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PAPER

Distributions of carbon and nitrogen in the products from hydrothermal liquefaction of low-lipid microalgae

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Microalgae are considered to be a promising alternative feedstock for next generation biofuels because of their rapid photosynthetic growth rates and less impact on land-use for food production compared with grain and other lignocellulosic biomass. In this study, a fast-growing, low-lipid, high-protein microalga species, *Chlorella pyrenoidosa*, was converted *via* hydrothermal liquefaction (HTL) into four products: bio-crude oil, aqueous product, gaseous product, and solid residue. The effects of operating conditions (reaction temperature and retention time) on the distributions of carbon and nitrogen in HTL products were quantified. Carbon recovery (CR), nitrogen recovery (NR) and energy recovery in the bio-crude oil fraction generally increased with the increase of reaction temperature as well as the retention time. The highest energy recovery of bio-crude oil was 65.4%, obtained at 280 °C with 120 min retention time. Both carbon and nitrogen tended to preferentially accumulate in the HTL bio-crude oil products as temperature and retention time increased, but the opposite was true for the solid residual product. The NR values of HTL aqueous product also increased with reaction temperature and retention time. 65–70% of nitrogen and 35–40% of carbon in the original material were converted into water soluble compounds when reaction temperature was higher than 220 °C and retention time was longer than 10 min. The CR of gas was less than 10% and is primarily present in the form of carbon dioxide. This study also introduces a novel treatment process (Environment-Enhancing Energy) that integrates algal growth for wastewater treatment with HTL of algal biomass, which provides synergistic recycling of carbon dioxide from the HTL gaseous product and the nutrients from HTL aqueous product to support multiple stages of algae production.

1 Introduction

Dependence on fossil fuel in the U.S. and other nations has a major impact on energy security and has given rise to serious environmental problems and concerns. Carbon dioxide is the major end-product of fossil fuel combustion, contributes to

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Broader context

Microalgae are viewed as next generation biofuel feedstocks because of their superior photosynthetic efficiencies and higher carbon capturing capabilities compared to terrestrial plants. Conventional algae-to-biodiesel technology mainly focuses on high-lipid species which typically have lower biomass productivities compared to low-lipid algal strains, and the extraction of lipids often requires energy-intensive drying processes. This research demonstrated that hydrothermal liquefaction (HTL) is suitable for converting different wet biomass including low-lipid algae into bio-crude oil. The carbon, nitrogen and energy balance of HTL of a fast-growing, low-lipid microalga (*Chlorella pyrenoidosa*) at different operating conditions were quantitatively investigated. About 70% of nitrogen and 35% of total energy in the original biomass remained in the post-HTL water. These nutrients in the post-HTL wastewater can be reused to grow algae, meanwhile to treat wastewater and capture carbon. This novel treatment process, refers to as Environment-Enhancing Energy (E^2-Energy), integrates algal growth for wastewater treatment with HTL of algal biomass, and provides synergistic recycling of carbon dioxide and the nutrients from HTL products to support multiple stages of algae production and biofuel conversion. Results of this research are useful to better understand the HTL reaction mechanisms and provide useful information for the entire E^2-Energy system life-cycle assessment.

concerns associated with increasing greenhouse gases (GHGs) concentrations in the atmosphere. In contrast, biofuel feedstocks from plants, algae, and some photosynthetic bacteria capture carbon dioxide during their growing cycle, and thus have the potential to be carbon neutral. However, biofuel feedstocks production from food crops and lignocellulosic biomass grown on arable land could compete for land-use with food production and lead to undesirable land clearing.[1] Land-use change from croplands for biofuels can result in higher food prices, and changes from non-arable to arable land typically lead to a net increase the GHGs emissions for many years because the carbon storage and sequestration by the cropland could be sacrificed by diverting land from its existing uses.[2]

Since microalgae usually have faster growth rates, shorter growing cycles, and higher photosynthetic efficiencies than terrestrial lignocellulosic biomass,[3] they are viewed as suitable feedstocks for next generation biofuel production.[4–7] In addition, algae production can occur on marginal lands and waterbodies, resulting in much less impact on current land-use for food production. Microalgae are usually composed of lipids, proteins, nucleic acids and non-cellulosic carbohydrates. Conventional algae-to-biodiesel technology requires drying of the algal biomass followed by solvent and/or mechanical extraction. The energy consumed in the drying process is more than 75% of the total energy consumption.[8] Additionally, this conventional approach leads to a focus on growing relatively pure cultures of high-lipid algae, which usually have lower biomass productivities compared to low-lipid algal strains.[9–11] In other words, focusing on lipid accumulation in microalgae under a stressed condition such as nitrogen depletion, sacrifices biomass productivity, reduces the net energy yield, and makes the process very sensitive to contamination, therefore could cause a net reduction in lipid productivity.[12] In contrast, it would be preferable to have an algal biofuel technology designed to work with wet biomass, low-lipid algae, and potential co-growth of non-target species.

