

THERMOCHEMICAL CONVERSION OF MICROALGAL BIOMASS FOR PRODUCTION OF BIOFUELS AND CO-PRODUCTS

by

UMAKANTA JENA

(Under the Direction of Keshav C. Das)

ABSTRACT

High oil prices, global warming, and emphasis on renewable technology are attracting new interest in a potentially rich source of biofuels, microalgae. Enormous potential of microalgae for generation of liquid transportation fuels (biodiesel, bioethanol and biocrude/ BioOil) is due to their high growth rate, ability to sequester CO₂ and the potential for producing lipids. However, low cell concentration, high harvesting and drying costs, and challenges in production of higher lipid strains are major hurdles in the choice of converting algae biomass into biofuels. In this study, we choose and compare two important thermochemical conversion processes for production of biocrude (BioOil) from a low lipid microalgae: thermochemical liquefaction (TCL) and pyrolysis. TCL is the wet conversion of biomass in hot compressed water whereas pyrolysis is the conversion of dry biomass at moderate temperature and atmospheric pressure. The objectives were to, 1) demonstrate the effect of operating temperature, holding time and solids concentration on product yield and characteristics in TCL process, 2) study the effect of addition of catalysts on biocrude yield and fuel properties, 3) evaluate and compare TCL with a conventional slow pyrolysis process, and 4) recycle the aqueous phase co-product (ACP) from TCL and evaluate it for algae cultivation.

TCL and pyrolysis experiments were conducted using batch type reactors and analyses were done by standard laboratory methods. TCL runs were performed at five levels of temperature, holding time, and solids concentration. Catalytic experiments were carried out using Na_2CO_3 , NiO , and $\text{Ca}_3(\text{PO}_4)_2$ catalysts at 300-350°C temperature and 30-60 min holding time. Pyrolysis runs were conducted at two temperatures 350°C and 500°C and 60 min holding time. Non-catalytic TCL reported a maximum biocrude yield of ~40%, which was improved to ~51% with Na_2CO_3 catalyst. Pyrolysis resulted in 23-29% biocrude yield compared to that of 40% by TCL process and even needed more energy than TCL. Biocrude from algae had energy content of $\sim 35 \text{ MJ kg}^{-1}$ which was ~82% of the fossil fuel. Nutrient balance showed that ~75% nitrogen and 29% phosphorous of raw algae were recovered in the ACP by TCL process. With the goal of recycling N and P, experiments were conducted in 250 mL flasks to evaluate the growth of *Chlorella minutissima*, a wastewater alga using ACP as growth medium. ACP at 500 dilutions reported a biomass growth of 50.5% of the BG11 growth medium. Based on the mass balance an integrated algae biorefinery was proposed for production of biofuels and value added co-products from algae along with carbon sequestration, nutrient recycling and global greenhouse gas reduction based on this study. The results obtained from this research may be used in further research and development of algae biofuels and co-products by the research communities and industries.

INDEX WORDS: Microalgae, Thermochemical liquefaction (TCL), Pyrolysis, Catalyst, Biofuels, Aqueous co-product (ACP), Nutrient cycling, Biorefinery

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UMAKANTA JENA

B.S., Orissa University of Agriculture and Technology, India, 2001

M.S., Indian Institute of Technology, India, 2003

A Dissertation Submitted to the Graduate Faculty of the University of Georgia in
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UMAKANTA JENA

Major Professor: Keshav C. Das

Committee: James R. Kastner
Sudhagar Mani

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2011

DEDICATION

To my parents and family members

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“It is the mark of an educated mind to be able to entertain a thought without accepting it”

-Aristotle

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FOREWORD

The goal of this research was to produce liquid fuel „biocrude“ (also known as „BioOil“) from microalgae biomass via thermochemical conversion pathways. Thermochemical liquefaction (TCL) and pyrolysis convert organics in the biomass into high energy dense biocrude through a series of chemical reactions. TCL converts high moisture biomass at moderate temperature and high pressure whereas pyrolysis converts dry biomass at moderate to high temperature and atmospheric pressure. Specific objectives within the scope of this dissertation are to: 1) to evaluate the effect of operating temperature, holding time and solids concentration on product yield and characteristics in TCL process, 2) to study the effect of addition of catalysts on biocrude yield and fuel properties, 3) to evaluate and compare TCL with a conventional slow pyrolysis process, and 4) to recycle the aqueous phase co-product from TCL and evaluate it for algae cultivation.

This dissertation is organized into seven chapters. The *first* chapter briefly describes the importance of microalgae for biofuel production, the important conversion pathways, and the goals and objectives of this research. The *second* chapter provides the reader with the fundamental principles, mechanisms, and current state-of-the-art in thermochemical liquefaction technology and also briefly discusses the pyrolytic conversion of biomass. In the *third* chapter, TCL is evaluated at different operating conditions of temperature, holding time and solids concentration. Results from this part of the research indicated that TCL operated at 350oC temperature, 60 min holding time and 20% organic solids concentration results in maximum biocrude yield with its fuel properties and composition similar to that of petroleum crude oil.

Chapter *four* outlines the effect of addition of chemical catalysts on TCL. Results obtained from this part indicated addition of Na_2CO_3 catalyst improved biocrude yield and TCL energy efficiency by 1.29 and 1.34 times respectively. Chapter *five* evaluates and compares TCL with a conventional pyrolysis process. Results indicated that converting high moisture algae biomass using TCL was more energy efficient than the pyrolysis process resulting higher in higher biocrude yield, lower solids yield, and higher energy recovery. The *sixth* Chapter evaluates cultivation of *Chlorella minutissima* using aqueous co-phase (ACP) effluents which is a co-product in TCL process. Results have shown that ACP growth media at 0.2% (v/v) produced highest growth with biomass productivity, 0.52 g L^{-1} . The summary, conclusion, and recommendations are covered in chapter *seven*.

This research is funded in part by the United States Department of Energy and State of Georgia. Portions of this research have been published in peer reviewed journals and presented in various scientific conferences. Chapter *three* has been published in *Bioresource Technology* (102(10): 6221-6229), and Chapter *six* has been published in *Bioresource Technology* (102(3): 3380-3387). Chapter *four* and Chapter *five* are in preparation and would be shortly submitted to the *Applied Energy* and the *Fuel processing Technology* journals respectively for publication.

CHAPTER 1

INTRODUCTION

Global climate change due to the emission of greenhouse gases, and the projected decline in the world oil production have placed energy as the single most important problem for the next 50 years (Wen et al., 2009). Fossil fuel accounts for 88% of the total estimated world primary energy consumption of 11,295 million tons of oil equivalent (mtoe) and 35% of the total energy is shared by oil consumption only (BP, 2008). Continuous depletion, uncertainties in long term availability, potential threat of greenhouse gas emissions due to the use of fossil fuels and associated global climate change arise questions on their sustainability (Brennan and Owende, 2010). The Kyoto Protocol 1997 has called for a 5.2% reduction in global GHG emissions from 1990 values (Wang et al., 2008). Securing clean, affordable energy for long term has been the biggest challenge in the recent times. To become replacement fuels for the future transport fuels, the candidate should have zero or low carbon emission with minimum detrimental effect to the environment, should be generated from abundant resources, and should be economically viable. Biomass is the only renewable candidate for the production of liquid fuels (Bridgwater, 2003; Wen et al., 2009). Currently biomass supplies 10% of the global energy needs and 3% of the total energy consumption of the United States as per the billion ton report (U.S. DOE-USDA. The national goal is set at deriving 25% of total energy from biomass resources by the year, 2017. Accomplishing this goal would require an increase in the biomass use and efficient conversion technologies.

Microalgae are envisaged as potential feedstock for sustainable biofuel production over the traditional lignocellulosic feedstock. Recently, there has been worldwide research interest, effort and investment in microalgae based biofuel technologies because of their capability of all year round production, higher photosynthetic activity, exponential growth rate and high biomass productivity compared to any other terrestrial plants (microalgae can double their biomass in as short as 3.5 h time), ability to fix higher oils (20-50%), easy adaptability to different growing conditions (fresh water as well as marine-waters) thus avoiding the use of arable lands unlike terrestrial biofuel crops, and ability to biomitigate CO₂ (1 kg dry algal biomass utilize 1.83 kg of CO₂) (Chisti, 2007; Brennan and Owende, 2010). However, harvesting microalgae cells from the growth medium is a tedious and energy consuming process (Grima et al., 2003). Most of the efficient harvesting methods result in wet biomass having very high initial moisture content (80-90%) (Grima et al., 2003). Hence microalgal feedstock can be energetically unsuitable for conversion in most kinds of conventional conversion technologies. Figure 1.1 shows various options for converting the wet and dry algal biomass into fuels and chemicals. Dry algae biomass can be converted into solids, liquids, and gases by thermochemical routes (pyrolysis, gasification, combustion) and mechanical methods (oil press, lipid extractions, densification) that can be subsequently upgraded into transport fuels and value added chemicals, or used in heat and power applications. Wet algae biomass can be converted into alcohols and improved fuels *via* biological routes by anaerobic digestion or fermentation of the carbohydrates. However, biological conversion takes a longer reaction time (Matsumura, 2002). Hydrothermal processing is another option for conversion of wet biomass into liquids, gases and solids via thermochemical liquefaction (TCL), supercritical water gasification (SCWG) and hydrothermal carbonization (HTC)/ wet torrefaction (WT) (Figure 1.1). TCL is a promising alternative to dry conversion

process and can transform high moisture feedstocks into liquid fuels that generates minimum amount of solid residues and it also requires lower energy than other processes (Aresta et al., 2005). The organic constituents of the biomass are converted into a hydrophobic liquid that has higher energy than the biomass itself along with gaseous co-products. Biomass constituents are converted through a series of depolymerization and polymerization reactions occurring in hot compressed water that serves as an excellent medium for reaction due to the change in density, solubility, polarity and dielectric constant at elevated temperature and pressure (Balat, 2008). Although liquefaction was initially developed for coal liquefaction (Li et al., 2008), it has been recently investigated for producing liquid fuels from a wide range of wet lignocellulosic feedstocks (Minowa et al., 1998), swine manure (He et al., 2000), sewage sludge (Suzuki et al., 1986; Dote et al., 1992), macroalgae (Zhou et al., 2010) and the microalgae biomass (Goldman et al., 1980; Dote et al., 1994; Matsui et al., 1997; Yang et al., 2004; Ross et al., 2010). The resulted bio-oil has been reported to have close fuel qualities to that of petroleum crude oil and can be potentially used as a fuel source. The yield of liquid (biocrude) and gaseous products are reported largely affected by temperature, and reaction time, and the presence of alkali and metal catalysts. Chapter 2 gives an overview of thermochemical liquefaction technology and current state of knowledge in microalgae liquefaction. Also, pyrolytic conversion of microalgae biomass has been briefly reviewed in Chapter 2.

Although many studies have been conducted on microalgae liquefaction and some progress has been made in the past decades (Chapter 2), many angles of research and development in this in TCL still remain unexplored. For example, 1) there is no information on stability and storage properties of algal biocrude; 2) not much is known about the variation of key compounds in the

biocrude obtained at different operating parameters; 3) also, information available on various aspects of TCL study reported in the literature is inconsistent and there is a wide variation in biocrude yields reported on TCL of microalgae (Dote et al., 1994; Matsui et al., 1997; Ross et al., 2010); 4) all studies have been conducted at low solids content (10% or less) and in small size reactors having working capacity of 100 mL, which are believed to be too low for economic scale-up and larger process volumes are required to obtain realistic estimates of yield and qualities of biocrude as further scale-up is evaluated; 5) information on detailed characteristics of other co-products in TCL is not clear (fate of nutrients and their final disposition is not well informed); 6) there is no information on its utilization and safe disposal of the aqueous phase effluents generated from TCL process; 7) also, it is not known, which pathway is more suitable for converting algae into an oil, „biocrude“, TCL or pyrolysis? Although, pyrolysis has been widely adopted for producing biocrude from lignocellulosic feedstocks (Mohan et al., 2006) and from microalgae (Miao et al., 2004), there is not much knowledge about the comparative process yields, energy efficiency and product qualities in TCL and pyrolysis for a single feedstock. Further studies are needed to fill the above gaps and inconsistencies and this research study attempts to address some of the above questions. The long term goal of this research work is to produce biocrude along with value added co-products from microalgae. Specific objectives are to:

1. study the effects of operating conditions on biocrude yield, products distribution and physicochemical properties of biocrude and other products in TCL
2. evaluate the influence of addition of catalysts on TCL products yield and properties, nutrient disposition and net energy ratio

3. compare and evaluate TCL and pyrolysis processes for biocrude production, specifically, the products yield distribution, storage characteristics of biocrude, mass and energy balance
4. evaluate the utilization of aqueous phase co-products generated from TCL process in algae cultivation and propose an integrated biorefinery for algal biofuel production along with carbon sequestration.

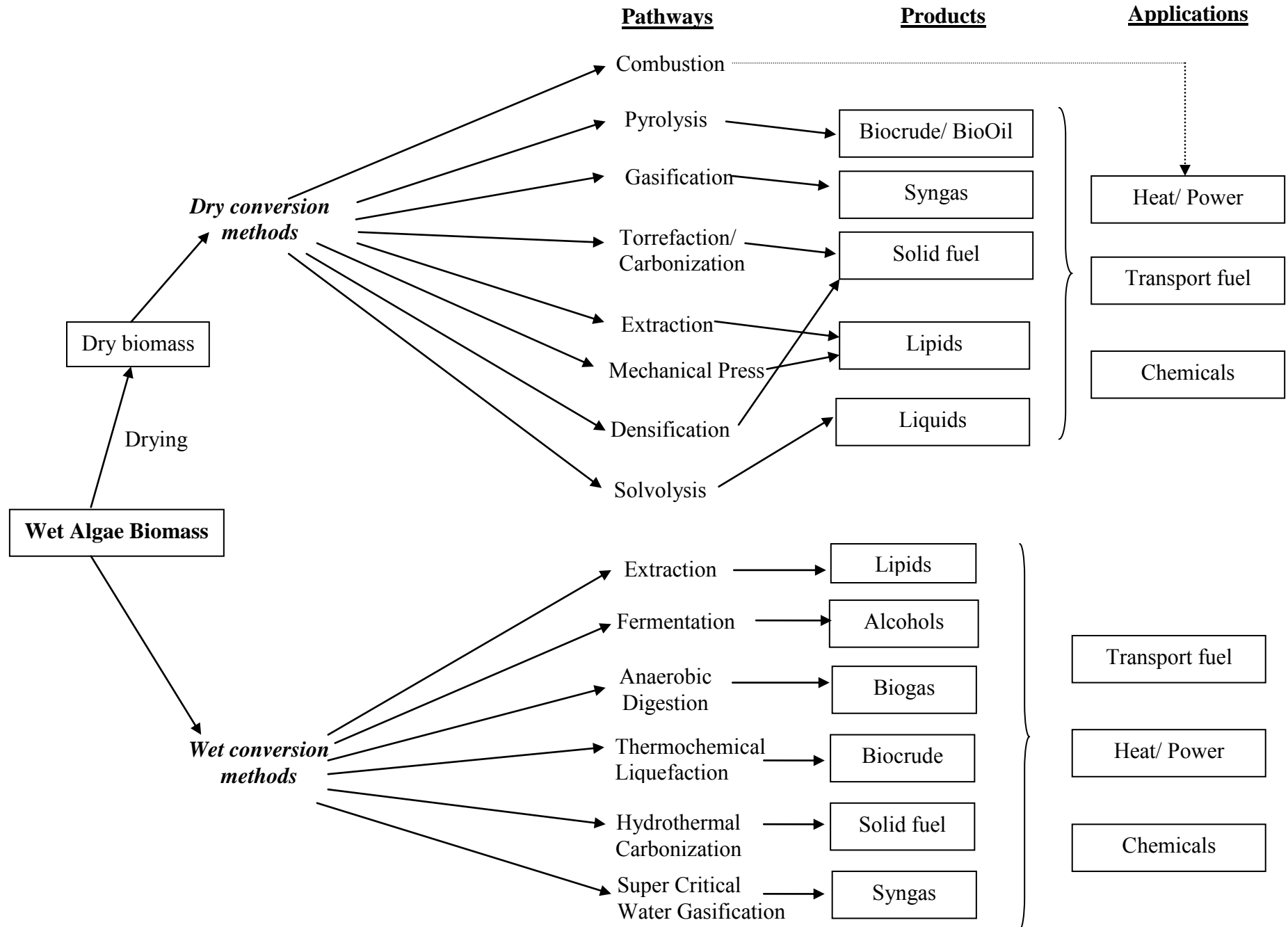
This research complements the present developments on sustainable algae biofuel conversion technologies; adds technical knowledge on liquid biocrude production and its properties; and opens scope for microalgae biorefinery and carbon sequestration approaches. In long run, outcomes of this research will help in building significant self-reliance on alternative energy sources by eliminating uncertainties in fluctuations in petroleum prices.

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CHAPTER 2

LITERATURE REVIEW

Microalgae are attractive options for biofuels production as they have a high biomass productivity, 40-80 dry tons ha⁻¹ year⁻¹ (Wijffels et al., 2010), they can grow in adverse climatic conditions and do not interfere with present food production systems. However, owing to low cell density and high harvesting costs there is a need for research and development of improved conversion technologies for processing wet microalgae biomass into biofuels. This review focuses on two thermochemical conversion technologies, thermochemical liquefaction (TCL) and pyrolysis for converting microalgae into an energy dense liquid fuel, „biocrude“ (also named as „BioOil“). The review chapter is divided into two main sections. The first section provides an overview of thermochemical liquefaction technology, role and changes in fundamental water properties at near-critical (or sub-critical) and supercritical conditions, fundamental process governing the thermochemical liquefaction, physical and chemical changes in the biomass in sub-critical water, and characterization of liquefaction products. A summary of the literature on algae liquefaction, methods and results has been provided in this section. The second section gives an overview of biomass pyrolysis and briefly discusses the current state of knowledge on pyrolysis of algae biomass.

2.1. Thermochemical liquefaction (TCL) technology: an overview

TCL is a thermochemical conversion technique which uses near-critical (sub-critical) to supercritical water as a reaction medium for conversion of biomass and waste streams. TCL is

an attractive option for wet conversion of biomass due to three main reasons (Peterson et al., 2008), i) the presence of water, ii) versatility of chemistry of hot compressed water, and iii) enhance reaction rates and efficient separations. Different terminologies have been used in literature for TCL under the names, hydrothermal upgrading (HTU), hydrothermal liquefaction (HTL), hydrothermal conversion (HTC), hydrothermal processing etc. The following sections detail the of role of hot compressed water at near critical and supercritical conditions, motivation behind biocrude production by TCL and historical overview, reaction chemistry and mechanisms of hydrothermal biomass conversion, and current state of knowledge on TCL of microalgae and other biomass.

2.1.1. Important properties of near-critical water (NCW) and supercritical water (SCW)

Water is ecologically safe and widespread in nature and is used as an excellent solvent in most of the green or environmentally benign chemical processes. However, liquid water at standard room temperature and pressure (25°C and 0.1 MPa) is poorly miscible with hydrocarbons and gases (Watanabe et al., 2004). Temperature and pressure have significant effect on water properties. Water at critical point ($T_c = 373.946^\circ\text{C}$, $P_c = 22.046\text{MPa}$, $\rho_c = 322\text{ kg m}^{-3}$) becomes a highly reactant medium due to drastic changes in many important properties such as dielectric constant, solubility, density, and ion product (Savage 1999; Watanabe et al., 2004; Peterson et al., 2008). Hot compressed water using near critical and supercritical conditions is attractive for many chemical reactions that would not have been possible in standard water conditions. Using hot compressed water instead of organic solvents offers environmental advantages and may lead to pollution prevention (Savage, 1999).

Figure 2.1 shows the changes in density, dielectric constant and ion product (K_w) of water with increase in temperature and hence the pressure. The dielectric constant decreases from about 80 for ambient water at 25°C to 10 at 400°C. Ion product which is defined as the product of concentrations of the acidic and basic forms of water, ($K_w = [\text{H}_3\text{O}^+][\text{OH}^-]$) increases from 10^{-14} at 25°C to $10^{-11} \text{ mol}^2 \text{ kg}^{-2}$ at 350°C and then increases towards higher temperature (Peterson et al., 2008). The K_w in SCW at higher pressure is some orders of magnitude higher than ambient water and in this region water may play the role of an acid or base catalyst (Watanabe et al., 2004). Acid- and base-catalyzed reactions at high pressures and high temperatures show a characteristic non-Arrhenius kinetic behavior near critical point of water. It has been found that the strong dissociation of water near the critical point generates a sufficiently high H^+ ion concentration for acid-catalyzed organic reactions to proceed without any added acid (Penninger, 1999). Density of water at critical temperature and pressure conditions lowers substantially than the normal water, and hydrogen bond tends to be disrupted. Hydrogen bond network of water at standard and supercritical conditions are explained in figure 2.2. In standard liquid water with higher density (1 g cm^{-3}), hydrogen ions are stabilized by complete hydrogen bond network, hence no reactive hydrogen ion is available for reaction with surrounding biomass components. In near-critical and supercritical water, hydrogen bond network is not developed well because of lower density ($0.3\text{-}0.7 \text{ g cm}^{-3}$). At this condition the compressed water reaction medium is conducive to rapid reactions and hydrogen is released from both water and biomass. Hydrogen ion with enhanced activity readily reacts with oxygen atom in biomass and other reactants to form intermediate compound of a positive charge, which ultimately turns into polymerized product through the migration of hydrogen via a chain of water molecules. Many possible chemical reaction pathways exist due to the radical \leftrightarrow ionic competition (Figure 2.3) and both

reactions proceed competitively around the critical point of water. NCW and SCW are characterized by large diffusion coefficients and low viscosities leading to high solubility and complete miscibility with many organic substances. The miscibility of organic compounds in water increases as it approaches 374°C and 22 MPa, that gives opportunity to conduct chemistry in a single phase (with co-existence of liquid and vapor phase) that otherwise would have to occur in a multiphase system. Enormous changes in solvation behavior of NCW and SCW transform it from a polar, highly hydrogen-bonded solvent to non-polar solvent such as hexane.

Hydrothermal processing in NCW and SCW can be divided into three main regions, liquefaction, catalytic gasification and high temperature gasification as shown in the phase diagram of water (Figure 2.4). Hydrothermal conversion *via* TCL occurs generally in the region 200 and 370°C, with pressures 4 and 20 MPa, where water remains as a compressed fluid in liquid state and this region favors the production of tarry material which forms the precursor to biocrude formation. Temperature in the region 370°C to 500°C is considered to favor catalytic gasification resulting in effective reforming and gasification reactions. At temperature above 500°C homogeneous gasification and thermolysis occur with co-existence of water's liquid-vapor behavior still extending until 700°C. Also, there is significant reduction in mass transfer limitations in NCW and SCW that help in preventing coke formation or catalyst poisoning (Watanabe et al., 2004).

2.1.2. Motivation and an overview on historical development of TCL technology

Motivation of TCL has been historically linked to the natural geological process of petroleum making in the earth's crust. Petroleum is widely believed to be originating from kerogen (which is formed from algae and other biodegraded organic compounds) under high pressure and

temperature in the submarine hydrothermal systems (Philippi, 1965; Simoneit, 1988) and the whole process takes thousands of years. High temperature and rapid fluid flow hydrothermal regimes in the earth's crust and hot circulating water (in the temperature range - warm to >400 degrees C) is responsible for the molecular alterations, expulsion and migration of the organic matter (Simoneit, 1995). The synthesized compounds are generally masked by the pyrolysis products formed from contemporary natural organic precursors. Heterocyclic sulfur compounds have been identified in high temperature zones in hydrothermal petroleum of the Guaymas Basin vent systems (Simoneit, 1995). TCL mimics the natural geological process of petroleum making but with substantial change in the time scale from years to several minutes. TCL was historically linked to hydrogenation (Li et al., 2009) and other high pressure thermal decomposition processes which employed hydrogen or carbon monoxide carrier gases to produce a hydrophobic liquid from organic matter at moderate temperature (300-400°C). The hydrophobic liquid is known as „biocrude“ or simply „BioOil“ and resembles to mother nature's crude oil.

The liquefaction research was started in 1971 by the US Bureau of Mines with the conversion of carbohydrates in hot compressed water in the presence of CO and Na_2CO_3 (Appel et al., 1971). In situ production of hydrogen by using combination of CO and Na_2CO_3 was prevalent in the early TCL developments until Molton et al. (1978) showed that combination of CO with alkali led to a limited increase in the oil yield which was later on confirmed by other research workers (Yokoyama et al., 1984; Osterman et al., 1981; Eager et al., 1981). Early work by the US Bureau of Mines led to the development of Albany pilot plant unit that was designed for processing of 18 kg wood per hour and used Douglas fir (Thigpen and Berry, 1979). Liquefaction was performed using the oil product itself (PERC process) or water (LBL process) as a carrier. The LBL process used slurries formed from acid pre-hydrolyzed wood chips and

water, as the feedstock. Several operating problems and unresolved issues in spite of process modifications (Berry, 1979), research on liquefaction was shifted to much smaller scale that used eleven continuous autoclaves to study large number of parameters associated with the process (Figuerola and Ergun, 1979; Figuerola 1982). TCL using biomass/water slurries of high organic/water ratios was studied at the University of Arizona (White et al., 1995) and the University of Saskatchewan (Eager et al., 1982; Eager et al., 1983; Eager et al., 1985) by using special feeding systems. STORS (Sludge to Oil Reactor System) evolved as an important development that involved sewage sludge treatment using hot compressed water technology. STORS process was performed in continuous operation mode using autoclaves with the capacity of 30 kg sewage sludge (@20 wt% solids) per hour in the Battelle Pacific Northwest laboratories of the US Department of Energy (Molton et al., 1988). Hydro-Thermal Upgrading (HTU®) process, developed during 1980's in the Shell Laboratories in Amsterdam, was restarted owing to worldwide renewed interest in biomass derived liquid fuels in 1990s. The HTU® process used a bench scale experimental setup (10 kg water-biomass slurry per hour) (Gourdriaan and Peferoen, 1990) and a pilot plant (20 kg dry matter per hour) (Gourdriaan, 2000). Among the several demonstration and (semi) commercial plants developed during this period the 5 tons per day demonstration plant was the one built in Japan (Itoh et al., 1994). This used STORS process for converting sewage sludge into a combustible energy which was later on successfully demonstrated in Colton, California for municipal wastewater treatment. An improved version of STORS process has been marketed by the company ThermoEnergy (US) under the name "Thermofuel process" (<http://www.thermoenergy.com/water-technologies/municipal-solutions/thermofuel-renewable-energy-process.aspx>) and "Slurrycarb process" marketed by EnerTech Environmental Inc (US) (<http://www.enertech.com/technology/slurrycarb.html>).

Thermofuel process modifies the waste sludge into an energy dense solid fuel at 277°C and 8.2 MPa. EnerTech Environmental Inc operates a 1 ton per day process development unit; a 20 tons per day demonstration unit in cooperation with Mitsubishi Corporation in Ube City (Japan) and is currently constructing a commercial scale facility in Rialto, California. The installation can convert more than 880 wet tons of bio-solids per day from five municipalities in the Los Angeles area into approximately 170 tons per day of the product called E-Fuel. Changing World technologies (US) also developed a process called „Thermo-Depolymerization“ for conversion of turkey waste to fuel products and fertilizer (http://ergosphere.files.wordpress.com/2007/04/cwt_genconflasvegas3_3_04.pdf). The company used a 15 ton per day pilot plant and 200 ton per day processing unit (the Renewable Environmental Solution unit in Carthage, Missouri). However, work has recently been discontinued (as of April 2011) due to financial difficulties (<http://www.carthagepress.com/news/x1092978749/Plant-closing-mixed-blessing-for-Carthage>). Many laboratory scale research on catalytic and non-catalytic TCL has also been performed around the world apart from the pilot plant studies. It appears that TCL of specific feedstocks to energy dense liquid and solid fuels is nearing commercial operation. However, application of TCL for broader range of feedstocks such as microalgae for production of transportation fuel precursors is still in research and development stage. Also, there are operational issues that still need to be addressed through further research. Research on hydrothermal conversion of microalgae *via* TCL or hydrothermal carbonization, wet gasification has been underway. Next sections give a brief review on reaction chemistry, mechanisms and previous results obtained from TCL biomass feedstocks.

2.1.3. Reaction chemistry in hydrothermal biomass conversion

Chemical reactions in hot compressed water are very complex and can be classified as ionic and free radical reactions (Chornet and Overend, 1985). The basic chemistry of formation of hydrocarbon products from carbonaceous compounds (Appleford et al., 2005; Demirbas 2000) can be summarized into a series of steps such as: (i) cracking and reduction of polymers, (ii) hydrolysis, (iii) hydrogenolysis in the presence of hydrogen, (iv) reduction of amino acids, (iv) new molecular rearrangements through dehydration, decarboxylation, C-O and C-C bond ruptures, and (vi) hydrogenation of functional groups. Depolymerization reactions lead to smaller molecules. Decarboxylation and dehydration leads to formation of new molecules and the formation of carbon dioxide through splitting off of carboxyl groups. In biomass materials oxygen removal occurs *via* internal dehydration and decarboxylation reactions during the initial pyrolytic stages. Generally, dehydration removes oxygen in the form of water and decarboxylation removes oxygen in the form of carbon dioxide (Peterson et al., 2008). When hydrogen is present (which comes from either the biomass and/ or the dissociation of water), hydrogenolysis and hydrogenation of functional groups, such as carboxyl-, keto-, and hydroxyl-groups also occur. Depending on the nature of biomass the constituents form different intermediates that form the resultant end products. Figure 2.5 shows the intermediates and the end products from the hydrothermal treatment of different biomass fractions and mixtures in supercritical water. Generally carbohydrates breakdown into glucose monomers, proteins are hydrolyzed into peptides and fats are simplified into fatty acids intermediates that finally produce hydrophobic liquids and sugars (King and Srinivas, 2009). Table 2.1 gives the structural information of the model biomass compounds, and their reaction intermediates encountered in hydrothermal processing of cellulose, hemicelluloses, lignin, fats and proteins. Thermal degradation of cellulose (Huber et al., 2006) shows different reaction pathways forming series of

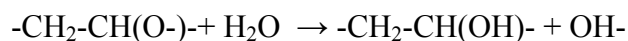
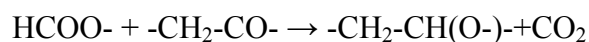
compounds (Figure 2.6). In a reaction of biomass in supercritical water, the hydrogen is generated from biomass as well as from the water. Below supercritical temperature condition (280-300°C) the reaction is mostly hydrothermal hydrolysis and produces majority of the water soluble products (sugar and non-sugars). When protein and/or oil are present in biomass, the degradation is much slower than the carbohydrates and their following products interfere with the degradation of carbohydrates, leading to inhibition of gas formation (Kruse et al., 2005). Protein degradation proceeds through aldol condensation reaction forming free radicals resulting in pyridine and pyroles (Kruse et al., 2005, Kruse et al., 2007). Phenol and furfurals are among the intermediates formed in hydrothermal liquefaction reactions from most of the carbonaceous feedstocks. A simplified reaction pathway for degradation of proteins and amino acids (Figure 2.5) has been reported by Yanik et al. (2007). Fat constituents are also believed to follow similar decomposition pathway to that of protein biomass and form biocrude and gas via formation of free fatty acids and carboxylic acid intermediates (King and Srinivas, 2009).

When catalysts are present, they enhance or inhibit the productions of different products. For example, alkali carbonates enhance the formation of liquids and inhibit the formation of char condensates and gaseous products whereas nickel catalysts enhance the formation of gases (Minowa, 1998). The reaction mechanism for a sodium carbonate catalyzed liquefaction of carbohydrate in the presence of carbon monoxide was described in different steps (Demirbas (2000):

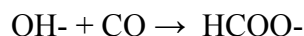
Step 1: First the sodium carbonate and water react with carbon monoxide to yield sodium formate, $\text{Na}_2\text{CO}_3 + 2\text{CO} + \text{H}_2\text{O} \rightarrow 2\text{HCOONa} + \text{CO}_2$

Step 2 & 3: Dehydration of vicinal hydroxyl groups in a carbohydrate to an enol, followed by isomerization to ketone, $-\text{CH}(\text{OH})-\text{CH}(\text{OH})- \rightarrow -\text{CH}=\text{C}(\text{OH})- \rightarrow -\text{CH}_2-\text{CO}$

Step 4: The reduction of newly formed carbonyl group to the corresponding alcohol with formate ion and water.



Step 5. The hydroxyl ion reacts with additional carbon monoxide to regenerate the formate ion,



In the catalyzed reactions, sodium carbonate and potassium carbonate act as catalysts for hydrolysis of macromolecules, such as cellulose and hemicelluloses, into smaller fragments. The fragments are further broken down to smaller compounds by dehydration, dehydrogenation, deoxygenation and decarboxylation. The compounds then rearrange through condensation, cyclization and polymerization leading to new compounds. Some of these new compounds are aromatic hydrocarbons. Similarly the reaction mechanism (Figure 2.6) by Minowa (1998), shows tertiary reactions leading to gaseous products in presence of nickel. Possible chemical reactions occurring in TCL of all kinds of biomass feedstocks and the respective products are assimilated and summarized in table 2.2 (Chornet, 1985; Demirbas, 2000; Balat, 2008). The mixture of reactants within the reaction is very complex, and do not necessarily take place in the order listed here.

2.1.4. Physical and chemical transformations occurring during TCL of biomass

When biomass is heated, thermal scission of its components (cellulose, hemicellulose, lignin protein and fats) occur. In chemical terms the key transformation occurs during biomass liquefaction is the removal of oxygen (Goudriaan et al., 2000). Biomass contains typically 40-45%wt (DAF basis) of oxygen. Oxygen removal results in a higher heating value and a product

with more hydrocarbon-like properties causing it to be immiscible with water. In thermochemical liquefaction oxygen is either removed as water or as carbon dioxide. Removal of carbon dioxide tends to leave a product with a higher H/C ratio and hence a higher heating value of the resulting biocrude is obtained. The ash or solid residue ranges from 6 to 20% for the lignocellulosic high ash content feed stocks (Minowa et al., 1998) and it can be up to a maximum of 5% in case of algae liquefaction (Yang et al. 2004). The pH of the aqueous phase is slightly alkaline (Yanik et al. 2007). The fundamental physical processes in thermal decomposition of biomass are the results of heat transfer, diffusion and the turbulence (Antal Jr., 1985). Heat transfer in the thermochemical reactor device is improved by the swirl device (stirrer) which helps in suspending the tarry material in the liquid phase rather than sticking to the non-reacted biomass particle (Matsumura et al., 2006). The suspension of biomass particles in a pressurized fluid (water) can be analogous to fluidized bed combustion/ gasification system (Matsumura et al., 2004). This phenomenon is advantageous in exchanging heat between the individual particles in the reaction phase as well as between the reactants and the products.

2.1.5. Earlier works on TCL of biomass

Thermal liquefaction of eighteen kinds of Indonesian biomass residues have been studied at 300°C and 10 MPa pressure (Minowa et al., 1998). An oil yield in the range 21-36% was reported from this study and the heating value of the oil was 30 MJ kg⁻¹. A study on non-catalytic thermal liquefaction of swine manure (He et al., 2000) has reported a maximum oil yield of 63% of the initial volatile solids weight at 285°C and the oil was having a heating value of 30.5 MJ kg⁻¹. They found that temperature had substantial effect on the process with depolymerization reaction which started at 220°C and the optimum temperature range for

maximizing the oil yield was 275-305°C. Liquefaction of a protein biomass, sewage sludge (75% moisture content) from municipality waste water treatment was reported by Suzuki et al. (1986). Biochemical composition of the sewage sludge was, 30% crude protein, 13% fat and 24% non-fibrous carbohydrates and produced an yield of 50% heavy oil at 340°C, having 31 MJ kg⁻¹ heating value. They reported that temperature did not affect the quality of oil Suzuki et al. (1986). Liquefaction of *botryococcus braunii* at 300°C, 10MPa, in the presence of Na₂CO₃ resulted in 57-64% oil yield with a 95% recovery of carbohydrates (Dote et al, 1994). Maximum oil yield was 64% at 300°C, which was 50% more than the oil yield resulted by organic solvent extraction method using hexane solvent. Liquefaction of *sprulina platensis* in hot compressed water at 350°C reportedly produced on oil yield of 78.3 wt% which was higher than the yield for liquefaction using toluene (Matsui et al., 1997). However, oil fraction obtained from liquefaction in toluene was characterized by higher carbon content, lower oxygen content, and higher heating value than that of oil the fractions obtained in water. The presence of moderate amount of water was considered to be effective for the production of oil of high heating value in high yield. FTIR spectroscopy and gel permeation chromatograph showed that production of oil fractions proceeded via thermal decomposition of polypeptides and hydrolysis by water produced during liquefaction in organic solvents. A similar study by Yang et al. (2004) on thermal liquefaction of *Mycrocystis viridis* has reported a maximum oil yield of 33% at 340°C, 30 min holding time, using sodium carbonate catalyst at 5wt% loading. The oil was reported having 3.7-7.5% nitrogen with heating value 31 MJ kg⁻¹ and was classified as similar to petroleum fuel having C₁₇-C₁₈ of n-alkane hydrocarbons and aromatic compounds with n-napthalene and benzothiophene. The gas product was having a major component of CH₄, with a heating value 6.6-10.1 MJ kg⁻¹ suggesting methanation favored by liquefaction. Solid yield was

less than 5% suggesting 95% conversion of total mass. The aqueous phase was having a pH of 7-8 and having total nitrogen of 998-1157 mg L⁻¹ and phosphates 2.45-5.38 mg L⁻¹. The composition of gas as a result of reaction of biomass under supercritical condition is strongly influenced by reaction temperature (Antal et al., 2000).

