Subcritical Ethanol Extraction of Lipid from Wet Microalgae Paste of *Nannochloropsis* *sp.*

Min Chen, Xiaolin Chen, Tianzhong Liu*, and Wei Zhang

Key Laboratory of Biofuels, Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China

In this paper, subcritical ethanol was applied to extract lipid from wet microalgae paste of *Nannochloropsis* *sp.* The effects of various operational parameters including moisture content, solvent (volume) to microalgae (dry weight) ratio, extraction temperature, and pressure, extraction time were investigated on the extraction performance of total lipids. The results showed that three factors, moisture content, solvent (volume) to microalgae (dry weight) ratio and extraction temperature, affected extraction efficiency obviously, but pressure and extraction time made little differences. The optimum extraction condition were 10% moisture, 40:1 ratio, 135 °C, 1.5 MPa and 50 min, which could produce 90.21% recovery rate of the total lipids. These results indicated that the subcritical ethanol extraction for lipid extraction from wet microalgae paste was efficient and economically successful due to ethanol's cheapness, safety, and easy recycling.

**Keywords:** Subcritical Ethanol, Wet Microalgae Paste, *Nannochloropsis* *sp.*, Total Lipids.

1. **INTRODUCTION**

Currently, the exploitation and utilization of renewable biofuels have been drawn researchers’ attentions with the appearing of the environmental problems of fossil energy and the depletion of fossil resources.\(^1\)–\(^3\) Biodiesel, a common term for long chain alkyl esters, shows its great potential because of its many advantages. Compared to petroleum diesel fuel, biofuel instead of gasoline will reduce carbon dioxide (CO\(_2\)) between 75–80% because biofuel sequesters carbon through the growth of the feedstock and biofuel emits lower levels of volatile organic compounds, particulate sulfur-compounds during combustion.\(^4\)–\(^5\)

Biofuel is made of biomass. Recent reports have found that more and more arable land for biofuel production from food crops, such as soybean, sunflower and palm could lead to deforestation worse, releasing more CO\(_2\) into the atmosphere than the biofuel would offset.\(^6\)–\(^7\) Therefore, finding an alternative feedstock for biodiesel production that does not use or use less farmland is imperative. Among variety of biomass, microalgae are suggested as a very suitable candidate for biomass energy production, due to their high growth rates, which are usually 50 times greater than the traditional plants and higher lipid content and easy growth in non-arable land.\(^1\)–\(^2\),\(^8\)–\(^10\) Thus, the advantage of high lipid content and high potential biomass productivity per unit surface area compared to traditional crops would make microalgae possible to satisfy the dramatically huge demands for fuels in limited area.

At present, the majority of the companies producing algae-based products focus on culturing algae on large-scale and increasing lipid productivity, while there have been only a few researches on lipid extraction.\(^8\) The extraction of lipid from microalgae just adopts conventional processing technology of oil such as compression, solvent extraction or supercritical CO\(_2\) extraction etc.\(^11\)–\(^13\) Most of them are laborious, time-consuming and have low lipid yield. And on the other hand, the use of chloroform/methanol, hexane, isopropanol and petroleum ether in conventional solvent extraction would bring out some safe problems, at least for the comprehensive utilization of algae residues after lipid extraction.

This paper utilized subcritical ethanol to extract lipid from *Nannochloropsis* *sp.* For this method, the non-toxic ethanol was chosen as solvent to avoid environment pollution and ethanol is soluble with water which made it easy contact with wet algae paste. And wet algae paste was chose as materials to avoid energy cost for dewatering process. Moreover, subcritical ethanol extraction (SEE) used elevated pressures to keep the solvent as liquid state when the temperature reached above the boiling point, which greatly improved extraction efficiency. We compared the
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2. MATERIALS AND METHODS

2.1. Samples, Chemicals

*Nannochloropsis* sp. gifted by Arizona State University Polytechnic Campus was grown in artificial sea water enriched with BG11 nutrients. The microalgae were cultivated at 25 ± 1 °C for 9 days in 15 liters glass plate into which ambient air and 2% CO$_2$ were used to agitate the culture. Illumination was provided by white fluorescent lamps at an irradiance of 90 mol photon m$^{-2}$ s$^{-1}$. After 9 days' culture, the irradiance was increased to 200 mol photon m$^{-2}$ s$^{-1}$ in order to stimulate lipid accumulation in the algae for another 9 days. Then, the biomass was harvested by centrifugation (10 min, 6000 rpm). And the wet microalgae paste (the moisture is about 65%) was stored at −70 °C in darkness until extraction. All other chemicals were purchased from HuaDa Co. (Guzhangzhou, China) and all chemicals were of analytical grade.

