see iron covering almost all the catalyst surface for both spent catalysts, which indicates that the iron in crude oil deposited in the catalyst.

Table 4-2 shows the EDS results for the spent catalysts. The result shows that for both of the catalysts, iron is the third most abundant element after C and O. The high carbon content from the spent Ni catalyst and the high oxygen content from the spent activated carbon catalyst could originate from the post-HTL solids products. The deposition of iron, carbon, and oxygen on the spent catalysts are similar.
Figure 4-4: SEM images of spent catalysts (left) and its iron mapping (right). Images on the top belong to spent activated carbon. Images on the bottom belong to Ni/Al$_2$O$_3$-SiO$_2$.

Table 4-2: Major elemental compositions of the spent catalyst

<table>
<thead>
<tr>
<th>Element</th>
<th>Activated Carbon</th>
<th>Ni/Al$_2$O$_3$-SiO$_2$</th>
<th>Hemin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt%   Atomic %</td>
<td>wt%   Atomic %</td>
<td>wt%   Atomic %</td>
</tr>
<tr>
<td>C</td>
<td>77.8  85.12</td>
<td>68.74 80.44</td>
<td>62.64 44.74</td>
</tr>
<tr>
<td>N</td>
<td>2.21  2.22</td>
<td>8.59  5.26</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>15.8  13.06</td>
<td>15.61 13.72</td>
<td>9.82  5.26</td>
</tr>
<tr>
<td>Fe</td>
<td>5.91  1.4</td>
<td>5.03  1.27</td>
<td>8.57  1.32</td>
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<tr>
<td>Ni</td>
<td>7.05  1.69</td>
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4.3.3 Comparison with microalgae

From our previous study in Chapter 2, iron content in the resulting algal biocrude is always above 1000 ppm even at 350 °C with 1 min of holding time. At such condition, the inner temperature of the reactor only reaches about 200 °C. However, hemin will not start decomposing
until the temperature reaches 300 °C. This difference could be possibly explained by the influence of other molecules in microalgae. At such mild conditions, hemin itself would not form any measurable amounts of DCM-soluble products. However, there are macromolecules in microalgae such as carbohydrates, proteins, and lipids that can depolymerize and form oil phase products at such conditions. Those molecules could potentially affect the behavior of heme reactions in microalgae under hydrothermal conditions. There are also non-porphyrin forms of iron existing in microalgae, such as Fe-S clusters which could transfer iron into oil-soluble products.

The trend that iron contents in hemin oil phase first increase and then decrease with increasing holding time is consistent with iron in microalgae. As for iron content in algal biocrude oil, iron concentration reaches maximum at 3 min and gradually decrease with holding time (Chapter 2). However, for hemin HTL, iron content at 60 min is higher than that at 3 min.

As for the catalytic effects, Ni/Al₂O₃-SiO₂ reduces most of the iron from hemin crude oil and algal crude oil. Activated charcoal can moderately reduce iron content in algal crude oil. However, with a high loading equivalent to the biomass, activated charcoal can be very effective at reducing iron contents in hemin oil. CoMo/Al₂O₃, in comparison, can moderately reduce iron content in hemin oil but is very effective in reducing iron content in algal oil. A severe processing condition (higher temperature and holding time) facilitates iron reduction for both microalgae and hemin crude oil.

**4.3.4 Molecular characterizations of hemin oil product**

To characterize the oil product produced from hemin, we loaded the reactors with hemin, water, and Ni/Al₂O₃-SiO₂ catalyst without blank oil. The reaction condition is 400 °C and 60 min.
We then characterized the hemin oil product from HTL of hemin with or without adding the catalyst. Figure 4-5 displays the GC chromatogram of the hemin oil with (blue) and without (black) adding the catalyst. As we could tell, catalytic HTL of hemin does not generate new molecules that differentiate from non-catalytic reactions. The positions of the main components in the oils overlap with each other. The one new peak generated when using the catalyst is long-chain alkene product whose retention time is 19.523 min.

Some major peaks identified in the GC mass spectra were shown in Table 4-3. The components include cyclopentenone and cyclohexenone derivatives, indoles, long-chain alcohols, aromatics, and amides. In comparison, algae derived crude oil includes more components such as organic acids, long-chain hydrocarbons (saturated and unsaturated), indoles, piperidine derivatives, cholestane, cholestene, amides, and other N-containing compounds.66,96

The products identified are mostly likely from hemin degradations. The hypothesis is that sub- and supercritical water can attack the methene bridges of the tetapyrrole rings, producing various pyrrole products in addition to releasing iron through hydroxylation. The pyrrole fragments will then have denitrogenation and oxidation reactions, forming products such as cyclopentenone and cyclohexenone derivatives.
Figure 4-5: GC-MS chromatogram of the original hemin oil (black) and hemin oil treated with Ni/Al\textsubscript{2}O\textsubscript{3}-SiO\textsubscript{2} catalyst (blue) at 400 °C and 60 min HTL conditions
Table 4-3: some major peaks identified from GC-MS analysis of the hemin crude oil

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<tr>
<th>Retention time (min)</th>
<th>NIST MS Library Hit</th>
<th>Similarity</th>
<th>NIST MS Library Hit</th>
<th>Similarity</th>
<th>NIST MS Library Hit</th>
<th>Similarity</th>
<th>NIST MS Library Hit</th>
<th>Similarity</th>
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<td>94</td>
<td>2,4-Dimethylfuran</td>
<td>90</td>
<td>4,4-Dimethyl-2-cyclopenten-1-one</td>
<td>83</td>
<td>2,4-Hexadiene, 2,3-dimethyl-</td>
<td>87</td>
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<tr>
<td>12.908</td>
<td>2-Cyclopenten-1-one, 3,4-dimethyl-</td>
<td>86</td>
<td>2,4-Hexadiene, 2,3-dimethyl-</td>
<td>83</td>
<td>4,4-Dimethyl-2-cyclopenten-1-one</td>
<td>83</td>
<td>2,4-Hexadiene, 2,3-dimethyl-</td>
<td>87</td>
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<td>13.926</td>
<td>2-Cyclopenten-1-one, 3,4-dimethyl-</td>
<td>92</td>
<td>2-Cyclopenten-1-one, 3,4-dimethyl-</td>
<td>89</td>
<td>2-Cyclopenten-1-one, 3,4-dimethyl-</td>
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<td>2,4-Hexadiene, 2,3-dimethyl-</td>
<td>87</td>
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<td>1,3-Hexadiene, 3-ethyl-2-methyl-</td>
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<td>Cyclohexane, (1-methylethylidene)</td>
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<td>17.503</td>
<td>5-Ethylcyclopent-1-enecarboxaldehyde</td>
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<td>2-Cyclopenten-1-one, 2,3,4,5-tetramethyl-</td>
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<td>2-Cyclopenten-1-one, 5-methyl-2-(1-methylpropyl)-</td>
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<td>2-Cyclopenten-1-one, 5-methyl-2-(1-methylpropyl)-</td>
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<td>1H-Indole, 2,6-dimethyl-</td>
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<td>1H-Indole, 2,5-dimethyl-</td>
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<td>Methyl 4, 6-decadienyl ether</td>
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<td>39.765</td>
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<td>45.227</td>
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</table>
FT-ICR MS analysis is used to identify Fe-containing organic species in the crude oil. FT-ICR has the ability to accurately determine the mass of thousands of ions within one sample to within 5 ppm. There are only a few published researches using FT-ICR MS to analyze bio-oil from HTL of microalgae,\textsuperscript{1,97-99} and only Jarvis et al.\textsuperscript{1} studied iron porphyrinic species in the algal biocrude oil. Kaczorowska et al.\textsuperscript{100} used Electron Induced Dissociation (EID) tandem mass spectrometry and observed charged iron (III) complexes. Figure -6 compares the positive ion ESI FT-ICR mass spectra of HTL biocrude without (top) and with (bottom) the Ni catalyst. No blank oil was added in those samples so that the spectra are all from post HTL products of hemin. The iron porphyrinic structures in the uncatalyzed hemin crude oil at m/z 400-500 are of low abundance or absent from the spectra using biocrude from catalytic HTL. As a result, the dominant species in the hemin crude oil from catalytic HTL are at m/z 200-400, which can contain many N\textsubscript{2} species. Those N\textsubscript{2} species are also present in the biocrude from uncatalyzed reactions. Jarvis et al.\textsuperscript{1} found N\textsubscript{1} species dominant after catalytic hydroprocessing of microalgal crude oil. The dominant species identified in this region are shown in Table 4-4. The absence of the iron porphyrinic species in the catalyzed hemin crude oil indicates that iron-porphyrin structures are undergoing ring opening and being degraded into smaller molecular weight fractions. Jarvis et al.\textsuperscript{1} compared FT-ICR spectrum before and after cyanobacteria HTL biocrude hydroprocessing, and also concluded that the porphyrin structures that are dominant for the unprocessed HTL biocrude are not present in the hydroprocessed HTL biocrude. These observations all together lead to the conclusion that iron has similar reaction pathways and fates in algal biocrude oil and in hemin crude oil. Both our previous study on algal biocrude demetallation\textsuperscript{75} and Jarvis et al.\textsuperscript{1} concluded the mechanisms for iron porphyrin degradation might be similar to the nickel and vanadium porphyrins in petroleum crude oil, where the porphyrin structure is first hydrogenated followed by hydrogenolysis that results in demetallation.\textsuperscript{101,102}
ESI FT-ICR MS identified more than 15,000 unique molecular formulae in each of the hemin crude samples as shown in Figure 4-6. In comparison, Faeth et al.99 identified over 25,000 unique molecular formulae in biocrude samples from fast and isothermal HTL of microalgae. Table 4-4 shows the iron porphyrin molecular formulae assignment to each FT-ICR spectrum of hemin HTL crude oil without catalysts. We used the mass spectrum library from Jarvis et al.1 and identified 52 iron porphyrinic ions in the crude oil. The dominant iron porphyrinic species are \( \text{N}_4\text{Fe}_1 \) species such as \( \text{C}_{27}\text{H}_{26}\text{N}_4\text{Fe}_1^+ \) at m/z 462.1500, \( \text{C}_{26}\text{H}_{24}\text{N}_4\text{Fe}_1^+ \) at m/z 448.1344, \( \text{C}_{28}\text{H}_{28}\text{N}_4\text{Fe}_1^+ \) at m/z 476.1658, \( \text{C}_{29}\text{H}_{30}\text{N}_4\text{Fe}_1^+ \) at m/z 490.1816. \( \text{N}_4 \text{-6O}_0 \text{Fe}_1 \) species were also identified. Those iron-containing porphyrin ions identified in hemin crude oil are similar to those identified in algal biocrude, indicating the good proximity of using hemin as a model compounds to study iron fates during HTL of microalgae.
Figure 4-6: ESI FT-ICR mass spectra of the hemin biocrude oil from uncatalyzed (top) and Ni/Al₂O₃-SiO₂ catalyzed (bottom) HTL. 400 °C and 60 min of HTL conditions are applied.

