UNIVERSITI PUTRA MALAYSIA

PRETREATMENT OF LIGNOCELLULOSIC MATERIALS BY DEEP EUTECTIC SOLVENTS FOR ENHANCED ENZYMATIC HYDROLYSIS

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By

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The potential of deep eutectic solvent (DES) for the process of oil palm biomass and cellulose conversion into glucose was investigated. DES has been studied extensively in various fields such as organic synthesis, biocatalysis, dissolution, electrochemistry and extraction process. Owing to its cheap and 'green' properties, we are interested in exploring the potential of DES for biomass processing. In order to achieve optimized glucose conversion via enzymatic hydrolysis, biomass must undergo pretreatment in order to break down the structure and this can enhance the access of cellulase to cellulose matrix which usually preferred amorphous or unorganized cellulose structure.

Herein, choline chloride:glycerol (ChCl:Gly), choline chloride:ethylene glycol (ChCl:EG), ethylammonium chloride:glycerol (EAC:Gly), ethylammonium chloride:ethylene glycol (EAC:EG) and choline chloride:urea (ChCl:U) with 1:2 molar ratio for all DESs were used as solvent for pretreatment of oil palm biomass and cellulose. The dissolution process was done by heating and stirring 5% (w/w) oil palm biomasses which were trunk (OPT), frond (OPF) and empty fruit bunch (EFB) in ChCl:Gly, ChCl:EG, EAC:Gly, EAC:EG and ChCl:U at 100 °C for 48 h and cellulose for 24 h at the same temperature. The chemical composition for pretreated oil palm biomass was investigated and it was found that EAC:EG gave major removal of hemicellulose in OPT, while significant lignin reduction was shown in all DESs for OPT. The highest percentage of dissolution for oil palm biomass was recorded by OPT pretreated in EAC:EG with 55.6% while the lowest dissolution recorded by OPF pretreated in ChCl:Gly with only 20%. The highest percentage of dissolution for cellulose was also recorded when pretreated in EAC:EG with 22.9% while ChCl:EG gives the lowest dissolution with only 7.6%. The regenerated or pretreated oil palm biomass was recovered by adding distilled water/ethanol to the pretreated sample. Recovered pretreated samples were analysed by optical microscopy for physical structure study. The result shows that EAC:EG provides better media for dissolution process as the solvent produced more small fragments which indicates that dissolution occurred for oil palm biomass and cellulose. From FTIR analysis, EAC:EG was recorded to be the best solvent for disruption of hydroxyl group in the biomass material while ChCl:U recorded the best solvent in removing lignin for OPT.

The screening of parameter study was carried out for enzymatic hydrolysis in order to determine the optimum conversion for the substrate pretreated in DES. Study of few crucial factors affecting the reaction rate such as hydrolysis media, enzyme loading and substrate concentration was conducted. The highest glucose release among pretreated oil palm biomass was when hydrolysing OPT pretreated in EAC:EG with 60% conversion. Cellulose hydrolysis recorded high amount of glucose release within range of 77-95% conversion. The optimisation study was carried out by using OPT pretreated in EAC:EG, not the cellulose because it considered to already achieved high conversion rate. The optimum condition was obtained by using 15 mg/ml as substrate concentration, 50 FPU/g and 100 CBU/mL as enzyme loading for Celluclast 1.5 L and Novozyme 188 respectively at 50 °C for 24 h with 73.5% glucose conversion.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PRARAWATAN BAHAN LIGNOSELLULOSIK OLEH PELARUT EUTEKTIK UNTUK PENINGKATAN HIDROLISIS BERENZIM

Oleh

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Potensi pelarut eutektik bagi proses penukaran biomass kelapa sawit dan selulosa kepada glukosa telah dikaji. Kajian terhadap pelarut eutektik sedang giat dijalankan di dalam beberapa bidang seperti sintesis organik, biopemangkinan, pemelarutan, elektrokimia dan proses pengekstrakan. Oleh kerana ia murah dan bersifat 'hijau', kami tertarik untuk mendalami potensi pelarut eutektik bagi pemprosesan hasil buangan. Untuk mencapai penukaran glukosa yang optima melalui hidrolisis berenzim, hasil buangan harus menjalani prarawatan untuk menguraikan strukturnya dan ini boleh meningkatkan laluan untuk selulasa ke matrik selulosa dimana kebiasaannya lebih cenderung kepada amorfos ataupun struktur selulosa yang tidak teratur.

