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Ethanol Fermentation of the Enzymatic Hydrolysates from the Products Pretreated using [EMIM]Ac and Its Co-Solvents with DMF

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Abstract

Ethanol fermentation of the enzymatic hydrolysates from the products pretreated using 1-ethyl-3-methyl-imidazolium acetate ([EMIM]Ac) and its co-solvents with dimethylformamide (DMF) was conducted using *Saccharomyces cerevisiae* (D452-2). The optical density change due to the yeast cell growth, the consumption amount of monosugars (glucose, xylose), the concentration of acetate, and ethanol production yield were investigated. The co-solvent system lowered inhibition of the growth of the cells. The highest concentration of glucose (7.8 g/L) and xylose (3.6 g/L) was obtained from the enzymatic hydrolysates of the pretreated product by pure [EMIM]Ac. The initial concentration of both monosugars in the enzymatic hydrolysates was decreased with increasing fermentation time. Ethanol of Approximately 3 g/L was produced from the enzymatic hydrolysates by pure [EMIM]Ac and co-solvent with less than 50% DMF.

Key Words: ethanol, fermentation, enzymatic saccharification, pretreatment, ionic liquid, organic solvent

Introduction

Biofuels from lignocellulosic biomass can be a suitable complement for petroleum-based fuel and bioethanol is one of the main biofuels especially in the transport sectors (Pacini and Strapasson 2012; Paulova et al. 2015). Bioethanol with high purity is also known as an excellent clean-burning fuel without generating by-products such as dioxide and metal oxide during combustion unlike petroleum (Chang et al. 2018; Muhaji and Sutjahjo 2018). The technology development of economically-feasible pretreatment, saccharification, and fermentation for bioethanol production has been achieved, resulting in the commercialization of bioethanol, e.g., producing more than 25 million gallons per

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year (Menon and Rao 2012; Brown and Brown 2013).

Bioethanol is now commercially produced mainly from starch sources such as corn starch. Because of the conflict with food security, however, the research on the bioethanol production from lignocellulosic biomass, which is a non-edible crop, has been actively conducted worldwide (Paulova et al. 2015). The cellulose, which is the main component of lignocellulosic biomass, can be hydrolyzed into glucose by acid or enzyme hydrolysis. The hemicellulose can also be hydrolyzed into pentoses such as xylose and arabinose, and hexose such as glucose, galactose and mannose (Jensen et al. 2010). The resulting sugars can be converted to ethanol by fermentation.

Pretreatment is an essential step for bioethanol production from lignocellulosic biomass. Its aim is to disrupt the complex cell wall structure of lignocellulosic biomass, enhancing enzyme accessibility and digestibility for saccharification (Galbe and Zacchi 2002; Mosier et al. 2005; Alvira et al. 2010). A number of pretreatment methods have been developed (Li et al. 2010; Kumar and Sharma 2017). Among these, the pretreatment using various ionic liquids (ILs) is known to be environmentally friendly and more effective than conventional pretreatment methods such as dilute sulfuric acid pretreatment (Dadi et al. 2007).

Saccharomyces cerevisiae is one of the famous traditional yeasts for ethanol fermentation and has been commercially used for bioethanol production from lignocellulosic biomass (Chu et al. 2013; Kang et al. 2014). It has strong resistance against many microorganisms inhibitory compounds such as organic acids, furan derivatives, and phenolic compounds, which can be generated during the pretreatment process of lignocellulosic biomass (Almeida et al. 2007).

In this study, the effect of pretreatment using [EMIM]Ac and its co-solvent with DMF on ethanol production was investigated. *Saccharomyces cerevisiae* (D452-2) was used for fermentation. It is the mutant yeast strain with a resistance against [EMIM]Ac developed by Ha's research team of Kangwon National University (Lee et al. 2017).

Materials and Methods

Material

Wood powder (40-80 mesh) of Pussy willow (*Salix gracilistyla* Miq.) treated by ethanol-benzene solution (1/2: v/v) for 8 h to remove the extractives and vacuum-dried at 40°C for 24 h before pretreatment. The [EMIM]Ac and DMF were purchased from IoLiTec (Heilbronn, Germany) and Daejung Chemicals & Metals Co., Ltd. (Gyeonggi-do, Daejeon, Korea), respectively. Acremonium cellulase and Optimash BG were purchased by Meiji Seika (Tokyo, Japan) and Genencor Kyowa (Tokyo, Japan), respectively, for enzymatic saccharification. *Saccharomyces cerevisiae* (D452-2) was used for the ethanol fermentation.

