THERMOCHEMICAL CONVERSION OF MICROALGAE FOR BIOFUEL PRODUCTION

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ABSTRACT

Concerns about diminishing fossil fuels and increasing greenhouse gas emissions are driving many countries to develop renewable energy sources. In this respect, biomass may provide a carbon-neutral and sustainable solution. Microalgae have received growing interest recently because of their high productivity, high oil content and the ability to grow in a wide range of climates and lands. Pyrolysis is a thermochemical process in which biomass is thermally decomposed to a liquid product known as bio-oil. In this dissertation, pyrolysis and hydrothermal conversion techniques were applied to microalgae for biofuel production and an integrated algae-based biorefinery was proposed which includes algal biomass production, hydrothermal pretreatment, catalytic pyrolysis of microalgae into biofuels, and recycling of the wastewater from conversion as low-cost nutrient source for algae cultivation.

In Chapter 3, Microwave-assisted pyrolysis (MAP) of *Chlorella* sp. was carried out with char as microwave reception enhancer. The results indicated that the maximum bio-oil yield of 28.6% was achieved under the microwave power of 750 W. The bio-oil properties were characterized with elemental, gas chromatography-mass spectrometry (GC-MS), gel permeation chromatography (GPC), Fourier transform infrared (FTIR) spectroscopy, and thermogravimetric (TG) analysis. The algal bio-oil had a density of 0.98 kg/L, a viscosity of 61.2 cSt, and a higher heating value (HHV) of 30.7 MJ/kg. The GC-MS results showed that the bio-oils were mainly composed of aliphatic hydrocarbons, aromatic hydrocarbons, phenols, long chain fatty acids and nitrogenated compounds, among which aliphatic and aromatic hydrocarbons (account for 22.18 % of the total GC-MS spectrum area) are highly desirable compounds as those in crude oil,
gasoline and diesel. The results indicate that fast growing algae are a promising source of feedstock for advanced renewable fuels production via MAP.

To further elucidate the pyrolysis mechanism of microalgae, the different roles of three major components (carbohydrates, proteins, and lipids) in microalgae were investigated on a pyroprobe. In Chapter 4, cellulose, egg whites, and canola oil were employed as the model compounds of the three components, respectively. Non-catalytic pyrolysis was used to identify and quantify some major products and several pyrolysis pathways of algal biomass were also postulated by analysis and identification of pyrolysis products from the model compounds. Algal bio-oil contains oxygenates and nitrogenates which can lower the heating values and lead to NOx emissions, and thus upgrading processes towards reducing nitrogen and oxygen are necessary. Catalytic pyrolysis was then carried out with HZSM-5 for the production of aromatic hydrocarbons at different temperatures and catalyst to feed ratios. The aromatic yields of all feedstocks were significantly improved when the catalyst to biomass ratio increased from 1:1 to 5:1. Egg whites had the lowest aromatic yield among the model compounds under all reaction conditions, which suggests that proteins can hardly be converted to aromatics with HZSM-5. Lipids, although only accounted for 12.33% of Chlorella, contributed about 40% of aromatic production from algal biomass.

Based on the preliminary catalytic pyrolysis results, a detailed catalyst screening study was carried out to evaluate the performance of different zeolites for the production of aromatic hydrocarbons in Chapter 5. Three zeolites with different crystal structures (H-Y, H-Beta and H-ZSM5) were used to study the effect of catalyst type on the aromatic yield. All three catalysts significantly increased the aromatic yields from pyrolysis of
microalgae and egg whites compared with non-catalytic runs, and H-ZSM5 was most
effective with a yield of 18.13%. Three H-ZSM5 with silica-to-alumina (Si/Al) ratios of
30, 80 and 280 were used to study the effect of Si/Al ratio on the aromatic yield. The
maximum yield was achieved at the Si/Al ratio of 80, which provides moderate acidity to
achieve high aromatic production and reduce coke formation simultaneously. Aromatic
production increased with the incorporation of copper or gallium to HZSM-5. However,
other metals studied either had no significant influence or led to a lower aromatic yield.

Based on the results in Chapter 4 and Chapter 5, nitrogenates are very resistant to
catalytic conversion and the aromatic hydrocarbon yield from proteins was the lowest
among the three major components of microalgae. However, since nitrogen is an essential
element for algal growth, recycling of this nutrient will be important to achieving
sustainable algal feedstock production. Therefore, hydrothermal pretreatment (HP) was
employed to reduce the nitrogen content in *Nannochloropsis oculata* feedstock by
hydrolyzing proteins without requiring significant energy inputs. The effects of reaction
conditions on the yield and composition of pretreated algae were investigated by varying
the temperature (150–225 °C) and reaction time (10–60 min). Compared with untreated
algae, pretreated samples had higher carbon contents and enhanced heating values under
all reaction conditions and 6–42% lower nitrogen contents at 200 °C–225 °C for 30–60
min. The pyrolytic bio-oil from pretreated algae contained less nitrogen-containing
compounds than that from untreated samples. The bio-oil contained mainly (44.9% GC-
MS peak area) long-chain fatty acids (C14–C18) which can be more readily converted
into hydrocarbon fuels in the presence of simple catalysts. Additionally, the feasibility of
using recovered nutrients from HP for cultivation of microalga *Chlorella vulgaris* was
investigated. Different dilution multiples of 50, 100 and 200 were applied to the recycled process water from HP and algal growth was compared among these media and a standard growth medium BG-11. Algae achieved a biomass concentration of 0.79 g/L on 50× process water after 4 days. Algae removed total nitrogen, total phosphorus and chemical oxygen demand by 45.5-59.9%, 85.8-94.6% and 50.0-60.9%, respectively, on different diluted process waters. The fatty acid methyl ester yields for algae grown on the process water were 11.2% (50×), 11.2% (100×) and 9.7% (200×), which were significantly higher than 4.5% for BG-11 grown algae. In addition, algae cultivated on process water had 18.9% higher carbon and 7.8% lower nitrogen contents than those on BG-11, indicating that they are very suitable as biofuel feedstocks. In summary, HP is a low cost and efficient way to reduce the nitrogen content in microalgae without significant energy inputs. The recovered aqueous nutrients from HP can be recycled for algal cultivation. Pretreated microalgae were very hydrophobic with reduced nitrogen content and retained 73 to 99% lipids of the starting microalgae. These lipids can be easily converted into hydrocarbon fuels in the presence of simple catalysts, such as H-ZSM5 zeolite.
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CHAPTER 1 INTRODUCTION

1.1 Background and significance of the research

Concerns about diminishing fossil fuels and increasing greenhouse gas emissions are driving many countries to develop renewable energy sources. In this respect, biomass may provide a carbon-neutral and sustainable solution, because the carbon dioxide generated during fuel combustion can be consumed by biomass growth. In addition, biomass can improve national energy security by reducing the reliance on foreign sources. The Vision for Bioenergy and Biobased Products in the United States developed by the Biomass Technical Advisory Committee established long-term goals that 20 percent of transportation fuels and 25 percent of chemicals and materials would be produced from biomass by 2030 (U.S. Department of Energy and Department of Agriculture, 2002).

Bioethanol and biodiesel, as the first generation biofuels, have been commercialized in many parts of the world. However, they have been blamed for their competition with food supply for the use of arable land. According to World Energy Outlook of International Energy Agency (IEA, 2006), about 14 million hectares (1% of the world’s arable land) of land are used for the production of biofuels, providing 1% of global transport fuels. Clearly, humans cannot produce enough transport fuels from first generation biofuels because of the limited capacity of arable lands on the earth. In light of this, vigorous research initiatives are going on for the pursuit of biofuel alternatives.
1.2 Algal biofuel production

Algae used in the field of biofuels are typically referred to as microalgae, which are usually unicellular plant-like microorganisms capable of performing photosynthesis. Microalgae as a biofuel source have gained much attention these days for a number of reasons. First, they have 5–30 times higher biomass production of oil crops per unit surface area. Second, they do not compete with traditional agricultural resources as they can be cultivated on non-arable land or on wastewater (Schenk et al., 2008). Third, they are rich in oil being 20-50% dry weight of biomass in many species (Chisti, 2007).

Bioethanol is produced by microbial fermentation of sugars and biodiesel by transesterification reaction between oils and alcohols. Most of the research on algal biofuels has been focused on biodiesel production because of the high oil content in algae. In fact, a number of thermochemical technologies have been developed and applied to other biomass for biofuels production, such as pyrolysis, gasification and carbonization (Huber et al., 2006). These technologies are highly efficient which usually take several seconds or minutes and can produce a range of solid, liquid and gaseous fuels. However, not enough attention has been paid to these technologies in the field of algal biofuels. Knowledge of the feasibility and potential of thermochemical conversion of microalgae is still lacking.

1.3 Objectives or hypothesis to be tested

The overall goal of this research is to develop and evaluate thermochemical processes for bioenergy production using microalgae as the feedstock. The specific objectives are as follows:
(1) Investigate microwave-assisted pyrolysis (MAP) of microalgae for biofuels production and characterize the physical and chemical properties of the produced bio-oil.

(2) Study the pyrolysis mechanism of microalgae by investigating the pyrolysis behavior and products of the model compounds, including cellulose, egg whites, and canola oil.

(3) Study the influence of the addition of catalysts on the profile of pyrolysis products from microalgae.

(4) Investigate the influence of low-temperature (150–225 °C) hydrothermal pretreatment on the profile of pyrolysis products from microalgae.
CHAPTER 2 LITERATURE REVIEW

This chapter will review previous research in renewable energy production from microalgae. There are several conversion technologies being developed as shown in Fig. 2.1. The choice of conversion methods depends on the desired form of the end-product and techno-economic considerations.

![Current strategies for production of biofuels from algal biomass](image)

**2.1 Algal lipids extraction**

Biodiesel is a mixture of fatty acid alkyl esters obtained by transesterification of lipid feedstock, such as oil crops and animal fats. Transesterification is a chemical reaction between triglycerides and alcohol catalyzed by acids or alkalis. Biodiesel can be used alone or blended with petrodiesel in standard diesel engines (Sharma et al., 2009). For algal biodiesel production, the algae biomass usually needs to be dewatered, dried and extracted with organic solvent to obtain the oil in algae cells. Various techniques, including sonication, bead milling, microwave treatment, have been investigated for more efficient lipid extraction from microalgae. However, these extraction methods are still on
a laboratory scale and none of them is proved to be practical and economical for commercial production (Chen et al., 2010).

2.2 Biological conversion

Bioethanol production is a very mature technology using sugars as the substrates. Some microalgae species contain high amount of starch (37% dry weight), which can be converted to ethanol by fermentation (Hirano et al., 1997). Anaerobic digestion (AD) is another biological conversion technique involving the breakdown of the organics in biomass into methane gas. Algae contain no lignin and are high in moisture content, making them a good material for AD (Vergara-Fernandez et al., 2008). Both technologies are under investigation and there are more reports emerging in this field.

2.3 Thermochemical conversion

Thermochemical conversion is the thermal decomposition of organic matter in biomass to produce fuels. A main advantage is that it is usually carried out in much shorter time than biological conversion. Moreover, thermochemical conversion has the potential to be integrated into the existing petroleum processing infrastructure.

2.3.1 Gasification

Gasification typically involves the partial oxidation of biomass into fuel gases at high temperatures (> 800 °C). It is usually carried out with air or steam to generate a mixture of CO, H₂, CO₂, and some light hydrocarbons (Demirbas, 2001). Although extensive research has been done on terrestrial biomass, scientific reports on gasification of microalgae are very limited and most of them used hydrothermal gasification.
Hydrothermal technologies broadly cover chemical and physical transformations in high-temperature (200–600 °C) and high-pressure (5–40 MPa) water (Peterson et al., 2008). They have energetic advantages for wet biomass such as algae, because water is heated in pressurized liquid form so that energy input for water removal by evaporation is eliminated. Gasification processes generally take place at higher temperatures (> 370 °C, supercritical water) with or without catalysts. Chakinala et al. (2010) investigated hydrothermal gasification of *Chlorella vulgaris* in the range of 400-700 °C. Gasification efficiency increased with higher temperature, lower algae concentrations and longer residence times. Complete gasification occurred at 700 °C with excess amounts of Ru/TiO$_2$ catalysts. Stucki et al. (2009) found that 60–70% of the heating value in microalgae (*Spirulina platensis*) was recovered as methane at 399–409 °C with catalysts Ru/C or Ru/ZrO$_2$.

**2.3.2 Hydrothermal liquefaction (HTL)**

Compared with gasification, there are more publications and reports on HTL of algae which could directly produce liquid fuels (bio-oil). It is usually carried out in the temperature range of 250–350 °C, which is referred to as the subcritical water condition. The yield and quality of bio-oil was found to be a function of temperature, retention time, biomass loading and presence of catalysts. The yield of bio-oil was reported in the range of 10%–50% with a heating value of 35–40 MJ/kg, slightly lower than that of petroleum crude oil of 43 MJ/kg (Biller et al., 2011; Duan et al., 2011; Zou et al., 2009). However, since a large amount of water exists in HTL, bio-oil is always emulsified within the water and large amount of organic solvent is needed for extraction.
2.3.3 Hydrothermal carbonization (HTC)

HTC, also known as wet torrefaction, is an exothermic process of combined dehydration and decarboxylation of a fuel to improve its carbon content and calorific value (Funke et al., 2010). In this process, biomass is usually heated up to around 200 °C in a suspension with water under saturated pressure for a certain period of time. Compared with hydrothermal gasification and liquefaction, the conditions for HTC are milder. After HTC at 260 °C for 5 min, the heating value of loblolly pine increased to 1.36 times of the untreated feedstock, and the carbon content increased significantly from 50.25% to 72.07%. In addition, the solid products were hydrophobic and suitable for storage and transportation (Yan et al., 2009). Compared with dry biomass, HTC is more suitable for the conversion of wet biomass such as algae, as water required is already contained in the biomass. Heilmann et al. (2010) studied HTC of several strains of green and blue-green algae. All species showed different levels of carbonization under different experimental conditions. The char products were of bituminous level quality in terms of carbon and energy content. The authors also compared the energy input/output for combustion of *Chlamydomonas reinhardtii* and its char product. An energy gain of 12.01 MJ/kg was obtained from the combustion of algal char compared with an energy loss of 5.27 MJ/kg for starting algae because HTC could greatly reduce the energy required for algae drying. Recently, another report by Heilmann et al. (2011) found that fatty acids products in HTC were adsorbed onto the char in high yield. Then, fatty acids in the char could be extracted and converted into liquid transportation fuels.
2.3.4 Pyrolysis

Pyrolysis is the conversion of biomass into bio-oil, biochar and syngas at elevated temperature (300–700 °C) in the absence of air (Bridgwater et al., 2000). The yield and properties of each fraction are dependent on the pyrolysis temperature, heating rate, residence time and catalysts. According to the vapor residence time, pyrolysis is always classified into slow pyrolysis and fast pyrolysis. Slow pyrolysis with a vapor residence time from several minutes to hours has been carried out for charcoal production for centuries. More recent exploitation has been focused on the optimization of liquid bio-oil, as it is easily storable and transportable with the potential of being upgraded to high quality drop-in fuels. The bio-oil can replace heavy and light fuel oils in industrial boiler for heat production (Mohan et al., 2006). However, the high level of oxygen (30–40%) makes bio-oil unstable and unable to be used as transportation fuels directly.

While most of traditional slow and fast pyrolysis processes use fixed and fluidized bed reactors whose heating is provided by heated surface, sands, etc. (Czernik et al., 2004; Meier et al., 1999; Mohan et al., 2006), others looked into alternative heating methods such as microwave heating (Wan et al., 2009; Yu et al., 2008). Microwave technology uses electromagnetic waves that pass through material and cause its molecules to oscillate and generate heat. The technical advantages of MAP over conventional pyrolysis include: First, microwave heating is uniform and can be applied to large particles of biomass; second, the syngas produced has higher heating value since it is not diluted by carrier gas used in some types of pyrolysis, such as fluidized bed pyrolysis. Thus, the syngas can be combusted to provide in-situ electricity for microwave
generation; third, the products are cleaner because of no agitation and fluidization in the process; fourth, microwave heating is a mature technology which is easy to scale up.

