Renewable Energy 65 (2014) 169–174

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Effects of ionic liquid mixtures on lipid extraction from *Chlorella vulgaris*

Sun-A Choi [a,b], You-Kwan Oh [a], Min-Ji Jeong [a], Seung Wook Kim [b], Jin-Suk Lee [a,*], Ji-Yeon Park [a,*]

[a] Department of Clean Fuel, Korea Institute of Energy Research, Daejeon 305-343, Republic of Korea
[b] Department of Chemical & Biological Engineering, Korea University, Seoul 136-701, Republic of Korea

ARTICLE INFO

Article history:
Received 15 February 2013
Accepted 15 August 2013
Available online 8 September 2013

Keywords:
Lipid extraction
Chlorella vulgaris
Microalgal oil
Ionic liquid mixtures
1-Ethyl-3-methyl imidazolium cation

ABSTRACT

In this study, pre-biodiesel-production lipid extraction from microalgae (*Chlorella vulgaris*) was performed. The yield-enhancing effects of ionic liquid blends on the lipid extraction were investigated. The initial fatty acids content of the *C. vulgaris* was 292.2 mg/g cell. The lipid extraction yield using single ionic liquids was compared with the yield obtained with organic solvents and ionic liquid mixtures, respectively. The yield by hexane–methanol solvent was 185.4 mg/g cell. Among the 12 ionic liquids, 1-ethyl-3-methyl imidazolium acetate, 1-ethyl-3-methyl imidazolium diethylphosphate, 1-ethyl-3-methyl imidazolium tetrafluoroborate, and 1-ethyl-3-methyl imidazolium chloride showed high (>200.0 mg/g cell) lipid extraction yields. Although the yields of 1-ethyl-3-methyl imidazolium ethyl sulfate and 1-ethyl-3-methyl imidazolium thiocyanate were only 60.5 and 42.7 mg/g cell, respectively, the yield for their mixture (weight ratio of 1:1) was improved to 158.2 mg/g cell. Similarly, whereas the lipid extraction yield of 1-ethyl-3-methyl imidazolium hydrogen sulfate was just 35.2 mg/g cell, that for its mixture with 1-ethyl-3-methyl imidazolium thiocyanate (weight ratio of 1:1) was boosted to 200.6 mg/g cell. Overall, the synergistic effects of the ionic liquid mixtures with different anions improved the lipid extraction yield of *C. vulgaris*.

1. Introduction

Biodiesel is a renewable alternative fuel for diesel engines. Biodiesel is produced from any number of various vegetable oils, according to agricultural policies, local crop availability, and feedstock prices in a specific country of origin [1–3].

In Korea, most of the biodiesel produced is obtained from imported vegetable oils (e.g., palm and soybean oils) via transesterification. Recently, a great deal of research effort has been devoted to biodiesel production that utilizes microalgae as a domestic oil source [4–7]. Microalgae are photosynthetic microorganisms capable of converting, under a variety of light conditions (400–700 nm), carbon dioxide and water into macromolecules such as lipids, polysaccharides and proteins [8]. Some microalgae result in a high oil productivity compared with those of plant oils and do not compete with food crops. For microalgae production purposes, wastewater or seawater can moreover be used instead of freshwater, thereby enhancing environmental and economic feasibilities [4,7,9]. Conversion of microalgae to biodiesel typically includes the four following steps: microalgae cultivation, cell harvesting, lipid extraction, and biodiesel conversion [9]. A number of methods, including solvent extraction, enzymatic hydrolysis, fractionation, pyrolysis, ionic liquid extraction, and osmotic shock, can be used to extract lipids from microalgae [10].

Ionic liquids are salts composed of relatively large asymmetric organic cations coupled with smaller inorganic or organic anions that remain liquid at moderate-to-room temperatures (0–140 °C). Ionic liquids differ from simple ionic solutions wherein ions are dissolved in a molecular medium [11]. Ionic liquids' cations generally are composed of a nitrogen-containing ring structure (e.g., imidazolium or pyrinidine) to which a broad range of functional side groups can be attached. The property of the resulting ionic liquid varies according to the side-group structure [12]. Ionic liquids have the interesting properties of non-flammability, electric conductivity, thermal stability, low vapor pressure, and high heat capacity [13]. Moreover, ionic liquids, owing to their synthetic flexibility, are referred to as "designer solvents" [14].

Ionic liquid treatment has several advantages including its non-usage of autoclave reactor due to low vapor pressure, short reaction

* Corresponding authors. Tel.: +82 42 860 3041; fax: +82 42 860 3495.
E-mail addresses: bmjslee@kier.re.kr (J.-S. Lee), yearn@kier.re.kr (J.-Y. Park).

0960-1481/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.renene.2013.08.015

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. The present study investigated the influence of ionic liquids on lipid extraction from microalgae. Extraction by single ionic liquids was compared with that by organic solvents and ionic liquid mixtures in order to determine ionic liquids offer good utilities in enhancing lipid extraction yields. The synergistic effects of the combinations of ionic liquid mixtures with different anions also were examined.

2. Experimental methods

2.1. Materials

Chlorella vulgaris (hereafter: *C. vulgaris*), a freshwater microalga, was isolated locally and cultured in a nutrient medium (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; $Al_2(SO_4)_3 \cdot 18H_2O$, 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 at a rate of 0.75 L/min. Cells were harvested by centrifugation (4000 rpm, 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 analyze its fatty acids content. Twelve ionic liquids (Sigma–Aldrich, USA) were summarized in Table 1 and used without any further purification.

