# Biocrude oil production from *Chlorella* sp. cultivated in anaerobic digestate after UF membrane treatment

Wang Meng[1], Wang Xinfeng[1], Zhu Zhangbing[1], Lu Jianwen[1], Yuanhui Zhang[1,2], Li Baoming[1], Lu Haifeng[1], Duan Na[1], Zhang Dongming[3], Dong Taili[3], Liu Zhidan[1*]

(1. *Laboratory of Environment-Enhancing Energy, and Key Laboratory of Agricultural Engineering in Structure and Environment, Ministry of Agriculture, College of Water Resources and Civil Engineering, China Agricultural University, Beijing 100083, China*;
2. *Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*;
3. *Shandong Minhe Biological Technology Co., Ltd, Penglai 265600, China*)

**Abstract:** Algae cultivation in animal wastewater could recover nutrient resources, and harvest considerable amount of algae biomass for biofuel conversion. In this study, *Chlorella* sp. cultivated in ultrafiltration (UF) membrane treated anaerobic digestion effluent of chicken manure was converted into biocrude oil through hydrothermal liquefaction (HTL). The potential of biocrude production from grown *Chlorella* sp. was studied through changing the operational conditions of HTL, i.e., holding temperature (HT, 250°C-330°C), retention time (RT, 0.5-1.5 h), and total solid (TS) (15 wt%-25 wt%) of the feedstock. The highest biocrude oil yield was 32.9% at 330°C, 1.5 h and 20 wt% TS. The single factor experiments of HT also suggested that the biocrude oil yield decreased when the temperature was higher than 330°C. There were no significant differences of elemental contents in biocrude samples. The maximum higher heating values (HHV) of *Chlorella* sp. biocrude was 40.04 MJ/kg at HT of 330°C, RT of 1 h and TS of 15 wt%. This study suggests the great potential for energy recovery from *Chlorella* sp. cultivated in UF membrane treated anaerobic digestion effluent via HTL.

**Keywords:** microalgae, wastewater, hydrothermal liquefaction, anaerobic digestion effluent
**DOI:** 10.3965/j.ijabe.20171001.2700

**Citation:** Wang M, Wang X F, Zhu Z B, Lu J W, Zhang Y H, Li B M, et al. Biocrude oil production from *Chlorella* sp. cultivated in anaerobic digestate after UF membrane treatment. Int J Agric & Biol Eng, 2017; 10(1): 148–153.

## 1 Introduction

Biofuel recovery via hydrothermal liquefaction (HTL) is attracting an increasing attention, partly because of its efficient conversion of wet feedstock, like microalgae, to renewable energy[1]. Meanwhile, wastewater can be cleaned via algae cultivation[2], since microalgae has the specific advantages of tremendous environmental benefits through the capture of $CO_2$ and nutrients[3,4].

Algae cultivation in cleaning wastewater like municipal wastewater[5,6], industrial effluent[7], animal wastewater[8,9] and anaerobic digestion effluent (AD effluent)[10-12] harvested considerable amount of algae biomass, generally ranged from 0.37 g/L to 4.3 g/L[11] due

---

**Received date:** 2016-09-28 **Accepted date:** 2016-12-02

**Biographies: Wang Meng**, Master student, research interests: bioenergy engineering, Email: wang2014@139.com; **Wang Xinfeng**, PhD student, research interests: algae technology, Email: xinfengw@cau.edu.cn; **Zhu Zhangbing**, PhD student, research interests: biomass energy, Email: zhuzhangbing@163.com; **Lu Jianwen**, PhD student, research interests: biomass energy, Email: jianwenlu2012@126.com; **Yuanhui Zhang**, PhD, Professor, research interests: environment-enhancing energy, Email: yzhang1@illinois.edu; **Li Baoming,** PhD, Professor, research interests: agricultural bio-environment engineering, Email: libm@cau.edu.cn; **Lu Haifeng**, PhD, Lecturer, research interests: wastewater treatment, Email: hflu@cau.edu.cn; **Duan Na,** PhD, Associate Professor, research interests: biogas engineering, Email: duanna@cau.edu.cn; **Zhang Dongming**, Engineer, research interests: biogas engineering, Email: 13583549333@163.com; **Dong Taili**, Engineer, research interests: biogas engineering, Email: dongtaili@126.com.