Hydrothermal liquefaction (HTL) is a thermochemical process to convert organic compounds of the feedstock into liquid products, most notably a bio-crude oil product. Feedstocks with high-lipid content or high-protein content can both be converted into bio-crude oil *via* HTL.[13,14] Compared with pyrolysis, HTL requires lower temperatures for conversion; and most importantly does not require energy intensive drying of the feedstock.[15,16] Although fast pyrolysis has the advantages of shorter residence times and lower capital costs because of lower operating pressures, the oil products from pyrolysis usually have higher moisture and oxygen content compared to oils from HTL.[17,18] Therefore, HTL oils typically have more desirable quantities than pyrolysis oils and can be produced with higher energetic efficiency,[18] especially for wet biomass feedstocks such as animal waste, human waste, food-processing waste and algae. In a typical HTL process, feedstock is converted into bio-crude oil, solid residue, gaseous product and aqueous product, in which the aqueous product contains water soluble constituents produced from HTL. Bio-crude oil from HTL needs further upgrading to be used as transportation fuels because of higher oxygen and nitrogen contents than conventional petroleum.[14,19] Previous studies have reported that, during the HTL process, only about 40% of the carbon and 35% of the hydrogen in algal feedstocks were converted into oil, which means most of the organic compounds in the feedstock were converted into water soluble or gaseous products.[20] Most of the nitrogen in the feedstock was converted into water soluble product though HTL process;[21] and homogeneous catalysts could affect nitrogen distribution in HTL products. For instance, the use of organic acids could result in increase of nitrogen in the bio-crude oil.[22] However, most previous studies have focused on high-lipid content algae,[14,23–26] and there is little known about the effect of operating conditions, such as reaction temperature and retention time, on the distribution of nutrients and energy recovery when fast-growing, low-lipid content algae are used as feedstocks in an HTL process.

Carbon and nitrogen remaining in the HTL aqueous product are generally not suitable to be discharged to the environment without additional treatment. Management strategies for the nutrients in the HTL products streams can significantly affect the viability of an HTL process. One approach to integrate bio-crude oil production *via* HTL and wastewater treatment through growing algae is an integrated process we refer to as Environment-Enhancing-Energy (E^2-Energy).[27] Fig.1 illustrates a simplified diagram of the E^2-Energy concept. In this approach, algae growing are used as a wastewater treatment method to uptake nutrients and capture CO_2 from HTL products. Subsequently, the resulting algal biomass will be further converted into bio-crude oil *via* HTL. Since nitrogen and carbon contents of the HTL aqueous stream are high, the algae species that grow in the post-HTL water are expected to be low-lipid content microalgae.[28]

Nutrient distribution in HTL products is one of the key research questions in the E^2-Energy paradigm because the amount of nutrients and energy remaining in the aqueous products and gas stream affects the wastewater treatment by algae growing. The recycling of nutrients to the algae growing ponds allows more algae to be grown and thus increases the amount biofuel created. The objective of the present work was to investigate the effects of reaction temperature and holding time (retention time) on the carbon and nitrogen distributions in the products from HTL of low-lipid microalgae. The elemental distribution under different operating conditions observed in this study can be used to better understand the reaction mechanism of HTL and provide useful information for optimizing the HTL process. This information can also be utilized in the life-cycle assessment to evaluate the feasibility of the entire E^2-Energy system.

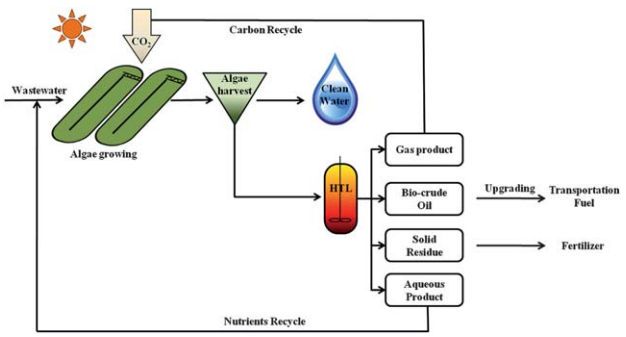

Fig. 1 Concept of Environment-Enhancing-Energy (E^2-Energy) technology.

2 Materials and methods

2.1. Feedstock

The raw material, microalgae *Chlorella pyrenoidosa*, was obtained from a health food store as food grade material. *C. pyrenoidosa* is a green unicellular alga with low-lipid and high-protein content that is found in both fresh and marine waters.[29] In the present study, the elemental compositions of the alga and HTL products were measured using a CHN analyzer (CE-440, Exeter Analytical Inc., North Chelmsford, MA). Other macro-molecular and chemical compositions of alga were analyzed according to the standard methods of the Association of Official Analytical Chemists (AOAC). Table 1 summarizes the characteristics of the *C. pyrenoidosa*. The dry alga was mixed with tap water to make a feedstock with 20% total solid content by weight for each HTL experiment.

2.2. HTL experiments

The HTL experiments were performed according to previously reported methods[30] using a stainless steel cylindrical reactor of 2L capacity with a magnetic drive stirrer (model 4534, Parr Instrument Co., Moline, IL). In a typical experiment, 800g of feedstock with 20% total solid content (80% water) was loaded into the reactor. After the reactor was sealed, pure nitrogen gas was used to purge the reactor three times, and then built up to 0.69 MPa gauge pressure to prevent water from boiling during the experiment. The reactor was then heated by an electric resistance heater up to the designated experimental temperature (defined as the reaction temperature), and the temperature was maintained for a predetermined time. At the end of the reaction, the reactor was rapidly cooled by flowing tap water through the cooling coil located inside the reactor. The retention time was defined as the elapsed time between when the reaction temperature is first achieved to the time when the reactor begins cooling down. Two or three replicates were conducted for each experiment and both the average and standard deviation values are presented in the figures.

