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Hydrothermal Liquefaction of Macroalgae *Enteromorpha prolifera* to Bio-oil

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Marine macroalgae *Enteromorpha prolifera*, one of the main algae genera for green tide, was converted to bio-oil by hydrothermal liquefaction in a batch reactor at temperatures of 220−320 °C. The liquefaction products were separated into a dichloromethane-soluble fraction (bio-oil), water-soluble fraction, solid residue, and gaseous fraction. Effects of the temperature, reaction time, and Na_2CO_3 catalyst on the yields of liquefaction products were investigated. A moderate temperature of 300 °C with 5 wt % Na_2CO_3 and reaction time of 30 min led to the highest bio-oil yield of 23.0 wt %. The raw algae and liquefaction products were analyzed using elemental analysis, Fourier transform infrared (FTIR) spectroscopy, gas chromatography−mass spectrometry (GC−MS), and ^{1}H nuclear magnetic resonance (NMR). The higher heating values (HHVs) of bio-oils obtained at 300 °C were around 28−30 MJ/kg. The bio-oil was a complex mixture of ketones, aldehydes, phenols, alkenes, fatty acids, esters, aromatics, and nitrogen-containing heterocyclic compounds. Acetic acid was the main component of the water-soluble products. The results might be helpful to find a possible strategy for use of byproducts of green tide as feedstock for bio-oil production, which should be beneficial for environmental protection and renewable energy development.

1. Introduction

Eutrophication of marine water bodies can cause marine algal blooms. Green tide, formed by green macroalgae, including *Ulva* sp. and *Enteromorpha* sp., has been one of the major marine environmental problems all over the world.[1−3] An unprecedented scale of *Enteromorpha prolifera* green tide broke out in China's Yellow Sea in Qingdao, Shandong province, during May and July, 2008.[4] A large number of drifting *E. prolifera* flocked to the shore, then became waste, and soon began to decay, resulting in negative effects to the coastal seawater quality and the ecological environment.[5] Up to 1 million tons of algal waste was collected after the cleanup of *E. prolifera*; however, more algal waste was produced in the following waves of green tides (which have spread to the coast of Jiangsu province). Therefore, it is important to study how to achieve better resource use for algal waste to protect the environment.

Algae for fuel production have received worldwide concern in recent years because of the high growth rate and efficient carbon dioxide fixation of algae. Direct thermochemical conversion of microalgae can produce gases and liquid fuels in a quick and efficient way. Because microalgae as-collected usually have a high moisture content (∼90 wt %), hydrothermal liquefaction has been developed to produce biofuel directly because of the excellent solvent properties of water as a reaction medium.[6] Dote et al. reported that *Botryococcus braunii* produced liquid oils at 57−64 wt % dry weight at 300 °C and 10 MPa in hot water, catalyzed by Na_2CO_3.[7] Minowa et al. reported that direct thermochemical liquefaction of *Dunaliella tortiolecta* cells with 21.6 wt % dry weight gave yield of oils up to 37 wt % of the total organic matter.[8] Yang et al. liquefied one kind of water-bloom genera microalgae, *Microcystis viridis*, at 300−340 °C and 20 MPa, and a maximum of 33 wt % oil yield was obtained.[9] Sawayama et al. studied the energy balance and CO_2-mitigating effect of a liquid fuel production process from *B. braunii* using thermochemical liquefaction and suggested that thermochemical liquefaction of wet biomass and waste to recover liquid fuel was feasible.[10]

Many efforts have been dedicated toward producing biofuels from microalgae;[11] however, less efforts have been

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(1) Kamer, K.; Boyle, K. A.; Fong, P. Macroalgal bloom dynamics in a highly eutrophic southern California estuary. *Estuaries* **2001**, *24*, 623–635.

(2) Blomster, J.; Bäck, S.; Fewer, D. P.; Kiirikki, M.; Lehvo, A.; Maggs, C. A.; Stanhope, M. J. Novel morphology in *Enteromorpha* (Ulvophyceae) forming green tides. *Am. J. Bot.* **2002**, *89*, 1756–1763.

(3) Vinares, R.; Carballeira, A. Nutrient limitation in macroalgae (*Ulva* and *Enteromorpha*) from the Rías Baixas (NW Spain). *Mar. Ecol.: Prog. Ser.* **2004**, *25* (3), 225–243.

(4) Liu, D. Y.; Keesing, J. K.; Xing, Q. U.; Shi, P. World's largest macroalgal bloom caused by expansion of seaweed aquaculture in China. *Mar. Pollut. Bull.* **2009**, *58*, 888–895.

(5) Hiraoka, M.; Ohno, M.; Kawaguchi, S.; Yoshida, G. Crossing test among floating *Ulva thalli* forming "green tide" in Japan. *Hydrobiologia* **2004**, *512*, 239–245.

(6) Peterson, A. A.; Vogel, F.; Lachance, R. P.; Fröling, M.; Antal, M. J.; Tester, J. W. Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies. *Energy Environ. Sci.* **2008**, *1*, 32–65.

(7) Dote, Y.; Sawayama, S.; Inoue, S.; Minowa, T.; Yokoyama, S. Y. Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. *Fuel* **1994**, *73*, 1855–1857.

(8) Minowa, T.; Yokoyama, S. Y.; Kishimoto, M.; Okakurat, T. Oil production from algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction. *Fuel* **1995**, *74*, 1735–1738.

(9) Yang, Y. F.; Feng, C. P.; Inamori, Y.; Maekawa, T. Analysis of energy conversion characteristics in liquefaction of algae. *Resour., Conserv. Recycl.* **2004**, *43*, 21–33.

(10) Sawayama, S.; Minowa, T.; Yokoyama, S. Y. Possibility of renewable energy production and CO_2 mitigation by thermochemical liquefaction of microalgae. *Biomass Bioenergy* **1999**, *17*, 33–39.

(11) Brennan, L.; Owende, P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable Sustainable Energy Rev.* **2010**, *14*, 557–577.

