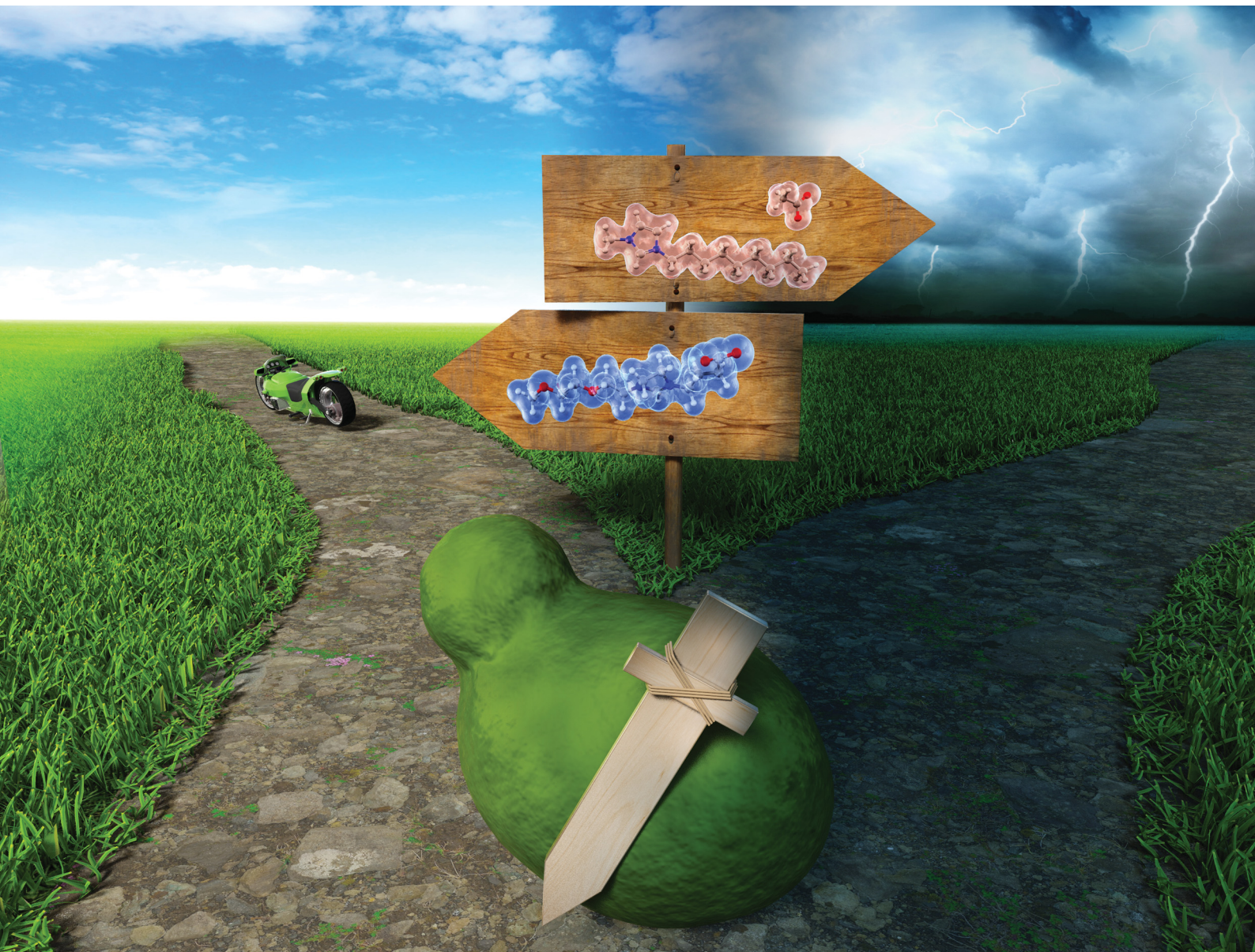


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Bioethanol fermentation in the presence of ionic liquids: mini review

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Ionic liquids are known as efficient pretreatment solvents for cellulosic biomass, but typical cellulose-dissolving ionic liquids are toxic to microorganisms, hindering the fermentation process for bioethanol production. Although the ionic liquids can be removed by washing after pretreatment, it negatively impacts the energy balance. Therefore, successive/simultaneous biomass pretreatment, hydrolysis, and fermentation processes with low-toxicity ionic liquids are desired. Herein, especially for the beginners and students, the toxicity of ionic liquids and their mechanisms by which the microorganisms are destroyed are simply discussed. Furthermore, two currently evolving solutions, low-toxicity ionic liquids and ionic liquid-resistant microorganisms for bioethanol production, are discussed. To the best of my knowledge, this is the first review specifically focusing on bioethanol fermentation in ionic liquid solutions, whereas there are many reports on cellulose dissolution.

