Effects of Electro-fermentation on Increasing Lipid Extraction from Schizochytrium

Kaiguo Huang¹, Lu Wang², Weikun Tian¹, Dian Wang¹, Yue Shi¹,³ and Kun Zhang¹*

¹College of Power and Energy Engineering, Harbin Engineering University, Harbin, Heilongjiang Province, China
²CSSC Cruise Technology Development Co., Ltd. Shanghai, China
³Qingdao HEU Zhenghe Environmental Protection Technology Co., Ltd., Qingdao, Shandong Province, China
Email: 664292904@qq.com

Abstract. In this study, electro-fermentation was used as one of the ways to break the wall of microalgal biomass. Schizochytrium is a marine microalga rich in lipids. Chloroform and methanol are excellent solvents for extracting lipids from microalgae in that they can surmount the obstacles of cell walls, but they are too contaminated and exorbitant and are not suitable for large-scale fuel production. For the purpose of improving the extraction rate of microalgae lipid, utilizing electro-fermentation, the crude lipids content was increased by 55.6%, the lipids extraction efficiency was increased by 1373% after extraction with a series of solvents. Compared with 100% Bligh & Dyer, 80% lipids yield can be obtained by using only a small amount of chloroform, methanol and isopropanol. This study demonstrates that electro-fermentation could be an effective and energy-saving method of increasing lipid extraction efficiency from Schizochytrium.

1. Introduction
To explore sustainable energy and less energy consumption [1, 2], the development of the global industry with its vast demand for fossil energy has stimulated the biofuel that excites scientists interest [3]. Carbon-neutral biofuel as a promising alternative green energy is expected to offer new opportunities to fossil fuels and developed at a large scale to cope with energy shortage and global warming [4]. The microalgae of high weight ratio of lipids and growth rate for the production of renewable biodiesel provide an excellent feed stock.

Microalgae are a versatile energy plant that produces plentiful lipids for sustainable biofuel production. Schizochytrium is a kind of marine microalgae rich in lipids. Due to its high lipid content (40-75% of dry weight) [5], and mainly exists in heterotrophic industrial fermentation in the form of triacylglycerols (TAGs), owing to its high DHA content, so it is beneficial to health [6]. After further processing, oils extracted can also become excellent biodiesel raw materials.

In order to efficiently extract intracellular lipids, it is essential to disrupt the protective barrier of the cytoderm. Anaerobic digestion (AD) can be regarded as a rising microalgae cell disruption technique, thanks to the dominating components of the microalgae cell wall such polysaccharide and diverse glycoproteins that can be hydrolyzed by anaerobic germ [7]. In the hydrolysis stage of AD, specific hydrolytic ferments can be produced by related anaerobic microorganisms to biodegradable
complex high-molecular substances such as proteolytic enzymes hydrolyzing proteins into peptides and amino acids, while carbohydrates are hydrolyzed into sugars by carbohydrase [8].

Recently, the MEC-based electro-fermentation system utilizes electrochemistry to affect the microbial fermentative metabolism, which is a new technology that enhances the fermentation through the regulation of redox potentials (triggered by the application of a small voltage). Electro-fermentation has been successfully applied to Scenedesmus fermentation and recovery of organics, phosphorus from Fe-based CEPS sludge [9, 10]. Electro-fermentation increases protein degradation by 4 times compared with SF, and it improved the wet extraction efficiency by 3 folds utilizing non-toxic solvents [10]. Electro-fermentation is a hopeful method to enhance the biodegradation of complex organic substrates. However, despite some good results, little concern has been gotten to the research of microalgae increasing the degradation rate of anaerobic hydrolysis stage to achieve the accumulation of lipids through electro-fermentation. The latest methods to make lipids extraction more sustainable and energy-saving include solvent-free extraction or "green" solvents (e.g. hexane and isopropanol). While avoiding contamination, the yields of these methods are relatively low, although cell fragmentation is effective. B & D has considered to the "gold standard" solvent that is a mixture of chloroform and methanol, which can completely extract all lipids from tissues.