***Corresponding author: Liu Zhidan**, PhD, Associate Professor, research interests: bioenergy and environment-enhancing energy, Mailing address: No.17 Qinghua East Road, Haidian District, Beijing 100083, China. Tel/Fax: +86-10-62737329; Email: zdliu@cau.edu.cn.

to different cultivating conditions. The algae biomass is a potential feedstock for biofuel production via HTL.

Accordingly, hydrothermal conversion of wastewater-cultivated microalgae indicated its high potential of biocrude oil production and environmental benefits both at home and abroad[13-20]. Wastewater mainly came from municipal wastewater treatment plant[13-16], only few researches focused on eutrophic freshwater[21] or post HTL wastewater[17]. However, there is little information available in literature about HTL of microalgae cultivated in AD effluent.

In this study, *Chlorella* sp. cultivated in AD effluent of chicken manure after UF membrane treatment was converted into biocrude oil via HTL. The optimum reaction parameters on HTL of this feedstock were investigated through an orthogonal experimental design. The potential for energy recovery from *Chlorella* sp. cultivated in UF membrane treated AD effluent via HTL was also investigated.

## 2 Materials and methods

### 2.1 Materials

The microalgae *Chlorella* sp. initially obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, was selected for this study due to its high productivity in land-based systems, resistance to high ammonia nitrogen, tolerance to environmental fluctuations, and bio-chemical profile suited to the development of bio-products. This microalga was cultivated in raceway pond in AD effluent in a greenhouse with an average room temperature of 22°C, an average water temperature of 19.6°C, 15% $CO_2$ aeration rate of 1 L/min, light intensity of 1600 lx to 9000 lx, and impeller rotating speed of 60 r/min. The *Chlorella* sp. was harvested every two days once its biomass concentration reached 1 g/L through filtration, centrifugation, drying to stable weight and then stored at room temperature in a laboratory glassware-desiccator.

The AD effluent was provided by Shandong Minhe Biological Technology Co., Ltd. (Penglai, China), of which suspended solids were removed via deposition. The wastewater was further treated by UF membrane, and diluted by adding 93% freshwater to get a suitable ammonia nitrogen concentration for algae cultivation. As a result, the concentrations of ammonia nitrogen, total nitrogen and total organic carbon in the effluent were 250 mg/L, 261 mg/L and 100 mg/L, respectively.

The HTL experiments were performed in a 100 mL batch reactor (Model 4593, Parr Instrument Company, Moline, Illinois, USA) according to a procedure in literature[21].

### 2.2 Analyses of feedstock and HTL products

Before HTL experiments, the characteristics of the feedstock were analyzed. Ash content of this *Chlorella* sp. was analyzed as dry residue at (105±2)°C and the combustion residue at 575°C. The crude fat was measured using the Soxhlet extraction method, and the crude protein was measured using the Kjeldahl method. The crude fiber of algae, such as acid detergent fiber, neutral detergent fiber and lignin were analyzed using the methods of Van Soest[21]. Non-fibrous carbohydrate was calculated by difference.

Elemental contents of C, H, and N of samples were determined using an Elemental Analyzer (Vario MICRO Cube, Elementar Analysensysteme GmbH, Germany), elemental content of O was calculated by difference. The samples were dried in (105±2)°C for 12 h to stable weight prior to measurement[21]. All measurements were in triplicate, and the average values were used. Helium and oxygen gases were used in elemental analysis during operation for proper condition, and the working pressure of these gases were 0.12 MPa and 0.2 MPa in room temperature, respectively. Analytical methods used in this study were described in a previous study[21].

