Successive Conversion of Lignocellulose to Bio-ethanol Using Zwitterions

Dissertation Abstract

Successive Conversion of Lignocellulose to Bio-ethanol Using Zwitterions

Graduate School of
Natural Science and Technology
Kanazawa University

Division of Natural System

Student ID No. : 1424062015
Name : Heri Satria
Chief advisor : Prof. Kenji TAKAHASHI
Date of Submission : September, 22nd 2017

Abstract

In this study, successive efficient process on conversion of lignocellulosic biomass into ethanol using ionic liquids (ILs) were developed as pretreatment solvents or hydrolytic catalysts. Since ILs are known toxic to fermentative microorganisms, we modified the structure of ILs into zwitterions (ZIs) that expected to be effective to overcome the toxicity. The ZIs were less toxic than ILs. By exploiting the low toxic of ZIs, we developed the two successive processes.

We developed a successive process with an IL as a hydrolysis catalyst. The catalytic IL, 1-(4-sulfobutyl)-3-methylimidazolium hydrogen sulfate ($[C_1imC_4SH]HSO_4$) is composed of H_2SO_4 and a ZI, 1-(4-sulfobutyl)-3-methylimidazolium (C_1imC_4S). Bagasse was pretreated by using H_2SO_4 that included in IL, for pretreatment, following by addition of ZI for i*n situ* synthesis of $[C_1imC_4SH]HSO_4$. This process improved the glucose yield at 77% for 40 minutes hydrolysis at 100 ºC. After hydrolysis, the $[C_1imC_4SH]HSO_4$ separated into C_1imC_4S and H_2SO_4 by electrodialysis to decrease the toxicity of IL because the remaining ZI no longer toxic. The electrodialysis separated H_2SO_4 from $[C_1imC_4SH]HSO_4$ by the recovery at 97%; the separation enabled the successive fermentation. This successive conversion of bagasse into ethanol has the ethanol yield at 52%.

We also developed a successive process with a ZI as a pretreatment solvent in one-pot process. A novel carboxylate-type zwitterion (1-(3-carboxypropyl)-3-(methoxyethoxyethyl) imidazolium, OE_2imC_3C) was synthesized. OE_2imC_3C dissolved 6 wt% of cellulose at 100 ºC. Since OE_2imC_3C does not show toxicity to *E. coli*, the one-pot process using 0.5 M of OE_2imC_3C obtained ethanol yield at 28%, while when using ILs no ethanol was obtained due to the toxicity. The viscosity of OE_2imC_3C was noted as a critical inhibition factor in this one-pot process. Dimethyl sulfoxide (DMSO) was added as a co-solvent to improve the efficiency of conversion. The addition DMSO into OE_2imC_3C accelerated dissolution of cellulose. Furthermore, OE_2imC_3C/DMSO (8/2) at 150 gL^{-1} was chosen as a solvent in the one-pot conversion and the ethanol yield was increasing at 51%.

This study successfully developed successive processes by exploiting ZIs to avoid the high toxicity of ILs while the superior ability of ILs maintained.

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Introduction

Utilization of lignocellulosic feedstock in bioethanol production is demanded. To convert biomass into ethanol, three main processes consist of biomass dissolution, hydrolysis, and fermentation are required. Recently, ionic liquids (ILs) have demonstrated to be promising pretreatment solvents or hydrolytic catalyst for lignocellulose. On the other hand, successive efficient process on conversion of lignocellulosic biomass into ethanol is needed for efficiency of cost and energy.

However, the application of ILs has been limited due to their inhibition effect on fermentable microorganisms, and this condition make the successive process of lignocellulosic biomass conversion became difficult to realize. The ILs are known to be toxic to microorganisms because they destruct cell membranes. The cation part first approaches to anionic phospholipid in membranes, followed by insertion of alkyl chain of the cation into hydrophobic part of the membranes. We attached the anion part of ILs to the alkyl chain of cation, namely zwitterions (ZIs) and it is expected to be effective to prevent the insertion of the alkyl chain of the cation to the membranes.