In this study, electro-fermentation system was used to promote the destruction of microalgae cells. Schizochytrium was used as the feedstock. The purpose of this work was to develop a cost-effective, powerfrugal, and environmentally cell-breaking mean in combination with green and non-toxic solvents for cell disruption to facilitate lipids extraction from microalgae.

2. Materials and Methods

2.1. Feedstock and Bacteria

Anaerobic sludge was purchased from Wenchang Sewage Treatment Plant in Harbin City, Heilongjiang Province, China. The total suspended solids (TS) and volatile solids (VS) were 32.0 g / L and 18.3 g / L, respectively. Schizochytrium is from Qingdao Xuneng Biological Engineering Co., Ltd, Shandong, China. The total suspended solids (TS) and volatile suspended solids (VSS) of Schizochytrium were 7.7 g / L and 7.2 g / L, respectively. The volume ratio of anerobic sludge: Schizochytrium biomass was 1:10 (10 mL inoculum: 240 mL Schizochytrium biomass).

2.2. The Electro-fermentation Set-Up and Operation

The H-type reactor consists of an anode and a cathode chamber, connected by titanium wires and separated by a PEM (Nafion 117, DuPont, USA). The working volume was 250ml and the cross section of PEM was 19.63cm². Two carbon felts were used as the electrodes (5*6 cm²). In the start-up process, anaerobic sludge and synthetic media were used as inoculums and substrates to enrich anode in MFC. When the exoelectrogens accumulated on the anode surface, an iterative maximum voltage was obtained. The EF reactor was fed Schizochytrium in fed-batch mode and a DC power source was supplied at 0.6 V. The cathode solution was 50mM NaCl solution without PBS. The anode solution in EF was continuously stirred with a magnetic stirrer (400 rpm). Before operation, nitrogen (99.999%) was used to spray at least 5 minutes on the Schizochytrium and inoculums to remove dissolved oxygen. Record the current of the EF reactor by measuring the voltage drop on the resistance (10 Ω) every 5 minutes using a data recorder.

Used as a control reactor, a serum bottle with a 250 mL work volume was fed with the same Schizochytrium and inoculum and operated as a routine fermentation reactor. The control reactors were well mixed with an incubator shaker at 150 rpm and kept at 37±1 °C.

2.3. Analytical Methods

Soluble chemical oxygen demand (SCOD) was determined in accordance with the Standard Methods. Lipid extraction followed the methods of Lai [11]. The solvents were Bligh and Dyer (Chloroform: methanol:water= 1:2:0.8, V/V), Folch (Chloroform: methanol = 2:1, V/V), H & I (Hexane: methanol:water= 2:1:0.8, V/V), and B & D (e.g. hexane and isopropanol). While avoiding contamination, the yields of these methods are relatively low, although cell fragmentation is effective. B & D has considered to the "gold standard" solvent that is a mixture of chloroform and methanol, which can completely extract all lipids from tissues.
Isopropanol = 1:1, V/V), Hexane and Isopropanol. The mixture was vortexed for 3 hours at room temperature using a vortex mixer. The weight of crude fat was obtained by subtracting the total dry weight from the empty tube weight.

2.4. Determination of Lipid Extraction Efficiency
The effect of electro-fermentation on the lipid extraction efficiency of Schizochytrium was determined by comparing the extracted lipid content before and after fermentation. The enhanced lipid extraction efficiency ($\eta$) was calculated based on equation (1).

$$\eta = \frac{r_{\text{lipid,fermentation}} - r_{\text{lipid,control biomass}}}{r_{\text{lipid,control biomass}}} \times 100\%$$