There are numerous papers on the pyrolysis of lignocellulosic biomass. However, very few reports are available for algal bio-oil production via pyrolysis. Miao et al. (2004b) performed fast pyrolysis of *Chlorella protothecoides* and *Microcystis areuginosa* at 500 °C, and bio-oil yields of 18% and 24% were obtained, respectively. The bio-oil exhibited a higher carbon and nitrogen content, lower oxygen content than wood bio-oil. When *Chlorella protothecoides* was cultivated heterotrophically, bio-oil yield increased to 57.9% with a heating value of 41 MJ/kg (Miao et al., 2004a).

Recently when microalgae become a hot research topic as the third generation of biofuel, pyrolysis has drawn more attention as a potential conversion method for algal biofuel production. Pan et al. (2010) investigated slow pyrolysis of *Nannochloropsis* sp. residue with and without the presence of HZSM-5 catalyst and obtained bio-oil rich in aromatic hydrocarbons from catalytic pyrolysis. Algal pyrolytic liquids separate into two phases with the top phase called bio-oil (Campanella et al., 2012; Jena et al., 2011a). The higher heating values (HHV) of algal bio-oil are in the range of 31–36 MJ/kg, generally higher than those of lignocellulosic feedstocks. Pyrolytic bio-oil consists of compounds with lower mean molecular weights and contains more low boiling compounds than bio-oil produced by hydrothermal liquefaction. These properties are similar to those of Illinois shale oil (Jena et al., 2011a; Vardon et al., 2012), which may indicate that pyrolytic bio-oil is suited for petroleum fuel replacement. In addition, the high protein content in microalgae led to a high N content in the bio-oil, resulting in undesirable NOx emissions during combustion and deactivation of acidic catalysts when co-processed in existing
crude oil refineries. Algal bio-oil had better qualities in many aspects than those produced from lignocellulosic biomass. For example, algal bio-oil has a higher heating value, a lower oxygen content and a greater than 7 pH value. However, upgrading towards the removal of nitrogen and oxygen in the bio-oil is still necessary before it can be used as drop-in fuels.

2.3.5 Bio-oil upgrading

The most common upgrading methods of bio-oil are hydrotreatment or catalytic cracking (Zhang et al., 2007). Hydrotreatment is performed in the presence of pressurized hydrogen with catalysts at moderate temperatures (300-600 °C). Oxygen is removed as H₂O and CO₂. Hydrotreatment of bio-oil has been carried out utilizing different noble metal and transition metal catalysts (Huber et al., 2006; Wildschut et al., 2009). However, coking of catalysts and the source of hydrogen for conversion still need to be addressed to make the technology economically viable. In catalytic cracking, oxygenated compounds in bio-oil are deoxygenated to hydrocarbons with oxygen removed as H₂O, CO and CO₂. Shape-selective catalysts, such as zeolites, are necessary for the cracking process. Different from hydrotreatment, catalytic cracking is carried out in atmospheric pressure and does not require the addition of external hydrogen (Azeez et al., 2011; Carlson et al., 2009; Foster et al., 2012). A recent publication suggested upgrading bio-oil by hydroprocessing of bio-oils over metal catalysts followed by zeolite-cracking for the production of commodity chemicals (Vispute et al., 2010). Recently, researchers became interested in catalytic pyrolysis, also known as in-situ upgrading, in which volatiles from thermal decomposition of organics react directly or immediately with catalysts.
Typically, catalysts are pre-mixed with biomass, or a catalyst bed is set up between the pyrolysis reactor and condensers. This approach could eventually eliminate the costly condensation and re-evaporation procedures used in traditional upgrading of pyrolytic oil (Stefanidis et al., 2011; Triantafyllidis et al., 2007; Zhang et al., 2010).
CHAPTER 3 MICROWAVE-ASSISTED PYROLYSIS OF MICROALGAE

A paper published in Bioresource Technology

3.1 Introduction

Fossil fuels are produced from unsustainable resources and their uses contribute significantly to greenhouse gas (GHG) emission to the environment. Biomass is an abundant and potentially carbon-neutral energy source widely available on the earth (McKendry, 2002; Mohan et al., 2006). Biomass feedstock can be converted into solid, liquid, and gaseous products through various thermochemical processes including pyrolysis (Huber et al., 2006). In pyrolysis, organics in biomass are thermally converted to bio-oil, combustible gases, and biochar (Bridgewater et al., 2000; Yaman, 2004). While most of traditional slow and fast pyrolysis processes use fixed and fluidized bed reactors whose heating is provided by heated surface, sands, etc. (Czernik et al., 2004; Meier et al., 1999; Mohan et al., 2006), others looked into alternative heating methods such as microwave heating. The new microwave-assisted pyrolysis (MAP) process that we developed offers several advantages over traditional processes, including uniform internal heating of large biomass particles, ease of control, and no need for agitation or fluidization and hence less particles (ashes) in the bio-oil. Studies of MAP of wood (Miura et al., 2004), corn stover (Wan et al., 2009; Yu et al., 2007), rice straw (Huang et al., 2010), coffee hulls (Dominguez et al., 2007), pine sawdust (Wang et al., 2009), and wheat straw (Budarin et al., 2009) have been recently reported, and suggest that MAP is highly scalable technology suitable for distributed conversion of bulky biomass.
Microalgae as an alternative biofuel source have gained much attention these days because they have numerous advantages compared with lignocellulosic feedstocks: (1) they have higher biomass production, 5-30 times of oil crops per unit surface area (Schenk et al., 2008); (2) they do not compete with traditional agricultural resources as they can be cultivated on non-arable land or on wastewater; (3) they are exceedingly rich in oil, over 60% by weight of dry biomass in some species (Gouveia et al., 2009). Up to today, various thermochemical conversion routes of microalgae have been investigated. There are few reports on pyrolysis of microalgae. Miao and Wu (2004a) performed fast pyrolysis of autotrophic and heterotrophic *Chlorella protothecoides* and reported bio-oil yields of 16.6% and 57.2%, respectively. In addition, the bio-oils obtained had better quality than those from wood in terms of bio-oil viscosity, density and heating value. Pan et al. (2010) investigated pyrolysis of *Nannochloropsis* sp. residue with and without the presence of HZSM-5 catalyst and obtained bio-oil rich in aromatic hydrocarbons from catalytic pyrolysis.

In this study, MAP of *Chlorella* sp. was conducted under different microwave power levels. Detailed compositional characterization and comparisons were carried out with elemental, gas chromatography-mass spectrometry (GC-MS), gel permeation chromatography (GPC), Fourier transform infrared (FTIR) spectroscopy, and thermogravimetric (TG) analysis. The components of the gaseous byproducts were also analyzed with gas chromatography (GC).
3.2 Materials and methods

3.2.1 Materials

*Chlorella* sp., a wild-type algae strain, was screened from local freshwater in Minnesota, USA, and then cultivated in a pilot-scale 1300 L photobioreactor filled with Tris-Acetate-Phosphorus (TAP) media (Harris, 1989). The photobioreactor was set up in the greenhouse located on the Saint Paul campus at the University of Minnesota, Twin Cities, where the average sunshine duration in May of Saint Paul was 14-15 hours per day and the temperature fluctuated between 22 °C to 30 °C, and stayed around 25 °C most of the time. When the biomass reached around 1g/L, a semi-continuous harvesting regimen, in which 450 L of the culture volume was harvested followed by supplementing with the same volume of tap water enriched with TAP media, was carried out. Algae paste with a water content of 85-90% was obtained after flocculation and filtration, and then subjected to natural drying to constant weight. The main characteristics of dry *Chlorella* sp. are listed in Table 3.1.

<table>
<thead>
<tr>
<th>Proximate analysisa (wt.%)</th>
<th>Elemental analysisb (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>C</td>
</tr>
<tr>
<td>Volatile</td>
<td>H</td>
</tr>
<tr>
<td>Fixed Carbon</td>
<td>N</td>
</tr>
<tr>
<td>Ash</td>
<td>Oc</td>
</tr>
</tbody>
</table>

*a Wet basis; b Dry basis; c Calculated by difference, O(%) = 100 – C – H – N – Ash.*
3.2.2 Pyrolysis

The pyrolysis of biomass was carried out in a Panasonic NN-SD787S microwave oven with the maximum incident power of 1250 W at a frequency of 2450 MHz. The schematic diagram of experimental apparatus is shown in Fig. 3.1. As biomass is poor microwave absorbent, mixing it with microwave absorption enhancers should improve biomass heating (Dominguez et al., 2007; Menendez et al., 2002). Since a higher absorber/biomass ratio leads to higher energy consumption per unit biomass, it is important to determine the minimum amount of absorber to create the required pyrolysis conditions. The char produced from pyrolysis of biomass feedstock is an excellent microwave absorber. Partial recycling of the char in a continuous MAP process is expected to recover some heat and improve microwave absorption and hence energy efficiency. In our study, the lowest effective ratio of absorber (char) to biomass was 1:5 determined through preliminary experiments. For consistent comparison, each sample was prepared by blending 30 g algae biomass with 6 g solid char in this study. The char for the first experiment at a certain power level was obtained by mixing Chlorella sp. with activated carbon (approximately 1.5mm diameter × 3mm), which was easily separated from the solid residue after pyrolysis. The char for the subsequent experiments was gleaned from previous experiments at the same power level. After the sample preparation, the mixture was placed in a 500 mL quartz flask, which was then subjected to microwave treatment with nitrogen used as inert carrier gas at a flow rate of 500 mL/min to maintain anoxic atmosphere before and during the experiment. Throughout the pyrolysis process, the evolution of reaction temperature was monitored with an infrared optical pyrometer, and the final temperature was measured by inserting a
thermocouple into the sample immediately at the end of reaction. Meanwhile, the condensable volatiles were continuously collected using five condensers with cooling water temperature around 0-2 °C, and the non-condensable gases in a gas bag. The reaction time was set for 20 min when no appreciable volatiles were observed later on. The solid and liquid fraction yields were calculated from the weight of each fraction, while the gas yield was calculated by difference based on the mass balance. All experiments were performed in triplicate to determine the uncertainties in the experimental results.

![Fig. 3.1 The schematic diagram of MAP system: (1) microwave cavity; (2) quartz reactor; (3) holder; (4) bio-oil collector; (5) condensers; (6) gas sampling](image)

3.2.3 Bio-oil and gaseous products analysis

The bio-oil properties were characterized with elemental, GC-MS, GPC, FTIR, and TG analysis, depicted as follows: The viscosity of the bio-oil was measured by a RVA Super 4 Visco Analyzer (Newport Scientific Pty Ltd., Australia). The elemental analysis was performed with an elemental analyzer (CE-440, Exerter Analytical Inc., USA).
The components of the liquid product were specified using an Agilent 7890-5975C gas chromatography/mass spectrometer with a HP-5 MS capillary column. Helium was employed as the carrier gas at a flow rate of 1.2 mL/min. The injection size was 1 μL with a split ratio of 1:10. The initial oven temperature was 40 °C held for 3 min and then increased to 290 °C at a rate of 5 °C/min, and held at 290 °C for 5 min, while the injector and detector were maintained at constant temperature of 250 °C and 230 °C, respectively. The compounds were identified by comparing their mass spectra with those from the National Institute of Standards and Technology (NIST) mass spectral data library.

GPC analysis of bio-oil was performed using a Varian Polaris HPLC system equipped with one Oligo-Pore GPC column (polystyrene-divinyl-benzene copolymer, 300 × 7.5 mm) at 35 °C. In this system, tetrahydrofuran (THF) was used as the eluent at a flow rate of 1 mL/min, and a differential refractometer was used as the detector. Bio-oil samples were dissolved in THF at a concentration of 10 mg/mL and molecular weight calibration was performed by nine polystyrene standards in the molecular weight range of 162-2900.

The FTIR spectra were collected in a Nicolet Series II Magna-IR System 750 spectrometer, equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector. The oil was deposited between two NaCl disks. The spectral range was selected at 400-650 cm⁻¹, with a resolution of 4 cm⁻¹.

TG and Derivative thermogravimetric (DTG) analysis were performed with a Perkin Elmer TG/DTA 6300 in both nitrogen and air atmospheres. Samples were heated from 30 °C to 700 °C with a heating rate of 30 °C/min. The gas flow rate was 20 mL/min.
The gaseous products were analyzed by a Varian Micro-GC CP4900/thermal conductivity detector (TCD) with a 5A molecular sieve column and a PPQ column. The temperatures of injector and detector were maintained at 110 °C. The oven temperatures of 5A molecular sieve and PPQ column were kept at 80 °C and 150 °C, respectively.

3.3 Results and discussion

3.3.1 Temperature profiles

The temperature profiles of pyrolysis determined by the infrared pyrometer at different microwave power levels are presented in Fig. 3.2. Accurate measurement of the evolution of the temperature during the process was very difficult (Dominguez et al., 2003), and hence the temperature profiles of the samples shown in Fig. 3.2 only serve the purpose of comparisons among different batches. The final temperatures measured with a thermocouple are shown in Table 3.2. As shown in Fig. 3.2 and Table 3.2, the final temperatures in the inner region of the sample were about 100-150 °C higher than those of the outer region monitored by the pyrometer. The increases in temperatures were observed in the first 8-9 min, after which the temperatures became relatively stable, probably due to the balance between heat loss and heat generated by microwave. It is obvious that the higher the microwave power, the higher the heating rate and the higher final temperature. To achieve the same final temperature, the microwave power required in our system was higher than that reported by Huang et al. (2010) and Domínguez et al. (2006). This may be caused by the poorer heat insulation and larger amount of samples used in our system.
Fig. 3.2 The temperature profiles under different microwave power levels

Table 3.2 The final temperatures under different microwave powers measured by a thermocouple

<table>
<thead>
<tr>
<th>Microwave power (W)</th>
<th>500</th>
<th>750</th>
<th>1000</th>
<th>1250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final temperature (°C)</td>
<td>462 ± 29</td>
<td>569 ± 42</td>
<td>600 ± 27</td>
<td>627 ± 17</td>
</tr>
</tbody>
</table>

3.3.2 Product fractional yields

The percentage yields of the pyrolysis products under different microwave power levels are presented in Fig. 3.3. The liquid product separated into oil phase (bio-oil) and water phase automatically. When the microwave power increased from 500 W to 1250 W, the yield of oil phase product increased to a maximum value of 28.6% at 750 W, and then decreased gradually. The yield of gas increased over the range of microwave power studied, while that of the char decreased from 500 W to 750 W, and then remained almost constant. The underlying reason is that the sample was not pyrolyzed adequately below 750 W, and it might just reach complete decomposition at 750 W and the maximum oil yield was obtained. Beyond 750 W the decrease in oil yield and increase in gas yield may
be caused by the secondary cracking of oil vapors into incondensable gases. In addition, constant char yield in the power range of 750 W-1250 W is possibly arising from the fact that the decomposition of the sample was complete or there was a balance between decomposition of solids and formation of char-like carbonaceous material through repolymerization. The water phase yield remained virtually constant at about 21% in the studied power range. As shown in Fig. 3.3, there was a tradeoff between heating rate and pyrolysis temperature. Lower power with lower heating rate always led to the formation of higher yield of char (Williams et al., 1996), while higher power with higher pyrolysis temperature favored gasification reactions which thus decreased the yield of bio-oil. These results are similar to those reported in the literature (Islam et al., 2010; Pan et al., 2010; Sensoz et al., 2006). The variations are mainly due to the compositional differences of feedstock and the specific characteristics of the microwave pyrolysis system. In this study, 750W was the optimum power to obtain bio-oil product from MAP of *Chlorella* sp.