2.3. Products separation and yields

When the temperature in the reactor attained room temperature, the gas phase in the reactor was carefully released through a control valve into a Tedlar® gas sampling bag, and the gas was analyzed using Varian CP-3800 Gas Chromatography equipped with an Alltech Hayesep D 100/120 column and a thermal conductivity detector (TCD). The carrier gas was helium with a flow rate at 30 ml min^{-1}. The temperature of both the injector and detector were 120 °C. The recovery procedure of the HTL products is shown in Fig.2. The moisture content of the water insoluble product was determined using a distillation apparatus based on ASTM Standard D95-99.[31] The solid residue fraction of the raw oil product was measured by using Soxhlet extraction, according to ASTM Standards D473-02[32] and D4072-98.[33] In this study, the toluene soluble fraction of the raw oil product was defined as the bio-crude oil. The calculations of product yields were performed according to previously reported methods.[34]

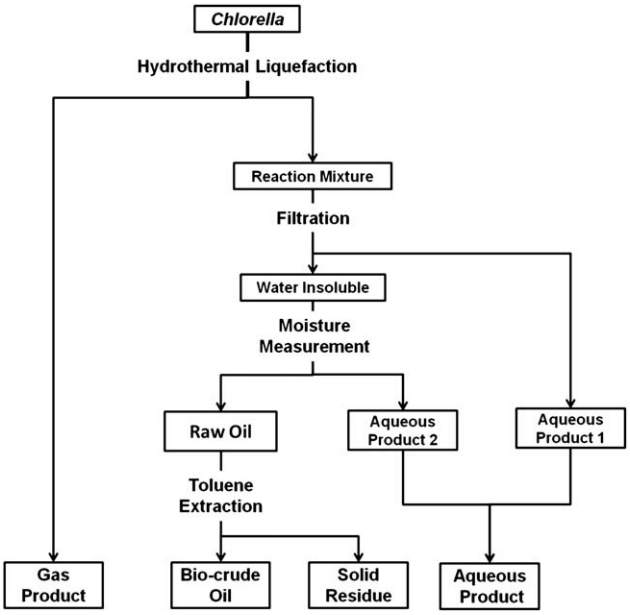

Fig. 2 HTL products recovery procedure.

2.4. Recoveries of elements and energy

Carbon, hydrogen and nitrogen content of both feedstock and HTL products were analyzed using a CHN analyzer; and the oxygen content was calculated by difference. Thus, the oxygen content is slightly larger than the true value since it includes the other minor elements. The elemental compositions of raw oil and solid residue were analyzed first, and then the elemental composition of the bio-crude oil product was calculated by difference between these two fractions. The recovery of carbon or nitrogen is defined as the ratio of carbon or nitrogen in the HTL product to the carbon or nitrogen in the original *C. pyrenoidosa* feedstock. The calculation equations are shown below. The

Table 1 Characteristics of *C. Chlorella* (wt % dry basis)

Chemical composition						
VS[a]	Crude Fat	Crude Protein	Cellulose	Hemi-cellulose	Lignin	Non-fibrous carbohydrate[b]
94.4	0.1	71.3	0.3	0.5	0.2	22.0

Elemental composition			
C	H	N	O[b]
51.4	6.6	11.1	30.9

[a] Volatile solid content. [b] Calculated by difference, assuming that the quantities of elements besides C, H, N, and O are relatively small.

carbon and nitrogen recoveries for the aqueous product were calculated by difference once the other three components were determined.

Carbon Recovery (CR):

$$\text{Carbon Recoveries of Product (\%)} = \frac{\text{C\% of the product} \times \text{weight of product}}{\text{weight of carbon in dry matter of Chlorella}} \times 100$$

Nitrogen Recovery (NR):

$$\text{Nitrogen Recoveries of Product (\%)} = \frac{\text{N\% of the product} \times \text{weight of product}}{\text{weight of nitrogen in dry matter of Chlorella}} \times 100$$

Energy recovery was defined as the higher heating value (HHV) of bio-crude oil divided by the higher heating value of the original algal biomass. HHV values in this study were calculated using the Dulong formula:

$$\text{HHV}(\text{MJ}\cdot\text{kg}^{-1}) = 0.3383\text{C} + 1.422(\text{H} - \frac{\text{O}}{8})$$

where C, H, and O are the mass percentages of carbon, hydrogen, and oxygen respectively.

$$\text{Energy Recovery of Biocrude oil (\%)} = \frac{\text{Heating value of Biocrude oil}}{\text{Heating value of dry Chlorella biomass}} \times 100$$

3 Results and discussion

3.1. Effects of reaction temperature

3.1.1. Reaction temperature effects on carbon recovery. Fig.3 illustrates the effect of reaction temperature on the carbon recoveries in different phases after HTL, including oil/solid phase (raw oil), gas phase and aqueous phase (aqueous product). The tests in Fig.3 were conducted at reaction temperatures ranging from 100 °C to 300 °C with a constant retention time of 30 min and 0.69 MPa initial pressure of nitrogen. Although the physical properties such as the viscosity and pour point of raw oil were not included in this study, the appearance of the raw oil product

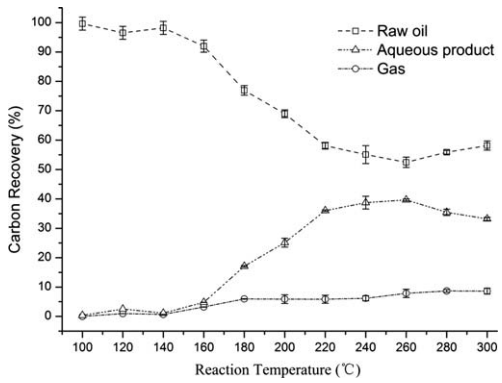

Fig. 3 CR in different phases *vs.* reaction temperatures with 30 min retention time.

