

Nitrogen distribution in the products from the hydrothermal liquefaction of *Chlorella* sp. and *Spirulina* sp.

Tianyi Bao¹, Yuanyuan Shao (✉)^{1,2}, Haiping Zhang¹, Jesse Zhu³

¹ School of Chemical Engineering of Technology, Tianjin University, Tianjin 300072, China

² Tianjin Key Laboratory of Membrane Science and Desalination Technology, Tianjin 300072, China

³ Department of Chemical & Biochemical Engineering, The University of Western Ontario, Ontario N6A 3K7, Canada

© Higher Education Press 2022

Abstract The high contents of nitrogen-containing organic compounds in biocrude obtained from hydrothermal liquefaction of microalgae are one of the most concerned issues on the applications and environment. In the project, *Chlorella* sp. and *Spirulina* sp. were selected as raw materials to investigate the influence of different reaction conditions (i.e., reaction temperature, residence time, solid loading rate) on the distribution of nitrogen in the oil phase and aqueous phase. Three main forms of nitrogen-containing organic compounds including nitrogen-heterocyclic compounds, amide, and amine were detected in biocrudes. The contents of nitrogen-heterocyclic compounds decreased with temperature while amide kept increasing. The effect of residence time on the components of nitrogen-containing organic compounds was similar with that of temperature. However, the influence of solid loading rate was insignificant. Moreover, it was also found that the differences of amino acids in the protein components in the two microalgae might affect the nitrogen distribution in products. For example, nitrogen in basic amino acids of *Spirulina* sp. preferred to go into the aqueous phase comparing with the nitrogen in neutral amino acids of *Chlorella* sp. In summary, a brief reaction map was proposed to describe the nitrogen pathway during microalgae hydrothermal liquefaction.

Keywords microalgae, hydrothermal liquefaction, biocrude, nitrogen distribution

1 Introduction

To deal with environmental problems caused by fossil fuels and in view of the renewable and zero carbon characteristics of biomass, it is crucial to research the

production of liquid transport fuel from biomass [1–3]. Generally, biomass conversion technology targeting liquid fuel as product is mainly divided into biochemical and thermochemical methods [4]. Biochemical conversion is mainly based on anaerobic fermentation and biological enzyme technology [5]. Anaerobic fermentation is mostly suitable for decomposing non-solid biomass such as industrial organic waste liquid and human manure into biogas [6]. Biological enzyme technology is to convert biomass into ethanol, but the large-scale production of biological enzymes is still difficult to achieve, and it also has problems such as slow conversion speed and the need for downstream treatment of waste liquid [7–9]. On the other hand, thermochemical conversion of biomass includes the chemical methods converting biomass into combustible gas or biocrude, such as combustion, gasification, pyrolysis and liquefaction [10]. As one of the most commonly-used thermochemical conversion methods, hydrothermal liquefaction (HTL) is a chemical recombination process of organic matter in sub/supercritical water under the conditions of 200–370 °C and 7–30 MPa to produce biocrude and other products including aqueous phase, solid residue and gas [11,12]. The whole process does not need to dry the feedstock, which reduces the energy consumption, especially suitable for the treatment of biomass with high water content [13]. On the other hand, microalgae biomass is generally high in water content, and has the advantages of fast growth, short growth cycle, high photosynthetic efficiency, and does not occupy agricultural land [14,15]. As a result, HTL using for wet microalgae to directly produce biocrude has received widespread attention.

However, the existence of nitrogen-containing organic compounds (NOCs) has always been a concern during utilization of microalgae as an energy source, in particular, via HTL process [16]. As the biocrude obtained by HTL needs further refining optimization, and the high concentration of nitrogen-heterocyclic compounds such as

quinoline, pyridine, etc. will make the catalyst used in the refining process toxic, so that the deactivation of the catalyst will cause great difficulties in refining the biocrude. In addition, NOCs and sulfides have a competitive relationship in the desulfurization process of biocrude which inhibit the desulfurization effect [17]. Moreover, NOCs could also cause atmosphere pollution due to producing considerable NO_x in industrial applications [18]. Therefore, various studies have proposed some methods to reduce the nitrogen content in biocrude. Chen et al. [19] found that water extraction could reduce the nitrogen content in microalgae biocrude from 6.83% to 5.75%, which increased the carbon and hydrogen content simultaneously. Gas chromatography and mass spectrometry (GC-MS) of the biocrude had further confirmed that water could separate the fatty acid derivatives from biocrude, and these derivatives can be seen as a surfactant to emulsify NOCs to the aqueous phase. As a result, water is not only a conventional solvent, but also participates in some reactions which occur in organic phase during HTL. A two-step HTL process was developed by Miao et al. [20,21], in which proteins and polysaccharides were separated from the microalgae at first, and then the remaining algae residue was as the feedstock for HTL. Comparing with the one-step method, the contents of nitrogen and oxygen in biocrude obtained by the two-step method was lower. It proved that the two-step HTL process could improve the quality of biocrude, because it was almost using lipids as the raw material for the second step which increased the yield of biocrude and reduced the nitrogen content in the oil phase. The way of using biological macromolecule model compounds as feedstock may be more effective to study the reaction mechanism and pathway of nitrogen. Montero-Hidalgo et al. [22] employed hydrothermal pretreatment at low temperatures of *Nannochloropsis gaditana* for reducing the contents of nitrogen and oxygen in biocrude. Reaction temperature and residence time were key factors which having a negative impact on the biomass yield while a positive impact on the heteroatom recovery in aqueous phase. However, the effect of solid loading rate in feedstock on the two aspects was opposite from reaction temperature and residence time. Therefore, different reaction conditions have a significant influence on the yield and distribution of heteroatom, and at the same time, the selection of microalgae may also affect the reaction conditions. An alga called *Enteromorpha prolifera* was investigated at different reaction conditions with using crude glycerol as solvent by Lu et al. [23]. They discovered that crude glycerol could reduce the contents of nitrogen in biocrude and improve the yield. Thus, changing the reaction solvent might improve the characteristics of biocrude. Tang et al. [24] researched flash heating method of *Nannochloropsis*. In the products separation procedure, the aqueous extract was separated from aqueous phase by adding dichloromethane into the water solution. Dichloromethane is a

commonly-used extraction solvent in the process of oil-water separation, because the boiling point of dichloromethane is lower than 40 °C which could be a benefit for the post separation and very cost-effective. Comparing with the conventional HTL, the biocrude yield obtained by the flash heating method was almost the same, but it could save the reaction time and improve the efficiency. And the separation of the aqueous extract could improve the biocrude yield by reducing the contents of NOCs. Therefore, the change of experimental method or separation procedure will have certain effects on the nitrogen distribution in products.

