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Hydrothermal Treatment for Production of Aqueous Co-Product and Efficient Oil Extraction from Microalgae

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Abstract**—**Hydrothermal liquefaction (HTL) 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 operating 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 HTL conditions to clarify suitable conditions for extracting bio-oil together with value-added co-products. Firstly, different microalgae loading rates (5-30%) were tested and found that this parameter has not much significant to product yield. Therefore, 10% microalgae loading rate was selected as a proper economical solution for conditioned schedule at 250°C and 30 min-reaction time. Next, a range of temperature (210-290°C) was applied to verify the effects of each parameter by keeping the reaction time constant at 30 min. The results showed no linkage with the increase of the reaction temperature and some reactions occurred that lead to different product yields. Moreover, some nutrients found in the aqueous product are possible to be utilized for nutrient recovery.

Keywords**—**Bio-oil, Hydrothermal Liquefaction, Microalgae, Aqueous co-product.

I. Introduction

THERMOCHEMICAL conversion (TCC) is a chemical amending process of biomass production under heated, generally pressurized, and oxygen deprived conditions in which long-chain organic compounds (solid biomass) are broken into short-chain hydrocarbons, such as syngas or oil [1]. TCC also includes gasification, pyrolysis, and liquefaction [1], [2]. Hydrothermal liquefaction (HTL) engages in direct liquefaction of biomass, with the presence of water and with or without a catalyst that directly transforms the biomass into liquid oil, which contains higher energy content than syngas or alcohol, with a temperature lower than 400°C for the reaction [1]. HTL is different from biomass gasification and pyrolysis, which require dried feedstock and an environmental temperature higher than 600°C to encourage the process and therefore consume a larger amount of energy [1], [3]. Water is an important factor in HTL 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], [2], [4]. HTL, under high temperature and pressure (generally carried out at 200-370°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 [2], [5]-[10].

HTL 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°C) to create a hydrophilic bio-oil with a reduction of 10-18% of oxygen content when compared to the parent material [11]. 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) [11]. Thereby, HTL processing does rely on the unique properties of water at high temperature and pressure. At elevated temperatures, hydrogen bonding of the 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 [1], [2], [4], [11]. Dielectric constant is a ratio of a permittivity of a substance to a 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), because a dipole moment of the water molecule and so the 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 [11]. Thereby water becomes a very good solvent.

Liquid biofuel from microalgae has drawn many attentions and beaten other biomasses due to it has been identified as a promising feedstock for scaling up to industrial-scale production of carbon-neutral biodiesel [1]-[4]. Moreover, HTL has been emphasized due to energy saving on dewatering process and economical viability on production of value-added co-products along with bio-oil.

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 [12]-[15]. Algae contain about 2-40% of lipids per weight [12], whereas microalgae hold about 15-77% of oil contents per weight [15]. This difference is because the entire cell surface of algae can be involved in the photosynthesis process. As a

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result, lipids are accumulated in the entire cell which is different from other oil crops where seeds can contain oil [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 [12]-[17]. 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 [8], [17]. Moreover, reference [7] indicated that large amounts of lipids found in green microalgae are polar lipids and polyunsaturated C16 and C18 fatty acids. 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.

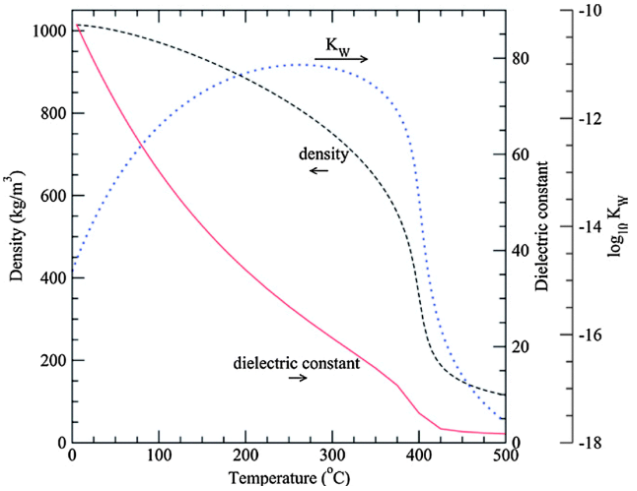

Fig. 1 Dielectric constant of water

In this study, we focused 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 aqueous co-product, nutrients for algae re-cultivation. The role of operating conditions, such as the biomass loading rate and the reaction temperature were investigated in regards to the composition and yield of the products. Firstly, different microalgae loading rates (5-30%) were tested then a range of temperature (210-290^{o}C) was applied.

