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Research Article

Repeated Utilization of Ionic Liquid to Extract Lipid from Algal Biomass

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In this work, different kinds of ionic liquids and reaction conditions for the extraction of lipid from microalgae biomass were optimized and repeated use of ionic liquids for microalgal lipid extraction was evaluated. Morphological changes of microalgae cells were compared in terms of pre- and post-treatment to understand the mechanisms of ionic liquid treatment. Ionic liquid [BMIM][$MeSO_4$] showed the best lipid extraction efficiency at 70°C and with reaction time of 2 hours. The ratios (ILs : methanol) of 1 : 7 and 1 : 3 were the optimum ratios to complete the extraction of the lipids from microalgae. The initial 50% volume fraction of [BMIM][$MeSO_4$] was 16.04% of dry weight, which showed the highest five average extraction rates. The loss of ionic liquid in the reaction system and the increase in water content of ionic liquids were considered as the main reasons for the decrease in the extraction rate. It is suggested that the potential of lipid extraction in this IL-methanol co-solvent system is promising due to the high efficiency, low cost, safety, environmental protection, and other characteristics.

1. Introduction

Biodiesel is considered as a green energy resource because of its less negative impact on the environment such as air pollution, minimizing the CO_2 footprint and thus ensuring sustainability and environment protection [1]. As an important potential feedstock for biodiesel, microalgae have significant advantages, including high photosynthetic efficiency, less requirement of land, and no or minimal competition of food supply. Thus, research and commercialization interest on microalgae is growing fast around the world [2–4]. Conversion of microalgae lipid into biodiesel typically includes the following steps: cultivation of algae, cell harvest, lipid extraction, and esterification of lipids [5, 6]. Lipid extraction, as one of the most important processes in microalgae biodiesel production, which faced problems such as high energy consumption, environmental pollution, and low extraction efficiency, has been identified as a major bottleneck for large-scale algal biodiesel production [7–9].

Concerns on flammability and high toxicity of organic solvent lead to seeking of alternative technologies with as much less hazard to human beings and the environment. Lipid extraction of microalgae mainly includes physical, chemical, and biological methods to lower the amount or to completely eliminate the use of toxic solvents and reduce lipid extraction time and temperature. Methods such as microwave [10] pretreatment have been proven to be effective, but require high-energy input. Alternative chemical and biological cell disruption [11, 12] can increase the extraction efficiency of total lipids from microalgae for further conversion to biodiesel. SC-CO_2 [13] extraction was superior to other extraction techniques, but exhibited significant variations in yield with changes in operating parameters such as temperature and pressure [14]. Continuous ultrasonication [15] also can be a potential method to release the lipids in rigid-walled microalgae species without expensive dewatering steps. Cell disruption with bead milling, homogenization, microwave, and ultrasonication prior to solvent extraction could reduce the solvent utilization as well as decrease the process time. Therefore, several groups have reported the use of supercritical CO_2, less toxic solvent mixtures, and ionic liquids to replace toxic organic solvents. Ionic liquid has the advantages of almost no toxicity, stability of lipid extraction rate, and easy operation and has become the most promising lipid for extraction of microalgae lipid.

Ionic liquids (ILs) are organic salts that melt below 100°C. ILs stem from their potential application as "green solvents." Specifically, their nonvolatile character and thermal stability make them alternatives for volatile organic solvents. Ionic liquid treatment has several advantages including its nonusage of the autoclave reactor due to low vapor pressure, short reaction time, recovery and reuse of ionic liquid, and high performance yield. Because the hydrogen bonds of microalgae cell walls are affected by the ions of ionic liquids, the enhancement of lipid extraction due to the modification of cell walls is expected. Moreover, the blending of two ionic liquids is expected to stimulate the change of cell walls and to have an effect on lipid extraction. Nowadays, the attention is focused on increasing the yield of extraction, while reducing extraction steps, energy requirements, and process costs [16].

