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Lipid extraction from *Chlorella vulgaris* by molten-salt/ionic-liquid mixtures

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ABSTRACT

In this study, lipid extraction from *Chlorella vulgaris* was performed by using mixtures of molten salt and ionic liquid. The yield-enhancing effects of blending of molten salt with ionic liquid were investigated. Among the three molten salts ($Zn(NO_3)_2 \cdot 6H_2O$, $Mg(ClO_4)_2 \cdot 6H_2O$, and $FeCl_3 \cdot 6H_2O$), $FeCl_3 \cdot 6H_2O$ showed a high lipid extraction yield (113.0 mg/g cell) and good reaction performance. When $FeCl_3 \cdot 6H_2O$ was mixed with [Emim]OAc (5:1, w/w), the lipid extraction yield increased to 227.6 mg/g cell, a performance similar to that of single [Emim]OAc (218.7 mg/g cell). When lipid was extracted by the $FeCl_3 \cdot 6H_2O$/[Emim]OAc mixture at a 5:1 (w/w) blending ratio, 90 °C temperature, and 1 h duration, the fatty acid content of the extracted lipid was 981.7 mg/g lipid, indicating less than 2% impurity. The lipid extraction from *C. vulgaris* was improved by the synergistic effects of molten salt and ionic liquid with different ions.

1. Introduction

Most of the biodiesels industries produce are derived from edible vegetable oils (e.g., palm, rapeseed, and soybean oils) via transesterification. Recently, a great deal of research effort has been devoted to biofuel production that utilizes microalgae as a domestic oil source [1–4]. Microalgae are photosynthetic microorganisms capable of converting carbon dioxide and water to macromolecules such as lipids, polysaccharides, and proteins under light conditions [5]. Some microalgae result in high oil productivity relative to the capacities of plant oils, and offer the additional advantage of saving plants for food-crop purposes. For microalgae-production purposes, wastewater or seawater can be used in place of freshwater to enhance their environmental and economic feasibilities [1,3,6]. Conversion of microalgae to biodiesel typically includes the following four steps: microalgal cultivation, cell harvesting, lipid extraction, and biodiesel conversion [6]. A number of methods can be used to achieve lipid extraction from microalgae, including solvent extraction, enzymatic hydrolysis, fractionation, and ionic-liquid extraction [7,8].

Ionic liquids are salts composed of relatively large organic cations coupled with smaller inorganic or organic anions that remain liquid at moderate-to-room temperatures (0–140 °C) [9]. Ionic liquids have attracted interest for their diverse range of applications including electrochemistry, catalysis, inorganic/organic chemistry, and biotechnology [10]. Their properties include good thermal and electrochemical stabilities, high conductivity, low vapor pressure, non-flammability, and a broad miscibility range [11,12]. Ionic liquids, owing to their synthetic flexibility, are referred to as "designer solvents" [13]. The extent to which researchers develop processes that utilize ionic liquids will depend not only on their chemical, physical, and biological properties but also on several other factors, including cost, availability, and recyclability. Ionic liquids are preferable to water and organic solvent as catalysts and media for the purposes of synthesis or extraction [14]. Some ionic liquids have, for example, excellent properties for cellulosic biomass dissolution and cellulose recovery uses. Fujita et al. [15] reported the successful dissolution of marine microalgae in polar ionic liquids under mild conditions. Lovejoy et al. [16] examined the potential of ionic liquids as novel biocompatible agents for extracting high-value products from microalgae. Ionic liquids are expected to be effective solvents for microalgae dissolution and lipid extraction purposes because the cell walls of microalgae contain cellulose.

Molten salts are salts that, though in the normal solid state at the standard temperature and pressure, are in the liquid phase due to elevated temperature. Technically, molten salts are a class of ionic liquid. Molten inorganic hydrates have gained attention as new, highly efficient solvents and media for use in cellulose modification [17,18]. A multitude of pure molten-salt hydrates as well as salt mixtures have been investigated to elucidate their interaction with cellulose. The results have shown that molten salts dissolve, swell, decompose cellulose, or have no effect on it [19].