HHV (Model 6200, Parr Instrument Co., Moline, Illinois, USA). The samples were dried in (105±2)°C for 12 h to stable weight prior to measurement[21]. To compare with the HHVs of biocrude oil in the literature, HHVs (MJ/kg) based on Dulong formula as Equation (1)[17,19,21] below were also mentioned. In the formula, $C$, $H$ and $O$ were the weight percentages of carbon, hydrogen, and oxygen in the feedstock and biocrude oil, respectively.

$$HHV = 0.338C + 1.428\left(H - \frac{O}{8}\right) \qquad (1)$$

Yields of HTL products were based on Equations (2) and (5), in which *M* is the mass of HTL products or feedstock as marked in subscripts. Dry and ash-free based biocrude oil yield was defined by Equation (6), to compare with that of reported literature as well. Liquefied fraction indicating HTL efficiency was defined as the difference between the mass of feedstock and the solid residue (Equation (7)). Energy recovery was defined as the ratio of HHV of biocrude oil against that of the algae feedstock, which was calculated using Equation (8).

$$\text{Solid residue (\%)} = \frac{M_{\text{solid residue}}}{M_{\text{feedstock}}} \times 100 \qquad (2)$$

$$\text{Biocrude oil yield (\%)} = \frac{M_{\text{biocrude oil}}}{M_{\text{feedstock}}} \times 100 \qquad (3)$$

$$\text{Gasesous phase (\%)} = \frac{M_{\text{gas}}}{M_{\text{feedstock}}} \times 100 \qquad (4)$$

$$\text{Aqueous phase (\%)} = 100 - \text{Gases} - \text{Biocrude oil yield} - \text{Solid residue} \qquad (5)$$

$$\text{Biocrude oil yield (daf\%)} = \frac{\text{Biocrude oil yield}}{(1 - \text{Ash})} \qquad (6)$$

$$\text{Liquefied feaction (\%)} = 100 - \text{Solid residue} \qquad (7)$$

$$\text{Energy recovery (\%)} = \frac{HHV_{\text{biocrude oil}} M_{\text{biocrude oil}}}{HHV_{\text{feedstock}} M_{\text{feedstock}}} \times 100 \qquad (8)$$

## 3 Results and discussion

### 3.1 Feedstock characterization

Physio-chemical characteristics of feedstock were listed in Table 1. The data showed that *Chlorella* sp. cultivated on AD effluent was a low-lipid, high-protein feedstock containing 13.08% of ash. It was high in quality as compared with algae harvested in eutrophic freshwater[21] or municipal wastewater[14] for their higher ash and lower lipid contents. The high protein content resulted from high ammonia nitrogen content, for that low-protein microalgae always resulted from lower nitrogen content in cultivation medium[7].

**Table 1 Physico-chemical characteristics of feedstock**

| Components | *Chlorella* sp. |
|---|---|
| Base | Dry based |
| Ash/% | 13.08±0.85 |
| Crude lipid/% | 5.40±0.21 |
| Crude fiber/% | 10.04±0.19 |
| Crude Protein/% | 53.84±0.69 |
| Non-fibrous carbohydrate/% | 17.64±1.71 |
| HHV/MJ·kg$^{-1}$ | 21.25±0.08 |

### 3.2 Production of biocrude oil from *Chlorella* sp. via HTL

The key operational parameters including holding temperature (HT, 250°C-330°C), retention time (RT, 0.5-1.5 h), and total solid content (TS, 15 wt%-25 wt%) were considered as the important factors in the HTL process[23]. The experimental results of HTL of *Chlorella* sp. conversion are listed in Table 2, focusing on liquefied fraction, oil yield, elemental content and HHV. The results suggested that the highest biocrude oil yield was achieved at 330°C, 1.5 h and 20% TS.

**Table 2 HTL conversion efficiency and characterization of biocrude oil obtained under optimal conditions (initial pressure=2.5 MPa) (*n*=3)**