Table 1. Characteristics of *E. prolifera*

proximate analysis (wt %)			ultimate analysis (wt %)[a]				
VM[b]	FC[c]	ash	C	H	N	O	H/C
42.35	19.54	30.10	28.75	5.22	3.65	32.28	2.18

inorganic composition of the ash (wt %)[a]												
Na	K	Mg	Ca	Al	Fe	Ni	Ti	Cl	Br	S	P	Si
31.5	9.64	9.05	2.02	4.19	1.32	0.13	0.10	27.1	0.44	6.24	1.27	7.04

[a] All measured on a dry basis. [b] VM = volatile matter. [c] FC = fixed carbon.

reported about macroalgae. Similar to microalgae, macroalgae have a strong photosynthetic capacity and high growth rate. The marine macroalgae *E. prolifera* contains plenty of polysaccharides, proteins, a low content of fats and cellulose, and also some essential mineral elements for human health; thus, they are mainly used as food or for medical purposes.[12] In fact, macroalgae can be used for fuel production by thermochemical conversion techniques. Aresta et al. produced biodiesel from a green macroalgae *Chaetomorpha linum* by a comparison of two techniques: supercritical carbon dioxide (sc-CO_2) and thermochemical liquefaction at 250−395 °C, and the results indicated that thermochemical liquefaction was more efficient, although the yield was still low.[13] Ross et al. classified macroalgae as fuel by investigating the combustion and flash pyrolysis behaviors of five macroalgae from the British Isles and analyzing the pyrolysis products.[14]

The objective of present work is to investigate the possibility of using macroalgae *E. prolifera* for bio-oil production by hydrothermal liquefaction. Effects of the temperature, reaction time, and alkali catalyst on product yields were studied, and the characters of liquid and solid products were analyzed using multiple analysis methods, such as elemental analysis, Fourier transform infrared (FTIR) spectroscopy, gas chromatography−mass spectrometry (GC−MS), and ^{1}H nuclear magnetic resonance (NMR).

2. Experimental Section

2.1. Raw Materials. The raw materials of macroalgae *E. prolifera* were collected from the coast of the East Sea, Zhejiang province, China, in June, 2009. After impurities and sea salts were removed, *E. prolifera* was dried at 60 °C for 12 h and then milled to 50−100 mesh for hydrothermal liquefaction. The ash content of *E. prolifera* was measured at 550 °C, and the volatile matter and fixed carbon were determined by means of thermogravimetric analysis (TGA). The organic composition (C, H, and N) of raw materials was measured by elemental analyzer Vario EL III, and the content of O was calculated by O (wt %) = 100 − (ash + C + H + N) (wt %). The inorganic composition of the ash was measured by X-ray fluorescence (XRF) using a S4 Explorer X-ray spectrometer. The analysis results were shown in Table 1.

2.2. Hydrothermal Liquefaction. The hydrothermal liquefaction of *E. prolifera* was performed using a hydrothermal reaction system (Figure 1), which consisted of a 250 mL GSH-0.25 zirconium cylindrical autoclave, an electrically heated furnace, a magnetic stirrer, a pressure holding circuit, and a controller, similar to the systems reported in the literature.[7,9] In a typical run, 20 g of *E. prolifera* powder, 150 mL of distilled water, and the desired quantities of Na_2CO_3 catalyst (0 or 5 wt %) were charged in the autoclave. After that, the autoclave was sealed firmly. Residue air was removed by purging with N_2 for 5 min. Then, the autoclave was pressurized to 2.0 MPa with N_2 to suppress the drastic boiling of water during the liquefaction process.[15] The reaction was started by heating the autoclave with stirring at 120 revolutions/min. When the temperature reached the set value, it was maintained for a certain time, defined as the reaction time, and then the autoclave was cooled with running water to room temperature. The gaseous product was vented, and the autoclave was opened. Some viscous tar-like matter attached to the cooling pipe and the inner wall of the autoclave, and some floated on the aqueous surface. The reaction mixture was carefully collected, and the cooling pipe and the wall of the autoclave were further washed with CH_2Cl_2. The separation procedure of liquefaction products was illustrated in Figure 2. About 200 mL of CH_2Cl_2 (including the part for washing) was added to the reaction mixture, and two phases were separated. The CH_2Cl_2 phase was filtrated 3 times and then evaporated at 40 °C under reduced pressure to remove the solvent, and the remaining liquid product was dichloromethane-soluble products, defined as bio-oil. The CH_2Cl_2-insoluble solid residues (SRs) were dried at 105 °C for 12 h and then weighed. The aqueous phases were evaporated at 65 °C for 12 h to completely remove water. The experimental errors for liquefaction products were lower than 5% by three duplicate runs at the same conditions.

The operating temperature and reaction time are two important parameters for the hydrothermal liquefaction process. Their effects on the liquefaction process were investigated in detail. Alkali carbonates were proven to be effective catalysts in biomass liquefaction, as reported in the literature;[16−20] thus, 5 wt % Na_2CO_3 was used to examine the effect on the liquefaction of *E. prolifera*. The liquefaction products included bio-oil, water-soluble products (WSPs), SR, and gaseous products. Yields of all products were calculated on the basis of the mass of feed (algae + catalyst) by the following equations:

$$\text{bio-oil (wt \%)} = \frac{W_{\text{oil}}}{W_{\text{feed}}} \times 100\% \quad (1)$$

$$\text{WSP (wt \%)} = \frac{W_{\text{water-soluble}}}{W_{\text{feed}}} \times 100\% \quad (2)$$

$$\text{solid residue (wt \%)} = \frac{W_{\text{residue}}}{W_{\text{feed}}} \times 100\% \quad (3)$$

$$\text{gas (wt \%)} = 1 - (\text{bio-oil} + \text{solid residue} + \text{WSP}) \text{ (wt \%)} \quad (4)$$

(12) Cai, C. E.; Yao, B.; Shen, W. R.; He, P. M. Determination and analysis of nutrition compositions in *Enteromorpha clathrata*. *J. Shanghai Ocean Univ*. **2009**, *18*, 155–159 (in Chinese).

(13) Aresta, M.; Dibenedetto, A.; Carone, M.; Colonna, T.; Fragale, C. Production of biodiesel from macroalgae by supercritical CO_2 extraction and thermochemical liquefaction. *Environ. Chem. Lett*. **2005**, *3*, 136–139.

