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Direct dissolution of wet and saliferous marine microalgae by polar ionic liquids without heating

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ABSTRACT

We successfully dissolved wet and saliferous microalgae (WSM) in polar ionic liquids (ILs) under mild conditions. The Kamlet–Taft parameters, especially β for the ILs, were good predictors of the ability to dissolve WSM. 1-Ethyl-3-methylimidazolium methylphosphate ([C2mim][MeO(H)PO_2]) was the IL that best dissolved WSM without heating. WSM (containing 95 wt% water) was mixed with [C2mim][MeO(H)PO_2]; the WSM had dissolved completely within 30 min at room temperature with gentle stirring. The IL maintained its chemical structure after removal of the microalgae component, suggesting recyclable use. The concentration of contaminant mineral salts in the [C2mim][MeO(H)PO_2] did not increase with increasing recycle number. The recycled [C2mim][MeO(H)PO_2] maintained its ability to dissolve WSM regardless of the number of recycling studied here.

1. Introduction

Alternative sources of energy to fossil fuels are increasingly sought. In particular, renewable energy sources have been advocated [1,2]. Candidates include biofuels produced from biomass. The first generation of biofuel was based on edible plants such as potato and sugar cane, because material conversion from starch is easy. It is, however, inadvisable to assign a share of edible crops to automobiles. Second generation biofuels have therefore been developed, deriving from non-edible biomass from lignocellulosic materials such as bagasse, which has a much reduced effect on the food supply [3]. Unfortunately the extraction of cellulosic components from biomass involves significant amounts of energy. Efforts have been made to extract cellulose under mild conditions. Polar ILs are promising solvents in this step. The third generation of biofuels comprises algae, which offer considerable benefits including reduction of net CO_2 production and the generation of renewable energy in nature. Microalgae have recently been considered as a third generation feedstock biofuel for synthesizing useful organic materials, because they grow in water and do not compete with land-based crops [4]. Microalgae, with their high growth rate and capability for mass cultivation, have recently been studied as a means of producing important compounds from CO_2 with the aid of sunlight [5]. Depending on the type of microalgae, various organic compounds can be produced and stored in the cells, including docosahexaenoic acid, eicosapentaenoic acid, and linolenic acid [1,6,7]. Furthermore, increasing yields have been reported by adjusting the treatment conditions for the cells [8]. Microalgae comprise a promising system for green energy sources including hydrogen, biodiesel, and bioethanol. Microalgae are also promising materials in bio-refining, generating organic compounds such as bio-plastics and fine chemicals [1,2,4,6].

Microalgae have been studied for many years. A strategy is needed for separating the particular organic compounds produced from the microalgae [9]. For the production of these compounds to be useful, their recovery should be a low-energy process. Conventional methods of separation of target compounds using extraction solvents generally involve several steps (see Scheme 1A) [10,11]. This is because the cell wall of the microalgae contains cellulose and peptidoglycans which are scarcely soluble in ordinary solvents. Steps in the conventional treatment include demineralization, dehydration, and drying or disruption of the cell wall by physical or chemical methods. These steps involve large amounts of energy and organic solvents, and must be performed at high efficiency and with recycling capability. Since, it is very difficult to reduce the energy consumption of existing methods, novel methods are strongly requested.

Abbreviations: WSM, Wet and saliferous microalgae; ILs, Ionic liquids; NMR, Nuclear magnetic resonance; [C2mim][MeO(H)PO_2], 1-Ethyl-3-methylimidazolium methylphosphate.

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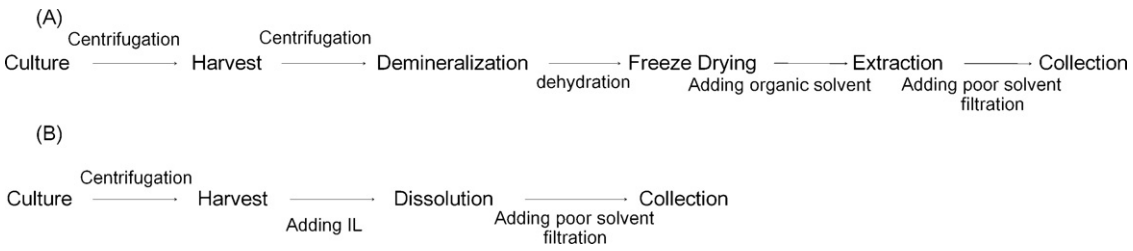

Scheme 1. Comparison of treatment procedure for WSM. Conventional process (A) and process proposed in the present study (B).

Ionic liquids (ILs) are increasingly studied as novel materials with a wide range of applications. Some ILs have excellent properties for use in cellulosic biomass treatment [12–14]. They are also expected to be effective solvents for microalgae. ILs should be effective at removing lignin and hemicelluloses, and preventing cellulose crystallization, because complete dissolution takes place at the molecular level [15]. High temperature is still needed in most cases, however. The dissolution of algae in ILs has recently been reported [16]. Unfortunately, all of the chloride salts used are solid at room temperature. To make them liquid and suitable for treatment of algae, it is necessary to heat them above 100 °C. Although it should be possible to treat them with super-cooled ILs, these would be highly viscous. We conclude that treatment under mild conditions and small energy expenditure is essential for viable extraction of energy from biomass.