II. Experimental Design

A. 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, purchased from OM Labtech (Tokyo, Japan) was employed. The stainless steel, SUS-316, autoclave with a capacity of 500 mL has a resistance of 300^{o}C and 20 MPa. Most chemicals used were purchased from Wako Chemicals (Tokyo, Japan).

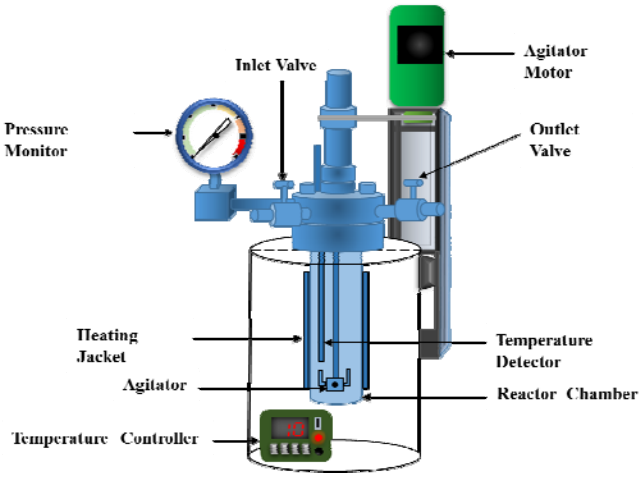

Fig. 2 A batch-type reactor autoclave (model MMJ500)

B. Experimental Procedures

In order to study the effect of the microalgae biomass loading rate, the fresh microalgae was prepared in a dried form to confirm an exact concentration of the microalgae biomass loading rate at each desired ratio. Then, different microalgae loading rates (5%, 10%, 15%, 20%, 25% and 30%) were tested with different distillated water quantities varied to the total amount of 100 mL-sample size. Firstly, this was conducted based on a conditioned schedule at 250^{o}C and 30 min-reaction time. This was first selected as prior research [18]-[20] showing that HTL at 250^{o}C 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] which means that more molecules will tend to dissociate in water at 250^{o}C. Secondly, a range of temperature (210^{o}C, 230^{o}C, 250^{o}C, 270^{o}C and 290^{o}C) was applied to verify the effects of this parameter by keeping the reaction time constant at 30 min and a 10% microalgae biomass loading rate was selected as a proper economical solution from the previously studied step. 30 min of the reaction time was first fixed for all experiments as the results in [19] were shown to be optimal. All of the experiments were conducted in duplicate batch reactions and the average values were reported.

For example, 5% microalgae biomass loading rate means that 5 g of powder microalgae and 95 g of distilled water were mixed thoroughly and added to the 500 mL-SiO_2 tube chamber and put into the autoclave. An argon gas was purged into the headspace of the reactor for 2 min in order to make oxygen-free environment in order to avoid any combustion. The agitator equipped with an impeller was run at the speed of about 200 rpm. After that the reactor was heated to the desired temperature with a ramped rate of 4.7°C/min and was kept at that point for 30 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 evaporated at 55-60°C using a shaking water bath 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 [21], [22]. The overall procedure for collecting and separating the HTL products is illustrated in Fig. 3.

C. Analysis

The microalgae feedstock was first determined to evaluate the chemical composition and conduct a proximate and ultimate analysis (Table I). The crude lipid content of the microalgae was determined by the Bligh and Dyer method used by TISTR. The moisture and ash content, and volatile matter were analyzed by the ASTM D3173, ASTM D5142 and ASTM D3175 method respectively, whereas fixed carbon was calculated by subtraction. The ultimate analysis, CHNOS, 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. 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. Trace elementals were measured by ICP emission spectroscopy (ICPS-8100, Shimadzu Inc.) after being digested by the MultiWave3000, Perkinelmer Inc. The total organic carbon (TOC) was measured by a total organic carbon analyzer, TOC-5000 (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.).