(14) Ross, A. B.; Jones, J. M.; Kubacki, M. L.; Bridgeman, T. Classification of macroalgae as fuel and its thermochemical behavior. *Bioresour. Technol*. **2008**, *99*, 6494–6504.

(15) Xu, C. B.; Lad, N. Production of heavy oils with high caloric values by direct liquefaction of woody biomass in sub/near-critical water. *Energy Fuels* **2008**, *22*, 635–642.

(16) Minowa, T.; Fang, Z.; Ogi, T. Cellulose decomposition in hot-compressed water with alkali or nickel catalyst. *J Supercrit. Fluids* **1998**, *13*, 253–259.

(17) Fang, Z.; Minowa, T.; Smith, R. L.; Ogi, T.; Koziński, J. A. Liquefaction and gasification of cellulose with Na_2CO_3 and Ni in subcritical water at 350 °C. *Ind. Eng. Chem. Res*. **2004**, *43*, 2454–2463.

(18) Song, C. C.; Hu, H. Q.; Zhu, S. W.; Wang, G.; Chen, G. H. Nonisothermal catalytic liquefaction of corn stalk in subcritical and supercritical water. *Energy Fuels* **2004**, *18*, 90–96.

(19) Karagöz, S.; Bhaskar, T.; Muto, A.; Sakata, Y.; Oshiki, T.; Kishimoto, T. Low-temperature catalytic hydrothermal treatment of wood biomass: Analysis of liquid products. *Chem. Eng. J*. **2005**, *108*, 127–137.

(20) Kumar, S.; Gupta, R. B. Biocrude production from switchgrass using subcritical water. *Energy Fuels* **2009**, *23*, 5151–5159.

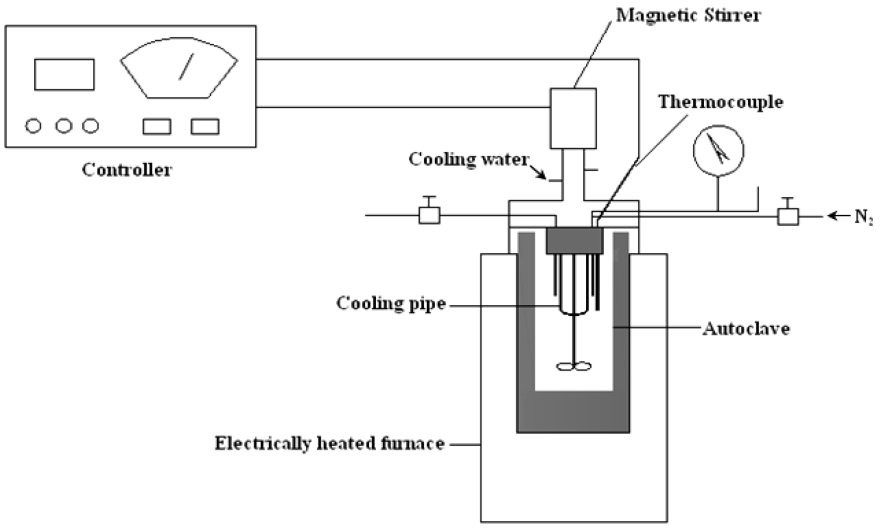

Figure 1. Schematic diagram of the hydrothermal reaction system.

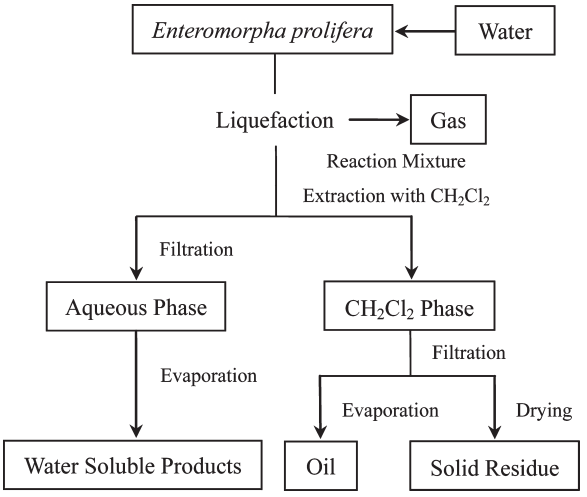

Figure 2. Product separation procedure.

where W_{oil} is the mass of bio-oil (g), W_{feed} refers to the total mass of feed, including algae and catalyst (if avilable), $W_{water\text{-}soluble}$ is the mass of WSPs, and $W_{residue}$ is the mass of SR. It should be noted that the yield of gaseous products was obtained by difference; therefore, the contribution of water vapor and other losses were also included.

2.3. Product Analysis. *2.3.1. Bio-oils.* The elemental compositions of bio-oils were analyzed by CHNO elemental analyzer Vario EL III. The higher heating value (HHV) of bio-oils was calculated by the Dulong formula[15]

$$\text{HHV (MJ/kg)} = 0.3383C + 1.422(H - O/8) \quad (5)$$

where C, H, and O are the weight percentages of carbon, hydrogen, and oxygen in the oil, respectively.

FTIR spectroscopic analysis of raw material *E. prolifera* and bio-oils were performed by Thermo Nicolet Nexus 470 over a range of 400−4000 cm^{-1}. All measurements were carried out by means of KBr plates.

GC−MS analysis of bio-oil was carried out using Agilent 6890N/5973 with a HP-5 ms column (5% phenyl and 95% dimethylpolysiloxane, 30 m × 0.25 mm × 0.25 mm). The carrier gas was helium, with a flow rate at 15 mL min^{-1}. A total of 1 μL of dichloromethane solution of bio-oil (0.2 g/10 mL CH_2Cl_2) was injected into the column. The injector was set at splitless mode, with an inlet temperature of 280 °C. The GC oven temperature program was as follows: hold at 40 °C for 3 min, raise to 300 °C with a heating rate of 5 °C min^{-1}, and hold for 8 min. Compounds were identified by means of the National Institute of Standards and Technology (NIST) library of mass spectra.