The aim of this study is to develop successive processes of lignocellulose conversion into ethanol using ZIs to avoid the high toxicity of ILs while the superior ability of ILs maintained as both of pretreatment solvents and hydrolysis catalysts.

Experimental Method

Selected ILs and ZIs was used in growth assay and fermentation assay. In growth assay, ILs and ZIs in the range concentration 0.0-1.0 M were applied in lysogeny broth (LB) as a medium for growing up *Escherichia coli* (*E. coli*).

An IL ($[C_1imC_4SH]HSO_4$) as a hydrolysis catalyst was used in a successive process conversion of bagasse into ethanol. Bagasse was pretreated by using sulfuric acid that included in IL, for pretreatment. Following the pretreatment, the ZI has added for i*n situ* synthesis of $[C_1imC_4SH]HSO_4$. Subsequent to hydrolysis, the $[C_1imC_4SH]HSO_4$ then was separated into C_1imC_4S and H_2SO_4 by electrodialysis. Eventually, the remained solution that including of sugar and C_1imC_4S was fermented by using recombinant *E. coli* to produce ethanol.

A ZI as a pretreatment solvent was used in one-pot successive process conversion of bagasse into ethanol. The novel zwitterion (1-(3-carboxypropyl)-3-(methoxyethoxyethyl) imidazolium, OE_2imC_3C) was used as a pretreatment solvent at 120 ºC for 8 hours. After the pretreatment, hydrolysis was done by using cellulase (Celic® CTec2 enzyme) in acetate buffer pH 5.0 at 50 ºC for 48 hours. Following the hydrolysis, fermentation was done by using recombinant *E. coli* to produce ethanol. The similar procedure was done by using mixture solution of OE_2imC_3C/DMSO.

Result and Discussion

The toxicity of ILs to *E. coli* was higher than ZIs. Relative OD_{600} at 24 hours value that describes relative growth up of *E.coli* in presence of ILs or ZIs to the control (without ILs or ZIs) was used to evaluate the trend of toxicity. The growth up of *E. coli* was lower in presence of ILs than ZIs (Figure 1). ILs show toxicity to microorganisms by destruction of cell membranes. The cation part first approaches to anionic phospholipid in membranes, followed by insertion of alkyl chain of the cation into hydrophobic part of the membranes. When the anion part of ILs introduced to the end of the cation, namely zwitterions (ZIs), the toxicity appeared decreasing. It expected to be effective to prevent the insertion of the alkyl chain of the cation to the membranes because ZIs no longer have the hydrophobic cation tail. As a result, the ZIs were not toxic to the growth of *E. coli*.

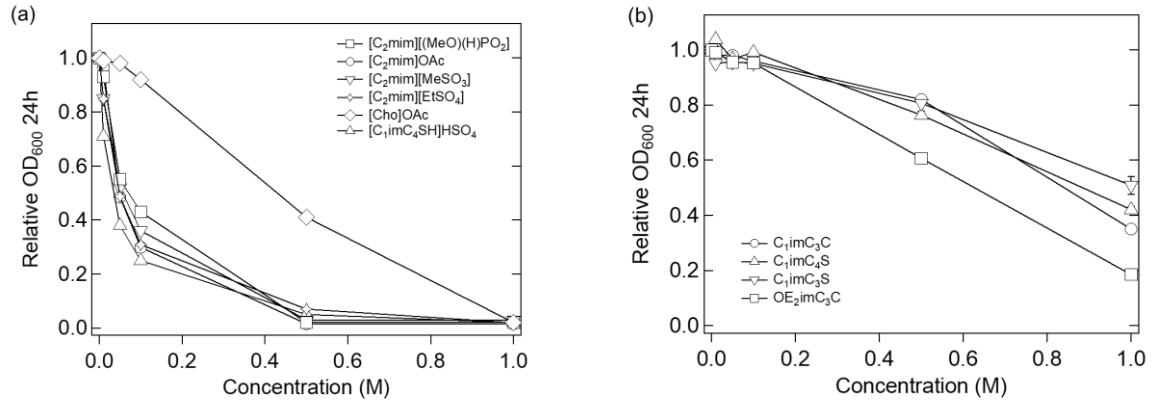

Figure 1 Relative OD_{600} 24h of ILs (a) and ZIs (b) that describe growth up *E. coli* in presence of ILs or ZIs.