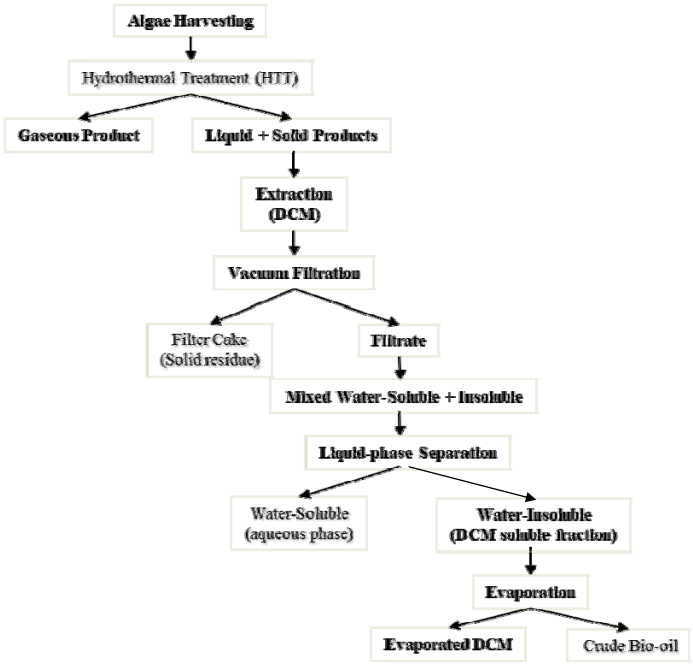

Fig. 3 HTL products collecting procedure

TABLE I
CHARACTERISTICS OF MICROALGAE TISTR-8511 STRAIN

Proximate analysis (wt%)				Chem. comp. (wt%)	Ultimate analysis (wt%)[a]							Higher Heating Value (MJ/Kg)
Moist	VM	FC	Ash	Lipids	C	H	N	O	S	P	K	HHV
5.57	65.42	2.69	26.32	22.55	38.26	4.96	4.51	51.02	0.36	0.48	0.41	16.61

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

Yields of all the products were calculated by (1), whereas the yield of the gas was defined following (2). Energy recovery was calculated by (3) and elemental recovery was calculated by (4).

$$\text{Yield (wt\%)} = (\text{Mass of product fraction} / \text{Mass of dry algae}) \times 100\% \quad (1)$$

$$\text{Yield of gas (wt\%)} = 100 - \text{Yield of (Bio-oil + Water-soluble + Solid residue)} \quad (2)$$

$$\text{Energy recovery (\%)} = (HHV_{oil} \times Mass_{oil} / HHV_{algae} \times Mass_{algae}) \times 100\% \quad (3)$$

$$\text{Elemental recovery (\%)} = (\text{Mass of element in aqueous product} / \text{Mass of element in dry algae}) \times 100\% \quad (4)$$

III. Results and Discussion

This study investigated the influence of the microalgae biomass loading rate (5-30%) at the constant reaction temperature of 250°C and 30 min reaction time and the influence of the reaction temperature (210-290°C) at the constant microalgae biomass loading rate of 10% and 30 min reaction time on the HTL products.

A. Effect of the HTL Operating Conditions on Product Yields

The effect of increasing the algae biomass concentration (10-50 wt%) has been previously studied [23] showing that it has no significant influence on the product properties and distributions, while [18] found that the increase of the microalgae biomass loading rate (5-35 wt%) also increased the oil yield from 36% to 46%. Different algae species lead to differences in the bio-oil yield even at the same concentration or condition [18], [23]. Therefore, this study is aimed to find the minimum microalgae biomass concentration required to possibly maximize the bio-oil yield. Fig. 4 shows the effect of the microalgae biomass loading rate on the product yields (oil, solid residue, water-soluble and gas) of the dry algae after HTL at 250°C and 30 min reaction time. The results show that the bio-oil yield seems slightly increased, but at 10% and 20% algae rates the yields jumped out of the trend. The maximum bio-oil yield was found at 17.0% when 20% algae rate was loaded into the reactor. Comparable to 10% algae rate, the bio-oil yield was 15.6%. Doubling the algae biomass concentration gave only 1.5% difference in the bio-oil yield. Hence, we found that 10% microalgae concentration rate is the most economical concentration for HTL at 250°C and 30 min reaction time on the basis of the bio-oil yield. Thereafter, we also selected 10% microalgae concentration rate for the next experiment in this study on the effect of the reaction temperature. The solid residue yield also followed the same increasing trend as the bio-oil yield. However, it appeared that this was not inversed with the bio-oil yield as the bio-oil yield increased the solid residue should have decreased. This is due to the macromolecules in microalgae being hydrolyzed into micromolecules and then turned into fatty acids and other substances. This may be explained by the higher yield of the water-soluble case since more water molecules would be produced during a dehydration reaction that was promoted by HTL [24].