Furthermore, the investigation of reaction mechanisms during microalgae HTL process is very meaningful. For example, Maillard reaction plays an important role in the nitrogen distribution, especially the formation of nitrogen-heterocyclic compounds. Qiu et al. [25] investigated Maillard reaction between protein and carbohydrate by using leucine and glucose as model compounds. They found that the main pathway of Maillard reaction during HTL had a certain relationship with the reaction of deaminated leucine and epoxy compounds in the degradation of glucose to produce pyrazine derivatives, because nitrogen-heterocyclic compounds, organic acids and amine derivatives were the principal components of both the oil and aqueous phase. Therefore, it is necessary to pay more attention on Maillard reaction when studying Nitrogen-related reaction pathway during microalgae HTL. Other reactions such as Mannich reaction was also investigated by Chen et al. [26] with using microalgae and sweet potato as feedstocks. The addition of sweet potato could promote the Mannich reaction. And this reaction could transfer more NOCs from oil phase to solid residue. As a result, the content of nitrogen decreased significantly in biocrude. Besides, it could also increase the content of low boiling point compounds in biocrude. Thus, this reaction links the conversion process of nitrogen between the oil phase and solid residue. Sheng et al. [27] proposed a yield model of biocrude, which considered the interaction between the macromolecules in microalgae during HTL. The interaction between different substances could increase biocrude yield as shown in the experimental results. They proposed that the content of nitrogen-heterocyclic compounds reached highest when the content of protein was triple of that of carbohydrate. The work applied simulation and modelling methods and could more accurately and conveniently predict the contents of NOCs in the products.

From the above, although there were many studies on tackling nitrogen-related problems and analyzing reaction mechanisms for some key reactions, there is still no complete and systematic investigation on the overall nitrogen pathway during HTL process. Thus, it is essential to detect the nitrogen distribution in different products and analyze the effects of reaction conditions on nitrogen distribution to explore its reaction pathway. In this project, *Chlorella* sp. and *Spirulina* sp. were selected as feedstock.

The effects of reaction temperature, residence time and solid loading rate on the yield, nitrogen recovery and chemical compositions of biocrude were comprehensively investigated. Nitrogen distribution in the oil phase and aqueous phase was analyzed, and a brief reaction map was proposed to describe the nitrogen pathway during microalgae HTL.

2 Experimental

2.1 Materials

Two microalgae, *Chlorella* sp. and *Spirulina* sp., were purchased from Shandong Jianchuan Biological Technology Co., Ltd., China. All reagents including dichloromethane are at a purity of 99.9%, which obtained from Tianjin Yuanli Chemical Engineering Co., Ltd., China. Table 1 presented the elemental and biochemical compositions of *Chlorella* sp. and *Spirulina* sp. The two microalgae were similar in compositions, in which the contents of carbon, nitrogen and oxygen were high and hydrogen was relatively low. Both of them had a high content of protein, a low content of lipid and carbohydrate, which belonged to high-protein low-lipid microalgae.

Table 1 Compositions of *Chlorella* sp. and *Spirulina* sp.

Microalgae	<i>Chlorella</i> sp.	<i>Spirulina</i> sp.
Ultimate/wt%		
Carbon	47.128	45.822
Hydrogen	6.826	7.692
Nitrogen	10.314	10.413
Sulfur	0.635	0.606
Oxygen ^{a)}	35.097	35.467
Biochemical/wt%		
Protein	87.319	84.156
Lipid	8.642	9.542
Ash	0.753	0.892
Carbohydrate ^{a)}	3.286	5.410

a) Calculated by difference.

2.2 Procedure

According to literatures [28–30], reaction temperature, residence time and solid loading rate were determined 240–290 °C, 30–90 min and 10–30 wt%, respectively. Figure 1 presents the experimental procedure in the project. A 500 mL steel reactor was adopted for the HTL process, which equipped with quick opening and mechanic stirring.

Based on the planned solid loading rate, microalgae powder and deionized water were added into the reactor. Air in the reactor was purged by injecting argon and the initial pressure was set at 0.5 MPa. Leaking testing was

carried out to ensure the reactor was sealed well before each single test. After that, the reactor started to be heated up by furnace at a heating rate of 3 °C·min⁻¹ till the inside temperature for the reactor reached the preset value. And then, keeping the reactor running for the desired residence time at the operational temperature. When the experiment was done, the reactor was quickly cooled down to room temperature by ice-water mixture for about 30 min. The pressure in the reactor was recorded before collecting gas with a gas bag, then down to atmospheric pressure. Afterwards, the reactor was opened and added a certain amount of dichloromethane for dissolving the water-insoluble substances. Then an ultrasonic vibration instrument was used to vibrate the oil products down from the solid residue. The mixture was filtered by vacuum filtration (20 µm) and the filter residue after drying was defined as the solid residue. The filtrate was poured into a separatory funnel for about 30 min in order to separate the aqueous phase and oil phase. The upper liquid in the separatory funnel was the aqueous phase product, and the lower organic liquid was transferred to the rotary flask. Finally, the biocrude was separated from dichloromethane via rotary evaporation for 0.5 h and weighed for the biocrude yield calculation.

2.3 Analysis methods

The elemental analysis (C, H, N and S) of feedstock and products was conducted by LECO CHN628 Elemental Analyzer, America. The determination of protein and lipid in feedstock were carried out using the Kjeldahl method (GB 5009.5-2010) and the solvent extraction method (GB/T 5009.6-2003), respectively. The ash content of feedstock was obtained by weighing the difference of weight before and after burning in the muffle furnace. The content of carbohydrate in feedstock was calculated by difference between microalgae and its other three components (protein, lipid and ash).

The chemical compositions of biocrude were analyzed by using a GC-MS (QP2010 ultra) equipped with a DB-5 column (30 m × 0.25 mm × 0.25 µm). The temperature of ion source was 280 °C. The carrier gas was Helium (1 mL·min⁻¹). Biocrude-dichloromethane solution (1 µL) was injected into the instrument at a split ratio of 10:1 at 280 °C. The initially temperature was set at 50 °C for 5 min, and it took 45 min to reaching 320 °C, and then run analyzing at this temperature for 10 min. The aqueous phase was also qualitatively analyzed by GC-MS with the same instrument but with a TR-WAXMS column. The temperature of ion source was 200 °C. The carrier gas kept unchanged. The initially temperature was set at 40 °C for 5 min, and it took 34 min to reaching the 240 °C, keeping at 240 °C for 20 min. The qualitative analysis report provided the normalized peak area in the sample with more than 1% content. Biocrude yield and nitrogen recovery (R_N) were calculated according to the following Eqs. (1)

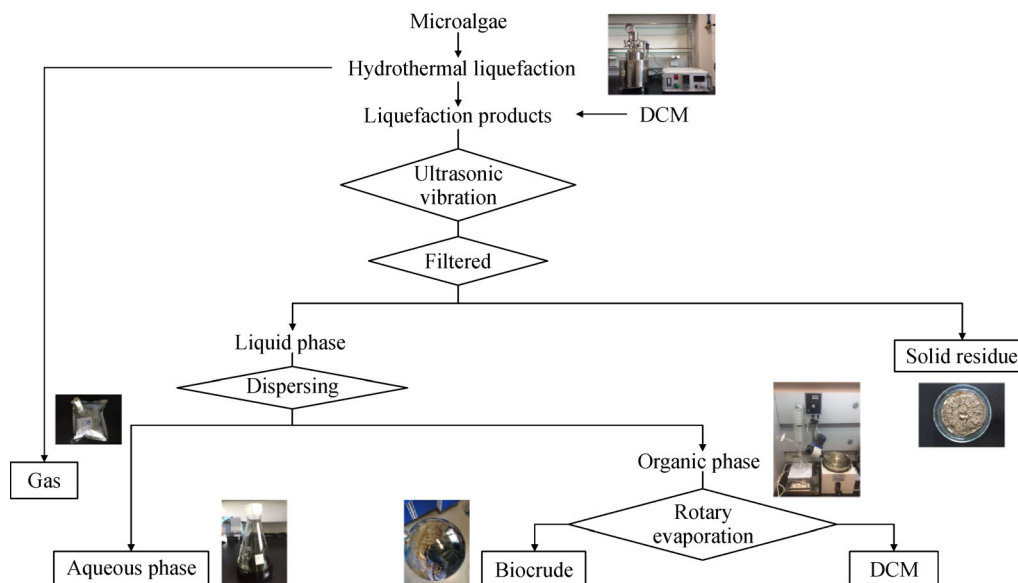


Fig. 1 The scheme of the experimental procedure.

