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Research article

Comparison between direct transesterification of microalgae and hydrochar

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Abstract: Hydrothermal carbonization (HTC) of microalgae is one of processes that can effectively remove moisture from microalgae. In addition, the hydrochar retains most of fatty acids from microalgae feedstock, and the content of fatty acids in hydrochar is doubled. This research concentrates on the comparison between direct transesterification of microalgae and hydrochar. The result shows that the biodiesel yields of hydrochar were higher than those of microalgae at the same reaction conditions due to the higher extraction rate of fatty acids from hydrochar. Finally, the amount of methanol and catalyst which is required for a given amount of microalgae can be reduced to a half through the direct transesterification of hydrochar.

Keywords: microalgae; hydrochar; hydrothermal carbonization; direct transesterification; biodiesel

1. Introduction

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 [1], 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 [2,3]. 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 [4,5]. Among them, the small increase of the moisture content can significantly affect the biodiesel yield [4,6]. The direct transesterification of wet microalgae was conducted in previous works. Chlorella sp. and N. oculata were used in the direct transesterification using sulfuric acid, sodium hydroxide, and sodium methoxide as catalyst [7]. The higher yields for N. oculata (73% d.b. w/w) and for Chlorella sp. (92% d.b. w/w) were achieved in the case of sulfuric acid. The yields decreased with the increase of moisture contents of 0% w/w, 1.5% w/w, and 10% w/w [7]. Sathish et al. also investigated the moisture content of microalgae on biodiesel yield. When the moisture content was higher than 20% w/w, the biodiesel yield significantly reduced. At the ratio of 100 mg dry microalgae/1 ml methanol (2% v/v H_2SO_4), 90 ℃, and 30 minutes of reaction, the biodiesel yield achieved 90.0% d.b. w/w. When the moisture content increased up to 84% w/w, the biodiesel yield decreased down to 22.0% d.b. w/w. However, the biodiesel yield could be improved up to 81.9% d.b. w/w when the concentration of sulfuric acid increased up to 10% (v/v) at the ratio of 25 mg microalgae/ml methanol [6]. 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% w/w [1]. Therefore, the complete removal of water from microalgae should be done to facilitate 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 dried microalgae [5,6] 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.

For the issue of high moisture content, there is one method called the hydrothermal carbonization (HTC) which is very effective in the water removal from microalgae paste. HTC of microalgae is a process in which microalgae react with water under a high temperature and pressure (about 200 ℃ and 2 MPa) to create one solid product called hydrochar and one aqueous phase [8]. After HTC, the removal of water from hydrochar can be done in an energy-effective way by filtration. Besides, the mass yield of hydrochar is around 40% while the hydrochar retains most of fatty acids (80–95%) in microalgae feedstock [8,9,10]. In other words, the content of fatty acids in hydrochar is approximately doubled compared to the microalgae feedstock, and the mass of 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 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 research through the comparison between the direct transesterification of microalgae and hydrochar.

2. Materials and Method

2.1. Materials

2.1.1. Chemicals

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.

2.1.2. Equipment

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. The confocal laser-scanning microscopy (LSM780, ZEISS) was used to detect the lipid droplets inside microalgae (or hydrochar) which were stained by Nile Red.

2.2. Method

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% w/w. 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 [8,11,12]. In the real application, the microalgae paste should be used in the HTC process. Then, argon gas was injected into the autoclave in order to purge the air inside. The prepared 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 ℃, 210 ℃, and 220 ℃. These conditions are appropriate for HTC of microalgae [8,9,12] which do not have the components of cellulose, hemicellulose, and lignin [8]. The experiments conducted at 190 ℃ or lower indicated that the average size of hydrochar particles was still small, so this led to the difficulty of filtering hydrochar. Meanwhile, the retention of fatty acids in hydrochar was considerably reduced at the HTC temperature of 230 ℃ or higher. 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 ℃.