Table 4-4: Molecular Formulae assignment of dominant species at m/z 400-500 from uncatalyzed and catalyzed HTL of hemin. 400 °C and 60 min of HTL condition is applied.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Theoretical mass</th>
<th>Experimental Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16H15N2</td>
<td>235.1230</td>
<td>235.1237</td>
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<tr>
<td>C16H17N2</td>
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<td>C17H17N2</td>
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<tr>
<td>C17H19N2</td>
<td>251.1543</td>
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</tr>
<tr>
<td>C18H19N2</td>
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Table 4-5: Molecular formula assignment of iron-containing porphyrins from uncatalyzed HTL of Hemin. 400 °C and 60 min of HTL condition is applied. Theoretical Mass data is acquired from Jarvis et al. DBE is Double Bond Equivalent.

<table>
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<th>Experimental mass</th>
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### 4.4 Conclusion

In this chapter, we used hemin as a model compound to represent iron fates during HTL of microalgae. We then characterized the spent catalysts and the crude oil products and were able to postulate a set of the demetallation pathways. Using hemin as a model compound provides an unprecedented opportunity to examine the products formed from iron-containing molecules in microalgae.

Hemin is very thermally stable and would not decompose until the reaction temperature is at 300 °C and above. Iron content in hemin crude oil first increases and then decreases with increasing reaction temperature and holding time. This indicates that hemin starts to decompose
as smaller iron porphyrinic fractions that are soluble in DCM and then those iron-containing porphyrins would further degrade and deposit into the solid phase.

As for the interventions attempted to reduce iron contents in crude oil, using Ni/Al₂O₃-SiO₂ catalyst at severe conditions during HTL is able to remove almost all the iron content in crude oil, although the penalty comes along with high nickel content in the resulting oil. The combination of using MTBE as a recovery solvent and using activated carbon or CoMo/Al₂O₃ during HTL could also remove most of the iron in crude oil, although using either MTBE or the catalyst alone would not have the same significant result. Iron is deposited onto the catalyst after HTL. The deposition of iron, carbon, and oxygen were similar on the spent catalyst of activated carbon and Ni/Al₂O₃-SiO₂.

GC-MS characterizations of oil from uncatalyzed and Ni/Al₂O₃-SiO₂ catalyzed HTL of hemin have shown the majority of crude oil molecules such as cyclopentenone and cyclohexenone derivatives, indoles, long-chain alcohols, aromatics, and amides. Those ring-containing molecules come from decomposition of hemin fractions. FT-ICR MS spectra has shown the presence of tens of unique iron-containing porphyrin ions in uncatalyzed hemin crude oil, with N₄Fe₁ being the dominant species. For catalyzed hemin crude oil, the porphyrin structures that are dominant for the unprocessed HTL biocrude are not present, indicating those iron porphyrin molecules have undergone ring opening and degradation with the introduction of catalysts.

Finally, the behavior of iron in hemin crude oil is generally in agreement with iron in HTL of microalgae, but a few differences were also pointed out. The similarity of the presence of iron-containing species and the effect of processing conditions on iron fates suggests hemin as an effective model compound to represent iron behavior during HTL of microalgae. However, with the introduction of elemental blank oil, the product yield could not be examined in this study.
Future studies are needed to focus on iron behavior in HTL of hemin as well as to optimize product yields and crude oil quality in terms of iron concentration.
Chapter 5
Reaction Network and Kinetic Modeling of Product Yields and Iron Distributions from Hydrothermal Liquefaction of Hemin

5.1 Introduction

To the best of our knowledge, there has been no attempt made to develop a quantitative model of reaction kinetics for hydrothermal liquefaction of hemin. There are some kinetic models for HTL of microalgae. Valdez et al. developed the first quantitative model for microalgae liquefaction and used the model to predict the temporal variations of each of the product yields. In another study from this group, a general kinetic model was developed which accounted separately for the initial reactions of protein, lipid, and carbohydrate in microalgae. Vo et al. used the same reaction network to model yields from HTL of Aurantiochytrium sp. KRS 101. Sheehan et al. included kinetic interactions between algal proteins, carbohydrates, and lipids in his model using experimental product fraction yields from published studies. Hietala et al. developed a microalgal HTL kinetic model that is valid for a broad range of holding times including fast HTL.

However, all of those past researches focused on modeling the behavior of product quantities (yields). None of the models above has focused on predicting the quality of products quantitatively. Hietala et al. mentioned that there were trade-offs between biocrude quantity and quality. In our previous study, we also concluded that there was a tradeoff between biocrude yield and iron content in biocrude. As iron is a crucial indicator for oil quality, being able to successfully predict the iron fates in HTL product is also of great importance. Microalgae is a complex system and has different types of biomolecules, ligands, and metals present, which could affect the kinetics for iron networks. Therefore, in this chapter, we seek to investigate the effects
of time and temperature on HTL of the iron model compound, hemin. We then use the data to develop a quantitative kinetic model to predict hemin product yields and iron distributions into post HTL products. The developed reaction networks and kinetic parameters are compared against each other and the data from iron distribution in microalgae HTL products. These results are valuable for exploring reaction pathways of product formation for different systems containing iron porphyrinic compounds.

5.2 Materials and Methods

5.2.1 Materials

Hemin (porcine) was purchased from Alfa Aesar with 98+% purity. Dichloromethane (HPLC grade) was purchased from HiPerSolv Chromanorm. Reactor components were purchased from Swagelok. Iron standard in aqueous phase (1000 µg/mL iron in 2% HNO₃), iron standard in oil phase (FEOMS 1000 µg/mL iron in mineral oil), and 10 µg/mL Indium in 2% HNO₃ were purchased from High-Purity Standards.

5.2.2 Procedure

15 wt.% hemin powder and DI water were loaded into 4.1 mL batch reactors made from a ½ in. Swagelok port connector and two caps, all of 316 stainless-steel construction. Water loading was calculated such that reactor pressures would be around 320 bar at the final temperature. We carefully chose the above reactor loadings such that the product weight would be large enough to determine accurately experimentally but would not be overfilling under all temperatures. The
reactors were put into sand bath at set-point temperatures of 325, 350, 400 °C for 10, 20, 40, 60, and 80 min respectively.

The product workup procedure is as described in Chapter 2. Gas mass was obtained as the difference between the two reactor masses before and after opening. The rest of the procedure involves pouring the reactor contents into a 50 mL conical centrifuge tube, rinsing the reactor with 30 mL of DCM and 10 mL of DI water in small aliquots, and collecting those rinsings. The tube was then subjected to centrifugation and filtration to facilitate phase separations.

The DCM soluble phase and the water phase were dried separately to obtain bio-oil yield and aqueous yield. Since the yields of gas, oil, and dried aqueous products could be measured accurately, and since it was not possible to recover all the solids from reactor walls and glass pipets, solid yields were calculated by difference. Material balances for total product yields ranged from 88.7% to 97.4%.

Metal contents in crude oil and aqueous phase were measured directly by ICP-OES. Due to the low ash content obtained after solid product calcination before being subjected to acid digestion, the iron residing in solids cannot be measured accurately. Therefore, iron in the solids was calculated by difference. The specific procedure for iron analysis in crude oil is described in Chapter 2. Dried aqueous phase was diluted with 2% HNO₃ matrix to 10 mL and then subjected to analysis for Fe content by ICP-OES. Indium was used as an internal standard for aqueous phase metal analysis.
5.3 Iron distribution and its kinetic modeling

5.3.1 Iron content in crude oil and aqueous phase

We studied the HTL reaction temperature from 300 °C to 400 °C. At 300 °C with variable holding times (up to 80 min), there was no HTL product oil obtained. Hemin remained largely unreacted in the reactors. As far as we know, this work is the first to study hemin and iron fates under sub- and supercritical water. However, there are studies on metalloporphyrin decompositions and stabilities without water environment. Those studies have consistent conclusions for the thermal stability of metalloporphyrins. Premovic et al.\textsuperscript{108} tested the thermal stability of asphaltene vanadyl porphyrin, which is abundant in petroleum and coals. They concluded that those porphyrins are stable up to 400 °C during laboratory heating, and at above this temperature their content sharply decreases. Lebedava et al.\textsuperscript{109} studied thermal oxidative decomposition of certain zinc porphyrin complexes, and most of the compounds would not start decomposition until temperatures exceed 335 °C. Due to the thermal stability of hemin at 300 °C, we chose some more severe HTL conditions and isothermal HTL to study the behavior of iron and HTL products of hemin.

Table 5.1 shows the iron concentration (ppm) in both the crude oil and dried aqueous phases under different HTL processing conditions. The lowest iron concentration in crude oil is obtained with 80 min of holding times at 350 °C and 400 °C reaction temperatures. The highest iron concentration in crude oil is obtained at 350 °C with 10 min of holding time, which is about 5.5 times of the lowest iron concentration. At 325 °C, iron concentration in crude oil increases with holding time. At 400 °C, in contrast, iron concentration in crude oil decreases monotonically with holding time. The opposite trends could be possibly attributed to the hemin decomposition characteristics. At 325 °C, the reaction condition is relatively mild for hemin decomposition, thus
resulting in more and more iron in crude as reaction proceeds. However, at 400 °C, hemin has already been converted and released most of the iron into the crude oil phase in a very short time, as is shown in later discussion. Therefore, as holding time increases, the iron porphyrinic compounds in oil would further break down and transfer to solid phases. At 350 °C, iron content in crude oil does not vary monotonically, meaning that the decompositions of hemin and iron porphyrinic species in crude oil are happening simultaneously within 10-80 min of holding time. This conclusion at 350 °C is consistent with what we observed in algal crude oil, which concludes that only a fraction of the Fe-containing structures (e.g., porphyrins) in the biomass are converted into DCM-soluble material (i.e., biocrude) at the mildest conditions. Although the iron concentration in dried aqueous phase can be very high, the actual iron mass in aqueous phase is just a few percent of that originally loaded into the reactor. However, it is noticeable that at 400 °C, iron in the aqueous phase is orders of magnitude lower than that in lower temperatures.