and (2) respectively:

$$\text{Biocrude yield (wt\%)} = \frac{\text{Weight of biocrude}}{\text{Weight of microalgae}} \times 100\%, \quad (1)$$

$$R_N(\%) = \frac{\text{Biocrude yield} \times \text{content of N in biocrude}}{\text{Content of N in microalgae}} \times 100\%. \quad (2)$$

3 Results and discussion

3.1 Effect of reaction temperature on the characteristics of biocrude

The influence of reaction temperature was shown in Fig. 2 and the biocrude yields of the two microalgae were gradually increasing with the reaction temperature. The result was consistent with the literature [17], especially in the range under 300 °C. The maximal biocrude yield was observed at 290 °C for both microalgae, which may be caused by some reactions occurring in small molecules at high temperatures and producing more oil products. Some studies already reported that protein and carbohydrate could not be fully decomposed to small molecules at low temperatures (< 230 °C) and only lipid could be hydrolyzed sufficiently [31,32].

Figure 3 presented the effect of reaction temperature on the R_N of the biocrude from *Chlorella* sp. and *Spirulina* sp. The R_N of *Chlorella* sp. increased first and then decreased,

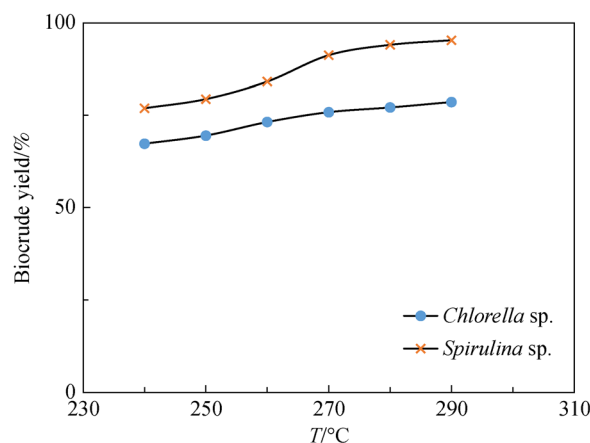


Fig. 2 Effect of reaction temperature on the biocrude yield from *Chlorella* sp. and *Spirulina* sp.

while *Spirulina* sp. changed slightly. According to the definition of R_N , the increase in R_N of the biocrude from *Chlorella* sp. between 240 and 270 °C might be caused by the increase of biocrude yield, but when the temperature exceeded 270 °C, R_N had a significant decrease. However, the biocrude yield of *Chlorella* sp. increased continuously, which indicating that the decrease in R_N of the biocrude from *Chlorella* sp. was due to the decrease in nitrogen content of biocrude. This result might be related to the decomposition of some NOCs such as nitrogen-heterocyclic compounds, which hydrolyzed and decarboxylated into some water-soluble amine derivatives at high temperatures. Nevertheless, this phenomenon was not observed in *Spirulina* sp. The biocrude yield of *Spirulina* sp. increased constantly from 240 to 290 °C. Maybe the

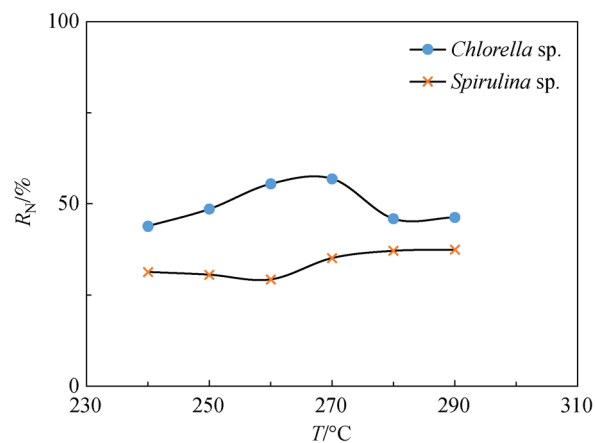


Fig. 3 Effect of reaction temperature on the R_N of biocrude from *Chlorella* sp. and *Spirulina* sp.

decrease in the nitrogen content of biocrude from *Spirulina* sp. caused the increase in R_N tend to be flat at high temperatures, which did not have a significant impact. These results indicated that the existing forms of nitrogen in the biocrude obtained from the two microalgae may be significantly different.

However, as shown in Table 1, *Chlorella* sp. and *Spirulina* sp. had similar compositions including protein and nitrogen contents. Owing to the biocrude yields of the two microalgae had similar trends with temperature, the nearly opposite trends in R_N could be only resulted from the difference of nitrogen distribution for two different microalgae. As shown in Fig. 3, the nitrogen in *Chlorella* sp. looked easier to be transferred to the oil phase with temperature increased. Gai et al. [17] reported that the protein compositions in *Chlorella* sp. and *Spirulina* sp. were not same. The representative of amino acids in *Chlorella* sp. and *Spirulina* sp. protein are leucine and arginine, respectively. In terms of chemical structure, leucine is a neutral amino acid while arginine is a basic amino acid. Therefore, it might be concluded that more nitrogen in basic amino acids is inclined to go into the aqueous phase and existed in the forms of water-soluble NOCs. Maybe this was the reason why the R_N in the case of *Chlorella* sp. was always higher than that of *Spirulina* sp.

In order to verify the above speculations, the chemical compositions of the oil phase and aqueous phase were detected by GC-MS, and the solid residue was analyzed by elemental analysis. Experimental results illustrated that the nitrogen content in the solid phase could be ignored due to the low yield. Therefore, the effect of reaction temperature on the distribution of NOCs in the oil phase and aqueous phase of the two microalgae was investigated as shown in Fig. 4. The proportion of nitrogen-heterocyclic compounds in the oil phase decreased with temperature but the amide and amine increased. This trend could be summarized from Fig. 4(a), it might be caused by more nitrogen-heterocyclic

macromolecules tended to convert into small molecules such as amide and amine compounds at high temperatures. However, Fig. 4(b) showed that the contents of nitrogen-heterocyclic and amide compounds were slightly increasing in the aqueous phase, while amine kept decreasing. This trend was different from the oil phase, which indicating that some water-soluble amine compounds preferred to condense or acylate to form nitrogen-heterocyclic and amide compounds with temperature. Therefore, the same type of compounds may exist in different forms in different phases. Similar results were also mentioned by Matayeva et al. [33]. They explored the reaction pathway of NOCs by using amino acids as protein model compounds. The carbon recovery of biocrude increased significantly owing to the acylation of the amino moiety was proposed. It caused many water-soluble substances to be transferred to the oil phase. Obviously, the distribution of amides in aqueous phase and oil phase is also a reflection of the different conversion process of nitrogen.