When we investigated the toxicity of ILs to fermentation, and the ILs which act as hydrolytic catalysts or cellulose solvents were highly toxic. On the other hand, ZIs were low-toxic regardless of the structure. Figure 2 describes the relative ethanol yield 48 hours (Relative Y_{EtOH} 48h) of ILs (a) and ZIs (b).

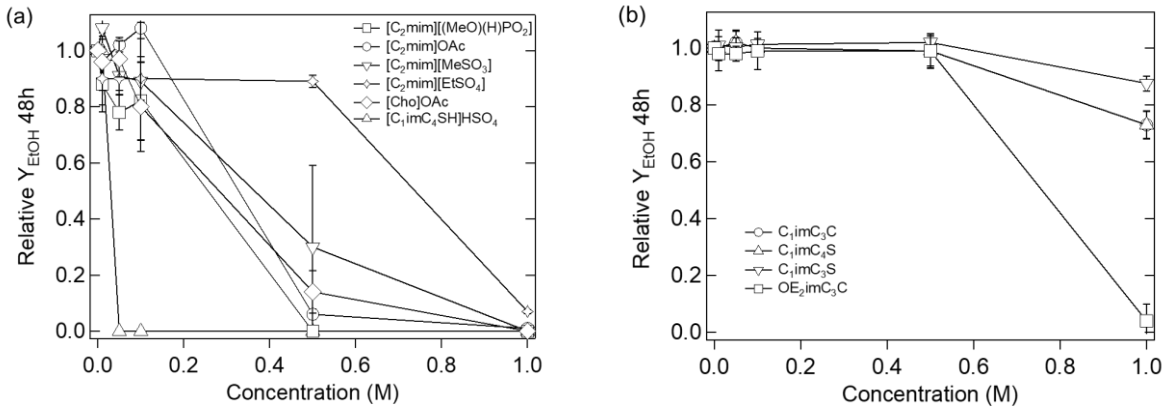

Figure 2 Relative Y_{EtOH} 48h of ILs (a) and ZIs (b) that describe toxicity of ILs or ZIs to *E. coli* in fermentation of glucose into ethanol.

The catalytic of an IL was applied to the successive conversion process of bagasse into ethanol. The IL, $[C_1imC_4SH]HSO_4$, is composed of sulfuric acid and a ZI, (C_1imC_4S), which sulfuric acid has pretreatment ability and $[C_1imC_4SH]HSO_4$ has high catalytic activity. Using sulfuric acid for pretreatment following by addition of ZI (C_1imC_4S) for *in situ* synthesis of $[C_1imC_4SH]HSO_4$ that use in hydrolysis was effective method to increase the glucose yield. This process improved the glucose yield of avicel hydrolysis to 77% for 40 minutes at 100 ºC (Figure 3).

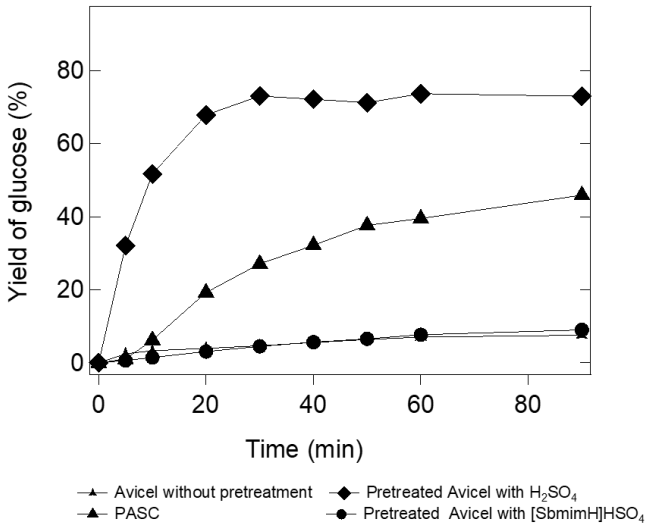

Figure 3 Time course of the glucose yield during the hydrolysis of differently pretreated cellulose (Avicel and PASC) using 1.0 M of $[C_1imC_4SH]HSO_4$ under microwave heating at 100 ºC.