Recent research reports [17, 18] demonstrated the feasibility of lipid extraction processes using mixtures and polar organic solvents. Kim et al. [19] investigated on the influence of a variety of ILs on lipid extraction efficiency. But optimization of reaction conditions for ionic liquid extraction and the reuse of ionic liquid have not been studied well. In our present study, the influence of reaction time, reaction temperature, ratio of methanol to ionic liquids, and different kinds of ionic liquids on extraction efficiency was analyzed. The synergistic effects of the combinations of ionic liquid mixtures with different anions also were examined. Finally, continuous extraction of microalgal lipid by recycling ionic liquid was studied.

2. Materials and Methods

2.1. Materials. Microalgal strain *Neochloris oleoabundans* was preserved in the culture collection center of Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, (Guangzhou, China). Microalgae were cultivated in BG11 medium which contains macronutrients such as $NaNO_3$ (1.5 g L^{-1}), $K_2HPO_4 \cdot 3H_2O$ (40 mg L^{-1}), $MgSO_4 \cdot 7H_2O$ (75 mg L^{-1}), $CaCl_2 \cdot 2H_2O$ (36 mg L^{-1}), $NaCO_3$ (20 mg L^{-1}) $FeCl_3 \cdot 6H_2O$ (3.15 mg L^{-1}), and citric acid (6 mg L^{-1}) and 1 mL of microelements composed of H_3BO_3 (2.86 mg L^{-1}), $MnCl_2 \cdot 4H_2O$ (1.81 mg L^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.22 mg L^{-1}), $Na_2MoO_4 \cdot 2H_2O$ (0.39 mg L^{-1}), $CuSO_4 \cdot 5H_2O$ (0.08 mg L^{-1}), and $Co(NO_3)_2 \cdot 6H_2O$ (0.05 mg L^{-1}) per liter. The inoculated medium was incubated at 25 ± 1°C in the presence of cool white fluorescent light (120-150 μmol m^{-2} s^{-1}) under a light/dark cycle of 12 h/12 h.

2.2. ILs and IL Recycling. ILs of 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-butyl-3-methylimidazolium methyl sulfate ([BMIM][$MeSO_4$]), 1-butyl-3-methylimidazolium dicyanamide ([BMIM][DCN]), and 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) were chosen to test their effects for lipid extraction. All ILs, chloroform, methanol, and n-hexane were purchased from Sigma–Aldrich (USA).

In experiments, mixtures of ILs and methanol at different volume ratios (1 : 7, 1 : 3, 1 : 2, 1 : 1, and 2 : 1, only ILs or methanol) were used to investigate the effect of IL and methanol on the extraction efficiency of lipids from microalgae. Methanol can be used as a reactant for transesterification after extraction of lipids from the biomass and was used to decrease the high viscosity of some ILs.

Phase separation and IL recycling were as follows: dry weight of 200 mg microalgae was mixed with a mixture 3 mL of IL and methanol under magnetic stirring at different temperatures and different times. The mixture was cooled at room temperature and centrifuged to separate liquid phase and algae residues. The methanol phase was evaporated from the mixture using a rotary evaporator. Then, 1 mL n-hexane was added to the mixture to dissolve the lipid. The mixture was separated between the organic phase and ILs by vibration and centrifugation. The recovered n-hexane phase was washed three times with water to remove polar compounds. Crude lipids were then obtained by evaporation of the n-hexane phase using a rotary evaporator. The residue was weighed to measure the gravimetric yield. The ILs, which were then filtered and added with methanol, continue to be reused.

2.3. Lipid Extraction by ILs and Organic Solvent Extraction Method. A microalgae dry weight of 200 mg was mixed with a mixture of 3 mL of ILs and methanol under magnetic stirring for 3 h at different temperatures. The mixture was cooled at room temperature and centrifuged to separate liquid phase and algae residues. A total of 2 mL n-hexane and 1 mL water were added to the mixture to dissolve the lipid and separate inorganic and organic phases. The organic phase was separated in the upper phase by vibration and centrifugation. Then, the upper phase was evaporated from the mixture using a rotary evaporator. Crude lipids were then obtained by evaporation of the n-hexane phase using a rotary evaporator. After washing in water, the residue was weighed to measure the gravimetric yield.

Organic solvent extraction method is according to Zhou et al. [20].

