Catalytic conversion of *Chlorella pyrenoidosa* to biofuels

in supercritical alcohols over zeolites

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Abstract: Microalgae have been considered as the feedstock for the third generation biofuels production, given its high lipid content and fast productivity. Herein, a catalytic approach for microalgae liquefaction to biocrude is examined in a temperature range of 250-300 °C in methanol and ethanol over zeolites. Higher biocrude yield was achieved in ethanol and at lower temperatures, while better quality biocrude with higher light biocrude ratio and lower average molecular weight (M_w) was favored in methanol and at higher temperatures. Application of zeolites improves the biocrude quality significantly. Among the catalysts, HY shows the strongest acidity and performs the best to produce high quality biocrude. Solid residues have been extensively explored with thermal gravity analysis and elemental analysis. It is reported for the first time that up to 99 wt.% of sulfur is deposited in the solid residue at 250 °C for both solvents.

Keywords: Microalgae, Biocrude, Methanol, Ethanol, Zeolites

1. Introduction

The third generation biofuels derived from algal biomass (Duan & Savage, 2011; Garcia Alba *et al.*, 2011; Yang *et al.*, 2014) have emerged as a promising alternative for traditional fossil fuel. Unlike terrestrial plant which requires arable land, fresh water and fertilizer, algae can be cultivated on nonarable land and grow in brackish or salt water. Moreover, microalgae has higher growth rate than terrestrial biomass (Yeh *et al.*, 2013).

Algae contain proteins, polysaccharides and lipids, among which lipids mainly contributes to the liquid fuel. Hydrothermal liquefaction is a favorable process for algae conversion to liquid fuel, because it saves energy for drying the algae (water content > 90 wt.%) compared to other processes, such as pyrolysis or lipid extraction. Biocrude obtained by hydrothermal liquefaction mainly contains fatty acids which are derived from lipids and need further decarboxylation to alkanes or esterification to esters for application as transportation fuels. Here, an one-step in situ algae transesterification to biocrude in alcohols is reported. Compared to water as solvent, alcohols offer several advantages: (1) milder reaction conditions because of their relatively lower critical temperature, (2) stabilizing intermediates including fatty acids in the reaction process, (3) dissolving high-molecular-weight liquid products to prevent char formation.

So far, there has been limited works on microalgae conversion in alcohols over heterogeneous catalysts. *Spirulina* conversion was investigated in sub- and supercritical ethanol with the catalysis of homogeneous catalysts, such as FeSO₄, FeS,

Na₂CO₃ and NaOH (Huang et al., 2011). The liquefaction of *Nannochloropsis* was explored in sub- and supercritical ethanol with heterogeneous catalysts, Raney-Ni and HZSM-5 (Zhang et al., 2013). In this work, investigation on the roles of zeolite catalysts and solvents (methanol and ethanol) on the conversion of *Chlorella pyrenoidosa* is reported. Analysis of solid residue is also examined for the first time.

2. Materials and Methods

2.1. Materials

Chlorella pyrenoidosa powder with non-cracked cell wall was purchased from Shandong Binzhou Tianjian Biotechnology Co., Ltd. It contains 4 wt.% moisture and 11.3 wt.% ash. Analytical grade chemicals and solvents, including ethanol, methanol, dichloromethane, n-hexane, and HPLC grade tetrahydrofuran (THF) as well as the catalysts (HZSM-5 (Si/2Al=25, 38 and 50), HY (Si/2Al=10), NaY (Si/2Al=10), H-USY (Si/2Al=12) zeolites), were purchased from Tianjin Guangfu Technology Development Co. Ltd. and used as received.

