Hydrothermal Liquefaction of Model Compounds Protein and Glucose: Effect of Maillard Reaction on Low Lipid Microalgae

Xiaohan Tang, Chao Zhang and Xiaoyi Yang*
School of Energy and Power Engineering, Beihang University, 37 Xueyuan Road, Haidian District, Beijing, P. R. China
*Corresponding Author

Abstract. Reaction between protein and carbohydrate is important for the hydrothermal liquefaction (HTL) of low lipid microalgae. Model compounds glucose and soy protein were used to simulate the HTL of low lipid microalgae under different conditions. Transfer of element and distributions of compounds were quantified to study the effect of Maillard reaction during HTL. Solid residue was mainly caused by dehydration of glucose. Deamination was the main reaction for protein, although most of the nitrogen was presented in aqueous or gas products, high protein content had bad effects on the quality of bio-crude. Interaction between protein and glucose at optimal ratio could prevent the production of solid residue from glucose and counteract bad effects of protein during HTL, thus promote the quality and yield of bio-crude.

1. Introduction
Microalgae have emerged as feedstocks for bio-energy production due to their high photosynthetic efficiency and fast growth rate [1]. Hydrothermal liquefaction (HTL) is believed as a cost-effective way because it avoids the drying process [2]. Chemical properties of the hydrothermal products mostly depend on the microalgal composition [3]. Current microalgae-to-biofuel technology focus on utilization of lipids rich strains because high lipids content can improve the yield and quality of bio-crude [4,5]. While lipids accumulation for most microalgae requires severe cultivation condition, such as phosphate and nitrogen limitation [6]. Potential conversion methods should prove the ability to convert cheap, low quality feedstock. Thus, the conversion research about low lipid microalgae is required.

Low lipid microalgae are rich in protein and carbohydrate. When protein and carbohydrate processes HTL reaction individually, either of them generated different products; but it may interplay each other and the products thus become complex when they process together [7,8]. The complex process of polymerization during HTL of carbohydrate and protein, named Maillard reaction, generate some polymeric products with different molecular weights, structures and element compositions [9]. Since the Maillard reaction is complex [10,11], it is simple to evaluate the performance of hydrothermal liquefaction by the element distribution and understand the reaction mechanism by the element transfer route instead of characterizing every single compounds of product.

Up to date, Maillard reactions between carbohydrate and protein were mostly investigated in the field of food chemistry, and most of the research was conducted at relative lower temperature of 60~120 °C, which is different from the high react temperature above 200 °C during HTL process. In addition, most of the HTL research focus on the quality and yield of bio-crude. The shortage of information of aqueous and solid products after HTL makes it difficult to understand the reaction mechanism. In this study,
glucose and soy protein were chosen as the model component to simulate low lipid microalgae, the mixture of them at different ratios were run HTL tests from 160 to 280 °C. Effects of reaction conditions on distribution of element and compounds in the bio-crude, aqueous fraction and solid residue were analyzed, respectively. The element and compounds distributions under different reaction conditions may be helped to explore the HTL reaction mechanism and optimizing the reaction process. With better knowledge of the reaction mechanism, the properties of HTL products can be improved by adjusting operating conditions or biomass substrate design.

2. Material and Methods

2.1. Materials

In order to reflect the reaction between carbohydrate and protein in actual microalgae, glucose and soy protein were chosen as the model compounds. Glucose and soy protein were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The feedstocks were used as received.

2.2. Hydrothermal Liquefaction Experiment

HTL experiments were carried out in a 500 mL stainless-steel autoclave batch reactor with a magnetic stirring. The reaction procedure can be found in previous published work [12]. In a typical experiment run, 100 g of feedstock (10 wt.% solid) were added into the reactor. The residue air was replaced by nitrogen gas after reactor was sealed. The reaction time for all the HTL experiments was 60 min. The reaction time didn’t include the heat-up times which took 45-60 min. for the reactor to increase to 280 °C.

2.3. Products Separation and Yields Calculation

After the reaction was finished, the reactor was cooled down to room temperature by compressed air. The reactor was opened and the products mixture was transferred to a flask. 100 mL of dichloromethane (DCM) was poured to the reactor to wash the reactor wall and stirrers and the washed fraction was also transferred to the flask. The reaction mixture was separated by filtration. The filter residue was defined as solid residue, the filtrate can be separated to DCM phase and aqueous phase. The DCM phase after solvent removal was defined as bio-crude, and the aqueous phase got after HTL reaction was dried at 70 °C, the left material was defined as involatiles. The yields of bio-crude, involatiles and solid residue were calculated as the mass of bio-crude, involatiles and solid residue dividing by the mass of dry substrate, respectively.