The optimum temperature for this process was at 100 ºC, and the glucose yield remains stable for up to 90 minutes. At 90 ºC, the increase of glucose yield was slowly and at 110 and 120 ºC, quite similar yields were generated (72.52% and 76.88%) at 30 and 10 minutes respectively, however a decrease in glucose yield appeared, caused by the relatively strong conditions (Figure 4 (a)). On the other hand, the initial yield of xylose noted already at 20.64%. The xylose yield at 90 and 100 ºC was 118.21% and 107.83% respectively (Figure 4 (b)).

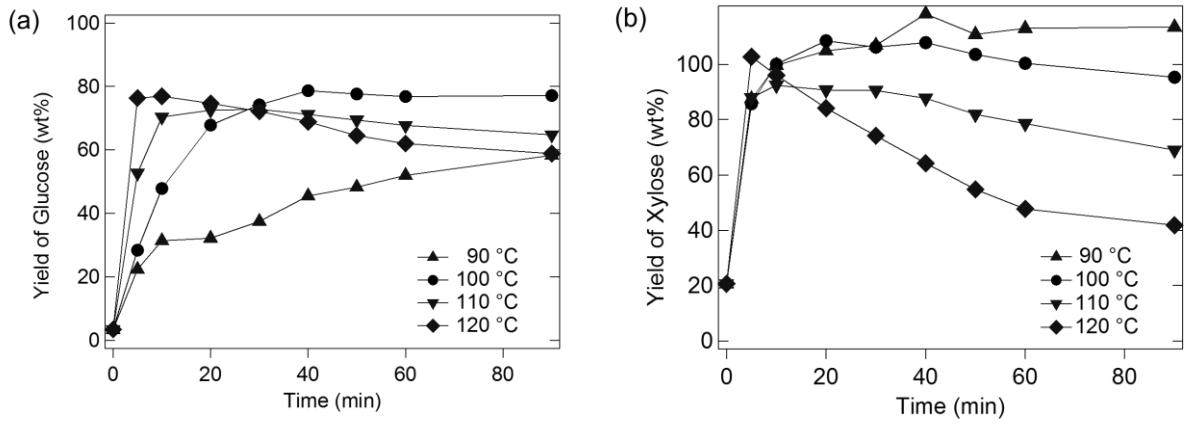

Figure 4 Time courses of glucose yield (a) and xylose yield (b) during hydrolysis of bagasse pretreated with 72% H_2SO_4 in 1.0 M $[C_1imC_4SH]HSO_4$ solution under microwave heating at 90,100, 110, and 120 ºC.

The decomposition of xylose into furfural appeared at 100 ºC (6.12%) since no HMF from the decomposition of glucose detected in this hydrolysis condition (Figure 5).

