Effects of the Ionic Liquid 1-Butyl-3-methylimidazolium Chloride on the Growth and Ethanol Fermentation of Saccharomyces cerevisiae AY92022

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Use of ionic liquids has provided a potential effective alternative in the conversion of carbohydrates in lignocellulosic materials into fermentable sugars for ethanol production. To evaluate how the remained ionic liquids in the fermentable sugars affect the subsequent ethanol fermentation process, the effects of ionic liquid 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) in the medium at different concentrations from $10^{-3}$ to $1 \text{ g L}^{-1}$ on the morphological structure, growth and ethanol fermentation of the yeast Saccharomyces cerevisiae AY92022 were investigated and compared with the control. First, the morphological structures of the yeast at different [Bmim]Cl concentrations were observed under an optical microscope. The results show that its single cell morphology remained unchanged at all [Bmim]Cl concentrations, but its reproduction rate by budding decreased with the [Bmim]Cl concentration increasing. Then its growth during ethanol fermentation process at different [Bmim]Cl concentrations was examined. The results indicated that the ionic liquid [Bmim]Cl inhibited the yeast growth. Its specific growth rate during the log phase and bacterial concentration during the stationary phase all decreased with the increase of [Bmim]Cl concentration. Finally, the ethanol fermentation process at different [Bmim]Cl concentrations was investigated and the results demonstrated that the ionic liquid [Bmim]Cl had a negative effect on ethanol production. When the [Bmim]Cl concentration increased, the final ethanol concentration and its yield from the fermentable sugars decreased, the finally remaining fermentable sugars increased, and it is interesting the ethanol specific formation rate at stationary phase remained unchanged at all [Bmim]Cl concentrations. It was also observed that when the [Bmim]Cl in the medium was $10^{-3} \text{ g L}^{-1}$, the ethanol fermentation process data was almost no different to that of the control. This suggests that the [Bmim]Cl in the fermentable sugars should be controlled below $10^{-3} \text{ g L}^{-1}$, thus it would not affect the subsequent ethanol fermentation process.

Key words: Ionic liquid, [Bmim]Cl, growth, ethanol fermentation, Saccharomyces cerevisiae AY92022

Introduction

Energy consumption has been increasing steadily with the population growth and industrial development. Conventional energy sources have difficulty in meeting the increasing energy demand. Therefore, there is great interest in exploring alternative energy sources to maintain the sustainable growth of society. Ethanol, as a clean and renewable energy, has drawn much attention in recent years. Lignocellulosic materials are the most economical and highly renewable natural resources in the world. Therefore, production of ethanol from lignocellulosic materials has become one of the potentially practical routes to solve the energy problems. The conversion of carbohydrates in lignocellulosic materials into ethanol includes two sub-processes: hydrolysis of carbohydrates in lignocellulosic materials to fermentable sugars, and then fermentation of the fermentable sugars to ethanol. The hydrolysis always becomes the bottleneck process because of the complex structure of lignin and hemicellulose with cellulose in lignocellulosic materials. Extensive researches have been carried out on the hydrolysis process, but few can be used in an industrial scale based on economical and environmental consideration. Use of ionic liquids has provided a potentially efficient alternative to convert the carbohydrates in lignocellulosic materials into fermentable sugars for ethanol production. In recent years, there have been many reports on the conversion of carbohydrates in lignocellulosic
materials into fermentable sugars by using ionic liquid technology.\textsuperscript{10–15}\textsuperscript{10} Some researchers used ionic liquids to pre-treat lignocellulosic materials for improvement of their enzymatic hydrolysis efficiency,\textsuperscript{10–12}\textsuperscript{12} while others directly obtained the fermentable sugars from lignocellulosic materials by their chemical hydrolysis in ionic liquid system with or without acid catalyst.\textsuperscript{13–15}\textsuperscript{15} Whatever methods were employed, it was inevitable that some ionic liquids remained in the obtained fermentable sugars. As far as is known, how the remaining ionic liquids in fermentable sugars will affect the subsequent ethanol fermentation process has not been reported before, but it is extremely important to evaluate the suitability of the use of ionic liquids for ethanol production from lignocellulosic materials. This work is to deal with the influence of the remained ionic liquids in the fermentable sugars on the subsequent ethanol fermentation process. To do this, the effects of ionic liquid 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) in the medium at different concentrations on the morphological structure, growth and ethanol fermentation of the yeast \textit{Saccharomyces cerevisiae} \textit{AY92022} were investigated.

\textbf{Materials and methods}

All experiments were carried out three times, and the given numbers are the mean values with relative error within \pm 5\%.

