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นายกีรติ ประไพวัชรพันธ์



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

# BIO-OIL PRODUCTION FROM ALGAE BY TWO-STEP HYDROTHERMAL LIQUEFACTION PROCESS

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
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| Thesis Title | BIO-OIL PRODUCTION FROM ALGAE BY TWO-STEP HYDROTHERMAL LIQUEFACTION PROCESS |
| By | Mr. Keerati Prapaiwatcharapan |
| Field of Study | Petrochemistry and Polymer Science |
| Thesis Advisor | Assistant Professor Napida Hinchiranan, Ph.D. |
| Thesis Co-Advisor | Assistant Professor Prapan Kuchonthara, Ph.D. |

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กีรติ ประไพวัชรพันธ์ : การผลิตน้ำมันชีวภาพจากสาหร่ายด้วยกระบวนการไฮโดรเทอร์มอลลิควิแฟกชันแบบสองขั้นตอน (BIO-OIL PRODUCTION FROM ALGAE BY TWO-STEP HYDROTHERMAL LIQUEFACTION PROCESS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.นพิดา หิญชีระนันทน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร.ประพันธ์ คูชลธารา, 92 หน้า.

จุลสาหร่ายเป็นชีวมวลที่สามารถถูกนำไปใช้เป็นแหล่งพลังงานทางเลือกได้ เนื่องจากมีความสามารถในการผลิตเชื้อเพลิงเหลวในปริมาณสูงและมีอัตราการเจริญเติบโตที่รวดเร็ว งานวิจัยนี้ได้นำเสนอวิธีการเปลี่ยนจุลสาหร่ายไปเป็นเชื้อเพลิงเหลวด้วยกระบวนการไฮโดรเทอร์มัลลิควิแฟคชันในเครื่องปฏิกรณ์แบบกึ่งต่อเนื่อง ภายใต้อุณภูมิและความดันสูงเพื่อสลายจุลสาหร่ายเป็นสารประกอบอินทรีย์ที่มีขนาดโมเลกุลเล็กลง วัตถุประสงค์ของงานวิจัยนี้ คือ ศึกษาการผลิตและปรับปรุงน้ำมันชีวภาพจากจุลสาหร่ายโดยใช้กระบวนการไฮโดรเทอร์มัลลิควิแฟคชันแบบสองขั้นตอนในการผลิตน้ำมันชีวภาพ เปรียบเทียบผลได้กับผลที่ได้จากกระบวนการไฮโดรเทอร์มัลลิควิแฟคชันขั้นตอนเดียว โดยใช้อุณหภูมิที่อยู่ในช่วง 280 ถึง 360 องศาเซลเซียส ความดันน้ำ 12 ถึง 20 เมกะปาสคาล และอัตราการไหลของน้ำที่ 0.25 ถึง 1.00 มิลลิลิตรต่อนาที พบว่าที่อุณภูมิ 320 องศาเซลเซียส ความดัน 20 เมกะปาสคาล และอัตราการไหลของน้ำ 0.50 มิลลิลิตรต่อนาที ทำให้ได้น้ำมันชีวภาพสูงสุดที่ 29.5% โดยน้ำหนัก และเมื่อพิจารณาธาตุองค์ประกอบของน้ำมันชีวภาพที่ได้พบว่ามีสารประกอบไนโตรเจนจำนวนมาก (8.7% โดยน้ำหนัก) ดังนั้นจึงได้ปรับปรุงกระบวนการผลิตน้ำมันชีวภาพจากกระบวนการไฮโดรเทอร์มัลลิควิแฟคชันแบบชั้นตอนเดียวมาเป็นแบบสองขั้นตอน โดยใช้อุณหภูมิในขั้นตอนแรกในช่วง 150 ถึง 225 องศาเซลเซียส และอุณหภูมิในขั้นที่สองในช่วง 280 ถึง 360 องศาเซลเซียส ความดันน้ำ 12 ถึง 20 เมกะปาสคาล และอัตราการไหลของน้ำที่ 0.25 ถึง 1.00 มิลลิลิตรต่อนาที พบว่ากระบวนการไฮโดรเทอร์มัลลิควิแฟคชันแบบสองขั้นตอนสามารถลดปริมาณสารประกอบไนโตรเจนที่อยู่ในน้ำมันชีวภาพได้เหลือเพียง 4.8% โดยน้ำหนัก นอกจากนี้ผลได้ของน้ำมันชีวภาพยังมีปริมาณเพิ่มขึ้นอีกด้วย (32.0% โดยน้ำหนัก) เมื่อเปลี่ยนวัฏภาคต่อเนื่องจากน้ำกลั่นเป็นสารละลายโซเดียมคาร์บอเนตหรือสารละลายโพแตสเซียมไฮดรอกไซด์ (ความเข้มข้น 10% โดยน้ำหนักของตัวทำละลาย) พบว่าการใช้สารละลายที่มีความเป็นด่างมากขึ้นสามารถลดปริมาณองค์ประกอบไนโตรเจนในน้ำมันชีวภาพได้มากขึ้น (3.1% โดยน้ำหนัก) แต่ผลได้ของน้ำมันชีวภาพจะลดลงเหลือเพียง 22.4% โดยน้ำหนัก

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORDS: BIO-OIL / ALGAE / HYDROTHERMAL LIQUEFACTION / SEMI-CONTINUOUS REACTOR

KEERATI PRAPAIWATCHARAPAN: BIO-OIL PRODUCTION FROM ALGAE BY TWO-STEP HYDROTHERMAL LIQUEFACTION PROCESS. ADVISOR: ASST. PROF. NAPIDA HINCHIRANAN, Ph.D., CO-ADVISOR: ASST. PROF. PRAPAN KUCHONTHARA, Ph.D., 92 pp.

Microalgae is biomass which can be used as an alternative energy source since it has high performance to produce high content of liquid fuel and it also has high growth rate. This research work presents the method to convert microalgae as the liquid fuel via hydrothermal liquefaction in semi-continuous reactor under high temperature and pressure to degrade microalgae as organic compounds with smaller molecules. The objective of this research was to study the production and quality improvement of bio-oil derived from two-step hydrothermal liquefaction and compared to one-step hydrothermal liquefaction operated at 280 to 360 $^{\circ}$C, water pressure of 12 to 20 MPa and water flow rate of 0.25 to 1.00 mL/min. The result showed that this process carried out at 320 $^{\circ}$C, 20 MPa and a water flow rate of 0.50 mL/min provided 29.5 wt% of bio-oil containing a large amount of nitrogen compounds (8.7 wt%). Thus, the two-step hydrothermal liquefaction was applied. In the first step, the temperature was controlled in the range of 150 to 225 $^{\circ}$C and the temperature used in the second step was 280 to 360 $^{\circ}$C. All process was under a pressure of 12 to 20 MPa with a water flow rate of 0.25 to 1.00 mL/min. It was found that the two-step hydrothermal liquefaction decreased the amount of nitrogen compounds to 4.8 wt% with high bio-oil yield (32.0 wt%). When the continuous phase was changed from water to basic solutions containing sodium carbonate or potassium hydroxide (10 wt% of solvent), the amount of nitrogen compounds in the obtained bio-oil decreased to 3.1 wt% with a lower bio-oil yield (22.4 wt%).

| | | |
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| Field of Study: | Petrochemistry and Polymer Science | Student's Signature ..... |
| | | Advisor's Signature ..... |
| Academic Year: | 2014 | Co-Advisor's Signature ..... |

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# CHAPTER I

# INTRODUCTION

## 1.1 Background and motivation

In the past, Thailand's energy consumption had a robust growth rate. The energy of Thailand was normally supplied and consumed in several ways as shown in Figure 1.1. The total primary energy consumption increased with robust rate by 6.8% year on year or defined as level of crude oil about 1,981 thousand barrels per day. The natural gas was the largest energy consumption as about 44% of all energy and its consumption increased about 9.6% per year. Oil consumption was the second largest that was consumed about 36% of all energy, increasing per year about 5.2%. (Ministry of Energy, 2013b).

The world has been confronted with the energy crisis due to depletion of limited resources of fossil fuels, which are now widely accepted as unsustainable and promote the production of greenhouse gases released into the environment. To decrease these problems, the renewable energy such as geothermal energy, solar cell, bio-gas and biomass is interesting to replace the use of conventional fossil fuels.

Figure 1.2 shows the alternative and renewable energy shares of Thailand final energy consumption, Q1/2013 (Ministry of Energy, 2013a). The fossil fuels was consumed about 70% of total energy consumption, which was higher than alternative energy. From this report, Thailand should either increase the use of the renewable energy or reduce the use of fossil fuels to enhance the energy stability of country.

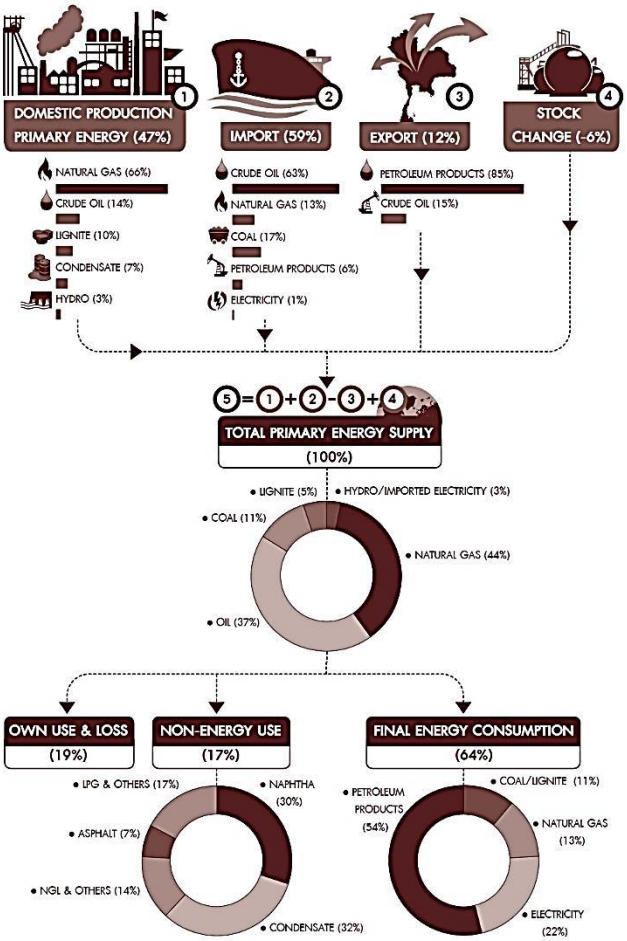


**Figure 1.1** Energy overview of Thailand 2012 (Ministry of Energy, 2013b).

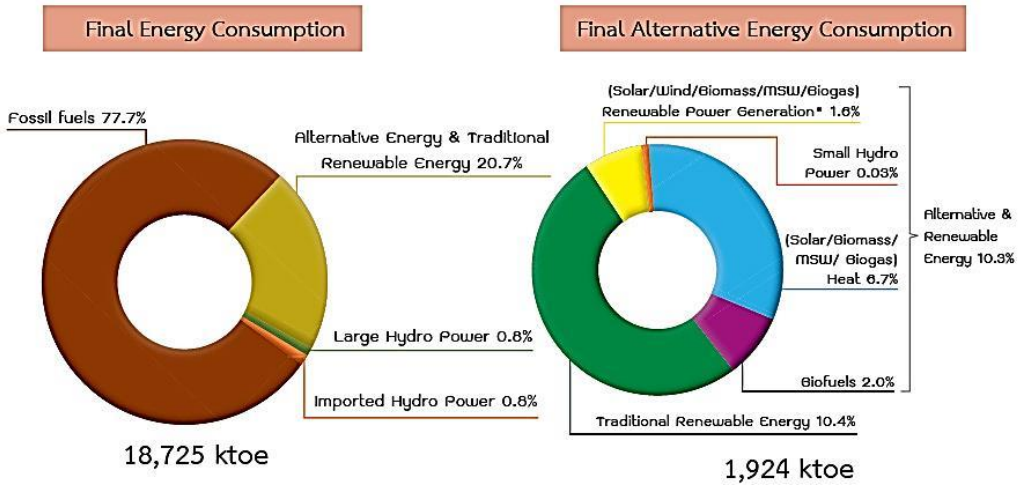


**Figure 1.2** Alternative and renewable energy shares of Thailand final energy consumption, Q1/2013 *including off grid power generation (Ministry of Energy, 2013a).

Biomass is one of several renewable energy sources that are in use today and has high potential for energy production. Biomass can be converted as bio-fuel in forms of solid, liquid and gas via both biochemical and thermochemical (Suali et al., 2012). Typically, the biofuels can be classified into four categories based on their resources and production technologies. First generation of biofuels production is normally referred to traditional ethanol and biodiesel that are derived from food feedstocks. However, the rising food prices and problems of greenhouse gases emission causes the unsustainable development of this biofuel type. Second and third generations of biofuels are developed to overcome these problems. They are produced by using non-edible agricultural feedstocks. The feedstocks for the second generation of biofuels production are lignocellulosic materials, including the agricultural waste and woody crops; whilst the third generation of biofuel production involves with the fast-growing algae or bacteria. The fourth generation of biofuel production is presently under development based on the conversion of vegetable oil and biodiesel into biogasoline using the most advanced technology (Demirbas, 2011).

Among the available feedstocks, the microalgae have potential for producing liquid fuels since their growth rate is about 100 times faster than terrestrial plants. Their production lies between 15 and 25 tonne/ha/year with an assumption of 30% lipid content in microalgae cells (Lam et al., 2012). Moreover, the algal feedstock has other unique advantages such as utilizing a wide variety of water sources, recycling stationary emissions of carbon dioxide and integrating the production of fuels and co-products within biorefineries (U.S. DOE, 2010). The hydrothermal liquefaction (HTL) is suggested to be a promising thermochemical conversion technique to produce a liquid fuel from microalgae (López Barreiro et al., 2013). HTL is an environmentally friendly technique for converting biomass as bio-oil under hot compressed water or subcritical water under moderate high temperature (100 – 375 $^{\circ}$C) and high pressure. Since it can directly convert wet feedstocks, it is possible to eliminate cost of drying process when compared to the pyrolysis generally used for bio-oil production (Akhtar et al., 2011, Hector et al., 2013).

However, HTL shows high content of nitrogen compounds in the bio-oil due to the high protein content in microalgae, resulting in undesirable nitrogen oxide ($NO_x$) emissions during combustion and deactivation of catalysts in crude oil refineries (Du et al., 2012).

The aim of this research was to study the production of bio-oil with low nitrogen content from the *Coelastrum sp.* via two-step hydrothermal liquefaction (THTL) in semi-continuous reactor. The first step of HTL was used to extract the nitrogen compounds in the microalgae and the second step of HTL was applied to produce the bio-oil. The effect of the first step temperature, the second step temperature, water pressure, water flow rate and alkali additives was investigated. The influence of difference THTL parameters on the bio-oil yield and the reduction of the content of nitrogen compounds in the bio-oil was compared to the single step hydrothermal liquefaction (SHTL).

## 1.2 Objectives

The objectives of this research were started as followed:

1. To investigate effects of process conditions of THTL on bio-oil yield and the amount of nitrogen compounds in the obtained bio-oil.
2. To evaluate the effect of alkali co-solvent on bio-oil production and the content of nitrogen compounds in the bio-oil

## 1.3 Scope of the research

The details of experimental procedure for this research were briefly presented as followed

1. Dry microalgae for using as a raw material for HTL process
2. Investigate the influence of parameters for the SHTL on bio-oil yield and the amount of nitrogen content in the obtained bio-oil as followed :
   - Temperature : 280 - 360 $^{\circ}$C
   - Water flow rate : 0.25 - 1.00 mL/min
   - Pressure : 12 - 20 MPa
3. Investigate the influence of parameters on the THTL on bio-oil yield and the amount of nitrogen content in the obtained bio-oil as followed :
   - Temperature in the first step : 150 - 225 $^{\circ}$C
   - Temperature in the second step : 280 - 360 $^{\circ}$C
   - Water flow rate : 0.25 - 1.00 mL/min
   - Pressure : 12 - 20 MPa
   - $Na_2CO_3$ concentration : 5 - 15 wt%
   - KOH concentration : 5 - 15 wt%
4. Analyze properties of bio-oil obtained from SHTL and THTL
   - Element compositions by CHN-analyzer
   - Chemical compositions by Gas Chromatography/ Mass Spectroscopy (GC/MS)
   - Boiling point distribution by Gas Chromatography Simulated Distillation (GC-SIMDIS)
5. Summarize the results

## 1.4 Expected benefits

Production of bio-oil with low nitrogen content using THTL process of microalgae.

# CHAPTER II
# THEORY AND LITERATURE REVIEWS

Before exploring the experiment work, some fundamental information related to biomass and its utilization techniques have been reviewed for basic understanding, which will be incorporated into the experimental analysis and discussion.

## 2.1 Biomass

The biomass resource is composed of a wide variety of forestry and agricultural resources, industrial processing residues, and municipal solid including urban wood residues (Table 2.1). The forest resources are consisted of residues produced during harvesting forest products, fuel-wood extracted from forestlands, residues obtained from the primary milling process during generating the forest products. The agricultural resources involves with grains used for biofuels production, animal manures and residues, and crop residues primarily derived from corn and small grains (e.g. wheat straw). A variety of regionally significant crops, such as cotton, sugar cane, rice, and fruit including nut orchards can also be classified as a source of crop residues. Municipal and urban wood residues obtained from variety of materials-yard and tree trimmings, land-clearing wood residues, wooden pallets, packaging materials, and construction and demolition debris are widely available (Perlack et al., 2005).

**Table 2.1** Biomass resources (Perlack et al., 2005)

| | Forest resources | Agricultural resources |
|---|---|---|
| Primary | • Logging residues from conventional harvesting operations and residue from forest management and land clearing operations<br>• Removal of excess biomass (fuel treatment) from timberlands and other forestlands<br>• Fuel-wood extracted from forestlands | • Crop residues from major crops – corn stover, small grain straw, and other<br>• Grains (corn and soybeans) used for the production of ethanol, biodiesel, and other bioproducts<br>• Perennial grasses<br>• Perennial woody crops |
| Secondary | • Primary wood processing mill residues<br>• Secondary wood processing mill residue<br>• Pulping liquors (black liquor) | • Animal manures<br>• Food/feed processing residue |
| Tertiary | • Urban wood residues – construction and demolition debris, tree trimmings, packaging waste and consumer durable | • Municipal solid waste and post-consumer residues and landfill gases |

## 2.2 Biofuels

Biofuels are referred to solid, liquid or gaseous fuels derived from organic matters. They are generally divided into primary and secondary biofuels (Figure 2.1). The primary biofuels are refered to the fuel-wood primarily used in an unprocessed for heating, cooking or electricity production; while, the secondary biofuels such as bioethanol and biodiesel are produced by converting biomass as forms that can be applied in vehicles and various industrial processes.

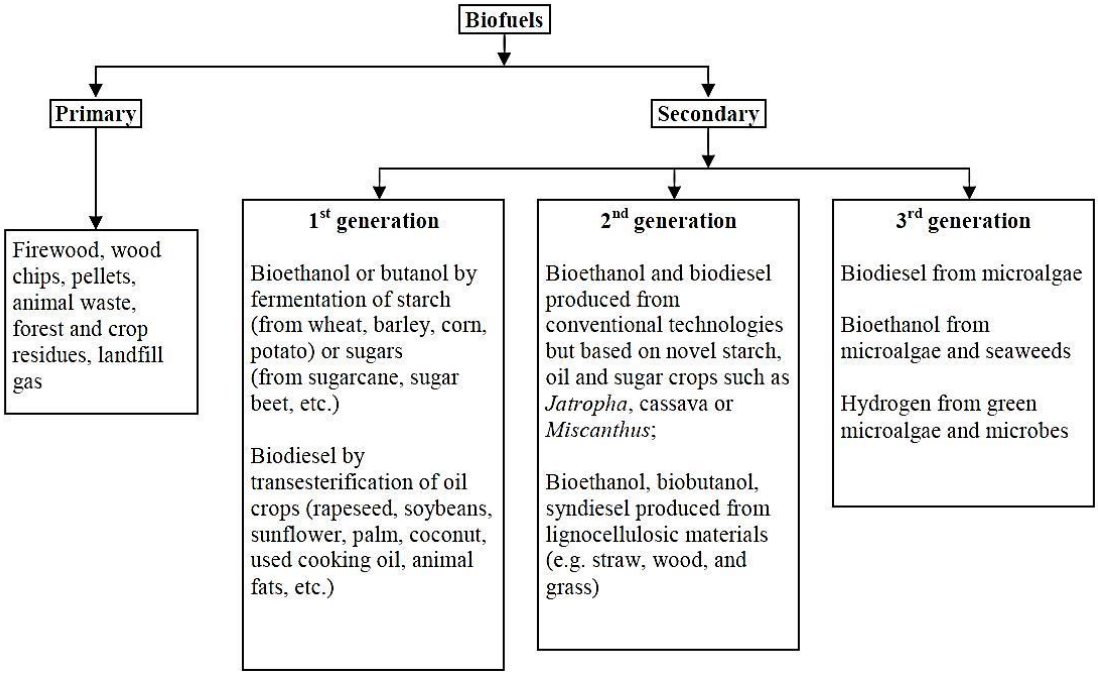


**Figure 2.1** Classification of biofuels (Dragone et al., 2010).

The secondary biofuels can be categorized as first, second and third generations based on parameters such as types of processing technology, feedstocks or the level of development (Nigam et al., 2010).

Although biofuels have a great potential to provide a carbon-neutral route for fuel production, the biofuels production in the first generation has considerable economic and environmental limitations in terms of productivity and the competition with edible plants for land extension which is not suitable for the developing countries suffering from insufficient fertile areas and high amount of population. In addition, the intensive use of land with high fertilizer and pesticide applications including water consumption causes significant environmental problems (Schenk et al., 2008).

The advent of second generation biofuels is intended to produce fuels from lignocellulosic biomass or the woody part of plants that do not compete with food production. The sources of biomass used in this generation include agricultural residues, forest harvesting residues or wood processing waste such as leaves, straw or wood chips as well as the non-edible components of corn or sugar cane. However,

the conversion of the woody biomass to fermentable sugars requires costly technologies involving pre-treatment with special enzymes (Brennan et al., 2010).