Disini, kolin klorida:gliserol (ChCl:Gly), kolin klorida:etilena glikol (ChCl:EG), etilammonia klorida:gliserol (EAC:Gly), etilammonia klorida:etilena glikol (EAC:EG) dan kolin klorida:urea (ChCl:U) dengan kadar molar 1:2 untuk semua DES telah digunakan sebagai pelarut untuk prarawatan biomass kelapa sawit dan selulosa. Proses pemelarutan telah dilakukan dengan memanas dan mengacau 5% (b/b) biomass kelapa sawit iaitu batang pokok kelapa sawit, pelepah pokok kelapa sawit dan tandan buah kosong kelapa sawit, pada suhu 100°C untuk 48 jam dan selulosa untuk 24 jam pada suhu yang sama. Komposisi kimia dalam bahan pra-rawat biomass kelapa sawit telah dikaji dan mendapati EAC:EG memberikan penyingkiran hemiselulosa yang banyak dalam OPT, sementara penyingkiran lignin yang besar telah ditunjukkan oleh semua DES untuk OPT. Peratus pemelarutan tertinggi untuk biomass kelapa sawit adalah direkodkan oleh OPT diprarawat di dalam EAC:EG dengan 55.6%, sementara pemelarutan terendah direkodkan oleh OPF diprarawat di dalam ChCl:Gly dengan hanya 20%. Peratusan pemelarutan paling tinggi bagi sellulosa adalah direkodkan apabila diprarawat di dalam EAC:EG dengan 22.9%, sementara ChCl:EG memberikan peratusan pemelarutan paling rendah dengan hanya 7.6%.

Penghasilan semula atau biomass kelapa sawit yang telah diprarawat diambil dengan menambahkan air suling/etanol pada sampel prarawat. Sampel yang diprarawat telah diambil untuk dianalisa oleh mikroskop optik untuk kajian struktur fizikal. Hasil menunjukkan bahawa EAC:EG menyediakan media yang lebih baik untuk proses pemelarutan dimana pelarut itu menghasilkan lebih banyak fragmentasi kecil,

menunjukkan bahawa pemelarutan berlaku untuk biomass kelapa sawit dan selulosa. Daripada analisis FTIR, EAC:EG merekodkan sebagai pelarut terbaik untuk penggangguan kumpulan hidroksil dalam bahan biomass sementara ChCl:U merekodkan pelarut terbaik dalam penyingkiran lignin untuk OPT.

Kajian saringan parameter telah dijalankan untuk hidrolisis berenzim bagi mengenalpasti penukaran yang optimum untuk substrat prarawat di dalam DES. Kajian terhadap beberapa faktor penting yang mempengaruhi kadar tindak balas seperti media hidrolisis, muatan enzim dan kepekatan substrat telah dijalankan. Penukaran glukosa yang tertinggi di kalangan hasil buangan kelapa sawit yang telah diprarawat adalah apabila menghidrolisiskan OPT yang diprarawat di dalam EAC:EG dengan penukaran sebanyak 60%.

Hidrolisis selulosa merekodkan kandungan pembebasan glukosa yang tinggi iaitu antara 77-95%. Kajian optimum diteruskan dengan menggunakan OPT yang telah diprarawat di dalam EAC:EG, bukan selulosa kerana ia dianggap sudah mencapai kadar penukaran yang tinggi. Keadaan optimum telah diperolehi dengan menggunakan 15 mg/ml sebagai kepekatan substrat, 50 FPU/g dan 100 CBU/g sebagai muatan enzim untuk Celluclast 1.5 L dan Novozyme 188, pada suhu 50°C untuk 24 jam dengan 73.5% penukaran glukosa.

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I certify that a Thesis Examination Committee has met on 6 May 2015 to conduct the final examination of Syarilaida binti Zulkefli on her thesis entitled "Pretreatment of Lignocellulosic Materials by Deep Eutectic Solvents for Enhanced Enzymatic Hydrolysis" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements of the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

DES	Deep eutectic solvent
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
LTTM	Low transition temperature mixture
ChCl	Choline chloride
Gly	Glycerol
EAC	Ethylammonium chloride
EG	Ethylene glycol
U	Urea
IL	Ionic liquid
OPT	Oil palm trunk
OPF	Oil palm frond
EFB	Empty fruit bunch
FT-IR	Fourier Transform Infrared Spectroscopy
HPLC	High Performance Liquid Chromatography
h	Hour
min	Minute
rpm	Rotation per minute
g	gram
FPU	Filter Paper Unit
CBU	Cellobiase Unit

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Deep eutectic solvent (DES) is a novel solvent which is a combination of two components giving the solvent a very low melting point compared to its constituting components (Abbott *et al.,* 2003). It is non-flammable, biodegradable, less toxic and inexpensive, which can be suitable for many applications. Generally, the components involve hydrogen bond donor (alcohols, amides, amino acids, monosaccharides) and ammonium salt (choline chloride, betaine, ethylammonium chloride, tetra butylammonium chloride) in certain molar ratio. Variety of combinations make the solvent more attractive as changing the pairing will alter the physical or chemical properties of DES. This can be advantageous because by changing the properties of DES will provide wider range of applications by satisfying the requirements in certain process.