Influence of catalysts

Transition metal oxides in the sedimentary rock have been supposed to play a major role in the generation of petroleum and natural by altering the reaction mechanism and increasing the reaction rates (Mango, 1997). Dispersion of metal catalysts such as Fe (iron), Mo (molybdenum) and Ru have reportedly to increase the conversion efficiency and oil yield in hydroliquefaction of coal by improving the hydrogen transfer (Ikenaga et al., 1997) and co-liquefaction of coal with a microalgae biomass (Ikenaga et al., 2001). Hydrogen is activated by the dispersed catalyst that contributes to an increase in oil yield. Using iron catalyst (Fe(CO)₅-S) in the liquefaction of the microalga spirulina reported an increased oil yield from 52.3 to 66.9 wt% catalyst at 350°C for 60 min in tetralin under 5.0 MPa of hydrogen (Matsui et al., 1997). Carbonyl type catalysts were effective in reducing the nitrogen content in the oil. Influence of alkali carbonates (Na₂CO₃, K₂CO₃, and Cs₂CO₃) on the biomass volatilization has been reported by Hallen et al. (1985). Addition of carbonates favored the yield of gases by increasing the reaction rates. Pressurized thermal decomposition of heavy oil at 600°C temperature and 4.2 Mpa pressure was favored by the addition of calcium hydroxide and calcium oxide (Sato et al., 2003). The liquid yield has been reported to be increased by the addition of alkali metals such as sodium carbonate, potassium carbonate, potassium hydroxide, sodium hydroxide, and metal catalysts in case of thermochemical liquefaction of lignocellulosic biomass (Minowa et al., 1995; Xu, et al., 2008)

and algae (Dote et al., 1994; Sawayama et al., 1999). An optimum oil yield having a heating value of 36 MJ kg^{-1} was resulted from the liquefaction of municipal solid at 4 wt% of sodium carbonate as catalyst (Minnowa et al., 1995).

Methods and properties of biocrude from TCL

Chemical composition and properties are evaluated by a gas chromatography (GC) or GC-MS (mass spectrometry) (qualitative and quantitative analyses of the volatile compounds), HPLC or HPLC/ electrospray MS (non-volatile compounds), Gel Permeation Chromatography (GPC) (molecular weight distribution), Fourier Transform Infrared (Spectroscopy) (functional groups) and nuclear magnetic resonance (NMR) (types of hydrogen or carbon specific natural groups, bonds, area integrations) (Mohan et al., 2006). Color, smell, water content, specific gravity, and viscosity are analyzed by standard laboratory methods using ASME standards for indicating the physical properties of biocrude/ BioOil.

Biocrude produced from liquefaction is usually a dark brown, free flowing liquid and has a distinctive odor (Huber et al., 2006) similar to the pyrolysis produced bio-oils and is a complex mixtures of oxygenated hydrocarbons, water from the original biomass moisture (Dote et al., 1994; Minowa et al., 1998; He et al., 2000; Brown et al., 2010). Aqueous phase and char particles have a distinct smoky smell. The biocrude processed by TCL had an approximate water content of 5.1% (Balat, 2008). Typically the biocrude obtained has a higher heating value of 16-31 MJ kg^{-1} for the most of the lignocellulosic feedstocks (Elliot et al., 1987; He et al., 2000) and was as high as 31-45 MJ kg^{-1} for microalgal biomass (Dote et al., 1994; Yang et al., 2004). The viscosity of the thermochemically processed biocrude is found to be typically 20-100cps (at 334K). Bio-oil is usually water insoluble and major fraction of it is soluble in benzene and other

non-polar solvents. The benzene solubility of the bio-oil is in the range of 81.6-89.8% (He et al., 2000) indicating the higher quality of bio-oil. Typically, bio-oil processed by thermochemical liquefaction from all sources contains C: 61-85%, H: 8-14%, N: 0.9-6.0%, O: 12-20%. The higher oxygen content leads to higher energy density. It has an atomic H/C ratio of 1.0-1.3, the average molecular weight being 300 (Balat, 2008).

Due to the large number of reactions occur during the bio-oil production such as hydrolysis, dehydration, isomerization, dehydrogenation, aromatization, retro-condensation and coking, the exact composition of bio-oil is largely dependent on feedstock as well as process parameters. Bio-oil is a complex mixture having more than 400 organic compounds found in it (Huber et al., 2006). The wide ranges of chemical compounds found in the bio-oil are acids, esters, alcohols, ketones, aldehydes, sugars, furans, phenols, methyl phenol, guaiacols, and syringols. Guaiacol and syringol are produced from the biomass that are having lignin component. Elliot et al. (1987) have reported the composition of bio-oil from the thermochemical liquefaction of five different high moisture biomass such as kelp, water hyacinth, spent grain, napier grass and sorghum. The bio-oil consisted of the following types of compounds: esters, aldehydes, alcohols, cyclic ketones, unsaturated and alkylated furans (dihydrofuranones, hydroxymethyl tetrahydrofuran), phenols, benzenediols, naphthols, aromatic oxygenates (bismethylguaiacol), phenylphenols, benzodioxin, cyclic hydrocarbons (alkylcyclopentenes, alkylbenzenes), alkylindans, phenanthrene, long-chain hydrocarbons, saturated and unsaturated acids, nitrogen cyclics (alkylpyrrolidinones, alkylaziridines, alkylpyrroles, alkylindoles), amines/amides. Analysis of bio-oil obtained by thermochemical liquefaction of sewage sludge (Dote et al., 1992) reported similar compounds representing weak acids, basic and neutrals.

2.2. A brief review on biomass pyrolysis

Pyrolysis is the thermal decomposition of biomass in the absence of oxygen or when significantly less oxygen is present than required for complete combustion (Mohan et al., 2006). Based on the operating temperature (low to high) and residence time (very long to short), the process is known as carbonization, slow pyrolysis and fast/ flash pyrolysis. Slow pyrolysis occurs at lower temperature, lower heating rate and longer residence time than that of the fast/ flash pyrolysis processes (Mohan et al., 2006; Brennan et al., 2010). Different products obtained in the pyrolysis process are liquid (called BioOil), solid char and gas. The proportions of BioOil, char and gas can vary depending on the type and nature of pyrolysis and biomass feedstocks. Pyrolysis is a complex phenomenon of heat, mass and momentum transfer. A summary the general changes that take place during general pyrolysis, especially during slow pyrolysis are (Mohan et al., 2006) explain the heat and mass transfer during pyrolytic reactions and are as follows:

- heat transfers into the biomass from a heat source through conduction and convection and increases the biomass temperature.
- pyrolysis reaction starts with the increased temperature in biomass matrix, that further increases the sample temperature resulting in breakdown of biomass constituents and release of volatiles and char.
- hot volatiles flow towards un-pyrolysed biomass and transfer heat to the relatively cooler surfaces.
- heat released from hot volatiles cause condensation on colder surfaces of the biomass followed by secondary reactions producing tar.

- auto-catalytic secondary and primary reactions occur simultaneously in the biomass along with reactions of biomass constituents
- further thermal decomposition, reforming, water gas shift reactions, recombination and dehydrations occur depending upon process residence time/ temperature/pressure profiles of the reactions zones.

2.2.1. Fundamental principles and mechanism

The thermal decomposition by pyrolysis process is governed by heat, mass and fluid transport phenomena and reaction can be explained by the basic mechanisms of heat transfer (conduction and convection), mass transfer (diffusion and convection), and turbulence (fluidization of biomass particles). Assuming that an individual biomass particle being dispersed in the fluid phase (water) and surrounded by gaseous phase (nitrogen) (Figure 2.9), the transport phenomena occurring in the process can be described by the fundamental physical processes (Antal Jr., 1985) as:

- heat transfer to the particle by radiation, convection and conduction.
- diffusion of gaseous reactants to the particle through the gas film separating the particle from the bulk fluid.
- heat transfer within the particle by conduction and radiation.
- diffusion of gaseous reactants through the uncovered virgin solid.
- chemical reaction of the convertible solid, forming the primary products (pyroligenous liquids).
- intra- and extra-particle homogenous and heterogeneous chemical reactions of the primary products.

- diffusion of gaseous products through the converted solid and liquid products.
- diffusion of gaseous products away from the particle through the boundary layer.

Heat transfer in the reactor can be improved by agitation devices to increase turbulence/fluidization and hence the heat and mass transfer (Matsumura et al., 2006). This phenomenon is advantageous in exchanging heat between the individual particles in the reaction phase as well as between the reactants and the products.

Multiple models are available for representing the chemical kinetics of pyrolysis and other thermochemical conversion processes. They can be simple thermal models and comprehensive models (Moghtaderi, 2006) or the multi-step models (Skala et al., 1990). A two-step global pyrolysis model for wood and cellulose pyrolysis has been explained in figure 2.10 by Moghtaderi (2006). The model considers both primary and secondary reactions during cellulose pyrolysis. A kinetic model explaining the hydrogenation of algae into bio-oil, gas and aqueous phase explains a multi-step reaction mechanism with asphaltene as the intermediate product (Chin and Engel, 1981). As the biomass is a complex structure of hydrocarbons, proteins and fats, the reaction of thermochemical liquefaction can be a multi-step process taking the tertiary reactions into account.

2.2.1. Earlier works on biomass pyrolysis

Pyrolysis has been widely adopted for generating BioOil from varieties forest biomass and agricultural wastes (Mohan et al., 2006). Yield of 60-95% BioOil has been reported from fast pyrolysis process depending the biomass variety and the processing conditions. BioOil obtained from lignocellulosic feedstock were characterized by high oxygen content, low heating value, acidic pH, high viscosity, poor flow properties, and poor stability (Mohan et al., 2006). These

technological challenges need to be sorted out and presently research on BioOil upgrading and improvement by hydrogenation, catalytic cracking are under progress for making them suitable for the end users (Osama et al., 1999; Czernik and Bridgewater, 2004; Zhang et al., 2007). Commercialization of lignocellulosic feedstock based pyrolysis technology are recently in progress. For example, Dynamotive, a Canadian company has patented a fast pyrolysis technology under the name “Bio-therm” for processing wood and agricultural wastes into BioOil (<http://www.dynamotive.com>). Dynamotive is presently operating two plants of 2 tons per day and 14 tons per day capacities in Canada.

Although till date pyrolysis process generating BioOil has been tested for a wide varieties of lignocellulosic feedstocks (sawdust, bark, wood savings, sugarcane bagasse, rice hulls, rice straw, peanut hulls, oat hulls, switchgrass, and wheat straw), only few studies have been reported on the pyrolysis of microalgae. Among all, studies by Miao and co-workers reported fast pyrolysis of autotrophic and heterotrophic species of *Chlorella protothecoids* (Miao and Wu, 2004) and *Microcystis aeuroginosa* (Miao et al., 2004) at 500°C and at heating rate 600°C min⁻¹. BioOil yield of 57.2% was reported from the metabolically controlled heterotrophic algae biomass compared to 16.6% for *C. protothecoids* in autotrophic cultivation (Miao and Wu, 2004) and 24% for *M. aeuroginosa* (Miao et al., 2004). Their comparison with BioOil obtained from wood had shown that algae derived BioOil was having superior fuel properties such as higher energy content, higher stability, and lower oxygen content. However, algal BioOil reported higher elemental nitrogen percentage than the wood derived BioOil (Miao et al., 2004). Demirbas reported a study on slow pyrolysis of *C. protothecoids* at temperature 274-413°C and showed that BioOil yield increased with increase in temperature from 5.7% at 274°C to 55.3% at 502°C and then dropped down with further increase in temperature (Demirbas, 2006).

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Table 2.1. Biomass model compounds and reaction intermediates in hydrothermal processing
(Source: Peterson et al., 2008)

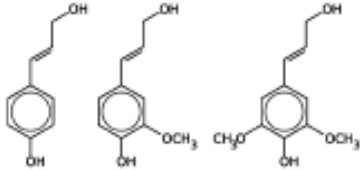
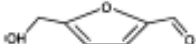

Substance	Chemical formula	Structural information
Feedstocks		
Cellulose	$[C_6H_{10}O_5]_n$	$n \approx 500\text{--}10\,000$; β (1 \rightarrow 4) linkages between glucose residues
Hemicellulose	Typical monomers: $[C_5H_8O_4]$, $[C_6H_{10}O_5]$	Branched with variable monosaccharide residues; degree of polymerization $\sim 500\text{--}3000$
Lignin	Typical monomers: 	Polymer of aromatic subunits in random structure (see Fig. 1); molecular weight: $>10\,000$ Da
Triacylglycerides (fats)	$RCOO-CH_2CH(R'COO)CH_2-(R''COO)$	$RCOO$, $R'COO$, $R''COO$ are fatty acids with ester linkages to the glycerol backbone
Proteins	$[NHCH(R)C(O)]_n$	Monomer is amino acid residue with various side (R) groups; $n \approx 50\text{--}2000$
Intermediates		
Glucose	$C_6H_{12}O_6$	Exists as 6-membered ring, 5-membered ring, and open chain (see Fig. 9)
Xylose	$C_5H_{10}O_5$	Exists as 6-membered ring, 5-membered ring, and open chain
Amino acid	$H_2NCH(R)COOH$	R is the side group, varies from H to heterocyclic group
Fatty acid	$RCOOH$	R is an alkyl group, typically of 12–20 carbons with 0–4 double bonds
5-Hydroxymethylfurfural		
Furfural		

Table. 2.2. Summary of the possible chemical reactions of organic compounds in hot compressed water (assimilated from various sources as referred in the text)

Reactions	Products	Remarks
Depolymerization	Micromolecules of acids (Levulinic acid)	Cracking and reduction of polymers like lignin and lipids
Hydrolysis	Glucose and simple sugars	Break down of carbohydrates
Dehydration	Oligomers, furfurals, Alkanes, Anhydroglucose	New molecular rearrangement at the end of each reaction
Decarbonylation	CO	Formation of carbon monoxide in catalyzed reactions
Decarboxylation	Carboxylic acids, Ketones, CO ₂	Removal of oxygen
Hydrogeolysis	Alcohols, alkanes	Occurs in the presence of hydrogen
Isomerization	Ketones	
Deamination	Amines	In case of protein containing biomass
Deoxygenation	Aldehydes, Ketones	Oxygen reduction from the biomass takes place
Deesterification	Fatty acids	
Aromatization	Phenols, benzenes , toluene, Furans	Repolymerization of small groups
Cyclization	Cyclohexene, Styrene	Molecular reaarangement
Aldol condensation and Condensation	Pyridine and pyroles, char	For biomass having protein and fat composition
Methanation	CH ₄	Basically enhanced in the presence of a catalytic reagent
Water-gas shift reaction	H ₂	Reaction of water with the CO ₂
Steam reforming reaction	H ₂	
Coking	Char, CO ₂	Reaction of the remaining solid with oxygen

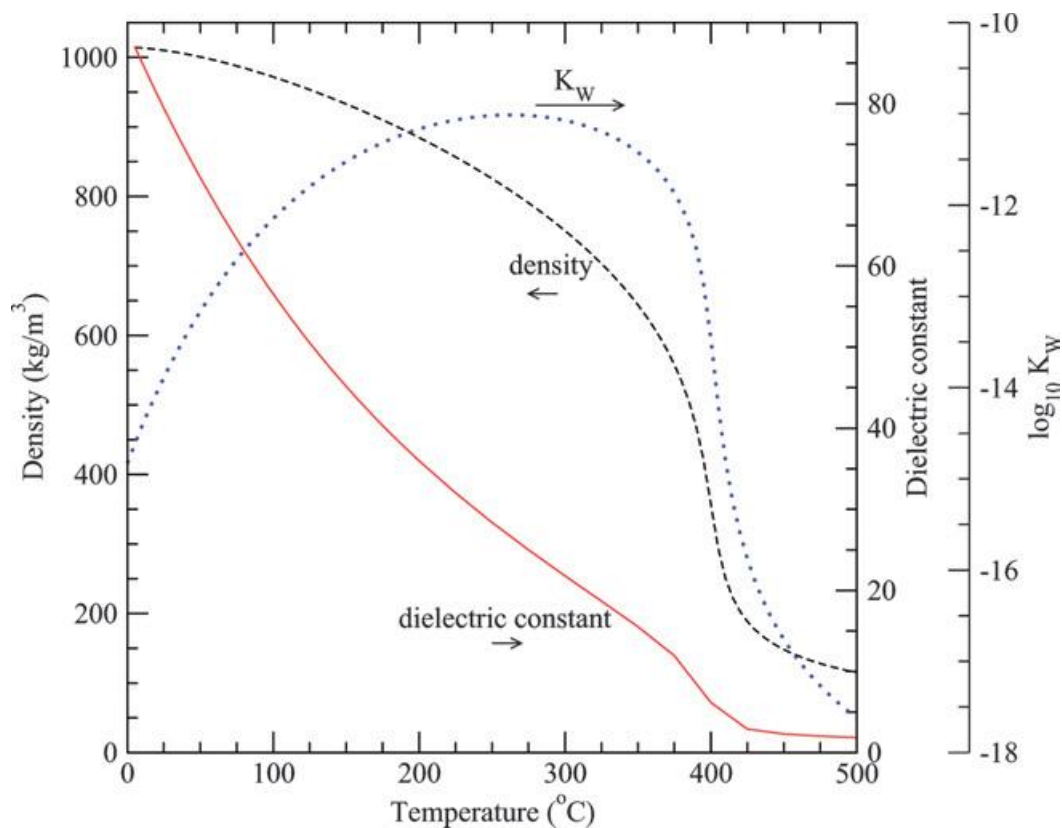


Figure 2.1. Density, dielectric constant, and ion product (K_w) of water as a function of temperature. The dielectric constant of water drops drastically as water is heated, and approaches that of a (room-temperature) non-polar solvent at supercritical conditions (Source: Peterson et al., 2008)

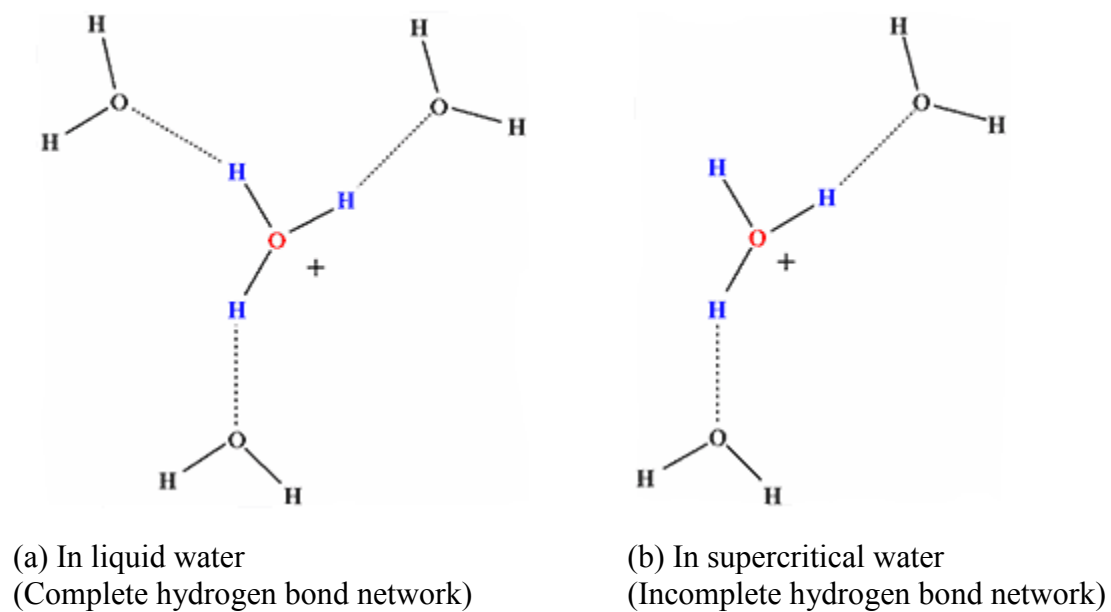


Figure 2.2. Hydrogen bond network surrounding hydrogen ion (its hydrated form H_3O^+)

(Source: http://www.aist.go.jp/aist_e/latest_research/2004/20040520/20040520.html)

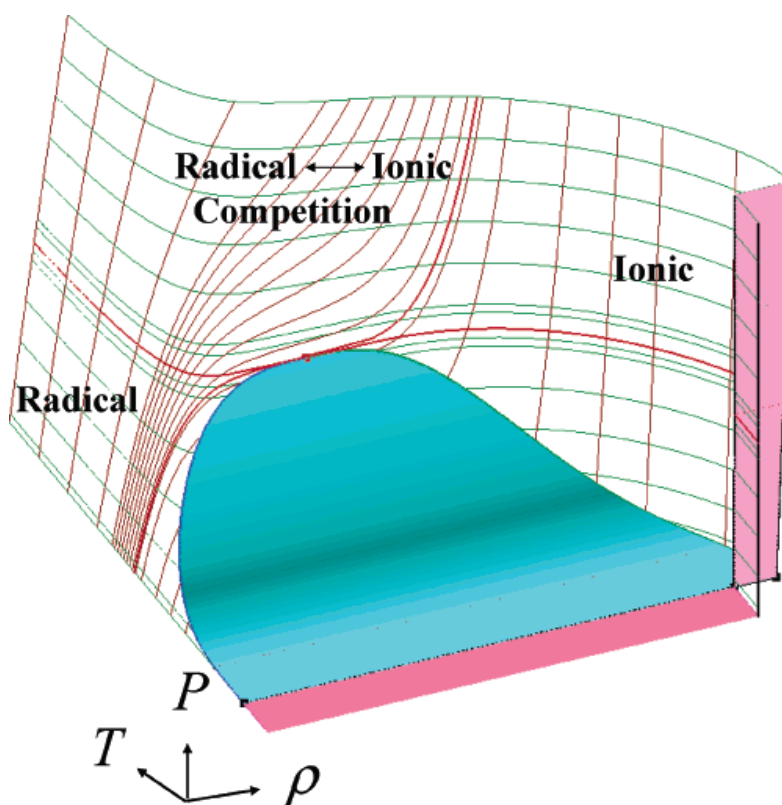


Figure 2.3. P- ρ -T surface of water showing important reaction regimes (Source: Watanabe et al., 2004)

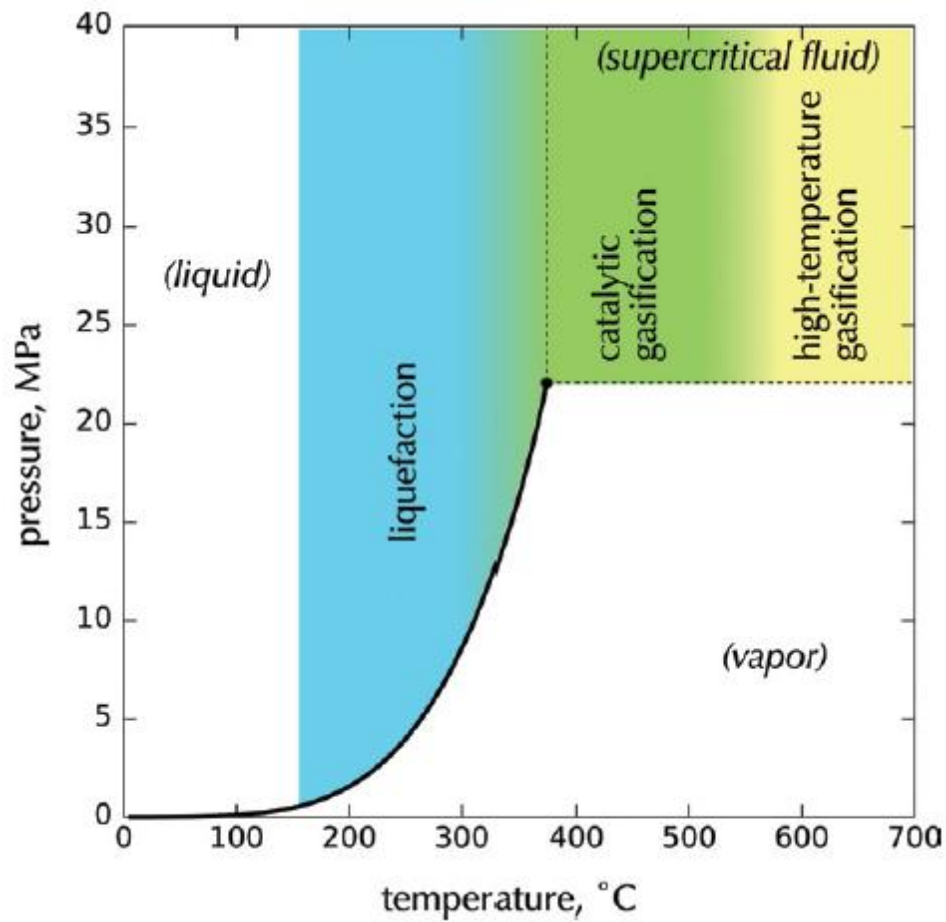


Figure 2.4. Reaction fields during the hydrothermal processing in NCW and SCW water as referenced to the pressure-temperature phase diagram of water (Source: Peterson et al., 2008)

Biomass Fractions:

Oils/Fats – Lipids \Rightarrow Oil \Rightarrow Free Fatty Acids (FFA)

Proteins \Rightarrow Hydrolyzates \Rightarrow Peptides \Rightarrow Amino Acids

Carbohydrates \Rightarrow Hydrolyzates \Rightarrow Monomers (Glucose)

Deoiled Biomass Fraction:

Proteins - Carbohydrate \Rightarrow Amino Acids, Organic Acids,
Sugars

Unfractionated Biomass:

Oil/Fat - Protein-Carbohydrate \Rightarrow Oil, FFA, Protein Hydrolyzate,
Sugars

Oil/Fat - Protein-Carbohydrate \Rightarrow FFA, Amino Acids, Sugars

Figure 2.5. Reactions of representative biomass compounds and mixtures with subcritical water

(Source: King and Srinivas, 2009)

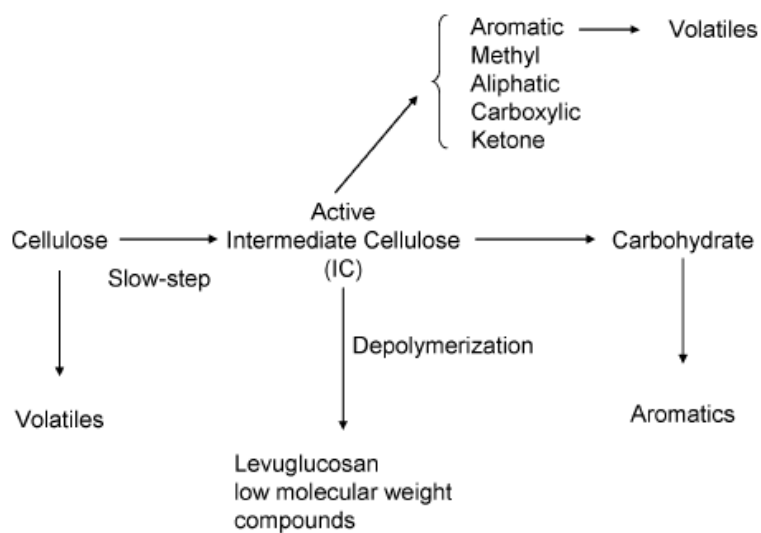


Figure 2.6. Mechanism of cellulose decomposition (Source: Huber et al. 2006)

Proteins → Amino acids → Low-molecular-weight
carboxylic acid → End products (formic acid, acetic
acid, etc.)

Figure 2.7. Mechanism of proteins and amino acid decomposition (Source: Yanik et al.2007)

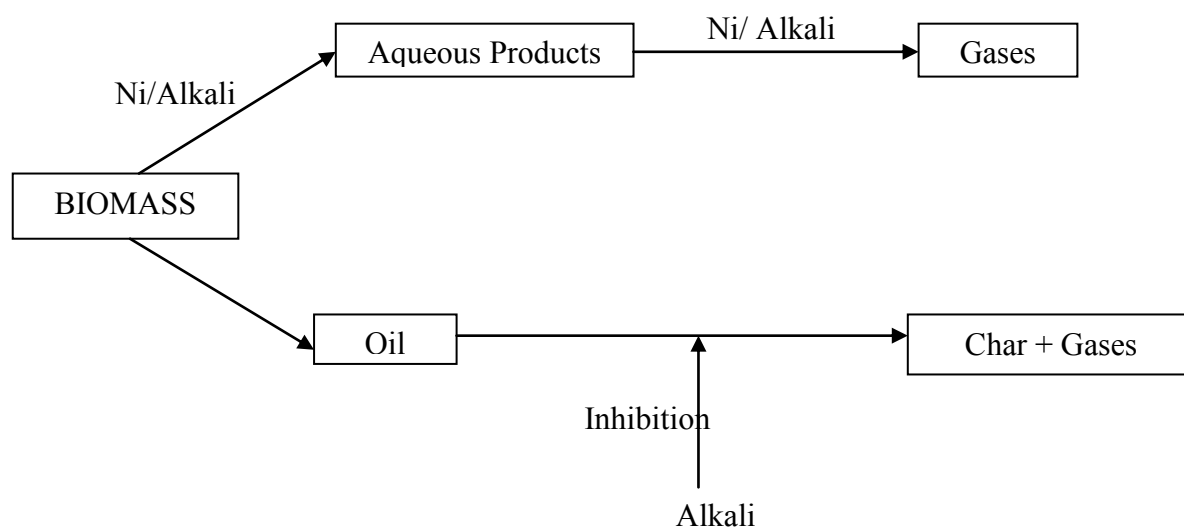


Figure 2.8. Proposed reactions for metal and base catalysts roles during a hydrothermal treatment operation. (Source: modified from Minowa, 1998)

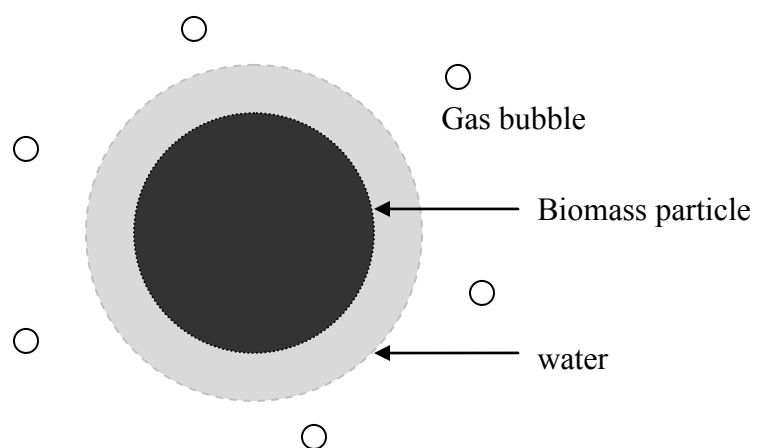


Figure 2.9. Individual biomass particle in a pyrolysis reactor (Source: modified from Antal, 1985)

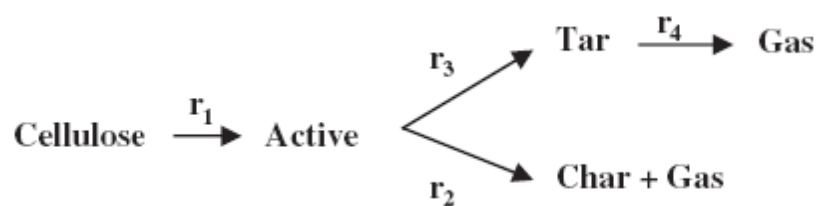


Figure 2.10. Two-stage semi-global reaction scheme for cellulose (Source: Moghtaderi, 2006)

CHAPTER 3

EFFECT OF OPERATING CONDITIONS OF THERMOCHEMICAL LIQUEFACTION ON
BIOCRUDE PRODUCTION FROM *SPIRULINA PLATENSIS*¹

¹Jena, U., Das, K.C., Kastner, J.R. 2011. *Bioresource Technology* 102(10): 6221-6229
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Abstract

This study investigated the optimum thermochemical liquefaction (TCL) operating conditions for producing biocrude from *Spirulina platensis*. TCL experiments were performed at various temperatures (200-380°C), holding times (0-120 min), and solids concentrations (10-50%). TCL conversion at 350°C, 60 min holding time and 20% solids concentration produced the highest biocrude yield of 39.9% representing 98.3% carbon conversion efficiency. Light fraction biocrude (B₁) appeared at 300°C or higher temperatures and represented 50-63% of the total biocrude. Biocrude obtained at 350-380°C had similar fuel properties to that of petroleum crude with energy density of 34.7-39.9 MJ kg⁻¹ compared to 42.9 MJ kg⁻¹ for petroleum crude. Biocrude from conversion at 300°C or above had 71-77% elemental carbon, and 0.6-11.6% elemental oxygen and viscosities in the range 40-68 cP. GC/MS of biocrude reported higher hydrocarbons (C₁₆-C₁₇), phenolics, carboxylic acids, esters, aldehydes, amines, and amides.

Keywords. Thermochemical Liquefaction (TCL), Microalgae, Biocrude, Biofuel

3.1. Introduction

Microalgae are attractive feedstocks for biofuel production because of their high productivities (40-80 tons ha⁻¹ year⁻¹), high lipid content (30 -60%) (Wijffels et al., 2010), their ability to grow in contaminated waters, their ability to sequester atmospheric CO₂, and because they can be cultivated on marginal lands and variable climatic conditions. Because microalgal biofuel development does not compete with food production systems, this feedstock has further appeal. In order to reduce overall energy and cost input, it is necessary to process algae without complete drying, which can be accomplished by the thermochemical liquefaction (TCL) process. The process converts organic constituents of algae into a liquid biocrude that can be refined to gasoline like fuels. In addition, a major part of the N and P from the original biomass is recovered in the aqueous phase co-product and can be used in downstream algae cultivation systems (Jena et al., 2011).

Hot compressed water is a highly reactive medium because of changes in properties such as solubility, density, dielectric constant and reactivity as water approaches its critical point (374°C, 22.1 MPa). These enhance depolymerization and repolymerization of lignins, celluloses, lipids, proteins and carbohydrates, transforming them into biocrude (also referred to as biooil in the literature), gas and char. Multiple reactions occur in three steps, namely, hydrolysis, depolymerization and repolymerization/self-condensation reactions (Yin et al., 2010). In lignocellulosic biomass, the lignin and cellulose components are hydrolyzed into unit structures of sugar monomers (Yin et al., 2010), whereas protein molecules are hydrolyzed into aminoacids followed by deamination and decarboxylation reactions to complex hydrocarbons (Sato et al., 2004). The biocrude is a dark viscous liquid with an energy value 70-95% of that of petroleum fuel oil (Brown et al., 2010; Dote et al., 1994; He et al., 2000; Minowa et al., 1998).

Thermochemical liquefaction has been investigated for producing liquid fuels from several types of biomass including lignocellulosic feedstocks (Minowa et al., 1998), swine manure (He et al., 2000), macroalgae (Zhou et al., 2010) and microalgae (Brown et al., 2010; Dote et al., 1994; Ross et al., 2010; Zou et al., 2009; Yang et al., 2004). TCL process is known to be effective for biomass feedstocks including algae that have lower percentage of net lipids. The oil is produced not only from the conversion of triglycerides but also from all other components such as proteins, fibers and carbohydrates that constitute the whole biomass (Duan and Savage, 2011). Also through TCL, greater amount of high quality biocrude is produced. Conversion of microalgae biomass was reported to be a function of operating variables such as temperature, holding time, and presence of catalysts and co-solvents (Duan and Savage, 2011; Huang et al., 2011; Zou et al., 2009). Highest TCL yield of 97% was reported for the liquefaction of *Dunaliella tertiolecta* at optimized operating conditions (Zou et al., 2009).

Although some information is available on liquefaction of algae, further studies are needed to fill the following gaps and inconsistencies in the literature: 1) Wide variation in biocrude yield and composition reported in the literature. For example, maximum biocrude yield was reported as 9.0% for *Spirulina* and 13.6% for *Chlorella vulgaris* (Ross et al., 2010), 42.0% for *D. tertiolecta* and 64.0% for *Botryococcus braunii* (Dote et al., 1994). Another study (Huang et al., 2011) reported biocrude yield from liquefaction of *Spirulina* to be 35-45%; 2) Studies have not attempted to vary key operating parameters with a view to identify an optimum. Particularly, all studies have been conducted at 10% or lower solids content, which we believe to be too low for economic scale-up. With developments of attached growth algal cultivation and advanced harvesting technologies, cell concentration of 20% or more can be achieved easily (Grima et al., 2003) and conversion processes need to be evaluated under these conditions; 3) Most of these

studies reported in literature (Biller & Ross, 2011; Brown et al., 2010; Ross et al., 2010; Zou et al., 2009) have used small reactor volume of less than 100 mL with dry biomass of <10 g. We believe that larger process volumes are required to obtain realistic estimates of yield and qualities of biocrude as further scale-up is evaluated and 4) There is no information on long term storage properties of biocrude obtained from algal biomass in any of the above studies. To fill these gaps, we studied TCL of the microalga, *S. platensis*, in 500-750 mL reactant volume (100-250 g dry biomass solids) at varying temperatures, holding times, and solids concentrations. We report biocrude yield, its properties, and characteristics of other co-products to better describe the effect of operating conditions.

