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Hydrothermal Treatment for Production of Value-Added Co-Products and Efficient Oil Extraction from Microalgae

A dissertation submitted in partial fulfillment of

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by

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Summary

Algae-based biofuels, also known as the 3rd generation biofuels, are believed to be a displacement of transportation fossil fuels in the near future. The alternative biofuels have been increasing public's attention regarding rising world's population, declining fossil fuels and worsening global environment. Algae are becoming increasing interesting as a numerous of advantages including the fastest growing species, improve carbon dioxide mitigation, no competition with agricultural land, water and food, and high lipid content species. A constrain has been concerning is economical issue comparing to fossil-derived fuels. Current technologies for wet biomass extraction, cultivation cost and downstream functioning are challenging still. A number of researchers have proven these are realistic and implemented on a commercial scale. However, there are some points lack of subjected. This study, thereafter, would like to introduce the proven technologies have been advised for decades to cope with microalgae-based biofuels' by-products in a practical way aims to economize the algae-based biofuels production.

The hydrothermal treatment (HTT), an effective technology for extraction liquid biofuel from wet biomass, was first employed on freshwater green microalgae TISTR-8511 strain (Chlorellaceae strain) to extract bio-oil. The 2 key parameters, operating temperature (190-250^{o}C) and retention time (30, 60 and 90 min), were applied over the HTT mechanism in order to observe their best relevance. Then 4 main products were obtained; bio-oil, gaseous, solid and aqueous co-products. The results revealed that the condition at 230^{o}C and 60 min retention time gave the best bio-oil yield of 3.54% and followed by the yield of 3.39% at 230^{o}C and 30 min retention time and 3.27% at 250^{o}C and 30 min retention time. The bio-oil was then analyzed for a feasibility to be upgraded to biodiesel and it showed a comparable for upgrading. The solid co-product, on the contrary, served the best yield with the lowest bio-oil yield. Nevertheless, the solid co-products were further analyzed for available nutrients and only the products employed by 60 min retention time were implemented on Komatsuna (Brassica rapa var. perviridis) planting at 3 applying ratios; 100%, 50% and 25%. Only the solid co-products of 60 min retention time were practiced as of the best bio-oil yield. The practical planting showed that nitrogen nutrient is the most essential factor for plant growth. Moreover, an organic fertilizer provides better yield than an inorganic fertilizer. Additionally, the highest dry weights were also observed at 230^{o}C on all applying ratios. However, most of the yields were lower than the standard fertilizer excluded when 100% applying ratio was applied on 190-230^{o}C. The aqueous co-products were next recovery as of a rich nutrient product. They were first analyzed for available nutrients required for algae growth. Next, they were diluted to 2%v/v with distilled water and implemented to the marine water green microalgae, Chlorella sp. The results showed the highest protein content was found at 5.75%wt when 250^{o}C and 60 min retention time was employed. Furthermore, they revealed that the yield of protein content behaved similar to the available organic nitrogen in the algae medium. In addition, the yield of carbohydrate content also revealed the similar performance with the available inorganic carbon in the algae medium where the highest yield was found at 4.89%wt when 190^{o}C and 60 min retention time was employed. Unlike the lipid content, the highest yield was observed at 230^{o}C and 90 min retention time of 2.06%wt with no correlation to any element. Nonetheless, most of these contents were produced at lower yield than when the Rodik standard algae medium was applied, unless the protein content.

The study can be concluded that the economical viability of microalgae-based biofuels production employing low temperature HTT (190-250^{o}C and 30-90 min retention time) is realistic with the implementation of co-products for solid bio-fertilizer and algae growth media.

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Chapter 1 Introduction

1.1 Algae as a liquid fuel source

9 billion world's population is expected to be reached in 2050 then an intensifying of energy demands of course is a result. The world's petroleum-derived fuels are predicted to be out sooner than later [1-1]. This predicted scenario has raised the global concerns. Biofuels, thereafter, have been proposed to replace this current transportation fossil fuel. This is not only the environmentally friendly issue but also a limited availability of fossil fuel on earth. Biofuels can be classified into 4 categories based on their technologies as; first generation biofuels (FGBs), second generation biofuels (SGBs), third generation biofuels (TGBs) and fourth generation biofuels [1-2]. The FGBs, produce energy from cellulosic material and other nonfood crops like sugar, starch and vegetable oil, in the forms of bioethanol (ETBE) and vegetable oil (FAME), whereas the SGBs use lignocellulosic crops such as waste biomass, wheat straw, corn, wood and nonfood crops to produce bioethanol, Fischer-Tropsh diesel and bio-oil. The TGBs, currently gain many attractions, is biodiesel derived from algae. The fourth generation biofuel definition is any engineering genetic crop that consumes more carbon dioxide from the atmosphere than they will produce during utilizing as a combustion fuel [1-2]-[1-4].

Microalgae-based biofuel, carbon-neutral renewable liquid biofuels, is a promising alternative biofuel as of many superiors including no competition with food crops for arable land nor fresh water, can theoretically be grown with much higher annual yields per acre, high carbon dioxide absorption and uptake rate, high lipid content species and lack of difficulty of cellulose and lignin structure breakdown [1-5], [1-6]. However, many economically constrains have been debated over current implementation of microalgae-based systems. An excessively high production cost is the most challenge compared to petroleum-derived fuels [1-7], thus a commercial-scale production of algae biodiesel will not occur unless the economical considerations are also favorable [1-8]. A selection of microalgae species, a developed technology for wet biomass conversion, a cost of cultivation and a downstream products waste management [1-5], [1-7], for example are also taking an essential account. In this chapter, proven technologies which have been advised for the decades for coping with microalgae-based biofuel are introduced. Then, a practical upgrading of by-products will be proposed.

1.2 Algal biofuels conversion technology

There are 2 categories for utilizing microalgae biomass to energy; thermochemical conversion and biochemical conversion. The adopted selection is based on the type and quantity of biomass feedstock, the desired form of the energy, economical consideration, project specific and the desired end form of the product [1-9], [1-10].

1.2.1 Thermochemical conversion

This technology covers the thermal decomposition of organic components in biomass to yield fuel products which can realizable by different processes as followed.

1.2.1.1 Gasification

Gasification involves the partial oxidation of biomass into a combustible gas mixture at high temperatures (800-1000^{o}C) with oxygen and steam to generate syngas, a mixture of CO, H_2, CO_2, N_2 and CH_4. A key advantage of this technology is that syngas can be produced from a variety of feedstock, nonetheless, an energy intensive is still a challenge.

1.2.1.2 Pyrolysis

Pyrolysis is a conversion of biomass to bio-oil, syngas and charcoal at medium to high temperature (350-700^{o}C) in the absence of air. An advantage of this technology is that the high liquid conversion ratio, however, the outcome products are acidic, unstable, viscous and contain solid and chemically dissolved water.

1.2.1.3 Thermochemical liquefaction

Thermochemical liquefaction is a process employing low-temperature (200-350^{o}C) and high pressure (5-20 MPa) to convert biomass in an aqueous medium to liquid fuel, called bio-oil, with or without a catalyst. An outstanding advantage of this technology is its ability to convert wet biomass into energy however the fuel-feed system and reactor are complex and expensive. The process utilizes a unique property of water at sub-critical conditions to decompose complex biomass materials down to shorter and smaller molecule materials with a high energy density.

1.2.1.4 Direct combustion

A direct combustion is a burning process of biomass in the presence of air so as the stored chemical energy inside the biomass can be converted into hot gases, usually used for generating electricity. It prefers low moisture biomass (<50% dry weight) and can burn any type of biomass. The drawbacks of this process are high energy demand and normally required pre-treatment of the biomass and the produced heat cannot be stored.

1.2.2 Biochemical conversion

The biological process for converting biomass to energy includes;

1.2.2.1 Anaerobic digestion

Anaerobic digestion (AD) is a conversion of organic waste into biogas, mainly consisting of CH_4 and CO_2, and some small trace gases. AD is suitable for high moisture organic waste (80-90% moisture) thus it can be employed for wet algae biomass. However, a high protein content in algae can lead to a low C/N ratio, inhibit anaerobic microorganisms by high ammonium containing produces, and also toxic to some anaerobic microorganisms who cannot stand for the sodium ions.

1.2.2.2 Alcoholic fermentation

Alcoholic fermentation is a conversion of biomass containing sugars, starch or cellulose into ethanol. A purification process (distillation) is absolutely required to remove water or other impurities in the diluted alcohol product. The solid residues from this process can be used for animal-feed or gasification, with the advantage of low-cost. However, microalgae recognized as high lipid containing do not have potential for this starch conversion as some pre-treatments are required.

1.2.2.3 Photobiological hydrogen production

Microalgae cultures for this technology must be subjected to anaerobic conditions where 2 approaches can be taken places. The first approach produces H_2 with limited usage time because H_2 can be leveled off after 60 hours. The second approach suffers severe hydrogenase inhibition, thus less attractive.

Hydrothermal treatment (HTT), a category of thermochemical conversion, uses water at lower temperature (200-350^{o}C) and its saturated steam pressure to convert algae into fuel. Thus, it is a wet process with no additional solvent. This is the best combination of cost and performances that needs no biomass dewatering and uses its water content at this subcritical region, so that the water still remains in the liquid state as a reactant. Therefore, it is not only the lipids will be converted, but also the protein and carbohydrate will be converted into bio-oil. As a consequence, the bio-oil yield is higher than other known extraction [1-11], [1-12]. Thus, HTT can change the focus of growing high lipid content-algae because HTT converts the whole algae cell into bio-oil. And since water is used as a solvent, bio-oil can be easily removed by just oil/water separation. Moreover, this is a clean process with almost no waste and the output water is clean, clear and sterile which can be simply recycled [1-11]. Therefore, high energy efficiency with clean bio-oil extraction from microalgae employing this HTT technology is expectable.

1.3 Hydrothermal treatment

The hydrothermal treatment (HTT) engages in direct decomposition of biomass, with the presence of water and with or without a catalyst that directly transforms the biomass into solid, liquid and gas with a temperature lower than 400^{o}C for the reaction [1-13]. HTT is different from biomass gasification and pyrolysis, which require dried feedstock and an environmental temperature higher than 600^{o}C to promote the process and therefore consume a larger amount of energy [1-13], [1-14]. Water is an important factor in HTT since its property is changed when temperature increases. First, its relative permittivity decreases rapidly, then the dissociation of water dramatically

increases. Therefore, water becomes a good solvent for hydrocarbon at a high temperature, typically non-polar under standard environmental conditions [1-13], [1-15]-[1-16]. HTT, under high temperature and pressure (generally carried out at 200-370^{o}C and 5-25 MPa), is ideal for energy recovery from high moisture content biomass since the water is still in a liquid state and acts as a reactant and catalyst and has been extensively studied [1-15], [1-17]-[1-22].

HTT involves an application of heat and pressure to biomass in an aqueous medium, therefore, high energy efficiency in terms of obviating biomass dewatering and drying is its distinct merit. Feedstock containing about 80% of water is subjected to subcritical temperature (250-350^{o}C) to create a hydrophilic bio-oil with a reduction of 10-18% of oxygen content when compared to the parent material [1-23]. This bio-oil can be used directly as a heavy petroleum oil replacement, for co-firing with coal, and is a candidate for upgrading to high quality distillate fuels (e.g., diesel and gasoline) [1-23]. Thereby, the HTT processing does rely on the unique properties of water at high temperature and pressure. At elevated temperatures, hydrogen bonding of water is diminished then the water dielectric constant is reduced, and thus its ion product increases. As a consequence, many organic compounds become completely miscible in the high temperature water [1-13], [1-15]-[1-16], [1-23]. The dielectric constant is the ratio of the permittivity of a substance to the permittivity of a free space. The water does have a very high dielectric constant of 80.10 at 20°C (as depicted in Fig. 1-1), because a dipole moment of the water molecule and so water can be polarized. The large dielectric constant means that substances whose molecules contain ionic bonds will tend to dissociate in water yielding solutions containing ions [1-23]. Thereby water becomes a very good solvent.

Liquid biofuel from microalgae has drawn many attentions and beaten other biomasses because it has been identified as a promising feedstock for scaling up to industrial-scale production of carbon-neutral biodiesel [1-13]-[1-16]. Moreover, HTT has been emphasized due to energy saving on dewatering process and economical viability in production of value-added co-products along with bio-oil.

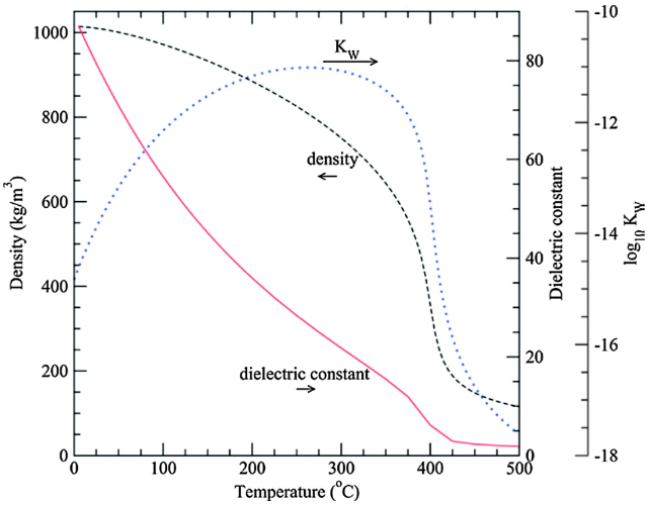

Fig. 1-1 Dielectric constant of water

1.4 Oil product from microalgae by HTT

The bio-oil from HTT cannot be directly used as a transportation fuel as it still contains high oxygen, nitrogen, and sulfur contents, and slightly acidic compared to conventional fossil fuel, however, its quality is better than the pyrolysis oil [1-11], [1-24]. The oxygen content from microalgae bio-oil can be as low as half of that from the pyrolysis process, thus the upgrading will be much easier and so lower cost [1-24]. Furthermore, the energy density is also higher than that of the pyrolysis since HTT has a capability to convert carbon content to as high as 85% [1-11]. A comparison of HTT bio-oil to pyrolysis of wood biomass and fossil fuel is shown in Table 1-1. For

transportation fuel production, nonetheless, the pre-treatment is necessary to remove the oxygen, nitrogen and sulfur contents still. In this study, the HTT bio-oil properties were investigated for feasibility to be upgraded to biodiesel.

Table 1-1 Property range of HTT bio-oil [1-24]

	HTT	Pyrolysis	Fossil oil
Carbon, wt%	68-81	56-66	83.0-87.0
Sulfur + Nitrogen, wt%	0.1	0.1	0.001-5
Oxygen, wt%	9-25	27-38	0.005-1.5
Water, wt%	6-25	24-52	<1
Density, Kg/L	1.10-1.14	1.11-1.23	0.75-1.0

1.5 Solid product from microalgae by HTT

The solid residue or biochar obtained from microalgae by HTT has been given much attraction recently. For example, it was proposed that char may be useful as re-enforcing additives in cement and organic polymers or even a carbon source for synthesis gas formation or as a coal coke alternative in steel manufacture when ash is found to be low [1-25]. Moreover, it was reported that the biochar has a potential for agricultural applications as a biofertilizer and for carbon sequestration [1-10]. There are few authors who have studied a feasibility of applying this biochar for biofertilizer, so far.

The global food consumption has been increased sharply as a result of continuously increased world's population. World demand for total fertilizer nutrients, therefore, is estimated to grow at 1.8% annually from 2014 to 2018, whereas the demand for nitrogen, phosphate and potash is forecasted to grow 1.4, 2.2 and 2.6% per annual respectively. Asia is the largest consumer of fertilizer in the world at 58.5%, there will be many regions lack of nutrient deficit then [1-26]. The price of fertilizer is related to the crude oil price and both of them are closely related to global economic activity and wealth. In the case of N fertilizers (ammonia, urea, ammonium nitrate) the direct feedstock for their production is natural gas whose price is generally correlated to crude oil. In short, the hydrogen atoms in natural gas are combined with atmospheric nitrogen to make ammonia. On the other hand, the P and K fertilizer are mined and processed, thus fuels take a significant part in their direct and indirect costs somehow [1-27]. From this point of view, utilizing HTT solid product for biofertilizer is a good idea. A challenge is there is no practical implementation of solid phase obtained from HTT as biofertilizer still. Thus, this study proposed this utilization with a practical usage.

1.6 Aqueous product from microalgae by HTT

The HTT aqueous residues have been proven to be rich in nutrients where nitrogen and phosphorus were found to be the major contents [1-14], [1-28]. It has been found that 40-80% of phosphorus can be recovered and it was in the form of orthophosphate [1-29] Moreover, 66-80% of nitrogen in biomass could also be found here where 20-60% was in the form of ammonia. It is not only high in nutrients, but also the recovery yield is relatively high to be recycled as up to 82% of the total mass [1-30]. Hence an idea to recycle this aqueous for algae cultivation has been proposed [1-31]. Furthermore, it has been found that a 20-fold dilution of the nutrients rich aqueous phase could support about half of the optimal nutrient required for algal growth [1-25]. However, it was observed that the HTT aqueous residues derived from Spirulina was highly toxic to mammalian Chinese hamster ovary cells when applying a high volume of 7.5% in the growth media [1-32]. Nonetheless, the characteristics of HTT aqueous products are mostly rely on the algae's biochemical composition and the conditions of HTT applied. According to these researches, HTT, therefore, is a promising process not only because of its ability to produce bio-oil, but also the nutrients in its aqueous residue can be facilitated for algae growth to improve the overall economic viability of the microalgae-based biofuels production. This study also would like to investigate the possibility of the usage of the aqueous product from HTT for algae growth media so as to study a practical usage of this implementation.

1.7 Research objective

To make economically viability of bio-oil extraction from microalgae, solid fertilizer and algae growth media co-production have been proposed to give high value to HTT co-products along with bio-oil upgrading feasibility. The overall concept of this study is depicted in Fig. 1-2.

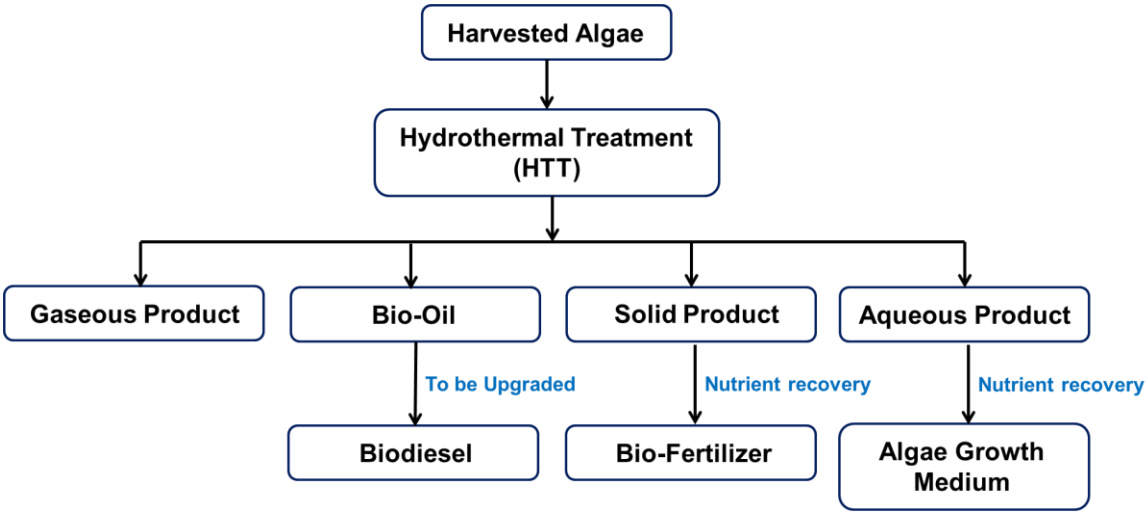

Fig. 1-2 Overall concept of the study

1.8 Thesis outline

To achieve the goal of this study, the content of this thesis is separated into 6 chapters as follows:

Chapter 1 Introduction
In this chapter, the root ideas of this research were brought in. A conversion of aquatic biomass to an alternative energy was reviewed and followed by the key principal of hydrothermal treatment technology. Later on, the optional by-product residues utilizations were proposed to add value to the residue and economize the bio-oil extraction from microalgae. And finally, the aims and originality of this study were stated.

Chapter 2 Influences of reaction conditions on HTT products
In this chapter, a lab-scale hydrothermal treatment (HTT) for bio-oil extraction was conducted. The yields of all products were certified prior to further investigation. The influences of the reaction temperature and the reaction time of HTT were determined for bio-oil, solid and liquid residues. The properties of bio-oil were firstly studied in terms of energy recovery and energy density. Next, the characteristics of solid residue were indicated against a standard of solid fertilizer. Furthermore, Rodik as an algae medium was set to examine an equivalence of liquid residue as to being used for algae re-cultivation. Lastly, the key nutrient elements; N, P and K; were investigated to show the effects of the operating conditions on the flows of these elements.

Chapter 3 Characterization and upgrading of the bio-oil
In this chapter, bio-oil's elemental composition was first analyzed for comparation with the petroleum crude oil. Next, the acid numbers and the intensity of free fatty acids (FFA) of the bio-oil were determined. The intensity of FFA over 5% is referred to acid catalyst preference for bio-oil transformation. A transmethylation process known as an effective reaction for bio-oil transforming to fatty acids methyl ester (FAME) was then employed with sulfuric acid catalyst. Later on, an analysis of FAME using GC/MS was conducted. As a result of changing the reaction temperature of HTT, the yields of saturated fatty acids (SAT), mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) were changed. Finally, iodine value was measured to know the degree of unsaturation of the bio-oil.

Chapter 4 Characterization and fertilizer application of the solid product
In this chapter, seed germination was conducted firstly to investigate the plant phytotoxicity. Next, the contents of nitrogen were evaluated to determine the available nitrogen for plant growth. Later, a practical planting of Komatsuna plant was implemented on a set of solid residue applying ratio. The shoot height, leaves width and dry weight of the planted Komatsuna were analyzed to evaluate the potential usage of solid product as solid fertilizer.

Chapter 5 Characterization and microalgae cultivation application of the aqueous product
In this chapter, pH of aqueous solutions was measured then dilution ratio could be trialed as of a significant factor for microalgae growth. A time period of 14 days was set for growth development of microalgae and harvested after 2 weeks. The harvested microalgae were biochemically analyzed for protein, carbohydrate and lipid contents to assess the aqueous product's potential usage for microalgae cultivation.

Chapter 6 Conclusion
The principal results achieved in this research are summarized.

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Chapter 2 Influences of reaction conditions on HTT products

Abstract

Hydrothermal treatment (HTT) is a technique for obtaining clean biofuel from biomass in the presence of heat and pressure in an aqueous medium which leads to a decomposition of this biomass to the formation of various products. A role of reaction conditions is essential for the bio-oil and other products' yield and also quality of the products. The effects of these parameters were investigated in regards to the composition and yield of the products. Chlorellaceae microalgae were tested under different HTT conditions to clarify suitable conditions for extracting bio-oil together with value-added co-products. Firstly, different reaction temperatures (190-250^{o}C) were tested at 60 min reaction time and found that the reaction temperature had a significant effect on the product yields. Therefore, this range of temperature was also employed for studying the influences of the reaction time. The results showed that the reaction time had not much influence on the physical product yields, however, it did on the chemical characteristics. On another hand, the reaction temperature was found to be a key parameter for both physical and chemical properties of the HTT products.

2.1 Introduction

Hydrothermal processing is categorized into 3 separated processes depends on the severity of the operating conditions as (1) the process takes place at the temperature below 247^{o}C, known as hydrothermal carbonization (HTC), (2) the process occurs at the intermediate temperature of 247-374^{o}C, defined as hydrothermal treatment (HTT), and (3) the process happens at higher temperatures above 374^{o}C which known as hydrothermal gasification (HTG) [2-1]. The main product of HTC is hydrochar and its properties are similar to a low rank coal. In case of microalgae HTC, the product is mainly converted from carbohydrate and protein contents thus it is possible to extract lipid content prior to apply to the process [2-2]. Whereas, the product from HTT is a liquid fuel known as bio-oil, has similar properties to petroleum crude and can be upgraded for distillation as petroleum-derived fuels [2-1]. The product of gasification reaction is defined as synthetic fuel gas or syngas which is superior to the HTT product in terms of high carbon efficiencies as the lower amount of organic carbon is presented in the water phase product [2-3]. In any case, the aim of hydrothermal processing is to increase the energy density of the product by removing of oxygen content [2-1].

Hydrothermal treatment (HTT) is a process of converting organic biomass in hot compressed water to a liquid biofuel [2-3], [2-4]. The process occurs at the temperature ranges of 200-350^{o}C with the pressures that keep the process in subcritical region so as the latent heat of vaporization can be avoided, usually around 5-20 MPa [2-4]. In this subcritical region the biomass is broken down and repolymerized to oily compounds [2-5]. This mechanism, therefore, is an ideal for wet biomass, usually high moisture content, like microalgae as drive off the water process is negligible [2-6]. In several publications [2-7]-[2-11], the mechanisms of HTT process are briefly described as:

1. Hydrolysis of biomass macromolecules (in the case of algae biomass-lipid, protein and carbohydrate) into smaller fragments;
2. Conversion of these fragmented molecules by other reactions such as dehydration or deoxygenation into others smaller compounds, and
3. Rearrangement of these compounds via condensation, cyclization and polymerization to produce new components.

However, the difference of biomass's biochemical compositions may take a significant part in these mechanisms case by case.

Microalgae is a sunlight-driven cell that efficiently converts solar energy, water and carbon dioxide into large amount of lipids, proteins and carbohydrates as membrane components, storage products, metabolites and sources of energy, in a shorter period of time compared to other terrestrial plants [2-12]-[2-15]. Algae contain about 2-40% of

lipids per weight [2-12], whereas microalgae hold about 15-77% of oil contents per weight [2-15]. This difference is because the entire cell surface of algae can be involved in the photosynthesis process. As a result, lipids are accumulated in the entire cell which is different from other oil crops where seeds can contain oil [2-16]. Hence, algae have high oil content potential, high productivity with the smallest land-use footprint and rapid lipid accumulation. Moreover, it can be grown on non-arable land and can survive in various types of water. So the production of liquid biofuel from microalgae has drawn much attention and has exceeded other biomasses without any conflict with food crop producers [2-2], [2-12]-[2-16]. There are many taxonomic groups of algae species that are able to accumulate lipids in high amounts. Many studies have shown that green microalgae strains are the biggest group with high potential to produce large quantities of lipids that are also capable of being grown in a mass culture [2-2], [2-17]. Based on these previous studies, a freshwater green microalgae, the Chlorellaceae strain, was selected for evaluation in this study. Not only for these reasons, but environmental toleration, ease of cultivation and also cost efficiency makes this strain the best candidate for bioenergy sources in this study.