2.2. Procedure

Fig. 1 illustrates the procedure of post-reaction processing. The reaction was carried out in an autoclave (Parr Instrument Company, 300 mL) in a temperature range of 250 to 300 °C for 1 h. For each experiment, algae (5 g) and solvent (100 mL) were put in the reactor. In the case of catalytic reactions, catalyst (0.5 g) was added into the reactor. The reactor was purged with nitrogen and sealed with an initial pressure (gauge) 0 MPa. After the reaction, the slurry was filtrated and the solid

residue was washed with dichloromethane until the filtrate is clear. The solid residue then was dried and weighed. The liquid was evaporated using a rotary evaporator at 45 °C to remove dichloromethane and ethanol. The remaining material that did not evaporate was biocrude. Firstly, the biocrude was weighed to decide the biocrude yield ($\text{biocrude yield} = (\text{mass of biocrude} / \text{mass of microalgae powder}) \times 100\%$). After that, n-hexane (15 mL) was added to the solvent-free biocrude to dissolve the “light” fraction. The insoluble portion that remained after removal of the hexane solution was the “heavy” biocrude. The light and heavy biocrude yields were determined gravimetrically. The light biocrude portion was calculated as: $(\text{mass of light biocrude} / \text{mass of biocrude}) \times 100\%$. Lastly, 1 mL of hexane solution of light biocrude was transferred to a vacuum evaporator to remove hexane, then 1 mL of THF was added to dissolve the hexane-free light biocrude, followed by GPC characterization. All experiments were repeated at least twice and the average value were taken.

2.3. Characterization

NH₃ temperature-programmed desorption (NH₃-TPD) was performed on a Micromeritics ASAP2920 instrument (USA). Typically, 50 mg of the sample loaded in a quartz reactor was pretreated first with high purity He at 500 °C for 1 h. After the sample was cooled to 120 °C, NH₃ adsorption was performed by switching He flow to a 10% NH₃/He mixture and then keeping at 120 °C for 30 min. Subsequently, the reactor was purged with high purity He to remove the gas phase or the weakly adsorbed NH₃ at the same temperature. The desorption of ammonia was measured

from 120 to 800 °C at a rate of 10 °C min⁻¹ with a TCD detector.

Thermal Gravimetric Analysis (TGA) was performed on a NETZSCH STA 449 F3 Jupiter (Germany) from 40 to 800 °C in 50 mL cm⁻¹ air at 10 °C/min. Both thermogravimetric (TG) and differential thermogravimetric (DTG) data were collected. Elemental analysis was performed on Elemental Analyzer Vario Micro Cube instrument (Germany).

The weight-average molecular weight (M_w) was measured with an Agilent 1200 HPLC (USA) equipped with a Waters Styrylgel HR1 column (USA) and a UV detector at 270 nm. The column was calibrated based on polystyrene standards for the molecular weight calibration curve. Analyses were carried out at 40 °C using THF as eluent with a flow rate of 1 mL min⁻¹. Weight-average molecular weight was determined by following the calculation strategy reported in the literature by whole curve integration (Baumberger et al., 2007).

3. Results and discussion

3.1. Effect of temperature

The biocrude yields were measured with varying the reaction temperature from 250 to 300 °C for 1 h without a catalyst. As shown in Fig. 2, the biocrude yields decrease with the increase of the reaction temperature. One reason for the yield decrease is because higher temperature promotes large molecules to convert to smaller and more volatile ones, causing the weight loss. The biocrude yield drops from 90 to 64 wt.% in methanol and from 83 to 72 wt.% in ethanol, as temperature increases

from 250 to 300 °C. The biocrude yield is more sensible to the reaction temperature in methanol than in ethanol.

M_w was measured to give a rough idea of biocrude quality. Smaller M_w is desired for better quality biocrude, which is easier for further refining to transportation fuels. M_w decreases with the increase of temperature, indicating better biocrude quality is preferred at higher temperature. M_w drops from 2498 to 1977 g/mol in ethanol and decreases from 1571 to 1362 g/mol in methanol, as temperature increases from 250 to 300 °C. These results confirm the conclusion above that higher temperature leads to deeper cracking content, which means lower yield and smaller M_w . In this case, methanol is a better solvent than ethanol, because the M_w is much smaller for the product obtained in methanol than in ethanol. Relatively high quality biocrude is achieved at 300 °C, so the following work was carried out at this temperature.