2.4. Element and Compounds Analysis of Each Fraction

Bio-crude was identified by GC-MS (Agilent 7890A-5975C, USA) equipped with a HP-5 capillary column (30 m × 0.25 mm i.d. × 0.25 μm). The inlet temperature was 250 °C, the split ratio was 10:1 and the flow rate was 1 mL/min. Oven temperature was initially maintained at 50 °C for 5 min. followed by heating to 260 °C (5 °C /min.) and maintained at this temperature for 5 min. Carbon, hydrogen and nitrogen content HTL products were analyzed by an element analyzer (Euro Vector EA3000, Italy), and the oxygen content was calculated by difference. All the analysis was conducted twice and the standard error was less than 0.5 wt.%.

3. Results and Discussion

3.1. HTL Products yields

Effects of temperature and the ratio between protein and glucose on the yields of each HTL products are presented in figure 1. As presented in figure 1 (a), the total recovery of bio-crude, involatiles and solid residue decreased with temperature increasing, which could be caused by the formation of more volatile or gas products at higher temperature. When temperature was increased from 160 °C to 280 °C, the yield of bio-crude gradually increased from 1.38% to 9.06%, while the yields of involatiles and solid residue decreased from 38.16% and 31.68% to 23.86% and 13.19%, respectively. There appeared to be some inter-conversion of material in the solid residue, involatiles and bio-crude during the reaction processes,
the aqueous phase and solid residue were converted into bio-crude phase at higher temperature. It can be deduced that high temperature could elevate products yields of Maillard reaction and promote the transformation of solid residue and aqueous products into bio-crude, while cause more mass loss due to more gas production.

Figure 1. Effect of (a) temperature and (b) ratio between protein and glucose on the yields of HTL products.

Figure 1 (b) depicts the change in yields of HTL products with respect to variant ratios of glucose and protein. The bio-crude and involatiles yields of glucose were much lower than those of protein. The solid residue yield of glucose was 37.2%, while trace of solid residue left for protein (0.97%). Bio-crude and involatiles products were the main products of protein. Over 60% of carbon from glucose of glucose was generated as Hydroxymethylfurfural (HMF) ring structure [13]. Considering the high solid residue of glucose, it can be deduced that dehydration and condensation was the main reaction for glucose during HTL process. The pH of the aqueous, which was measured right after HTL test, was 1.5 (table 1) for pure glucose. That means some acids were produced during the HTL of glucose and thus accelerated the polymerization of dehydration products of glucose. Therefore, we hypothesized that the formation of solid residue during HTL of glucose was caused by the polymerization of furan material, such as 5-HMF, and the formation of solid residue inhibited the transfer of glucose to bio-crude.

| Ratio glucose 0.5:1 1:0:1 1.5:1 2:0:1 3:0:1 4:0:1 5:0:1 protein | pH 1.5 6.4 7.5 7.4 7.3 8.2 8.2 8.3 9.5 |
|----------------------------------------------------------|------------------------------------------|

For the mixture of glucose and protein, the yield of bio-crude increased with the ratio between protein and glucose and achieved maximum of 21.23% at the ratio of 3:1, then decreased slightly. Peterson et al. reported that the reaction between glycine and glucose was correlated with the glucose concentration [14]. This result may suggest that there was an optimum ratio between glucose and protein to achieve the highest yield of bio-crude. The yield of involatiles followed almost the same trend with bio-crude. The involatiles yield, 7.61% for individual glucose, increased with the ratio between protein and glucose and achieved 37.96% at the ratio of 5:1. It seemed like the reaction between glucose and protein can promote the generation of aqueous products compared with individual glucose. The solid residue significantly decreased from 37.15% to 2.30% when the ratio between protein and glucose increased from 0 to 5:1. The presence of protein in HTL process made more solid residue converted to aqueous phase and bio-crude. The results indicated that the reaction between protein and glucose can reduce the reaction rate of polymerization, resulted in decrease of solid residue yield. In addition, the total recovery of bio-crude, solid residue and involatiles increased gradually with the ratio, suggested the decrease of gas yield. Each fraction yields of Maillard reaction could be controlled by adjusting the reaction temperature and the ratio between carbohydrate and protein.