Similar to the bio-oil yield found at 10% microalgae concentration as shown in Fig. 4, the bio-oil yield at 250°C shown in Fig. 5 gave a yield of about 15.4%. Fig. 5 shows the effect of the temperature on the HTL product yield at 10% microalgae biomass loading rate and 30 min reaction time. The trend of bio-oil yield is very noticeable and it increased with the temperature but then turned down after increasing beyond 250°C. The maximum bio-oil yield was marked at 15.4% when the reaction temperature was 250°C. A previous study [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 after that the maximum yield was decreased.

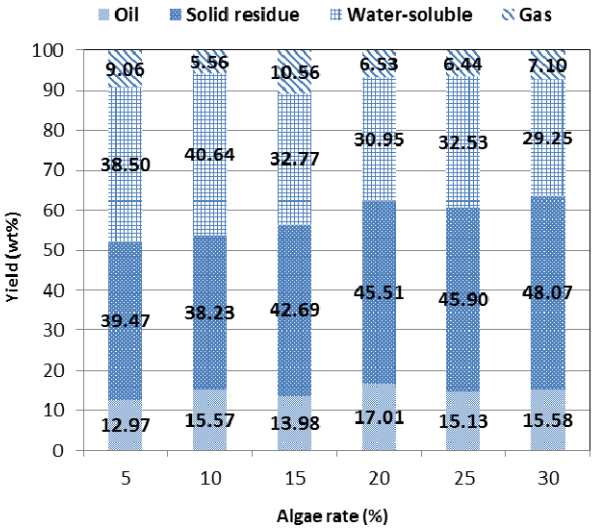

Fig. 4 Effect of the algae biomass loading rate on HTL products yield at 250°C, 30 min reaction time

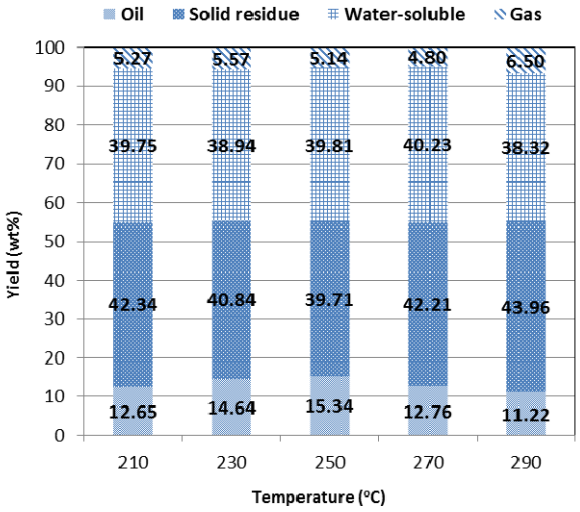

Fig. 5 Effect of the temperature on HTL products yield at 10% microalgae biomass loading rate, 30 min reaction time

The reaction scheme of biomass liquefaction was described in [18]-[25]. 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 dehydration, deoxygenation, decarboxylation, and 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.

An example of this hypothesis can be seen in Fig. 5. Increasing temperatures from 210°C to 250°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, dehydration, deoxygenation and 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. 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. 5.