![Fig. 3.3 Product fractional yields under different microwave power levels](image)

Fig. 3.3 Product fractional yields under different microwave power levels
3.3.3 Physical properties and elemental analysis of bio-oil

The characteristics of the algal bio-oil in comparison with wood bio-oils and diesel fuel are showed in Table 3.3. The bio-oil from *Chlorella* sp. had a lower oxygen content, higher carbon, hydrogen content and HHV than bio-oil produced from lignocellulosic materials. These values are close to the results of Miao and Wu (2004a). Algal bio-oil has a lower density than lignocellulosic bio-oil, and a viscosity in the typical range of wood bio-oil. The presence of nitrogen bases, including indole, pyridine, amides, ammonia, etc., rendered the algal bio-oil pH alkaline (9.7), which is very different from that for lignocellulosic bio-oil (typically 2-3). However, the elemental composition and HHV of the bio-oil from *Chlorella* sp. are still not comparable to (quite different from) those of fossil oil.
Table 3.3 Comparison among No. 2 diesel fuel, bio-oil from MAP of *Chlorella* sp. and other lignocellulosic feedstocks

<table>
<thead>
<tr>
<th>Properties</th>
<th>Bio-oils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Chlorella</em> sp.</td>
</tr>
<tr>
<td>Elemental composition (wt.%)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>65.4</td>
</tr>
<tr>
<td>H</td>
<td>7.84</td>
</tr>
<tr>
<td>N</td>
<td>10.28</td>
</tr>
<tr>
<td>O</td>
<td>16.48&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>HHV (MJ/kg)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>30.7</td>
</tr>
<tr>
<td>Density (kg/L)</td>
<td>0.98&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>9.7</td>
</tr>
<tr>
<td>Viscosity, at 40 °C (cSt)</td>
<td>61.2</td>
</tr>
</tbody>
</table>

* Derived from the reference (Sensoz et al., 2002);<sup>a</sup> Derived from the reference (Budarin et al., 2009);<sup>b</sup> Derived from the reference (Bridgwater et al., 2000);<sup>c</sup> Derived from the reference (Tat et al., 1999);<sup>d</sup> Calculated by difference;<sup>e</sup> Calculated using the equation (Friedl et al., 2005) HHV (MJ/kg) = (3.55×C²−232×C−2230×H+51.2×C×H+131×N+20600)×10⁻³;<sup>f</sup> at 30 °C.

### 3.3.4 GC-MS characterization of bio-oil

The GC-MS profile of algal bio-oil is shown in Fig. 3.4. The identified compounds were categorized into the following groups: aliphatic hydrocarbons, aromatic hydrocarbons (including benzene and benzene alkyl derivatives), nitrogenated compounds (including nitriles, amides and N-heterocyclic compounds), phenols, polycyclic aromatic hydrocarbons (PAHs), and others (such as fatty acids, alcohols and esters). A semi-quantitative analysis was performed by calculating the relative percentage of area of the chromatographic peaks with results shown in Table 3.4. Among these
compounds, hydrocarbons are valuable components in bio-oil from the point of view of fuel application. Specifically, aromatic hydrocarbons serve as important industrial chemicals and transportation fuel additives to increase octane number. Table 3.4 shows that both aliphatic and aromatic hydrocarbons were higher than those obtained from other biomasses (Adam et al., 2006; Wang et al., 2009; Zhang et al., 2009). This might result from the larger amount of lipids in microalgae being cracked into hydrocarbons during pyrolysis. Phenol and its alkylated derivatives, which are of great commercial importance, represented 6.20% of the bio-oil. N-containing compounds formed during the decomposition of proteins in algae cells, and they may account for potential NO\textsubscript{x} emissions during fuel combustion. In addition, about 3.38% PAHs were also detected. As the composition of liquid product is so complex, further upgrading, such as denitrogenation and deoxygenation, will be necessary to make the bio-oil suitable as engine fuels.

![Fig. 3.4 GC-MS profile of algal bio-oil obtained under 750 W microwave power](image-url)
Table 3.4 Relative proportions (area%) of the main compounds of algal bio-oil

<table>
<thead>
<tr>
<th>Categories</th>
<th>Compounds</th>
<th>Area/%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aliphatics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)-</td>
<td>2.02</td>
</tr>
<tr>
<td></td>
<td>2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>Dodecane, 2,6,10-trimethyl-</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>1-Tridecane</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Aromatics</strong></td>
<td></td>
<td>6.99</td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>Ethylbenzene</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>Styrene</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>o-Xylene</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Nitrogenates</strong></td>
<td></td>
<td>28.39</td>
</tr>
<tr>
<td></td>
<td>Indole</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td>Hexadecanamide</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>Pentadecanenitrile</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>1H-Pyrrole, 3-methyl-</td>
<td>1.11</td>
</tr>
<tr>
<td><strong>Phenols</strong></td>
<td></td>
<td>6.20</td>
</tr>
<tr>
<td></td>
<td>Phenol, 4-methyl-</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td>Phenol</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>Phenol, 2-ethyl-</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>PACs</strong></td>
<td></td>
<td>3.38</td>
</tr>
<tr>
<td></td>
<td>Naphthalene</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Anthracene</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td>17.90</td>
</tr>
<tr>
<td></td>
<td>n-Hexadecanoic acid</td>
<td>5.04</td>
</tr>
<tr>
<td></td>
<td>Oleic Acid</td>
<td>4.73</td>
</tr>
<tr>
<td></td>
<td>Hexadecanoic acid, Z-11-</td>
<td>2.09</td>
</tr>
<tr>
<td></td>
<td>9,12-Octadecadienoic acid, methyl ester</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Unidentified</strong></td>
<td></td>
<td>21.95</td>
</tr>
</tbody>
</table>
3.3.5 Molecular weight distribution and functional group compositional analysis

GPC was used to obtain the molecular weight distribution of the bio-oil from MAP under 750 W. The oil showed a molecular weight distribution ranging from 80 to over 1500. The number average molecular weight ($M_n$) and the weight average molecular weight ($M_w$) of the bio-oil were 244 and 299, respectively. The polydispersity ($PD = M_w/M_n$), which is a measure of the homogeneity of the bio-oil, was 1.23. These values were lower than those reported for lignocellulosic material (Hassan et al., 2009; Mullen et al., 2010b). The lower molecular weight and higher homogeneity might result from the fact that microalgae contain no lignin, which is the major source of phenolic oligomeric species in bio-oil from lignocellulosic biomass. Those pyrolytic lignin macromolecules, which constitute 25% - 30% of the whole bio-oil, have molecular weight range from several hundred to as high as 5000 or higher (Mohan et al., 2006).

Functional group compositional analysis was carried out using FTIR spectrometry. The functional groups identified from FTIR spectrum are shown in Table 3.5 and the spectrum is shown in Fig. 3.5. According to the interpretation of the main bands by (Socrates, 1994), FTIR functional groups indicated the presence of alkanes, aromatic compounds, nitriles, amides, phenol, and etc. These results are complementary to GC-MS analysis since compounds with high molecular weight could not be detected by GC-MS.
Table 3.5 FTIR functional groups of bio-oil obtained under 750 W microwave power

<table>
<thead>
<tr>
<th>Frequency range (cm⁻¹)</th>
<th>Groups</th>
<th>Class of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>3600-3200</td>
<td>O-H stretching</td>
<td>Phenols, Alcohols</td>
</tr>
<tr>
<td>3100-3010</td>
<td>N-H stretching</td>
<td>Amines</td>
</tr>
<tr>
<td>3000-2800</td>
<td>C-H stretching</td>
<td>Aromatics</td>
</tr>
<tr>
<td>2300-2000</td>
<td>C≡N stretching</td>
<td>Nitriles</td>
</tr>
<tr>
<td>1775-1650</td>
<td>C=O stretching</td>
<td>Carboxylic acids, esters, ketones</td>
</tr>
<tr>
<td>1680-1575</td>
<td>C=C stretching</td>
<td>Alkenes</td>
</tr>
<tr>
<td>1550-1490</td>
<td>N-H bending</td>
<td>Amides</td>
</tr>
<tr>
<td></td>
<td>C=C stretching</td>
<td>Aromatics</td>
</tr>
<tr>
<td>1470-1325</td>
<td>C-H bending</td>
<td>Alkanes</td>
</tr>
<tr>
<td>1300-950</td>
<td>C-O stretching</td>
<td>Alcohols, phenols, esters</td>
</tr>
<tr>
<td></td>
<td>O-H bending</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3.5 FTIR spectrum of algal bio-oil obtained under 750 W microwave power
3.3.6 Thermogravimetric analysis

The TG and DTG profiles of bio-oil in flow medium of nitrogen (evaporation profile) and air (combustion profile) are presented in Fig. 3.6. Two profiles exhibited similarities before 350 °C. Between 300 and 660 °C, the weight loss in air was lower than that in nitrogen, probably due to the aging reactions to form non-volatiles. Above 670 °C, the combustion of char residues was complete. In addition, the two DTG peaks represent the light and heavy organic compounds in the corresponding boiling point range. An estimate of the boiling point distribution was obtained using the evaporation profiles of the bio-oil. It was found that 23% of the bio-oil had a boiling point below 160 °C, 11% between 160-200 °C and 51% between 200-350 °C, which are similar to the boiling point range of light naphtha, heavy naphtha and middle distillate, respectively (Laresgoiti et al., 2004). The existence of high amount of middle distillate indicates that bio-oil from microalgae is very promising as kerosene and diesel fuels. The result also shows that there was about 50% of the bio-oil with a boiling point < 250 °C. This further demonstrates that compounds analyzed by GC-MS represented a fraction of the bio-oil since high molecular weight compounds could not be gasified and identified with GC-MS.

(A)
3.3.7 Analysis of gaseous products

The permanent gas collected was comprised of H₂, CO, CO₂ and gaseous hydrocarbons. The quantitative results of the four main components (H₂, CO, CO₂ and CH₄) are presented in Fig. 3.7. With the increase of microwave power, the CO₂ concentration decreased significantly, while the H₂ and CO concentrations increased gradually. The highest concentration of H₂ + CO (syngas) was 49.8%. The trends of the components were in agreement with the literature and indicated that the following reactions were favored at higher temperatures (Domínguez et al., 2007; Wang et al., 2009):

\[
C(s) + CO_2(g) \leftrightarrow 2CO \quad \Delta H_{298K} = 173 \text{ KJ/mol}
\]

\[
C(s) + H_2O \leftrightarrow CO + H_2 \quad \Delta H_{298K} = 132 \text{ KJ/mol}
\]
3.4 Conclusions

Chlorella sp. was pyrolyzed in a microwave cavity. The microwave power of 750 W was found to be the optimum microwave power as the maximum bio-oil yield of 28.6% was obtained. Several analyses indicate that the algal bio-oil exhibit a better quality than lignocellulosic bio-oils in terms of physical and chemical properties. The algal bio-oil was characterized by low oxygen content with aliphatic and aromatic hydrocarbons constituting 22.18% of the total ion chromatogram of GC-MS. However, further upgrading to remove N and O from bio-oil is necessary to make it suitable as engine fuels.
CHAPTER 4 PYROLYSIS MECHANISMS OF MICROALGAE AND THEIR
THREE MAJOR COMPONENTS

A paper accepted for publication in Bioresource Technology

4.1 Introduction

Microalgae have received growing interest recently because of their high productivity, high oil content and the ability to grow in a wide range of climates and lands (Schenk et al., 2008). However, conversion of microalgae to biofuels is still a challenge. Traditional algal biodiesel is produced via costly extraction of oil by organic solvents followed by transesterification reaction. Since microalgae contain a significant amount of free fatty acids, acid catalysts need to be used firstly to convert free fatty acids into fatty acids esters, which require much longer conversion time than basic catalysts. Another issue with algal biodiesel is that only oil portion can be utilized and the rest is considered as waste in terms of fuel use.

Recently, another conversion route, pyrolysis, has received growing attention in conversion of whole microalgae. During pyrolysis, organic materials are thermally decomposed in the absence of oxygen and the condensed liquid is referred as bio-oil (Mohan et al., 2006). Although pyrolysis has been studied extensively on lignocellulosic biomass, few studies have been carried out using microalgae as the feedstock. The reported results (Du et al., 2011; Du et al., 2012b; Maddi et al., 2011; Thangalazhy-Gopakumar et al., 2012a) showed that algal bio-oil has better qualities in many aspects than that produced from lignocellulosic biomass. For example, algal bio-oil has higher heating value, lower oxygen content, and desirable pH. However, algal bio-oil contains many oxygenates and nitrogenates (Maddi et al., 2011; Pan et al., 2010), which make it
difficult for direct use as drop-in fuels. Thus, both homogeneous (Babich et al., 2011) and heterogeneous catalysts (Thangalazhy-Gopakumar et al., 2012a) were applied to pyrolysis of microalgae, aiming to improve the quality of algal bio-oil. It was found that zeolite HZSM-5 increased the carbon yield of aromatic hydrocarbons from 0.9 to 25.8% for pyrolysis of *Chlorella vulgaris* (Thangalazhy-Gopakumar et al., 2012a).

These previous studies focused primarily on the influence of process variables on the quantity and quality of algal bio-oil. No previous report on algal pyrolysis has provided insight into the pyrolysis mechanism. Different from terrestrial biomass which is basically composed of cellulose, hemicellulose, and lignin, the major constituents of microalgae are carbohydrates, proteins, and lipids. Therefore, the objective of this study was to study the production pathways of the major compounds in algal bio-oil via pyrolysis of model compounds. Microcrystalline cellulose, dried egg whites and canola oil were used as the model compounds of carbohydrates, proteins and lipids, respectively. In addition, the influence of catalysts on the distribution of pyrolytic products from each model compounds was also studied.

**4.2 Materials and methods**

**4.2.1 Materials**

Dried algal biomass (*Chlorella vulgaris*) powder was collected from our laboratory and the detailed cultivation process was described in our previous study (Du et al., 2011). Laboratory grade microcrystalline cellulose was obtained from Sigma Aldrich (St Louis, MO). Egg white powder was purchased from Rose Acres farms, Inc (Seymour, IN). Canola oil was bought as food-grade materials from a local grocery store. The elemental
and chemical compositions of algal biomass and the model compounds are listed in Table 4.1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Elemental analysis (wt.% dry basis)</th>
<th>Chemical composition (wt.% dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C  H  N  O&lt;sup&gt;a&lt;/sup&gt;  Ash</td>
<td>Lipids  Proteins  Others&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>49.16 6.28 8.05 28.91 7.60</td>
<td>12.33 50.31 29.76</td>
</tr>
<tr>
<td>Cellulose</td>
<td>43.69 6.08 0.00 50.23 0.00</td>
<td>0.00 0.00 100.00</td>
</tr>
<tr>
<td>Egg whites</td>
<td>47.71 6.34 13.30 26.73 5.92</td>
<td>0.00 83.13 10.95</td>
</tr>
<tr>
<td>Canola oil</td>
<td>77.86 10.96 0.05 11.13 0.00</td>
<td>100.00 0.00 0.00</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated by difference, O(%) = 100 – C – H – N – Ash.

<sup>b</sup> Calculated by difference, Others (%) = 100 – Lipids – Proteins – Ash.

**4.2.2 Catalyst**

ZSM-5 zeolite powder in the ammonium form (Si/Al = 30, surface area = 405 m²/g) was purchased from Zeolyst International (Conshohocken, PA). Prior to use, the catalyst was calcined to its active hydrogen form HZSM-5 at 550 °C in air for 5 hours.

