Pretreatment with Deep Eutectic Solvents for Improved Hydrolysis and Fermentation for Aroma Compound Production

Kraipat Cheenkachorn[1,*], Prapakorn Tantayotai[2], Malinee Sriariyanun[3]

1 Department of Chemical Engineering, Faculty of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand; 2 Department of Microbiology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand; 3 Biorefinery and Process Automation Engineering Center, The Sirindhorn International Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

**Corresponding author: kraipat.c@eng.kmutnb.ac.th*

Highlights

- Three types of deep eutectic solvents were applied for pretreatment of Nepier grass
- Inhibitory effects of DESs on cellulase and fermentation activities were evaluated
- Phenylethyl alcohol and isoamyl alcohol were produced from fermentation of Nepier grass hydrolysate

1. Introduction

The biorefinery process, encompassing pretreatment, hydrolysis, fermentation/catalysis, and product recovery, faces a bottleneck in hydrolysis due to the resilient nature of cellulose, hemicellulose, and lignin. To address this, a pretreatment step is essential to modify biomass properties. Deep Eutectic Solvent (DES), considered an environmentally friendly and non-corrosive green solvent, is applied in the biorefinery process. DES has low volatility, a high flash point, and is recyclable [1]. Although DES is currently more cost-effective than ionic liquids (IL), it has not been extensively researched in the context of biomass pretreatment, specifically its impact on hydrolysis or fermentation processes.

Previous research using ILs in the pretreatment of lignocellulose showed improved hydrolysis efficiency with over 90% conversion. However, residual ILs in the pretreated straw negatively affected the efficiency of cellulase, reducing the conversion by more than 65% [2]. Additionally, studies indicated that residual IL negatively affected the growth of Saccharomyces cerevisiae yeast, impacting ethanol production from lignocellulose. Unlike IL, DES applications in biorefinery is still in earlier stage of development. Therefore, this study aims to thoroughly investigate of DES related impacts to design and control the overall process for aroma compounds from lignocellulose biomass effectively.

2. Methods

2.1 DES pretreatment of Nepier grass

Three types of Deep Eutectic Solvents (DES) were utilized, namely Chlorine chloride: Lactic acid (1:4) (ChCl/LA), Chlorine chloride: Glycerol (1:2) (ChCl/G), and Chlorine chloride: Urea (1:2) (ChCl/U), for the pretreatment of napier grass biomass [3]. Subsequently, the enzyme cellulase (CTec2, 20 FPU/g-biomass) was added into pretreated biomass for enzymatic hydrolysis, and the quantity of sugars produced from this reaction was measured through the DNS standard method.

2.2. Inhibitory effect of DES on cellulase

The impact of DES on the activity of CTec2 cellulase enzyme was investigated. The experimental setup included testing with substrates such as Carboxymethyl cellulose (CMC) and Avicel at different concentrations [S]. Subsequently, the mixtures were incubated at 50°C for 1 hour. The sugars in hydrolysate were utilized to plot the Michaelis-Menten equation in the form of a Line-Weaver plot between 1/[S] and 1/v for calculating enzyme kinetic parameters, namely Km and Vm.

2.3. Fermentation for aroma compounds

Cultivation of yeast strain, namely Kluyveromyces marxianus, was performed in a basic minimum media liquid formulation, with the primary carbon source being hydrolyzed biomass. Yeast inoculum for each strain was added to the media containing lignocellulosic hydrolysate with a concentration of 5%. Samples of the fermentation broth were then collected for fractionation, and the resulting products were analyzed using GC-MS [4].

3. Results and discussion

Three types of DESs, including ChCl/LA, ChCl/G and ChCl/U, which are acidic, neutral and alkaline DESs, were applied for pretreatment of nepier grass. After pretreatment, the samples were enzymatic hydrolysis and the sugar yields released from ChCl/LA-, ChCl/U- and ChCl/G-pretreated samples were increased for 1.82, 2.21 and 1.20 folds, respectively, compared to untreated sample. Then, the compositions of pretreated samples were analyzed and the results showed that lignin contents were removed, which correlated well with the FTIR analysis. Furthermore, it was observed from XRD analysis that DES pretreatment increased the crystallinity index (CrI).

After pretreatment optimization, the effects of DESs on Ctec2 activities were evaluated by testing with DES concentration of 5-15%. The productions of reducing sugars were reduced for 20%, whereas ChCl/G had no effect on Ctec2 activity. The kinetic parameters, Km and Vm, of Ctec2 showed that the DES residues caused the reduction of Vm, suggesting the deactivation of Ctec2. However, ChCl/U and ChCl/G residues also decreased Km, implying the higher enzyme affinity to substrate.

Then, the consolidated process combining pretreatment and hydrolysis was conducted in one reactor by diluting the DES concentration after pretreatment to be 5%. The results showed that the sugar yields obtained from consolidated reactor and separated reactor were indifferent. The DES pretreated hydrolysate was subsequently fermented by K. marxianus, and the fermented products were analyzed by GCMS. The results showed that aroma compounds, including Phenylethyl Alcohol, Isoamyl alcohol, 2-methyl-Propanal, which are rose, banana and malt smell, were increased in DES pretreated hydrolysate, especially isoamyl alcohol at 356.75 mg/L was achieved.

Figure 1. Productions of fermentation products by K. marxianus from hydrolysates of Nepier grass

Products	Samples			
	Untreated	ChCl/L pretreated	ChCl/U pretreated	ChCl/G pretreated
Ethanol (g/L)	3.24	5.65	5.12	4.06
Phenylethyl Alcohol (mg/L)	98.46	222.56	203.67	210.13
2-methyl-Propanal (mg/L) (mg/L)	150.77	218.23	228.98	175.46

4. Conclusions

Investigating three DESs for napier grass pretreatment, we optimized the process to boost sugar yields and enhance enzymatic hydrolysis. DES effectively removed lignin, increased cellulose crystallinity, and produced minimal inhibitory byproducts. DES residues influenced Ctec2 enzyme activity but improved substrate affinity. ChCl/U and ChCl/G DES-pretreated hydrolysate had no significant impact on yeast survival rates. Combining pretreatment and hydrolysis in a consolidated process yielded comparable results. Fermented products showcased enhanced aroma compound production, underscoring DES's potential in biomass processing for bio-based products.

References

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Keywords

Aroma compound; Biorefinery; Deep eutectic solvent; Lignocellulose biomass