| No. | HT/°C | RT/h | TS/ wt% | Liquefied fraction/% | Biocrude oil yield/%daf | HHV/MJ·kg$^{-1}$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | Calculated | Tested |
| 1 | 250 | 0.5 | 15 | 76.94±0.62 | 20.46±1.01 | 37.73±0.02 | 34.60±0.22 |
| 2 | 250 | 1.0 | 20 | 78.44±1.41 | 24.51±1.16 | 38.67±0.95 | 36.18±0.24 |
| 3 | 250 | 1.5 | 25 | 80.73±1.73 | 28.69±1.75 | 37.28±0.18 | 36.83±1.03 |
| 4 | 290 | 0.5 | 20 | 83.84±0.09 | 29.57±0.37 | 37.62±0.28 | 37.35±0.76 |
| 5 | 290 | 1.0 | 25 | 82.79±0.19 | 30.68±0.23 | 38.94±0.66 | 37.01±0.81 |
| 6 | 290 | 1.5 | 15 | 80.68±0.60 | 30.29±2.27 | 39.90±0.11 | 36.52±0.28 |
| 7 | 330 | 0.5 | 25 | 84.70±0.60 | 30.75±0.73 | 40.01±0.11 | 38.62±0.96 |
| 8 | 330 | 1.0 | 15 | 84.70±0.40 | 32.50±0.41 | 39.95±0.12 | 38.01±0.22 |
| 9 | 330 | 1.5 | 20 | 84.17±0.40 | 32.90±0.24 | 39.56±0.30 | 36.77±0.92 |

Note: C, H, N, and O are carbon, hydrogen, nitrogen, oxygen content, respectively. HHVs of feedstock and biocrude oil were both discussed in two definitions, the calculated version based on the elemental content by Dulong formula, and the tested version was analyzed using a bomb calorimeter mentioned in Section 2.2.

To analyze the effect of key operational parameters in all HTL products, the range analysis of orthogonal experiment was conducted as shown in Figure 1. Gaseous product was hardly influenced by these three parameters with range less than 0.86%, because the gasification merely occurred at 320°C or higher[24].

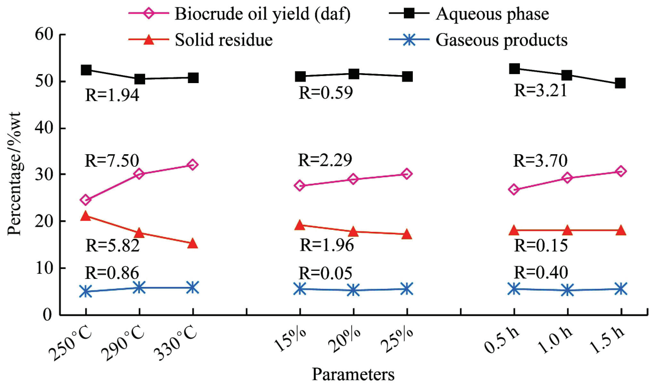


Figure 1 Range analysis of orthogonal experiment for HTL products (R: range)

As temperature rising, the amount of aqueous products had a much smaller range (1.94%) as compared to those of biocrude oil and solid residue (7.50% and 5.82%). Biocrude oil and solid residue had totally an opposite trend. With temperature increased from 250°C to 330°C, a higher production yield of biocrude oil was achieved, while the quantity of solid residue decreased.

When TS of feedstock increased from 15% to 25%, higher biocrude oil yield and lower solid residue were achieved, the same trend was as their performance as in rising temperature. With increase of TS, the aqueous yield increased in the first stage of 15%-20% TS, and then decreased in the second stage of 20%-25% TS, they were both in very little range less than 0.59%, compared with that of biocrude oil yield in 2.29%. This suggested that increasing TS had a limited effect on aqueous product yield. Meanwhile, TS had less influence than HT in biocrude oil production.

It was observed that a long retention time cannot achieve a higher liquefied fraction. And in this process, small compounds in aqueous phase recombined into long-chain compounds in biocrude oil[25].

The orthogonal experimental suggested that the increase of HT had a more remarkable influence on the performance of HTL, achieving a higher biocrude oil yield up to 7.5 wt%. However, there was no necessary relationship between TS and the biocrude oil yield[21]. According to the literature, more issues related to mass transfer, thermochemical conversion and energy consumption may occur if the TS are too high, whereas the volume efficiency of the HTL reactor is reduced if TS is too low[18]. Increasing RT may result in gasification and achieve higher biocrude oil yield, however, with lower quality.