In this study, the influence of molten salts and ionic liquids on lipid extraction from a microalga was investigated. Extraction by single molten salts and single ionic liquids was compared with that by molten-salt/ionic-liquid mixtures in order to identify synergistic effects

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for enhanced lipid extraction and to decrease the amount of expensive ionic liquids used. The molten salts' and ionic liquids' synergistic effects on lipid extraction also were examined.

2. Experimental methods

2.1. Materials

Chlorella vulgaris (hereafter: *C. vulgaris*), a freshwater microalga, was isolated locally and cultured in nutrient media (constituents: KNO_3, 3 mM; KH_2PO_4, 5.44 mM; Na_2HPO_4, 1.83 mM; $MgSO_4 \cdot 7H_2O$, 0.20 mM; $CaCl_2$, 0.12 mM; FeNaEDTA, 0.03 mM; $ZnSO_4 \cdot 7H_2O$, 0.01 mM; $MnCl_2 \cdot 4H_2O$, 0.07 mM; $CuSO_4$, 0.07 mM; and $Al_2(SO_4)_3 \cdot 18H_2O$, 0.01 mM) adjusted to a pH of 6.5. The *C. vulgaris* was then cultivated at 30 °C in a Pyrex bubble-column reactor (working volume: 6 L) equipped with 12 fluorescent lamps at the front and right/left sides (light intensity: 80 μmol/m^2/s) and kept in a constant-temperature room. The reactor was supplied with 10% (v/v) CO_2 in air at a rate of 0.75 L/min. Cells were harvested by centrifugation (4000 rpm and 10 min), washed with deionized water (three times), freeze-dried (FD5512, IlShin BioBase Co., Korea) for 4 days or longer, and finally stored at −20 °C preparatory to an analysis of their fatty acid contents and for lipid-extraction experiments.

Three molten salts, namely zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), magnesium perchlorate hexahydrate ($Mg(ClO_4)_2 \cdot 6H_2O$), and iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), were purchased from Sanchun Pure Chemical (Korea) and used without any further purification. Five ionic liquids, specifically 1-ethyl-3-methyl imidazolium acetate ([Emim]OAc, 90%), 1-ethyl-3-methyl imidazolium hydrogen sulfate ([Emim]HSO_4, 95%), 1-ethyl-3-methyl imidazolium diethylphosphate ([Emim]DEP, 98%), 1-ethyl-3-methyl imidazolium thiocyanate ([Emim]SCN, 95%), and 1-ethyl-3-methyl imidazolium bis(trifluoromethylsulfonyl)imide ([Emim]$(CF_3SO_2)_2N$, 98%), were purchased from Sigma-Aldrich (USA) and used without any further purification.

2.2. Lipid extraction by single molten salts and single ionic liquids

Single molten-salt treatment using three molten salts – $Zn(NO_3)_2 \cdot 6H_2O$, $Mg(ClO_4)_2 \cdot 6H_2O$, and $FeCl_3 \cdot 6H_2O$ – and single ionic-liquid treatment using five ionic liquids – [Emim]OAc, [Emim]HSO_4, [Emim]DEP, [Emim]SCN, and [Emim]$(CF_3SO_2)_2N$ – were performed at 110 °C for 2 h (5% *C. vulgaris* loading, w/w).

After the molten-salt and ionic-liquid treatments, lipid was collected from the treatment solution by the following procedure: mixing with hexane (96%, Junsei, Japan) as the organic solvent for 4 h at room temperature; separation of the organic solvent layer from the cell debris layer by centrifugation at 4000 rpm for 10 min; evaporation of the lipid-containing organic solvent layer using a vacuum evaporator (EZ2 PLUS, Genevac, UK); and recovery of the lipid. The lipid extraction yield was quantified according to the weight of the recovered lipid. All of the experiments were performed in duplicate.

2.3. Lipid extraction by molten-salt/ionic-liquid mixtures

$FeCl_3 \cdot 6H_2O$ was mixed with [Emim]OAc at 110 °C for 2 h (5% *C. vulgaris* loading, w/w). Different blending ratios ($FeCl_3 \cdot 6H_2O$:[Emim]OAc = 1:1, 2:1, 3:1, 4:1, 5:1, and 6:1, w/w) were compared to determine the optimal one. [Emim]OAc was injected into $FeCl_3 \cdot 6H_2O$ once solid $FeCl_3 \cdot 6H_2O$ had transitioned to the liquid phase under increasing temperature.