In this study, the focus was put on identifying the suitable conditions for converting microalgae biomass to bio-oil and also the possibility to convert microalgae biomass residue into high value added solid and aqueous co-products, i.e., solid fertilizer and nutrients for algae cultivation. The role of the reaction conditions, the reaction temperature and the reaction time of HTT were investigated in regards to the composition and yield of the products. Firstly, a series of the reaction temperature (190-250^{o}C) was conducted then a set of the reaction time was applied.

2.2 Materials and methods

2.2.1 Materials

The microalgae TISTR-8511 strain (Chlorellaceae strain) was obtained from TISTR (Thailand Institute of Scientific and Technological Research) in a powder form. The powder form was selected for ease of transportation and also because the microalgae concentration itself is relatively low at 0.406 g/L (dry basis). Hence, the fresh microalgae was gravimetrically precipitated before being harvested and then naturally dried and finally grinded by a juicer grinder before transport to Japan. A batch-type reactor autoclave (model MMJ500) equipped with a magnetic drive agitator, an electrically heated furnace, and a SiO_2 tube chamber as illustrated in Fig. 2-1, purchased from OM Labtech (Tokyo, Japan) was employed. The stainless steel, SUS-316, autoclave with a capacity of 500 mL has an operable temperature and pressure up to 300^{o}C and 20 MPa. Most chemicals used were purchased from Wako Chemicals (Tokyo, Japan).

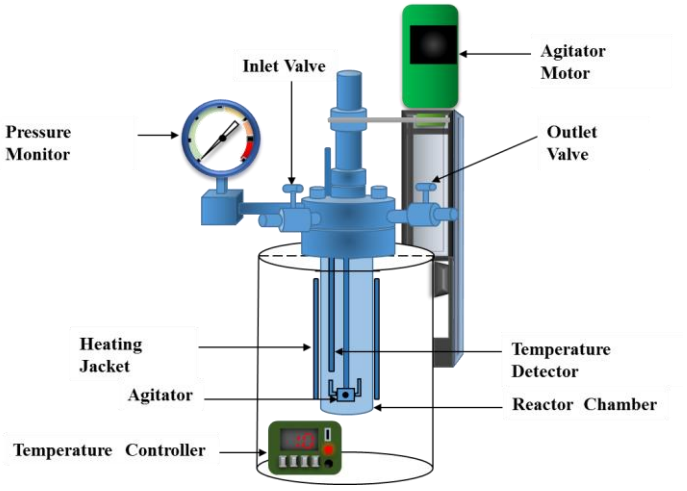


Fig. 2-1 A batch-type reactor autoclave (model MMJ500)

2.2.2 Methods

In order to study the effect of the reaction temperature, a series of the reaction temperature change (190^{o}C, 210^{o}C, 230^{o}C and 250^{o}C) was firstly conducted at a constant reaction time of 60 min to observe only the influence of the reaction temperature. Then, a range of the reaction time (30 min and 90 min) was employed followed that of the 60 min reaction time set. The 250^{o}C reaction temperature was selected to be the maximum temperature applied as prior research [2-8], [2-18]-[2-19] showed that HTT at this temperature is possible to optimize the bio-oil yield. Moreover, the dielectric constant (relative permittivity) of subcritical water at 250^{o}C, 27.1 F/m, shows a lower value than for water at normal standard environment, 78.5 F/m at nearly one-third [2-20] which means that more molecules will tend to dissociate in water at 250^{o}C. Besides, the 30 min was fixed as the minimum reaction time because the results in [2-8] showed that this reaction time was optimal.

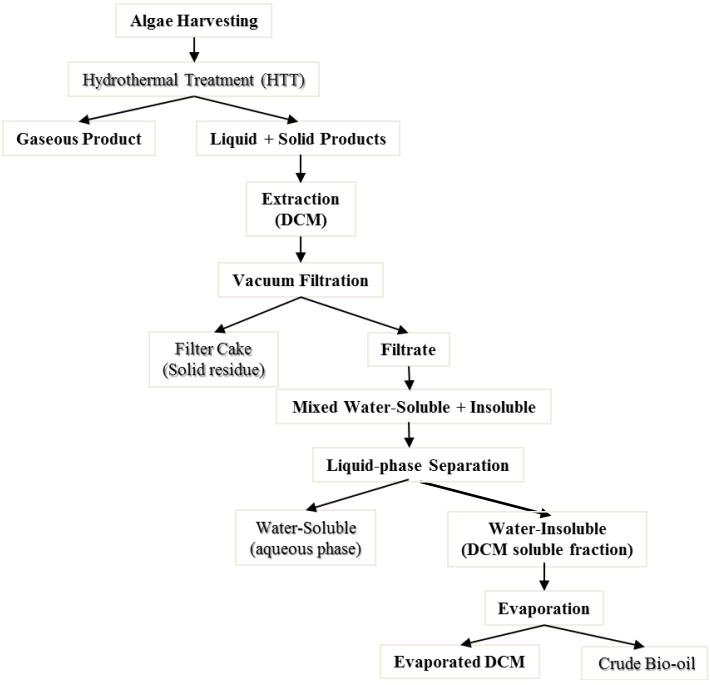


Fig. 2-2 HTT products collecting procedure

The algae concentration was fixed at 20 wt% for the entire study. An amount of 80 g distilled water was added to each batch-type reactor tube in order to meet a 100 g – sample size, thus, there was no interruption of the algae concentration. Then the mixture was thoroughly mixed and put into the autoclave. Argon gas was purged into the headspace of the reactor for 2 min in order to make an oxygen-free environment so as to avoid the combustion reaction. The agitator equipped with an impeller was operated at the speed of about 200 rpm. After that the reactor was heated to the desired temperature with a ramped rate of 4.7^{o}C/min and was kept at that temperature for 60 min and then allowed to cool down to the room temperature. The gaseous product was then depressurized before opening the reactor. The product was collected and mixed with 100 mL-dichloromethane (DCM; Sigma-Aldrich, 99% purity), which included the part for washing and then transferred for vacuum filtration using a 1.2μm pore size-glass microfiber filter paper (GF/C, Whatman). The filtered algal residue was defined as solid residue and was separated for further analysis. The two-phase mixture was then separated for the DCM-soluble phase (bottom part) and the DCM-insoluble phase (top part) by an auto-pipette (Gilson Pipetteman). The aqueous phase (DCM-insoluble phase) was further analyzed for nutrient recovery. The DCM-soluble phase was naturally evaporated at the room temperature in a fume hood for about 3 days to remove the DCM-solvent and the remaining product (DCM-soluble liquid) was defined as the bio-oil and was further characterized. All of the experiments were conducted in triplicate

batch reactions and the average values were reported. The overall procedure for collecting and separating the HTT products is illustrated in Fig. 2-2.

2.2.3 Analysis

The microalgae feedstock was first determined to evaluate the chemical composition and the proximate and the ultimate analysis were done (Table 2-1). The crude lipid content of the microalgae was determined by the Bligh and Dyer method used by TISTR. The moisture and the ash content, and the volatile matter were analyzed by the ASTM D3173, ASTM D5142 and ASTM D3175 method respectively, whereas the fixed carbon was calculated by subtraction. The ultimate analysis, CHNS, was characterized by the elemental analyzer (Vario MICRO Cube, Elementar Inc.) following the ASTM D5291, D3176 method. Higher heating value was analyzed by the bomb calorie meter, OSK200-model (Ogawa Sampling Inc., Tokyo), following the ASTM D5864 method. Trace elementals were measured by the ICP emission spectroscopy (ICPS-8100, Shimadzu Inc.) after being digested by the MultiWave3000, Perkinelmer Inc and the results are listed in Table 2-2. The electrical conductivity (EC) and pH of the aqueous product were measured by the desk-type meters (F-70/DS-70 series, Laqua Inc.). The functional groups of the bio-oil were analyzed by the Fourier transform-infrared (FT-IR) spectroscopic analyzer (SPX200, JEOL Inc.)

Table 2-1 Characteristics of microalgae TISTR-8511 strain

Proximate analysis (wt%)			Chem. comp. (wt%)		Ultimate analysis (wt%)[a]					High Heating Value (MJ/Kg)
Moist	VM	FC	Ash	Lipids	C	H	N	O	S	HHV
5.57	65.42	2.69	26.32	22.55	38.26	4.96	4.51	51.02	0.36	16.17

[a] On dry basis, VM = volatile matter, FC = fixed carbon, Chem. comp. = biochemical composition

Table 2-2 Available nutrients in algae feedstock

Feedstock	Available nutrient, %											
TISTR-8511	P	K	Mg	Ca	Fe	Na	Si	B	Mn	Zn	Cu	Co
	0.50	0.41	2.47	11.59	0.27	0.99	3.45	0.0271	0.0715	0.0247	0.0104	0.0047

Yields of the bio-oil, solid and aqueous residues were calculated by Eq.2-1, whereas the yield of the gas was defined followed Eq.2-2, and loss was defined as Eq.2-3. Energy recovery was calculated by Eq.2-4 and elemental distribution was calculated by Eq.2-5.

Yield (wt%) = (Mass of product fraction / Mass of initial feed) x 100% Eq.2-1

Yield of gas (wt%) = Total weight after HTT - Yield of (Bio-oil + Aqueous residue + Solid residue) Eq.2-2

Loss (wt%) = 100 – Yield of (Bio-oil + Aqueous residue + Solid residue + Gas) Eq.2-3

Energy recovery (%) = (HHV_{oil} x $Mass_{oil}$ / HHV_{algae} x $Mass_{algae}$) x 100% Eq.2-4

Elemental distribution (%) = (Mass of element in product fraction / Mass of element in dry algae) x 100% Eq.2-5

2.3 Results and Discussions

This study investigated the influences of the reaction temperature (190-250^{o}C) and the reaction time (30, 60 and 90 min) on the HTT products.

2.3.1 Effects of the HTT reaction conditions on products yield

Fig. 2-3 shows the effects of the reaction conditions on the products' yield (bio-oil, solid residue, aqueous residue, gas and loss) where (a) presents the effects at 30 min reaction time, (b) at 60 min reaction time and (c) at 90 min reaction time. Fig 2-3-(a) shows that the bio-oil yield was increased with the increase of the reaction temperature when the reaction time was kept stable at 30 min. However, at too high temperature, the bio-oil yield was declined. It can clearly be seen that the bio-oil yield was increased from 2.45% at 190^{o}C to 3.04% at 210^{o}C and 3.39% at 230^{o}C then slightly dropped to 3.27% at 250^{o}C. A similarity was found on the aqueous yield as well. The aqueous

yield showed the increasing trend from 69.87% at 190°C to 72.16% at 230°C, and turned to 72.0% at 250°C. Nonetheless, the aqueous yield at 210°C showed an unusually high apart from others, thus the gas yield at this temperature was also irregularly lower than others. Anyhow, the gas yield showed an increasing trend with the increase of the reaction temperature except for the one at 210°C. The solid yield, on a contrary, showed a reversal trend with all those products' yield. It started to decrease from 9.97% at 190°C to 8.85% at 210°C, and 8.54% at 230°C, and finally went back up to 9.15% at 250°C.

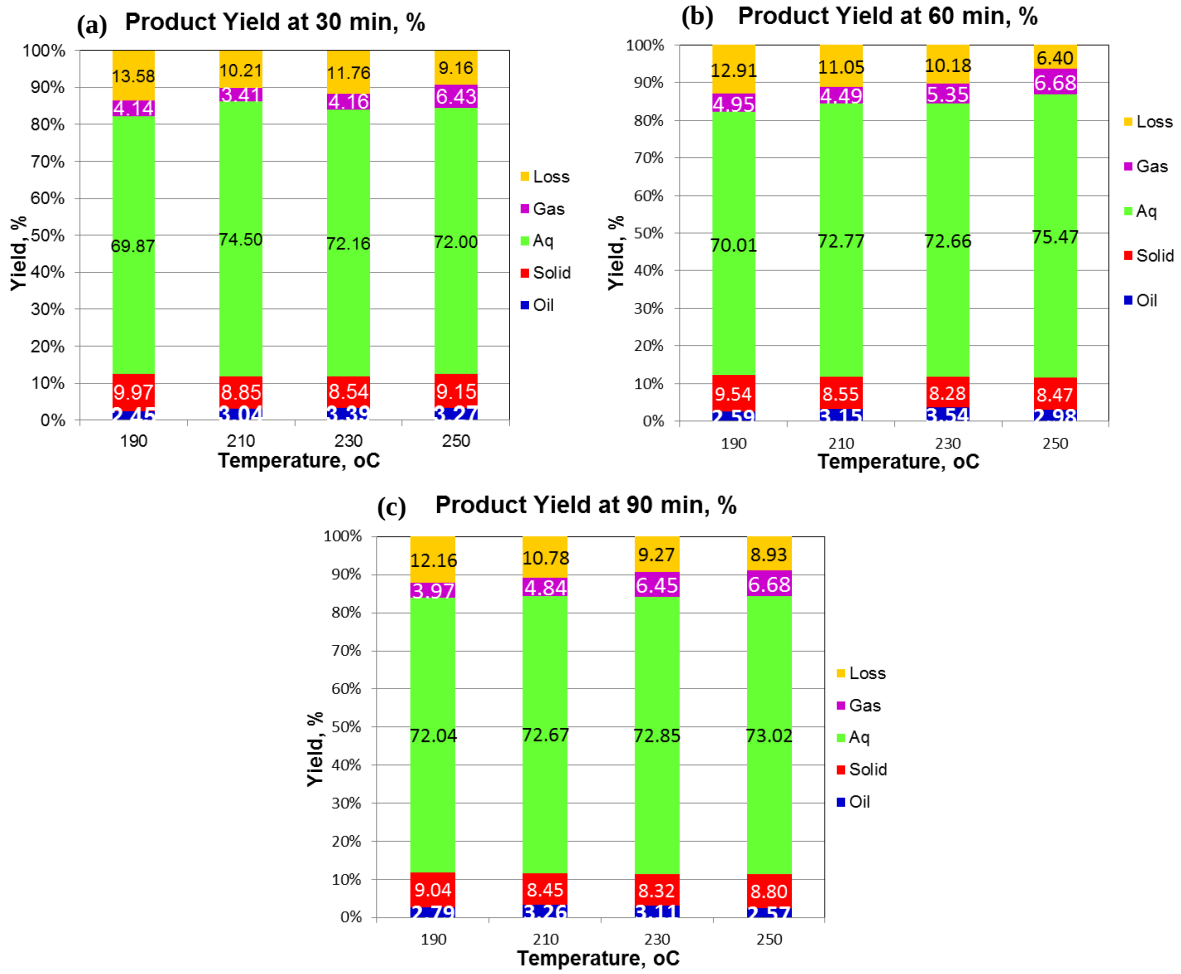


Fig. 2-3 Effects of the reaction conditions on HTT products at (a) 30 min, (b) 60 min and (c) 90 min reaction time

This is due to the reaction scheme of biomass liquefaction described in [2-8], [2-10]-[2-11], [2-18]-[2-19], [2-21]-[2-23]. When the temperature increases, it simultaneously increases the active energy of the bonds inside the biomass; hence, the depolymerization occurs at first. As a result, the concentration of the free radicals and the probability of repolymerization of the fragmented molecules also increase. Therefore, the formation of the bio-oil and char are promoted, where the formation of the bio-oil tends to be higher than that of the char at the intermediate temperatures. After the hydrolysis of biomass macromolecules such as lipids, proteins and carbohydrates into smaller molecules, some conversion reactions may occur such as the dehydration, the deoxygenation, the decarboxylation and the deamination. Then the fragmented molecules are rearranged to produce new compounds via condensation, cyclization and polymerization. However, the variety of the biomass (varying in biochemical composition) leads to the different suitable temperature for the bio-oil production. Besides, a previous study [2-20] based on experiments of temperatures ranging from 250°C to 550°C showed that the bio-oil yield were maximum at subcritical temperatures within this range but over this range, the maximum yield was decreased. Additionally, Fig.

2-4 showing the FT-IR spectra band of bio-oil at 60 min reaction time also indicated that the intensity of the C-H stretch group which belongs to the presence of lipid and is shown in the orange bands, was first increased from 190°C (in the red line) to 230°C (in the blue line), and then decreased at 250°C (in the dark green line). This means that the oil yield was firstly increased from 190°C and after reached the maximum at 230°C, it decreased at 250°C.

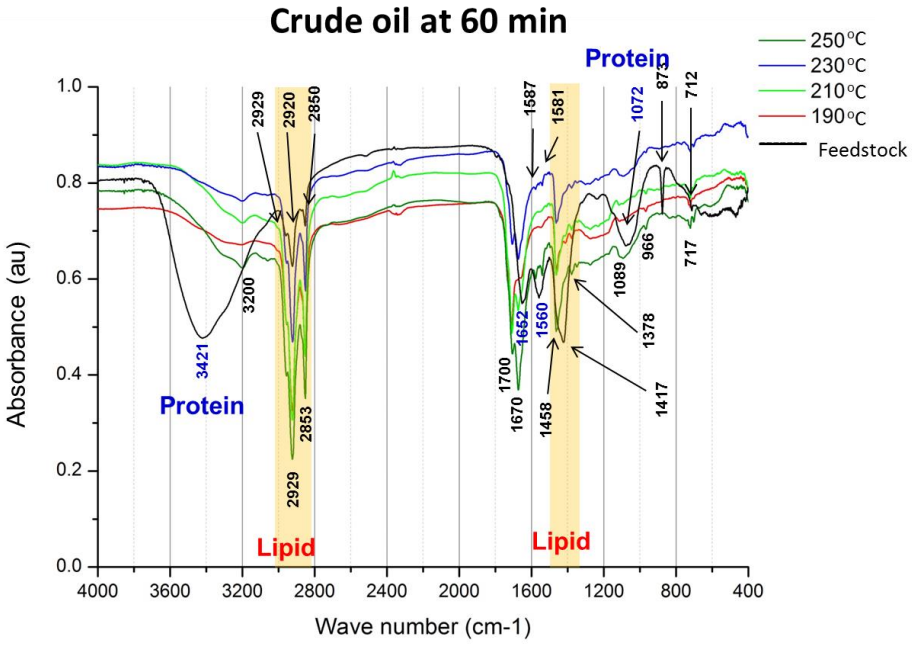


Fig. 2-4 FT-IR spectra band of crude bio-oil at 60 min reaction time

Table 2-3 FT-IR functional group

Conditions	Wave number (cm^{-1})	Functional group
Feedstock	3421, 1072	N-H Stretch/2° Amines
	2929, 2920, 2850	C-H Stretch/Alkyl
	1652, 1560	N-O asymetric stretch/Nitro compounds
	1417	C-H Stretch/Alkane
	873, 712	=C-H Bend/Alkenes
Bio-oil	3200	O-H Stretch/Alcohol
	2929, 2853	H-C-H Stretch/Alkyl
	1700, 1670	C=O Stretch/Ketones, Aldehydes, Carboxylic acids
	1587, 1581	C-C=C Stretch aromatic ring, N-H bend
	1458, 1378	C-H Stretch/Alkanes
	1089, 966	C-O Stretch/1°, 2°, 3° Alcohols
	717	O-H bend/Phenol, Esters, Ethers, Aromatic compounds

Moreover, Table 2-3 also shows the functional groups found in the bio-oil compared to the feedstock. It was obviously seen that most of the peaks found in the feedstock belonged to protein and lipid. Two big peaks of N-H stretch found at 3421 and 1072 cm^{-1} and another two medium peaks found at 1652 and 1560 cm^{-1} showed the protein content in the algae feedstock whereas, one big peak at 1417 cm^{-1} and several small peaks at 2929, 2920 and 2850 cm^{-1} were found as the lipid content. There were also various peaks of lipid found as C-H stretch in the bio-oil at 2929, 2853, 1458 and 1378 cm^{-1}. Furthermore, there were found N-compounds and carboxylic acids as a result of protein conversion. In the same time, ketones, phenol and alkyl groups were also found in the bio-oil as a

consequence of carbohydrate conversion. Finally, the O-containing compounds represent the bio-oil formation such as alcohols, aldehydes, carboxylic acid, ketones and esters were found at 1700, 1670, 1089, 966 and 717 cm^{-1}.

More examples of this hypothesis can be observed in Figs. 2-3-(b) and (c). Increasing the reaction temperature from 190^{o}C to 250^{o}C showed that the macromolecules in the algae biomass were hydrolyzed into smaller fragmented species in the water-soluble phase. These smaller fragmented species were then converted to a new species in bio-oil and gas (as the bio-oil yield was increased, whereas the solid residue was decreased) via some of these reactions, the dehydration, the deoxygenation and the decarboxylation. After reaching the maximum, the bio-oil yield decreased as other reactions occurred to inhibit the formation of the bio-oil. At higher temperatures, the secondary decomposition and the Bourdard gas reaction play more significant roles in gas formation [2-19]. The free radicals, which have high concentration, are recombined to form the char. Consequently, the production of bio-oil is reduced at high temperatures as can be seen in Fig. 2-3-(b) when 60 min reaction time was employed. However, at high temperature accompanied with a long reaction time, these phenomenon were more promoted as noticeable at 230^{o}C and 250^{o}C conducted at 90 min reaction time that the oil yield was already decreased.

Figs. 2-3 (a), (b) and (c) show that the oil yields were increased with a longer reaction time (at 190^{o}C and 210^{o}C), however, at a too high temperature, they were declined (at 230^{o}C and 250^{o}C). At 190^{o}C, the oil yields were gradually increased from 2.45% to 2.59% and finally reached 2.79% at 30 min, 60 min and 90 min reaction time respectively. Likewise the oil yield at 210^{o}C also increased from 3.04% to 3.15% and stopped at 3.26% when the reaction time increased from 30 min to 90 min. Similar to the yield at 230^{o}C, it showed a slight increase from 3.39% to 3.54% when the reaction time was prolonged from 30 min to 60 min. However, beyond 60 min reaction time, the yield was decreased to 3.11% (at 90 min reaction time). Unlike the one at 250^{o}C, the oil yield was reversed. It was surprising that the oil yield at this temperature was declined step by step, from 3.27% to 2.98% and laid down at 2.57% with the increase of the reaction time. It might be a result of severe condition that promoted the 2nd decomposition and the Bourdard gas reaction faster than the mild condition. Hence the bio-oil yield of 250^{o}C was gradually declines. Anyhow, the solid yields at all reaction times were found similar to that of the bio-oil yield but on the reverse side. The yield of aqueous residues, on a contrary, was found to show reversal behavior. It did decrease with the increase of the reaction time at 190^{o}C and 210^{o}C, whereas it increased at 230^{o}C and 250^{o}C. The aqueous yield at 250^{o}C and 60 min reaction time showed an unusually high value which might be a result of the lower loss. Nonetheless, most of the gas yields showed that they were increased with the reaction time except for the one at 190^{o}C, 90 min reaction time, which might be caused by a slightly higher aqueous yield than those at a shorter reaction time.

The longer reaction time could prolong the time for the macromolecules in microalgae being hydrolyzed into micromolecules and then turned into other substances. It seems that the yields of the HTT products were increased while the raw material was decreased. The increased aqueous yield observed at 230^{o}C and 250^{o}C may similarly be explained by the same reactions promoted by HTT [2-11] that more water molecules would be produced during the dehydration reaction since the oil yield was decreased.

2.3.2 Effects of the HTT reaction conditions on bio-oil compositions

Table 2-4 shows the elemental compositions and properties of the bio-oil after HTT for both the reaction time and the reaction temperature parameters. At 30 min reaction time, the carbon and hydrogen contents showed slight increase with the temperature increase, whereas the oxygen content was decreased. This is likely due to the deoxygenation reaction which is the key mechanism of HTT. Moreover, the decrease of oxygen content was more significant at a high temperature as can be seen that the oxygen content at 250^{o}C was lower than that at 190^{o}C as a consequence of the deoxygenation reaction at a higher temperature. Thereafter, the carbon and hydrogen contents were simultaneously increased as a promotion of the dehydrogenation and decarboxylation reactions. The oxygen content, moreover, was significantly decreased almost 2 times of that in the algae feedstock. On another hand, the carbon and hydrogen contents were 2 times increased. As a result, the high heating value (HHV) was doubling increased from the algae feedstock as well. Nevertheless, the HHV did not increase with the decrease of the oxygen content as a consequence of the increase of the ash content, which was due to the easy formation of char at a higher

temperature. The sulfur content showed a slight increase with the increase of the reaction temperature, while its content was relatively low, ranged in 0.4-0.6 wt%. It was found that the sulfur content may be inherent to the algal species [2-18]. The nitrogen content, on the other hand, increased significantly from 1.98 to 4.72 wt% when the reaction temperature increased from 190°C to 250°C. It can be assumed that the deamination reaction preferably occurred at a high temperature which promoted the recombination of these nitrogenous compounds to form hydrocarbon chains [2-20], [2-24]. The increase of the nitrogen content in the bio-oil is undesirable since it promotes NOx formation when used as a transportation fuel. So some pre-treatments should be applied. The energy recovery (ER), like neither elements, it did not followed any element but just the oil yield as of its definition in Eq.2-4stated in the analysis section above. Anyhow, the ER relied more on the oil yield rather than its HHV as illustrated in Fig. 2-5.