3.2. Materials and Methods

3.2.1. Raw materials

S. platensis biomass was provided by Earthrise Nutritionals LLC (Calipatria, CA) in dry powder form with defined properties (Table 3.1), and was stored in airtight packages at room temperature prior to use. Laboratory grade acetone (99.5% purity) was purchased from Sigma Aldrich. Nitrogen and helium gases were obtained from the Universal Gas and Electric Corp. (USA).

3.2.2. Thermochemical liquefaction (TCL) and separation of products

TCL experiments were performed in a 1.8-L batch reactor system (Parr Instruments Co. Moline, PA) at different temperatures (200, 250, 300, 350 and 380°C), holding times (0, 30, 60, 90 and 120 min), and solids concentrations in the slurry (10, 20, 30, 40 and 50%). In a typical experimental run 500 mL to 750 mL premixed algal slurry was prepared by adding defined

amounts of algal powder to deionized water to achieve the desired solids concentration and placed in the reactor. The reactor was sealed, thoroughly purged and pressurized to 2 MPa (289 ± 3 psi) using nitrogen. Then, the reactor was heated to the desired temperature using an electrical heating jacket and held at that temperature ($\pm 3^\circ\text{C}$) for the predefined holding time period. The reactor was stirred by an impeller type agitation device at 300 rpm speed for all experimental runs to ensure homogeneous reactions. At the end of the reaction, the reactor was cooled to room temperature by passing tap water through the cooling coils of the system. TCL runs were performed in triplicates for all experimental runs except that at 200-250°C, where duplicate runs were performed. The yield results were reported as average of two or three runs.

Following liquefaction, the process gas was sampled using Tedlar sampling bags for further analysis and the remaining gas was vented to the atmosphere. Then the various products were separated by following a series of filtration and extraction procedure (Figure 3.1). The liquid products mixture was poured into a separatory funnel and the top phase consisting of light fraction biocrude was separated by decantation. The remaining two phases were filtered under vacuum to obtain water soluble components (aqueous phase), and water insoluble components (solid residues and heavy fraction biocrude). Water soluble components obtained as filtrates were sampled and analyzed. The total aqueous phase is henceforth referred to as aqueous phase co-product (ACP). The water insoluble components retained on the filter and wall of the reactor were washed thoroughly with laboratory grade acetone and vacuum filtered. The solids recovered on the filter were oven dried at 105°C for 24 hrs and obtained as solid residue (SR). The acetone soluble fraction was vacuum evaporated at 55°C to evaporate acetone and separate the heavy fraction biocrude. The light fraction biocrude and heavy fraction biocrude thus obtained are referred to as light biocrude (B_1) and heavy biocrude (B_2). Heavy fraction biocrude

(B₂) obtained from the all the TCL runs had similar physical and chemical properties and hence properties of the sample B₂ obtained for TCL run at 350°C temperature, 60 min holding time and 20% solids concentration are presented for the purpose of discussion.

3.2.3. Analytical methods

Yield of each product fraction was determined as the ratio of their mass to the initial mass of the biomass used. Biocrude yields were reported on a dry basis and reported as the weight of total biocrude fraction (B₁+B₂). The gaseous products (denoted as “Gas”) yield was quantified by measuring the weight difference of the reactor and contents before and after the experiment. The water soluble products of liquefaction were the products in the aqueous phase (deionized water) and were quantified from the mass balance as follows:

$$\text{Yield of water solubles, \%} = 100 - \text{Yield (\%)} \text{ of (Biocrude + Gas + Solid residue)}$$

Untreated biomass, solid residues and biocrude samples were analyzed for elemental C, H, N, S, O (ultimate analysis) using a LECO brand (Model CHNS-932) following methods outlined in ASTM D 5291 and D 3176. The analyzer was calibrated using sulfamethazine (C – 51.78 %, H – 5.07 %, N – 20.13 %, and S – 11.52 %) as the standard material. Atomic ratio and empirical formula of raw feedstock and the biocrudes were derived from the elemental results. Higher heating values (HHV) of solid and biocrude samples were measured using an isoperibol bomb calorimeter Model 1351 (Parr Instruments Co., Moline, Illinois) following ASTM D 5865 and D 4809 standard methods. Carbohydrate content of the algal biomass was measured using the DuBois method (DuBois et al., 1956), protein content was estimated by multiplying the elemental N content by a factor of 4.58 (Lourenco et al., 1998), and lipid content was measured by gravimetric method using an ANKOM^{XT10} automated extraction system (ANKOM

Technology, Macedon, NY) where hexane was used as extraction solvent. The metal composition of *S. platensis* and the biocrude were analyzed using an inductively coupled plasma (argon) spectrometer equipped with a mass spectral (ICP-MS) detector system (Isaac & Johnson, 1985). Water content of the biocrude samples was measured using a Mettler-Toledo titrator (Model-DL31, Columbus, OH) following the ASTM D 1744 and ASTM E 203. The titrant used was manufactured by AquaStar (Composite 5K) designed for materials containing aldehydes and ketones and the solvent was AquaStar-Solvent KC containing dichloromethane for enhanced solubility with oils and fats. Specific gravity values (g mL^{-1}) were determined gravimetrically using 2 mL Gay-Lussac pycnometers. Dynamic viscosities of biocrude samples were measured at using a Brookfield DV-I+Viscometer with a UL/YZ spindle adapter using a modified version of ASTM D 2983. The modification was the use of a higher temperature (60°C) than the standard, because of the very high viscosity of biocrude at lower temperatures. The storage stability of biocrude was assessed by storing it for 120 days at room temperature ($20\pm 2^{\circ}\text{C}$) and measuring the change in dynamic viscosity at 40°C at approximately 7 days interval.

Biocrude composition was determined by GC-MS analysis using a Hewlett-Packard (Model HP-6890) gas chromatograph in conjunction with a Hewlett-Packard mass spectrometer (Model HP-5973) with a mass selective detector. The GC contains an HP-5 MS column of the following dimensions: 30 m length, 0.25 mm internal diameter, and $0.25\ \mu\text{m}$ film thickness. The method used was as follows: inlet temperature 230°C , detector temperature 280°C (mass spectrometer interface temperature), flow at $1\ \text{mL min}^{-1}$ He, oven at 40°C for 2.5 min followed by a ramp at $8^{\circ}\text{C min}^{-1}$ to 250°C (held for 5 min). The mass spectrometer scan range was from 15-500 mass units. Sample size was $1\ \mu\text{L}$ and samples were prepared for GC-MS analysis by diluting the biocrude to 2.5 % (v/v) with acetone. The compounds were identified using Agilent

Technologies software (MSD ChemStation D.03.00.611) which uses a probability-based matching (PBM) algorithm to match unknown spectra to those found in a mass spectral library using National Institute of Standards and Technology's 1998 version (NIST 98). The quality of a match determined by ChemStation is defined as the probability that the unknown is correctly identified as the reference. Obtained quality of a match was between 1 and 100 and values above 90 were considered as very good matches in our analysis. Fourier transform infrared (FT-IR) analyses of biocrude, SR and raw feedstock were performed using a Varian model Scimitar 2000 (Palo Alto, CA, USA) to identify the structural groups. All the samples were analyzed in triplicates in the range $4000\text{--}600\text{ cm}^{-1}$ and averaged for each sample. Solid samples were analyzed using KBr as transparent pellets.

The gaseous products were analyzed by GC (Agilent 3000A micro-GC) to determine the concentration of gases (H_2 , CO, CO_2 , CH_4 , and other $\text{C}_2\text{--C}_5$). Columns on the GC included an MS 5A PLOT (10 m length \times 32 mm diameter), a PLOT U (8 m \times 0.32 mm), an alumina PLOT (10 m \times 0.32 mm), and an OV-1 (10 m \times 0.15 mm \times 2.0 μm). Compounds were detected and quantified with thermal conductivity detectors that were calibrated using a refinery gas calibration mix (Agilent, part #5184-3543) after column separation. Helium at $0.55\pm 0.01\text{ MPa}$ ($80\pm 2\text{ psi}$) pressure was used as the carrier gas for the analysis. The water soluble components in the aqueous phase co-product were analyzed by a high performance liquid chromatography (LC-20 AT, Shimadzu Corp., USA) equipped with a RID-10A refractive index detector and a 7.8 \times 300 mm Corezel 64-H transgenomic analytical column for glucose, succinate, formate and acetate. About 2 mL of the aqueous phase co-product was centrifuged at 5000 rpm for 5 min, and then decanted. The supernatant was filtered through a 0.25- μm filter into 2-mL auto-sampling vials. The samples of 5 μL sizes were injected into the column using the LC-20 AT

Shimadzu auto-injector. The samples were analyzed at 6.89 MPa (1000 psi) pressure and 60°C internal oven temperature with an eluent (4 mN H₂SO₄) flow rate of 0.6 mL min⁻¹ for 30 minutes retention time. The compounds in the liquid samples were identified by comparing retention times with standards and they were quantified by comparing the peak area with that of the standards. Chemical oxygen demand (COD) was analyzed by the Reactor Digestion Method (Method 8000) using HACH DRB 200 spectrophotometer (HACH Corporation, Loveland, CO) (Jirka & Carter, 1975). Samples were diluted 100-fold with deionized water (v/v) and COD data were corrected to this dilution factor. The pH values of liquid samples were measured using a portable meter (Fischer Scientific, Accumet- AP 62). The carbon conversion efficiency (E_{CE}) was determined by measuring the unconverted energy in solids residue (SR) and was calculated as follows:

$$E_{CE}(\%) = \frac{\text{weight of feedstock} \quad \text{HHV of feedstock} - \text{weight of SR} \quad \text{HHV of SR}}{\text{weight of feedstock} \quad \text{HHV of feedstock}} \times 100$$

3.3. Results and Discussion

3.3.1. Feedstock composition

Spirulina had higher organic matter (78.15% volatiles and 15.25% fixed carbon) that comprised 93.40% of the total biomass weight and had 6.60% ash content. The major inorganic composition of the ash consisted of 1.98% K, 0.12% Ca, 1.18% P, 0.07% Na, 0.05% Mg, and 0.05% Fe. The ash content of *Spirulina* was lower compared to many other lignocellulosic biomass materials such as rice husk, rice straw, and oil-palm shell (Minowa et al., 1998) and other microalgae biomass such as *Nanochloropsis oculata*, *Porphyridium cruentum* (Biller & Ross, 2011), *Dunaliella tertiolecta* (Zou et al., 2009) and *Botryococcus braunii* (Dote et al., 1994). Biochemically, *Spirulina* consisted of 49.23% protein, 31.20% carbohydrates and 11.20

% lipids. The lipid content of *Spirulina* was lower than other species of algae such as *C. vulgaris*, *N. oculata* (Biller & Ross, 2011) and *B. braunii* (Dote et al., 1994) was expected to affect the biocrude yield and composition.

3.3.2. Effect of operating conditions on TCL

Effect of operating temperature on TCL products distribution

The effects of temperature on the product yields can be explained by Figures 3.2a & 3.2b, which present the results obtained at 200–380°C (subcritical water to supercritical water) for 60 min holding time, and 20% solids concentration. The yield of biocrude increased with increase in temperature reaching a maximum of 39.9% at 350°C and then dropped down to 36.0% at 380°C (Figure 3.2a). Similar results of increasing biocrude yield with increase in temperatures were earlier reported for liquefaction of algae (Brown et al., 2010; Zou et al., 2009; Yang et al., 2004). Except at low temperatures (200°C and 250°C), in all the experimental runs three distinct phases were observed during the separation of products, namely a dark brown oily phase at the top (B₁), a medium density phase (aqueous phase), and a heavy phase (B₂) at the bottom of the separatory funnel. The yield of B₁ fraction ranged between 14.5% and 27.2% of initial biomass weight (Fig.2b) representing 49.5% to 63.5% of the total biocrude yield. With increase in temperature from 300°C to 350°C, yield of B₁ fraction increased, but reduced thereafter when temperature increased to 380°C which was the highest temperature evaluated. Yield of B₂ fraction reduced with increase in TCL temperature. This behavior could be due to further decomposition of intermediate compounds into gas and condensation of light volatile compounds into solids at higher temperature (Brown et al., 2010). No similar information is available on two fractions (B₁ and B₂) of biocrude in the literature, as the earlier studies on algae liquefaction

reported results based on very small sample sizes (≤ 10 g) and biocrude separation was achieved by solvent extraction. In the present study, use of larger sample size (100-250 g dry biomass) produced enough biocrude allowing us to do such separation and its evaluation.

Temperature is a critical parameter for organic conversion with hot-compressed water. With increase in process temperatures, the ionic properties of water changes and this catalyzes the various reactions during liquefaction. The ionic product of water ($K_w = [H^+][OH^-]$; $[H^+] = [OH^-]$) increases drastically near the critical point (374°C , 22.1 MPa), and hot compressed water in this condition has the ability to hydrolyze complex carbonaceous compounds catalyzed by the H^+ and OH^- ions (Shuping et al., 2010). Proteins, carbohydrates and lipid macromolecules are known to undergo isomerization, defragmentation/ depolymerization and condensation reactions to form biocrude. Beyond the critical point of water, conditions favor the formation of gaseous products due to further decarboxylation, cracking, steam reforming and gasification reactions of liquid/char intermediates. Hence, in our study with temperature increased to 380°C , yield of gases increased to 28.0% relative to a value of 23.2% at 350°C . We observed a drop in the yield of solid residues from 22.0% at 200°C to 5.7% at 380°C indicating higher organic conversion (defined as the percentage mass of organics converted into liquid/gas products with respect to organics in the initial solids) at increased temperatures. Also, a decrease in the yield of water solubles from 56.3% at 200°C to 30.2% at 380°C indicated the conversion of intermediate water soluble products into gases and biocrude.

Effect of holding time on TCL products distribution

The holding time, reported here, was the actual time of TCL reaction once it reached 350°C . With increase in holding time, biocrude yield increased until 60 min and thereafter decreased

with further increase in the holding time (Figures 3.3a & 3.3b). Similar results have been reported in the literature with maximum biocrude yield obtained in the holding time range of 30-60 min (Zou et al., 2009; Zhou et al., 2010). The decrease in biocrude yield at higher holding times can be attributed to the conversion of the lighter hydrocarbon compounds in the biocrude into gaseous products. This conclusion is supported by the observed increase in gaseous products yield in this study from 18.2% at 60 min to 24.8% at 90 min and 27% at 120 min holding times. Longer holding time showed an increase in yield of B₁ fraction and decrease in B₂ fraction. Generally there was no significant change in the yields of solids residues, but the yield of water-solubles decreased from 44.4% to 34.9% as the holding time increased from 0 to 120 min. Initial increase in biocrude yield with prolonged holding time (up to 60 min) could be explained in the similar way to that of the effect of temperature where increased dehydration of carbohydrates and formic acid/ acetic acid intermediates resulted by hydrolysis reactions that might account for formation of additional biocrude.

Effect of solids concentration on TCL products distribution

TCL of *Spirulina* was investigated for the range, 10-50% of organic solids in the slurry. Generally solids concentration of algal slurry had no significant effect on the product yields at solids contents greater than 20% (Figures 3.4a & 3.4b). Biocrude yield increased from ~32.5% to ~39.9% when the solids concentrations increased from 10 to 20% and then remained more or less constant with further increase to 50% solids. The variation of yield results could be ascribed to the availability of optimum quantities of organics to the H⁺ and OH⁻ ions in hydrothermal reactions at higher solids concentration. There was no variation in the yields of B₁ and B₂ fractions due to solids concentration, although there was a slight decrease in the yield of B₁ at

higher solids (Figure 3.4b). Yields of water solubles dropped from 44.6% to 30.9% in the solid concentration of 10-50%. Yields of gases and solids residues were not markedly different for liquefaction at 10-50% solids concentrations and were 18.0-19.4% and 5.4-7.0%, respectively (Figure 3.4a).

3.3.3. Biocrude fuel properties

Physical and chemical properties of biocrude

The physical and chemical properties of the biocrude are presented and compared with the untreated *Spirulina* feedstock and petroleum crude in Table 3.1. Biocrudes from all TCL runs had HHV in the range of 25.2-39.9 MJ kg⁻¹, which were significantly higher than the original feedstock (20.5 MJ kg⁻¹). HHVs of biocrudes obtained in this study were in good agreement with earlier studies reported in the literature (Brown et al., 2010; Ross et al., 2010; Zou et al., 2009; Yang et al., 2004). HHV increased with the increase in temperature and at 380°C was 39.9 MJ kg⁻¹ which was 92.7% of the energy content of petroleum crude (42.9 MJ kg⁻¹). Generally, biocrudes obtained at all TCL runs had higher carbon and hydrogen content (55-83% and 8.3-9.9% respectively) and lower oxygen content than that of the original feed stock (Table 3.1). Temperature had significant effect on the carbon and hydrogen content, which showed an increase with the increase in temperature. Importantly, biocrude from *Spirulina* had significantly higher nitrogen in the range 5.3% to 6.6%, which was due to higher protein in the biomass (~50%) and consistent with results reported in the literature (Ross et al., 2010). A sulfur content of 0.50% to 1.55% was found in B₁ fraction biocrude and was 2.34% for the heavy fraction biocrude (Table 3.1). The atomic ratios H/C, O/C and N/C of the biocrude obtained from TCL of *Spirulina* at 20% solids concentration, at 350 °C and 60 min holding time were 1.44, 0.10 and

0.07, respectively, compared to the respective values of 1.77, 0.56 and 0.19 of the original biomass. The empirical formula of B₁ and B₂ obtained from TCL at 350°C temperature, 60 min holding time and 20% solids concentration were CH_{1.44}O_{0.10}N_{0.07}S_{0.004} and CH_{1.63}O_{0.12}N_{0.09}S_{0.012}, respectively, and that of the raw feedstock was CH_{1.77}O_{0.56}N_{0.19}S_{0.004}.

The specific gravities of B₁ and B₂ ranged between 0.96-1.05 g L⁻¹ and 1.17-1.19 g L⁻¹, respectively, compared to 0.86 g L⁻¹ for petroleum crude. There was a slight decrease in the specific gravity of light biocrude with increase in TCL temperature (data not shown). The light biocrude was dark brown in color, with a strong odor and an alkaline pH (9.13±0.63), while the heavy biocrude was similar to tar. The viscosity of the light fraction biocrude measured at 60°C was found to be 40.2-67.8 cP, while that of the heavy crude was 210.4 cP (Table 3.1). The light fraction biocrude obtained at higher temperatures had lower viscosity values than that obtained at lower TCL temperatures. Viscosities of algal biocrude observed in this study were similar to previously reported values (Dote et al., 1994). Although these values were significantly higher than that for light and medium petroleum crude (5.14 cP and 22.7 cP, respectively), it was lower than the heavy petroleum crude 88.03 cP (Al-Besharah et al., 1987). Higher viscosity data suggest that algal biocrudes will need suitable modification for engine uses.

Identification of chemical compounds using GC-MS and FTIR analyses

A typical chromatogram of the biocrude obtained from TCL run at 350°C is presented as Fig.A-1. More than 60 compounds have been identified and 21 of them are listed and labeled here. The main compounds include: furans, phenolic compounds, indole, benzaldehyde, higher alkanes (heptadecane, hexadecane), alkenes (hexadecene, pentdecene), aldehydes, amides, carboxylic acids (hexadecanoic acids), pyrrolidines and amides. Heterocyclic nitrogen

compounds are attributed to the high protein content in the *Spirulina* feedstock which hydrolyzes into aminoacids further forming amines and amides in a series of reactions like decarboxylation and deaminations (Ross et al., 2010). The compounds identified from the GC/MS were categorized into five classes of compounds based on the functional groups: 1) monoaromatics and single ring heterocyclic compounds such as benzene, toluene, and phenol, 2) aliphatic compounds such as alkenes, alkanes and their derivatives, 3) oxygenated compounds such as long chain carboxylic acids, esters, aldehydes and ketones, 4) nitrogenated compounds such as amines and amides, and 5) polyaromatics such as naphthalene, indene and their derivatives. Variation of key groups of compounds for different TCL experiments is presented in Figure 3.5. Biocrudes obtained at lower temperatures had higher oxygenated compounds, lower monoaromatics and lower aliphatics than that obtained at higher temperatures such as 350°C and 380°C. Nitrogenated compounds initially increased with increase in temperature from 200 to 250°C after which it decreased. Abundance of polyaromatics was similar for all TCL runs although it slightly increased from 200°C to 250°C. Presence of oxygenated and nitrogenated compounds in the biocrude would adversely affect its quality by interfering with the storage stability and catalytic upgrading. Abundance of the above key compounds was not markedly different due to the variation in holding time and solids concentration (data not shown).

Some of the major trends revealed by GC-MS analysis were confirmed by FTIR results which also provides the nature of functional groups present in the samples (Fig.A.2-a & b). The spectra in the bands between 3505-3065 cm^{-1} in Fig.A.2-a, are assigned to either O–H or N–H stretching bonds representing hydroxy or amine group compounds in the biocrude. Carboxylic acids and esters correspond to the C=O stretching band in COOH and are characterized by bands at 1672-1631 cm^{-1} . Biocrudes obtained at higher TCL temperatures are characterized by lower

intensity at the above bands (Fig.A.2-a) suggesting less abundance of oxygenated compounds as observed in GC-MS results (Fig.3.5). The absorption bands at 2931, 2918, and 2845 cm^{-1} are related to C–H stretching vibrations indicating the presence of methylene and methyl groups. Bands at 1435 cm^{-1} and 2331 cm^{-1} are due to C=C stretching vibrations in aromatic hydrocarbons and nitriles (C≡N), respectively. Biocrudes obtained at higher temperature have higher abundance of aromatic compounds characterized by higher peaks at 1445-1335 cm^{-1} (Fig.A.2-a). FTIR of SR shows the presence of an intense peak at 1018 cm^{-1} ascribed to C=S (thiocarbonyl) or S=O (sulfoxides) or P–OR (esters) (Fig.A.2-b). FTIR spectra of SR obtained from TCL at 200°C were quite similar to that of the raw biomass (not shown). Absorption bands at 1535 cm^{-1} of raw feedstock and SR (200°C) are ascribed to polypeptides, which do not appear in the spectra of biocrudes (Fig.A.2-a), indicating the scission of peptide linkages. Gradual disappearance of bands at 2931-2841 cm^{-1} , 2335-2270 cm^{-1} and bands at 1672-1375 cm^{-1} in the raw biomass and SR (at 200°C) (Fig.A.2-b) suggested that temperature had significant effect on the TCL process. FTIR of biocrudes obtained at different holding times were quite similar (data not shown).

Metals in biocrude from S. platensis

Majority of metal elements analyzed in algal biocrude were, calcium (Ca), iron (Fe), magnesium (Mg), copper (Cu), zinc (Zn), nickel (Ni), molybdenum (Mo), sodium (Na), potassium (K), aluminum (Al), chromium, boron (B), lead (Pb), manganese (Mn) and cadmium (Cd). Metals in biocrude could have originated from *Spirulina* biomass (Jena et al., 2011), and are attributed to their uptake as minor nutrients during biomass growth. Majority of the above metals were also reported to be present in petroleum crude (Gondal et al., 2006). The values of

the major trace elements in algal biocrude in our study were 848 mg kg^{-1} , 116.0 mg kg^{-1} , 69.3 mg kg^{-1} , 11.1 mg kg^{-1} , 8.2 mg kg^{-1} , 72.1 mg kg^{-1} , 14.6 mg kg^{-1} , 22.4 mg kg^{-1} , 19.4 mg kg^{-1} , 5.9 mg kg^{-1} and 1.17 mg kg^{-1} , for Fe, Ca, Mg, Cu, Zn, Ni, Na, K, Mo, Cr and Mn, respectively and were higher than those of petroleum crude (Gondal et al., 2006). Trace amounts of Pb, Cd and B were also found. Biocrude and biooil derived from algae and lignocellulosic biomass are foreseen as future drop in liquid fuel replacements for petroleum oils after suitable upgradation. Though the presence of some metals in the biocrude may require further downstream refining and hence increasing costs of fuel production, others (e.g. Fe, Cu, Zn, Ni, Cr, Mn and Mo) can be useful and be available as catalysts for the upgradation process.

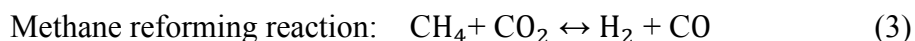
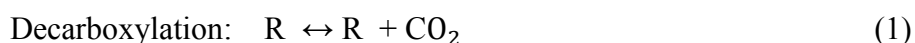
Storage stability of algal biocrude

Storage stability of biocrude samples obtained from TCL of *Spirulina* (at 350°C , 20% solids concentration and 60 min holding time) is reported here. The viscosity was found to increase linearly (Viscosity, cP = $5.16 \times \text{Days} + 191.5$, $R^2 = 0.99$, $n = 10$) with increase in storage time up to 75 days after which it remains more or less unchanged with the slope of the line reducing more than 10-fold from 5.16 to 0.54 (Viscosity, cP = $0.54 \times \text{Days} + 191.5$, $R^2 = 0.71$, $n = 3$) (data not shown). Although there is no information available on storage behavior of biocrude obtained from algae biomass, biooil generated from fast pyrolysis of wood were reported to increase in viscosity during storage (Diebold, 2002). This phenomenon is also known as “aging”. The pattern of viscosity change of biocrude in this study was similar to biooils obtained from fast pyrolysis of wood as reported by Diebold (2002). Rapid increase in viscosity of algal biocrude during the initial phase could be either due to loss of volatiles (low molecular weight

compounds) or chemical reactions of oxygenated compounds found in the biocrude (3.3.2) or a combination of the above.

3.3.4. Analysis of gaseous products

Analyses of gases showed the presence of N₂, H₂, CH₄, CO, CO₂ and traces of higher hydrocarbons (C₂-C₅). As the TCL runs were conducted in excess N₂, the resulting gaseous products had 70-92% N₂ and hence concentration of gases relative to N₂ is presented here (Table 3.2). Among different gaseous products CO₂ was a major product in all the experiments and constituted 22.1-28.2 mol%. At lower temperatures (200-250°C), the gas products were characterized by negligible amount of H₂ and C H₄ (data not shown), which increased with the increase in TCL temperature in temperature window, 300-380°C. Also, at 380°C the concentration of higher hydrocarbon gases (C₂-C₅) were found be higher than the rest of the TCL runs. The formation of H₂, CO₂ and CO can be explained by one or combination of hydrothermal reactions such as decarboxylation, water gas shift reaction, methane forming reaction, and gasification of solid residues (char) and are favored by highly reactive hot compressed water:



In the temperature window 300-350°C, more CH₄ was produced than at 380°C, which could be due to the methane forming reactions favored at low temperature conditions. As the temperature was increased to super-critical temperature (380°C), the reactions favored formation

of H_2 and CO via water shift reactions and gasification reactions. Higher concentration of CO_2 was observed at higher temperatures as a result of enhanced decarboxylation and gasification reactions. With increase in holding time beyond 60 min, the resulting gases reported higher concentration of H_2 , and CO_2 and lower CO suggesting higher holding time favored the decarboxylation and water gas shift reaction (Table 3.2).

3.3.5. Analysis of aqueous phase co-products (ACP)

Table 3 displays the results of key properties of ACP obtained from different TCL runs. The COD was found to be in the range of 63,800-190,966 $mg\ L^{-1}$ at the different operating conditions. The higher values of COD suggest presence of high percentage of organics that could pose problems for safe discharge of this process co-product. Effluent ACP from experiments with higher initial solids resulted in higher COD. The COD decreased with increase in the reaction temperature suggesting further conversion of water soluble compounds into gaseous products, thus increasing the gas production. These information are supported by the higher biocrude and gas yield and lower water soluble yield at higher temperatures (Figure 3.2a). The COD decreased with increase in retention time from 0-60 min, and then remained constant with further increase in time. Elemental analyses of the ACP indicate that O is the dominant element present (~81%) with H, C and N consisting of the balance. The ACP obtained at conversion temperatures of 300°C and higher temperatures were light brown in color, whereas the ACP obtained at 200°C and 250°C were dark in color, viscous and having an acute odor. Generally, the aqueous fractions obtained from the liquefaction had a basic pH around 8.3-9.5, which was in agreement with earlier studies (Ross et al., 2010). HPLC analysis of the ACP showed the presence of acetates, formates and small amounts of succinates. The largest

concentration of formates was found at 250°C and was 8110 mg L⁻¹, while the largest concentration of acetates (3662 mg L⁻¹) was found at 380°C (data not shown). *Spirulina* biomass has 31% of carbohydrates (3.1) and the majority of this is glucose (Richmond, 1988), which contributes to the production of succinates and formates due to the hydrolysis reaction at 200-300°C. ACP from TCL at 200°C only reported the presence of low concentrations of glucose (90 mg L⁻¹), however it was not present at higher temperatures. Previous studies have reported that hydrolysis of *Spirulina* yields glucose, sucrose, glycerol, and polyols (Richmond, 1988). Acetates were also reportedly found in the aqueous phase in algae liquefaction (Ross et al., 2010).

3.3.6. Analysis of solids and carbon conversion efficiency (E_{CE}) in TCL process

The solid residues obtained at lower conversion temperatures (200°C) had elemental C, H, N, and O values close to that of the original biomass (Table 3.4). The solid residue obtained at 200°C had an energy value (HHV) of 19.5 MJ kg⁻¹ compared to 20.5 MJ kg⁻¹ for the original biomass. This suggests that this temperature favors the carbonization of the feedstock rather than liquefaction, which was confirmed from the dark char like color of the residue. The corresponding E_{CE} was 79.1% which increased with increase in liquefaction temperature and characterized by low solid yields (Figure 3.2a). Higher temperature and holding time favored liquefaction and gasification reactions (3.3.1). Carbon, hydrogen, nitrogen and sulfur content in the solid residue generally decreased with increase in temperature and holding time, and the oxygen content increased resulting in low HHV (Table 3.4). Solids concentration did not have any effect on the carbon conversion efficiency of the TCL runs and the elemental composition of solid residues.

3.4. Conclusion

This study has shown that up to 39.9% yield of biocrude could be produced from the TCL of microalgae *S. plantensis*. Biocrude obtained from TCL runs at 350-380°C had fuel properties similar to that of petroleum crude and could be further refined to a liquid transportation fuel. Our results have shown that TCL process parameters of 350°C temperature, 60 min holding time, and 20% solids was optimum for liquefaction of *S. plantensis*. The biocrude obtained under different conditions had 50-63% light biocrude fraction. Carbon conversion efficiency of 98.3% was obtained under these preferred conditions.

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Table 3.1. Properties of raw feedstock and biocrudes obtained from liquefaction of *S. platensis* at different operating conditions

Conditions	Elemental analysis (wt%)					^f HHV, (MJ kg ⁻¹)	^g Viscosity (cP)	Atomic ratio (mol mol ⁻¹)			Empirical Formula
	C	H	N	S	[†] O			H/C	O/C	N/C	
^a <i>S. platensis</i>	46.87	6.98	10.75	0.54	34.86	20.52	-	1.77	0.56	0.19	CH _{1.77} O _{0.56} N _{0.19} S _{0.004}
^b Temperature, °C											
200	55.55	8.57	5.48	1.55	28.85	25.24	-	1.84	0.39	0.09	CH _{1.84} O _{0.39} N _{0.08} S _{0.010}
250	68.31	8.36	5.90	1.33	16.10	33.16	-	1.46	0.18	0.07	CH _{1.46} O _{0.18} N _{0.07} S _{0.007}
300	71.81	8.94	6.34	1.34	11.57	34.68	67.83	1.48	0.12	0.08	CH _{1.48} O _{0.12} N _{0.07} S _{0.007}
350	73.73	8.90	6.30	0.90	10.17	35.27	49.90	1.44	0.10	0.07	CH _{1.44} O _{0.10} N _{0.07} S _{0.004}
380	82.11	9.84	6.53	0.87	0.64	39.89	40.20	1.43	0.01	0.07	CH _{1.43} O _{0.00} N _{0.06} S _{0.003}
^c Holding time, min											
0	73.07	9.58	5.29	0.95	11.11	34.08	-	1.46	0.11	0.06	CH _{1.56} O _{0.11} N _{0.06} S _{0.004}
30	73.28	9.50	5.94	0.96	10.32	36.44	-	1.44	0.11	0.07	CH _{1.54} O _{0.10} N _{0.07} S _{0.004}
60	73.73	8.90	6.30	0.90	10.17	35.30	49.90	1.34	0.10	0.07	CH _{1.43} O _{0.12} N _{0.07} S _{0.004}
90	75.15	9.79	6.10	0.50	8.47	36.77	49.53	1.45	0.08	0.07	CH _{1.55} O _{0.08} N _{0.06} S _{0.002}
120	77.15	8.57	4.56	0.57	9.14	36.80	47.47	1.33	0.09	0.05	CH _{1.32} O _{0.08} N _{0.05} S _{0.002}
^d Solids concentration, %											
10	72.33	9.06	5.74	1.13	11.74	35.25	-	1.49	0.12	0.07	CH _{1.49} O _{0.12} N _{0.07} S _{0.006}
20	73.73	8.90	6.30	0.90	10.17	35.27	49.90	1.44	0.10	0.07	CH _{1.44} O _{0.10} N _{0.07} S _{0.004}
30	73.81	8.98	5.72	1.03	10.46	35.86	48.47	1.45	0.10	0.06	CH _{1.45} O _{0.10} N _{0.07} S _{0.005}
^e B ₂	70.16	9.59	7.23	2.34	10.68	37.15	210.40	1.62	0.09	0.11	CH _{1.63} O _{0.12} N _{0.09} S _{0.012}
Petroleum crude	83-87	10-14	0.1-1.5	0.1-2	0.5-6	42.9	23.00	-	-	-	-

^aMeasured at room temperature, ^bTCL performed at 20% organic solids, and 60 min holding time, ^cTCL performed at 20% organic solids, and 350 °C temperature ; ^dTCL performed at 350 °C temperature, and 60 min holding time, ^eHeavy fraction biocrude obtained at non-catalytic run at 20% organic solids, 350 °C temperature, for 30 min holding time, ^fHHV: higher heating value, ^gViscosity measured at 60 °C, [†]By difference

Table 3.2. Concentration of evolved gaseous species (relative to N₂) in liquefaction of *Spirulina* at 20% solids concentration, 60 min holding time, and 2 Mpa initial N₂ pressure

Temperature, °C	Time, min	Yield of gas species (mol%)				
		H ₂	CH ₄	CO	CO ₂	^a C ₂ -C ₅
300	60	0.45	0.00	0.10	22.14	0.48
350	60	0.36	0.79	1.10	26.00	0.75
380	60	1.51	1.05	1.42	28.24	1.24
350	0	0.24	0.14	1.21	22.86	0.87
350	30	0.37	0.82	1.35	27.95	0.81
350	90	0.87	0.96	1.03	28.95	0.83

^aHigher hydrocarbon gases: Ethane, propane, butane, pentane, acetylene, butane, propylene

Table 3.3. Analysis of aqueous co-products from liquefaction of *S. platensis* at various operating conditions

Conditions	Elemental analysis (wt%)					pH	COD, mg/L
	C	H	N	S	O		
^a Temperature, °C							
200.00	8.96	9.37	2.12	0.07	78.81	7.2	190966
250.00	7.97	10.70	2.13	0.06	78.58	8.6	158500
300.00	5.56	9.99	1.73	0.04	82.33	9.0	115400
350.00	4.07	10.18	1.91	0.05	83.39	9.4	90766
380.00	4.57	9.11	1.42	0.04	84.48	8.5	67230
^b Holding time, min							
0.00	5.14	9.68	1.63	0.05	83.08	9.3	130100
30.00	5.38	10.24	1.76	0.05	82.14	9.4	100533
60.00	4.07	10.18	1.91	0.04	83.39	9.4	90766
90.00	4.84	10.10	2.15	0.07	82.23	8.5	93600
120.00	4.11	10.05	1.40	0.03	84.11	8.3	94600
^c Solids concentration, %							
10.00	3.67	10.14	1.22	0.05	84.50	8.3	63800
20.00	4.07	10.18	1.91	0.04	83.39	9.4	90766
30.00	6.98	10.23	2.28	0.05	80.50	9.3	139966
40.00	8.13	10.25	3.21	0.05	77.92	9.0	161900
50.00	10.30	9.86	3.95	0.08	75.08	9.3	159933

^a TCL performed at 20% organic solids, and 60 min holding time,^b TCL performed at 20% organic solids, and 350 °C temperature,^c TCL performed at 350 °C temperature, and 60 min holding time,[†] By difference

Table 3.4. Analysis of solid residues and energy conversion efficiency of liquefaction at various operating conditions