2.4. Analytical Method. The cell-wall and surface ultrastructures of microalgae cells were identified by scanning electron microscopy (SEM) (Hitachi S-4800) on a copper substrate at an acceleration voltage of 25 kV. The FTIR spectra of *N. oleoabundans* ILs' pretreated and untreated biomass were recorded in the spectral range of 4000–400 cm^{-1} using a PerkinElmer spectrum GX FTIR spectrometer, to understand structural changes that occurred during pretreatment.

3. Results and Discussion

3.1. Effect of the Types of ILs. Mixtures of ILs and methanol can penetrate into the cells or can break down the cell wall

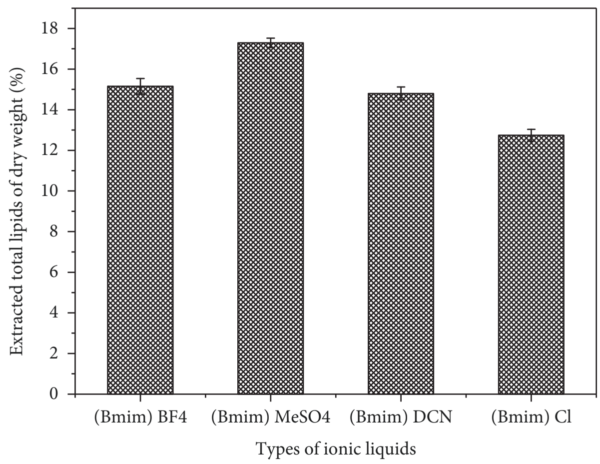
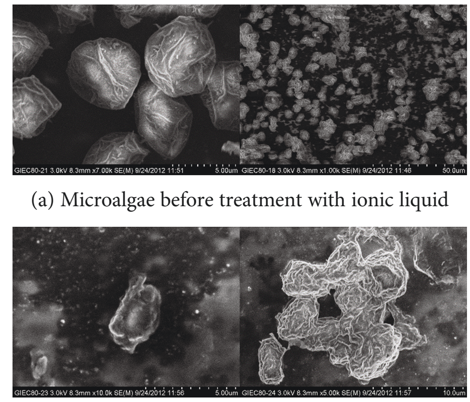


FIGURE 1: The influence of types of ionic liquids on lipid extraction.



(a) Microalgae before treatment with ionic liquid

(b) Microalgae after treatment with ionic liquid

FIGURE 2: The influence of types of ionic liquids on lipid extraction.

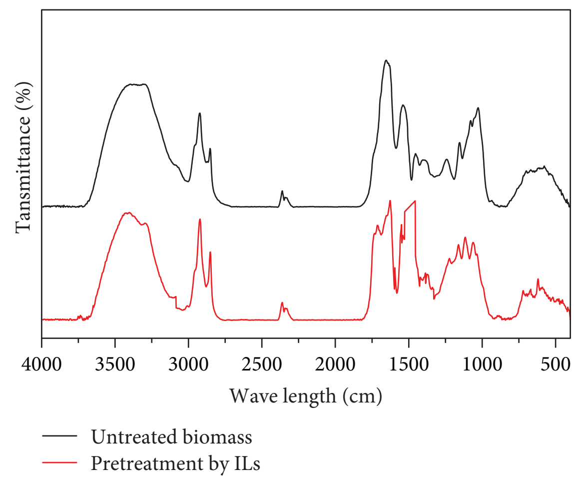


FIGURE 3: FTIR of microalgae before and after treatment with $[BMIM]MeSO_4$.