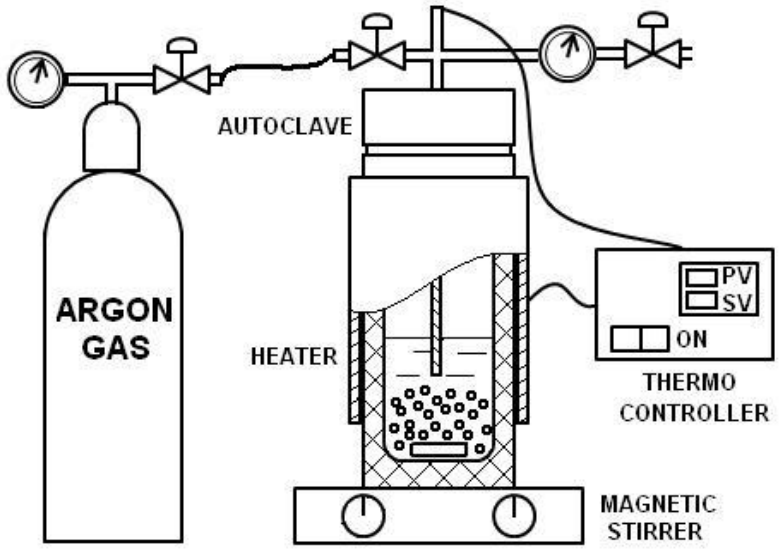

Figure 1. The HTC process of microalgae in the autoclave.

The mixture after the HTC process was filtered by the GF/A paper having pore size of 1.6 μm. The hydrochar was dried at 100 ℃ for 4 h and stored at 4 ℃. The mass of hydrochar was weighed, and the mass yield of hydrochar was determined by the formula 1 below.

$$\mathrm{H_M}, \% = \frac{\text{Mass of hydrochar}}{\text{Mass of treated microalgae}} \times 100 \text{ (formula 1)}$$

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 hydrochar was also conducted by the direct transesterification method [3]. 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 ℃, 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 ℃ prior to 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 GCMS machine were set as follows: the injection temperature of 220 ℃, the pressure of 80.8 kPa, the column flow rate of 1.46 mL min^{-1}, and the splitless mode. The column temperature was increased from 40 ℃ to 190 ℃ at the heating rate of 20 ℃ min^{-1}, increased from 190 ℃ to 220 ℃ at the heating rate of 2 ℃ min^{-1}, and held at 220 ℃ for 2.5 minutes.

The content of fatty acids in the sample was calculated by the following formula 2.

$$C, \% = \sum \left(\frac{A_n}{A_{IS}} \times \frac{1}{r_n} \times \frac{m_{IS}}{M} \times 100 \right) \text{(formula 2)}$$

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 response factors of each FAME are shown as the slopes in Figure 2.

The retention of fatty acids in hydrochar was calculated by the following formula 3, where C_{HC} is the content of fatty acids in hydrochar, and C_{AL} is the content of fatty acids in microalgae.

$$R, \% = \frac{C_{HC}}{C_{AL}} \times H_M \text{ (formula 3)}$$

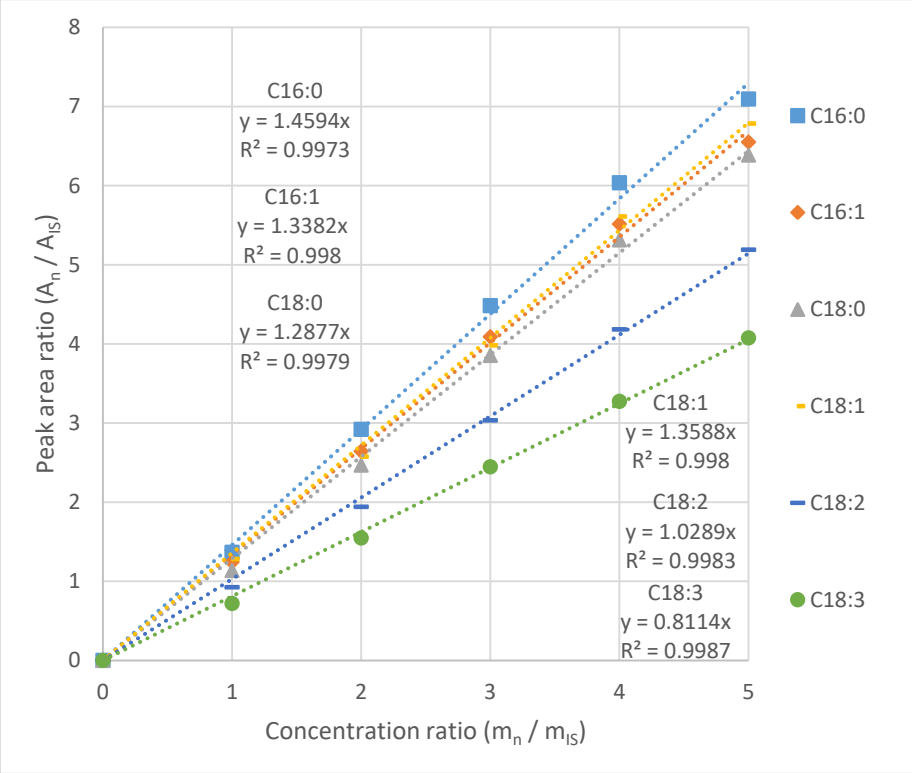

Figure 2. The response factors of standards of fatty acid methyl esters.