Table 2 Appearance of raw oil products from different temperatures

Reaction Temperature	Raw oil product appearance	Stage
<180 °C	Green algal cake	Stage 1
180–200 °C	Black solid, looking like a bio-char product	Stage 2
200–240 °C	High viscosity asphalt/ bitumen-like product	Stage 2
>240 °C	Self-separating, flowable oil phase on the top of the aqueous phase	Stage 3

were changed substantially by increasing reaction temperature, which is summarized in Table 2.

The raw oil is the combination of bio-crude oil and the solid residue (Fig.2), which are considered together in some of the data below, because these components are often mixed together in the reaction products such as the char and bitumen-like products, and can be used as the substitute of asphalt.[35] The trend of CR in the raw oil with increasing temperature can be divided into three stages as shown in Fig.3. First, when temperature was lower than 160 °C, the CR values of raw oil were all higher than 90%, which may be due in part to unreacted solids given the green appearance of the reaction products. Secondly, it decreased from 92.0% to 52.5% when the reaction temperature increased from 160 °C to 260 °C. Finally after the CR in the raw oil reached the minimum value at 260 °C, its values were slightly increased from 52.5% to 58.2%.

In contrast, the CR of the aqueous product initially increased with reaction temperature; but the value reached at peak of 39.7% at 260 °C, after which it started to decrease slightly. This graph shows that more than one-third of the total carbon in the original materials was converted into water soluble organic matters at temperatures above 220 °C. This was likely due to hydrolysis of proteins in the algal biomass and the resulting formation of hydrophilic amino acids, organic acids and their derivatives.

The bio-crude oil was defined as the toluene soluble fraction of the raw oil and it is the most important fraction for determining the potential of HTL to produce petroleum-like products. The bio-crude oil yield, based on the dry matter of feedstock, increased from 0.4% to 35.4%, when the reaction temperature increased from 100 °C to 300 °C. Fig.4 shows the CR of bio-crude oil and the solid residue *vs.* reaction temperature. The changes of CR for the bio-crude oil and solid residue can also be divided into three stages, as was noted above for the raw oil product. For Stage 1, when the reaction temperature was lower than 160 °C, most of carbon in raw oil was in solid residue fraction and the CR of bio-crude oil was less than 5%. Considering that the toluene soluble fraction of the original *C. pyrenoidosa* material was less than 0.1%, it indicates bio-crude oil formation did not occur when the temperature was lower than 160 °C. For Stage 2, as the temperature increased from 180 °C to 240 °C, CR in the bio-crude oil increased from 3.1% to 43.2%. On the other hand, CR in the solid residue rapidly decreased from 73.9% to 11.8%. Thus some of the solid residue was converted into the aqueous fraction at the same time when oil formation was occurring. At Stage 3, when the temperature increased from

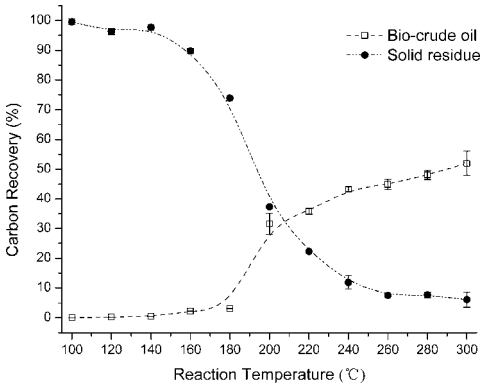

Fig. 4 CR of bio-crude oil & solid residue *vs.* reaction temperature with 30 min retention time.

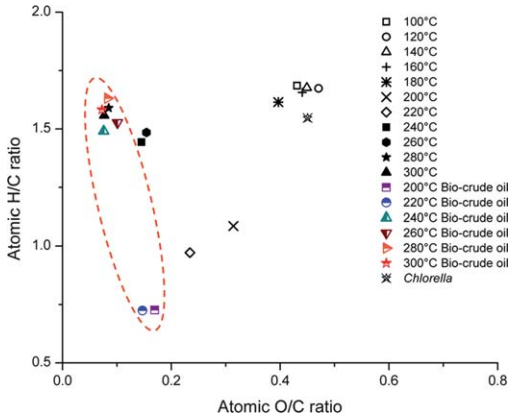

Fig. 5 Van Krevelen diagram of raw oil and bio-crude oil.

240 °C to 300 °C, the change of CR for both bio-crude oil and the solid residue slowed down and tended to plateau. The highest CR for the bio-crude oil was 55.3% at 300 °C. Compared with other previous studies that reported CR values for HTL oil in the range of 20 to 40%, the carbon recovery of the bio-crude oil in the present work was higher.[20,22,36] This is probably due to the differences in algae species, operating conditions and solvent extraction methods. For reaction temperatures higher than 200 °C, the carbon content of bio-crude oil was consistently around 74% based on the total mass, and did not change much with increasing temperature. Therefore, the increase in CR for the bio-crude oil above 200 °C was mainly due to the increase of bio-crude oil yield.