Operating condition	Elemental analysis (wt%)					HHV, MJ kg ⁻¹	E _{CE} , %
	C	H	N	S	[†] O		
^a Temperature, °C							
200	44.82	5.90	6.80	1.01	41.53	19.50	79.1
250	28.91	3.70	3.80	1.70	62.01	12.60	93.1
300	21.92	3.10	2.80	0.60	71.63	9.31	97.2
350	11.82	2.41	1.61	1.81	82.43	6.32	98.3
380	9.41	1.20	0.80	1.01	87.62	3.80	99.0
^b Holding time, min							
0	8.72	1.40	1.40	0.61	87.82	7.11	97.8
30	8.42	1.81	1.40	0.61	87.81	8.64	97.6
60	11.82	1.81	1.41	0.61	84.41	6.32	98.3
90	9.84	1.71	1.41	0.62	86.48	6.20	98.4
120	8.81	1.81	1.32	0.71	87.42	5.60	98.8
^c Solids concentration, %							
10	12.84	2.21	1.31	0.60	83.11	6.00	98.3
20	11.82	2.41	1.61	0.81	83.42	6.32	98.3
30	11.91	2.80	2.10	0.60	82.51	5.81	98.0
40	11.55	1.77	2.11	0.71	84.11	5.11	98.2
50	12.72	2.0	1.60	0.70	82.92	5.20	98.2

^a TCL performed at 20% organic solids, and 60 min holding time,^b TCL performed at 20% organic solids, and 350 °C temperature,^c TCL performed at 350 °C temperature, and 60 min holding time

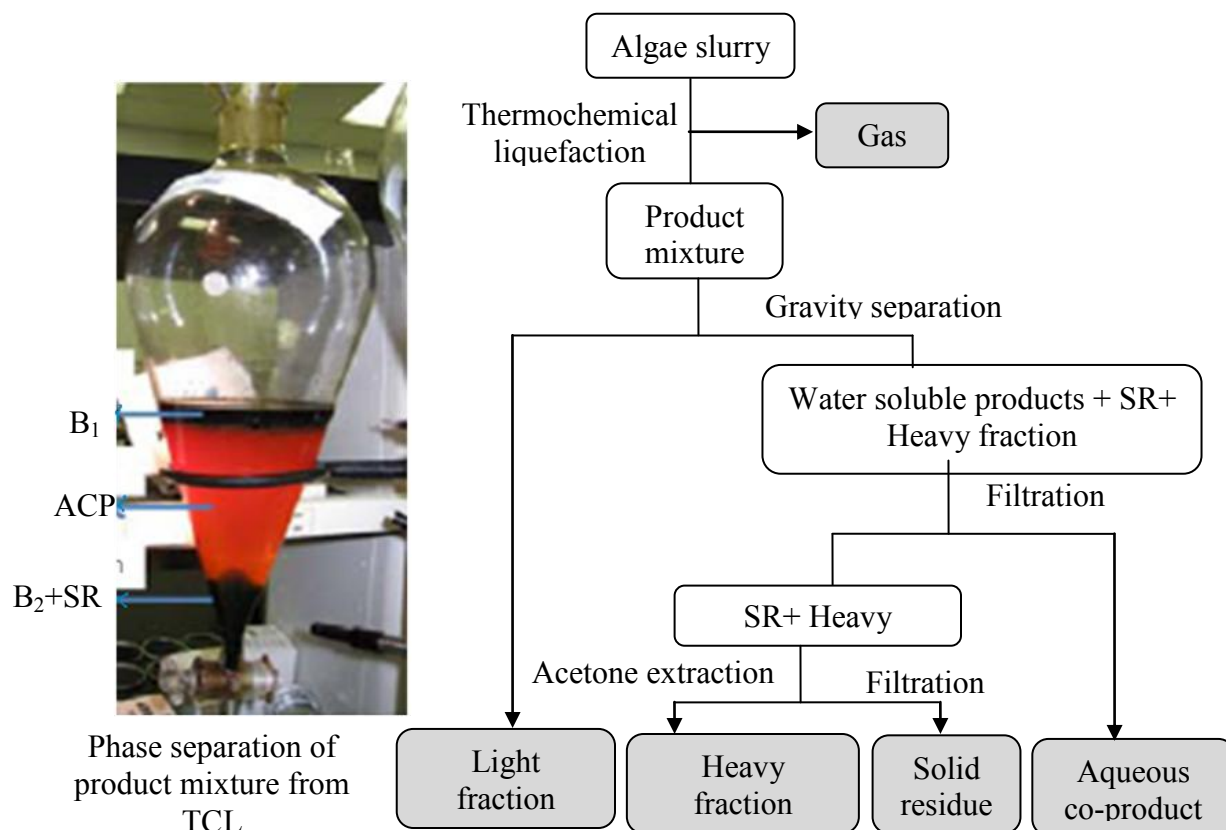


Figure 3.1. Separation of biocrude (B_1+B_2), aqueous co-product (ACP) and solids residues (SR) from TCL product mixture

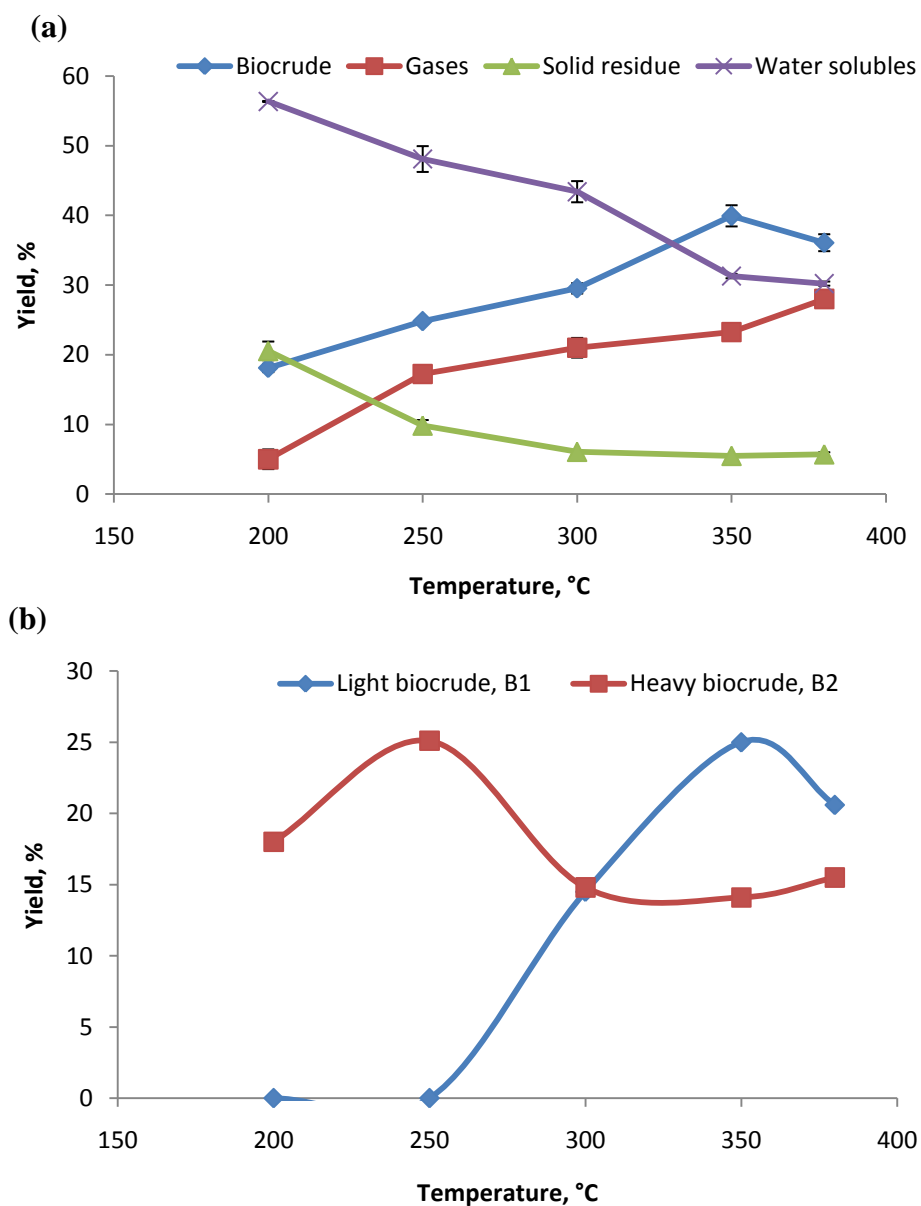


Figure 3.2. Effect of temperature on (a) Products distribution in TCL and (b) Distribution of light and heavy biocrude fractions (at 20% solids concentration, and 60 min holding time)

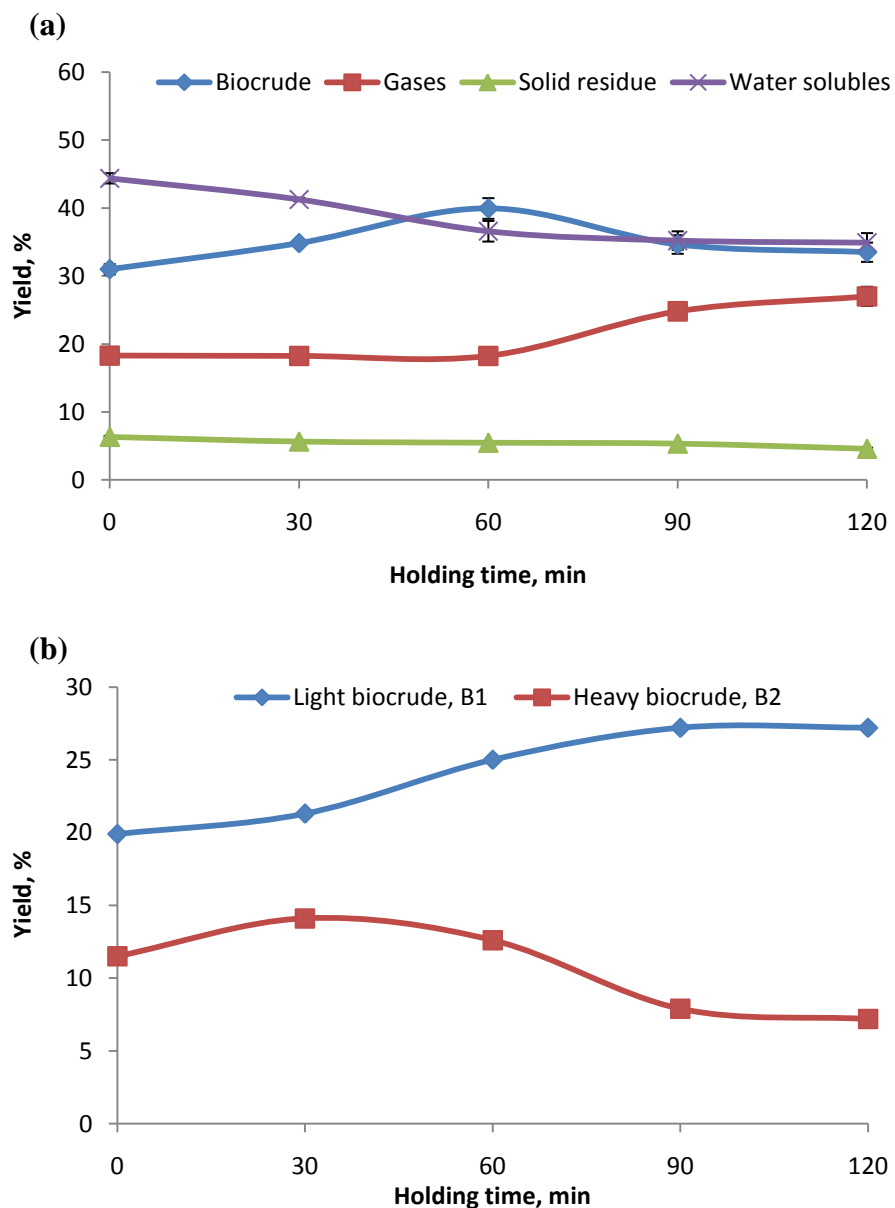


Figure 3.3. Effect of holding time on **(a)** Products distribution in TCL and **(b)** Distribution of light and heavy biocrude fractions (at 20% solids concentration, and 350°C temperature)

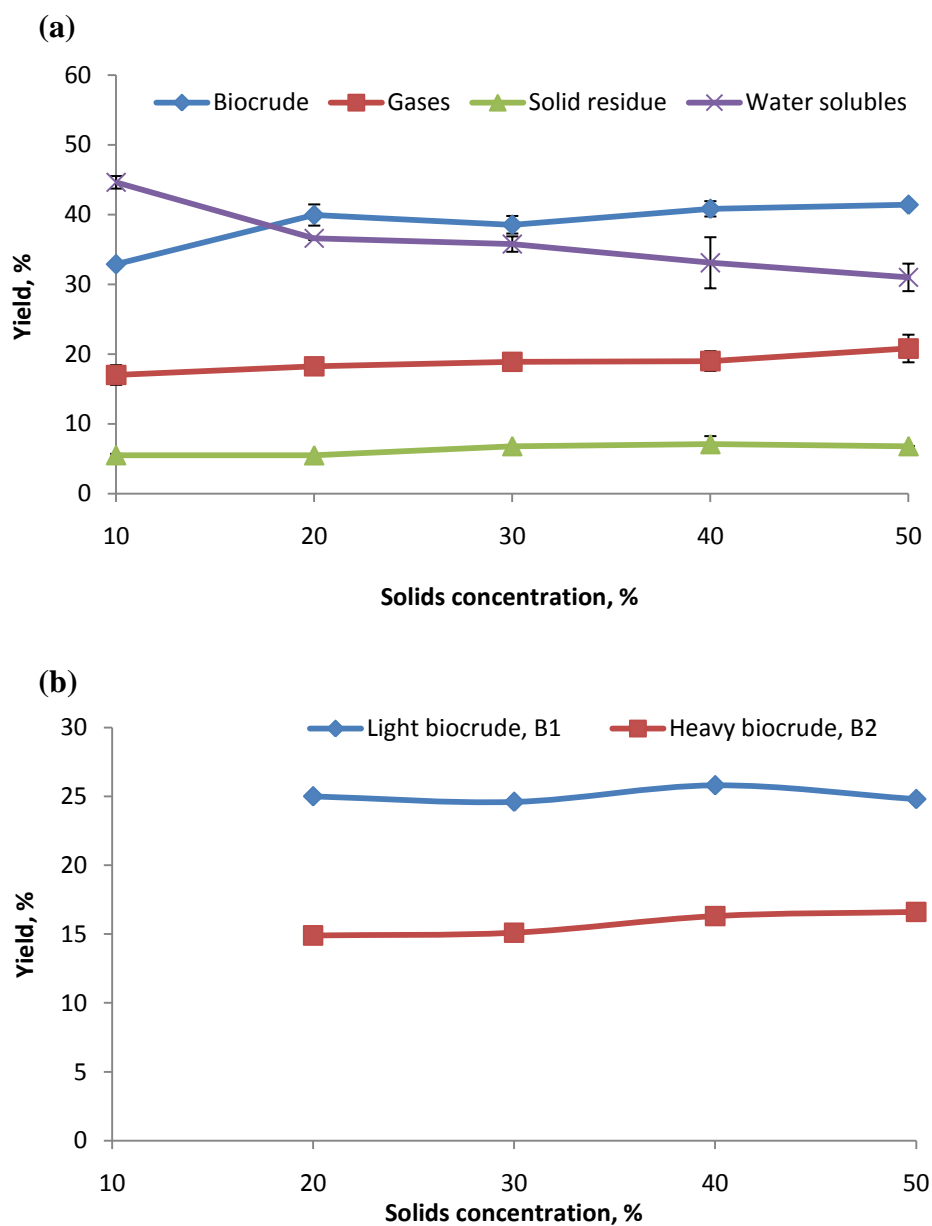


Figure 3.4. Effect of solids concentration on (a) Products distribution in TCL and (b) Distribution of light and heavy biocrude fractions (at 350°C temperature, and 60 min holding time)

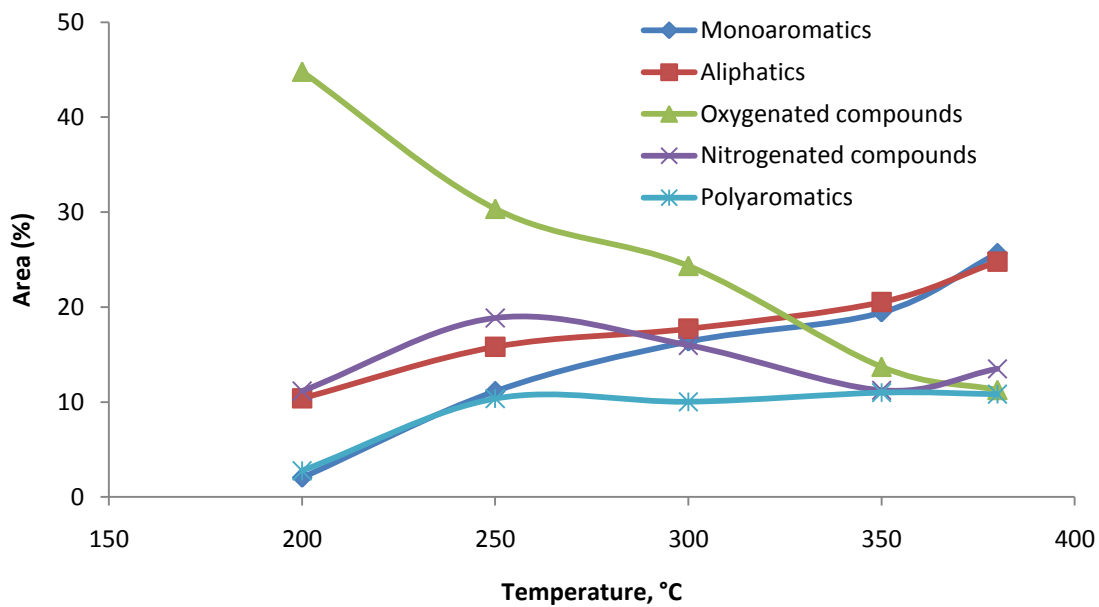


Figure 3.5. GC-MS analysis of distribution of key chemical compounds in biocrudes obtained at different TCL temperatures

CHAPTER 4

INFLUENCE OF ADDITION OF CATALYSTS ON THERMOCHEMICAL LIQUEFACTION
OF MICROALGA *SPIRULINA PLATENSIS*

¹Jena, U., Das, K.C., Kastner, J.R. 2011. To be submitted to *Applied Energy*.

Abstract

This study investigated the effect of chemical catalysts on the algal biocrude yield in thermochemical liquefaction (TCL) of the microalga, *Spirulina platensis*. TCL experiments were performed in a 1.8 L batch reactor using Na_2CO_3 , NiO , and $\text{Ca}_3(\text{PO}_4)_2$ catalysts and compared with non-catalytic TCL results. Among the three catalysts, Na_2CO_3 was found to favor biocrude yield resulting in 51.6% biocrude which was ~29% higher than the non-catalytic TCL. Addition of NiO , and $\text{Ca}_3(\text{PO}_4)_2$ was found to favor the yields of gaseous products. GC-MS and FTIR of biocrudes showed the key variations in chemical composition of biocrudes obtained from TCL in presence of different catalysts. TCL using Na_2CO_3 was found to consume the lowest energy among all treatments. Algal biocrude had similar fuel properties to that of petroleum crude with energy density of 34.7-39.9 MJ kg^{-1} compared to 42.9 MJ kg^{-1} for petroleum crude. In all cases, the solids conversion was more than 94%. Analysis of solids revealed that 40-60% of the initial catalysts could be retained in the solid char.

Keywords. Thermochemical Liquefaction (TCL), Microalgae, Biocrude, Catalysts, Hydrothermal media, Energy consumption ratio (ECR)

4.1. Introduction

Thermochemical liquefaction (TCL) is a low-temperature and high-pressure thermochemical process in which macromolecule compounds in biomass are degraded into small molecules with or without catalyst in an aqueous medium or using organic solvent (Balat, 2008). At near-critical and supercritical temperature and pressure (374°C, 3205 Psi) water undergoes significant changes in physical properties such as solubility, density, dielectric constant, ionic properties and reactivity (Savage, 1999). Dramatic changes in phase behavior and higher H^+ concentration makes hot compressed water a powerful hydrothermal media for depolymerization and repolymerization of lignins, celluloses, lipids, and proteins and result in production of biocrude, water soluble organics, gas and char (Jena et al., 2011). Biocrude is a dark viscous energy dense liquid fuel having close properties to that of petroleum fuel oil. Production of biocrude from microalgae has been of much interest in the recent times because of their ability to produce higher net weight of biomass than any other terrestrial crops, grow in adverse climatic conditions, and to reduce green house gases. TCL is a probable technology for sustainable conversion of low energy dense wet biomass including microalgae to high energy dense fuel “biocrude”. Maximizing the yield and qualities of biocrude from algae biomass have been of primary interest in most of the studies (Matsui et al. 1997; Ross et al., 2010; Jena et al., 2011). Operating temperature, holding time, solids concentration and addition of chemical catalysts and solvents are known to improve the yield and fuel properties of biocrude obtained from TCL of microalgae. Catalysts have been found to be one of the important operating parameter that affects TCL reactions significantly.

Role of catalysts in liquefaction is historically linked with natural process of petroleum generation. Transition metal oxides such as Ni, V, Ti, and Co present in sedimentary rock are

known to catalyze the production of petroleum and natural gas (Mango, 1992). Various catalysts have been tested in TCL to see their effect on product yields and properties. Metal catalysts derived from Fe, Mo and Ru have been reported to improve the hydrogen transfer and thereby increase in oil yield and the overall conversion efficiency in co-liquefaction of coal and microalgae biomass (Ikenaga et al., 2001). A study by Matsui et al. (1997) showed that iron catalyst ($\text{Fe}(\text{CO})_5\text{-S}$) increased an oil yield by ~28% in the thermal liquefaction of the microalga *Spirulina* at 350°C and 60 min holding time (Matsui et al., 1997). Alkali metal salts using carbonates and hydroxides of Na and K have substantially increased biocrude yield from algae and other carbonaceous feedstocks in various studies (Ogi et al., 1985; Yang et al., 2004; Ross et al., 2011; Huang et al., 2011; Shuping et al. 2011). The liquid yield has been reported to be increased by the addition of alkali metals such as sodium carbonate, potassium carbonate, potassium hydroxide, sodium hydroxide, and metal catalysts in case of thermochemical liquefaction of lignocellulosic biomass (Minowa et al., 1995; Xu, et al., 2008) and algae (Dote et al., 1994; Sawayama et al., 1999). This is due to formation of formate salt (HCOOK) by KOH while reacting with CO , which later reacts with water to form hydrogen. Noble metal catalysts like Ru (ruthenium), Ni (nickel) and Rh (rhodium) reportedly improved the conversion efficiency in low temperature gasification under a pressurized environment (Hao et al., 2005).

The goal of the present study was to evaluate the influence of addition of Na_2CO_3 , NiO , and $\text{Ca}_3(\text{PO}_4)_2$ catalysts on biocrude yield and properties from TCL of microalga *Spirulina platensis*. The present study is a continuation of the earlier work by the authors, which evaluated the effect of temperature, holding time and solids concentration on non-catalytic TCL of *S. platensis* (Jena et al., 2011). An optimum operating condition of 350°C temperature, 60 min holding time and 20% organic solids concentration in the slurry produced the best biocrude yield. In the present

study catalytic TCL is investigated in these operating conditions and the results are compared with the non-catalytic TCL runs. This paper also reports a detailed energy balance and nutrient distribution at all TCL conditions.

4.2. Materials and Methods

4.2.1. Raw materials

S. platensis biomass was provided by Earthrise Nutritionals LLC (Calipatria, CA) in dry powder form with defined properties (Table 4.1), and was stored in airtight packages at room temperature prior to use. The catalysts Na_2CO_3 , NiO and $\text{Ca}_3(\text{PO}_4)_2$ were purchased from J.T. Baker Inc., Fischer Scientific and Sigma-Aldrich, respectively in anhydrous powder form. Laboratory grade acetone (99.5% purity) was purchased from Sigma Aldrich. Nitrogen and helium gases were obtained from the Universal Gas and Electric Corp. (USA).

4.2.2. Thermochemical liquefaction

TCL experiments were performed in a 1.8-L Parr reactor system (Figure 4.1a) at 300-350°C temperatures for 30-60 min holding times, with 10-20% organic solids concentrations in the slurry, and three different catalysts, namely Na_2CO_3 , NiO , and $\text{Ca}_3(\text{PO}_4)_2$. In a typical experimental run 500 mL premixed algal slurry with 20% solids concentrations was prepared by adding 100 g dry algae powder with 400 g deionized water and placed in the reactor. In the catalytic liquefaction runs, 5% of catalyst on the basis of initial biomass weight was added to the slurry. The reactor was sealed, thoroughly purged using nitrogen at 289 ± 3 psi (2 MPa) initial pressure and heated to the desired temperature using an electrical heating jacket. The heating time for achieving 350°C liquefaction temperature was approximately 105 min and a hold time

of 30-60 min was maintained which was later considered as actual holding time for all TCL runs (Figure 4.1b). The reactor was continuously stirred at 300 rpm by an impeller type agitation device in all experimental runs. At the end of the reaction, the reactor was cooled to room temperature by passing tap water through the cooling coils. Process gas was sampled using polyethylene gas sampling bags for further analysis. Products mixture was separated using a definitive separation protocol as described by Jena et al. (2011). The separation procedure involved a series of steps such as decantation, vacuum filtration of light phase oil and acetone extraction and filtration of heavy phase oil and SR. The solids recovered on the filter were oven dried at 105°C for 24 hrs and obtained as solid residue (SR). The acetone soluble fraction was vacuum evaporated at 55°C to evaporate acetone and separate the heavy fraction biocrude. Biocrude yield reported in this study is the total weight of light fraction biocrude and heavy fraction biocrude.

4.2.3. Products analysis

Product yields were defined as weight percentages of biocrude, solids, gases and water soluble products relative to the initial weight of raw material. All yields were related to the initial weight of catalyst free raw feedstock. Weight of gaseous products was determined by measuring the weight difference of the reactor and contents before and after the experiment. Water soluble products from TCL runs were quantified from the mass balance as follows:

$$\text{Yield of water solubles, \%} = 100 - \text{Yield (\%)} \text{ of (Biocrude + Gas + Solid residue)}$$

Yield of solid residue (SR) represents the catalyst free solids in each of TCL runs, weight calculated by $m_{SR} = m_{SR+catalyst} - m_{catalyst}$.

Product analyses were performed using standard ASTM methods. Elemental C, H, N, S, O of solids and biocrude samples were analyzed by a LECO brand (Model CHNS-932) ultimate analyzer using ASTM D 5291 and D 3176 methods. Atomic ratio and empirical formula were derived from the elemental results. An isoperibol bomb calorimeter (Model 1351, Parr Instruments Co., Moline, Illinois) was used for measurement of higher heating values (HHV) of solid and biocrude samples. Chemical contents of raw *Spirulina* were analyzed for carbohydrate content using the DuBois method (DuBois et al., 1956), protein content by multiplying the elemental N by a factor of 4.58 (Lourenco et al., 1998), and lipid content by gravimetric method using an ANKOM^{XT10} automated extraction system (ANKOM Technology, Macedon, NY) where hexane was used as solvent. Metal composition of solid residues were analyzed using an inductively coupled plasma (argon) spectrometer equipped with a mass spectral (ICP-MS) detector system (Isaac & Johnson, 1985). Specific gravity values (g mL^{-1}) were determined gravimetrically using 2 mL Gay-Lussac pycnometers. Brookfield DV-I + Viscometer with a UL/YZ spindle adapter was used for measuring the viscosities of biocrude samples. A modified ASTM D 2983 method that involved higher temperature (60°C) was used for viscosity measurements. Carbon and hydrogen recovery (CHR) was calculated from the elemental composition of biocrude and raw feedstock and was defined as:

$$CHR = \frac{\text{weight of C and H in biocrude}}{\text{weight of C and H in the raw feedstock}} \times 100\%$$

GC-MS spectra of biocrude samples were obtained using a Hewlett-Packard (Model HP-6890) GC-MS system with a HP-5 MS column (30 m length \times 0.25 mm internal diameter \times 0.25 μm film thickness). Helium (99.99% purity) at 1 mL min^{-1} flow rate was used as a carrier gas. The method followed is described herewith. An inlet temperature of 230°C , and a detector temperature of 280°C (mass spectrometer interface temperature) was considered. The column

temperature was programmed from 40°C to 250°C at 8°C min⁻¹ after an initial for 2.5 min isothermal period. The column was kept at the final temperature for five minutes. The inlet temperature and detector temperature were set to 230°C, and 280°C respectively. The mass spectrometer was set at m/z 15-500. Samples were prepared by mixing 2.5% (v/v) biocrude in acetone and 1 µL sample volume was used for GC-MS analysis. Identification of organic compounds was accomplished by comparing the mass spectra to the resolved components using an electronic mass spectral library „NIST 98“. The quality of a match determined by ChemStation is defined as the probability that the unknown is correctly identified as the reference. Obtained quality of a match was between 1 and 100 and values above 90 were considered as very good matches in our analysis. Fourier transform infrared (FT-IR) analyses of biocrude and the raw feedstock were performed using a Varian model Scimitar 2000 (Palo Alto, CA, USA) to identify the structural groups. All the samples were analyzed in triplicates in the range 3600-600 cm⁻¹ and averaged for each sample. Solid samples were analyzed using KBr as transparent pellets.

GC (Agilent 3000A micro-GC) using thermal conductivity detector was used to analyze the gaseous composition. Three columns namely MS 5A PLOT (10 m length x 32 mm diameter), a PLOT U (8 m x 0.32 mm), an alumina PLOT (10 m x 0.32), and an OV-1 (10 m x 0.15 mm x 2.0 µm) were used for separation of gases. Following column separation, calibration was performed using a refinery gas calibration mix (Agilent, part #5184-3543). Helium at 0.55 ± 0.01 MPa (80 ± 2 psi) pressure was used as the carrier gas.

4.3. Results and Discussion

4.3.1. TCL products distribution in catalytic and non-catalytic experimental runs

Distribution of biocrude, gases, solids and water solubles from different TCL runs are presented in Figure 4.2. Among the three catalysts evaluated in our study, Na_2CO_3 was found to be effective in increasing the biocrude yield and decreasing gas yield. Sodium carbonate is known to enhance liquefaction yield and favor biocrude production in hydrothermal conversion reactions (Ross et al., 2010; Shuping et al., 2011; Yang et al., 2004; Zou et al., 2009; Sawayama et al., 1999). In the present study, catalytic liquefaction using 5% Na_2CO_3 resulted in a biocrude yield of 51.5% which was 29.2% higher than the non-catalytic liquefaction (Figure 4.2). In high pressure hydrothermal reactions, Na_2CO_3 breaks down to $\text{CH}_3\text{-COO-Na}$ (sodium formate) in the presence of CO which is generated from the hydrocarbons in the biomass (Ogi et al., 1985). A series of subsequent reactions such as dehydration, isomerization, decarbonylation and hydroxylation result in generation of OH^- and HCOO^- ions that favor further decomposition of hydrocarbon macromolecules. The compounds then rearrange through condensation, cyclization and polymerization leading to complex groups forming the biocrude. The other two catalysts, NiO and $\text{Ca}_3(\text{PO}_4)_2$ were found to reduce biocrude yield and favor gas yield. Although no information is available in literature on direct use of NiO as a catalyst in TCL, nickel in the form of NiSO_4 , (Goldman et al., 1980) have been reported to favor biocrude yield. Generally, nickel is known to enhance gasification in hydrothermal reactions with higher methane composition in the product gas (Elliott, 2008; Waldner & Vogel, 2005). Presence of nickel leads to tertiary reactions of the hydrocarbon intermediates leading to production of gases whereas presence of alkali metal inhibits the production of gases (Minowa et al., 1997). Using NiO a decrease in biocrude yield to 30.2% and an increase in gas yield to 30.5% was observed (Figure 4.2). Although calcium in forms of CaCO_3 and $\text{Ca}(\text{OH})_2$ have been reported to catalyze hydrothermal reactions by increasing biocrude yield from sewage sludge (Ogi et al., 1985), no information is

available on the use of $\text{Ca}_3(\text{PO}_4)_2$ in TCL reactions. Use of $\text{Ca}_3(\text{PO}_4)_2$ in the present study resulted in a reduction in biocrude yield to 34.5% and increase in gas yield to 25% (Figure 4.2). There was no significant difference in yields of water-soluble products and solid residues when using different catalysts and their yields were 30-34% and 5-6%, respectively.

TCL runs were also performed in different combination of reaction temperature (300°C and 350°C) with holding time (30 min and 60 min) and Na_2CO_3 catalyst. Catalytic TCL using Na_2CO_3 produced increased biocrude yield in all cases (Table 4.2). For example, TCL runs operated at 300°C using 5 N Na_2CO_3 produced 37.5% and 40.3% biocrude compared to that of 31.3% and 32.65% biocrude under non-catalytic conditions at 30 min and 60 min holding time respectively (Table 4.2). Similarly catalytic TCL run at 350°C reported 42.5 and 51.6 biocrude yield compared to 34.5% and 39.9% biocrude for non-catalytic TCL at 30 min and 60 min holding time respectively (Table 4.2). Thus it can be inferred that addition of Na_2CO_3 was found to lower the TCL temperature and holding time to achieve the similar biocrude yield as that of TCL without catalyst. For all the experimental runs, the biocrude yield was higher than the lipids content (11%) in the original biomass, which is in agreement with earlier studies (Biller & Ross, 2011; Brown et al., 2010). This confirms that hydrocarbons other than lipids, namely proteins and carbohydrates contribute significantly to biocrude formation and therefore TCL is a preferred conversion option for liquid crude production from low lipid algae. In summary, biocrude yield higher than the lipid content of *Spirulina* confirms biocrude formation by decomposition of all biomass components including proteins, carbohydrates and lipids with most of them (wt%) converted to oily compounds at favorable conditions of temperature, time, solids and catalysts. Among the operating parameters studied in this study, temperature and use of catalysts affected the products in a significant way.

4.3.2. *Physical and chemical properties of biocrude*

Biocrude obtained from all TCL runs was a dark viscous liquid characterized by a smoky odor and had a specific gravity in the range 0.93-1.08 g mL⁻¹. Elemental carbon, hydrogen and sulfur content in biocrude from non-catalytic and catalytic runs resembled to that of the fossil crude oil (Table 4.3), however they had higher percentage of nitrogen and oxygen than that of crude oil suggesting further upgradation of the biocrude for using as engine or furnace fuels. Higher oxygen percentage in biocrude results in lower energy content compared to fossil fuels. In general, TCL produced a higher energy dense liquid fuel in terms of biocrude and for all non-catalytic and catalytic runs higher heating value (HHV) was in the range 35-38 MJ kg⁻¹ compared to HHV of 20.52 MJ kg⁻¹ for raw feedstock and 83-91% of HHV of fossil crude oil. Biocrudes obtained from all TCL runs had similar HHV values and elemental compositions, although biocrude obtained from TCL using NiO catalyst reported the highest HHV (38.4 MJ kg⁻¹) and was characterized by lower oxygen content. Higher nitrogen in biocrude was due to higher protein in the biomass (49.2%) (Table 4.1) and consistent with the results reported in the literature (Matsui et al., 1997; Ross et al., 2010). Generally, biocrudes obtained at all TCL runs had higher carbon and hydrogen content and lower oxygen content than that of the original feed stock. Viscosities (measured at 60°C) for biocrudes obtained from non-catalytic run were found lower than that obtained in catalytic TCL runs using Na₂CO₃ and Ca₃(PO₄)₂. However, biocrude obtained from catalytic run using NiO was lower than the other two catalysts, which was due to lower oxygen content in the biocrude obtained at this condition. Highest CHR of 78.3% was obtained in catalytic TCL using Na₂CO₃ indicating that most of the organics were converted into biocrude compared to that of 61.2% CHR for non-catalytic TCL and 48.0% and 52.1% CHR for TCL using NiO and Ca₃(PO₄)₂ respectively.

GC-MS analysis revealed that biocrude obtained from algae was a complex mixture of furans, phenol and derived phenolic compounds, indole and derivatives, benzaldehyde, higher alkanes (heptadecane, hexadecane), alkenes (hexadecene, pentdecene), aldehydes, amides, carboxylic acids (dodecanoic acids), pyrrolidines and amides. N-heterocycle compounds were attributed to the high protein content in the *Spirulina* feedstock which hydrolyzed into aminoacids further forming amines and amides in a series of reactions like decarboxylation and deaminations (Ross et al., 2010). Above sixty compounds were identified by GC-MS and were categorized into five classes: 1) monoaromatics and single ring heterocyclic compounds (benzene, toluene, and phenol), 2) aliphatic compounds (alkenes, alkanes and their derivatives), 3) oxygenated compounds (carboxylic acids, esters, aldehydes and ketones), 4) N-heterocycles, amines and amides, and 5) polycyclic aromatic compounds (PACS) such as naphthalene, indene and their derivatives. Variation in relative abundance of key compounds in non-catalytic and catalytic TCL experiments are presented in figure 4.3. Among three catalysts used in this study, Na_2CO_3 was found to result in higher monoaromatics, higher aliphatics and lower oxygenated compounds. NiO resulted in the higher nitrogenous compounds (15.22%) compared to 10.86% for Na_2CO_3 and 10.06% for $\text{Ca}_3(\text{PO}_4)_2$ and lower abundance of oxygenated compounds (12.1%) compared to 12.2% and 18.73% for Na_2CO_3 and $\text{Ca}_3(\text{PO}_4)_2$ respectively. This data was supported by higher elemental N and lower O in biocrude from TCL run with NiO catalyst (Table 4.3). Figure 4.4 shows the FT-IR spectra of *Spirulina* biomass, and biocrudes obtained from non-catalytic and catalytic liquefaction of *Spirulina* at 20% solids and 60 min holding time. Predominant bands of biocrudes from non-catalytic and catalytic runs at $2935\text{--}2848\text{ cm}^{-1}$ (Figure 4b & c) were due to the C-H stretching vibrations, indicating the alkyl C-H bonds. Absorption bands at 1631 and 1541 cm^{-1} and a broad band at 1018 cm^{-1} ascribed to polypeptides

and carboxyl groups in the biomass (Figure 4a) does not appear in the spectra of biocrudes (Figure 4b & c), indicating the scission of peptide linkages. A wide spectra at band width 3340-3050 cm^{-1} in the biomass (Figure 4a) represents polymeric O-H group and water/ impurities. Disappearance of this band suggested that TCL proceeds through the scission of polymeric hydroxyl (O-H) bonds, or amines (beyond 3300 cm^{-1}) which are indicated by the appearance of spectra at 1489-1361 cm^{-1} (C-H stretching representing alkanes) and 1234-1141 cm^{-1} (C=O in primary and secondary alcohols). Absorbance spectra at 796-731 cm^{-1} and 691 cm^{-1} in biocrudes from non-catalytic run (Figure 4b) and catalytic runs (Figure 4c) indicate the presence of aromatic rings. The difference in the absorption spectra between biocrudes from non-catalytic TCL and catalytic TCL using Na_2CO_3 (Figure 4b & c) suggests the presence/ absence of key functional groups.