Therefore, the third generation for the production of biofuels derived from microalgae has been considered as a viable alternative energy resource to solve the major drawbacks associated with first and second generation for biofuel production (Chisti, 2007, Li et al., 2008, Nigam et al., 2010). Microalgae are able to produce the higher volume of liquid fuels as 15–300 times for biodiesel production than traditional crops on an area basis. Furthermore, the conventional crops are usually harvested once or twice a year; whereas, microalgae have a very short harvesting cycle (~1– 10 days depending on the process), and allow multiple or continuous harvests with significantly increasing yields (Schenk et al., 2008).

## 2.3 First-generation biofuels

The most well-known first-generation biofuel is ethanol produced by fermentation of sugar extracted from sugar cane or sugar beets, or sugar extracted from starch contained in maize kernels or other starch-laden crops. The different fermentation organisms yield various kinds of alcohols. Commercialization for butanol production is ongoing, while ethanol is already a well-established industry.

Biodiesel produced from oil-seed crops is the other well-known first-generation biofuel. Interest in palm biodiesel is growing, especially in South-East Asia (Malaysia, Indonesia and Thailand) where is the majority of the world's palm oil production. Jatropha, a non-edible-oil tree, is drawing attention due to its ability to produce oil seeds on lands with widely various quality. In India, Jatropha biodiesel is being pursued as a part of a wasteland reclamation strategy. From the perspective of petroleum substitution or carbon emissions reductions potential, biodiesel derived from oil-bearing seeds is like starch-based alcohol fuels – limited, as concluded in Table 2.2 (United Nations Conference On Trade And Development, 2008).

**Table 2.2** First-generation biofuels (United Nations Conference On Trade And Development, 2008)

| Pros | Cons |
|---|---|
| • Simple and well-known production methods<br>• Familiar feedstocks<br>• Scalable to smaller production capacities<br>• Fungibility with existing petroleum-derived fuels<br>• Experience with commercial production and use in several countries | • Feedstocks directly compete with crops grown for food<br>• Production of by-products need markets<br>• High-cost feedstocks lead high-cost production (except Brazilian sugar cane ethanol)<br>• Low land-use efficiency<br>• Modest net reduction in fossil fuel use and greenhouse gas emissions with current processing methods (except Brazilian sugar cane for ethanol production) |

## 2.4 Second-generation biofuels



Second-generation biofuels are produced from lignocellulosic biomass, which is a kind of non-edible feedstocks with lower cost and it can eliminate the problem of food vs. fuel competition. The second-generation biofuels can be further classified following the process used to convert the biomass to fuels: biochemical and thermochemical processes. The second-generation ethanol or butanol would be produced via biochemical processing, while all other second-generation fuels discussed here would be generated via thermochemical processing. The second-generation thermochemical biofuels may be less familiar to most readers than second-generation ethanol because there are no first-generation analogs. On the other hand, many second-generation thermochemical biofuels are already for commercial production by using several processes, which some cases are identical to those used for biofuel production (Figure 2.2).

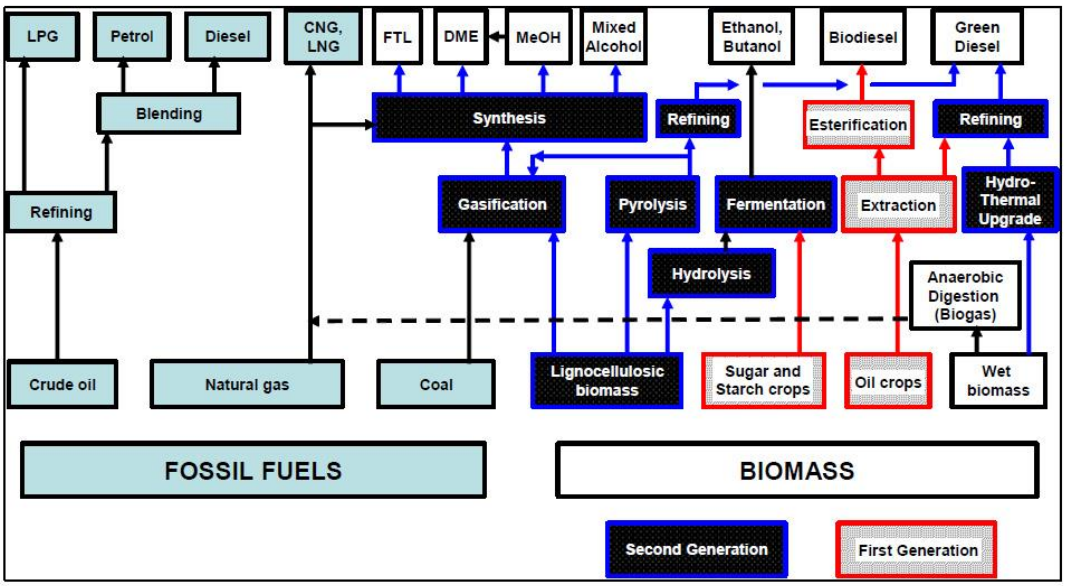


**Figure 2.2** Production pathways to liquid fuels from biomass and from fossil fuels (United Nations Conference On Trade And Development, 2008).

These biofuels include methanol, refined Fischer-Tropsch liquids (FTL), and dimethyl ether (DME). Mixed alcohols can also be produced from fossil fuels, but there is no commercial production today due to the immature state of some systems for producing them. The another thermochemical biofuel in Figure 2.2 is green diesel, which there is no obvious in fossil fuel analog. Unrefined fuels, such as pyrolysis oils, are also thermochemically produced, but they require considerable refining before applying in engines (United Nations Conference On Trade And Development, 2008).

## 2.5 Third-generation biofuels (Wang, 2013)

The third generation biofuel feedstocks are aquatic microorganisms. Microalgae are used to produce the third-generation biofuel. The advantages of microalgae are high carbon adsorption ability, high lipid contents and simple growth in any environment with short time. Among them, the lipid content of microalgae is 25 to 200 times higher than that of soybeans. The extracted oil from microalgae can produce biodiesel and their carbohydrate can be fermented as alcohol, as well as their nitrogen and phosphorus contents that can be recycled as fertilizer.

To compare with terrestrial plants, microalgae can save much more arable land and fresh water sources than other biofuel feedstocks. In the opposite, the immature production techniques for microalgae bioenergy production including unknown environmental impacts are still the disadvantage.

In Figure 2.3, algae 50% TAG means microalgae containing 50% lipid contents and they can be cultivated in the close photobioreactors. Although oil palm is the best feedstock for the first and second generation of biofuel production due to its highest oil yield and the lowest land use, algae 50% TAG can produce oil content at least 10 times higher than oil palm. They also use only 1/160 of land area used for palm oil production. However, the large scale production for microalgae biofuel is the biggest challenge.

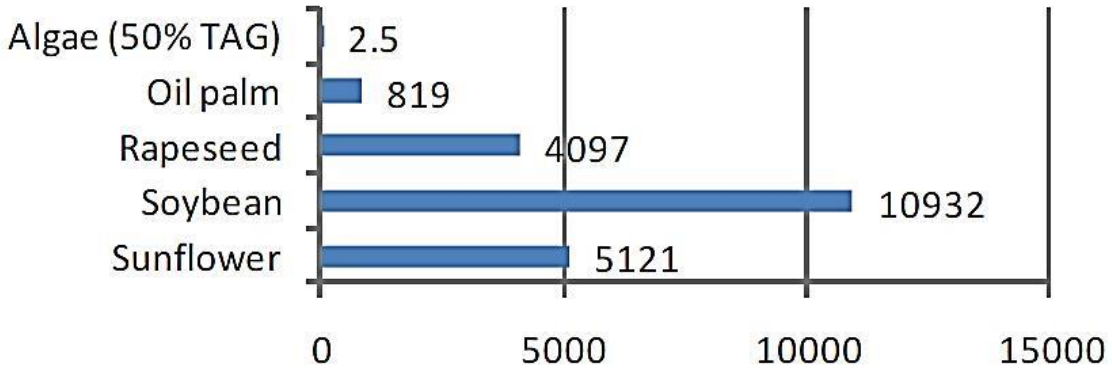


**Figure 2.3** Area which to produce global demand (hectares × $10^6$) (Wang, 2013).

## 2.6 Microalgae

Microalgae are microscopic photosynthetic organisms that are found in both marine and fresh water environments. Their photosynthetic mechanism is similar to land-based plants due to a simple cellular structure. Since they are submerged in an aqueous environment, where they have efficient access to water, carbon dioxide ($CO_2$) and other nutrients, they are generally more efficient to convert solar energy in their cellular structure. The absence of non-photosynthetic supporting structures (roots, stems, etc.) also favours the microalgae in aquaculture (John et al., 2010).

Microalgae appear to represent the only current renewable way to generate biofuels (Chisti, 2007, Schenk et al., 2008). They have much lower impact on the environment and on the world's food supply than conventional biofuel-producing crops. To compare with other plants biofuels, microalgal biomass has a high caloric value, low viscosity and low density. These properties induce microalgae to be an appropriate renewable energy resource for biofuel production than lignocellulosic materials (Miao et al., 2004). Moreover, microalgae has inherently high-lipid content and semi-steady state production with suitability in various climates (Clarens et al., 2010). One unique aspect of algae as compared to other advanced feedstocks is the several species that are available for amenability for biofuel production. Various algae species may be selected to optimize the production of different kinds of biofuels. Algae offer a diversity of valuable products and pollution solutions, such as food, nutritional compounds, omega-3 fatty acids, animal feed, energy sources (including jet fuel, aviation gas, biodiesel, gasoline, and bioethanol), organic fertilizers, biodegradable plastics, recombinant proteins, pigments, medicines, pharmaceuticals, and vaccines (Pulz, 2004, Pienkos et al., 2009).

Microalgae is expected that they may soon be one of the Earth's most important renewable fuel crops (Campbell, 1997). The main advantages of microalgae are listed below (Campbell, 1997, Huntley et al., 2007, Li et al., 2008, Schenk et al., 2008, Khan et al., 2009, Rodolfi et al., 2009):

- Higher photon conversion efficiency (approximately 3–8% against 0.5% for terrestrial plants), which represents higher biomass yields per hectare and high growth rates (e.g. 1–3 doublings/day)
- Higher $CO_2$ sequestration capacity
- Ability to grow in various kinds of liquid media including salt and waste water streams (saline/brackish water/coastal seawater), which can decrease the freshwater consumption

- Ability to utilize nitrogen and phosphorous from a variety of wastewater sources (e.g. agricultural run-off, concentrated animal feed operations and industrial and municipal wastewaters) providing the additional benefit of wastewater bioremediation
- Ability to use marginal areas which are unsuitable for agricultural purposes (e.g. desert and seashore lands) and thereby does not compete with arable land for food production
- No requirement of seasonal production and ability to be harvested all-year-round
- Ability to be cultured for inducing a high concentration of feedstock (oil, starch, biomass)
- No requirement of fertilizers and preticides, resulting in less waste and pollution
- Minimum of environmental impact such as deforestation
- Ability to produce value-added co-products or by-products (e.g. proteins, polysaccharides, pigments, biopolymers, animal feed and fertilizers).

## 2.7 Bio-oil production technology

Figure 2.4 shows the energy production by microalgae. Two main types of processes for production of bio-oils from biomass are pyrolysis and liquefaction. Pyrolysis involves the rapid thermal decomposition of organic compounds by heat in the absence of oxygen, resulting in the production of charcoal, bio-oil, and gaseous products. Liquefaction is also called as direct liquefaction, hydrothermal upgrading/pyrolysis, hydrothermal liquefaction (HTL), depolymerization, and solvolysis. This process is conducted under elevated pressure and temperature to keep water in either liquid or supercritical state. The use of water as a solvent obviates the need to dry biomass and permits reactions to be carried out at lower temperatures in comparison with flash pyrolysis. The primary product of HTL is bio-oil or bio-crude, and the main by-products are the solid residue, bio-char and

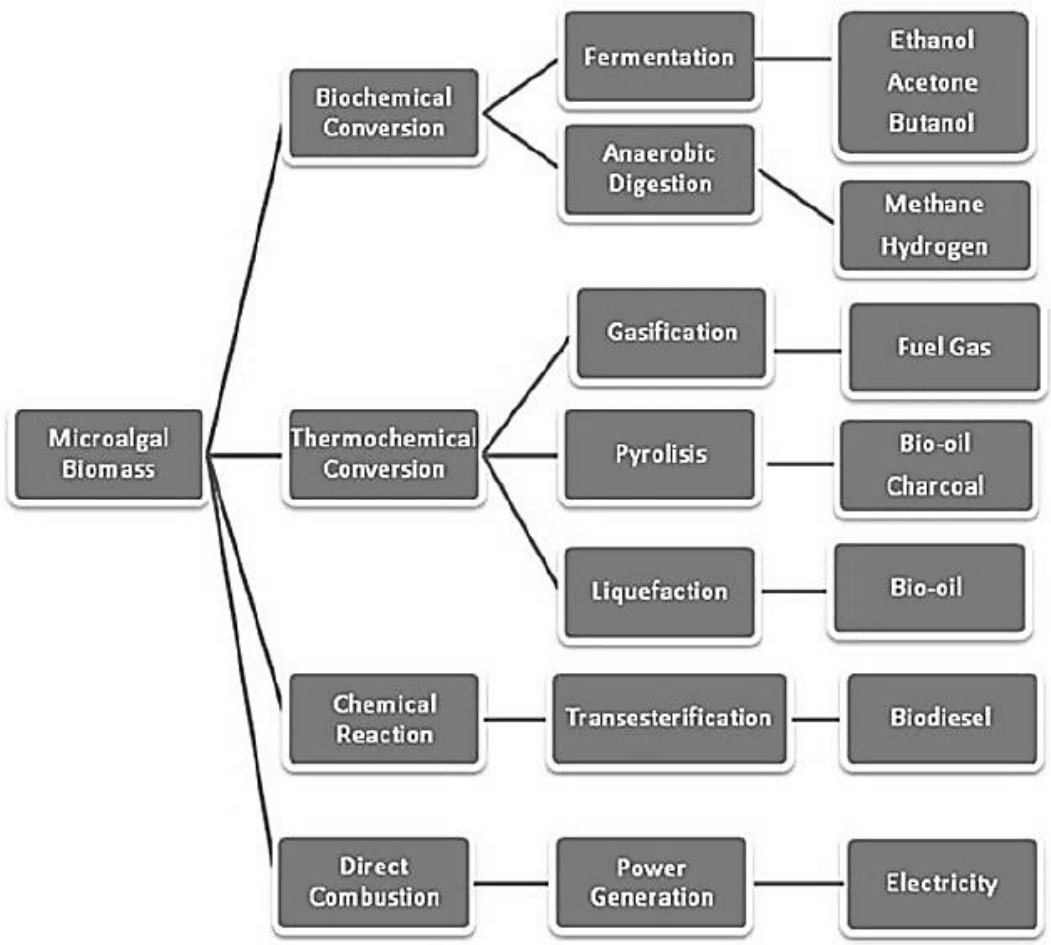


**Figure 2.4** Energy production by microalgae biomass conversion using biochemical, thermochemical, chemical and direct combustion process (Gouveia, 2011).



water containing soluble organic compounds. Both processes belong to the thermochemical technologies in which feedstock organic compounds are converted as bio-oil products. The thermochemical process is advantageous since it is relatively simple, usually requiring only one reactor. Thus, the capital cost is low. However, this process is non-selective, producing a wide range of products including a large amount of char (Huber et al., 2006).

The characteristic and technique feasibility of the two thermochemical processes for bio-oil production are compared in Table 2.3. Flash pyrolysis has a short gas residence time (~1 s), atmospheric pressure, and a relatively high temperature (450–500 $^{\circ}$C). Furthermore, feedstock drying is necessary. Hydrothermal liquefaction is usually performed at lower temperatures (300–400 $^{\circ}$C),

**Table 2.3** Comparison of two typical thermochemical processes for bio-oil production (Xiu et al., 2012)

| Methods | Treatment condition/requirement | Reaction mechanism/process description | Technique feasibility | |
|---|---|---|---|---|
| | | | Pros. | Cons. |
| Flash/fast pyrolysis | • Relatively high temperature (450-500°C)<br>• A short residence time (~1s);<br>• Atmosphere pressure<br>• Requirement of drying process | The light small molecules are converted to oily products through homogeneous reaction in the gas phase | - High oil yield up to 80% on dry feed<br>- Lower capital cost<br>- Commercialized already | - Poor fuel quality. |
| Hydrothermal liquefaction (HTL) | • Lower temperature (300-400 °C)<br>• Longer residence time (0.2-1.0 h)<br>• High pressure (5-20 MPa)<br>• Non-requirement of drying process | Occurs in aqueous medium which involves complex sequences of reactions | - Better quality of bio-oil (high heating value, low moisture content | - Relatively low oil yield (20-60%);<br>- Need high pressure equipment<br>- higher capital cost |

longer residence times (0.2–1.0 h), and relatively high operating pressure (5–20 MPa). Contrary to flash pyrolysis and gasification processes, drying process of the feedstock is not needed in the HTL process, which makes it especially suitable for naturally wet biomass. However, a reducing gas and/or a catalyst are often included in the process in order to increase the oil yield and quality.

The reaction mechanisms of the two processes are different, which have been studied by many investigators (Minowa et al., 1998, Demirbas, 2000). The hydro-thermal process occurred in aqueous media involve complex sequences of reactions including solvolysis, dehydration, decarboxylation and hydrogenation of functional groups, etc. (Chornet et al., 1985). The decomposition of cellulose was studied by Minowa et al. (1995). The effects of adding a sodium carbonate catalyst, a reduced nickel catalyst, and no catalyst addition in the decomposition of cellulose in hot-compressed water were investigated. They found that hydrolysis could play an important role to form glucose/ oligomer, which can be quickly decomposed as non-glucose aqueous products, oil, char and gases (Figure 2.5a). Without the addition of catalyst, char and gases were produced via oil consumption. However, oil intermediates were stabilized in the presence of an alkali catalyst resulting in the reduction of char production.

For the flash pyrolysis, the light small molecules are converted to oily products through homogeneous reactions in the gas phase. A number of pyrolysis mechanisms have been proposed, such as Broido-Shafizadeh model (Allan et al., 1979). A comprehensive review of the myriad models, which is available for describing the biomass pyrolysis reactions, is beyond the scope of this communication. However, those models have been thoroughly reviewed by other researchers (Blasi, 1998, White et al., 2011). For example, Blasi (1998) has reviewed many semi-global mechanisms that have been proposed in the literature and compared them in terms of kinetics and species selectivity (Blasi, 1998). The relatively simple single particle pyrolysis model is shown in Figure 2.5b. Biomass is rapidly heated in the absence of air, vaporizes, and quickly condenses to obtained bio-oil, yields upto 80 wt% on dry feed, with char and gas as by-products (Bridgwater et al., 2000).

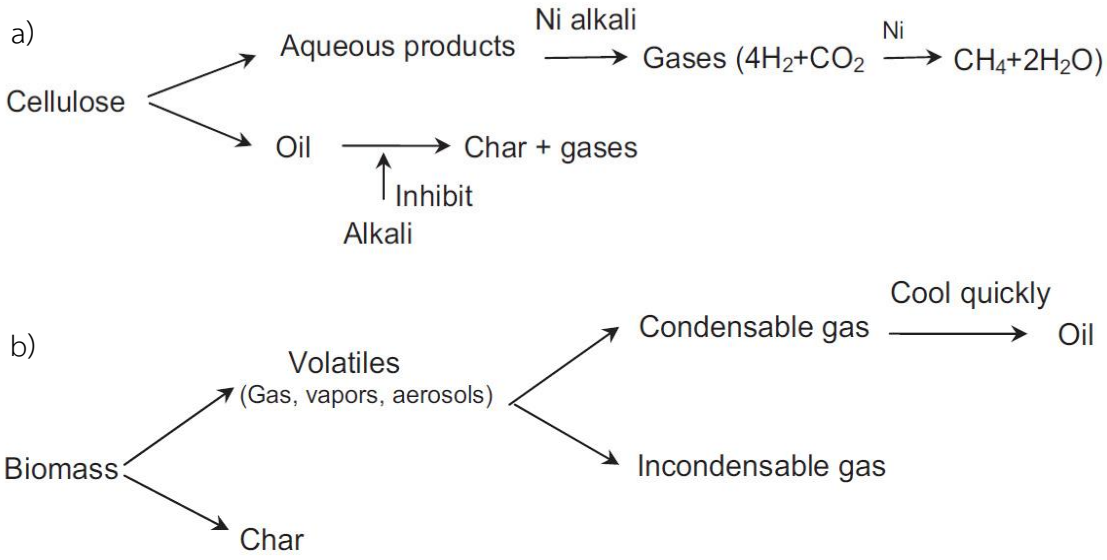


**Figure 2.5** Reaction pathway for the biomass a) hydrothermal liquefaction of cellulose and b) flash pyrolysis process of biomass (Xiu et al., 2012).

## 2.8 Hydrothermal liquefaction

Hydrothermal liquefaction processes involve physical and chemical transformations carried out in the presence of water at elevated pressure and temperature. In the past, these systems have been called as hot compressed water, sub- and supercritical water, or superheated water. Since the hydrothermal processes are generally operated above 100°C and under sufficient pressure to remain water as a liquid, pure water is in the supercritical state when the operating condition is above the critical point (374°C and 221 bar). Hydrothermal systems are unique in a number of dimension. The surroundings can be turned to affect numerous properties, such as density or dielectric constant. By changes in these properties, different reaction pathways are possible to be occured that they are not accessible in a liquid water or steam vapor phase at atmospheric conditions. Numerous reviews have been written about reactions in hydrothermal systems (Johnson, 2012).

## 2.9 Water properties (Johnson, 2012)

Hydrothermal liquefaction (HTL) conditions have two distinct regions: subcritical and supercritical regions. Water remains in a liquid phase in the range of 100 to 374$^{\circ}$C above the vapor pressure in a term of subcritical water. Above the critical pressure and temperature, water is again a single phase but in a supercritical state. Property changes in water under various temperatures at 300 bar are the ion product, density, and dielectric constant as shown in Figure 2.6. The change of these properties of water occurs when it is heated in the liquid state. To compare with ambient water which has the dissociation constant of water ($K_w$) of $10^{-14}$, the ions produced in the subcritical state are on the order of $10^{-11}$. The higher ionic content environment provides many base and acid catalyzed reactions without adding catalysts. The $K_w$ is dropped as it approaches the critical point. This includes the reduction of water density since the near-critical region generates higher gas phase-like reaction behavior and promotes radical reactions. This gas-like behavior is evidenced by the dielectric constant, which is monotonically dropped with increasing temperature. This changes water from a polar properties at ambient conditions to an organic-like solvent at near-critical conditions.