^{1}H NMR was performed using a Bruker DPX-500 spectrometer to determine the percentage of functional groups in bio-oil. A total of 0.08 g of bio-oil was dissolved in 0.6 mL of $CDCl_3$, which contained 0.03% (v/v) tetramethylsilane (TMS) as an internal reference. The ^{1}H spectra of bio-oil were acquired at 500 MHz, with a 90° pulse angle and a sweep width of 8000 Hz.

2.3.2. WSPs. The WSPs obtained at 300 °C with and without catalyst were analyzed. The WSPs (sampling 3.0 g) were washed with 25 mL of methanol (HPLC grade) to remove the water-soluble salts. Compounds in water-soluble organics were analyzed using GC−MS by Thermo Finnigan Voyager with a VF-wax ms column (PEG 20000, 30 m × 0.25 mm × 0.25 mm), and N_2 was used as a carrier gas, with a flow rate at 10 mL min^{-1}. The injector was set at splitless mode, with an inlet temperature of 230 °C. A total of 1 μL of methanol solution of WSPs was injected into the column. The GC oven temperature program was as follows: hold at 60 °C for 2 min, raise to 250 °C with a heating rate of 15 °C min^{-1}, and hold for 5 min. Compounds were identified by means of the NIST library of mass spectra.

2.3.3. SRs. FTIR analysis was performed for the SRs to study the effect of liquefaction with and without catalyst, and the method was illustrated in section 2.3.1.

3. Results and Discussion

3.1. Effects of the Temperature and Reaction Time on Liquefaction Yields. Figure 3 shows the effect of the temperature on liquefaction product yields at 220−320 °C, with a reaction time of 30 min. The product yields were calculated on the weight of feed. The yields of WSPs were in the range of 34.3−45.2 wt %, which were much higher than any other product yields in each run. This is reasonable because there is a large fraction of water-soluble sea salts in the raw materials, which could be confirmed by the composition analysis results of raw materials in Table 1. From Table 1, we could find that the ash content was 30.10 wt % raw materials and most components of ash were water-soluble, such as 31.5 wt % Na, 9.64 wt % K, 9.05 wt % Mg, and 27.1 wt % Cl. The yield of WSPs decreased with the increase of the liquefaction temperature.

The bio-oil yield increased with the temperature at 220−300 °C and then decreased at 320 °C, and the yield

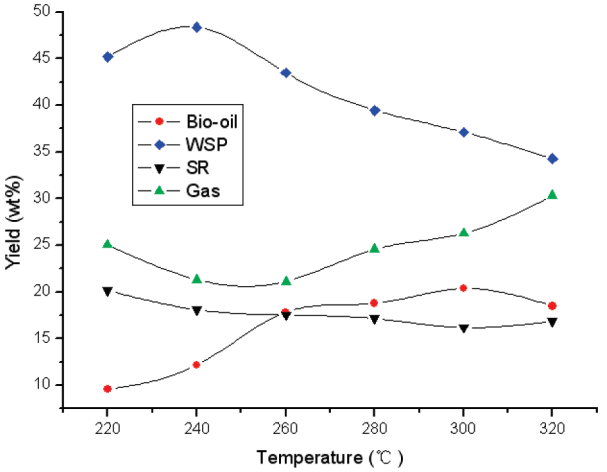

Figure 3. Product yields of hydrothermal liquefaction of *E. prolifera* at 220−320 °C, with a reaction time of 30 min.

ranged from 9.6 to 20.4 wt %. The gas yield decreased with the temperature initially at 220−260 °C but climbed as the temperature increased further to 320 °C, and the yield of gas ranged from 21.1 to 30.3 wt %. The yield of SR decreased slowly with an increasing temperature, and the yield was in the range from 16.9 to 20.2 wt %.

Minowa et al.[16,17] studied the liquefaction of cellulose in hot compressed water, and their results indicated that temperature could significantly influence the liquefaction process. The reaction scheme of cellulose liquefaction could be briefly illustrated as follows: hydrolysis to WSPs, convert to oil and gas by reactions such as dehydration, deoxygenation, and decarboxylation, and then polymerize to char. Yuan et al.[21] studied the process of straw liquefaction in hot compressed water by comparisons of products obtained at different temperatures and proposed that hydrolysis and repolymerization (refers to the formation of oil) existed simultaneously at 220−300 °C, and with the increase of the temperature, the reaction of repolymerization was more drastic compared to hydrolysis. The WSPs were decomposed and repolymerized into gases and oily products. Polysaccharides and proteins are rich in algal cells, and they decompose easily at low temperatures. As shown in Figure 3, the hydrolysis dominated at 220 and 240 °C. During this process, polysaccharides, proteins, and cellulose broke up to small molecular fragments, and further reactions of these substances, such as dehydration, deoxygenation, and decarboxylation occurred but were not drastic, which resulted in a low yield of bio-oil. With the increase of the temperature, these reactions for bio-oil formation were greatly enhanced, especially with temperatures at 260−300 °C, more WSPs were converted to bio-oil, and the gas yield also increased. The yield of SR decreased with the temperature because of the gradual conversion of algal material. With the further increase of the temperature to 320 °C, the gasification was enhanced, some oily products decomposed, and more SR was formed, which led to the decrease of the bio-oil yield. The highest yield of bio-oil was 20.4 wt %, obtained at 300 °C.

The reaction time is also an important parameter for hydrothermal liquefaction. Figure 4 shows yields of liquefaction

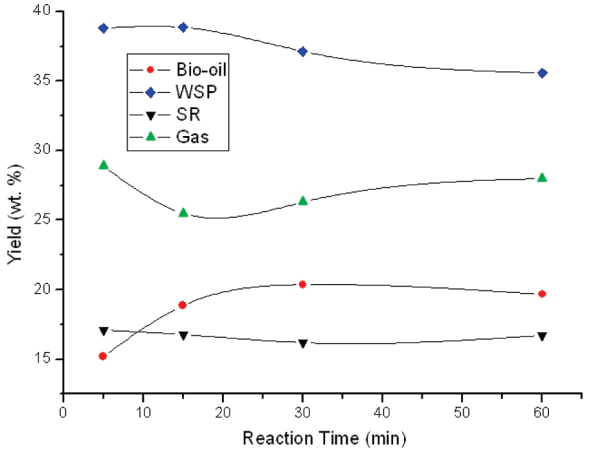

Figure 4. Product yields of hydrothermal liquefaction of *E. prolifera* at 300 °C, with a reaction time of 5−60 min.