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Lignocellulose as a resource of bioethanol

The global demand for energy is increasing, and concerns about climate change and the instability of oil resources have led to renewed interest in alternative energy sources to replace fossil fuels. As a result, many countries have been focusing on sustainable and renewable biofuels.^{1,2} Currently, corn starch, sugarcane, and beet monosaccharides are directly converted to ethanol, but these edible sugars compete with food and feed. Therefore, lignocellulose, a major component of plant cell walls, is recognized as one of the most promising resources for ethanol production.³ Specifically, cellulose and hemicellulose in lignocellulose are the resources of bioethanol. Although hemicellulose is relatively easy to convert, cellulose is not; it is one of the most chemically and physically robust and recalcitrant natural polymers.^{3,4}

Significance of bioethanol fermentation in the presence of ionic liquids

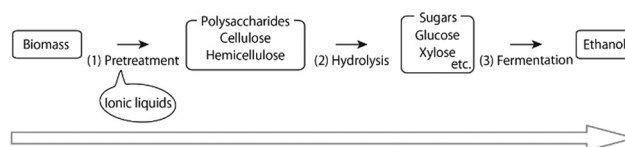
Typical ionic liquids, which are liquid salts below 100 °C,^{5–14} are toxic to microorganisms.^{15–20} Then, why is it necessary to

conduct microbial fermentation in the presence of ionic liquids? To figure this out, we first look at the overall picture of the bioethanol production process (Fig. 1).

Polysaccharides can be converted to ethanol by the following three processes: (1) Pretreatment with ionic liquids to improve enzyme accessibility of the polysaccharides; (2) enzymatic hydrolysis of the polysaccharides to sugars, such as glucose and xylose; (3) ethanol fermentation by microorganisms, such as yeasts.

Energy cost is one of the most important factors in the production of ethanol as a fuel. It is essential to reduce energy costs during production and thus maximize the energy obtained from ethanol. Although there are several cellulose solvents such as LiCl/*N,N*-dimethylacetamide and ethylene diamine/salt,^{21–23} they do not efficiently dissolve cellulose at room temperature and ambient pressure. The high temperature and/or pressure are very energy intensive, hindering the practical industrialization of bioethanol.

On the other hand, ionic liquids, especially those containing carboxylate, dialkylphosphate, or alkylphosphonate anions, efficiently dissolve cellulose/biomass even at room temperature.^{24–31}



To proceed these steps successively or simultaneously,
 (2) Hydrolysis and (3) Fermentation have to be carried out in the presence of ionic liquids.

Fig. 1 Overview of the bioethanol production process and the significance of fermentation in the presence of ionic liquids.

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2. Use of ionic liquid-tolerant microorganisms

2-(1) Search for ionic liquid-tolerant microorganisms. *Yarrowia lipolytica* (*Y. lipolytica*), a type of yeast, is highly tolerant to ionic liquids.⁵² For example, *Y. lipolytica* is stress tolerant by nature and has the ability to adapt to a wide range of pH (2–11) and high salt concentration such as 12% NaCl. Therefore, it can grow and ferment even in 10% [C₂mim]OAc. Although it is not capable of producing ethanol, it can produce useful substances such as α -ketoglutaric acid and eicosapentaenoic acid. Among yeasts capable of ethanol fermentation, *Kazachstania telluris* and *Wickerhamomyces anomalus* exhibit high ionic liquid tolerance;⁵³ however, it is not as high as that of *Y. lipolytica*.

2-(2) Incubation in ionic liquids to confer ionic liquid tolerance. Walker *et al.* succeeded in improving the ionic liquid tolerance of *Y. lipolytica*.⁵⁴ They started culturing *Y. lipolytica* in 5% [C₂mim]OAc and gradually increased the concentration of [C₂mim]OAc to obtain a *Y. lipolytica* strain that could grow in an 18% [C₂mim]OAc solution. This strain showed an increase in the phospholipid content and changes in its composition, as well as an increase in sterol content after incubation. In particular, it has been suggested that the decrease in cell membrane fluidity associated with the increase in sterol content is important.

2-(3) Gene modification to confer ionic liquid tolerance. To confer ionic liquid tolerance to microorganisms *via* genetic modification, it is necessary to identify factors that are important for ionic liquid tolerance. Ionic liquids affect the mitochondria in *S. cerevisiae* because of the cation uptake transporters.⁵⁵ Therefore, attempts have been made to knock out its related gene, *ptk2*, to avoid the uptake of ionic liquids. The *ptk2* knockout greatly improved the resistance to [C₂mim]OAc and [C₂mim]Cl.⁵⁵ Furthermore, *E. coli* with improved ionic liquid resistance by genetic recombination has been reported.⁵⁶

Other mechanisms of ionic liquid resistance have been investigated in *Enterobacter lignolyticus*.⁵⁵ These include: (1) decreased intracellular permeability of ionic liquids due to changes in the phospholipid composition and reduction of porin (a passive transporter that penetrates through the plasma membrane), (2) extracellular efflux of ionic liquids by multidrug efflux pumps, and (3) accumulation of sugars and amino acids to confer osmotolerance. It can be expected that ionic liquid tolerance will be conferred by using these mechanisms.

