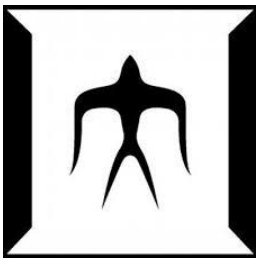
論文 / 著書情報
Article / Book Information

題目(和文)	微細藻類ペーストの水熱炭化を前処理として用いた微細藻類からのバイオディーゼル製造
Title(English)	Biodiesel production from microalgae through hydrothermal carbonization of microalgae paste as the pretreatment step
著者(和文)	VOTHANH PHUOC
Author(English)	Phuoc Vo Thanh
出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第10656号, 授与年月日:2017年9月20日, 学位の種別:課程博士, 審査員:吉川 邦夫,竹下 健二,日野出 洋文,加茂 徹,高橋 史武,時松 宏治
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第10656号, Conferred date:2017/9/20, Degree Type:Course doctor, Examiner:,,,,,
学位種別(和文)	博士論文
Type(English)	Doctoral Thesis

BIODIESEL PRODUCTION FROM MICROALGAE THROUGH HYDROTHERMAL CARBONIZATION OF MICROALGAE PASTE AS THE PRETREATMENT STEP

Vo Thanh Phuoc

Supervisor: Professor Kunio Yoshikawa



A doctoral thesis submitted to the

Department of Environmental Science and Technology

Interdisciplinary Graduate School of Science and Engineering

Tokyo Institute of Technology

Yokohama, Japan

August 2017

Acknowledgements

First of all, I would like to express my sincere gratitude to my academic advisor, Professor Kunio Yoshikawa, for giving me the opportunity to study, research and complete this dissertation. Without his patience, academic guidance and support in the most difficult times, this dissertation is impossible to be completed. Again, I would like to send my deep thanks to Professor Kunio Yoshikawa.

Also, I would like to acknowledge the thesis committee including Professor Tohru Kamo, Professor Hirofumi Hinode, Professor Kenji Takeshita, Professor Koji Tokimatsu, and especially Professor Fumitake Takahashi, for the time and the efforts that Professors spent on examining the whole dissertation. Through comments and criticisms, the professors helped me expand and understand more my research topic as well as improve the quality of this dissertation.

This dissertation also cannot be completed without the help of Monotsukuri Center (in Suzukakedai campus) where I was supported and provided necessary equipment and devices for experiment. I really thank the staffs of the center, especially Ms. Kyoko Sato and Ms. Ryoko Urakawa, who helped me use each equipment and device. I also want to thank Dr. Kiyoshi Tsuji, Dr. Ayako Takada, and Mrs. Eriko Ohno for the dedicated support during the experiments.

I would like to thank my laboratory colleagues for their help during the experiments. Many thanks go to Abooali Golzary, Department of Environmental Engineering, Faculty of Environment, University of Tehran, for his enthusiastic guidance and support in microalgae cultivation. I also cannot forget discussions, encouragements, and experiences which I received from other colleagues, especially Mohammad Saber and Muhammad Kunta Biddinika.

In addition, I would like to thank my family members, my parents and my younger sister, for their spiritual support during the most important periods, especially at the time of writing this dissertation. Their encouragement gave me more motivation to complete this thesis.

Last but not least, I am grateful for JICA's support through the program "Doctoral Degree in Japan" of AUN/SEED-Net. Not only financial support, JICA also created a good environment for learning and research. In particular, I would like to acknowledge the program coordinator, Ms. Tomomi Nishigaki, who supported and gave me helpful advice when I faced difficulties in my research and daily life as well.

Contents

Tables

Figures

Abstract

In recent years, the biofuels are studied with the aim of gradually replacing fossil fuels. In particular, the biodiesel production from microalgae gains much attention. Microalgae have specific characteristics such as the high lipid content, the rapid growth rate, and the good adaptation to non-agricultural land, and these make microalgae more competitive than other types of feedstock in the production of biodiesel. The biodiesel production from microalgae has two main approaches. In the first one called the two-step method, the lipids are firstly extracted by organic solvents, and then the extracted-lipids undergo the transesterification process for biodiesel synthesis. In the other called the direct transesterification method, the lipids in microalgae are extracted and converted into biodiesel in one step. This method has some advantages such as the elimination of using extraction solvents and the higher biodiesel yield. However, there are still some obstacles hindering the application of these methods in the actual production. After the harvesting, the moisture content of microalgae paste is in the range of 75 – 85%. This high moisture level significantly affects the extraction yield of lipid from microalgae and the biodiesel yield of the direct transesterification reaction. Therefore, the complete removal of water from microalgae must be done before subsequent conversion steps for biodiesel production. Because the drying process consumes so much energy, another pretreatment of microalgae paste is necessary.

Hydrothermal carbonization (HTC) is an energy-effective method to remove water from microalgae paste. HTC of microalgae is a process in which microalgae react with water at a high temperature and pressure (around 200 ^{o}C and 2 MPa). After the process, the solid hydrochar can be filtered from the aqueous phase, and most of lipid in microalgae feedstock is retained in the hydrochar. The production of biodiesel from hydrochar was investigated in this thesis by the methods that have been applied to microalgae. In each method, the biodiesel yields of microalgae and hydrochar were compared to assess the advantages that can be achieved when using hydrochar as a feedstock for biodiesel production.

In the two-step method, the yield of lipid extraction from the hydrochar (about 80%) was higher than from microalgae (about 30%). These following factors facilitated the penetration of solvent into hydrochar to extract lipid. After the hydrothermal carbonization process, the microalgae cells were impacted and contractive. Moreover, the ratio of FFAs/TFAs of the hydrochar (at HTC 200 ^{o}C) was higher than microalgae (48.0% and 24.9%). The appropriate conditions for the esterification pretreatment of extracted lipid were as follows: the catalyst Amberlyst 15 of 30% wt. oil, the reaction temperature of 80 ^{o}C, 12:1 molar ratio of methanol / lipid. Next, the biodiesel yield of 93.6% can be achieved after the transesterification of pretreated lipid at the conditions of 12:1 molar ratio of methanol / lipid, the amount of KOH of 1.5% wt. oil, the reaction temperature of 65 ^{o}C and the reaction time of 60 minutes.

In the direct transesterification method, the yields of hydrochar were higher than the yields of microalgae at the same reaction conditions. The appropriate conditions for biodiesel

production from microalgae were the ratio of 200 mg microalgae / ml methanol, 2% v/v acid H_2SO_4, reaction temperature of 80 ^oC, and 90 minutes of the reaction. To convert lipids in 400 mg microalgae into biodiesel, a volume of 2 ml methanol with the H_2SO_4 concentration of 2% v/v was required. On the other hand, if this amount of lipids exists in the hydrochar through HTC of microalgae, a volume of only 1 ml methanol with the H_2SO_4 concentration of 2% v/v was necessary. The amounts of methanol and catalyst which were required for a given amount of microalgae can be reduced to a half through the direct transesterification of the hydrochar.

Finally, an entire process, from the storage of fresh algae, HTC of microalgae paste to the direct transesterification of the hydrochar using the acid catalyst H_2SO_4, was investigated for biodiesel production. Instead of doing HTC of fresh microalgae immediately after harvesting, the storage of fresh microalgae for one day at the room temperature (25 °C) before conducting HTC can improve the total biodiesel yield of 19.3% at the same conversion conditions. This additional step is very meaningful in the real production and highly practical because there is no consumption of much energy and chemicals required in the storage of microalgae.

Chapter 1. Introduction

In this chapter, the background of the research is explained. The overview of biodiesel production from microalgae is firstly introduced. There, the advantages of microalgae over other feedstock in biodiesel production are highlighted, and the main challenge of water removal from microalgae paste is mentioned as well. After that, the overview of hydrothermal treatment of microalgae is presented. Finally, the purpose of study and the thesis outline are stated.

1.1. Biodiesel production from microalgae

Biodiesel can be produced from vegetable oils or animal fats. However, these feedstock are also consumed by human, so the utilization of these feedstock for biodiesel production makes the rival with the food sector and higher price of them. Moreover, the land area requirement for both food and biofuel production is more than the potential available area [1.1]. Beside of vegetable oils and animal fats, biodiesel can be produced from waste oils such as frying oils or greases. However, these feedstock are just suitable to small scale production, and these cannot meet the demand. In recent years, the biodiesel production from microalgae gains much attention because microalgae have many advantages compared to other feedstock.

Microalgae are prokaryotic microorganisms (cyanobacteria) or eukaryotic microorganisms (green algae) which have the unicellular or simple multicellular structure. Microalgae can survive and develop in a wide range of environment. The number of microalgae is over 50,000 species, but only around 30,000 species have been already studied [1.2].

1.2. Advantages of microalgae in biodiesel production

Compared to other feedstock, microalgae have many advantages in biodiesel production as follows.

a/ The lipid content of microalgae is higher than other feedstock. For some special species, the lipid content can reach 75% at a low growth rate (Botryococcus braunii). Most of species have the lipid content in the range of 20 – 50% at a normal growth rate. Besides, most of

components of fatty acids are C14:0, C16:0, C18:1, C18:2, and C18:3 which are suitable for biodiesel production [1.3].

b/ Microalgae can grow very fast, and they can be harvested in a short time after a few days of cultivation [1.4]. Therefore, microalgae have much higher productivity of lipid and much less land area requirement compared to other plants such as soybean, sunflower, palm. For example, the microalgae with the lipid content of 30% w/w require land area 49 or 132 times less than the requirement of rapeseed or soybean [1.5]. Therefore, the microalgae cultivation does not have a large effect on the land area for food crops.

c/ The cultivation medium is quite simple. Microalgae just need water, some simple nutrients (nitrogen, phosphorous), CO_2, and sunlight to make the photosynthesis [1.6,1.7,1.8].

d/ There are a numerous types of microalgae species, so the best adapted species can be selected for a specific environment. This selection cannot be made in other plants such as soybean, sunflower and palm [1.9].

e/ Other purposes can be achieved through the microalgae cultivation. Because microalgae need CO_2 for photosynthesis, so the CO_2 in the flue gas can be eliminated in the combination of microalgae cultivation [1.10]. Moreover, NH_4^+, NO_3^- and PO_4^{3-} in wastewater are necessary for the growth of microalgae, so the wastewater can be also treated through the microalgae cultivation [1.10]. Because the microalgae also contain protein and carbohydrate, the lipid-extracted microalgae can be used for methanol production, animal feed or organic fertilizer [1.10]. For some special microalgae species, other valuable compounds can be also extracted such as pigments, antioxidants, biological derivatives [1.11,1.12,1.13]. Therefore, microalgae will have an important contribution in pharmaceutical, food and cosmetic sectors in the future.

1.3. Transesterification and esterification reactions for biodiesel production

Biodiesel is a mixture of fatty acid methyl esters which are products of the transesterification reaction of triglycerides (the major composition of vegetable oils or animal fats). Transesterification is a 3-step reaction. First, triglycerides are converted into diglycerides. Next, diglycerides are converted into monoglycerides. Finally, monoglycerides are converted into fatty acid methyl esters (biodiesel). The steps of the transesterification reaction are

illustrated in **Figure 1.1**. Where, radicals R_1, R_2, R_3 represent hydrocarbon chains of fatty acids [1.9].

In the transesterification reaction, methanol is commonly used as a reagent at 6/1 molar ratio of methanol / oil (catalyst KOH) to obtain the complete conversion of oil. Although acid or base, homogenous or heterogeneous catalysts may be used for transesterification reaction, the homogeneous base catalyst KOH are commonly used in industrial production in the form of stirred reactors [1.9].

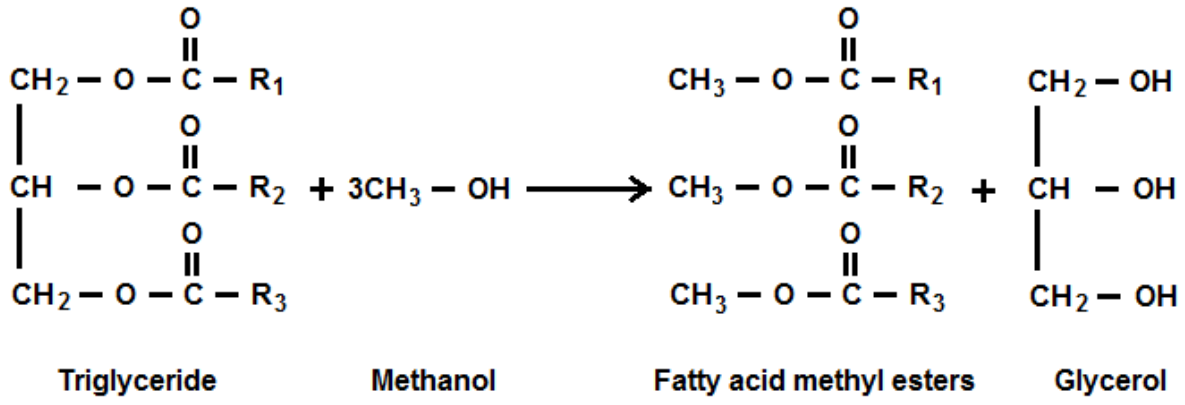


Figure 1.1. Transesterification of triglycerides for biodiesel production [1.14]

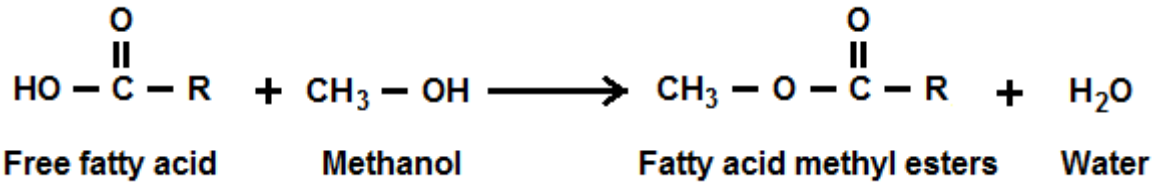


Figure 1.2. Esterification of free fatty acids for biodiesel production [1.14]

However, the transesterification reaction is only suitable for the oil with low content of free fatty acids with the acid value lower than 2 mg KOH / g oil [1.15]. For oils with a high level of free fatty acid (frying oil), a combination method including the esterification pretreament shown in **Figure 1.2** and the transesterification has been developed [1.15]. In the esterification pretreatment step, free fatty acids react with methanol to create fatty acid methyl esters under the presence of an acid catalyst. When the amount of free fatty acids in the oil is reduced to the required level (acid value lower than 2 mg KOH / g oil), the acid

catalyst is removed. Subsequently, the transesterification reaction of oil was performed to convert triglycerides into fatty acid methyl esters.

1.4. Hydrothermal treatment of microalgae

Because the concentration of microalgae in the culture is very low, a large amount of water must be removed in microalgae harvesting. The harvesting cost accounts for 20 – 30% of the total microalgae production cost [1.16]. Generally, the harvesting methods are combined from different physical, chemical and biological steps to achieve the maximum efficiency. The main harvesting methods are sedimentation, filtration, and centrifugation. Flocculation is a method that coagulates microalgae cells to increase the block size of microalgae, so it is usually combined with three methods above to support the separation process.

After the harvesting, the moisture content of microalgae paste is in the range of 75 – 85% [1.9]. This high moisture level is the inherent characteristic of microalgae paste. However, the moisture content significantly affects the extraction yield of lipid from microalgae [1.17] and the biodiesel yield of the direct transesterification reaction [1.18,1.19]. Therefore, the complete removal of water from microalgae must be done before subsequent conversion steps for biodiesel production. The drying process consumes so much energy. Even the sunlight can be used to dry microalgae in a small scale, but it looks like not effective for an industrial scale. Moreover, the availability and the stability of sunlight as well as the degeneration of microalgae must be taken into account. Therefore, instead of the drying process, another pretreatment of microalgae paste is necessary. There are two different processes to treat microalgae and water at a high temperature as follows.

1.4.1. Hydrothermal carbonization

Hydrothermal carbonization (HTC) of microalgae is a process in which microalgae react with water at a high temperature and pressure (around 200 ^{o}C and 2 MPa) [1.20]. After the process, two main products are created: the solid hydrochar and the aqueous phase. Among hydrothermal treatment methods, HTC is the process occuring at the mildest conditions of temperature and pressure. It means that the enery requirement and the equipment investment are reduced. Heilmann et al. conducted HTC of many microalgae species and proved that HTC of microalgae happened at milder temperature and pressure (200 ^{o}C in the holding time

of 30 minutes) compared to other biomass feedstocks. The reason is that the microalgae do not have cellulose, hemicellulose and lignin [1.20].

Although the low value of solid hydrochar, especially compared to the char produced from other biomass feedstock, the HTC of microalgae has some important features. The lipid component of microalgae does not take part in the HTC process [1.21]. In other words, most of lipid in microalgae feedstock is retained in the hydrochar [1.22,1.23,1.24]. Therefore, the HTC of microalgae can be used as the pretreatment step to create the lipid-high hydrochar for biodiesel production. Moreover, the aqueous phase contains high content of nutrients (nitrogen and phosphorus), so this can bring additional benefits for the HTC process [1.20].

1.4.2. Hydrothermal liquefaction

Hydrothermal liquefaction (HTL) of microalgae occurs at higher temperatures (280 – 370 ^{o}C) and at higher pressures (10 – 25 MPa). The main product is a biocrude oil, and the by-products are the gas, the aqueous phase and the solid [1.25]. The biocrude oil cannot be used directly, but it can be used as the co-feedstock in the petroleum refinery. At the hydrothermal treatment temperature of 375 ^{o}C, the biocrude oil yield can be up to 49.4% [1.26]. However, the oil has high viscosity and high content of nitrogen (up to 6%) mainly from protein derivatives [1.26]. It means that there is an additional cost required for denitrogenation.

1.5. The purpose of this study

Obviously, HTC process is more preferable than HTL process for the purpose of biodiesel production from microalgae. The energy requirement and the investment cost of HTC are lower than HTL process. After HTC, most of lipid from microalgae is concentrated in the hydrochar. This makes the conversion of lipid into biodiesel easy. Therefore, the hydrothermal carbonization of microalgae paste should be used as a pretreatment step for biodiesel production from microalgae.

However, the production of biodiesel from hydrochar has not been carried out in previous studies. Thus, the production of biodiesel from hydrochar was investigated in this thesis through the methods that have been applied to microalgae. In each method, the biodiesel yields of microalgae and hydrochar were compared to assess the advantages that can be achieved when using hydrochar as a feedstock for biodiesel production.

The objectives of this research are stated as follows.

1. Comparison between the lipid extraction from microalgae and the hydrochar, and the transesterification of extracted lipid for biodiesel production.