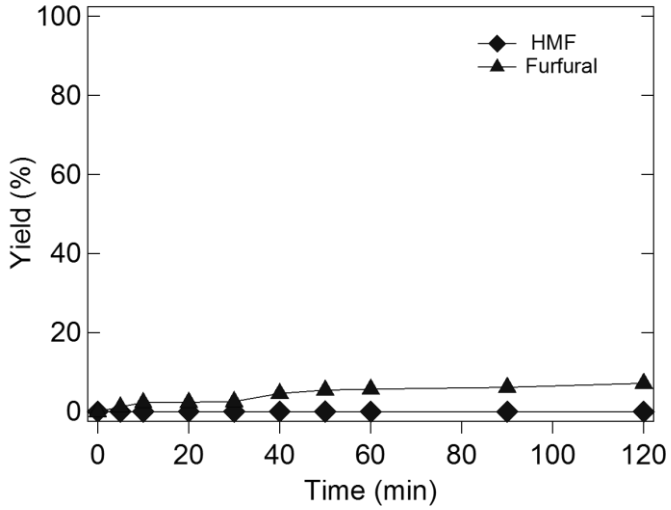

Figure 5 Time courses of yield of HMF and furfural during hydrolysis of the bagasse, pretreated using H_2SO_4 in 1.0M $[C_1imC_4SH]HSO_4$ solution under microwave heating at 100 ºC.

Subsequent to hydrolysis, the $[C_1imC_4SH]HSO_4$ then separated into C_1imC_4S and H_2SO_4 by electrodialysis to decrease the toxicity of IL because the remaining ZI no longer toxic. In addition, electrodialysis successfully separated H_2SO_4 from $[C_1imC_4SH]HSO_4$ by the recovery at 97% (Figure 6). The separation enabled the successive fermentation, and this successive conversion of bagasse into ethanol has the ethanol yield at 52%.

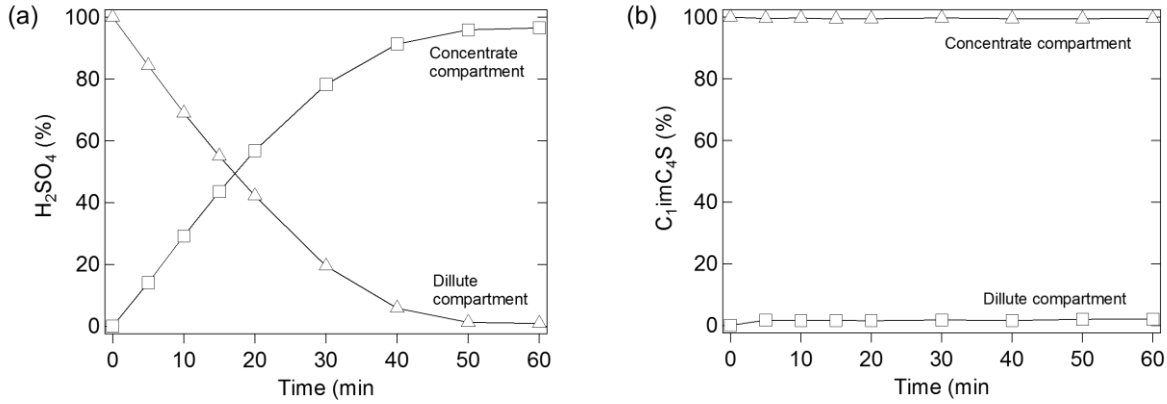

Figure 6 Time courses of concentration of H_2SO_4 (a) and C_1imC_4S (b) in dilute and concentrate compartment during electrodialysis of $[C_1imC_4SH]HSO_4$.

A novel zwitterion, OE_2imC_3C, dissolves 6 wt% of cellulose. Using OE_2imC_3C in one-pot conversion of bagasse into ethanol as a pretreatment solvent increased the ethanol yield at 28% while when using IL ($[C_2mim]OAc$) no ethanol was obtained due to the its toxicity (Figure 7).