TABLE II
Oil Elemental Composition and Properties after HTL at Each Condition

Algae rate (%)	C (wt%)	H (wt%)	O (wt%)	N (wt%)	S (wt%)	HHV (MJ/Kg)	ERC (%)
5	70.51	9.40	15.23	4.36	0.50	33.24	25.95
10	69.99	9.41	15.90	4.30	0.40	32.98	30.92
15	68.28	9.44	16.75	4.68	0.85	33.45	28.16
20	71.20	9.83	13.28	4.97	0.72	33.34	34.15
25	69.97	9.78	14.42	5.44	0.39	32.56	29.66
30	69.52	9.69	14.69	5.70	0.40	30.97	29.05
T (°C)							
210	69.97	10.07	16.67	2.93	0.37	35.49	27.02
230	65.98	9.25	20.37	4.00	0.41	33.67	29.69
250	69.22	9.77	16.22	4.39	0.42	33.34	30.79
270	64.71	9.26	20.01	5.50	0.53	34.00	24.66
290	73.41	9.64	11.42	4.97	0.57	36.12	21.71

ERC = Energy recovery

B. Effect of the HTL Operating Conditions on Oil Composition

Table II shows the elemental composition and properties of the bio-oils after HTL for the algae concentration rate variation and the temperature variation series experiments. It can be seen from the algae concentration rate variation series that most of the elemental compositions (C, H, O, N and S contents) were nearly constant. This means that the algae concentration has no significant effect on the bio-oil elemental compositions, even at 10 and 20% algae concentration. The carbon and hydrogen contents were greater than the parent algae feedstock by about two times leading to increased higher heating values (HHV) of the oil. In addition, the energy recovery ratio into oil ranged from 26% to 34% for which the maximum was obtained at 20% microalgae concentration and followed by 10% microalgae concentration. This was similar to the bio-oil yield shown in Fig. 4. Moreover, the oxygen content was significantly lower from the parent algae feedstock, which showed that the dehydration and decarboxylation reactions also play an important role in HTL. There is some variation in the sulfur content at 15 and 20% algae concentration where the highest value was found to be 0.85 wt%. The sulfur content may be inherent to the algal species [18] even though its content is relatively low. The nitrogen content in the algae rate series shows nearly constant with the slight increase from 4.36-5.70 wt% when the algae concentration increased. This range is similar to the nitrogen content in the parent algae feedstock.

For the temperature series, the carbon content slightly increased as the temperature increased, whereas the hydrogen content did not vary much. Thereafter, the HHV also did not change much. The largest decrease was found in the oxygen content from 16.7 wt% to 11.4 wt% when the temperature increased from 210°C to 290°C, showing that deoxygenation preferably occurred at the higher temperature. The nitrogen content, on the other hand, increased significantly from 2.9 to 5.5 wt% when the temperature increased from 210°C to 270°C then gradually dropped down. It can be assumed that nitrogenous compounds were preferably recombined to form hydrocarbon chains at higher temperatures. However, at too high temperature (over 270°C), these nitrogenous compounds were decomposed again and dissolved in another phase. The increase of the nitrogen content in the bio-oil is an undesirable substance since it promotes NOx formation when used as a transportation fuel. The energy recovery, in the same manner as in the algae rate series, ranged from 21.7% to 30.8% and the highest was found at the highest bio-oil yield.

C. Effect of the HTL Operating Conditions on Fatty Acids Composition

The fatty acids found in microalgae are mostly in a range of C12-C22 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 [26]. Large portions of fatty acids found are polyunsaturated C16 and C18 fatty acids [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, medium composition, light intensity, ratio of the light and dark cycle and the aeration rate [5], [26]. From Table III, the fatty acid composition of the bio-oil of both of the algae concentrations varied and the reaction temperature varied are shown. Despite showing all few hundred substances found in the bio-oil, the compounds usually created in the microalgae fatty acids (C12-C22) are elucidated. These total fatty acids (TFA) cover more than 80% by the peak area of the entire TFA in algae.

In the algae rate series, the largest portion belongs to the C18, and followed by the C16 fatty acids. The algae concentration showed no significant influence on the C18 fatty acids, whereas the C16 fatty acids had some small changes. The highest TFA yield is seen at 10% algae concentration, which is about 88.6% by the peak area, and followed by 87.8% by the peak area of 5% algae concentration. This supports the first selection of 10% algae concentration as the possible optimum algae solution for HTL at 250°C and 30 min reaction time. Increasing the algae concentration has no significant effect on the TFA composition. C16:0, C18:1 and C18:2 fatty acids appear to be good candidates for the fatty acids conversion to high-quality biodiesel [26]. Additionally,

using a higher unsaturated fatty acid content than 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 [26]. On another hand, a relatively high amount of polyunsaturated fatty acids (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 [26].