These changes in water properties allow for interesting reactions to be occured. Non-polar chemicals are soluble in subcritical water higher than the ambient condition. The dense solvents with considerable thermal energy and solvation capacity allow high energy configurations of reactants. By utilizing water, not only these interesting physical properties are available, but it also happens in a solvent that is readily available, and without the addition of necessary chemicals.

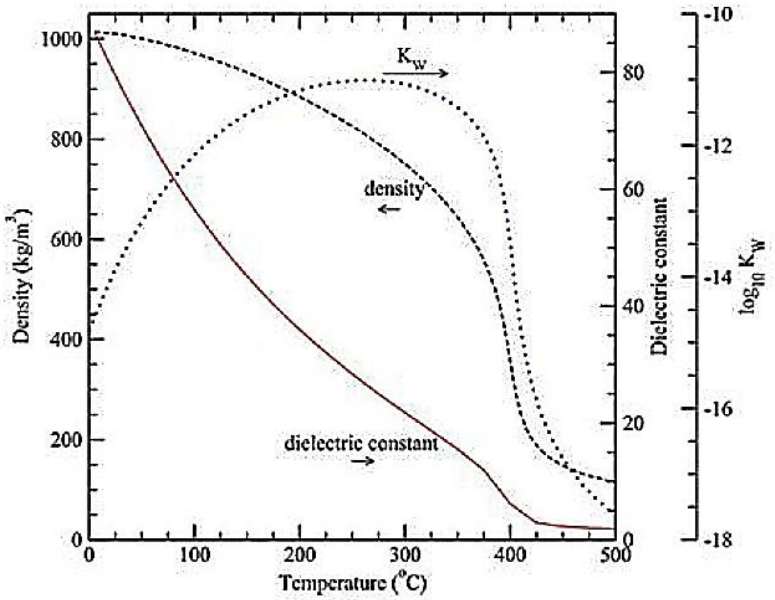


**Figure 2.6** Water properties as a function of temperature at 300 bar (Johnson, 2012).

## 2.10 Literature reviews

Sereewatthanawut et al. (2008) investigated the production of value-added proteins and amino acids from deoiled rice bran by using hydrolysis in subcritical water (SW) operated in the temperature range between 100 and 220 °C for 0-30 min. The amount of produced protein increased with increasing temperature. The highest yields of protein and amino acids were 219 ± 26 and 8.0 ± 1.6 mg/g of dry bran which were obtained at 200 °C at hydrolysis time of 30 and 20 min, respectively. This study demonstrated that SW could be used to potentially hydrolyze biomass to reduce proteins and amino acids.

Ross et al. (2010) investigated the conditions for producing high quality and low molecular weight bio-crude from microalgae and cyanobacteria containing low lipid content such as *Chlorella vulgaris* and *Spirulina*. Liquefaction experiments were performed in a high pressure batch reactor at 300 and 350 °C. The influence of process variables such as temperature and catalyst types on the yield of bio-crude and its properties was studied. The employed catalysts were alkali such as potassium hydroxide and sodium carbonate and the organic acids such as acetic acid and formic acid. The results showed that the yield of bio-crude obtained from the use of

organic acids was higher than that of alkali catalysts. The nitrogen content in the bio-crude (4-6%) was significantly higher than a petroleum crude oil. This implied that use of organic acids did not reduce the nitrogen content in the obtained bio-crudes.

Jena et al. (2011) investigated the optimum thermochemical liquefaction (TCL) operating condition for producing bio-oil from *spirulina platensis.* TCL experiment were performed at various temperatures (200-380 $^{\circ}$C), holding time (0-120 min), and solids concentrations (10-50%). This results showed that the highest bio-oil yield of 39.9% was achieved at 350 $^{\circ}$C, 60 min of holding time and 20% solids concentration. Light fraction of bio-oil appeared at 300 $^{\circ}$C or higher temperature was 50 – 63% of the total bio-oil. Bio-oil with energy density of 34.7-39.9 MJ $kg^{-1}$ obtained at 350-380 $^{\circ}$C had similar fuel properties to petroleum crude (42.9 MJ $kg^{-1}$). Bio-oil from every condition contained 71-77% element carbon, 0.6-11.6% element oxygen and 5.5-7.2% element nitrogen. These results demonstrated that the bio-oil obtained from TCL had higher nitrogen content than crude petroleum.

Valdez et al. (2012) investigated a hydrothermal liquefaction of *Nannochloropsis sp.* with systematic process variables and analysis of the product fractions. The experiment was performed at different temperatures (250-400 $^{\circ}$C), time (10-90 min), water densities (0.3-0.5 g/mL), and biomass loading (5.35 wt%). The results showed that the liquefaction produced a bio-oil with light and heavy fractions including gaseous, aqueous and solid product. The gravimetric yields of the product fractions were independent on water density at 400 $^{\circ}$C. The increase in the biomass loading increased the bio-oil yield from 36 to 46 wt%. The yields of light and heavy bio-oil were dependent on reaction time and temperature. These results demonstrated that microalgae could produce bio-oil via hydrothermal liquefaction and the bio-oil yield was dependent on process conditions.

Du et al. (2012) studied the hydrothermal treatment for reducing the nitrogen content in *Nannichloropsis oculata* feedstock by removing proteins without requiring significant energy input. The effects of reaction conditions and composition of algae on the yield were inveastigated by varying the temperature (150-225 $^{\circ}$C) and reaction time (10-60 min). To compare with untreated algae, the pretreated samples had 6-42% lower nitrogen content at 200-225 $^{\circ}$C for 30-60 min. The pyrolytic bio-oil from

pretreated algae contained less amount of nitrogen compounds than untreated samples. Therefore, hydrothermal treatment was an effective pretreatment strategy for high-quality bio-oil production from algal biomass.

Elliott et al. (2015) reviewed the recent results in hydrothermal liquefaction (HTL) of biomass in continuous flow processing systems. The current reviews on continuous HTL of biomass lead to the conclusion that there was significant potential for commercialization of the technology. Several feedstocks had successfully been processed at high feed concentrations resulting in high energy recoveries and carbon efficiencies. The current sizes of continuous systems were not adequate for demonstration scale of operation. The technoeconomic assessment (TEA) calculations nevertheless highlight the potential of the technology especially for wet waste and algae feedstock.

# CHAPTER III

# EXPERIMENTAL METHODOLOGY

## 3.1 Materials

*Coelastrum sp.* was used as a raw material for all experiments. Dry microalgae was provided by PTT Research and Technology Institute. It was stored at room temperature before used. Dichloromethane (DCM) (Earth Cheme Lab Ltd., Thailand) was used for bio-oil extraction. Sodium carbonate anhydrous ($Na_2CO_3$) (CT Chemical Co., Ltd., Thailand) and potassium hydroxide (KOH) (Ajax Finechem) were used as additive.

## 3.2 Experimental procedures

Hydrothermal liquefaction of microalgae was performed in single-step and two-step semi-continuous processes. The schematic layout of the experimental system is presented in Figure 3.1. The tubular reactor was made of stainless steel (SUS316, 0.5 in. OD, 29.5 in. length and 0.083 in. thickness). An analytical isocratic HPLC pump (PU-2080, JASCO Ltd.) was used to control a water flow rate. The pressure and temperature were adjusted using a back-pressure regulator (BP-66, GO regulator) and tube furnace (CTF12/65/550, Carbolite Ltd.), respectively.

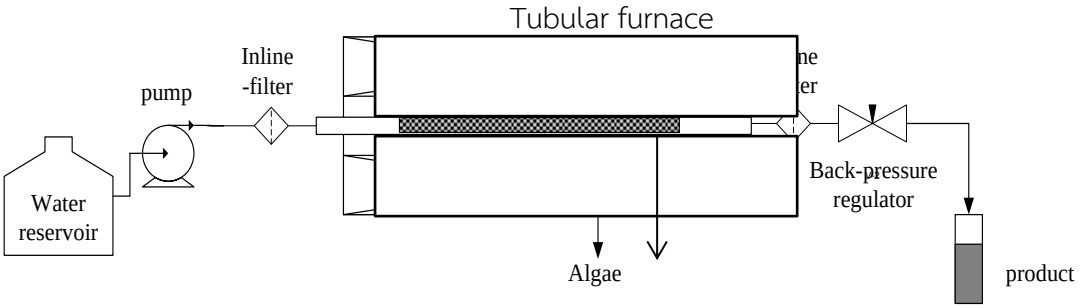


**Figure 3.1** Schematic diagram of hydrothermal liquefaction process.

### 3.2.1 The single step hydrothermal liquefaction (SHTL)

Microalgae (10 g) mixed with some of inert materials (30 g of gravel) were packed in the tubular reactor. The water was continuously introduced into the reactor. Then, pressure and temperature were adjusted to the desired setting values. The time interval of sample collected was dependent on the applied water flow rate. The operating time was defined as the elapse time starting from the reactor reached the setting temperature and pressure. The experiment was initiated when the temperature and pressure reached to the desired point. Then, this condition was maintained for a desired resident time. To terminate the process, the pressure was released to atmosphere and the reactor was cooled down to room temperature. The gases generated during the reaction were vented before disassembly the reactor. The liquid product collected at the outlet of back pressure regulator was separated as bio-oil and aqueous phase by using DCM in separation funnel. The DCM soluble fraction was defined as the bio-oil phase. The DCM containing bio-oil phase was recovered by using vacuum evaporator at 50 $^{\circ}$C, and then the weight of the residual bio-oil was measured. The solid residue remained inside the reactor was collected. Then, it was dried at 110 $^{\circ}$C overnight, weighted and kept at room temperature. The schematic of SHTL was shown in Figure 3.2.

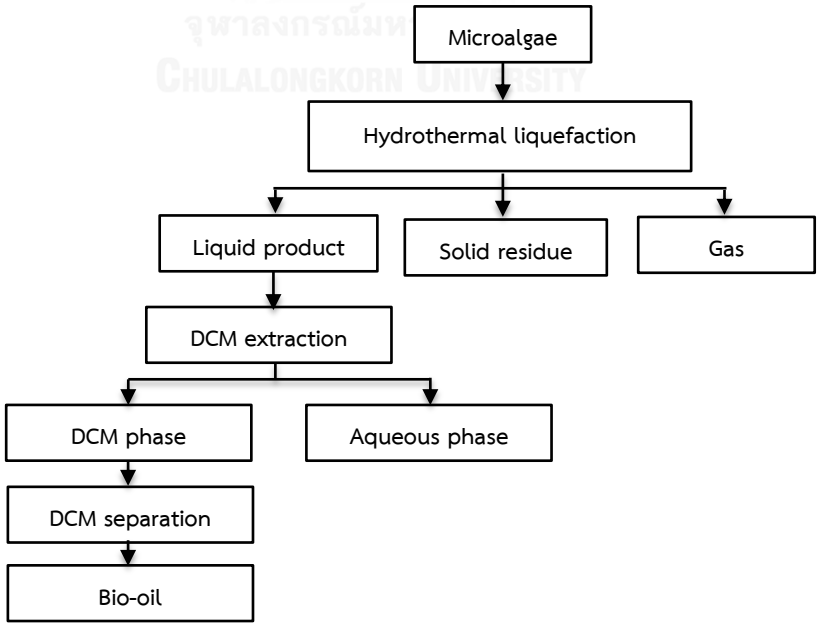


**Figure 3.2** Schematic of SHTL.

### 3.2.2 The two-step hydrothermal liquefaction (THTL)

The hydrothermal liquefaction was performed in two-step. The first step was carried out as described in Section 3.2.1 at lower temperature and pressure. The first step was terminated by the operating time. When the liquid product obtained from the first step was collected, the reactor was not disassembled. The temperature and pressure were adjusted again to the new setting values. The solid residue obtained from the first step was used as the raw material for the second step. The liquid product was collected again when the reactor reached to the desired temperature and pressure. The liquid products obtained from both steps were separated as bio-oil and aqueous phases by using DCM. The procedures for the product separation and collection were the same as described in Section 3.2.1. The yields were determined following dry ash-free basis as shown in Section 3.3. The schematic of THTL was shown in Figure 3.3

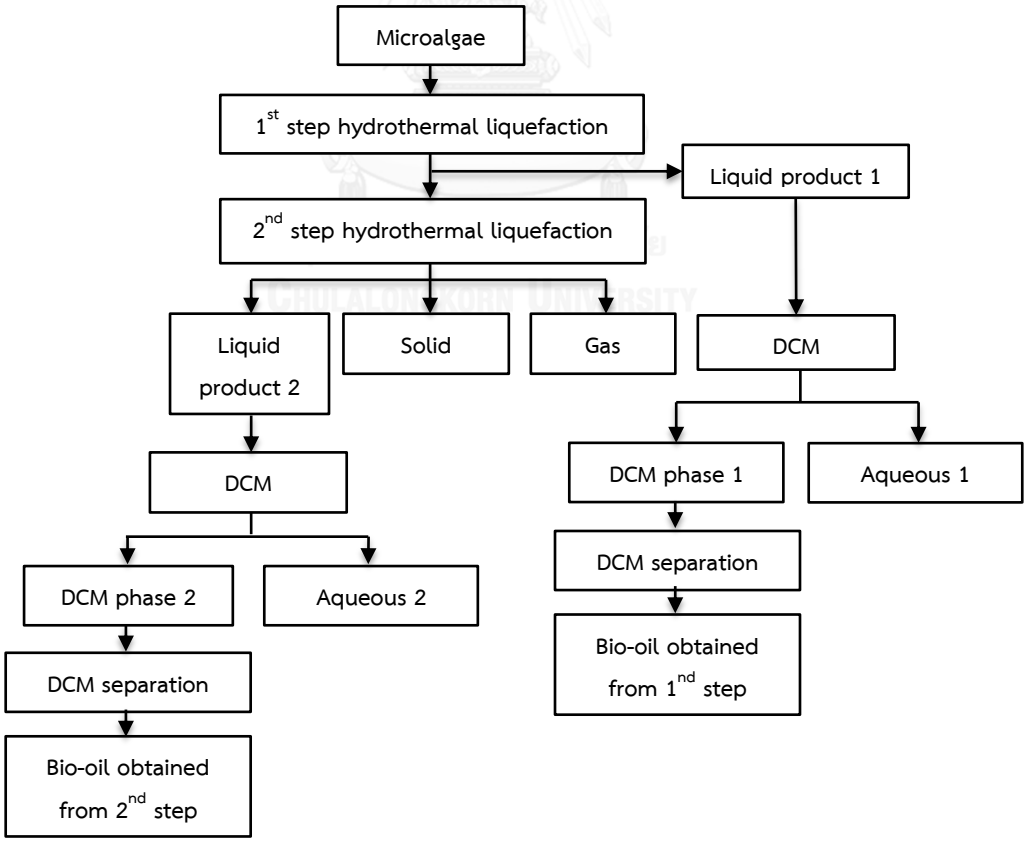


**Figure 3.3** Schematic of THTL.

## 3.3 Experimental conditions

### 3.3.1 Single step hydrothermal liquefaction (SHTL)

The experiments were conducted in the temperature range of 280 to 360 °C, under 12 to 20 MPa and a water flow rate of 0.25 to 1.00 mL/min. Table 3.1 shows the condition used for SHTL. The data of these experiments were compared with THTL.

**Table 3.1** Experimental conditions for SHTL

| Parameters | |
|---|---|
| Temperature (°C) | 280, 300, 320, 340, 360 |
| Pressure (MPa) | 12, 16, 20 |
| Water flow rate (mL/min) | 0.25, 0.50, 0.75, 1.00 |

### 3.3.2 Two-step hydrothermal liquefaction (THTL)

In the first step, the effect of temperature on yields and properties of bio-oil was investigated. The temperature in the first step was in the range of 150 to 225 °C with a constant water pressure of 7 MPa; whilst, the condition used in the second step was fixed at 320 °C, 20 MPa and a water flow rate of 0.50 mL/min. In the second step, the effects of the temperature, water pressure and water flow rate on yields and properties were investigated. The temperature in the second step was in the range of 280 to 360 °C under the water pressure of 12 to 20 MPa and the water flow rate of 0.25 to 1.00 mL/min; whilst, the condition used in the first step was fixed at 200 °C and 7 MPa because this condition could promote the production of highest bio-oil yield with low nitrogen content from the second step. Moreover, the effect of alkali additives on yields and properties of bio-oil was investigated. This experiment was carried out at first step temperature of 200 °C, the second step temperature of 320 °C, water pressure of 20 MPa and a water flow rate of 0.50 mL/min. Sodium carbonate and potassium hydroxide were used as additives and their concentrations were in range of 5 to 15 wt% based on the content of solvent. The condition of experiments in THTL was shown in Table 3.2.

**Table 3.2** the condition of experiments in THTL

| Parameters | |
|---|---|
| First step temperature ($^{O}$C) | 150, 175, 200, 225 |
| Second step temperature ($^{O}$C) | 280, 300, 320, 340, 360 |
| Pressure (MPa) | 12, 16, 20 |
| Water flow rate (mL/min) | 0.25, 0.50, 0.75, 1.00 |
| Additives ($Na_2CO_3$ and KOH) (wt%) | 5, 10, 15 |

## 3.5 Analysis method

### 3.5.1 Characterization of microalgae

The proximate analysis was evaluated following ASTM D3173 – D3175 to detect the levels of moisture, volatile matter, ash and fixed carbon of microalgae. Quantification methods of evaluating the crude protein, crude lipid and total carbohydrate were described in Table 3.3.

The ultimate analysis was evaluated following ASTM D5291 - D5296 to detect the total carbon, hydrogen and nitrogen contents by using CHN analyzer (Leco CHN-2000) as shown in Figure 3.4. The oxygen content was then calculated by the percentage of difference.

The thermal analysis of microalgae was evaluated by using the thermogravimetric/differential thermal analyzer (TG/DTA) (Mettler Toledo TG Analyzer 851e model) as shown in Figure 3.5. The microalgae was milled, the particle size was in the range of 0.15 to 0.25 mm. The weight precision of the instrument is 0.1 µg. The microalgae weighting about 10 mg was placed in an open platinum sample pan. The microalgae was heated to 1,000 $^{O}$C at a constant heating rate of 10 $^{O}$C/min, under a constant nitrogen flow rate of 20 mL/min through the sample chamber.

The high heating value (HHV) of microalgae was calculated from the formula as shown in Eq. 3.1 based on the elemental composition of samples (Chun-Yang, 2011).

$$HHV_{biomass}\,(MJ/kg) = 0.3536FC + 0.1559VM - 0.0078ash \qquad (3.1)$$

where:

FC = Fix carbon (wt%)

VM = Volatile matter (wt%)

**Table 3.3** Quantification method for microalgae analysis

| Analysis | Method |
|---|---|
| Crude protein (N×6.25) | ISO 5983-2 (2005) |
| Crude lipid | AOAC (2012), 922.06 |
| Total carbohydrates | Compendium of methods for food analysis (2003), p. 2-9 |


**Figure 3.4** CHN Analyzer (CHN-2000, LECO Instrument (Thailand) Ltd.).

**Figure 3.5** Thermogravimetric/differential thermal analyzer (Mettler Toledo TG Analyzer 851e model).

### 3.5.2 Bio-oil analysis

#### 3.5.2.1 High heating value (HHV)

The carbon, hydrogen and nitrogen content in produced bio-oil were analyzed using a CHN analyzer (CHN-2000, LECO Instrument (Thailand) Ltd.). The HHV of bio-oil was calculated from the Dulong formula as shown in Eq.3.2 based on the elemental composition of samples (Chen et al., 2012).

$$HHV_{bio-oil} = 0.3383C + 1.422(H - \frac{O}{8}) \quad (3.2)$$

#### 3.5.2.2 Nitrogen reduction

The efficiency of THTL for the reduction of nitrogen content in the bio-oil obtained from the second step of this process was compared to SHTL and exhibited as %N reduction that was calculated following Eq.3.3:

$$\%N\,\text{reduction} = \left[\frac{N_0 - N_1}{N_0}\right] \times 100$$
$$\text{Weight of nitrogen (g)} = W_{oil} \times (\%N\,\text{reduction}) \quad (3.3)$$

Where:

$N_0$ = weight of N content in bio-oil from SHTL (g)

$N_1$ = weight of N content in bio-oil from the second step of THTL (g)

$N_{\%}$ = %Nitrogen content in bio-oil (wt%)

$W_{oil}$ = weight of bio-oil (g)

#### 3.5.2.3 Ammonium in aqueous phase

The total $NH_4^+$ level was spectrophotometrically determined following the reaction of ammonia with salicylate and hypochlorite to produce indophenol blue following the method of salicylate-hypochlorite (Bower et al., 2011) and measuring the absorbance at 660 nm. The $NH_4^+$ yields was reported in terms of the %nitrogen recovery compared to the amount of nitrogen content in the original algae, as presented in Eq.3.4.

$$\%N-NH_4^+ = \frac{\frac{NH_4^+ (mg/L)}{mw.NH_4^+} \times mw.N \times \text{vol. of aqueous phase}}{\text{wt. of N in original algae}} \times 100 \quad (3.4)$$

### 3.5.2.4 Nitrate in aqueous phase

Total $NO_3^-$ was determined using the spectrophotometric screening method as reported (APHA et al., 1998), measuring the absorbance at 220 nm and then dividing this by twice the absorbance reading at 275 nm. The $NO_3^-$ yields are reported in terms of the %nitrogen recovery compared to the amount of nitrogen content in the original algae, as presented in Eq.3.5.

$$\%N-NO_3^- = \frac{\frac{NO_3^- (mg/L)}{mw.NO_3^-} \times mw.N \times \text{vol. of aqueous phase}}{\text{wt. of N in original algae}} \times 100 \quad (3.5)$$

### 3.5.2.5 Polypeptide in aqueous phase

The total polypeptide level was determined by Lowry's method using Bovine Serum Albumin (BSA) as a standard (Lowry et al., 1951). The sample was diluted and mixed well with the analyzing Folin and Ciocalteu's phenol reagent, and the absorbance was then measured at 750 nm with a UV spectrophotometer. The extractable polypeptide yield was reported in terms of the %nitrogen recovery compared to the amount of nitrogen content in original alga, as demonstrated in Eq.3.6.

$$\%N-\text{polypeptide} = \frac{\text{wt.Protein from lowry assay}}{6.25 \times \text{wt. of N in original algae}} \times 100 \quad (3.6)$$

### 3.5.2.6 Gas Chromatography/Mass Spectroscopy (Shimadzu, GC-2010)

The bio-oil was diluted in DCM (0.01 wt%) and performed on a gas chromatography mass spectrometer (Shimadzu, GC-2010) as shown in Figure 3.6. It used DB-5 column for separation and indication of the type of multiple compounds in the bio-oil. The sample (1 μL) was injected at 200 $^{\circ}$C and oven temperature was programmed to rise from 40 $^{\circ}$C (hold 2 min) to 280 $^{\circ}$C (hold 8 min) at a heating rate of 15 $^{\circ}$C/min. The detector operated at 250 $^{\circ}$C and the carrier gas was helium (1 mL/min). The detail for operating condition of GC-MS was presented in Table 3.4.