**4.2.3 Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS)**

Pyrolysis experiments were performed using an analytical Pyroprobe 2000 pyrolyzer (CDS Analytical Inc.) coupled with an Agilent 7890-5975C GC-MS. About 0.5 mg of samples were filled and held in the center of a quartz tube (25 mm long, 2 mm outside diameter) with packed quartz wool. For catalytic pyrolysis experiments, samples were prepared by physically mixing the biomass and the catalyst in defined ratios. The tube was then inserted into a platinum coil, and pyrolyzed in a CDS1500 valved interface which was maintained at 250 °C. Samples were heated at a heating rate of 1000 °C/s to the set temperature and then maintained isothermally for 30 s to ensure complete
pyrolysis. Upon pyrolysis, the pyrolysis vapors were directly swept into the GC-MS with a HP-5MS (30 m × 0.25 m × 0.25 µm) column. An injector temperature of 250 °C and a split ration of 1:100 were used. The oven was programmed to hold at 40 °C for 5 min, and then the temperature was increased to 200 °C at a rate of 10 °C /min, followed by a 20 °C/min ramp up to 290 °C. Major products were quantified with the calibration curves of standards in known concentrations. A four-point calibration curve with the R² above 0.98 was obtained by plotting the peak area against the mass of each compound. Residue yields were determined gravimetrically by weighing the whole quartz tube unit before and after pyrolysis. Data were analyzed using one-way analysis of variance (ANOVA, at 0.05 significance level) using software SPSS 18.0.

4.3 Results and discussion

4.3.1 Py-GC/MS of Chlorella and model compounds

Temperatures between 450 °C to 600 °C were used, in which range the highest bio-oil yield from microalgae was obtained in the literature (Du et al., 2011; Maddi et al., 2011). The yield of each compound at each temperature is shown in Table 4.2. Since some compounds, such as mono-sugars and long chain fatty acids, have inconsistent quantification results on the HP-5 capillary column, they are listed as identified but not quantified. The composition of algal bio-oil is very different from lignocellulosic materials derived pyrolysis products which are mainly composed of oxygenates (Huber et al., 2006). According to the pyrolytic products of model compounds, furfural and levoglucosan were resulted from the decomposition of carbohydrates in microalgae cells, such as cellulose and starch. The aromatic hydrocarbons were mainly derived from the
protein fraction in algae and their yields increased with temperature. Li et al. (2008) suggested that decarboxylation, deamination and concerted rupturing of C-C bonds (radical formation) pathways exist during the primary decomposition of phenylalanine and tyrosine. This is confirmed in this study that ammonia, CO₂ and other non-condensable gases were identified as one peak which eluted firstly from the GC column. Based on these results, the postulated mechanism of the formation of toluene and styrene from phenylalanine is shown in scheme 1 of Fig. 4.1. Although aromatics are desirable products in terms of fuel use, nitrogenated compounds were also produced from proteins. Compounds, including amines, nitriles, pyridine and indole, were detected in the pyrolysis products of microalgae and egg whites. The formation of benzyl nitrile and indole from phenylalanine and tryptophan was proposed in scheme 1 and 2 of Fig. 4.1. Since microalgae contain no lignin, lignin derivatives, such as guaiacols and syringols, were not detected. However, phenol and cresol were found in the bio-oil of microalgae and egg whites, indicating phenols are formed from the proteins. Radical mechanism of phenol formation from tyrosine is shown in scheme 3 of Fig. 4.1. Long chain fatty acids were derived from the pyrolysis of triglycerides in microalgae. Although thermal cracking of triglycerides produced short chain hydrocarbons (Maher et al., 2007), long chain fatty acids still existed because of the short residence time in the quartz tube. Small amount of aromatic hydrocarbons were also produced from canola oil at relatively high temperatures in which range cyclization and aromatization reaction occur. In addition, the high amount of residue (in liquid form) from canola oil pyrolysis is due to the re-condensation of vaporized triglycerides and fatty acids on the two cold ends of the quartz tube. Since these quantified compounds had very different formation mechanisms, they
showed different optimal temperatures for their formation. In fact, these compounds showed no statistically significant change or changed very slightly with temperature.

Table 4.2 Composition of main identified products at different temperatures (wt.% of feedstock on a dry basis)

<table>
<thead>
<tr>
<th>Bio-oil compounds</th>
<th>Chlorella vulgaris</th>
<th>Cellulose</th>
<th>Egg whites</th>
<th>Canola oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>0.35±0.04</td>
<td>0.36±0.05</td>
<td>0.37±0.09</td>
<td>0.40±0.08</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>0.03±0.00</td>
<td>0.06±0.01</td>
<td>0.05±0.02</td>
<td>0.06±0.00</td>
</tr>
<tr>
<td>Styrene</td>
<td>0.09±0.00</td>
<td>0.12±0.04</td>
<td>0.10±0.03</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.47±0.19</td>
<td>0.45±0.13</td>
<td>0.38±0.06</td>
<td>0.45±0.08</td>
</tr>
<tr>
<td>Phenol, 4-methyl-</td>
<td>0.85±0.27</td>
<td>1.03±0.20</td>
<td>0.96±0.12</td>
<td>0.90±0.23</td>
</tr>
<tr>
<td>Pyridine</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
</tr>
<tr>
<td>Benzyl nitrile</td>
<td>0.12±0.04</td>
<td>0.13±0.03</td>
<td>0.14±0.03</td>
<td>0.17±0.04</td>
</tr>
<tr>
<td>Pyrrole</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
</tr>
<tr>
<td>Benzenepropanenitrile</td>
<td>0.09±0.02</td>
<td>0.11±0.01</td>
<td>0.13±0.04</td>
<td>0.16±0.05</td>
</tr>
<tr>
<td>Indole</td>
<td>0.22±0.04</td>
<td>0.21±0.05</td>
<td>0.20±0.02</td>
<td>0.17±0.07</td>
</tr>
<tr>
<td>1-Undecene</td>
<td>0.11±0.04</td>
<td>0.13±0.02</td>
<td>0.15±0.02</td>
<td>0.16±0.04</td>
</tr>
<tr>
<td>Furfural</td>
<td>0.17±0.03</td>
<td>0.23±0.02</td>
<td>0.18±0.05</td>
<td>0.19±0.06</td>
</tr>
<tr>
<td>Levoglucosan</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
</tr>
<tr>
<td>Hexadecanoic acid</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
<td>× × × ×</td>
</tr>
</tbody>
</table>

- The number in each cell indicates mean values ± standard deviation of three replicates.
- The symbol “×” means the corresponding compound was identified but not quantified.
- The blank cell means that the corresponding compound was not detected.
4.3.2 Catalytic pyrolysis

The products from pyrolysis of Chlorella vulgaris and the model compounds were very complex and the maximum yield of quantified aromatic hydrocarbons only accounted for 0.89 wt.% for egg whites at 600 °C. Thus, HZSM-5 was employed as the catalyst to study its effect on the profile of pyrolysis products.

4.3.2.1 Effect of catalyst to feed ratio

Fig. 4.2 shows the product yields for catalytic pyrolysis of Chlorella, cellulose, egg whites and canola oil as a function of the catalyst to feed ratio. Besides toluene, ethylbenzene and styrene in Table 4.2, many other aromatic hydrocarbons were formed when catalysts were added. Since the chromatogram peak of indole was merged into that of naphthalene, indole was not quantified for the catalytic pyrolysis runs. The identified
compounds are listed as: benzene, toluene, xylene (including \textit{p-}/\textit{o-}xylene, ethylbenzene and styrene), trimethylbenzene (including trimethyl-benzene and methyl-ethyl-benzene), indane (including indane, indene and their alkyl derivatives), naphthalene (including naphthalene and its alkyl derivatives), 3-methyl-1H-Indole, nitriles (including benzyl nitrile and benzenepropanenitrile), phenols (including phenol and 4-methyl-phenol) and furfural.

![Graphs](image)

Fig. 4.2 Effect of catalyst to feed ratio at 550 °C on the aromatic yield from different materials. (a) Chlorella vulgaris; (b) cellulose; (c) egg whites; (d) canola oil

For all materials studied, significantly more aromatic hydrocarbons were produced from catalytic pyrolysis than non-catalytic pyrolysis. This is consistent with many other studies that show lignocellulosic derived organics can be deoxygenated and cracked to
produce aromatics over HZSM-5 (Mihalcik et al., 2011; Mullen et al., 2010a). In addition, the aromatic yield increased significantly as the catalyst to feed ratio increased from 1:1 to 5:1. This is probably because a ratio of 1:1 cannot provide enough surface contact between the pyrolysis vapors and catalysts. Chen et al. (1988) defined the effective hydrogen index (EHI), which is an indicator of hydrogen/carbon ratio after debiting the compound’s hydrogen content for complete conversion of heteroatoms to NH₃, H₂S and H₂O. In this study,

\[ EHI = \frac{(H - 2O - 3N)}{C}, \]

where H, C, O and N are the number of moles of hydrogen, carbon, oxygen and nitrogen in the feedstock. Feedstocks with the same EHI should have similar theoretical yields of aromatics and a higher EHI leads to a higher aromatic yield. In this study, EHI values for Chlorella, cellulose, egg whites and canola oil are 0.23, 0, 0.22 and 1.47, respectively. However, egg whites, rather than cellulose, produced the least amount of aromatics under the same reaction conditions. This could be because that nitrogen-containing heterocyclic compounds, such as indole and pyridine, are more stable than oxygenates, which makes denitrogenation much more difficult than deoxygenation. Canola oil had the highest aromatic yield of 53.57% due to its low oxygen content and relatively simpler chemical structure for catalytic cracking. Among the individual aromatic compounds quantified, higher percentage of naphthalene (45.3% of the total aromatic yield) was produced from cellulose than from other materials, which suggests that cellulose is more selectively converted to polycyclic aromatic compounds (PACs). For Chlorella, more furfural and 3-methyl-1H-indole were formed from catalytic pyrolysis at 1:1 ratio than non-catalytic pyrolysis, suggesting that low amounts of catalysts could increase the efficiency of
biomass depolymerization producing more thermally stable organics, such as furfural and indole derivatives. When the catalyst to biomass ratio increased further to 5:1, both compounds decreased significantly with furfural undetectable. The similar trends were found for furfural produced from cellulose and 3-methyl-1H-indole from egg whites. The yields of nitriles and phenols decreased significantly for catalytic versus non-catalytic pyrolysis, which suggests they were catalytically converted to other compounds. We propose that benzyl nitrile and benzenepropanenitrile can be cracked into aromatics and HCN, which were detected on GC. Since simple phenolics were hard for conversion and tightly bound to the acidic active sites of HZSM-5 (Graca et al., 2009), their reduction could be attributed to the coke formation on the catalysts. Based on the chemical composition of Chlorella as shown in Table 4.1, the additive aromatic yield from cellulose, egg whites, and canola oil at 5:1 catalyst to fuel ratio is 16.32%, which is very close to the aromatic yield of 16.72% observed for Chlorella. This suggests that the individual components in microalgae behaved additively with respect to the production of aromatics.

The residue yield of non-catalytic and catalytic pyrolysis with the catalyst to feed ratio of 5:1 is summarized in Fig. 4.3. The coke formation can come from homogeneous gas phase thermal decomposition reactions and from heterogeneous reactions on the catalyst (Carlson et al., 2009). With the addition of catalyst, the solid residue yields of Chlorella, cellulose and egg whites all increased significantly compared with that from non-catalytic runs. The net increase in residue could be attributed to the coke deposit on the added catalyst. However, the residue yield of canola oil decreased significantly to only 3.7%. This is because the vaporized oil was catalytically cracked down to lower
molecular weight compounds with lower boiling points, which did not condense on the tail portion of the quartz tube. Our data are consistent with Chen et al. (1988) who suggested that oxygenates having EHI values below 1 produced excessive amounts of coke.

Fig. 4.3 Yield of residue from non-catalytic and catalytic pyrolysis at the catalyst to feed ratio of 5:1.

4.3.2.2 Effect of pyrolysis temperature

Fig. 4.4 shows the effect of temperature on the yield of different compounds at the catalyst to feed ratio of 5:1. The total aromatic yield of *Chlorella* increased with temperature to a maximum value of 16.72% at 550 °C and then decreased slightly when the temperature increased to 600 °C, which could be because pyrolytic vapors were further cracked into gaseous products at higher temperatures. The similar trend was also observed for cellulose, egg whites and canola oil. For *Chlorella*, nitriles and furfural were
not found at all temperatures, indicating they were completely transformed. Significantly less phenolic compounds were found in catalytic runs than non-catalytic ones, but their amounts remained almost constant at different temperatures in catalytic pyrolysis. About 65% of 3-methyl-1H-indole from non-catalytic pyrolysis was still detected in catalytic runs, which suggests that indole derivatives were very stable and can be hardly converted by catalysts. Similar trends were noticed for egg whites except that nitriles were still found. Furfural yield from cellulose showed a significant yield with temperature, which was also observed by another study with sawdust as the feedstock (Olazar et al., 2000), which indicates that a lower temperature favors the conversion of furfural.

Fig. 4.4 Effect of temperature on the aromatic yield from different materials at the catalyst to feed ratio of 5:1. (a) *Chlorella vulgaris*; (b) cellulose; (c) egg whites; (d) canola oil.
4.3.2.3 Postulated reaction mechanism

Many studies have been carried out on the catalytic pyrolysis of carbohydrates and lignocellulosic biomass on HZSM-5 (Carlson et al., 2010; Williams et al., 1994), and the reaction pathways of deoxygenation have been proposed based on the transformation of key model components in bio-oil. Light organics, including alcohols, aldehydes, acids and ketones, derived from carbohydrates in biomass are deoxygenated and cracked into C2-C6 olefins. Then these olefins undergo a series of aromatization reactions to produce benzene followed by alkylation and isomerization to produce other aromatics as shown in scheme 1 of Fig. 4.5 (Adjaye et al., 1995; Gayubo et al., 2004a; Gayubo et al., 2004b). The catalytic pyrolysis of triglycerides on HZSM-5 has also been studied extensively (Idem et al., 1996; Katikaneni et al., 1996). These studies proposed that canola oil is thermally decomposed to heavy oxygenated hydrocarbons, such as long chain fatty acids, ketones, esters, etc., which are then converted to heavy hydrocarbons by deoxygenation. These heavy hydrocarbons are cracked down to olefins, which subsequently undergo a series of oligomerization, cyclization and aromatization to give aromatics (Scheme 2, Fig. 4.5).

On the other hand, very limited numbers of reports are available for the catalytic pyrolysis of proteins on HZSM-5. Sec-butylamine was converted to butenes through deamination reactions catalyzed by HZSM-5 (Lequitte et al., 1992). In our study, HCN was detected in the pyrolysis products, which indicates that C-C bond in nitriles can be broken by HZSM-5 zeolite. In addition, some oxygenates, such as ketones and aldehydes were also found but not quantified. They can also undergo the same pathways as those
Fig. 4.5 Postulated pathways for catalytic pyrolysis of carbohydrates (scheme 1), lipids (scheme 2) and proteins (scheme 3).

derived from cellulose to produce aromatics. However, as discussed in section 3.2.2, indole derivatives are relatively stable on HZSM-5, and thus they are not considered as
the major source of aromatics. Based on the literature and our results, the postulated mechanism of catalytic pyrolysis of proteins is shown as scheme 3 of Fig. 4.5. Further studies are still required to elucidate the exact reaction pathway of each individual compounds.