It can be concluded that the minimum reaction temperature for bio-crude oil formation should be in the range of 180 °C to 240 °C. However, further temperature increase was also important for changing the physical property of the product because raw oil could be turned from an asphalt/bitumen-like product into a self-separating, flowable oil product at higher temperature. The mechanism of HTL can be briefly explained as large molecular compounds being decomposed into fragments of small molecules, while at the same time these unstable and reactive fragments can repolymerize into oily compounds.[37] The repolymerization of these reactive fragments is influenced by the temperature, which subsequently affects the physical properties of the oil product.

Fig.5 shows the classification of *C. pyrenoidosa* feedstock, raw oil and bio-crude oil from different reaction temperatures based on a Van Krevelen diagram. Since bio-crude oil yield was lower than 5% at 180 °C, it is expected that formation of bio-crude oil would mainly occur at temperature higher than 180 °C. At the same time Fig.5 also shows the H/C and O/C ratios of bio-crude oil produced in the temperature ranges from 200 °C to 300 °C. When the reaction temperature was lower than 180 °C, the atomic H/C and O/C ratios of raw oil were similar to the algal feedstock. Then both H/C and O/C ratios of the raw oil decreased substantially when the reaction temperatures were 200 °C and 220 °C. Since amino acids and organic acids could be produced through protein hydrolysis, decarboxylation and deamination could be the main pathways of amino acids decomposition under hydrothermal conditions.[38] Under this assumption, amine and carboxyl groups were removed from larger protein molecules at the relatively low temperatures.

The deamination could remove nitrogen and hydrogen from the amino acids and produce hydroxyacid. For example, the glycine could be converted into glycolic acid through deamination.[39] The deoxygenation could occur through decarboxylation[40] as the carbon was removed as CO_2. When the temperature was increased from 220 °C to 300 °C, the O/C ratio still decreased while the H/C ratio started to increase. In this temperature range, the H/C ratios of the raw oil were around 1.5 and very similar to the original *C. pyrenoidosa*. On the other hand, the O/C ratios of the raw oil were much lower compared to the algal feedstock. This finding implies that the oxygen was continuously removed *via* HTL as the reaction temperature increased from 100 °C to 300 °C, while the hydrogen was removed from the feedstock initially at temperature below 220 °C, and then the addition of hydrogen atoms occurred at higher temperatures. The H/C and O/C ratios of bio-crude oil produced from temperatures higher than 220 °C were similar to those found in another study.[22] Increasing temperature tended to decrease O/C ratio and increase H/C ratio of the bio-crude oil (colored data points enclosed by dashed line in Fig.5).

The change of the atomic H/C and O/C ratios of raw oil with temperature in this study was different from biochars produced from pistachio shell biomass *via* slow pyrolysis, in which both the H/C and O/C ratios of char produced at high temperature were much lower than the raw material and the chars produced from low temperature.[41] Additionally the H/C ratios of raw oil product across the reaction temperature range were all higher than the chars from pyrolysis of lignocellulosic biomass.[41,42] Furthermore, when the reaction temperature was higher than 220 °C, O/C ratios of bio-crude oil were all smaller than O/C ratios of bio-oil produced from microalgae *via* fast pyrolysis process.[43] These observations imply that bio-crude oils produced from HTL usually have lower oxygen contents than the biofuels produced from pyrolysis processes, which is advantageous for biofuel applications because of higher energy density.[16,18]

Carbon monoxide, carbon dioxide and methane were three carbon-source gaseous products analyzed in this study, and carbon dioxide was the predominant gas. The CR of gaseous product increased from 0% to 8.7% as the reaction temperature increased. Another study indicated the CR from the gas and oil could be equal at 500 °C, and gaseous product would contain more carbon at even modestly higher temperatures (>500 °C).[24]

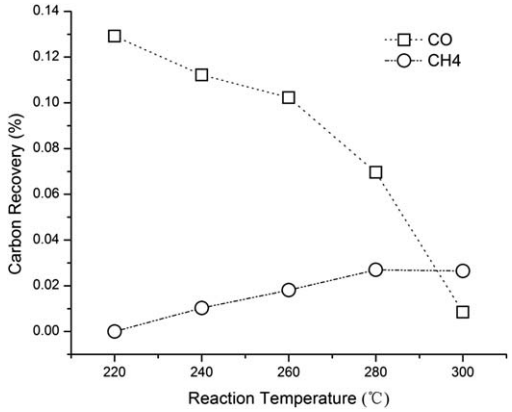

Fig. 6 CR of CO and CH_4.

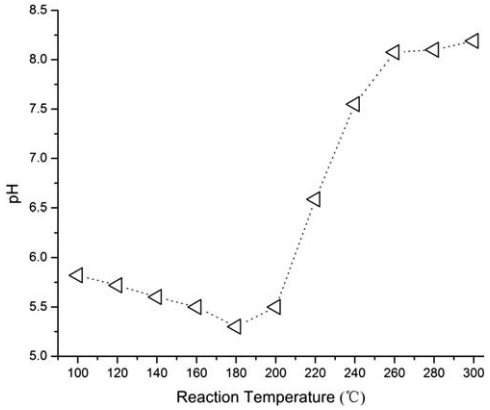

Fig. 7 pH of post-HTL water.