Pretreatment

Pretreatment using [EMIM]Ac and co-solvent with DMF was conducted at 120°C for 2 h with 15% solid loading of wood powder. The co-solvent was prepared by adding 30, 50, and 70% DMF to [EMIM]Ac. The pretreated products were regenerated by precipitating with distilled water at room temperature for 1 h and then filtered using a PTEF filter.

Enzymatic saccharification and Fermentation

The pretreated sample was added to an enzyme cocktail with 2.5% solid loading. An enzyme cocktail was prepared with 0.1% (w/v) Acremonium cellulose, 0.2% (v/v) Optimash BG and 50 mM sodium acetate buffer (pH 5.0). Enzymatic saccharification was performed in a shaking incubator (VS-101Si, Vision scientific Co., Ltd., Daejeon, Korea) at 50°C for 72 h. The enzymatic hydrolysate was inactivated using a heating block (Wise Therm HB-48-Set, Daihan Scientific, Seoul, Korea) at 95°C for 15 min and additionally inactivated again for 15 min in a dryer at 95°C and stored at 4°C, and then filtrated using a syringe filter (0.2 μm of pore size). The hydrolysates (900 μL) was fermented for 24 h in a shaking incubator at 30°C and 200 rpm using 100 μL of YP 10X (Yeast extract 100 g/L, Peptone 200 g/L).

Measurements

Yeast strain growth was measured by optical density at wavelength 600 nm using a UV-visible spectrophotometer (Biomate 5, Thermo Fisher Scientific Inc., Waltham, USA). The concentration of glucose, xylose, acetate, and ethanol was determined by high-performance liquid chromatography (Agilent 1200 infinity series, Agilent Technologies, CA, USA), equipped with a refractive index detector, using

a Rezex ROA-Organic Acid H+ (8%) column (Phenomenex Inc., CA, USA). The column was eluted with 5 mmol of H_2SO_4 at a flow rate of 0.6 mL/min and 50°C.

Results and Discussion

Fig. 1 shows the dependency of the optical density of fermentation products on fermentation time. The optical density is related to the number of microbial cells, which will be increased when the yeast is growing. The optical density of all samples was initially increased to 3 h, thereafter gradually decreased for 6 h. The optical density of the products pretreated with only [EMIM]Ac or DMF was lower than using co-solvent, indicating that the co-solvent system lowers the inhibition of the growth of the cells. There was no big difference between the samples pretreated co-solvents.

Fig. 2 shows the effect of the fermentation time on glucose and xylose consumption of the enzymatic hydrolysates. With increasing fermentation time, the concentration of glucose was decreased and then almost depleted for 6 h of fermentation time. The glucose concentration of enzymatic hydrolysates from the products pretreated using the co-solvent with less than 50% DMF was more than 7 g/L, which is around half value for 3 h of fermentation time. The glucose concentration by only DMF was 1.7 g/L and depleted for 3 h. The xylose concentration using the co-solvent with more than 50% DMF was over 3 g/L, thereafter decreased to about 2 g/L for 6 h of the fermentation. However, the xylose concentration using co-solvent with 70% DMF was 2.3 g/L and decreased to 1 g/L for 6 h. In the case of hydrolysates by only DMF, xylose concentration was an only small amount (0.2 g/L), showing almost depleted value with increasing fermentation time.

Fig. 3 shows the change of the acetate concentration of the enzymatic hydrolysates with fermentation time. Acetate can be produced by the yeast to gain additional energy during ethanol fermentation (Wiegel 1982). The original ace-

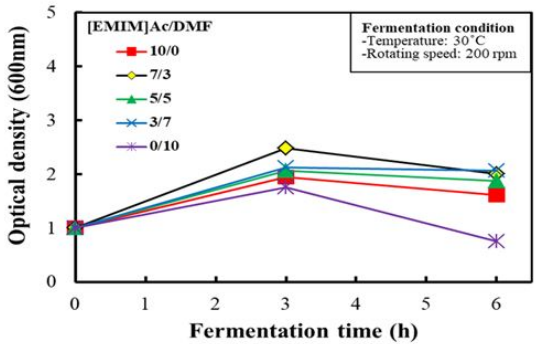

Fig. 1. The change of the optical density of the fermented product from the enzymatic hydrolysates for different fermentation times.