3. Results and Discussion

3.1. Properties of Microalgal Biomass Feedstock after Electro-fermentation
As revealed in figure 1, SCOD of all groups presented the same trend throughout the fermentation process. For electro-fermentation, the SCOD contents tardily increased in the first 3 days, then increased rapidly in the next 5 days. However, for batch fermentation, the SCOD contents increased gently during the first six days and then suddenly increased during the last two days. The tendency that SCOD usually increased throughout the whole digestion process was the same with some reports [12]. The final SCOD concentrations of electro-fermentation and batch fermentation were 3970 and 4645 mg/L, respectively. The related hydrolytic bacteria grew slowly in the earlier phase, and microalgae are complex substrates not easily to be hydrolyzed. These results indicate that the electrochemically adjusted extracellular and intracellular redox potentials in electro-fermentation enhance the hydrolysis ability of the fermenters, enabling the rapid hydrolysis of organic components such as carbohydrates and proteins, and shortens the reaction time. Compared with the batch fermentation, SCOD contents of electro-fermentation increased to 17.0%, increasing the degree of microalgae cell disruption.

3.2. Comparison of Lipids Recovery before and after Electro-fermentation
On account of the thick and stiff cell walls of microalgae, the extent of cell wall destruction may directly affect lipids extraction efficiency. In this study, electro-fermentation and batch fermentation were used, and it compared the lipids extraction efficiency of Schizochytrium that they work upon. Figure 2a displays that the lipid recovery correlates with electro-fermentation and batch fermentation and a series of solvents. Compared to control group, electro-fermentation improved crude-lipid recovery about 55.6%, 11.0%, 15.2%, 4.7% and 17.6%, while batch fermentation improved about 53.2%, 1.6%, 6.89%, -11.5% and 3.5% for B&D, Folch, H&I, hexane, and isopropanol, respectively. Electro-fermentation improved accessibility of these green solvents (e.g., hexane and isopropanol) to the lipids. B&D is considered to the strong solvents are compounds of chloroform and methanol that can completely extract all lipids in the tissue. As shown in figure 2b, the lipid extraction efficiency of electro-fermentation ($\eta$=1373%) was obtained at B&D compared to control biomass, while $\eta$=713% was obtained at isopropanol, probably by reason of the mass transfer limitation caused by electro-fermentation, the enhancement of lipids extraction was remarkable. However, chloroform is a hazardous, toxic, and expensive solvent. Isopropanol is a green solvent suitable for electro-fermentation, which can potentially replace toxic solvents.

This may be thanks to the biological decomposition of the microalgae cell wall during fermentation, resulting in cell leakage, thus facilitating lipids extraction owing to easier solvent penetration. However, the mechanism of the cell damage caused by electro-fermentation remains unclear. Therefore, further research is needed to reveal this mechanism, which is the next step in our future research.
3.3. Solvent Requirement Reduced by Electro-fermentation

The B&D solvent plays a key effect in solubilizing lipids from the membrane matrix. Reducing the use of toxic solvents can reduce environmental pollution. Figure 3 shows that the crude lipids extracted increased with the increase of B&D solvent volume ratio in B&D + isopropanol compounds. An apparent excellence of using electro-fermentation is that it decreased the quantity of B&D solvent demanded to get a majority of lipids yield. For the EF-treated group, the lipids yield received by appending 33.3% B&D was 80% of the lipids yield obtained by extraction with 100% B&D. Therefore, Electro-fermentation significantly reduced the quantity for toxic B&D solvents in order to obtain satisfactory yields, which indicated that electro-fermentation destroyed the intermolecular forces and made it easier for green solvents to extract lipids.

Furthermore, figure 4 displays that electro-fermentation lessens the vortex time by approximately 36 folds to obtain the same recovery of lipids. The downstream process of microalgae production of biodiesel is often accompanied by a large amount of energy input. Thus, electro-fermentation reduced the power input of the mechanisms needed for mixing and shorten the extraction time.
Figure 3. Extracted lipid to biomass ratio (% of dry weight) for the solvent mixtures of B&D and isopropanol with a series of different ratios for EF-treated Schizochytrium.

Figure 4. Extracted lipid to biomass ratios (% of dry weight) varying from vortex times for EF-treated Schizochytrium.