4.3.3. Analysis of gaseous products

Gases were analyzed for determination of relative concentrations of N_2 , H_2 , CH_4 , CO , CO_2 and higher hydrocarbons ($\text{C}_2\text{-C}_5$). Gaseous products from TCL runs were characterized by 70-92% nitrogen and 8-30% of gaseous products consisted of other gases. Concentration of other gases from non-catalytic and catalytic reaction conditions (as evaluated relative to nitrogen concentration) is shown in table 4.4. CO_2 was a major gaseous product in most TCL runs (22.1-26.2 mol%). The catalytic TCL runs using NiO produced the lowest concentration of carbon dioxide (8.2 mol%) followed by Na_2CO_3 (21.05 mol%) and $\text{Ca}_3(\text{PO}_4)_2$ (26.2 mol%), respectively. The formation of H_2 , CO_2 and CO can be explained by one or a combination of hydrothermal reactions such as decarboxylation, water gas shift reaction, methane forming reaction, and gasification of solid residues (char) and are favored by highly reactive hot

compressed water (Jena et al., 2011). TCL run using Ca catalyst resulted in similar gaseous composition as that of non-catalytic TCL except that it produced negligible amount of CO. When NiO was used as a catalyst, concentration of higher hydrocarbon gases (C₂-C₅) increased and was 2.35 mol% compared to 0.75 mol% for non-catalytic TCL run at 350°C.

4.3.4. Carbon (C), nitrogen (N) and phosphorous (P) balance in TCL process

Distributions of C, N, and P in various TCL product fractions were evaluated for non-catalytic TCL run (at 350°C, 60 min holding time and 20% solids concentration) and catalytic TCL run at above conditions using 5% Na₂CO₃ catalyst. Carbon, nitrogen and phosphorous distributions were determined from the elemental analysis and metal analyses of biocrude, solids and the aqueous phase co-product. The fractions in the gas phase were determined as the balance. Most of the C present in the original feedstock was converted into biocrude and the fractions were 62.9% and 79.2% for non-catalytic and catalytic TCL runs respectively (data not shown). Fractional distributions of C in ACP, SR and gas were 20.9%, 0.7% and 15.4% respectively, for the non-catalytic run and were 10.2%, 1.9% and 8.6% respectively, for the catalytic run. In both non-catalytic and catalytic TCL, largest fractions of the initial N (74.6% and 68.4%) of *Spirulina* biomass ended up in the ACP and 23.4% and 26.1% of N ended up in the biocrude. From the previous research it is known that majority of N retained in the ACP are in the form of ammonia (Jena et al., 2011). Approximately, 1% and 29% of P ended up in biocrude and aqueous phase co-product with 70% of P ending up in solid char and gas in non-catalytic TCL process. N and P in the aqueous phase co-product can be further recycled that contributes to the economy of the process. Reuse of aqueous phase co-product in algae cultivation process has been earlier demonstrated by Jena et al. (2011) for recovering N & P.

4.3.5. Analysis of SR and catalyst recovery

The solids obtained from non-catalytic and catalytic liquefaction runs were analyzed for metals to assess the fate of catalysts. Recovery indicates the recovery of Na, Ni, or Ca in SR calculated on the basis of initial weights of Na_2CO_3 , NiO , and $\text{Ca}_3(\text{PO}_4)_2$ catalysts added to algal biomass respectively and normalized to the respective metals in SR from non-catalytic TCL run. Table 4.6 shows the solids conversion (=100-% yield of SR) and percentage recovery of Na, Ni, or Ca from the catalytic TCL runs using Na_2CO_3 , NiO , and $\text{Ca}_3(\text{PO}_4)_2$ catalysts respectively. All TCL runs reported very high solid conversion in the range 93-95%. The HHV of SR was 4.5-7.0 MJ kg^{-1} (data not shown) that indicated that approximately 98% of the total energy in the raw feedstock was converted into various products in TCL process. Metal data in SR reveals that ~38% Ni, ~55% Na and ~63% Ca of the initial Na_2CO_3 , NiO , and $\text{Ca}_3(\text{PO}_4)_2$ catalyst weight can be recovered in the solids.

4.3.6. Energy analysis

Energy balance in TCL and pyrolysis processes was determined by two approaches: 1) energy Recovery (ER), and 2) energy consumption ratio (ECR). ER was calculated from the biocrude yield data and was defined as the percentage of the total energy recovered in BioOil as follows:

$$\text{ER} = \frac{\text{HHV of BioOil} \times \text{mass of BioOil}}{\text{HHV of raw feedstock} \times \text{mass of raw feedstock}} \times 100$$

About 69% of energy in original biomass could be recovered in the form of biocrude obtained from non-catalytic TCL. Use of catalyst has reported an increase or decrease in ER. TCL with Na_2CO_3 catalyst resulted an ER of 91% where as NiO and $\text{Ca}_3(\text{PO}_4)_2$ reported lower ER values than that of non-catalytic TCL and catalytic TCL (Na_2CO_3 catalyst) (Table 4.3).

ECR was estimated from the experimental results and was calculated by the method described in literature (Biller and Ross, 2011; Sawayama et al., 1999; Minowa et al., 1995; Murakami et al., 1990; Suzuki et al., 1986) as:

$$\text{ECR} = \frac{\text{energy required TCL reaction}}{\text{available energy in biocrude}}$$

$$= \frac{W \times C_{pw} \times (T - 20) + (1 - W) \times C_{ps} \times (T - 20)}{r \times Y \times (1 - W) \times VS \times H_0}$$

where W is the moisture content, C_{pw} , C_{ps} are the average specific heats of water and dry solid, T is the process temperature, r is the efficiency of available combustion energy, Y is the BioOil yield, VS is the organic content (=100-ash), and H_0 is the HHV of algal biomass. C_{pw} , C_{ps} and r were taken as $4.18 \text{ kJ kg}^{-1} \text{ K}^{-1}$, $4.18 \text{ kJ kg}^{-1} \text{ K}^{-1}$, and 0.6 respectively (Minowa, 1995). An ECR value less than 1.0 indicates that the process is a net energy producer, where as ≥ 1 ECR indicates that the process consumes more energy than it produces. ECR values obtained for TCL runs indicated that TCL was net energy producer in all cases. TCL using Na_2CO_3 was the most efficient (ECR=0.56) followed by non-catalytic run with 0.75 ECR (Table 4.3) indicated that catalytic TCL using Na_2CO_3 consumed 75% of total energy as required for non-catalytic TCL. Also, ECR values suggested that energy required for TCL using Na_2CO_3 was 39% and 36% less than the energy required for TCL using NiO and $\text{Ca}_3(\text{PO}_4)_2$ catalysts respectively. Higher value of ECR in case of TCL using Na_2CO_3 catalyst was because of its higher yield which (Figure 4.2).

4. 4. Conclusion

This study has shown that addition of chemical catalysts influences the product yields from TCL of algae. Catalytic TCL using Na_2CO_3 was found to produce higher biocrude yields and lower gaseous yields than that of non-catalytic TCL. NiO and $\text{Ca}_3(\text{PO}_4)_2$ are favorable for

gaseous yields. Addition of Na_2CO_3 was found to lower the TCL temperature and holding time to achieve the similar biocrude yield as that of TCL w/o any catalyst. Also, biocrude composition has been reportedly changed due to addition of catalysts. In all cases of catalytic liquefaction tested in this study resulted in biocrude having close fuel properties and composition as that of petroleum crude oil. Carbon conversion efficiency of 94% or more was obtained under all TCL conditions.

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Table 4.1. Proximate analysis, chemical composition and concentrations of major inorganic elements of *S. platensis* biomass used in the thermochemical liquefaction studies

Proximate analysis (wt%)		Organic matter composition (wt%)		Ultimate analysis (wt%)	
Volatiles	78.15	Proteins	49.23	C	46.87
Fixed carbon	15.25	Carbohydrates	31.20	H	7.00
Ashes	6.60	Lipids	11.10	N	10.75
HHV, MJ/kg	20.52	[†] Others	8.47	[†] O	35.38

[†] *By difference*

Table 4.2. Biocrude yields in TCL at different temperatures (T) and holding times (t) using Na_2CO_3 catalyst

T, °C	t, min	Yield of biocrude (wt%)	
		Non-catalytic	Catalytic
300	30	31.3	37.5
	60	32.65	40.3
350	30	34.5	42.5
	60	39.9	51.6

Table 4.3. Properties of biocrude and energy balance obtained in thermochemical liquefaction of *S. platensis* (at 350 °C temperature, 60 min residence time and 20% solids concentration)

Catalysts	Elemental analysis (wt%)					^a HHV, (MJ kg ⁻¹)	^b Viscosity (cP)	CHR (%)	ER (%)	ECR
	C	H	N	S	[†] O					
<i>None</i>	73.73	8.90	6.30	0.90	10.17	35.27	49.90	61.35	68.64	0.75
Na ₂ CO ₃	72.00	9.88	5.44	0.87	11.81	36.29	74.60	78.27	91.30	0.56
NiO	75.98	9.76	6.41	1.35	6.50	38.41	48.63	48.06	56.50	0.91
Ca ₃ (PO ₄) ₂	72.33	9.06	4.74	1.13	12.74	35.07	n.d.	52.12	58.98	0.87

^aHHV: higher heating value,

^bViscosity measured at 60 °C,

[†]By difference

CHR: carbon and hydrogen recovery

ER: energy recovery

ECR: energy consumption ratio

Table 4.4. Concentration of evolved gaseous species (relative to N₂) in thermochemical liquefaction of *Spirulina* (at 350°C, 60 min time and 20% solids concentration)

Catalyst	Yield of gas species (mol%)				
	H ₂	CH ₄	CO	CO ₂	^a C ₂ -C ₅
None	0.36	0.49	1.10	26.00	0.75
Na ₂ CO ₃	0.96	0.55	0.19	21.05	1.14
NiO	0.58	0.67	0.18	8.21	2.35
Ca ₃ (PO ₄) ₂	0.36	0.49	0.00	26.21	1.04

^aHigher hydrocarbon gases: Ethane, propane, butane, pentane, acetylene, butane, propylene

Table 4.5. Carbon (C), nitrogen (N) & phosphorous (P) distribution in TCL products obtained from non-catalytic and catalytic reactions (at 350°C, 60 min time and 20% solids concentration)

TCL condition		C, N, P distribution (wt%)			
		Biocrude	Aqueous co-product	Solid residue	[†] Gas
Non-catalytic					
	C	62.92	20.99	0.65	15.44
	N	23.44	74.62	1.41	0.53
	P	0.85	28.69	0.55	69.91
Catalytic, (Na ₂ CO ₃)					
	C	79.27	10.24	1.88	8.61
	N	26.11	68.40	0.82	4.66
	P	<i>n.d</i>	<i>n.d</i>	<i>0.42</i>	<i>n.d</i>

[†]By difference,

n.d: Not determined

Table 4.6. Analysis of solids conversion and metals in SR for catalyst recovery in the solid residue from the TCL process as analyzed by ICP-MS

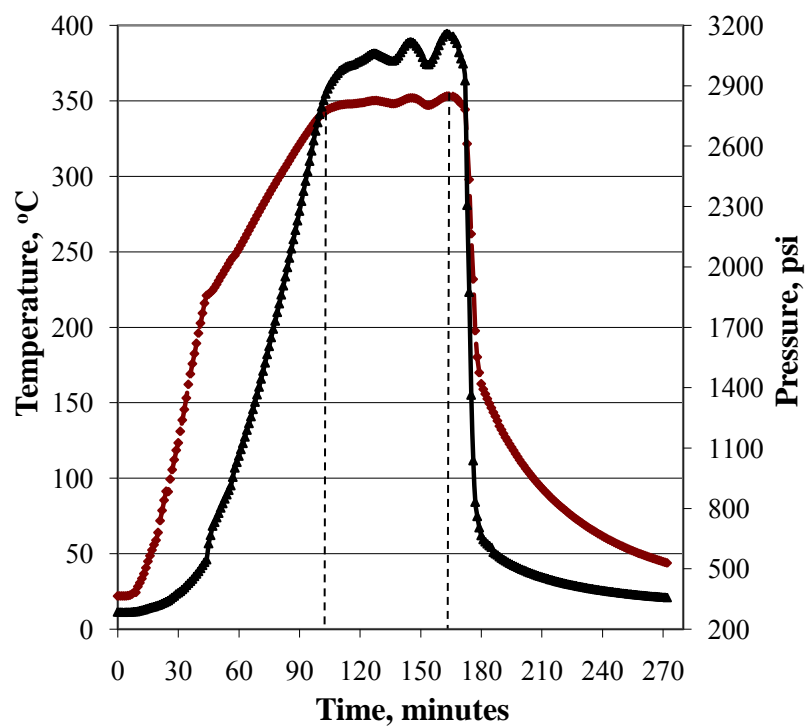
	#Solids conversion, %	Metals, %				##Recovery, %
		Na	Ni	Ca	P	
None	94.50	2.24	0.23	10.00	13.10	-
Na ₂ CO ₃	94.51	23.72	0.31	7.52	10.60	54.38
NiO	94.27	1.04	26.20	6.04	7.39	38.01
Ca ₃ (PO ₄) ₂	93.58	2.05	0.17	29.10	21.40	63.27

[#]Solids conversion = 100- % yield of SR,

^{##}Recovery indicates the recovery of Na, Ni, or Ca in SR calculated on the basis of initial weights of Na₂CO₃, NiO, and Ca₃(PO₄)₂ catalysts added to algal biomass respectively and normalized to the respective metals in SR from non-catalytic TCL run



A



B

Figure 4.1. A) Experimental set up for TCL runs, B) Temperature and pressure profile in a typical TCL run

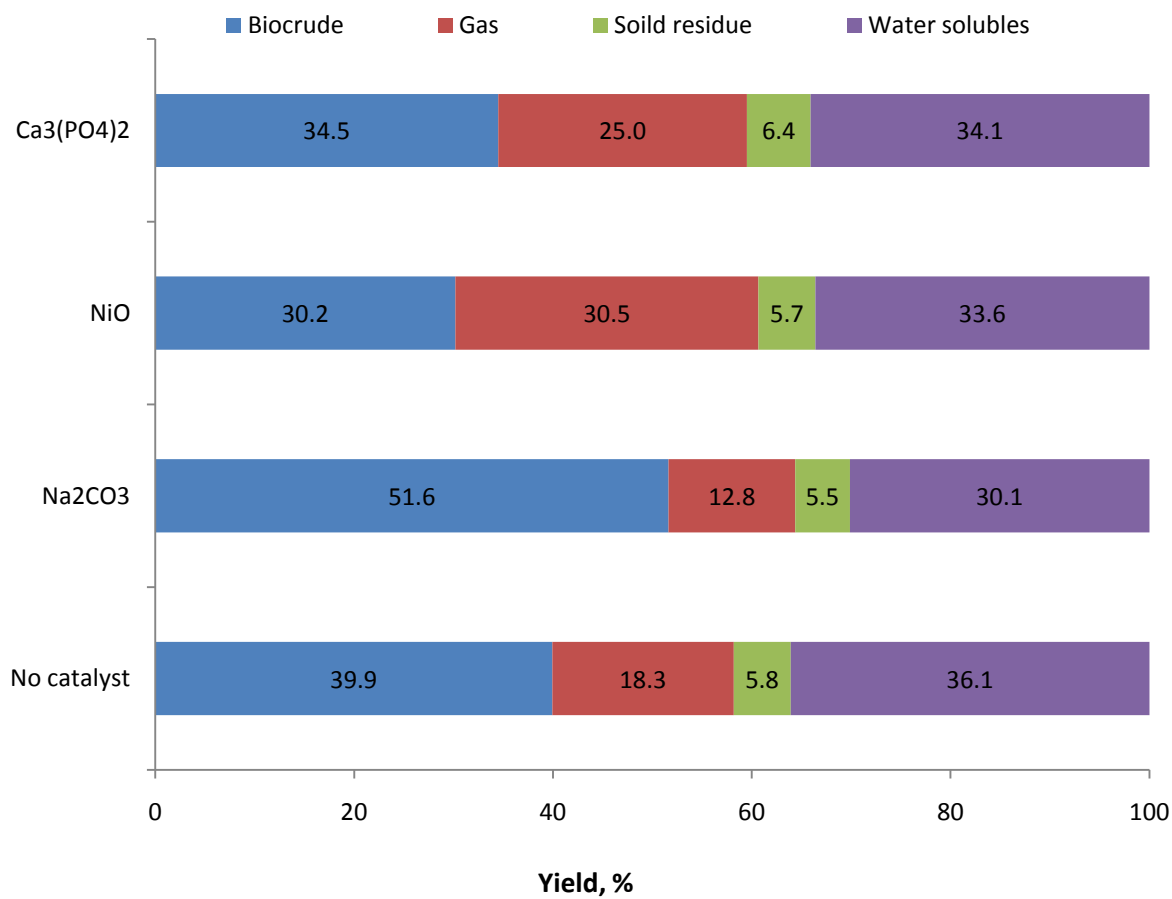


Figure 4.2. Effect of catalysts on yields of biocrude and co-products on TCL of *S. platensis* (at 350 °C temperature, 60 min residence time and 20% solids concentration)

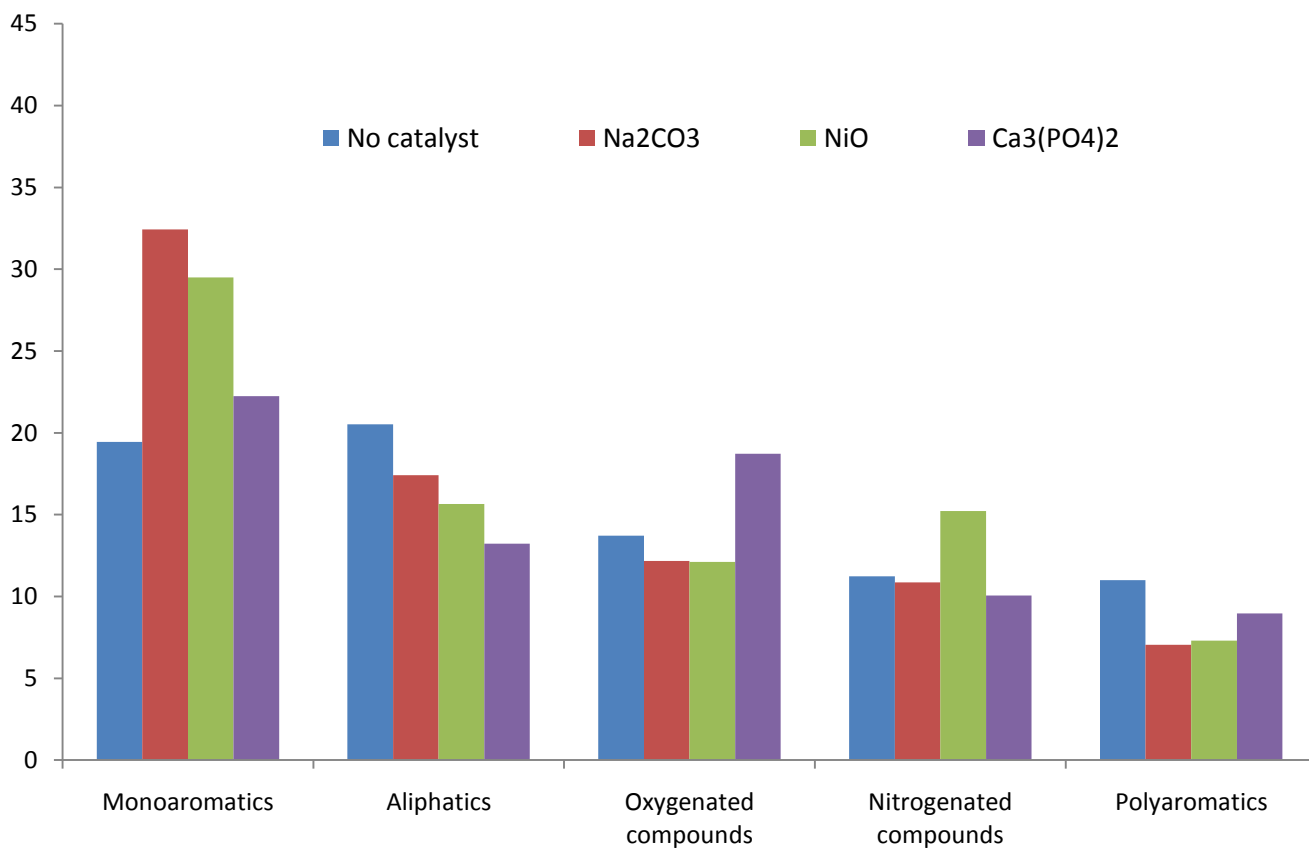


Figure 4.3. Effect of catalysts on distribution of key chemical compounds in TLC of *S. platensis*

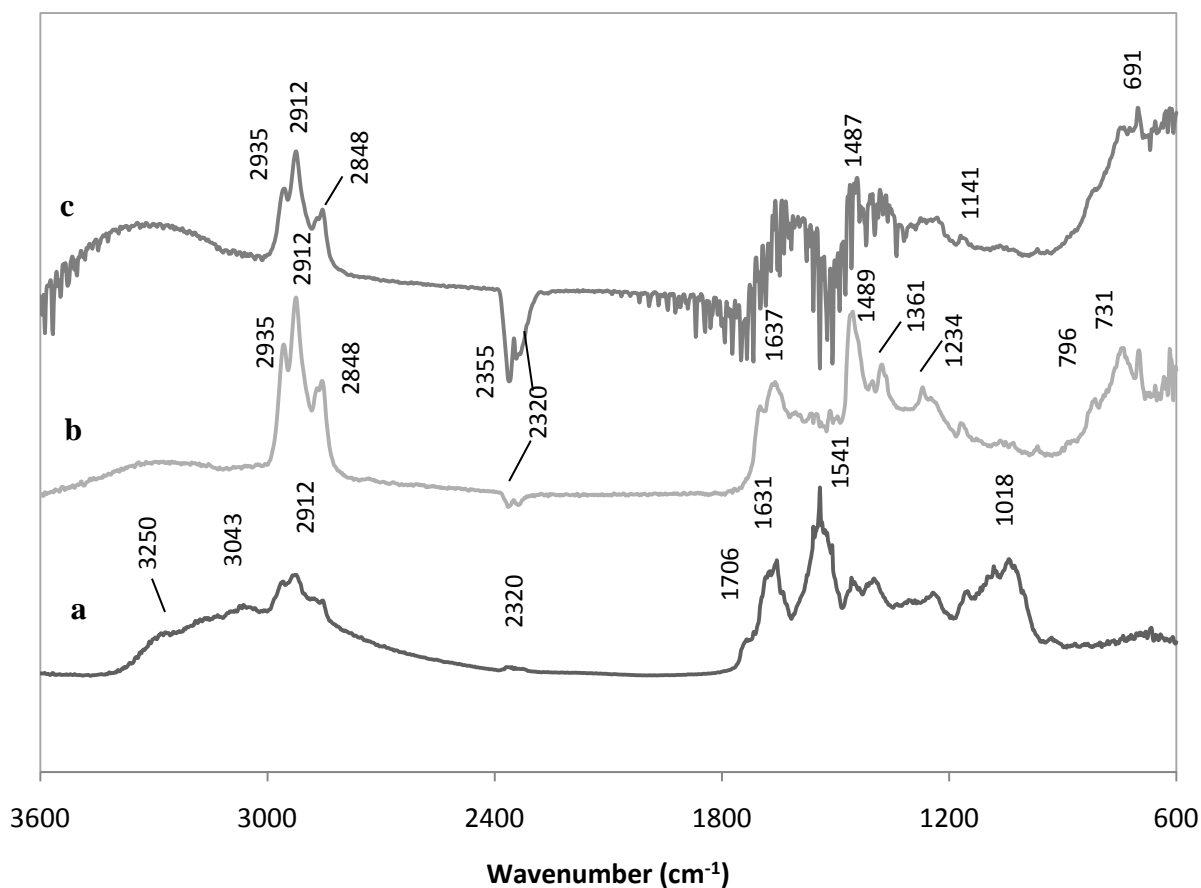


Figure 4.4. FTIR absorbance spectra of (a) *Spirulina* biomass, (b) biocrude obtained in non-catalytic TCL, and (c) biocrude obtained in catalytic TCL using Na_2CO_3 catalyst. Other reaction conditions were 20% solids concentration and 60 min holding time and 350°C temperature.

CHAPTER 5

COMPARATIVE EVALUATION OF THERMOCHEMICAL LIQUEFACTION AND
PYROLYSIS PROCESSES FOR BIOOIL PRODUCTION FROM MICROALGAE¹

¹Jena, U., Das, K.C. 2011. To be submitted to *Fuel Processing Technology*.

Abstract

BioOil is a marketable liquid product that is produced from biomass feedstocks by thermochemical liquefaction and pyrolysis processes. Thermochemical liquefaction (TCL) is a low temperature and high pressure process where as pyrolysis is accomplished at moderate to high temperature and atmospheric pressure and is restricted to dry feedstock. The goal of the present study was to evaluate and compare TCL and pyrolysis processes for producing BioOil from algae. TCL experiments were performed in a 1.8 L Parr reactor using algae slurry (80% moisture) and pyrolysis runs were carried out in a mild steel cubical reactor of $0.20 \text{ H} \times 0.20 \text{ W} \times 0.20 \text{ D m}$ internal dimensions and using dry algae powder as received (~4% moisture). Yields and composition of BioOil, char, gases and aqueous phase were evaluated and compared for TCL and pyrolysis. TCL process resulted in higher BioOil yields (~41%), lower char yields (~6.3%) and higher energy compared to pyrolysis process that resulted 23-29% BioOil, and 28-40% solids yields. BioOil obtained from TCL was found to have higher energy content and superior fuel properties such as thermal and storage stabilities than that of pyrolysis processes. Energy analysis had shown that TCL was more efficient than the pyrolysis processes

Keywords: Thermochemical liquefaction, Pyrolysis, BioOil, Stability, Energy analysis

5.1. Introduction

Pyrolysis and thermochemical liquefaction (TCL) are two important thermochemical conversion processes that directly convert the organic constituents in the biomass into a liquid fuel, namely „BioOil“ or „biocrude“ [1-3]. BioOil has got an upper edge over direct combustion and gasification because of its higher energy density [4], and is easier for transportation and storage purposes compared to gases. BioOil has close composition and properties to that of gasoline fuels, and hence can be used in engine, turbine and burner applications as fossil fuel substitutes [5-6]. They can also be upgraded to superior quality biofuels using the current technologies such as hydroxygenation, catalytic cracking, emulsification and steam reforming [3, 7]. In addition to the above qualities, BioOil is biodegradable and does not contribute to a net rise in the level of CO₂ in the atmosphere; hence sustainable production of BioOil has been of significant research interest for last two decades. In both pyrolysis and TCL processes, the organic constituents of biomass breakdown into smaller components in excess hydrogen (H⁺) produced either from the biomass or from water, that further undergo a series of depolymerization and repolymerization reactions to form liquids [1-2] in an oxygen free environment. The major differences between pyrolysis and TCL are;

- 1) TCL occurs at 725-2900 psi pressure (5-20 MPa) and moderate temperature 250-350°C whereas pyrolysis proceeds at atmospheric pressure (14.5-72.5 psi i.e. 0.1-0.5 MPa) and 400-600°C temperature [1, 3].
- 2) TCL process can convert high moisture biomass (>80% moisture) using the excess water in biomass as a highly reactant medium, hence eliminating the need for drying whereas pyrolysis is restricted to dry biomass feedstocks [1-2].

- 3) Also, TCL results in higher yield of oily products whereas pyrolysis in most cases (except fast pyrolysis) leads to production higher solid char than other products [1-3].

Microalgae are among the aquatic biomass feedstocks that are considered to be one of the best sources of liquid fuels. They can accumulate net lipids that can be converted into biofuels and have drawn significant attention for research and business ventures in recent times because of many-fold reasons [8]. Algae have higher photoconversion efficiency in the range 3-8% compared to 0.5% for terrestrial, higher oil productivity (can accumulate 10-70% lipids), ability to grow in water rather than land, and ability to sequester CO₂ from the atmosphere [9-10]. In literature, various conversion technologies including thermochemical conversion such as pyrolysis and TCL have been suggested for production of biofuel oil and gas from algae [11-12]. Although pyrolysis has been extensively reported for a wide variety of lignocellulosic biomass including different species of wood [13], there is not much information about laboratory scale study on pyrolysis of algal biomass except the one on fast pyrolysis of microalgae, *Chlorella protothecoides*, and *Microcystis aeruginosa* [14]. BioOil yields of 17.5% and 23.7% were reported from the fast pyrolysis of *C. protothecoides*, and *M. aeruginosa* respectively in the above study. Also, fuel properties of BioOils from both the algae biomass were found superior than that obtained from wood. TCL for microalgae biomass has been widely reported to result higher BioOil yields [15-17]. BioOil yields vary from species to species and the operating conditions and have been reported as 22-43% for *Nanochloropsis sp.* [15] and 18.3- 46% for *Spirulina platensis* [17]. Although an overall comparison on fuel properties of BioOil from TCL and pyrolysis were reviewed [1, 3], there has not been a single study reported on comparative evaluation of yields and fuel properties of BioOil obtained from TCL and pyrolysis processes on a single feedstock. Considering the ongoing research attention centered on liquid fuel production

from biomass and fast developments in the fields of pyrolysis/ liquefaction technology, further information is needed. In the present study effort has been made to do a comparative assessment of the BioOil yield and qualities from a microalgal feedstock using dry conversion process, slow pyrolysis and TCL. Slow pyrolysis is heating biomass at a low heating rate and longer residence time (>30 min) compared to high heating rate and short residence time (10-20 s) in case of fast pyrolysis [12]. To maintain uniform residence for both the processes, slow pyrolysis has been adopted in this study. The goals of the present study were to, (1) compare the process yields and product distribution from TCL and Pyrolysis of *Spirulina platensis*, (2) make a detailed evaluation of BioOil fuel properties, and storage characteristics, and (3) to evaluate energy and mass balance in TCL and pyrolysis processes. Other co-products such as solid char, gases and the aqueous phase have been evaluated and reported in this paper.

5.2. Experimental methodology

5.2.1. Raw material

Microalga, *S. platensis* was shipped vacuum packaged in dry powder form (4-6% moisture content) from Earthrise Naturals LLC (Calipatria, CA, USA) and was stored in a dry cool ventilated place until further use. Initial ultimate, proximate and biochemical compositing analysis were done following the standard laboratory procedures as described in section 5.2.3 and presented in table 5.1.

5.2.2. TCL and pyrolysis processes and methods

Liquefaction and slow pyrolysis experiments were conducted in the Bioconversion laboratory at The University of Georgia, Athens, USA using batch reactors (Figure 5.1). For

thermochemical liquefaction runs, a slurry with 20% solids was prepared by mixing 150 g of algae (dry basis) to 600 g of deionized water and was processed in a 1.8-L batch stirred reactor (Parr Instruments Co., Moline, PA) (Figure 5.1a). The reactor was purged thoroughly with nitrogen and pressurized to 290 psi nitrogen pressure to prevent evaporation of water. TCL runs were accomplished in two stages: i) by heating the reactor and contents to 350°C temperature (corresponding water pressure was 3000 psi) by an electrical heater at a heating rate of $\sim 3.3^{\circ}\text{C min}^{-1}$ and ii) an isothermal reaction step, where the temperature was held at 350°C ($\pm 1^{\circ}\text{C}$) for 60 min. Separation of BioOil, solids (char) and aqueous phase was accomplished by a series of filtration and acetone washing as detailed in our previous study [18]. Gaseous products were sampled using a Tedler sample bag for further analysis works before the reactor was cooled down.

Pyrolysis of algae was performed at two different temperatures 350°C and 500°C in nitrogen atmosphere using an unstirred type mild steel reactor with internal dimensions of 0.20 H \times 0.20 W \times 0.20 D m. The reactor was provided with two ports each having 0.013 m internal diameter for introduction of nitrogen gas and removal of evolved gases and vapors and arrangements for thermocouple for measuring sample biomass temperature and nitrogen flow during the experiment (Figure 5.1b). Pyrolysis runs were started by placing the reactor in programmable muffle furnace. In a typical run 500 g of dry algae sample was loaded into the reactor, and sealed airtight. A ceramic mesh was covered to prevent biomass powder from flowing along with the gas. Pyrolysis runs were completed in two steps: i) a non-isothermal step of heating the sample at $\sim 3.5^{\circ}\text{C min}^{-1}$ and $\sim 7^{\circ}\text{C min}^{-1}$ for the reaction temperatures of 350°C and 500°C respectively, and ii) an isothermal reaction step, where the reaction was held at the desired temperature for 60 min. Nitrogen gas flow rate was maintained at 0.25 L min^{-1} . Then the

furnace was turned off and the reactor was allowed to cool down to room temperature. The vapors were collected and condensed in the condenser set up consisting of a series of stainless steel traps in an ice bath. Gas samples were collected at the vent at the end of pyrolysis runs. The total liquids collected in the condensers were weighed. The liquids were separated by gravity separation by separator funnel into two phases (oily phase called „BioOil“ and aqueous phase called „water solubles“). The solid residues remaining in the reactor were collected and weighed, which gave the solid char yield. BioOil and water solubles samples obtained from different runs of pyrolysis and TCL experiments were stored in firmly closed glass bottles in darkness at 4°C for further analysis.

Treatments for TCL and pyrolysis runs have been represented as TCL350, Pyro350 and Pyro500 for the rest of the paper.

5.2.3. Product analysis

Yields of different products were determined from the ratio of the weight of the respective product to the initial weight of raw material and expressed as percentage yields. Different physicochemical properties and composition of BioOil, gaseous products, water solubles and solid char have been described in this section.

Ultimate analysis

Elemental C, H, N, S, and O were analyzed by ASTM D 5291 and D 3176 method using a LECO brand (Model CHNS-932). The analyzer was calibrated using sulfamethazine (C–51.78 %, H–5.07 %, N–20.13 %, and S–11.52 %) as the standard material.

Proximate analysis

Moisture, volatiles, ash and fixed carbon of solid and BioOil samples were measured using a LECO TGA-701 proximate analyzer (Leco Corp., St. Joseph, MI) following the ASTM D 5142 method.

Biochemical composition and metal analyses

Carbohydrate contents of algal biomass were analyzed by using DuBois method [19], protein content was approximated by multiplying elemental N by a factor of 4.58 [19] and lipids composition in the algal biomass were analyzed by gravimetric method respectively as described elsewhere [17]. The metal composition of algal biomass and the BioOil samples were analyzed using an inductively coupled plasma (argon) spectrometer (ICP) equipped with a mass spectrometer (MS) detector system [20].

Higher heating value

Measurement of higher heating values (HHV) of algae, BioOil and char samples were accomplished using an isoperibol bomb calorimeter (Parr, Model 1351) following ASTM D 5865 and D 4809. The bomb calorimeter was calibrated using benzoic acid as the standard material.

Viscosity

Dynamic viscosities of BioOil samples were determined using a Brookfield DV-I+ Viscometer with a UL/YZ spindle adapter. The method used is a modified version of ASTM D 2983 using higher temperatures than the standard due to the very high viscosity of BioOil at low temperature. A circulating bath using ethylene glycol as coolant was used to maintain constant temperatures from 10°C to 60°C with an accuracy of $\pm 1^\circ\text{C}$. Viscosity tests were replicated three times and showed an average value with precision of $\pm 3.5\%$. Most samples were analyzed for

viscosity at 40°C and 60°C. However, one stability analysis method involved measuring viscosity at 40°C before and after an accelerated aging procedure.

Density

Density of BioOil samples were obtained from the specific gravity data. Specific gravity (g mL^{-1}) was determined gravimetrically by laboratory methods using 2 mL Gay-Lussac pycnometers.

pH analysis

The pH of BioOil and solid samples was measured with a portable digital pH meter (AP62, Thermo Fisher Scientific, Accumet AP 62). For measurement of pH values of solid chars, finely ground samples were suspended in deionized water using a 1: 100 (w/w) ratio [21]. Samples were thoroughly mixed in a vibratory shaker and allowed to equilibrate for 1 h.