4.4 Conclusions

Several pyrolysis pathways of algal biomass were postulated by analysis and identification of pyrolysis products from the model algal biomass compounds. Catalytic pyrolysis of microalgae *Chlorella vulgaris* and the model algal biomass compounds demonstrated the potential of aromatic production using HZSM-5 catalytic pyrolysis. The aromatic yields of all four feedstocks increased significantly when the catalyst to biomass ratio increased from 1:1 to 5:1. The aromatic yield followed the trend that lipids > carbohydrates > proteins under all reaction conditions. To understand the low yield of aromatics from proteins, further studies on the reaction pathways of different groups of compounds are required.
CHAPTER 5 CATALYST SCREENING FOR CATALYTIC PYROLYSIS OF MICROALGAE

5.1 Introduction

Due to the higher growth rate and biomass productivity than terrestrial plants, microalgae have been receiving increasing attentions in recent years (Ahmad et al., 2011; Schenk et al., 2008). Among various conversion technologies, pyrolysis has been investigated extensively because of its ability to directly convert solid biomass into liquid fuels known as bio-oil. The few reported results from pyrolysis of microalgae (Campanella et al., 2012; Du et al., 2011; Maddi et al., 2011) showed that algal bio-oil has better qualities in many aspects than that produced from lignocellulosic biomass. For example, algal bio-oil has a higher heating value, a lower oxygen content and a pH value greater than 7. However, algal bio-oil contains many oxygenates and nitrogenates (Du et al., 2012b; Maddi et al., 2011; Pan et al., 2010), and thus upgrading processes to reduce nitrogen and oxygen are necessary.

Catalytic pyrolysis, also known as in-situ upgrading, incorporates catalysts directly into the pyrolysis reactions by mixing biomass with catalysts or setting up an upgrading fixed bed right at the outlet of pyrolysis vapors. Catalytic pyrolysis of lignocellulosic materials has been examined by several researchers and zeolites have been shown to be effective in the deoxygenation of pyrolysis vapors and production of aromatic hydrocarbons (Azeez et al., 2011; Carlson et al., 2009; Foster et al., 2012; French et al., 2010; Mihalcik et al., 2011; Mullen et al., 2010a). During catalytic pyrolysis, the primary pyrolysis products enter the pores of zeolites where they are catalytically converted to aromatics via a series of reactions such as decarbonylation, decarboxylation, dehydration.
and aromatization reactions (Li et al., 2012). In addition, different metals, such as nickel (Valle et al., 2010) and gallium (Cheng et al., 2012) were incorporated into zeolite to increase the aromatic yield, because some transition metals have dehydrogenating activity and can change the acidity of zeolite. However, the major constituents of microalgae are carbohydrates, proteins, and lipids, which are very different from those for lignocellulosic biomass. To our knowledge, the only paper on catalytic pyrolysis of microalgae with zeolite is from Thangalazhy-Gopakumar et al. (2012a) who reported that HZSM-5 increased the carbon yield of aromatic hydrocarbons from 0.9 to 25.8% for pyrolysis of *Chlorella vulgaris*. Very limited information is known on the influence of different types of zeolite catalysts on the profile of pyrolysis products from microalgae. Also, as a major constituent of microalgae (around 50 wt.%), proteins did not receive enough attention in previous studies as a pyrolysis feedstock. Thus, dried egg white powders were employed as the model compounds of proteins in this study. A series of zeolites with different frameworks and silica-to-alumina ratios (Si/Al) were screened using Pyrolysis-Gas Chromatography/ Mass spectrometry (Py-GC/MS) to determine their effects on the pyrolysis product profiles of *Chlorella* and egg whites. In addition, the effects of metal incorporation into zeolite on the yield of aromatic hydrocarbons were studied.

### 5.2 Materials and methods

#### 5.2.1 Materials

Dried algal biomass (*Chlorella vulgaris*) powder was obtained from our laboratory and the detailed cultivation and harvesting processes were described in our previous
study (Du et al., 2011). Egg white powder was purchased from Rose Acres farms, Inc (Seymour, IN). The elemental and chemical compositions of algal biomass and egg whites are listed in Table 5.1.

Table 5.1 Elemental compositions of *Chlorella vulgaris* and egg whites

<table>
<thead>
<tr>
<th>Compound</th>
<th>Elemental analysis (wt.% dry basis)</th>
<th>Chemical composition (wt.% dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>C 49.16  H 6.28  N 8.05  O 28.91  Ash 7.60</td>
<td>Lipids 12.33  Proteins 50.31  Others 29.76</td>
</tr>
<tr>
<td>Egg whites</td>
<td>C 47.71  H 6.34  N 13.30  O 26.73  Ash 5.92</td>
<td>Lipids 0.00  Proteins 83.13  Others 10.95</td>
</tr>
</tbody>
</table>

*a* Calculated by difference, O(%) = 100 − C − H − N − Ash.

5.2.2 Catalysts

Four types of zeolites were purchased from Zeolyst International (Conshohocken, PA), including ZSM-5, Beta and Y zeolite. ZSM-5 and Beta zeolites were supplied in their ammonium form and they were activated to their hydrogen form in a furnace at 550 °C for 5 hours before use. HZSM-5 with a Si/Al ratio of 30 was selected for metal impregnation using the incipient wetness method. Salt solutions, including cobalt nitrate (Co(NO$_3$)$_2$), copper nitrate (Cu(NO$_3$)$_2$), iron nitrate (Fe(NO$_3$)$_3$), gallium nitrate (Ga(NO$_3$)$_3$), ammonium heptamolybdate ((NH$_4$)$_6$Mo$_7$O$_{24}$), and nickel nitrate (Ni(NO$_3$)$_2$), were slowly added to HZSM-5 powders to achieve 5 wt.% of metal loadings. The mixture was then stirred until a paste was formed. The paste was then dried at 105 °C for 24 hours and subsequently calcined at 550 °C for 5 hours. The catalysts were grounded and reduced in a flow of 10 vol.% hydrogen in nitrogen gas at 450 °C for 3 hours. The Brunauer-Emmett-Teller (BET) surface areas of these metal-impregnated catalysts were
measured from nitrogen adsorption on a Micromeritics ASAP 2020 system. The physicochemical properties of the zeolites in this study are summarized in Table 5.2.

Table 5.2 Physicochemical properties of zeolites used in this study

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Product name</th>
<th>Crystal structure</th>
<th>Si/Al ratio</th>
<th>Pore size (Å)</th>
<th>BET surface area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-Y (80)</td>
<td>CBV780</td>
<td>FAU</td>
<td>80</td>
<td>7.4×7.4</td>
<td>780⁹</td>
</tr>
<tr>
<td>H-Y (5.1)</td>
<td>CBV400</td>
<td>FAU</td>
<td>5.1</td>
<td>7.4×7.4</td>
<td>730⁹</td>
</tr>
<tr>
<td>H-Beta (38)</td>
<td>CP814C</td>
<td>BEA</td>
<td>38</td>
<td>7.6×6.4, 5.6×5.6</td>
<td>710⁹</td>
</tr>
<tr>
<td>H-ZSM5 (280)</td>
<td>CBV28014</td>
<td>MFI</td>
<td>280</td>
<td>5.6×5.3, 5.5×5.1</td>
<td>400⁹</td>
</tr>
<tr>
<td>H-ZSM5 (80)</td>
<td>CBV8014</td>
<td>MFI</td>
<td>80</td>
<td>5.6×5.3, 5.5×5.1</td>
<td>425⁹</td>
</tr>
<tr>
<td>H-ZSM5 (30)</td>
<td>CBV3024E</td>
<td>MFI</td>
<td>30</td>
<td>5.6×5.3, 5.5×5.1</td>
<td>405⁹</td>
</tr>
<tr>
<td>Co-ZSM5</td>
<td>MFI</td>
<td>30</td>
<td></td>
<td></td>
<td>276.1</td>
</tr>
<tr>
<td>Cu-ZSM5</td>
<td>MFI</td>
<td>30</td>
<td></td>
<td></td>
<td>309.4</td>
</tr>
<tr>
<td>Fe-ZSM5</td>
<td>MFI</td>
<td>30</td>
<td></td>
<td></td>
<td>302.7</td>
</tr>
<tr>
<td>Ga-ZSM5</td>
<td>MFI</td>
<td>30</td>
<td></td>
<td></td>
<td>300.8</td>
</tr>
<tr>
<td>Mo-ZSM5</td>
<td>MFI</td>
<td>30</td>
<td></td>
<td></td>
<td>254.1</td>
</tr>
<tr>
<td>Ni-ZSM5</td>
<td>MFI</td>
<td>30</td>
<td></td>
<td></td>
<td>275.0</td>
</tr>
</tbody>
</table>

⁹ The blank cell means the parameter not measured in this study; ⁹ Data provided by the supplier.

5.2.3 Py-GC/MS

Pyrolysis experiments were performed using an analytical Pyroprobe 2000 pyrolyzer (CDS Analytical Inc.) interfaced with an Agilent 7890-5975C GC-MS. For catalytic pyrolysis experiments, samples were prepared by physically mixing the feedstock and the catalyst in the weight ratio of 1:5. About 0.5 mg of the mixture was filled and held in the center of a quartz tube (25 mm long, 2 mm outside diameter) with packed quartz wool. The tube was then inserted into a platinum coil, and pyrolyzed in a CDS1500 valved interface which was maintained at 250 °C. Samples were heated at a heating rate of 1000
°C/s to 550 °C and then maintained isothermally for 30 s to make sure that biomass was completely pyrolyzed. Upon pyrolysis, the pyrolysis vapors were directly swept into the GC-MS with a HP-5MS (30 m × 0.25 m × 0.25 µm) column. An injector temperature of 250 °C and a split ration of 1:100 were used. The oven was programmed to hold at 40 °C for 5 min, and then the temperature was increased to 200 °C at a rate of 10 °C /min, followed by a 20 °C/min ramp up to 290 °C. Major products were quantified with the calibration curves of standards in known concentrations. A four-point calibration curve with the R² above 0.98 was obtained by plotting the peak area against the mass of each compound. Data were analyzed using one-way analysis of variance (ANOVA, at 0.05 significance level) using software SPSS 18.0.

5.3 Results and discussion

5.3.1 Effect of catalyst type

Consistent with our previous study (Du et al., 2011) and other reports (Campanella et al., 2012; Maddi et al., 2011), the composition of algal bio-oil is very complex and more than 150 compounds were detected in this study. Thus, only some major compounds identified were quantified and listed as: benzene, toluene, C8 aromatics (including p-/o-xylene, ethylbenzene and styrene), C9-C11 aromatics (including C3-C5 alkyl derivatives of benzene), indane (including indane, indene and their alkyl derivatives), napthalene (including napthalene and its alkyl derivatives), 3-methyl-1H-Indole, nitriles (including benzyl nitrile and benzenepropanenitrile) and phenols (including phenol and 4-methyl-phenol).
Three catalysts with a close Si/Al ratio, H-Y (80), H-Beta (38) and H-ZSM5 (80),
were used to study the effect of catalyst framework on the product profiles of *Chlorella*
and egg whites as shown in Fig. 5.1a and Fig. 5.1b, respectively. When no catalyst was
added to *Chlorella*, the only prominent aromatic hydrocarbon detected was toluene with a
yield of 0.37 %. Many other types of aromatic hydrocarbons, including benzene, xylene,
styrene, naphthalene and their alkyl derivatives, were detected during catalytic runs. The
total aromatic yields increased dramatically to 3.93%, 3.43% and 18.13% for H-Y (80),
H-Beta (38) and H-ZSM5 (80), respectively. This is consistent with previous findings in
catalytic pyrolysis of lignocellulosic materials (Jae et al., 2011; Mihalcik et al., 2011; Yu
et al., 2012). Beta zeolite has straight channels of 7.6 Å×6.4 Å and tortuous channels of
5.6 Å ×5.6 Å, and Y zeolite has a cubic structure with circular 12-membered rings (7.7 Å×7.7 Å). These two catalysts are considered as large pore zeolites compared with ZSM-5,
which is composed of 10-membered rings with straight channels (5.5 Å×5.1 Å)
connected by zigzag channels (5.6 Å×5.3 Å). Jae et al. (2011) explained the aromatic
yields on different catalysts with the concept of constraint index (CI), which is defined as
the ratio of the cracking rate constants of n-hexane to 3-methylpentane (Frillette et al.,
1981), and a higher CI value means a larger steric hindrance. They found that a higher CI
value of 6.9 for ZSM-5 favors aromatic formation than zeolites with a lower CI value,
such as 0.6 for Beta. Also, Yu et al. (2012) suggested that large pore zeolites have more
severe coke formation due to polymerization reactions and coke can block active sites in
catalysts. On the other hand, phenols were reduced but not very effectively during
catalytic runs. Simple phenols are very resistant to catalytic conversion and are likely to
bind to the acidic sites leading to coke formation (Gayubo et al., 2004a; Mullen et al.,
The yields of 3-methyl-1H-Indole and nitriles increased during catalytic runs unfortunately, since they are undesired nitrogenates in algal bio-oil. This suggests that they can be produced from the secondary cracking of other protein-derived compounds. The detailed mechanism for the transformation of nitrogenates needs to be further elucidated by using pure model components in the future. However, the overall denitrogenation effect of zeolite cannot be determined in this study since it is too expensive and impossible to quantify all the identified nitrogenates. Elemental analysis of algal bio-oil obtained from larger-scale catalytic runs can be used to investigate the nitrogen removal on zeolites in future studies.

Similar trend of the yield of each compound was observed for egg whites as shown in Fig. 5.1b. Overall, the yield of nitrogenates was higher than that for Chlorella, due to the much higher protein content in egg whites. Interestingly, egg whites have slightly higher aromatic yield during catalytic runs with H-Y (80) and H-Beta (38) than Chlorella. This is probably because that some amino acids, such as phenylalanine and tyrosine, contain benzene ring in their molecules. They can produce aromatics with low-activity catalysts or even without catalysts. However, when high-activity catalyst H-ZSM5 (80) was used, lipids and carbohydrates in Chlorella were also converted to aromatics, leading to a significant higher aromatic yield (18.13 %) than egg whites (11.11 %).

5.3.2 Effect of Si/Al ratio

To investigate the effects of acidity of zeolites, biomass samples were mixed with H-ZSM5 (30), H-ZSM5 (80) and H-ZSM5 (280) in the catalytic pyrolysis tests. Fig. 5.2
shows the product yields for catalytic pyrolysis of *Chlorella* and egg whites as a function of Si/Al ratio. H-ZSM5 contains Brønsted acid sites where aromatics are formed through a series of dehydration, decarbonylation, decarboxylation and aromatization reactions.

Fig. 5.1 Effect of catalyst type on the yields of pyrolysis products from: (a) Chlorella vulgaris and (b) egg whites.
Thus a low Si/Al ratio (high acidity) should favor the production of aromatics. However, the maximum aromatic yield (18.13% for *Chlorella* and 11.11% for egg whites) occurred at a moderate Si/Al ratio of 80, and a similar phenomenon was also observed by Foster et al. (2012) for the catalytic pyrolysis of glucose. They explained that the concentration of acid sites in close proximity to one another increase as the Si/Al ratio decreases. This increase may facilitate secondary reactions converting aromatics to coke. On the other hand, the yields of 3-methyl-1H-Indole and nitriles increased with the Si/Al ratio, which is probably because that these nitrogen bases can be more efficiently converted or absorbed on more acidic catalysts.

### 5.3.3 Effect of metal incorporation

To investigate the effect of metal substitution, 6 different metals, including Co, Cu, Fe, Ga, Mo, and Ni, were impregnated into H-ZSM5 (30). Fig. 5.3 shows the influence of metal incorporation on the aromatic yields of *Chlorella* and egg whites. Among the catalysts, Cu and Ga enhanced the aromatic yields significantly from 16.72% for normal HZSM5 (30) to 21.16% and 18.03%, respectively. A recent study (Cheng et al., 2012) also found that Ga can promote desired decarbonylation reactions and olefin aromatization, which thus resulted in a higher aromatic yield. On the other hand, Co, Fe and Mo have an adverse effect on the aromatic yield of *Chlorella*, while Ni does not have a significant effect. Ni-Mo and Co-Mo have been used as bimetallic catalysts for the hydrodeoxygenation of bio-oil (Huber et al., 2006). However, they seem to work only
under high pressure with the addition of hydrogen. Thangalazhy-Gopakumar et al. also found (2012b) that Mo-impregnated catalyst was not as active as normal HZSM-5.