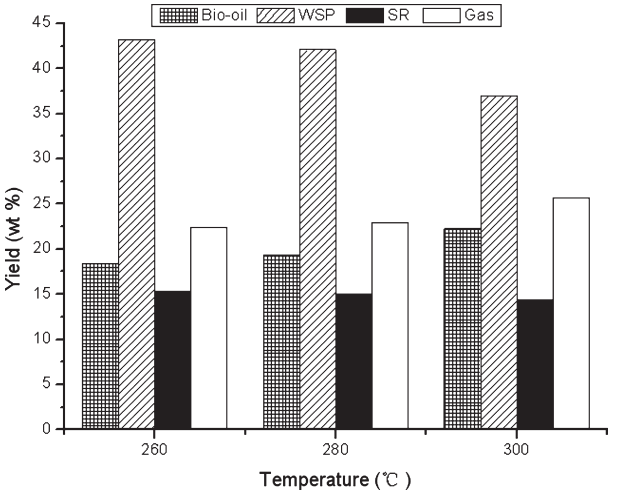

Figure 5. Product yields of hydrothermal liquefaction of *E. prolifera* at 260, 280, and 300 °C for 30 min with 5 wt % Na_2CO_3 added.

products at 300 °C for 5−60 min. The yield of WSP (35.5−39.0 wt %) was much greater than that of any other products, and it decreased with the reaction time. It should, however, be noted that the decrease of the gaseous product yield from 5 to 15 min was possibly caused by the error of the WSP yield at a reaction time of 5 min, because some small molecular organics were lost during the evaporation of the aqueous phase. The effect of the reaction time on the bio-oil yield was significant. The bio-oil yield increased from 15.2 to 20.4 wt % for 5−30 min, but no further increase was observed as the time increased to 60 min. The yield of SR was at around 16.2−17.1 wt %. Too short of a reaction time was negative for the oil formation process, but extending the reaction time too long might promote the formation tendency of gas and char and caused a decrease of the bio-oil yield. Therefore, a reaction time of 30 min is favorable for hydrothermal liquefaction of *E. prolifera*.

3.2. Effects of the Catalyst on Liquefaction Yields. The liquefaction product yields at three low temperatures (260, 280, and 300 °C) for 30 min with 5 wt % Na_2CO_3 added are shown in Figure 5. The yield of WSP decreased with an increasing temperature, while the gas yield increased. The bio-oil yield at 300 °C was relatively higher than the other two runs, but the SR yields at three temperatures changed slightly, at around 14.4−15.3 wt %. Alkali carbonates can

(21) Yuan, X. Z.; Tong, J. Y.; Zeng, G. M.; Li, H.; Xie, W. Comparative studies of products obtained at different temperatures during straw liquefaction by hot compressed water. *Energy Fuels* **2009**, *23*, 3262–3267.

Table 2. Elemental Analysis Result of Bio-oils Obtained at 300 °C with or without Catalyst

bio-oil	C	H	N	O[a]	H/C	HHV (MJ/kg)
none	64.45	7.68	5.42	22.45	1.43	28.74
catalyst	66.33	7.76	5.76	20.15	1.40	29.89

[a] By difference.

effectively promote the liquefaction and bio-oil yield of biomass, such as cellulose and woody plants,[15−19] because alkali carbonates react with water to form their bases and bicarbonates, which can enhance the yield of oil and suppress the formation of char.[15,18] However, in the present work, the bio-oil yield increased slightly from 20.4 to 23.0 wt % when 5 wt % Na_2CO_3 was added, while the yield of SRs decreased slightly from 16.2 to 14.4 wt %. The comparison of bio-oil yields obtained at 300 °C with and without catalyst indicated that sodium carbonate has little catalytic effect for the bio-oil yield. Moreover, similar results were reported in previous studies on the liquefaction of microalgae, such as *B. braunii* and *D. tortiolecta* at 300 °C.[7,8] By comparing the composition of ash in macroalgae *E. prolifera* and microalgae *D. tortiolecta*,[7] we found that both of them contained a considerable amount of sodium, almost the same content (31.5 wt %, in Table 1; 31.6 wt %, in ref 8), and it is suspected that the high content of sodium in algae might have an effect on the catalytic role of sodium carbonate; therefore, further studies are needed to clarify whether this is the cause. However, it is clear that the main components of algae are quite different from that of woody plants,[7] because woody plants are composed of lignocelluloses, while algae are composed of polysaccharides, proteins, and fats.[12]

3.3. Analysis of Bio-oils. The elemental compositions of bio-oils obtained at 300 °C with or without 5 wt % Na_2CO_3 added are shown in Table 2. It can be seen that the compositions of both bio-oils are similar. The content of carbon and hydrogen of bio-oils had greatly increased compared to that of raw materials (Table 1), but the oxygen content was still high. The HHVs of bio-oils are around 28−30 MJ/kg, which are similar to that of oils obtained from microalgae *M. viridis* by liquefaction at 300−340 °C.[9] It seems that sodium carbonate had little catalytic effect on the HHV of bio-oil because the changes of carbon and oxygen content were very little after the addition of sodium carbonate. As indicated in previous studies on liquefaction of microalgae,[8,9] a high nitrogen (protein) content of algal feedstock usually results in a high nitrogen content of oils. Therefore, for further use of bio-oil as a fuel, upgrading, including denitrogenation and deoxygenation, is necessary.