GC-MS analysis

GC-MS was used to identify the key chemical compounds in BioOil samples, water solubles and gaseous products. GC-MS analyses were performed by a Hewlett-Packard (Model HP-6890) gas chromatograph using Hewlett-Packard mass spectrometer (Model HP-5973) with a mass selective detector and a 30 m length \times 0.25 mm i.d. HP-5 MS column. Sample size of 1 μL was injected at 230°C inlet temperature, 280°C detector temperature, and at a He flow rate of 1 mL min^{-1} . The oven temperature was maintained at 40°C for 2.5 min followed by a ramp at 8°C min^{-1} to 250°C (held for 5 min). The mass spectrometer scan range was from 15-500 mass units and compounds were identified by mass spectral library using NIST 98. For BioOil analysis, samples were prepared by diluting BioOil to 2.5 % with acetone (v/v). For analysis of water solubles, samples were prepared by adding 10% of water soluble products to a solvent mixture made of acetone and methanol at 1:1 (v/v). Analysis of gaseous products were accomplished

by a portable micro GC using Agilent Technologies (G2858A) having a thermal conductivity detector (TCD), a molecular sieve column (5A PLOT) of 10 m \times 0.32 mm size using He as carrier gas. The GC was calibrated using a refinery gas calibration mix (Agilent, part # 5184-3543). Hydrocarbons in the gas samples were analyzed by the Hewlett-Packard GC-MS and injecting 150 μ L of sample at a He flow rate 1.51 mL min⁻¹, and holding it for 1 min at 200°C oven temperature with initial oven temperature 50°C (held for 2.5 min). The mass spectrometer scan range was from 15-500 mass units for analyzing the gas samples.

FT-IR analysis

FT-IR of the BioOil and solid char samples was performed using a Varian model Scimitar 2000 (Palo Alto, CA, USA) to identify the structural groups. All the samples were analyzed in triplicates in the range 3600-600 cm⁻¹. Solid samples were analyzed using KBr as transparent pellets.

BioOil stability assessment

Assessment of stability of algal BioOil samples from TCL and pyrolysis process was accomplished by analyzing their storage behavior, thermal stability and oxidative stability. Thermal stability of BioOil was determined by measuring the viscosity during an accelerated aging procedure [22]. Using this procedure, preweighed and analyzed BioOil samples were heated in a forced air oven at 80°C for 24 h. Then the samples were allowed to cool down at room temperature and weighed again to assess any weight change due to loss of volatiles. Viscosities of the samples were measured at 40°C prior to the aging procedure and after aging. Change in viscosity was evaluated for assessment of thermal stability.

Oxidative stability of BioOil was determined by measuring oxidation onset temperature (OOT). OOT is a temperature, at which oxidation (combustion) begins. Samples with higher

OOT are more stable. The oxidation onset test for the algal BioOil samples obtained from TCL and pyrolysis processes were performed using ASTM E 2009. The oxidation onset temperature were measured using a diffraction scanning calorimeter (DSC) by placing the approximately 3 mg of sample into the measuring cell of DSC and heating the sample from 25°C to 350°C at 10°C min⁻¹ in an oxygen atmosphere (50 mL min⁻¹). The OOT was the temperature at which combustion began (marked by the exothermic peak) and was determined as the point of intersection of by extrapolating the tangent line from the peak and the base line as described by Roger et al. [22].

Storage behavior was determined by measuring dynamic viscosity at regular time intervals until 120 days and then a final point measurement at 270 day during the storage of 9 month-time period. The BioOil from different treatments were collected and stored at room temperature (21°C) for the storage stability assessment.

HPLC analysis

The water solubles were analyzed for formates and acetates, ethanol and propionates by a high performance liquid chromatography (LC-20 AT, Shimadzu Corp., USA) using a RID-10A refractive index detector and a 7.8×300 mm Corezel 64-H transgenomic analytical column. About 5 µL of the centrifuged and filtered samples were injected into the column using the LC-20 AT Shimadzu auto-injector. The samples were analyzed at an eluent (4 mN H₂SO₄) flow 0.6 mL min⁻¹ for 30 minutes retention time at 1000 psi pressure and 60°C internal oven temperature.

Statistical analysis

One-way analysis of variance (ANOVA) was performed for BioOil yields using Excel spreadsheet. Differences between the treatments were determined at $\alpha = 0.05$ level of significance. Yield data were analyzed from five experimental runs for all the treatments.

5.3. Results and Discussions

5.3.1. Product yield and distribution in TCL and pyrolysis processes

Yields of BioOil and other products obtained in TCL and pyrolysis has been shown in Figure 5.2. ANOVA test on the BioOil yields showed that for all the treatments (TCL and pyrolysis), yields were significantly different from each other ($p < 0.0001$, $F = 123.30$). Among the two processes taken in this study, TCL resulted in significantly higher BioOil yield and lower solids yield than that of the pyrolysis processes. TCL resulted in 40.7% BioOil and 6.7% solids yield compared to 23-29% BioOil and 28-40% solids yield in case of pyrolysis. Pyrolysis performed at lower temperature (350°C) resulted in higher yield of solids (39.7%), lower yield of BioOil (24.8%) and lower gaseous yield (19.2%) compared to 25.6% solid yield, 28.5% BioOil yield and 28% gaseous yield for pyrolysis performed at higher temperature i.e. 500°C. Generally, BioOil yield from pyrolysis is known as to be a function of pyrolysis temperature and heating rate [3, 13]. Pyrolysis of wood at low temperature (<400°C) and low heating rate has earlier reported lower BioOil yield and more solid char yield than that at higher temperature and higher heating rate [13]. Yield of water solubles (=100-yields of {BioOil + solids}) was also high for TCL process (31.9%) compared to 17.8% and 20.2% for pyrolysis performed at 350°C and 500°C respectively. Higher yield of total liquids (BioOil + water solubles) in case of TCL could be due to hydrogenation reactions occurring in presence of excess H^+ and OH^- ions that are resulted from break down of water as it approaches near critical conditions of water (374°C temperature and 22.1 MPa pressure) and thus favoring the conversion of total net organics in the algal biomass. Our pyrolysis results were similar to the fast pyrolysis oil yields from *Chlorella protothecoides* and *M. aeruginosa* (14.57% and 12.5% lipid in biomass) which were 17.5% and 23.7%, respectively [14]. In the present study, slow pyrolysis of *Spirulina* (13.1% lipid in

biomass, Table 5.1) reported 23-29% BioOil yield, similar to Miao; however, TCL on the same *Spirulina* had 40.7% yield. Supporting this conclusion, solids yield in pyrolysis was higher than TCL indicating low conversion efficiency for pyrolysis. We achieved solid conversion efficiency (=100-%yield of solid char) of ~93% in TCL vs. only 60-72% in pyrolysis.

5.3.2. BioOil properties and composition

Physical properties of algal BioOil

The algal BioOil samples obtained from both TCL and pyrolysis processes were viscous liquids. BioOil produced from TCL350 was dark in color and smoky in odor, where as Pyro350 and Pyro500 processes resulted in BioOil with reddish brown color and acrid smoky odor and caused eye irritation. If left standing for longer periods, BioOil obtained from low temperature pyrolysis (Pyro350) further became a gum and ultimately a carbonaceous clot. The gum formation was not observed in case of BioOil obtained from TCL process and also pyrolysis performed at 500°C indicated that BioOil obtained at low temperature pyrolysis had poor stability and could be due to the presence of more oxygenated compounds, which will be discussed in later in this section. Generally, algal BioOil from both TCL and pyrolysis processes had alkaline pH (>9) compared to acidic pH (2-3.8) of the BioOil obtained from most of the lignocellulosic biomass feedstocks [13]. The alkaline pH could be due to higher protein content (49.2%) in the algae biomass (Table 5.1) resulting in higher abundance of nitrogenous compounds in BioOil as supported by GC-MS results in Table 5.3. Alkaline property of algal BioOil is a desirable quality and it will reduce the risk of corrosion fuel storage tanks, burners and fuel injectors. Density of algal BioOil obtained from TCL (0.97 kg L⁻¹) was comparatively lower than that obtained from pyrolysis processes (1.16-1.20 kg L⁻¹) (Table 5.2). Results

obtained for pyrolysis processes in our study were comparable to the results reported in earlier studies for the BioOil from fast pyrolysis of microalgae biomass [14]. The density of algal BioOil in our study was less than that of wood BioOil (1.20 kg L^{-1}) and close to density of fossil oil ($0.75\text{-}1.00 \text{ kg L}^{-1}$). Viscosity of BioOil in our study was measured at two temperatures, 40°C and 60°C . Viscosity values for BioOil samples obtained from TCL were 189.8 cP and 51.2 cP at 40°C and 60°C respectively, compared to that of 100.6 cP and 34.3 cP for BioOil obtained from pyrolysis at 350°C (Pyro350) and 79.2 cP and 23.1 cP for BioOil obtained at 500°C (Pyro 500) respectively (Table 3). In general, viscosity of algal BioOil in our study was reported to be higher than the BioOil obtained from pyrolysis of wood and was higher than the viscosity of petroleum crude (23.0 cP) and was in good agreement with earlier studies on fast pyrolysis of *C. protothecoides* [14]. Viscosity is one of the key properties for use of BioOil as fossil fuel substitute. Higher viscosity of BioOil will require more robust fuel filters and injectors for the engine use and hence will need suitable upgradation.

Elemental composition of algal BioOil

The elemental C, H, N, S and O analyses (Table 5.2) show that BioOil produced from TCL350 had lower nitrogen content (6.30%) than the BioOil obtained from pyrolysis processes (7.13-10.71%). High nitrogen contents in algal BioOil are due to nature of algal biomass composition dominated by the presence of chlorophyll and proteins (Miao et al., 2004). BioOil from high temperature pyrolysis (Pyro500) had similar elemental composition as that obtained from TCL350 process and was superior to pyrolysis performed at low temperature (Pyro350). Generally, BioOil obtained from low temperature pyrolysis treatment was characterized by higher elemental O leading to lower energy content (29.30 MJ kg^{-1}) than that from pyrolysis at 500°C (33.62 MJ kg^{-1}) and TCL performed at 350°C (34.21 MJ kg^{-1}). Elemental composition

and energy content of BioOil obtained from *Spirulina* biomass in our study were similar to that of the BioOil obtained from fast pyrolysis of heterotrophic *C. Protothecoids* [14]. The energy content of BioOil from *C. Protothecoids* was reported as 30-41 MJ mg⁻¹. O/C ratio of algal BioOil in our study was lower and H/C ratio was higher compared to the BioOil samples obtained from pyrolysis of wood and other lignocellulosic feedstocks [13]. The sulfur content of algal BioOil was less than 1% in all cases compared to 0.05-5.0% for fossil oil.

Major inorganic elements analyzed for algal BioOil were Ni, Fe, Ca, P, Si, Al, Mg, and Na and were presented in Table 5.2. In general, algal BioOil reported higher amounts of inorganic elements compared to that of wood pyrolysis oil as reported by Mohan et al., 2006 [13]. The inorganic elements in BioOil could have originated from *Spirulina* biomass [18]. BioOil obtained from TCL process had higher amounts of inorganic elements than that of pyrolysis processes, which was basically due to the fact that TCL is a high pressure process and leads to more intense reactions. Also, the liquids and solids generated in TCL were allowed to remain in pressurized reactor (Figure 5.1a) till the cooling and subsequent separation of the products were performed and in the process, more inorganic elements could have leached from solids and ended up mixing with the liquids/ BioOil, where as in pyrolysis process BioOil vapor was collected in a set of condensers leaving the solids inside the main reactor (Figure 5.1b) as the reaction further proceeded.

Chemical composition of algal BioOil

Results of GC-MS analysis reveal that algal BioOil is an extremely complex mixture of numerous compounds. Among the compounds indentified in Table 5.3, main components of the BioOil included aromatic hydrocarbons, N-heterocyclics, amides, amines, carboxylic acids, esters, ketones, and straight chain hydrocarbon compounds. Higher abundance of heterocyclic N

compounds in all BioOil samples were due to the chlorophyll and protein composition in algae biomass. In general, BioOils generated from pyrolysis runs especially at 350°C were characterized by abundance of higher percentage of nitrogenous compounds and aromatic heterocycles than that of TCL BioOil, which was characterized by higher abundance of straight chain compounds. Algal BioOil generally reported less oxygenated compounds than that of the BioOil obtained from lignocellulosic biomass. Different kinds of biomass possess different reactions in pyrolytic process resulting in complexity and difference in BioOil composition.

FT-IR of the BioOil samples presents normalized absorbance plotted against wavelengths (Figure 5.3). BioOils obtained from TCL and pyrolysis had revealed similar spectra indicating the presence of same functional groups. However, samples from Pyro350 and Pyro500 had higher peaks at certain band width compared to that of TCL350 and suggested the higher abundance of particular compounds. The spectra in the wave length 3150-3500 cm^{-1} are assigned to O-H functional groups represent hydroxy groups. A distinct band at about 3300 cm^{-1} for BioOil from pyrolysis runs correspond to N-H functional groups and represent higher abundance of nitrogenous compounds than that of TCL350 BioOil. Band widths of 2850-3100 cm^{-1} represent C-H stretching vibrations for alkyl derivatives. Lower peaks in BioOil from TCL350 at 1670 cm^{-1} (C=O functional groups) represents less abundance of carboxylic acids, esters or aryl ketones and polar components (C=C) in the bandwidth 1450-1670 cm^{-1} than that of pyrolysis BioOils. Stretching band at 1165 cm^{-1} in all samples correspond to functional groups CH_3 , CH_2 and CH.

BioOil stability assessment

Table 5.4 presents the results of thermal and oxidative stability of BioOils from TCL and pyrolysis of algae. There was no significant change in weight of samples during the accelerated

aging process as confirmed by weight measurements before and after aging. BioOil samples obtained from TCL runs showed the best thermal stability characteristics followed by BioOil from Pyro500 and Pyro350. BioOil from Pyro350 showed the worst thermal stability characteristics due to accelerated aging and characterized by 232.7% change in viscosity in 24 h and ranked 3 (Table 5.4). OOT evaluated for oxidative stability for the samples from TCL and pyrolysis showed that TCL BioOil had the highest OOT (190.8°C) and ranked 1 compared to 121.2°C for BioOil from Pyro350 and 155.4°C for BioOil from Pyro500. Higher OOT value of the TCL BioOil indicated better stability of TCL BioOil than that obtained from pyrolysis processes at 350°C and 500°C. Pattern of change in viscosities of the BioOil samples stored over 270 days and measured at 60°C is shown in Figure 5.4. Change in viscosities of BioOils during storage is also known as aging and occurs due to the oxidation reactions of oxygenated compounds present in BioOil samples. In initial 50 days, the viscosity change was drastic for all BioOil samples and was characterized by steep slope. However, TCL BioOil showed better storage stability over the pyrolytic BioOils and characterized by plateau at the end of 90 days, whereas viscosities of BioOils from Pyro350 and Pyro500 processes still kept on changing until 120 days. In summary, BioOil generated from TCL had better stability characteristics than that of pyrolysis oils and BioOil obtained at 350°C had the least stability that led to gum formation as described earlier in 5.3.2.

5.3.3. Gaseous products analysis

In all the samples more than 90% of the total gas could be analyzed for TCL and pyrolysis runs and gaseous products were analyzed for determination of volumetric abundance of N₂, H₂, CO, CO₂, and other hydrocarbons (HC) in the range C₁-C₄ compounds (CH₄, C₂H₆, C₃H₈, C₄H₁₀, C₂H₆ and C₃H₄). Nitrogen consisted of majority of gaseous components for both TCL and

pyrolysis, as both processes were performed in nitrogen atmosphere. Gases from TCL process reported 89% N₂, 4.8-% CO₂, 0.23% H₂ with negligible amount of CO and 1.0-1.5% HC gases, whereas gases from pyrolysis processes measured 75-83% N₂, 7.0-8.5% CO₂, 0.37-0.73% H₂, 1.2-2.5% CO and 8.2-10.5% HC gases (data not shown). Gases resulted from Pyro350 runs were characterized by more percentage of N₂, and CO₂, and less percentage of CO, H₂ and HC than that of pyrolysis performed at higher temperature (Pyro500). Higher hydrocarbons in gases (higher than C₅) as analyzed by GC-MS are presented in Table 5.5. Majority of the higher hydrocarbons in the gases consisted of N-heterocyclics, substituted furans, toluenes, styrenes, xylenes, benzenes and substituted benzenes, as well as aliphatic C-chains. Abundance of aromatic ring compounds in gases from pyrolysis runs was higher than that of TCL runs (Table 5.5).

5.3.4. *Characteristics of water soluble products*

Water solubles generated from TCL was pale yellow to light brown in color and was characterized by light smoky odor, whereas water solubles from pyrolysis runs were deep brown in color and had a strong smell. HPLC analysis of the water solubles from both TCL and pyrolysis processes identified the presence of formates, acetates, ethanol and propionates. Water solubles from TCL had lower abundance of formates and acetates (2.03 g L⁻¹, and 3.36 g L⁻¹ respectively) compared to Pyro350 (9.28 g L⁻¹, and 16.16 g L⁻¹) and Pyro500 (10.67 g L⁻¹, and 29.12 g L⁻¹) respectively (Table 5.6). This indicated that both TCL and pyrolysis process proceeded via hydrolysis, decarboxylation and deoxygenation reactions. This argument is further supported by GC-MS identification of organic compounds in the water solubles (Fig 5.5) that are characterized by the presence of substituted acetate compounds such as acetic acids and

acetamides. Also, identification of several amides and N-heterocyclics suggests that both TCL and pyrolysis of algae proceeds through scission of peptide linkages in protein and subsequent condensation/ repolymerization of amide polymers.

5.3.5. Properties of solid char

Table 7 shows various physicochemical properties of solids obtained from TCL and pyrolysis processes. Solid char obtained from pyrolysis processes had higher percentage of volatiles, higher fixed carbon and lower ash content indicating lower organic conversion than that of the TCL process. Also, the solid char obtained from the pyrolysis experiments had higher energy value 23.77 MJ kg^{-1} and 26.12 MJ kg^{-1} compared to that of 10.98 MJ kg^{-1} for the char from TCL. It indicated that most of the organic constituents of the original biomass were converted into valuable products such as liquids and gases in case of TCL process, whereas in pyrolysis methods most of them remained unconverted in char form. Among the two pyrolysis treatments pyrolysis performed at 350°C (Pyro350) and $3.5^\circ\text{C min}^{-1}$ is more like a carbonization process resulting in energy dense char, higher volatiles, less ashes compared to pyrolysis at 500°C (Table 5.7).

Solid char obtained from TCL had almost neutral pH (6.77) compared to the alkaline pH values of 11.06 and 11.69 for chars obtained from algae pyrolysis at 350°C and 500°C respectively. The slightly acidic pH value of char obtained from TCL over pyrolytic biochar could be due to the presence of ethers as represented by the distinct peak in the wave length range $860\text{-}1200 \text{ cm}^{-1}$ as indicated by the FTIR spectra in Fig 5.6. Solid char obtained from pyrolysis of biomass is also named as “biochar” and has been a major area of research interest in the recent times as biochar is reportedly known to enhance the soil fertility there by increasing

the crop productivity. Application of biochar helps in ameliorating soil conditions for crop production by increasing soil porosity, available soil water content, C-organic, soil pH, available P, CEC, exchangeable K, and Ca [21]. However, biochar alkalinity is a key factor in controlling the liming effect on acid soils. Biochar with high pH value is useful for amendments of acid soils. Solid chars resulted from the TCL and pyrolysis runs in our study had similar pH value and C elements to that of the biochar obtained from many other lignocellulosic feedstocks [23]. Especially, the solids obtained from pyrolysis runs can be further tested as biochar for acidic soils as they have other comparable properties such as higher C and H percentages (Table 5.7).

5.3.6. Energy and mass balance

Energy balance in TCL and pyrolysis processes was determined by the term energy recovery (ER). ER was calculated from the yield data and expressed in percentage of the total energy recovered in BioOil as:

$$ER = \frac{\text{HHV of BioOil} \times \text{mass of BioOil}}{\text{HHV of raw feed} \times \text{mass of raw feedstock}} \times 100$$

About 67.9% of energy in original biomass could be recovered in BioOil in TCL350 whereas pyrolysis processes reported low ER values with 33.9% for Pyro350 and 46.7% for Pyro500 (Table 5.2).

Mass balance in TCL and pyrolysis processes was expressed in two ways: 1) carbon and hydrogen recovery (CHR), and 2) nitrogen disposition in products. CHR was calculated from the elemental composition of BioOil and raw feedstock (Table 5.2) and was defined as:

$$CHR = \frac{\text{weight of } C \text{ and } H \text{ in BioOil}}{\text{weight of } C \text{ and } H \text{ in the raw feedstock}} \times 100$$

TCL process reported the highest CHR of 71.7%, whereas CHR values for pyrolysis processes were very low and were 39.3% and 51.3% for Pyro350 and Pyro500 processes respectively.

Disposition of nitrogen in various products was defined as follows:

$$\text{N disposition} = \frac{\text{weight of } N \text{ in products fraction}}{\text{weight of } N \text{ in the raw feedstock}} \times 100$$

Disposition of nitrogen BioOil and other co-products are presented in Fig. 5.7. Most nitrogen present in initial feedstock were distributed in water solubles and BioOil (74.5% and 23.45% respectively) where as in 63-74% nitrogen of initial feedstock were disposed to the solid char and gaseous products. Only 7-13% of nitrogen was recovered in water solubles.

5.4. Conclusion

The present study concludes that both TCL and pyrolysis can be employed for generating high quality BioOil from algal biomass. However, yield of BioOil from TCL process was significantly higher (~41%) than that of pyrolysis processes (23-29%). Also, pyrolysis performed at 350°C temperature (Pyro350) produced lower quality BioOil compared to the BioOil obtained from TCL runs performed at same temperature and heating rate conditions (350°C and ~3.5°C min⁻¹). However, pyrolysis runs at higher temperature (500°C) and relatively higher heating rates (~7°C min⁻¹) produced BioOil having close qualities to that of TCL process. Also, pyrolysis performed at higher temperature resulted in higher BioOil yield, and higher gaseous yields than the one performed at lower temperature. TCL has reported the highest solids conversion (~93%) compared to that of 60-72% for pyrolysis processes. TCL produces more than energy in form of BioOil than the pyrolysis process. In summary, as per the process yield, fuel quality and energy consumption ratio are concerned TCL is better suited for the algal biomass than the pyrolysis process.

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Table 5.1. Composition of raw algae biomass samples (as received basis wt%)

<i>Proximate analysis</i>	
Moisture	4.54±0.32%
Volatiles	79.14±0.20%
Ashes	6.56±0.24%
[†] Fixed carbon	15.24±0.41%
Higher heating value (MJ kg ⁻¹)	20.52±0.23
<i>Element composition</i>	
C	46.16±0.79%
H	7.14±0.20%
N	10.56±0.14%
S	0.74±0.21%
[†] O	35.44±0.31%
<i>Chemical content</i>	
Carbohydrates	30.21±0.26%
Lipids	13.30±1.50%
Proteins	48.36±0.50%
Ashes	6.56±0.24%
[†] By difference	

Table 5.2. Physical properties, ultimate analysis, inorganic elements of algal BioOil samples, and energy and mass balance in TCL and pyrolysis processes.

Properties	Conversion processes		
	TCL350	Pyro350	Pyro500
Color	Black	Reddish brown	Reddish brown
Odor	Smoky	Acrid smoky	Acrid smoky
pH	9.60	9.35	9.52
ρ , kg L ⁻¹	0.97	1.20	1.05
Viscosity, cP			
At 60°C	51.20	34.30	23.10
At 40°C	189.80	100.67	79.20
C	73.73%	67.52%	74.66%
H	8.90%	9.82%	10.57%
N	6.30%	10.71%	7.13%
S	0.90%	0.45%	0.81%
O	10.17	11.34%	6.81%
H/C ratio	1.44	1.73	1.68
O/C ratio	0.10	0.13	0.06
HHV, MJ kg ⁻¹	34.21	29.30	33.62
Inorganics in ash, mg kg ⁻¹			
Na	14.6	14.6	14.0
Mg	69.3	11.3	2.3
Al	60.1	58.8	10.7
Si	115	54.8	15.5
P	249	63.2	39.6
Ca	116	35.4	7.6
Fe	848	135	180
Ni	72.1	6.4	24.1
Energy and mass balance			
ER, %	67.9	33.9	46.7
CHR, %	71.7	39.3	51.3

TCL: Thermochemical liquefaction,
Pyro: Pyrolysis, ρ : Density,
HHV: Higher heating values,
ER: Energy recovery,
CHR: Carbon and hydrogen recovery

Table 5.3. Main components of the algal BioOils obtained from TCL and pyrolysis processes as identified by GC-MS

RT, min	Chemical compound	Area, %		
		TCL350	Pyro350	Pyro500
3.38	Pentanenitrile	-	0.92	1.06
3.77	4-Penten-2-one, 4-methyl-	0.63	0.59	-
3.93	Pyrrole	-	0.77	1.09
4.05	Toluene	2.00	6.78	11.90
4.76	3-Penten-2-one, 4-methyl-	2.24	0.59	-
5.75	Butanenitrile, 3-methyl-	-	1.61	2.01
6.16	Ethylbenzene	2.36	1.01	2.41
6.85	Styrene	1.86	0.46	1.57
9.55	2(1H)-Pyridinone, 4-hydroxy-1,6-dimethyl-	-	26.94	13.98
9.81	Phenol	1.83	0.50	0.91
10.52	Benzene, (2-methylpropyl)-	0.70	0.41	0.41
11.76	4-Piperidinone, 2,2,6,6-tetramethyl-	-	3.34	-
14.20	Benzenepropanenitrile	0.93	0.37	-
15.25	1H-Indole, 6-methyl-	1.67	2.24	-
18.32	Pentadecane	1.14	1.03	1.7965
19.80	Hexadecane	-	0.45	0.7147
21.22	Heptadecane	7.78	5.65	8.17
23.00	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	2.23	1.00	1.78
23.62	E-6-Octadecen-1-ol acetate	-	1.72	2.99
23.89	Hexadecanenitrile	-	6.64	7.45
24.17	Pentadecanoic acid, 14-methyl-, methyl ester	0.75	1.44	-
24.72	Hexadecanoic acid	18.14	-	-
26.19	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	-	1.67	0.87
27.25	Hexadecanamide	6.95	12.23	17.43
29.24	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-	5.19	2.12	2.69
31.77	Pyrrolidine, 1-(1-oxopentadecyl)-	2.61	0.96	-

RT: Retention time,

TCL: Thermochemical liquefaction,

Pyro: Pyrolysis

Table 5.4. Stability analysis of BioOils obtained from TCL and pyrolysis processes

Treatments	Viscosity increase		OOT	
	%	Rank	°C	Rank
TCL350	72.88±1.74	1	190.87±3.23	1
Pyro350	232.67±1.52	3	121.22±3.23	3
Pyro500	118.36±6.37	2	155.41±3.49	2

OOT: Onset oxidation temperature,

TCL: Thermochemical liquefaction,

Pyro: Pyrolysis

Table 5.5. Compositions of hydrocarbons in the gaseous products identified by GC-MS

RT	Compound name	% Area		
		TCL 350	Pyro350	Pyro500
1.37	Cyclopentene	-	0.32	1.20
1.43	Propanenitrile	-	5.45	5.38
1.50	2-Butanone	10.81	9.13	12.26
1.56	Furan, 2-methyl-	-	22.64	4.31
1.84	Cyclopentene, 4-methyl-	2.76	1.49	-
1.92	1-Pentanamine	-	3.62	4.47
1.95	Benzene	-	-	6.76
2.17	1-Heptene	-	1.45	0.35
2.26	Heptane	-	2.44	1.44
2.28	Heptane	1.42	-	1.90
2.83	1H-Pyrrole, 1-ethyl-	10.63	-	-
3.18	Toluene	6.85	28.63	55.00
3.82	Styrene	3.42	-	-
4.74	Ethylbenzene	-	-	3.85
4.88	p-Xylene	0.95	-	2.99
5.31	1-Butylpyrrolidine	2.47	-	-
5.91	2-Pyrrolidinone, 1-propyl-	1.97	-	-

RT: Retention time,

TCL: Thermochemical liquefaction,

Pyro: Pyrolysis

Table 5.6. HPLC analysis of water solubles in the aqueous phase obtained from TCL and pyrolysis processes

Treatments	Concentration, g L ⁻¹			
	Formate	Acetate	Ethanol	Propionate
TCL350	2.03±0.00	3.36±0.00	0.80±0.00	BDL
Pyro350	9.28±0.32	16.16±0.07	BDL	2.84±0.03
Pyro500	10.67±0.88	29.12±0.09	BDL	2.14±0.04

BDL: Below detection limit,

TCL: Thermochemical liquefaction,

Pyro: Pyrolysis

Table 5.7. Analysis of solid char obtained from algae in TCL and pyrolysis processes

Parameters	TCL350	Pyro350	Pyro500
V.M., % (db)	10.73	36.67	16.58
[†] F.C., % (db)	0.23	43.43	57.29
Ash, % (db)	88.42	18.61	25.98
C, %	11.85	59.24	47.80
H, %	2.62	4.83	2.78
N, %	2.07	10.96	9.51
S, %	0.89	0.91	0.84
HHV, MJ/kg	7.98	26.12	23.77
pH	6.77	11.06	11.69

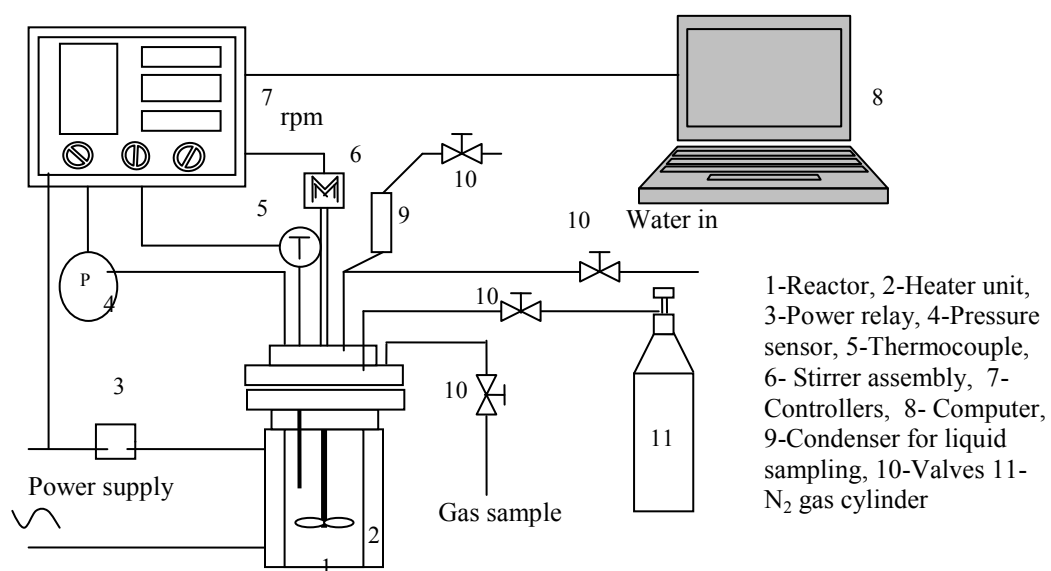
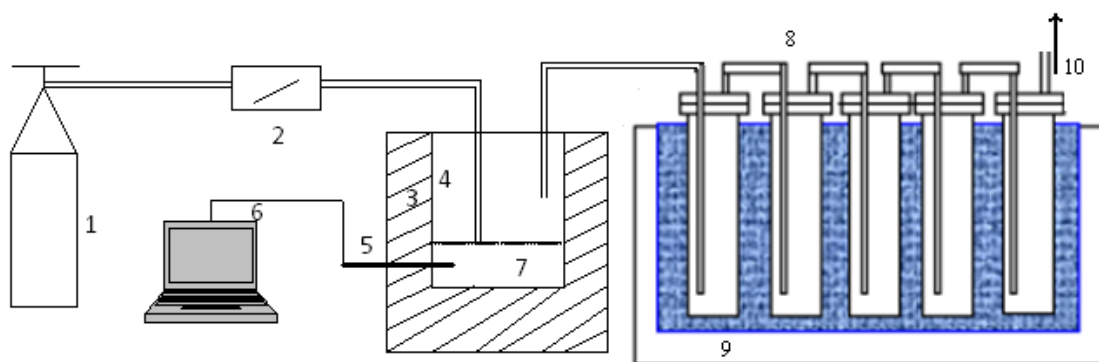
[†]Fixed carbon (F.C.) calculated by difference, $F.C. = 100 - (Ash + V.M.)$,

db: dry basis,

V.M.: volatile matter

TCL: Thermochemical liquefaction,

Pyro: Pyrolysis

(a)**(b)**

1. N₂ gas cylinder, 2. Flow meter, 3. Programmable furnace, 4. Reactor, 5. Thermocouple, 6. Data logger, 7. Sample, 8. Condenser set up, 9. Ice bath, 10. Gas vent

Figure 5.1. The experimental set up for **(a)** TCL run, and **(b)** pyrolysis runs

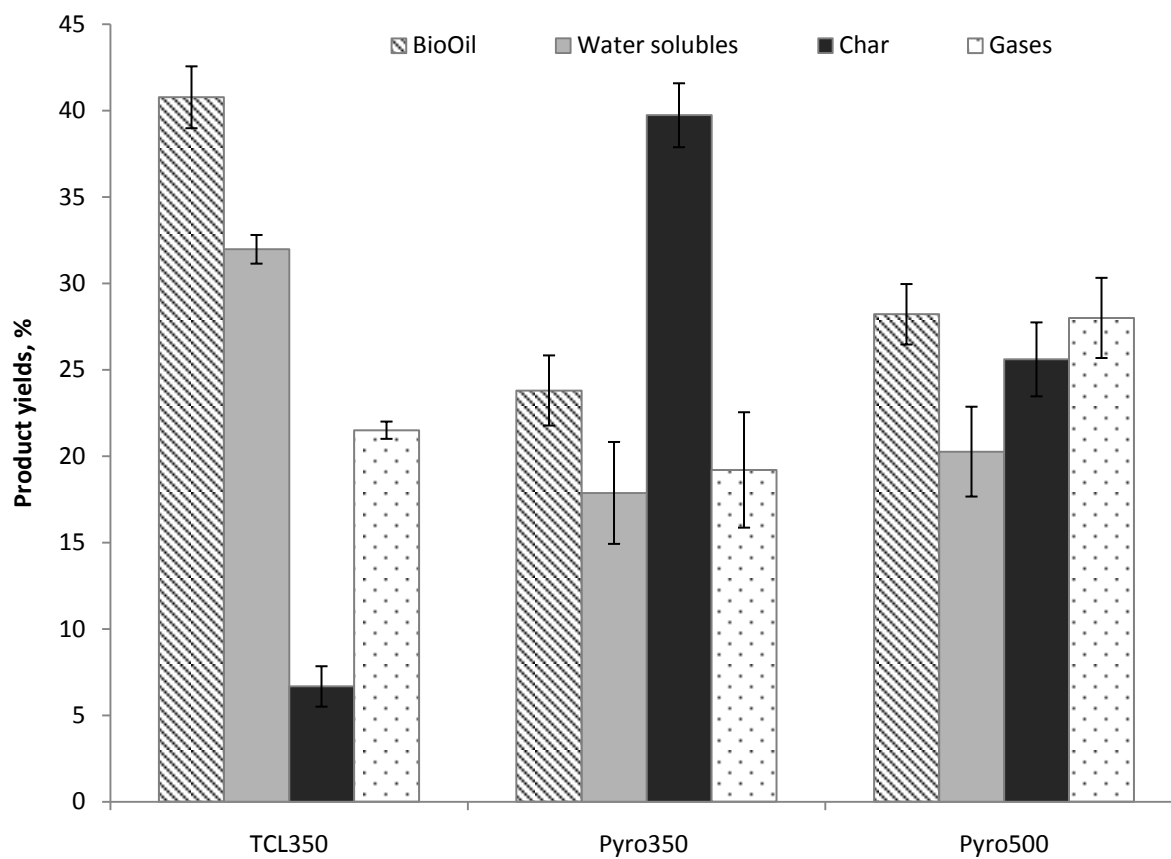


Figure 5.2. Comparison of product yields from algae in TCL process (at 350°C) with pyrolysis processes performed at two different temperatures (350°C and 500°C)