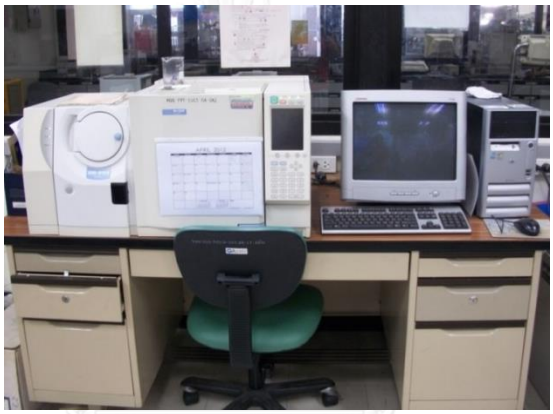

**Figure 3.6** Gas chromatography - mass spectrometry, GC/MS (Shimadzu, GC-2010).



**Table 3.4** Condition of GC-MS analysis

| | |
|---|---|
| Carrier gas | Helium (He) |
| Column | DB-5 column |
| Injector temperature | 200 $^{\circ}$C |
| Oven column temperature | 40 – 280 $^{\circ}$C |
| Transfer line temperature | 220 $^{\circ}$C |
| Ion source temperature | 220 $^{\circ}$C |

### 3.5.2.7 Gas Chromatography Simulated Distillation (GC-SIMDIS)

The Agilent Technology 6890N Simulated Distillation Gas Chromatograph (SIMDIST-GC), conformed with ASTM D2887 method for true boiling point curves, was employed to identify the classification of petroleum fractions. The equipment is consisted of a flame ionization detector and simdis HT capillary column (5 m × 530 μm inner diameter (ID), 2.65 μm film thickness) to separate individual hydrocarbon components following their boiling points range. The boiling point range of different fractions for hydrocarbon liquid fuels was shown in Table 3.5. The sample was diluted in DCM (1:10 w/w). The sample (0.1 μL) was injected at 298 $^{o}$C and oven temperature was programmed to rise from 35 $^{o}$C to 350 $^{o}$C at a heating rate of 15 $^{o}$C/min. The detector was operated at 320 $^{o}$C and carrier gas was helium (1.5 mL/min). The SIMDIST-GC was shown in Figure 3.7.

**Table 3.5** Boiling point range of different fractions (Reddy et al., 1998)

| Fraction | Atmospheric equivalent boiling point ($^{o}$C) |
|---|---|
| Light naphtha | IBP – 130 |
| Heavy naphtha | 130 – 220 |
| Atmospheric gas oil | 220 – 340 |
| Light gas oil | 340 – 450 |
| Heavy vacuum gas oil | 450 – 540 |
| Super heavy gas oil | 540 – 847 |
| Nondistillable residue | >847 |



**Figure 3.7** Simulated distillation gas chromatograph (Agilent Technology 6890N).

# CHAPTER IV
# RESULTS AND DISCUSSIONS

Based on the objectives of this research, the experiments were separated as two parts. The first part was conducted on the single step hydrothermal liquefaction (SHTL). The second part was related to the two-step hydrothermal liquefaction (THTL). The experimental data obtained from the first part in terms of bio-oil yield and its composition would be used to compare with the THTL. The effect of the addition of additives in the continuous water phase on the bio-oil yield and its quality was also observed.

## 4.1 Microalgae characterization

### 4.1.1 Proximate and ultimate analysis of dry microalgae

The data in Table 4.1 showed the proximate and ultimate analysis of microalgae. The results from proximate analysis demonstrated that the microalgae had moisture content, volatile matter, ash content and fixed carbon as 9.0, 46.7, 34.4 and 10 wt% as received, respectively. The high ash content would decrease the bio-oil yield by reducing the volatile content which was then converted as bio-oil (Tian et al., 2014) and it might induce problems during biofuels production such as the process design and operation, the product purification process and product quality (Bi et al., 2012). Moreover, the high ash content in microalgae decreased the heating value of the biomass following Eq. 3.1 because of the lower volatile and fixed carbon fraction, which were the source of heating value for any materials.

From the ultimate analysis, the elemental contents of carbon (C), hydrogen (H), nitrogen (N) and oxygen (O) were 35.9, 7.0, 5.3 and 51.8 wt% dry ash-free (daf), respectively. Bi et al. (2013) reported that the nitrogen content in microalgae was 1.6-6.8% which was defined as proteins and it might be released as nitrogen compounds during hydrothermal liquefaction (HTL). These nitrogen compounds released odor and negatively affected combustion and other properties of bio-oil (Tian et al., 2014). Moreover, the oxygenated compounds derived from HTL of microalgae negatively

affected the heating value of the obtained bio-oil following Eq. 3.2. It was essential to fully understand the beneficial effect of using the microalgae as a biofuel feedstock before further processing. Since the carbon content of the biomass proportionally correlated to the heating value of the biofuel (Bi et al., 2012), this implied that the microalgae containing high carbon content could be used to produce bio-oil with high energy content.

From the test of carbohydrates, proteins, and lipid contents, the microalgae contained very high level of protein (42.7 wt%) which could promote $NO_x$ formation during combustion of bio-oil obtained from HTL of microalgae (Maddi et al., 2011). Thus, the additional treatment to decrease the nitrogen content in the microalgae before using it as the raw material for the bio-fuels production was required.

**Table 4.1** Proximate and ultimate analysis of dry microalgae (*Coelastrum sp.*)

| Analysis | Values |
|---|---|
| Proximate analysis (wt% as received ) | |
| Moisture | 9.0 |
| Volatile matter | 46.7 |
| Ash | 34.3 |
| Fixed carbon | 10.0 |
| Ultimate analysis (wt% daf) | |
| C | 35.9 |
| H | 7.0 |
| N | 5.3 |
| O[a] | 51.8 |
| High heating value (MJ/kg) | 10.5 |
| Chemical composition (wt% daf) | |
| Crude proteins | 42.7 |
| Crude lipid | 17.3 |
| Total carbohydrates | 40.0 |

[a] By difference

### 4.1.2 Thermal gravimetric analysis (TGA)

TGA is a technique to provide thermal property information of microalgae. Figure 4.1 showed TGA profile of *Coelastrum sp.,* which had three degradation stages. The first stage (50-100 $^{\circ}$C) was associated with a small amount of weight loss due to dehydration of the moisture in the microalgae. Above 110 $^{\circ}$C, there was two more stages of mass loss at approximately in the ranges of 160-340 $^{\circ}$C and 340-500 $^{\circ}$C due to the losses of organic compounds and volatile matters, respectively (Silva et al., 2008). Moreover, this region could be classified as two sub-stages of degradation such as the degradation of proteins and carbohydrates in the range of 160 to 340 $^{\circ}$C and degradation of lipid in the range of 340 to 500 $^{\circ}$C (Agrawal et al., 2013). The last degradation region started after 500 $^{\circ}$C resulting from the existence of the combustion of solid residue from stage 2 and lignin (about 1.8 wt% daf as shown in Table 4.2). The component of microalgae was also determined following method forage fiber analysis (Agricultural Research Service, 1970) as presented in Table 4.2. For the woody biomass, cellulose is the only skeletal polysaccharide providing cell wall with high tensile strength.

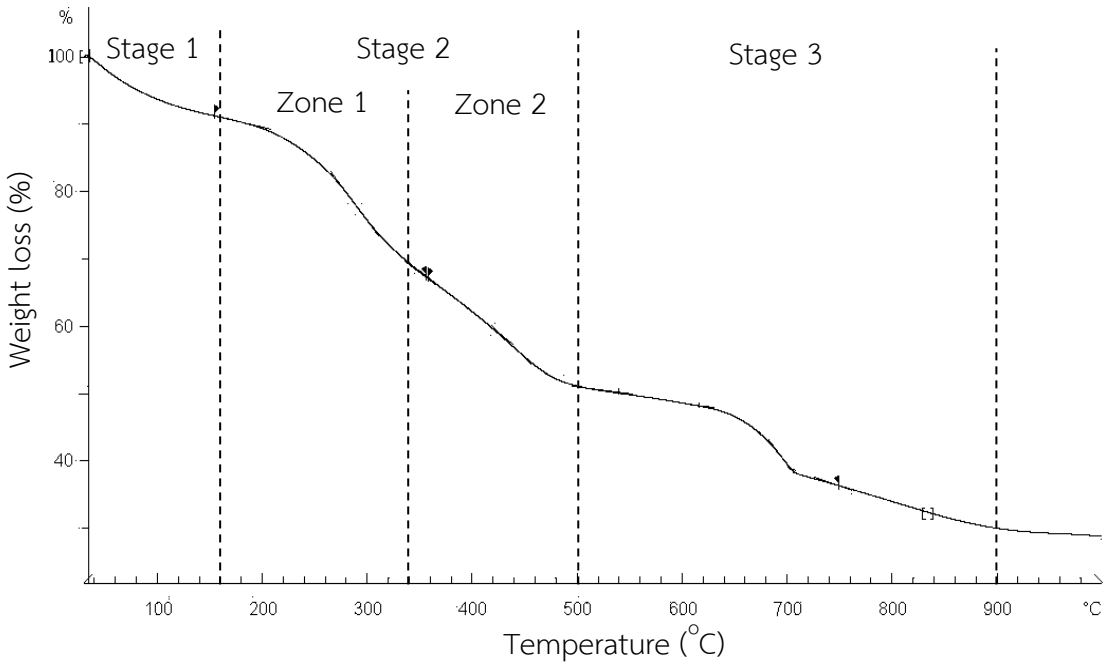


**Figure 4.1** Thermal degradable behavior of microalgae under nitrogen gas ($N_2$) (Heating rate of 10 $^{\circ}$C/min).

**Table 4.2** The component of microalgae (*Coelastrum sp.*)

| Components (wt% as received) | Values |
|---|---|
| Cellulose | 3.2 |
| Hemicellulose | 7.2 |
| Lignin | 1.8 |

For microalgae, the amount of cellulose was lower and more variable proportion than the woody plants. Moreover, some species of microalgae might not have cellulose (Baldan et al., 2001).

From the previous studies, the hemicellulose was more thermally labile than cellulose and it was decomposed in the range of decomposition temperature at 220 $^{\circ}$C to 315 $^{\circ}$C in oxygen free atmosphere followed by cellulose at 315 $^{\circ}$C to 400 $^{\circ}$C. Therefore, lignin, which was a relatively more heterogeneous polymer than hemicellulose and cellulose, was decomposed in the wider range of decomposition temperature at 160$^{\circ}$C to 900$^{\circ}$C (Maddi et al., 2011). However, the TGA showed the lower ash content (~30 wt%) in microalgae than that obtained from the proximate analysis (34.3 wt%) possibly due to the use of small sample for TGA analysis resulted in the heterogeneous ash content in the sample. Since the TGA result demonstrated that the thermal degradation of proteins in the microalgae appeared in the range of 160 to 340 $^{\circ}$C. Since the *Coelastrum sp.* had high protein content, the pre-treatment to decompose nitrogen in the microalgae before producing bio-oil via HTL was operated at the temperature in range of 280 $^{\circ}$C to 360 $^{\circ}$C.

### 4.2 Two-step hydrothermal liquefaction

The experiments of hydrothermal liquefaction were conducted in two systems. The first system was a single step hydrothermal liquefaction process (SHTL), which directly produced the bio-oil without pre-treatment of microalgae. The second system was a two-step hydrothermal liquefaction (THTL), which produced the bio-oil by using two stages of HTL: the first stage used for treatment and the second stage applied for bio-oil production. The results obtained from the variation of process

parameters such as temperature, water pressure and water flow rate used in the THTL were compared those obtained from SHTL.

### 4.2.1 Effect of the first step temperature

To study the effect of the first step temperature in THTL, the SHTL was controlled to be operated at 320 $^{o}$C, in the presence of 20 MPa water pressure and 0.5 mL/min water flow rate. The results obtained from THTL were compared with the SHTL as shown in Table 4.3 and 4.4 in terms of product yields and the composition in the derived bio-oil, respectively. The bio-oil yield produced from SHTL was 29.5 wt% daf with nitrogen content of 8.7 wt%. Since the elemental distribution in the bio-oil was important for the quality of the bio-oil, it was expected that the obtained bio-oil should have high carbon content and low amount of nitrogen in order to minimize the deactivating of acidic catalysts when co-processed in existing crude oil refineries (Du et al., 2012).

The results from Table 4.4 showed that the bio-oil obtained from SHTL had high nitrogen content. Thus, THTL was developed to extract proteins and amino acids from the microalgae in the first step. The effect of the first step temperature on the extraction of nitrogen was shown in Table 4.5. The %nitrogen content in the aqueous co-products obtained from the first step of THTL was determined in terms of the level of ammonium (%-$NH_4^+$), nitrate (%N-$NO_3^-$) ions and polypeptide (%N-PP). The results indicated that the increase in the temperature from 150 $^{o}$C to 200 $^{o}$C increased the levels of $NH_4^+$ and $NO_3^-$ about two-fold, while the polypeptide content was increased about three-fold. The resulting solid residue from the first step was then decomposed to produce bio-oil at elevated temperature in the second step. The effect of the first step temperature of THTL on bio-oil yield and compositions was studied in the range of 150-225 $^{o}$C under water pressure of 7 MPa. Whereas, the second step of THTL was performed at 320 $^{o}$C and the water pressure of 20 MPa. The water flow rate of all experiments was kept constant at 0.50 mL/min. The bio-oil obtained from the first step of THTL increased from 9.9 wt% to 12.7 wt% with increasing temperature from 150 to 225 $^{o}$C.

**Table 4.3** Effect of the first step temperature of THTL on bio-oil yield and solid residue content compared with SHTL

| Process | Temperature | Bio-oil yield (wt% daf) | | | Solid residue |
|---|---|---|---|---|---|
| | ($^{o}$C) | 1$^{st}$ step | 2$^{nd}$ step | overall | (wt%$^{b}$) |
| SHTL | 320 | - | - | 29.5 | 20.6 |
| THTL$^{a}$ | 150 | 9.9 | 23.6 | 33.5 | 16.2 |
| | 175 | 11.3 | 19.9 | 31.2 | 18.2 |
| | 200 | 11.5 | 20.5 | 32.0 | 16.0 |
| | 225 | 12.7 | 19.9 | 32.6 | 16.2 |

[a] THTL condition for 2$^{nd}$ step : temperature = 320 $^{o}$C; water flow rate = 0.5 mL/min; water pressure = 20 MPa

**Table 4.4** Effect of the first step temperature of THTL on elemental composition in bio-oil

| Process | Temperature | Elemental composition in bio-oil$^{a}$ (wt%) | | | | %N | HHV$^{a}$ |
|---|---|---|---|---|---|---|---|
| | ($^{o}$C) | C | H | N | O$^{c}$ | reduction$^{b}$ | (MJ/kg) |
| SHTL | 320 | 64.7 | 7.0 | 8.7 | 19.6 | - | 28.3 |
| THTL | 150 | 65.8 | 7.7 | 5.8 | 20.6 | 45.5 | 29.5 |
| | 175 | 70.8 | 6.1 | 4.9 | 18.2 | 61.7 | 29.4 |
| | 200 | 70.9 | 7.0 | 4.8 | 15.9 | 61.3 | 33.2 |
| | 225 | 67.9 | 6.5 | 4.9 | 20.6 | 61.1 | 28.5 |

[a] Bio-oil for THTL was the 2$^{nd}$ step of THTL content in Table 4.3

[b] %N reduction calculated following Eq. 3.3

[c] By difference

**Table 4.5** Nitrogen compounds in aqueous phase from the 1$^{st}$ step of THTL

| 1$^{st}$ | Nitrogen compounds in aqueous phase obtained from 1$^{st}$ step (wt%) | | |
|---|---|---|---|
| temperature$^{a}$ | $NH_4^{+}$ $^{b}$ | $NO_3^{-}$ $^{c}$ | Polypeptides$^{d}$ |
| 150 | 1.2 | 0.3 | 4.9 |
| 200 | 2.5 | 0.9 | 15.3 |

[a] THTL condition for 2$^{nd}$ step : temperature = 320 $^{o}$C; water flow rate = 0.5 mL/min; water pressure = 20 MPa

[b] $NH_4^{+}$ calculated followed Eq. 3.4

[c] $NO_3^{-}$ calculated followed Eq. 3.5

[d] Polypeptide calculated followed Eq. 3.6

The bio-oil obtained from the second step of THTL was slightly decreased with increasing the first step temperature. The total bio-oil yield obtained from THTL was in the range of 31-33 wt% with the solid residue content of 16-18 wt%. This exhibited that the effect of the first step temperature was insignificant on the yields of bio-oil and solid residue. This observation was similar to Miao et al. (2012). However, the total bio-oil yield obtained from THTL was higher than that from SHTL. This was possible that the pre-treatment of the algal biomass at lower temperature in the first step of THTL induced the algal cell wall to be more fragile and porous resulting in the low energy requirement used in the second step of THTL to disrupt the cell (Miao et al., 2012).

Table 4.4 showed the effect of the first step temperature on the elemental content in the bio-oil obtained from the second step of THTL. It could be seen that the bio-oil obtained from the pretreated algae had lower nitrogen content (ca. 5 wt%) than one obtained from SHTL (8.7 wt%). The results were related to the elemental composition in the pretreated microalgae as shown in Table 4.5. The nitrogen content in biomass decreased from 5.3 to 3.0 wt% at the first step temperature of 225 $^{\circ}$C. This indicated that the pre-treatment step had potential to reduce the nitrogen content in the biomass before producing the bio-oil in the second step of THTL. Moreover, the carbon, hydrogen and nitrogen contents in the solid residue generally decreased with increasing the temperature in the second step (Table 4.6). The oxygen content in the solid residue increased from pretreated microalgae because the reduction of carbon fraction resulted in the enhancement of the oxygen fraction (Chen et al., 2014).

**Table 4.6** Effect of the first step temperature of THTL on elemental composition in pretreated microalgae

| 1st step temperature of THTL (°C) | Elemental composition[a] (wt%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pretreated microalgae | | | | Solid residue obtained from 2nd step of THTL | | | |
| | C | H | N | O[b] | C | H | N | O[b] |
| 150 | 30.2 | 5.6 | 3.5 | 60.7 | 24.5 | 4.3 | 1.7 | 69.5 |
| 175 | 30.3 | 5.3 | 3.3 | 61.2 | 24.7 | 3.5 | 1.2 | 70.6 |
| 200 | 30.0 | 5.2 | 3.1 | 61.7 | 24.1 | 3.4 | 0.9 | 71.6 |
| 225 | 29.0 | 5.0 | 3.0 | 63.0 | 23.7 | 3.0 | 0.9 | 72.4 |

[a] Dry ash-free

[b] By difference

From Table 4.4, the nitrogen content in the bio-oil obtained from the second step of THTL slightly decreased from 5.8 wt% to 4.8-4.9 wt% when the first step temperature of THTL increased from 150 °C to 225 °C. This was possible that the higher first step temperature of THTL increased the solubility of protein in water resulting from the increase in the ionization constant (or called as dissociation constant or ion product constant) of water ($K_w$) from 1 x $10^{-14}$ at ambient temperature to 7 x $10^{-12}$ at 220 °C, which enhanced the concentration of hydronium and hydroxide ions (Sereewatthanawut et al., 2008). In the presence of hydronium and hydroxide ions, peptide bonds were broken down as smaller soluble proteins or amino acids molecules, which could be further degraded as low molecular weight carboxylic acids such as formic acid, acetic acid, propionic acids, etc. (Sereewatthanawut et al., 2008). To compare the efficiency of THTL for the nitrogen reduction in the obtained bio-oil with one obtained from SHTL, it was found that the THTL provided 45.5-61.7 wt% nitrogen reduction of the obtained bio-oil when the first step temperature was in the range of 175-225 °C. The carbon content in the bio-oil from the second step of THTL was in range of 65.8 to 70.9 wt%, which was higher than that from the SHTL (64.7 wt%). This resulted the higher heating value of the bio-oil obtained from the second step of THTL (28.5-33.2 MJ/kg) than one obtained

from the SHTL (28.3 MJ/kg). This indicated that the pre-treatment process (the first step of THTL) provided the bio-oil with higher quality in terms of lower nitrogen content, higher amount of carbon with higher heating value than the one obtained from SHTL. However, the first step temperature higher than 200 $^{\circ}$C could degrade the obtained bio-oil by reducing the carbon content resulting in the lower heating value. Thus, the first step temperature of THTL was controlled as 200 $^{\circ}$C for further experiments.

### 4.2.2 Effect of the second step temperature

From the section 4.2.1, the first step temperature showed the synergetic effect on the bio-oil yield due to the extension of biomass fragmentation. The temperature was also suggested as the most important factor influencing the product yields by many literatures. To compare with THTL, the effect of temperature on bio-oil yield and nitrogen content in the obtained bio-oil derived from the SHTL was investigated.

For the SHTL, it was performed in range of 280 to 360 $^{\circ}$C under water pressure of 20 MPa for 2 h. The yield of the bio-oil obtained from SHTL was shown in Table 4.7. It indicated that the yield of bio-oil slightly increased from 27.8 wt% to 29.5 wt% with increase in the temperature from 280 $^{\circ}$C to 320 $^{\circ}$C due to extended biomass fragmentation. Above this point, the bio-oil yield decreased to 22.9 wt% when the temperature was 360 $^{\circ}$C. This might be explained that the high temperature could decompose the bio-oil to be gas product. It was also possibly corresponded to the decrease in the solid residue from 25.5 wt% at 280 $^{\circ}$C to 17.6 wt% at 360 $^{\circ}$C resulting in the higher organic conversion (defined as the percentage mass of organics converted as liquid or gas products) at elevated temperature (Akhtar et al., 2011).