Carbon dioxide was usually the predominant gaseous product at relatively low temperature (<350 °C) in a HTL process.[20,24,36] Although there were some molecules with short carbon chain existing in the gaseous product, the fractions of these gases were small and even negligible when the reaction temperature was lower than 300 °C.[24] In the present study, when the reaction temperature was lower than 220 °C, CO_2 was the only detected carbon-source gas. After the reaction temperature was increased above 220 °C, small amounts of CO and CH_4 were detected. CR contributed by CO_2 in the gaseous products increased from 0% to 8.61% when the temperature increased from 100 °C to 300 °C, which indicated more than 98% of the carbon in the gaseous product was attributed to CO_2. This result indicates that the idea of E^2-Energy to utilize HTL gaseous product with high CO_2 concentration as carbon source for algae growing is a desirable approach (Fig.1). On the other hand, CR of CO reached a peak value at 220 °C and then decreased quickly with the increase of reaction temperature. This finding suggested that CO was produced during the HTL process and then consumed. At the same time, from 240 °C to 300 °C the CR of CH_4 increased with increasing reaction temperature. The relationship of CR of CO and CH_4 with reaction temperature is shown in Fig.6.

Other study also found the concentration of CO in the HTL gaseous product was negligible and speculated that the consumption of CO and formation of CH_4 in the HTL process could be involved in a water-gas shift reaction and a methanation reaction.[24] However the water-gas shift reaction is usually favorable in supercritical water, therefore more experimental evidences are needed to study whether it could occur in the subcritical water.

Fig.7 shows the change of pH value of the post-HTL water with reaction temperature. The pH value of the liquid first decreased when the reaction temperature increased from 100 °C to 180 °C, and then started to increase with reaction temperature. This trend indicated some acids were produced first in the HTL process and then decomposed; some free hydrogen ions were produced and then consumed. This is consistent with the results shown in Fig.5, in which addition of hydrogen to the oil product occurred at high temperatures. Since the protein content of the raw algal material was high, it was reasonable to assume that the peptide bonds in the protein molecules were broken down under relatively low temperature so that amino acids or other short carbon chain organic acids were produced. These organic acids could be further broken down to produce carbon monoxide and hydrogen ions, therefore enabling other recombination reactions as the temperature continued to increase. Some previous studies found that the "Fischer–Tropsch" (FT) reaction could occur in a hydrothermal system, with water or other short carbon chain organic acids acting as hydrogen sources, where CO can apparently reacted with H_2 to produce alkanes under milder temperatures (150–250 °C) in a stainless steel reactor with 2–3 days retention time.[44,45] Since the reaction intermediates produced from the HTL of microalgae are complex, more research efforts are required to study the reactions between the intermediates produced from protein, lipid and carbohydrate in the HTL system.

3.1.2. Reaction temperature effects on nitrogen recovery. Deamination is usually accompanied by liberation of ammonia from the amino acids molecules.[38] Although ammonium could be one of the sources of nitrogen in the HTL aqueous product when alkali catalysts were used[22] and at higher temperature (350 °C) than this study,[46] there was substantial nitrogen recovered by organic compounds containing nitrogen with the presence of organic acids and amino acids under the hydrothermal condition at temperature of 300 °C.[47] This could explain why, in many algae HTL studies including the present study, there was no ammonia gas detected in the gaseous products.[20,24,48] The authors therefore assume that all of the nitrogen in the algal feedstock is recovered in the aqueous and raw oil products. The relationship of the NR in the raw oil and the aqueous product to reaction temperature is shown in Fig.8. NR of the raw oil product kept decreasing with the increase of reaction temperature, while the nitrogen recovery in the aqueous product increased from 1.1% to 73.5%. This result implies that the majority of the nitrogen in the original material would be converted into water soluble product if the reaction temperature increased. It also was evidence for the deamination of the amino acids, and for the susceptibility of amino acid degradation under hydrothermal conditions corresponding to the decomposition temperature.[38] The rate of change of the nitrogen recovery for both raw oil and the aqueous product decreased at the high reaction temperature; the NR of those two products reached respective plateaus when the reaction temperature increased above 240 °C.

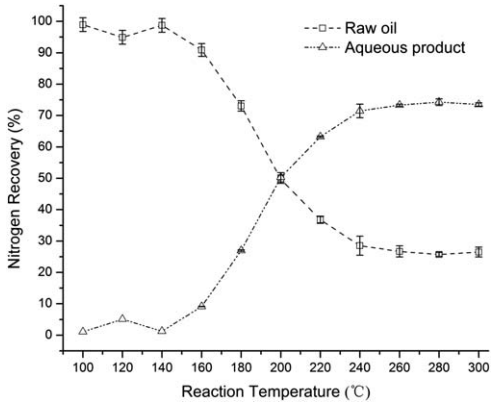

Fig. 8 NR of raw oil and aqueous product *vs.* reaction temperature with 30 min retention time.

To further understand the distribution of nitrogen content in the raw oil, Fig.9 presents the relationship of NR of the bio-crude oil and the solid residue to reaction temperature. The NR of bio-crude oil increased from 0.4% to 23.4% when the reaction temperature increased from 100 °C to 300 °C, and the NR of solid residue decreased substantially from 98.6% to 3.1%. Therefore, even though the total nitrogen recovery of the raw oil decreased as the temperature increase, more nitrogen was recovered by the bio-crude oil instead of the solid residue. In general, oils from algae *via* thermochemical conversion contain a higher proportion of nitrogen compared to oils from lignocellulosic biomass because of the high protein content in algae.

