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 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} 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} 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.[1,2] 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.[1–4]

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.[1,5,6] Use of ionic liquids has provided a potentially efficient alternative to convert the carbohydrates in lignocellulosic materials into fermentable sugars for ethanol production.[7–9] In recent years, there have been many reports on the conversion of carbohydrates in lignocellulosic

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materials into fermentable sugars by using ionic liquid technology.[10–15] Some researchers used ionic liquids to pre-treat lignocellulosic materials for improvement of their enzymatic hydrolysis efficiency,[10–12] while others directly obtained the fermentable sugars from lignocellulosic materials by their chemical hydrolysis in ionic liquid system with or without acid catalyst.[13–15] Whatever methods were employed, it was inevitable that some ionic liquids remained in the obtained fermentable sugars. As far as is known, how the remained 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 *Saccharomyces cerevisiae* AY92022 were investigated.

Materials and methods

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

Microorganism, medium, and culture conditions

The yeast *Saccharomyces cerevisiae* 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^{-1}):

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

The inoculum medium: D-glucose 20, peptone 20, yeast extract 10.

The ethanol fermentation medium: D-glucose 100, peptone 20, yeast extract 10.

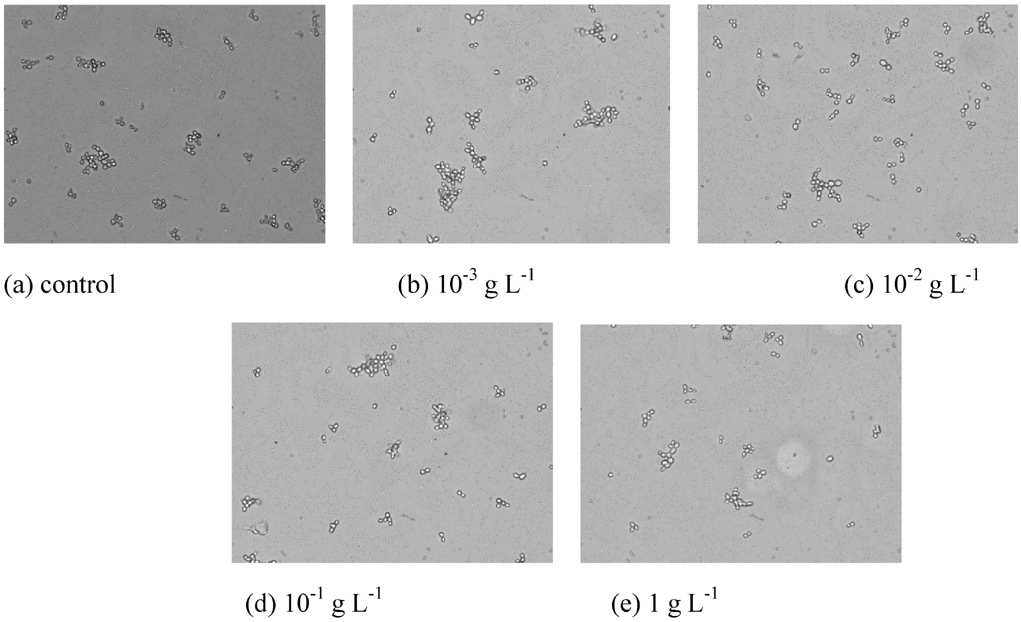
Each medium was autoclaved at 121 °C for 20 minutes after the suitable amount of ionic liquid [Bmim]Cl was added to a given concentration.

Analytical methods

The samples taken from the ethanol fermentation process were used to observe the bacterial morphological structure and determine concentration of yeast, ethanol and the fermentable sugars. The bacterial morphological structure was observed using an OLYMPUS CX41 microscope. Yeast concentration was determined by the dry weight method.[16] Ethanol content was determined by gas chromatography[17] and the fermentable sugars concentration was estimated using the 3,5-dinitrosalicylic acid method.[18]

Results and discussion

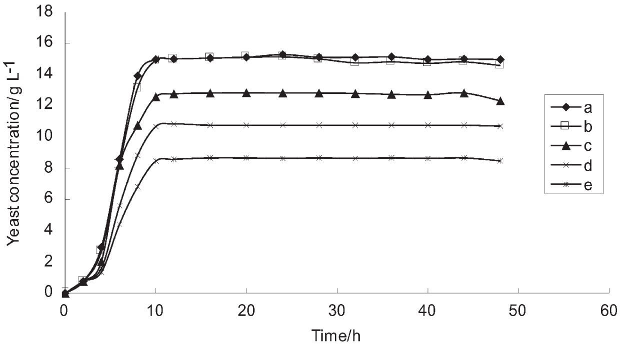
The morphological structure is one of the most important physiological properties for a microorganism. Some recent researches on the toxicity of ionic liquids have shown that ionic liquids could lead to changes in the morphological structure of some microorganisms.[19,20] Observation of the changes to the morphological structure of microorganisms in the medium containing ionic liquids under a microscope can provide useful information on the toxicity of ionic liquids to them. The morphological structures of the yeast *Saccharomyces cerevisiae* AY92022 at different [Bmim]Cl concentrations in the medium during ethanol fermentation were observed using an OLYMPUS CX41 microscope in this study and Fig. 1 shows the morphological images of the yeast *Saccharomyces cerevisiae* AY92022 at different [Bmim]Cl concentrations in the medium when ethanol fermentation time was 8 h. As indicated in Fig. 1, the single cell morphology of the yeast *Saccharomyces cerevisiae* AY92022 kept almost unchanged at all [Bmim]Cl concentrations in comparison with the control, but its reproduction rate by budding decreased with the increase of [Bmim]Cl concentration from 10^{-3} to 1 g L^{-1} although its budding rate had no obvious difference between the control and the [Bmim]Cl concentration at 10^{-3} g L^{-1}. This suggests that the ionic liquid [Bmim]Cl at higher concentration (greater than 10^{-3} g L^{-1}) could inhibit the growth of the yeast *Saccharomyces cerevisiae* AY92022 by decreasing its budding rate. However, the inhibition of ionic liquid [Bmim]Cl at lower concentration (less than 10^{-3} g L^{-1}) on the growth of the yeast