2.2. Lipid extraction from microalgae by organic solvents

In an investigation of the lipid extraction characteristics of *C. vulgaris*, lipid was extracted from microalgae using three organic solvents: hexane (96%, Junsei, Japan); hexane:methanol (99.6%, Junsei, Japan) = 7:3 (v/v); and chloroform (99%, Junsei, Japan): methanol = 2:1 (v/v). The *C. vulgaris* loading was 5% (w/w). 1 g of the cells was mixed with 19 g of solvent. The mixture was stirred at 1000 rpm for 6 h at room temperature to extract the lipids. Afterwards, it was separated into organic solvent layer and cell debris layer by centrifugation at 4000 rpm. Finally, the organic solvent layer containing lipids was evaporated using a vacuum evaporator (EZ2 PLUS, Genevac, UK), and the lipid was recovered. The lipid extraction yield was determined with reference to the weight of the recovered lipid. All of the experiments were performed twice.

Table 1
Twelve ionic liquids used for lipid extraction.

Ionic liquids	Name, purity
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 tetrachloroaluminate	[Emim]$AlCl_4$, 95%
1-ethyl-3-methyl imidazolium diethylphosphate	[Emim]DEP, 98%
1-ethyl-3-methyl imidazolium ethyl sulfate	[Emim]$EtOSO_3$, 95%
1-ethyl-3-methyl imidazolium thiocyanate	[Emim]SCN, 95%
1-ethyl-3-methyl imidazolium methanesulfonate	[Emim]CH_3SO_3, 95%
1-ethyl-3-methyl imidazolium bis (trifluoromethylsulfonyl)imide	[Emim]$(CF_3SO_2)_2N$, 98%
1-ethyl-3-methyl imidazolium tetrafluoroborate	[Emim]BF_4, 98%
1-ethyl-3-methyl imidazolium chloride	[Emim]Cl, 95%
1-Allyl-3-methylimidazolium chloride	[Amim]Cl, 95%
1-butyl-3-methyl imidazolium chloride	[Bmim]Cl, 95%

2.3. Lipid extraction from microalgae using ionic liquids

Ionic liquid treatment using three ionic liquids – [Emim]OAc, [Emim]BF_4, and [Amim]Cl – was performed at 65 °C for 18 h and at 120 °C for 2 h (5% *C. vulgaris* loading, w/w). 1 g of the cells was mixed with 19 g of ionic liquid. After the treatment, lipid was extracted at 1000 rpm for 4 h at room temperature using hexane as the organic solvent. The organic solvent layer was separated from the cell debris layer by centrifugation at 4000 rpm.

Ionic liquid treatment using the ten ionic liquids of the [Emim] series, [Amim]Cl, and [Bmim]Cl was performed at 120 °C for 2 h (5% *C. vulgaris* loading, w/w). Afterwards, lipid was extracted from the ionic liquid using hexane, and the organic solvent layer was separated from the cell debris layer by centrifugation. In the case of [Emim]Cl, hexane:methanol = 7:3 (v/v) was used instead of hexane due to the fact that [Emim]Cl becomes solid after mixing with hexane.

Ionic liquid treatments using mixtures of [Emim]OAc with three organic solvents including methanol, chloroform, and hexane as the co-solvents, were performed at 65 °C for 18 h (5% *C. vulgaris* loading, [Emim]OAc:co-solvent = 1:1, w/w). 1 g of the cells was mixed with each 9.5 g of ionic liquid and co-solvent. Thereafter, lipid was extracted from the ionic liquid using hexane, and the organic solvent layer was separated from the cell debris layer by centrifugation.

Ionic liquid treatments using mixtures of two ionic liquids were performed by means of various couplings at 120 °C for 2 h (5% *C. vulgaris* loading, 1:1, w/w). 1 g of the cells was mixed with each 9.5 g of the two ionic liquids. Thereafter, lipid was extracted from the ionic liquid using hexane, and the organic solvent layer was separated from the cell debris layer by centrifugation.

Finally, the organic solvent layer containing lipids was evaporated using a vacuum evaporator, whereupon the lipid was recovered. The lipid extraction yield was determined with reference to the weight of the recovered lipid.

2.4. Analyses

The intracellular content of the fatty acids was analyzed using the modified direct transesterification method [15]. Approximately 10 mg of the cells was put inside an 11 mL Pyrex-glass tube with a Teflon-sealed screw-cap. Two mL of the newly prepared chloroform–methanol mixture (2:1, v/v) was added to the cells, whose solution was then vigorously agitated using a vortex mixer (Vorex Genius 3, Ika, Italy) at room temperature for 10 min. One mL of chloroform solution containing heptadecanoic acid (Sigma, USA) as an internal standard (500 μg/L; Sigma, USA), 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 100 °C bath-water for 10 min, after which it was cooled to room temperature, supplemented with 1 mL of distilled water, and intensely mixed for 5 min. It was 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.

FAME conversion was performed following the modified direct transesterification method cited above [15]. Initially, recovered microalgal lipid was used for the transesterification instead of the cells. Around 10 mg of microalgal lipid was put inside an 11 mL