**Table 4.7** Effect of the second step temperature of THTL on bio-oil yield and solid residue content compared with SHTL

| 2nd step Temperature[a] | Bio-oil yield (wt%) | | | | Solid residue (wt%) | |
|---|---|---|---|---|---|---|
| | SHTL[b] | THTL | | | SHTL | THTL[c] |
| | | 1st step | 2nd step | overall | | |
| 280 | 27.8 | 10.3 | 14.7 | 25.0 | 25.5 | 19.0 |
| 300 | 27.2 | 10.0 | 17.6 | 27.6 | 22.7 | 17.6 |
| 320 | 29.5 | 11.5 | 20.5 | 32.0 | 20.6 | 16.0 |
| 340 | 24.7 | 10.4 | 17.3 | 27.7 | 18.8 | 13.7 |
| 360 | 22.9 | 10.5 | 15.9 | 26.4 | 17.6 | 13.6 |

[a] The first step temperature of THTL = 200 °C

[b] SHTL operated at temperature used for the 2nd step in THTL process

[c] Bio-oil was obtained from the 2nd step of THTL

For the THTL, the experiments of the first step temperature of THTL were controlled at 200 °C under water pressure of 7 MPa; whilst, the second step of THTL was performed in the temperature range of 280 to 360 °C under water pressure of 20 MPa and the water flow rate of 0.50 mL/min, which was applied for both two steps. The bio-oil yield and solid residue content of THTL were shown in Table 4.7. The overall yield of bio-oil increased from 25.0 wt% to 32.0 wt% when the 2nd step temperature increased from 280 °C to 320 °C due to the change of water properties. The increase in temperature induced water to behave like an organic solvent and enhanced the extraction of organic compounds from pretreated microalgae (Miao et al., 2012).

Above 320 °C, the overall bio-oil yield decreased to 26.4 wt% at 360 °C because the high HTL temperature might convert liquid and solid products as gaseous products (Eboibi et al., 2014). It could be noted that the amount of solid residue decreased with increasing the temperature of the second step because of gradual conversion of organic matters in raw material (Eboibi et al., 2014). Table 4.7 also showed the comparison of total bio-oil yield obtained from SHTL and THTL. The total bio-oil yield obtained from THTL was higher than one obtained from SHTL for applied conditions. These results could be explained that the pretreatment step

affected the change in the solid structure of biomass and enhanced the performance of the second step of THTL to increase the bio-oil yield (Kumar et al., 2011, Du et al., 2012). The highest bio-oil yield (32.0 wt%) was obtained at 320 $^{O}$C for THTL. Thus, the second step temperature at 320 $^{O}$C of THTL was used to investigate for further experiments.

To consider the composition in the obtained bio-oil, Table 4.8 showed the elemental composition in the bio-oils. The nitrogen content in the bio-oil obtained from SHTL was independent on the applied temperature. The nitrogen content in the bio-oil obtained from SHTL was ca. 7-8 wt%. These results was similar to the reported in the previous liturature conducted in the temperature range of 275-375 $^{O}$C (Alba et al., 2012). Wang (2011) reported that the distribution of nitrogen compounds in the oil occurred since 150 $^{O}$C to 250 $^{O}$C. For the THTL, it was found that the nitrogen content in the obtained bio-oil was in the range of 4.8-5.7 wt%, which was lower than that in one derived from SHTL (7.2-8.8 wt%). The lowest nitrogen content (4.8 wt%) was achieved at the first step temperature of 200$^{O}$C and the second step temperature of 320 $^{O}$C.

**Table 4.8** Effect of the second step temperature of THTL on elemental composition in bio-oil

| 2nd step Temperature[a] | Element composition in bio-oil (wt%) | | | | | | | | %N reduction[e] | HHV (MJ/kg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | SHTL[b] | | | | THTL[c] | | | | | | |
| | C | H | N | O[d] | C | H | N | O[d] | | SHTL | THTL[c] |
| 280 | 63.0 | 7.3 | 8.7 | 21.1 | 62.5 | 7.2 | 5.0 | 25.4 | 67.6 | 27.9 | 26.8 |
| 300 | 63.8 | 7.5 | 7.2 | 21.5 | 66.4 | 7.5 | 5.0 | 21.1 | 57.1 | 28.4 | 29.3 |
| 320 | 64.7 | 7.0 | 8.7 | 19.6 | 70.9 | 8.5 | 4.8 | 15.9 | 61.9 | 28.3 | 33.2 |
| 340 | 66.5 | 7.7 | 8.8 | 17.1 | 66.1 | 8.4 | 5.3 | 20.2 | 59.0 | 30.3 | 30.7 |
| 360 | 69.0 | 8.1 | 7.9 | 15.1 | 63.9 | 6.9 | 5.7 | 23.5 | 51.1 | 32.1 | 27.2 |

[a] The first step temperature of THTL = 200 $^{O}$C

[b] SHTL operated at temperature used for the 2nd step in THTL process

[c] Bio-oil was obtained from the 2nd step of THTL

[d] By difference

[e] %N reduction calculated following Eq. 3.3

To compare the efficiency to reduce the nitrogen content in the bio-oil obtained from both processes, the results in Table 4.8 exhibited that the THTL provided 51.1-67.6 %nitrogen reduction based on the nitrogen content in the bio-oil derived from SHTL. For the carbon content and heating value of the obtained bio-oils, it was observed that the increase in the temperature in SHTL from 280 $^{\circ}$C to 360 $^{\circ}$C increased the carbon content of the bio-oil resulting in the increase in the heating value from 27.9 to 32.1 MJ/kg due to the decrease in oxygen fraction by dehydration (Akhtar et al., 2011). In the case of the bio-oil obtained from the second step of THTL, it had the highest carbon content as 70.9 wt% at the second step temperature of 320 $^{\circ}$C and then it was decreased with an increase in the temperature to 340-360 $^{\circ}$C due to the cracking reaction that was similar to previous reports (Ogi et al., 1985b, Anastasakis et al., 2011). Therefore, the suitable operating temperature for the second step of THTL was 320 $^{\circ}$C to provide both the highest bio-oil yield and heating value.

### 4.2.3 Effect of water pressure

The water pressure is another parameter affecting the degradation of biomass in HTL. The water pressure maintains single-phase media under subcritical liquefaction condition. By maintaining the water pressure above the critical pressure of media, the rates of hydrolysis and biomass dissolution have to be controlled to enhance thermodynamically favorable reaction pathways for production of liquid fuels (Akhtar et al., 2011).

Table 4.9 shows the effect of water pressure on the bio-oil yield and the content of solid residue. For the SHTL, the experiments were performed at 320 $^{\circ}$C, water flow rate of 0.50 mL/min and water pressure varying from 12 to 20 MPa. The yields of bio-oil and solid residue increased from 22.1 to 29.5 wt% and 12.4 to 20.6 wt%, respectively. These phenomena of increasing bio-oil yield could be explained by the properties of hot compressed water.

**Table 4.9** Effect of water pressure in the 2nd step of THTL on bio-oil yield and solid residue content compared with SHTL

| Water pressure (MPa) | Bio-oil yield (wt%) | | | | Solid residue (wt%) | |
|---|---|---|---|---|---|---|
| | SHTL[a] | THTL[b] | | | SHTL | THTL |
| | | 1st step | 2nd step | overall | | |
| 12 | 22.1 | 12.0 | 18.0 | 30.0 | 12.4 | 19.9 |
| 16 | 23.5 | 11.9 | 19.0 | 30.9 | 19.3 | 18.3 |
| 20 | 29.5 | 11.5 | 20.5 | 32.0 | 20.6 | 16.0 |

[a] SHTL operated at water pressure used for the 2nd step in THTL process, temperature of 320 °C and water flow rate of 0.50 mL/min

[b] 1st step temperature of THTL = 200 °C, 1st step water pressure = 7 MPa, 2nd temperature of THTL = 320 °C and water flow rate = 0.50 mL/min

The increase in the water pressure increased the solvent density (Akhtar et al., 2011). The media with higher density had more efficiency to penetrate into the molecules of biomass component resulting in the enhancement of decomposition and extraction (Deshande et al., 1984). It was also found that the content of solid residue increased with increasing the water pressure. These phenomena could be explained by the diffusion limitation or cage effect. Under high temperature and high water pressure atmosphere, the formation of water around the intermediate products was occurred. This water cage inhibited the C-C bonds breakage and favored their repolymerization instead (Akiya et al., 2002, Kruse et al., 2007, Akhtar et al., 2011).

For the THTL, the first step was performed at 200 °C, under water pressure of 7 MPa; whilst, the second step were carried out at 320 °C and the water pressure was varied from 12 to 20 MPa. The bio-oil yield obtained from the second step also slightly increased from 18.0 wt% to 20.5 wt% with increasing in water pressure. The total bio-oil yield obtained from THTL was higher than one obtained from SHTL for all conditions and achieved the highest bio-oil yield as 32.0 wt% at the water pressure of 20 MPa. The solid residue content derived from THTL decreased from 19.9 wt% at 12 MPa to 16.0 wt% at 20 MPa. This was different from the SHTL, which

the solid residue content increased with increasing the water pressure. These results could be explained that no cage effect occurred in the second step of THTL. This might be due to the partial decomposition of microalgae in the first step of THTL resulting in the higher penetration of media into the microalgae structure framework.

The effect of water pressure on nitrogen content in the bio-oil obtained from both processes was shown in Table 4.10. In the case of SHTL, the nitrogen content in the bio-oil increased from 6.9 wt% to 8.7 wt% with increasing the water pressure from 12 MPa to 20 MPa at the same temperature (320 $^{O}$C) and water flow rate (0.50 mL/min). These results might be explained that the high water pressure increased the reaction rate of nitrogen compound formation such as indole which could be more formed in the bio-oil than at lower water pressure (Kruse et al., 2007, Wang, 2011). The nitrogen content in the bio-oil obtained from the second step of THTL was relatively constant (4.8-5.2 wt%) with varying the water pressure. The results could be explained that the extraction of proteins in the first step of THTL had enough capability to remain a small amount of nitrogen content in the second step of THTL. Therefore, the nitrogen content in the bio-oil obtained from the second step of THTL was not different in all applied water pressures.

**Table 4.10** Effect of water pressure on elemental composition in bio-oil

| Water pressure (MPa) | | Element composition in bio-oil (wt%) | | | | | | | | %N Reduction[d] | HHV (MJ/kg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SHTL[a] | | | | THTL[b] | | | | | | |
| 1st step | 2nd step | C | H | N | O[c] | C | H | N | O[c] | | SHTL | THTL[b] |
| 7 | 12 | 62.8 | 6.5 | 6.9 | 23.8 | 65.9 | 7.9 | 5.2 | 21.0 | 47.2 | 26.2 | 29.8 |
| 7 | 16 | 65.5 | 7.7 | 7.3 | 19.5 | 67.7 | 7.2 | 4.9 | 20.2 | 49.8 | 29.7 | 29.5 |
| 7 | 20 | 64.7 | 7.0 | 8.7 | 19.6 | 70.9 | 8.5 | 4.8 | 15.9 | 61.9 | 28.3 | 33.2 |

[a] SHTL operated at water pressure used for the 2nd step in THTL process

[b] Bio-oil was obtained from the 2nd step which the THTL condition was similar to Table 4.8

[c] By difference

[d] %N reduction calculated following Eq. 3.3

To compare the carbon content in the bio-oil obtained from SHTL and the second step of THTL, it was found the carbon content in the bio-oil derived from the second step of THTL (70.9 wt%) was higher than that derived from SHTL (64.7 wt%). This could be explained that the high water pressure increased the extraction efficiency, resulting in many hydrocarbon compounds in the bio-oil obtained from the second step of THTL. The higher carbon content and lower oxygen content in the bio-oil obtained from the second step of THTL led the higher heating value (33.2 MJ/kg) than one obtained from SHTL (28.3 MJ/kg) at water pressure of 20 MPa.

Since the high water pressure could produce the highest bio-oil yield, the water pressure of 20 MPa was selected for using in further experiments.

### 4.2.4 Effect of water flow rate

The water flow rate directly affected the residence time of both SHTL and THTL. The water flow rate of 0.25, 0.50, 0.75 and 1 mL/min reflected the residence time as 80, 40, 30 and 20 min, respectively. Table 4.11 showed the effect of water flow rate on the yields of bio-oil and solid residue. For the SHTL, it was performed at 320 $^{\circ}$C under a water pressure of 20 MPa. The water flow rates were varied from 0.25 to 1.00 mL/min. It was found that the yield of bio-oil derived from SHTL increased with increase in the water flow rate and reached to the maximum as 29.5 wt% at the water flow rate of 0.50 mL/min because the increase in the water flow rate increased the mass transfer of fed water resulting in the higher effective degradation biomass (Liu et al., 2003). When the water flow rate increased to 1.00 mL/min, the bio-oil yield decreased to 16.5 wt% since the over water flow rate reduced the residence time to produce the bio-oil. The solid residue content seemed to be constant at ca. 20-22 wt% with varying the water flow rates. As mentioned above, the microalgae was packed in the reactor and heated with the same operating temperature for all water flow rates. Therefore, the amount of solid residue was not different for all applied water flow rates.

**Table 4.11** Effect of water flow rate of THTL on bio-oil yield and solid residue content compared with SHTL

| Water flow rate (mL/min) | Bio-oil yield (wt%) | | | | Solid residue (wt%) | |
|---|---|---|---|---|---|---|
| | SHTL[a] | THTL[b] | | | SHTL | THTL[c] |
| | | 1st step | 2nd step | overall | | |
| 0.25 | 27.3 | 9.7 | 18.4 | 28.1 | 22.4 | 19.2 |
| 0.50 | 29.5 | 11.5 | 20.5 | 32.0 | 20.6 | 16.0 |
| 0.75 | 25.0 | 11.3 | 18.4 | 29.7 | 21.4 | 18.9 |
| 1.00 | 16.5 | 9.0 | 15.7 | 24.7 | 21.3 | 19.4 |

[a] SHTL operated at 320 °C under a water pressure of 20 MPa

[b] 1st step temperature of THTL = 200 °C, 1st step water pressure = 7 MPa, 2nd temperature of THTL = 320 °C and water pressure = 20 MPa

[c] Bio-oil was obtained from the 2nd step

For the THTL, the first step of THTL was performed at 200 °C under the water pressure of 7 MPa; whilst, the second step of THTL was conducted at 320 °C, a water pressure of 20 MPa and the water flow rate varying from 0.25 to 1.00 mL/min. From Table 4.11, it was found that the increase in the water flow rate from 0.25 mL/min to 1.00 mL/min slightly affected the bio-oil yield obtained from the first step of THTL. The bio-oil yield obtained from the both step of THTL increased with increasing the water flow rate from 0.25 to 0.50 mL/min resulting in the increase in the overall bio-oil yield from 28.1 to 32.0 wt%. When the water flow rate increased from 0.50 to 1.00 mL/min, the overall bio-oil yield decreased from 32.0 to 24.7 wt% same as in the SHTL process. The reduction of solid residue content was corresponded to the enhancement of the total bio-oil yield.

The effect of water flow rate on the nitrogen content in the bio-oil obtained from SHTL was shown in Table 4.12. The nitrogen content in the bio-oil was maximum at 8.7 wt% when the water flow rate was 0.50 mL/min. This implied that the water flow rate of 0.50 mL/min provided the suitable residence time (40 min) for degradation of biomass as the bio-oil resulting in the high amount of nitrogen compounds in the bio-oil as described above. At the water flow rate of 0.25 mL/min, the long residence time induced higher probability to decompose proteins as

gaseous phase such as ammonia ($NH_3$) resulting in the low nitrogen content in the bio-oil. Whilst, the high water flow rate (0.75-1.00 mL/min) decreased the efficiency of hydrolysis of the nitrogen compounds obtained from biomass in the bio-oil (Liu et al., 2003, Tian et al., 2014).

In the case of THTL, the nitrogen content in the bio-oil obtained from the second step of THTL was lowest as 4.8 wt% when the water flow rate was 0.50 mL/min or 40 min of residence time. The increase in the water flow rate provided shorter residence time to decrease the extraction efficiency for removing the nitrogen compounds in the first step of THTL as shown in terms of nitrogen reduction (the highest N reduction (61.9%) was achieved at 0.50 mL/min). Du et al. (2012) reported that the suitable residence time to treat the microalgae in the HTL was about 40 to 60 min. The carbon content in the bio-oil from SHTL was varied in the range of 60-65 wt% when the water flow rate was increased from 0.25 to 1.00 mL/min. However, it was lower than that in bio-oil obtained from the second step of THTL (70 wt% at water flow rate of 0.50 and 1.00 mL/min). This indicated that the THTL process could extracted the oxygen compounds such as polysaccharide resulted in the reduction of the oxygen content in the biomass before producing the bio-oil.

**Table 4.12** Effect of water flow rate on elemental composition in bio-oil

| Water flow rate (mL/min) | Element composition in bio-oil (wt%) | | | | | | | | %N reduction[e] | HHV (MJ/kg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | SHTL[a] | | | | THTL[b,c] | | | | | | |
| | C | H | N | O[d] | C | H | N | O[d] | | SHTL | THTL[c] |
| 0.25 | 60.6 | 6.7 | 6.8 | 25.9 | 66.0 | 6.9 | 6.6 | 20.5 | 39.0 | 25.5 | 28.5 |
| 0.50 | 64.7 | 7.0 | 8.7 | 19.6 | 70.9 | 8.5 | 4.8 | 15.9 | 61.9 | 28.3 | 33.2 |
| 0.75 | 64.0 | 6.7 | 6.9 | 22.4 | 64.0 | 6.9 | 6.5 | 22.6 | 31.8 | 27.2 | 27.5 |
| 1.00 | 65.6 | 7.0 | 6.9 | 20.5 | 70.9 | 7.6 | 6.2 | 15.2 | 6.1 | 28.6 | 32.1 |

[a] SHTL operated at 320 °C under a water pressure of 20 MPa

[b] 1st step temperature of THTL = 200 °C, 1st step water pressure = 7 MPa, 2nd temperature of THTL = 320 °C and water pressure = 20 MPa

[c] Bio-oil was obtained from the 2nd step of THTL

[d] By difference

[e] %N reduction was calculated follow Eq. 3.3

Thus, the fraction of carbon content in the bio-oil obtained from the second step of THTL was higher than one obtained from SHTL. Therefore, the water flow rate of 0.50 mL/min was suitable for both SHTL and THTL to provide the maximum bio-oil yield with lowest nitrogen content.

### 4.2.5 Effect of alkaline additives for THTL

The alkaline additives were only applied to THTL process for improving the efficiency of bio-oil production and reduction of nitrogen content in bio-oil. $Na_2CO_3$ and KOH were used as the additives because they could produce the bio-oil with low nitrogen content (Ross et al., 2010). The concentration of additives was varied in range of 5 to 15 wt% based on the content of microalgae. The experiments of alkali additives were performed in THTL with the first step temperature of 200 $^{\circ}C$, water pressure in the first step of 7 MPa, the second step temperature of 320 $^{\circ}C$ and water pressure in the second step of 20 MPa. This process was under the constant water flow rate as 0.50 mL/min in both two steps.

The yields of bio-oil and solid residue obtained from the THTL with alkali additives were shown in Table 4.13. It was found that the addition of the additives into the water media significantly decreased the overall bio-oil yield obtained from THTL without the addition of additive because the carbohydrates in microalgae was hydrolyzed by alkali into the gaseous phase instead of the bio-oil (Biller et al., 2011).

To compare the overall bio-oil yield obtained from THTL using $Na_2CO_3$ and KOH, it was found that the bio-oil obtained from THTL using $Na_2CO_3$ (22.4 wt%) was higher than that using KOH (16.3 wt%) for 10 wt% of additive concentration.

**Table 4.13** Effect of additives on the yields of bio-oil and solid residue of THTL

| Additives | Concentration (wt%[a]) | Bio-oil yield (wt%) | | | Solid residue (wt%) |
|---|---|---|---|---|---|
| | | 1st step | 2nd step | overall | |
| None | - | 11.5 | 20.5 | 32.0 | 16.0 |
| $Na_2CO_3$ | 5 | 5.3 | 12.2 | 17.5 | 19.6 |
| | 10 | 7.5 | 14.9 | 22.4 | 20.4 |
| | 15 | 4.9 | 6.7 | 11.6 | 17.7 |
| KOH | 5 | 3.4 | 6.4 | 9.8 | 19.8 |
| | 10 | 7.1 | 9.1 | 16.3 | 19.7 |
| | 15 | 5.1 | 8.6 | 13.7 | 20.1 |

[a] Based on weight solution

Anderson et al. (1977) proposed the mechanism for sodium carbonate-catalyzed conversion of carbohydrate to bio-oil followed:

1) Reaction of sodium carbonate, water and carbon monoxide generated sodium formate :

$$Na_2CO_3 + 2CO + H_2O \longrightarrow 2HCOONa + CO_2$$

2) Dehydration of vicinal hydroxyl groups in a carbohydrate to an enol, followed by isomerization to ketone :

$$\text{-CH(OH)-CH(OH)-} \longrightarrow \text{-CH=C(OH)-} \longrightarrow \text{-}CH_2\text{-CO-}$$

3) Reduction of newly formed carbonyl group to the corresponding alcohol with formate ion and water :

$$HCOO^- + \text{-}CH_2\text{-CO-} \longrightarrow \text{-}CH_2\text{-CH}(O^-)\text{-} + CO_2$$

$$\text{-}CH_2\text{-CH}(O^-)\text{-} + H_2O \longrightarrow \text{-}CH_2\text{-CH(OH)-} + OH^-$$

4) The hydroxyl ion reacted with additional carbon monoxide to regenerate the formate ion :

$$OH^- + CO \longrightarrow HCOO^-$$

Whilst, the potassium hydroxide could easily converted to the carbonate form in the presence of $CO_2$ as demonstrated below :

$$2MOH + CO_2 \longrightarrow M_2CO_3 + H_2O$$

The conversion of potassium hydroxide to be carbonate form before had lower activity than sodium carbonate (Ogi et al., 1985a).

To consider the effect of alkali concentration, it found that the overall bio-oil yield obtained from both additives increased with increasing the concentration of alkali additive up to 10 wt%. However, the bio-oil yield decreased with increasing the concentration of additives to 15 wt%. These results could be explained that the overload of additives provide high content of sodium carbonate, which it could react with some fraction of the bio-oil to form alkali salts. They could be dissolved in the aqueous phase (Yokoyama et al., 1986). The results was similar to the previous literature reported by Ogi et al. (1985b).

The nitrogen content in the bio-oil obtained from the second step of THTL with alkali additives was exhibited in Table 4.14. To compare with THTL without the addition of additives, the bio-oil obtained from THTL with the addition of alkali had lowest nitrogen content (3.3 wt%) when 5 wt% $Na_2CO_3$ was applied.