Table 5-1: Iron concentration (ppm) in hemin crude oil and aqueous phase (dried)

<table>
<thead>
<tr>
<th></th>
<th>10 min Oil</th>
<th>Aqueous</th>
<th>20 min Oil</th>
<th>Aqueous</th>
<th>40 min Oil</th>
<th>Aqueous</th>
<th>60 min Oil</th>
<th>Aqueous</th>
<th>80 min Oil</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>325 °C</td>
<td>40,941</td>
<td>191,046</td>
<td>53,931</td>
<td>188,238</td>
<td>64,048</td>
<td>186,150</td>
<td>63,561</td>
<td>181,000</td>
<td>65,545</td>
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<tr>
<td>350 °C</td>
<td>75,006</td>
<td>202,900</td>
<td>49,653</td>
<td>63,112</td>
<td>64,927</td>
<td>103,842</td>
<td>28,017</td>
<td>164,485</td>
<td>13,287</td>
<td>24,996</td>
</tr>
<tr>
<td>400 °C</td>
<td>51,346</td>
<td>7,315</td>
<td>39,373</td>
<td>2,121</td>
<td>28,832</td>
<td>791</td>
<td>24,550</td>
<td>1,605</td>
<td>16,338</td>
<td>2,041</td>
</tr>
</tbody>
</table>

5.3.2 Kinetic model development

In this section, we use the experimental data (discrete points Figure 5-2) to derive a general reaction network and a corresponding kinetic model for iron distributions in post hemin HTL products. We then use the network to determine values for rate constants and Arrhenius parameters for each pathway in the network.
The reaction network is depicted as in Figure 5-1. The reaction network is developed after examining varying possibilities. In this network, we posit a newly formed solid product (N) that is distinct from the unreacted hemin. The network comprises the irreversible formation of iron in aqueous phase (A) and oil phase (B) directly from the hemin reactant (S), a reversible pathway between aqueous (A) and the new solids (N), and an irreversible pathway from oil (B) to new solids (N). We assume each reaction pathway to be pseudo-first-order and follow Arrhenius kinetics, as in Equation 7.1.

$$k_{ij} = k_b \exp \left( \frac{E_{ij}}{RT_b} \left(1 - \frac{T_b}{T} \right) \right)$$  \hspace{1cm} (7.1)

Here, $k_{ij}$ is the rate constant for reaction pathway from reactant i to product j. $E_{ij}$ is the corresponding activation energy. R is the gas constant, T is reaction temperature. $k_b$ and $T_b$ are the base rate constant and base temperature respectively. In our modeling, $T_b$ is set as 623.15 K.

Coupling the rate laws with the batch reactor design leads to equations (7.2)-(7.5). The subscripts on each rate constant refer to specific reaction pathways in Figure 5-1.

$$\frac{dX_S}{dt} = -(k_{sb} + k_{sa})X_S$$ \hspace{1cm} (7.2)

$$\frac{dX_A}{dt} = k_{sa}X_S - k_{an}X_A + k_{na}X_N$$ \hspace{1cm} (7.3)

$$\frac{dX_B}{dt} = k_{sb}X_S - k_{bn}X_B$$ \hspace{1cm} (7.4)

$$\frac{dX_N}{dt} = k_{an}X_A + k_{bn}X_B - k_{na}X_N$$ \hspace{1cm} (7.5)
\[ X_{S,\text{total}} = X_S + X_N \] (7.6)

Here, \( X_i \) is iron distribution (wt. %) of the corresponding product (i). It means that \( X_i \% \) of the iron from the hemin reactant resides in product i. Note that \( X_i \) is an indicator of iron distribution instead of the absolute iron content in product, which was discussed in section 5.3.1. As only the total solids content of the HTL products was available experimentally, we used equation (7.6) to relate the calculated newly formed solids and hemin reactants with the total solids.

We performed the control experiment at room temperature (25 °C), following the same procedure outlined to determine the initial conditions for the model. Since hemin dissolved in or reacted with neither water nor DCM, there were only solids at room temperature. Therefore, the boundary conditions were set such as \( X_S \mid t=0 = 100, X_A \mid t=0 = X_B \mid t=0 = X_N \mid t=0 = 0 \). We simultaneously solved the system of ordinary differential equations and estimated the values for rate constants \((k_{ij})\) and activation energies \((E_{ij})\) using a non-linear least-squares objective function. The objective function is defined as the summation of the squared differences between the experimental value for each product fraction \((x_i^{exp})\) and the model value \((x_i^{model})\) at each reaction temperature and time.

\[ Q = \sum_i \sum_t \left[ x_i^{exp}(t) - x_i^{model}(t) \right]^2 \]

Athena Visual Studio is used to minimize the objective function and estimate the parameters for each reaction rate constant. Experimental data at different temperatures are used to estimate both the activation energy and base rate constant at the same time.
5.3.3 Kinetic parameter analysis

Figure 5-2 shows the ability of the model (solid lines) to correlate experimental data (discrete points) for iron from HTL of hemin at 325 °C, 350 °C, and 400 °C. Agreement is very good at the conditions investigated, thus the model is capable of capturing the iron fates after HTL of hemin. Under all temperatures examined, most of the iron in hemin was recovered in a solid phase. At 325 °C, iron in solids phase decreases slowly with holding time. At 350 °C and 400 °C, iron in solids both decrease and then increase to almost 100% at 80 min. That is to say, iron increasingly partitions to the solid phase as reaction severity increases. Iron mass in both oil and aqueous phase counted for 20% or less respectively. Opposite to the trend of solids, iron mass in aqueous and oil phase decreases with increasing reaction severity. As many iron porphyrinic species were detected in the hemin crude oil phase by FT-ICR MS (data not shown here), and porphyrinic species are very thermally stable, it is likely that iron porphyrinic species in oil break down at more severe conditions, making it form oil insoluble fractions and then precipitate in solids phase.

Table 5-2 shows the optimized rate constants and activation energies for iron reaction pathways in the HTL of hemin. Pathways 1&2 have the highest activation energies, which indicate the recalcitrance of hemin to decompose in hot and compressed water. Iron conversion between solid and aqueous phase is the only reaction pathway assumed to be reversible. Within the model, this pathway can be one-directional but a reversible route is of importance for better mathematical fitting.

In comparison, the thermal decomposition of both nickel and vanadyl petroporphyrins is also a first order process. The Arrhenius activation energies calculated from the rate constants are 180 kJ/mol for the vanadyl porphyrin and 192 kJ/mol for the nickel porphyrin.110
Figure 5-2: Experimental iron distributions (discrete points) and model calculations (solid lines) over time at different temperatures for solids (red rectangles), aqueous (brown triangles), oil (blue rhombus) for HTL of hemin. Top left, top right, and bottom left correspond to reaction temperatures at 325 °C, 350 °C, and 400 °C respectively.

Table 5-2: Optimized rate constants and Arrhenius parameters for iron distribution pathways from HTL of hemin

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Reaction</th>
<th>$k_{ij}$ (min$^{-1}$)</th>
<th>$E_{ij}$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>325 °C</td>
<td>350 °C</td>
</tr>
<tr>
<td>1</td>
<td>S → A</td>
<td>0.017</td>
<td>0.112±0.0081</td>
</tr>
<tr>
<td>2</td>
<td>S → B</td>
<td>0.006</td>
<td>0.034±0.0019</td>
</tr>
<tr>
<td>3</td>
<td>A → N</td>
<td>0.077</td>
<td>0.382±0.1601</td>
</tr>
<tr>
<td>4</td>
<td>B → N</td>
<td>0.009</td>
<td>0.014±0.0007</td>
</tr>
<tr>
<td>5</td>
<td>N → A</td>
<td>0.012</td>
<td>0.019±0.0015</td>
</tr>
</tbody>
</table>
5.3.4 Calculated iron distributions

Having shown that the kinetic model can describe iron fates as a function of temperature and time, we can now use it to improve our understanding of the kinetics governing this process. Figure 5-3 displays contour plots of iron distribution into each product fraction as a function of temperature and time for isothermal HTL.

Figure 5-3 (a) shows the calculated iron distribution in the original hemin reactant as a function of temperature and time. Iron mass fraction in hemin decreases monotonically with increasing temperature and holding time during HTL. Temperature is more influential on iron fractions in hemin than time, as iron converts more than 90% in a few minutes when the temperature is above 360 °C.

Figure 5-3 (b) shows the calculated iron distribution in the solids other than hemin during HTL. The iron fraction in the new solids increases monotonically with increasing temperature and time. Temperature and time contribute fairly evenly on iron fractions. The highest iron fraction in the new solids occurs in the most severe reaction conditions (360 °C < T < 400 °C, 30 min < t < 80 min). The formation of iron in the new solids corresponds mainly with decomposition of hemin at mild conditions, and with decomposition of iron porphyrinic species in crude oil at severe conditions.

Figure 5-3 (c) shows the model calculated iron distribution in the solid phase (both hemin & new solids), which is the summation of figure 5-3 (a) and (b). The highest iron fractions in solids occur in two regions: 1) under very short holding times (t < 5 min) when hemin just starts decomposition. 2) under severe conditions (380 °C < T < 400 °C, 50 min < t < 80min). The second region coincides with the region where iron has the lowest distribution in crude oil phase, which is reasonable as most of the iron fractions from hemin are in the solid phase.
Figure 5-3 (d) shows the calculated iron distribution in the aqueous phase. In a rather wide region, the iron fraction in the aqueous phase only accounts for less than 4% of the total mass \((T > 360 \, ^\circ C, t > 10 \text{min})\). At low reaction severity, iron in aqueous phase increases with increasing temperature and time as hemin is converted and then decreases with increasing reaction severity. Temperature is significantly more influential than time on iron fates in aqueous phase. As when temperature is above 385 °C, there will be no iron in aqueous phase at all. There is a very narrow area where iron fraction in aqueous phase can be as high as 18%. This area corresponds to a very short reaction time \((t < 5 \text{min})\) and a temperature range \((365 \, ^\circ C < T < 380 \, ^\circ C)\) around the critical temperature.

Figure 5-3 (e) shows the calculated iron distribution in crude oil phase. The iron fraction in the crude oil is equally sensitive to temperature and time. In general, iron in crude oil phase increases and then decreases with reaction severity. The increase of iron in crude oil in the mild region coincides with the corresponding decrease of iron in solids. This observation trend aligns well with our previous study on iron content in algal biocrude oil\(^{81}\). Iron mass fraction can be achieved as low as 4% in crude oil at severe conditions \((380 \, ^\circ C < T < 400 \, ^\circ C, 60 \text{min} < t < 80 \text{min})\). The region where iron fraction in crude oil achieves the highest (18%) is a widely used region for HTL operations. It includes fast HTL at high temperatures (e.g. HTL at 400 °C with \(t < 5 \text{min}\)) and isothermal HTL at subcritical temperatures (e.g. HTL at 350 °C with 10 min < \(t < 20 \text{min}\)). Having a knowledge of the conditions under this region is of importance to avoid high iron fractions in the resulting crude oil. Compared with 5-3 (d), we can conclude that the conversion of iron from hemin to aqueous phase is faster than that to crude oil phase.
Figure 5-3: Iron distribution (%) in (a) Hemin, (b) New solids, (c) Solids in total, (d) Aqueous phase, (e) Crude oil as functions of time, and isothermal temperature. Solid black lines correspond to the yield contours marked by numbered ticks on the bar legend.
5.3.5 Model comparison with iron in HTL products of microalgae

In this section, we will compare the fates of iron from HTL of hemin and microalgae. As a result, we will examine the robustness of using hemin as a model compound to represent iron fates during algal biocrude conversion. Using the iron distribution data on HTL of microalgae from our previous study, we modified the reaction network (Figure 5-1) and simplified it as shown in Figure 5-4. We eliminated the reversible pathways between iron in aqueous (A) and in the new solid phase (N) because the rate constants for those pathways are minimal.