2.2. Extraction of Lipids

2.2.1. Determination of the Total Lipids Content for Microalgae Samples

The content of total lipids in microalgae samples was determined according to B&D method, in which total lipids in algae cells were extracted with chloroform/methanol/water (2/2/1.8, v/v/v) first and then evaporated all solvent to weight the obtained lipids. The obtained lipid content was as the standard for the following calculation.

2.2.2. Hot Ethanol Extraction at Atmosphere Pressure (HEE)

The process was performed with wet algae paste at atmosphere pressure and under continuous agitation and reflux condensation. 9 g of wet algae paste and 70 ml ethanol (95%, v/v) were filled into the flask. Heat to 70 °C and then extract for 100 min for one cycle. After a cycle, the mixture was centrifuged and collected the supernatant. And the supernatant was concentrated by a rotary evaporator. Then the recovery of the total lipids can be calculated by the following equation:

\[
\text{The recovery rate of the total lipids} = \frac{\text{the lipid extracted in one cycle}}{\text{total lipid content by B & D method}}
\]

The extraction process consisted of four cycles. Every cycle followed the similar steps as mentioned.

2.2.3. Subcritical Ethanol Extraction (SEE)

Subcritical ethanol extraction was performed with wet algae paste using high pressurized extractor (Xin Yuan Co., China) equipped with a stirrer and 100 mL stainless steel extraction cell. The wet algae paste and ethanol was filled into the extraction cell. An alipuot of the solution was taken for water content determination by Moisture Meter 870F. The extraction process consisted of the following steps: Seal and pressurization; sample cell heating; static extraction; cooling and depressurizing; transfer the mixture. The mixture was centrifuged and collected the supernatant. And the supernatant was concentrated by a rotary evaporator. Then the concentrate was dried under a gentle stream of nitrogen at 65 °C, and the content of lipid was measured by weight. Then the recovery of the total lipids can be calculated by the following equation:

\[
\text{The recovery rate of the total lipids} = \frac{\text{the lipid extracted in SEE}}{\text{total lipid content by B & D method}}
\]

3. RESULTS AND DISCUSSION

3.1. The Effect of Hot Ethanol Extraction (HEE) to the Recovery of Total Lipids

Figure 1 showed the multiple extractions of wet microalgae by hot ethanol under 70 °C, atmosphere pressure. It can be found that most of the total lipid (about 45.8%) was extracted in the first cycle. After 2 to 4 cycle’s extraction, the total recovery rates of total lipid could reach 65%, 80%, and 90%. It means that hot ethanol has good lipid extraction ability from wet algal pastes. However, with this procedure, the total solvent (volume) to microalgae (dry weight) ratios consumed in all four cycles was 160:1; and total time required 400 minutes. Such large solvent consumption and extraction time made the extraction process high cost and low efficiency for future industrial application.

3.2. The Effect of Moisture Content on the Recovery of Total Lipids

Figure 2 showed the effect of moisture content on the recovery of total lipids. The moisture content was produced by adding various concentrations of ethanol to finish...
Fig. 1. The effect of multiple extractions from wet microalgae by hot ethanol extraction (HEE) on the recovery rate of total lipids (Extraction conditions: phase ratio of ethanol (95%, v/v) to dry algae: 40:1, temperature: 70 °C, extraction time: 100 min for each cycle, the total lipid content of the sample is 38.5%).

The results in Figure 3 showed that the solvent (ethanol (95%, v/v)) to microalgae (dry weight) ratio (v/w) was also an important factor to the effect of total lipids. Higher ratio will lead to higher lipid yield since the contact surface between the microalgae and the liquid phase becomes greater which favors good mass transfer.17 By changing the ratio from 5:1 up to 40:1, the increased lipid recovery was 24.4%. However, little increment of lipid can be obtained by increasing the ratio from 40:1 to 50:1. The reasons deduced that the ratio of 40:1 was just the diffusion limit between the solvent and the lipids and sufficient to extract the maximum amount of lipids under this condition.