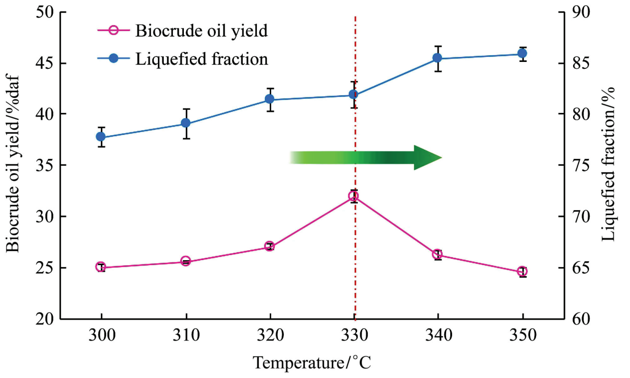


Figure 2 HTL conversion efficiency under different temperatures (TS=15%, RT=60 min, initial pressure of HTL=2.5 MPa)

To explore whether temperature still had a remarkable influence on the performance of HTL when it is higher than 330°C, a single factor experiment of temperature was conducted. The experimental results showed that the highest biocrude oil yield was achieved at 330°C, the same temperature as that obtained from orthogonal experiments. However, in the second stage of 330°C-350°C, the amount of liquefied fraction continued to increase while that of biocrude oil decreased. According to literature[24], gasification occurs when temperature is higher than 320°C and low molecule hydrocarbon gases (e.g., $CH_4$, $C_2H_6$, etc.) are produced.

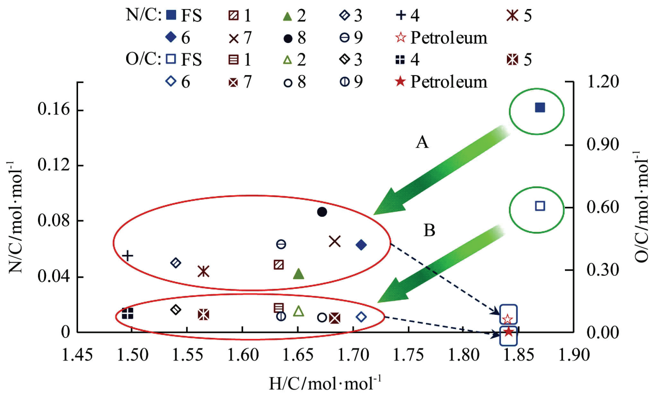
### 3.3 Characterization of biocrude oil

#### 3.3.1 HHVs of biocrude oil

Table 2 shows the results of elemental analysis, and HHVs of feedstock and the biocrude oil produced in orthogonal experiments. To compare with those in literatures[17,19,20], HHVs of feedstock and biocrude oil were discussed in two definitions, the calculated version based on the elemental content (Equation (1)), and the tested version that was analyzed using a bomb calorimeter. The tested HHVs were lower than the calculated values based on Dulong formula. One reason, ash content (metal-salt, for example) of biocrude oil has no contribution to HHV, even absorbs heat, when samples was tested. Compared to HHVs of 33.3-39 MJ/kg calculated in the literatures[14,15,17,21] of biocrude oil from wastewater-cultivated algae, the highest calculated HHV of biocrude oil from *Chlorella* sp. cultivated in AD effluent has a better performance at 40 MJ/kg, while the tested version of the same sample is 38.62 MJ/kg.

### 3.3.2 Deoxygenation/denitrogenation during HTL

Deoxygenation and denitrogenation during HTL are two important reactions in order to understand the formation of biocrude oil from harvested algae biomass through HTL. Figure 3 illustrates the relations of H/C, N/C and O/C of biocrude oil under different conditions via a Van Krevelen diagram. Through a hydrothermal conversion, the algae achieved lower N/C and O/C ratios, but H/C ratio did not increase as expected. No.6 (290°C, 90 min, 15% TS) achieved the highest H/C and lower O/C ratios, while No.2 (250°C, 60 min, 20% TS) achieved the lowest N/C ratio. Typical oxygen content of petroleum is less than 0.01%, much lower than that of the biocrude oil. Thus deoxygenation is a main task in converting biomass into hydrocarbon fuels. In deoxygenation, oxygen could be preferentially removed as $H_2O$ through dehydration, and $CO_2$ and CO through decarboxylation. The high nitrogen content of the biocrude oil is contributed by the high protein content of algae feedstock, which affects the properties of biocrude oil, such as smell and combustion. The reforming of nitrogen via HTL has the advantage of reducing the potential for $NO_x$ emissions during the combustion of biocrude oil[18].