The undesirable high nitrogen content indicates upgrading of the HTL bio-crude oil should include nitrogen removal. Zou *et al.*, reported the nitrogen content in the oil product could be significantly decreased to less than 1% if the liquefaction was performed in ethylene glycol medium with sulphuric acid as a catalyst.[49] While the nitrogen result is promising, in this case, economic constraints would suggest the need to re-use the solvent media and catalyst, which is not easily accomplished. Thus, additional work on the removal of nitrogen from HTL oil products is an important topic for the future work.

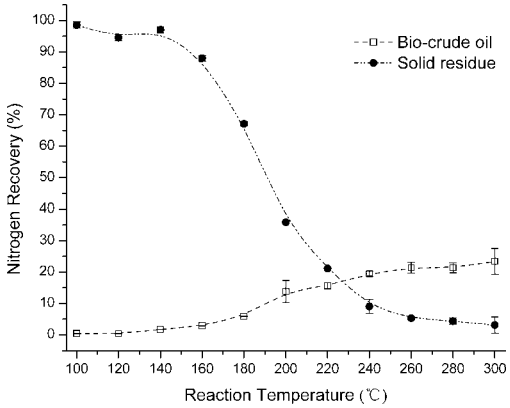

Fig. 9 NR of bio-crude oil & solid residue *vs.* reaction temperature with 30 min retention time.

3.2. Effects of retention time

For investigating the retention time effects on CR and NR values of HTL products, HTL tests were conducted under 200 °C, 220 °C, 240 °C, 260 °C and 280 °C with 0, 10, 30, 60 and 120 min of retention time using the same 0.69 MPa initial pressure of nitrogen. Zero minute retention time means as soon as the temperature reached the designated reaction temperature, the cooling water was introduced into the cooling coil inside the reactor to cool down the reactor to room temperature. The bio-crude oil yield increased from 3.2% to 22.6%, from 16.7% to 30.5%, from 26.0% to 33.4%, from 25.8% to 35.9%, and from 29.6% to 39.4% when the retention time increased from 0 to 120 min at 200 °C, 220 °C, 240 °C, 260 °C and 280 °C, respectively. Thus, increasing retention time had the most impact at lower temperatures (200 °C and 220 °C). It was observed that at any given retention time, higher oil yields were achieved with higher reaction temperature.

3.2.1. Retention time effects on carbon recovery. Fig.10 shows how the retention time affected the CR of bio-crude oil and solid residue. At five reaction temperature levels, CR of bio-crude oil all increased with the increasing of retention time, at the same time the solid residue CR values all decreased. Prolonging the retention time helps to convert toluene insoluble organic carbon into toluene soluble organic carbon. It was also found that the effects of retention time on CR and NR values at high temperatures were less substantial than that at low temperatures. For instance, at 280 °C CR of bio-crude oil increased from 39.0% to 55.3%, while it increased from 4.0% to 31.9%% at 200 °C.

Meanwhile, CR of raw oil decreased from 62.9% to 52.5% at 240 °C, and increased from 49.9% to 56.9% at 280 °C when the retention time increased from 0 min to 120 min. However, when the temperature was 260 °C, CR of raw oil appeared to be stable around 52% regardless of how long the retention time was. The result indicates that 260 °C could be a threshold value or turning point of reaction temperature, above which the relationships between raw oil carbon recovery and the reaction temperature were opposite to each other. This conclusion is consistent with the result in Fig.3, which showed that when the reaction temperature was lower than 260 °C, the CR of the raw oil decreased with increasing of reaction temperature but when the reaction temperature was higher than 260 °C, the CR of the raw oil started to increase. The carbon in the feedstock was removed as CO_2 through decarboxylation; thus, the carbon recovery of raw oil first decreased with increasing of reaction temperature. Since deamination could introduce hydroxyl groups by removing

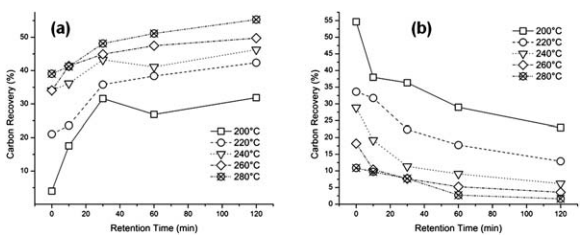

Fig. 10 CR *vs.* retention time: (a) for bio-crude oil; and (b) for solid residue.

nitrogen from amino acids, it is possible that amino acids containing hydroxyl groups were produced. On the other hand, amino acids with aldehyde groups can also be produced through deamination. These organic molecules with hydroxyl, aldehyde and carboxyl groups may react with each other through aldol condensation or esterification. These recombination reactions could produce water insoluble organic molecules; therefore the carbon recovery of raw oil could increase as the temperature was further increased. Testing of this hypothetical mechanism is beyond the scope of this paper, but it could be accomplished in the future by analysis of the organic molecules and functional groups in the aqueous and oil phases.

3.2.2. Retention time effects on nitrogen recovery. The retention time effects on the nitrogen recoveries of bio-crude oil and solid residue are shown in Fig.11.