For reaction temperature and time determination, $FeCl_3 \cdot 6H_2O$ was mixed with [Emim]OAc at 70, 90, and 110 °C for 2 h and at 90 °C for 30 min, 1 h, 1 h 30 min, and 2 h (5% *C. vulgaris* loading, w/w and $FeCl_3 \cdot 6H_2O$:[Emim]OAc = 5:1, w/w).

After the treatments with the mixtures of molten salt and ionic liquid, lipid was collected from the treatment solution by mixing it with hexane for 4 h at room temperature followed by the separation of the organic solvent layer from the cell debris layer by centrifugation.

To investigate the effects of lipid extraction on the $FeCl_3 \cdot 6H_2O$/ionic liquid mixtures containing different anions, $FeCl_3 \cdot 6H_2O$ was mixed with [Emim]HSO_4, [Emim]SCN, [Emim]DEP, and [Emim]$(CF_3SO_2)_2N$ at 90 °C for 1 h ($FeCl_3 \cdot 6H_2O$:ionic liquid = 5:1, w/w), respectively. Afterwards, lipid was collected from the mixture using hexane, and the organic solvent layer was separated from the cell debris layer by centrifugation.

Finally, the lipid-containing organic solvent layer was evaporated using a vacuum evaporator, whereupon the lipid was recovered. The lipid extraction yield was quantified, again, according to the weight of the recovered lipid. All of the experiments were performed in duplicate.

2.4. Analyses

The fatty acid content of *C. vulgaris* was analyzed using the modified direct transesterification method [20]. First, approximately 10 mg of cells was placed inside a Pyrex-glass tube with a Teflon-sealed screw-cap. Two milliliters of the newly prepared chloroform/methanol mixture (2:1, v/v) was added to the cells, whose solution was then vigorously mixed using a vortex mixer (Vortex Genius 3, Ika, Italy) at room temperature for 10 min. One milliliter of chloroform solution containing heptadecanoic acid (Sigma, USA) as an internal standard (500 μg/L), 1 mL of methanol, and 300 μL of sulfuric acid were sequentially added to the glass tube and vortex-mixed for 5 min. The tube was then placed in a 100 °C water bath for 10 min, after which it was cooled to room temperature, supplemented with 1 mL of distilled water, mixed thoroughly for 5 min, and centrifugally layer-separated at 4000 rpm for 10 min. The lower layer (organic phase) was extracted using a syringe and filtered with a 0.22 μm PVDF syringe filter (Millex-GV, Millipore, USA). Fatty acid methyl ester (FAME) was analyzed using a gas chromatograph equipped with an automatic injector (Model 7890, Agilent, USA). Mix RM3, Mix RM5, GLC50, GLC70 (Supelco, USA), and heptadecanoic acid were used as the standards. The other reagents used were of analytical grade.

The fatty acid content of microalgal lipid was analyzed following the modified direct transesterification method noted above [20]. Initially, recovered microalgal lipid was used in place of the cells for transesterification. Around 10 mg of microalgal lipid was placed inside a Pyrex-glass tube. The following procedure was the same as above.

3. Results and discussion

3.1. Fatty acid content of microalgae

The fatty acid contents and compositions of *C. vulgaris* are summarized in Table 1. The total fatty acid content was 334.7 mg/g cell. The palmitic acid content was 75.8 mg/g cell (22.6% of total fatty acids), the linoleic acid content was 64.8 mg/g cell (19.4% of total fatty acids), and the oleic acid content was 60.3 mg/g cell (18.0% of total fatty

Table 1
Fatty acid contents and compositions of lyophilized *C. vulgaris*.

Fatty acids	Content (mg/g cell)	Composition among total fatty acids (%)
Myristic acid (C14:0)	0.9	0.3
Palmitic acid (C16:0)	75.8	22.6
Palmitoleic acid (C16:1)	0.6	0.2
Stearic acid (C18:0)	21.1	6.3
Oleic acid (C18:1)	60.3	18.0
Linoleic acid (C18:2)	64.8	19.4
Linolenic acid (C18:3)	29.5	8.8
Others	81.6	24.4
Total	334.7	100.0