Note: A: Denitrogenation during algae HTL; B: Deoxygenation during algae HTL. The arrows designate the changes of H/C, O/C and N/C from algae feedstock to algae biocrude oil through HTL.

Figure 3 Van Krevelen diagram of feedstock, petroleum and biocrude oil under different operational conditions (FS: feedstock)

## 3.4 Energy recovery

Energy recovery is an important parameter to determine the reaction efficiency of HTL. The energy converted from feedstock was mainly stored in the biocrude oil during the HTL[26], while little of them was stored in gases, solid residue and aqueous phase. Figure 4 illustrates the energy recovery of HTL from AD effluent cultivated *Chlorella* sp. The HHV and the biocrude oil yield are two primary factors affecting the energy efficiency of HTL. The highest energy recovery of biocrude oil from *Chlorella* sp. was 56.92% according to Equations (3), (6) and (8), and HHV tested values in Tables 1 and 2 under the optimal conditions in run No.9. Apparently, HTL process conditions affected the energy recovery of biocrude oil, because the value of energy recovery based on yields and the HHVs of biocrude oil. Holding temperature have main impact on the energy recovery due to higher energy consume during reaction process.

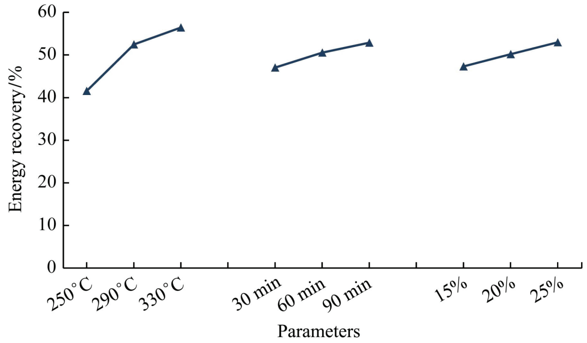


Figure 4 Range analysis of energy recovery for biocrude oil

## 4 Conclusions

This study demonstrated that *Chlorella* sp. cultivated in AD effluent can be efficiently converted to biocrude oil via HTL. The highest biocrude oil yield was 32.9% at 330°C, 90 min retention time and 25% TS. The operational conditions for the highest oil yield were not the same as those for the best oil quality. The yield and HHV of biocrude oil were higher than those in previous reports using algae grown in wastewater except for catalytic conversion. This study suggested that HTL coupled with algae cultivation is a potential approach for recovering energy and nutrients from AD effluent.

## Acknowledgements

This study was financially supported by Natural Science Foundation of China (U1562107, 51576206), Beijing Science and Technology Program (Z161100001316009) and Beijing Youth Top-notch Talents Program (2015000026833ZK10). Mr. Wang Meng would like to thank Shandong Minhe Biological Technology Co., Ltd. (Penglai, China) for the supply of UF membrane treated anaerobic digestion effluent of chicken manure.

## [References]

[1] Savage P E. Algae under pressure and in hot water. Science, 2012; 338(6110): 1039–1040.

[2] Zhu L D, Wang Z M, Shu Q, Takala J, Hiltunen E, Feng P, et al. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. Water Research, 2013; 47(13): 4294–4302.

[3] Biller P, Ross A B. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. Bioresource Technology, 2011; 102(1): 215–225.

[4] Zhou D, Zhang S C, Fu H B, Chen J M. Liquefaction of macroalgae enteromorpha prolifera in sub-/supercritical alcohols: direct production of ester compounds. Energy & Fuels, 2012; 26(4): 2342–2351.

[5] Sreesai S, Pakpain P. Nutrient recycling by chlorella vulgaris from septage effluent of the Bangkok city, Thailand. ScienceAsia, 2007; 33(3): 293.

[6] Sydney E B, Da Silva T E, Tokarski A, Novak A C, De Carvalho J C, Woiciecohwski A L, et al. Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. Applied Energy, 2011; 88(10): 3291–3294.

[7] Tsolcha O N, Tekerlekopoulou A G, Akratos C S, Bellou S, Aggelis G, Katsiapi M, et al. Treatment of second cheese whey effluents using a Choricystis-based system with simultaneous lipid production. Journal of Chemical Technology & Biotechnology, 2016; 91(8): 2349–2359.