Figure 6 shows the FTIR spectra of raw materials and bio-oils. The spectra of bio-oils obtained at 300 °C without or with 5 wt % Na_2CO_3 (spectra b and c of Figure 6, respectively) are similar. The O−H stretching vibrations at 3400 cm^{-1} in the spectrum (Figure 6a) indicate the presence of polysaccharides and proteins in raw materials. For the infrared spectrum of bio-oil (Figure 6b), the absorption at around 3300 cm^{-1} is possibly from O−H or N−H stretching vibrations.[22] The intense absorbance peaks in 2860−2950 cm^{-1} are ascribed to C−H stretching vibrations of CH_3 and CH_2 groups, and the

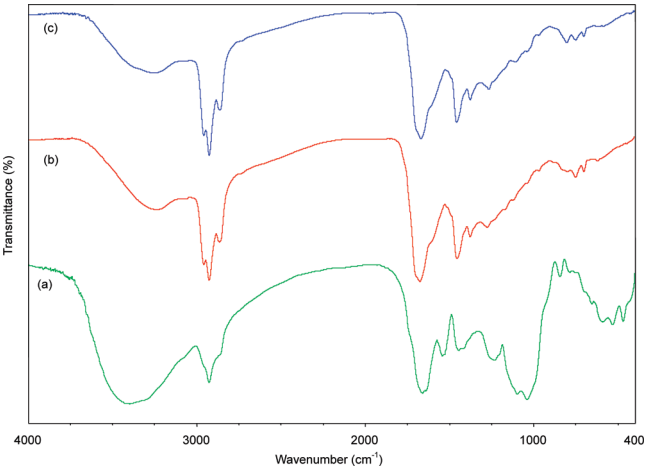

Figure 6. FTIR spectra of (a) raw material and bio-oil obtained at 300 °C (b) without and (c) with 5 wt % Na_2CO_3.

C═O vibrations at 1674 cm^{-1} [22] indicate the presence of ketones, aldehydes, or carboxylic acids.[23] The C−H bending vibrations at 1377 and 1456 cm^{-1}, together with the C−O bending vibration at 1265 cm^{-1}, suggest the presence of fats and esters.[24] In addition, some other absorbance peaks appearing at the band of 650−900 cm^{-1} are ascribed to the C−H bending vibrations from aromatics.

Bio-oil produced from *E. prolifera* is a very complex mixture, and more than 180 compounds were detected in the bio-oil. When mass spectra were compared to the NIST library data, 40 compounds with varying molecular weights were identified, and the result is presented in Table 3. The bio-oil was composed of ketones, aldehydes, phenols, alkenes, fatty acids, esters, aromatics, and a few nitrogen-containing heterocyclic compounds. This result is consistent with the FTIR result of bio-oil. The main compounds in bio-oil are fatty acids: hexadecanoic acid (retention time = 33.29 min) and oleic acid (retention time = 36.35 min), which contribute to 32.63% of the total area. Only one ester, hexadecanoic acid methyl ester (retention time = 32.08 min), was detected. Different types of cyclic ketones and phenols (retention time = 4.55−11.97 min) were identified, and they mostly converted from polysaccharides and cellulose by reactions of hydrolysis, dehydration, cyclization, etc. Alkenes, such as 1-pentadecene (retention time = 22.73 min), 2-hexadecene, 3,7,11,15-tetramethyl-, [*R*-[*R**,*R**-(*E*)]]- (retention time = 30.22 min), and 8-heptadecene (retention time = 26.97 min) were detected, but their total content was only 6.73%. These alkenes possibly generated from the conversion of unsaturated fatty acids in algal cells. The nitrogen-containing heterocyclic compounds, such as indole (retention time = 17.77 min) and quinoline, 4-methyl- (retention time = 19.74 min), were mainly from the decomposition of proteins, and pyrazines (derivatives), such as pyrazine, methyl- (retention time = 4.11 min), pyrazine, 2,5-dimethyl- (retention time = 7.07 min), are typical products from Maillard reactions, which are reactions of amines with sugars.[25,26] In addition, trace-element-containing compounds, such as *p*-iodophenyl phenyl ether (retention time = 50.42 min),

(22) Matsui, T.; Nishihara, A.; Ueda, C.; Ohtsuki, M.; Ikenaga, N.; Suzuki, T. Liquefaction of microalgae with iron catalyst. *Fuel* **1997**, *76*, 1043–1048.

(23) Qian, Y. J.; Zuo, C. J.; Tan, J.; He, J. H. Structural analysis of bio-oils from sub-and supercritical water liquefaction of woody biomass. *Energy* **2007**, *32*, 196–202.

(24) Zou, S. P.; Wu, Y. L.; Yang, M. D.; Li, C.; Tong, J. M. Thermochemical catalytic liquefaction of the marine microalgae *Dunaliella tertiolecta* and characterization of bio-oils. *Energy Fuels* **2009**, *23*, 3753–3758.

Table 3. Identification of Compounds in Bio-oil Obtained at 300 °C without Catalyst by GC−MS Analysis

number	retention time (min)	compound name	MW	area (%)
1	4.11	pyrazine, methyl-	94	1.25
2	4.55	cyclopentanone, 2-methyl-	98	0.2
3	4.71	cyclopentanone, 3-methyl-	98	0.19
4	6.32	2-cyclopenten-1-one, 2-methyl-	96	5.24
5	7.07	pyrazine, 2,5-dimethyl-	107	0.35
6	7.89	benzaldehyde	106	0.32
7	8.04	2-cyclopenten-1-one, 3-methyl-	96	4.12
8	8.70	2(3*H*)-furanone, dihydro-5,5-dimethyl-	114	0.34
9	8.90	2-cyclopenten-1-one, 3,4-dimethyl-	110	0.78
10	9.06	phenol	94	1.18
11	9.77	4,4-dimethyl-2-cyclopenten-1-one	110	0.47
12	10.31	2-cyclopenten-1-one, 2,3-dimethyl-	110	3.88
13	11.06	2-cyclopenten-1-one, 3,4,4-trimethyl-	124	0.35
14	11.42	2-cyclopenten-1-one, 3-ethyl-	110	0.83
15	11.56	pyrazine, 3-ethyl-2,5-dimethyl-	136	0.52
16	11.97	phenol, 4-methoxy-	108	2.17
17	12.15	cyclopentanecarboxaldehyde, 2-methyl-3-methylene-	124	0.62
18	12.62	phenylethyl alcohol	122	0.22
19	16.02	quinoline	129	0.44
20	17.77	indole	117	1.33
21	18.22	1-methylindan-2-one	146	0.14
22	19.15	naphthalene,1,2-dihydro-1,1,6-trimethyl-	172	0.55
23	19.74	quinoline, 4-methyl-	143	0.54
24	20.12	1*H*-indole, 2-methyl-	131	0.14
25	22.73	1-pentadecene	210	3.18
26	24.42	2,3,7-trimethylindole	159	0.45
27	25.64	fluorene, 1,2,3,4,4a,9a-hexahydro-, *cis*-	172	0.24
28	26.97	8-heptadecene	238	2.43
29	30.22	2-hexadecene, 3,7,11,15-tetramethyl-, [*R*-[*R**,*R**-(*E*)]]-	280	1.12
30	30.33	2-butanone, 4-(2,2,6-trimethylcyclohexyl)-	196	0.65
31	32.08	hexadecanoic acid, methyl ester	270	0.59
32	33.29	hexadecanoic acid	256	19.57
33	35.34	2(3*H*)-furanone, 5-dodecyldihydro-	254	0.53
34	36.35	oleic acid	282	13.06
35	36.64	2(1*H*)-naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4aα,7*β*,8a*β*)-	208	0.07
36	36.87	octadecanamide	283	2.75
37	37.69	2,5-piperazinedione, 3-benzyl-6-isopropyl-	246	3.74
38	43.07	pyrrolidine, 1-(1-oxo-5,8,11,14-eicosatetraenyl)-	357	0.42
39	50.42	*p*-iodophenyl phenyl ether	296	0.91
40	54.70	demecolcine	371	0.19
		total		76.07