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

The content of FFAs in the samples (microalgae or 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) [13]. After that, the solvents were completely evaporated at 60 ℃. Next, FFAs were selectively converted into FAMEs by 1 ml of pyridine/N, N-Dimethylformamide dimethyl acetal (1:1 v/v) at 60 ℃ in 15 minutes [14]. 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 ℃ before GCMS analysis.

2.2.4. Photos of lipid distribution in microalgae and hydrochar

The lipid droplets inside microalgae (or hydrochar) were detected by Nile Red [15]. The stock solution of Nile Red (200 μg ml^{-1}) 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^{-1} in 15 minutes. The Nile Red signals were detected by an 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 [16].

2.2.5. Comparison between direct transesterification of microalgae and hydrochar

The hydrochar which has the highest retention of fatty acids after HTC was used in the direct transesterification for comparison. Because the moisture content of microalgae (or hydrochar) significantly affects the reaction yield, the moisture was completely removed by drying microalgae (or hydrochar) at 105 ℃ 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 [17]. Wahlen 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, reaction times of 10 and 20 minutes, and reaction temperature in the range of 60–110 ℃. The biodiesel yield was very high at 80 ℃, and there was no significant increase in the reaction yield when the temperature was up to 110 ℃ [5]. Sathish 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, reaction temperature of 90 ℃, and reaction time of 30 minutes [6]. When the ratio of microalgae/methanol was up to 200 mg microalgae/ml methanol, the reaction yield decreased down to 63.5% d.b. w/w [6]. 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 ℃, and the biodiesel yields were measured at the times of 30, 60 and 90 minutes. The hydrochar samples which have the equivalent mass of fatty acids were also subjected to the direct transesterification at the same conditions.

The experimental procedure can be referred above. 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 hydrochar (Y_{HC}) at different times were calculated by the following formula 4, where $C_{(t)}$ is the content of fatty acids in samples measured at the time of (t).

$$Y_{AL} = \frac{C_{(t)}}{C_{AL}} \times 100, Y_{HC} = \frac{C_{(t)}}{C_{HC}} \times 100 \text{ (formula 4)}$$

3. Results and Discussion

3.1. The retention of fatty acids in hydrochar

The content of fatty acids in microalgae Chlorella sp. was 11.6% w/w. The composition of fatty acids in microalgae is shown in Table 1. This commercial type of microalgae contains a large amount of polyunsaturated fatty acids.

The retentions of fatty acids in hydrochar are presented in Table 2. There is a high retention (95.0% w/w) of fatty acids in hydrochar after HTC at 200 ℃. This result is consistent with the previous researches [9,10,11,18]. Lu et al. stated that the retention of fatty acids which have up to 3 double bonds was very high after HTC at 200 ℃ [9].

When the HTC temperature increased from 200 ℃ to 210 ℃ and 220 ℃, 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 [11]. A small amount of these free fatty acids was dissolved in the aqueous phase. 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. Heilmann et al. proved that triacylglycerides and free fatty acids did not take part in the HTC process [19].

Table 1. The composition of fatty acids in microalgae.

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

Table 2. The retention of fatty acids in hydrochar.

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

The hydrochar (HTC 200 ℃) having the highest retention of fatty acids was used in the direct transesterification reaction for comparison. The content of fatty acids in hydrochar was more than double compared to the content in microalgae (25.4% and 11.6% respectively). The masses of

hydrochar (HTC 200 ℃) having the equivalent amount of fatty acids in 100, 200, 300, 400, and 500 mg microalgae are calculated in Table 3.

Table 3. The masses of hydrochar having the equal amount of fatty acids in microalgae.