Table 2-4 Bio-oil elemental compositions and properties after HTT

Reaction time	T (°C)	C (wt%)	H (wt%)	O* (wt%)	N (wt%)	S (wt%)	Ash (wt%)	HHV (MJ/Kg)	ER (%)
30 min	190	70.92	10.84	14.47	1.98	0.43	1.47	35.61	26.91
	210	69.66	10.56	14.14	3.16	0.47	1.67	34.05	32.09
	230	68.98	10.24	13.71	4.00	0.53	2.27	33.61	35.16
	250	67.00	9.68	13.74	4.72	0.47	4.40	32.66	33.03
60 min	190	70.35	10.52	14.46	2.46	0.41	1.13	36.13	28.90
	210	70.04	10.50	13.93	3.60	0.47	1.40	34.51	33.62
	230	68.89	10.14	13.31	4.25	0.51	2.90	34.12	37.30
	250	69.49	9.86	11.89	5.43	0.58	2.80	33.24	30.62
90 min	190	70.16	10.84	14.48	2.76	0.43	1.20	33.60	28.96
	210	70.42	10.38	13.76	3.84	0.50	1.37	32.92	33.23
	230	69.12	10.08	12.35	4.60	0.51	3.33	32.74	31.47
	250	70.96	10.14	10.77	5.47	0.56	2.37	32.49	25.83

* calculated by difference, ER = Energy recovery

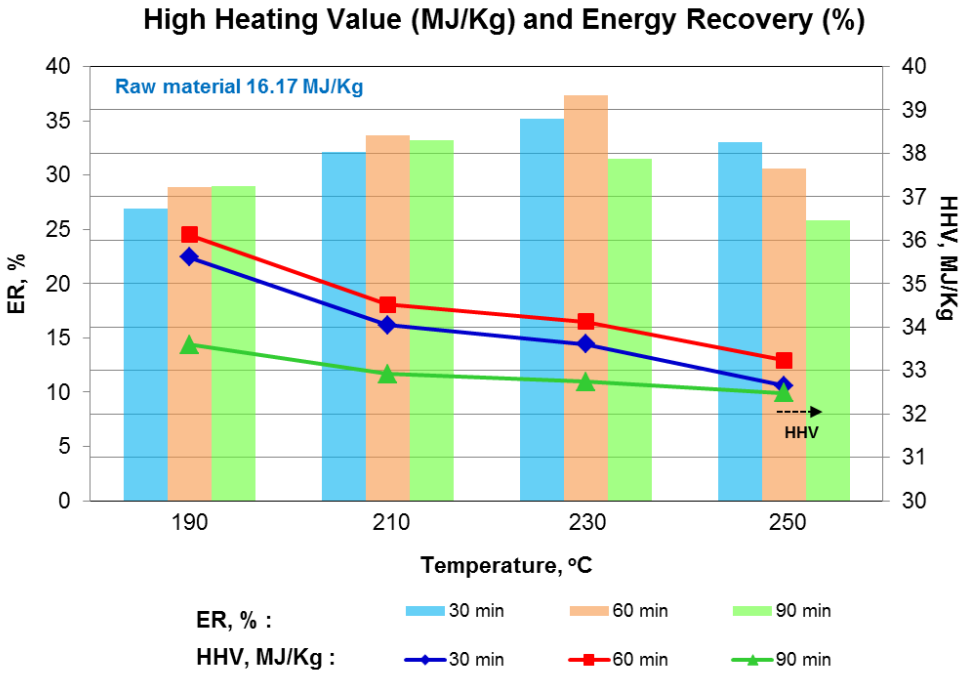


Fig. 2-5 HHV and ER of bio-oil after HTT

Regarding the increasing of the reaction time (from 30 min to 90 min), the oxygen and nitrogen contents solely showed the incisively decreasing and increasing respectively, whereas the others showed some fluctuations of the

increasing and decreasing. However, the HHV firstly showed the increasing trend with the reaction time as the decrease of the oxygen content from 30 min to 60 min reaction time. Later, the HHV slid down when the reaction time was prolonged to 90 min influenced by various factors. Additionally, most of the ER values merely followed their oil yields except for the one at 210°C that was slightly decreased when the reaction time was extended from 60 min to 90 min.

2.3.3 Effects of the HTT reaction conditions on aqueous residue

Fig. 2-6 shows the pH and EC values of the aqueous residue after HTT. This figure distinctively displays that the EC and the pH values were found to be increasing with the increase of the reaction temperatures. Since high temperature promotes high active energy of macromolecules in the algae biomass, it contributed more for the aqueous. Therefore, high EC values were noticeable when the temperature increased as the decomposed ionic molecules were water-soluble. An explanation for the pH value is similar, but its change was more. The relatively low pH value at 190°C showed that some free hydrogen ions were desirably formed at low temperatures. According to the previous studies [2-24]-[2-28], the protein, lipid and carbohydrate contents of algae were hydrolyzed into amino acids, fatty acids, organic acids and their derivatives via some of the reactions explained in section 2.3.1, hence, the pH was first low in the acidic. Later, these fragmented molecules were consumed by other reactions, thus, the pH were gradually increased to the basic.

The longer the reaction time, the higher the pH was as a result of longer time for the fragmented molecules to be released, dissolved and consumed. However, the reaction time had more influence on the pH value at a higher temperature as can be seen from Fig. 2-6, more severity of the pH was found at 250°C and followed by 230°C rather than at 190°C and 210°C. This was not only due to the prolonging time of the reaction, but also due to the more active energy that the algae received at a higher temperature. Unlike the EC value, the reaction time seemed to show no distinct influence. At a lower temperature (190°C), the reaction time gave a negative effect on the EC value, whereas it gave a positive one at a higher temperature (230°C). The reason is that some elements exhibited lower ability to conduct an electrical current through it, i.e. P, S, Si and Fe, as a result, it may prohibit the dissolved materials into the water somehow [2-24]-[2-28].

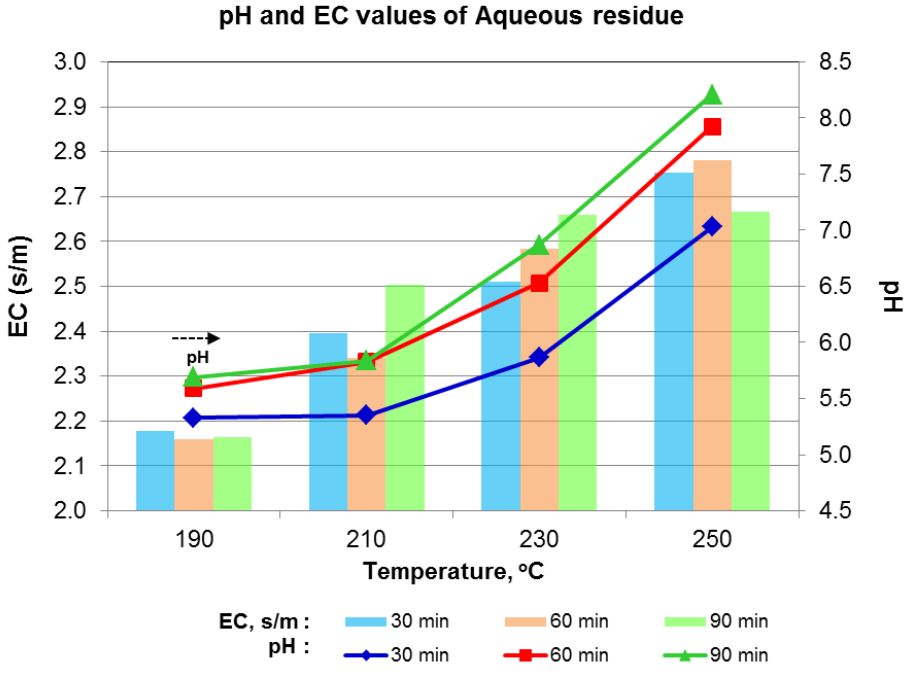


Fig. 2-6 pH and EC values of the aqueous residue after HTT

Table 2-5 Available nutrients in aqueous residue after HTT

		30 min, °C				60 min, °C				90 min, °C			
	Rodik	**190**	**210**	**230**	**250**	**190**	**210**	**230**	**250**	**190**	**210**	**230**	**250**
EC (S/m)		2.18	2.40	2.51	2.75	2.16	2.34	2.58	2.78	2.16	2.50	2.66	2.67
pH		5.33	5.35	5.86	7.03	5.59	5.83	6.53	7.92	5.69	5.84	6.87	8.21
		(mg/L)											
N	49.41	7260	7891	8134	9588	7437	8177	8516	9484	7899	8064	8503	8444
K	13.72	371	375	384	393	497	499	517	522	498	513	522	526
P	8.93	808	553	163	16	821	547	72	3	703	413	28	1
Mg	1.97	1600	1500	1400	1200	1600	1500	1200	1100	1500	1500	1200	910
Ca	17.09	1200	1100	1100	1000	1100	1100	1000	1200	1200	1100	1100	1300
Fe	3.52	46	40	28	16	46	40	19	11	44	34	20	9
Na	7.86	2400	2500	2400	2500	2500	2500	2500	2600	2100	2500	2700	2700
B	0.05	48	40	42	42	44	44	42	42	44	43	43	42
Mn	0.49	37.0	27.0	14.0	5.7	32.0	22.0	9.4	5.9	28.0	18.0	7.7	4.8
Zn	0.02	5.50	3.10	1.30	0.46	4.40	2.90	0.68	0.19	3.50	2.20	0.53	0.11
Mo	0.02	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Cu	0.02	0.12	0.08	0.13	0.11	0.20	0.16	0.14	0.15	0.14	0.19	0.17	0.17
Co	0.06	0.30	0.23	0.17	0.14	0.24	0.30	0.12	0.14	0.26	0.19	0.12	0.17

* EC = Electrical conductivity, Rodik is an algal medium, n/d = not detected

The available nutrients in aqueous residue are shown in Table 2-5 where the Rodik algal media was referred for a comparison of the required nutrients for algae growth. The result shows that the concentrations of most of the available nutrients left in the aqueous residue were higher than the concentration in the Rodik medium except for the P-major nutrient at 250°C and the Mo-trace metal nutrient. Therefore, this aqueous residue is expected to be used for algae cultivation. However, most of the nutrient concentrations decreased when the reaction temperature was increased except for N, K and Na contents. These decreasing nutrients, finally, were found in the solid phase. The increase of the N-major nutrient with the rise of the temperature is likely due to more hydrophilic nitrogenous compounds were released from proteins after the hydrolysis at a high reaction temperature in a no oxygen environment. Ammonia, a derivative form of amino acids, then was converted to ammonium rather than nitrate, and dissolved into the water phase [2-29]. Hence, the N concentration in the aqueous phase was increased with the increase of the reaction temperature. Regarding this phenomenon, the hydroxide ions (OH^-) were simultaneously produced and released into the aqueous phase. Thereafter, the pH of the aqueous was gradually increased which was eminently observable in Fig. 2-6. As a result, the concentration of the P major-nutrient was drastically decreased with the increase of the reaction temperature as the pH value. Since phosphate is transported by the phosphate specific transport (Pst) system in the form of $H_2PO_4^-$ and HPO_4^{2-} (the predominant phosphate species over the pH range from 5.0 to 9.0), the proportion of $H_2PO_4^-$ decreased with the increase of pH [2-30]. Unlike neither N nor P, K was always presented in minerals as a single-charged cation (K^+) which was rapidly dissolved into the aqueous phase then there was mostly found in the aqueous phase [2-31]. When more active energy was introduced as a result of increasing the reaction temperature, K^+ could be more dissolved as the polarity of water is increased by heating. The Ca nutrient is an essential part of plant cell wall structure [2-33], and it was found relatively high regardless of the reaction temperature or the reaction time. The Mg nutrient which is a part of chlorophyll in all green plants and essential for photosynthesis [2-32], also was found to be high. However, it showed a little declination with the increase of the reaction temperature as a higher temperature shifted it to the solid phase.

In terms of the reaction time, the N and K nutrients were influenced still. The longer reaction time had more influence on the K nutrient when a shorter reaction time was applied and its concentration was much increased from 30 min to 60 min reaction time, whereas the increasing was small when 60 min was prolonged to 90 min. However, all of them had been tried to reach the maximum amount of the available K nutrient in the algae feedstock at 544 mg/L. Therefore, it could be understood that when the released concentration was nearly reached the available concentration (at 60 min), prolonging the reaction time would have less effect (at 90 min). As for the N nutrient, it could be assumed that the peptide bonds in the proteins were decomposed more when a longer reaction time was available then released some more amino acids and other short-chain organic acids. This amino acids could be further decomposed into ammonia, combined with water then released the ammonium ion and also shifted the pH value. Thus, the increasing value could be observed step by step. Due to the increase of pH when the reaction time was further prolonged, the P nutrient was gradually decreased.

2.3.4 Effects of the HTT reaction conditions on solid residue

Table 2-6 Available nutrients in solid residue after HTT

Nutrients, %	Australian std.*	30 min, °C				60 min, °C				90 min, °C			
		190	210	230	250	190	210	230	250	190	210	230	250
N	0.5	3.89	2.83	2.18	1.74	3.32	2.53	1.96	1.53	3.06	2.33	1.91	1.68
P	0.5	0.54	0.73	0.96	1.09	0.57	0.82	1.04	1.08	0.70	0.99	1.22	1.28
K	0.5	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S	0.5	0.40	0.28	0.31	0.29	0.47	0.39	0.60	0.34	0.53	0.31	0.31	0.35
Ca	0.5	17.98	21.48	21.16	21.04	18.98	21.24	23.26	21.30	19.93	21.94	22.46	21.08
Mg	0.5	2.64	2.86	3.13	3.63	2.53	3.46	3.96	4.06	3.03	3.45	4.20	4.46
Si	0.5	5.75	7.40	6.49	7.25	6.83	2.71	7.92	8.37	5.80	4.93	3.95	2.48
Fe	0.1	0.70	0.76	0.77	0.75	0.58	0.69	0.62	0.86	0.68	0.64	0.72	0.64
Cu	0.05	0.019	0.004	0.020	0.023	0.030	0.020	0.032	0.030	0.033	0.032	0.037	0.035
Mn	0.05	0.115	0.134	0.142	0.128	0.127	0.140	0.139	0.147	0.124	0.138	0.153	0.154
Zn	0.05	0.050	0.053	0.055	0.060	0.051	0.059	0.059	0.058	0.048	0.052	0.059	0.060
B	0.02	0.024	0.024	0.016	0.044	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Mo	0.001	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Co	0.001	0.0024	0.0222	0.0029	0.0041	0.0038	0.0037	0.0037	0.0033	0.0043	0.0042	0.0044	0.0042
Se	0.001	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

* Australian government standard for solid fertilizer, Fertilizer working group, Department of Agriculture, 2011.

Table 2-6 shows the available nutrients left in the solid residue after HTT compared to a solid fertilizer standard of the Australian government (Aus. std.) which are shown in wt%. For the primary nutrients, N, P and K, it can obviously be seen that only K had lower concentration than the Aus. std. which almost shifted from the solid phase, whereas, N and P had higher concentrations. However, N showed the decreasing trend with the increase of the reaction temperature, while P showed the opposite trend. The decreasing trend of N was likely due to the form of N which was in a water-soluble form hence it was moved into the liquid phase, and some parts shifted to the oil phase. The presence of P was mostly dependent on pH of the product. With the increase of pH of the aqueous product, P shifted from the aqueous phase to the solid phase. The vanishing of the K nutrient, nonetheless, was a result of its form (K^+) that was quickly dissolved into the liquid phase. For the case of secondary nutrients, S, Ca, Mg and Si, the result showed that just the S nutrient had lower concentration than the Aus. std. and showed no relationship with the reaction temperature. Since the available S nutrient in the algae feedstock was relatively low, as a consequence, the left concentration was also low. On the contrary, the Mg nutrient showed an increasing trend with the increase of the reaction temperature while the Ca was increased to the maximum at 230^{o}C and slightly dropped beyond that temperature. The correlation between these secondary nutrients and the reaction temperature, so far, has not yet clearly been studied. Regarding to the micro nutrients, Fe, Cu, Mn, Zn, B, Mo, Co and Se, they varied more or less with the reaction temperature, which Fe, Mn, Zn and Co were found to have higher amount than the Aus. std.

For the influences of the reaction time, the N and K nutrients were behaved similar to that of the influence of the reaction temperature where N decreased and P increased with the increase of the reaction time. In any case, the reaction time showed no significant effect on the macro and micro nutrients.

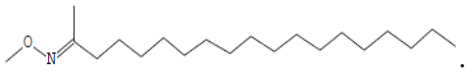
2.3.5 Effects of the HTT reaction conditions on elemental distribution

2.3.5.1 Nitrogen distribution

Fig. 2-7 illustrates the distribution of elemental nitrogen in HTT products at (a) 30 min, (b) 60 min and (c) 90 min reaction time. As a consequence of increasing the reaction temperature, for example, Fig.2-7-(a), the N-compounds macromolecules were hydrolyzed more and small fragmented N-micromolecules like amino acids were hydrolyzed and further repolymerized into ammonia. Without the presence of oxygen, the ammonia was combined with water molecules then ammonium ion was produced and released hydroxide ion. The ammonium ion is an exchangeable form which can be stayed in either liquid or solid state. However, it prefers staying in an aqueous state. The nitrogen concentration in the aqueous phase (blue bar), thus, was increased and the solid phase (red bar) was decreased.

$$\text{Protein} \rightarrow \text{Amino acids} \rightarrow NH_{3\,(g)} + H_2O \rightarrow NH_4^{+}{}_{(aq)} + OH^-$$

Unlike the solid and liquid states, more severe conditions promote the Fischer-Tropsch type reaction to repolymerize some small organic nitrogen to longer chain HC and aromatic ring-types structure, therefore, higher N content could be found in the oil phase at a high temperature [2-33]. All possible N-compound structures having the similarity index of ≥ 75% found in the bio-oil are shown in Table 2-7. For example, there was one N-compound found at 230°C and 30 minutes. This compound was found at the order of 24th of the total 50 similarity compounds search. There was only one possible N-compound hint found for this compound and it was found as the 1st hint from the total 5 hints search with 81% similarity index with the peak area of 0.23%. It was named as 2-Nonadecanone, O-methyloxime (2E)-2-Nonadecanone o-meth and had the molecular structures as



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The N concentration in the aqueous phase increased more or less when the reaction time was prolonged, whereas it gradually decreased in the solid phase except for the case at 250°C, 90 min reaction time as can be observed in Fig.2-7-(a)-(c). This may be caused by the irregular decrease of N at 90 min reaction time in the aqueous phase that was 10% lower from the previous 60 min reaction time. Therefore, the N concentration in the solid phase was unusually increased rather than decreased. The N concentration in the oil phase, nonetheless, was step by step increased with the reaction time. Despite that, the N concentrations at 230°C and 250°C were slightly decreased when the reaction time was further increased from 60 min to 90 min. This was likely due to the less oil yield at more severe conditions (higher temperature and longer time) as previously shown in Fig. 2-3.

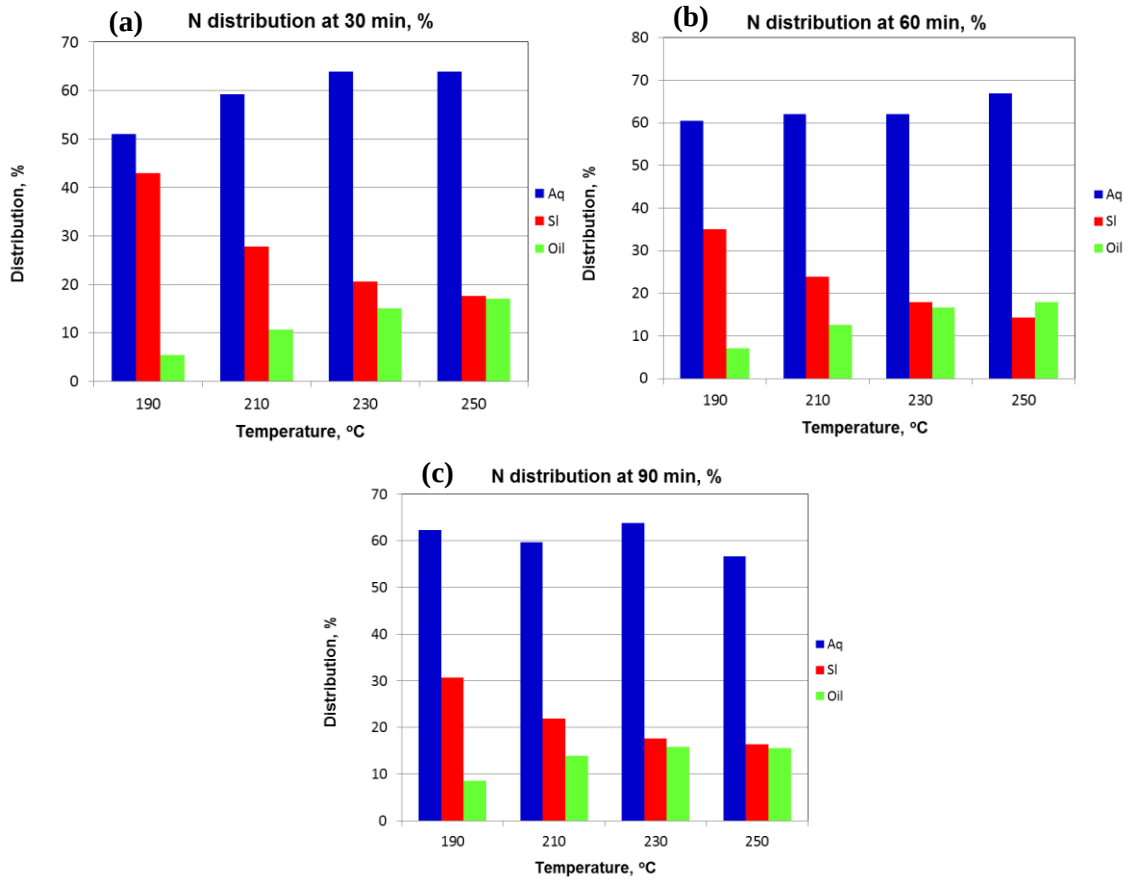
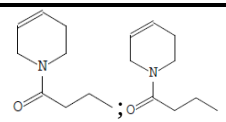
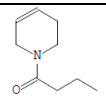
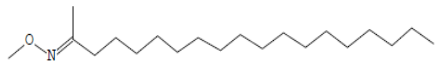
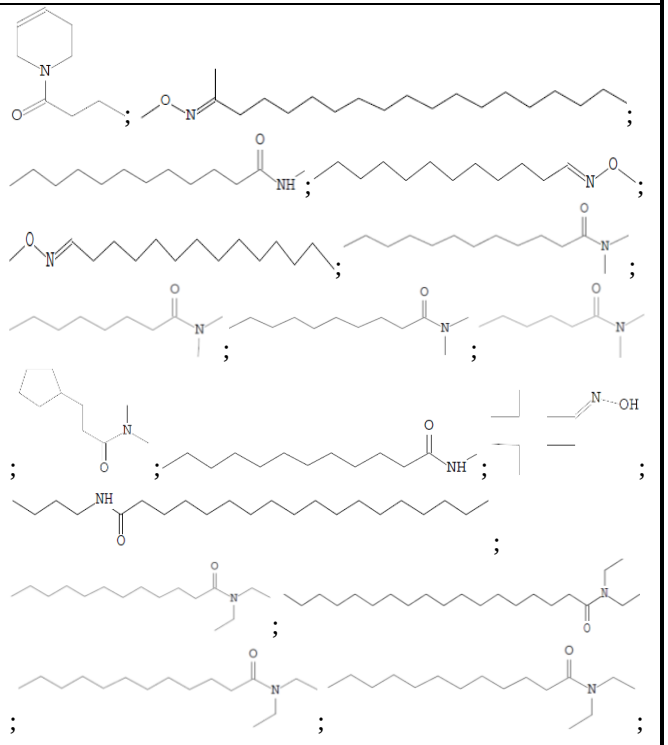
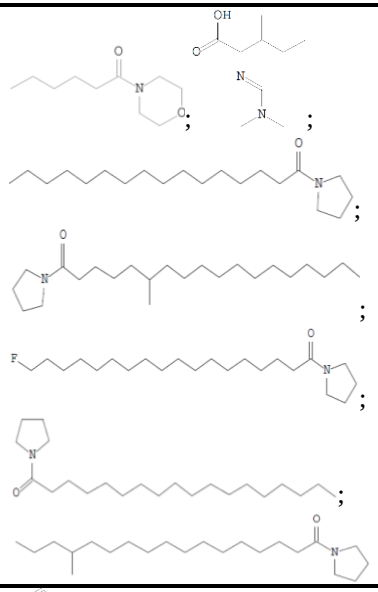
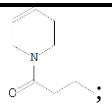
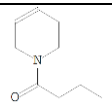
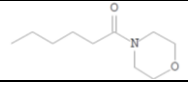
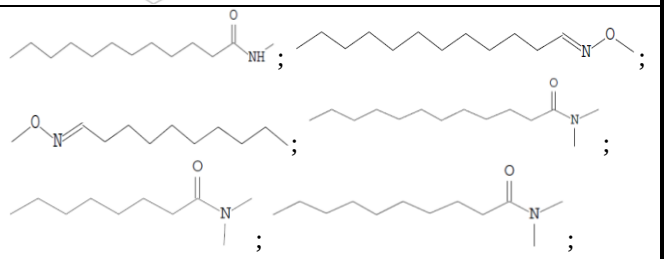
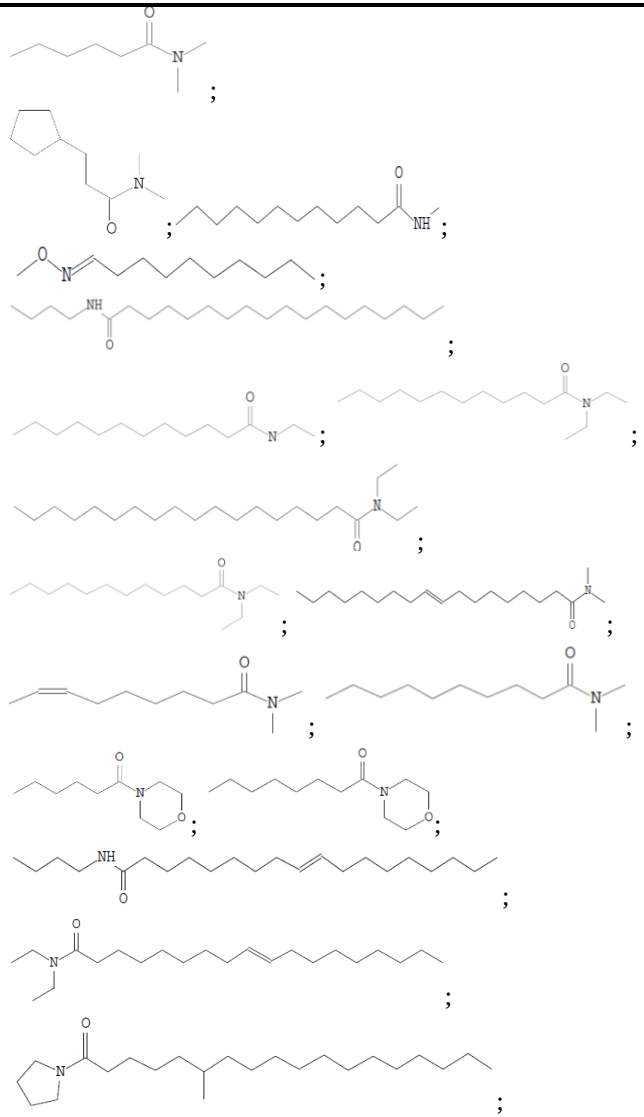