2. Comparison between the direct transesterification of microalgae and the hydrochar for biodiesel production.

3. Effect of the storage condition of microalgae paste on the hydrochar lipid and the direct transesterification of the hydrochar lipid for biodiesel production.

1.6. Thesis outline

In the chapter 1, the background of the research is explained. This includes the overview of biodiesel production from microalgae, the advantages of microalgae over other feedstock, and the main challenge of water removal from microalgae. Next, the overview of hydrothermal treatment of microalgae which includes hydrothermal carbonization (HTC) and hydrothermal liquefaction (HTL) is presented. Finally, the purpose of study and the thesis outline are stated.

In the chapter 2, HTC of microalgae with 85% moisture (at 200, 210 and 220 ^{o}C) was conducted to create the hydrochar. The lipid retention in the hydrochar, the ultimate analysis of the hydrochar, and the nutrition distribution in the aqueous phase and the hydrochar were measured. The hydrochar (HTC at 200 ^{o}C) which had the highest lipid retention was used in the subsequent conversion steps for biodiesel production.

The lipid extraction from microalgae and the hydrochar (HTC at 200 ^{o}C) was also performed at the same conditions of the volume of solvent, the extraction temperature and the extraction time. Next, the esterification pretreatment and the transesterification of the extracted oil from hydrochar (HTC at 200 ^{o}C) were also conducted for biodiesel production.

In the chapter 3, the direct transesterification of microalgae and the hydrochar (at HTC 200 ^{o}C) was conducted at the same conditions of the amount of methanol, the acid H_2SO_4 concentration, the reaction temperature, and the reaction time for comparison.

In the chapter 4, the effect of the additional storage step of microalgae paste on the hydrochar lipids was investigated. Finally, an entire process (from the storage of fresh algae, HTC of microalgae paste to the direct transesterification of the hydrochar using the acid catalyst H_2SO_4) was developed for biodiesel production.

In the chapter 5, the results of each chapter were summarized and compared.

Reference

1.1. Scarlat N, Dallemand J, GALLEGO-PINILLA F. Impact on agricultural land resources of biofuels production and use in the European Union; 2008.

1.2. Richmond A (2008) Handbook of microalgal culture: biotechnology and applied phycology: John Wiley & Sons.

1.3. Thomas WH, Tornabene TG, Weissman J (1984) Screening for lipid yielding microalgae: activities for 1983. Final subcontract report. Solar Energy Research Inst., Golden, CO (USA).

1.4. Sheehan J, Dunahay T, Benemann J, et al. (1998) A look back at the US Department of Energy's aquatic species program: biodiesel from algae. National Renewable Energy Laboratory 328.

1.5. Chisti Y (2007) Biodiesel from microalgae. Biotechnology advances 25: 294-306.

1.6. Aslan S, Kapdan IK (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecological Engineering 28: 64-70.

1.7. Pratoomyot J, Srivilas P, Noiraksar T (2005) Fatty acids composition of 10 microalgal species. Songklanakarin J Sci Technol 27: 1179-1187.

1.8. Renaud SM, Thinh L-V, Parry DL (1999) The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. Aquaculture 170: 147-159.

1.9. Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. Renewable and sustainable energy reviews 14: 217-232.

1.10. Wang B, Li Y, Wu N, et al. (2008) CO2 bio-mitigation using microalgae. Applied microbiology and biotechnology 79: 707-718.

1.11. Li Y, Horsman M, Wang B, et al. (2008) Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans. Applied microbiology and biotechnology 81: 629-636.

1.12. Li Y, Horsman M, Wu N, et al. (2008) Biofuels from microalgae. Biotechnology progress 24: 815-820.

1.13. Raja R, Hemaiswarya S, Kumar NA, et al. (2008) A perspective on the biotechnological potential of microalgae. Critical reviews in microbiology 34: 77-88.

1.14. Corro G, Tellez N, Jimenez T, et al. (2011) Biodiesel from waste frying oil. Two step process using acidified SiO 2 for esterification step. Catalysis today 166: 116-122.

1.15. Zhang Y, Dube M, McLean D, et al. (2003) Biodiesel production from waste cooking oil: 1. Process design and technological assessment. Bioresource technology 89: 1-16.

1.16. Grima EM, Belarbi E-H, Fernández FA, et al. (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnology advances 20: 491-515.

1.17. Halim R, Gladman B, Danquah MK, et al. (2011) Oil extraction from microalgae for biodiesel production. Bioresource technology 102: 178-185.

1.18. Ehimen E, Sun Z, Carrington C (2010) Variables affecting the in situ transesterification of microalgae lipids. Fuel 89: 677-684.

1.19. Sathish A, Smith BR, Sims RC (2014) Effect of moisture on in situ transesterification of microalgae for biodiesel production. Journal of Chemical Technology and Biotechnology 89: 137-142.

1.20. Heilmann SM, Davis HT, Jader LR, et al. (2010) Hydrothermal carbonization of microalgae. Biomass and Bioenergy 34: 875-882.

1.21. Heilmann SM, Jader LR, Sadowsky MJ, et al. (2011) Hydrothermal carbonization of distiller's grains. Biomass and Bioenergy 35: 2526-2533.

1.22. Heilmann SM, Jader LR, Harned LA, et al. (2011) Hydrothermal carbonization of microalgae II. Fatty acid, char, and algal nutrient products. Applied Energy 88: 3286-3290.

1.23. Levine RB, Pinnarat T, Savage PE (2010) Biodiesel production from wet algal biomass through in situ lipid hydrolysis and supercritical transesterification. Energy & Fuels 24: 5235-5243.

1.24. Lu Y, Levine RB, Savage PE (2014) Fatty acids for nutraceuticals and biofuels from hydrothermal carbonization of microalgae. Industrial & Engineering Chemistry Research 54: 4066-4071.

1.25. Toor SS, Rosendahl L, Rudolf A (2011) Hydrothermal liquefaction of biomass: a review of subcritical water technologies. Energy 36: 2328-2342.

1.26. Garcia Alba L, Torri C, Samorì C, et al. (2011) Hydrothermal treatment (HTT) of microalgae: evaluation of the process as conversion method in an algae biorefinery concept. Energy & Fuels 26: 642-657.

Chapter 2. Comparison between the lipid extraction from microalgae and the hydrochar and transesterification of extracted lipid for biodiesel production

2.1. Introduction

The biodiesel production from microalgae has two main approaches. In the first one called the two-step method, the lipids are firstly extracted by organic solvents, and then the extracted lipids undergo the transesterification process for biodiesel synthesis. In the other called the direct transesterification method, the lipids in microalgae are extracted and converted into biodiesel in one step.

This chapter is divided into 2 parts. In the first part, HTC of microalgae was conducted to create the hydrochar. The method of HTC (200, 210 and 220 ^{o}C) of microalgae with 85% moisture was described, and the results were evaluated and compared to the previous researches in terms of the lipid retention in the hydrochar, the ultimate analysis of the hydrochar, and the nutrition distribution in the aqueous phase and the hydrochar. In the second part, the microalgae and the hydrochar which had the highest retention of lipid were used in the lipid extraction and the transesterification steps for biodiesel production.

2.2. Materials and method

2.2.1. Materials

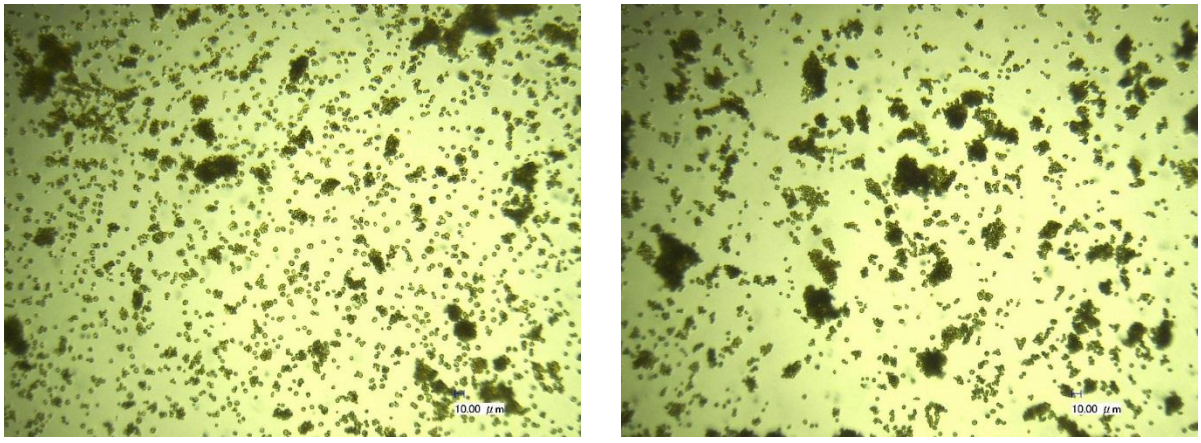
The microalgae species of Chlorella sp. (unbroken cell) was purchased from Sunrise Nutrachem Group Co., Limited. The chemicals of methanol, sulfuric acid, and hexane were purchased from Wako Pure Chemical Industries, Limited. The standards of methyl hexadecanoate (C16:0), methyl Z-9-hexadecenoate (C16:1), methyl octadecanoate (C18:0), methyl Z-9-octadecenoate (C18:1), methyl (Z,Z)-9,12-octadecadienoate (C18:2), methyl (Z,Z,Z)-9,12,15-octadecatrienoate (C18:3), and methyl nonadecanoate (C19:0) were purchased from Sigma-Aldrich.

One 50 ml autoclave was purchased from Taiatsu Techno Corporation. The Tomy Multipurpose Refrigerated Centrifuge EX-126 was used to centrifuge samples. The Shimadzu GCMS-QP2010 SE was used to analyze fatty acid methyl esters (FAMEs). The Shimadzu Total Organic Carbon Analyzer was used to measure the contents of carbon and nitrogen in the aqueous sample.

2.2.2. Method

2.2.2.1. Hydrothermal carbonization of microalgae to create hydrochar

The experiments of HTC of microalgae were conducted in the 50 ml autoclave. The feedstock (3 g of microalgae and 17 g water) was weighed and put into the autoclave to simulate the real microalgae paste with the moisture content of 85%. The use of the dried microalgae ensured the accurate mass of microalgae in each experiment as well as the stabilization of microalgae properties during the storage period. The dried microalgae were used in many previous researches [2.1,2.2,2.3]. In the real application, the microalgae paste should be used in the HTC process. Then, argon gas was added to the autoclave to displace the air inside.



Hydrochar (at HTC 190 ^{o}C) Hydrochar (at HTC 200 ^{o}C)

Figure 2.1. The sizes of hydrochar at HTC of 190 ^{o}C and 200 ^{o}C

The mixture was heated to the desired temperature by the heating band covered outside the autoclave, and this temperature was held for 30 minutes. The HTC temperatures were 200 ^{o}C, 210 ^{o}C, and 220 ^{o}C. These conditions are appropriate for HTC of microalgae [2.1,2.3,2.4]

which do not have the components of cellulose, hemicellulose and lignin [2.1]. The experiments conducted at 190 ^{o}C or lower indicated that the size of hydrochar was still small, so this led to the difficulty of filtering the hydrochar. Meanwhile, the retention of fatty acids in the hydrochar was considerably reduced at the HTC temperature of 230 ^{o}C or higher.

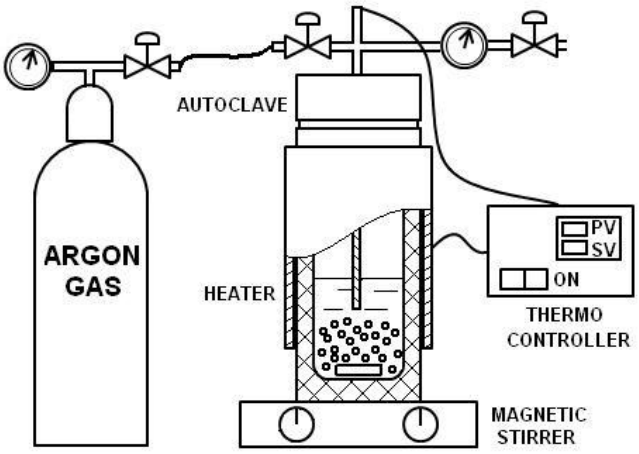


Figure 2.2. The HTC process of microalgae in the autoclave

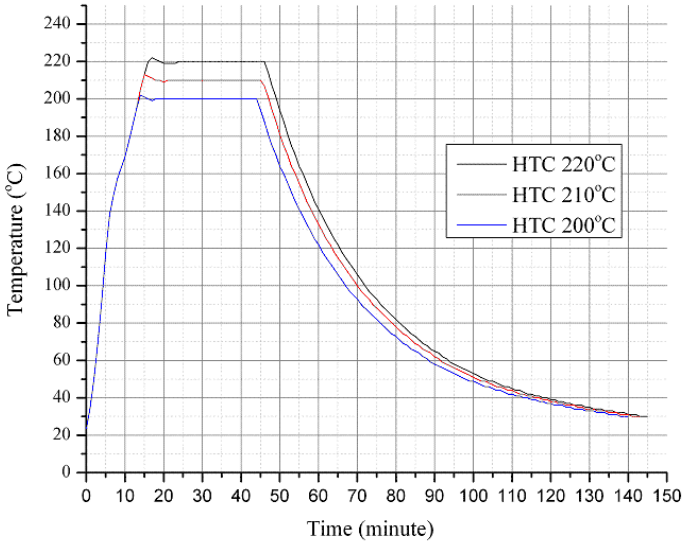


Figure 2.3. The temperature profiles of the HTC experiments

One magnetic stirring bar was used to agitate the mixture during the HTC process at the speed of 180 rpm. After kept at the HTC temperature for 30 minutes, the autoclave was cooled down to the room temperature of 25 ^{o}C. The temperature profiles of the HTC experiments are shown in **Figure 2.3**.

The mixture after the HTC process was filtered by the GF/A paper with the pore size of 1.6 µm. The hydrochar was dried at 100 ^{o}C for 4 h and stored at 4 ^{o}C. The mass of the hydrochar was weighed, and the mass yield of the hydrochar was determined by the formula below.

$$H_M, \% = \frac{\text{Mass of hydrochar}}{\text{Mass of treated microalgae}} \times 100$$

2.2.2.2. Determination of the content of total fatty acids (TFAs) in the samples

The determination of the content of total fatty acids in microalgae or the hydrochar was also conducted by the direct transesterification method [2.5]. The samples (10.0 mg microalgae or 4.5 mg hydrochar) were put into the bottom of 10 ml glass tubes. After that, 1 ml methanol solution of 2% v/v H_2SO_4 containing 0.1 mg of methyl nonadecanoate (C19:0) as an internal standard was added into the glass tube. Next, the tube was placed in an oil bath preheated at 85 ^{o}C, and the direct transesterification occurred in 90 minutes. The mixture was agitated by one magnetic bar at the speed of 180 rpm. When the reaction time reached, a volume of 1 ml water was added to stop the reaction, and a volume of 5 ml hexane was also added to extract FAMEs. Then, the mixture was shaken strongly for 5 minutes, centrifuged at the speed of 2000 rpm for 5 minutes to completely separate the hexane phase and methanol – water phase. A volume of 1 ml of the hexane phase was pipetted into vials which were stored at 4 °C prior to GCMS analysis.

2.2.2.3. Determination of the content of free fatty acids (FFAs) in the samples

The content of FFAs in the samples (microalgae or the hydrochar) was determined by the selective conversion of FFAs into FAMEs, and the content of FAMEs was measured by GCMS analysis. The samples (10.0 mg microalgae or 4.5 mg hydrochar) were grinded by mortar, and the lipid was extracted by a mixture of hexane / isopropanol (3:2, v/v) [2.6]. After that, the solvents were completely evaporated at 60 ^{o}C. Next, FFAs were selectively converted into FAMEs by 1 ml of pyridine/N, N-Dimethylformamide dimethyl acetal (1: 1 v/v) at 60 ^{o}C in 15 minutes [2.7]. After the reaction, 4 ml hexane containing 0.1 mg of methyl nonadecanoate (C19:0) was added. The mixture was then centrifuged at 2000 rpm in 5 minutes, and 1 ml solution was pipetted into the vial which was stored at 4 ^{o}C before the GCMS analysis.

2.2.2.4. GCMS analysis

The samples were analyzed by the GCMS-QP2010 SE, Shimadzu. The column Stabilwax which has the length of 30 m, the diameter of 0.25 mm and the film thickness of 0.25 µm was used to separate the FAME components. The operating parameters of the GCMS machine were as follows: the injection temperature of 220 ^{o}C, the pressure of 80.8 kPa, the column flow rate of 1.46 mL / min, and the splitless mode. The column temperature was increased from 40 ^{o}C to 190 ^{o}C at the heating rate of 20 ^{o}C / min, increased from 190 ^{o}C to 220 ^{o}C at the heating rate of 2 ^{o}C / min, and held at 220 ^{o}C for 2.5 minutes.

The content of fatty acids in the sample was calculated by the following formula.

$$C, \% = \sum(\frac{A_n}{A_{IS}} \times \frac{1}{r_n} \times \frac{m_{IS}}{M} \times 100)$$

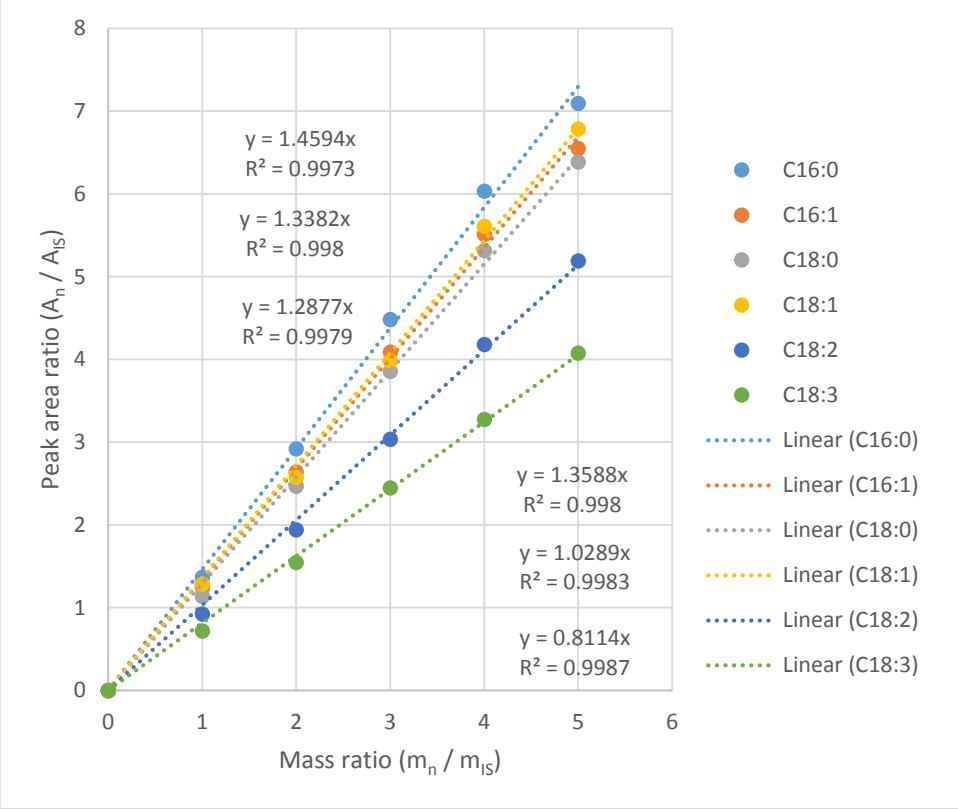


Figure 2.4. The response factors of standards of fatty acid methyl esters

Where A_n represents the peak area of each FAME, A_{IS} represents the peak area of internal standard (C19:0), r_n represents the response factor of each FAME, m_{IS} represents the mass of internal standard and M represents the mass of sample.