3.2 Effect of residence time on the characteristics of biocrude

Figure 5 illustrated that the biocrude yield of *Chlorella* sp. increased first and then decreased slightly with residence time, while *Spirulina* sp. increased slightly and was higher than *Chlorella* sp. under the same conditions. The result implied that prolonging the residence time could increase the biocrude yield to some extent, but too long the residence time caused more secondary reactions to occur, thereby reducing the biocrude yield. This inference had been mentioned in other literatures before [32,34]. In fact, different microalgae correspond to different optimal residence times for yield, but generally the yield of biocrude increases first and then decreases with the residence time. Therefore, the optimal residence time of the biocrude of *Spirulina* sp. may not appear under current experimental conditions.

As the residence time increasing, the R_N of the biocrude from *Chlorella* sp. increased first and then decreased, while *Spirulina* sp. changed slightly as shown in Fig. 6. The increase in R_N of the biocrude from *Chlorella* sp. between 30–60 min might be caused by the increase of biocrude yield, but when the residence time exceeded 80 min, R_N had a significant decrease. However, the biocrude yield of *Chlorella* sp. decreased slightly from 70 to 90 min, which indicating that the decrease in R_N of the biocrude from *Chlorella* sp. was mainly due to the significant reduction in nitrogen content of biocrude. The result implied that some unstable NOCs would undergo secondary reactions such as ring opening or decomposition under a long residence time. Therefore, part of the nitrogen was transferred to the other phase. Interestingly, this phenomenon was not observed in *Spirulina* sp. The biocrude yield and R_N of *Spirulina* sp. both changed slightly, which indicated that

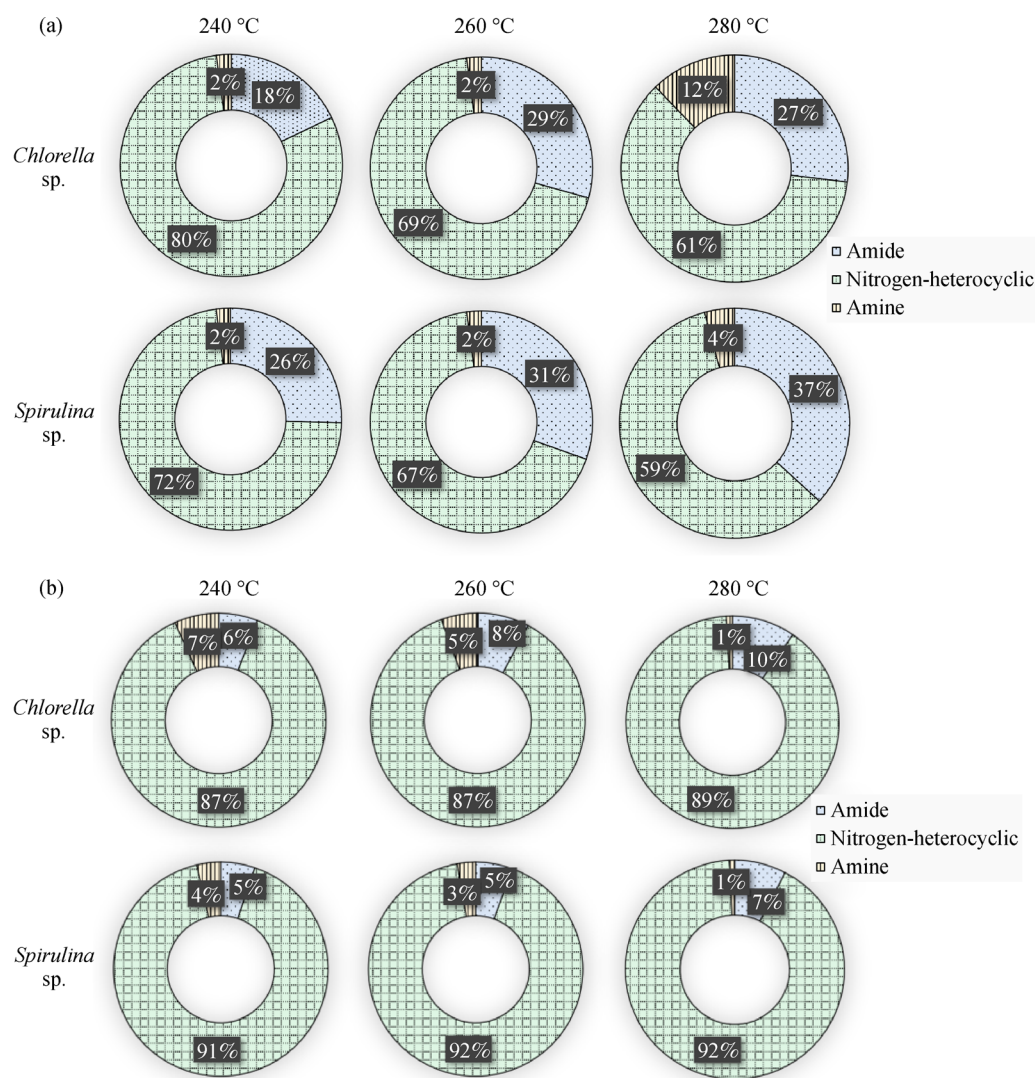


Fig. 4 Effect of reaction temperature on the distribution of NOCs in (a) oil phase and (b) aqueous phase of *Chlorella* sp. and *Spirulina* sp.

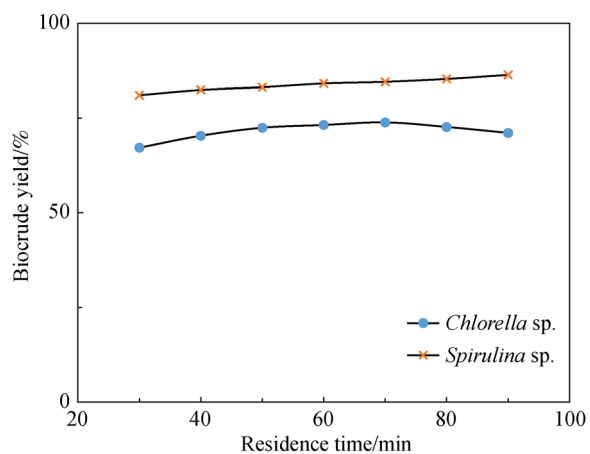


Fig. 5 Effect of residence time on the biocrude yield of *Chlorella* sp. and *Spirulina* sp.

