



## Article Effective Concentration of Ionic Liquids for Enhanced Saccharification of Cellulose

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**Abstract:** In an aqueous enzymatic saccharification using cellulase, the dissolution of crystalline cellulose is one of the rate-limiting steps. Insoluble cellulose powder was preliminarily heat-treated with ionic liquids (ILs), such as [Bmim][Cl] (1-butyl-3-methylimidazolium chloride) and [Amim][Cl] (1-allyl-3-methylimidazolium chloride), which enable the production of soluble cellulose. On the other hand, the presence of ILs leads to a denaturation of enzymes. Using cellulase from *Trichoderma viride*, the effects of [Bmim][Cl] and [Amim][Cl] in the enzymatic saccharification were compared. The production of glucose was optimized with 5 wt%-ILs, both for [Bmim][Cl] and for [Amim][Cl]. The significant inhibiting effects of ILs (IL concentration >10 wt%) could be due to the denaturation of cellulase, because the peak shifts of intrinsic tryptophan fluorescence were observed in the presence of 7.5 wt%-ILs. To analyze kinetic parameters, the Langmuir adsorption model and the Michaelis-Menten model were employed. The investigation suggests that [Amim][Cl] can provide soluble cellulose more efficiently, and can promote enzymatic saccharification below 5 wt%.

Keywords: ionic liquid; cellulase; insoluble cellulose; hydrolysis; kinetic analysis

## (a) 10 9 OAMIMCI only 8 AMIMCI + cellulose 7 Viscosity [cp] 6 5 4 3 Φ 2 1 0 0 10 15 20 5 IL [wt%]

## Supplementary



Figure S1. Viscosities of solutions including (a) [Amim][Cl] and (b) [Bmim][Cl], in the presence of cellulose.

The viscous environment, coursed by the presence of IL and dissolved cellulose, might inhibit the diffusion of substrate and enzyme. The viscosity of the reaction mixture (in the absence of enzyme) was analyzed as a function of IL concentration (Figure S1). With a treatment of [Amim][Cl], the viscosity slightly increased in proportion to IL concentration. In contrast, the [Bmim][Cl] samples showed almost similar viscosities in the absence or presence of cellulose. In both cases, the increase of the viscosity was only slightly, suggesting that the influence of solution viscosity on saccharification reaction could be negligible.



**Figure S2.** Correlation between IL concentration and reaction rate constant. (**a**) [Amim][Cl]; 5wt% (*open square*); 10wt% (*open triangle*); and 15wt% (*open circle*). (**b**) [Bmim][Cl]; 5wt% (*closed square*); 10wt% (*closed triangle*); 15wt% (*closed square*).



**Figure S3.** Lineweaver-Burke plot in region  $\alpha$  which IL concentration from 1 to 5wt%. (**a**) [Amim][Cl],  $V_{\text{max}} = 5.70 \times 10^{-3}$  (M/h),  $K_{\text{m}} = 5.27 \times 10^{-1}$  (wt%). (**b**) [Bmim][Cl],  $V_{\text{max}} = 5.80 \times 10^{-3}$  (M/h),  $K_{\text{m}} = 8.70 \times 10^{-1}$  (wt%).