3.2. Characterization of Bio-crude

Bio-crude of HTL was a complicated mixture and various compounds were detected by GC-MS.
Temperature had a significant influence on compounds distribution of Maillard reaction products. At 160 °C, the molecular weight of the products was small and peaks were shown before 10 min, most of which was heterocyclic oxygen, such as furan. With temperature increasing, more N-heterocyclic compounds with heavy molecular weight or complicated structure were generated.

![Figure 2](image-url) Composition of bio-crude got at different (a) temperatures and (b) ratio between protein and glucose.

Chemical compounds characterized by GC-MS were categorized according to the functionalities. All the experiments presented in figure 2(a) were conducted for the mixture of soy protein and glucose at the ratio of 1:1 and the results indicated that reaction temperature significantly affected the chemical composition of bio-crude. About half of the peak area was taken by the heterocyclic oxygen content at 160 °C. With temperature increasing to 200 °C, heterocyclic oxygen content sharply decreased to 6.03% and kept small variation as temperature continuing rising. While the N-heterocyclic content consecutively increased as temperature rising. The N&O heterocyclic content increased from 25.83% to 64.06% as temperature rising from 160 °C to 240 °C, and decreased to 43.62% at 280 °C. These results indicated that most bio-crude was derived from glucose at 160 °C and less protein was decomposed to ammonia acid that can react with glucose at this temperature. With temperature increasing, protein start to decompose, resulted in the higher content of N-heterocyclic and N&O heterocyclic in bio-crude. Heterocyclic oxygen and phenol were the main products in bio-crude from glucose, while straight & branched amides and N&O heterocyclic were the main component in bio-crude from protein HTL due to the generation of amines and aldehydes through decarboxylation and deamination reactions of amino acids. The mixture of glucose and protein generated more N-heterocyclic and N&O heterocyclic but less heterocyclic oxygen than the individual feedstock. The maillard reaction between protein and glucose lead to nitrogen containing cyclic compounds [15]. Most part of the heterocyclic oxygen was furan, such as 5-HMF. Under sub-critical condition, 5-HMF processed via two routes simultaneously: decomposing to gas or polymerizing to char [16]. The results presented in figure 2 (b) suggested that the reaction between glucose and protein at an appropriate ratio could inhibit the polymerization of 5-HMF and thus decreased the solid residue yield. In addition, it also made more protein transfered into bio-crude but not aqueous or gas products during HTL process, which could be deduced from the increase in N-heterocyclic and N&O heterocyclic content of the mixture feedstock.

3.3. Element Recovery
The element recoveries of each fraction for different substrate after HTL (280 °C for 1 hour) are presented in figure 3 and they were calculated as below:
Element recovery of each fraction= (element content of each fraction × yield of each fraction)/the element content of raw material
The element recoveries of glucose and protein were totally different. For glucose, most part of the carbon
was left in solid residue, thus the carbon recovery of bio-crude and involatiles was just 2.25% and 9.51%, respectively. The carbon of protein was mainly recovered in bio-crude (31.49%) and involatiles (32.65%). Little solid residue was generated from HTL of protein, resulted in just 0.75% of carbon recovery. The carbon recovery in bio-crude gradually increased with the ratio between protein and glucose and reached the maximum of 34.57% at the ratio of 3:1, then decreased to 30.68% at the ratio of 5:1. With the ratio between protein and glucose increasing, the carbon recovery of solid residue kept decreasing and the involatiles performed inversely. When glucose mixed with protein, the condensation of glucose reduced, therefore, the solid residue rapidly decreased (figure 1) and the pH of aqueous phase sharply increased. It can be concluded that the reaction between glucose and protein favored the transfer of carbon from the solid residue to gas, bio-crude or aqueous products.

\[ \text{Figure 3. C and N recovery of HTL products.} \]