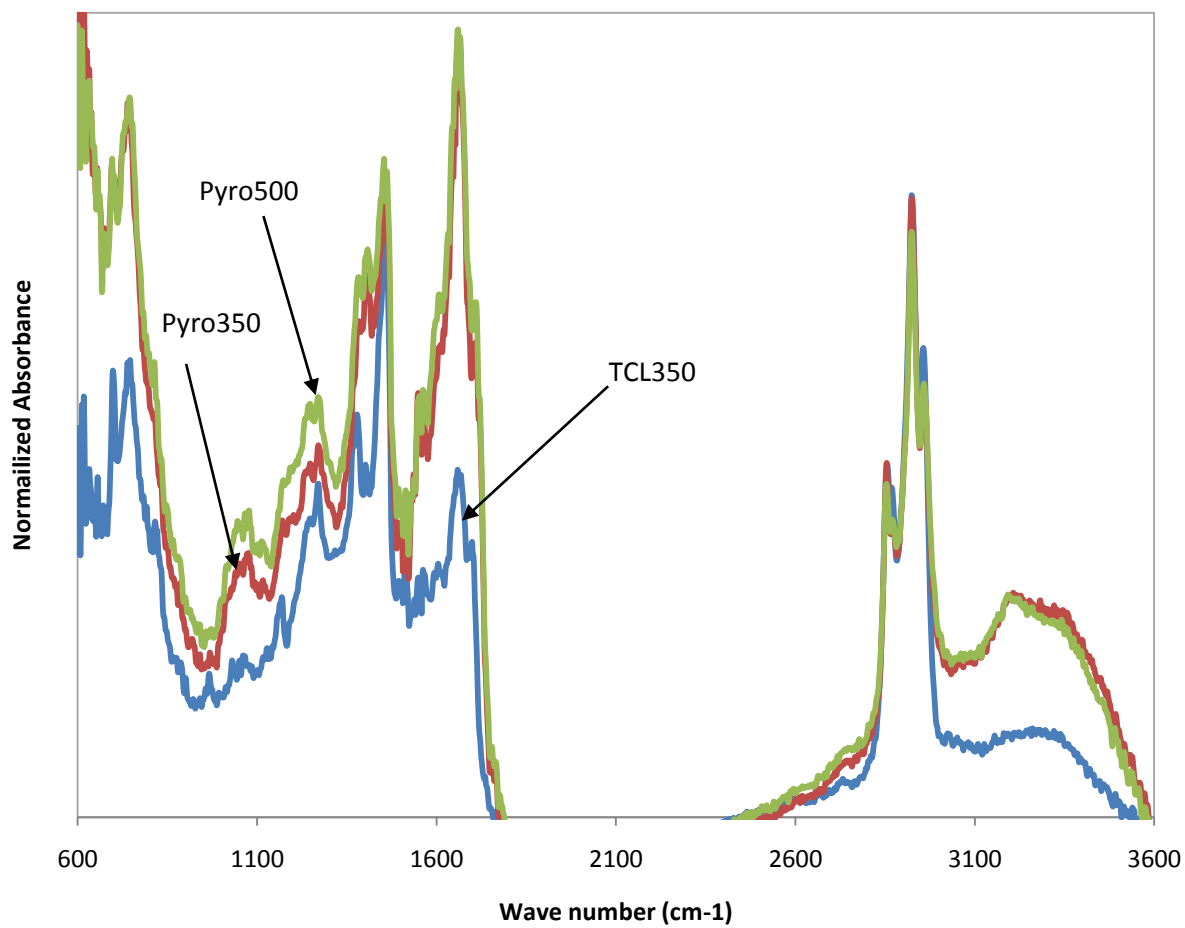


Figure 5.3. Infrared Spectra of BioOil samples obtained from TCL and pyrolysis processes

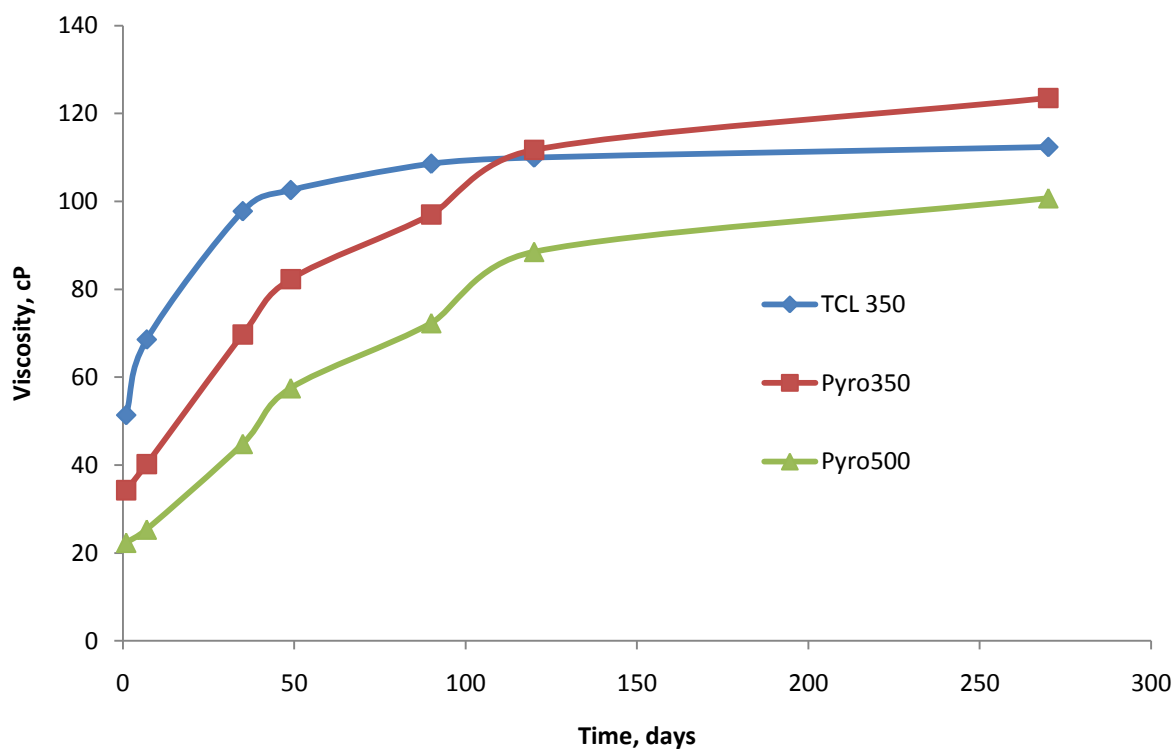


Figure 5.4. Storage stability characteristics (viscosity variation) of algal BioOil obtained from TCL and pyrolysis processes

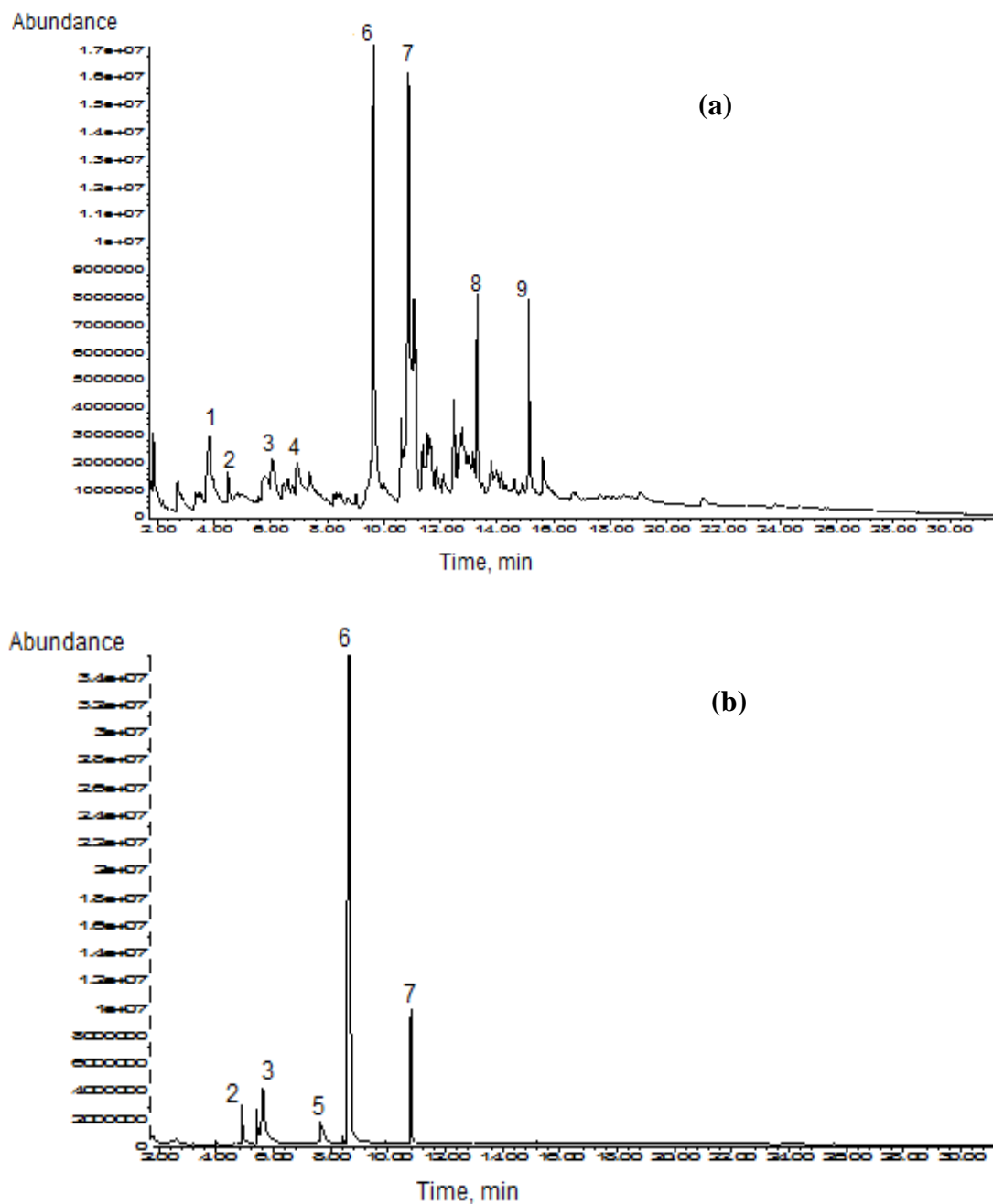


Figure 5.5. GC-MS analysis of aqueous phase; (a) from TCL process, and (b) from pyrolysis performed at 350°C. (The labeled peaks are: 1. Acetic acid, 2. Pyrazine, methyl-, 3. Acetamide, N-methyl, 4. Acetamide, N-methyl, 5. 2-Propanamine, N-(1-methylpropylidene)-, 6. 2-Pyrrolidine, 1-methyl, 7. 2-Pyrrolidinone, 8. Piperidine, 1-butyl-)

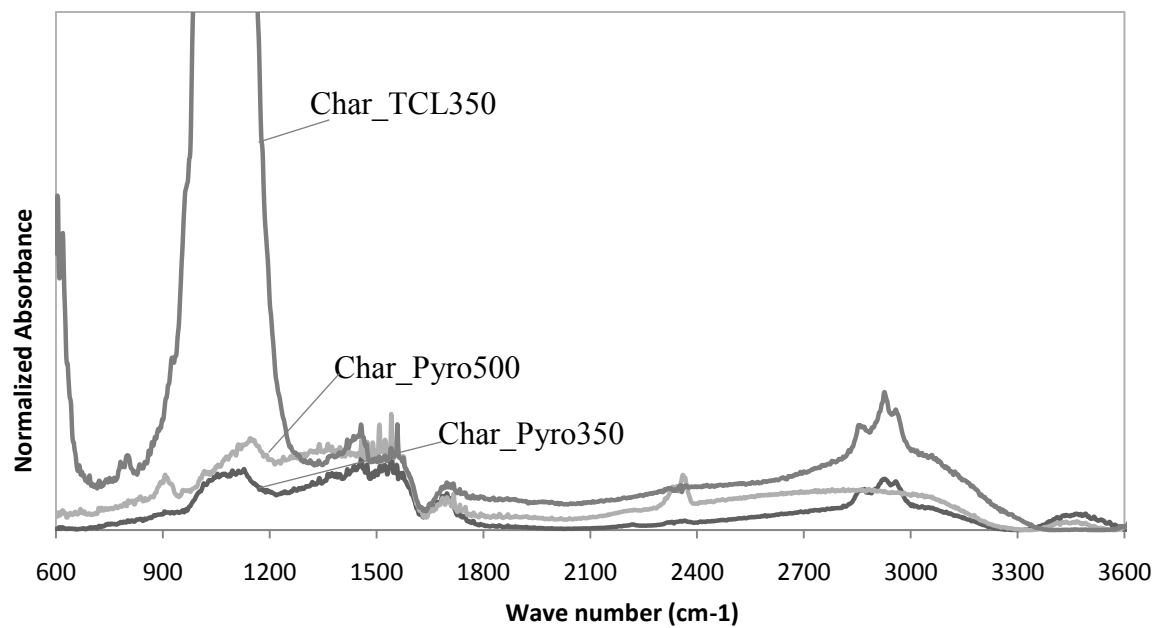


Figure 5.6. Infrared Spectra of solids char samples obtained from TCL and pyrolysis processes and raw feedstock

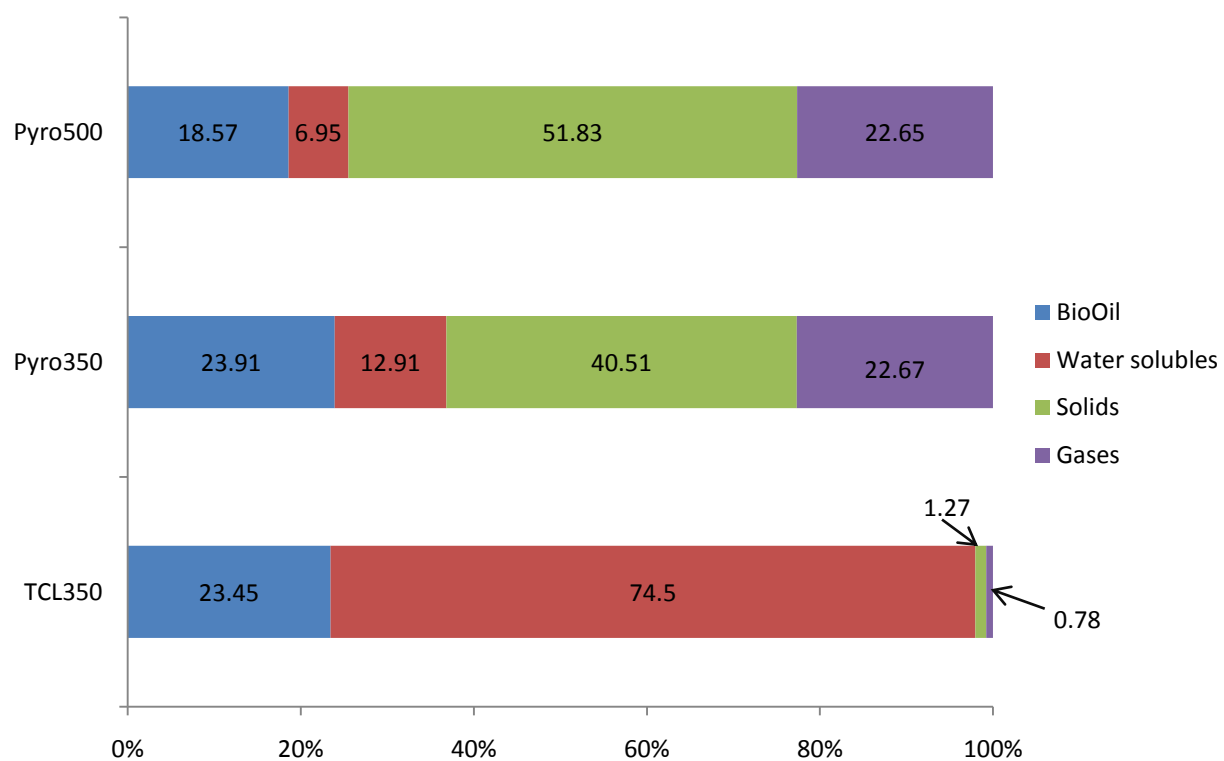


Figure 5.7. Nitrogen disposition in TCL and Pyrolysis processes (expressed in percentage weight distribution of initial nitrogen in dry algae biomass)

CHAPTER 6

EVALUATION OF MICROALGAE CULTIVATION USING RECOVERED AQUEOUS CO-PRODUCT FROM THERMOCHEMICAL LIQUEFACTION OF ALGAL BIOMASS¹

¹Jena, U., Vaidyanathan, N., Chinnasamy, S., Das, K.C. 2011. *Bioresource Technology*, 102(3): 3380-3387.

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Abstract

This study characterized the ACP stream from the TCL of *Spirulina* and evaluated its potential as a nutrient source for cultivation of microalgae. TCL of 100 g of dry *Spirulina* resulted in 40% BioOil and 429.80% ACP. The ACP was found to have high nitrogen ($16,200 \text{ mg L}^{-1}$), phosphorus (795 mg L^{-1}), potassium ($11,260 \text{ mg L}^{-1}$) and secondary and micronutrients. Growth media were prepared using ACP as sole nutrient source in deionized water at 0.2%, 0.33%, 1%, and 10% v/v concentration and compared with a standard growth medium (BG 11) for algal cultivation. *Chlorella minutissima* was grown in these media for 12 days and monitored for biomass concentration, total chlorophyll and lipids. Biomass productivities with the ACP added media at 0.2% and 0.1% concentration were 0.035 and $0.027 \text{ g L}^{-1} \text{ d}^{-1}$, respectively compared to $0.07 \text{ g L}^{-1} \text{ d}^{-1}$ in BG 11.

Keywords: Aqueous Co-Product (ACP), BioOil, Microalgae, Nutrients, Thermochemical liquefaction (TCL).

6.1. Introduction

Biomass, a source of renewable and carbon neutral fuels, is produced through photosynthesis by plants using carbon dioxide, water, nutrients and sunlight. In recent times microalgae have emerged as a potential energy crop for liquid biofuel production due to their high annual biomass productivity ($175 \text{ tons ha}^{-1} \text{ year}^{-1}$) and ability to grow in poor quality waters and wastewaters. In addition, they do not compete for arable lands used for cultivating food crops (Ginzburg, 1993; Chisti 2007& 2008). Recently “BioOil” production from biomass through thermochemical conversion processes is gaining importance, because of its similarity to petroleum crude oil. BioOil can be produced in a thermochemical process via pyrolysis (Bridgewater, 2000) or hydrothermal liquefaction (Elliot et al, 1996). Pyrolysis is the processing of biomass by heating it in an oxygen free environment at atmospheric pressure, where as biomass is heated under high pressure (5 to 40 times atmospheric pressure) in the presence of a solvent (generally excess water) and a chemical catalyst in hydrothermal liquefaction process. Hydrothermal liquefaction is also referred as thermochemical liquefaction (TCL) and supercritical water gasification (SCWG) by many researchers. In all of the above processes organic constituents of solid biomass are converted into liquid BioOil and gas through a series of polymerization, depolymerization and condensation reactions (Demirbas, 2000). Among these options, pyrolysis is more suitable for dry biomass feedstocks. In pyrolysis, the first step of the process is drying of the biomass, which requires significant heat energy and can be energetically inefficient if the moisture content of biomass high. In contrast, TCL is the aqueous conversion of biomass in hot compressed water (Balat, 2008) and hence better suited for the biomass with high moisture content (~80-98%) such as algae. The main advantages of aqueous conversion of wet biomass through TCL are given below:

- 1) No need for drying of the biomass Carbon conversion efficiency is very high.
- 2) Like dry gasification and pyrolysis, the reaction times (processing times) in the above methods can be in the order of few seconds to few minutes.
- 3) They can facilitate the recovery and nutrient recycling of the inorganic constituents of biomass such as sulfates, nitrates and phosphates.
- 4) Products generated from the above processes are completely sterilized with respect to possible pathogens including bacteria, fungi and viruses.

Microalgae are ideal feedstocks for TCL and SCWG processes because of high moisture content and smaller particle size (3-30 μ diameter), (Grima et al., 2003). Also, they would not need any further preconditioning such as grinding or drying which are required with other conventional biomass feedstocks. In contrast to other thermal conversions, such as pyrolysis or gasification, liquefaction does not require energy for vaporization of water. Sawayama et al. (1999) describes the method of calculating energy required for liquefaction and concludes that energy requirements for algae liquefaction is 6.69 MJ kg^{-1} of oil produced under the assumptions of organic content 50%, oil yield 64%, heating value 45.9 MJ kg^{-1} , $C_{pw} 4.18 \text{ kJ kg}^{-1}\text{K}^{-1}$ and $C_{ps} 1.25 \text{ kJ kg}^{-1}\text{K}^{-1}$. If pyrolysis were used as a conversion method, all of the water would have to be evaporated, thus consuming latent heat of vaporization of water at $2,260 \text{ kJ kg}^{-1}$. This translates to 28.25 MJ kg^{-1} of oil for the evaporation component only in a sample that has 80% water content and using other assumptions as described in Sawayama et al. (1999). Therefore liquefaction is energetically and economically preferred for biomass that are high in water content.

A large quantity of aqueous phase containing organics, nitrogen, phosphorus and other nutrients remains after the separation of the BioOil as the TCL occurs at very high moisture

levels (in excess of 80-90%). The aqueous co-product from TCL of swine manure was reported as having high concentrations of N, P, and K and found not suitable for conventional wastewater treatment for safe disposal (He et al., 1999). The recovered solution from the catalytic gasification of *Chlorella vulgaris* (at 87% moisture content) was reported to have ammonia concentrations as high as 9,000 mg L⁻¹ (Minowa and Sawayama, 1999). The beneficial use of the ACP stream is critical to the overall economic viability of the liquefaction process. Considering TCL as a potential technology for conversion of biomass feedstocks with high moisture levels such as microalgae, sewage sludge and other similar feedstocks, evaluating beneficial use options for ACP is considered as an important short-term goal.

Algae cultivation using wastewater effluents from various sources such as agricultural farms (Woertz et al., 2009; Wang et al., 2010), piggery and aquaculture farms (Aziz and Ng, 1992; Mallick, 2002; An et al., 2003), paper industry (Tarlan et al., 2002) and carpet industry (Chinnasamy et al., 2010) has been reported in the literature. Biomass production potential (measured in terms of chl *a*) for various freshwater and marine strains of algae grown in treated and untreated carpet wastewater was 16% to 247% higher than the growth reported in a standard BG 11 medium (Chinnasamy et al., 2010). Algae cultivation has been well established for wastewater treatment coupled with algal biomass production. Limited information is available on the use of TCL recovered ACP. Although Minowa and Sawayama (1999) briefly reported the growth of *C. minutissima* in the recovered ACP from the gasification of *Chlorella*, there is not a single systematic study available on detailed characterization of the aqueous co-product from the TCL process. The objective of the present study was to characterize the ACP generated from the thermochemical liquefaction of microalga *Spirulina platensis* and evaluate the potential of ACP at different concentrations as growth medium for the cultivation of *Chlorella minutissima*.

6.2. Methods

6.2.1. Recovery of aqueous co-product from thermochemical liquefaction

S. platensis was procured from a commercial farm (Earthrise Nutritionals, LLC, Calipatria, CA) as dry powder and mixed with deionized water to make a slurry with 20% solid content. This solids content was chosen as representative of the upper bound of physical dewatering methods such as centrifuging (Grima et al., 2003). Thermochemical liquefaction was carried out in a 1.8-L batch stirred reactor (Parr Instruments Co., Moline, PA) with a nitrogen atmosphere at 350°C for 60 minutes reaction time (hold time after reaching target temperature). At the end of the reaction, the reactor was cooled by passing tap water through the cooling coils and process gas was vented to the atmosphere. The end products mixture was poured out of the reactor and filtered under vacuum into two components: water soluble components and water insoluble components (solids residues and BioOil). Water soluble components (ACP) were weighed, sampled and analyzed. The water insoluble components retained on the filter and wall of the reactor were washed with laboratory grade acetone (99%) and vacuum filtered. The solids recovered in the filter were oven dried at 105°C for 24 hrs. The acetone soluble fraction was vacuum evaporated at 55°C to evaporate acetone and obtain the BioOil fraction.

6.2.2. Analysis of aqueous co-product and BioOil

Once recovered, ACP was stored at 4°C until further use. It was then filtered through a 25 μ Whatman filter to remove the solid residues prior to analysis of physical and chemical constituents. Electrical conductivity and pH were measured using a portable meter (Fischer Scientific, Accumet AP 62). Elemental carbon, hydrogen, nitrogen, sulfur, and oxygen (ultimate analysis) of ACP and BioOil were analyzed using a LECO Model CHNS-932 (Leco Corporation,

St. Joseph, MI) with optional pyrolysis furnace (Model VTF-900) using ASTM D 5291 and D 3176 methods. Measurements of ammonia nitrogen, nitrate nitrogen, total Kjeldahl nitrogen (TKN), phosphates, and analyses for anions, cations, micronutrients and phenol of ACP were performed using Standard USEPA Test Methods (USEPA 1983). Total solids and total volatile solids of ACP were measured using a LECO TGA-701 proximate analyzer (Leco Corp., St. Joseph, MI) following the ASTM D5142 method and chemical oxygen demand (COD) was analyzed by the Reactor Digestion Method (Method 8000) using HACH DRB 200 spectrophotometer (HACH Corporation, Loveland, CO) (Jirka and Carter, 1975).

Samples of BioOil were analyzed for water content by Karl Fischer titration using a Mettler-Toledo titrator (Model DL31), following ASTM E203 guidelines. Higher heating value (HHV, in MJ kg⁻¹) was measured using an isoperibol bomb calorimeter (Parr, model 1351) following ASTM D240 and dynamic viscosity was measured at 60°C using a dynamic viscometer (Brookfield, model DV-I+ with UL/YZ spindle adapter) using a modified version of ASTM D2983. Specific gravity (SG) was measured using 2 mL Gay-Lussac pycnometer.

6.2.3. Algae cultivation

Chlorella minutissima, a freshwater strain was isolated from an industrial wastewater following the method described by Chinnasamy *et al.* (2010). The cultures were enriched and maintained in BG 11 medium (Stanier *et al.*, 1971).

Inoculum preparation

A sample of 50 mL of the sub-cultured strain was centrifuged at 8,000 rpm for 15 minutes and the cell pellet was collected and re-suspended in Erlenmeyer flasks containing 500 mL of BG 11 medium to enhance the cell concentration in mother inoculum. Flasks were placed in a

temperature and humidity controlled growth chamber ($25 \pm 1^\circ\text{C}$) with a light intensity of 80 to 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and supplemented with 5% of carbon dioxide from an air- CO_2 mixing system. The initial pH of the culture was adjusted to 7.5 by adding 0.1 M NaOH solution.

Standard growth medium

Standard BG 11 medium was prepared in a 2 L Erlenmeyer flask, with pH adjusted to 7.5 and 75 mL of the medium were transferred to each of 24 standard 250-mL Erlenmeyer flasks. The flasks were closed with foam lids, wrapped with aluminum foils and sterilized in autoclave.

ACP growth medium

The concentrated ACP from TCL of *S. platensis* was diluted with deionized water to strengths of 0.20%, 0.33%, 1%, and 10% v/v (i.e. diluted to 500, 300, 100 and 10 times volume). After each medium was prepared, pH was adjusted to 7.5 by adding 0.1 M NaOH solution and the flasks were sterilized in an autoclave. Sterilized media were cooled to room temperature and inoculated with 7.5 mL of a seven days old culture of *C. minutissima*. Inoculated flasks were placed in the growth chamber for 12 days for further growth study. A one way ANOVA (analysis of variance) was used for the statistical analysis of algal biomass growth and chlorophyll concentration for five different growth media and one algal strain accounting for a total of five treatments. Henceforth, these treatments will be referred to as „DL 500“, „DL 300“, „DL 100“ and „DL 10“. The data were recorded in triplicate for the parameters in various treatments and were analyzed using SAS statistical package to evaluate the variation among different treatments. For the treatment DL 10, all parameters were monitored for initial and final day only.

6.2.4. Analyses

Biomass and chlorophyll analyses

Biomass (g L^{-1}) was determined by filtering 40 mL of the algal culture using pre-weighed 4.7 cm Whatman GF/C glass fiber filters. The filters with algal biomass were dried overnight at 60°C, cooled to room temperature in a vacuum desiccator and weighed. Sampling was done by sacrificing three flasks per treatment at each measurement point. There were a total of 4 sampling points and 3 flasks per treatment.

Chlorophyll 'a', 'b' and total chlorophyll were estimated following the method described by Porra *et al.* (1989). A 10 mL sample of the algal suspension was centrifuged at 5,000 rpm for 10 minutes. The supernatant was discarded and 10 mL of 95% methanol was added to the cell pellet. The tubes were covered with aluminum foil and placed in a water bath at 60°C for 45-60 minutes to ensure complete chlorophyll extraction. The cells devoid of chlorophyll were centrifuged again at 5,000 rpm for 10 minutes. Absorbance of the supernatant containing the chlorophyll extracted in methanol was measured at 652 nm and 665 nm using a Varian Cary 50-Bio UV visible spectrophotometer (**Varian**, Palo Alto, California). Chlorophyll content was calculated using the following formula (Porra *et al.*, 1989):

$$\text{Chlorophyll 'a'} (\mu\text{g mL}^{-1}) = 16.29 \times A_{665} - 8.54 \times A_{652}$$

$$\text{Chlorophyll 'b'} (\mu\text{g mL}^{-1}) = 13.58 \times A_{665} + 30.66 \times A_{652}$$

$$\text{Total Chlorophyll} = \text{Chlorophyll 'a'} + \text{Chlorophyll 'b'}$$

Biomass and chlorophyll analyses were done for all samples on days 0, 4, 8, and 12 in triplicate.

Specific growth rate, divisions per day and generation time

Specific growth rates, divisions per day and generation time were used as indicators for comparing the growth between different treatments and were determined based on methods

described by Levasseur et al. (1993) and Lee and Shen (2004). Plots of biomass growth and total chlorophyll against time were used to identify the beginning and end of the exponential growth and stationary growth phases respectively, in the above calculations.

Lipids estimation

Gravimetric measurement of lipids was done using an ANKOM^{XT10} automated extraction system (ANKOM Technology, Macedon, NY) where hexane was used as extraction solvent. Biomass (g L^{-1}) was determined for the samples as described earlier in section 2.4.1. The biomass filters were weighed (W_1 , empty filter weight= W_0), inserted carefully into the ANKOM^{XT10} extraction bags and sealed using an impulse sealer. The extraction bags were dried, vacuum desiccated, weighed (W_2) and placed inside the automated system. The system was then set up for 1 hour extraction at 90°C . After the extraction was done, the wet bags were dried at 60°C overnight, vacuum desiccated while cooling to room temperature and weighed (W_3). The following equation was used to calculate the lipid content (%) of algae samples:

$$\text{Lipids (\%)} = (W_2 - W_3) / (W_1 - W_0) \times 100$$

6.3. Results and discussion

6.3.1. TCL product distribution

Thermochemical liquefaction of *S. platensis* resulted in generation of $39.93 \pm 1.49\%$ of BioOil (the target product of the TCL process), $23.60 \pm 0.50\%$ of gaseous products, and $4.67 \pm 0.17\%$ solid residues. Approximately 17 to 23% of the organics in the initial feedstock were converted to water soluble components that ended up in the aqueous phase. The total aqueous phase recovered was approximately 420% of the starting biomass, i.e. the TCL processing of one dry ton of algae along with 4 tons of water, could generate as much as 4.2 tons (1,140 gallons) of

aqueous phase. Mass balance of materials reported 10-15% mass losses during the unit operations involved in product separation. The BioOil was a dark dense liquid fuel and had an energy value of 31.5 MJ kg⁻¹ (Table 6.1) compared to 42 MJ kg⁻¹ for petroleum crude oil (Matar and Hatch, 2001) and water content in the range 9% to 15% compared to 9.9% of that the crude oil (Margolis and Hagwood, 2003). Though the viscosity and elemental nitrogen were higher than the crude oil (Table 6.1), it could be either upgraded to a gasoline like fuel or directly used as fuel oil in heating furnaces with suitable modifications. The other products were gases (23.6%) and solid residues (4.6%). Gas chromatography of the gaseous products shows carbon dioxide as the major constituent (68-75%) along with carbon monoxide, hydrogen, methane, acetylene and traces of other higher hydrocarbons such as ethane and propane (data not shown). The carbon dioxide could be separated from the gaseous product mixture and recycled for algae cultivation and other gases could be potentially used as fuel gases for industrial applications or upgraded to synfuels.

6.3.2. Aqueous co-product characteristics

Recovered ACP had large quantities of total Kjeldahl nitrogen (TKN) (16,200 mg L⁻¹), and 78.39% of the TKN was in the form of ammonia and nitrates. High amount of TKN was typically expected from the thermal decomposition of proteins which were major constituents (65% w/w) of *S. platensis* (Raouf et al., 2006). The nitrogen in biomass was converted into ammonia during TCL and remained in the dissolved form in the ACP (Minowa and Sawayama, 1999). Also the TCL process was conducted in nitrogen atmosphere and at high pressure, which could have possibly resulted in the diffusion of nitrogen into the aqueous product. Higher ammonia concentration (12,700 mg L⁻¹) observed in our study compared to the ACP reported by

Minowa and Sawayama (1999) was due to higher algal solids concentration (20%), and variation in the feedstock and process parameters. Other properties of ACP included electrical conductivity of $2.13 \mu\text{S cm}^{-1}$, hardness of 23.31 mg L^{-1} and nitrate concentration of 26.76 mg L^{-1} (as N). The ACP was reported to have 795 mg L^{-1} of phosphate phosphorus, which translated to 28.3% w/w of phosphorus recovery from the original biomass. Carbon, nitrogen and phosphorus are the three most important nutrients for autotrophic growth of algae (Grobbelaar, 2004) and typical algal dry biomass can contain up to 10% nitrogen and 1% phosphorus. Presence of phenols (50.9 mg L^{-1}) in the recovered aqueous phase can be attributed to the depolymerization and repolymerization reactions forming a series of hydrocarbon compounds during the liquefaction process (Demirbas, 2000; Balat, 2008). Phenols and phenolics are toxic compounds and have been shown to have inhibitory effects on algal growth (Nakai et al., 2001; Scragg, 2006). Studies by Nakai et al. (2001) have shown that only particular group of polyphenols such as catechol and hydroquinone inhibited the growth of alga *Mycrocystis aeruginosa* even when the concentration of the chemical was low (i.e. less than 7.5 mg L^{-1}). Other kinds of phenols did not show any growth inhibition. The inhibitory effect of phenols was attributed to the auto-oxidation of the polyphenols in alkaline solution in the presence of di- or trivalent metal ions (such as Mg^{2+}) forming radicals and thus making it toxic. However, it is known that some algal strains have tolerance to certain concentration of phenols. Addition of phenol to the cultivation media of *C. vulgaris* and *Chlorella* VT-1 showed very little growth inhibition at 100 mg L^{-1} phenol concentration, but increased to 44% and 100% inhibition at a phenol concentration of 400 mg L^{-1} for the above two strains of *Chlorella*, respectively (Scragg, 2006). We believe that the phenol concentration ($<5.1 \text{ mg L}^{-1}$) in the DL10 growth media in this study might not be toxic and ACP could be used for developing algal growth media to cultivate *C. minutissima* after

suitable dilution with deionized water. The pH of the aqueous phase was 8.67, which was significantly higher than the recommended pH of 7.5 for algae cultivation (Grobbelaar, 2004). The ACP was reported to have 2.58% total solids, 1.95% volatile solids, with a chemical oxygen demand (COD) value, 80,000 mg L⁻¹.

Most of the mineral nutrients needed for algal growth were found in the ACP (Table 6.2). Grobbelaar (2004) reported a list of nutrients present in the algal biomass viz. Sodium (Na), Potash (K), Calcium (Ca), Sulfur (S), Magnesium (Mg), Chlorides (Cl), Iron (Fe), Zinc (Zn), Manganese (Mn), Bromides (Br), Silicon (Si), Boron (B), Molybdenum (Mo), Vanadium (V), Strontium (Sr), Aluminum (Al), Rubidium (Rb), Lithium (Li), Copper (Cu), Cobalt (Co), Iridium (I), and Selenium (Se) apart from the major nutrients such as C, N and P. The minerals present in the ACP originated from the biomass of *S. platensis* (Table 6.2) and they were distributed into different products during the TCL process. Concentrations of essential nutrients measured in this study indicated that they were present in significant quantities when compared to the standard growth culture media such as BG 11, modified Allen's medium, Bold's Basal, Sorkin/Krauss, Zarrouck, Ben-Amotz and Avron medium (Grobbelaar, 2004). Table 6.3 shows the recovery of elemental carbon, nitrogen, sulfur and oxygen in the ACP. Elemental conversion ratio (ECR) was calculated as the ratio of weight fraction of a particular element in ACP to the weight fraction of that element in the original feedstock. The higher values of ECR for hydrogen and oxygen were due to the low percentage of total solids (2.58%) in the ACP. ECR indicated that about 75% (w/w) of total elemental nitrogen is recovered from the biomass (Table 6.3).