The retention of fatty acids in the hydrochar was calculated by the following formula, where C_{HC} is the content of fatty acids in the hydrochar, and C_{AL} is the content of fatty acids in microalgae.

$$R, \% = \frac{C_{HC}}{C_{AL}} \times H_M$$

2.2.2.5. Photos of lipid distribution in microalgae and the hydrochar

The lipid droplets inside microalgae (or the hydrochar) were detected by Nile Red [2.8]. The stock solution of Nile Red (200 µg/ml) was prepared in acetone and stored in dark. The samples were then stained by Nile Red at the final concentration of 0.1 µg/ml in 15 minutes. The Nile Red signals were detected by a confocal laser-scanning microscopy (LSM780, ZEISS) with an argon laser for excitation at 488 nm and a filter in the range of 537 nm to 599 nm [2.9].

2.2.2.6. Comparison between the lipid extraction from microalgae and the hydrochar

The solvents for lipid extraction from microalgae must have some important characteristics. Firstly, these solvents must be volatile for the low energy consumption in the evaporation step after oil extraction. Next, they have ability to extract all lipids including neutral and polar lipids. Therefore, a mixture of non-polar solvent and polar solvent is frequently used in the lipid extraction [2.10].

A mixture of chloroform and methanol is commonly used in lipid extraction from living tissue. The advantage of this method is the complete drying of microalgae is not necessary because water can work as the third component. After the extraction process, more chloroform and water were added to make the phase separation. The top methanol-chloroform phase contained most of lipid, when the bottom methanol-water phase mainly contained non-lipid components [2.11]. However, the chloroform is very toxic and not applicable to be used in a big scale [2.10].

Later, a mixture of hexane/isopropanol (3/2 v/v) was also used to extract lipid from microalgae [2.6]. This mixture also works based on the layer separation. After the extraction process, water and hexane were added into the mixture, and the mixture was separated into two phases: a top hexane phase and a bottom isopropanol – water phase. The role of

isopropanol (polar solvent) is to support the extraction of polar lipids, so it made the extraction yield of lipid from microalgae by this mixture higher than by hexane only. The extraction yield of the hexane phase was 0.048 g lipid / g dried microalgae, the additional yield from the isopropanol – water phase was 0.020 g lipid / g dried microalgae. Meanwhile, the extraction yield by hexane only was 0.015 g lipid / g dried microalgae [2.6]. The extraction conditions were as follows: 300 ml solvent / 4 g dried microalgae (or 75 ml solvent / 1 g dried microalgae), at the room temperature (25 ^{o}C) in 7.5 h [2.6].

Based on these data, the extraction conditions for microalgae and the hydrochar were selected as follows. The microalgae lipid was extracted by a mixture of hexane / isopropanol (3/2 v/v) at the ratios of 1, 2, 3 and 4 ml solvent / 100 mg microalgae at the room temperature (25 ^{o}C) for 8 h. The solvent contained 1 mg of methyl nonadecanoate (C19:0) as the internal standard for each experiment. After the extraction, the mixture was filtered, and the filtrate was dried at 60 ^{o}C overnight to entirely remove the solvent. After that, the lipid portion of oil was converted into FAME by the direct transesterification using 1 ml methanol solution of 2% v/v H_2SO_4 at 85 ^{o}C in 90 minutes. The FAME components were then extracted by hexane and measured by the GCMS analysis. The experimental procedure can be referred in the section 2.2.2, and the fomula for the content of fatty acids in the sample can be referred in the section 2.2.4.

The main originality of this work is the lipid extraction of hydrochar pre-treated by autoclave at 200 ^{o}C. At this temperature, the average size of hydrochar particles is large enough in order to remove water from hydrochar by the filtration. In the previous works, the pretreatment of microalgae was conducted by an autoclave at lower temperatures (for example, 125 ^{o}C), and the method used to remove water from the pretreated microalgae was not mentioned.

The extraction yields of lipid from microalgae (or the hydrochar) were determined by the following formulas, where C_{AL2} and C_{HC2} are the contents of lipid in algae and the hydrochar determined through the lipid extraction step.

$$Y_E, \% = \frac{C_{AL2}}{C_{AL}} \times 100 \text{ or } Y_E, \% = \frac{C_{HC2}}{C_{HC}} \times 100$$

2.2.2.7. Conversion of extracted lipid into biodiesel

The contents of TFA (C_{TFA}) and FFA of extracted oil were determined by the procedure mentioned in the section 2.2.2, 2.2.3 and 2.2.4. Based on the previous researches, the extracted oils from the hydrochar had the high content of FFA [2.2,2.12]. Therefore, the esterification pretreatment of FFA using the acid catalyst must be conducted before the transesterification process to effectively convert TFAs into biodiesel. For the esterification process, the homogenous acid catalyst H_2SO_4 were commonly used [2.13,2.14,2.15]. However, the use of homogenous catalyst led to the washing step of catalyst by water, and it caused the difficulty in catalyst recovery and wastewater treatment.

On the other hand, the use of solid catalyst brings many advantages. The catalyst can be easily separated and recovered after the esterification process. The acid solid catalyst Amberlyst was commonly used in the esterification process of FFAs in many previous researches. In the esterification reaction of FFAs in soybean oil and trap grease, Amberlyst 15 and Amberlyst BD20 were used at the 1:6 molar ratio of oil to methanol, catalyst of 20% wt. oil, and the reaction time of 80 ^oC for 6 h. Around 90% of FFAs was converted into biodiesel. The catalyst activity remained high after five times of reuse [2.16]. Amberlyst 15 was also used in the esterification of palm fatty acid distillate (PFAD) [2.17]. The optimum reaction conditions were as follows: the amount of methanol of 18% wt. PFDA, the amount of Amberlyst 15 of 30% wt. PFDA, the reaction temperature of 60 ^oC, the agitation speed of 250 rpm, and the reaction time of 7 h [2.17].

Based on the previous data, the esterification conditions of FFAs were determined as follows. The amount of catalyst was in the range of 10 – 50% wt. oil, and the molar ratios of methanol/lipid were in the range of 6:1 – 24:1. The esterification reaction took place at 80 ^oC in 180 minutes, and the content of FFAs/TFAs was measured in the interval of 30 minutes. The optimum reaction conditions were determined with the aim that the content of FFAs/TFAs was lower than 1% (or the acid value lower than 2 mg KOH / g oil). After the esterification, the Amberlyst catalyst was recovered by washing by the mixture of hexane/isopropanol two times and dried at 60 ^oC. This catalyst was reused in the esterification reaction three times.

After the esterification pretreatment of oil, the oil was dried at 105 ^{o}C to completely eliminate water. Next, this oil underwent the transesterification using the KOH catalyst to convert all fatty acid into FAMEs. The transesterification reaction conditions were as follows: the molar ratio of methanol / lipid of 12:1, the amount of KOH of 0.5, 1.0, and 1.5% wt. oil, the reaction temperature of 65 ^{o}C and the reaction time of 60 minutes [2.13].

2.3. Results and discussion

2.3.1. The retention of fatty acids in the hydrochar

Each peak in the chromatograph was analyzed and compared to the data in the library. The retention time and the name of each FAME detected by the high similarity with the data in the library are presented in **Table 2.1**.

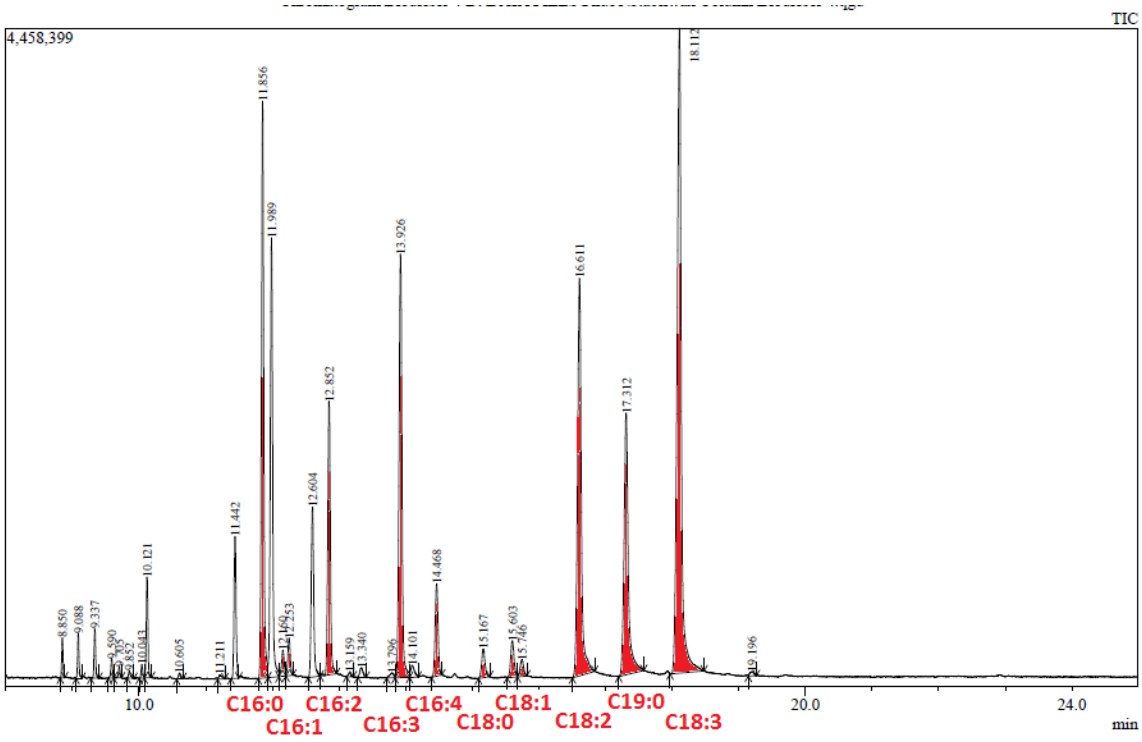


Figure 2.5. The chromatograph of FAMEs in the samples (microalgae or hydrochar)

Moreover, FAME standards (from C14 to C19) were also analyzed. Compared to the retention times of standards of C14:0, C14:1 and C15:0, the peaks in samples at these times were not detected. On the other hand, the peaks of C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3 in the samples have the similar retention times with FAME standards.

Table 2.1. The retention time of FAMEs in the samples (microalgae or hydrochar)

Time (min.)	---	---	---	11.856	12.160	12.852	---	13.926	14.468
Components	---	---	---	C16:0	C16:1	C16:2	---	C16:3	C16:4
Time (min.)	15.167	15.603	16.611	17.312	18.112				
Components	C18:0	C18:1	C18:2	C19:0	C18:3				

Table 2.2. The retention times of FAME standards

Time (min.)	9.581	9.954	10.591	11.831	12.218	---	13.321	---	---
Components	C14:0	C14:1	C15:0	C16:0	C16:1	---	C17:0	---	---
Time (min.)	15.125	15.555	16.516	17.227	17.969				
Components	C18:0	C18:1	C18:2	C19:0	C18:3				

The content of fatty acids in microalgae Chlorella sp. was 11.6 %. The composition of fatty acids in microalgae is shown in **Table 2.3**.

Table 2.3. The composition of fatty acids in microalgae

	Fatty acids		**Percentage, %**
1	Methyl hexadecanoate (C16:0)	C16:0	12.14
2	Methyl Z-9-hexadecenoate (C16:1)	C16:1	1.44
3	Methyl 7,10-hexadecadienoate (C16:2)	C16:2	7.95
4	Methyl 7,10,13-hexadecatrienoate (C16:3)	C16:3	17.86
5	Methyl 4,7,10,13-hexadecatetraenoate (C16:4)	C16:4	4.11
6	Methyl octadecanoate (C18:0)	C18:0	0.78
7	Methyl Z-9-octadecenoate (C18:1)	C18:1	0.94
8	Methyl (Z,Z)-9,12-octadecadienoate (C18:2)	C18:2	17.36
9	Methyl (Z,Z,Z)-9,12,15-octadecatrienoate (C18:3)	C18:3	37.42
Total			100

This commercial type of microalgae contains a large amount of polyunsaturated fatty acids.

The retentions of fatty acids in the hydrochar are presented in **Table 2.4**. There is a high retention (95.0%) of fatty acids in the hydrochar after HTC at 200 ^{o}C. This result is consistent with the previous researches [2.8,2.9,2.10,2.17]. Yingda Lu and et al. stated that the retention of fatty acids which have up to 3 double bonds was very high after HTC at 200 ^{o}C [2.8].

Table 2.4. The retention of fatty acids in the hydrochar

	Content of fatty acids C, %	**Mass yield H_M, %**	**Retention of fatty acids R, %**	**Ratio of FFAs/TFAs, %**
Microalgae	11.6 ± 0.2	---	---	24.9
Hydrochar (HTC 200 ^{o}C)	25.4 ± 0.7	43.2	95.0	48.0
Hydrochar (HTC 210 ^{o}C)	25.9 ± 0.8	37.9	85.1	47.1
Hydrochar (HTC 220 ^{o}C)	25.7 ± 1.1	34.8	77.4	48.5

When the HTC temperature increased from 200 ^{o}C to 210 ^{o}C and 220 ^{o}C, the retention of fatty acids significantly decreased. The reason is that during the HTC process, a part of bound fatty acids inside microalgae was hydrolyzed into free fatty acids [2.10]. When the HTC temperature increased, more free fatty acids were created and dissolved in the aqueous phase, and this led to the higher loss of fatty acids.

Table 2.5. The total content of each fatty acid in the hydrochar (HTC 200 ^{o}C) and the aqueous phase

	HTC of microalgae (200 ^{o}C)	**Percentage, %**		
	Fatty acids	**Hydrochar**	**Aqueous phase**	**Total**
1	Methyl hexadecanoate (C16:0)	95.0	4.5	99.5
2	Methyl Z-9-hexadecenoate (C16:1)	96.7	3.0	99.7
3	Methyl 7,10-hexadecadienoate (C16:2)	96.7	3.0	99.7
4	Methyl 7,10,13-hexadecatrienoate (C16:3)	94.0	4.8	98.8

5	Methyl 4,7,10,13-hexadecatetraenoate (C16:4)	86.5	9.4	96.0
6	Methyl octadecanoate (C18:0)	93.9	5.5	99.4
7	Methyl Z-9-octadecenoate (C18:1)	97.9	1.9	99.8
8	Methyl (Z,Z)-9,12-octadecadienoate (C18:2)	94.4	5.0	99.4
9	Methyl (Z,Z,Z)-9,12,15-octadecatrienoate (C18:3)	92.5	6.0	98.5

Table 2.6. The total content of each fatty acid in the hydrochar (HTC 210 ºC) and the aqueous phase

	HTC of microalgae (210 ºC)	**Percentage, %**		
	Fatty acids	**Hydrochar**	**Aqueous phase**	**Total**
1	Methyl hexadecanoate (C16:0)	94.0	5.4	99.4
2	Methyl Z-9-hexadecenoate (C16:1)	92.6	6.6	99.3
3	Methyl 7,10-hexadecadienoate (C16:2)	86.7	12.0	98.7
4	Methyl 7,10,13-hexadecatrienoate (C16:3)	77.3	18.1	95.5
5	Methyl 4,7,10,13-hexadecatetraenoate (C16:4)	71.3	20.0	91.4
6	Methyl octadecanoate (C18:0)	93.0	6.3	99.3
7	Methyl Z-9-octadecenoate (C18:1)	92.0	7.2	99.2
8	Methyl (Z,Z)-9,12-octadecadienoate (C18:2)	92.0	7.2	99.2
9	Methyl (Z,Z,Z)-9,12,15-octadecatrienoate (C18:3)	81.4	15.0	96.3

At the high temperature of 220 ºC, there was a lower retention of fatty acids having many double bonds (C16:4) in the hydrochar due to the low thermal stability. The total content of each fatty acid in the hydrochar and the aqueous phase was in **Table 2.5, 2.6 and 2.7**.

Table 2.7. The total content of each fatty acid in the hydrochar (HTC 220 ºC) and the aqueous phase

	HTC of microalgae (220 ºC)	**Percentage, %**		
	Fatty acids	**Hydrochar**	**Aqueous phase**	**Total**

1	Methyl hexadecanoate (C16:0)	85.5	13.0	98.6
2	Methyl Z-9-hexadecenoate (C16:1)	81.7	16.5	98.2
3	Methyl 7,10-hexadecadienoate (C16:2)	79.7	16.3	95.9
4	Methyl 7,10,13-hexadecatrienoate (C16:3)	70.8	20.4	91.2
5	Methyl 4,7,10,13-hexadecatetraenoate (C16:4)	64.4	21.4	85.8
6	Methyl octadecanoate (C18:0)	89.7	9.3	99.0
7	Methyl Z-9-octadecenoate (C18:1)	89.2	9.7	98.9
8	Methyl (Z,Z)-9,12-octadecadienoate (C18:2)	84.6	12.4	96.9
9	Methyl (Z,Z,Z)-9,12,15-octadecatrienoate (C18:3)	76.4	16.5	92.9

The hydrochar (HTC 200 ^{o}C) having the highest retention of fatty acids was used in the direct transesterification reaction for comparison. The content of fatty acids in the hydrochar was more than double compared to microalgae (25.4% and 11.6%). The mass of the hydrochar (HTC 200 ^{o}C) having the equivalent amount of fatty acids in 100 mg microalgae is calculated in **Table 2.8**.