to release lipids. ILs of $[BMIM][BF_4]$, $[BMIM][MeSO_4]$, [BMIM][DCN], and [BMIM][Cl] were chosen to test their effects for lipid extraction. Figure 1 shows the extraction of lipids using different mixtures of ILs and methanol (ratio 1 : 1) under the reaction condition of 65°C and 2 hours. The lipid extraction efficiency of ILs followed the order [BMIM] $[MeSO_4]$ >$[BMIM][BF_4]$ >[BMIM][DCN] > [BMIM][Cl]. This indicates that the anionic structure of ionic liquids highly correlated with the extraction of lipids. Previous results showed that the extraction efficiency of lipids generally increased with decreasing dipolarity/polarizability, with increasing acidity values of ILs; however, hydrophobic and water immiscible ILs showed a low extraction efficiency, while hydrophilic and water miscible ILs showed a high extraction efficiency [8]. Our experimental results showed that [BMIM][Cl] was not ideal for microalgae lipid extraction which is a highly absorbent ionic liquid. Previous reports also showed that the addition of CO_2 (acidity values) to $[BMIM][BF_4]$ increased the lipid yield of *Chlorella vulgaris* from 68.0% to 75.6%, and for [BMIM] Cl, the effect of CO_2 addition on lipid yield was negligible [21]. [BMIM][Cl] was also found to have the capability to damage the membrane structure of *Skeletonema marinoi* and *Phaeodactylum tricornutum* [22]. Therefore, different cell wall structures (cellulose, glycoprotein, silica, and peptidoglycan) of microalgae may play a crucial role in the choice of ionic liquid species. The wall structure of *N. oleoabundans* rich in fibrous structures used in this paper might be more susceptible to $[BMIM][MeSO_4]$ [16, 23, 24].

Figure 2 shows the surface changes of microalgae cells before and after lipid extraction by $[BMIM][MeSO_4]$. After comparison, in the SEM photos of microalgae treated by ionic liquid, we observed that a portion of the cell barrier structure of the cell was destroyed; however, the cell wall structure remains largely intact. The cytoplasm flows out of the cell and is dispersed in the field of view, and the pretreated cells were reduced by about 20% compared to intact cells. This result suggests that ionic liquid mainly acts on the dissolution of cell wall polysaccharides (cellulose, hemicellulose analogs) and lipids can more easily release from cells. Our study also found that low concentrations of xylose and glucose appeared which may be related to the dissolution of cell wall polysaccharides. In some references, the FTIR spectroscopy method was used to determine structural changes in biomass of pretreatments. Major components of microalgal biomass, i.e., carbohydrate, show a peak near 1100–900 cm^{-1}, lipids show peak near 2970-2850 cm^{-1}, and proteins show peak near 1750–1500 cm^{-1} [25, 26]. Compared with untreated biomass, there is no certain decline in the above three wavelengths, which suggested that ionic liquid treatment has no significant break of cell inclusion (Figure 3). The peak near 2970-2850 cm^{-1} increased after ionic liquid pretreatment, potentially caused by the dissolution of intracellular lipid composition; fatty acids are eluted out of the cell by methanol. Solubility of ionic liquid for cellulose and polysaccharide analogues in the cell wall may result in peak-type change of 1760~980 cm^{-1}. The depolymerization and allosteric structure of protein may be the reason for the large change of the peak shape of 1100–900 cm^{-1} [27].

3.2. Optimization of Lipid Extraction Condition by $[BMIM][MeSO_4]$. Reaction temperature and time were the main factors that affected hydrogen bond network damage

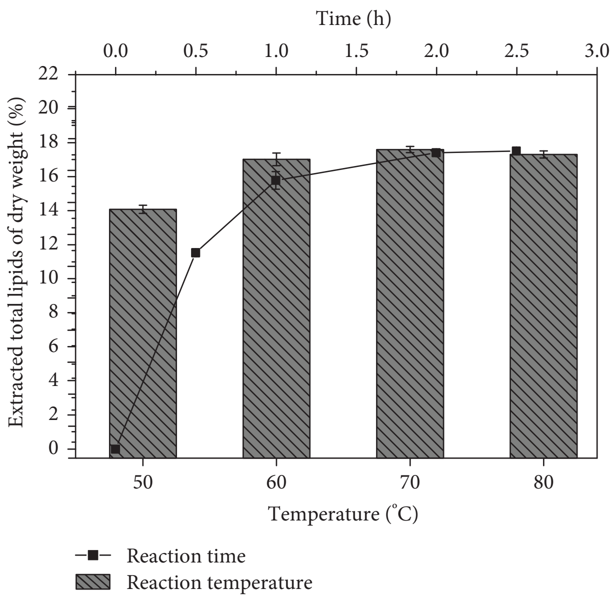


Figure 4: The influence of reaction time and temperature on lipid extraction.