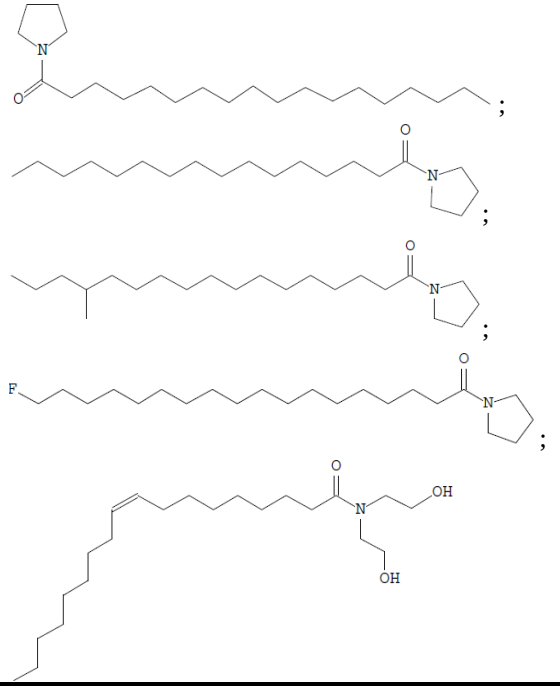
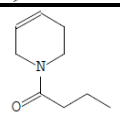
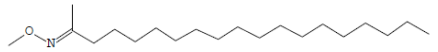
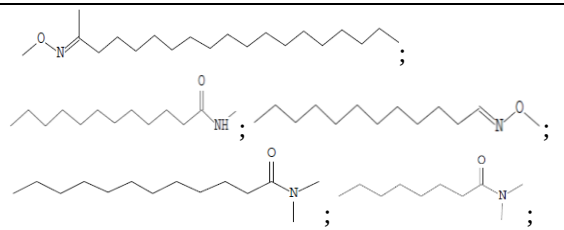
Fig. 2-7 Nitrogen distribution in HTT products at (a) 30 min, (b) 60 min and (c) 90 min reaction time

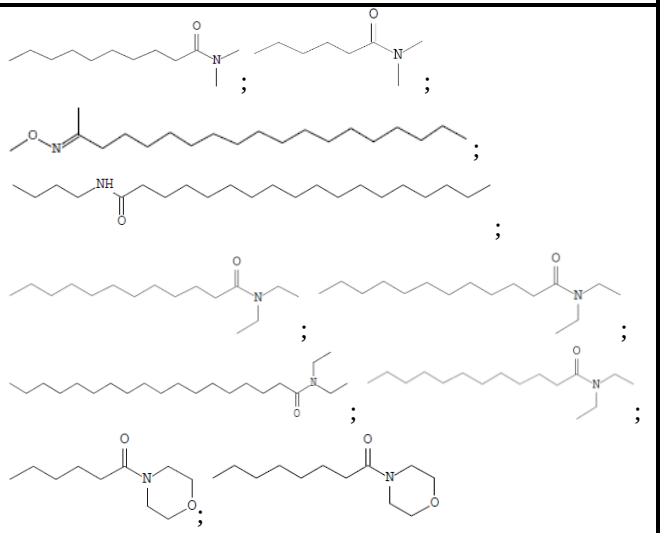
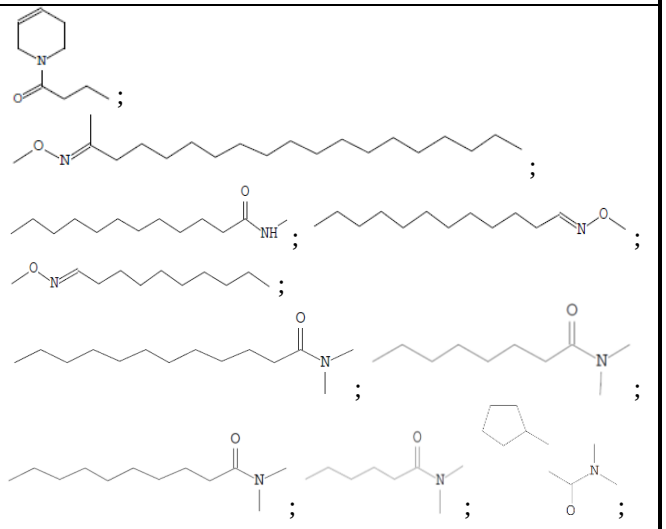
Table 2-7 Possible N-compounds found in the bio-oil

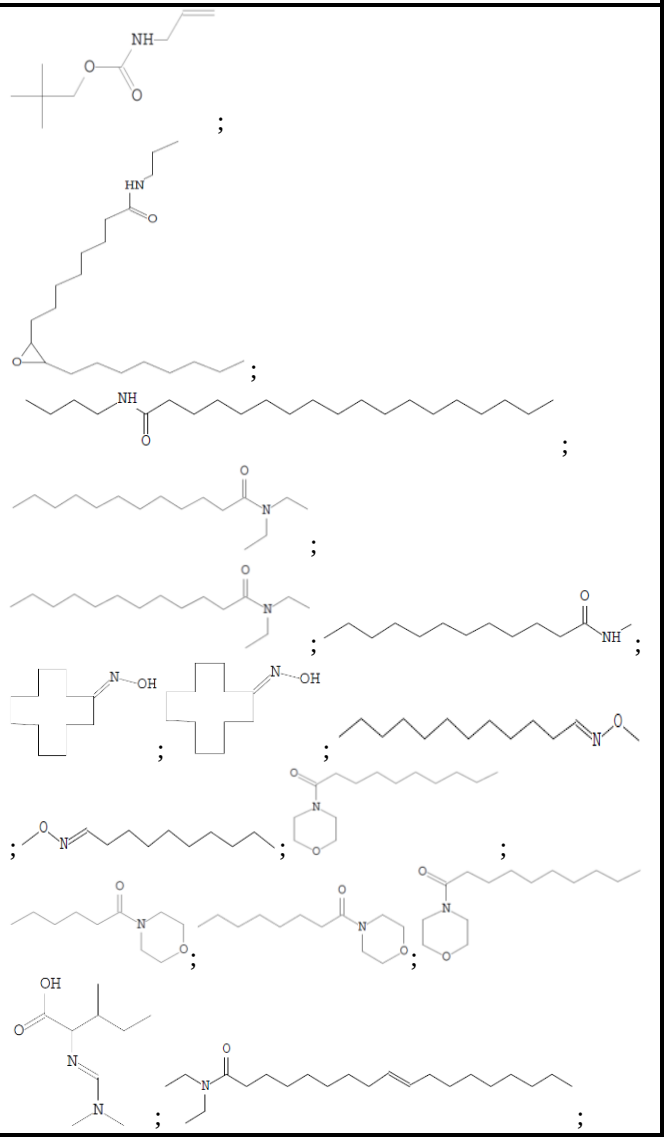
Condition	No. of comp. found	Order of the comp.	No. of hints	Order of hints	SI	%A	Name	Structure
30min 190°C	2	9; 14	1; 1	4; 5	82; 83	0.13; 0.96	Pyridine, 1,2,3,6-tetrahydro-1-(1-oxobutyl)-1-Butyryl-1,2,3,6-tetrahydropyridine; Pyridine, 1,2,3,6-tetrahydro-1-(1-oxobutyl)-1-Butyryl-1,2,3,6-tetrahydropyridine	
30min 210°C	1	13	1	4	82	0.47	Pyridine, 1,2,3,6-tetrahydro-1-(1-oxobutyl)-1-Butyryl-1,2,3,6-tetrahydropyridine	
30min 230°C	1	24	1	1	81	0.23	2-Nonadecanone, O-methyloxime(2E)-2-Nonadecanone o-meth	
30min 250°C	8	11; 24; 37; 39; 44; 45; 47; 49	1; 1; 3; 5; 2; 5; 2; 5	5; 1; 1, 4, 5; 1-5; 1-2; 1-5; 1-2; 1-5	79; 80; 91, 81, 80; 87, 87, 87, 84, 81; 78, 75; 84, 80, 79, 78; 77, 75; 89, 89, 88, 88, 88	0.28; 0.36; 0.22; 0.26; 0.15; 0.27; 0.57; 0.13	Pyridine, 1,2,3,6-tetrahydro-1-(1-oxobutyl)-1-Butyryl-1,2,3,6-tetrahydropyridine; 2-Nonadecanone, O-methyloxime(2E)-2-Nonadecanone o-methyloxime; N-Methyldodecanamide / Dodecanal, O-methyloxime (1E)-Dodecanal o-methyloxime / Tetradecanal, O-methyloxime (1E)-Tetradecanal o-methyloxime; N,N-Dimethyldodecanamide Dodecanamide, N,N-dimethyl-Hallcomid M 12 Lauryl N,N-dimethylamide N,N-Dimethyldode / Octanamide, N,N-dimethyl-N,N-Dimethylcaprylamide N,N-Dimethyloctanamide / N,N-Dimethyldecanamide Decanamide, N,N-dimethyl-N,N-Dimethylcapramide N,N-Dimethylcapylamide N,N-Dimethylde / N,N-DimethylhexanamideHexanamide, N,N-dimethyl-N,N-Dimethylcaproamide / 3-Cyclopentylpropionamide, N,N-dimethyl-; N-Methyldodecanamide / Cyclododecanone, oxime Cyclododecanone oxime; Octadecanamide, N-butyl-n-Butyloctadecanamide / Dodecanamide, N,N-diethyl- Diethyllauramide N,N-Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide / N,N-Diethyloctadecanamide / Dodecanamide, N,N-diethyl-Diethyllauramide N,N-	

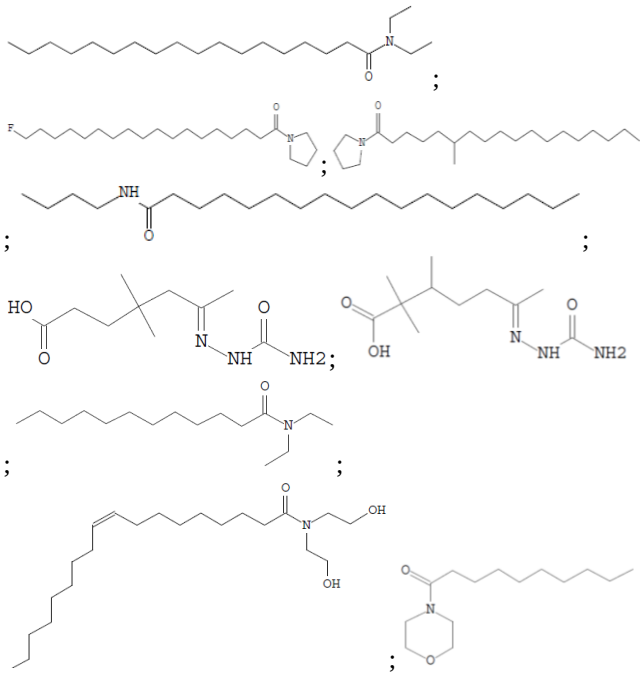
Condition	No. of comp. found	Order of the comp.	No. of hints	Order of hints	SI	%A	Name	Structure
							Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide / Dodecanamide, N,N-diethyl-Diethyllauramide N,N-Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide; Hexanoic acid, morpholide / Octanoic acid, morpholide ; Hexadecanoic acid, pyrrolidide / Pyrrolidine, 1-(6-methyl-1-oxooctadecyl)-1-(6-Methyloctadecanoyl)pyrrolidine / 18-Fluoro-octadecanoic acid, pyrrolidide / Pyrrolidine, 1-(1-oxooctadecyl)-Pyrrolidine, 1-stearoyl-1-Stearoylpyrrolidine 1-Octadecanoylpyrrolidine / 14-Methyl-heptadecanoic acid, pyrrolidide	
60min 190°C	1	10	1	4	82	0.45	Pyridine, 1,2,3,6-tetrahydro-1-(1-oxobutyl)-1-Butyryl-1,2,3,6-tetrahydropyridine	
60min 210°C	1	13	1	4	81	0.32	3-Methyl-5-(1,4,4-trimethylcyclohex-2-enyl) pentan-1-ol	
60min 230°C	1	47	1	1	79,	0.29	Hexanoic acid, morpholide / Octanoic acid, morpholide	
60min 250°C	9	34; 35; 40; 41; 42; 44; 46; 47; 48	3; 5; 2; 5; 3; 2; 2; 5; 1	1, 2, 5; 1-5; 1-2; 1-5; 1-3; 1-2; 1-2; 1-5; 1	91, 82, 80; 88, 87, 87, 84, 82; 80, 75; 83, 80, 79, 79, 77; 85, 80, 75; 76, 75;	0.55; 0.57; 0.3; 0.65; 0.22; 1.36; 0.26; 0.58; 0.53	N-Methyldodecanamide / Dodecanal, O-methyloxime (1E)-Dodecanal o-methyloxime / Decanal, O-methyloxime(1E)-Decanal o-methyloxime; N,N-Dimethyldodecanamide Dodecanamide, N,N-dimethyl-Hallcomid M 12 Lauryl N,N-dimethylamide N,N-Dimethyldode / Octanamide, N,N-dimethyl-N,N-Dimethylcaprylamide N,N-Dimethyloctanamide / N,N-	

Condition	No. of comp. found	Order of the comp.	No. of hints	Order of hints	SI	%A	Name	Structure
					78, 75; 89, 89, 88, 88, 88; 81		Dimethyldecanamide Decanamide, N,N-dimethyl-N,N-Dimethylcapramide N,N-Dimethylcapylamide N,N-Dimethylde / N,N-Dimethylhexanamide Hexanamide, N,N-dimethyl-N,N-Dimethylcaproamide / 3-Cyclopentylpropionamide, N,N-dimethyl; N-Methyldodecanamide / Decanal, O-methyloxime (1E)-Decanal o-methyloxime; Octadecanamide, N-butyl-n-Butyloctadecanamide / Dodecanamide, N,N-diethyl-Diethyllauramide N,N-Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide / Dodecanamide, N,N-diethyl-Diethyllauramide N,N-Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide / N,N-Diethyloctadecanamid / Dodecanamide, N,N-diethyl-Diethyllauramide N,N-Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide; 9-Octadecenamide, N,N-dimethyl-(9E)-N,N-Dimethyl-9-octadecenamide / Non-7-enoic acid, dimethylamide(7Z)-N,N-Dimethyl-7-nonenamide / N,N-Dimethyldecanamide Decanamide, N,N-dimethyl- N,N-Dimethylcapramide N,N-Dimethylcapylamide N,N-Dimethylde; Hexanoic acid, morpholide / Octanoic acid, morpholide; 9-Octadecenamide, n-butyl-(9E)-n-Butyl-9-octadecenamide / 9-Octadecenamide, N,N-diethyl-(9E)-N,N-Diethyl-9-octadece; Pyrrolidine, 1-(6-methyl-1-oxooctadecyl)-1-(6-Methyloctadecanoyl)pyrrolidine / Pyrrolidine, 1-(1-oxooctadecyl)-Pyrrolidine, 1-stearoyl-1-Stearoylpyrrolidine 1-Octadecanoylpyrrolidine / Hexadecanoic acid, pyrrolidide / 14-Methyl-heptadecanoic acid, pyrrolidide / 18-Fluoro-octadecanoic acid, pyrrolidide;Oleic diethanolamide 9-	

Condition	No. of comp. found	Order of the comp.	No. of hints	Order of hints	SI	%A	Name	Structure
							Octadecenamide, N,N-bis (2-hydroxyethyl) -, (Z)-Alkamide DO-280 Amidex O Emid 6545Incrom	
90min 190°C	1	14	1	4	82	0.49	Pyridine, 1,2,3,6-tetrahydro-1-(1-oxobutyl)-1-Butyryl-1,2,3,6-tetrahydropyridine	
90min 210°C	1	27	1	2	80	0.14	2-Nonadecanone, O-methyloxime(2E)-2-Nonadecanone o-methyloxime	
90min 230°C	5	21; 38; 40;45; 47	1; 2; 5; 5; 2	1; 1,4; 1-5; 1-5; 1-2	80; 81, 80; 85, 85, 85, 82, 79; 84, 80, 79, 79, 78; 77, 75	0.24; 0.14; 0.19; 0.25; 0.55	2-Nonadecanone, O-methyloxime(2E)-2-Nonadecanone o-methyloxime; N-Methyldodecanamide / Dodecanal, O-methyloxime (1E)-Dodecanal o-methyloxime; N,N-Dimethyldodecanamide Dodecanamide, N,N-dimethyl-Hallcomid M 12 Lauryl N,N-dimethylamide N,N-Dimethyldodecamide / Octanamide, N,N-dimethyl-N,N-DimethylcaprylamideN,N-Dimethyloctanamid / N,N-	

Condition	No. of comp. found	Order of the comp.	No. of hints	Order of hints	SI	%A	Name	Structure
							Dimethyldecanamide Decanamide, N,N-dimethyl-N,N-Dimethylcapramide N,N-Dimethylcapylamide N,N-Dimethylde / N,N-Dimethylhexanamide Hexanamide, N,N-dimethyl-N,N-Dimethylcaproamide / 2-Nonadecanone, O-methyloxime (2E)-2-Nonadecanone o-methyloxime; Octadecanamide, N-butyl-n-Butyloctadecanamide / Dodecanamide, N,N-diethyl-Diethyllauramide N,N-Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide / Dodecanamide, N,N-diethyl-Diethyllauramide N,N-Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide / N,N-Diethyloctadecanamide / Dodecanamide, N,N-diethyl-Diethyllauramide N,N-Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide; Hexanoic acid, morpholide / Octanoic acid, morpholide	
90min 250°C	13	10; 19; 32; 33; 36; 38; 39; 41; 43; 44; 45; 46; 47	1; 1; 3; 5; 2; 3; 5; 4; 2; 2; 5; 1; 1	5; 1; 1, 2, 5; 1-5; 2, 3; 1-3; 1-5; 1-4; 1-2; 1-2; 1-5; 1; 1	79; 81; 92, 82, 81; 88, 88, 87, 85, 81; 76, 76; 82, 75, 75; 84, 80, 80, 80, 77; 85, 79, 75, 75; 77, 75; 78, 75; 88, 88, 88, 87, 87; 75; 82	0.4; 0.28; 0.93; 1.08; 1.16; 0.22; 0.2; 0.52; 1.62; 0.35; 0.6; 1.39; 2.71; 0.46; 0.87; 0.21; 1.11	Pyridine, 1,2,3,6-tetrahydro-1-(1-oxobutyl)-1-Butyryl-1,2,3,6-tetrahydropyridine; 2-Nonadecanone, O-methyloxime (2E)-2-Nonadecanone o-methyloxime; N-Methyldodecanamide / Dodecanal, O-methyloxime (1E)-Dodecanal o-methyloxime / Decanal, O-methyloxime (1E)-Decanal o-methyloxime; N,N-Dimethyldodecanamide Dodecanamide, N,N-dimethyl-Hallcomid M 12 Lauryl N,N-dimethylamide N,N-Dimethyldod / Octanamide, N,N-dimethyl-N,N-Dimethylcaprylamide N,N-Dimethyloctanamide / N,N-Dimethyldecanamide Decanamide, N,N-dimethyl-N,N-Dimethylcapramide N,N-Dimethylcapylamide N,N-Dimethylde / N,N-DimethylhexanamideHexanamide, N,N-dimethyl-N,N-Dimethylcaproamide / 3-Cyclopentylpropionamide, N,N-dimethyl-; Carbonic acid, monoamide, N-allyl-, neopentyl ester / cis-9,10-Epoxy-n-propyloctadecanamide 8-(3-Octyl-2-oxiranyl)-n-propyloctanamide;	

Condition	No. of comp. found	Order of the comp.	No. of hints	Order of hints	SI	%A	Name	Structure
							N-Methyldodecanamide / Cyclododecanone, oxime Cyclododecanone oxime / Cyclododecanone, oxime Cyclododecanone oxime; 9-Octadecenamide, N,N-dimethyl-(9E)- N,N-Dimethyl-9-octadecenamide / Non-7-enoic acid, dimethylamide (7Z)-N,N-Dimethyl-7-nonenamide / N,N-Dimethyldecanamide Decanamide, N,N-dimethyl-N,N-Dimethylcapramide N,N-Dimethylcapylamide N,N-Dimethylde / N,N-Dimethyldodecanamide Dodecanamide, N,N-dimethyl-Hallcomid M 12 Lauryl N,N-dimethylamide N,N-Dimethyldode / Nonanamide, 8-azido-7-bromo-N,N-dimethyl-; Octadecanamide, N-butyl-n-Butyloctadecanamide / 4,4-Dimethyl-6-semicarbazonoheptanoic acid (6E)-6-[(Aminocarbonyl)hydrazono]-4,4-dimethylheptanoic acid / 2,2,3-Trimethyl-6-semicarbazonoheptanoic acid (6E)-6-[(Aminocarbonyl)hydrazono]-2,2,3-trimethylheptanoic acid / Dodecanamide, N,N-diethyl-Diethyllauramide N,N-Diethyldodecanamide N,N-Diethyllaurylamide N,N-Diethyllauramide; Hexanoic acid, morpholide / CompName:Octanoic acid,; 9-Octadecenamide, n-butyl-(9E)-n-Butyl-9-octadecenamide / 9-Octadecenamide, N,N-diethyl-(9E)-N,N-Diethyl-9-octadecenamide; Hexadecanoic acid, pyrrolidide / 18-Fluoro-octadecanoic acid, pyrrolidide / Pyrrolidine, 1-(6-methyl-1-oxooctadecyl)-1-(6-Methyloctadecanoyl)pyrrolidine / 12-Methyltridecanoic acid, pyrrolidide / 14-Methyl-heptadecanoic acid, pyrrolidide; N,N-Diethyloctadecanamide; Oleic diethanolamide 9-Octadecenamide, N,N-bis (2-hydroxyethyl)-, (Z)-Alkamide DO-280 Amidex O Emid 6545 Incromide	

Condition	No. of comp. found	Order of the comp.	No. of hints	Order of hints	SI	%A	Name	Structure
								

No. of comp. found = number of compounds found, Order of the comp. = order of the compounds, SI = similarity index, %A = percentage by peak area

2.3.5.2 Phosphorus distribution

Phosphorus has been known to promote energy storage of bacteria, yeasts and plants, which helps with the transformation of solar energy into chemical energy and transfer this energy to drive another reaction within the cell of microorganisms [2-30], [2-34]. It is a linear, unbranched polymer of orthophosphate residues linked by phosphor-anhydride bonds (Fig. 2-8), and usually named as the polyphosphate $(PO_4^{3-})_n$. In the liquid phase, the orthophosphate mostly exists as $H_2PO_4^-$ in acidic condition or as HPO_4^{2-} in alkaline condition and transported by phosphate specific transport (Pst) system in the form of either of the above two forms. These 2 forms are the predominant phosphate species over the pH range from 5.0 to 9.0, thus the high pH at the higher temperature could drive the fate of phosphorus [2-32]. In alkalinity condition (pH > 7.3), Ca/Mg are the dominant cation (positive ion) that will react with phosphate and forms a new compound that is solid which results in a decrease in solubility and availability of phosphate. In acidic condition (pH < 5.5), on another hand, Al/Fe are the dominant ion that will react with phosphate forming a new compound that also low in solubility [2-34]. According to this fact, there was no presence of P at 250°C, 60 min and 90 min reaction time as the pH values of these 2 conditions were higher than 7.3 (Fig. 2-6) as presented in Fig. 2-9. At 250°C, 60 min reaction time, the pH value was 7.9 while it increased to 8.2 when the reaction time was further increased to 90 min. Thus, P at these 2 conditions were reacted with the available Mg ion in the aqueous phase and formed new compounds that was very insoluble, which can be observed that the concentration of the Mg in the aqueous (Table 2-4) at theses 2 conditions were decreased, whereas the concentrations in the solid phase (Table 2-5) were increased. As a result, the available P at these 2 conditions in the aqueous phase was reduced (Fig. 2-9).

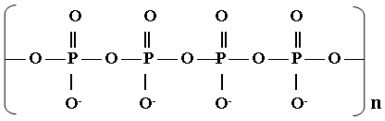


Fig. 2-8 Linear structure of polyphosphate

2.3.5.3 Potassium distribution

Potassium is always presented in minerals as a single-charged cation (K^+) which is easily dissolved into the aqueous phase therefore there was merely no presence in the solid phase as apparently illustrated in Fig. 2-10 [2-31].

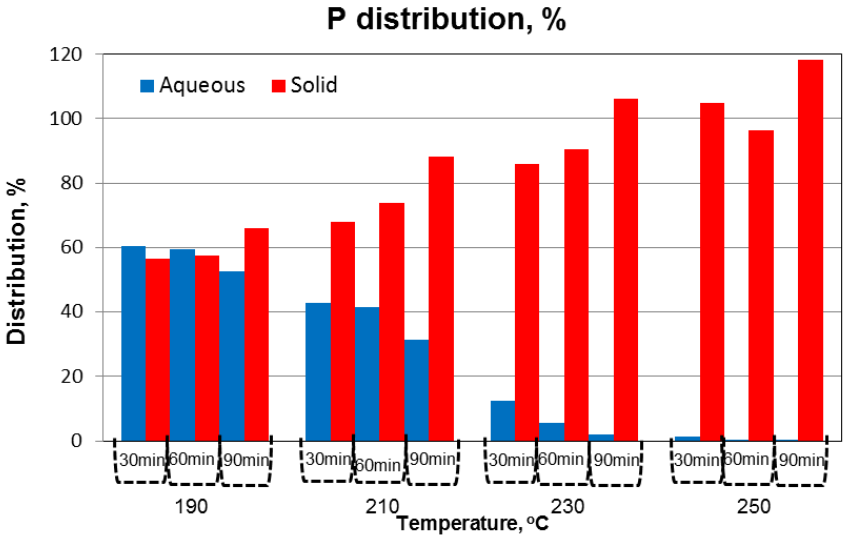


Fig. 2-9 Phosphorus distribution in HTT products

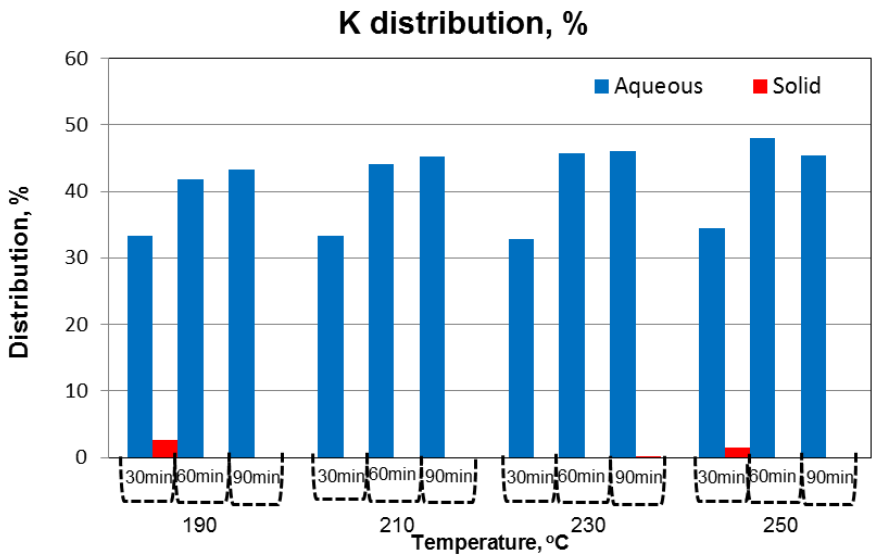


Fig. 2-10 Potassium distribution in HTT products

2.4 Conclusion

This study demonstrated that low temperature HTT (190-250^{o}C) of microalgae has a potential to utilize the solid and aqueous co-products to be a solid fertilizer and algae growth media along with bio-oil extraction. The series of reaction temperature (190^{o}C, 210^{o}C, 230^{o}C and 250^{o}C) and the reaction time (30 min, 60 min and 90 min) were employed to investigate the influences of these 2 parameters on the HTT products' yield. Increasing the reaction temperature raised the yield of bio-oil to the maximum at 230^{o}C, whereas it enhanced the yields of the aqueous co-product and the gaseous product all through the reaction time of 250^{o}C. On the contrary, the increase of the reaction time did increase the oil yield only at a low temperature (190^{o}C and 210^{o}C), when severe conditions were introduced. Unlike the aqueous product, extending the reaction time gave a small increase in its yield, whereas, the gaseous product perceived this positive effect.