Table 2.8. The mass of the hydrochar having the equal amount of fatty acids in 100 mg microalgae

	Microalgae (mg)	**Hydrochar (mg)**
Equal amount of fatty acids	100	45.5

In addition, after the HTC process, the aqueous phase contained a half of carbon and most of nitrogen in the microalgae feedstock. The reason is that the components of carbohydrate and protein took part in the HTC process [2.7]. The main compositions of microalgae include carbohydrate, protein and lipid. During the HTC process, the carbohydrates are hydrolyzed into glucose and fructose which decompose into carbon containing compounds. On the other hand, the proteins are hydrolyzed into amino acids which decompose into nitrogen containing compounds. Besides, the lipids are hydrolyzed into free fatty acids [2.18].

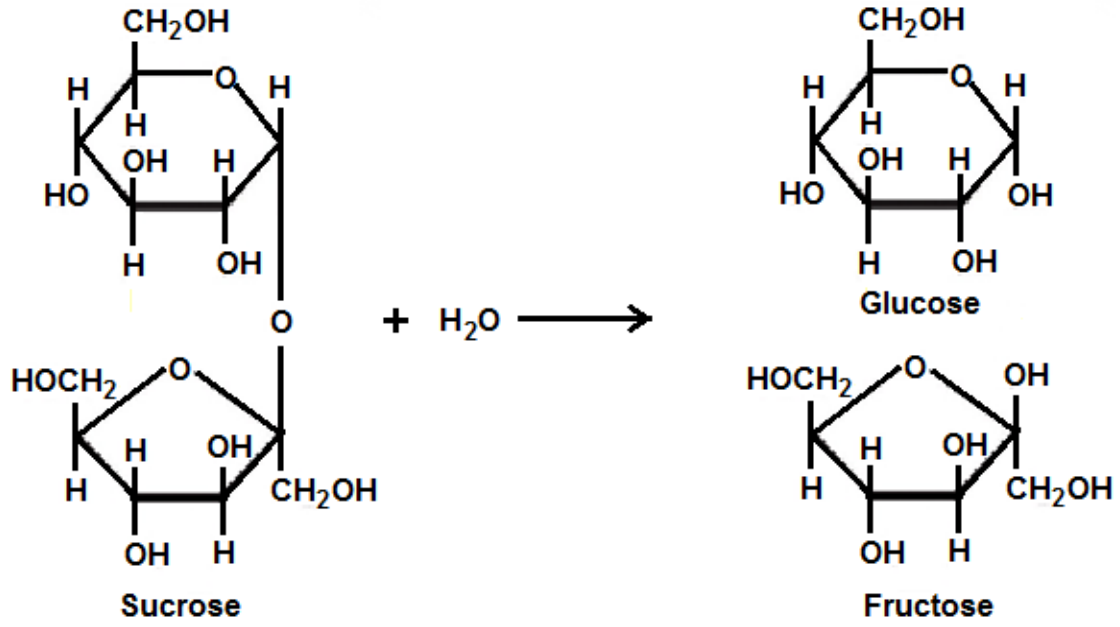


Figure 2.6. Hydrolysis of sucrose (carbohydrate) into glucose and fructose

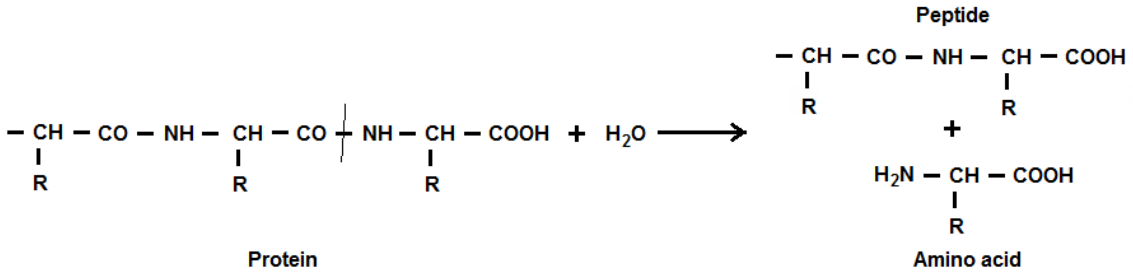


Figure 2.7. Hydrolysis of protein into peptide and amino acid

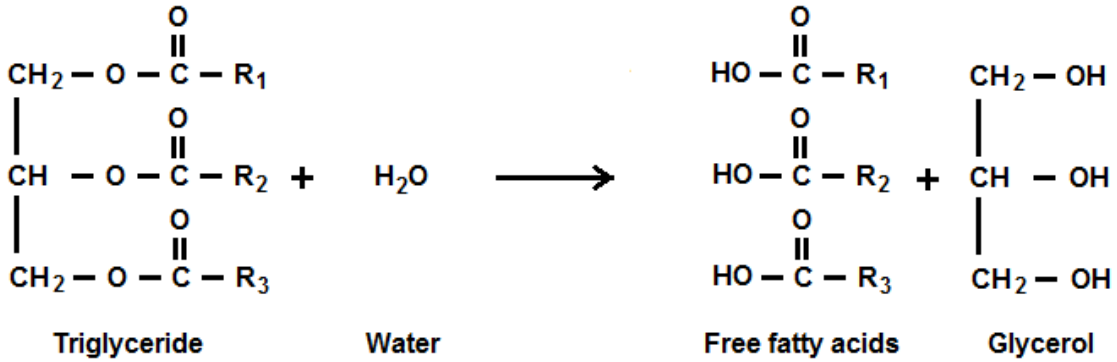


Figure 2.8. Hydrolysis of triglyceride into free fatty acids and glycerol

The ultimate analyses of microalgae and the hydrochar (HTC 200 ^{o}C) are listed in **Table 2.9**. The carbon content of the hydrochar increased up to 63.28%, and this led to the higher high heating value (HHV) of the hydrochar compared to microalgae (29.9 and 21.9 MJ/kg). The ash content of the hydrochar also reduced to 1.6%.

Table 2.9. Ultimate analyses of microalgae and the hydrochar (HTC 200 ^{o}C)

Sample	C	H	N	O	S	Ash	HHV (MJ/kg)
Microalgae	50.35	7.00	9.61	28.88	0.86	3.7	21.9
Hydrochar	63.28	8.32	7.53	19.11	0.61	1.6	29.9

Dulong formula: HHV (MJ/kg) = 0.338 x C + 1.428 × (H − O/8) + 0.095 × S

The additional benefit of this aqueous phase which can be used as fertilizer should be considered. These data are presented in **Table 2.10**.

Table 2.10. The distribution of carbon and nitrogen in the hydrochar (HTC 200 ^{o}C) and the aqueous phase

Element	Hydrochar, %	Aqueous phase, %
Carbon, C	51.9	45.1
Nitrogen, N	32.3	65.9

The comparison between the energy requirement for the drying process and for the HTC process of microalgae is calculated for 10 kg microalgae paste with the moisture content of 85% w/w as follows.

The latent heat of evaporation of water at T (K) is calculated by the formula below [2.19].

$$\Delta H_v(T) = 52053000 \times (1 - T_r)^{(0.3199 - 0.212 \times T_r + 0.25795 \times T_r^2)} \text{ (J kmol}^{-1}\text{)}$$

$$T_r = \frac{T \text{ (K)}}{647.096}$$

The latent heat of evaporation of water at 100 ℃ (or 373.15 K):

$$\Delta H_v(373.15 \text{ K}) = 40798295 \text{ (J kmol}^{-1}\text{)} = 2.265 \text{ (MJ kg}^{-1}\text{)}$$

The heat capacity C_p(J $kmol^{-1}$ K^{-1}) is calculated by the formula below [2.19].

$$C_p = 276370 - 2090.1 \times T + 8.125 \times T^2 - 0.014116 \times T^3 + 9.3701 \times 10^{-6} \times T^4$$

The energy requirement Q_1 for heating 1 kg water from 25 ℃ (298.15 K) to 100 ℃ (373.15 K)

$$Q_1 = \int_{298.15}^{373.15} C_p dT = 5.66 \times 10^6 \text{ (J kmol}^{-1}) = 0.314 \text{ (MJ kg}^{-1})$$

The energy requirement Q_2 for heating 1 kg water from 25 ℃ (298.15 K) to 200 ℃ (473.15 K)

$$Q_2 = \int_{298.15}^{473.15} C_p dT = 13.4 \times 10^6 \text{ (J kmol}^{-1}) = 0.747 \text{ (MJ kg}^{-1})$$

The energy requirement for drying microalgae

The energy requirement for drying microlagae paste

$$E_1 = (0.314 \text{ MJ kg}^{-1} + 2.265 \text{ MJ kg}^{-1}) \times 8.5 \text{ kg} = 21.92 \text{ (MJ)}$$

The energy requirement for HTC of microalgae and drying hydrochar

The heat capacity of 21 different biomasses varies from 1.3 to 2.0 kJ kg^{-1} K^{-1} in the temperature range of 313–353 K [2.20]. The heat capacity of marine microalgae Nannochloropsis salina is calculated to be around 1.5 kJ kg^{-1} K^{-1} in the range of 298.15–328.15 K from the mixture of microalgae and water [2.21]. The heat capacity of water is around 4.2 kJ kg^{-1} K^{-1} in the range of 298.15–473.15 K. Assuming that the heat capacity of microalgae is half of water in the range of 298.15–473.15 K [2.22], the energy requirement for HTC of microalgae E_2 is as follows:

$$E_2 = (0.747 \text{ MJ kg}^{-1} \times 8.5 \text{ kg} + 0.747 \text{ MJ kg}^{-1} \times 0.5 \times 1.5 \text{ kg}) = 6.91 \text{(MJ)}$$

The moisture content of hydrochar is around 50% w/w, so the mass of water equals to the mass of hydrochar. The energy requirement for drying hydrochar (HTC 200 ℃) is

$$E_3 = (0.314 \text{ MJ kg}^{-1} + 2.265 \text{ MJ kg}^{-1}) \times 1.5 \text{ kg} \times 43.2\% = 1.67 \text{ (MJ)}$$

The total energy requirement for HTC of microalgae and drying hydrochar is

$$E_4 = (6.91\ MJ + 1.67\ MJ) = 8.58\ (MJ)$$

In conclusion, the energy requirement for the HTC process is much lower than the drying process.

Energy balance

The high heating value of biodiesel is 40.1 MJ/kg. Because the fatty acid content of hydrochar is 25.4% w/w, the energy balance is calculated as follows.

Δ = (29.9 MJ/kg × 1.5 kg × 43.2% − 40.1 MJ/kg × 1.5 kg × 43.2% × 25.4%) − 8.58 MJ = 4.22 MJ.

The energy balance is positive, so the heating value of lipid-extracted hydrochar is higher than the energy requirement for the HTC process and drying hydrochar.

2.3.2. Comparison between the lipid extraction from microalgae and the hydrochar

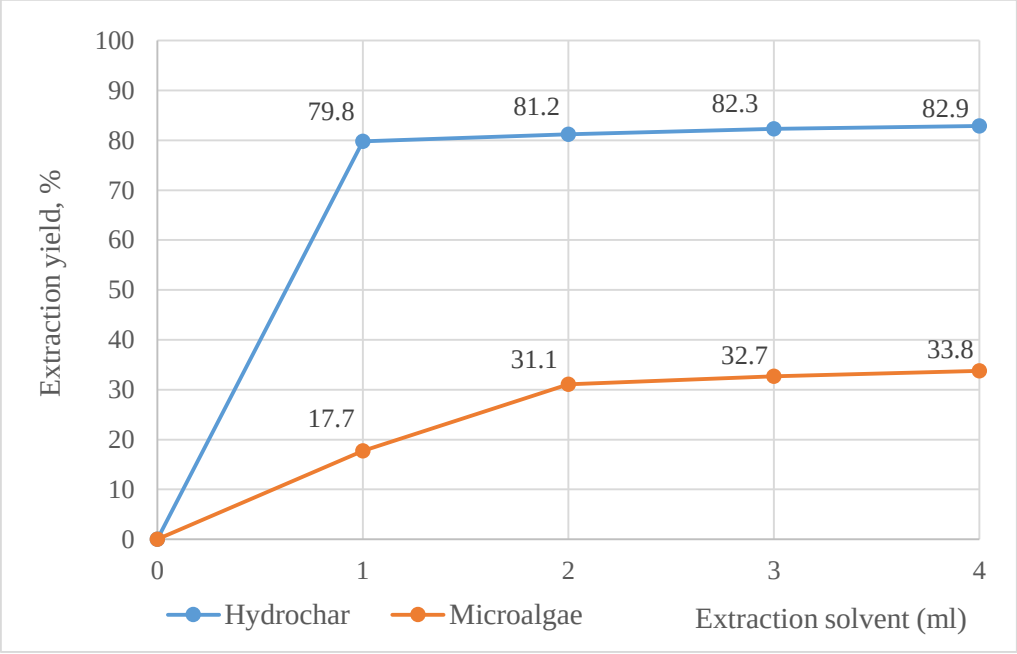
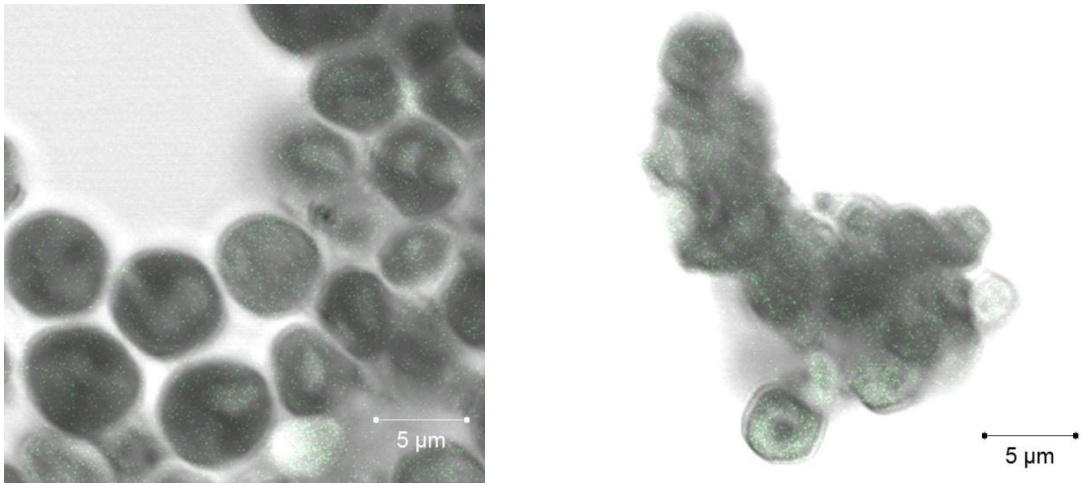


Figure 2.9. Yields of lipid extraction from microalgae and the hydrochar by a mixture of hexane/isopropanol (3:2 v/v)



Microalgae Hydrochar

Figure 2.10. Photos of microalgae and the hydrochar (at HTC 200 ºC) by confocal laser-scanning microscopy (LSM780, ZEISS)

The result is that the yield of lipid extraction from the hydrochar (about 80%) is higher than from microalgae (about 30%). The yield of lipid extraction from microalgae was quite low. The microalgae cells were unbroken, so this hindered the penetration of solvent into microalgae to extract lipid (see **Figure 2.10**). After the hydrothermal carbonization process, the microalgae cells were impacted and contractive. This facilitated the penetration and lipid extraction by solvent. Moreover, the ratio of FFAs/TFAs of the hydrochar (at HTC 200 ºC) was higher than microalgae (48.0% and 24.9%). The higher content of FFAs also supported the lipid extraction by solvent. The oil extracted from the hydrochar by solvent at the ratio of 1 ml solvent / 45.5 mg hydrochar was used for biodiesel production in the next step.

2.3.3. Conversion of extracted lipid into biodiesel

In the esterification pretreatment step, the amount of catalyst was firstly investigated at the fixed molar ratio of methanol / lipid of 24:1. With the amount of catalyst of 10% wt. oil, the content of FFAs/TFAs was quite high (18.9%) after 180 minutes of the reaction. The aim here was to reduce the content of FFAs/TFAs lower than 1% or the acid value lower than 2 mg KOH / g oil [2.23]. Therefore, the higher amounts of catalyst were used in this pretreatment step. At the amount of catalyst of 20% wt. oil, the content of FFAs/TFAs reduced to 4.0% at the reaction time of 180 minutes, but it was still higher than 1%.

Table 2.11. The content FFAs/TFAs after the esterification pretreatment using Amberlyst 15 (molar ratio of methanol / lipid of 24:1, reaction temperature of 80 ºC, catalyst in the range of 10 – 50% wt. oil)

	Reaction time (minutes)						
Catalyst	**0**	**30**	**60**	**90**	**120**	**150**	**180**
10% wt. oil	44.9	33.7	28.4	25.6	23.1	21	18.9
20% wt. oil	44.9	26.9	16.5	10.7	7.8	5.3	4.0
30% wt. oil	44.9	26.1	14.5	6.2	2.2	0.5	0.2
40% wt. oil	44.9	20.3	8.9	3.4	1.1	0.5	0.3
50% wt. oil	44.9	11.8	3.6	1.5	0.5	0.3	0.3

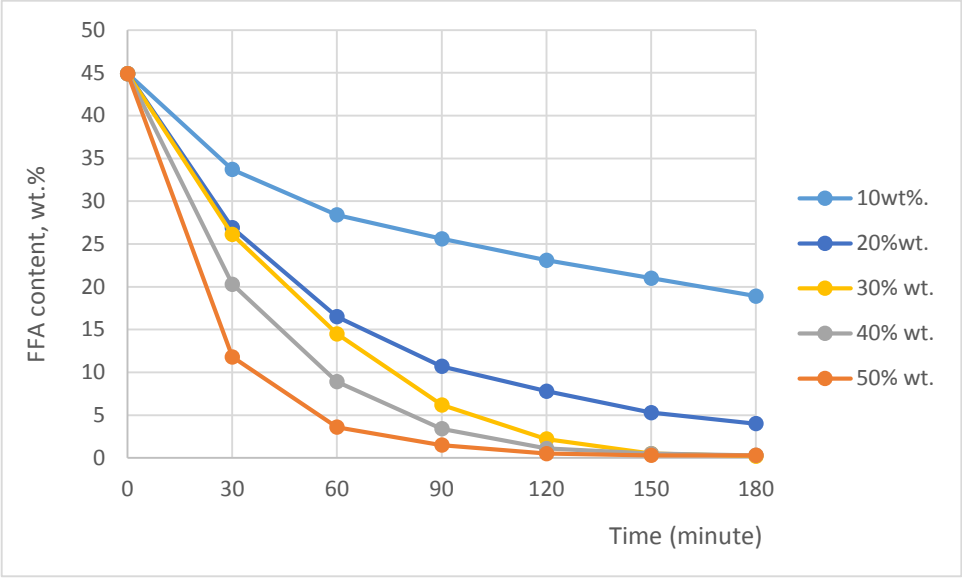


Figure 2.11. The content FFAs/TFAs after the esterification pretreatment using Amberlyst 15 (molar ratio of methanol / lipid of 24:1, reaction temperature of 80 ºC, catalyst in the range of 10 – 50% wt. oil)

At the amount of catalyst of 30, 40, and 50% wt. oil, the contents of FFAs/TFAs were lower than 1% at the reaction time of 180 minutes, and there was no significant difference in the contents of FFAs/TFAs from the time of 120 minutes. Therefore, the optimum amount of catalyst was 30% wt. oil. This result is similar to the previous works [2.16,2.17]. These data

of the effect of catalyst on the content of FFAs/TFAs were listed in **Table 2.11** and graphed in **Figure 2.11**.

