Synergistic action of deep eutectic solvents and cellulases for lignocellulosic biomass hydrolysis

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The increased awareness of environmental and economic issues has led many researchers to seek green and low-cost solvent for the conversion of lingocellulosic biomass to bioenergy. In this context, deep eutectic solvents, a new class of ionic liquids, have been regarded as greener substitute to conventional solvents for pretreatment of biomass. This paper is concerned with the stability and synergicity action of deep eutectic solvents for the digestion of biomass. The stability was studied by incubating commercial cellulases to different concentration of ethylene glycol–choline chloride-based deep eutectic solvent. The synergistic tests were studied by performing enzymatic saccharification after pretreatment of rice husk with deep eutectic solvent at various temperatures. The stability test showed that, the commercial cellulase activity retained more than 90% of relative activity after 1 hour of incubation in 10(%,v/v) deep eutectic solvent at 30°C. The prepared deep eutectic solvent in combination with commercial cellulases study showed that the higher pretreatment temperature improved the production of simple sugar from rice husk.

Keywords: Ionic liquids, Cellulases, Lignocellulosic biomass, DESs, Rice husk

Introduction

Lignocellulosic biomass is a plant-based material, which primarily consist of cellulose, hemicelluloses and lignin. These biopolymers are recognized as one of the promising substrates for the production of renewable energy. A key step in the conversion of biomass to bioenergy lies on the pretreatment of biomass.[1] The pretreatment process helps in the deconstruction of biomass to improve the hydrolysis of polysaccharides into simple sugars that subsequently can be fermented to produce biofuel. Cost-effective lignocellulosic biomass pretreatment is major challenge of cellulose-based fuel research and technology.[2] Ionic liquids (ILs) have been identified as new potential solvent for effective lignocelluloses pretreatment.[3] However, conventional ILs have some drawbacks including costly, complexity of the solvent synthesis and purification, toxicity and poor biodegradability.[4,5] As an alternative, a new class of ILs known as deep eutectic solvents (DESs) was introduced. In comparison to the conventional ILs, this new class of ILs offers advantages in terms of easy to synthesize and made entirely from cheap and safe materials.[6,7] As DES shares the benefits of conventional ILs with additional improvement and advantages, DES has been identified as novel solvent for deconstruction of lignocellulosic biomass.[8] In this work, DES of ethylene glycol–choline chloride was introduced as potential solvent for lignocellulosic biomass pretreatment. This type of DES was selected for the lignocellulose pretreatment due to its low characteristic as compared with other types of DES.[2,9] The low viscosity solvent is preferred in the pretreatment process as it is easy to handle, does not need additional force to pump in the solvent and thus reduced the operating cost in industrial applications. Besides, it has been reported that the establishment of low viscous cellulose/ILs system improved the thermodynamic and kinetics of the reaction.[10] Thus, the low viscosity ILs improved the dissolution of cellulose. In this work, rice husk (RH) was used as a lignocellulosic material for studying the synergistic action between cellulases and ethylene glycol–choline chloride-based DES for biomass hydrolysis.

Experimental methods

Preparation of ethylene glycol–choline chloride-based DES (EG-ChCl-based DES)

Choline chloride (hygroscopic) was dried under vacuum at 80°C for 6 hours before use. Subsequently, choline chloride and ethylene glycol were mixed in 1:2 molar ratio. The mixture was heated and stirred at certain

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temperature in a closed flask until a homogenous colourless solution was formed. Then, the prepared DES was kept in a vacuum desiccator with silica gel until further used.

Stability of cellulases in EG-ChCl-based DES

The stability of cellulases in EG-ChCl was measured by incubating the cellulases (Celluclast, 1·5 L from Novozyme) in different concentration (5, 10, 15 and 20% v/v) of EG-ChCl-based DES in citrate buffer (50 mM, pH 4·8) at 30°C for 1 hour. The total cellulase activity was determined by filter paper assay (FPase) using Whatman No. 1 filter paper strip as recommended by Ghose[11] and expressed as an international unit. One FPase is the concentration of cellulases that can release 2·0 mg of glucose from 50 mg of cellulose over a 60-minute period. One unit of enzyme activity was defined as the amount of enzyme capable of releasing 1 μmol of reducing sugar per minute under the assay conditions. The amount of reducing sugar was determined by dinitrosalicylic acid (DNS) according to the standard method[12] and glucose was used as a standard.