Enhancing the reaction temperature affected the HHV of the bio-oil in a negative way while the ER (energy recovery) was positively affected as a result of better oil yield. However, prolonging the reaction time could help increasing of these HHV and ER values only when the condition was not severity. The nutrients recovery from the aqueous residue have found a superior to be utilized for algae growth media with the increasing of the N and K major nutrients with the increase of both the reaction time and the reaction temperature. Even though the P major nutrient was found to show an adverse result, it could also be recovered in a satisfied amount. Likewise, the solid residue gratified the solid fertilizer standard requirements as well even though that there was almost no presence of the K nutrient.

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Chapter 3 Characterization and Upgrading of the Bio-Oil

Abstract

Carbon-neutral renewable liquid biofuels are needed to replace current fossil transport fuels in the near decades. It is not only the environmental concern, but also the limited availability of fossil fuels. The third generation biofuels, recognized as microalgae-based biofuels, have been proved its stability to meet the global demands. The best productive aquatic biomass compared to other terrestrial biomasses, promotes the microalgae superior to others. However, the economically conveying this wet biomass to transport biofuels is still an issue. Herein, the hydrothermal treatment employing low temperature (190-250^{o}C) was proposed. A feasibility was conducted under 3 reaction times, 30 min, 60 min and 90 min. A possibility of upgarding this microalgae bio-oil for transport fuel is comparable to transportation fuels. A proper pre-treatment is needed still to improve its quality.

3.1 Introduction

An economically constrain of converting algae biomass into liquid biofuels is still needed an improvement particularly an excessive high cost compared to petroleum derived fuels additionally, an efficacy process of extracting liquid dense in energy from the wet biomass [3-1]. Multiple approaches have been proposed, one of those known as hydrothermal treatment (HTT). More studies are required still to manage this technology's by-products and of course, scaling up to industrial scale.

HTT involves an application of heat and pressure to biomass in an aqueous medium, therefore, high energy efficiency in terms of obviating biomass dewatering and drying is its distinct merit [3-2]. In HTT, biomass is converted into liquid products with high energy content. It is usually carried out in water at 250-374^{o}C under pressure of 4-22 MPa and an organic liquid called bio-oil is obtained [3-3]. However, HTT under 200-370^{o}C and 5-25 MPa is ideal for energy recovery from high moisture containing biomass since the water is still in a liquid state and acts as a reactant and catalyst and has been extensively studied [3-4], [3-5]-[3-10]. This bio-oil can be used directly as a heavy petroleum oil replacement, for co-firing with coal, and is a candidate for upgrading to high quality distillate fuels (e.g., diesel and gasoline) [3-2]. Thereby, HTT processing does rely on the unique properties of water at high temperature and pressure. At elevated temperatures, hydrogen bonding of water is diminished then the water dielectric constant is reduced, and thus its ion product is increased. As a consequence, many organic compounds become completely miscible in the high temperature water [3-2], [3-4], [3-11]-[3-12]. Many complex reactions take place during the transformation of biomass into crude oil-like products. The complexity of the chemical reactions which occur during hydrothermal treatment is due to the complex structure of biomass. Biomass components mainly consist of carbohydrates, lignin, protein and fats. The decomposition of these components in subcritical water conditions yields different products, but degradation mechanisms comprise the following steps: the depolymerization of the biomass; the degradation of monomers (the cleavage, the dehydration and the decarboxylation reactions); and recombination of fragmented components. Macromolecules in the structure of biomass first dissociate into water-soluble oligomers and monomers by the hydrolysis. The monomers and oligomers may also undergo further degradation, re-polymerization or have their functional groups reduced [3-13], [3-14].

To produce biofuels such as biodiesel via transesterification of algal bio-oil is more energy-efficient than fermentation to produce ethanol [3-15]. However, the current algal oil production is still far more expensive than petroleum-diesel fuels. For example, it was estimated that the production cost of algal oil from a photobioreactor with an annual production capacity of 10,000 tons per year would be as high as $10.50 per gallon compared to $3.80-$4.50 per gallon of the petroleum diesel price in Virginia [3-16]. Moreover, HTT bio-oil still contains high amounts of O and N in comparison to conventional fossil fuel which in turn become a challenge for subsequent upgrading before replacing the current transportation fuel.

In this study, it was focused to determine the characteristics of algal bio-oil obtained by employing HTT for upgrading as transportation biofuels. The bio-oils obtained from HTT at different conditions (190-250^{o}C and 30-90

min reaction time) were evaluated for upgrading feasibility by investigating the atomic ratios, the acid value, the fatty acid profile and the iodine value.

3.2 Materials and methods

3.2.1 Materials

A batch-type reactor autoclave (model MMJ500, OM Labtech, Tokyo, Japan) equipped with a magnetic drive agitator, an electrically heated furnace with a glass tube chamber as illustrated in Fig. 2-1 in chapter 2 was employed for HTT experiments using the powder freshwater green microalgae obtained from TISTR in this study. Most chemicals used were purchased from Wako Chemicals (Tokyo, Japan).

3.2.2 Methods

A series of temperature (190^{o}C, 210^{o}C, 230^{o}C and 250^{o}C) and a set of reaction time (30, 60 and 90 min) was firstly conducted to investigate the influence of the reaction temperature and the reaction time. The concentration of algae biomass was fixed at 20 wt% and mixed with 80 g of distilled water to meet a 100 g – sample size for the entire of the study. The mixture was mixed and placed into the autoclave then the reactor was closed. Then the headspace was purged by argon for about 2 min to limit the oxygen that could make any combustion. 200 rpm was set for the mixing speed then heated to the desired temperature and was kept at that temperature for each desired reaction time. The reactor was cooled down to the room temperature when the reaction times were reached then depressurized the inside gaseous. The mixture product was collected and firstly mixed with 100 mL-dichloromethane (DCM; Sigma-Aldrich, 99% purity) then transferred to vacuum filtrate by a 1.2µm pore size-glass microfiber filter paper (GF/C, Whatman). The filtered algal residue defined as solid residue was separated for further analysis while the two-phase mixture was separated for the DCM-soluble phase and the DCM-insoluble phase using an auto-pipette (Gilson Pipetteman). The aqueous phase was also kept for further analysis, whereas the DCM-soluble phase was naturally evaporated at room temperature in a fume hood for about 3 days to remove the DCM-solvent and the remaining product (DCM-soluble liquid) was defined as the bio-oil and was further characterized. A transesterification reaction is considered to be the best existing technology for converting the bio-oil to its respective esters and was conducted to transform the bio-oil into the fatty acid methyl ester (FAME) form. The method used was adapted from [3-17], [3-18]. The HTT experiments were triplicated and reported by the average values. The overall procedure for collecting and separating the HTT products is illustrated in Fig. 2-2 in chapter 2.

3.2.3 Analysis

The proximate and the ultimate analysis of the microalgae feedstock was first determined using the elemental analyzer (Vario MICRO Cube, Elementar Inc.) followed the ASTM standard test method D3173, D5142, D3175, D5291 and D3176, whereas the lipid content was provided from TISTR. Higher heating value was analyzed by the bomb-type calorie meter, OSK200-model, Ogawa Sampling Inc., Tokyo, was employed for higher heating value followed the standard of ASTM D5864 method. Trace elementals were measured by ICP emission spectroscopy (ICPS-8100, Shimadzu Inc.) after being digested by the MultiWave3000, Perkinelmer Inc and listed in Table 3-1.The fatty acid composition of the bio-oil was analyzed by a GC/MS equipped with Rtx-5MS, 30 m-long, 0.25 mm-ID, and 0.25 µm-film thickness column (GCMS-QP2010 SE, Shimadzu Inc.) using the NIST98 library for quality matching at 85% or more. An acid value (AV) is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralized the acidic constituents in one gram of sample was measured followed the ASTM D664 [3-19]. Thus, the free fatty acid (FFA) values were calculated using the mathematical formulas found in the American Oil Chemists' Society (AOCS) method Ca 5a-40 [3-20]. An iodine value (IV) is a measurement of total unsaturation of fatty acids measured in gram iodine per 100 grams of biodiesel sample was measured followed the ASTM standard test method D5768 [3-21].

Table 3-1 Trace elements in algae feedstock

	Trace elements, %											
Feedstock	P	K	Mg	Ca	Fe	Na	Si	B	Mn	Zn	Cu	Co
	0.50	0.41	2.47	11.59	0.27	0.99	3.45	0.0271	0.0715	0.0247	0.0104	0.0047

3.3 Results and Discussions

The bio-oil obtained from HTT at each condition was determined to judge whether its properties were feasible to be upgraded for transportation fuels or not, thus, the elemental composition, the acid value, the free fatty acid value and the iodine value were analyzed.

3.3.1 Determination by elemental composition

Elemental analysis was used to determine the elemental compositions (C, H, O, N and S) of the algae feedstock and the bio-oil. It usually includes the atomic ratios of H/C, O/C and N/C. The O/C ratio is a significant factor used to estimate the degree of the deoxygenation occurring during HTT of biomass, whereas, the H/C ratio provides a clue regarding the aromatic content of the bio-oil. In another word, when the H/C ratio is high, the aromatic content is low. Moreover, the elemental composition can be used to determine the heating value of the bio-oil by using the Dulong Formula [3-3]. Table 3-2 shows the elemental compositions of the bio-oil produced from HTT at different operating conditions, where the algae feedstock and the crude oil are compared. The analysis showed that the C content of bio-oil always exceeded the one of the feedstock and almost 2 times higher. However, the increasing was lower with both the reaction temperature and the reaction time as of the HTT mechanisms [3-13], [3-14] which is decarboxylation. Similarly, the H content always exceeded the one of the feedstock and behaved similar manners with the C content. The O content, on the other hand, decreased steadily with the increase of the reaction temperature and the reaction time which was much lower than the one of the feedstock. A significant increase of the N content, nonetheless, started from the lower content than the feedstock but then step by step increased with the increase of the temperature and the reaction time. Much concern needs to be taken care for the denitrogenation treatment. Furthermore, the S content also showed the increasing trend with the increase of both the reaction temperature and the reaction time which was higher than the feedstock. It can eminently be seen that HTT helps to enrich the C and H contents of algae feedstock to be able to be compared with the crude oil. However, the upgrading by the deoxygenation, the denitrogenation and the desulfurization are necessary still to make this bio-oil competitive with transportation fossil fuels [3-22].

Table 3-2 Elemental composition and properties of the bio-oil obtained at different operating conditions

Reaction time	**T (^{o}C)**	**C**	**H**	**O***	**N**	**S**	**H/C**	**O/C**	**N/C**	**HHV**
				(wt%)				**(mol/mol)**		**(MJ/Kg)**
Feedstock		38.26	4.96	51.02	4.51	0.36	1.56	1.00	0.10	16.17
30 min	190	70.92	10.84	14.47	1.98	0.43	1.83	0.15	0.02	35.61
	210	69.66	10.56	14.14	3.16	0.47	1.82	0.15	0.04	34.05
	230	68.98	10.24	13.71	4.00	0.53	1.78	0.15	0.05	33.61
	250	67.00	9.68	13.74	4.72	0.47	1.73	0.15	0.06	32.66
60 min	190	70.35	10.52	14.46	2.46	0.41	1.79	0.15	0.03	36.13
	210	70.04	10.50	13.93	3.60	0.47	1.80	0.15	0.03	34.51
	230	68.89	10.14	13.31	4.25	0.51	1.77	0.14	0.05	34.12
	250	69.49	9.86	11.89	5.43	0.58	1.70	0.13	0.07	33.24
90 min	190	70.16	10.84	14.48	2.76	0.43	1.85	0.15	0.03	33.60
	210	70.42	10.38	13.76	3.84	0.50	1.77	0.15	0.05	32.92
	230	69.12	10.08	12.35	4.60	0.51	1.75	0.13	0.06	32.74
	250	70.96	10.14	10.77	5.47	0.56	1.72	0.11	0.07	32.49
Crude oil		83-87	10-14	0.1-1.5	0.1-2	0.5-6				42.9

* calculated by difference

The atomic ratios, another criterion often used for fuels characterization, can be interpreted from the elemental composition analysis. The H/C ratios of the bio-oil were slightly higher than that of the feedstock (1.56), nevertheless, they were favorably comparable with the ratio of the crude oil. The slight decrease of this H/C ratio with the increase of the reaction temperature shows that it contained more aromatic compounds. The N/C ratio of the bio-oil was ranged from 0.02 to 0.07 with the increasing trend with the increase of the reaction temperature and the reaction time, and was lower than that of the feedstock. Compare to the N/C ratios of the crude oil, of course, they were comparable still. The O/C ratio of the bio-oil showed steady at 0.15 when the reaction time was 30 min, but gradually decreased with the increase of the reaction temperature when 60 min and 90 min reaction times were adopted. The O/C ratio which was 6 times lower than that of the feedstock showed that a significant deoxygenation was performed. However, more deoxygenation is required still to produce a replaceable oil for the fossil fuels.

3.3.2 Determination of the acid value (AV)

The acid value (AV), also called as the neutralization number or the acid number, is a mass of potassium hydroxide (KOH) in milligrams required to neutralize the acidic constituents in one gram of oil-sample [3-23]. The AV is used to quantify the presence of acid in a biodiesel sample which could be found as a result of (1) residual mineral acids from production process, (2) residual free fatty acid from the hydrolysis process or the post-hydrolysis process of the esters and (3) oxidation by-products in the form of other organic acids [3-24]. As illustrated in Fig. 3-3, the AV, shown in the blue bar, of the bio-oil were relatively high and much higher than the biodiesel standard at 0.5 mg KOH/g oil. Fig. 3-3-(a), the AV of the bio-oil at 30 min reaction time showed a continuously decrease with the increase of the reaction temperature from 216 at 190^{o}C^{o}C to 51 mg KOH/g oil at 250^{o}C, whereas the AV at 60 min (Fig. 3-3-(b)) showed a slight decrease from 126 mg KOH/g oil at 190^{o}C to 95 mg KOH/g oil at 230^{o}C and a sharply decreased to 48 mg KOH/g oil when the reaction temperature further increased to 250^{o}C. The AV at 90 min, shown in Fig.3-3-(c), firstly showed no change at 120 mg KOH/g oil from 190^{o}C to 210^{o}C, thereafter, decreased rapidly to 51 mg KOH/g oil at 230^{o}C and kept decreasing to 31 mg KOH/g oil at 250^{o}C. A too high value of AV may reflect the residue of any acids mentioned previously. There is a possibility that the acids came from the algal feedstock minerals as listed earlier in Table 3-1 or the organic acids produced during HTT. The decreasing trend of the AV with the increase of the reaction temperature means that the acids were reduced by the increase of the reaction temperature, thus it is possible that the acids came from the HTT process as discussed in chapter 2 showing that pH of the HTT aqueous product increased with the increase of the reaction temperature and the reaction time. Despite that, a corrosiveness of this bio-oil and a filter clogging when functioning at reduced temperatures must be carefully concerned [3-23].

3.3.3 Determination of the free fatty acids (FFA)

The FFA of the bio-oil is shown in Fig. 3-3 in the red line. It is so obvious that the FFA of all conditions was very high and when it is higher than 5 wt%, the formation of soap during the esterification reaction will occur. A difficulty in separation of soap, thereafter, will increase and leads to a yield loss [3-25]. Thus, an acid catalyst is suggested in order to reduce these FFA, 70-80% is possible [3-26]. A transesterification reaction was employed to convert the bio-oil, in the form of Triglyceride to Fatty Acid Methyl Esters (FAMEs) by using H_2SO_4 acid as a catalyst. The reaction is depicted in Fig. 3-4. FAME is a derivative of biodiesel that usually used to determine a quality of biodiesel, thenceforth, the fatty acid profile analysis by GC/MS was conducted so as to analyze its compositions.

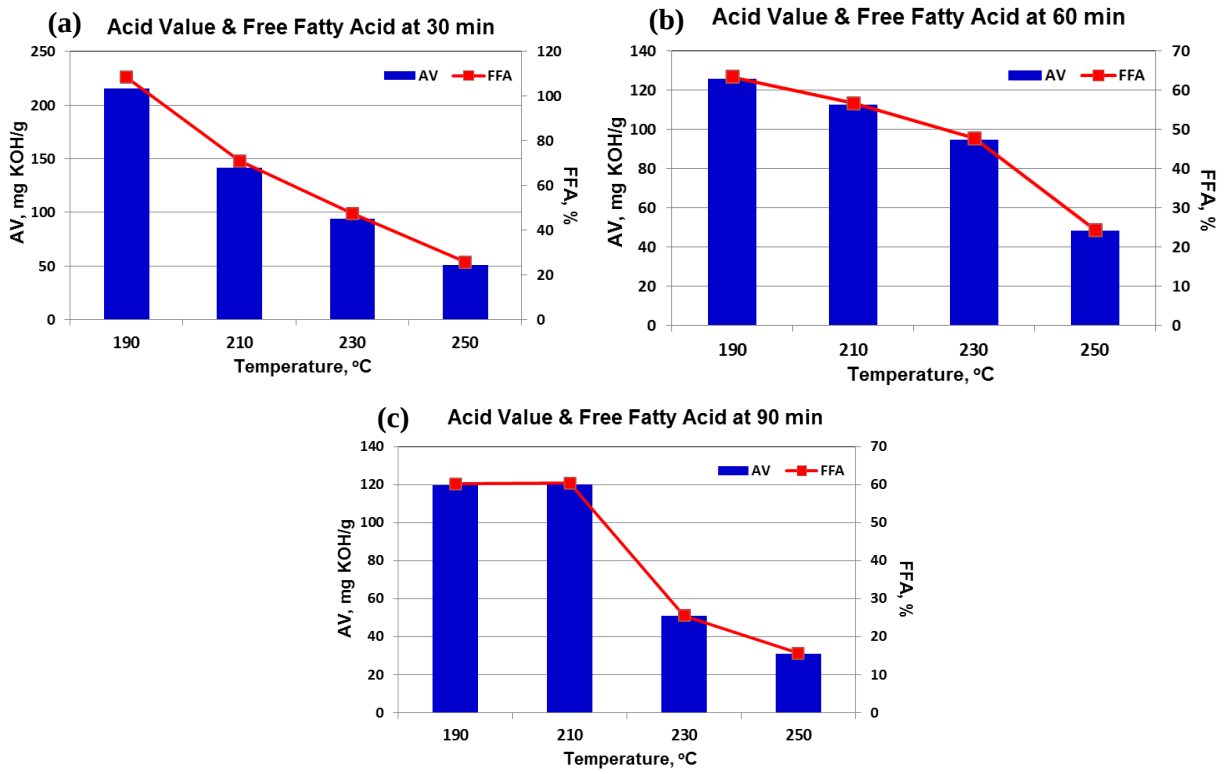

Fig. 3-3 Acid value and free fatty acids of the bio-oil obtained at (a) 30 min, (b) 60 min and (c) 90 min reaction time

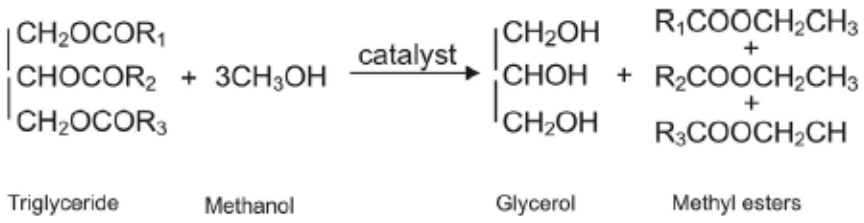

Fig. 3-4 Esterification reaction

The fatty acids found in microalgae are mostly in a range of C12-C24 in length and can either be saturated or unsaturated fatty acids. However, the number of double bonds in the fatty acid chain never exceeds 6 and most of the unsaturated fatty acids are cis isomers [3-27]. Large portions of fatty acids found are polyunsaturated C16 and C18 fatty acids [3-5]. In that way the composition and fatty acid profile of the extracted lipids from a particular species are essentially affected by the microalgae life cycle itself and the cultivation conditions such as the temperature, the medium composition, the light intensity, the ratio of the light and dark cycles and the aeration rate [3-5], [3-27]. In Table 3-3, the fatty acid composition of the bio-oil at different conditions is shown. Despite the fact that several hundreds substances are found in the bio-oil, the compounds usually created in the microalgae fatty acids (C12-C24) are elucidated. These total fatty acids (TFA) cover more than 80% by the peak area of the entire TFA in algae. The result showed that the amount of saturated fatty acid (SFA), C14:0, C15:0, C16:0, C18:0, C20:0, C22:0 and C24:0, decreased when the reaction temperature increased, whereas the mono unsaturated fatty acids (MUFA), C16:1 and C18:1 increased as a result of hydrogen removal by the deoxygenation reaction when the reaction temperature increased. This finding was also affirmed by the decrease of the H/C ratio with the increase of the reaction temperature as previously discussed in Table 3-2. Moreover, the quantities of poly unsaturated fatty acid

(PUFA), C16:3 and C18:2, were found to be decreased when the reaction temperature increased which is likely due to the unstable state of H atom when the reaction temperature increases. Hence, H atoms were removed whereas the C atoms were still constant. However, the TFA profile at 230^{o}C, 90 min reaction time performed quite differently from others but closed to the performance of 210^{o}C, 90 min. Nonetheless, the bio-oil obtained at 230^{o}C, 90 min also showed the highest quantities of TFA.

Anyhow, C16:1, C18:1 and C18:2 fatty acids appear to be good candidates for the fatty acids conversion to high-quality biodiesel [3-27], additionally, using a higher unsaturated fatty acid content than the saturated fatty acid content is more preferable. This is due to the great advantage of FAME derived from the cis unsaturated fatty acids based on the cold flow properties (a low cloud point and a low pour point). As a consequence of the cis unsaturated fatty acids that are prevented from forming regular molecular packing when the bends are imposed by the cis double bonds and consequentially freeze at a much lower temperature [3-27]. In another hand, a relatively high amount of PUFA is responsible for purification before it can be transesterified according to its poor volatility, the low oxidation stability and the tendency for gum formation as observed in some oilseed-derived biodiesel [3-27].

Table 3-3 Total fatty acids of the bio-oil at different conditions

TFA (% by peak area)													
Lipid Numbers	Systematic Name	30 min				60 min				90 min			
		190	210	230	250	190	210	230	250	190	210	230	250
C14:0	Tetradecanoic acid	2.67	2.46	2.21	1.54	1.89	1.62	1.32	0.83	1.73	1.4	1.44	
C15:0	Pentadecanoic acid	2.32	3.17	2.83	1.71	1.79	2.27	1.26	1.09	1.66	1.7	2.09	0.94
C16:0	Hexadecanoic acid								0.5				0.8
C16:1	Palmitoleic acid	15.79	17.98	22.77	25.52	19.24	24.24	29.87	30.92	23.06	33.51	26.94	20.5
C16:3	Hexadecatrienoic acid		1.42	0.86						1.1			
C18:0	Octadecanoic acid	8.34	7.49	6.61	7.12	6.75	6.03	4.59	4.91	6.08	4.05	6.2	4.91
C18:1	Oleic acid	46.11	48.19	50.43	51.92	51.65	51.54	52.44	56.79	51.08	48.99	54.59	52
C18:2	Linoleic acid	6	5.54	4.65	4.06	5.22	4.54	3.24		4.82	3.64	3.53	2.15
C20:0	Eicosanoic acid	3.98	3.31	2.99		2.83	2.48	1.84	0.68	2.3	1.6	1.9	
C22:0	Docosanoic acid	1.49	1.34	1.1	0.52	1.21	1.07	0.74		1	0.61	0.63	
C24:0	Tetracosanoic acid						1.13	0.87					
C12-C24		86.7	90.9	94.45	92.39	90.58	94.92	96.17	95.72	92.83	95.5	97.32	81.3

3.3.4 Determination of the iodine value (IV)

The iodine value (IV), sometimes called as the iodine number, is used to evaluate its stability to oxidation. Thus, it is a measurement of total unsaturation of fatty acids which were measured in g-I per 100 g of oil sample [3-23]. The results in Fig. 3-5 shows that all the bio-oil's IV exceeded the IV of biodiesel standard at 120 mg-I/100 g oil except for the one at 190^{o}C, 30 min reaction time which had the IV of 120.45 mg-I/100 g oil. The IV at 30 min reaction time showed a steady increase with the increase of the reaction temperature from 120.45 mg-I/100 g oil at 190^{o}C to 177.45 mg-I/100 g oil 250^{o}C which is likely due to the increase of unsaturated fatty acids. Whereas, the bio-oil at 60 min and 90 min showed a similar trend that the IV from 190^{o}C to 230^{o}C were more or less the same, around 130 mg-I/100 g oil and 140 mg-I/100 g oil for 60 min and 90 min respectively, but a particular increase was found when the reaction temperature was further increased to 250^{o}C, 168.63 and 174.19 mg-I/100 g oil for 60 min and 90 min respectively. Nevertheless, these high IV indicated that these bio-oils can be easily oxidized in contact with air. Furthermore, they have more tendencies to be polymerized than other temperatures (190-230^{o}C at the same reaction time) [3-23].