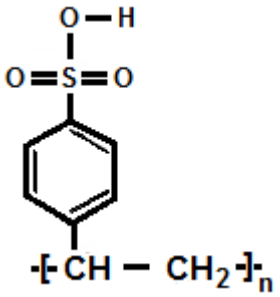


Figure 2.12. Acid site (–SO_3H) of Amberlyst-15 catalyst

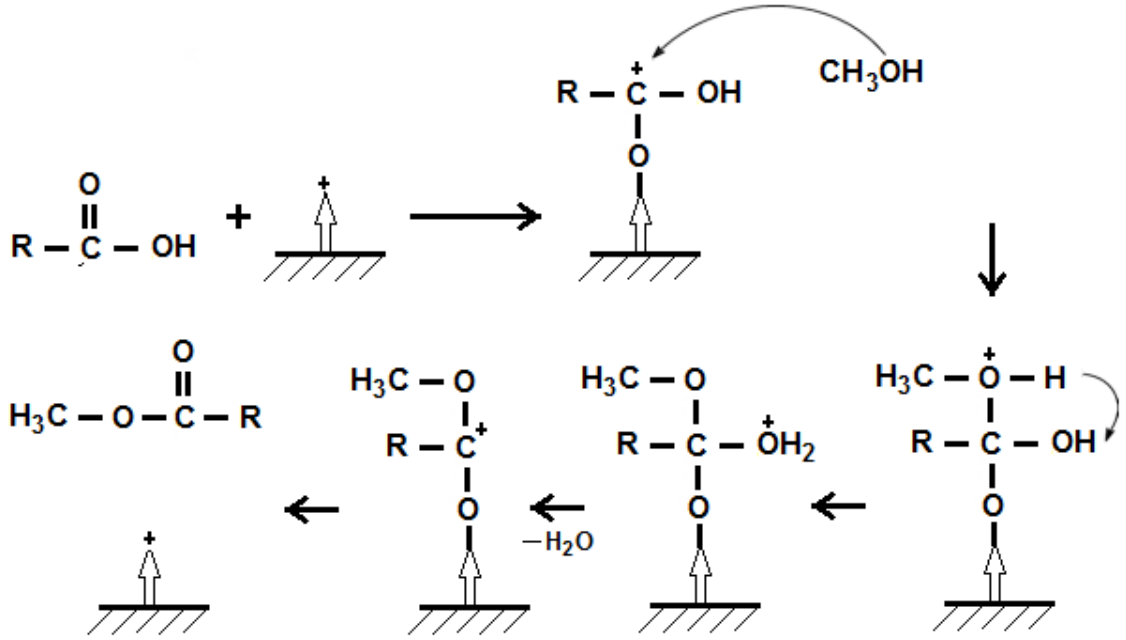


Figure 2.13. Mechanism of Amberlyst-15 catalyzed esterification of FFAs [2.24]

In the esterification of oleic acid with ethanol catalyzed by sulfuric acid at 45 °C, the reaction rate was rapid and achieved the equilibrium after 60 minutes of reaction at sulfuric acid concentrations of 2.261% and 5.139 w/w of oil [2.25]. In this research, the esterification of free fatty acids using Amberlyst-15 catalyst reached the equilibrium in a longer time of 150 minutes of reaction in spite of the higher reaction temperature of 80 ^oC. Obviously, the adsorption rate of FFAs and methanol on acid sites of Amberlyst-15 was the limiting rate, and the reaction rate depended on this adsorption rate. Therefore, the reaction rate was

proportional to the number of acid sites or the amount of Amberlyst catalyst used in the esterification reaction.

Next, the effect of the molar ratio of methanol / lipid were investigated at the fixed amount of catalyst of 30% wt. oil.

Table 2.12. The content FFAs/TFAs after the esterification pretreatment using Amberlyst 15 (catalyst of 30% wt. oil, the reaction temperature of 80 ºC, the molar ratio of methanol / lipid in the range of 24:1, 18:1, 12:1, and 6:1)

	Reaction time (minutes)						
Molar ratio	**0**	**30**	**60**	**90**	**120**	**150**	**180**
24:1	44.9	26.1	14.5	6.2	2.2	0.5	0.2
18:1	44.9	24.0	16.7	11.5	4.1	2.6	0.6
12:1	44.9	23.8	15.4	9.7	4.6	0.2	0.4
6:1	44.9	27.9	20.7	15.1	10.4	6.4	5.5

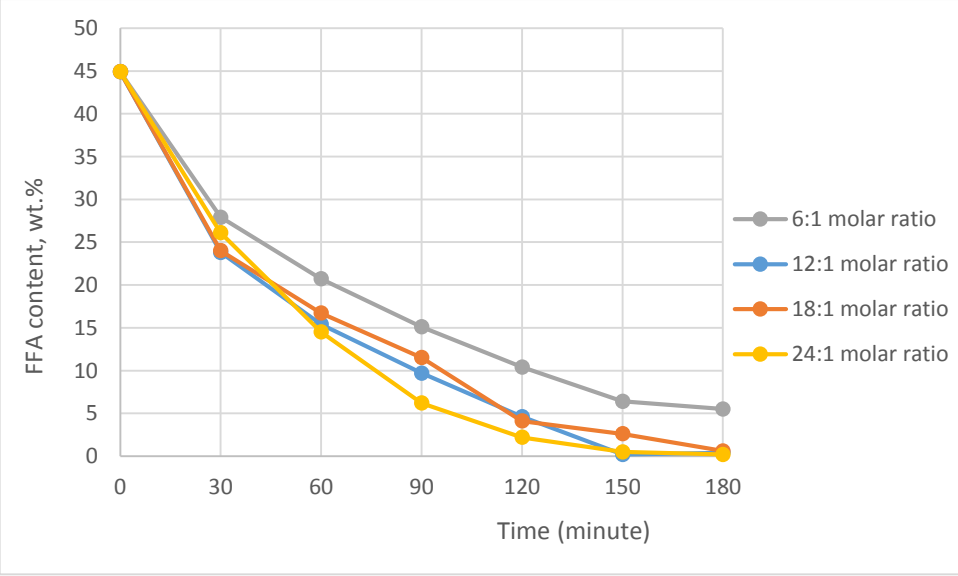


Figure 2.14. The content FFAs/TFAs after the esterification pretreatment using Amberlyst 15 (catalyst of 30% wt. oil, the reaction temperature of 80 ºC, the molar ratio of methanol / lipid in the range of 24:1, 18:1, 12:1, and 6:1)

The molar ratios were decreased from 24:1 to 18:1, 12:1 and 6:1 to reduce the amount of methanol, but the content of FFAs/TFAs after 180 minutes of the reaction must be kept lower than 1% [2.23]. At the molar ratio of 12:1, the content of FFAs/TFAs was still lower than 1%. However, the content of FFAs/TFAs was higher than 1% (5.5%) at the molar ratio of 6:1. Therefore, the molar ratio of methanol to lipid 12:1 was selected in this esterification pretreatment step. These data of the effect of the molar ratio of methanol / lipid on the content of FFAs/TFAs were listed in **Table 2.12** and graphed in **Figure 2.14**. The Amberlyst 15 was reused for the esterification pretreatment up to three times, the contents of FFAs/TFAs were still lower than 1% after 180 minutes of the reaction.

After the esterification pretreatment of oil, the oil underwent the transesterification using the catalyst KOH. The biodiesel yield achieved 93.6% at the amount of KOH of 1.5% wt. oil.

Table 2.13. The biodiesel yields through the esterification pretreatment and the transesterification (the molar ratio of methanol / lipid of 12:1, the amount of KOH of 0.5, 1.0, and 1.5% wt. oil, the reaction temperature of 65 ^{o}C and the reaction time of 60 minutes)

Catalyst KOH (% wt. of oil)	0.5	1	1.5
Biodiesel yield, %	72.2	85.7	93.6

2.4. Conclusion

After the hydrothermal carbonization process, the microalgae cells were impacted and contractive. Moreover, the ratio of FFAs/TFAs of the hydrochar (at HTC 200 ^{o}C) was higher than microalgae (48.0% and 24.9%). These factor facilitate the penetration of solvent into microalgae to extract lipid. This leads to the yield of lipid extraction from the hydrochar (about 80%) higher than from microalgae (about 30%). The appropriate conditions for the esterification pretreatment were as follows: the catalyst Amberlyst 15 of 30% wt. oil, the reaction temperature of 80 ^{o}C, 12:1 molar ratio of methanol / lipid. Next, the biodiesel yield of 93.6% can be achieved after the transesterification at the conditions of 12: 1 molar ratio of methanol / lipid, the amount of KOH of 1.5% wt. oil, the reaction temperature of 65 ^{o}C and the reaction time of 60 minutes.

Reference

2.1. Heilmann SM, Davis HT, Jader LR, et al. (2010) Hydrothermal carbonization of microalgae. Biomass and Bioenergy 34: 875-882. (Please add 2. for each reference number)

2.2. Levine RB, Pinnarat T, Savage PE (2010) Biodiesel production from wet algal biomass through in situ lipid hydrolysis and supercritical transesterification. Energy & Fuels 24: 5235-5243.

2.3. Broch A, Jena U, Hoekman SK, et al. (2013) Analysis of solid and aqueous phase products from hydrothermal carbonization of whole and lipid-extracted algae. Energies 7: 62-79.

2.4. Lu Y, Levine RB, Savage PE (2014) Fatty acids for nutraceuticals and biofuels from hydrothermal carbonization of microalgae. Industrial & Engineering Chemistry Research 54: 4066-4071.

2.5. Griffiths M, Van Hille R, Harrison S (2010) Selection of direct transesterification as the preferred method for assay of fatty acid content of microalgae. Lipids 45: 1053-1060.

2.6. Halim R, Gladman B, Danquah MK, et al. (2011) Oil extraction from microalgae for biodiesel production. Bioresource technology 102: 178-185.

2.7. Thenot J-P, Horning E, Stafford M, et al. (1972) Fatty acid esterification with N, N-dimethylformamide dialkyl acetals for GC analysis. Analytical Letters 5: 217-223.

2.8. Greenspan P, Mayer EP, Fowler SD (1985) Nile red: a selective fluorescent stain for intracellular lipid droplets. The Journal of cell biology 100: 965-973.

2.9. Iwai M, Ikeda K, Shimojima M, et al. (2014) Enhancement of extraplastidic oil synthesis in Chlamydomonas reinhardtii using a type‐2 diacylglycerol acyltransferase with a phosphorus starvationxtraplastidic oil synthesis in Chlamydomonas reinhardtii usin

2.10. Halim R, Danquah MK, Webley PA (2012) Extraction of oil from microalgae for biodiesel production: A review. Biotechnology advances 30: 709-732.

2.11. Folch J, Lees M, Sloane-Stanley G (1957) A simple method for the isolation and purification of total lipids from animal tissues. J biol Chem 226: 497-509.

2.12. Heilmann SM, Jader LR, Harned LA, et al. (2011) Hydrothermal carbonization of microalgae II. Fatty acid, char, and algal nutrient products. Applied Energy 88: 3286-3290.

2.13. Chen L, Liu T, Zhang W, et al. (2012) Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresource technology 111: 208-214.

2.14. Berchmans HJ, Hirata S (2008) Biodiesel production from crude Jatropha curcas L. seed oil with a high content of free fatty acids. Bioresource technology 99: 1716-1721.

2.15. Ghadge SV, Raheman H (2005) Biodiesel production from mahua (Madhuca indica) oil having high free fatty acids. Biomass and Bioenergy 28: 601-605.

2.16. Park J-Y, Kim D-K, Lee J-S (2010) Esterification of free fatty acids using water-tolerable Amberlyst as a heterogeneous catalyst. Bioresource technology 101: S62-S65.

2.17. Talukder MR, Wu J, Lau S, et al. (2008) Comparison of Novozym 435 and Amberlyst 15 as heterogeneous catalyst for production of biodiesel from palm fatty acid distillate. Energy & Fuels 23: 1-4.

2.18. Costanzo W, Hilten R, Jena U, et al. (2016) Effect of low temperature hydrothermal liquefaction on catalytic hydrodenitrogenation of algae biocrude and model macromolecules. Algal Research 13: 53-68.

2.19. Rowley R, Wilding W, Oscarson J, et al. (2006) DIPPR data compilation of pure chemical properties. Design Institute for Physical Properties.

2.20. Dupont C, Chiriac R, Gauthier G, et al. (2014) Heat capacity measurements of various biomass types and pyrolysis residues. Fuel 115: 644-651.

2.21. Schneider N, Fortin TJ, Span R, et al. (2016) Thermophysical properties of the marine microalgae Nannochloropsis salina. Fuel Processing Technology 152: 390-398.

2.22. Heilmann SM, Davis HT, Jader LR, et al. (2010) Hydrothermal carbonization of microalgae. Biomass and Bioenergy 34: 875-882.

2.23. Canakci M, Van Gerpen J (2001) Biodiesel production from oils and fats with high free fatty acids. Transactions-American Society of Agricultural Engineers 44: 1429-1436.

2.24. Lee D-W, Lee K-Y (2014) Heterogeneous solid acid catalysts for esterification of free fatty acids. Catalysis Surveys from Asia 18: 55-74.

2.25. Marchetti J, Errazu A (2008) Esterification of free fatty acids using sulfuric acid as catalyst in the presence of triglycerides. Biomass and Bioenergy 32: 892-895.

Chapter 3. Comparison between the direct transesterification of microalgae and the hydrochar

3.1. Introduction

The biodiesel production from microalgae has two main approaches. In the first one called the two-step method which was mentioned in Chapter 2, the lipids are firstly extracted by organic solvents, and then the extracted lipids undergo the transesterification process for biodiesel synthesis. In the other called the direct transesterification method, the lipids in microalgae are extracted and converted into biodiesel in one step. This method has some advantages such as the elimination of using extraction solvents and the higher biodiesel yield [3.1,3.2]. However, there are still some obstacles hindering the application of this method in the actual production.

In the direct transesterification method, the important parameters affecting the biodiesel yield are the moisture content of microalgae, the ratio of alcohol / microalgae, the amount of catalyst, the reaction temperature, the reaction time and the agitation [3.3,3.4]. Among them, the small increase in the moisture content can significantly affect the biodiesel yield [3.3,3.5]. However, the high moisture level is the inherent characteristic of microalgae, and the moisture content of microalgae paste after the centrifugation is in the range of 75 – 85% [3.6]. Therefore, the complete removal of water from microalgae must be done before the direct transesterification process. The sunlight can be used to dry microalgae in a small scale, but it looks like not effective for an industrial scale. Moreover, the availability and the stability of sunlight as well as the degeneration of microalgae must be taken into account.

In addition to the moisture content of microalgae, the ratio of alcohol / microalgae also plays a crucial role in the direct transesterification reaction. Here, the alcohol plays both roles as the extraction solvent and the reactant. The appropriate ratios of alcohol / microalgae are in the range of 1 – 2 ml methanol / 100 mg microalgae [3.4,3.5] to obtain the high biodiesel yield. Obviously, these ratios are quite high, and the reduction of the amount of alcohol used in the direct transesterification for a given type of microalgae is necessary.

In Chapter 2, it was proved that the energy requirement for the HTC process is much lower than the drying process. Besides, the hydrochar (HTC 200 ^{o}C) retains 95.0% of fatty acids in microalgae, and the content of fatty acids in the hydrochar is more than double compared to the microalgae feedstock (25.4% and 11.6%). In other words, the mass of the hydrochar is around a half of the mass of microalgae for an equal amount of fatty acids. Therefore, in the direct transesterification process, the use of the hydrochar as a feedstock instead of microalgae may lead to a superior extraction of fatty acids by alcohol, and the reduction of the amount of alcohol can be achieved. This idea will be investigated in this chapter through the comparison between the direct transesterification of microalgae and the hydrochar.

3.2. Materials and method

3.2.1. Materials

The microalgae species of Chlorella sp. (unbroken cell) was purchased from Sunrise Nutrachem Group Co., Limited. The chemicals of methanol, sulfuric acid, and hexane were purchased from Wako Pure Chemical Industries, Limited. The standards of methyl hexadecanoate (C16:0), methyl Z-9-hexadecenoate (C16:1), methyl octadecanoate (C18:0), methyl Z-9-octadecenoate (C18:1), methyl (Z,Z)-9,12-octadecadienoate (C18:2), methyl (Z,Z,Z)-9,12,15-octadecatrienoate (C18:3), and methyl nonadecanoate (C19:0) were purchased from Sigma-Aldrich.

One 50 ml autoclave was purchased from Taiatsu Techno Corporation. The Tomy Multipurpose Refrigerated Centrifuge EX-126 was used to centrifuge samples. The Shimadzu GCMS-QP2010 SE was used to analyze fatty acid methyl esters (FAMEs).

3.2.2. Method

3.2.2.1. Hydrothermal carbonization of microalgae to create the hydrochar

The experimental procedures for the hydrothermal carbonization of microalgae at 200 ^{o}C, the determination of the content of TFAs and FFAs in microalgae and the hydrochar can be referred in the sections 2.2.1, 2.2.2, 2.2.3, and 2.2.4.

3.2.2.2. Comparison between the direct transesterification of microalgae and the hydrochar

The hydrochar at HTC 200 ^{o}C and microalgae were used in the direct transesterification for comparison. Because the moisture content of microalgae (or the hydrochar) significantly affects the reaction yield, the moisture was completely removed by drying microalgae (or the hydrochar) at 105 ^{o}C for 4 h.