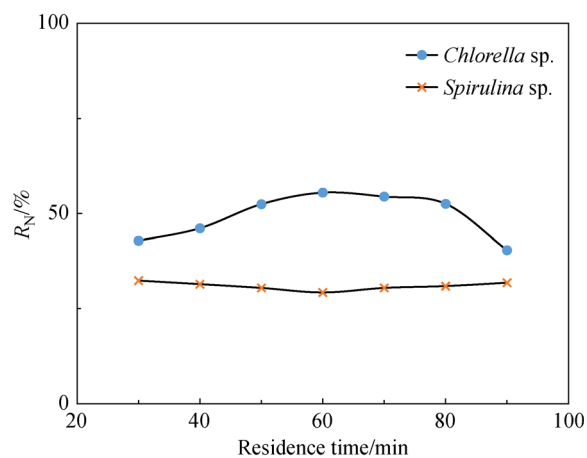


Fig. 6 Effect of residence time on the R_N of biocrude from *Chlorella* sp. and *Spirulina* sp.

the nitrogen content in biocrude from *Spirulina* sp. was almost unchanged. It demonstrated that the chemical structures of NOCs in the biocrude from *Spirulina* sp. were very stable. Secondary reactions would not occur with the extend of residence time. In fact, as mentioned before, the types of amino acids in two microalgae leads to the difference in nitrogen distribution of biocrude. This may be the reason why the content and existing forms of nitrogen in two microalgae was really different.

Figure 7 presented the effect of residence time on the distribution of NOCs in the oil phase and aqueous phase of the two microalgae. As shown in Fig. 7(a), the proportion of nitrogen-heterocyclic compounds in the oil phase decreased and amide increased with residence time. However, Fig. 7(b) showed that the distribution of NOCs in aqueous phase was almost unchanged. The content of amide kept increasing, and nitrogen-heterocyclic com-

pounds kept decreasing in biocrude, especially between 30 and 60 min, which mainly caused by the decomposition of diketopiperazines (DKPs). As the substituents of DKPs are complex and diverse, it can be speculated that after hydrolysis, some water-soluble and oil-soluble amides are formed. The increasing of amides in the oil phase may be due to the fatty acids from lipid hydrolysis which have a large non-polar group prefer to reacting with the free ammonia from amino acid deamination. This finding was agreed well with those reported on the literatures [35,36]. However, the three main existing forms of nitrogen varied limited in the aqueous phase. The similarity of aqueous phase at 30 and 90 min might imply that the different chemical structures of NOCs were converted to each other with the residence time. Considering engineering scaling up, too long residence time may cause unwanted reactions occurring, leading to high energy consumption and high

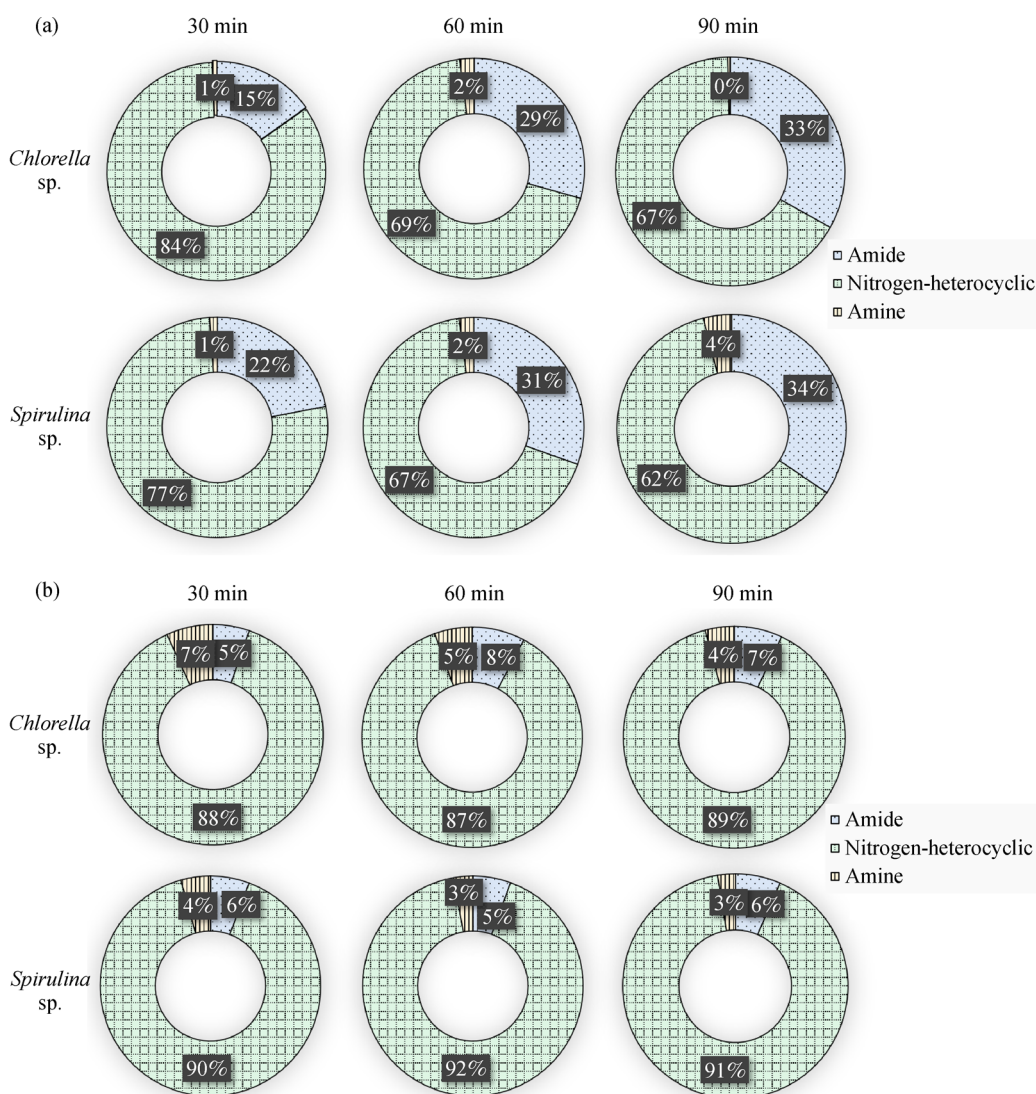


Fig. 7 Effect of residence time on the distribution of NOCs in (a) oil phase and (b) aqueous phase of *Chlorella* sp. and *Spirulina* sp.

equipment cost [37]. Therefore, setting a moderate residence time may be beneficial to the yield and quality of biocrude.

3.3 Effect of solid loading rate on the characteristics of biocrude

Figure 8 showed that the biocrude yield of *Chlorella* sp. decreased volatility with the solid loading rate of feed solution, while *Spirulina* sp. kept decreasing and was constantly higher than *Chlorella* sp. The result illustrated that the increase in solid content of feedstock was not conducive to increasing the biocrude yield, which was different from the reaction temperature and residence time. It might be caused by the limited solvent content in the feed solution, which led to some macromolecules dissolved and hydrolyzed incompletely [38]. However, there was no significant change in R_N of the biocrude from *Chlorella* sp. and *Spirulina* sp. as shown in Fig. 9, which indicated that the water content of solution had no significant effect on the conversion of nitrogen during HTL process as literatures [39,40] mentioned.