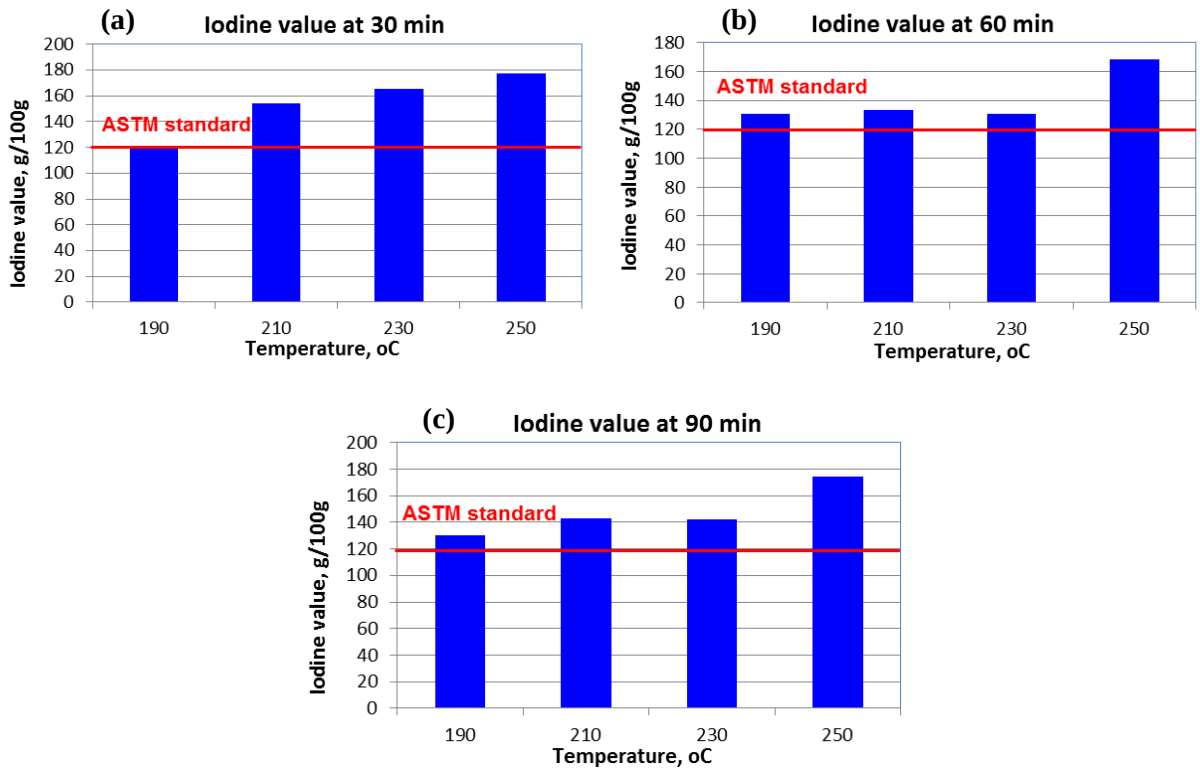

Fig. 3-5 Iodine value of the bio-oil at (a) 30 min, (b) 60 min and (c) 90 min reaction time

3.4 Conclusions

Microalgae bio-oils obtained by HTT at a low reaction temperature (190-250^{o}C) had a feasibility to be upgraded for transportation fuels with some treatments due to their comparable performances to petroleum crude oil and biodiesel in regards to the atomic ratio and the TFA profile. The proper pre-treatments to reduce oxygen, nitrogen and sulfur contents, residual acids and degree of unsaturation are necessary for fungible transportation fuel production.

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Chapter 4 Characterization and fertilizer application of the solid product

Abstract

The biofuel derived from microalgae has been the only promising biofuel that could meet the world energy demand due to its various merits. One great merit is the environmentally-friendly fuel. However, an economical technological solution for the oil extraction from microalgae and downstream management are challenge to make this a socio-economic fuel. The HTT solid co-product accounted for 10% has been proposed to be utilized as solid fertilizer due to its rich nutrients. A practical utilization on planting showed a satisfied result. This biofertilizer performed better than a chemical fertilizer and yielded better more or less.

4.1 Introduction

The increasing world population and rapid industrial evolving have driven the world energy demand sharply. The current available fossil fuels such as coal, oil and natural gas, of course cannot meet these increasing demands for long. Biofuels have become the only alternative to fix this problem with less harm to the environment. However, there are still some obstacles promoting this alternative fuel. Microalgae-based biofuel, therefore, has been recognized as the only currently available source of oil that could meet the global demand for transport fuels [4-1]. Many researchers have proposed the hydrothermal processing as an important thermochemical conversion process used to convert this microalgae biomass into valuable products known as bio-oil. The hydrothermal treatment (HTT) is usually performed in a hot compressed water at 200-374^{o}C under the pressure of 2-22 MPa. At these conditions, the microalgae are degraded into small components in water forming new compounds known as bio-oil, gaseous product, solid and liquid products [4-2]. However, one challenge for microalgae derived biofuels is performing economic extraction of the bio-oil from wet biomass and by-products management. A number of scientists have shown that the aqueous phase from HTT is capable of nutrients recovery and proposed microalgae cultivation as a choice [4-3]. The solid phase, mostly called as biochar, on another hand, has not yet been attracted. Nonetheless, it was proposed that the biochar may also be useful as re-enforcing additives in cement and organic polymers or even a carbon source for synthesis gas formation or as a coal coke alternative in steel manufacture when ash is found to be low [4-4]. Moreover, it was reported that the biochar has a potential for agricultural applications as a biofertilizer and for carbon sequestration [4-5]. There are few authors have studied a feasibility of applying this biochar for biofertilizer. So far, there is no practical utilization of the solid phase from HTT for biofertilizer. Thus, this study proposed this utilization with a practical usage.

4.2 Materials and methods

4.2.1 Materials

The powder form of freshwater green microalgae TISTR-8511 strain (Chlorellaceae strain) obtained from TISTR (Thailand Institute of Scientific and Technological Research) was selected for employing HTT experiment in a batch-type reactor autoclave (model MMJ500) equipped with a magnetic drive agitator, an electrically heated furnace, and a 500 mL glass tube chamber (OM Labtech,Tokyo, Japan). Most chemicals applied in this study were purchased from Wako Chemicals (Tokyo, Japan).

For planting test, the Komatsuna (Brassica rapa var. perviridis) seeds were purchased from Tohoku seed Inc., Japan. A special dot-filter paper (Tanepita) for germination test was purchased from FHK Inc., Japan while the petri dish dia. 90x20 was purchased from Ikeda Inc., Japan. Neubauer pot was used as a no hole container to prevent no water nor nutrients to come out.

4.2.2 Methods

To clarify the influences of reaction temperature and reaction time, the first variable was focused on 190^{o}C, 210^{o}C, 230^{o}C and 250^{o}C, and 30 min, 60 min and 90 min for the second variable, in line. The pressure was varied followed its saturated steam pressure of each temperature. 20 g of powder microalgae was firstly added to a glass tube

chamber, followed by 80 g of distilled water to meet a 100 g-sample size then thoroughly mixed and put into the autoclave. Argon gas was then introduced to the autoclave for approximately 2 min to remove the air in the headspace so as any combustion could be avoided. About 200 rpm was set as a mixing speed then the reactor was heated to the set temperatures with a ramped rate of 4.7^{o}C/min. When reached the set temperature, the reactor was kept at that temperature for 30 min, 60 min and 90 min, case by case and then left for cooling down to the room temperature. The inside gaseous was released to depressurize then opened and collected the product. The two-phase mixture product was mixed with 100 mL-dichloromethane (DCM; Sigma-Aldrich, 99% purity) and transferred for vacuum filtration using a 1.2µm pore size-glass microfiber filter paper (GF/C, Whatman). The filtered algal residue defined as solid residue was separated for further nutrients analysis, whereas the two-phase mixture was later separated for the DCM-soluble phase and the DCM-insoluble phase using an auto-pipette (Gilson Pipetteman). The DCM-insoluble phase defined as aqueous residue was further analyzed and the DCM-soluble phase was next left for DCM removal in a fume hood at room temperature for 3 days and the remaining product (DCM-soluble liquid) was defined as the bio-oil and was further characterized. All of HTT experiments were conducted in triplicate and the average values were reported.

In order to utilize the solid residue as a solid fertilizer, the seed germination test was firstly conducted so as to evaluate the phytotoxicity to the plant. Later, the planting test was done. 50 seeds of Komatsuna without any treatment were placed on a special dot-filter paper used for the germination test and put into a petri dish. 10 mL of distilled water was given as a food source and moisture for a control, whereas, 1:1 ratio of solid residue to water was employed instead of pure water in the case of the sample. All the dishes were kept in dark for 72 hours at 25^{o}C, 80% humidity for incubation. Three replicates were set for each condition. After 72 hours, germinated seeds were counted and the root length was measured.

In the planting test, 500 g of decomposed granite soil was mixed with a solid sample differently prepared for each condition as listed in Table 4-1 and watered with 150 mL (based on 30% water content [4-6]) of distilled water on the first day. Then 20 seeds of Komatsuna were sown in a pot and kept under the room temperature (23-28^{o}C), 50-70% humidity, using a fluorescent lamp as an artificial light source for 25 days.

Table 4-1 Experimental design for the planting test

Group	**Conditions,** mg/pot		
	TN	**TP**	**TK**
Blank	Only pure water, no additive		
Standard	100 mg	100 mg	100 mg
N	100 mg	0	0
S100	100 mg	Make-up to 100 mg	Make-up to 100 mg
S50	50 mg + 50 mg-N	Make-up to 100 mg	Make-up to 100 mg
S25	25 mg + 75 mg-N	Make-up to 100 mg	Make-up to 100 mg

For the planting test, the solid residue obtained from HTT at 60 min reaction time only was employed for the test due to the highest bio-oil yield was found at this reaction time. As listed in Table 4-1, there was no fertilizer added to the Blank and only pure distilled water was supplied for the seeds. In Standard, 100 mg of TN, TP and TK chemical fertilizer were added, whereas only 100 mg of TN chemical fertilizer was applied for N. In the case of the sample, the solid residues obtained at each reaction temperature were divided into 3 groups as S100, S50 and S25 for 100%, 50% and 25% solid residue applied respectively. Thus, S100 means 100 mg of TN from solid residue was applied and TP and TK chemical fertilizers were made-up to meet 100 mg of each nutrient calculated by the difference from the available TP and TK in the solid residue. S50 and S25 mean 50 mg and 25 mg of TN respectively from the solid residues were applied and also made-up by TP and TK fertilizers to meet 100 mg of each nutrient.

4.2.3 Analysis

The chemical composition, proximate and ultimate analysis of the microalgae feedstock listed in Table 4-2 were first analyzed for comparison followed the ASTM standard test method D3173, ASTM D5142, D3175, D5291 and

D3176 respectively, whereas the lipid content was provided by TISTR. The elemental analyzer (Vario MICRO Cube, Elementar Inc.) was employed for elemental analysis and the bomb calorie meter, OSK200-model (Ogawa Sampling Inc., Tokyo) was used to conduct the higher heating value followed the ASTM standard test method D5864. ICP emission spectroscopy (ICPS-8100, Shimadzu Inc.) was employed for trace elementals after being digested by the MultiWave3000, Perkinelmer Inc and listed in Table 4-2. The desk-type meters (F-70/DS-70 series, Laqua Inc.) were used for electrical conductivity (EC) and pH of the sample product.

Table 4-2 Characteristics of microalgae feedstock

Proximate analysis (wt%)				**Biochemical composition (wt%)**	**Ultimate analysis (wt%)[a]**					**HHV (MJ/Kg)**
Moist	VM	FC	Ash	Lipids	C	H	N	O	S	
5.57	65.42	2.69	26.32	22.55	38.26	4.96	4.51	51.02	0.36	16.17

Trace elements, %											
P	K	Mg	Ca	Fe	Na	Si	B	Mn	Zn	Cu	Co
0.50	0.41	2.47	11.59	0.27	0.99	3.45	0.0271	0.0715	0.0247	0.0104	0.0047

[a]On dry basis, VM = volatile matter, FC = fixed carbon, HHV = higher heating value

Evaluation of the seed germination was done with the followed equations Eq.4-1, Eq.4-2 and Eq.4-3 [4-7], whereas the evaluation criteria for the planting test were adapted from [4-8], [4-9] and are defined in the equations of Eq.4-4, Eq.4-5, Eq.4-6 and Eq.4-7.

SGR (Seed Germination Ratio) = Number of germinated seeds x 100% / Number of seeds Eq.4-1

MRL (Mean Root Length) = Sum of all root length / Number of germinated seeds Eq.4-2

$$GI\ (\text{Germination Index}) = \frac{(\text{Mean of germinated seeds x Mean of root length})_{sample}}{(\text{Mean of germinated seeds x Mean of root length})_{control}} \times 100\% \quad \text{Eq.4-3}$$

SR (Sprout ratio) = Number of growth plants in each pot x 100% / Number of all seeds Eq.4-4

MLW (Mean Leaf Width) = Sum of all leaves width in each pot / Number of all the growth plants Eq.4-5

MGH (Mean Growth Height) = Sum of the shoot height x 100% / Sum of the growth plants Eq.4-6

$$GI\ (\text{Growth Index}) = \frac{(\text{Mean of growth plants number x Mean of the leaf width})_{sample}}{(\text{Mean of growth plants number x Mean of the leaf width})_{control}} \times 100\% \quad \text{Eq.4-7}$$

4.3 Results and Discussions

HTT solid residues obtained from chapter 2 were utilized for solid fertilizer in this chapter, therefore, seed germination was first conducted to evaluate any phytotoxicity to the plant. The available nutrients in the solid residues are shown in Table 2-6 in chapter 2.

4.3.1 Seed germination test

The solid residues obtained at each condition were first dissolved with distilled water at 1:1 ratio before being applied to the seeds in each petri dish. The pH and EC values of these solutions were illustrated in Fig. 4-1.

The pH values of these solutions were located around 8 which were slightly more or less than the distilled water's pH value at 8.153. However, these pH values were gradually increased with the increase of both the reaction temperature and the reaction time. In another hand, the EC values of these solutions were quite varied from 4 to 7.5 mS/m. Nonetheless, these EC values were much higher than the distilled water's EC value at 0.08 mS/m. It might be guessed that the plant could be safe with these solutions. In fact, the reason for increasing the pH value has not much been cleared and rarely been studied since there are several forms of minerals inside and could not exactly be identified.

After 72 hours, all the germinated seeds were counted, the root length was measured and the seed germination ratio (SGR), the mean root length (MRL) and the germination index (GI) were determined. The results are illustrated in Table 4-3and Fig. 4-2.

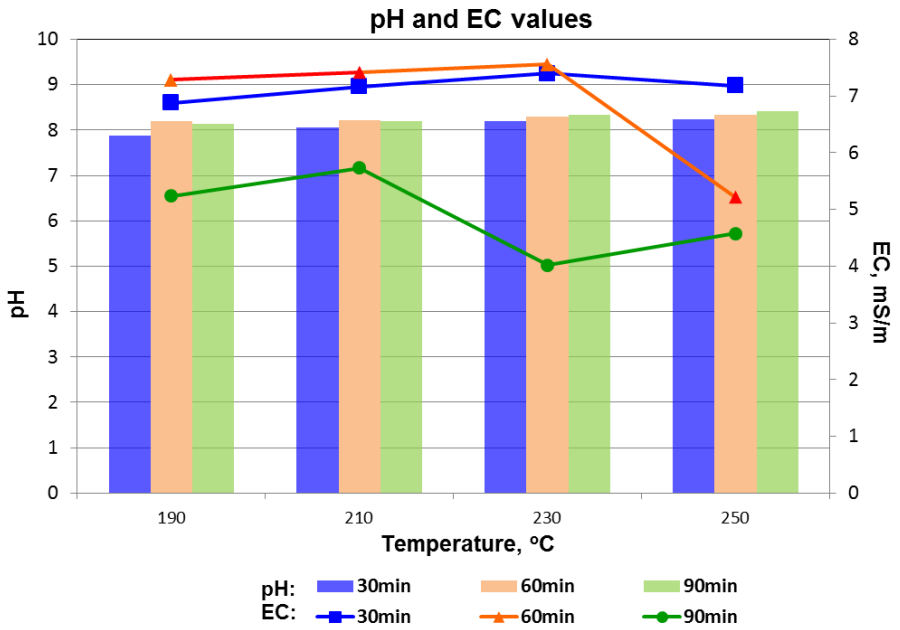

Fig. 4-1 pH and EC values of solid residue at 1:1 solid to water ratio

Table 4-3 Seed germination's results

	30 min				60 min				90 min				Blank
	190°C	**210°C**	**230°C**	**250°C**	**190°C**	**210°C**	**230°C**	**250°C**	**190°C**	**210°C**	**230°C**	**250°C**	
SGR, %	92.0 ±0.00	99.3 ±0.67	94.0 ±1.15	98.7 ±1.33	94.0 ±1.15	98.0 ±2.00	98.0 ±1.15	100.0 ±0.00	99.3 ±0.67	98.0 ±2.00	98.7 ±0.67	94.7 ±2.40	97.3 ±1.33
MRL, cm	1.57 ±0.25	1.86 ±0.29	1.34 ±0.19	1.48 ±0.20	1.59 ±0.19	1.42 ±0.16	1.32 ±0.14	1.23 ±0.12	1.73 ±016	1.87 ±0.17	1.84 ±0.15	1.66 ±0.13	1.83 ±0.08
GI, %	76.4	106.0	68.3	83.0	80.9	78.9	73.4	70.9	98.2	103.7	103.2	85.7	

The results are reported by the means of triplicates ± standard error of the mean.

In Fig. 4-2 (a), when the solution of 30 min reaction time was applied, the SGR was varied from 92% to 99% whereas the MRL was varied around 1.4-1.9 cm. In either case, the highest value was found at 210°C and the lowest was at 230°C. Similarly, the GI was also found to be the highest and lowest at the same temperature but the differences were greater. Nonetheless, when 60 min reaction time was applied (Fig. 4-2 (b)), the SGR showed gradual increase with the increase of the reaction temperature from 94% to 98% while the MRL showed opposite. The MRL showed the decreasing trend from 1.6 cm to 1.2 cm when the reaction temperature increased from 190°C to 250°C. Therefore, the GI was also decreased, started from 80% to 70%. Unlike 60 min reaction time, the data for 90 min reaction time shown in Fig. 4-2 (c) indicated the gradual decrease as well. However, the MRL was different. It firstly increased from 1.73 cm to 1.87 cm, then beyond 210°C, it decreased and reached 1.66 cm when 250°C was applied, likewise the GI performance. The GI showed the increasing trend from 98% first then decreased to 85%. Overall, the GI was varied from 70-106% which indicated that these solid residues had no phytotoxicity to the plants, though they could not provide 100% maturity.

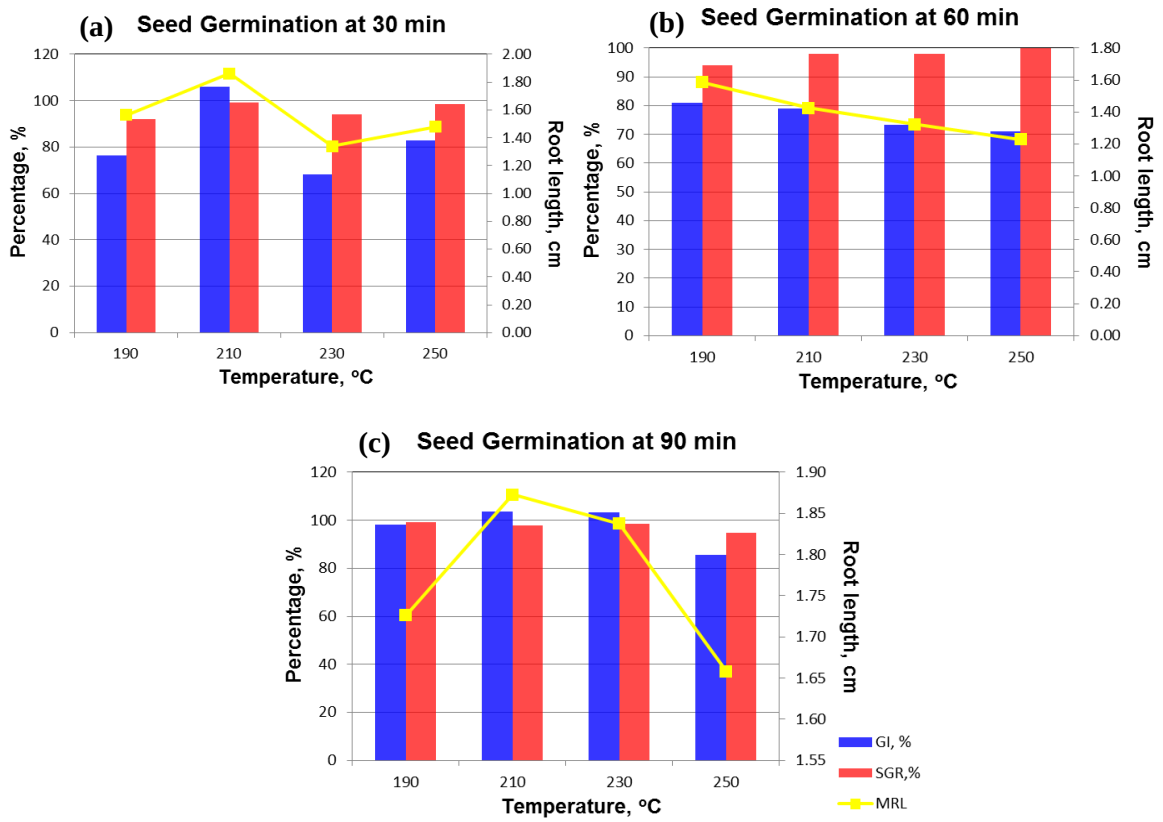

Fig. 4-2 Seed germination evaluation at (a) 30 min, (b) 60 min and (c) 90 min reaction time

4.3.2 Planting test

In order to determine the effects of the solid residues on the plants, the decomposed granite soil (DGS) was selected for this study due to very low macro nutrient available in the soil. DGS is made from the granite that was decomposed in a period, not the humus, thus it mainly contains mineral-like nutrients. The characteristics of the DGS soil are shown in Table 4-4. Table 4-5 listed the available nutrients of the solid residue after HTT at 60 min reaction time as for the solid biofertilizer whereas, Table 4-6 shows the available TN, TP and TK nutrients in the solid residues applied to the Komatsuna seeds.

Table 4-4 DGS soil characteristics

Moist,%	pH	EC, mS/cm	Available nutrient, %													
1.23	7.589	7.22	C	N	P	K	S	Si	Fe	Al	Ca	Ti	Mg	Mn	Ba	Sr
			0	0	0	7.75	0	47.5	20.9	13.4	5.46	2.33	1.44	0.384	0.296	0.0902

Table 4-5 Elemental compositions as for solid biofertilizer

Nutrients, %	Australian std.*	60 min, °C			
		190	210	230	250
N	0.5	3.32	2.53	1.96	1.53
P	0.5	0.57	0.82	1.04	1.08
K	0.5	0.00	0.00	0.00	0.00
S	0.5	0.47	0.39	0.60	0.34
Ca	0.5	18.98	21.24	23.26	21.30
Mg	0.5	2.53	3.46	3.96	4.06
Si	0.5	6.83	2.71	7.92	8.37

* Australian government standard for solid fertilizer, Fertilizer working group, Department of Agriculture, 2011.

Table 4-6 Available TN, TP and TK nutrients in the solid residues applied to the Komatsuna seeds.

mg	TN	TP	TK	TN	TP	TK	TN	TP	TK
T, ^{o}C		100%			50%			25%	
190	100	0.0173	0	50	0.0086	0	25	0.0043	0
210	100	0.0325	0	50	0.0162	0	25	0.0081	0
230	100	0.0531	0	50	0.0266	0	25	0.0133	0
250	100	0.0707	0	50	0.0354	0	25	0.0177	0

After 25 days, the plants as shown in Figs. 4-3 and 4-4 were cut, the shoot length and the leaves width were measured, and oven dried at 65^{o}C for 3 days then the dry weight was measured. The results are shown in Figs. 4-5 and 4-6.

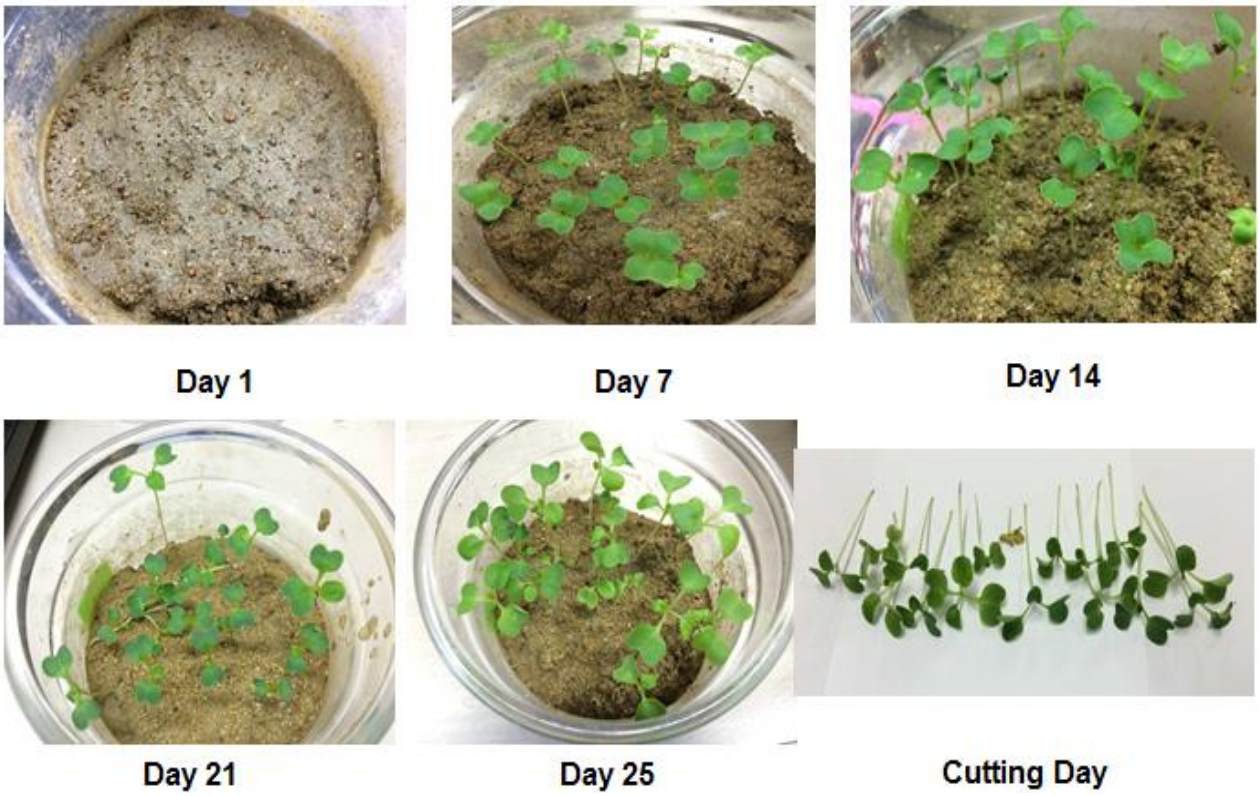

Fig. 4-3 The planted Komatsuna

It can be seen from Fig. 4-3 that about half of the seeds could be germinated and the root came out on the first day. After 7 days, two primary leaves became bigger and most of the third leaf came out after 14 days. After 21 days, the size of the third leaf was almost the same as the first 2 primary leaves while the forth leaf had been developed. After 25 days, the forth leaf became a little bigger. However, these developments were varied case by case depending on the solid residues applied in regards to the color, the size, the number of leaves and plants as depicted in Fig. 4-4. It can obviously be noticed from Fig. 4-4 that not all the seeds could be germinated and all the germinated seeds could not be growth and all the growth plants could not be matured. These results, so far, could support the seed germination test's conclusion.