In the present study, we first deployed polar ILs for the direct dissolution of marine microalgae without heating. These microalgae, in both marine and fresh water, are candidates for producing effective compounds from CO_2 with the aid of sunlight. Since the factors facilitating the dissolution of marine microalgae are similar to those for fresh water microalgae, we studied marine microalgae. We have reported previously the dissolution of cellulose under mild conditions [17,18]. With the aid of polar ILs, it should be possible to omit several steps such as drying and demineralization, as shown in Scheme 1(B). We propose below a novel method for treating WSM so as to achieve the separation of useful organic compounds.

2. Materials and methods

2.1. Materials

A series of commercially available ILs was used to examine the dissolution of WSM. The following ILs were purchased from Kanto Chemical Co: 1-ethyl-3-methylimidazolium chloride ([C2mim]Cl), 1-ethyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide ([C2mim][Tf_2N]), 1-ethyl-3-methylimidazolium tetrafluoroborate ([C2mim]BF_4), and 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]). All were used as received. 1-Ethyl-3-methylimidazolium methylphosphate ([C2mim] [$MeO(H)PO_2$]) was passed through an aluminum column before use. All other chemicals were purchased from Sigma–Aldrich.

2.2. Microalgae cultivation and harvesting

Two species of microalgae were used: *Synechocystis* sp. PCC 6803, which is commonly used in the production of organic compounds [19], and *Synechocystis* st. NKBG 042902, an original species originating with our collaborator [20]. Both species were grown in 500 mL BG-11 medium containing 3% of NaCl under continuous aeration and illumination. The cells were harvested after 1 week of culture, by centrifuging until the water content was less than 95 wt%. The resulting WSM were used in the experiments.

2.3. Dissolving of wet and saliferous microalgae in ILs

To investigate the effect of polarity of ILs on the dissolution of WSM, we used five different ILs, as shown in Fig. 1. The polarity of the ILs was determined from the solvatochromism of dye molecules [21]. Three dyes were used: 4-nitroaniline, *N,N*-diethyl-4-nitroanilne, and Reichardt's dye; the Kamlet–Taft parameters were calculated according to the equations in Ref. [21]. Dissolution of WSM in chloroform was also studied as a reference, since chloroform is a conventionally used solvent for these extractions [10,11].

2.4. Deposition of the microalgae component and recycling of IL

After the WSM had been dissolved in the polar ILs, a small amount of a poor solvent, namely water, was added to the solution in order to precipitate the microalgae component. The precipitated microalgae component was removed by filtration. The supernatant, comprising the mixture of IL and poor solvent, was then evaporated to remove the latter. After vacuum drying, the IL structure was checked by NMR. Contamination of mineral salts in recycled IL was confirmed by ion chromatography. The solubility of WSM was tested with the recycled IL via the same procedure specified above.

3. Results and discussion

3.1. Dissolving of wet and saliferous microalgae

The solubility of both *Synechocystis* sp. PCC 6803 and *Synechocystis* st. NKBG 042902 was almost identical to each other regardless of solvent used. Fig. 2 shows pictures of the mixture of *Synechocystis* sp. PCC6803 (0.5 wt%) with ILs or chloroform. The mixture comprises two separate phases in chloroform (Fig. 2(A)), and WSM did not dissolve. In contrast, complete dissolution of WSM was observed in [C2mim][$MeO(H)PO_2$] after 30 min of stirring at room temperature (Fig. 2(B)). [C2mim]Cl also dissolved WSM completely after 1 h of stirring at 80 °C. In the case of [C2mim][OAc], some parts of WSM had dissolved after 24 h of stirring at room temperature (Fig. 2(C)). When [C2mim][Tf_2N] was used, two separate phases were observed without dissolution. This was similar to the results with chloroform (see Fig. 2(A) and (D)). In the case of [C2mim]BF_4, the microalgae were simply dispersed in the IL (Fig. 2(E)). No dissolution was observed in this IL even after stirring for 24 h at room temperature.

The Kamlet–Taft parameters were calculated for the ILs used in this study in order to analyze the relation between polarity of the ILs and WSM solubility. Table 1 shows the values of α, β and π^* for these ILs, which respectively are measures of hydrogen bonding donating ability, hydrogen bonding accepting ability, and dipolarity/polarizability. Polar ILs have been reported to dissolve biopolymers such as cellulose that are scarcely soluble in molecular liquids. ILs with large β value have considerable capability to dissolve cellulose [17,18,22]. In the case of microalgae, the major components of the cell wall are reported to be cellulose and

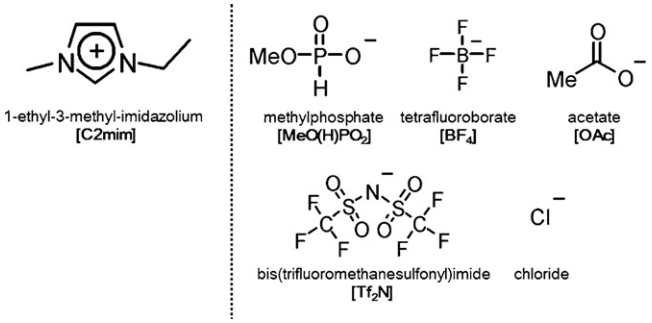

Fig. 1. Structure of cations and anions of ILs examined in this study.