Fig. 5.2 Effect of Si/Al ratio of H-ZSM5 on the yields of pyrolysis products from: (a) *Chlorella vulgaris* and (b) egg whites.
Fig. 5.3 Effect of metal impregnation in H-ZSM5 (30) on the aromatic yield from: (a) Chlorella vulgaris and (b) egg whites.

between 100-300 psi; higher aromatic yield was obtained for Mo-ZSM-5 when the pressure increased to 400 psi.
5.4 Conclusions

A series of zeolites were evaluated for their influence on the aromatic production performance of pyrolysis of *Chlorella* and egg whites. The H-ZSM5 had the highest aromatic yield of 18.13% among the three zeolites (H-ZSM5, H-Beta and H-Y) with different crystal structures. For H-ZSM5, the maximum aromatic yield of *Chlorella* was obtained at the Si/Al ratio of 80. The impregnation of copper or gallium to HZSM-5 (30) increased the aromatic yield from 16.72% to 21.16% and 18.03% respectively, suggesting that some transition metals can promote the aromatization function of H-ZSM5.
CHAPTER 6 EFFECTS OF HYDROTHERMAL PRETREATMENT ON PYROLYTIC BIO-OIL PRODUCTION FROM MICROALGAE

A paper published in Bioresource Technology

6.1 Introduction

Various conversion routes have been developed for the production of liquid fuels from biomass (Huber et al., 2006). Among those technologies, pyrolysis and hydrothermal liquefaction are two prevailing thermochemical processes which convert biomass into liquid fuels commonly named bio-oils (Mohan et al., 2006; Toor et al., 2011). Although there has been extensive research conducted on a wide variety of terrestrial biomass resources using these two technologies, few studies have investigated microalgae. Pyrolysis decomposes dry algal biomass into condensable vapors under inert atmosphere at 450–600 °C. Algal pyrolytic liquids separate into two phases with the top phase called bio-oil (Campanella et al., 2012; Du et al., 2011; Jena et al., 2011a). The higher heating values (HHV) of algal bio-oil are in the range of 31–36 MJ/kg, generally higher than those of lignocellulosic feedstocks. In contrast to pyrolysis, hydrothermal liquefaction can be applied to wet biomass. By converting the biomass slurry in pressurized vessels, enthalpic energy losses can be avoided by eliminating the need to dry the biomass before conversion (Biller et al., 2011; Brown et al., 2010; Zou et al., 2010). Microalgae conversion experiments were usually carried out in a batch reactor in sub- or super-critical water (i.e. 300–400 °C and 15–30 MPa). After cooling, the bio-oil was usually separated from the large amount of water involved in the reaction using organic solvents which were subsequently removed through evaporation. The HHVs of the bio-oils under these conditions were in the range of 33–39 MJ/kg.
Pyrolytic bio-oil consists of compounds with lower mean molecular weights and contains more low boiling compounds than bio-oil produced by hydrothermal liquefaction. These properties are similar to those of Illinois shale oil (Jena et al., 2011a; Vardon et al., 2012), which may indicate that pyrolytic bio-oil is suited for petroleum fuel replacement. However, hydrothermal liquefaction showed a more favorable energy balance due to energetic losses by water volatilization in pyrolysis. In addition, the high protein content in microalgae led to a high N content in the bio-oil, resulting in undesirable NOx emissions during combustion and deactivation of acidic catalysts when co-processed in existing crude oil refineries.

Since N is an essential element for algal growth, recycling of this nutrient will be important to achieving sustainable algal feedstock production. Hydrothermal hydrolysis has been explored as an extraction method for proteins and amino acids from biomass (Lamoolphak et al., 2006; Sereewatthanawut et al., 2008). The process temperature was in the range of 150–250 °C, lower than that for hydrothermal liquefaction, and the hydrolysates were suitable for yeast growth. Therefore, a hydrothermal pretreatment (HP) step is proposed, prior to pyrolysis of the algae for algal bio-oil production. This approach may provide the following benefits: (1) HP does not need significant energy inputs compared with direct thermal drying; (2) HP could hydrolyze the protein into peptides and amino acids, which would stay in the aqueous phase and thus reduce the N content in the pretreated algal feedstock; (3) pretreated algae with lower N would lead to a pyrolytic bio-oil with lower N; (4) the hydrolysates have the potential of being recycled back for algae cultivation. In the current study, reaction temperature and time were investigated for their effects on the composition of microalgae in HP. In addition, the
pyrolysis products of untreated and pretreated algae were compared to evaluate the effect of HP on the quality of the bio-oils.

6.2 Materials and methods

6.2.1 Materials

*Nannochloropsis oculata* slurry (80% moisture content) was purchased from Reed Mariculture, Inc. (Campbell, CA), stored in a refrigerator at 4 °C, but was used within three months before spoilage occurred. The main characteristics of dry *Nannochloropsis oculata* are listed in Table 6.1.

<table>
<thead>
<tr>
<th>Biochemical composition (wt.%)</th>
<th>Elemental analysis (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>39</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>20</td>
</tr>
<tr>
<td>Lipids</td>
<td>17</td>
</tr>
<tr>
<td>Ash</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated by difference, \( O(\%) = 100 - (C + H + N + Ash) \).

6.2.2 Apparatus and experimental procedure

Six-inch long T316 stainless steel tubes (3/8 inch outside diameter) with one plugged and one capped end were used as the batch reactors. Each reactor was loaded with 5.0 g of algal slurry (1.0 g on a dry basis) and flushed with nitrogen to ensure an inert atmosphere before sealing. The reactors were inserted in a preheated Techne IFB-52 sand bath (Burlington, NJ) which can provide fast, uniform and stable heat transfer. After a pre-determined period of time, the reactors were removed from the sand bath and quenched in a cold water bath to terminate the reactions. The total residence time in the
tubular reactors spanned the time period from inserting reactors in the sand bath until quenching. Upon cooling, the reactors were opened and weighed after the gas was released. The weight of gaseous products was recorded as the total weight difference of reactor and biomass before and after the reaction. The inner chambers, caps and plugs were rinsed with 50 mL deionized water to ensure complete collection of adhering products. The reaction mixtures and rinsates were filtered through a Whatman 0.45 µm glass fiber filter and the pretreated algae solids were freeze-dried to remove the remaining water. The pretreated algae samples were weighed before and after drying to determine the moisture content. The weight of the aqueous phase products was calculated according to mass balance, i.e.

\[
\text{Weight of aqueous phase products} = \text{Weight of algae before treatment} - \text{Weight of gaseous products} - \text{Weight of pretreated algae}
\]

### 6.2.3 Analysis of products

Untreated and pretreated algae were analyzed for their C, H and N contents with an Exeter AnalyticalCE-440 Elemental Analyzer (Chelmsford, MA). Ash content was determined by weighing the residues after complete combustion of the biomass in a furnace at 550 °C. All measurements were performed in duplicate and the mean values are reported. HHVs were calculated according to the following equation (Friedl et al., 2005):

\[
HHV (MJ/kg) = (3.55 \times C^2 - 232 \times C - 2230 \times H + 51.2 \times C \times H + 131 \times N + 20600) \times 10^{-3}
\]
Total organic carbon and nitrogen in the aqueous phase were measured using Hach analysis kits on a Hach 5000 Spectrophotometer. Lipids in untreated and pretreated algae were analyzed to determine the fatty acid retention after HP. Fatty acid composition and content were analyzed using acid-catalyzed in-situ transesterification (Indarti et al., 2005). Dried untreated and pretreated algae (ca. 0.1 g) were weighed in 25-ml screw-top glass tubes, and 10 ml of a mixture of methanol, concentrated sulfuric acid and chloroform (volume ratio 4.25:0.75:5) was added. The glass tubes were sealed and placed into a 90 °C water bath for 90 min. Upon cooling, the tubes were shaken and centrifuged at 7000 rpm for 5 min after adding 3 ml of distilled water into the reaction mixture. The chloroform layer containing fatty acid methyl esters (FAME) was carefully collected and subjected to gas chromatography-mass spectrometry (GC-MS). An Agilent 7890-5975C GC-MS with a HP-5MS (30 m × 0.25 m × 0.25 µm) capillary column was used for FAME analysis. Helium was employed as carrier gas at a flow rate of 1.2 mL/min. The initial oven temperature was 80 °C. After 1 min the temperature was increased to 290 °C at a rate of 4 °C /min, and held at 290 °C for 5 min, while the injector and detector were maintained at constant temperature of 250 °C and 230 °C, respectively. Compounds were identified with the National Institute of Standards and Technology (NIST) mass spectral data library and quantified with external standard calibrations of C14–C22 standards (Sigma-Aldrich). Each reaction was conducted in duplicate and average values of results are reported.

Equilibrium moisture contents (EMC) of untreated and pretreated algae were measured using the static desiccator method (Bellur et al., 2009). Solid samples were exposed to an environment with constant relative humidity and temperature over a period
of time, until the moisture content in the samples reached equilibrium. The humidity in
the chamber was maintained at a constant value by keeping the air in equilibrium with
saturated potassium acetate, magnesium nitrate and sodium chloride solutions, to achieve
humidity levels of 22.5, 52.9 and 75.3% at 30 °C.

The micro-structures of untreated and pretreated algae were analyzed with a Hitachi
scanning electron microscope (SEM, model S3500N) at the Imaging Center of University
of Minnesota, Twin Cities. Samples were placed in 2% gluteraldehyde and 0.1 M
sodium cacodylate buffer for 2 h, rinsed in buffer, then placed in 1% osmium tetroxide
and 0.1 M sodium cacodylate buffer for 2 h. Specimens were rinsed in ultrapure water
and dehydrated in an ethanol series. After the samples were in 100% ethanol, they were
suspended in two changes of hexamethyldisilazane (HMDS) for 5 min each. Drops of
the suspension were placed on individual round glass coverslips mounted on aluminum
stubs, allowed to dry, and sputter-coated with gold-palladium.

Pyrolysis experiments were performed using an analytical pyrolyzer coupled with a
GC-MS (Py-GC-MS). About 0.5 mg of untreated or pretreated algae was filled into a
quartz tube, inserted into a platinum coil, and pyrolyzed in a CDS2000 pyrolyzer.
Samples were heated at a heating rate of 1000 °C/s to 500 °C and then isothermally for
30 s to ensure complete pyrolysis. Upon pyrolysis, the pyrolysis vapors were directly
swept into the Varian 3400 CX-Saturn 3 GC-MS with a DB-5MS (30 m × 0.25 m × 0.25
µm) column. An injector temperature of 250 °C and a split ration of 1:100 were used.
The initial oven temperature was 40 °C. After 3 min, the temperature was increased to
250 °C at a rate of 5 °C /min, and held at 250 °C for 10 min.
6.2.4 Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA, at 0.05 significance level) using software SPSS 18.0.

6.3 Results and discussion

6.3.1 Pretreated algae yields and elemental composition

The yields of the pretreated algae under different temperatures and reaction times are shown in Fig. 6.1. Two-way ANOVA revealed that the pretreated algae yield was significantly influenced by temperature \((p<0.05)\) and time \((p<0.05)\) with no interaction between the two \((p>0.05)\). The mass of recovered pretreated algae dropped significantly with increasing temperatures and treatment times, although pretreated algae yield started to level off after 30 min of reaction. These results are consistent with those from other studies involving microalgae and lignocellulosic materials (Heilmann et al., 2010; Hoekman et al., 2011).
Fig. 6.1 Effects of temperature and reaction time on the yields of pretreated algae.

As shown in Table 6.2, higher carbon contents and energy densifications were achieved with increasing temperatures and time as a consequence of carbonization resulting from the removal of oxygen by dehydration and decarboxylation (Funke et al., 2010). In all cases, the heating values of pretreated algae increased between 22–95% compared with those of the untreated algae. These increases are comparable to those from other reports (Heilmann et al., 2010; Yan et al., 2009). The N retention in the pretreated algae decreased with increasing process severity, possibly due to the hydrolysis of proteins and nucleic acids. The N remaining in the pretreated algae could be from hydrophobic peptides, amino acids or Maillard reaction products, as the pretreated algae had a rich brown color. The organic C and N in the water phase could be recycled for algal. Fig. 6.2 shows the C and N distribution in the products of HP at 200 °C for 40 min. C in algal proteins could be lost in the form of water-soluble peptides and amino acids. Some carbohydrates, like starch, could be hydrolyzed to monosugars and oligosugars under hydrothermal conditions, and some C could be removed with the water. Also, CO₂ could be produced because of decarboxylation reactions. The ash content in the pretreated algae was greatly reduced, which could be due to the “wash out” effects of water on the minerals. This phenomenon is important because the high ash content in microalgae can cause slagging and fouling problems in thermochemical conversions (Ross et al., 2008).
Table 6.2 Comparison of the elemental composition between untreated and pretreated algae

<table>
<thead>
<tr>
<th>Pretreated Temperature (°C)</th>
<th>Time (min)</th>
<th>Elemental composition (%)</th>
<th>HHV(MJ/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C  H  N  Ash  C retention  N retention</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td>39.9 5.5 6.2 24.4</td>
<td>16.8</td>
</tr>
<tr>
<td>150</td>
<td>10</td>
<td>51.0 7.0 8.3 14.4 81.6</td>
<td>84.9 21.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>51.0 7.0 8.0 14.3 83.9</td>
<td>84.5 21.7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>51.1 6.9 7.8 12.4 74.3</td>
<td>73.0 21.7</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>50.5 6.9 7.7 14.7 77.7</td>
<td>76.2 21.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>51.3 7.0 7.7 14.6 75.9</td>
<td>73.1 21.8</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>52.0 7.0 7.6 12.8 77.8</td>
<td>73.2 22.1</td>
</tr>
<tr>
<td>175</td>
<td>10</td>
<td>49.9 6.9 7.0 18.8 64.0</td>
<td>57.8 21.0</td>
</tr>
<tr>
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<td>20</td>
<td>52.7 7.2 6.7 17.2 60.7</td>
<td>49.5 22.4</td>
</tr>
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<td>42.4 22.9</td>
</tr>
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<td>48.3 20.5</td>
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<tr>
<td></td>
<td>20</td>
<td>54.0 7.1 5.7 16.7 49.5</td>
<td>33.5 23.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>51.1 6.8 5.8 19.9 48.4</td>
<td>35.1 21.4</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>57.5 7.6 5.1 13.6 44.8</td>
<td>25.7 25.1</td>
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<tr>
<td></td>
<td>50</td>
<td>56.9 7.6 4.8 13.5 43.8</td>
<td>23.9 24.7</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>54.4 7.2 5.1 12.8 43.4</td>
<td>26.0 23.1</td>
</tr>
<tr>
<td>225</td>
<td>10</td>
<td>51.1 6.8 6.1 18.8 45.8</td>
<td>34.9 21.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>58.2 7.7 5.4 13.9 45.9</td>
<td>27.1 25.6</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>60</td>
<td>67.5 9.1 3.6 13.5 39.6</td>
<td>13.4 32.7</td>
</tr>
</tbody>
</table>
6.3.2 Fatty acid retention after HP

Lipids are desirable compounds in microalgae, because they can be converted to diesel-range hydrocarbons during pyrolysis (Idem et al., 1996; Schwab et al., 1988). Hydrocarbons in the algal pyrolytic bio-oil have been generally considered as the derivatives of lipids (Miao et al., 2004a; Vardon et al., 2012). Therefore, it is important to determine lipid retention (i.e. percentage of lipids in the untreated algae that still retained in the pretreated algae). Since hydrophobic compounds may be produced from proteins and polysaccharides by HP (Torri et al., 2012), lipid quantification by gravimetric analysis of organic solvent-extracts is not accurate. For this reason, the method described in section 2.2 was used for the determination of fatty acids. Detected fatty acids included C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:4 and C20:5. As can be seen in Fig. 6.3, the fatty acid retention ranged from 73 to 99%. Generally, there was a decreasing trend.
for fatty acid retention with increasing temperature, possibly because of greater emulsification between intracellular fatty acids and water when algal cells were more severely destroyed at higher temperatures. Also, polyunsaturated fatty acids could be decomposed to some extent in HP (Holliday et al., 1997). A similarly high lipid retention has been reported for heterotrophically cultivated *Chlorella vulgaris* with 45.6% lipid content (Levine et al., 2010). Although other studies showed that thermal treatment could hydrolyze triglycerides in microalgae into free fatty acids (FFA) (Levine et al., 2010; Torri et al., 2012) and generate undesirable soaps during transesterification, pyrolysis of microalgae after HP did not occur in the current study.