The NR of the bio-crude oil increased with retention time, while the NR of solid residue decreased substantially. At all reaction temperatures, the nitrogen recoveries for raw oil were decreasing as reaction temperatures increased (from 47.5% to 29.7%. from 41.0% to 26.4%, from 42.7% to 23.1%, from 31.3% to 23.5% and from 29.1% to 23.7% when the retention time increased from 0 min to 120 min at 200 °C, 220 °C, 240 °C, 260 °C and 280 °C reaction temperature, respectively). Thus, nitrogen generally accumulated in the bio-crude oil with longer retention time. Note that at 200 °C and 220 °C, the nitrogen content in bio-crude oil increased from 1.6% to 5.9% and from 1.8% to 6.5% respectively, when the retention time increased from 0 min to 120 min. However, at higher temperatures, nitrogen content in bio-crude oil did not change much with retention time. These results implied that at low temperature, some of the toluene insoluble nitrogen in organic molecules was converted into toluene soluble molecules when the retention time increased; but increased NR of bio-crude oil at high temperature were mainly due to the increasing of its yield.

Combined with the results in the previous section, it is evident that neither increasing reaction temperature nor prolonging retention time helps to decrease the NR of the bio-crude oil. Even so, at the five reaction temperature levels, most of the nitrogen in the original material was converted into aqueous product. The nitrogen recoveries of aqueous product all increased with the retention time, and could be expected to approach more than 75% with a sufficiently long retention time. Unfortunately, there is a strong correlation between reaction temperature/retention time conditions that increase CR in the bio-crude oil and those that increase NR in the bio-crude oil. Thus, other approaches, such as catalysts, would appear to be more promising for finding

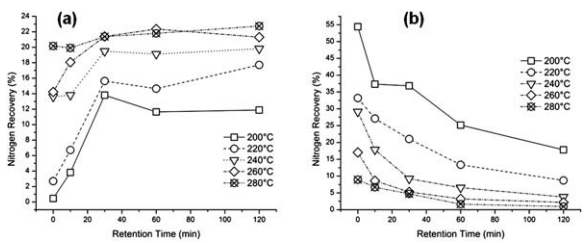

Fig. 11 NR *vs.* retention time: (a) for bio-crude oil; and (b) for solid residue.

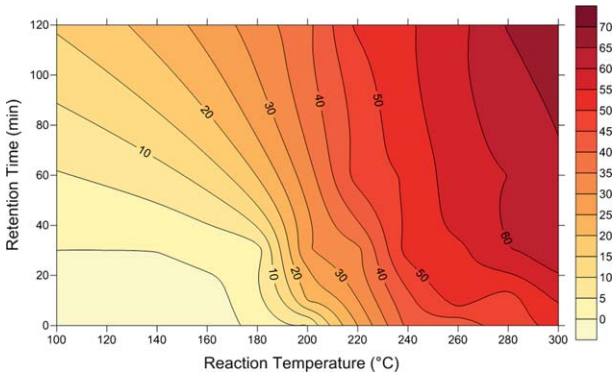

Fig. 12 Energy recovery (%) of bio-crude oil.

ways to reduce the nitrogen content of HTL oil. Otherwise additional upgrading to remove the nitrogen from bio-crude oil will be needed to produce transportation grade fuel from high protein feedstocks.[50]

3.3. Effects of reaction temperature and retention time on energy recovery

Fig.12 is a contour plot of the energy recovery of bio-crude oil at different reaction temperatures and retention times. The figure indicates that, with the temperature ranging from 100 °C to 300 °C and retention time ranging from 0 min to 120 min, energy recovery of bio-crude oil increased with reaction temperature and retention time.

The highest energy recovery of bio-crude oil was 65.4%, achieved at 280 °C reaction temperature and 120 min retention time. This result is comparable with one study using a higher reaction temperature (350 °C) and high-lipid content microalgae (*Nannochloropsis*) as HTL feedstock,[51] which indicated that about two thirds of the total energy in the algal feedstock was contained in the HTL oil product. Finding an economical way, such as the concept of E^2-Energy, to recover the fraction of energy contained in other HTL products would enhance the viability of the HTL process.

Conclusions

This study demonstrated that hydrothermal liquefaction is an effective way to convert low-lipid content microalga *C. Chlorella* into bio-crude oil. Reaction temperatures higher than 220 °C and retention time longer than 10 min were necessary to achieve desirable performance in terms of nutrient recycle from post-HTL wastewater. With these favorable combinations of temperature and retention time, there was approximately 40% of the carbon and 75% of nitrogen remained in aqueous phase (water soluble organic compounds). Meanwhile, more than 98% of carbon in HTL gaseous product was attributed to CO_2.

The increasing of carbon and nitrogen recoveries in the bio-crude oil with increasing reaction temperature and retention time implies that most of the carbon and nitrogen in the oil/solid phase (raw oil) tends to accumulate in the toluene soluble fraction at more intensive operating conditions. Further upgrading or applying catalysts in the HTL process would be necessary to remove nitrogen in the bio-crude oil. Energy recovery of bio-

crude oil increased with reaction temperature and retention time. The highest energy recovery (65.4%) was obtained at 280 °C with 120 min retention time.

About one third of the total energy and more than 70% of nitrogen in the original algal feedstock remained in the post-HTL aqueous stream. It would be desirable to develop an economical way to recover and reuse the energy and nutrients in the post-HTL wastewater. The integrated treatment concept of E^2-Energy as introduced in this paper provides a promising approach for solving these problems concurrently.

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