TABLE III
FATTY ACIDS COMPOSITION OF THE BIO-OIL

Lipid Number	Common Name	TFA (% by peak area) at Algae Rate (%)						at Temperature (°C)				
		5	10	15	20	25	30	210	230	250	270	290
C12:0	Lauric acid											0.85
C13:0	Tridecylic acid							0.54				3.74
C14:0	Myristic acid	0.82	0.68	0.74	0.58	0.63	0.55	0.6	0.65	0.87	0.67	1.88
C15:0	Pentadecylic acid	0.74	0.8	0.83		0.86	0.88	0.6	0.93	0.31	0.81	0.51
C16:0	Palmitic acid	25.25	21.61	23.68	23.5	20.7	17.61	38.07	38.89	35.61	21.98	15.65
C16:1	Palmitooleic acid	1.17	1.79	1.21	2.99	2.78	1.69	3.22	1.15	4.59	3.7	
C17:0	Margaric acid	3.64	3.63	7.15	6.65	8.24	11.1	2.83	4.31	2.38	4.47	10.16
C18:0	Stearic acid											
C18:1	Oleic acid	55.67	60.07	54.27	56.35	55.96	56.84	44.37	42.65	47.17	59.19	55.53
C18:2	Linoleic acid	6.89	6.96	6.03	5.56	6.48	6.39	2.48	4.79	2.72	2.66	4.44
C18:3	Linolenic acid											
C19:0	Nonadecylic acid	2.29	2.05	2.04	2.24	1.62	1.43	5.11	5	3.18	1.9	2.14
C20:0	Arachidic acid	0.52	0.46	0.42	0.42			0.95				
C20:1	Paulinic acid			0.78								
C22:0	Behenic acid							0.51	0.44		0.64	1.35
C22:6	Decosahex aenoic acid		0.88		0.67	0.78	0.74			0.61		

For the temperature series, the saturated C16 fatty acids illustrated the essential changes that relied on the temperatures. C16:0 decreased gradually when the temperatures increased from 210°C to 290°C. On the contrary, the unsaturated C16:1, C18:1 and C18:2 fatty acids presented some slimly increasing trends even though they were not very clear. The amounts of the TFA found were mostly higher than 80% by the peak area except for the one at 290°C. However, the saturated C16:0 fatty acids showed a decreasing trend as the temperature increased, whereas polyunsaturated C18:1, C18:2 fatty acids showed opposite trend. Although, this relationship was not clearly explained, this was likely due to the increase of the carbon content when temperature increased since the hydrogen content was constant.

D.Effect of the HTL Operating Conditions on Aqueous Products

HTL at different microalgae biomass loading rates led to the difference in aqueous product yield as shown in Table IV. The gravimetric yields were gradually decreased from 84.6 g at 5% algae rate to 58.1 g at 30% algae rate. This can be clearly understood as there was less water added for HTL as the algae loading rates were increased to the total of 100 g-sample size. However, a recovery rate of the aqueous product was nearly stable at about 87-90%. Likewise, an aqueous recovery in the temperature series was quite stable at 90% and the gravimetric yields were almost the same ranging from 81.0-83.2g as shown in Table IV. The disappeared water was due to its participated in the HTL reaction as a reactant. Table V shows the physical properties and an elemental recovery from the aqueous products. From this table, an electrical conductivity (EC) value showed an increasing trend from 0.93 s/m to 3.21 s/m when the algae concentration increased, then slightly decreased to 2.94 s/m at 30% algae concentration. This could be the result when more macromolecules in the algae biomass were hydrolyzed to form new small molecules hence, more ionic were released into the water-soluble phase as a result of a higher algae concentration. According to the previous studies [27]-[31], 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 A, hence, the EC values were affected. Too high concentration (30% algae rate) may prohibit the dissolved materials into the water somehow since some elements exhibited lower ability to conduct an electrical current through it, i.e. P, S, Si and Fe. This is also corresponded to the dissolved elements that found high in concentration in the aqueous product where concentration of the algae was high. It can obviously be seen that most of the elements recovered from the aqueous product were higher than the macro/micro nutrients required for algae growth when compared to the 3N-BBM+V medium excluding the P-macro nutrient and the Mn-micro nutrient. Additionally, the N and K-macro nutrients were found satisfying with their great concentration. It can be assumed that more hydrophilic nitrogenous compounds were released from proteins after the hydrolysis of the high algae biomass concentration. Moreover,