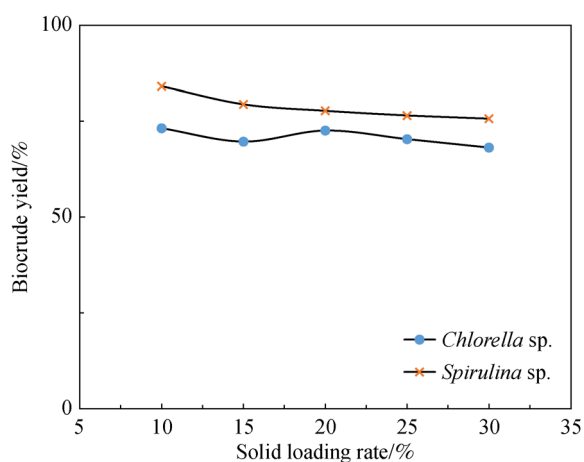


Fig. 8 Effect of solid loading rate on the biocrude yield of *Chlorella* sp. and *Spirulina* sp.

The effect of solid loading rate on the distribution of NOCs in the oil phase and aqueous phase of the two microalgae was shown in Fig. 10. Figure 10(a) illustrated that the proportion of nitrogen-heterocyclic compounds in biocrude decreased, whereas amide increased with the solid loading rate. However, the solid loading rate had limited effect on the N distribution in aqueous phase as shown in Fig. 10(b). It can be inferred that few nitrogen-heterocyclic compounds in the oil phase was converted into amide or amine derivative compounds with smaller molecular weight. But on the whole, the impact of solid loading rate on the biocrude yield and N distribution of two microalgae was limited.

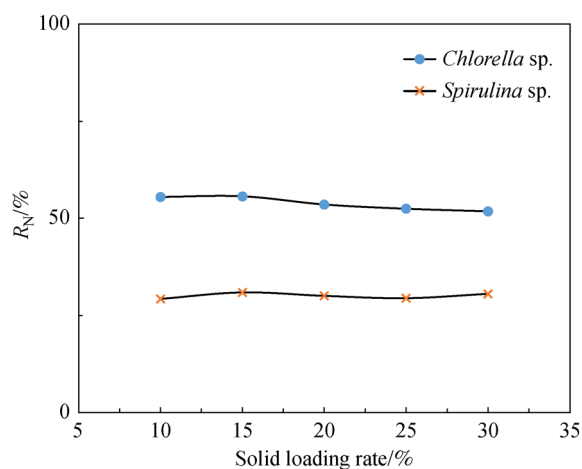


Fig. 9 Effect of solid loading rate on the R_N of biocrude from *Chlorella* sp. and *Spirulina* sp.

3.4 The reaction pathway of nitrogen

In summary, the nitrogen distribution map during microalgae HTL with temperature was prosed as shown in Fig. 11. Below 240 °C, the hydrolysis reaction of macromolecules occurred. Protein was hydrolyzed into polypeptides and amino acids, lipid was hydrolyzed into long-chain fatty acids and glycerol, and carbohydrate was hydrolyzed into small molecule monosaccharides such as glucose. Obviously, nitrogen was almost derived from protein. As the temperature further increasing, the amino acids partially formed into ammonia by deamination; partially formed into DKPs by intramolecular cyclization; partially formed into amines by decarboxylation. And then, the ammonia reacted with fatty acids to form amides or fatty amines through acylation. Some nitrogen-heterocyclic compounds such as pyrrole, pyridine etc. were formed by Maillard reaction of amines and reducing sugars such as glucose [25,41]. Moreover, based on analyzing the nitrogen distribution in the oil and aqueous products under different reaction conditions, it could be found that as the reaction proceeds, the microalgae is pyrolyzed to form a few oil-soluble macromolecules and water-soluble small molecules. The oil-soluble macromolecules further undergo decarboxylation, deamination, ring-opening and other reactions to form water-soluble substances such as alcohols, esters and other organic acids derivatives. Therefore, part of nitrogen is gradually transferred to the aqueous phase. Afterwards, the water-soluble small molecules are recombined through cyclization or condensation and polymerized to form the oil-soluble compounds in a short period of time. As a result, part of nitrogen is re-transferred to the oil phase. In short, the nitrogen is transferred back and forth between the aqueous phase and the oil phase during HTL.

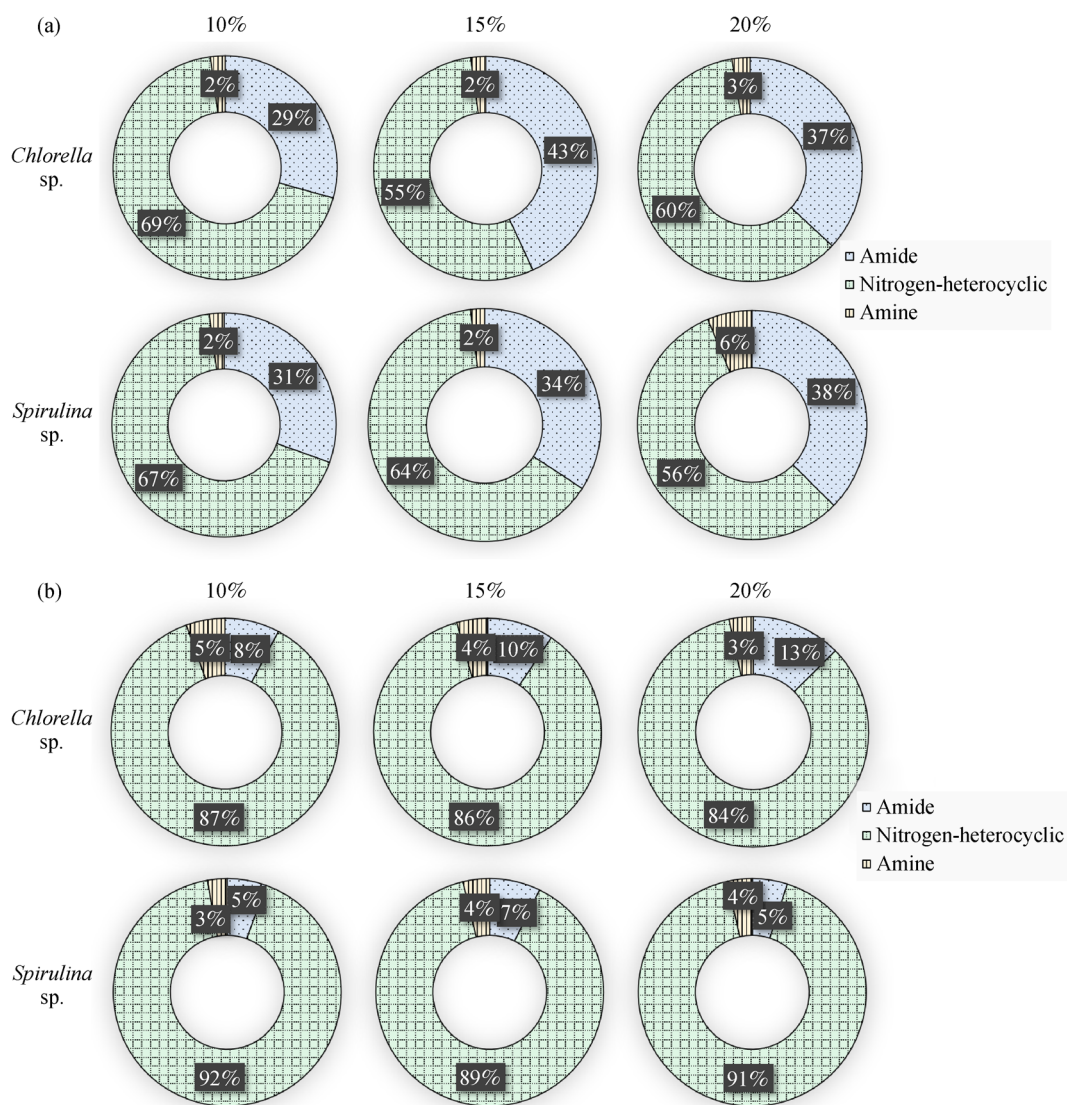


Fig. 10 Effect of solid loading rate on the distribution of NOCs in (a) oil phase and (b) aqueous phase of *Chlorella* sp. and *Spirulina* sp.