3.3. The Effect of Solvent (Volume) to Microalgae (Dry Weight) Phase Ratio on the Recovery of Total Lipids

The effect of extraction temperature on the recovery of lipids was shown in Figure 4, which indicated that when the temperature was below 135 °C, the recovery increased with the elevating of temperature. Extraction at 90 °C, it gave less than 75% of lipid yield, however at 135 °C, the recovery could be over 90%. This was because as the temperature increasing, the viscosity of solvent decreased, thus allowing better penetration of solid and enhancing lipid yield.18 While the temperature rose to 150 °C, the yield of the lipid could be improved and the lipid yield could be rose as seen from these results. On the other hand, the lower moisture content, the cost of extraction was higher. When the moisture content was 10%, the lipid recovery was over 85%. Therefore, the 10% of moisture content was elected to as the optimal concentration for the next investigation.

3.4. The Effect of Extraction Temperature on the Recovery of Total Lipids

The effect of extraction temperature on the recovery of lipids was shown in Figure 4, which indicated that when the temperature was below 135 °C, the recovery increased with the elevating of temperature. Extraction at 90 °C, it gave less than 75% of lipid yield, however at 135 °C, the recovery could be over 90%. This was because as the temperature increasing, the viscosity of solvent decreased, thus allowing better penetration of solid and enhancing lipid yield.18 While the temperature rose to 150 °C, the yield of the lipid could be improved and the lipid yield could be rose as seen from these results. On the other hand, the lower moisture content, the cost of extraction was higher. When the moisture content was 10%, the lipid recovery was over 85%. Therefore, the 10% of moisture content was elected to as the optimal concentration for the next investigation.
3.5. The Effect of Pressure on the Recovery of Total Lipids

Pressure was investigated on the extraction yield as displayed in Figure 5. All the pressures shown in Figure 5 were the reaction pressure. It was known that in order to maintain the solvent as liquids above its atmospheric boiling point, higher pressure was needed. For example, 0.75 MPa was needed to keep ethanol as liquid at 135 °C. In Figure 5 when the pressure changed from 0.75 MPa to 1.5 MPa, there was obvious movement in the recovery of total lipid, from 81.7% to 87.9%. However, exerting the pressure to 4.0 MPa and 6.0 MPa, the recoveries of the total lipid were reduced by 5.8% and 8.3%. These results may be explained by that limited pressure should facilitate extraction process because pressure forced the solvent to contact the solid well. But excessively high pressure may lead to the thermal degradation of some compounds. Obviously, the pressure should be chose 1.5 MPa as one of optimal extraction condition.

3.6. The Effect of Extraction Time on the Recovery of Total Lipids

To determine the effect of extraction time on the extraction efficiency, microalgae lipid was extracted at fixing the preheating time for 25 min and the cooling time for 25 min. As shown in Figure 6, only slight increase was observed with the extraction process from 50 min to 150 min. When the extraction was finished in 50 min, which included just the preheating time and the cooling time, the lipid yield could achieve 86.3%. When the extraction time prolonged to 100 min, the recovery of lipids rose just by 4.3%. For 150 min, the recovery of lipids was only about 91%. From these results it can be deduced that during the preheating process, most of the lipid was solubilized into the fresh solvent at this extraction condition and as time passing by, a little lipid got extracted gradually, which meant the SEE can be completed for a very short time just involving the preheating and cooling process.

4. CONCLUSION

In this paper, subcritical ethanol extraction and hot ethanol extraction were utilized to extract lipids from Nannochloropsis sp. Compared to the hot ethanol extraction, SEE was more efficient and less-time consuming. Five
operating parameters were optimized for the extraction of lipids. The optimum conditions were as followed: 10% moisture, 40:1 ratio, 135 °C, 1.5 MPa, and 50 min. The experimental data demonstrated that SEE could be an alternative to the classic toxic extraction and has good potential for the industrial application.

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