Microalgae (mg)	Hydrochar (mg)
100	45.5
200	91.0
300	136.5
400	182.1
500	227.6

The ultimate analyses of microalgae and hydrochar (HTC 200 ℃) are listed in Table 4. The carbon content of hydrochar increased up to 63.28% d.b. w/w, and this led the higher high heating value (HHV) of hydrochar compared to microalgae (29.9 and 21.9 MJ kg^{-1}). The ash content of hydrochar also reduced to 1.6% d.b. w/w. 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 [8]. The additional benefit of this byproduct which can be used as fertilizer should be considered. These data are presented in Table 5.

Table 4. Ultimate analyses of microalgae and hydrochar (HTC 200 ℃).

Sample	C (% d.b. w/w)	H (% d.b.w/w)	N (% d.b. w/w)	O (% d.b. w/w)	S (% d.b. w/w)	Ash (% d.b. w/w)	HHV (MJ kg^{-1})
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^{-1}) = 0.338 × C + 1.428 × (H − O/8) + 0.095 × S [20].

Table 5. The distribution of carbon and nitrogen in the hydrochar (HTC 200 ℃) and the aqueous phase.

Element	Hydrochar, % d.b. w/w	Aqueous phase, % d.b. w/w
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 5 below [21].

$$\Delta H_v(T) = 52053000 \times (1 - T_r)^{(0.3199 - 0.212 \times T_r + 0.25795 \times T_r^2)} \text{ (J kmol}^{-1}\text{)} \text{ (formula 5)}$$

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

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

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

The heat capacity C_p(J kmol^{-1} K^{-1}) is calculated by the formula 6 below [21].

$$C_p = 276370 - 2090.1 \times T + 8.125 \times T^2 - 0.014116 \times T^3 + 9.3701 \times 10^{-6} \times T^4 \quad (\text{for. } 6)$$

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 [22]. The heat capacity of marine microalgae Nannochloropsis salina is calculated around 1.5 kJ kg^{-1} K^{-1} in the range of 298.15–328.15 K from the mixture of microalgae and water [23]. 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 a half of water in the range of 298.15–473.15 K [8], 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

$$E_4 = (6.91\ \text{MJ} + 1.67\ \text{MJ}) = 8.58\ (\text{MJ})$$

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

3.2. Comparison between direct transesterification of microalgae and hydrochar

The biodiesel yields at various ratios of 100, 200, 300 and 400 mg microalgae/ml methanol are respectively presented in the Figures 3, 4, 6, and 7.

In Figure 3, the biodiesel yields of microalgae at the times of 30, 60, and 90 minutes were 85.7% d.b. w/w, 95.1% d.b. w/w, and 95.9% d.b. w/w respectively. At the similar conditions, Sathish et al. obtained the biodiesel yield of 90.0% d.b. w/w at the time of 30 minutes [6]. In this work, the biodiesel yields at the times of 60 and 90 minutes were higher than 90% d.b. w/w. Therefore, the results in this research are consistent with the previous one. For hydrochar, the biodiesel yields at the times of 30, 60, and 90 minutes were 93.6% d.b. w/w, 94.5% d.b. w/w, and 95.9% d.b. w/w respectively. There was no significant difference in the biodiesel yields of microalgae and hydrochar at this ratio, and all yields were very high. Therefore, higher ratios of microalgae (or hydrochar)/ml methanol were investigated.