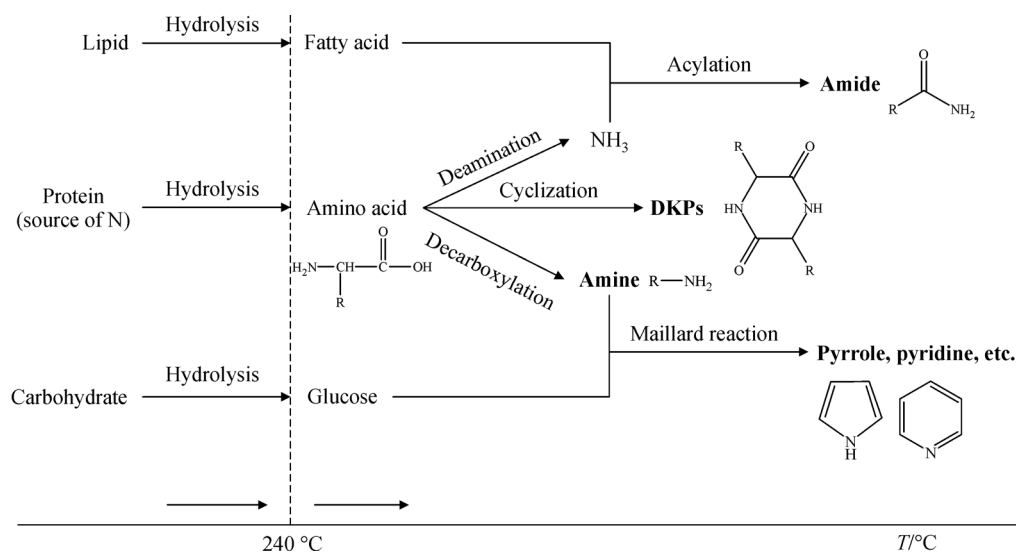


Fig. 11 The nitrogen distribution map during microalgae HTL.

4 Conclusions

Chlorella sp. and *Spirulina* sp. were selected as raw materials to investigate the influence of different reaction conditions (i.e., reaction temperature, residence time, solid loading rate) on the distribution of nitrogen in the oil phase and aqueous phase. Reaction temperature has a positive effect on the yield and quality of biocrude. When reaction temperature increased from 240 to 290 °C, the biocrude yields were gradually increasing, and NOCs became easily decompose resulting into that the biocrude is thus more suitable for subsequent treatment and refining. Moreover, the optimal residence time for different types of microalgae is different. Excessive extending the residence time has negative effect on the biocrude yield, but it can reduce the nitrogen content in the biocrude from *Chlorella* sp. Furthermore, solid loading rate seems less influence on the characteristics of biocrude, and it may not be a key factor for the reaction pathway of nitrogen.

Above all, the selected two microalgae with different representative of amino acids have significant effects on the content and existing forms of nitrogen in products. Compared with neutral amino acids (*Chlorella* sp.), the nitrogen from basic amino acids (*Spirulina* sp.) prefer to go into the aqueous phase.

Acknowledgements This project was sponsored financially by the National Natural Science Foundation of China (Grant No. 21606170).

Electronic Supplementary Material Supplementary material is available in the online version of this article at <https://dx.doi.org/10.1007/s11705-021-2126-y> and is accessible for authorized users.

References

- Monari C, Righi S, Olsen S. Greenhouse gas emissions and energy balance of biodiesel production from microalgae cultivated in photobioreactors in Denmark: a life-cycle modeling. *Journal of Cleaner Production*, 2016, 112: 4084–4092
- Chopra J, Mahesh D, Yerrayya A, Vinu R, Kumar R, Sen R. Performance enhancement of hydrothermal liquefaction for strategic and sustainable valorization of de-oiled yeast biomass into green bio-crude. *Journal of Cleaner Production*, 2019, 227: 292–301
- Egesa D, Chuck C, Plucinski P. Multifunctional role of magnetic nanoparticles in efficient microalgae separation and catalytic hydrothermal liquefaction. *ACS Sustainable Chemistry & Engineering*, 2018, 6(1): 991–999
- Cheng S, Wei L, Alsowij M, Corbin F, Boakye E, Gu Z, Raynie D. Catalytic hydrothermal liquefaction (HTL) of biomass for bio-crude production using Ni/HZSM-5 catalysts. *AIMS Environmental Science*, 2017, 4(3): 417–430
- Koley S, Khadase M, Mathimani T, Raheman H, Mallick N. Catalytic and non-catalytic hydrothermal processing of *Scenedesmus obliquus* biomass for bio-crude production—a sustainable energy perspective. *Energy Conversion and Management*, 2018, 163: 111–121
- Hu Y, Gong M, Xu C, Bassi A. Investigation of an alternative cell disruption approach for improving hydrothermal liquefaction of microalgae. *Fuel*, 2017, 197: 138–144
- Alba L, Torri C, Samori C, Van der Spek J, Fabbri D, Kersten S, Brilman D. Hydrothermal treatment (HIT) of microalgae: evaluation of the process as conversion method in an algae biorefinery concept. *Energy & Fuels*, 2012, 26(1): 642–657
- Saravanan A, Mathimani T, Deviram G, Rajendran K, Pugazhendhi A. Biofuel policy in India: a review of policy barriers in sustainable marketing of biofuel. *Journal of Cleaner Production*, 2018, 193: 734–747
- Mata T, Martins A, Caetano N. Microalgae for biodiesel production and other applications: a review. *Renewable & Sustainable Energy Reviews*, 2010, 14(1): 217–232
- Tekin K, Karagoz S, Bektas S. A review of hydrothermal biomass processing. *Renewable & Sustainable Energy Reviews*, 2014, 40: 673–687
- Dimitriadis A, Bezergianni S. Hydrothermal liquefaction of various biomass and waste feedstocks for biocrude production: a state of the art review. *Renewable & Sustainable Energy Reviews*, 2017, 68: 113–125
- Peterson A, Vogel F, Lachance R, Froling M, Antal M Jr, Tester J. Thermochemical biofuel production in hydrothermal media: a review of sub- and supercritical water technologies. *Energy & Environmental Science*, 2008, 1(1): 32–65
- Biller P, Sharma B, Kunwar B, Ross A. Hydroprocessing of biocrude from continuous hydrothermal liquefaction of microalgae. *Fuel*, 2015, 159: 197–205
- Duan P, Wang B, Xu Y. Catalytic hydrothermal upgrading of crude bio-oils produced from different thermo-chemical conversion routes of microalgae. *Bioresource Technology*, 2015, 186: 58–66
- Hogon C, Delrue F, Texier J, Grateau M, Thiery S, Miller H, Roubaud A. Comparison of pyrolysis and hydrothermal liquefaction of *Chlamydomonas reinhardtii*. Growth studies on the recovered hydrothermal aqueous phase. *Biomass and Bioenergy*, 2015, 73: 23–31
- Guo Y, Yeh T, Song W, Xu D, Wang S. A review of bio-oil production from hydrothermal liquefaction of algae. *Renewable & Sustainable Energy Reviews*, 2015, 48: 776–790
- Gai C, Zhang Y, Chen W, Zhang P, Dong Y. An investigation of reaction pathways of hydrothermal liquefaction using *Chlorella pyrenoidosa* and *Spirulina platensis*. *Energy Conversion and Management*, 2015, 96: 330–339
- Gai C, Zhang Y, Chen W, Zhou Y, Schideman L, Zhang P, Tommaso G, Kuo C, Dong Y. Characterization of aqueous phase from the hydrothermal liquefaction of *Chlorella pyrenoidosa*. *Bioresource Technology*, 2015, 184: 328–335
- Chen W, Tang L, Qian W, Scheppe K, Nair K, Wu Z, Gai C, Zhang P, Zhang Y. Extract nitrogen-containing compounds in biocrude oil converted from wet biowaste via hydrothermal liquefaction. *ACS Sustainable Chemistry & Engineering*, 2016, 4(4): 2182–2190
- Miao C, Chakraborty M, Chen S. Impact of reaction conditions on the simultaneous production of polysaccharides and bio-oil from heterotrophically grown *Chlorella sorokiniana* by a unique sequential hydrothermal liquefaction process. *Bioresource Technology*, 2012, 110: 617–627