The reaction conditions were determined based on the previous works. The direct transesterification of microalgae which used the acid catalyst H_2SO_4 had the higher biodiesel yield compared to the alkaline catalyst NaOH [3.7]. B.D. Wahlen and et al. conducted the direct transesterification of C. gracilis at the conditions of 100 mg microalgae / 2 ml methanol, 2% v/v H_2SO_4, the reaction times of 10 and 20 minutes, and the reaction temperature in the range of 60 – 110 ^{o}C. The biodiesel yield was very high at 80 ^{o}C, and there was no significant increase in the reaction yield when the temperature was up to 110 ^{o}C [3.4]. Ashik Sathish and et al. also obtained the high biodiesel yields (about 90%) at the similar conditions: 25 – 150 mg microalgae / ml methanol, 2% v/v H_2SO_4, the reaction temperature of 90 ^{o}C, and the reaction time of 30 minutes [3.5]. When the ratio of microalgae / methanol was up to 200 mg microalgae / ml methanol, the reaction yield decreased down to 63.5% [3.5]. Based on these data, the reaction conditions were selected as follows. The ratios of microalgae / methanol were 100, 200, 300, and 400 mg microalgae / ml methanol. To achieve the high biodiesel yield, the minimum acid H_2SO_4 concentration should be 2% v/v. The reaction temperature was 80 ^{o}C, and the biodiesel yields were measured at the times of 30, 60 and 90 minutes. The main originality is that the higher ratios of microalgae / methanol are investigated (> 200 mg microalgae / ml methanol). The hydrochar samples which have the equivalent mass of fatty acids were also subjected to the direct transesterification at the same conditions.

Table 3.1. The masses of the hydrochar having the equal amount of fatty acids in microalgae

	Microalgae (mg)	Hydrochar (mg)
1	100	45.5
2	200	91.0
3	300	136.5
4	400	182.1
5	500	227.6

The experimental procedure can be referred in the section 2.2.2. However, the amounts of internal standard (C19:0) were 1, 2, 3, and 4 mg respectively corresponding to the ratios of 100, 200, 300, and 400 mg microalgae / ml methanol. The biodiesel yields of microalgae (Y_{AL}) and the hydrochar (Y_{HC}) at different times were calculated by the following formulas, where $C_{(t)}$ is the content of fatty acids in samples measured at the time of (t).

$$Y_{AL} = \frac{C_{(t)}}{C_{AL}} \times 100, Y_{HC} = \frac{C_{(t)}}{C_{HC}} \times 100$$

3.3. Results and discussion

The biodiesel yields at various ratios of 100, 200, 300 and 400 mg microalgae / ml methanol are respectively presented in **Figures 3.1, 3.2, 3.4 and 3.5**.

In **Figure 3.1**, the biodiesel yields of microalgae at the times of 30, 60 and 90 minutes were 85.7%, 95.1% and 95.9%, respectively.

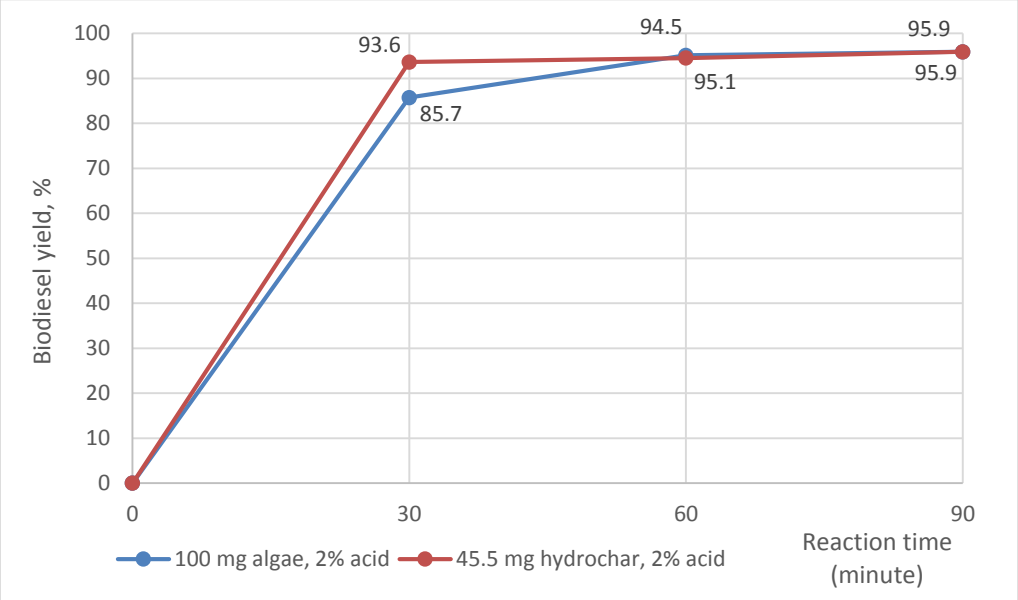


Figure 3.1. The biodiesel yields of the direct transesterification at the conditions of 100 mg microalgae (or 45.5 mg hydrochar) / ml methanol, 2% v/v acid H_2SO_4, 80 °C

At the similar conditions, Ashik Sathish and et al. obtained the biodiesel yield of 90.0% at the time of 30 minutes [3.5]. In this work, the biodiesel yields at the times of 60 and 90 minutes were higher than 90%. Therefore, the results in this research are consistent with the previous

one. For the hydrochar, the biodiesel yields at the times of 30, 60 and 90 minutes were 93.6%, 94.5% and 95.9%, respectively. There was no significant difference in the biodiesel yields of microalgae and the hydrochar at this ratio, and all yields were very high. Therefore, higher ratios of microalgae (or the hydrochar) / ml methanol were investigated.

As shown in **Figure 3.2**, the biodiesel yields of microalgae at the times of 30, 60 and 90 minutes were 55.7%, 84.3% and 88.8%, respectively. The biodiesel yield of 63.5% was also obtained by Ashik Sathish and et al. at the similar conditions at the time of 30 minutes [3.5]. Therefore, the biodiesel yields of microalgae are comparable with the previous study. However, all biodiesel yields at the ratio of 200 mg microalgae / ml methanol were lower than the yields at the ratio of 100 mg microalgae / ml methanol. Meanwhile, the biodiesel yields of the hydrochar did not change significantly compared to the yields shown in **Figure 3.1**.

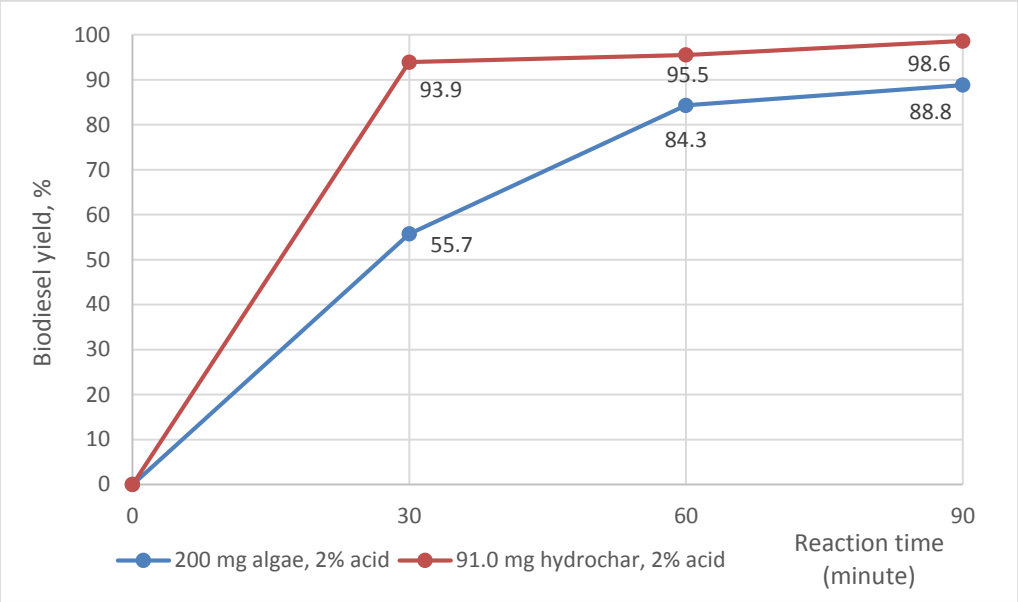
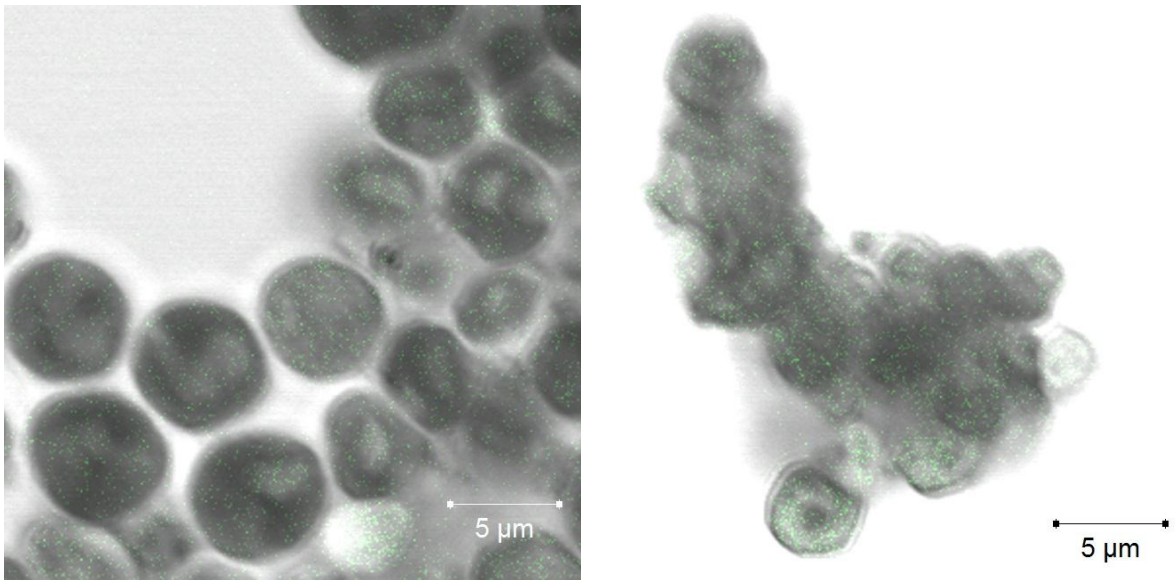


Figure 3.2. The biodiesel yields of the direct transesterification at the conditions of 200 mg microalgae (or 91.0 mg hydrochar) / ml methanol, 2% v/v acid H_2SO_4, 80 °C

These results can be explained as follows. The higher biodiesel yield of the hydrochar at the time of 30 minutes (93.9%) proves that the reaction rate of the hydrochar was faster than microalgae at the same conditions. In the direct transesterification, the methanol acts as the solvent and the reactant [3.4]. Therefore, the reaction rate depends on the extraction rate of fatty acids by methanol and the transesterification rate, and the extraction rate of fatty acids is

the limiting factor [3.8]. Siew Hoong Shuit and et al. reduced the size of Jatropha curcas L. seeds to increase the extraction rate of lipid by methanol, and this led to an increase in the direct transesterification rate [3.8]. During the HTC of microalgae, the components of carbohydrates and proteins were carbonized [3.9], and a large amount of carbon (45.1%) and nitrogen (65.9%) was extracted into the aqueous phase (Chapter 2). Moreover, the microalgae cells were weaken and contractive (see **Figure 3.3**). In addition, a portion of bound fatty acids was hydrolyzed to create the free fatty acids [3.10,3.11]. In this work, the ratios of FFAs/TFAs of microalgae and the hydrochar (HTC 200 ^{o}C) were 24.9% and 48.0%, respectively (Chapter 2). These phenomena led to a higher extraction rate of fatty acids as well as the higher biodiesel yields of the hydrochar compared to microalgae. Because the biodiesel yields of the hydrochar were still high, so the higher ratios of microalgae (or the hydrochar) / ml methanol were conducted to determine the maximum amount of fatty acids which can be converted into biodiesel.



Microalgae Hydrochar

Figure 3.3. Photos of microalgae and the hydrochar (HTC 200 ^{o}C) by confocal laser-scanning microscopy (LSM780, ZEISS)

As shown in **Figure 3.4**, when the mass of microalgae was up to 300 mg, the extraction of fatty acids from microalgae by methanol was more limited. There was a big reduction in the biodiesel yields only 60.5% at the time of 90 minutes. Even more acid H_2SO_4 was used (3% v/v), the biodiesel yield just achieved 81.6% at the time of 90 minutes. Meanwhile, the

biodiesel yield of the hydrochar were still high, 96.4% at the acid concentration of 2% v/v after 90 minutes of the reaction.

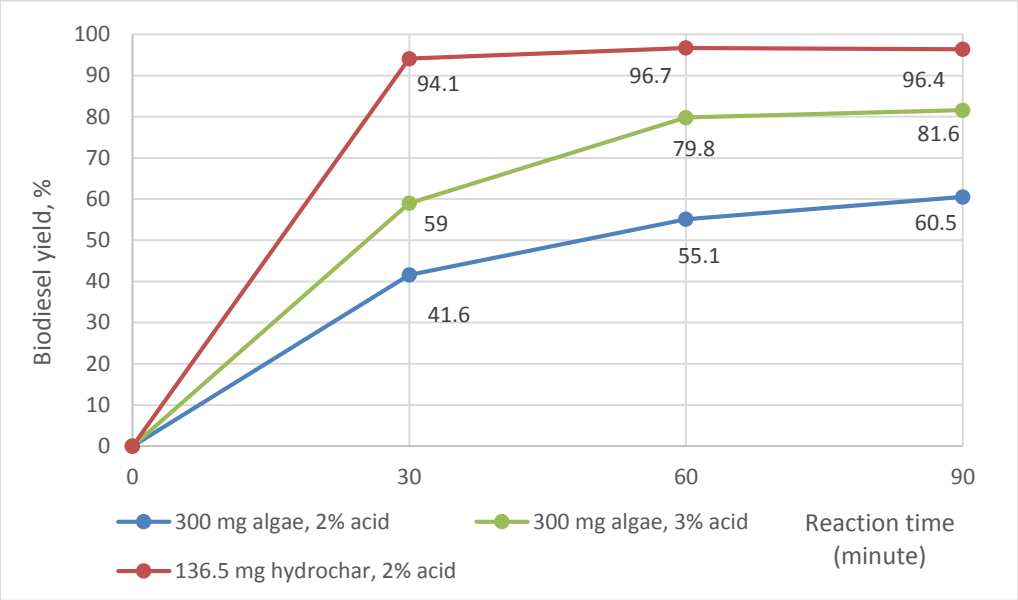


Figure 3.4. The biodiesel yield of the direct transesterification at the conditions of 300 mg microalgae (or 136.5 mg hydrochar) / ml methanol, 2% v/v (or 3% v/v) acid H_2SO_4, 80 ^oC

In **Figure 3.5**, it is shown that when the mass of microalgae was up to 400 mg, the microalgae was not completely covered by methanol. The biodiesel yield was very low, only 41.1% at 2% v/v of acid H_2SO_4 and 65.0% at 3% v/v of acid H_2SO_4 after 90 minutes of the reaction. Meanwhile, 182.1 mg hydrochar was still totally covered by methanol. The biodiesel yield was 91.1% at the time of 90 minutes. When the higher amount of the hydrochar was used (227.6 mg), the biodiesel yield decreased to 81.5%.

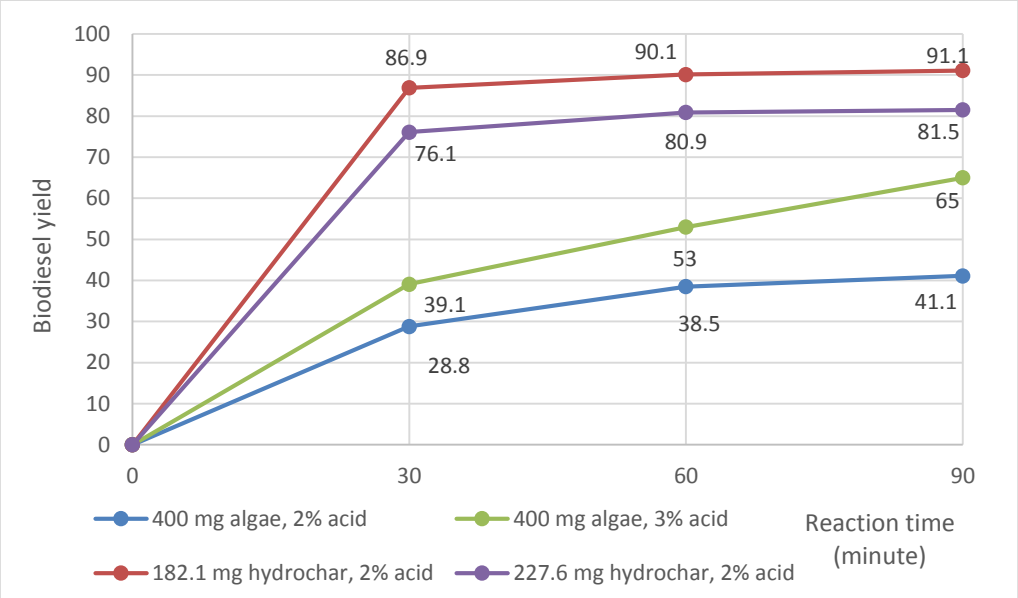


Figure 3.5. The biodiesel yield of the direct transesterification at the conditions of 400 mg microalgae (or 182.1 mg hydrochar) / ml methanol, 2% v/v (or 3% v/v) acid H_2SO_4, 80 ^oC

From the results above, the appropriate conditions for biodiesel production from microalgae were the ratio of 200 mg microalgae / ml methanol, 2% v/v acid H_2SO_4, 80 ^oC, and 90 minutes of the reaction. To convert lipids in 400 mg microalgae into biodiesel, a volume of 2 ml methanol with the H_2SO_4 concentration of 2% v/v is required. On the other hand, if this amount of lipids exists in the hydrochar through HTC of microalgae, a volume of only 1 ml methanol with the H_2SO_4 concentration of 2% v/v is necessary. It is only a half of the amount which is required for microalgae.

3.4. Conclusion

The combination of the HTC pretreatment of microalgae and the direct transesterification of the hydrochar is the effective method to convert lipids in microalgae into biodiesel. In a big scale, the water removal from microalgae paste by the HTC process is more energy-effective than the drying process. During HTC of microalgae, the components of carbohydrate and protein are carbonized, and the microalgae cells are weaken as well. In addition, a portion of bound fatty acids are hydrolyzed into free fatty acids. These phenomena facilitate the lipid extraction from the hydrochar by methanol, and this leads to the higher biodiesel yield of the hydrochar. The amounts of methanol and catalyst which are required for a given amount of

microalgae can be reduced to a half through the direct transesterification of the hydrochar. The additional benefit of the byproduct, the aqueous phase, which can be reused to cultivate microalgae [3.10,3.12] must be taken into account.