6.3.3. Algae cultivation

Biomass productivity: algal cell density and total chlorophyll

Algal growth for *C. minutissima* was determined by monitoring the cell density at 4 days intervals over the 12 days test period. *C. minutissima* was selected for these studies because of its ability to fix higher amount of lipids (57%) as reported by Gouveia and Oliveira (2009). Maximum biomass growth was reported in the first phase (0-4 days) of growth for all the treatments in ACP growth media (Figure 6.1). The biomass productivity on 12th day for the standard growth medium BG 11 was the highest (1.03 g L⁻¹) among all the treatments. Among the ACP enhanced media, the DL 500 treatment (with 0.2% ACP addition) was found to produce significantly higher biomass productivity than the rest of the treatments and it was 0.52 g L⁻¹ (p=0.05), which was 50.5% of the productivity reported in BG 11. Algal growth in a mixed media (1:1 volume ratio of diluted ACP and nitrogen less standard growth medium) was 85% of that in the standard medium, but it reduced to 12.5% when only ACP (diluted to 300 factor) was used as growth medium (Minowa and Sawayama, 1999). In our study ACP with dilution factor 300 (DL 300) showed a growth of 42.7% (0.43 g L⁻¹) of that of BG 11 medium. There was no significant difference in productivities between the treatments DL 100 (0.41 g L⁻¹) and DL 300 (0.44 g L⁻¹) (media with 1% and 0.33% ACP, respectively) although both were significantly lower in biomass productivities compared to DL 500 treatment (p =0.05). For the treatment DL 10, there was no significant increase in net cell density over the initial biomass data indicating no growth. Over the 12 days, growth of alga in BG 11 showed 509% increase over the initial biomass compared to 376% observed in DL 500 (Figure 6.1). Phenol toxicity was not an issue in the present investigation at 0.2% to 1% v/v concentrations of ACP due to dilution effect in resulting phenol concentration less than 0.51 mg L⁻¹ which was in agreement with earlier studies (Scragg, 2006). When the concentration of ACP in the growth medium was increased to 10%

(for the treatment DL 10), it resulted in the death of algal cells indicated by white discoloration in the medium. This could be due to higher phenol or higher concentration of secondary and micronutrients at this dilution that resulted in toxicity causing the death of the cells.

The total chlorophyll (Chl *a* + Chl *b*) was highest on day 8 and reached a plateau thereafter (Figure 6.2) although the productivity continued to increase till day 12 (Figure 6.1.). This indicated that after day 8 though there was no increase in the chlorophyll content, the cells continue to grow resulting in the net increase in the biomass concentration. Among all the treatments, BG 11 recorded the highest total chlorophyll (34.66 mg L^{-1}) production, which showed a 230% increase over the initial chlorophyll (day 0), followed by DL 100 where 140% increase was observed. The treatments DL 300 and DL 500 recorded an increase of only 60% and 120%, respectively, over the initial chlorophyll levels. DL 10 showed only 6% increase over the initial chlorophyll, indicating that there was no significant growth in the medium (Figure 6.2).

Based on the total biomass productivity and total chlorophyll data, the daily productivity, specific growth rate, divisions per day and generation time were calculated (Table 6.4). Daily biomass productivity for the best treatment condition observed in this study was $0.033 \text{ g L}^{-1} \text{ d}^{-1}$ compared to $0.117 \text{ g L}^{-1} \text{ d}^{-1}$ for the raceway ponds (Chisti, 2007). The specific growth rate for the BG 11 medium was the highest (0.13 d^{-1}) followed by DL 100 (0.09 d^{-1}). For DL 300 and DL 500 the specific growth rates were 0.05 d^{-1} and 0.08 d^{-1} respectively, while it was negative for the treatment DL 10. In a study of response of *Chlorella* to the nutrient conditions (Illman et al., 2000), the specific growth rates for different species of *Chlorella* grown in low-nitrogen growth media was reported to have a lower value than the standard growth media and the lowest value in this study was 0.19. The lower value for specific growth rates our study can be explained by

the variation in the experimental conditions such as inoculum, light intensity, pH of the culture and ACP dilutions. The generation time (time for cell doubling) was found to be the least for BG 11 medium among all treatments followed by DL 100 and DL 500 and it was of the order of 0.7 times that of the latter two treatments (Table 6.4).

Biomass growth per unit nutrient supply of N and P

Availability of nutrients is one of the major parameters that directly affect the algal growth (Wang et al., 2010) with nitrogen and phosphorus reported as limiting nutrients. Therefore, the biomass productivity per unit of the nitrogen and phosphorus supplied to the growth media was calculated. Table 6.5 summarizes the N and P concentrations in the algal growth media for growing *C. minutissima*. They were calculated on the basis of presence of total Kjeldahl nitrogen (TKN) and phosphate phosphorus in the original aqueous co-product. Biomass productivity per unit nitrogen and phosphorous for the DL 500 and DL 300 media were significantly higher than that of BG 11 for *Chlorella* even though the N and P concentrations of the standard growth medium (BG 11) were much higher than the DL 500 and DL 300. For all the treatments taken in the study, biomass productivities (mg-biomass) per mg nitrogen and phosphorous were in the following order: DL 500 ($0.89 \text{ mg mg}^{-1}\text{-N}$ and $18.33 \text{ mg mg}^{-1}\text{-P}$) > DL 300 ($0.48 \text{ mg mg}^{-1}\text{-N}$ and $9.75 \text{ mg mg}^{-1}\text{-P}$) > BG 11 ($0.33 \text{ mg mg}^{-1}\text{-N}$ and $8.25 \text{ mg mg}^{-1}\text{-P}$) > DL 100 ($0.17 \text{ mg mg}^{-1}\text{-N}$ and $3.51 \text{ mg mg}^{-1}\text{-P}$). Since the specific growth rate for DL 10 was negative (Table 6.4), the productivity per unit supply of N and P was negative for this treatment and hence not reported.

Lipid Analysis

There were no significant changes in lipid concentration in the biomass grown in different growth media (Figure 6.3). In general, 8 to 12% of lipids was reported for the alga *C. minutissima* in the present study. Lipid content monitored over 8 days had shown a decrease in initial four days and later it increased for all the treatments (Figure 6.3). The decrease in the lipid content might be attributed to initial lag phase. There was not much difference in the lipid data between the treatments.

6.3.4. A proposed integrated biorefinery system for renewable biomass and BioOil production

An enhanced medium with 0.2% nutrient rich ACP added to a nutrient depleted wastewater could be an attractive option for effective recycling of wastewater and production of renewable biomass and biofuels. Based on the results of the present study, an integrated biorefinery system is proposed which includes algal biomass production, processing of algal biomass to BioOil through TCL, and recycling of the ACP as low-cost nutrient source for algae cultivation for (Figure 6.4). Nitrogen and phosphorus are the major nutrients required for the growth and metabolism of the majority of plants including algae. In recent times, phosphorus depletion has raised worldwide concern and as per recent estimates world's readily available phosphorus supplies will be inadequate to meet the agricultural demand within 30 to 40 years (Anderson, 2010). The recycling of phosphorus for processes such as algae production is therefore critical as we move towards limited availability of phosphorus. The proposed biorefinery (Figure 6.4) shows a mass flow for cultivating and processing one dry ton (5 tons wet slurry, 20% solids) of algae biomass based on our results (Section 3.1). It can generate 0.4 tons (3,921 gallons) of BioOil for further refining into liquid fuels and chemicals. The ACP generated from TCL of one dry ton of algae biomass could retain 3.4 kilograms of phosphorus and 70 kilograms of total nitrogen along with significant amounts of other secondary and micronutrients. A part of the

ACP can be recycled to the cultivation system as N, and P rich growth medium and the rest can be concentrated to high value fertilizer (N, P, and K) and sold to fetch revenue. Also, about 0.18 tons of CO₂ (75% of the total gas) is generated as the co-product in the TCL process. The CO₂ gas can be recycled for algae cultivation after separation from the gaseous co-products (Figure 6. 4) and the rest of the fuel gas can be used for thermal applications or for upgradation to synfuels.

6. 4. Conclusion

The aqueous co-product (ACP) from the thermochemical liquefaction (TCL) was found to be high in nitrogen, phosphorus and potassium along with other minerals and essential micronutrients required for algae growth. Algal growth medium developed by using this ACP as a nutrient additive to deionized water at 0.2% v/v concentration resulted in the best growth (0.52 g L⁻¹) for *C. minutissima*. This study established the proof of concept for combining algae cultivation with TCL for recycling the nutrients. However, an overall life cycle analysis of microalgae cultivation integrated with TCL would be useful for the development of algae based biorefineries.

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Table 6.1. Physical properties and elemental composition of BioOil produced from thermochemical liquefaction of *S. platensis* compared with that of the petroleum crude oil.

Properties	Algal BioOil	Crude Oil
Higher Heating Value, MJ kg ⁻¹	31.5 ± 0.57	42 ^a
Water content, %	9.0-15.0	9.9± 0.06 ^b
Specific gravity, g mL ⁻¹	1.02 ± 0.02	0.87-1.00 ^a
pH	9.63 ± 0.10	n.a.
Viscosity, cP (at 60°C)	53.8 ± 4.17	23 ^a
C, %	68.3 ± 1.1	84.6 ^a
H, %	8.3 ± 0.1	12.5 ^a
N, %	6.2 ± 0.1	0.4 ^a
S, %	0.6 ± 0.0	1.5 ^a
O, %	*16.4 ± 0.1	0.5 ^a

n.a.- not available

* Elemental oxygen, 'O' was calculated by difference,

^aMatar and Hatch, (2001),

^bMargolis and Hagwood, (2003),

Table 6.2. Elemental analysis of the aqueous phase derived from thermochemical liquefaction of *S. platensis* and that of original feedstock.

Elements	Aqueous phase (ppm)	Original feedstock (ppm)
K	11,260	19,821 \pm 458.6
Cl	659.9	n.a.
Fl	141.5	n.a.
Al	12.95	83.2 \pm 6.9
B	3.97	n.a.
Ca	<5	1165.4 \pm 0.0
Cd	<0.5	0.1 \pm 0.0
Cr	0.86	3.3 \pm 0.9
Cu	<0.5	4.7 \pm 0.6
Fe	25.43	568.8 \pm 7.6
Mg	5.6	4247.8 \pm 893.3
Mn	<0.5	36.6 \pm 0.6
Mo	9.35	n.a.
Na	2035	730.7 \pm 19.6
Ni	2.42	0.4 \pm 0.0
P	791.2	11823.3 \pm 513.7
Pb	<2.5	0.1 \pm 0.0
S	2780	n.a.
Si	443.9	206 \pm 20.7
Zn	<0.5	12.3 \pm 1.7

n.a.: not determined

Table 6.3. Ultimate analysis of the original biomass and the aqueous co-product of liquefaction.

Elements,	<i>S. platensis</i> (%)	Aqueous co-product (%)	*Elemental conversion Ratio (ECR)
C	45.16±0.19	3.92 ± 0.14	0.37
H	7.14±0.20	10.35 ± 0.48	6.23
N	10.56±0.04	1.84 ± 0.05	0.75
S	0.74±0.01	0.05 ± 0.01	0.29
O [†]	36.39±0.27	83.74±0.53	9.89

*ECR is the weight fraction of the element present in the aqueous phase with respect to the original feedstock,

† by difference

Table 6.4. Productivity, specific growth, divisions per day and generation time of *C. minutisima* grown in different growth media: BG 11 (Control), DL 500 (0.2% ACP), DL 300 (0.33% ACP), DL 100 (0.1% ACP) and DL 10 (10% ACP).

Treatments	Daily productivity ^a , (g L ⁻¹ d ⁻¹)	Specific growth rate, K', (d ⁻¹)	Div./day ^b ,	Gen. time ^c , (d)
1. BG 11	0.070	0.13	0.18	5.44
2. DL 500	0.035	0.08	0.13	7.88
3. DL 300	0.023	0.05	0.08	12.24
4. DL 100	0.027	0.09	0.13	7.71
5. DL 10	-0.006	-0.02	-0.03	-33.84

(-) Data reported as negative value,

*All values were reported as average of three replications, standard deviations ranged from 0.001 to 0.02,

^bGen.. time: Generation time

^cDiv./day.: Divisions per day

Table 6.5. Nutrient (total nitrogen and phosphorous) distributions in algal growth media: BG 11 (Control), DL 500 (0.2% ACP), DL 300 (0.33% ACP), DL 100 (0.1% ACP) and DL 10 (10% ACP).

Growth medium	Volume of ACP per 1.0 L volume of the medium	N (mg L ⁻¹)	P (mg L ⁻¹)
BG 11	0 mL	250	10
DL 500	2 mL	32.4	1.59
DL 300	3 mL	48.6	2.385
DL 100	10 mL	162	7.95
DL 10	100 mL	1620	79.5

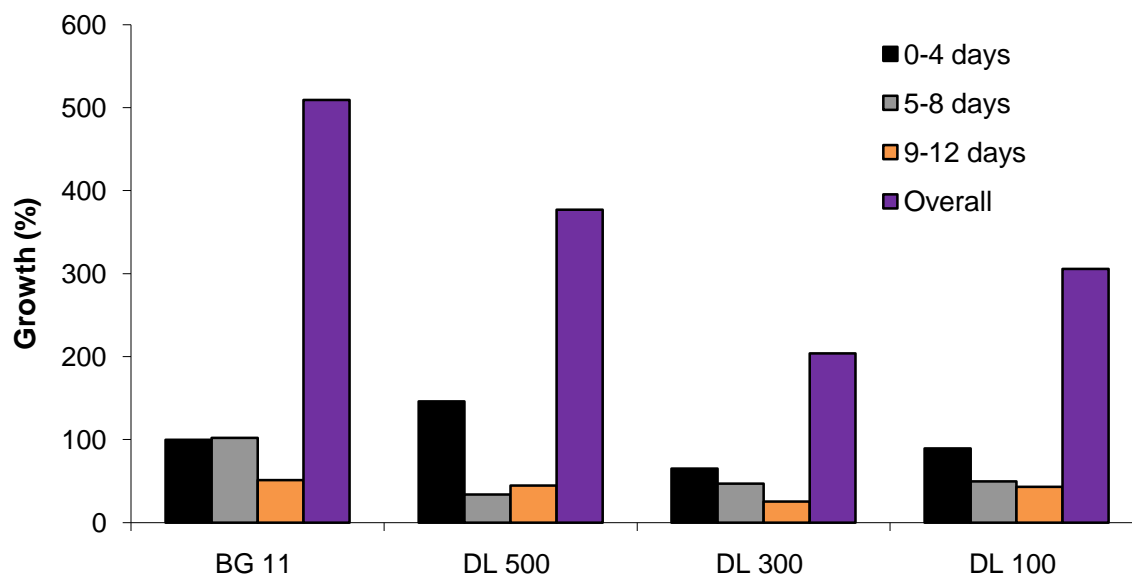


Figure 6.1. Biomass growth profile of *Chlorella minutissima* monitored in segments of every 4 days over 12 days of total cultivation period. The growth (%) in the Y-axis for any segment was,

$$\text{Growth, \%} = \frac{\text{Final day concentration, } g L^{-1} - \text{initial day concentration, } g L^{-1}}{\text{initial day concentration, } g L^{-1}} \times 100.$$

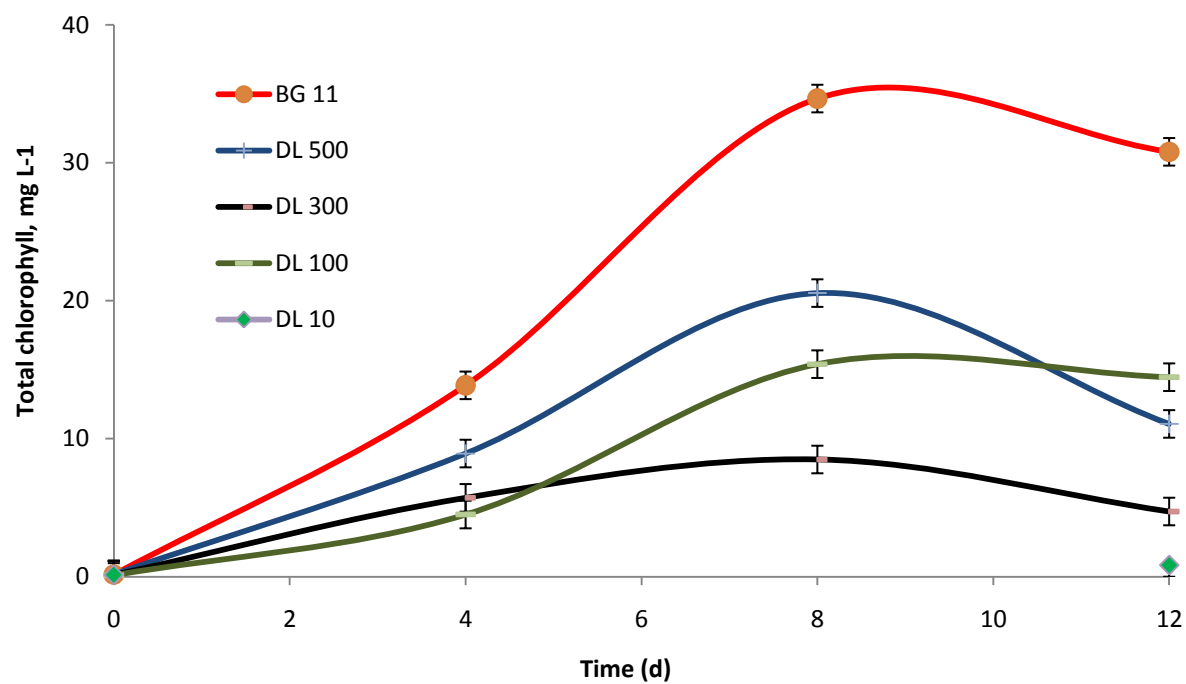


Figure 6.2. Growth response in terms of changes in total chlorophyll content of *Chlorella minutissima* grown in BG 11 and growth media with different dilutions of aqueous co-product from the TCL process.

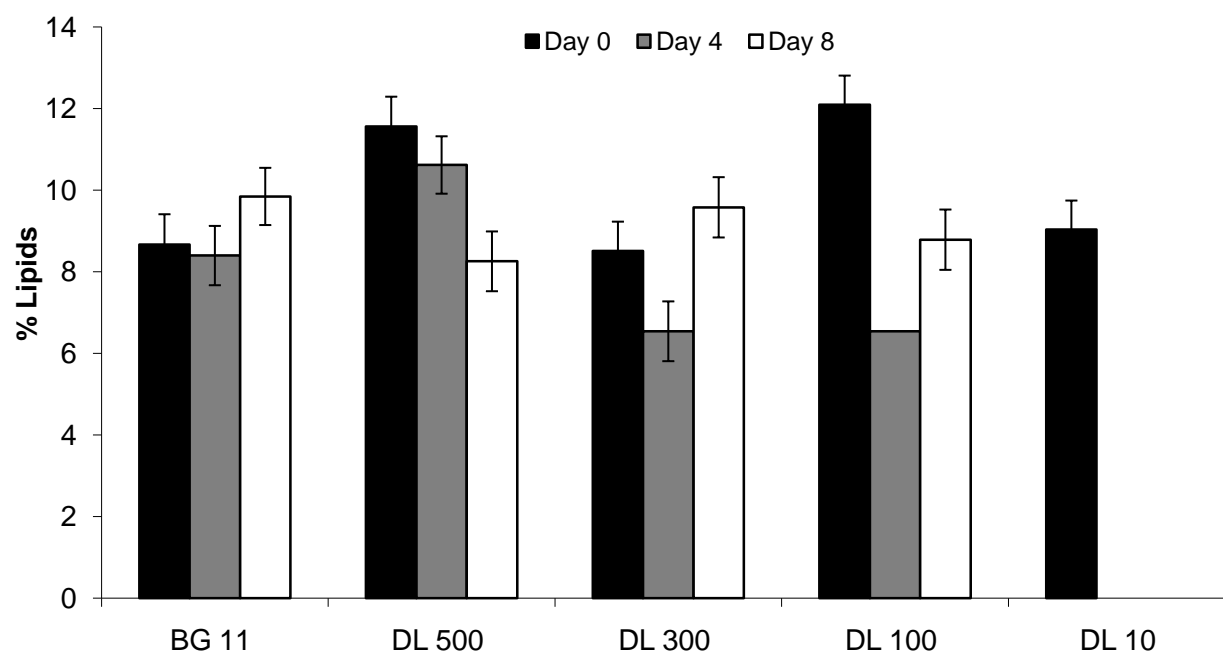


Figure 6.3. Changes in lipid content of *Chlorella minutissima*. (Lipids profile for day 12 was not determined).

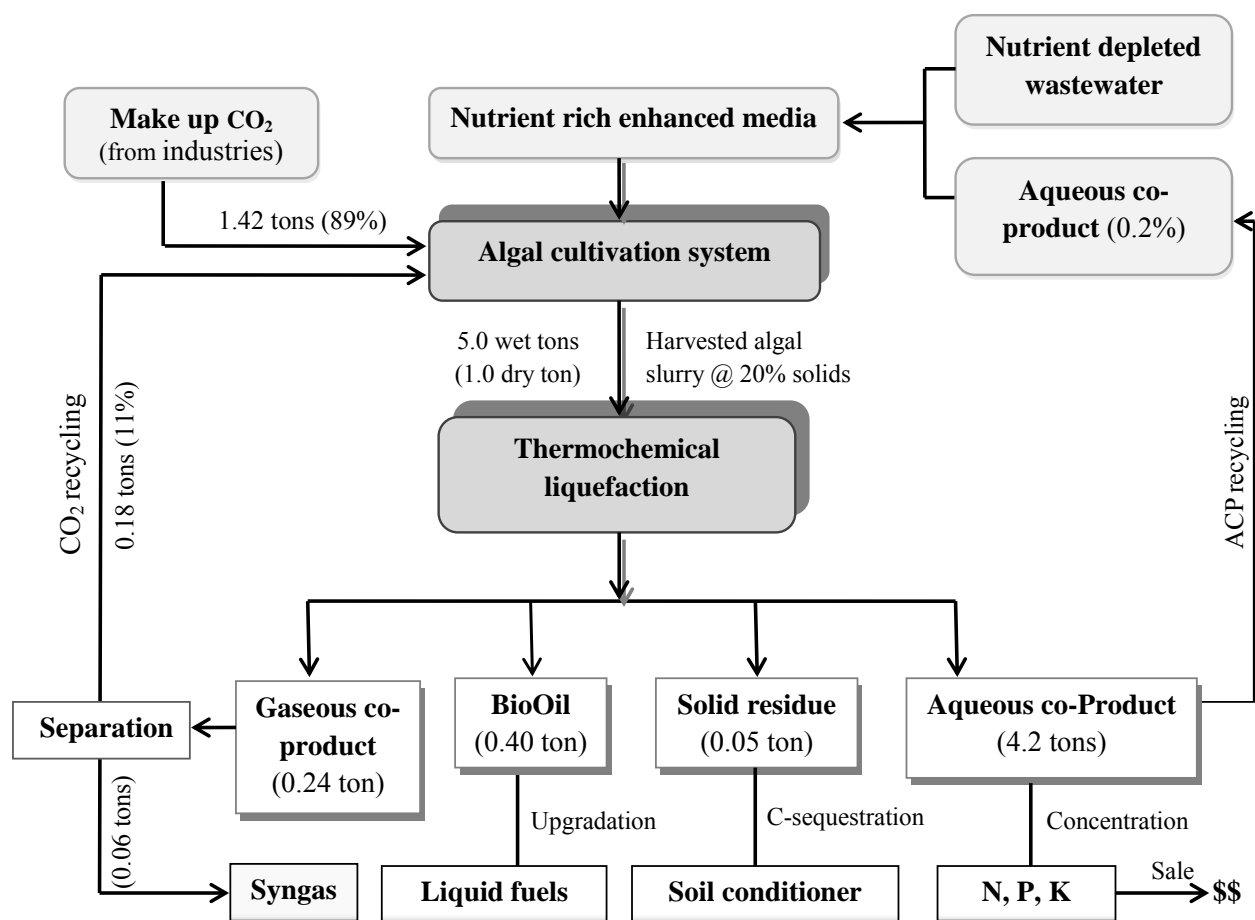


Figure 6.4. A proposed biorefinery with thermochemical liquefaction of the biomass into BioOil and recycling of the aqueous co-products and CO₂ gas for algal biomass production.

CHAPTER 7

SUMMARY AND CONCLUSIONS

Summary

With an overall goal to produce high energy liquid fuel from wet algae biomass this dissertation investigated the effects of operating conditions on thermochemical liquefaction (TCL), effect of addition of catalysts on biocrude yield and properties, comparative evaluation of TCL and pyrolysis processes, and evaluation of aqueous phase co-products (ACP) for algae cultivation. The goal was divided into four research studies. The first study (*effects of operating conditions on thermochemical liquefaction*) not only revealed that an energy dense liquid, biocrude could be obtained from a low lipid microalgae, but also showed that the best combination of temperature, holding time and solids concentration could maximize biocrude yield with improved fuel properties. The second study (*effect of addition of catalysts on TCL*) showed that catalysts altered the product distribution and net energy conversion ratio in TCL. The third study (*comparative evaluation of TCL and pyrolysis processes*) confirmed that TCL was a better option for converting wet algae biomass into biocrude than the pyrolysis where high amount energy was required for drying the feedstock and overall energy conversion ratio was higher compared to TCL. The fourth study (*evaluation of ACP for algae cultivation*) revealed that majority of the nutrients (N and P) were recovered in the ACP, a co-product in TCL process and could be recycled for algae cultivation and ACP enhanced growth medium resulted in ~51% biomass productivity of that obtained with a standard algae growth medium. The following sections summarize the conclusions drawn from each study.

Effects of operating conditions on TCL Study

This study explained the effects of operating temperature, holding time and solids concentration on yields of biocrude and other products from TCL and characterization of products. TCL of *S. plantensis* was conducted in a 1.8 L batch reactor using 290 psi nitrogen pressure at 200-380°C temperature, 0-120 min holding time and 10-50% solids concentration each at five levels. Products were separated by a series of filtration, acetone washing, and drying of solids. Yield products was evaluated and products were analyzed for various physical and chemical properties such as elemental C, H, N, S, O, higher heating value, specific gravity, viscosity, with identification of chemical compounds in biocrude, gas and aqueous phase co-product. Following were major conclusions drawn from this study:

- 1) Up to ~40% biocrude could be produced from TCL and the biocrude had an energy content of 35.2 MJ kg^{-1} and had fuel properties similar to petroleum crude oil.
- 2) Optimum operating conditions were found as 350°C temperature, 60 min holding time and 20% solids concentration.
- 3) Light fraction biocrude consisted 50-63% of total biocrude yield and was a complex mixture of benzenes, toluenes, furans, aldehydes, ketones, alcohols, esters, alkanes, carboxylic acids, amines, amides, indole, and pyrrolidines.
- 4) Algal biocrude was characterized by higher nitrogen content (4.5-7.3%) suggesting the need for upgradation.
- 5) Carbon conversion efficiency of 98.3% was obtained at the optimum TCL conditions.

Effect of addition of catalysts on TCL yield and properties

This study investigated the effect of addition of Na_2CO_3 , NiO , and $\text{Ca}_3(\text{PO}_4)_2$ catalysts on biocrude yield and properties from microalgae. TCL experiments were performed in a 1.8 L batch reactor at 350°C temperature, 60 min holding time and 20% solids concentration using the above catalysts. Product yields, biocrude properties and net energy ratio were compared with non-catalytic TCL. This study concluded as follows:

- 1) Addition of Na_2CO_3 was found to favor biocrude yield while NiO , and $\text{Ca}_3(\text{PO}_4)_2$ favored gaseous yield resulting ~51% BioOil yield compared to ~40% for non-catalytic TCL
- 2) In all the cases, solids conversion was more than 94%. Metal analyses of solids revealed that 40-60% of the initial catalysts could be retained in the solid char.
- 3) TCL using Na_2CO_3 was found to consume the lowest energy among all treatments reporting a energy consumption ratio of 0.56 and was 75% of the energy required for non-catalytic TCL.

Comparative evaluation of TCL and pyrolysis processes

This study evaluated and compared TCL and pyrolysis processes as options for producing BioOil (also known as biocrude) from algae. TCL and pyrolysis experiments were performed in batch type reactors in nitrogen atmosphere. Pyrolysis runs were carried out at two temperatures, 350°C and 500°C using dry algae powder with ~5% moisture, whereas TCL of algae slurry (80% moisture) was carried out at 350°C . Yields and composition of BioOil, char, gases and aqueous phase were evaluated and compared for TCL and pyrolysis processes. The following conclusions were made:

- 1) TCL process resulted in significantly higher BioOil yields (~41%), and lower solid char yields (~6.3%) than pyrolysis processes that resulted in 23-29% BioOil yields, and 28-40% solid char yield.
- 2) BioOil obtained from TCL was found to have higher energy content and superior fuel properties such as chemical composition and had better thermal and storage stabilities than the BioOil obtained from pyrolysis processes.
- 3) TCL was found to be more energy efficient and recovering more combustible energy from raw algae feedstock compared to pyrolysis process.
- 4) Pyrolysis at higher temperature resulted in higher BioOil yield, lower solids yield and was more energy efficient than that of pyrolysis at lower temperature.

Evaluation of ACP for algae cultivation

This study evaluated the ACP recovered from TCL process and tested its potential use as algae cultivation medium. ACP obtained from the separation of product mixtures from TCL of *S. platensis* was further analyzed for physical and chemical constituents. Using different concentrations of ACP growth media were prepared and *Chlorella minutissima* was grown in these media for 12 days. Biomass productivity was monitored and compared with BG 11 growth medium. The following conclusions were made from this study:

- 1) ACP recovered from TCL of algae was characterized by high nitrogen, phosphorus, and potassium contents, and showed that ~75% nitrogen and ~28% phosphorous of raw algae were recovered in the ACP by TCL process

- 2) Algae growth was possible using ACP as sole nutrient source at 0.2%, 0.33% and 1% (v/v) concentrations with 0.2% (v/v) concentration resulting in highest biomass productivity 0.51 g L^{-1} that was 50% of productivity obtained in BG 11 medium.
- 3) Mass balance showed that an integrated biorefinery could be possible using TCL technology for production along with nutrient recycling, carbon sequestration and wastewater treatment.

Benefits and Scope for Future Research

The results of this dissertation will not only benefit the algae based biofuel industries but also provide important information to the research world. Algae based biorefineries will generate a wide range of fuels and chemicals in forms of biocrude (BioOil), fuel gas, solid char for land use and liquid fertilizer in form of ACP. Biocrude is an energy dense liquid fuel and can be used as liquid fuel directly in the boilers, and industrial furnaces for steam generation, heating applications or can be co-combusted with gasoline, diesel and alcohol fuels in engines and turbines for generation of electricity. A pilot scale study on biocrude production from wet algae biomass will provide more information on sustainability and economic feasibility of the TCL technology. For design of a pilot scale reactor, information on reaction rate and activation energy is necessary. Some fundamental research on rate kinetics and TCL mechanism will improve the efficiency of the pyrolysis process. Also, energy consumption in TCL process (biomass procurement, processing and separation of products) and complete feasibility analysis for TCL process is required which calls for a complete energetics evaluation of microalgae liquefaction. In view of the above research needs, some additional work may broaden its impact. Following recommendations are made for future research studies:

- 1) develop a thermo-chemical kinetic model to predict decomposition and energy consumption.
- 2) assess unit cost of production of biocrude in a pilot scale facility and life cycle analysis of TCL for co-production of biofuels and co-products in an integrated biorefinery approach.
- 3) upgrade algal biocrude using currently available technologies adopted in petro refinery processes.
- 4) study the co-combustion of biocrude as boiler fuel and/ or engine fuel.

APPENDICES

Appendix A

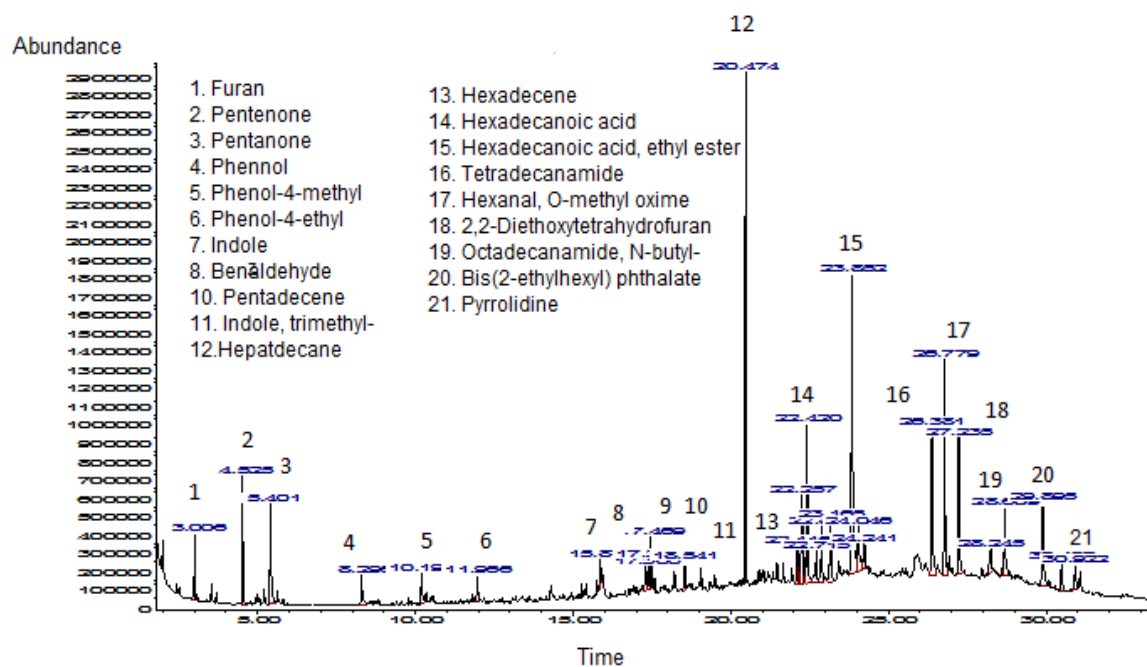


Figure A.1. A typical GC-MS chromatograms of light biocrude (B_1) obtained at 350°C temperature, 60 min holding time and 20% solids concentration.

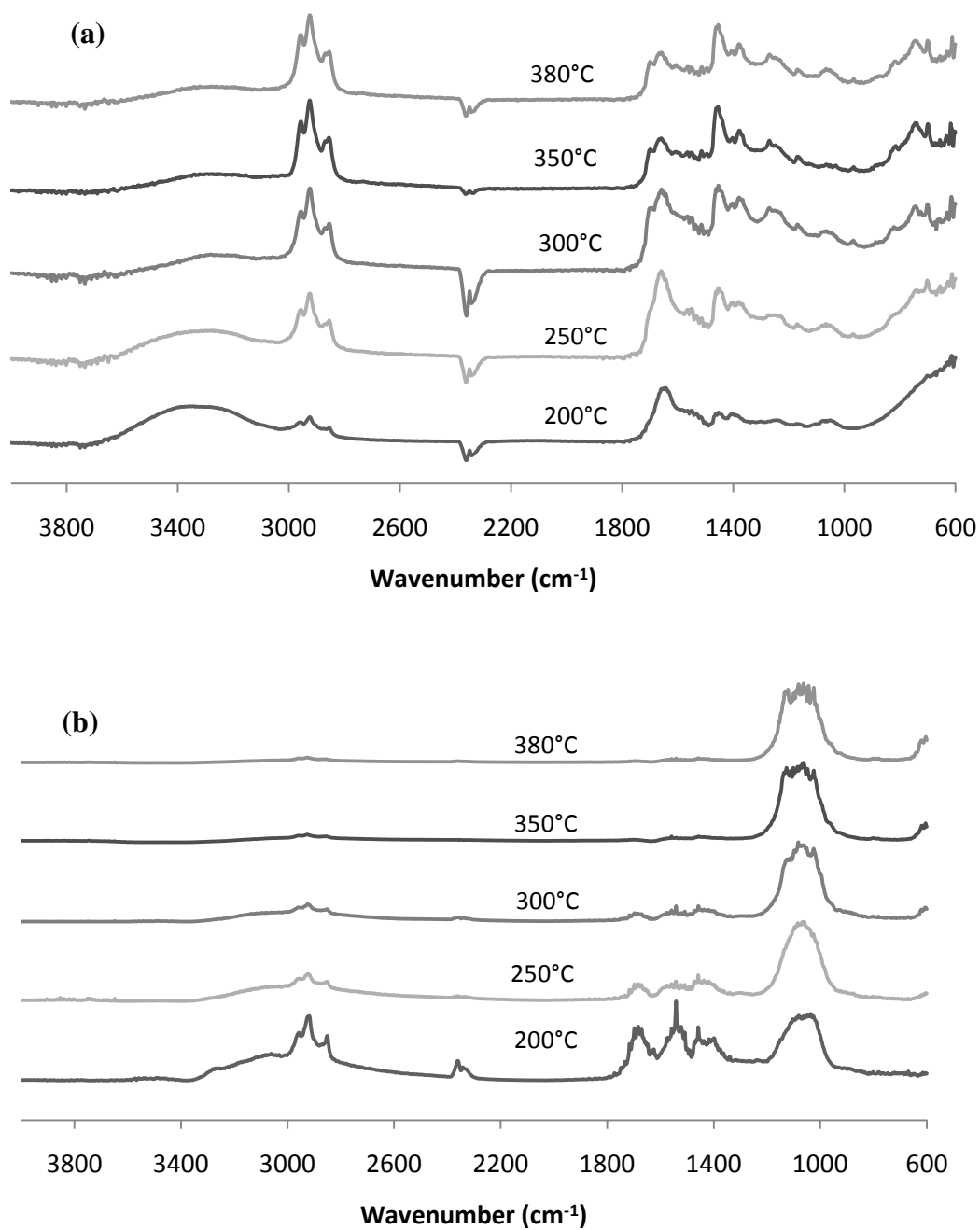


Figure A.2. FTIR absorbance spectra of; (a) Algal biocrudes and (b) Solid residues from TCL of *S. platensis* at varying temperatures, 60 min holding time and 20% solids concentration.