The study of nitrogen recovery is important for the optimization of HTL process, because nitrogen has become the biggest obstacle during the bio-crude upgrading process. After mixed with glucose, the nitrogen recovery of involatiles was around 40% for all the samples at different ratio of protein and glucose, much higher than that of individual protein (22.80%). The nitrogen recovery of solid residue at the ratio of 0.5:1 (protein: glucose, wt./wt.) was as high as 35.62%. The presence of glucose may inhibit the nitrogen transferred to bio-crude and aqueous. With the ratio between protein and glucose increasing, the nitrogen recovery of solid residue decreased to 1.89% at the ratio of 5:1. Nitrogen recovery of bio-crude performed the same trend with carbon and hydrogen recoveries. The maximum value of nitrogen recovery in bio-crude was 18.53%, gained at the ratio of 3:1. Besides the element recovery of each product fraction, the total recovery contained bio-crude, solid residue and involatiles are also presented in figure 3. The gap between 100% and the total recovery was caused by the gas and volatile products. The gas products were not considered because carbon recovery of gas was less than 10% and over 90% of carbon in HTL gas was CO₂ [17]. The total carbon recovery decreased from 77.99% to 62.09% when the ratio between protein and glucose increased from 0 to 1:1 and then increased with the ratio. Pure glucose had the highest total carbon recovery but the lowest hydrogen recovery. Reactions such as decarboxylation of protein resulted in about 35% loss of carbon.

The total nitrogen recovery of protein was the least (37.50%) compared with other substrates. The exist of glucose increased the total nitrogen recovery. For the mixture feedstock, the highest total nitrogen recovery achieved at the ratio of 0.5 and then decreased with the ratio increasing. The pH of the HTL aqueous for pure protein was 9.5. Yang et al. [18] reported that half of the total nitrogen in the aqueous was determined to be NH₃-N, little NO₃-N or NO₂-N. The NH₃-N caused the high pH of the aqueous and the loss of nitrogen because NH₃ is alkaline and easily soluble in water.

4. Conclusions
Maillard reaction had a predominant role in HTL process of low lipid microalgae. Temperature can affect the severity of Maillard reaction during HTL, and thus determines the products yield. The ratio of glucose and protein determined the distribution of compounds and element among different product...
fractions. Glucose and protein at an appropriate ratio during HTL can inhibit the polymerization of glucose and thus decreased the solid residue yield. The reaction between protein and glucose can promote more C content but less N content transfer from involatiles into bio-crude.

Acknowledgments
This project was supported by National key research and development program (2018YFB1501505).

References
[1] Chisti Y, 2007, Biotechnol. Adv., 25, 294-306.
[2] Peterson A A, Vogel F, Lachance R P, Fröling M, Antal J M J and Tester J W, 2008, Energ. Environ. Sci., 1, 32-65.
[3] Biller P and Ross A B, 2011, Bioresource Technol., 102, 215-225.
[4] Li H, Liu Z, Zhang Y, Li B, Lu H, Duan N, Liu M, Zhu Z and Si B, 2014, Bioresource Technol., 154, 322-329.
[5] Yoo G, Min S P, Yang J W and Choi M, 2015, Appl. Energ., 156, 354-361.
[6] Chen W, Zhang Y, Zhang J, Yu G, Schideman L C, Zhang P and Minarick M, 2014, Bioresource Technol., 152, 130-139.
[7] Teri G, Luo L and Savage P E, 2014, Energ. Fuel, 28, 7501-7509.
[8] Zhang C, Tang X, Sheng L and Yang X, 2016, Green Chem., 18, 2542-2553.
[9] Yaylayan V A and Kaminsky E, 1998, Food Chem., 63, 25-31.
[10] Valdez P J and Savage P E, 2013, Algal Research, 2, 416-425.
[11] Torri C, Garcia L, Samori C, Fabbri D and Brilman D W F W, 2012, Energ. Fuel., 26, 658-671.
[12] Tang X, Zhang C, Li Z, Yang X, 2016, Bioresource Technol., 2016, 202: 8-14
[13] Baccile N, Laurent G, Babonneau F, Fayon F, Titirici M and Antonietti M, 2009, J. Phys. Chem. C., 113, 9644-9654.
[14] Peterson A A, Lachance R P, Tester J W, 2010, Ind. Eng. Chem. Res, 49(5) 2107-2117
[15] Kruse A, Maniam P, Spieler F, 2007, Ind. Eng. Chem. Res., 46(1) 87-96
[16] Chuntanapum A and Matsumura Y, 2009, Ind. Eng. Chem. Res., 48, 9837-9846.
[17] Yu G, Zhang Y, Schideman L, Funk T and Wang Z, 2011, Energ. Environ. Sci., 4, 4587.
[18] Yang Y F, Feng C P, Inamori Y and Maekawa T, 2004, Res. Cons. Recyc., 43, 21-33.