3.2. Analysis of solid residue

3.2.1. TG and DTG curves of the solid residue

Fig. 3 represents the TG and DTG curves of the reaction solid residue in ethanol without a catalyst. It is defined that T_M as the corresponding temperature of the maximum decomposition rate. In previous works (Sun *et al.*, 2000; Wen *et al.*, 2013), T_M was found have relationship with the product molecular weight. The solid residue obtained at 250 °C shows two maximum decomposition rate peaks at 380 and 505 °C (Fig. 3), indicating that the solid consists of small M_w fraction (380 °C) and high M_w fraction (505 °C). For the residue obtained at 275 °C, the small M_w fraction almost

vanished, leaving a small shoulder peak at about 330 °C. Meanwhile, the T_M of high M_w fraction shifts from 505 to 510 °C. With further increase of the reaction temperature, the peak of the small M_w fraction disappeared completely, leaving a sole peak of high M_w fraction at 530 °C. The disappearance of the small M_w fraction with the increase of the reaction temperature illustrates they converted to high M_w fraction through condensation at higher temperature. Furthermore, the T_M of high M_w fraction shifts to higher temperature with the increase of the reaction temperature, indicating condensation is more severe at higher temperature resulting to an increased M_w .

The general trend of the DTG curves of the solid residue obtained in the reaction with methanol is the same with those obtained with ethanol as the solvent, except that the corresponding T_M of the curves is lower in methanol than that in ethanol. The two rate peaks are at 300 and 465 °C (Fig. 4), respectively, for the solid residue obtained at 250 °C. For the residue obtained at 275 °C, the low temperature peak almost vanished, leaving a sole peak at 460 °C. With the further increase of the reaction temperature, the sole high temperature peak shifts to 475 °C. The corresponding lower T_M in methanol indicates that molecular weight of solid residue is smaller than that in ethanol. Integrated with the results in section 3.1, it can be concluded that methanol favors lower molecular weight of both biocrude and solid residue.

For the TG curves, the weight loss increases with the increase of the reaction temperature. The weight loss increased from 40 to 46 wt.% in ethanol and increased from 22 to 46 wt.% in methanol as the reaction temperature increased from 250 to 300 °C. In general, the weight loss of the individual samples was smaller for the residue

obtained in methanol, indicating methanol tends to suppress the formation of solid residue.

3.2.2. Elemental analysis of the solid residue

Table 1 compares the elemental compositions of the solid residues. The elemental compositions of the original algae are 46.8 wt.% C, 6.9 wt.% H, 8.4 wt.% N, 0.6 wt.% S and 17.2 wt.% O. In Table 1, with the increase of reaction temperature from 250 to 300 °C, C content increases from 56.7 to 72.2 wt.% in methanol and from 57.6 to 68.8 wt.% in ethanol after the reaction, while H, S and O content decreases with the increase of the reaction temperature. Compared to the value in the original algae, a dramatic increase of the S content is noticed, indicating that the S-containing compounds are more likely the char precursors. The residue takes up to 99 wt.% of the S in the algae at 250 °C for both solvents.

3.3. Effect of catalysts

3.3.1. Effect of catalysts on the biocrude yields

Table 2 depicts the effect of catalyst addition on the biocrude yield in methanol and ethanol. In general, higher yield of biocrude was obtained in ethanol than in methanol. In the case of HZSM-5 as the catalyst, the achieved biocrude yields are in the order of HZSM-5 (50) > HZSM-5 (38) > HZSM-5 (25). In the case of Y zeolites as the catalysts, the achieved biocrude yields are in the order of NaY(10) > USY (12) > HY(10). However, compared to the results obtained without a catalyst, there is no significant improvement in the biocrude yield. One possible reason is that the oil yield is already near the upper bound, i.e., all the lipids, proteins and polysaccharides have

come out of the cell completely.