21. Jazrawi C, Biller P, He Y, Montoya A, Ross A, Maschmeyer T, Haynes B. Two-stage hydrothermal liquefaction of a high-protein microalga. *Algal Research-Biomass Biofuels and Bioproducts*, 2015, 8: 15–22
22. Montero-Hidalgo M, Espada J, Rodriguez R, Morales V, Bautista L, Vicente G. Mild hydrothermal pretreatment of microalgae for the production of biocrude with a low N and O content. *Processes* (Basel, Switzerland), 2019, 7(9): 1–14
23. Lu J, Liu Z, Zhang Y, Li B, Lu Q, Ma Y, Shen R, Zhu Z. Improved production and quality of biocrude oil from low-lipid high-ash macroalgae *Enteromorpha prolifera* via addition of crude glycerol. *Journal of Cleaner Production*, 2017, 142: 749–757
24. Tang X, Zhang C, Yang X. Optimizing process of hydrothermal liquefaction of microalgae via flash heating and isolating aqueous extract from bio-crude. *Journal of Cleaner Production*, 2020, 258: 120660
25. Qiu Y, Aierzhati A, Cheng J, Guo H, Yang W, Zhang Y. Biocrude oil production through the Maillard reaction between leucine and glucose during hydrothermal liquefaction. *Energy & Fuels*, 2019, 33(9): 8758–8765
26. Chen X, Peng X, Ma X, Wang J. Investigation of Mannich reaction during co-liquefaction of microalgae and sweet potato waste. *Bioresource Technology*, 2019, 284: 286–292
27. Sheng L, Wang X, Yang X. Prediction model of biocrude yield and nitrogen heterocyclic compounds analysis by hydrothermal liquefaction of microalgae with model compounds. *Bioresource Technology*, 2018, 247: 14–20
28. Hossain F, Kosinkova J, Brown R, Ristovski Z, Hankamer B, Stephens E, Rainey T. Experimental investigations of physical and chemical properties for microalgae HTL bio-crude using a large batch reactor. *Energies*, 2017, 10(467): 1–16
29. Toor S, Rosendahl L, Rudolf A. Hydrothermal liquefaction of biomass: a review of subcritical water technologies. *Energy*, 2011, 36(5): 2328–2342
30. Elliott D, Hart T, Schmidt A, Neuenschwander G, Rotness L, Olarte M, Zacher A, Albrecht K, Hallen R, Holladay J. Process development for hydrothermal liquefaction of algae feedstocks in a continuous-flow reactor. *Algal Research-Biomass Biofuels and Bioproducts*, 2013, 2(4): 445–454
31. Huang R, Cheng J, Qiu Y, Zhang Z, Zhou J, Cen K. Solvent-free lipid extraction from microalgal biomass with subcritical water in a continuous flow reactor for acid-catalyzed biodiesel production. *Fuel*, 2019, 253: 90–94
32. Yu G, Zhang Y, Guo B, Funk T, Schideman L. Nutrient flows and quality of bio-crude oil produced via catalytic hydrothermal liquefaction of low-lipid microalgae. *BioEnergy Research*, 2014, 7(4): 1317–1328
33. Matayeva A, Bianchi D, Chiaberge S, Cavani F, Basile F. Elucidation of reaction pathways of nitrogenous species by hydrothermal liquefaction process of model compounds. *Fuel*, 2019, 240: 169–178
34. Akiya N, Savage P. Roles of water for chemical reactions in high-temperature water. *Chemical Reviews*, 2002, 102(8): 2725–2750
35. Obeid R, Lewis D, Smith N, Van Eyk P. The elucidation of reaction kinetics for hydrothermal liquefaction of model macromolecules. *Chemical Engineering Journal*, 2019, 370: 637–645
36. Abbat S, Dhaked D, Arfeen M, Bharatam P. Mechanism of the Paal-Knorr reaction: the importance of water mediated hemiacetal pathway. *RSC Advances*, 2015, 5(107): 88353–88366
37. Chen W, Zhang Y, Zhang J, Yu G, Schideman L, Zhang P, Minarick M. Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil. *Bioresource Technology*, 2014, 152: 130–139
38. Li D, Chen L, Zhang X, Ye N, Xing F. Pyrolytic characteristics and kinetic studies of three kinds of red algae. *Biomass and Bioenergy*, 2011, 35(5): 1765–1772
39. Tian C, Liu Z, Zhang Y, Li B, Cao W, Lu H, Duan N, Zhang L, Zhang T. Hydrothermal liquefaction of harvested high-ash low-lipid algal biomass from Dianchi lake: effects of operational parameters and relations of products. *Bioresource Technology*, 2015, 184: 336–343
40. Yu G, Zhang Y, Schideman L, Funk T, Wang Z. Distributions of carbon and nitrogen in the products from hydrothermal liquefaction of low-lipid microalgae. *Energy & Environmental Science*, 2011, 4(11): 4587–4595
41. Li H, Liu Z, Zhang Y, Li B, Lu H, Duan N, Liu M, Zhu Z, Si B. Conversion efficiency and oil quality of low-lipid high-protein and high-lipid low-protein microalgae via hydrothermal liquefaction. *Bioresource Technology*, 2014, 154: 322–329