The comparison between two processes is presented in **Table 3.2**.

Table 3.2. Comparison between the process of lipid extraction, esterification, transesterification and the process of direct transesterification

	Lipid extraction + esterification + transesterification (1)	Direct transesterification (2)
Process	1. Lipid Extraction 2. Esterification 3. Transesterification	1. Direct transesterification
Lipid Extraction	Solvent: A mixture of hexane / isopropanol 1 mililiter solvent / 45.5 mg hydrochar or 4 litters solvent / 182.1 g hydrochar	
Esterification Transesterification	❖ For 182.1 g hydrochar, m lipid = 182.1 x 25.4% x 79.8% = 36.9 g m oil = 36.9 / 57.5% = 64.2 g • For esterification, n methanol = 12 x 36.9/839.3 = 0.53 mol V methanol = 0.53 x 32 / 0.792 = 21.3 ml • For transesterification, V methanol = 0.53 x 32 / 0.792 = 21.3 ml	1 mililiter methanol / 182.1 mg hydrochar or 1 liter methanol / 182.1 g hydrochar
Catalyst	Amberlyst = 64.2 x 30% = 19.3 g (reuse) KOH = 64.2 x 1.5% = 0.96 g	H_2SO_4 (2% v/v) – 20 ml
Yield	79.8 x 93.6 = 74.7%	91.1%
FAME extraction	Extract FAME from biocrude oil (high content of fatty acid 57.5%)	Extract FAME from the hydrochar

The advantages of the first method are that the amount of methanol, catalyst in the esterification pretreatment and the transesterification is lower than the second method. The advantage of the second method is the simple process. Furthermore, the large amount of solvent used for lipid extraction is eliminated.

Reference

3.1. Hidalgo P, Toro C, Navia R (2013) Advances in direct transesterification of microalgal biomass for biodiesel production. Reviews in Environmental Science and Bio/Technology 12: 179-199. (Please add 3. for each reference number)

3.2. Griffiths M, Van Hille R, Harrison S (2010) Selection of direct transesterification as the preferred method for assay of fatty acid content of microalgae. Lipids 45: 1053-1060.

3.3. Ehimen E, Sun Z, Carrington C (2010) Variables affecting the in situ transesterification of microalgae lipids. Fuel 89: 677-684.

3.4. Wahlen BD, Willis RM, Seefeldt LC (2011) Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. Bioresource technology 102: 2724-2730.

3.5. Sathish A, Smith BR, Sims RC (2014) Effect of moisture on in situ transesterification of microalgae for biodiesel production. Journal of Chemical Technology and Biotechnology 89: 137-142.

3.6. Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. Renewable and sustainable energy reviews 14: 217-232.

3.7. Velasquez-Orta S, Lee J, Harvey A (2012) Alkaline in situ transesterification of Chlorella vulgaris. Fuel 94: 544-550.

3.8. Shuit SH, Lee KT, Kamaruddin AH, et al. (2010) Reactive extraction and in situ esterification of Jatropha curcas L. seeds for the production of biodiesel. Fuel 89: 527-530.

3.9. Heilmann SM, Jader LR, Sadowsky MJ, et al. (2011) Hydrothermal carbonization of distiller's grains. Biomass and Bioenergy 35: 2526-2533.

3.10. Heilmann SM, Jader LR, Harned LA, et al. (2011) Hydrothermal carbonization of microalgae II. Fatty acid, char, and algal nutrient products. Applied Energy 88: 3286-3290.

3.11. Levine RB, Pinnarat T, Savage PE (2010) Biodiesel production from wet algal biomass through in situ lipid hydrolysis and supercritical transesterification. Energy & Fuels 24: 5235-5243.

3.12. Du Z, Hu B, Shi A, et al. (2012) Cultivation of a microalga Chlorella vulgaris using recycled aqueous phase nutrients from hydrothermal carbonization process. Bioresource technology 126: 354-357.

Chapter 4. Effect of the storage condition of microalgae paste on the hydrochar lipid and the direct transesterification of the hydrochar lipid for biodiesel production

4.1. Introduction

HTC of microalgae is a process in which microalgae react with water at a high temperature (around 200 °C, 2 MPa) to form one solid product called the hydrochar. Compared to the drying process, HTC of microalgae is an energy-effective process. After this process, the main product of the hydrochar retains most of fatty acids from microalgae, and it is a promising feedstock for biodiesel production [4.1,4.2]. Fatty acids in the hydrochar are bound fatty acids (BFAs) and free fatty acids (FFAs) [4.3]. One of the most effective methods to produce FAMEs from the hydrochar is the direct transesterification. Fatty acids (FAs) inside the hydrochar (both BFAs and FFAs) will directly react with methanol in the presence of a catalyst to form fatty acid methyl esters (FAMEs) without oil extraction. This approach eliminates a big amount of hazardous solvents required for oil extraction from microalgae such as chloroform and methanol [4.4], hexane and isopropanol [4.5]. Moreover, the biodiesel yield will be also improved compared to the traditional methods including the oil extraction and the subsequent transesterification [4.6,4.7,4.8]. Acid catalysts (the commonly used one is H_2SO_4) are usually used to catalyze both the esterification of FFAs and the transesterification of BFAs in one step.

Because microalgae paste is used in the HTC process, the storage time will affect the levels of TFAs and FFAs in the microalgae. In reality, there is a period of time from harvesting step to HTC of microalgae. This time period can be used for the shipment to the factory or the storage of feedstock at the factory. From the previous work, the contents of TFAs and FFAs in microalgae paste increase after one-day storage. In detail, there is an increase in FFAs and a decrease in triacylglycerides (TGs, a form of BFAs) due to the hydrolysis of triacylglycerides (TGs) under the effect of the enzyme lipase in microalgae [4.9,4.10]. The microalgae paste should undergo the HTC process to obtain the hydrochar used as a

feedstock for biodiesel production. If the TFA content of microalgae is higher, the TFA content of the created hydrochar will be higher at the same HTC condition. However, if the FFA content of microalgae is higher, more FFAs will be extracted into the aqueous phase during the HTC due to the high solubility of FFAs in water at a high temperature [4.11]. The higher the FFA content of microalgae is, the higher the possibility of losing these FFAs in the aqueous phase is. In other words, the TFA retention in the hydrochar will be lower. There are two opposite effects on the TFA content of the created hydrochar. Therefore, in this work, HTC of stored microalgae has been conducted to see whether the amount of TFAs in the hydrochar increases compared to the hydrochar from microalgae without storage. In addition, the percentage of FFAs/TFAs is also evaluated in both cases. Because the acid catalyzed esterification of FFAs is much faster than the acid catalyzed transesterification of BFAs, the higher percentage of FFAs/TFAs will make the biodiesel formation faster at the same reaction condition. This additional storage step does not require chemicals or much energy, so it should be highly applicable.

4.2. Materials and Method

4.2.1. Materials

4.2.1.1. Microalgae cultivation and harvest

The marine microalgae species, Chlorella (PTCC 6010), was cultivated in the Rudic medium with the total volume of 120 liters [4.12]. The medium recipe was as follows, $NaNO_3$: 300 mg/l, K_2HPO_4: 80 mg/l, KH_2PO_4: 20 mg/l, $MgSO_4.7H_2O$: 10 mg/l, $CaCl_2$: 47 mg/l, EDTA: 7.5 mg/l, NaCl: 20 mg/l, H_3BO_3: 0.3 mg/l, $MnSO_4.H_2O$: 1.5 mg/l, $ZnSO_4.7H_2O$: 0.1 mg/l, $(NH_4)_6Mo_7O_{24}.4H_2O$: 0.3 mg/l, $CuSO_4.5H_2O$: 0.08 mg/l, $Co(NO_3)_2.6H_2O$: 0.26 mg/l, $FeCl_3.6H_2O$: 17 mg/l. The salinity of the medium was 30 g NaCl/l. The temperature was kept at around 25 °C, and pH was adjusted at around 8 by gaseous CO_2. The microalgae density was determined by the UV-Vis spectrometer, and the microalgae medium reached to the stationary phase after 2 weeks and were ready for harvest. Microalgae were flocculated by $FeCl_3$ with an amount of 0.3 g/l of the culture medium [4.13]. The flocculated microalgae were washed 2 times with water and then filtered to get microalgae paste.

4.2.1.2. Chemicals

All chemicals used in the microalgae cultivation, the harvesting and the direct transesterification reaction were purchased from Wako Pure Chemical Industries, Ltd. The fatty acid methyl ester standards including cis-9-tetradecenoic acid, methyl ester (C14:1); tetradecanoic acid, methyl ester (C14:0); pentadecanoic acid, methyl ester (C15:0); cis-9-hexadecenoic acid, methyl ester (C16:1); hexadecanoic acid, methyl ester (C16:0); heptadecanoic acid, methyl ester (C17:0) were purchased from Sigma-Aldrich.

4.2.1.3. Equipment

The UV-Vis spectrometer model was Shimadzu UVmini 1240. The 50 ml autoclave was purchased from Taiatsu Techno Corporation. The Tomy Multipurpose Refrigerated Centrifuge EX-126 was used to centrifuge samples. The Shimadzu GCMS-QP2010 SE was used to quantitatively analyze fatty acid methyl esters.

4.2.2. Method

4.2.2.1. Storage of microalgae paste after harvesting

The microalgae paste was divided into 3 parts after harvesting. The first one was dried immediately at 105 °C for 4 hours. The second and the third ones were stored in the incubator at 25 °C respectively for 1 and 2 days, and then dried at 105 °C after finishing the storage. The determination of the content of TFAs and FFAs in microalgae can be referred in the sections 2.2.2, 2.2.3 and 2.2.4.

4.2.2.2. Hydrothermal carbonization of microalgae to create the hydrochar

The experimental procedures for the hydrothermal carbonization of microalgae (at 200 ^{o}C, 210 ^{o}C and 220 ^{o}C), and the determination of the content of TFAs and FFAs in microalgae and the hydrochar can be referred in the sections 2.2.1, 2.2.2, 2.2.3 and 2.2.4.

4.2.2.3. Direct transesterification of the hydrochar lipids

The direct transesterification of the hydrochar lipids was performed in a 10 ml glass tube shown in **Figure 4.1**. Firstly, the hydrochar (0.1 g or 0.2 g) and a magnetic bar were gently put into the glass tube, and the samples were prevented from sticking on the top of the tube.

An amount of 1 ml methanol solution containing H_2SO_4 and the internal standard hexadecanenoic acid methyl ester (C17: 0) of 100 µg was then added, and the tube was sealed by a teflon cap. The tube was then placed in a pre-heated silicone oil bath and underwent the transesterification at 80 °C for 90 min with the stirring speed of 180 rpm. To reduce the amount of methanol, the maximum amount of the hydrochar (0.2 g) were used corresponding to 1 ml of methanol, so two levels of 0.1 g and 0.2 g hydrochar/ml methanol were investigated. The effect of the sulfuric acid concentration was investigated at 1, 2, 3 and 4% v/v of methanol.

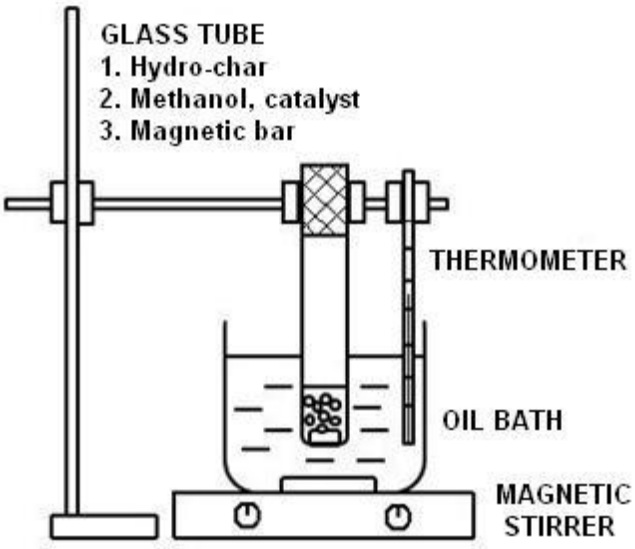


Figure 4.1. Direct transesterification of the hydrochar lipids in the glass tube heated by the oil bath

When the reaction time was reached, 1 ml of distilled water was added to stop the reaction. Subsequently, an amount of 5 ml hexane was added into the tube to extract FAMEs. The mixture was shaken strongly and centrifuged at 2000 rpm for 5 minutes to completely separate the aqueous phase and the hexane phase. An amount of 1 ml hexane phase was pipetted to vials which were stored at 4 °C before the GC-MS analysis.

4.2.2.4. Definition

Microalgae (0): fresh algae, microalgae (1): one-day-stored algae, microalgae (2): two-day-stored algae

Hydrochar (0) from the microalgae (0), hydrochar (1) from the microalgae (1)

Mass yield of the hydrochar H(0), H(1)

H(0), % = (mass of the hydrochar (0) × 100) / mass of treated microalgae (0)

H(1), % = (mass of the hydrochar (1) × 100) / mass of treated microalgae (1)

M(0): TFAs of the microalgae (0) (mg/g algae)

N(0): TFAs of the hydrochar (0) (mg/g hydrochar)

$\mathbf{Y(0)_1}$: TFA retention of the hydrochar (0), %

$$\mathbf{Y(0)_1 = N(0) \times H(0) / M(0)}, \%$$

$\mathbf{Y(0)_2}$: yield of the transesterification reaction of the hydrochar (0), %

Y(0): total biodiesel yield of the whole process from the microalgae (0) to biodiesel

$$\mathbf{Y(0) = M(0) \times Y(0)_1 \times Y(0)_2} \text{ (mg / g algae)}$$

M(1): TFAs of the microalgae (1) (mg/g algae)

N(1): TFAs of the hydrochar (1) (mg/g hydrochar)

$\mathbf{Y(1)_1}$: TFA retention of the hydrochar (1), %

$$\mathbf{Y(1)_1 = N(1) \times H(1) / M(1)}, \%$$

$\mathbf{Y(1)_2}$: yield of the transesterification reaction of the hydrochar (1), %

Y(1): total biodiesel yield of the whole process from the microalgae (1) to biodiesel

$$\mathbf{Y(1) = M(1) \times Y(1)_1 \times Y(1)_2} \text{ (mg/g algae)}$$

4.3. Results and discussion

4.3.1. Effect of the storage time on total fatty acids (TFAs) and free fatty acids (FFAs) in microalgae

The major fatty acids of the microalgae (0) are tetradecanoic acid, methyl ester (C14:0); cis-9-hexadecenoic acid, methyl ester (C16:1); hexadecanoic acid, methyl ester (C16:0). This composition is similar to a marine Chlorella sp. in the previous research [4.13].

Table 4.1. The effect of the storage time (one and two days) on total fatty acids (TFAs) and free fatty acids (FFAs) in microalgae

Fatty acid methyl esters	**Microalgae (0)** (mg/g algae)	**Microalgae (1)** (mg/g algae)	**Microalgae (2)** (mg/g algae)
Cis-9-Tetradecenoic acid, methyl ester (C14:1)	0.68 ± 0.02	0.88 ± 0.02	0.87 ± 0.04
Tetradecanoic acid, methyl ester (C14:0)	11.73 ± 0.27	13.28 ± 0.27	12.53 ± 0.61
Pentadecanoic acid, methyl ester (C15:0)	0.47 ± 0.01	0.49 ± 0.02	0.50 ± 0.02
Cis-9-Hexadecenoic acid, methyl ester (C16:1)	9.60 ± 0.19	11.64 ± 0.17	11.97 ± 0.53
Hexadecanoic acid, methyl ester (C16:0)	2.89 ± 0.08	3.00 ± 0.06	3.09 ± 0.15
Total fatty acids (TFAs), **M(0), M(1), and M(2)**	25.37 ± 0.56	29.28 ± 0.49	28.95 ± 1.10
Free fatty acids / Total fatty acids (FFAs/TFAs), %	14.7 ± 0.6	17.7 ± 0.3	18.4 ± 0.3

The percentage of FFAs/TFAs of the microalgae (0) was 14.7%. Inherently, the microalgae have a certain concentration of FFAs. Robert B. Levine and et al. claimed that the FFA content in the dry microalgae (65 °C for 24 h) was 1–2% [4.3]. On the other hand, Tao Dong and et al. also reported that the FFA content of the lyophilized C. Sorokiniana was very high (46.85% of the TFAs) [4.14].

According to the data shown in **Table 4.1**, after the storage period of one day, the amount of TFAs in the microalgae (1) increased approximately 15.4% (25.37 to 29.28 mg/g microalgae) compared to the microalgae (0). In the second day, the TFAs gradually reduced a little. Meanwhile, there was a slight increase in FFAs/TFAs from 14.7% to 18.4%.

The slight increase in TFAs in the microalgae (1) is consistent with the previous study [4.9]. The total lipid content of microalgae increased from 33.4% (stored at −80 °C) to 36.6% (stored at 25 °C) [4.9]. The phenomenon of slightly increased TFA content was also reported by E. Montaini and et al. when the microalgae Tetraselmis suecica paste was stored at 4 °C

[4.15]. On the other hand, the increase in FFAs/TFAs is caused by the hydrolysis of triacylglycerides (TGs) in the presence of enzyme lipase inside the microalgae [4.9,4.10].

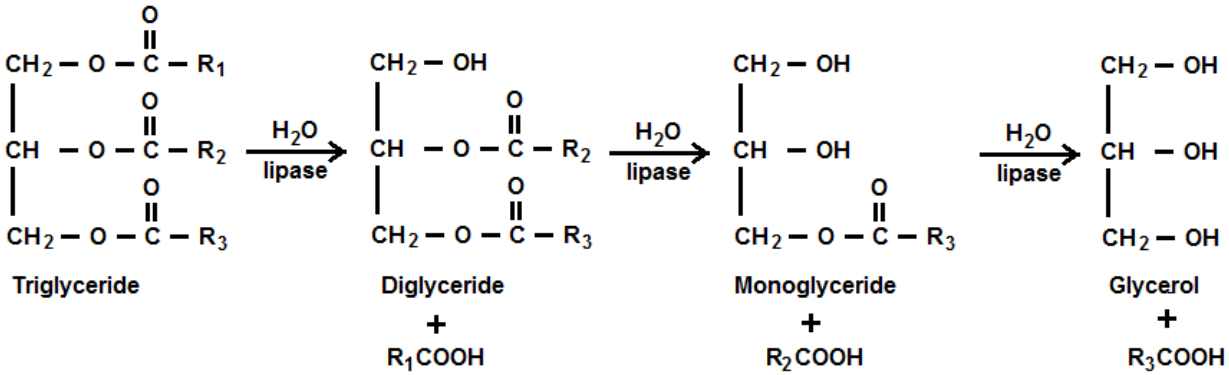


Figure 4.2. The hydrolysis of triglyceride by enzyme lipase to create free fatty acids

Due to the small difference between the microalgae (1) and the microalgae (2), the microalgae (1) and the microalgae (0) were used in the subsequent HTC for the comparison.