![Iron distribution reaction network for HTL of microalgae](image)

Using the same methodology and objective function, we write the batch reactor design equations as:

\[
\frac{dX_S}{dt} = -(k_{sb} + k_{sa})X_S
\]  (7.7)

\[
\frac{dX_A}{dt} = k_{sa}X_S
\]  (7.8)

\[
\frac{dX_B}{dt} = k_{sb}X_S - k_{bn}X_B
\]  (7.9)

\[
\frac{dX_N}{dt} = k_{bn}X_B
\]  (7.10)

Figure 5-5 shows the ability of the model (solid line) to correlate experimental data (discrete points) of iron reactions during HTL of microalgae at 350 °C and 400 °C, respectively. The trend for iron variation in different microalgae HTL product phases is consistent with that from HTL of hemin. Like hemin, most of the iron fractions end up in solid phase. Furthermore, the trends that iron in solids decreases and then increases and that iron in algal bio-oil increases...
and then decreases with holding time are consistent with iron from hemin products. However, algal bio-oil has a much higher iron fraction than hemin crude oil. This is due to the higher iron percentage loading of hemin reactants than microalgae and the higher solids yields in hemin post HTL products. Iron fraction in crude oil can be as high as 60% and thus becoming a concerning factor when hydroprocessing crude oil. Iron in aqueous phase is almost zero in all conditions examined compared to 20% or less as in hemin aqueous product. Another difference lies in the holding time when iron content reaches maximum in oil phase, i.e., minimum in solid phase. For HTL of hemin, iron fraction in solid phase reaches its minimum at about 5 min at 350 °C and almost immediately at about 400 °C. In contrast, iron fraction in solid phase reaches its minimum at about 20 min and 5 min respectively at 350 °C and 400 °C. Nevertheless, both studies show that maximum/minimum point occurs much faster at 400 °C than at 350 °C.

The observation that iron fates in microalgae and hemin HTL products have the same temporal trend but different proportions can be explained by the similarity and differences between reactants. Since iron is mostly in porphyrin forms in both microalgae and hemin, the trends of iron fates in different products can be very similar. The differences between the relative fractions in different product phases could possibly due to the following reasons. First of all, iron content in hemin is only about 0.1% in microalgae but 8.6% in hemin solids. The fact that hemin is orders of magnitude more concentrated in iron could lead to the distribution of iron shifting to solids phase, since reactant concentration is decisive for kinetic behavior. Second, solid yields from HTL of hemin are much higher than that from HTL of microalgae. Solid yields of hemin at 350 °C with different holding times are greater than 50%. Solid yields from HTL of microalgae at the same temperature are about 5% or less. The higher solid yields will favor a higher iron mass fraction correspondingly. Third, microalgae is a complexed mixture with different macromolecules, other metals, and heteroatoms present. The interactions between other
molecules with porphyrinic species, between electrolytes and non-electrolytes, between iron and other ligands present can affect iron fates in different products.

Using the same reaction network frame leads to a very good model fitting for iron fates in microalgal post HTL products as shown in Figure 5-5. This network emphasizes more on fast HTL such that we could be able to see iron distributions at very short holding times (< 10 min). Thus, Figure 5-2 together with Figure 5-5 gives us a more comprehensive examination of iron fates under fast and isothermal HTL with different model compounds.

Table 5-3 shows the optimized rate constants and activation energies for iron reaction pathways during HTL products of microalgae. Pathway 3 has the highest activation energy, comparing to the highest activation energies for hemin decomposition in Table 5-2. The activation energies of pathway 1&2 are lower than those from Table 5-2, perhaps because iron porphyrins (heme) only constitute a small fraction of the initial microalgae biomass. In general, the estimated rate constants and Arrhenius parameters of these two models agree with other within an acceptable range.
Table 5-3: Optimized rate constants and Arrhenius Parameters for iron distribution pathways from HTL of microalgae

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Reaction</th>
<th>$K_i$ (min$^{-1}$)</th>
<th>$E_i$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S → A</td>
<td>0.0002</td>
<td>0.042</td>
</tr>
<tr>
<td>2</td>
<td>S → B</td>
<td>0.08</td>
<td>0.1168</td>
</tr>
<tr>
<td>3</td>
<td>B → N</td>
<td>0.026</td>
<td>0.038</td>
</tr>
</tbody>
</table>

The facts that iron variations have similar trends and that the kinetic networks are similar between hemin and microalgae networks also demonstrates the capability of utilizing hemin as a model compound to represent and study iron fates in HTL of microalgae.

5.4 Product yields from hemin and its kinetic modeling

In this session, we are further building a reaction network and calculating the parameters from kinetic modeling for product yields. We then relate this model with the iron distribution model in order to find optimized conditions for higher yields and low iron contents in crude oil.

5.4.1 Kinetic model development

The reaction network from hemin HTL is depicted as in Figure 5-6. In this network, we propose the same concept of a newly formed solid product (N) that is used in Figure 5-1. The network comprises the formation of aqueous phase (A), oil phase (B), new solids (N), and gas (G) directly from hemin reactant (S). There is also an additional pathway from new solids (N) to gas product (G). This reaction network does not overlap with the network for iron distribution (figure 7-1) because although iron is relatively concentrated in hemin reactants and the crude oil products, it only constitutes about 5-10% of both hemin and crude oil mass. The reaction network
for product fraction yields from hemin HTL will be dominated by the masses of atoms other than iron. Therefore, it is reasonable that product yields and iron distributions will have different reaction pathways under the same reaction conditions.

![Reaction network](image)

**Figure 5-6: Reaction network for product yields from HTL of hemin**

Having the same assumptions and objective functions as described earlier in this chapter, we couple the rate law equations with reactor design equations and write them as:

\[
\frac{dX_S}{dt} = -(k_{sb} + k_{sa} + k_{sg} + k_{sn})X_S
\]

(7.11)

\[
\frac{dX_A}{dt} = k_{sa}X_S
\]

(7.12)

\[
\frac{dX_B}{dt} = k_{sb}X_S
\]

(7.13)

\[
\frac{dX_N}{dt} = k_{sn}X_S - k_{ng}X_N
\]

(7.14)

\[
\frac{dX_G}{dt} = k_{sg}X_S + k_{ng}X_N
\]

(7.15)

We then solve these equations together with the Arrhenius equations simultaneously using the methods stated in the earlier session.

### 5.4.2 Yields and model fitting

As shown in figure 7-8, the model fitting is in good agreement with the experimental yields. Solid has the highest yield among all phases of products (> 40%). Solid yield decreases
steadily with holding time and decreases with increasing temperatures. Aqueous product yields remain at about 5% at all temperatures and holding times examined. Gas product increases with temperature. Gas yield increases from less than 10% at mild conditions to about 40% at the most severe conditions. The aqueous yield seems to be rather low comparing to that from HTL of microalgae (about 50%), and the solid and gas yields are higher.62

Figure 5-7: Experimental yields (discrete points) and model calculation (solid lines) over time at different set temperatures for solids (red rectangles), aqueous (brown triangles), oil (blue diamond) for HTL of hemin. Top left, top right, and bottom left corresponds to reaction temperatures at 325 °C, 350 °C, and 400 °C respectively.
5.4.3. Kinetic parameter analysis

Table 5-4 displays the optimized rate constants and activation energies for different reaction pathways within the proposed network. The activation energies for hemin decomposition (Pathway 1-4 in Table 5-4) are comparable with those for iron distribution (Pathway 1-2 in Table 5-2), indicating that iron is released into other phases simultaneously when hemin starts to break down and form other products.

Table 5-4: Optimized rate constants and Arrhenius Parameters for product yields from HTL of hemin

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Reaction</th>
<th>$k_{ij}$ (min$^{-1}$)</th>
<th>$E_{ij}$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>325 °C</td>
<td>350 °C</td>
</tr>
<tr>
<td>1</td>
<td>S → A</td>
<td>0.0034</td>
<td>0.018±0.016</td>
</tr>
<tr>
<td>2</td>
<td>S → B</td>
<td>0.0113</td>
<td>0.068±0.061</td>
</tr>
<tr>
<td>3</td>
<td>S → G</td>
<td>0.0067</td>
<td>0.053±0.044</td>
</tr>
<tr>
<td>4</td>
<td>S → N</td>
<td>0.0274</td>
<td>0.157±0.154</td>
</tr>
<tr>
<td>5</td>
<td>N → G</td>
<td>0.0005</td>
<td>0.0009±0.0016</td>
</tr>
</tbody>
</table>

Figure 5-8 shows the instantaneous selectivity for a series of parallel reactions (Pathway 1-4 in Table 5-4). Instantaneous selectivity is defined here as $S_{Sj} = r_{Sj}/r_S = k_{Sj}/k_S$, where $j = A, B, G, N$ and $k_S = k_{SA} + k_{SB} + k_{SG} + k_{SN}$. $r_{Sj}$ and $k_{Sj}$ are rate and rate constant from solid conversion reactions $S \rightarrow j$ respectively. The selectivity of hemin to new solids and aqueous products are the highest and lowest respectively, at all times. The selectivity of solids to crude oil slightly decreases with increasing temperature. The selectivity of solids to gas increases monotonically and exceeds that of crude oil at around 375 °C. Therefore, this figure suggests that heating the reactants below 375 °C would maximize crude oil production. Heating the reaction mixture to 375 °C and higher would intensity the formation of gases.
Figure 5-8: Instantaneous selectivity of hemin product conversion with respect to temperature. Wine red, green, yellow, and blue correspond to new solids (N), gas (G), Oil (B), and aqueous products (A) respectively.

5.4.4 Calculated yields

Figure 5-9 displays contour plots of product yields as functions of temperature and time for isothermal HTL of hemin.

Figure 5-9 (a) shows the calculated yields of hemin reactants as a function of reaction temperature and holding time. Hemin decomposes readily during HTL and more than 90% conversion is complete by 50 min at all temperatures examined. Comparing with Figure 5-3 (a), the two contour plots coincidentally have the same trends, indicating again iron is transferred to other products simultaneously as hemin decomposes. However, hemin decompose rate is higher than iron participating rate. For instance, at 350 °C and 10 min, 90% of hemin is already converted while 70% of iron from hemin transferred to other products.

Figure 5-9 (b) shows the calculated yields of the new solids formed directly from hemin. The new solid yield increases with increasing reaction severity, reaches a maximum within a
wide region, and then slowly decreases with reaction severity due to the formation of gas products.

Figure 5-9 (c) shows the effect of temperature and holding time on yields of the total solids in the reactor. Solid yields remain about 40-60% for most of the conditions, and decrease slowly as reaction severity increases. We would prefer a low solid yield and gather as much as iron in the solid phase. The most severe conditions would result in the lowest solid yields as well as the highest iron distribution in the solid products (shown in Figure 5-3 (c)). Therefore, severe conditions are ideal for enriching iron level in the solids.