\textbf{Microorganism, medium, and culture conditions}

The yeast \textit{Saccharomyces cerevisiae} \textit{AY92022} was used throughout this study. The stock cultures were maintained on YPD agar plates at 4\°C and transferred to fresh plates every 4 weeks to avoid microorganism degradation. The inoculum was prepared by means of transferring the microorganism from stock cultures to a fresh plate and growing it for 48 h at 30\°C. Following this period, single colonies were transferred to a 250 mL flask with 100 mL inoculum medium. The flask was placed on an orbital shaker with a shaking diameter 5 cm and shaking frequency 200 rpm, and incubated at 30\°C for 24 h. This was used as the inoculum for ethanol fermentation. The ethanol fermentation was carried out in a 500 mL flask with 190 mL ethanol fermentation medium and 10 mL inoculum at 30\°C and 200 rpm for 48 h. During the fermentation, small samples were taken at regular intervals for later analytical use. The compositions of culture medium were as follows (g L\textsuperscript{–1}):

The YPD agar medium: D-glucose 20, peptone 20, yeast extract 10, agar 15.

![Image](image-url)
Saccharomyces cerevisiae AY92022 could be negligible.

The growth of the yeast Saccharomyces cerevisiae AY92022 is closely related to ethanol production. To evaluate the influence of ionic liquid [Bmim]Cl on its ethanol fermentation process, it is essential to know how ionic liquid [Bmim]Cl affects its growth. Fig. 2 shows the growth curves of the yeast Saccharomyces cerevisiae AY92022 at different [Bmim]Cl concentrations for ethanol fermentation process. As illustrated in Fig. 2, the growth curves of the yeast Saccharomyces cerevisiae AY92022 at all [Bmim]Cl concentrations were fit for the typical batch bacterial growth curves, which included 4 different periods: lag phase, log phase, stationary phase and decline phase. The ionic liquid [Bmim]Cl inhibited the growth of the yeast Saccharomyces cerevisiae AY92022 with the increase of [Bmim]Cl concentration. As shown in Table 1, its specific growth rate at log phase and its concentration at stationary phase all decreased with the increase of [Bmim]Cl concentration from $10^{-3}$ to 1 g L$^{-1}$. From Fig. 2 and Table 1, it could also be observed that the ionic liquid [Bmim]Cl at $10^{-3}$ g L$^{-1}$ had almost no inhibitory effect on the growth of the yeast Saccharomyces cerevisiae AY92022 in comparison with the control. This verified the result of the morphological structure observation of the yeast Saccharomyces cerevisiae AY92022 at different [Bmim]Cl concentrations in the above section. In previous work, Docherty and Kulpa found that the ionic liquid [Bmim]Br could slightly inhibit the growth of yeast when they investigated the toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids.21 Their work was consistent with our results perfectly. All this work suggests that the remained ionic liquids in the fermentable sugars at higher concentration (greater than $10^{-3}$ g L$^{-1}$) could inhibit the growth of the yeast Saccharomyces cerevisiae AY92022. However, the

\[ \gamma_i (\text{g L}^{-1}) \]

<table>
<thead>
<tr>
<th>$i$</th>
<th>0</th>
<th>$10^{-3}$</th>
<th>$10^{-2}$</th>
<th>$10^{-1}$</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma_b$ (g L$^{-1}$)</td>
<td>15.1</td>
<td>15.0</td>
<td>12.7</td>
<td>10.7</td>
<td>8.6</td>
</tr>
<tr>
<td>$\mu$ (h$^{-1}$)</td>
<td>0.428</td>
<td>0.427</td>
<td>0.403</td>
<td>0.380</td>
<td>0.346</td>
</tr>
</tbody>
</table>

$\gamma_i$ is the [Bmim]Cl concentration (g L$^{-1}$), $\gamma_b$ is the average concentration of the yeast Saccharomyces cerevisiae AY92022 at stationary phase (g L$^{-1}$), $\mu$ is the specific growth rate of the yeast Saccharomyces cerevisiae AY92022 at log phase (h$^{-1}$).
remained ionic liquids in the fermentable sugars at lower concentration (less than $10^{-3}$ g L$^{-1}$) would not affect its growth.