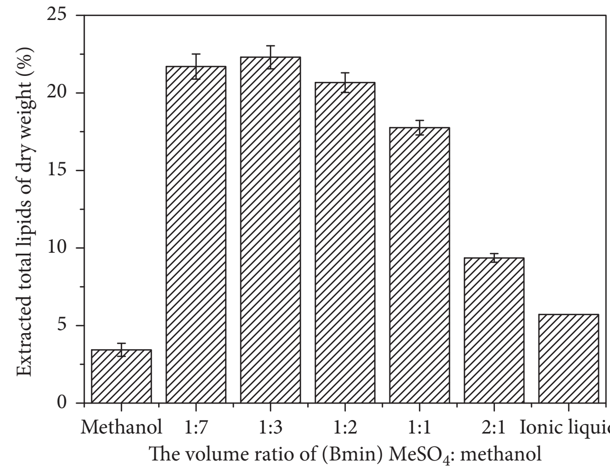


Figure 5: The influence of volume ratio of [BMIM]$MeSO_4$ and methanol on lipid extraction.

of cellulose [28, 29] and the efficiency of extraction of total lipids. In Figure 4, there is a significant increase in lipid extraction during the first 2 hours, and when temperature increased from 50°C to 70°C, the lipid extraction efficiency was improved from 13.5% to 17%. As the reaction temperature was more than 70°C and the time achieved was more than 2.5 hours, total lipid extraction was stable at around 17% for the proportion of dry weight. However, too long time and too high temperature would not lead to more lipids obtained. Conclusively, this result implicated that more than 70°C and 2 hours is the optimal reaction condition for low cost expected.

The volume ratio of methanol and ionic liquids has a crucial impact on lipid extraction efficiency. Several mixing ratios were chosen to evaluate their potential extraction value. Figure 5 shows the difference in the volume ratios from 1 : 7 to 2 : 1 of [BMIM][$MeSO_4$] and methanol. [BMIM][$MeSO_4$] and methanol were used separately as extraction solvents for comparative purposes. The experimental results suggested that the ratios of 1 : 7 and 1 : 3 had higher extraction efficiency. The high concentration of ionic liquid was not conducive for the extraction of microalgal lipids. Ionic liquid was proposed to disrupt the structure of the fiber bundle in the cell wall which made it easy to release the lipid from inside the microalgae cells [16]. Ionic liquid at low concentrations in methanol can reduce its viscosity to increase the probability to form hydrogen bonds between fibers and ionic liquid. On the other hand, most of the methanol molecules tended to be isolated from each other by interacting with the anion of ILs via H bonding; the action of the ionic liquid-methanol system made a hydrophobic environment to facilitate the transfer of lipids [8, 30]. Therefore, existence of a certain amount of ionic liquid guaranteed the thermodynamic activity, and there was also more methanol to play its role in parameter extraction. H bonding occurs between the OH groups of molecules and the anion of ILs. It is also proposed that the action of the polar covalent molecule (methanol) is largely to disrupt the cytomembrane and to improve the efficiency at which the lipid is being extracted from the biomass. When the permeability of the cell wall increases, excess methanol facilitates the precipitation of lipid from cells.

3.3. Ionic Liquid Water Stability and Continuous Extraction. Ionic liquids are more stable than conventional solvents and are prone to yield problems. In the experiment, a certain amount of ionic liquid was recovered after a single extraction of lipid. Some components of microalgae may cause changes in the catalytic effect of ionic liquids, and this subtle change may be amplified on multiple lipid extractions. Therefore, we defined the initial amount of ionic liquids, studied the recovery of ionic liquids for microalgal lipid extraction, and calculated the average lipid extraction rate. As shown in Figure 6, ionic liquids of 12.5% (A), 25% (B), 33% (C), and 50% (D) for 3-5 times of lipid extraction experiments were selected (Figures 6(a) and 6(b)). Ionic liquid was recovered after each experiment. The overall volume of the reaction was kept constant by adding methanol. Three replicate experiments were carried out with the same solution (12.5%), and we observed that the lipid extraction rate was reduced from 21.8% to 5.8% (dry weight ratio). Subsequent traces of ionic liquid cannot be recovered. Five times lipid extractions were completed at ionic liquid concentrations of 25%, 33.3%, and 50%. The lipid extraction efficiency basically maintains above 15% dry weight of B and C, and the lipid extraction rate gradually decreased from 20.4% and 21.2 to 6.2% and 5.2%, respectively. The average conversion rates of A, B, C, and D (three times) were 16.04%, 15.84%, 14.54%, and 14.59%, respectively. The average loss rates of the ionic liquids of A, B, and C were 7.3%, 6.8%, and 6.4%, respectively.