![Fig. 6.3 Fatty acid retention in pretreated algae.](image-url)
6.3.3 Microstructure of algal cell before and after HP

The observed variations in yield and lipid retention under different reaction conditions could be related to structural changes of the algal cells, considering that intracellular compounds may become more accessible when the cell wall is destroyed. Fig. 6.4 shows SEM images of algal cells obtained before and after HP. Cells exposed to 150 °C/50 min were intact and their morphology was very similar to that of untreated algae. At 200 °C, cells started to cluster and compact, although some cells were still recognizable. Cell deformation and compaction became stronger with time, which could lead to a greater release of intracellular compounds. At 225 °C, an abrupt cellular morphological change occurred and individual cells could no longer be observed even when cells were exposed to this temperature for only 10 min. These results could explain the decreasing yield of pretreated algae and lipid retention with increasing process severity, as greater cell wall breakage occurred and more intracellular materials decomposed and were released. More accessible intracellular proteins and polysaccharides could be hydrolyzed and dissolved in the aqueous phase. Lipids, although insoluble in water, can be hydrolyzed to FFAs, which are easier to be emulsified, leading to a lower lipid retention in pretreated algae.
6.3.4 EMC analysis

Previous studies have suggested that hydrothermally pretreated biomass is relatively hydrophobic (Acharjee et al., 2011). The hydrophobicity of solids can be characterized with EMC, which is defined as the moisture content in the biomass in equilibrium with that in the surroundings at a certain temperature and relative humidity. The EMCs of freeze-dried untreated and pretreated algae of 200 °C/40 min and 225 °C/40 min were analyzed in this study. Fig. 6.5 shows that pretreated algae was more hydrophobic than
untreated algae and the difference was more evident at higher relative humidity. The EMC of pretreated algae decreased with increasing pretreatment temperatures, perhaps because greater cell wall degradation reduced moisture absorption by hydrogen bond formation with hydrophilic hydroxyl groups of cell wall components (Andersson et al., 1989). The relatively hydrophobic pretreated algae could be beneficial since these algae can be easily dried after filtration. The dry pretreated algae can be stored with less biological deterioration to solve the seasonal availability problem, especially in northern regions where climate dependent year-around algal production facilities are impractical and transportation of the dry biomass would be less expensive.

Fig. 6.5 EMC of untreated and pretreated algae. Each data point represents the average value of duplicate measurements.
6.3.5 Energy balance

The energy consumption ratio (ECR), which was defined as the ratio of energy required in a process to the available energy of the products, was calculated to determine the process energy balance.

For HP,

\[
ECR_{HP} = \frac{W_1 C_{pw} T + (1 - W_1) C_{pa} T + W_2 C_{pw} \times 75^\circ C + W_2 L [1 - HR]}{Y Q_1 (1 - W_1) R}
\]

For thermal drying,

\[
ECR_{\text{combustion}} = \frac{W_1 C_{pw} \times 75^\circ C + W_2 L [1 - HR]}{Q_2 (1 - W_1) R}
\]

where \( W_1 \) is the initial moisture content of the algae slurry (80%), \( T \) is the temperature increase (assume an initial temperature of 25 °C), \( C_{pw} \) and \( C_{pa} \) are the specific heats of water and algae (4.18 kJ/kg/K and 1.00 kJ/kg/K assumed for algae according to Grierson et al. (2009)), \( HR \) and \( R \) are the efficiencies of the heat recovery after the reaction and combustion energy (assumed to be 0.5 and 0.7 respectively (Minowa et al., 1998)), \( W_2 \) is the residual moisture content in pretreated algae after filtration, \( L \) is the latent heat for water vaporization, \( Y \) is the yield of pretreated algae, \( Q_1 \) is the HHV of pretreated algae and \( Q_2 \) of untreated algae.

For HP at 200 °C for 40 min, \( W_2 \) is around 37% and the \( ECR_{HP} \) value is 0.31, compared with 0.44 for direct drying of algae. The ECR values less than 1 indicate that both processes had a net energy gain. In addition, HP shows more favorable results than direct drying of algae, because of the large amount of heat required for water removal.
6.3.6. Py-GC-MS

Py-GC-MS was used to study the differences between pyrolytic bio-oils derived from untreated and pretreated algae. Consistent with the results of the elemental analysis, the effects of hydrothermal pretreatment were more obvious under more severe conditions. Pyrolytic products from pretreated algae obtained at 150 °C and 175 °C (less severe conditions), although displaying some differences from those of original algae, still showed many N-containing compounds. However, pyrolytic products from more severe conditions (temperature 200 °C or above, time 30 min or above) showed very similar product profiles dominated by long chain fatty acids and hydrocarbons. The total ion chromatograms of untreated and pretreated algae obtained at 200 °C with 40 min reaction time are shown in Fig. 6.6 in supplementary data and major compounds with >1% peak area are listed in Table 6.3. Although peak area does not represent the actual concentration of each compound, it can illustrate the influence of HP on pyrolytic bio-oil composition.
Table 6.3 Major chemical compounds present in pyrolytic bio-oil of untreated and pretreated algae

<table>
<thead>
<tr>
<th></th>
<th>Untreated algae</th>
<th>Pretreated algae</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RT</strong> (min)</td>
<td>Are a/%</td>
<td>Compound</td>
</tr>
<tr>
<td>34.1</td>
<td>5.1</td>
<td>Pentadecanenitrile</td>
</tr>
<tr>
<td>22.9</td>
<td>4.8</td>
<td>1-Decanol, 2-methyl-</td>
</tr>
<tr>
<td>32.9</td>
<td>4.4</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
</tr>
<tr>
<td>1.6</td>
<td>4.1</td>
<td>L-Alanine, N-methyl-</td>
</tr>
<tr>
<td>20.2</td>
<td>4</td>
<td>Indole</td>
</tr>
<tr>
<td>39.3</td>
<td>3.9</td>
<td>Octadecanamide</td>
</tr>
<tr>
<td>33.7</td>
<td>3.7</td>
<td>9, 17-Octadecadienal</td>
</tr>
<tr>
<td>38.9</td>
<td>3.4</td>
<td>cis-11-Eicosenamide</td>
</tr>
<tr>
<td>29.8</td>
<td>2.9</td>
<td>2-Cyclohexen-3-ol-1-one, 2-[11-tetradecenoyl]-</td>
</tr>
<tr>
<td>20.3</td>
<td>2.7</td>
<td>Indolizine</td>
</tr>
<tr>
<td>4.5</td>
<td>2.6</td>
<td>Toluene</td>
</tr>
<tr>
<td>25.4</td>
<td>2.3</td>
<td>Pentadecane</td>
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<tr>
<td>38.2</td>
<td>2.3</td>
<td>Oleanitrile</td>
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<td>33</td>
<td>1.9</td>
<td>1,9-Tetradecadiene</td>
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<td>27.6</td>
<td>1.9</td>
<td>1-Dodecanol, 3,7,11-trimethyl-</td>
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<tr>
<td>25.2</td>
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<td>7-Tetradecene</td>
</tr>
<tr>
<td>48.7</td>
<td>1.6</td>
<td>9,17-Octadecadienal, (Z)-</td>
</tr>
<tr>
<td>30.1</td>
<td>1.6</td>
<td>1,4-Benzenedioli,2,5-dimethyl-</td>
</tr>
<tr>
<td>18.7</td>
<td>1.6</td>
<td>Benzenepropanenitrile</td>
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<tr>
<td>27.7</td>
<td>1.6</td>
<td>1-Dodecanol, 3,7,11-trimethyl-</td>
</tr>
<tr>
<td>22.7</td>
<td>1.5</td>
<td>Z-10-Pentadecen-1-ol</td>
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<tr>
<td>27.3</td>
<td>1.3</td>
<td>Pyrrolizidine-3-one, 5-acetyl methyl-</td>
</tr>
<tr>
<td>17.3</td>
<td>1.3</td>
<td>1-Teidecene</td>
</tr>
<tr>
<td>Compound</td>
<td>Retention time</td>
<td>Peak Area</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------</td>
<td>-----------</td>
</tr>
<tr>
<td>5,8,11,14,17-Eicosapentaenoic acid</td>
<td>44.9</td>
<td>1.3</td>
</tr>
<tr>
<td>2-Piperidinone</td>
<td>17.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>37.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Hydroxylamine, O-decyl-</td>
<td>17.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Fig. 6.6 GC-MS profile of bio-oil from (a) untreated algae and (b) pretreated algae obtained at 200 °C with 40 min reaction time
Consistent with other reports on pyrolysis of microalgae (Campanella et al., 2012; Du et al., 2011; Vardon et al., 2012), pyrolytic bio-oil of untreated algae contained very complex compounds, including hydrocarbons, alcohols, fatty acids, N-containing compounds, etc. Compounds, such as indole, 2-piperidinone and indolizine, are typical derivatives of protein pyrolysis (Moldoveanu, 1998); however, they were not found in the pyrolytic bio-oil of pretreated algae, which indicates that proteins resulting in the formation of these compounds were removed during HP. The bio-oil composition of pretreated algae was much simpler and dominated by long-chain fatty acids that did not decompose to smaller molecules because of fast heating in the pyroprobe. However, fatty acids can be easily converted to hydrocarbons in the presence of catalysts during pyrolysis (Milne et al., 1990; Twaiq et al., 1999). Also, amides of fatty acids were detected, most likely formed by reaction of fatty acids and ammonia released from protein or amino acids in pretreated algae. Overall, pretreated algae produced higher quality pyrolytic bio-oil with reduced percentages of heteroatomic (N) compounds compared with untreated algae. The influence of HP on the overall yield of bio-oil was not investigated in this study because of the small amount of pretreated algae from batch reactions; future studies utilizing scaled-up pyrolysis facilities will be employed to investigate this question.

6.4 Conclusions

Hydrothermally pretreated algae featured increased C contents and HHVs and reduced N and ash contents compared with untreated algae, which is beneficial to further downstream processing such as pyrolysis. More than 70% of the initial lipids were
retained in the relatively hydrophobic pretreated algae. In addition, pyrolytic bio-oil from
pretreated algae with reduced protein content contained less N-containing compounds
and was dominated by long-chain fatty acids which can be readily converted to
hydrocarbons in the presence of catalysts. Therefore, HP is an effective pretreatment
strategy for high-quality pyrolytic bio-oil production from algal biomass.
CHAPTER 7 SUMMARY AND FUTURE WORK

7.1 Summary

The research work presented in this dissertation offers preliminary insight into pyrolysis of microalgae and the three major constituents: carbohydrates, proteins and lipids. As one of the few studies on algae pyrolysis, comprehensive and systematic characterizations of algal bio-oil were carried out. The results showed that algal bio-oil had better qualities in many aspects than those produced from lignocellulosic biomass. For example, algal bio-oil has a higher heating value, a lower oxygen content and a pH value greater than 7. To further elucidate the pyrolysis mechanism of microalgae, cellulose, egg whites, and canola oil were pyrolyzed as the model compounds on a pyroprobe. Several pyrolysis pathways of algal biomass were postulated by analysis and identification of pyrolysis products from the model compounds. Catalytic pyrolysis of microalgae and the model compounds with zeolite catalysts were also investigated to improve the quality of algal bio-oil. Egg whites had the lowest aromatic yield among the model compounds under all reaction conditions, which suggests that proteins can hardly be converted to aromatics with HZSM-5. Another innovative method, i.e. hydrothermal pretreatment (HP), was studied and the results showed that pretreated algae had higher carbon contents and enhanced heating values under all reaction conditions and 6–42% lower nitrogen contents at 200 °C–225 °C for 30–60 min compared with untreated samples. Also, the recovered aqueous nutrients from HP can be recycled for algae cultivation. Based on the results in this dissertation, an integrated algae-based biorefinery was proposed (Fig. 7.1), which includes algal biomass production, hydrothermal
pretreatment, catalytic pyrolysis of microalgae into biofuels, and recycling of the wastewater during conversion as low-cost nutrient source for algae cultivation. The harvested algae are hydrothermally pretreated to reduce the protein contents. The pretreated algae can be converted into high-quality bio-oils via catalytic pyrolysis. Also, the aqueous phase products obtained during hydrothermal pretreatment can be recycled for algae cultivation.

![Diagram of the proposed biorefinery](image)

Fig. 7.1 Proposed biorefinery with bio-oil production and recycling of APP for algae cultivation

### 7.2 Future work

#### 7.2.1 Development of Microwave-assisted fast pyrolysis

Microwave-assisted pyrolysis (MAP) has received interest and is being commercialized for the pyrolysis of plastics, waste tires and rubbers (TekGar, 2012). All the reported processes, including the one in this dissertation, used microwave to directly heat up biomass and this process is considered as slow pyrolysis since the heating rate
was around 50 °C/min. There are many issues with slow pyrolysis, because the slow heating rate can lead to severe secondary cracking reactions which lower the yield of bio-oil. The low heating rate can be an important reason for the low algal bio-oil yield (~25%) in Chapter 3. We are currently developing a continuous fast microwave pyrolysis process. The results from the new system will be compared with those obtained in Chapter 3.

7.2.2 Mechanistic study of catalytic pyrolysis

Although many reaction pathways were postulated in Chapter 4, the evolution of many compounds, especially those nitrogenates, cannot be explained. For examples, nitrogenates, including nitriles and 3-methyl-1H-Indole, increased with the addition of catalysts in Chapter 5. The transformation of pure model components of bio-oil from wood materials were investigated before and these studies provided important insights and basis for bio-oil upgrading (Gayubo et al., 2004a; Gayubo et al., 2004b). However, detailed studies on algae-derived compounds have not been given enough attention. This part of work would be very important for the utilization of algal bio-oil.

7.2.3 Interaction effects between the major components

In both hydrothermal pretreatment and pyrolysis processes, the major components interact with each other and influence the final products. For example, long chain amines were detected in the pyrolysis products which can be the products of reactions between long chain fatty acids and ammonia. Also, the hydrothermally pretreated algae had a rich brown color, which could be because of the Maillard reaction between dissolved amino
acids and sugars. Characterizing these interactions can be very important for understanding the overall process.

7.2.4. Catalytic hydrothermal pyrolysis of wet algal biomass

Instead of using hydrothermal as pretreatment, higher pressure and temperature could be applied in the hydrothermal treatment process in addition to the catalysts to directly liquefy the wet algal biomass into desirable fuels and chemicals. This could reduce the drying cost and prevent the cooling and reheating energy loss in the additional processing/upgrading steps. However, fouling and slow heating rate during hydrothermal liquefaction has been a challenge for its application. Currently a hydrothermal liquefaction using ohmic fast heating method is being developed to address these problems.
BIBLIOGRAPHY


preparation of high-grade bio-oils through the controlled, low temperature microwave activation of wheat straw. Bioresource Technology, 100, 6064-6068.


APPENDIX A Experimental materials, equipment and samples

Fig. A.1 *Chlorella* sp. powder used in the pyrolysis experiments.

Fig. A.2 Algal bio-oil obtained from Microwave-assisted pyrolysis.
Fig. A.3 Hydrothermal pretreatment process.
Fig. A.4 Quartz tube and pyroprobe used in Py-GC/MS experiments.