4.3.2. Effect of the storage time on total fatty acids (TFAs) and free fatty acids (FFAs) in the hydrochar

The mass yields of the hydrochar after HTC of microalgae are shown in **Table 4.2**. The trend is that the higher the HTC temperature was, the less hydrochar could be obtained. However, the difference is negligible.

Table 4.2. Mass yields of the hydrochar after HTC of algae

HTC (°C)	Mass yields of hydrochar, %	
	Microalgae (0)	Microalgae (1)
200	57.1 ± 0.1	53.6 ± 0.3
210	55.3 ± 0.2	54.7 ± 0.5
220	52.7 ± 0.2	52.7 ± 0.3

The TFA retention in the hydrochar after HTC is presented in **Table 4.3**. In general, the higher the reaction temperature was, the lower the TFA retention was due to the enhanced hydrolysis of triacylglycerides (TGs) and the easy extraction of FFAs from microalgae into the aqueous phase under the HTC condition. At 200 °C, the retention level of TFAs was about 85%. This result is consistent with previous studies [4.1,4.16,4.17] which proved that

the hydrochar of HTC at around 200 °C retained most of TFAs of microalgae, from 80% to 95% depending on the type of microalgae.

Table 4.3. The TFA retention and FFAs/TFAs in the hydrochar after HTC

HTC (°C)	Total fatty acids (TFAs) retention		$[(M(1) \times Y(1)_1)- (M(0) \times Y(0)_1)] \times 100 /(M(0) \times Y(0)_1)$, %	Percentage of FFAs/TFAs, %	
	Hydrochar (0), $Y(0)_1$, %	Hydrochar (1), $Y(1)_1$, %		Hydrochar (0)	Hydrochar (1)
200	85.3 ± 1.1	83.2 ± 1.2	12.6	29.4 ± 0.8	37.1 ± 1.2
210	82.9 ± 0.5	78.4 ± 1.3	9.2	31.8 ± 2.7	38.0 ± 0.5
220	73.1 ± 2.4	70.8 ± 2.0	11.9	36.5 ± 0.8	39.3 ± 2.7

According to **Table 4.3**, it can also be seen that the hydrochar (1) had a little bit lower TFA retention compared to the hydrochar (0) ($\mathbf{Y(1)_1 < Y(0)_1}$) at all HTC conditions. The reason is that compared to the microalgae (0), the microalgae (1) contained more FFAs which were more freely and easily extracted to water phase during HTC of microalgae due to the high solubility of FFAs in water at a high temperature [4.11]. The higher percentages of FFAs/TFAs in the hydrochar (1) also proved that. On the other hand, the amount of TFAs in the microalgae (1) were 15.4% higher than the amount of TFAs in the microalgae (0) [$\mathbf{M(1) > M(0)}$] as mentioned above. Finally, the amount of TFAs in the hydrochar (1) $\mathbf{[M(1) \times Y(1)_1]}$ was still about 10% higher than the amount of TFAs in the hydrochar (0) $\mathbf{[M(0) \times Y(0)_1]}$ at all HTC temperatures. This result means that the additional storage step of microalgae paste can enhance the amount of TFAs in the subsequent hydrochar which favors for biodiesel production.

Meanwhile, the percentage of FFAs/TFAs tends to increase up to 40% as the temperature increases from 200 °C to 220 °C due to enhanced hydrolysis at a higher temperature. This is beneficial for the esterification-transesterification process because FFAs can be converted to FAMEs rapidly under the efffect of the acid catalysts. However, it should be noted that when the temperature increased up to 220 °C, the TFA retention went down. Therefore, the hydrochar (0) and the hydrochar (1) formed at 200 °C were used in the direct transesterification process for biodiesel production.

Besides, it can be seen that the percentages of FFAs/TFAs of the hydrochar (1) were higher than those of the hydrochar (0). This is because the hydrolysis of BFAs occurs slowly at first and then can be accelerated by the self-catalytic effect of formed FFAs [4.3]. Due to a higher

percentage of FFAs/TFAs in the microalgae (1) (17.7% > 14.7%), the hydrolysis rate of BFAs in the microalgae (1) was faster than that in the microalgae (0) at the same HTC condition, and more FFAs were formed in the hydrochar (1) (37.1% > 29.4% at 200 °C). This facilitated the esterification-transesterification using the acid catalyst due to a rapid conversion of FFAs to FAME [4.18].

4.3.3. Direct transesterification of the hydrochar

Table 4.4. Yields of the direct transesterification of the hydrochar (0) (HTC 200 °C) and the hydrochar (1) (HTC 200 °C) at 80 °C, 90min

No.	Hydrochar (g) / ml methanol	H_2SO_4 conc. % v/v	Hydrochar (0) $Y(0)_2$, %	Hydrochar (1) $Y(1)_2$, %	$Y(1)_2 - Y(0)_2$ %	$[Y(1) - Y(0)] \times 100 / Y(0)$, %
1	0.1	1	85.5 ± 0.3	94.4 ± 2.5	8.9	24.4
2		2	97.2 ± 0.4	98.0 ± 1.0	0.8	13.5
3	0.2	1	63.7 ± 0.8	72.6 ± 1.3	8.9	28.3
4		2	82.6 ± 0.6	88.7 ± 1.2	6.1	21.1
5		3	93.7 ± 0.4	99.2 ± 0.3	5.5	19.3
6		4	96.5 ± 0.5	99.0 ± 0.9	2.5	15.5

$\mathbf{Y(0) = M(0) \times Y(0)_1 \times Y(0)_2}$ (mg/g algae), $\mathbf{Y(1) = M(1) \times Y(1)_1 \times Y(1)_2}$ (mg/g algae)

The yield of the esterification-transesterification reactions is presented in **Table 4.4**. Generally, the higher the H_2SO_4 concentration was, the higher the reaction yield was. To obtain the reaction yield exceeding 95%, the required H_2SO_4 concentration was 2% v/v in the case of 0.1 g char/ml methanol and 3% v/v in the case of 0.2 g char/ml methanol. The methanol solution of 2% v/v H_2SO_4, with the same effect as the methanol solution of 5% v/v HCl, is normally used to prepare fatty acid methyl esters for chromatographic analysis [4.19]. This H_2SO_4 concentration was still sufficient for the case of 0.1 g char/ml methanol. However, when more hydrochar was used like 0.2 g char/ml methanol, the H_2SO_4 concentration should be higher (3% v/v) to get the high conversion. Therefore, the reaction condition (no.5) was selected in the direct transesterification of hydrochar.

Moreover, at the same reaction condition, the reaction yields of the hydrochar (1) were always higher than the hydrochar (0) [$\mathbf{Y(1)_2 > Y(0)_2}$]. This is because the percentage of FFAs/TFAs of the hydrochar (1) is higher than that of the hydrochar (0) (37.1% > 29.4%) as mentioned above, and the acid catalyst H_2SO_4 can catalyze the esterification of FFAs much faster than the transesterification of BFAs [4.18]. Furthermore, the difference of the reaction

yield [$\mathbf{Y(1)_2 - Y(0)_2}$] being around the difference of the percentage of FFAs/TFAs (37.1% − 29.4% = 7.7%) also proves this explanation, excluding the cases using an amount of sulfuric acid more than necessary for the hydrochar (1) (No.2 and 6).

Finally, the total yields **Y(1)** and **Y(0)** are compared as the ratio of [**Y(1) – Y(0)**] × 100 / **Y(0)** in **Table 4.4**. **Y(1)** is around 15% – 25% higher than **Y(0)** in all conditions of the esterification and the transesterification reactions. For the case of 0.2 g char/ml methanol and 3% v/v H_2SO_4 concentration, the difference is 19.3%. In summary, the additional storage step of microalgae not only increased the amount of total fatty acids (TFAs) in the hydrochar but also made the percentage of FFAs/TFAs higher as well as a higher reaction yield at the same reaction condition. This eventually leads to a higher total yield in the case (1) which includes the storage of microalgae paste. The comparison of both cases is briefly illustrated in **Figure 4.3.**

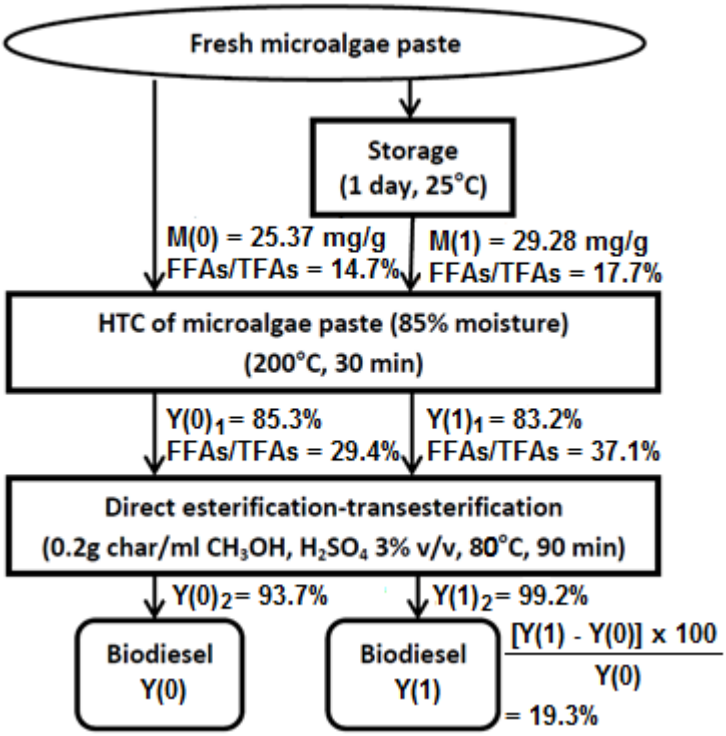


Figure 4.3. The whole process of biodiesel production from fresh microalgae in both cases of (0) and (1)

4.4. Conclusion

An entire process, from the storage of fresh algae, HTC of microalgae paste to the direct esterification-transesterification of the hydrochar using the acid catalyst H_2SO_4, was investigated for biodiesel production. Instead of doing HTC of fresh microalgae immediately after harvesting, the storage of fresh microalgae for one day at the room temperature (25 °C) before conducting HTC can improve the total biodiesel yield of 19.3% at the same conversion conditions. This additional step is very meaningful in the real production and highly practical because there is no consumption of much energy and chemicals required in the storage of microalgae.

Reference

4.1. Heilmann SM, Davis HT, Jader LR, et al. (2010) Hydrothermal carbonization of microalgae. Biomass and Bioenergy 34: 875-882. (Please add 4. for each reference number)

4.2. Heilmann SM, Jader LR, Harned LA, et al. (2011) Hydrothermal carbonization of microalgae II. Fatty acid, char, and algal nutrient products. Applied Energy 88: 3286-3290.

4.3. Levine RB, Pinnarat T, Savage PE (2010) Biodiesel production from wet algal biomass through in situ lipid hydrolysis and supercritical transesterification. Energy & Fuels 24: 5235-5243.

4.4. Folch J, Lees M, Sloane-Stanley G (1957) A simple method for the isolation and purification of total lipids from animal tissues. J biol Chem 226: 497-509.

4.5. Halim R, Gladman B, Danquah MK, et al. (2011) Oil extraction from microalgae for biodiesel production. Bioresource technology 102: 178-185.

4.6. Griffiths M, Van Hille R, Harrison S (2010) Selection of direct transesterification as the preferred method for assay of fatty acid content of microalgae. Lipids 45: 1053-1060.

4.7. Tran H-L, Hong S-J, Lee C-G (2009) Evaluation of extraction methods for recovery of fatty acids from Botryococcus braunii LB 572 and Synechocystis sp. PCC 6803. Biotechnology and Bioprocess Engineering 14: 187-192.

4.8. Lewis T, Nichols PD, McMeekin TA (2000) Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs. Journal of Microbiological Methods 43: 107-116.

4.9. Chen L, Liu T, Zhang W, et al. (2012) Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresource technology 111: 208-214.

4.10. Singh A, Nigam PS, Murphy JD (2011) Mechanism and challenges in commercialisation of algal biofuels. Bioresource technology 102: 26-34.

4.11. Khuwijitjaru P, Adachi S, Matsuno R (2002) Solubility of saturated fatty acids in water at elevated temperatures. Bioscience, biotechnology, and biochemistry 66: 1723-1726.

4.12. Golzary A, Imanian S, Abdoli MA, et al. (2015) A cost-effective strategy for marine microalgae separation by electro-coagulation–flotation process aimed at bio-crude oil production: optimization and evaluation study. Separation and Purification Technology 147: 156-165.

4.13. Sanyano N, Chetpattananondh P, Chongkhong S (2013) Coagulation–flocculation of marine Chlorella sp. for biodiesel production. Bioresource technology 147: 471-476.

4.14. Dong T, Wang J, Miao C, et al. (2013) Two-step in situ biodiesel production from microalgae with high free fatty acid content. Bioresource technology 136: 8-15.

4.15. Montaini E, Zittelli GC, Tredici M, et al. (1995) Long-term preservation of Tetraselmis suecica: influence of storage on viability and fatty acid profile. Aquaculture 134: 81-90.

4.16. Lu Y, Levine RB, Savage PE (2014) Fatty acids for nutraceuticals and biofuels from hydrothermal carbonization of microalgae. Industrial & Engineering Chemistry Research 54: 4066-4071.

4.17. Du Z, Mohr M, Ma X, et al. (2012) Hydrothermal pretreatment of microalgae for production of pyrolytic bio-oil with a low nitrogen content. Bioresource technology 120: 13-18.

4.18. Kail BW, Link DD, Morreale BD (2012) Determination of free fatty acids and triglycerides by gas chromatography using selective esterification reactions. Journal of chromatographic science: bms093.

4.19. Christie WW (1993) Preparation of ester derivatives of fatty acids for chromatographic analysis. Advances in lipid methodology 2: e111.

Chapter 5. Conclusion

HTC of microalgae is energy-effective for removing water from microalgae paste in a big scale. After HTC, most of lipid from microalgae is retained in the hydrochar. During the HTC of microalgae, the components of carbohydrate and protein are carbonized, and the microalgae cells are weaken and contractive. Moreover, the ratio of FFAs/TFAs of the hydrochar (at HTC 200 ^{o}C) was higher than microalgae (48.0% and 24.9%). These factor facilitate the penetration of solvent into hydrochar to extract lipid. This has the effect on subsequent conversion steps for biodiesel production from hydrochar.

1. In the lipid extraction and transesterification method, the yield of lipid extraction from the hydrochar (79.8%) is higher than that from microalgae (17.7%) at the ratio of 1 ml mixture of hexane / isopropanol (3/2 v/v) / 100 mg microalgae. The appropriate conditions for the esterification pretreatment were as follows: catalyst Amberlyst 15 of 30% wt. oil, the reaction temperature of 80 ^{o}C, 12:1 molar ratios of methanol / lipid. Next, the biodiesel yield of 93.6% can be achieved after the transesterification at the conditions of 12: 1 molar ratio of methanol / lipid, the amount of KOH of 1.5% wt. oil, the reaction temperature of 65 ^{o}C and the reaction time of 60 minutes. (chapter 2)

Although HTC of microalgae (at 200 ^{o}C) consumes much more energy compared to autoclave pretreatments at lower temperatures as in the previous studies, water can be removed from the hydrochar in an energy-effective way by the filtration. Most of lipid is still retained in the hydrochar, and the yield of lipid extraction from hydrochar is much higher than microalgae as shown in the previous studies. However, the main drawback of this method is the use of a large amount of extraction solvents (1 ml solvent for 100 mg microalgae or 45.5 mg hydrochar).

2. In the direct transesterification method, the yield of the direct transesterification of hydrochar is higher than microalgae at the same reaction condition. The appropriate conditions for biodiesel production from microalgae were the ratio of 200 mg microalgae / ml methanol, 2% v/v acid H_2SO_4, 80 ^{o}C and 90 minutes of the reaction. To convert lipids in 400 mg microalgae into biodiesel, a volume of 2 ml methanol with the H_2SO_4 concentration of

2% v/v is required. On the other hand, if this amount of lipids exists in the hydrochar through HTC of microalgae, a volume of only 1 ml methanol with the H_2SO_4 concentration of 2% v/v is necessary. It is only a half of the amount which is required for microalgae. The amounts of methanol and catalyst which are required for a given amount of microalgae can be reduced to a half through the direct transesterification of hydrochar. (chapter 3)

From the results presented in Chapters 2 and 3, the yield of the direct transesterification of the hydrochar (182.1 mg hydrochar / ml methanol) is higher than the yield of the lipid extraction from the hydrochar (45.5 mg hydrochar / ml mixture of hexane / isopropanol). The reason is that all fatty acids (FAs) inside the hydrochar (both BFAs and FFAs) will directly react with methanol in the presence of catalyst H_2SO_4 to form fatty acid methyl esters (FAMEs) which is easier to be extracted by solvents. The additional advantages of the direct transesterification are the simple process and the elimination of a large amount of solvent used for lipid extraction.

The direct transesterification is recommended to be used in the biodiesel production. In general, the samples must be completely covered by methanol in the direct transesterification. Otherwise, the biodiesel yield will become low due to the poor contact between methanol and lipids in samples. In addition, if the lipid content of the sample is higher than normal, it leads to a higher efficiency of using methanol and catalyst. For example, the content of fatty acids in the hydrochar doubled, the amount of methanol and H_2SO_4 was reduced to a half. Therefore, the content of fatty acids in samples should be increased by an improved cultivation technology or a pretreatment step.

3. An entire process, from the storage of fresh algae, HTC of microalgae paste to the direct esterification-transesterification of the hydrochar using the acid catalyst H_2SO_4, was investigated for biodiesel production. Instead of doing HTC of fresh microalgae immediately after harvesting, the storage of fresh microalgae for one day at the room temperature (25 °C) before conducting HTC can improve the total biodiesel yield of 19.3% at the same conversion conditions. This additional step is very meaningful in the real production and highly practical because there is no consumption of much energy and chemicals required in the storage of microalgae.

This procedure should be applied and tested for many other types of microalgae and similar aquatic plants that can be used as feedstock for biodiesel production. The aqueous phase containing nutrients should be evaluated as fertilizer used in plant or microalgae cultivation. In addition, the potential of using lipid-extracted hydrochar as an adsorbent or a catalyst support should be evaluated.