The experimental results showed that the average lipid extraction efficiency in A is the highest. Due to the small amount of initial ionic liquid, only three lipid extraction

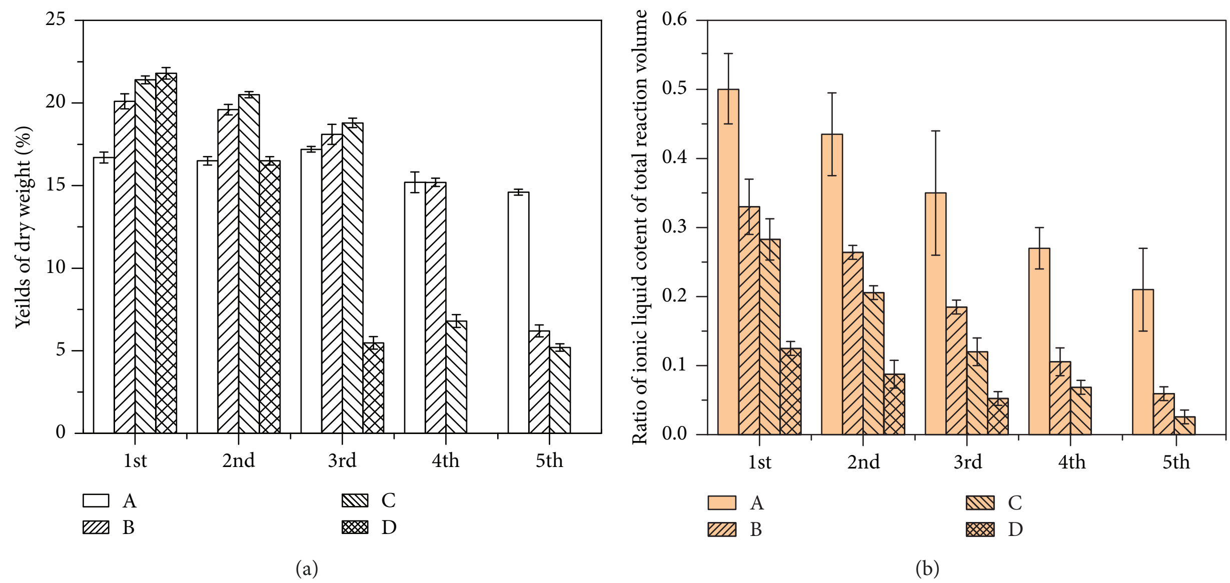


Figure 6: The influence of recycle times of [BMIM]$MeSO_4$ on lipid extraction and ionic liquid loss.

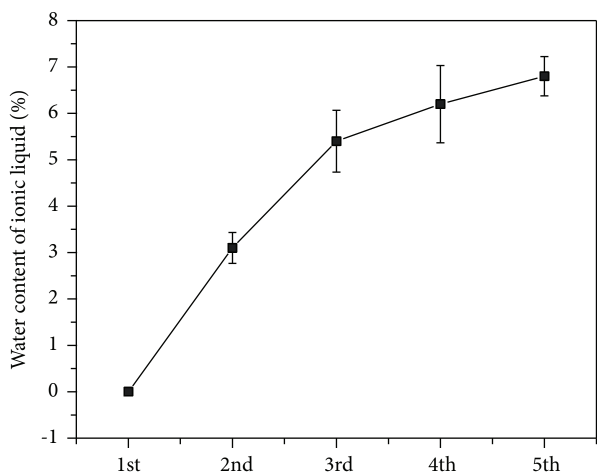


Figure 7: The water content of [BMIM]$MeSO_4$ in different cycle times.