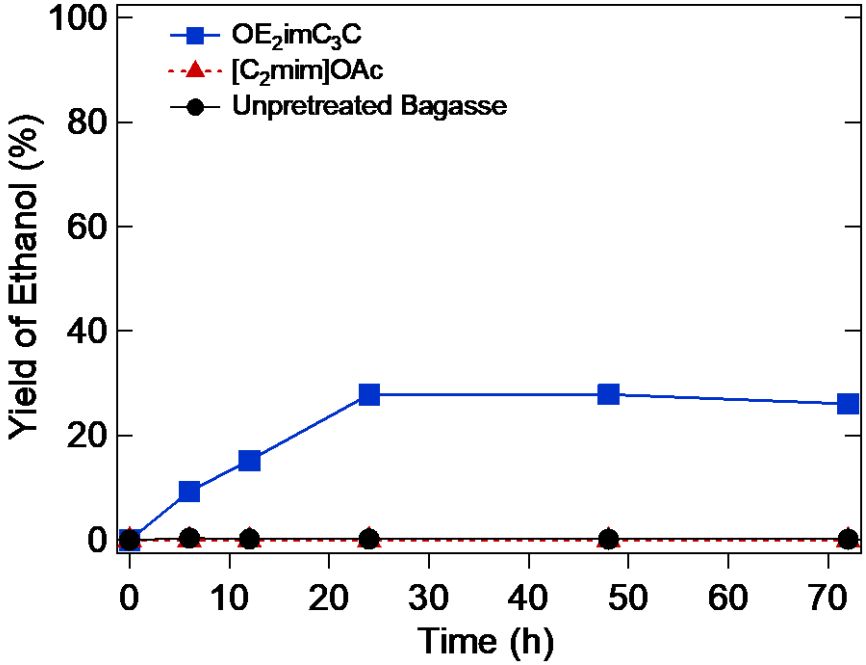

Figure 7 Time courses of ethanol yield in one-pot fermentation in the presence of OE_2imC_3C and $[C_2mim]OAc$, the initial of OD_{600} *E. coli* KO11 was 1.0.

To decrease the viscosity of OE_2imC_3C, DMSO was used as co-solvent in OE_2imC_3C/DMSO solution. Addition of DMSO to OE_2imC_3C accelerated dissolution of cellulose. A higher of DMSO concentration (40%) in OE_2imC_3C/DMSO (6/4) showed a peak of cellulose solubility (14 wt%). In contrast, the addition of DMSO over 40% to OE_2imC_3C decreased dissolution of cellulose (Figure 8).

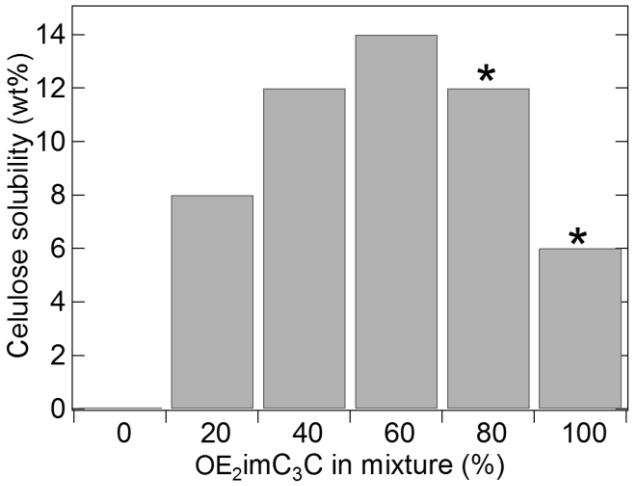

Figure 8 Dissolution of cellulose in OE_2imC_3C/DMSO mixture.

Furthermore, the activity of cellulase in presence of the mixture was investigated on enzymatic saccharification assay. The result of examination presents in Figure 9. It showed at 150 gL^{-1} of mixture, concentration DMSO gave higher inhibition than OE_2imC_3C, but at

250 gL^{-1} the opposite trend was appearing although for both of concentration the relative cellulase activity was not considerably different. Even though the inhibition effect of DMSO was higher than OE_2imC_3C but the addition of DMSO the OE_2imC_3C showed inhibition effect of mixture not too different from the pure OE_2imC_3C inhibition.