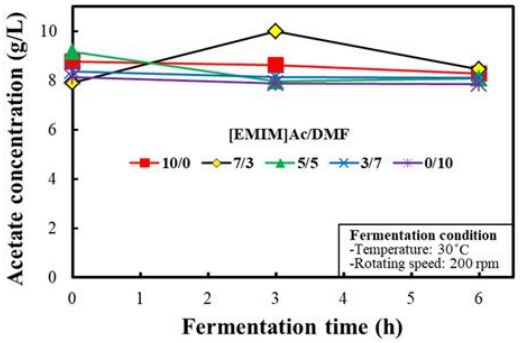

Fig. 3. Changes of the acetate concentration of the enzymatic hydrolysates by fermentation.

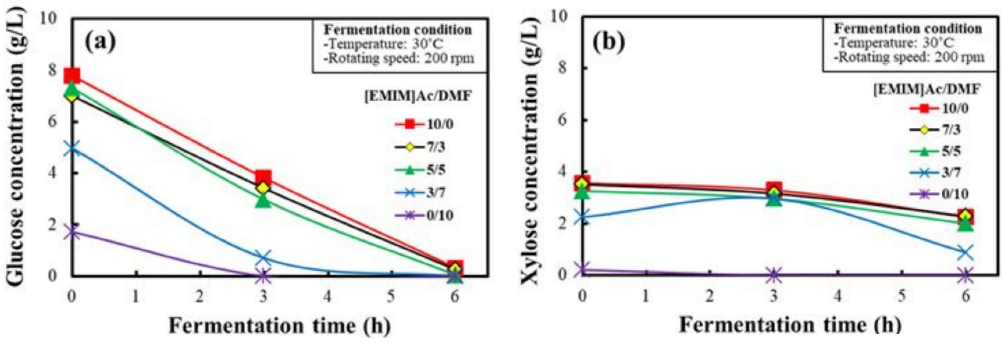

Fig. 2. Dependency of fermentation time on the consumption amount of glucose (a) and xylose (b) from the enzymatic hydrolysates.

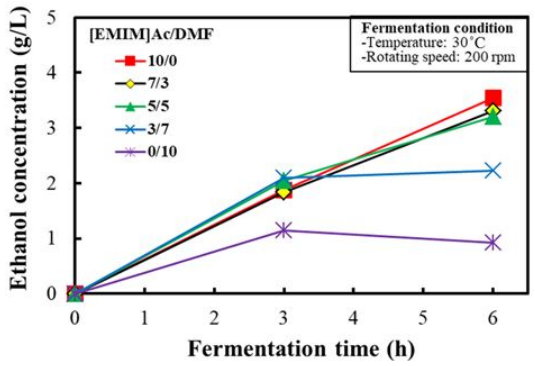

Fig. 4. Effect of fermentation time on ethanol concentration from the enzymatic hydrolysates.

tate concentration of the enzymatic hydrolysates indicates the value due to the acetate of [EMIM]Ac. There was no big change of the values of 7.9-9.2 g/L with fermentation time, excepting for the samples with the co-solvent with 30% DMF for 3 h of fermentation time.

Depending on fermentation time, the change of ethanol concentration is shown in Fig. 4. With increasing fermentation time, the ethanol concentration in all samples was increased. The ethanol concentration in the fermented product from the enzymatic hydrolysates by only [EMIM]Ac and its co-solvent with less than 50% was in the range from 3.2 to 3.5 g/L for 6 h of fermentation time. However, the ethanol concentration was lower in the fermented product from the hydrolysates by the co-solvent with more than 70% DMF and only DMF. Lienqueo et al. (2016) reported that the ethanol of 3.7 g /L was obtained from the pretreated product by [EMIM]Ac by simultaneous saccharification and fermentation. This value is similar with this study.

Conclusion

Ethanol fermentation using *Saccharomyces cerevisiae* (D452-2) was successfully conducted using the enzymatic hydrolysates from the pretreated products using [EMIM]Ac and its co-solvents with DMF. The addition of DMF to [EMIM]Ac lowered the inhibition of the growth of the cells. The enzymatic hydrolysates from the product pretreated by pure [EMIM]Ac showed the highest concentration of glucose (7.8 g/L) and xylose (3.6 g/L). The initial concentration of both monosugars was decreased with increasing fermentation time. Approximately 3 g/L of ethanol was produced from the enzymatic hydrolysates by pure [EMIM]Ac and co-solvent with less than 50% DMF.

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