F i g . 1 – *Morphological structure of the yeast Saccharomyces cerevisiae AY92022 at different [Bmim]Cl concentrations*

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 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



F i g . 2 – *Growth curves of the yeast Saccharomyces cerevisiae AY92022 for ethanol fermentation at different [Bmim]Cl concentrations; (a) control (b) 10^{-3} g L^{-1} (c) 10^{-2} g L^{-1} (d) 10^{-1} g L^{-1} (e) 1 g L^{-1}*

T a b l e 1 – *Effect of [Bmim]Cl concentration on the growth process parameters of the yeast Saccharomyces cerevisiae AY92022*

γ_i (g L^{-1})	0	10^{-3}	10^{-2}	10^{-1}	1
γ_b (g L^{-1})	15.1	15.0	12.7	10.7	8.6
μ(h^{-1})	0.428	0.427	0.403	0.380	0.346

γ_i is the [Bmim]Cl concentration (g L^{-1}), γ_b is the average concentration of the yeast *Saccharomyces cerevisiae* AY92022 at stationary phase (g L^{-1}), μ 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, the finally remaining fermentable sugars increased, 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

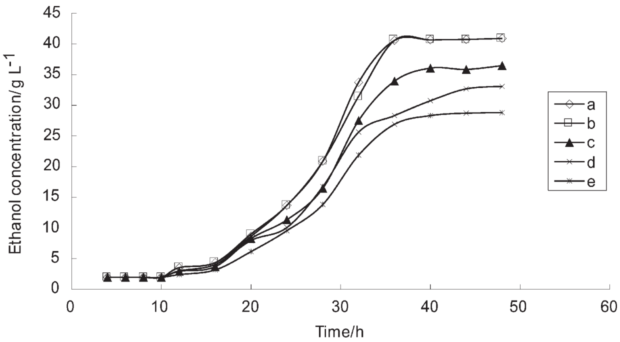


Fig. 3 – *Time courses of ethanol concentration for ethanol fermentation at different [Bmim]Cl concentrations; (a) control (b)* 10^{-3} *g* L^{-1} *(c)* 10^{-2} *g* L^{-1} *(d)* 10^{-1} *g* L^{-1} *(e) 1 g* L^{-1}

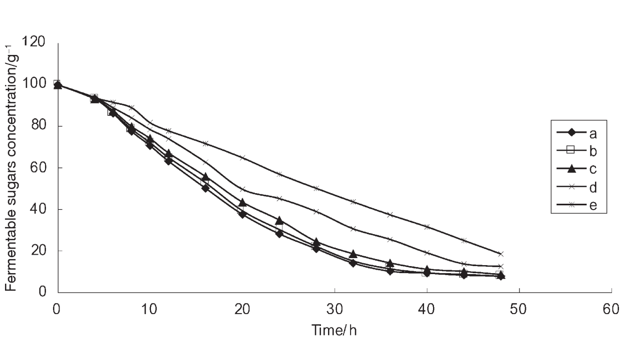


Fig. 4 – *Time courses of the fermentable sugars concentration for ethanol fermentation at different [Bmim]Cl concentrations; (a) control (b)* 10^{-3} *g* L^{-1} *(c)* 10^{-2} *g* L^{-1} *(d)* 10^{-1} *g* L^{-1} *(e) 1 g* L^{-1}

Table 2 – *Effect of [Bmim]Cl concentration on the ethanol fermentation process parameters*

γ_i (g L^{-1})	0	10^{-3}	10^{-2}	10^{-1}	1
γ_p(g L^{-1})	40.9	40.5	36.5	33.1	28.8
γ_s(g L^{-1})	8.0	8.3	8.7	12.6	18.6
q(h^{-1})	0.12	0.12	0.119	0.113	0.122
Y	0.445	0.442	0.400	0.379	0.354

γ_i is the [Bmim]Cl concentration (g L^{-1}), γ_p is the final ethanol concentration (g L^{-1}), γ_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.

(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.[22–23] 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, the finally remaining fermentable sugars also increased, and it was interesting that the etha-

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.

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