3.3.2. Effect of catalysts on molecular weight

The M_w of the light and heavy biocrudes were measured and listed the data in Table 2. In both methanol and ethanol solvent, the M_w of the light biocrude followed the order: no catalyst > HZSM-5 (50) > HZSM-5 (38) > HZSM-5 (25) > NaY > USY > HY. Once again, strong acidity helps to decrease the M_w of the light biocrude, because cracking reactions are favored by the acidic catalysts. However, for heavy biocrude, the M_w obtained in the presence of catalyst is higher than that without a catalyst. This is probably due to the fact that the acid catalyst not only accelerates cracking processes but also promotes polymerization reactions.

Furthermore, it is obvious that the M_w obtained in the solvent of ethanol is larger than that obtained in the solvent of methanol. This may be attributed to the polarity of methanol being larger than that of ethanol, therefore methanol can better decompose the large molecular compounds and have a better solubility for polar molecules.

3.3.3. Effect of catalysts on light biocrude ratio

Fig. 5 illustrates the effect of catalyst addition on the light biocrude ratio in methanol and ethanol solvents. In general, higher light biocrude ratio was obtained in methanol than in ethanol. This may be attributed to the polarity of methanol being larger than that of ethanol and larger polarity favors cracking reactions. In the case of HZSM-5 as the catalyst, the light biocrude ratio is in the order of HZSM-5 (50) < HZSM-5 (38) < HZSM-5 (25). This is because stronger acidity favors cracking reactions. In the case of Y zeolite as the catalyst, the light biocrude ratio is in the order

of NaY(10) < USY (12) < HY(10). The presence of a catalyst does promote the light biocrude ratio significantly. With HY as the catalyst, the light biocrude ratio is more than doubled compared to that obtained without a catalyst.

Fig. 6 (a) plots the NH₃-TPD curves of the Y zeolites, which shows the amount and the strength distribution of the acid sites. There are two peaks for the HY sample, one showing up at 240 °C, correlating to the weak acid sites and the other showing the peak at 400 °C reflecting the amount of the strong acid sites. USY zeolite presents two peaks at the similar but lower temperatures, but both peaks are smaller, with the peak related to the strong acid sites lowers very much. NaY zeolite only shows one peak at 210 °C, indicating that it contains only weak acid sites. As can be seen, the total peak areas are in the sequence of HY>USY>NaY, indicating the acidity of the catalysts follows the sequence of HY>USY>NaY, which explains the results that more light biocrude ratio is achieved with the catalysts of higher acidity. Fig. 6 (b) gives the acidity comparison of the HZSM-5 zeolites with different Si/Al ratios. Two peaks appear for HZSM-5 (25), indicating the existence of both weak acid and strong acid sites. However, there is only one peak for HZSM-5 (38) and HZSM-5 (50), which relates to the weak acid sites. Again, lower Si/Al ratio shows higher acidity as expected according to the peak areas.

3.3.4 Effect of catalyst on the pressure

Table 3 lists reaction temperature and the post-reaction pressure recorded at room temperature of 25 °C with and without addition of catalysts. There is a fluctuation of reaction pressure during the reaction and here the peak values are listed. Both the

reaction and post-reaction pressure in methanol are always higher than those in ethanol. Methanol owns a lower supercritical temperature than ethanol. Thus, higher reaction pressure is formed in methanol than in ethanol at the same temperature above the supercritical point. Post-reaction pressure is relevant to gas yield. The higher post-reaction pressure in methanol indicates more volatile molecules are formed. This is probably because methanol owns a larger polarity which favors cracking reactions.

4. Conclusions

This work demonstrates the successful liquefaction of microalgae with elevated biocrude quality, i.e., more light biocrude ratio and lower M_w , by introducing zeolites in the system, which motivate the cracking of large molecular compounds to small molecular compounds. Methanol displays a better feature to obtain better quality biocrude compared to ethanol, probably because methanol owns a stronger polarity. Besides, the solid residue formed in methanol shows a lower M_w . The detailed information of solid residues has been extensively explored and up to 99 wt.% of sulfur is deposited in the solid residue at 250 °C for both solvents.