4. Conclusion
Electro-fermentation increased the SOCD content by 17% compared to batch fermentation, causing greater damage to microalgal cells and making intracellular lipids more easily extracted. Electro-fermentation greatly improves lipids yield and lipids extraction efficiency for all solvent conditions. Therefore, lipids are more easily extracted for biodiesel production after electro-fermentation treatment. Electro-fermentation also reduces the use of toxic solvents (chloroform) and significantly reduces the need for mixed energy. Therefore, electro-fermentation provides a sustainable strategy for the extraction of fuel materials from microalgae.

Acknowledgments
This research was financially supported by the National Key R&D Plan of China (2017YFC1404605), the Natural Science Foundation of China (Grant No. 51579049), the Natural Science Foundation of Heilongjiang Province (E2017020), and the Fundamental Research Funds for the Central Universities (HEUCFG201820).

References
[1] Lin A, Zheng Q, Jiang Y, Lin X and Zhang H 2019 Sensitivity of air/mist non-equilibrium phase
transition cooling to transient characteristics in a compressor of gas turbine *International Journal of Heat and Mass Transfer* **137** 882-894.

[2] Lin A, Sun Y, Zhang H, Lin X, Yang L and Zheng Q 2018 Fluctuating characteristics of air-mist mixture flow with conjugate wall-film motion in a compressor of gas turbine *Applied Thermal Engineering* **142** 779-792.

[3] Yin Z, Zhu L, Li S, Hu T, Chu R, Mo F, Hu D, Liu C and Li B 2020 A comprehensive review on cultivation and harvesting of microalgae for biodiesel production: Environmental pollution control and future directions *Bioresour. Technol.* **301** 122804.

[4] Mathimani T and Mallick N 2018 A comprehensive review on harvesting of microalgae for biodiesel – Key challenges and future directions *Renewable and Sustainable Energy Reviews* **91** 1103-1120.

[5] Patil K P and Gogate P R 2015 Improved synthesis of docosahexaenoic acid (DHA) using *Schizochytrium limacinum* SR21 and sustainable media *Chemical Engineering Journal* **268** 187-196.

[6] Zeb L, Wang X-D, Zheng W-L, Teng X-N, Shafiq M, Mu Y, Chi Z-Y and Xiu Z-L 2019 Microwave-assisted three-liquid-phase salting-out extraction of docosahexaenoic acid (DHA)-rich oil from cultivation broths of *Schizochytrium limacinum* SR21 *Food and Bioproducts Processing* **118** 237-247.

[7] Patel A, Mikes F and Matsakas L 2018 An overview of current pretreatment methods used to improve lipid extraction from oleaginous micro-organisms *Molecules* **23**.

[8] Lin R, Deng C, Cheng J, Xia A, Lens P N L, Jackson S A, Dobson A D W and Murphy J D Graphene facilitates biomethane production from protein-derived glycine in anaerobic digestion *Science* **10** (2018) 158-170.

[9] Lin L, Tam L H, Xia X and Li X Y 2019 Electro-fermentation of iron-enhanced primary sedimentation sludge in a two-chamber bioreactor for product separation and resource recovery *Water Res.* **157** 145-154.

[10] Y Liu, Lai Y-J S, Barbosa T S, Chandra R, Parameswaran P and B E Rittmann 2019 Electro-selective fermentation enhances lipid extraction and biohydrogenation of *Scenedesmus acutus* biomass *Algal Research* 38.

[11] Lai Y S, Parameswaran P, Li A, Baez M and Rittmann B E 2014 Effects of pulsed electric field treatment on enhancing lipid recovery from the microalga, *Scenedesmus* *Bioresour. Technol.* **173** 457-461.

[12] Mahdy A, Mendez L, Ballesteros M and González-Fernández C 2014 Autohydrolysis and alkaline pretreatment effect on Chlorella vulgaris and *Scenedesmus* sp. methane production *Energy* **78** 48-52.