Figure 5-9 (d) shows the calculated yields for the aqueous-phase products. The highest yields for aqueous-phase products occur in the region with lower temperatures (\( T < 350 \, ^\circ C \)) and longer holding time (\( t > 20 \, \text{min} \)).

Figure 5-9 (e) shows the calculated yields for crude oil. The formation of crude oil corresponds with the decomposition of hemin solids. The highest yields for crude oil occur in the region where temperature is below 370 °C while holding time is more than 10 min. This is a rather wide area that composes iron fractions in biocrude from 4% to 20% according to the Figure 5-3 (e).

Figure 5-9 (f) shows the calculated yields for the gas products. Gas product yields increase with reaction severity and can be as high as 35% - 40%.
Figure 5-9: (a) Hemin, (b) New solids, (c) Solids in total, (d) Aqueous phase, (e) Crude oil product yields calculated as functions of time, t, and isothermal temperature, T. Solid black lines correspond to the yield contours marked by numbered ticks on the bar legend.
5.4.5 Optimization between yields and quality

Having examined the iron fates and product yields from HTL of hemin as well as building a kinetic modeling for both iron distributions and product yields, we will now take both models collectively to optimize product yields and quality. As an optimized result, we hope the HTL products will have the following characteristics: 1) We would prefer as low iron concentrations in the crude oil as possible. This is a direct indicator of oil quality and is of importance. This information is shown in Table 5-1. 2) We would prefer as low iron mass fraction (%) as possible in crude oil since iron in aqueous or solid phase products can be more easily recycled. This information is shown in Figure 5-4. 3) We would prefer high yields of crude oil, although this is of less importance since in microalgae, the crude oil compositions are initially from macromolecules such as lipids, proteins, and polysaccharides. The yield portion that hemin contributes to is much smaller.

We will then discuss separately under which scenarios those characteristics occur.

1. Crude oil with the lowest iron concentration is obtained with the longest holding time (80 min) under 350 °C and 400 °C. Iron contents are about 13,000 ppm and 16,000 ppm respectively, which is about 20% when iron concentration is the highest in crude oil. Even a 60 min of holding time under the same temperature (350 °C) can double iron concentrations in crude oil.

2. The lowest iron mass distribution in crude oil occurs during the most severe HTL conditions (T > 380 °C, t > 60 min). Lowest iron mass distribution in crude oil can be as low as 2% to 4%. The region corresponds to the top right corner region in Figure 5-4 (e). Remember that this region also gives us the desirable solid products, i.e., lowest solid yields and highest iron fractions in solids.
3. There is a trade-off between the highest crude oil yield and lowest iron fractions in crude oil. Two operating conditions might be considered in this scenario: one is ensuring the highest yield (22.5% - 25%) with a little higher iron fraction (4% - 6%), for instance, 365 °C and 80 min. This overlapping area is rather small with a temperature range from 364 °C to 368 °C and a time range from 75 min to 80 min. Another condition is ensuring the lowest iron fraction (2% to 4%) while losing some yields (20% - 22.5%), for instance, 390 °C and 70 min. This is the same region as the second scenario listed above.

Therefore, being restricted mostly by the first scenario, and considering the second and third scenarios collectively, we recommend the optimal processing conditions consisting an 80 min of holding time, and a temperature range from 364 °C to 368 °C or from 380 °C to 400 °C. Future studies are encouraged to examine iron fates with a holding time longer than 80 min.

5.5 Conclusion

In this study, we used hemin as a representative model compound to study iron fates during biocrude production from microalgae. We first analyzed iron contents in HTL products at each reaction conditions. We then developed reaction networks and quantitative kinetic models for product yields and iron distributions from HTL of hemin. This is the first study that offers models for product quantity and quality together from the same reactions. Calculated model values correlate well with observed values for networks presented in this study.

The similarity of iron participation trends between HTL of hemin and microalgae indicates that hemin is an appropriate model compound to represent iron compounds in microalgae. The fact that we use microalgae to study iron fates during fast HTL and use hemin to
study iron fates during isothermal HTL gives us a more comprehensive understanding of iron fates in both systems under all conditions.

We observed very comparable activation energies in both the quantity and quality model for solid conversion reactions. Relating this and the contour plots for yields and iron fractions in hemin together, we conclude that iron releases into other products as hemin breaks down.

Taking all the observation collectively, we also proposed optimal HTL conditions for a higher yield and crude oil quality in terms of iron presence. Severe conditions consisting an 80 min of holding time, and a temperature range from 364 °C to 368 °C and from 380 °C to 400 °C are recommended. Overall, this study is helpful to gain insights into the reaction pathways in hemin HTL products formation and quality.
Chapter 6

Conclusions and Recommendations for Future Research

6.1 Conclusions

In this dissertation, we addressed the metal contamination issues in algal biocrude oil obtained from hydrothermal liquefaction, explored the influence of processing conditions on all the metal distributions, and employed interventions to reduce iron content in the bio-oil. We were able to reduce the iron content to the same level of that in petroleum crude oil, so that we can potentially minimize capital cost by using the same downstreaming refinery infrastructures. We then use hemin as a model compound to represent iron fates in microalgae during HTL. We were able to identify the classes of molecular products in the hemin crude oil. Last but not the least, we postulated reaction networks and developed kinetic models that describe both the product yields and iron distributions after HTL of hemin.

In Chapter 2, we determined the effects of different hydrothermal liquefaction (HTL) process variables (temperature, holding time, algae loading, and water loading) on 13 different elements in biocrude and the aqueous phase co-product. Reaction conditions included both fast and isothermal HTL. The concentrations of P, Mg, Na, and Ca in the biocrude had their highest values at the mildest HTL conditions explored, and the concentrations decreased precipitously as even moderate HTL conditions were used. The concentrations of Zn, Cu, and Ni do not show much variation with HTL severity, whereas the concentration of Fe in the biocrude first increases and then decreases. P and Na were the most abundant elements in the biocrude from fast HTL, but Fe was the most abundant from isothermal HTL. These results show that the HTL conditions
can influence the concentrations of different metals and inorganic species in algal biocrude from HTL.

In chapter 3, we produced biocrude oil from microalgae at two hydrothermal liquefaction conditions and used eight organic solvents with different polarities and boiling points to explore their influence on biocrude yields and metal content. We determined how preventing contact between the organic solvent and the aqueous-phase product influenced the metal contents in the biocrude, as this approach would likely be employed at scale. For all solvents tested save dichloromethane, the bio-oil yield is higher when the solvent and aqueous phase do not come into contact. For all solvents tested, the alkali metal contents of the biocrudes are one or two orders of magnitude higher when there was no contact between solvent and aqueous phase product. The choice of solvent can reduce the biocrude iron content by half and make a significant difference in other metals as well. Using MTBE alone or the combination of hexane and ethanol and not allowing the solvents to contact the aqueous phase gives the best results in terms of high biocrude yield and low metal contents. The effect of the solvent on biocrude metals content cannot be attributed to any single solvent property. Rather multiple factors such as polarity, solubility, and dielectric constant need to be considered.

Also, in Chapter 3, we assessed the effect of adding heterogeneous catalyst on the metal content in the biocrude. The presence of the Ni catalyst reduced the iron content to just 3% of its value from noncatalytic HTL. These materials are promising catalysts for in situ demetallation of algae biocrude during its production via HTL.

In Chapter 4, catalytic studies with Fe-containing model compounds permitted post-reaction product characterization, and thereby shed light on some of the chemical details in this process. GC-MS characterizations of crude oil from HTL of hemin identified crude oil molecules such as cyclopentenone and cyclohexenone derivatives, indoles, long-chain alcohols, aromatics,
and amides. FT-ICR MS spectra have shown the presence of tens of unique iron-containing porphyrin ions in crude oil from uncatalyzed HTL of hemin, with $N_4Fe_1$ being the dominant species. For catalyzed hemin crude oil, the porphyrin structures that are dominant for the unprocessed HTL biocrude are not present, indicating those iron porphyrin molecules have undergone ring opening and degradation with the introduction of catalysts. The molecular product distribution allowed us to postulate a set of the hemin reaction and demetallation pathways. Our hypothesis is that a part of the hemin reactants would degrade such that the methene bridges of the tetapyrrole rings are attacked by water molecules, producing various pyrrole products in addition to releasing iron through hydroxylation. The pyrrole fragments will then have denitrogenation and oxidation reactions, forming products such as cyclopentenone and cyclohexnone derivatives. The other hemin molecules would still maintain the iron porphyrine structures without catalyst and will decompose when the demetallation catalyst is applied.

In Chapter 5, we postulated reaction networks for HTL of hemin and then developed quantitative kinetic models to predict hemin product yields and iron distributions into post HTL products. The developed reaction networks and kinetic parameters are compared against each other and against the data from modeling iron distribution in microalgae HTL products. These results are valuable for exploring reaction pathways of product formation under different systems containing iron porphyrinic compounds. Chapter 4 and Chapter 5 together provided the first known studies on HTL of iron model compound.

### 6.2 Recommendations for Future Research

In this dissertation, we laid the groundwork to shed light on metal behaviors during HTL of microalgae, employed successful demetallation methods, and conducted detailed studies on
iron model compounds. This work has contributed to resolving metal contamination issues in algal biocrude oil. However, since there are inadequate published researches in this area, and that we do see the unraveled knowledge required while conducting the studies in this dissertation, future studies of metal fates during biomass to biofuel conversion are still of importance in addition to our work. In the following sections, we would point out the research areas and challenges that deserve the attention to better understand the demetallation issues and broaden the demetallation applications.

6.2.1 Recycling and reusing the iron in solids product

Most of the iron either resided in the crude oil or the solid residue phase after HTL of microalgae or hemin compound without applying catalysts. When using heterogeneous catalysts, most of the iron is enriched in the solid phase. Although iron is undesirable in the crude oil, the rich iron content in solid phase can potentially be reused as it possesses magnetic properties.

Magnetic activated carbons (MACs) exhibit magnetic characteristics with great efficiency for the adsorption of contaminants. Compared to traditional carbon absorbents, MACs are more easily removed from the concentrated reaction solution using a magnetic separator. MACs have been traditionally synthesized by impregnating a magnetic medium (such as $\gamma$-Fe$_2$O$_3$) with carbon-based absorbents by a pyrolysis activation or chemical coprecipitation. However, such process can be costly due to the nonrenewable precursors and be unfeasible in large scale applications. Several researchers have used biomass waste to produce MACs for various adsorption purposes. There are also studies on activating biochar product from hydrothermal treatment of renewable organic materials into MACs, which take advantage of
the high iron content in the solid product without adding new iron substances when making MACs.