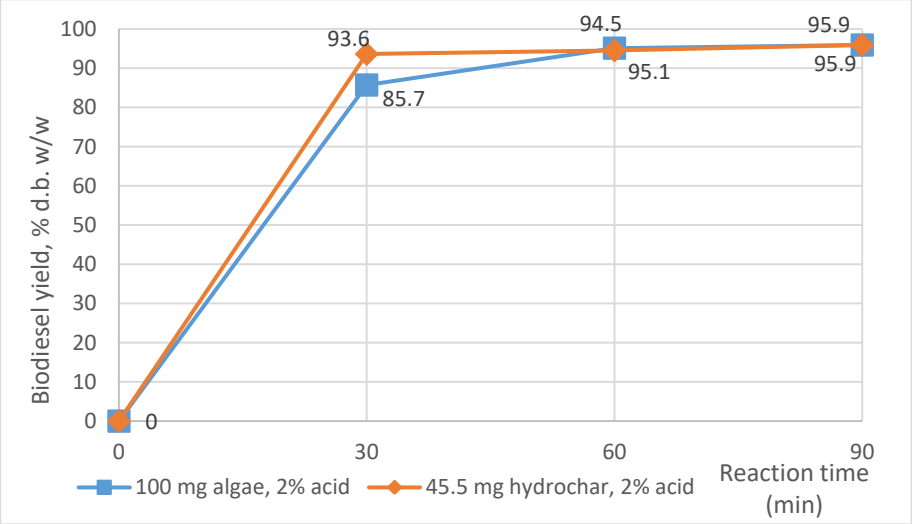

Figure 3. The biodiesel yields of direct transesterification at the conditions of 100 mg microalgae (or 45.5 mg hydrochar)/ml methanol, 2% v/v acid H_2SO_4, 80 ℃.

In Figure 4, the biodiesel yields of microalgae at the times of 30, 60, and 90 minutes were 55.7% d.b. w/w, 84.3% d.b. w/w, and 88.8% d.b. w/w. The biodiesel yield of 63.5% d.b. w/w was also obtained by Sathish et al. at the similar conditions at the time of 30 minutes [6]. Therefore, the biodiesel yields of microalgae are suitable 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 hydrochar did not change significantly compared to the yields in Figure 3. These results can be explained as follows: the higher yield of hydrochar at the time of 30 minutes (93.9% d.b. w/w) proves that the reaction rate of hydrochar was faster than microalgae at the same conditions. In the direct transesterification, the methanol acts as the solvent and the reactant [5]. 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 [24]. Shuit et al. reduced the size of Jatropha curcas L. seeds to increase the extraction rate of lipid by methanol, and this led an increase of the direct

transesterification rate [24]. During the HTC of microalgae, the components of carbohydrates and proteins were carbonized [19], and a large amount of carbon (45.1% d.b. w/w) and nitrogen (65.9% d.b. w/w) was extracted into the aqueous phase. Moreover, the microalgae cells were weaken and contractive (see Figure 5). In addition, a portion of bound fatty acids was hydrolyzed to create the free fatty acids [11,18]. In this work, the ratios of FFAs/TFAs of microalgae and hydrochar (HTC 200 ℃) were 24.9% d.b. w/w and 48.0% d.b. w/w respectively. These phenomena led the higher extraction rate of fatty acids as well as the higher biodiesel yields of hydrochar compared to microalgae. Because the biodiesel yields of hydrochar were still high, so the higher ratios of microalgae (or hydrochar)/ml methanol were conducted to determine the maximum amount of fatty acids which can be converted into biodiesel.

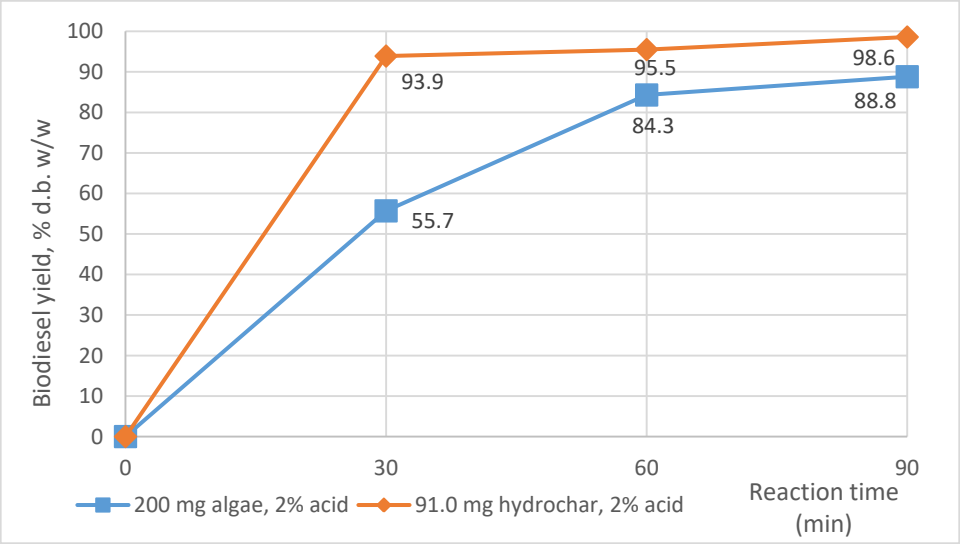

Figure 4. The biodiesel yields of direct transesterification at the conditions of 200 mg microalgae (or 91.0 mg hydrochar)/ml methanol, 2% v/v acid H_2SO_4, 80 ℃.