**Table 4.14** Effect of additives on elemental composition in bio-oil obtained from THTL

| Additive | Concentration (wt%) | Element composition in bio-oil[a] (wt%) | | | | %N reduction[c] | HHV[a] (MJ/kg) |
|---|---|---|---|---|---|---|---|
| | | C | H | N | O[b] | | |
| No | - | 70.9 | 8.5 | 4.8 | 15.9 | - | 33.2 |
| $Na_2CO_3$ | 5 | 76.7 | 10.5 | 3.3 | 9.5 | 59.1 | 35.1 |
| | 10 | 78.2 | 10.1 | 3.7 | 8.0 | 44.1 | 35.2 |
| | 15 | 75.3 | 6.4 | 4.1 | 14.2 | 72.0 | 28.2 |
| KOH | 5 | 73.8 | 8.0 | 3.6 | 14.6 | 76.6 | 29.7 |
| | 10 | 78.0 | 8.3 | 3.9 | 9.8 | 63.7 | 32.5 |
| | 15 | 64.8 | 7.9 | 4.0 | 23.3 | 65.0 | 25.7 |

[a] Bio-oil obtained from the 2nd step of THTL

[b] By difference

[c] %N reduction was calculated follow Eq. 3.3

Alkali treatment of protein has been known to form some unusual cross-linked amino acids (Friedman, 1999), which were easy to be removed by HTL. However, the nitrogen content in the bio-oil obtained from the second step of THTL with the addition of $Na_2CO_3$ and KOH was not significantly different. To consider the effect of concentration of the additives on nitrogen content in the bio-oil obtained from the second step of THTL, the nitrogen content in bio-oil increased with increasing the additive loading since the alkali additives increased the decomposition of biomass into small molecules, which could be dissolved in aqueous phase. Then, the small nitrogen compounds in the aqueous phase could be re-polymerized into bio-oil (Akhtar et al., 2010, Biller et al., 2011).

The carbon and hydrogen contents of the bio-oil obtained from THTL with additives were greatly higher than those of the bio-oil obtained from the system without additives due to the large reduction of oxygen fraction. The oxygen content in the bio-oil obtained from $2^{nd}$ step of THTL with alkali additives (about 13.2 wt%) were lower than one without the addition of additives (15.9 wt%) since the alkali additives were efficient to promote decarboxylation (Tian et al., 2014).

## 4.3 Quality of bio-oil

### 4.3.1 Gas Chromatography/Mass Spectroscopy (GC/MS)

GC/MS technique is consisted of two procedures in sequence, a gas chromatography (GC) separation followed by mass spectroscopy (MS) detection. The bio-oils obtained from SHTL, THTL and THTL with additives were analyzed by GC/MS for separation and indication of the types of multiple compounds. More than 30 compounds were identified for each bio-oil sample that was comprised >50% of the total ion chromatogram (TIC) peak area. The spectra and identified peaks provided in the supplemental information as shown in Figure 4.2 and the compounds represented in Table 4.15. The GC/MS chromatogram of the bio-oil obtained from SHTL (Figure 4.2a) showed the most abundant compounds with high peak intensity such as toluene (3.542 min) and tetradecane (17.017 min). The nitrogen compounds obtained from the decomposition of proteins were methylethylketone diethylhydrazone (9.975 min), N-butyl acetamide (10.367 min), 3-methyl-3-propyl-2,5-pyrrolidinedione (11.433 min), indole (12.933 min), and 1-propyl-2,5-pyrrolidinedione (14.108 min). Moreover, the straight chain hydrocarbons were identified among the major compounds such as tetradecane (17.017 min) and decane (8.076).

Although the THTL had the first step to remove the proteins from microalgae via hydrolysis, the nitrogen compounds such as methylethylketone diethylhydrazone (9.975 min) and 3-methyl-3-propyl-2,5-pyrrolidinedione (11.433 min) were still observed in the bio-oil obtained from the second step with lower intensity than those found in the bio-oil derived from the SHTL. Moreover, the new nitrogen compounds such as methyl-pyrazine (4.592 min), 2,5-dimethyl-pyrazine (6.356 min) were appeared. They might be formed through the reaction between small nitrogen compounds and carbonyl compounds which could be easily formed through the condensation of glucose. The pyrazine derivatives only appeared in the bio-oil obtained from the 2$^{nd}$ step of THTL. They could be formed via HTL in the first step of THTL, which extracted the large molecules of nitrogen compounds from microalgae and the small nitrogen compounds molecules still in the solid residue,

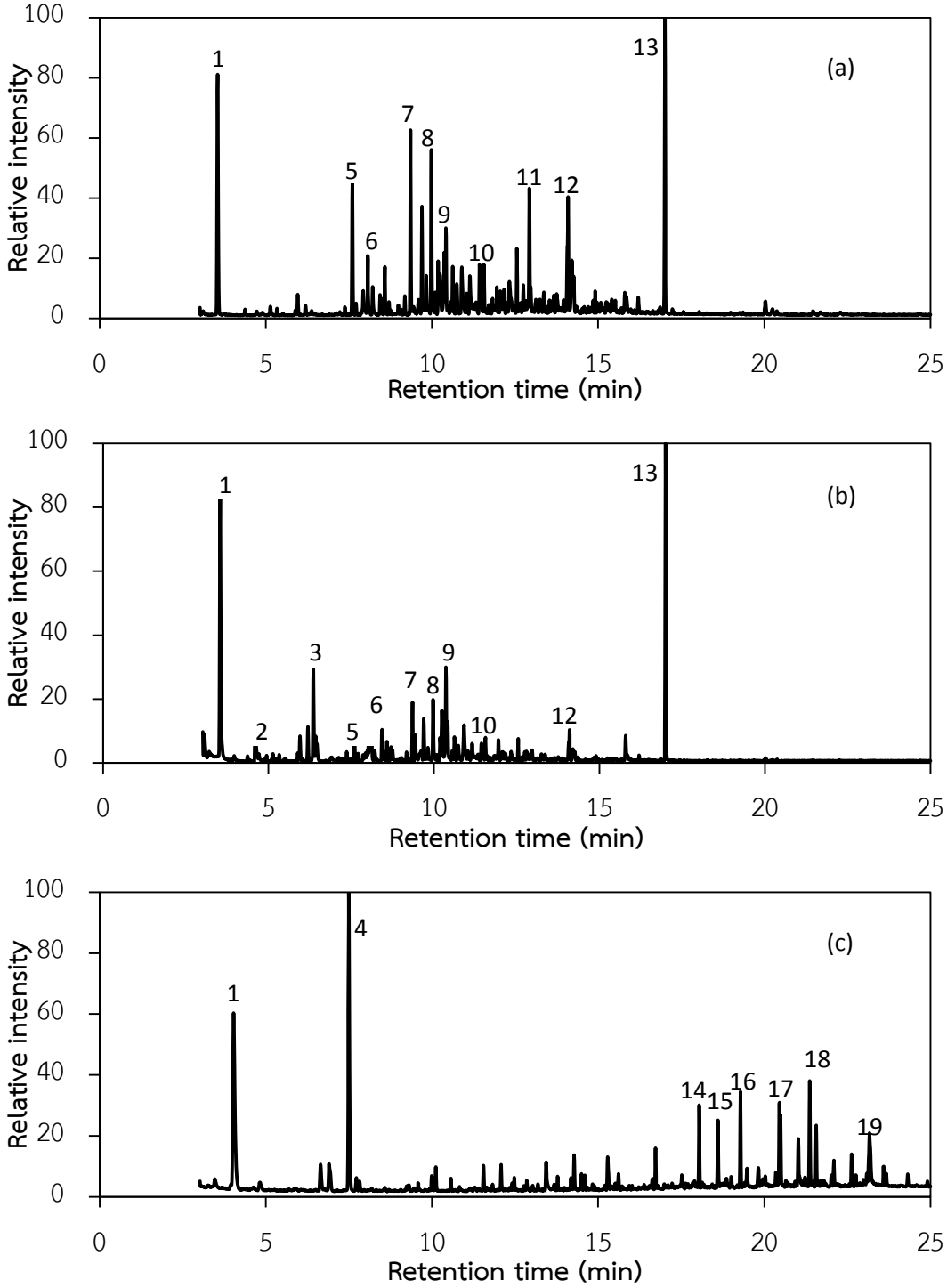


**Figure 4.2** GC/MS chromatograms of bio-oils obtained from (a) SHTL (8.7 wt% of nitrogen content), (b) THTL (4.8 wt% of nitrogen content) and (c) THTL with 10 wt% $Na_2CO_3$ (3.7 wt% of nitrogen content).

**Table 4.15** Tentative GC/MS characterization of bio-oil obtained from SHTL and THTL with and without the $Na_2CO_3$ additive

| Peak number | Retention time (min) | Tentative assignment | Relative intensity | | |
|---|---|---|---|---|---|
| | | | SHTL | THTL | $Na_2CO_3$ |
| 1 | 3.542 | Toluene | 75.7 | 83.4 | 60.3 |
| 2 | 4.592 | Methyl pyrazine | - | 14.7 | - |
| 3 | 6.356 | 2,5-dimethyl-pyrazine | - | 29.3 | - |
| 4 | 7.500 | styrene | - | - | 100 |
| 5 | 7.592 | Phenol | 17.1 | 11.9 | - |
| 6 | 8.067 | Decane | 20.9 | 14.8 | - |
| 7 | 9.358 | 4-methyl Phenol | 43.7 | 19.0 | - |
| 8 | 9.975 | Methylethylketone diethylhydrazone | 44.5 | 19.8 | - |
| 9 | 10.367 | N-butyl acetamide | 21.9 | 26.4 | - |
| 10 | 11.433 | 3-methyl-3-propyl-2,5-Pyrrolidinedione | 17.9 | 6.2 | - |
| 11 | 12.933 | Indole | 43.2 | - | - |
| 12 | 14.108 | 1-propyl-2,5-Pyrrolidinedione | 40.4 | 8.7 | - |
| 13 | 17.017 | Tetradecane | 100 | 100 | - |
| 14 | 18.042 | Pentadecane | - | - | 30.1 |
| 15 | 18.608 | Benzofuranone | - | - | 25.2 |
| 16 | 19.283 | Hexadecane | - | - | 34.5 |
| 17 | 20.450 | Octadecane | | | 30.5 |
| 18 | 21.367 | 1,2:8,9-diepoxy-p-Methane | - | - | 38.0 |
| 19 | 23.167 | Pyrrole | - | - | 20.9 |

which was use as the raw material for the 2nd step to occur the Maillard reaction (Wang, 2011) as shown in Figure 4.3.

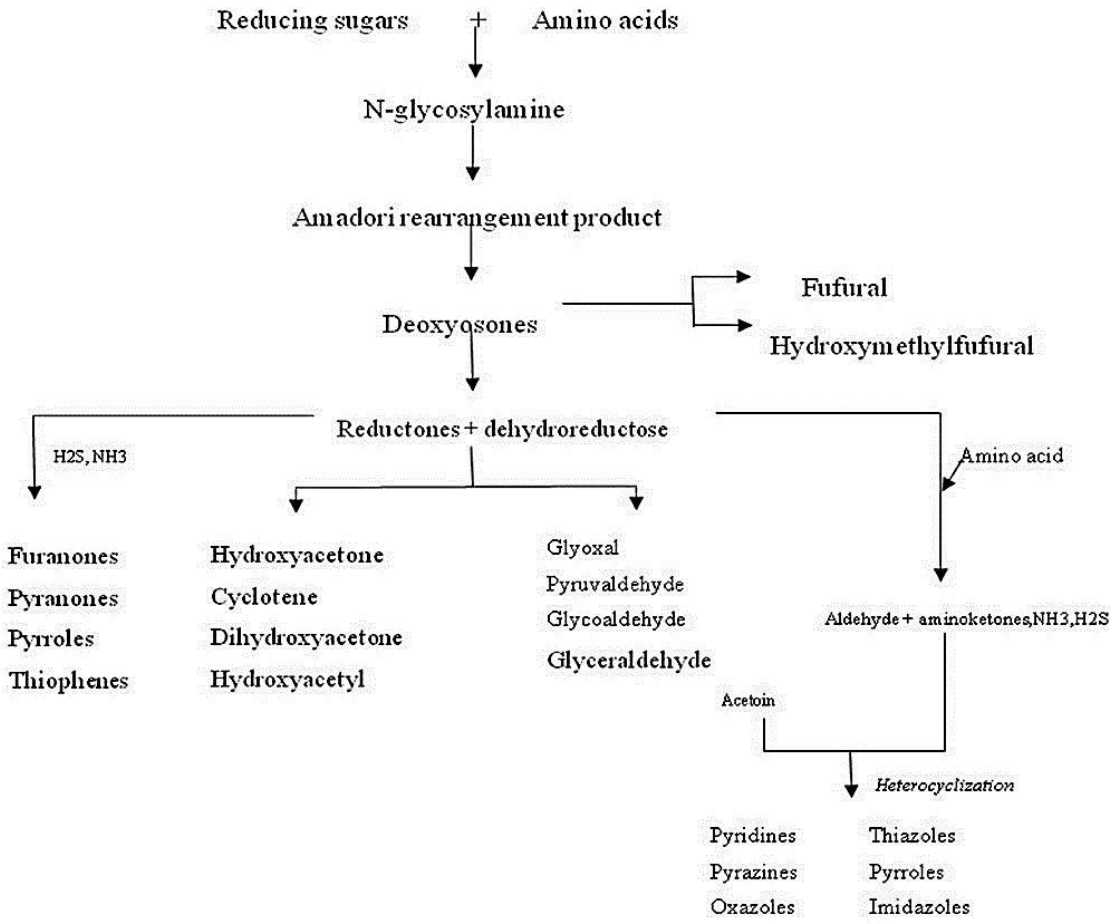




**Figure 4.3** General stage of Maillard reaction showing the formations of flover compounds. (van Boekel, 2006)

The GC/MS chromatogram of SHTL also showed the high intensity of oxygenated compounds such as phenol (7.592 min), 4-methyl phenol (9.358 min), methylethylketone diethylhydrazone (9.975 min), etc., which were derived from the decomposition of the carbohydrates (Anastasakis et al., 2011). Whilst, the THTL demonstrated the reduction of oxygenated compounds intensity as shown in Figure 4.2b (peak no. 5, 7 and 8) and Table 4.15.

Figure 4.2c showed the GC/MS chromatogram of the bio-oil obtained from the second step of THTL using 10 %.wt $Na_2CO_3$ solution as a media. To compare with the GC/MS chromatogram of bio-oil obtained from the second step of THTL (Figure 4.2b), it was found that the peak intensity of the most peaks appeared in the bio-oil obtained from the THTL without the addition of $Na_2CO_3$ additive significantly decreased especially, tetradecane (17.017 min). These results could be explained that the heavy oil was produced instead of light oil when alkali additives was applied (Ross et al., 2010, Wang, 2011). The styrene (7.500 min) only appeared in THTL using $Na_2CO_3$ with high intensity. The results was similar to previous work using the alkali additives resulted to the production of high monoaromatic compounds and lower aliphatic compounds (Jena et al., 2012). However, some straight chain hydrocarbons in bio-oil obtained from THTL using $Na_2CO_3$ solution were only observed such as pentadecane (18.042 min) and hexadecane (19.283 min). The compounds with high molecular weight were appeared in bio-oil obtained from $2^{nd}$ of THTL with $Na_2CO_3$. This might be due to the re-polymerization of small molecules, which was decomposed into aqueous phase (had been described in section 4.3.5).

For the nitrogen compounds obtained from THTL with additives, the pyrrole (23.167 min) was only one of nitrogen compounds appeared in bio-oil derived from THTL using $Na_2CO_3$ solution since the sodium carbonate improved the pyrroles formation by generating the furan compounds (Hwang et al., 1995, Wang, 2011).

### 4.3.2 Gas Chromatography Simulated Distillation (GC-SIMDIS)

The hydrocarbons in the bio-oil derived from SHTL and the 2nd step of THTL without the addition of the additives were analyzed by GC-SIMDIS following their boiling points range and compared to the commercial diesel and gasoline. The type of diesel and gasoline were high speed diesel with 0.05 wt% sulphur content (HSD 0.05%S) and unleaded premium gasoline with octane number of 95 (ULG 95 RON), respectively.

The GC-SIMDIS chromatograms and the boiling point distribution of diesel, gasoline and bio-oils were shown in Figure 4.4 and Table 4.16, respectively. The bio-oil obtained from SHTL was consisted of the light naphtha of 3.1 wt%, heavy naphtha of 18.6 wt% and atmosphere gas oil of 78.3 wt%. For THTL, the bio-oil consisted of the light naphtha of 3.6 wt%, heavy naphtha of 16.1 wt% and atmosphere gas oil of 67.4 wt%. Moreover, the high boiling point component such as light gas oil and heavy vacuum gas oil were only found in bio-oil obtained from THTL. It was possible that the high efficiency of pretreatment resulted in the high decomposition of microalgae in the second step. Thus, the more small molecules in aqueous phase could be re-polymerized to form the large molecules in the bio-oil via the second step of THTL. This result was similar to the previous report (Miao et al., 2012). To compare the boiling point of diesel and gasoline with the bio-oils obtained from SHTL and THTL (Figure 4.4), it was found that the boiling point range of both bio-oils obtained from SHTL and THTL had more similar to diesel than gasoline.

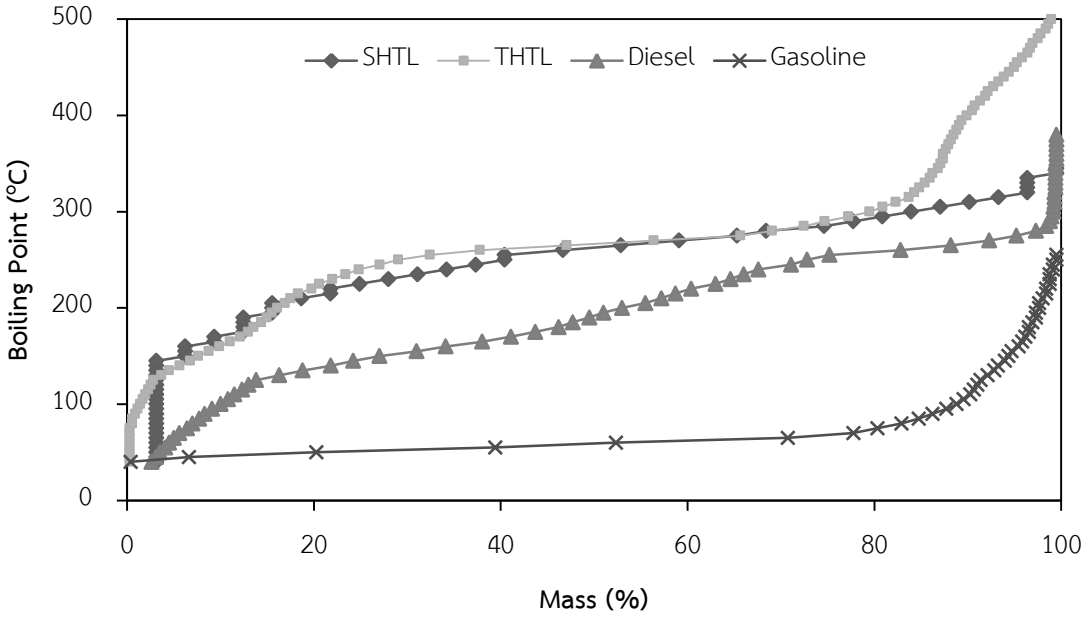


**Figure 4.4** GC-SIMDIS chromatograms of Diesel, Gasoline, Bio-oil obtained from SHTL and the second step of THTL. The SHTL operated at 320 $^{o}$C under the water pressure of 20 MPa and the water flow rate of 0.50 mL/min. The THTL operated at 200 $^{o}$C for 1$^{st}$ step and 320 $^{o}$C for the 2$^{nd}$ step under the water pressure of 20 MPa and the water flow rate of 0.50 mL/min. Commercial diesel (HSD 0.05%S) and gasoline (UGR 95 RON) were purchased from PTT public company limited.



**Table 4.16** Composition of bio-oil obtained from SHTL and the second step of THTL analyzed by GC-SIMDIS

| Component | Boiling point ($^{o}$C) (Reddy et al., 1998) | %Mass | |
|---|---|---|---|
| | | SHTL | THTL |
| Light naphtha | IBP - 130 | 3.1 | 3.6 |
| Heavy naphtha | 131 – 220 | 18.6 | 16.1 |
| Atmosphere gas oil | 221 – 340 | 78.3 | 67.4 |
| Light gas oil | 341 – 450 | - | 7.9 |
| Heavy vacuum gas oil | 451 – 540 | - | 5 |
| Super heavy gas oil | 541 – 847 | - | - |

# CHAPTER V
# CONCLUSIONS

## 5.1 Conclusions

This research work aimed to reduce the nitrogen content in the bio-oil obtained from hydrothermal liquefaction (HTL) of microalgae by using the two-step hydrothermal liquefaction (THTL) in a semi-continuous reactor. The effects of reaction parameters: the first step temperature, the second step temperature, water pressure, water flow rate, type and concentration of additives on the yields of bio-oil and solid residue including the composition in the obtained bio-oil were investigated. The results of this research work could be summarized as followed:

### 5.1.1 Characterization of microalgae

The microalgae had a large content of volatile matter of 46.7 wt% with high ash content of 34.3 wt%. The microalgae had heating value of 10.5 MJ/kg with a large amount of proteins (26.1 wt%). The microalgae had the decomposition temperature in a range of 200 to 500 $^{o}$C and the maximum decomposition temperature was about 320 $^{o}$C.



### 5.1.2 Single step hydrothermal liquefaction (SHTL)

The bio-oil yield was depended on temperature, water pressure and water flow rate. The increase in the temperature to 320 $^{o}$C increased the bio-oil yield to 29.5 wt%. Similar to the effect of water flow rate, the bio-oil yield increased to 29.5 wt% when the water flow rate was 0.50 mL/min and then decreased with increasing in the water flow rate. For the effect of water pressure, the bio-oil yield increased from 22.1 to 29.5 wt% with increasing water pressure from 12 to 20 MPa. The nitrogen content in the bio-oil obtained from SHTL only increased with increasing the water pressure due to the increasing rate of nitrogen compound formation. The maximum bio-oil yield (29.5 wt%) with nitrogen content of 8.7 wt% was achieved at

temperature of 320 $^{\circ}$C, water pressure of 20 MPa and water flow rate of 0.50 mL/min.