the recovery rates of N and K were quite high where K recovery was as high as 60-80% and N recovery was at 45-68% as illustrated in Fig. 6. The K and N recovery was first increased to the highest at 10% algae concentration then lowered with some fluctuations between 60% and 70% in the case of K, whereas it gradually decreased in the case of N. Likewise, the pH values were influenced by those reactions as well, and the values were gradually increased. It may be caused by the decomposition of some substances to produce some free hydrogen ions first and later consumed by another reaction [27].

TABLE IV
YIELD AND RECOVERY RATE OF THE AQUEOUS PRODUCT

	at Algae Rate (%)						at Temperature (°C)				
Aqueous	**5**	**10**	**15**	**20**	**25**	**30**	**210**	**230**	**250**	**270**	**290**
Yield (g)	84.56	79.67	74.14	70.82	67.58	58.1	82.59	81.57	80.95	83.23	81.24
Recovery (%)	88.92	88.53	87.23	88.52	90.11	83	91.77	90.63	89.95	92.48	90.27

TABLE V
PHYSICAL PROPERTIES AND ELEMENTAL COMPOSITION OF THE AQUEOUS PRODUCT

		at Algae Rate (%)						at Temperature (°C)				
	3N-BBM+V	**5**	**10**	**15**	**20**	**25**	**30**	**210**	**230**	**250**	**270**	**290**
EC (s/m)		0.93	1.59	2.04	2.79	3.21	2.94	1.45	1.516	1.595	1.676	1.779
pH	6.8	6.65	6.72	6.87	7.08	7.39	7.89	5.85	6.39	6.77	8.2	7.44
						(ppm)						
C	-	10053	16544	22997	32992	42011	51414	18192	18793	16211	15039	14012
N	124	2111	4512	6224	8527	10426	12549	4599	4979	4674	4214	4079
P	51	15.4	21.3	40.8	22.9	17.4	7.2	28.47	11.66	4.01	0.17	0.1
K	63	207.5	414.1	613.2	851.8	1139.3	1315.1	42.34	46.54	41.6	43.76	46
S	-	75.7	142.5	221.9	266.1	343.7	334.3	20.23	16.17	14.4	10.1	7.02
Na	77	65	120	170	240	320	380	110	120	130	130	130
Mg	7.39	25	52	83	96	110	120	97	69	62	31	2.8
Si	-	8.2	8.4	7.3	9.4	6.8	4.8	12	7.15	7.45	7.95	6.15
B	-	0.72	1.4	2	2.8	3.5	3.9	1.55	1.45	1.6	1.4	1.2
Fe	1.03	0.28	0.74	1.1	1.6	2.5	2.3	2.15	1.1	0.675	0.22	n/d
Mn	11.6	0.13	0.22	0.36	0.49	0.71	0.96	1.15	0.475	0.29	0.19	n/d

EC = Electrical conductivity, 3N-BBM+V = algae medium cultivation, n/d = not detected

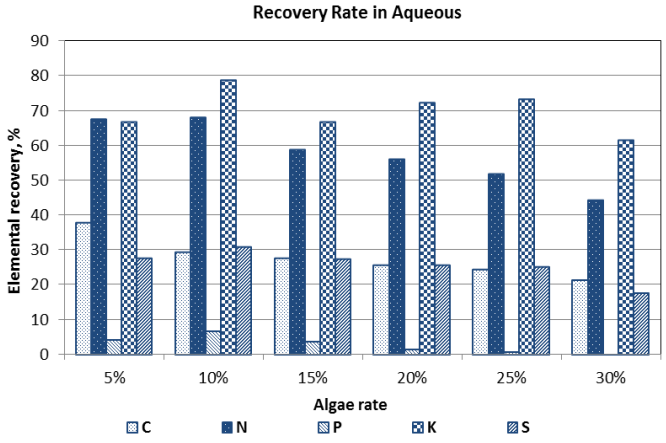

Fig. 6 Recovery rate in the aqueous product for microalgae biomass in the loading rate series at 250°C, 30 min reaction time