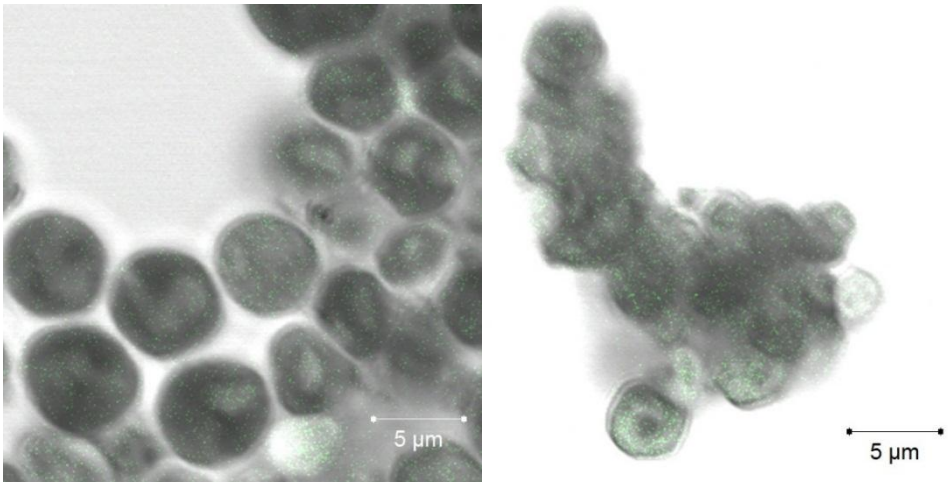

Figure 5. Photos of microalgae and hydrochar (HTC 200 ℃) by confocal laser-scanning microscopy (LSM780, ZEISS).

In the Figure 6, 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% d.b. w/w at the time of 90 minutes. Even more acid H_2SO_4 was used (3% v/v), the biodiesel yield just achieved 81.6% d.b. w/w at the time of 90 minutes. Meanwhile, the biodiesel yields of hydrochar were still high, 96.4% d.b. w/w at the acid concentration of 2% v/v after 90 minutes of reaction.

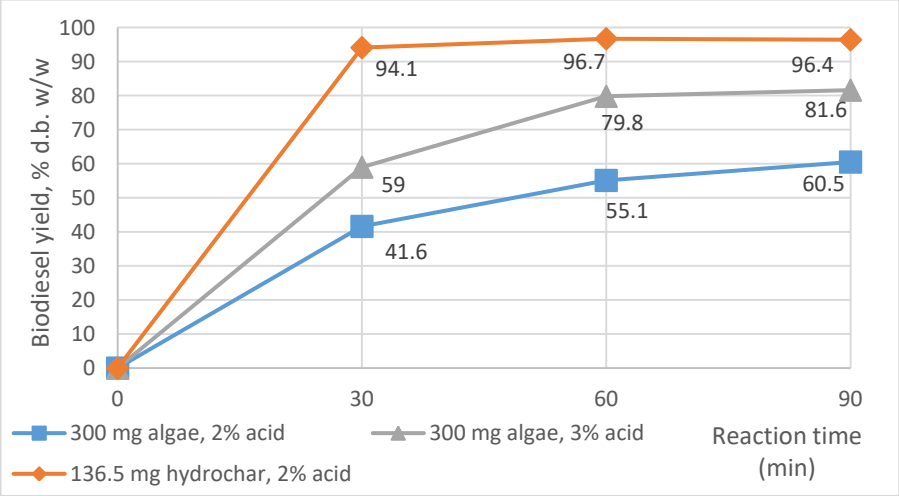

Figure 6. The biodiesel yields of direct transesterification at the conditions of 300 mg microalgae (or 136.5 mg hydrochar)/ml methanol, 2% v/v (or 3% v/v) acid H_2SO_4, 80 °C.

In Figure 7, when the mass of microalgae was up to 400 mg, the microalgae was not completely covered by methanol. The biodiesel yields were very low, only 41.1% d.b. w/w at 2% v/v of acid H_2SO_4 and 65.0% d.b. w/w at 3% v/v of acid H_2SO_4 after 90 minutes of reaction. Meanwhile, 182.1 mg hydrochar was still totally covered by methanol. The biodiesel yield was 91.1 % d.b. w/w at the time of 90 minutes. When the higher amount of hydrochar was used (227.6 mg), the biodiesel yield decreased to 81.5% d.b. w/w.