We have used AC as a demetallation catalyst and successfully reduced the iron content in the crude oil. The spent AC with high concentrations of iron could potentially be a great precursor for making MACs. As is shown in Chapter 4, about 5.9 wt% of iron is in the spent activated carbon catalyst and solid phase after HTL of microalgae. In comparison, the solid residue from hydrotreatment of one of the lignin sources has an iron content of 2.9 wt%, and an iron content between 4.7 wt% and 6.4 wt% after being made into MACs. This range of iron content is proven to be effectively separated by applying a magnetic field. Thus, future studies are recommended on recycling and reusing the spent catalyst and solid phase after HTL of algal biocrude. Challenges might lie in the low surface area and porosity of the biochar product for exploitation of its application. Different recovering procedures and characterizations can be performed to select excellent and easily recoverable absorbents. The recycling process together with the demetallation of biocrude can further reduce the carbon footprints and enhance the energy recovery from utilizing algal biomass.

6.2.2 Fast HTL of hemin and severe conditions for demetallation

In Chapter 4 and 5, we studied isothermal HTL of hemin and its kinetic modeling. Those conditions are selected with a temperature range between 325 °C to 400 °C and time range from 10 min to 100 min. Both iron content and yield variations were observed in this range. However, being the first known work studying hemin under HTL conditions, we have not examined the processing conditions thoroughly. Some future studies that are conducted outside the scope of
those conditions experimented, such as fast HTL, can help fill the discrepancies we observed for the model compound.

In Chapter 5, we observed that the iron distribution trends at 325 °C and 350 °C are very different, and that hemin at 300 °C does not decompose at all. Future studies on narrowing this 25 °C temperature gap would be informative on the iron behaviors in hemin product. Chemistry between 300 °C and 325 °C can also be very intriguing as intermediate products can be observed during hemin reaction under HTL conditions.

Furthermore, as we mentioned, HTL of hemin at short reaction times (less than 10 min) and fast heating rates have not yet been explored in this study. As is shown in Chapter 5.3.3, at 350 °C, iron content in the solids reaches its minimum in less than 10 min, and then starts increasing with holding time. In Chapter 5.4.2, we also showed that the hemin crude oil yields have reached its maximum in less than 10 min at both 350 °C and 400 °C. Experiments at fast HTL conditions are necessary to provide a better understanding of iron distributions and kinetics of hemin reactions.

Last but not the least, we have concluded that the optimal conditions for a higher yield and lower iron participation in crude oil phase after HTL of hemin is at 80 min, with a temperature range from 364 °C to 368 °C or from 380 °C to 400 °C. Those conditions are of the most severe ones we have examined. More severe conditions such as increasing the reaction holding times can be examined.

6.2.3 Hemin demetallation mechanism and reaction pathways

We have attempted to construct some of the possible reaction pathways that take place during HTL of hemin by analyzing the products. We observed that some of hemin molecules are
decomposed and formed smaller molecules, while some of the hemin molecules were only slightly modified and kept the iron porphyrin structures. We postulated how hemin molecules are degraded and form lighter products such as cyclopentenone derivatives. However, we are not able to test the hypothesis. A more powerful in-situ analysis of the hemin crude oil product and comprehensive characterizations of the solids products and spent catalyst would facilitate a deeper understanding of the reaction pathways. One challenge we encounter after heterogeneous catalytic HTL of hemin is to separate the solids products with the spent catalysts.

Furthermore, we analyzed the products under only one HTL processing condition (400 °C and 60 min). Information regarding product compositions at different conditions can be beneficial to understand if hemin molecules are prone to decompose or to keep the porphyrin structures at certain conditions.
APPENDICES
Appendix A

Asphaltenes from algal biocrude
A.1 Introduction

In petrochemistry, asphaltene is defined as the heaviest component of petroleum, and it is soluble in solvents such as benzene, toluene, and DCM, but insoluble in light paraffinic solvents such as n-pentane and n-heptane at ambient conditions.\textsuperscript{122-124} Asphaltene content plays an important role in determining the viscosity and density of crude oil.\textsuperscript{125} This material usually is hydrogen deficient (low H/C ratio, lots of aromatic structures) and contains undesired heteroatoms like S and N and metals such as V and Ni. Asphaltenes have been called “the cholesterol of petroleum” because they can precipitate from the crude oil, deposit on pipelines, and eventually block the flow of petroleum (e.g., from an off-shore production well).\textsuperscript{126,127} Previous literatures have shown that the coke formation tendency is in the increasing order of saturates < aromatics < resin < asphaltenes in crude oil fractions.\textsuperscript{128,129}

Asphaltene has one of the most complicated structures in nature and there has been no well-known asphaltene molecular structures revealed yet. A hypothetical structure of asphaltene molecule in petroleum is shown is figure A-1. It has been reported that in general, it is a high molecular weight molecule with a large aromatic sheet stacking on each other to form a unit cell. Additionally, metallolporphyrins are also associated with asphaltene molecule via $\pi$-electronic interaction.\textsuperscript{130}
Figure A-1: Hypothetical asphaltene molecule and its interaction with metalloporphyrins in petroleum

Biocrude from HTL of algae is a dense, viscous fluid and it looks more like a tar than an oil. It appears to be similar to a heavy crude oil, so the problems encountered with petroleum asphaltenes might also be encountered with algae biocrude, were it to be used on a large scale. There has been very little work done that is relevant to the issue of asphaltenes in bio-oil. Valdez et al. used hexane to separate light and heavy biocrude oil. Yoo et al. used a larger amount of n-hexane and referred to the heavy biocrude oil as asphaltenes. Bjelic et al. investigated the composition of asphaltene fraction produced from lignocellulosic biocrude. Asphaltenes are precursors of coke formation. Coke can deactivate catalyst during hydrotreatment in petroleum processing. Since coke formation varies with thermal maturity, extensive studies have been conducted to study petroleum asphaltenes. Those asphaltene caused problems in petroleum could also appear in algal crude oil derived asphaltene, since the ultimate purpose of utilizing biocrude is to co-process it with petroleum crude oil. However, to the best of our knowledge, there has been no work published on pyrolysis of the asphaltene obtained from biocrude. This study
determines the amount of asphaltenes present in algae biocrude produced by hydrothermal liquefaction. We then examine the molecular structures of separated products and determine whether they contain a high proportion of undesired heteroatoms and metals. We also obtain what chemical structures are present in the asphaltene pyrolysates, comparing the results with petroleum asphaltenes.

**A.2 Procedure**

Figure A-2: A schematic of procedures in this chapter

Figure A-2 describes more specifically overview of the experimental procedure on producing biocrude from HTL of microalgae, separating biocrude into asphaltene and maltene, pyrolysis of asphaltene products obtained, and product characterizations. After HTL, microalgae is converted into four phases of products: biocrude, aqueous, solids, and gas. Biocrude recovered is then subjected to separation into asphaltene and maltene products via n-pentane extraction. The original biocrude, asphaltene, and maltene products have been analyzed for their elemental composition (CHNS), metal contents, and functional groups. Pyrolysis of the asphaltene fraction have been conducted at different temperatures and molecular compositions have been investigated using GC-MS.
A.2.1 Separation of asphaltene and non-asphaltene components from biocrude

Batch reactors with 40 mL internal volume was constructed using two 1” OD caps and a tubing of 4.7” of length and 1”x0.095” wall from stainless steel Swagelok tube fittings. The reactors were used to produce large quantities of biocrude for separating asphaltene products. Those reactors were typically loaded with 4.8 g of Sapphire microalgae and 21.84 g of DI water. After tightening the cap carefully, the reactors were then put into a preheated sand bath at 350 °C for 60 min. The product workup procedure is similar to the one used for small batch reactors, except that the biocrude product were scraped out using a stainless steel spatula first before it is subjected to DCM extraction. The solid residue was separated by filtration after centrifuge. The organic phase containing biocrude was then dried on a heating plate at 45°C overnight.

N-pentane (50:1 weight ratio) was added to the dried crude oil and stirred for 2 h. The solution was then centrifuged to precipitate asphaltenes. The mixture was allowed to settle overnight prior to separation. The dissolved solution (maltene solution) was taken out carefully using pipet and was dried at 36°C for 3h at 30 psi by evaporating n-pentane under N₂ stream using a Labconco evaporator. The remaining asphaltene was kept drying on a heating plate overnight at 55°C. Dried asphaltene were collected and ground prior to analyze. The elemental compositions and molecular structural characterizations of original biocrude, maltene, and asphaltene samples were conducted by ICP, elemental analysis and ¹H and ¹³C NMR analysis.

A.2.2 Pyrolysis of asphaltenes

Asphaltene pyrolysis reactions were carried out in 4.1 mL batch reactors equipped with Swagelok tube fittings. The reactor was loaded with 0.35g of asphaltenes and purged with N₂ to provide an inert environment for reactions. The reactors were then put into a preheated fluidized
sandbath at 350 °C, 400 °C, 450 °C, and 500 °C respectively. After 60 min, the reactors were removed from the sandbath and quenched immediately into ice water. After the reactors were cooled at room temperature, caps were opened and the gas mass was recorded by weight difference. The reactor contents were next subjected to subsequent solvent extractions to separate and collect the pyrolysis product fractions.

Maltenes were collected in aliquots of n-heptane until the heptane extract remained colorless upon withdrawal from the reactor. The yields of maltenes were obtained by evaporating n-heptane from the solution. The molecular components in maltenes were then subjected to gas chromatography. The remaining products in the reactor were transferred into a conical tube by first being scrapped out using a metal spatula and then washed with DCM aliquots until the DCM extract remained colorless. The conical tube was vortexed and sonicated to ensure complete asphaltene recovery before asphaltene solution was separated by centrifuge. Asphaltene was recovered by evaporating DCM under flowing N₂ stream at 40 °C. The dried asphaltenes were then weighted. The insoluble solids remaining in the conical tube after drying was referred to as coke. Since the product yields for gas, maltene, and asphatene can be determined reasonably accurately, and it’s not possible to completely recover the coke products out of the reactor walls, coke product yield was calculated by difference.

**A.2.3 Yield and elemental analysis**

The gravimetric yield of algal biocrude was calculated as the mass of biocrude divided by the dry, ash-free mass of the algal biomass loaded into the reactor. The yields of pyrolysates were calculated as the mass of products divided by the asphaltene reactants. Elemental (C, H, N, S) analysis of biocrude was performed by a CEInstruments (Thermo Electron Corp) Elemental
Analyzer EA 1110 equipped with a TCD. We calculated the O composition by difference. The higher heating value (HHV) was calculated by using Dulong’s formula\(^8\) where C, H, S, and O represent the weight percentage of each element in the sample.

\[
HHV \text{ (MJ/kg)} = 0.338C + 1.428 \frac{H-O}{8} + 0.095S
\]

A.2.4 Molecular and functional group characterizations

Maltene obtained from biocrude was analyzed with a Shimadzu GC-MS QP-2010 Ultra with a 0.25 mm inner diameter Agilent DB-SMS column (30 m x 0.25 µm). The molecules in the sample were identified by comparing their mass spectra against the NIST mass spectral library. We tentatively identified 40 peaks with the largest peak areas for each sample, except for the one that was obtained at 500 °C where only 27 large peaks were in the total ion chromatogram.