In order to evaluate the suitability of the remained ionic liquids in the fermentable sugars for ethanol production, the influence of the ionic liquid [Bmim]Cl at different concentrations on the ethanol fermentation process was investigated. The time courses of ethanol and the fermentable sugars at different [Bmim]Cl concentrations for ethanol fermentation process are shown in Figs. 3 and 4 respectively. As shown in Figs. 3 and 4, the ionic liquid [Bmim]Cl at $10^{-3}$ g L$^{-1}$ had almost no effect on the ethanol fermentation processes in comparison with the control. However, the ionic liquid [Bmim]Cl at higher concentration (greater than $10^{-3}$ g L$^{-1}$) had a negative effect on ethanol fermentation process. Table 2 lists some important ethanol fermentation process parameters. As indicated in Table 2, when the [Bmim]Cl concentration increased, the final ethanol concentration and its yield from the fermentable sugars decreased, and it was interesting that the ethanol specific formation rate at stationary phase kept unchanged at all [Bmim]Cl concentrations, suggesting that the negative effect of ionic liquid [Bmim]Cl at higher concentration (greater than $10^{-3}$ g L$^{-1}$) on ethanol fermentation process resulted from its inhibitory effect on growth of the yeast *Saccharomyces cerevisiae* AY92022.

This is somewhat different from the research of Matsumoto *et al.* on the lactate production. In their work, the ionic liquids not only hampered the growth rate of microorganisms but also interfered with their lactate productivity. Anyway, the ionic liquid [Bmim]Cl at higher concentration (greater than $10^{-3}$ g L$^{-1}$) will inhibit the yeast growth and thus affect the subsequent ethanol fermentation process. Therefore, it is essential that the remained ionic liquid concentration in the fermentable sugars is controlled below $10^{-3}$ g L$^{-1}$ to avoid affecting the subsequent ethanol fermentation process.

### Conclusions

The effects of ionic liquid [Bmim]Cl at different concentrations from $10^{-3}$ to $1$ g L$^{-1}$ on the morphological structure, growth, and ethanol fermentation of the yeast *Saccharomyces cerevisiae* AY92022 were investigated and compared with the control. The main conclusions are as follows:

1) The single cell morphology of the yeast *Saccharomyces cerevisiae* AY92022 remained unchanged at all [Bmim]Cl concentrations, but its reproduction rate by budding decreased with increasing [Bmim]Cl concentration.

2) The ionic liquid [Bmim]Cl at higher concentration (greater than $10^{-3}$ g L$^{-1}$) inhibited the yeast growth. Its specific growth rate during the log phase and bacterial concentration during the stationary phase all decreased with the increase of [Bmim]Cl concentration.

3) The ionic liquid [Bmim]Cl at higher concentration (greater than $10^{-3}$ g L$^{-1}$) had a negative effect on ethanol production. When the [Bmim]Cl concentration increased, the final ethanol concentration and its yield from fermentable sugars decreased, and it was interesting that the etha-

### Table 2 — Effect of [Bmim]Cl concentration on the ethanol fermentation process parameters

<table>
<thead>
<tr>
<th>$\gamma_i$ (g L$^{-1}$)</th>
<th>0</th>
<th>$10^{-3}$</th>
<th>$10^{-2}$</th>
<th>$10^{-1}$</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma_p$ (g L$^{-1}$)</td>
<td>40.9</td>
<td>40.5</td>
<td>36.5</td>
<td>33.1</td>
<td>28.8</td>
</tr>
<tr>
<td>$\gamma_s$ (g L$^{-1}$)</td>
<td>8.0</td>
<td>8.3</td>
<td>8.7</td>
<td>12.6</td>
<td>18.6</td>
</tr>
<tr>
<td>$q$ (h$^{-1}$)</td>
<td>0.12</td>
<td>0.12</td>
<td>0.119</td>
<td>0.113</td>
<td>0.122</td>
</tr>
<tr>
<td>$Y$</td>
<td>0.445</td>
<td>0.442</td>
<td>0.400</td>
<td>0.379</td>
<td>0.354</td>
</tr>
</tbody>
</table>

$\gamma_i$ is the [Bmim]Cl concentration (g L$^{-1}$), $\gamma_p$ is the final ethanol concentration (g L$^{-1}$), $\gamma_s$ is the final fermentable sugars concentration (g L$^{-1}$), $q$ is the ethanol specific formation rate at stationary phase (h$^{-1}$), $Y$ is the ethanol yield from the fermentable sugars.
nol specific formation rate at stationary phase kept unchanged at all [Bmim]Cl concentrations.

4) When the [Bmim]Cl concentration was \(10^{-3}\) g L\(^{-1}\), the yeast growth and ethanol fermentation was almost no different to the control throughout this study. This suggests that the remained ionic liquid concentration in the fermentable sugars should be controlled below \(10^{-3}\) g L\(^{-1}\), thus it would not affect the subsequent ethanol fermentation process.

ACKNOWLEDGEMENTS

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References