Fig. 4-4 The incomplete development of planted Komatsuna

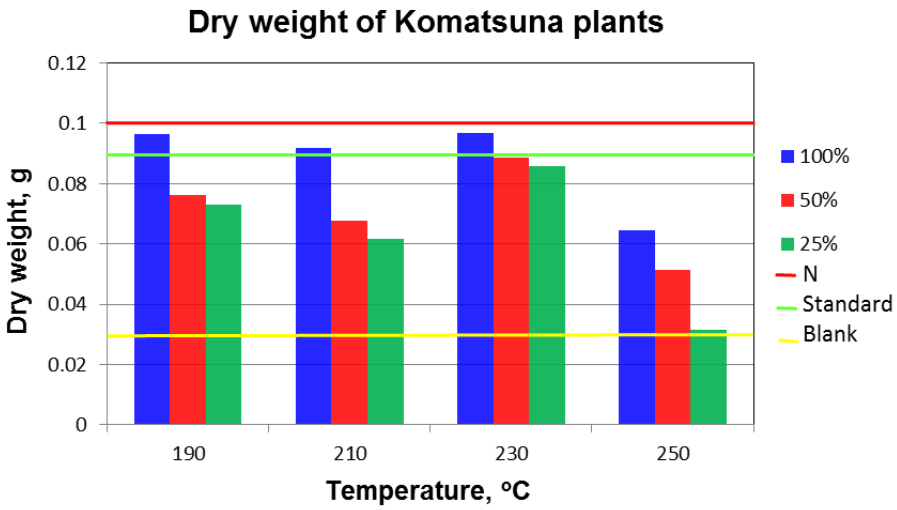

Fig. 4-5 Dry weight of planted Komatsuna

In Fig. 4-5, dry weight of the Komatsuna plants were compared in bar graphs where the blue bar represents for the 100% ratio, the red for the 50% ratio, the green for the 25% ratio. The red line shows the weight of the N fertilizer, the green line shows the standard fertilizer and the yellow line shows the blank. It is obviously seen that the N fertilizer is the most important factor for plant growth since the weight of the Komatsuna with only N fertilizer applied at 0.1 g was higher than the one with the standard fertilizer applied at 0.089 g and was the highest one, whereas, the blank gave the lowest yield at 0.033 g. As is known, N is a part of all living cells and is the necessary part of metabolic processes involved in the synthesis and transfer of energy (chlorophyll) [4-10]. It can also be said

that the chemical fertilizer resulted in the lower product yield than the organic fertilizer since the 100% ratio showed a higher yield than the standard fertilizer even they received the same amount of 100 mg-N nutrient. Moreover, when compared at the same temperature but different solid residue ratios, it is eminently showed that the 100% ratio gave the higher yield than the 50% and 25% ratios even they all had the same 100 mg of N, where the 100% ratio was applied with the organic-N only. Also the 50% ratio yielded higher than the 25% ratio. However, the standard fertilizer which had no organic-N fertilizer did not show the lower yield than the 25% ratio as the proportional trend. In contrast, it did yield higher than both the 50% and 25% ratios. In this case, it might be resulted from the mixing of the organic and inorganic nitrogen that would result in lower efficacy than the pure inorganic-N fertilizer. One more noticeable result is that the TP nutrient did not give a positive effect on plant growth as can be seen in each ratio group, where the higher the TP was provided, the lower the plants weight was. However, these amounts of the available P nutrient were relatively low compared to the make-up P fertilizer to meet 100 mg TP. The influence of the organic and inorganic fertilizers may be bigger than the influence of the P fertilizer. Like the N nutrient, P is also an essential part of the photosynthesis process which involved in the formation of oils, sugar, starched, etc. and also helps with the proper plant maturation [4-10].

Additionally, it is perceptible that the yield at 230^{o}C was higher than other temperatures. It might be a result of higher S (the secondary macronutrient) that was highest at 230^{o}C and followed by 190^{o}C, 210^{o}C and lowest at 250^{o}C as shown in Table 4-5. Because of S is the essential plant food for production of protein which promotes activity and development of enzymes and vitamins and also helps chlorophyll formation. Moreover, S also improves root growth and seed production. In addition, it does help vigorous plant growth and resistance to cold [4-10]. Thus it is possible that the yield at 230^{o}C was higher than others.

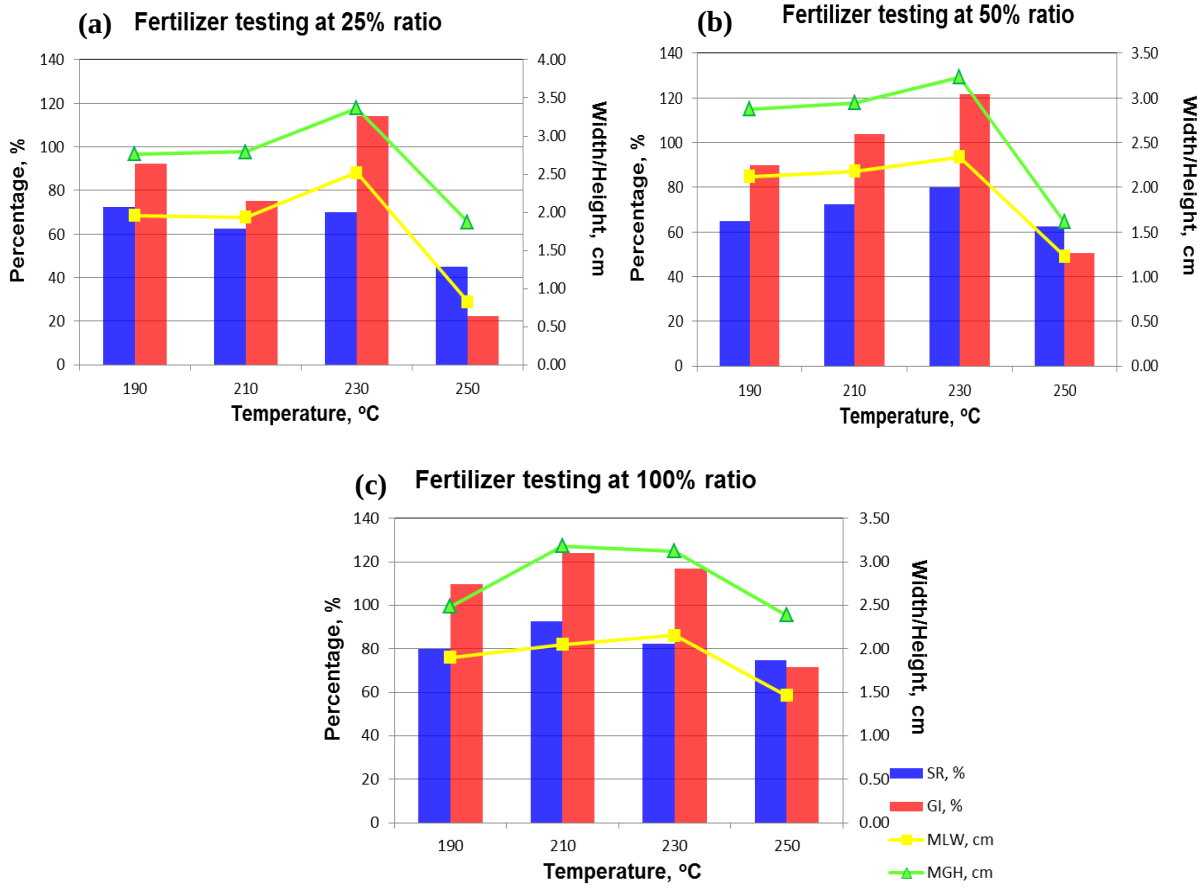

Fig. 4-6 Planting evaluation at (a) 30 min, (b) 60 min and (c) 90 min reaction time

A number of plant growth tests were performed in terms of SR and the results are shown in Fig. 4-6 in the blue bar. The ratio was ranged in 45-72% in the case of the 25% ratio where the highest ratio was found at 230^{o}C as shown in Fig. 4-6(a). The MLW was presented in the yellow line where 230^{o}C was found to have the widest leaf and the narrowest one was found at 250^{o}C. Similar for the MGH, the highest one was found at 3.37 cm and the shortest one was at 1.87 cm. For the GI, it had the similar trend with both MLW and MGH, not the SR. The best GI was at 114% when 230^{o}C was applied while the worst GI was at 22.55% when 250^{o}C was applied. For the 50% ratio shown in Fig. 4-6 (b), the best condition for all SR, MLW, MGH and GI was also found when 230^{o}C was supplied at 80%, 2.34 cm, 3.24 cm and 121.5% respectively, whereas the worst condition was found at 62.5%, 1.23 cm, 1.62 cm and 50.65 % when 250^{o}C was applied. In Fig. 4-6 (c), the best SR, MGH and GI were shifted to 210^{o}C and found at 92.5%, 3.19 cm and 124.18% respectively, whereas the best MLW was still at 230^{o}C and found at 2.16 cm. The worst condition at this ratio was at 250^{o}C still. It is clearly shown that the solid residue produced at 250^{o}C, at all reaction time have not good effect for plant growth, whereas the best fertilizer that helps to promote the plant growth is varied between 210^{o}C and 230^{o}C. Nevertheless, all the results here showed no correlation with the TN, TP and TK nutrients contained in the solid residue. The evaluation of the planting test is also tabulated in Table 4-6.

Table 4-6 Evaluation of the planting test

		SR, %	MLW, cm	MGH, cm	GI, %
100%	190^{o}C	78.5±1.53	1.90±0.78	2.49±0.36	109.6
	210^{o}C	94.8±0.14	2.05±0.16	3.19±0.41	124.2
	230^{o}C	81.8±0.76	2.16±0.11	3.12±0.46	116.8
	250^{o}C	74.5±1.00	1.47±0.05	2.39±0.04	71.7
50%	190^{o}C	63.5±1.61	2.12±0.10	2.87±0.39	89.9
	210^{o}C	73.0±0.76	2.18±0.10	2.94±0.37	103.8
	230^{o}C	78.8±1.26	2.34±0.11	3.24±0.24	121.6
	250^{o}C	62.5±0.29	1.23±0.18	1.62±0.08	50.7
25%	190^{o}C	72.3±0.43	1.96±0.02	2.76±0.04	92.5
	210^{o}C	63.0±0.76	1.94±0.21	2.79±0.42	75.3
	230^{o}C	69.3±0.87	2.52±0.17	3.37±0.49	114.2
	250^{o}C	44.8±0.50	0.83±0.13	1.87±0.29	22.6
Blank		70.0±0.58	1.97±0.01	1.98±0.07	90.1
N-fertilizer		72.3±2.29	2.71±0.04	4.45±0.002	128.2
Standard		63.0±0.76	1.94±0.03	2.46±0.55	78.9

The results are reported by the means of triplicates ± standard error of the mean.

4.4 Conclusions

The HTT solid residue has a potential to be utilized as a solid fertilizer because they showed no phytotoxicity to the plant but could not provide a complete maturity, thus some nutrients may need to be applied. However, the N nutrient has found to be a key nutrient for plant growth, whereas the P nutrient showed no positive effect. Nonetheless, the organic fertilizer was the best solution for plant growth.

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Chapter 5 Characterization and microalgae cultivation application of the aqueous product

Abstract

The hydrothermal treatment (HTT), a new promising technology for clean energy production, has attracted many potential customers including researchers and commercial sectors due to its great merit on the energy efficiency. An involvement of heat and pressure in an aqueous medium to a biomass leads to a decomposition of this biomass to form various products, bio-oil, gaseous, and solid and liquid residue. The liquid residue accounting for a major part has been regarded as waste but attracts a number of interests recently. Recirculation of this liquid residue for algae cultivation has been proposed with no detail on its characteristics so far. This point, therefore, is introduced. However, the HTT condition is the most essential factor for all HTT's products. The reaction temperature (190-250^{o}C) and the reaction time (30, 60 and 90 min), as a result, were first changed to study their effects on the HTT products. Then, the linkage between the outcome liquid residue employed for algae cultivation and the cultivated algae was discussed in regards to the biochemical composition. The protein content has found to have a correlation with the total organic nitrogen in its aqueous medium, whereas, the carbohydrate content has found to relate with the inorganic carbon.

5.1 Introduction

To date, microalgae-based biofuels have been recognized as the "third-generation of biomass energy" [5-1] and the "only current renewable source of oil that could meet the global demand for transport fuels" [5-2] due to superior photosynthetic efficiencies and high carbon dioxide fixation ability [5-3]. Algae are the fastest growing photosynthetic organisms on earth and capable to transform solar energy to chemical energy so as to drive its metabolism and have no competition for arable land. Its productivity could be as high as 50 times over the fastest growing terrestrial plant like switchgrass [5-4]. However, some considerations have been arisen. The cost effectiveness on the algae cultivation compared to some terrestrial plants regarding the energy required for necessary nutrients production such as nitrogen, is firstly concerned [5-5]. Besides, the energy efficiency of the microalgae-based biofuels production is another issue. Therefore, the energy intensive dewatering of the algae slurry and the nutrient management has been intensively studied [5-6]. Here, HTT has a potential to cope with these considerations. HTT, one of the thermochemical processes, involves an application of heat and pressure to biomass in an aqueous medium with or without a catalyst that directly transforms the biomass into liquid oil containing higher energy content than syngas or alcohol, in an environment temperature lower than 400^{o}C [5-7]. At elevated temperatures, the properties of this hot compressed water such as the solubility, the density, the dielectric constant and the reactivity, change much as it approaches its critical point (374^{o}C, 22.1 MPa) [5-8]. As a result, the depolymerization and the repolymerization of the biomass components like lignin, cellulose, lipids, proteins and carbohydrates, to transform them into bio-oil are enhancing. The reactions occurring are namely the hydrolysis, the depolymerization and the repolymerization/self-condensation [5-9]. The main products of HTT are bio-oil, solid residues, aqueous residues and gas product. The HTT aqueous residues have been proved to be rich in nutrients hence an idea to recycle this aqueous for algae cultivation has been proposed [5-10]. It is not only high in nutrients, but also high in the recovery yield to be recycled up to 82% of the total mass [5-11]. Moreover, it has been found that 20-fold dilution of the nutrients rich aqueous phase could support about half of the optimal nutrient required for algal growth [5-12]. According to these researches, HTT, therefore, is a promising process not only because of its ability to produce bio-oil, but also the nutrients in its aqueous residue can be facilitated for algae growth to improve the overall economic viability of the microalgae-based biofuels production.

There are many taxonomic groups of algae species that are able to accumulate lipids in high amounts. Many studies have shown that green microalgae strains are the biggest group with high potential to produce large quantities of lipids that are also capable of being grown in a mass culture [5-12], [5-13]. The green microalgae, the Chlorellaceae strain and Chlorella sp., were therefore selected for studying in this research based on these previous studies. In this study, we focused on the characteristics of harvested algae employing the HTT aqueous residue as a medium growth. The role of the operating conditions, the reaction temperature and the reaction time, were investigated in regards to the nutrients composition and yield of the aqueous residues. Firstly, HTT reaction temperature (210-290^{o}C) and the reaction time (30, 60 and 90 min) were changed. Then the cultivation of microalgae, Chlorella sp., employing the aqueous residues obtained from the previous step as a growth medium was studied.

5.2 Materials and methods

5.2.1 Materials

A 500 mL glass tube chamber reactor autoclave equipped with a magnetic drive agitator, an electrically heated furnace (model MMJ500, OM Labtech, Japan) was occupied for HTT experiment using powder freshwater green microalgae TISTR-8511 strain (Chlorellaceae strain) obtained from TISTR (Thailand Institute of Scientific and Technological Research) (Fig. 2-1, Chapter 2). Most chemicals used were purchased from Wako Chemicals (Tokyo, Japan).

The marine water green microalgae Chlorella sp. was provided from Iranian Research Organization for Science and Technology (IROST). The 900 mL laboratory scale glass bottles equipped with a 12 L/min air pump (MAS-1 model, As one Inc.) for stirring were used for algae re-cultivation. The 60 cm-length, 18 W-fluorescent lamps were used as an artificial light source (Panasonic FL20SS, W/18).

5.2.2 Methods

The reaction temperatures were varied for 190^{o}C, 210^{o}C, 230^{o}C and 250^{o}C for investigating the influences of reaction temperature, while the reaction times were set to 30 min, 60 min and 90 min for studying the influences of the reaction time. The pressure was then varied followed the saturated steam pressure of each temperature. 20 g of powder microalgae was firstly introduced to a glass tube chamber, followed by 80 g of distilled water to make a 100 g-sample size then thoroughly mixed and placed into the autoclave. Approximately 2 min of argon gas was purged into the headspace of the autoclave so as any combustion could be kept away. A mixing speed of 200 rpm was set and then heated to the desired temperature with 4.7^{o}C/min-ramped rate. When the target temperature was reached, the reactor was kept stable at that temperature for 30 min, 60 min and 90 min, and then let it cooled down to the room temperature. The pressure inside was then released and the product was collected. The two-phase mixture product was gently mixed with 100 mL-dichloromethane (DCM; Sigma-Aldrich, 99% purity) and transferred to vacuum filtered by a 1.2µm pore size-glass microfiber filter paper (GF/C, Whatman). The filtered algal residue, defined as solid residue, was separated for further analysis, whereas the two-phase mixture was separated for the DCM-soluble phase and the DCM-insoluble phase using an auto-pipette (Gilson Pipetteman). The DCM-insoluble phase defined as aqueous residue was further analyzed for nutrient recovery while the DCM-soluble phase was next left in a fume hood at room temperature for 3 days to remove DCM-solvent and the remaining product (DCM-soluble liquid) was defined as the bio-oil and was further characterized. The overall procedure was demonstrated in Fig. 2-2 in Chapter2. The average values of the triplicated experiments were reported.

For algae re-cultivation, 500 mL-sample size was designed for all culturing and divided into 5 groups as for blank, standard, aqueous residue of 30 min reaction time (S30), aqueous residue of 60 min reaction time (S60) and aqueous residue of 90 min reaction time (S90). The aqueous residues were applied at 1:50 aqueous residue to water ratio, whereas, the Rodik medium was added at 0.1 L/1 L of algae solution. The details of each group are shown in Table 5-1. 15 g of sodium chloride was added to each bottle so as to adjust the salinity environment of culturing as the Persian Gulf seawater condition where the Chlorella was brought from. The 60 cm-length, 18 W-fluorescent lamps were applied for 12 hr-dark and light cycles. After 2 weeks, algae were harvested, centrifuged to separate algae paste and oven dried at 65^{o}C for 2 days to prepare for further analysis.

Table 5-1 Experimental design for algae re-cultivation

Group	Conditions
Blank	500 mL distilled water
Standard	450 mL distilled water + 50 mL Rodik medium
S30	490 mL distilled water + 10 mL aqueous residue of 30 min reaction time
S60	490 mL distilled water + 10 mL aqueous residue of 60 min reaction time
S90	490 mL distilled water + 10 mL aqueous residue of 90 min reaction time

5.2.3 Analysis

The chemical composition, the proximate and the ultimate analysis of the microalgae feedstock were first analyzed and reported in Table 2.1 in Chapter 2, whereas the trace elementals were presented in Table 5-2 measuring by ICP emission spectroscopy (ICPS-8100, Shimadzu Inc.) after being digested by the MultiWave3000, Perkinelmer Inc. The total nitrogen (TN) and inorganic nitrogen (IN) were measured by QuAAtro 2-HR (BLTEC Inc.) The total organic carbon (TOC) and organic carbon were measured by a total organic carbon analyzer, TOC-L (Shimadzu Inc.). The electrical conductivity (EC) and pH of the aqueous product were measured by the desk-type meters (F-70/DS-70 series, Laqua Inc.). A UV-Vis spectrophotometer (UV-2550, Shimadzu Inc.) was employed for the biochemical composition analysis. The protein content and the carbohydrate content were analyzed followed the Bradford method [5-14] and the Dubois method [5-15] respectively, whereas the lipid content was followed the Bligh and Dyer method [5-16].

Yields of the products were determined by Eq.5-1, whereas, recovery rate was calculated by Eq.5-2.

Yield (wt%) = (Mass of product fraction / Mass of initial feedstock) x 100% Eq.5-1
Aqueous residue recovery (%) = (Mass of aqueous residue / Mass of water added for reaction) x 100% Eq.5-2

Table 5-2 Trace elements in microalgae feedstock

	Trace elements, %											
Feedstock	P	K	Mg	Ca	Fe	Na	Si	B	Mn	Zn	Cu	Co
	0.50	0.41	2.47	11.59	0.27	0.99	3.45	0.0271	0.0715	0.0247	0.0104	0.0047

5.3 Results and Discussion

5.3.1 Influences of HTT operating conditions on aqueous residue

5.3.1.1 Influences of the reaction temperature

HTT at different reaction temperatures led to different aqueous residue yields as shown in Table 5-3. However, these gravimetric yields were not much different, ranged from 70g to 75g, and showed slightly increasing trend with the increase of the reaction temperature. The slightly increasing trend of these yields were likely due to more fragmented molecules had been decomposed and released into the aqueous phase as of the increase of the reaction temperature. Later, they were consumed by other reactions [5-17]. Water molecules, hence, could be produced and disappeared. The recovery rate was found to have a similarity with that of the gravimetric yield and also shown in Table 5-3.

Table 5-3 Yields and recovery rate of the aqueous residues

	30 min, ^{o}C				60 min, ^{o}C				90 min, ^{o}C			
Aqueous	190	210	230	250	190	210	230	250	190	210	230	250
Yield (g)	69.94	74.22	72.21	72.13	70.02	72.78	72.71	75.50	72.05	72.75	72.88	73.05
Recovery (%)	88.96	92.75	89.88	89.92	86.25	90.46	90.67	94.25	89.04	90.50	90.42	88.38

Table 5-4 shows the physical properties and elemental compositions of the aqueous residues at each condition. From this table, we can see that the pH value was first in the acidic side as a result of the decomposition of macromolecules to produce some free H-ions, which later consumed by other reactions then the pH was gradually increased to the basic side. Additionally, the released nitrogenous compounds in a no oxygen environment were likely to form ammonium and released the hydroxide ions then the pH was increased [5-18]. Regarding the EC values, those molecules were in the forms of water-soluble organics such as, amino acids, fatty acids and their derivatives, hence, the EC values were affected. However, some elements exhibited lower ability to conduct an electrical current through it, i.e. P, S, Si and Fe. As a result, it may prohibit the dissolved materials into the water somehow [5-19]-[5-23].

It can obviously be seen from Table 5-4 that most of the nutrients recovery from the aqueous residues had found to be high and higher than the required nutrients of Rodik algae medium except for P at 250^{o}C of 60 min and 90 min that found to be lower. This finding has been satisfying the aim of converting this aqueous residue for algae cultivation. Moreover, N, K and Na were found to be increased when the reaction temperature increased. In the case of N, it is likely due to more hydrophilic nitrogenous compounds were released from proteins after the hydrolysis at a high reaction temperature in no oxygen environment. Ammonia, a derivative form of amino acids, then was converted to ammonium rather than nitrate, and dissolved into the water phase [5-18]. Hence, the N concentration in the aqueous phase increased with the increase of the reaction temperature. Potassium is always present in minerals as a single-charged cation (K^{+}) which is rapidly dissolved into the aqueous phase and then there was an increasing trend with the increase of the reaction temperature [5-24]. The concentration of phosphorus drastically decreased with the increase of the reaction temperature as a result of the increasing pH. Since phosphate is transported by the Phosphate specific transport (Pst) system in the form of $H_2PO_4^{-}$ and HPO_4^{2-}, the predominant phosphate species over the pH range 5.0 to 9.0 [5-25].

Table 5-4 Physical properties and elemental compositions of the aqueous residues

		30 min, ^{o}C				60 min, ^{o}C				90 min, ^{o}C			
	Rodik	190	210	230	250	190	210	230	250	190	210	230	250
EC (s/m)		2.18	2.40	2.51	2.75	2.16	2.34	2.58	2.78	2.16	2.50	2.66	2.67
pH		5.33	5.35	5.86	7.03	5.59	5.83	6.53	7.92	5.69	5.84	6.87	8.21
						(mg/L)							
N	49.41	7260	7891	8134	9588	7437	8177	8516	9484	7899	8064	8503	8444
K	13.72	371	375	384	393	497	499	517	522	498	513	522	526
P	8.93	808	553	163	16	821	547	72	3	703	413	28	1
Mg	1.97	1600	1500	1400	1200	1600	1500	1200	1100	1500	1500	1200	910
Ca	17.09	1200	1100	1100	1000	1100	1100	1000	1200	1200	1100	1100	1300
Fe	3.52	46	40	28	16	46	40	19	11	44	34	20	9
Na	7.86	2400	2500	2400	2500	2500	2500	2500	2600	2100	2500	2700	2700
B	0.05	48	40	42	42	44	44	42	42	44	43	43	42
Mn	0.49	37.0	27.0	14.0	5.7	32.0	22.0	9.4	5.9	28.0	18.0	7.7	4.8
Zn	0.02	5.50	3.10	1.30	0.46	4.40	2.90	0.68	0.19	3.50	2.20	0.53	0.11
Mo	0.02	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Cu	0.02	0.12	0.08	0.13	0.11	0.20	0.16	0.14	0.15	0.14	0.19	0.17	0.17
Co	0.06	0.30	0.23	0.17	0.14	0.24	0.30	0.12	0.14	0.26	0.19	0.12	0.17

EC = Electrical conductivity, Rodik = algae medium, n/d = not detected

5.3.1.2 Influences of the reaction time

The longer reaction time seemed to promote no change in either the gravimetric yield or the recovery rate as shown in Table 5-3. However, it did a promotion on the pH value and the EC value as shown in Table 5-4. Likewise the reaction temperature, the longer reaction time could prolong the time for substances to be decomposed. Hence, more fragmented molecules were released, dissolved and consumed, and brought about the slightly higher pH. Besides, it had also a positive effect on the concentration of N, K and Na but in less significance than that of the reaction temperature.