\(^1\)H and \(^{13}\)C NMR spectra of oil and asphaltene samples were obtained from staff at the NMR facility on Penn State campus. The Bruker Advance-III-850 MHz was used for \(^1\)H and \(^{13}\)C NMR analysis. The chemical shifts were referenced to tetramethysilane (TMS) for both spectra. The samples were dissolved in CDCl\(_3\) for \(^1\)H NMR acquisitions and in CDCl\(_3\) containing Cr(acac)_3 as relaxation reagent for \(^{13}\)C NMR spectra.

A.3 Results and Discussions

A.3.1 Asphaltenes and maltenes

Biocrude yield (%) from the 40mL batch reactor is 28.52 ±1.36. Maltene content is defined as maltene mass/biocrude mass. Asphaltene content is defined as asphaltene
mass/biocrude mass. Maltene content (%) is found to be 46.01±2.73. Yoo et al.\textsuperscript{131} separated about 80% maltene and 20% asphaltene using \textit{N. Oceanica}, and had about 60% maltene and 40% asphaltene using \textit{Gloenkinia sp.}, both at HTL condition of 350 °C with 60 min holding time. At the same HTL condition, Valdez et al.\textsuperscript{62} had about 70% of light biocrude (maltene) and 30% of heavy crude (asphaltene) using \textit{Nannochloropsis}. Biller et al.\textsuperscript{58} had an approximately 50% asphaltene fraction from HTL bio-crude using Chlorella. Bjelic et al.\textsuperscript{132} obtained 60% of asphaltene fraction from aspen wood crude oil. In petroleum, in comparison, asphaltenes usually range from 0 to 40% of normal crude oil.\textsuperscript{133} Algal biocrude oil thus is a heavy oil with high asphaltene content.

The elemental compositions of products are shown in Table A-1. The O content was calculated by difference from the experimental C, H, N, and S values. The compositions of these products are important because it relates to the quality of the product obtained. Biocrudes with more H and C and less O, N, S are more desirable products. The results show an increasing carbon content and heating value and a decreasing oxygen content in the order of asphaltene, biocrude, and maltene. This suggests that maltene is energy denser than biocrude and asphaltene is the opposite. Though the trends are as expected, the differences of elemental distribution and HHV between each sample are not significant. Thus, the inorganic elements and metals might be a better indicator of oil quality in this case.

Table A-1: Elemental compositions and HHVs of microalgae, biocrude, maltene, and asphaltene. HTL condition of 350 °C and 60 min was applied.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>S</th>
<th>O</th>
<th>HHV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalgae</td>
<td>44.2</td>
<td>6.7</td>
<td>6.6</td>
<td>1.2</td>
<td>41.3</td>
<td>17.2</td>
</tr>
<tr>
<td>Biocrude</td>
<td>76.70</td>
<td>9.78</td>
<td>4.66</td>
<td>0.22</td>
<td>8.63</td>
<td>38.4</td>
</tr>
<tr>
<td>Maltene</td>
<td>78.13</td>
<td>10.96</td>
<td>3.33</td>
<td>0.18</td>
<td>7.38</td>
<td>40.8</td>
</tr>
<tr>
<td>Asphaltene</td>
<td>75.47</td>
<td>9.48</td>
<td>4.74</td>
<td>0.27</td>
<td>10.04</td>
<td>37.3</td>
</tr>
</tbody>
</table>
Metal contents in maltene, biocrude, and asphaltene are shown in Figure A-3. As expected, most of the metals were in the asphaltene fraction as metal contents were reduced significantly in maltene compared to the original biocrude. Iron content was reduced from 2500 ppm to about 800 ppm. The most abundant metals in the original biocrude are Fe, Na, and Ca. The most abundant metals in maltene fraction are Fe and Na. Heavy metal contents such as Ni, Mn, Zn, Cu, Fe in asphaltene were also compared. Those metals contents are higher in asphaltene than the original biocrudes. Fe content is 3250 ppm compared to 2500 ppm in asphaltene. In addition, ash content in asphaltene is 3.57%. This extraction potentially allows lower metal contents in lighter oils (maltene), but also results in lower biocrude yields. Thus, further studies will focus on identifying suitable solvents and engineering solvents (mixing them) for improved metal separations in the oil fractions. The solvent study on metal contents was discussed in Chapter 3.

Figure A-3: Metals in biocrude and maltene product. HTL condition of 350 °C and 60 min was applied.
We conducted $^1$H and $^{13}$C NMR analysis of biocrudes, maltene, and asphaltene to identity the types of structures and functional groups present, as is shown in Figure A-4. All the $^1$H and $^{13}$C spectra show the same resonance respectively. This work has a special focus on the aromatic ring systems. The following regions of chemical shift are of interest in a $^1$H NMR spectrum: aromatic hydrogens (9 to 6 ppm) and aliphatic hydrogens (4.5 to 0.5 ppm). For $^{13}$C NMR spectrum, the following different integration domains are identified: aliphatic carbons (10 to 65 ppm) and aromatic carbons (100-170 ppm).$^{134}$ The aliphatic region contains discrete sharp peaks overlapping a broad hump. These sharp peaks are commonly attributed to the straight-chain alkanes and methyl substituents on aromatics, alkyl chains, and naphthenic units. For $^1$H NMR, the peaks at 0.8 and 1.2 ppm are characteristic of terminal methyl groups and methylene groups in alkyl chains respectively.$^{135}$ Table A-2 shows the average molecular parameters for the samples from the integration results. Aromaticity increases in the order of biocrudes, maltene, and asphaltene. Xu et al.$^{80}$ revealed the presence of alkyl moieties, carbonyl functionalities, and aromatic molecules in all of the biocrudes.
Figure A-4: $^1$H and $^{13}$C NMR spectrum for which different integration domains are shown, from top to bottom are biocrude, asphaltene, and maltene samples. HTL condition of 350 °C and 60 min was applied.

Table A-2: Average molecular parameters obtained by $^1$H and $^{13}$C NMR.

<table>
<thead>
<tr>
<th>Molecular Parameters</th>
<th>Biocrude</th>
<th>Asphaltene</th>
<th>Maltene</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of aromatic carbons</td>
<td>10.67</td>
<td>12.98</td>
<td>14.25</td>
</tr>
</tbody>
</table>
A.3.2 Pyrolysis of asphaltenes

The data in figure A-5 show the asphaltene pyrolysate yields under different pyrolysis temperatures at 350 °C, 400°C, 450°C, and 500°C respectively with 60 min of holding time. Asphaltene pyrolysis yielded gas, maltene, coke, and unreacted asphaltene. Maltene and gas are produced from decomposition of asphaltene, and condensation takes place to form coke products. The yield of gas products increased with increasing temperature, and the yield of coke products decreased with temperature. The yield of maltene product from pyrolysis is about 5% at 350 °C, 400 °C, and 500 °C, but is much higher at 450 °C, which is about 18%. The gas product yield is rather high compared to gas product yield of petroleum asphaltene pyrolysis. Savage et al. had a gas yield at about 1.3% and a maltene yield at 35% at 400 °C pyrolysis condition. Han et al. obtained gas yield less than 1% when pyrolyzed coal-tar asphaltene. Savage et al. pyrolyzed petroleum asphaltene and concluded that coke was not formed at 350 °C but was formed at 400 °C and above. Asphaltene conversion increases with reaction temperature, from about 75% conversion at 350 °C to 97% conversion at 500 °C. The yield data provides an insight into product distributions. However, more severe conditions are needed to tell when the asphaltene conversion will be completed.

<table>
<thead>
<tr>
<th></th>
<th>86.97</th>
<th>83.06</th>
<th>82.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of saturated carbons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of aromatic hydrogens</td>
<td>5.20</td>
<td>5.92</td>
<td>7.34</td>
</tr>
<tr>
<td>% of saturated hydrogens</td>
<td>94.68</td>
<td>93.98</td>
<td>92.43</td>
</tr>
</tbody>
</table>
Figure A-5: Product yields from pyrolysis of asphaltene with 60 min reaction time

Figure A-6 shows the different molecular compositions in maltene pyrolysate at different pyrolysis temperatures. At 350 °C and 400 °C, nitrogenous compounds and alkanes comprised over 90% of the total chromatogram area. The alkane fraction includes a series of long, straight-chain molecules such as C\textsubscript{13}, C\textsubscript{14}, C\textsubscript{15}, C\textsubscript{16}, C\textsubscript{17}, C\textsubscript{19}, C\textsubscript{20}, C\textsubscript{21}, C\textsubscript{32}, and C\textsubscript{40}. There is also a small fraction of palmitic acid from the maltene product at 350 °C. The nitrogen containing compounds identified include long and single-chained nitriles and amides. At 450 °C, alkanes are the dominant components, which is over 80% of the total molecular species in maltane. Notably, the nitrogenous compounds are only about 3% of the total compounds, which includes indoles and long-chain nitriles. At 500 °C, the nitrogenous components have the biggest product fraction, and include mostly indole molecules. Aromatic compounds observed include naphthalene and benzene derivatives, comprising about 20% of the chromatogram area. Thus, aromatization reactions during pyrolysis predominately happen only at 500 °C among the temperatures.
examined. Phenols also comprised about 15% of the molecular components in maltene at 500 °C. In addition, the maltene fraction produced at 450 °C and 500 °C revealed the presence of alkenes and branched alkanes, which is similar to the observation of Savage et al.\textsuperscript{138} As for alkanes and alkenes, a possible reaction route is the thermal decomposition of aromatic or naphthenic structures with peripheral aliphatic substituents or aliphatic linkages connecting molecular units. The possible formation concerning alkyl aromatics and possible reaction pathways in petroleum asphaltenes were documented in literature.\textsuperscript{139–142}

Figure A-6: Molecular compositions in maltene product from biocrude asphaltene pyrolysis

**A.4 Conclusions**

Asphaltenes and maltenes were separated further from algal biocrude oil and their properties were studied. Most of the metals were separated out by removing the asphaltenes. Fe
content in asphaltene is 3250 ppm versus 800 ppm in maltene fractions. Maltene also has less heteroatom contents compared to biocrude and asphaltene, resulting in a higher HHV. The results from $^1$H and $^{13}$C NMR show that aromaticity increases in the order of biocrudes, maltene, and asphaltene. All the $^1$H and $^{13}$C spectra show the same resonance respectively.

Asphaltene pyrolysis yielded gas, maltene, coke, and unreacted asphaltene. The yield of gas products increased with increasing temperature, and the yield of coke products decreased with temperature. Higher temperature drives to a more complete conversion of asphaltene reactants. The gas product yield is significantly higher than that from pyrolysis of petroleum asphaltenes. Different molecular compositions in maltene pyrolysate at different pyrolysis temperatures were also compared. Nitrogenous compounds and alkanes are the major products. The alkane fraction includes a series of long, straight-chain molecules. The nitrogen containing compounds identified include long and single-chained nitriles, amides, and indoles. There is a big fraction of phenols and aromatics products at 500 °C, which is not observed at other temperatures.
Appendix B

Additional data and figures for Chapter 2
B.1 Metals in microalgae and HTL products

Metal contents in the original biomass are shown in table B-1. Na, K, P, S, Mg, and Fe were the most abundant in both algal biomass samples, with all of these elements being present at at least 1000 ppm. Metal concentrations in algae from Reed Mariculture Inc. decreased in the order Na>K>P>S>Mg>Fe; As for metals in algae from Sapphire Energy Inc. the sequence was Na>K>P>Ca>S>Mg>Fe. These sequences were comparable to each other despite a few differences. For the Sapphire algae, Ca was also present in excess of 1000 ppm. Na and Fe were the two most abundant metals in the biocrude from HTL at 350 °C and 60 min of both algal biomass samples. All other elements in Table B-1 were below 300 ppm.