were also identified. However, bio-oil produced at 300 °C with Na_2CO_3 as a catalyst has almost the same composition.

According to those previous GC−MS analyses of oils obtained by liquefaction of microalgae (*B. bruunii* and *M. viridis*),[9,27] oils obtained from different algae are different. Fatty acids (e.g., C14−C20), long-chain hydrocarbons (e.g., C17−C22), and aromatics had been found in oils, also including some other polar substances, such as phenols, alcohols, and nitrogen-containing compounds. In the recent work of Ross et al. on hydrothermal liquefaction of microalgae *Chlorella vulgaris* and *Spirulina* at 300 and 350 °C using alkali and acids as catalysts, the biocrude obtained from two microalgae contains aromatic hydrocarbons, nitrogen heterocycles, and long-chain fatty acids and alcohols.[28] Although similar compounds, such as fatty acids and hydrocarbons, in the biocrude are also found in the present study, the main compositions of bio-oils are different from those in previous studies and influenced not only by the components of algal feedstocks[28] but also by the liquefaction method.

To have a clearer understanding of the compound distribution of the whole bio-oil, a semi-quantitative analysis was carried out using ^{1}H NMR. The results of functional group distribution in bio-oil obtained at 300 °C without catalyst are summarized in Table 4. It should be noted that the residual water signal is very low; therefore, it is not a significant feature in the spectra.[29] Aromatic protons, aldehyde protons, and also those in heteroaromatics containing oxygen and nitrogen occur at 9.5−6.5 ppm,[29,30] and this portion contains 10.08% of the protons in bio-oil. However, few typical compounds were detected by GC−MS. Phenolic OH and non-cojugated olefinic protons are found from 4.2 to 6.5 ppm,[30,31] with the content of 3.64%. Methoxy, $-CH_2O-$, and nitrogen connected to methylene groups in

(25) Kruse, A.; Krupka, A.; Schwarzkopf, V.; Gamard, C.; Henningsen, T. Influence of proteins on the hydrothermal gasification and liquefaction of biomass. 1. Comparison of different feedstocks. *Ind. Eng. Chem. Res.* **2005**, *44*, 3013–3020.

(26) Kruse, A.; Maniam, P.; Spieler, F. Influence of proteins on the hydrothermal gasification and liquefaction of biomass. 2. model compounds. *Ind. Eng. Chem. Res.* **2007**, *46*, 87–96.

(27) Inoue, S.; Sawayama, S.; Ogi, T. Components of oil derived from liquefaction of hydrocarbon-rich microalgae. *Prepr. Pap.−Am. Chem. Soc., Div. Fuel Chem.* **1996**, *41*, 720–723.

(28) Ross, A. B.; Biller, P.; Kubacki, M. L.; Li, H.; Lea-Langton, A.; Jones, J. M. Hydrothermal processing of microalgae using alkali and organic acids. *Fuel* **2010**, *89*, 2234–2243.

(29) Charles, A. M.; Gary, D. S.; Akwasi, A. B. Characterization of various fast-pyrolysis bio-oils by NMR spectroscopy. *Energy Fuels* **2009**, *23*, 2707–2718.

(30) Ingram, L.; Mohan, D.; Bricka, M.; Steele, P.; Strobel, D.; Crocker, D.; Mitchell, B.; Mohammad, J.; Cantrell, K.; Pittman, C. U. Pyrolysis of wood and bark in an auger reactor: Physical properties and chemical analysis of the produced bio-oils. *Energy Fuels* **2008**, *22*, 614–625.

(31) Özbay, N.; Apaydin, V. E.; Uzun, B. B.; Pütün, A. E. Characterization of bio-oil obtained from fruit pulp pyrolysis. *Energy* **2008**, *33*, 1233–1240.

Table 4. ^{1}H NMR Analysis Results of Bio-oil Obtained at 300 °C without Catalyst

chemical shift (ppm)	type of protons	area (%)
9.5−6.5	ArH, −CHO	10.08
6.5−4.2	ArOH, HC=C− (non-conjugated)	3.64
4.2−3.0	CH_3O−, $-CH_2O-$, $-CH_2-N-$	7.49
3.0−1.8	$CH_3-C{=}O$, CH_3-N, $Ar-CH_3$, $Ar-CH_2-$	33.85
1.8−0.5	$-CH_3$, $-CH_2-$	44.94

the region of 4.2−3.0 ppm[30] account for 7.49% of the total area. Many groups, including $CH_3-C{=}O$, nitrogen connected to methyl, and CH_2 and CH_3 in an aromatic ring, are found between 1.8 and 3.0 ppm (33.85%), and typical compounds with these functional groups were confirmed by GC−MS. The region in 1.8−0.5 ppm represents aliphatic protons, and these protons contribute 44.94% of the protons in bio-oil, indicating a high aliphatic content of bio-oil. These results were in good agreement with the results of GC−MS. However, functional group distribution in bio-oil obtained with catalyst is almost the same as that in bio-oil obtained without catalyst.