### 5.1.3 Two-step hydrothermal liquefaction (THTL)

THTL provided higher total bio-oil yield and less content of solid residue than SHTL. Moreover, the bio-oil obtained from the 2$^{nd}$ step of THTL had lower nitrogen content than SHTL due to the partial removal of protein in the first step of THTL. This step improved the performance of bio-oil yield and its quality obtained from the second step of THTL. The increase in the first step temperature to 225 $^{\circ}$C of THTL and the water pressure of the second step of THTL increased the bio-oil from 9.9 to 12.7 wt% and 18.0 to 20.5 wt%, respectively. However, the bio-oil yield obtained from the second step of THTL decreased when the temperature of the second step of THTL was higher than 320 $^{\circ}$C. These results were similar to those obtained from the effect of the water flow rate that decreased the bio-oil yield when the water flow rate was higher than 0.50 mL/min. The nitrogen content in the bio-oil obtained from the second step was only depended on the first step temperature. The increase in the first step temperature increased the level of nitrogen reduction in the obtained bio-oil and then it was leveled off when the first step temperature was higher than 175 $^{\circ}$C. Moreover, the use of alkali additives also decreased the nitrogen content in the bio-oil obtained from the second step of THTL, while the overall bio-oil yield decreased. The suitable condition suggested by this work for THTL would be 200 $^{\circ}$C under the water pressure of 7 MPa for the first step and 320 $^{\circ}$C under the water pressure of 20 MPa for the second step and the water flow rate of 0.50 mL/min to achieve the total bio-oil yield of 32.0 wt% with 4.8 wt% nitrogen content.

### 5.1.4 Quality of bio-oil

The high heating value of bio-oil obtained from the second step of THTL was slightly higher than that obtained from SHTL due to the higher carbon content. The results from GC-MS indicated that the bio-oil from SHTL had higher nitrogen compounds than one derived from THTL process. The most nitrogen compounds were methylethylketone-diethylhydrazone and indole. Moreover, the results from GC-SIMDIST indicated that the bio-oils from SHTL and the second step of THTL had boiling point closed to diesel.

## 5.2 Recommendation

- The evaluation of the effect of co-solvent such as alcohol and organic acids on bio-oil production should be further studied.
- The effect of process condition of THTL with co-solvents on bio-oil yield and its properties should be investigated.
- The vacuum distillation of the bio-oil obtained from the second step THTL should be investigated for reduction of oxygen, metallic content and boiling point ranges in bio-oil.
- The minerals in solid residue should be investigated to use as a fertilizer.

# REFERENCES


Agrawal, A. and Chakraborty, S. (2013). "A kinetic study of pyrolysis and combustion of microalgae *Chlorella vulgaris* using thermo-gravimetric analysis." Bioresource Technology 128: 72-80.

Agricultural Research Service (1970). Forage Fiber Analyses. Agriculture Handbook No.379. Washington, D.C., Superingtendent of documents**:** 1-20.

Akhtar, J. and Amin, N. A. S. (2011). "A review on process conditions for optimum bio-oil yield in hydrothermal liquefaction of biomass." Renewable and Sustainable Energy Reviews 15(3): 1615-1624.

Akhtar, J., Kuang, S. K. and Amin, N. S. (2010). "Liquefaction of empty palm fruit bunch (EPFB) in alkaline hot compressed water." Renewable Energy 35(6): 1220-1227.

Akiya, N. and Savage, P. E. (2002). "Roles of water for chemical reactions in high-temperature water." Chemical Reviews 102: 2725-2750.

Alba, L. G., Torri, C., Samori, C., Spek, J. v. d., Fabbri, D., Kersten, S. R. A. and Brilman, D. W. F. (2012). "Hydrothermal Treatment (HTT) of Microalgae : Evaluation of the process As Conversion Method in an Algae Biorefinery Concept." Energy and Fuels 26: 642-657.

Allan, G., Bradbury, W., Sakai, Y. and Shafizadeh, F. (1979). "A kinetic model for pyrolysis of cellulose." Journal of Applied Polymer Science 23(11): 3271-3280.

Anastasakis, K. and Ross, A. B. (2011). "Hydrothermal liquefaction of the brown macro-alga *Laminaria Saccharina*: Effect of reaction conditions on product distribution and composition." Bioresource Technology 102: 4876-4883.

Anderson, L. and Tillman, D. A., Eds. (1977). Fuels from waste. New York, Academic Press.

APHA, AWWA and WEF, Eds. (1998). Standard Methods for the Examination of Water and Wastewater. 20th ed. Washington, D.C., APHA.

Baldan, B., Andolfo, P., Navazio, L., Tolomio, C. and Mariani, P. (2001). "Cellulose in algae cell wall : an "in situ" localization." European Journal of Histochemistry 45: 51-56.

Bi, Z. and He, B. B. (2012). "Characterization of Microalgae for The Purpose of Biofuel Production." Food and Process Engineer Institute of ASABE.

Bi, Z. and He, B. B. (2013). "Characterization of microalgae for the purpose of biofuel production." American Society of Agricultural and Biological Engineers 56(4): 1529-1539.

Biller, P. and Ross, A. B. (2011). "Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content." Bioresource Technology 102: 215-225.

Blasi, D. (1998). "Comparison of semi-global mechanisms for promary pyrolysis of lignocellulosic fuels." Journal of Analytical and Applied Pyrolysis 47: 43-64.

Bower, C. E. and Holm-Hansen, T. (2011). "A Salicylate-Hypochlorite Method for Determining Ammonia in Seawater." Canadian Journal of Fisheries and Aquatic Sciences 37(5): 794-798.

Brennan, L. and Owende, P. (2010). "Biofuels from microalgae--A review of technologies for production, processing, and extractions of biofuels and co-products." Renewable and Sustainable Energy Review 14: 557-577.

Bridgwater, A. and Peacocke, G. (2000). "Fast pyrolysis processes for biomass." Renewable and Sustainable Energy Review 4(1): 1-73.

Campbell, C. (1997). The coming oil crisis. Multi-science Publishing Company and Petroconsultant, S.A. Essex.

Chen, W. T., Zhang, Y., Zhang, J., Yu, G., Schideman, L. C., Zhang, P. and Minarick, M. (2014). "Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil." Bioresour Technol 152: 130-139.

Chen, Y., Wu, Y., Zhang, P., Hua, D., Yang, M., Li, C., Chen, Z. and Liu, J. (2012). "Direct liquefaction of Dunaliella tertiolecta for bio-oil in sub/supercritical ethanol-water." Bioresour Technol 124: 190-198.

Chisti, Y. (2007). "Biodiesel from microalgae." Biotechnology Advances, 25: 294-306.

Chornet, E. and Overend, R. (1985). "Biomass liquefaction : An overview." Fundamentals of Themochemical Biomass Conversion: 967-1002.

Chun-Yang, Y. (2011). "Prediction of higher heating values of biomass from proximate and ultimate analysis." Fuel 90: 1128-1132.

Clarens, A., Resurreccion, E., White, M. and Colosi, A. (2010). "Evironmental life cycle comparision of algae to other bioenergy feedstocs." Environmental Science Technology 44: 1813-1819.

Demirbas, A. (2000). "Mechanism of liquefaction and pyrolysis reactions of biomass." Energy Conversion and Management 41: 633-646.

Demirbas, A. (2011). "Competitive liquid biofuels from biomass." Applied Energy 88(1): 17-28.

Deshande, G., Holder, G. and Shah, Y. (1984). Effect of solvent density on coal liquefaction under supercritical condition. Department of Chemical and Petroleum Engineering. Pittsburgh, PA, University of Pittsburgh.

Dragone, G., Fernandes, B., Vicent, A. A. and Teixeira, J. A. (2010). Third genertion biofuels from microalgae. Portugal, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology**:** 12.

Du, Z., Mohr, M., Ma, X., Cheng, Y., Lin, X., Liu, Y. and Zhou, W. (2012). "Hydrothermal pretreatment of microalgae for production of pyrolytic bio-oil with a low nitrogen content." Bioresource Technology 120: 13-18.

Eboibi, B. E., Lewis, D. M., Ashman, P. J. and Chinnasamy, S. (2014). "Effect of operating conditions on yield and quality of biocrude during hydrothermal liquefaction of halophytic microalga Tetraselmis sp." Bioresour Technol 170: 20-29.

Elliott, D. C., Biller, P., Ross, A. B., Schmidt, A. J. and Jones, S. B. (2015). "Hydrothermal liquefaction of biomass: developments from batch to continuous process." Bioresour Technol 178: 147-156.

Friedman, M. (1999). "Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidnoalamine in food and othe protein." Journal of Agricultural and Food Chemistry 47(4): 1295-1319.

Gouveia, L. (2011). Microalgae as a Feedstock for biofuels, SpringerBriefs in Microbiology**:** 2-4.

Hector, A. R., Rosa, M. R.-J., Bruno, D. F., Antonio, A. V. and Jose, A. T. (2013). "Hydrothermal processing, as an alternative for upgrading agriculture residues and marine biomass according to the biorefinery concept : A review." Renewable and Sustainable Energy Review 21: 35-51.

Huber, G. and Dumesic, J. (2006). "An overview of aqueous-phase catalytic process for production of hydrogen and alkanes in biorefinery." Catalysis Today 111(1-2): 119-132.

Huntley, M. and Redaje, D. (2007). "$CO_2$ mitigation and renewable oil from photosynthetic microbes: a new appraisal." Mitig Adapt Strat Glob Change 12: 573-608.

Hwang, H. I., Hartman, T. G. and Ho, C. T. (1995). "Relative reactivities of amino-acid in the formation of pyridines, pyrroles, and oxazoles." Journal of Agricultural and Food Chemistry 43(11): 2917-2921.

Jena, U., Das, K. C. and Kastner, J. R. (2011). "Effect of operating condition of therochemical liquefaction on biocrude production from *Spirulina platensis*." Bioresource Technology 102: 6221-6229.

Jena, U., Das, K. C. and Kastner, J. R. (2012). "Comparison of the effects of Na2CO3, Ca3(PO4)2, and NiO catalysts on the thermochemical liquefaction of microalga Spirulina platensis." Applied Energy 98: 368-375.

John, R., Anisha, G., Nampoothiri, K. and Pendey, A. (2010). "Micro and macroalgal biomass: a renewable source for bioethanol." Bioresource Technology.

Johnson, M. C. (2012). Hydrothermal Processing of High-Lipid Biomass to Fuels. Department of Chemical Engineering, Massachusetts Institute of Technology. Degree of Doctor of Philosophy in Chemical Engineering: 187.

Khan, S., Rashmi, H. M., Prasad, S. and Banerjee, U. (2009). "Prospects of biodiesel production from microalgae in India." Renewable and Sustainable Energy Review 13: 2361-2372.

Kruse, A. and Dinjus, E. (2007). "Hot compressed water as reaction medium and reactant. 1. Properties and synthesis reactions." Journal of Supercritical Fluids 39: 362-380.

Kumar, S., Kothari, U., Kong, L., Lee, Y. and Gupta, R. (2011). "Hydrothermal pretreatment of switchgrass and corn stover for production of ethanol and carbon microspheres." Biomass and bioenergy 35: 956-968.

Lam, M. K. and Lee, K. T. (2012). "Microalgae biofuels: A critical review of issues, problems and the way forward." Biotechnology Advances 30(3): 673-690.

Li, Y., Horsman, M., Wu, N., Lan, C. and Dubois-Calero, N. (2008). "Biofuels from microalgae." Biotechnology Progress 24(815-820).

Liu, C. and Wyman, C. E. (2003). "The Effect of Flow Rtae of Compressed Hot Water on Xylan, Linin, and Total Mass Removeal from Corn Stover." Industrial and Engineering Chemistry Research 42: 5409-5416.

López Barreiro, D., Prins, W., Ronsse, F. and Brilman, W. (2013). "Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects." Biomass and bioenergy 53: 113-127.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). "Protein measurement with the Folin phenol reagent." Journal of Biological Chemistry 193(1): 265-275.

Maddi, B., Viamajala, S. and Varanasi, S. (2011). "Comparative study of pyrolysis of algal biomass from natural lack bloom with lignocellulosic biomass." Bioresource Technology 102(23): 11018-11026.

Miao, C., Chakraborty, M. and Chen, S. (2012). "Impact of reaction conditions on the simultaneous production of polysaccharides and bio-oil from heterotrophically grown Chlorella sorokiniana by a unique sequential hydrothermal liquefaction process." Bioresour Technol 110: 617-627.

Miao, X. and Wu, Q. (2004). "High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*." Journal of Biotechnology 110: 85-93.

Ministry of Energy (2013a). "Energy in Thailand : Facts and Figures Q1/2013." 3.

Ministry of Energy (2013b). Energy statistics of Thailand 2013**:** 11.

Minowa, T., Kondo, T. and Sudirjo, S. (1998). "Thermochemical liquefaction of Indonesian biomass residue." Biomass and bioenergy 14(5-6): 517-524.

Minowa, T., Murakami, M., Dote, Y., Ori, T. and Yokoyama, S. (1995). "Oil production from garbage by thermochemical liquefaction." Biomass and bioenergy 8(2): 117-120.

Nigam, P. and Singh, A. (2010). "Production of liquid biofuels from renewable resources." Progress in Energy and Combustion Science.

Ogi, T., Yokoyama, S.-y. and Koguchi, K. (1985a). Direct liquefaction of wood by alkali and alkaline earth salt in an aqueous phase. Chemistry Letter, The Chemical Society of Japan: 1199-1202.

Ogi, T., Yokoyama, S. and Kogushi, K. (1985b). "Direct liquefaction of wood by catalyst. Part I. Effect of pressure, temperature, holding time and wood/catalyst/water ratio on oil yield." Sekkiyu Gakkaishi 28: 239-245.

Parikh, J., Channiwala, S. and Ghosal, G. (2005). "A correlation for calculating HHV from proximate analysis of solid fuels." Fuel 84(5): 487-494.

Perlack, R. D., Wright, L. L., Turhollow, A. F. and Graham, R. L. (2005). Biomass as Feedstock for a Bioenergy and Bioproducts Industry : The Technical Feasibility of a Biollion-Ton Annual Supply. U.S., Department of Energy Office of Science and Technical Information.

Pienkos, P. and Drazins, A. (2009). "The promise and challenges of micro-algal derived biofuels." Biofuel Bioproducts Biorefine 3: 431-440.

Pulz, O. (2004). "Valuable products from biotechnology of microalgae." Applied Microbiology Biotechnology 65(635-648).

Reddy, K. M., Wei, B. and Song, C. (1998). "High-temperature simulated distillation GC analysis of petroleum resids and their products from catalytic upgrading over Co-Mo/$AL_2O_3$ catalyst." Catalysis Today 43: 187-202.

Rodolfi, L., Zitelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredic, M. (2009). "Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor." Biotechnology and Bioengineering 102: 100-112.

Ross, A. B., Biller, P., Kabucki, M. L., Li, H., Lea-Langton, A. and Jones, J. M. (2010). "Hydrothermal processing of microalgae using alkali and organic acid." Fuel 89: 2234-2243.

Schenk, P., Thomas-Hall, S., Stephens, E., Marx, U., Mussgnug, J., Posten, C., Kruse, O. and Hankamer, B. (2008). "Second generation biofuels: high efficiency microalgae for biodiesel production." BioEnergy Research 1: 20-43.

Sereewatthanawut, I., Prapintip, S., Watchiraruji, K., Goto, M., Sasaki, M. and Shotipruk, A. (2008). "Extraction of protein and amino acid from deoiled rice bran by subcritical water hydrolysis." Bioresource Technology 99: 555-561.

Silva, V., Silva, L., Andrade, J. d., Veloso, M. and Santos, G. (2008). "Determination of moisture content and water activity in algae and fish by thermoanalytical techniques." Quimica Nova 31(4): 901-905.

Suali, E. and Sarbatly, R. (2012). "Conversion of microalgae to biofuel." Renewable and Sustainable Energy Reviews 16(6): 4316-4342.

Tian, C., Li, B., Liu, Z., Zhang, Y. and Lu, H. (2014). "Hydrothermal liquefaction for algal biorefinery: A critical review." Renewable and Sustainable Energy Reviews 38: 933-950.

U.S. DOE (2010). National Algae Biofuels Technology Roadmap. U. S. D. o. Energy. Office of Energy Efficiency and Renewable Energy, Bimass Program.

United Nations Conference On Trade And Development (2008). Biofuel production technologies : status, prospects and implications for trade and development New York and Geneva, United Nations: 5-9.

Valdez, P. J., Nelson, M. C., Wang, H. Y., Lin, X. N. and Savage, P. E. (2012). "Hydrothermal liquefaction of *Nannochloropsis sp.* : Systematic study of process variables and analysis of the product." Biomass and bioenergy 46: 317-331.

van Boekel, M. A. (2006). "Formation of flavour compounds in the Maillard reaction." Biotechnol Adv 24(2): 230-233.

Wang, Y. (2013). Microalgae as the Third Generation Biofuel : Production, Usage, Challenges and Prospects. Department of Earth Science. Department of Earth Science, Uppsala University. Master degree in sustainable development: 31.

Wang, Z. (2011). Reaction mechanisms of hydrothermal liquefaction of model compounds and biowaste feedstocks. Department of Agricultural and Biological Engineering. Urbana, Illinois, University of Illinois at Urbana-Champaign. Doctor of Philosophy in Agricultural and Biological Engineering: 116.

White, J. and Catallo, W. (2011). "Biomass pyrolysis kinetic : A comparative critical review with relevant agricultural residue case studies." Journal of Analytical and Applied Pyrolysis 91(1): 1-33.

Xiu, S. and Shahbazi, A. (2012). "Bio-oil production and upgrading research : A review." Renewable and Sustainable Energy Review 16: 4406-4414.

Yokoyama, S.-y., Suzuki, A., Murakami, M., Ogi, T., Koguchi, K. and Nakamura, E. (1986). Liquid fuel production from sewagw sludge by catalytic conversion using sodium carbonate. N. R. I. f. P. a. Resources. Yatabe, Tsukuba, Ibaraki 305, Japan.

# APPENDIX

# APPENDIX A

# CALCULATION OF PRODUCT YIELDS

The total conversion and product yields were calculated by the following expression:

% Bio-oil yield = $100 \times (W_{Oil} / W_{Daf})$

% Solid residue = $100 \times (W_M - W_{Ash} / W_{Daf})$

Where:

$W_{oil}$ = weight of bio-oil

$W_{Daf}$ = weight of dry-ash free microalgae

$W_M$ = weight of dry microalgae before HTL

$W_{Ash}$ = weight of ash in microalgae

Example:

The two step hydrothermal liquefaction condition:

- The first step temperature: 200 $^{o}$C
- The second step temperature: 320 $^{o}$C
- The first step water pressure: 7 MPa
- The second step water pressure: 20 MPa
- The water flow rate: 0.50 mL/min

Calculation

Weight of dry microalgae = 8.57 g

Weight of bio-oil obtained from the first step = 0.59 g

Weight of bio-oil obtained from the second step = 1.05 g

Weight of solid residue = 4.26 g

Weight of ash = 3.43 g

% The bio-oil yield in the first step THTL = 100 x (0.59/5.14) = 11.48

% The bio-oil yield in the second step THTL = 100 x (1.05/5.14) = 20.42

% Total bio-oil yield = 11.48 + 20.42 = 31.9

% Solid residue = 100 x [(4.26-3.43)/5.14] = 16.1

# APPENDIX B

# CALCULATION OF HIGH HEATING VALUE

The high heating value (also known as gross calorific value) of a fuel is defined as the amount of heat released by a specified quantity (initially at 25°C) once it is combusted and the products have returned to a temperature of 25°C, which takes into account the latent heat of vaporization of water in the combustion products.

The high heating value of microalgae was determined following proximate analysis data (Parikh et al., 2005). Calculate the high heating value using the following equation:

$$HHV_{algae} = 0.3536FC + 0.1559VM - 0.0078ASH$$

Where:

$HHV_{algae}$ = high heating value (MJ/kg)

FC = fixed carbon (wt%)

VM = volatile matter (wt%)

ASH = ash (wt%)



The high heating value of bio-oil was determined following ultimate analysis data. Calculate the high heating value using the following Dulong formula (Chen et al., 2012):

$$HHV_{oil} = 0.3383C + 1.442(H - \frac{O}{8})$$

Where:

$HHV_{oil}$ = high heating value (MJ/kg)

C = carbon (wt%)

H = hydrogen (wt%)

O = oxygen (wt%)

# APPENDIX C

## CALCULATION OF %NITROGEN REDUCTION

The efficiency of THTL for the reduction of nitrogen content in the bio-oil obtained from the second step of this process was compared to SHTL and exhibited as %N reduction. It was calculated using the following equation:

$$\%N\,\text{reduction} = \left[\frac{N_0 - N_1}{N_0}\right] \times 100$$

$$\text{Weight of nitrogen (g)} = W_{oil} \times N_{\%}$$

Where:

$N_0$ = weight of N content in bio-oil from SHTL (g)

$N_1$ = weight of N content in bio-oil from the second step of THTL (g)

$N_{\%}$ = %Nitrogen content in bio-oil (wt%)

$W_{oil}$ = weight of bio-oil (g)

**Table C-1** The influence of the first step temperature on N content in bio-oil obtained the second step of THTL

| Processes | Temperature (°C) | Weight of bio-oil (g) | %Nitrogen content | Weight of nitrogen | %Nitrogen reduction |
|---|---|---|---|---|---|
| SHTL[a] | 320 | 1.7 | 8.7 | 0.146 | - |
| THTL[b] | 150/320 | 1.3 | 5.8 | 0.079 | 45.5 |
| | 175/320 | 1.1 | 4.9 | 0.055 | 61.7 |
| | 200/320 | 1.2 | 4.8 | 0.056 | 61.3 |
| | 225/320 | 1.1 | 4.9 | 0.056 | 61.1 |

[a] The SHTL operated in temperature of 320 °C, water pressure of 20 MPa and water flow rate of 0.50 mL/min.

[b] The THTL operated in vary of the first step temperature of 150 to 225 °C, the second step temperature of 320 °C, water pressure in the second step of 20 MPa and water flow rate of 0.50 mL/min

[c] Bio-oil obtained from the second step

%Nitrogen reduction of bio-oil obtained from the second step of THTL for temperature of 200/320 was calculated as followed:

$$\%\text{Nitrogen reduction} = \left[\frac{N_0 - N_1}{N_0}\right] \times 100$$

$$\%\text{Nitrogen reduction} = \left[\frac{0.146 - 0.055}{0.146}\right] \times 100$$

$$= 61.3\ \%$$

# APPENDIX D

# FORAGE FIBER ANALYSES

The chemical composition of microalgae such as cellulose, hemicellulose and lignin were determined by Chemical-fiber determination method. This method was separated into three paths following the different reagent which used to extract the composition of microalgae. Then, the received data from three paths were calculated to measure the value of the chemical composition.