For the temperature series, similar trends of the EC and the pH values were found to be increasing due to an impact of the increasing temperatures. Since high temperature promotes high active energy of macromolecules in the algae biomass, it contributed more for the aqueous. Therefore, high electrical current were noticeable when the temperature increased. The explanation for the pH value is similar, but its change was more. The relatively low pH value at 210°C showed that some free hydrogen ions were desirably formed at low temperatures. This could be assumed that the peptide bonds in the proteins were decomposed more at low temperatures and released some amino acids and other short-chain organic acids. The organic acids could be further decomposed into other substances and hydrogen ions then the pH value was relatively low. Later on, these hydrogen ions were consumed by other reactions, so the pH values were increased from the acid side to the basic side. Different from the algae rate series, most of the elemental concentrations decreased when severe temperatures were introduced except for the K and Na contents. Compared to the elemental composition in the bio-oil yield, the C, N and S contents were transformed into the bio-oil more via some forms of fatty acids and nitrogen-compounds at high temperature where it could be the explanation of the decreasing in these contents in the aqueous product. Despite that, the K content was kept stable at about 41.6 to 46.54 ppm where the Na content slightly increased from 110 ppm to 130 ppm. The P and Mn contents were lower than those values in the 3N-BBM+V medium. Moreover, the K content was lower than in the 3N-BBM+V medium. Anyhow, some of the Fe contents were found higher than the 3N-BBM+V medium where the temperatures were beyond 230°C. A further study on the solid side should have been conducted since these un-passed standard values of the 3N-BBM+V medium might be exhibited there. Even though that most of the major nutrients in the temperature series were not qualified the algae medium standard they are satisfying to find some nutrients are acceptable for the algae re-cultivation with some nutrients modifying. The elemental recovery rate as depicted in Fig. 7 shows that the N content first increased

from 80% to 86% at 230°C then gradually decreased to 72% at 290°C as a consequence of the proteins were hydrolyzed into nitrogenous compounds and later transferred into the bio-oil phase and some probably transferred into gaseous when temperature increased.

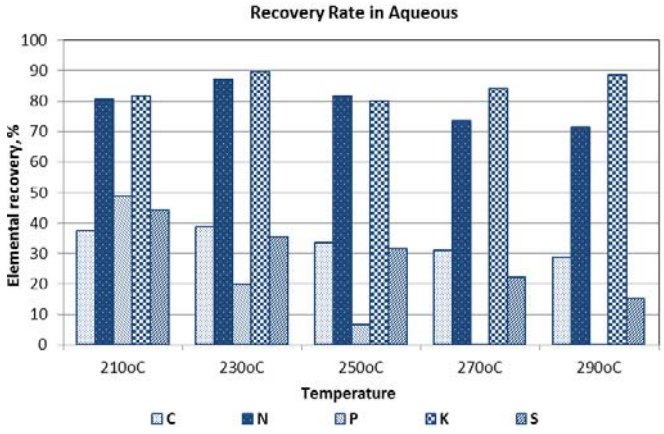

Fig. 7 Recovery rate in the aqueous product in the temperature series at 10% algae loading rate and 30 min reaction time

IV. Conclusion

The most economical microalgae biomass loading rate in this study was found at 10% when the HTL operating condition was scheduled at 250°C and 30 min-holding time, whereas the maximum oil yield was discovered at 15.4% with 85.5% principal fatty acids required for biodiesel conversion. The bio-oil yield and its composition are dominantly influenced by the temperature, whereas the concentration of the microalgae biomass plays some roles. In all algae concentration rate variation and temperature variation cases, the nutrients recovery from theirs aqueous products is possible with some nutrients adding depending on each case. Further study on the solid side should be included to understand the overall flow of the elemental compositions in more detail. However, the differences of the HTL product yields and theirs compositions could be found vary depend on the algae biomass species.

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