[8] Chen R, Li R, Deitz L, Liu Y. Freshwater algal cultivation with animal waste for nutrient removal and biomass production. Biomass and Bioenergy, 2012; 39: 128–138.

[9] Wang L, Wang Y, Chen P, Ruan R. Semi-continuous cultivation of *chlorella vulgaris* for treating undigested and digested dairy manures. Applied Biochemistry and Biotechnology, 2010; 162(8): 2324–2332.

[10] Liu Z, Liu Y. Synergistic integration of electrocoagulation and algal cultivation to treat liquid anaerobic digestion effluent and accumulate algal biomass. Process Biochemistry, 2016; 51(1): 89–94.

[11] Cheng J, Ye Q, Xu J, Yang Z, Zhou J, Cen K. Improving pollutants removal by microalgae *Chlorella* PY-ZU1 with 15% $CO_2$ from undiluted anaerobic digestion effluent of food wastes with ozonation pretreatment. Bioresource Technology, 2016; 216: 273–279.

[12] Kim G, Yun Y, Shin H, Kim H S, Han J I. *Scenedesmus*-based treatment of nitrogen and phosphorus from effluent of anaerobic digester and bio-oil production. Bioresource Technology, 2015; 196: 235–240.

[13] Wang Z, Adhikari S, Valdez P, Shakya R, Laird C. Upgrading of hydrothermal liquefaction biocrude from algae grown in municipal wastewater. Fuel Processing Technology, 2016; 142: 147–156.

[14] Chen W T, Zhang Y H, Zhang J X, Yu G, Schideman L C, Zhang P, et al. Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil. Bioresource Technology, 2014; 152: 130–139.

[15] Roberts G W, Fortier M P, Sturm B S M, Stagg-Williams S M. Promising pathway for algal biofuels through wastewater cultivation and hydrothermal conversion. Energy & Fuels, 2013; 27(2): 857–867.

[16] Neveux N, Magnusson M, Mata L, Whelan A, de Nys R, Paul N A. The treatment of municipal wastewater by the macroalga *Oedogonium* sp. and its potential for the production of biocrude. Algal Research, 2016; 13: 284–292.

[17] Zhou Y, Schideman L, Yu G, Zhang Y. A synergistic combination of algal wastewater treatment and hydrothermal biofuel production maximized by nutrient and carbon recycling. Energy & Environmental Science, 2013; 6(12): 3765.

[18] Tian C, Li B, Liu Z, Zhang Y, Lu H. Hydrothermal liquefaction for algal biorefinery: A critical review. Renewable and Sustainable Energy Reviews, 2014; 38: 933–950.

[19] Brown T M, Duan P, Savage P E. Hydrothermal liquefaction and gasification of *nannochloropsis sp*. Energy & Fuels, 2010; 24(6): 3639–3646.

[20] Zhou D, Zhang L A, Zhang S C, Fu H, Chen J. Hydrothermal liquefaction of macroalgae enteromorpha prolifera to bio-oil. Energy & Fuels, 2010; 24: 4054–4061.

[21] Tian C Y, Liu Z D, Zhang Y H, Li B, Cao W, Lu H, et al. 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.

[22] Li H, Liu Z, Zhang Y, Li B, Lu H, Duan N, et al. 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.

[23] Zou S P, Wu Y L, Yang M D, Li C, Tong J. Bio-oil production from sub- and supercritical water liquefaction of microalgae *Dunaliella tertiolecta* and related properties. Energy & Environmental Science, 2010; 3(8): 1073–1078.

[24] Duan P, Savage P E. Hydrothermal liquefaction of a microalga with heterogeneous catalysts. Industrial & Engineering Chemistry Research, 2011; 50(1): 52–61.

[25] Toor S S, Rosendahl L, Rudolf A. Hydrothermal liquefaction of biomass: A review of subcritical water technologies. Energy, 2011; 36(5): 2328–2342.

[26] Duan P G, Savage P E. Catalytic hydrotreatment of crude algal bio-oil in supercritical water. Applied Catalysis B: Environmental, 2011; 104(1-2): 136–143.