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Figures and tables

Fig. 1. Procedure for reaction and product work-up.

Fig. 2. Effect of reaction temperature on the yields and M_w of the biocrude [without a catalyst](#) at 1 h in N_2 without a catalyst.

Fig. 3. TG and DTG curves of solid residue obtained in ethanol at 1 h in N_2 without a catalyst.

Fig. 4. TG and DTG curves of solid residue obtained in methanol at 1 h in N_2 without a catalyst.

Fig. [5](#). Effect of catalysts on light biocrude portion (300 °C, 1h).

Fig. [6](#). NH_3 -TPD patterns of the catalysts.

Table 1. Elemental compositions (wt.%) of solid residue.

Table 2. [Yield and](#) M_w of biocrude at 300 °C for 1 h.

[Table 3. Effect of catalyst on the pressure](#)

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Fig. 4. TG and DTG curves of solid residue obtained in methanol at 1 h in N₂ without a catalyst.

Fig. 5. Effect of catalysts on light biocrude ratio (300 °C, 1h).

Fig. 6. NH_3 -TPD patterns of the catalysts.

Table 1. Elemental compositions (wt.%) of solid residue.

	T/°C	C	N	H	S	S ^① /g	S ^② /%
Algae		46.8	8.4	6.9	0.6	0.03	--
	250	13.1	1.4	1.7	4.9	0.0297	99.0
Methanol	275	24.9	2.5	2.5	3.9	0.0293	97.7
	300	33.2	2.7	3.0	3.2	0.0282	94.0
	250	22.8	2.8	2.5	3.8	0.0299	99.7
Ethanol	275	28.0	2.9	2.6	3.5	0.0291	97.0
	300	31.7	3.7	2.8	3.2	0.0264	88.0

① Mass of sulfur in the algae or solid residue, calculated as: mass of sulfur = algae
(solid residue)× S%.

② S% = (mass of sulfur in the solid residue/mass of sulfur in the original algae) ×
100%.

Table 2. Yield and M_w of biocrude at 300 °C for 1 h.

Solvent	Catalyst	Biocrude yield (wt.%)	M_w (g/mol)	
			Light biocrude	Heavy biocrude
Ethanol	No catalyst	72	1802	2198
	HZSM-5 (50)	73	1638	5280
	HZSM-5 (38)	72	1572	4978
	HZSM-5 (25)	68	1480	4725
	NaY(10)	68	1173	5973
	USY(12)	66	980	5140
	HY (10)	64	959	4133
Methanol	No catalyst	64	1209	1364
	HZSM-5 (50)	69	1101	2568
	HZSM-5 (38)	65	983	2022
	HZSM-5 (25)	65	982	1792
	NaY(10)	66	962	3271
	USY(12)	65	925	1789
	HY (10)	60	874	1262

Table 3. Effect of catalysts on pressure

	Methanol		Ethanol	
	Reaction pressure /MPa	Post-reaction pressure (25 °C) /MPa	Reaction pressure /MPa	Post-reaction pressure (25 °C) /MPa
300 °C, no catalyst	13.2	0.05	10.7	0.048
275 °C, no catalyst	11.8	0.027	8.7	0.025
250 °C, no catalyst	9.0	0.007	6.7	0.070
300 °C, HY (10)	14.5	0.075	10.9	0.061
300 °C, USY (12)	14.7	0.076	10.8	0.054
300 °C, NaY (10)	14.6	0.075	10.8	0.054
300 °C, HZSM (50)	14.5	0.075	10.7	0.052
300 °C, HZSM (38)	14.5	0.075	10.7	0.052
300 °C, HZSM (25)	14.5	0.075	10.7	0.052

Graphical Abstract