3.4. Analysis of Water-Soluble Organics. The aqueous fractions obtained without catalyst showed a weak acidity around 6.5−7.0 by pH testing, while those with 5 wt % Na_2CO_3 showed a weak basicity around 7.5−8.0. The WSP was a strong polar mixture, and those organics were recovered with methanol. Table 5 lists compounds identified in the water-soluble organics produced at 300 °C without catalyst. As can be seen, most compounds identified have low molecular weight in the range of 60−150. Acetic acid was the compound of highest content, contributing 34.72% of the total area, while a much higher content (56.84%) of acetic acid was found in the water-soluble organics when 5 wt % Na_2CO_3 was added. The probable reason is that acetic acid had formed salts with the basic ingredients, which were relatively stable and less volatile during the separation and storage. Some other organic acids, such as propanoic acid (retention time = 8.41 min), pentatoic acid, 3-methyl- (retention time = 10.56 min), levulinic acid (retention time = 14.26 min), and the aromatic acid, benzenepropanoic acid (retention time = 16.16 min), were also detected. Glycerol, another valuable compound (12.78%), is a typical product generated from the hydrolysis of fats (i.e., triglycerides) contained in algal cells. In addition, the dimethyl sulfoxide (retention time = 8.83 min) detected usually came from the degradation of the metabolites of the algae. Various nitrogen-containing compounds were found in the organics, and they mainly came from the decomposition of proteins. In addition, marine macroalgae usually contain a high content of ash (sea salts) in cells, most of which were released into the water during liquefaction, therefore, a substantial amount of sea salts can be separated from the aqueous phase and used for other operations.

3.5. Analysis of SRs. Figure 7 shows the FTIR spectra of the raw material and SRs. Remarkable changes have been found for the FTIR spectra of SRs (spectra b and c of Figure 7) compared to that of the raw material (Figure 7a), which indicated an effective conversion of the algae raw material under hydrothermal conditions. The absorbance intensity at around 3400 cm^{-1} sharply decreased, as well as that between 2860 and 2950 cm^{-1}. The absorbance peak of residues at the band of 1600−1700 cm^{-1} had shifted upward, while some peaks between 1000 and 1600 cm^{-1} disappeared. It can also be observed that the absorbance of peaks between 650 and 900 cm^{-1} in the spectra of residues became much weaker than that of the raw material. The similar FTIR spectra of SRs obtained at 300 °C with and without catalyst indicated that there was no obvious change for the structure of residues obtained with 5 wt % Na_2CO_3.

Table 5. Identification of Compounds in Water-Soluble Organics Obtained at 300 °C without Catalyst by GC−MS Analysis

number	retention time (min)	compound name	MW	area (%)
1	7.60	acetic acid	60	34.72
2	8.41	propanoic acid	74	3.53
3	8.66	propanoic acid, 2-methyl-	88	0.58
4	8.83	dimethyl sulfoxide	78	0.51
5	9.22	acetamide, *N*-ethyl-	87	0.76
6	9.27	acetamide, *N*-methyl-	73	0.86
7	10.27	acetamide	59	3.15
8	10.56	pentanoic acid, 3-methyl-	116	0.57
9	10.62	propanamide	73	1.42
10	12.44	2-pyrrolidinone	85	5.42
11	13.10	2-piperidinone	99	1.81
12	13.80	2(1*H*)-pyridinone, 3,6-dimethyl-	123	1.36
13	13.98	phenol, 2-amino-	109	1.62
14	14.10	glycerol	92	12.78
15	14.26	levulinic acid	116	3.79
16	14.62	phenol, 3-amino-	109	5.36
17	14.77	3-pyridinol	95	8.77
18	15.40	phenol, 4-amino-	109	0.69
19	16.16	benzenepropanoic acid	150	1.51
		total		89.21

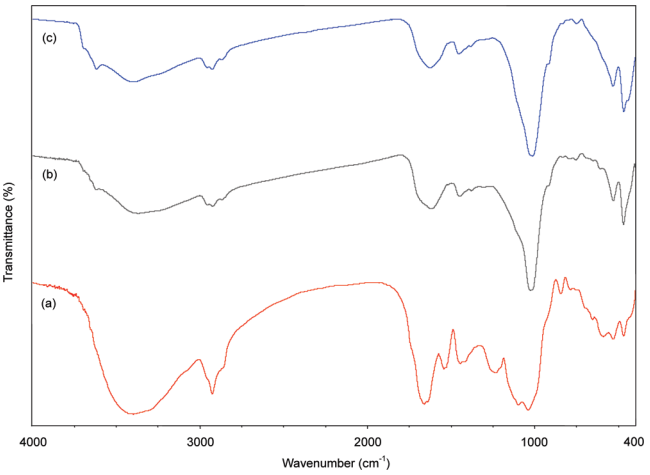

Figure 7. FTIR spectra comparison of (a) raw material and SRs obtained at 300 °C (b) without and (c) with 5 wt % Na_2CO_3.

4. Conclusions

In this study, one of the main algae genera for green tide, macroalgae *E. prolifera*, had been converted to bio-oil by hydrothermal liquefaction. The temperature and reaction time can significantly influence the liquefaction process, especially for the bio-oil formation. The bio-oil yield up to 23.0 wt % (calculated on the feed) was obtained at 300 °C, with a reaction time of 30 min and the addition of 5 wt % Na_2CO_3. Bio-oils produced from *E. prolifera* were composed of ketones, aldehydes, phenols, alkenes, fatty acids, esters, and nitrogen-containing heterocyclic compounds, and the bio-oils had a high aliphatic content. Valuable chemicals, such as acetic acid and glycerol, were recovered from water-soluble

organics, and various nitrogen-containing compounds were also found. This preliminary study has shown that hydrothermal liquefaction of macroalgae *E. prolifera* can potentially produce bio-oil and possible value-added chemicals. However, to improve the yield and quality of bio-oil, more work is needed, especially using different catalysts and other solvents for the liquefaction of *E. prolifera*.

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