Many works have been done in exploring the potential of DES which includes metal dissolution, extraction and purification, electrochemistry, biocatalysis, organic synthesis and biomass processing. However, little work was done in exploring the potential of DES in biomass processing. Francisco *et al.,* (2012) worked on cellulose, starch and lignin dissolution by using LTTMs mixture and showed that most of the solvents can dissolve lignin such as malic acid:proline, lactic acid:betaine, lactic acid:choline chloride and lactic acid:histidine, from wheat straw. However, cellulose and starch did not give promising result as the percentage of dissolution was very low. From this report, it opens up more room to improve the LTTMs or DESs as the solvent for lignocellulosic biomass pretreatment. Thus, in this study, DESs was utilised for pretreatment of lignocellulosic biomass with aim to deconstruct the highly crystalline cellulose and remove or dissolve the lignin and hemicellulose to achieve optimum conversion of fermentable sugar.

There are 2 types of bioethanol, first generation (FGB) and second generation bioethanol (SGB). Currently, bioethanol is produced from food source such as starch and sugar-based feedstock which is categorized as FGB (Tan *et al.,* 2010). However, debate arises on the feedstock of FGB where competition between human-fuel supplies should be avoided. Therefore, SGB can be the alternative to FGB because it can be produced from lignocellulosic biomass. Biomass or wastes that can be converted into valuable end-products will contribute huge benefits on environment and able to avoid using human food source as feedstock for fuel production. Nevertheless, the lignocellulosic biomass is a complex material consists of highly crystalline cellulose, hemicellulose and lignin that could hinder the efficiency of the production (Silverstein *et al.,* 2007). Thus, lignocellulosic biomass must undergo few processes in order to obtain bioethanol.

Pretreatment process is the crucial step to break down the highly crystalline cellulose within the lignocellulosic biomass. The most common pretreatment process of lignocellulosic biomass usually involves the physical and chemical pretreatment. However, the pretreatment process for lignocellulosic biomass is still in research scale. Extensive studies have been done on both physical and chemical pretreatment where usually involves steam explosion and alkali pretreatment respectively (Holm and Lassi, 2011). By changing the crystalline structure to amorphous, enzyme or microbe will easily hydrolyse the polysaccharide into monosaccharide (glucose or xylose) via enzymatic hydrolysis. Enzymatic route is the preferred method as it can be carried out in mild condition and this could reduce consumption of energy, cost and corrosive chemicals. Due to the extensive development of these processes, there are many types of pretreatment and hydrolysis has been introduced. Chemical pretreatment by using ionic liquid (IL) has attracted many chemists since it provides good quality of solvent for lignocellulosic biomass. However, IL has few limitations regarding its toxicity and cost to synthesise where it may not be efficient for large-scale process. On the other hand, DES has low toxicity and can be produced using cheap material such as choline chloride. Therefore, the potential of DES in biomass processing is enormous.

1.2 Problem Statement

In this study, the concern is to achieve access of cellulase and cellobiase to cellulose from the oil palm biomass in order to obtain high yield of glucose. In lignocellulosic biomass, cellulose is hindered by heteropolymer of hemicellulose and lignin. Removal of lignin and hemicellulose can assist the access of enzymes on cellulose. The highly crystalline cellulose structure is also one of the issues where although removal of lignin and hemicellulose can enhance the enzymatic hydrolysis, the cellulose must be in amorphous or less packed structure in order for enzymes to be effective.

Thus, the lignocellulosic biomass should undergo pretreatment process to break or open up the cell wall and to decrease the recalcitrant by using physical, chemical or biological pretreatment. However, the common pretreatment process uses harsh chemicals such as sodium hydroxide (NaOH) solution that could cause the reactor to corrode. IL is reported the new cellulose solvent for the last few decades and researches are extensively carried out. Unfortunately, IL is very costly in terms of its preparation and materials, thus it is not suitable for practical scale. DES is one of the ionic solvent where it is cheap, biodegradable and environmental friendly. However, limited work was done on DES related to biomass processing. In order to fill the gap, DESs were used in this study to pretreat the lignocellulosic biomass prior to enzymatic hydrolysis.

1.2 Aim and Objectives

The aim of this study is to explore the potential of DES in pretreatment and enzymatic hydrolysis of oil palm biomass and cellulose. The objectives were set as follows:

1) To pretreat oil palm biomass and cellulose in DES and determine the physico-chemical changes of the pretreated materials.
2) To enhance the enzymatic hydrolysis of oil palm biomass by pretreatment using DES.

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