**Neutral-detergent (cell-wall)** (Agricultural Research Service, 1970)

The neutral-detergent procedure for cell-wall constituents is a rapid method for analyzing the total fiber in vegetable feedstuffs, It appears to divide the dry matter of feeds very near the point that separates the nutritively available (98 percent) and soluble constituents from those that are incompletely available and dependent on a microbial fermentation.

1. Weigh 0.5 to 1.0 g air-dry sample ground to pass 20 to 30 mesh (1 mm) or equivalent into a beaker of the refluxing apparatus.
2. Add in order, 100 mL cold (room temperature) neutral-detergent solution, 2 mL decahydronaphthalene, and 0.5 g sodium sulfite with a calibrated scoop. Heat to boiling in 5 to 10 minutes. Reduce heat as boiling begins, to avoid foaming. Adjust boiling to an even level and reflux for 60 minutes, timed from onset of boiling.
3. Place previously tared Gooch crucibles on filter manifold. Swirl beaker to suspend solids, and fill crucible. Do not admit vacuum until after crucible has been filled. Use low vacuum at-first and increase it only as more force is needed. Rinse sample into crucible with minimum of hot (90-100 °C) water. Remove vacuum, break up mat, and fill crucible with hot water. Filter liquid and repeat washing procedure.
4. Wash twice with acetone in same manner and suck dry. Dry crucibles at 100° C for 8 hours or overnight and weigh.

5. Report yield of recovered neutral-detergent fiber as percent of cell-wall constituents. Estimate cell soluble material by subtracting this value from 100.
6. Ash residue in the crucible for 3 hours at 500° to 550° C and weigh. To report ash content as ash insoluble in neutral-detergent.

**Acid-detergent fiber** (Agricultural Research Service, 1970)

The acid-detergent fiber procedure provides a rapid method for lignocellulose determination in feedstuffs. The residue also includes silica. The difference between the cell walls and acid-detergent fiber is an estimate of hemicellulose; however, this difference does include some protein attached to cell walls. The acid detergent fiber is used as a preparatory step for lignin determination.

1. Weigh 1 g air-dry sample ground to pass 20 to 30-mesh (1-mm) screen or the approximate equivalent of wet material into a beaker suitable for refluxing.
2. Add 100 mL cold (room temperature) acid detergent solution and 2 mL decahydronaphthalene. Heat to boiling in 5 to 10 minutes. Reduce heat as boiling begins, to avoid foaming. Reflux 60 minutes from onset of boiling; adjust boiling to a slow, even level.
3. Filter on a previously tared Gooch crucible, which is set on the filter manifold; use light suction. Break up the filtered mat with a rod and wash twice with hot water (90-100 °C). Rinse sides of the crucible in the same manner.
4. Repeat wash with acetone until it removes no more color; break up all lumps so that the solvent comes into contact with all particles of fiber.
5. Optional wash with hexane. Hexane should be added while crucible still contains some acetone (Hexane can be omitted if lumping is not a problem in lignin analysis). Suck the acid-detergent fiber free of hexane and dry at 100° C for 8 hours or overnight and weigh.

6. Calculate acid-detergent fiber:

$$ADF\ (wt\%) = [(W_o - W_t) \times 100]/S$$

where: $W_o$ = weight of oven-dry crucible including fiber
$W_t$ = tared weight of oven-dry crucible
S = oven-dry sample weight

**Permanganate lignin, cellulose, insoluble, ash, and silica** (Agricultural Research Service, 1970)

An indirect method to determine lignin was developed that makes possible the preparation of cellulose and insoluble ash in the same sample. The insoluble ash is an estimate of total silica content, which in many grasses is a primary factor in reducing digestibility. The permanganate lignin method is an alternative procedure to the 72 percent sulfuric acid method; each has its own advantages. Choice of methods depends on materials analyzed and on the purpose for which the values are to be used. Advantages of the permanganate method over the 72 percent acid method include a shorter procedure for lignin *per se* while the residue is reserved for further analysis of cellulose and silica. The permanganate reagents are much less corrosive and require no standardization. The residue requires no filter aids, and lignin values are not subject to some interferences that affect 72 percent sulfuric acid lignin. Values are less affected by heat-damage artifacts and are closer to a true lignin figure.

However, cutin, which is important in many seed hulls, is not measured. A variation for the analysis of seed hulls is to prepare the permanganate cellulose and treat with 72 percent $H_2SO_4$ and asbestos for 3 hours as described in the acid-detergent lignin procedure. This results in the partitioning of crude lignin into two fractions as described in the acid-detergent cutin procedure. One disadvantage to permanganate lignin is that large particles are poorly penetrated by the reagents and yield low values. Consequently, all materials must be dried and ground to pass through a 20 to 30-mesh (less than 1 mm) screen, and the method is not applicable

to fresh feces and forages that have been ground in a meat grinder. Because of high sensitivity to heat damage, 72 percent acid is preferred for assaying artifact lignin. Theory of the method Interfering matter is removed by preparing acid-detergent fiber, which is chiefly composed of lignin, cellulose, and insoluble minerals. Lignin is oxidized with an excess of acetic acid-buffered potassium permanganate solution, containing trivalent iron and monovalent silver as catalysts. Deposited manganese and iron oxides are dissolved with an alcoholic solution of oxalic and hydrochloric acids, which leaves cellulose and insoluble minerals. Lignin is measured as the weight lost by these treatments; whereas, cellulose is determined as the weight loss upon ashing. The ash residue is mainly silica and much of the non-silica matter can be removed by leaching with concentrated hydrobromic acid.

1. Dry samples at less than 65° C and grind through 20 to 30-mesh (1 mm) screen. Prepare and determine acid-detergent fiber according to standard procedure. Use a 1 g sample, except on samples containing a high amount of lignin (15 percent or more) use 0.5 g sample. Place previously weighed crucibles in a shallow enamel pan containing cold water to a depth of about 1 cm Fiber in crucibles should not be wet.
2. Add about 25 mL of combined saturated potassium permanganate and lignin buffer solution (2:1 by volume) to the crucibles in the enamel pan containing cold water. Adjust level (2-3 cm) of water in pan to reduce flow of solution out of crucibles. Place a short glass rod in each crucible to stir contents, to break lumps, and to draw permanganate solution up on sides of crucibles to wet all particles.
3. Allow crucibles to stand at 20 to 25° C for 90 ± 110 minutes; add more mixed permanganate solution if necessary. Purple color must be present at all times.
4. Remove crucibles to filtering apparatus. Suck dry. Do not wash. Place in a clean enamel pan, and fill crucibles no more than half full with demineralizing solution. Demineralizing solution may be added directly to crucibles in case filtering is difficult. Care must be taken to avoid spillage by foaming. After about 5 minutes, suck dry on filter and refill half full with

demineralizing solution. Repeat after second interval if solution is very brown. Rinse sides of crucibles with solution from a wash bottle with a fine stream. Treat until fiber is white. Total time required is 20 to 30 minutes.

5. Fill and thoroughly wash crucible and contents with 80 percent ethanol. Suck dry and repeat two times. Wash twice in similar manner with acetone. Suck dry.
6. Dry at 100° C overnight and weigh. Calculate lignin content as loss in weight from acid-detergent fiber.
7. Ash at 500° C for 3 hours, cool, and weigh. Calculate residual ash as the difference between this weight and original tare of crucible. Calculate cellulose by weight loss upon ashing.
8. A presumptive analysis for silica may be obtained by hydrobromic acid treatment of the ashed permanganate lignin or ADF residue. This determination has its greatest value when the residual ash is greater than 2 percent. Ash and weigh, then add enough drops of 48 percent HBr to moisten all particles. Use no more than 4 mL acid. Allow to stand 1 to 2 hours. Add more drops of HBr if much red color forms. Suck off excess acid on vacuum and wash once with acetone. Use no water. Dry and ash briefly at 500° C Cool and weigh. Report silica as the difference between this weight and the original tare.

The data which received from the chemical-fiber determinations method was used to calculate the value of cellulose, hemicellulose and lignin content in microalgae as followed:

$$\text{Cellulose (wt\%)} = \frac{W_{KMnO_4} - W_{ash}}{W_i} \times 100$$

$$\text{Hemicellulose (wt\%)} = \%NDF - \%ADF$$

$$\text{Lignin (wt\%)} = \frac{W_{acid} - W_{KMnO_4}}{W_i} \times 100$$

where:

$W_{KMnO_4}$ = weight of microalgae after treatment with potassium permanganate

$W_{acid}$ = weight of microalgae after treatment with potassium permanganate

$W_{ash}$ = weight of ash

$W_i$ = weight of initial microalgae

$$\%NDF = \frac{W_{neutral} - W_{crusible}}{W_i} \times 100$$

# APPENDIX E

# DATA OF EXPERIMENTS

Table E-1 The bio-oil yield and solid residue obtained from SHTL

| Temperature ($^{O}$C) | Water pressure (MPa) | Water flow rate (mL/min) | Bio-oil yield (wt%) | Solid residue (g) |
|---|---|---|---|---|
| 280 | 20 | 0.50 | 27.8±0.7 | 25.5±1.5 |
| 300 | 20 | 0.50 | 27.2±1.5 | 22.7±2.1 |
| 320 | 20 | 0.50 | 29.5±2.0 | 20.6±3.0 |
| 340 | 20 | 0.50 | 24.7±3.3 | 18.8±0.7 |
| 360 | 20 | 0.50 | 22.9±1.9 | 17.6±1.2 |
| 320 | 12 | 0.50 | 22.1±0.2 | 12.4±3.3 |
| 320 | 16 | 0.50 | 23.5±1.1 | 19.3±2.4 |
| 320 | 20 | 0.25 | 27.3±2.3 | 22.4±0.9 |
| 320 | 20 | 0.75 | 25.0±2.8 | 21.4±1.0 |
| 320 | 20 | 1.00 | 16.5±1.6 | 21.3±0.7 |

Table E-2 The elements composition and heating value of bio-oil obtained from SHTL

| Temperature ($^{O}$C) | Water pressure (MPa) | Water flow rate (mL/min) | Element composition (wt%) | | | | HHV[b] (MJ/kg) |
|---|---|---|---|---|---|---|---|
| | | | C | H | N | O[a] | |
| 280 | 20 | 0.50 | 63.0±3.5 | 7.3±0.6 | 8.7±0.7 | 21.1±3.9 | 27.9±1.0 |
| 300 | 20 | 0.50 | 63.8±3.1 | 7.5±0.3 | 7.2±0.6 | 21.5±3.9 | 28.4±2.1 |
| 320 | 20 | 0.50 | 64.7±5.4 | 7.0±0.4 | 8.7±2.7 | 19.6±6.5 | 28.3±4.2 |
| 340 | 20 | 0.50 | 66.5±1.6 | 7.7±1.0 | 8.8±0.3 | 17.1±3.2 | 30.3±2.0 |
| 360 | 20 | 0.50 | 69.0±2.0 | 8.1±0.4 | 7.9±2.5 | 15.1±2.6 | 32.1±0.3 |
| 320 | 12 | 0.50 | 62.8±0.2 | 6.5±0.1 | 6.9±0.9 | 23.8±1.0 | 26.2±0.1 |
| 320 | 16 | 0.50 | 65.5±0.6 | 7.7±0.1 | 7.3±0.1 | 19.5±0.5 | 29.7±0.1 |
| 320 | 20 | 0.25 | 60.6±1.7 | 6.7±0.2 | 6.8±0.8 | 25.9±0.7 | 25.5±0.2 |
| 320 | 20 | 0.75 | 64.0±3.3 | 6.7±0.1 | 6.9±0.9 | 22.4±4.0 | 27.2±2.6 |
| 320 | 20 | 1.00 | 65.6±1.2 | 7.0±0.5 | 6.9±0.0 | 20.5±1.7 | 28.6±0.7 |

[a] By difference

[b] Calculated followed Eq. 3.2

Table E-3 Nitrogen compounds in aqueous phase from the 1st step of THTL

| 1st temperature[a] | Nitrogen compounds in aqueous phase obtained from 1st step (wt%) | | |
|---|---|---|---|
| | $NH_4^+$ [a] | $NO_3^-$ [b] | Polypeptides[c] |
| 150 | 1.2±0.0 | 0.3±0.032 | 4.9±0.012 |
| 200 | 2.5±0.0 | 0.9±0.078 | 15.3±0.0 |

[a] $NH_4^+$ was calculated followed Eq. 3.4

[b] $NO_3^-$ was calculated followed Eq. 3.5

[c] Polypeptides was Calculated followed Eq. 3.6

Table E-4 Effect of the first step temperature of THTL on elemental composition in pretreated microalgae

| 1st step temperature of THTL (°C) | Elemental composition[a] (wt%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pretreated microalgae | | | | Solid residue obtained from 2nd step of THTL | | | |
| | C | H | N | O[b] | C | H | N | O[b] |
| 150 | 30.2±1.2 | 5.6±0.4 | 3.5±0.1 | 60.7±1.7 | 24.5±0.8 | 4.3±0.2 | 1.7±0.1 | 69.5±1.1 |
| 175 | 30.3±0.2 | 5.3±0.6 | 3.3±0.1 | 61.2±0.5 | 24.7±1.1 | 3.5±0.4 | 1.2±0.1 | 70.6±0.4 |
| 200 | 30.0±1.3 | 5.2±0.2 | 3.1±0.0 | 61.7±1.5 | 24.1±0.9 | 3.4±0.1 | 0.9±0.0 | 71.6±1.0 |
| 225 | 29.0±0.9 | 5.0±0.1 | 3.0±0.1 | 63.0±1.1 | 23.7±0.6 | 3.0±0.1 | 0.9±0.1 | 72.4±0.7 |

[a] Dry ash-free

[b] By difference

Table E-5 The bio-oil yield and solid residue obtained from THTL

| Temperature (°C) | | Water pressure (MPa) | Water flow rate (mL/min) | Bio-oil yield (wt%) | | | Solid residue (wt%) |
|---|---|---|---|---|---|---|---|
| 1st step | 2nd step | | | 1st step | 2nd step | Overall | |
| 150 | 320 | 20 | 0.50 | 9.9±0.6 | 23.6±0.9 | 33.5±1.6 | 16.2±3.1 |
| 175 | 320 | 20 | 0.50 | 11.3±2.1 | 19.9±1.3 | 31.2±3.4 | 18.2±0.2 |
| 200 | 320 | 20 | 0.50 | 11.5±2.8 | 20.5±3.1 | 32.0±6.2 | 16.0±2.2 |
| 225 | 320 | 20 | 0.50 | 12.7±3.1 | 19.9±2.1 | 32.6±1.0 | 16.2±2.9 |
| 200 | 280 | 20 | 0.50 | 10.3±3.1 | 14.7±1.4 | 25.0±2.3 | 19.0±0.04 |
| 200 | 300 | 20 | 0.50 | 10.0±2.8 | 17.6±2.9 | 27.6±2.9 | 17.6±0.1 |
| 200 | 340 | 20 | 0.50 | 10.4±3.1 | 17.3±3.8 | 27.7±3.4 | 13.7±0.3 |
| 200 | 360 | 20 | 0.50 | 10.5±2.0 | 15.9±3.1 | 26.4±2.6 | 13.6±0.1 |
| 200 | 320 | 12 | 0.50 | 12.0±3.0 | 18.0±0.1 | 30.0±1.5 | 19.9±1.1 |
| 200 | 320 | 16 | 0.50 | 11.9±0.9 | 19.0±1.2 | 30.9±2.1 | 18.3±0.4 |
| 200 | 320 | 20 | 0.25 | 9.7±2.2 | 18.4±0.5 | 28.1±2.7 | 19.2±2.3 |
| 200 | 320 | 20 | 0.75 | 11.3±0.9 | 18.4±0.2 | 29.7±0.6 | 18.9±3.2 |
| 200 | 320 | 20 | 1.00 | 9.0±0.6 | 15.7±1.2 | 24.7±1.7 | 19.4±2.8 |

Table E-6 The elements composition and heating value of bio-oil obtained from THTL

| Temperature (°C) | | Water pressure (MPa) | Water flow rate (mL/min) | Element composition[a] (wt%) | | | | HHV[a,c] (MJ/kg) |
|---|---|---|---|---|---|---|---|---|
| 1st step | 2nd step | | | C | H | N | O[b] | |
| 150 | 320 | 20 | 0.50 | 65.8±2.5 | 7.7±1.1 | 5.8±0.7 | 20.6±2.9 | 29.5±0.8 |
| 175 | 320 | 20 | 0.50 | 70.8±0.1 | 6.1±0.9 | 4.9±1.1 | 18.2±2.0 | 29.4±1.7 |
| 200 | 320 | 20 | 0.50 | 70.9±2.5 | 7.0±0.4 | 4.8±2.7 | 15.9±6.5 | 33.2±4.2 |
| 225 | 320 | 20 | 0.50 | 67.9±4.9 | 6.5±0.5 | 4.9±1.6 | 20.6±2.8 | 28.5±1.4 |
| 200 | 280 | 20 | 0.50 | 62.5±2.7 | 7.2±0.7 | 5.0±0.1 | 25.4±2.9 | 26.8±0.9 |
| 200 | 300 | 20 | 0.50 | 66.4±2.9 | 7.5±1.4 | 5.0±1.3 | 21.1±2.0 | 29.3±4.0 |
| 200 | 340 | 20 | 0.50 | 66.1±0.8 | 8.4±0.2 | 5.3±0 | 20.2±0.9 | 30.7±0.6 |
| 200 | 360 | 20 | 0.50 | 63.9±0.3 | 6.9±0.7 | 5.7±0.1 | 23.5±0.7 | 27.2±0.8 |
| 200 | 320 | 12 | 0.50 | 65.9±1.5 | 7.9±0.4 | 5.2±1.5 | 21.0±3.4 | 29.8±1.6 |
| 200 | 320 | 16 | 0.50 | 67.7±6.2 | 7.2±0.8 | 4.9±0.1 | 20.2±9.1 | 29.5±5.6 |
| 200 | 320 | 20 | 0.25 | 66.0±6.6 | 6.9±0.5 | 6.6±1.1 | 20.5±6.2 | 28.5±4.6 |
| 200 | 320 | 20 | 0.75 | 64.0±3.5 | 6.9±0.6 | 6.5±0.6 | 22.6±4.0 | 27.5±0 |
| 200 | 320 | 20 | 1.00 | 70.9±1.6 | 7.6±0.5 | 6.2±1.0 | 15.2±2.6 | 32.1±2.0 |

[a] Bio-oil was obtained from the 2nd step of THTL

[b] By difference

[c] Calculated followed Eq. 3.2

Table E-7 Weight of bio-oil yield and solid residue obtained from THTL* with additive

| Additives | Concentration (%wt solvent) | Bio-oil yield (wt%) | | | Solid residue (wt%) |
|---|---|---|---|---|---|
| | | 1st step | 2nd step | Overall | |
| $Na_2CO_3$ | 5 | 5.3±0.9 | 12.2±0.6 | 17.5±0.8 | 19.6±0.6 |
| $Na_2CO_3$ | 10 | 7.5±0.5 | 14.9±0.3 | 22.4±0.4 | 20.4±1.3 |
| $Na_2CO_3$ | 15 | 4.9±0.7 | 6.7±0.8 | 11.6±0.8 | 17.7±2.3 |
| KOH | 5 | 3.4±0.6 | 6.4±1.4 | 9.8±1.0 | 19.75±0.1 |
| KOH | 10 | 7.1±0.7 | 9.1±2.3 | 16.3±1.5 | 19.7±1.3 |
| KOH | 15 | 5.1±1.8 | 8.6±1.6 | 13.7±1.7 | 20.1±0.04 |

* THTL operated at 200 °C of 1st step, 320 °C of 2nd step, 20 MPa and 0.50 mL/min

Table E-8 The elements composition and heating value of bio-oil obtained from THTL* with additives

| Additives | Concentration (%wt solvent) | Element composition[a] (wt%) | | | | HHV[a,c] (MJ/kg) |
|---|---|---|---|---|---|---|
| | | C | H | N | O[b] | |
| $Na_2CO_3$ | 5 | 76.7±6.7 | 10.5±2.3 | 3.3±0.7 | 9.5±7.4 | 35.1±8.2 |
| $Na_2CO_3$ | 10 | 78.2±8.0 | 10.1±1.3 | 3.7±0.9 | 8.0±6.9 | 35.2±5.8 |
| $Na_2CO_3$ | 15 | 75.3±2.8 | 6.4±0.5 | 4.1±0.4 | 14.2±3.7 | 28.2±2.2 |
| KOH | 5 | 73.8±1.2 | 8.0±1.2 | 3.6±0.1 | 14.6±0.1 | 29.7±1.3 |
| KOH | 10 | 78.0±3.2 | 8.3±0.8 | 3.9±0.1 | 9.8±2.2 | 32.5±1.2 |
| KOH | 15 | 64.8±5.2 | 7.9±0 | 4.0±1.4 | 23.3±7.5 | 25.7±3.2 |

* THTL operated at 200 °C of 1st step, 320 °C of 2nd step, 20 MPa and 0.50 mL/min

[a] Bio-oil for THTL was the 2nd step of THTL

[b] By difference

[c] Calculated followed Eq. 3.2

# VITA

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1. Publications

1.1) Korean Journal of Chemical Engineering

Sunphorka S., Prapaiwatcharapan K., Hinchiranan N., Kangvansaichol K. and Kuchonthara P. (2014). "Biocrude oil production and nutrient recovery from algae by two-step hydrothermal liquefaction using a semi-continuous reactor." Korean J. Chem. Eng. DOI:10.1007/s11814-014-0165-5.

1.2) Bioresource Technology (revised)

Prapaiwatcharapan K., Sunphorka S., Kuchonthara P., Kangvansaichol K., Hinchiranan N., "Single- and two-step hydrothermal liquefaction of microalgae in a semi-continuous reactor : effect of the operating parameters." Bioresource Technology.

2. Proceeding

Prapaiwatcharapan K., Kucholthara P., Hinchiranan N., "Bio-oil production via two-step hydrothermal liquefaction of microalgae", Poster presentation, Pure and applied chemistry international conference (PACCON2014), 8-10 Janury 2014, Khon Kaen University, Thailand.