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 °C, and 90 minutes of 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 the 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. This also leads to the reduced energy requirement for the direct transesterification of hydrochar.

For the feedstock of 10 kg microalgae paste with the moisture content of 85% w/w, the mass of microalgae is 1.5 kg. The methanol volume required in the direct transesterification of microalgae is $V = 1.5 \times 10^3$ g microalgae/0.2 g microalgae = 7.5×10^3 ml methanol = 7.5 liters methanol.

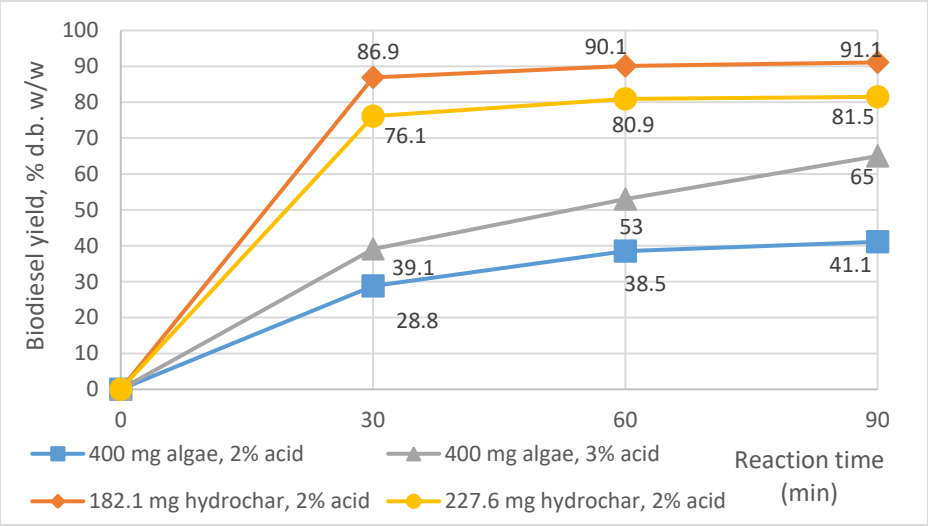

Figure 7. The biodiesel yields of direct transesterification at the conditions of 400 mg microalgae (or 182.1 mg hydrochar)/ml methanol, 2% v/v (or 3% v/v) acid H_2SO_4, 80 ℃.

Through the direct transesterification of hydrochar, the methanol volume required is only 3.75 liters. Assuming that the heat capacities of algae and hydrochar are the same, the decrease in energy requirement in the direct transesterification of hydrochar is mainly due to the less used amount of methanol (3.75 liters). It is calculated as follows.

The heat capacity of methanol C_p(J $kmol^{-1}$ K^{-1}) is calculated by the formula 7 below [21].

$$C_p = 105800 - 362.23 \times T + 0.9379 \times T^2 \text{ (formula 7)}$$

The energy requirement Q_3 for heating 1 kg methanol from 25 ^oC (298.15 K) to 80 ^oC (353.15 K)

$$Q_3 = \int_{298.15}^{353.15} C_p dT = 4.81 \times 10^6 \text{ (J kmol}^{-1}\text{)} = 0.150 \text{ (MJ kg}^{-1}\text{)}$$

The energy requirement for heating 3.75 liters (or 2.96 kg) methanol from 25 ℃ (298.15 K) to 80 ℃ (353.15 K)

$$E_5 = 0.150 \text{ MJ kg}^{-1} \times 2.96 \text{ kg} = 0.44 \text{ (MJ)}$$

In summary, the decrease in energy requirement in the direct transesterification of hydrochar is 0.44 MJ.

4. Conclusion

The combination of the HTC pretreatment of microalgae and the direct transesterification of hydrochar is the effective method to convert lipids in microalgae into biodiesel. In an industrial scale, the water removal from microalgae paste by the HTC process is more energy-effective than the drying process. During the 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 hydrochar by methanol, and this leads to the higher biodiesel yields of hydrochar. The amounts of methanol and catalyst which are required for a given amount of microalgae can be reduced to a half through direct transesterification of hydrochar. The additional benefit of the byproduct, the aqueous phase, which can be reused to cultivate microalgae [18,25] must be taken into account.

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Conflict of Interest

All authors declare no conflicts of interest in this paper.

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