Pretreatment of rice husk using EG-ChCl-based DES

A solution of 4% (w/v) RH was prepared by combining 0·4 g of RH with 10 mL DES in three sterilized bottles, respectively. Each biomass/DES mixtures were heated at 100, 130 and 160°C, respectively, for 4 hours. After the pretreatment, the mixtures were cooled to room temperature. The residues were washed several times using ionized water and filtered under vacuum. Then, the residues were dried at 60°C overnight.

Enzymatic saccharification of pretreated rice husk

0·1 g of the dried pretreated RH from each pretreatment condition was mixed with 10 mL citrate buffer. The cellulases were added to each mixture and the enzymatic hydrolysis was carried out at 50°C and 180 rev min^{-1}. The sugar yield was monitored over time (1, 3, 6, 9, 12 and 24 hours) using DNS method.

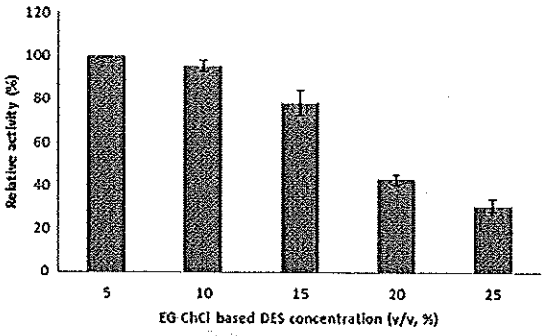
Result and discussions

Stability of cellulases in the presence of DES

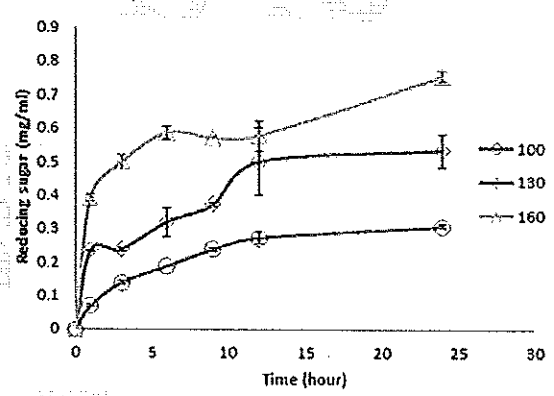
The effects of different concentration EG-ChCl-based DES is shown in Fig. 1. It is apparent that 90% of relative cellulose activity could be retained in the presence of 10% (v/v) DES. IL is the salt in the liquid form at the room temperature. Being a salt, ILs are expected to inhibit enzyme activity. However, as compared with other ILs,[13,14] the newly designed of ILs showed less inhibiting effects on cellulases. This finding is useful in the subsequent enzymatic hydrolysis step because it will minimize the inhibition of the enzyme caused by residual ILs during saccharification.[15] Additionally, this finding could significantly reduce the amount of water required during washing step after pretreatment process.[16,17]

Enzymatic saccharification of DES pretreated rice husk

The effect of DES pretreatment temperature on the enzymatic saccharification was studied by treating RH for



1 The cellulases relative activity after pre-incubation with various concentration of EG-ChCl-based deep eutectic solvent



2 Reducing sugar concentration obtained from enzymatic reaction after pretreatment of DES at different temperature conditions

4 hours at 100, 130 and 160°C. Figure 2 shows the saccharification results in the form of reducing sugar produced over period of time. It is apparent that the produced reducing sugars tend to increase with the pretreatment temperature. After 24 hours of enzymatic hydrolysis, the pretreatment temperature of 160°C seem to be the highest reducing sugar of 0·74 mg ml^{-1} produced while at 130 and 100°C seem to reduce to 0·5 and 0·3 mg ml^{-1}, respectively. These results probably attributed to the increase of biomass pore size and redistribution of lignin and at higher temperature.[18,19] These results are inline with other findings[20,21] that revealed, higher pretreatment temperature accelerated the deconstruction of biomass and in turn improved the production of reducing sugar.

Conclusions

This study showed that the cellulases could retain more than 90% of relative activity in the presence of 10% (v/v, %) EG-ChCl-based deep eutectic solvent. This demonstrated the characteristic of the solvent to have less inhibiting effects towards cellulases. Additionally, this gives benefit to the pretreatment process where it reduces the significant amount of water required for washing in after pretreatment process. Hence, this significantly improved the pretreatment process where the deep eutectic solvent and cellulases work synergically to

increase the efficacy of pretreatment process. Apart from that, the effects of pretreatment temperature on the sugar production from the deep eutectic solvent pretreated lignocellulose revealed that the higher preteratment temperature showed improvement of production of reducing sugar. However, with concern to DES stability, it is necessary to find the optimum conditions for deep eutectic solvent to function properly. This study is undergoing in our laboratory.

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