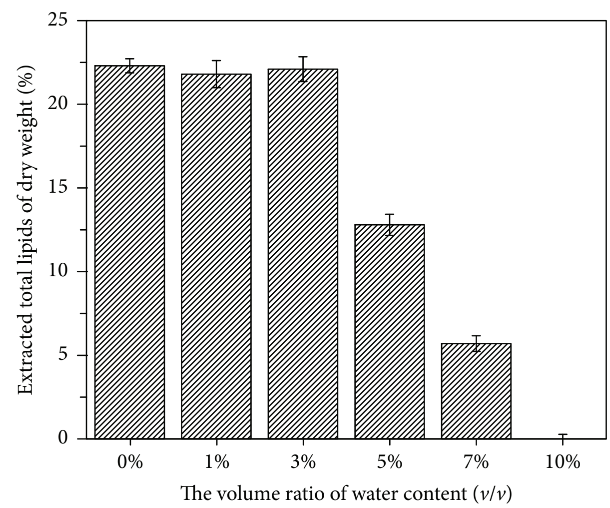


Figure 8: Effect of water addition on lipid yield extracted by [BMIM][$MeSO_4$]-methanol.

cycles can be completed in D. Residues of ionic liquids were found in microalgal lipids during gas-mass spectrometry monitoring. It was also found that loss of ionic liquids mainly originates from the adherence of algal residue after lipid extraction. This residual ionic liquid is currently difficult to recycle.

The experimental results showed that the first lipid extraction conversion rate in C is 21.2% higher than the fifth lipid yield rate of 14.6% in A. This indicates that the repeated circulation of ionic liquid leads to a decrease in the extraction efficiency of microalgae lipids. The darkening of the ionic liquid after repeated use may be a manifestation of the dissolution of the algal cytochrome phospholipids in the ionic liquid. Under mild reaction conditions, water dissolution in the reaction may also be responsible for low lipid extraction efficiency. Figure 7 shows water content in the ionic liquid for different cycles. The relationship between lipid yields and water content with 0 to 9% (v/v) of total reaction volume was investigated (Figure 8). This range was selected considering the stability of IL lipid extraction of the aqueous environment. The amount of lipid extracted was kept high at the addition of less than 3% of water (based on the total reaction volume). When the moisture content increases to 5%, the lipid extraction efficiency drops by about 40%. The reaction system almost stopped when the water content was more than 10%. Obviously, the water content has great influence on the extraction rate of the lipid, but it is gratifying that the trace amount of moisture did not cause the performance of ionic liquid to subside. It can be speculated that the presence of excess methanol may counteract the dissociation effect of low concentrations of water on hydrogen bonding of ionic liquids. Otherwise, Liu et al. found that cellulose was hydrolyzed and effectively catalyzed by solid acid in ionic liquid [AMIN][Cl] at a low content of 5% water content [31]. Trace moisture may contribute to the hydrolysis of polysaccharides on the microalgae cell wall. This discovery is undoubtedly good news for the reduction of microalgae energy demand and process cost.

4. Conclusion

Ionic liquids of lipid extraction of Scenedesmus sp. WZKMT is a reliable approach on destroying the cells' structures and improving the recovery of microalgae lipids. In this work, different kinds of ionic liquids, reaction time, reaction temperature, and the ratio of methanol and ionic liquids of lipid extraction have been optimized. More than 70°C and 2 hours is the optimal reaction condition for low cost expected by [BMIM][$MeSO_4$]-methanol of 1 : 3. The amount of lipid extracted was kept high at the addition of less than 3% of water. Five times average lipid extraction by [BMIM][$MeSO_4$]-methanol of 1 : 7 showed the highest extraction effectiveness. Together, the reuse of IL–methanol lipid extraction systems can be regarded as a feasible and environmentally friendly way to extract lipids from algal biomass.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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