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Ionic liquid pretreatment and in situ enzymatic saccharification using biocompatible ionic liquid

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Ionic liquids (ILs) have received much attention recently for pretreatment of lignocellulosic biomass, because ILs can dissolve cellulose and that cellulose re-precipitated after being dissolved in ILs exhibits a much greater efficiency of enzymatic hydrolysis due to its decreased crystallinity. Moreover, it has been extended this IL-assisted pretreatment method to various lignocellulosic biomasses and demonstrated that the IL-assisted pretreatment methods are more effective than the conventional methods using diluted acid or ammonia. In the IL-assisted pretreatment process, the pretreated biomass has to be washed extensively so as to remove the residual IL because the residual IL in pretreated biomass causes inhibition of cellulolytic enzymes and fermentative microorganisms during saccharification and fermentation. However, this extensive washing of pretreated biomass results in large amounts of diluted IL aqueous solution, which leads to a high cost for concentrating IL from its diluted aqueous solution by evaporation and for treating the resultant wastewater. The cost issue can be an obstacle for scale-up of IL-assisted pretreatment processes. Recently, we suggested a simple process comprising IL-assisted pretreatment and in situ enzymatic saccharification without washing out IL from the pretreated biomass and with the addition of a smaller amount of water for diluting IL in order to save costs derived from the extensive washing step. To reduce the water use for diluting IL and to achieve the higher sugar yield/concentration in the in situ enzymatic saccharification, the following factors are important: 1) high loading of biomass to IL (less IL amount per unit biomass) during the IL pretreatment step, reducing water use per unit biomass; 2) use of biocompatible IL and/or IL-tolerant cellulase enzymes, which reduce water use for diluting IL before enzymatic saccharification because a higher IL concentration is possible. Some in situ enzymatic saccharification has even been performed by employing biocompatible IL as well as conventional IL. Our previous study demonstrated that choline acetate (ChOAc) was less toxic for fermentative microorganisms than imidazolium ionic liquids. We will report on in situ enzymatic saccharification using cholinium IL as a candidate for biocompatible IL. The data for pretreatment capability, inhibition to commercial cellulase, and performance of the in situ saccharification process will be then compared with data obtained using the standard imidazolium IL. Moreover, we will demonstrate an electrodialysis-assisted separation of IL and monomeric sugar obtained by in situ enzymatic saccharification. This separation technique is very important to use ILs in pretreatment processes.

Biological pretreatment of modified wheat straw for ethanol production

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Wheat straw is the most abundant lignocellulosic residues in UK. And it has been identified as a cheap and effective raw material for bioethanol production. However at this moment based on current technology, lignocellulosic bioethanol production process is not ready to be commercialised due to the cost of enzyme required and high energy needed in pretreatment process. The University of Nottingham has investigated a number of pretreatment strategies and developed a process, which can be applied with wheat straw. Biological pretreatment is one of pretreatment methods that have been studied. In this study, biological pretreatment of wheat straw using *Aspergillus niger* was performed based on solid state fermentation strategy to convert wheat straw to fermentable hydrolysate. *A. niger* was cultured on the wheat straw firstly for the cellulolytic enzyme production and then the enzymes were used to hydrolyse the wheat straw. In a biological pretreatment using autoclaved wheat straw, an enzyme activity of 9.5 FPU/g was achieved. When 0.5% yeast extract and mineral solution were added, the enzyme activities increased to 24.0 FPU/g after 5 days of cultivation. While using an alkali modified wheat straw (1% NaOH at 121°C for 30 minutes), the enzyme activity reached 17.2 FPU/g at the first day of culture. Surprisingly an alkali soaked wheat straw (1% NaOH at room temperature for overnight) led to even higher enzyme activity (23.3 FPU/g) just after 1 day of culture. Biological pretreated wheat straw was characterized by X-ray diffraction analysis and the effectiveness of pretreatment was evaluated by enzymatic hydrolysis and fermentation. It was found that biological pretreatment by *A. niger* has altered wheat straw structure by decreasing crystallinity region in biomass. Therefore during hydrolysis of biological pretreated wheat straw, glucose yield has reached 73.26% which much higher than non-treated wheat straw. The hydrolysate was applied to fermentation process to produce ethanol. The ethanol yield from hydrolysate reached 82.13%.It can be conclude that biological pretreatment by *A. niger* is a potential method of pretreat wheat straw for ethanol production.

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