5.3.2 Influences of HTT aqueous residue on algae growth

This study aims to promote a utilization of aqueous residue from HTT which accounted for the largest portion to algae medium for re-cultivation so as to minimize the discharged waste causing the water pollution and optimize oil extraction process. Algae are photosynthesis organisms growing in the water medium, thus most of their life time are rely on the water quality. The pH is the most crucial factor for their alive, therefore, the aqueous residue dilution ratio was first justified regarding this matter. The 1:50 aqueous residue to water ratio was then employed as listed in Table 5-1. The adjusted pH values of the solutions are shown in Fig. 5-1. As can be seen in Fig. 5-1, the pH values of the aqueous residues were more close to neutral than before the dilution and the EC values were also nearly 0 S/m, which were similar to that of the distilled water. However, at lower temperatures, the pH values were far more neutral, about 5.5, still. Additionally, the available nutrients required for algae growth were found to be sufficient and satisfactory that they were much higher than the Rodik-standard algae medium as presented in Table 5-5.

Nevertheless, there were K and Fe that could rarely be recovered and then insufficiently available for algae growth. Also, P could be recovered only at a low reaction temperature, thus it was unable to supply P to the culturing with a high reaction temperature, 230°C and 250°C.

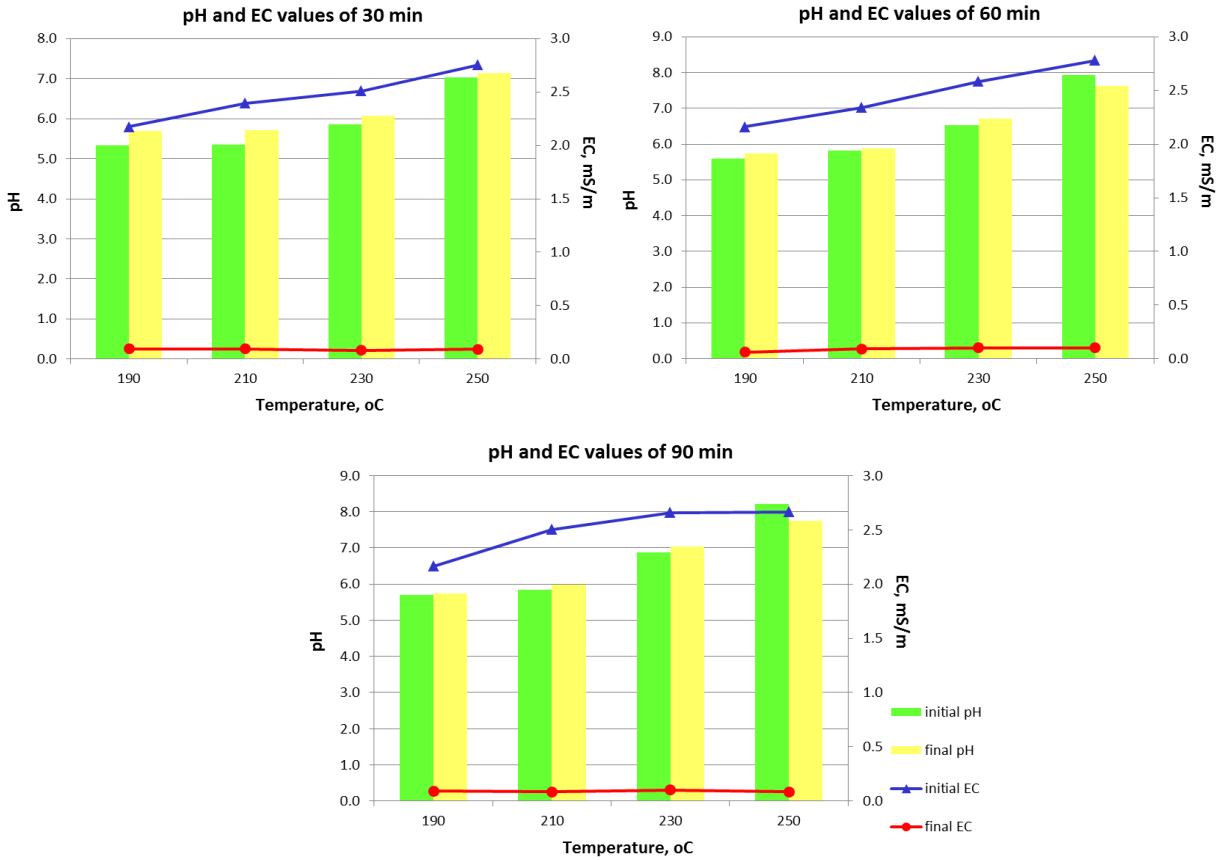

Fig. 5-1 pH and EC values of aqueous residues for algae re-cultivation at (a) 30 min, (b) 60 min and (c) 90 min

Table 5-5 Available nutrients in 1:50 aqueous solution required for algae growth

		30 min, °C				60 min, °C				90 min, °C			
	Rodik	190	210	230	250	190	210	230	250	190	210	230	250
		(mg/L)											
N	49.41	145.19	157.83	162.68	191.77	148.74	162.33	170.33	189.68	157.98	161.29	170.07	168.88
K	13.72	7.42	7.50	7.68	7.86	9.94	9.99	10.34	10.44	9.96	10.25	10.45	10.51
P	8.93	16.16	11.07	3.26	0.31	16.43	10.94	1.44	0.06	14.05	8.27	0.56	0.02
Mg	1.97	32.00	30.00	28.00	24.00	32.00	30.00	24.00	22.00	30.00	30.00	24.00	18.20
Ca	17.09	24.00	22.00	22.00	20.00	22.00	22.00	20.00	24.00	24.00	22.00	22.00	26.00
Fe	3.52	0.92	0.80	0.56	0.32	0.92	0.80	0.38	0.22	0.88	0.68	0.40	0.18

After 2 weeks of culturing, all the algae solutions shown in Fig. 5-2 were harvested and analyzed for protein, carbohydrate and lipid contents produced.

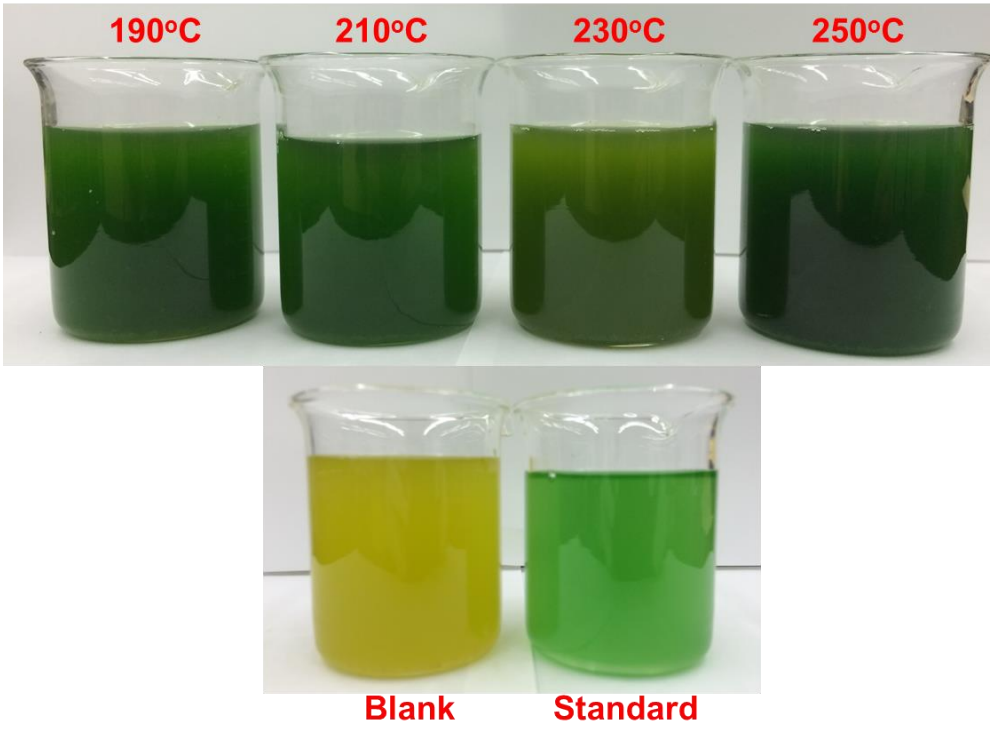

Fig. 5-2 Harvested algae solution

5.3.2.1 Influences of HTT conditions of the aqueous residue on the protein content

In Fig. 5-3, the protein concentrations in algae are shown in the blue bars on the left-hand side, whereas the red line shows the concentration of the standard at about 2.86 wt% and the green line shows the concentration of the blank at 2.24 wt%. In Fig. 5-3 (a1) at 30 min reaction time, it is apparently shown that the protein concentration of 2.50 wt% at 190°C was the lowest one and lower than the standard, while, it was higher than the blank's one. As temperature increased, the protein contents did not show the increasing trend as the available N nutrients shown in Table 5-5 does. It first increased from 190°C to 210°C then decreased at 230°C and increased again at 250°C. Nonetheless, this behavior was found to have a correlation with the total organic nitrogen available in the aqueous solution as shown in Fig.5-3 (a2) on the right. The right figures of Fig. 5-3, (a2, b2 and c2) illustrate the available nitrogen nutrients in the aqueous residue employed for algae re-cultivation where the blue line represents the total organic nitrogen (TON), the red line represents the inorganic nitrogen (IN) and the green bars represents the total nitrogen (TN) available. The concentration of TON was found to be more than 2 times of the concentration of IN. Hence, it can be expected that the protein produced was also performed as TON even though algae would uptake IN first and later converted this IN to TON. In Fig.5-3 (b1), the protein content of 60 min reaction time is shown. It clearly shows that the concentration at 230°C was the lowest one at about 2.39 wt%, however, it was higher than the blank but lower than the standard still. It is noticeable that the protein contents at 60 min reaction time were performed alike their TON as shown in Fig.5-3 (b2). Likewise the 90 min reaction time, the protein contents shown in Fig.5-3 (c1) and the available TON in aqueous residue shown in Fig.5-3 (c2) were performed a similar correlation with the 30 and 60 min reaction time. As a result, a hypothesis that TON in algae medium is a key factor of the protein content in algae can be stated.

In Fig. 5-3 (b1), in addition, the lowest protein content found at 230°C might be a result of the lowest Ca (the secondary macronutrient) at 230°C which gradually increased at 190°C, 210°C and highest at 250°C as shown in Table 5-5. Ca is the essential part of the cell wall structure which helps strengthen the plant. Moreover, Ca also provides normal transport and retention of other elements [5-26]. Because of the low available Ca and TON in the algae medium at 230°C thus, it might be possible that the transportation and retention of this TON required for algae growth was also lower than others. Finally, it brought about

lower protein production than other conditions. However, this behavior was not found at 30 and 90 min reaction time which may be a result of more significant of the available TON primary-macronutrient than the Ca secondary-macronutrient. The produced protein was preferable to follow the available amount of TON rather than the little available amount of the Ca.

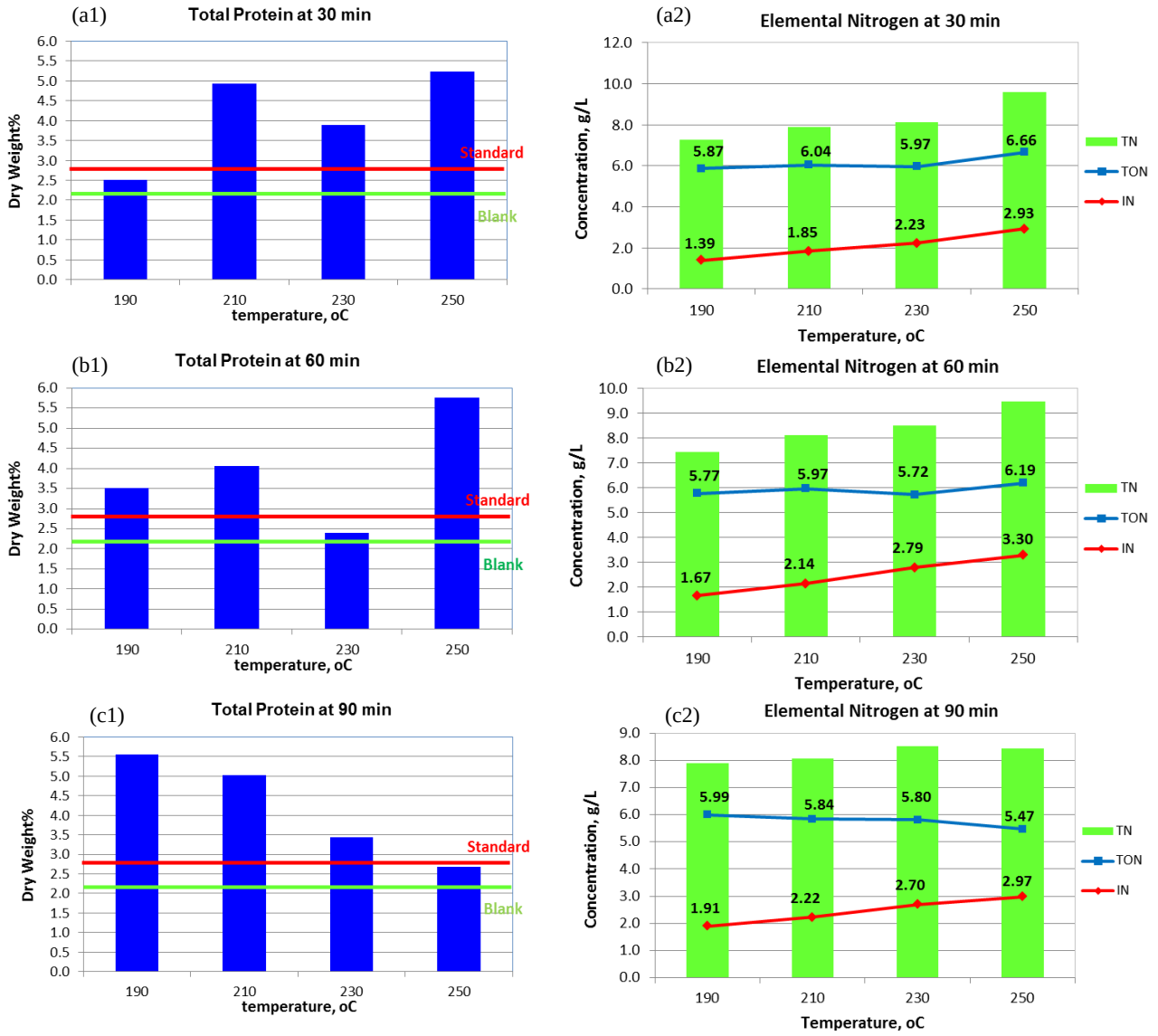

Fig. 5-3 Total protein of harvested algae solution at (a1) 30 min, (b1) 60 min and (c1) 90 min, and the elemental nitrogen available in aqueous residues at (a2) 30 min, (b2) 60 min and (c2) 90 min

5.3.2.2 Influences of HTT conditions of aqueous residue on the carbohydrate content

The carbohydrate contents in algae are shown in Fig. 5-4 on the left where the blue bars shows the carbohydrate concentration, while the standard is shown by the red line at 5.04 wt% and the blank is shown by the green line at 2.95 wt%. In Fig.5-4 (a1) where the concentration of 30 min reaction time was illustrated, it was obviously seen that only the concentration of 4.03 wt% at 190^{o}C was higher than the blank's one, but there was none higher than the standard. Moreover, there was no similarity with the total carbon which is the major component of the carbohydrate. Nevertheless, its behavior had been found to have a relationship with the inorganic carbon available in its algae

solution as shown in Fig.5-4 (a2). Fig. 5-4 (a2, b2 and c2), illustrated the available carbon dissolved in the aqueous residue employed for algae re-cultivation where the blue line represents the total organic carbon (TOC), the red line represents the inorganic carbon (IC) and the green bars represented the total carbon (TC) available. The explanation was similar to that of the protein content since the IC was available 2-3 times higher than TOC in the solution, the behavior of glucose, represented the total carbohydrate content, was selectively performed the same. Besides, the 60 min and 90 min reaction times have found show a similar performance as well. There showed no relationship between the reaction time and the carbohydrate content anyhow.

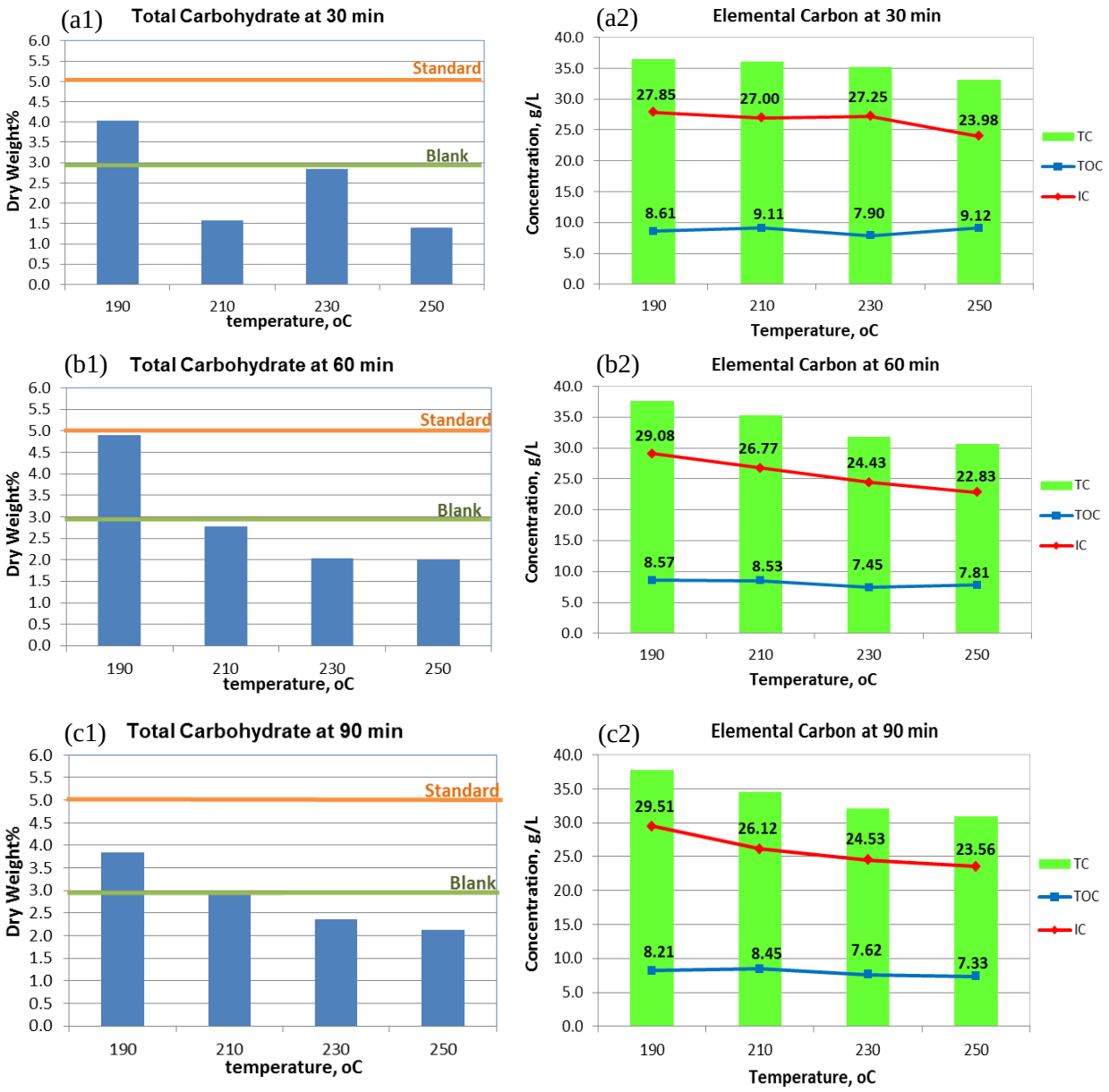

Fig. 5-4 Total carbohydrate of harvested algae solution at (a1) 30 min, (b1) 60 min and (c1) 90 min, and the elemental carbon available in the aqueous residues at (a2) 30 min, (b2) 60 min and (c2) 90 min

5.3.2.3 Influences HTT conditions of aqueous residue on lipid content

Total lipid content is shown in the blue bar while that of the standard is shown at 2.51 wt% in the red line and that of the blank is shown at 0.75 wt% in the green line in Fig. 5-5. In Fig. 5-5 (a), the total lipid content of 30 min reaction time is presented. It can be observed that all the temperatures could produce higher lipid content than the blank, but lower than the standard still. Additionally, all 30 min, 60 min and 90 min reaction times showed lower lipid concentration than the standard. However, 90 min reaction time showed higher lipid concentrations than others, where no linkage between the lipid content and the reaction temperature can be observed. Anyway, the total lipid content has found to have no relationship with any element in the algae medium.

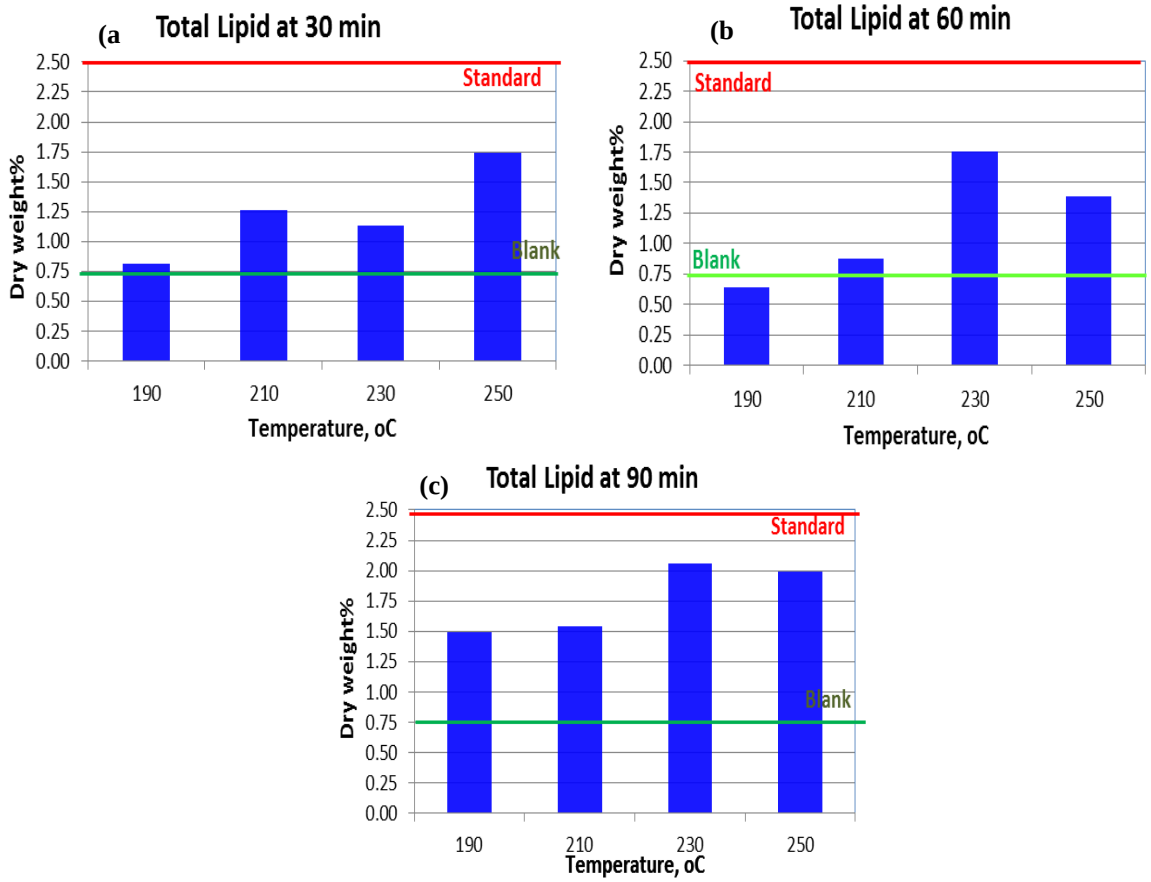

Fig. 5-5 Total lipids of harvested algae solution at (a) 30 min, (b) 60 min and (c) 90 min

5.4 Conclusions

This study demonstrated that HTT aqueous residue has a potential to be utilized for algae cultivation in order to minimize the waste emission and add values to the residue. The low temperature HTT, 190-250^{o}C, was first employed to extract the bio-oil while the residues left were still rich in nutrients. The N, K and Na nutrients were found to increase in the residue with the increase of the reaction temperature regardless of the reaction time. However, neither the reaction temperature nor the reaction time had a significant influence on the aqueous residue yields.

The cultivated algae employing the HTT aqueous residue have found to have satisfactory biochemical characteristics. Most of the protein contents were higher than that of the standard, whereas, the carbohydrate and lipid contents were lower. However, it has been discovered that TON and IC in the algae medium are the most significant factors for the protein content and the carbohydrate content respectively.

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Chapter 6 Conclusions and Recommendations

The aim of this study is to effectively extract oil from microalgae together with efficacious utilization of by-products by employing the hydrothermal treatment (HTT) technology. Therefore, the overall conclusions of this study are as followed.

1. Low temperature HTT (190-250^{o}C) of microalgae has a potential to utilize the solid and aqueous co-products as solid bio-fertilizer and algae growth media along with a bio-oil extraction. Increasing the reaction temperature and the reaction time positively affected the bio-oils' yield when the conditions were not severe. On the contrary, the solid product showed the opposite trend. Unlike the aqueous product, the yield showed more or less steady. Different from other products, the gaseous product gradually increased with the increase of both operating parameters.
2. Microalgae bio-oils employing low temperature HTT showed a comparable performance to petroleum derived fuels. However, some pre-treatments are necessary for fungible transportation fuel production.
3. HTT solid product has a potential to be utilized as solid bio-fertilizer because they showed no phytotoxicity to the plant and rich nutrients could be recovered. The N fertilizer was found to be a key nutrient for plant growth. The organic fertilizer promoted better growing than the inorganic fertilizer.
4. HTT aqueous product also demonstrated an effective algae re-cultivation utilization performance. A new finding was a correlation between the characteristic of algae growth media and the biochemical characteristic of harvested algae. The protein content of harvested algae showed similar behavior with the total organic nitrogen of the algae media, whereas the carbohydrate content behaved similar to the inorganic carbon. However, the lipid content showed no relationship with any elements.

Nevertheless, a contribution from this study would be fruitful if practical usage is adopted. Recommendations on the future work are also welcome. Anyhow, current recommendations would be on the variety of algae species as of the primary predominant to the product yields' composition. Moreover, a bigger laboratory scale should have been conducted in order to gain larger amount of HTT products for better analysis. Additionally, more varieties of solid product employed for fertilizer testing would provide clearer result whereas, more dilution trials of the aqueous product applied for algae media should be adopted to learn more relationship between the harvested algae and the algae solution. Lastly, the distribution of the potassium in the oil phase should further be analyzed for a completed tracking of this major nutrient.