3. Use of low toxicity ionic liquids

Although [C₂mim]⁺-based ionic liquids effectively delignify and reduce cellulose crystallinity in biomass,^{10,57,58} they are basically highly toxic. Therefore, low-toxicity ionic liquids are being developed. One strategy for developing low-toxicity ionic liquids is to use bio-derived ions such as choline acetate ([Ch]OAc). As expected, [Ch]OAc has been reported to be less toxic than [C₂mim]OAc.⁵⁹ Although its pretreatment capacity is somewhat less than that of [C₂mim]OAc, it is reported to be adequate.^{60–62} Pretreatment with [Ch]OAc also occurs through the mechanisms of cellulose crystallinity reduction and delignification. However, despite its low toxicity toward microbial growth, it

Table 2 Half maximal effective concentration (EC₅₀) of zwitterions, an ionic liquid, organic solvents against *E. coli* KO11 and relative ethanol concentration in 0.5 mol L⁻¹ solutions.^{45,48}

		EC ₅₀ (g L ⁻¹)	Relative ethanol concentration (%)	Pretreatment ability
Zwitterion	OE ₂ imC ₃ C	158	96	Yes
	C ₁ imC ₃ C	141	100	Yes
	C ₁ imC ₃ S	> 200	104	No
Ionic liquid	[Ch]OAc	70	15	Yes
	Dimethyl sulfoxide	91	—	No
	Ethanol	17	—	No
Control	—	—	100	—

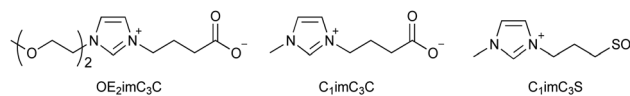


Fig. 4 Structures of zwitterions described in this review.

is highly toxic to fermentation, resulting in low ethanol yields (Table 2).⁴⁸

Ionic liquids with choline cations and amino acid anions have also been reported.^{60,63–66} These ionic liquids are capable of pretreating biomass, with choline lysinate being particularly effective. The primary pretreatment mechanism of choline lysinate is delignification, but it can also slightly reduce cellulose crystallinity.

Artificial ions can also exhibit low toxicity. Zwitterionic liquids (*e.g.*, OE₂imC₃C; Fig. 4), in which the anion and cation are covalently bonded, have been reported to be much less toxic than the corresponding ionic liquids.^{45,48,67,68} The greatest advantage of zwitterionization is that zwitterionic liquids exhibit low toxicity without loss of their functionality, because the constituent cations or anions are the same as the original ionic liquids. OE₂imC₃C, which combines an imidazolium cation with a carboxylic acid anion, can not only perform delignification and reduce cellulose crystallinity, but also dissolution of cellulose.^{45,48,68–70} In addition, microbial fermentation is possible even in OE₂imC₃C solutions of more than 50 wt%, showing its extremely low toxicity.⁴⁸

Feasibility of continuous ethanol production

One-pot ethanol production from plant biomass is at the edge of realization through the various innovations described above. In particular, the Joint BioEnergy Institute and its affiliated research institutes in the United States are vigorously pursuing research toward practical application, including protic ionic liquids and deep eutectic solvents.^{66,71–75} They have already been continuously converting tens of kilograms of woody biomass into ethanol with relatively high yields.⁷² The one-pot ethanol production process also reduces the capital cost and wastewater treatment cost. They then estimate the



production cost of ethanol to be approximately \$5.3 per gallon, which can be lowered to \$1.8 per gallon with further improvements.⁷² The price is similar or a little bit higher than the case produced by the other pretreatment methods,⁷⁶ although precise comparison of the values from different literature is difficult. Reducing the price of ethanol to match that of gasoline will facilitate the practical application of bioethanol.

Conclusions

The practical application of cellulosic bioethanol is an extremely difficult task. It is evidenced by the fact that it has not yet been economically industrialized on a large scale, despite knowing that “cell walls are made of glucose units” for 100 years. However, as mentioned in the previous section, the possibility of practical application is gradually emerging. In Europe, a venture company, Lixea, has been established, and its practical application is expected in the future.

On the other hand, bioethanol production includes chemical, enzymatic, and microbial processes, requiring interdisciplinary insights and techniques such as those from biochemistry, genetics, organic chemistry, and chemical engineering. Agriculture is also important considering the cultivation of biomass. Thus, new ideas and innovation are urgently required from areas other than of ionic liquids and biomass pretreatment.

While this paper mainly focuses on ethanol production by yeast, a wide variety of fermentation products can be obtained from glucose and other sugars. For example, limonene⁵⁶ and isopentenol⁶⁶ were produced by *E. coli*, which can be easily genetically modified. The technologies described in this review are expected to expand into many other areas, not limited to bioethanol production.

Conflicts of interest

There are no conflicts to declare.

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