Fig. A.5 Catalysts used in Py-GC/MS experiments. From left to right: H-ZSM5 (30), Co-ZSM5, Cu-ZSM5, Fe-ZSM5, Ga-ZSM5, Mo-ZSM5, Ni-ZSM5.
Fig. A.6 GC-MS chromatograms of pyrolytic runs at 550 °C on the aromatic yield from different materials with the catalyst to feed ratio of 5:1. (a) *Chlorella vulgaris*; (b) cellulose; (c) egg whites; (d) canola oil.
APPENDIX B Cultivation of a microalga *Chlorella vulgaris* using recycled aqueous phase nutrients from hydrothermal carbonization process

A paper published in *Bioresource Technology*

1. Introduction

Hydrothermal conversion is a process in which biomass is converted to liquids, solids or gases in hot pressurized water. Hydrothermal technologies broadly cover chemical and physical transformations in high-temperature (200–600 °C) and high-pressure (5–40 MPa) water (Peterson et al., 2008). They have energetic advantages for wet biomass such as algae due to the elimination of energy inputs for water removal by evaporation. Many recent studies have shown that a bio-oil with a high heating value can be obtained from the hydrothermal liquefaction (HTL) of microalgae (Biller et al., 2011; Brown et al., 2010; Torri et al., 2012). A large amount of the process water containing carbon, nitrogen, phosphorus and minerals is produced as a co-product in this process. It is expected that these nutrients can be recycled for cultivation of microalgae to enhance the overall economic viability of algal biofuel process. There are very few reports on recycling process water from HTL for algae cultivation. Some previous studies (Biller, 2012; Jena et al., 2011b) compared algae growth on diluted HTL process water and standard media, such as BG-11 and 3N-BBM+V. Algae grew slower and reached a lower final concentration on the diluted process water than on standard media. This is probably due to the high concentration of inhibitors, including nickel, fatty acids, phenols and other toxic compounds produced in HTL under high temperatures ranging from 250–400 °C. Hydrothermal carbonization (HTC) is another technology carried out in compressed water but at lower temperature (subcritical condition, ~200 °C) than HTL. HTC has been
reported to produce energy densified solid fuels (Heilmann et al., 2010) and pretreat biomass for subsequent thermochemical processing, such as pyrolysis and gasification (Du et al., 2012a; Du et al., 2011; Hoekman et al., 2011). Our previous study utilized HTC as a pretreatment step to reduce the nitrogen content by hydrolyzing proteins in microalgae feedstock (Du et al., 2012a). Polysaccharides and proteinaceous materials were hydrolyzed to monosaccharides, and carboxylic acids and amino acids, respectively, under HTC, and the hydrolysates were found suitable for yeast growth (Lamoolphak et al., 2006; Pourali et al., 2009). Many algae species can grow mixotrophically on substrates such as simple carboxylic acids and amino acids as the carbon and nitrogen sources (Perez-Garcia et al., 2011). In this context, the current study was carried out to evaluate the feasibility of recycling process water from HTC as the growth media for cultivation of *Chlorella vulgaris*.

2. Materials and methods

2.1. Characteristics of process water from HTC

The process of HTC was described in details in our previous work (Du et al., 2012a). Process water obtained under 200 °C/40 min was recycled for use in this study. The main characteristics of process water are listed in Table B.1. Chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN), ammonia (NH₄-N), nitrate (NO₃-N), nitrite (NO₂-N) and total phosphorous (TP) of the process water were determined using specific test kits on a Hach DR 5000 Spectrophotometer (Loveland, CO). Metal ion concentrations were analyzed on an inductively coupled plasma atomic emission
spectrometer (Perkin Elmer Optima 3000, Waltham, MA) by Soil Testing Laboratory at University of Minnesota, St Paul.

Table B.1 Characteristics of process water from HTC

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration (mg/L)</th>
<th>Metals</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>134800 ± 2287</td>
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</tr>
<tr>
<td>TOC</td>
<td>45700 ± 1513</td>
<td>Mg</td>
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</tr>
<tr>
<td>TN</td>
<td>9650 ± 1582</td>
<td>Mn</td>
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</tr>
<tr>
<td>Ammonia</td>
<td>1343 ± 75</td>
<td>Fe</td>
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<tr>
<td>Nitrate</td>
<td>211 ± 20</td>
<td>Na</td>
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<tr>
<td>Nitrite</td>
<td>3.63 ± 0.73</td>
<td>B</td>
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<tr>
<td>TP</td>
<td>343 ± 43</td>
<td>Ni</td>
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</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

* Each data point indicates the mean ± standard deviation for three independent measurements; data for metal analysis were the average value of duplicate measurements.

2.2. Algal strain and culture conditions

Algal strain was a wild-type *Chlorella vulgaris* isolated from local freshwater and its selection procedure was described in details in our previous study (Li et al., 2011). The algal strain was enriched in 100 ml BG-11 medium (Stanier et al., 1971) with 2 g/L glucose to obtain enough starting cultures for this study. Since the nutrient levels were too high for microalgae to survive, the process water was diluted to 50×, 100× and 200× times’ volume with distilled water. BG-11 medium was used as the control to evaluate the growth efficiency of *C. vulgaris* on diluted process water. Algae were inoculated with the starting dry weight of 0.15 g/L in 250 mL Erlenmeyer flasks containing 150 mL autoclaved medium. The flasks were placed on a shaker with 100 rpm rotation speed. All cultures were kept at 25 ± 2 °C under continuous cool-white fluorescent light.
illumination at 100 µmol m\(^{-2}\)s\(^{-1}\). Each growth condition was carried out in triplicates and a fourth culture was used to supplement the medium after sampling.

2.3. Algae growth and chemical analysis

Algal growth was determined daily by measuring total volatile suspended solids (TVSS) using 4 mL algae suspension collected from each flask. The biomass productivity was calculated from the equation:

\[
P \left( g \, L^{-1} \, day^{-1} \right) = \frac{(TVSS - TVSS_0)}{t}
\]

where \( t \) (day) was the time between the two measurements, TVSS and TVSS\(_0\) were the concentration of biomass at day \( t \) (growth curve leveling off) and day 0, respectively. A one-way analysis of variance (ANOVA, at 0.05 significance level) and the least significant difference method (LSD) was carried out for the statistical analysis of algal growth on the four different media.

A volume of 8 mL algae suspension was collected daily from each flask for nutrient analysis. The samples were centrifuged at 7000 rpm for 10 min and supernatants were collected and diluted to suitable concentrations for analysis. TN, TP and COD were measured using test kits on a Hach DR 5000 Spectrophotometer.

Algae harvested at the end of the 5-day batch culture were analyzed for their C, H and N contents with an Exeter Analytical CE-440 Elemental Analyzer (Chelmsford, MA).

Fatty acid content and composition were analyzed using acid-catalyzed in-situ transesterification method (Indarti et al., 2005). Dried algae (ca. 0.05 g) were weighed in
25-ml screw-top glass tubes, and 10 ml of a mixture of methanol, concentrated sulfuric acid and chloroform (volume ratio 4.25:0.75:5) was added. The glass tubes were sealed and placed into a 90 °C water bath for 90 min. Upon cooling, the tubes were shaken and centrifuged at 7000 rpm for 5 min after adding 3 ml of distilled water into the reaction mixture. The chloroform layer containing fatty acid methyl esters (FAME) was carefully collected and subjected to gas chromatography-mass spectrometry (GC-MS). An Agilent 7890-5975C GC-MS with a HP-5MS (30 m × 0.25 m × 0.25 µm) capillary column was used for FAME analysis. The carrier gas was helium at a flow rate of 1.2 mL/min. The oven temperature was initially 80 °C for 1 min, then increased to 290 °C at a rate of 4 °C /min, and held at 290 °C for 5 min. The injector and detector were maintained at constant temperature of 250 °C and 230 °C, respectively. Compounds were identified with the National Institute of Standards and Technology (NIST) mass spectral data library and quantified with external standard calibrations of C14–C22 FAME standards (Sigma-Aldrich).

3. Results and discussion

3.1. Algal growth

Fig. B.1 shows the growth curves for the four different media. The *C. vulgaris* could survive in all media as evidenced by the increase of biomass concentration. The biomass productivities were 0.013, 0.160, 0.092 and 0.054 g L⁻¹ d⁻¹ for BG-11, 50×, 100× and 200× process water, respectively. Algae had significant higher productivities and biomass concentrations on the three dilutions of process water than BG-11 medium. Different from HTL carried out at high temperatures with many growth inhibitors produced,
polysaccharides and proteins were mainly hydrolyzed to mono-sugars and amino acids in HTC. These mono-sugars and amino acids provided adequate carbon and nitrogen nutrients which can be readily used by algae. However, algae need to sequester CO₂ as the sole carbon source when grown on inorganic BG-11 medium photoautotrophically. Many reports showed that mixotrophic growth can result in higher biomass production than phototrophic growth (Bhatnagar et al., 2011; Zhou et al., 2012).

Fig. B.1 (a) Growth curves for algae grown on the four different media; (b) pH values.

3.2. Nutrient removal
Among the three dilutions of the process water, both biomass productivity and final biomass concentration were in the following order: $50\times > 100\times > 200\times$. This indicates that algae can endure the higher concentration of potential growth inhibitors in the more concentrated process water. It is noticed that algae grew rapidly in the first 4 days and then decreased significantly on the fifth day on the $50\times$ process water. However, algae grew at a lower rate on the $100\times$ and $200\times$ process water and then leveled off after the third day. The stationary phase occurred so early mainly due to the limitation of phosphorus which will be discussed later.

Variations of TN, COD and TP in different media for the 5-day batch culture are depicted in Fig. B.2. TN was significantly reduced by 45.5-59.9% on different dilutions of process water. As shown in Table B.1, most of the nitrogen in the process water was in the form of soluble organic nitrogen. This again indicates that algal proteins were hydrolyzed into simple amino acids during HTC, since algae can only use ammonia, nitrate and simple organic nitrogen, such as urea and amino acids as the nitrogen source (Barsanti et al., 2006). However, only 5.4% of TN was used in BG-11 medium, which coincides with the slight increase of algal biomass concentration under autotrophic growth. CODs also decreased drastically by 50.0-60.9% in the process water. This indicates that TOCs hydrolyzed from polysaccharides and proteins can be efficiently used by algae. A significant reduction (85.8-94.6%) of TP was achieved for all of three diluted process water media. Since the concentration of TP was relatively low compared with other nutrients, it was quickly consumed in the first two days of growth. For green algae, the suitable N/P ratio should be in the range of 5:1 to 12:1 so that both N and P can be efficiently used (Li et al., 2010). However, the N/P ratio was 28:1, and P concentration
reached to the range of 1.96 to 6.23 mg/L, leading to a P starvation condition. The depletion of the TP was at the end of the cultivation period could be the main reason that biomass growth leveled off.

Fig. B.2 Nutrient removal profiles. (a) TN removal; (b) COD removal; (c) TP removal.
3.3. Lipid and elemental compositional analysis

Fatty acids in algae can be converted to biodiesel via transesterification reaction (Li et al., 2011) or pyrolyzed into simple hydrocarbons (Du et al., 2012a). Thus, it is very important to determine the fatty acid content of algae grown on recycled process water. Table B.2 shows the fatty acid profiles of algae grown on different media.

Table B.2 FAME profile for algae cultivated in different media.

<table>
<thead>
<tr>
<th>FAME composition</th>
<th>BG-11</th>
<th>50×</th>
<th>100×</th>
<th>200×</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fatty acids subtotal (% of total FAME)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>34.5</td>
<td>24.5</td>
<td>25.2</td>
<td>22.5</td>
</tr>
<tr>
<td>18:0</td>
<td>0.2</td>
<td>1.4</td>
<td>1.0</td>
<td>0.4</td>
</tr>
<tr>
<td>20:0</td>
<td>0.7</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:0</td>
<td></td>
<td></td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>24:0</td>
<td></td>
<td></td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>Monoenoic fatty acids subtotal (% of total FAME)</td>
<td>5.4</td>
<td>11.9</td>
<td>9.0</td>
<td>10.0</td>
</tr>
<tr>
<td>16:1</td>
<td>1.3</td>
<td>5.5</td>
<td>4.4</td>
<td>4.9</td>
</tr>
<tr>
<td>18:1</td>
<td>4.1</td>
<td>6.4</td>
<td>4.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Polyenoic fatty acids subtotal (% of total FAME)</td>
<td>59.9</td>
<td>61.5</td>
<td>61.5</td>
<td>67.1</td>
</tr>
<tr>
<td>16:2</td>
<td>1.9</td>
<td>8.5</td>
<td>6.8</td>
<td>7.0</td>
</tr>
<tr>
<td>16:3</td>
<td>11.1</td>
<td>5.1</td>
<td>7.1</td>
<td>10.5</td>
</tr>
<tr>
<td>18:2</td>
<td>17.6</td>
<td>28.7</td>
<td>23.5</td>
<td>23.9</td>
</tr>
<tr>
<td>18:3</td>
<td>29.3</td>
<td>17.5</td>
<td>22.2</td>
<td>24.0</td>
</tr>
<tr>
<td>20:5</td>
<td>1.7</td>
<td>1.8</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Total (% of TVSS)</td>
<td>4.5</td>
<td>11.2</td>
<td>11.2</td>
<td>9.7</td>
</tr>
</tbody>
</table>

The FAME yields for algae grown on the process water were 11.2% (50×), 11.2% (100×) and 9.7% (200×), which were significantly higher than that of 4.5% for BG-11.
This is probably because microalgae were in the exponential phase on BG-11 medium when harvested at the fifth day, but lipid accumulation mainly happens at the stationary phase for algae. The fatty acid composition of algae is very similar among all these media. Hexadecanoic acid (C16:0), octadecadienoic acid (C18:2) and octadecatrienoic acid (C18:3) were the most abundant fatty acids under all conditions. Long chain fatty acids (C22:0 and C24:0) were detected in 100× process water in small quantities. Algae cultivated on BG-11 contained higher amount of saturated fatty acids and lower monoenoic fatty acids than those on the process water.

The elemental analysis results of the algae are shown in Table B.3. The elemental composition of the algae had no significant difference among the three dilution multiples of the process water, which is because the nutrient ratios (C: N: P) were the same, although their concentrations were different. Algae grown on the process water were higher in carbon and hydrogen and lower in nitrogen contents. This is in accordance with the lipid analysis results, since carbon denser products such as lipids will lead to higher carbon content. Also, lower nitrogen content indicates that algae grown on the process water had lower protein content. These properties are desirable for fuel application since high lipid and low protein will lead to better fuel quality and simpler downstream processing needs.
Table B.3 Elemental composition of algae cultivated in different media.

<table>
<thead>
<tr>
<th>Elemental composition (%)</th>
<th>BG-11</th>
<th>50×</th>
<th>100×</th>
<th>200×</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>42.9 ± 0.1</td>
<td>51.0 ± 2.6</td>
<td>49.9 ± 0.4</td>
<td>50.8 ± 1.3</td>
</tr>
<tr>
<td>H</td>
<td>6.1 ± 0.0</td>
<td>7.4 ± 0.2</td>
<td>7.2 ± 0.1</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>N</td>
<td>9.7 ± 0.8</td>
<td>9.0 ± 0.6</td>
<td>8.9 ± 0.3</td>
<td>8.8 ± 0.1</td>
</tr>
</tbody>
</table>

4. Conclusions

Algae grew much faster and achieved significantly higher biomass concentrations on the process water from HTC than those on BG-11 medium. 45.5-59.9% of TN, 85.8-94.6% of TP and 50.0-60.9% of COD were utilized by algae, on differently diluted process water. The results proved the feasibility and great potential of using the process water from HTC. The recycling of the process water for algae production could significantly increase the overall nutrient efficiency and reduce the production cost for the algae based biorefineries.