The unusually high iron content in biocrude is the main reason causing problems in later processing. This content is orders of magnitude higher than iron in petroleum crude oil, which is found to be 5-50 ppm. 48%-75% of the iron in crude oil exists as organic salts, and 6%-40% exists as an iron complex.37

Table B-1: Metal concentration (ppm) in dried Nannochloropsis sp. algae biomass and biocrude from HTL at 350 °C, 60 min, using DCM as a recovery solvent

<table>
<thead>
<tr>
<th>Metal</th>
<th>Sapphire Algae</th>
<th>Reed Algae</th>
<th>HTL biocrude from Reed algae</th>
<th>HTL biocrude from Sapphire algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>21</td>
<td>323</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>B</td>
<td>77</td>
<td>12</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Ca</td>
<td>796</td>
<td>9450</td>
<td>240</td>
<td>48</td>
</tr>
<tr>
<td>Cr</td>
<td>2.3</td>
<td>1.7</td>
<td>3</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>6</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Fe</td>
<td>1096</td>
<td>1245</td>
<td>2276</td>
<td>1673</td>
</tr>
<tr>
<td>K</td>
<td>11112</td>
<td>11391</td>
<td>260</td>
<td>23</td>
</tr>
<tr>
<td>Mg</td>
<td>2327</td>
<td>3991</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>Mn</td>
<td>242</td>
<td>178</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Na</td>
<td>95588</td>
<td>38575</td>
<td>1596</td>
<td>726</td>
</tr>
<tr>
<td>Ni</td>
<td>1</td>
<td>1</td>
<td>43</td>
<td>65</td>
</tr>
<tr>
<td>P</td>
<td>7934</td>
<td>10988</td>
<td>--</td>
<td>21</td>
</tr>
<tr>
<td>S</td>
<td>5422</td>
<td>7731</td>
<td>--</td>
<td>214</td>
</tr>
<tr>
<td>Si</td>
<td>140</td>
<td>123</td>
<td>52</td>
<td>12</td>
</tr>
<tr>
<td>Zn</td>
<td>33</td>
<td>93</td>
<td>59</td>
<td>133</td>
</tr>
</tbody>
</table>

We then compared iron and sodium concentrations in aqueous phase and biocrude oil phase under the same reaction conditions. Those conditions were taken under fast HTL, a rapid heating, non-isothermal HTL process that produces similar biocrude yields. Algae biomass from Sapphire Energy was used. The heating profile obtained by connecting the dummy reactor with thermocouple is shown in Figure B-1. The heating up time was taken when the temperature of reactants reached 90% of the desired temperature (400 ºC). According to the data plotted on Figure B-1, the heating up times under 400 ºC, 500 ºC, and 600 ºC are 180s, 100s and 83s.
Figure B-1: Reactor temperature profiles at different sand bath set point temperatures for fast HTL

**B.2 Influence of heating rate on metal contents**

Table B-2 summarizes the effect of HTL temperature and holding time on Fe and Na concentrations in the biocrude. The holding times for these runs were chosen such that the reactor contents would just reach 400 °C and then be removed from the sandbath. Increasing the reaction severity drives more of the Fe-containing molecules out of the biocrude phase. Also, noticeably, the biocrude yield at 600 °C was decreased significantly compared to the other two temperatures.

Metal concentration data in aqueous phase after HTL are shown in Table B-3. Three sets of data were compared under different heating rates. Each set of data was done in triplicate experiments. As we could tell, most of the metals did not go into aqueous phase after HTL. The
most abundant metals in aqueous phase are Na and K, while the other metals are in trace amounts.

Analysis of the Na and Fe content in the biocrude and aqueous phase products from fast HTL (400 °C set point, 3 min holding time) showed that 64% of the Fe in the algal biomass resided in the bio-crude with very little (2%) residing in the aqueous phase. In contrast, 95% of the sodium was in the aqueous phase after HTL, whereas the biocrude contained only 0.4%.

These results gave us information on heating rate effect on HTL biocrude yields and metal distributions. The effect of HTL processing conditions on metal contents were examined in details in Chapter 2.

Table B-2: Fe and Na concentrations and yields for biocrude produced from HTL under different conditions (microalgae from Sapphire)

<table>
<thead>
<tr>
<th>Set point Temp °C</th>
<th>Batch Holding Time min</th>
<th>Biocrude Recovery Solvent</th>
<th>Fe conc. (ppm)</th>
<th>Na conc. (ppm)</th>
<th>Biocrude Yield (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>3</td>
<td>DCM</td>
<td>2222±400</td>
<td>607±285</td>
<td>41.7±4.4</td>
</tr>
<tr>
<td>500</td>
<td>1.67</td>
<td>DCM</td>
<td>2156±128</td>
<td>620±221</td>
<td>44.2±2.6</td>
</tr>
<tr>
<td>600</td>
<td>1.38</td>
<td>DCM</td>
<td>2038±485</td>
<td>736±530</td>
<td>34.1±2.0</td>
</tr>
</tbody>
</table>

Table B-3: Concentration of metals (unit: ppm) in aqueous phase with different heating rates during HTL

<table>
<thead>
<tr>
<th></th>
<th>400°C 3min</th>
<th>500°C 100s</th>
<th>600°C 83s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>0.10±0.03</td>
<td>0.10±0.04</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>B</td>
<td>1.72±0.61</td>
<td>2.80±0.46</td>
<td>3.74±1.65</td>
</tr>
<tr>
<td>Ca</td>
<td>3.64±1.51</td>
<td>5.07±3.91</td>
<td>3.02±0.25</td>
</tr>
<tr>
<td>Cu</td>
<td>0.02±0.008</td>
<td>0.03±0.003</td>
<td>0.03±0.007</td>
</tr>
<tr>
<td>Fe</td>
<td>4.17±0.49</td>
<td>4.09±0.34</td>
<td>3.89±0.28</td>
</tr>
<tr>
<td>K</td>
<td>1617±164</td>
<td>1495±81</td>
<td>1518±94</td>
</tr>
<tr>
<td>Mg</td>
<td>2.07±0.70</td>
<td>3.0±1.4</td>
<td>1.2±0.08</td>
</tr>
</tbody>
</table>
Biocrudes from HTL of two different sources of *Nannochloropsis sp.* microalgae were compared in terms of metal concentrations. Fe is the most concentrated metal in biocrudes from both sources and significantly higher than Fe in petroleum crude oil. Over 60% of Fe at HTL condition of 350 °C and 60 min ended up in biocrude phase.

Three non-isothermal HTL conditions were examined in this Appendix in order to examine the effect of heating rate on metal distribution. For the conditions examined, increasing the reaction severity drives more of the Fe-containing molecules out of the biocrude phase.
B.3 Additional plot for temperature profile

Figure B-2: Temperature profiles in proxy reactors with highest water vol% loading for different sand bath set point temperatures

B.4 Additional plot for metal distributions
Figure B-3: Recoveries (%) of different biomass elements in the aqueous phase (blue) and biocrude phase (black) after HTL at 350 °C (15 wt% algae loading, highest water vol %, Runs 1-7 from Table 2-2)
Figure B-4: Recoveries (%) of different biomass elements in the aqueous phase (blue) and biocrude phase (black) after HTL at 450 °C (15 wt% algae loading, Runs 25-29 from Table 2-2)
Figure B-5: Recoveries (%) of different biomass elements in the aqueous phase (blue) and biocrude phase (black) after HTL with different algae loadings at 350 and 400 °C for 30 min (Runs 6, 8, 9, 18, 20, 21 from Table 2-2)
Figure B-6: Recoveries (%) of different biomass elements in the aqueous phase (blue) and biocrude phase (black) after HTL with different water loadings ($\rho_w = 0.15, 0.30, 0.50 \text{ g cm}^{-3}$) at 350 and 400 °C for 30 min (Runs 6,10,11,18,22,23 from Table 2-2)
B.5 Additional plots of metal concentration in biocrude vs Ro

Figure B-7: Variation of the Zinc and Copper concentration (ppm) in biocrude with the reaction ordinate (HTL with 15 wt% algae loading and highest water vol% loading, Runs 1-7, 12-19, and 25-29 from Table 2-2)

Figure B-8: Variation of the Sulfur concentration (ppm) in biocrude with the reaction ordinate (HTL with 15 wt% algae loading and highest water vol% loading, Runs 1-7, 12-19, and 25-29 from Table 2-2)
Figure B-9: Variation of the Nickel concentration (ppm) in biocrude with the reaction ordinate (HTL with 15 wt% algae loading and highest water vol% loading, Runs 1-7, 12-19, and 25-29 from Table 2-2)

Figure B-10: Variation of the Sodium and Potassium concentration (ppm) in biocrude with the reaction ordinate (HTL with 15 wt% algae loading and highest water vol% loading, Runs 1-7, 12-19, and 25-29 from Table 2-2)
Figure C-1: Biocrude yield from algae HTL at 400 °C and 5 min with no contact between solvent and aqueous phase co-product

Figure C-2: Biocrude yield from Sapphire algae HTL at 400 °C and 5 min
Figure C-3: Biocrude yield from algae HTL at 350 °C and 60 min
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PUBLICATIONS

- **Jiang, J.; Savage, P. E.** "Using Solvents to Reduce Metal Content in Crude Bio-oil from Hydrothermal Liquefaction of Microalgae." *Ready for submission to I&CE.*
- **Jiang, J.; Savage, P. E.** “Interventions on metal content in hemin oil and study on hydrodemetallation mechanisms”. *Manuscript in preparation.*

SELECTED PRESENTATIONS

- Presenting author at BASF North American Research Forum, Iselin, NJ 08/2018
- Presenting author at AIChe Annual Meeting, Pittsburgh, PA 10/2018
- Presenting author at AIChe Annual Meeting, Minneapolis, MN 10/2017
- Presenting author at AIChe Annual Meeting, San Francisco, CA 11/2016

AWARDS

- **AIChe CRE Division Graduate Student Travel Grant Award** 2018
- **College of Engineering Travel Grants, Penn State** 2017 & 2016
- **George A. and Ruth S. Robb Graduate Fellowship** 2015
- **China Scholarship Council Full Scholarships for Exchange Study** 2014 & 2013
- **1st Class Honor (top 5%), Dalian University of Technology** 2013 & 2012
- **Merit Student of Dalian University of Technology** 2012