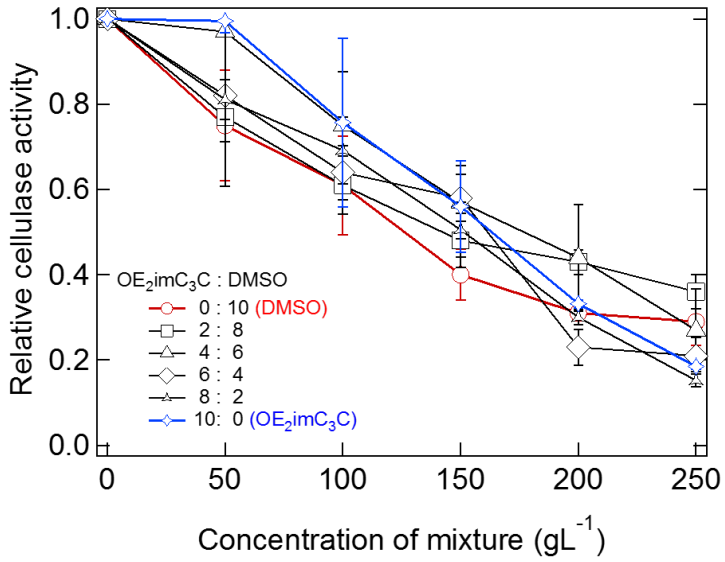

Figure 9 Relative activity of cellulase in the presence of OE_2imC_3C/DMSO mixture.

Base on the investigation of inhibition OE_2imC_3C/DMSO to the enzyme activity and toxicity the *E. coli* growth including in fermentation, the OE_2imC_3C/DMSO (8/2) at 150 gL^{-1} was chosen as a solvent to conduct one-pot conversion of bagasse into ethanol. The pretreatment using OE_2imC_3C/DMSO (8/2) was effective to enhance the fermentable sugar, so that in fermentation, the ethanol yield was achieved 51%. Figure 10 presents the ethanol yield in this one-pot fermentation.

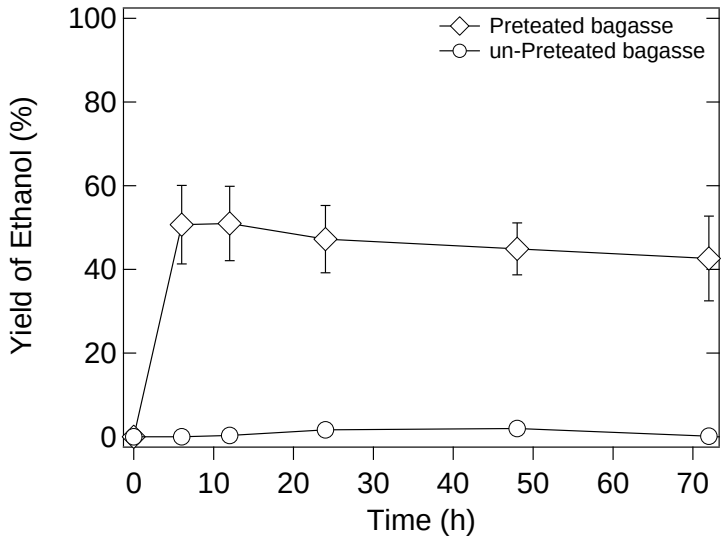

Figure 10 Time courses of ethanol yield in one-pot conversion of bagasse into ethanol using OE_2imC_3C/DMSO (8/2).

Conclusion

This study successfully developed successive processes by exploiting ZIs to avoid the high toxicity of ILs while the superior ability of ILs maintained. Using approach of ILs on biomass processing, the novel process enabled successive processes in biomass conversion consists of pretreatment, hydrolysis, and fermentation. The keys of successful conversion were how to overcome the crystallinity of biomass in pretreatment, efficient hydrolysis process, and reduce toxicity effect that allowed fermentation using microorganisms.

平成 29 年 8 月　3 日

学位論文審査報告書（甲）

1．学位論文題目（外国語の場合は和訳を付けること。）

Successive Conversion of Lignocellulose to Bio-ethanol using Zwitterions

双性イオン液体を用いたリグノセルロースからバイオエタノールへの逐次変換

2．論文提出者　(1) 所　　属　　自然システム学専攻

(2) 氏（ふり）　名（がな）　Heri SATRIA (へり　さとりあ)

3．審査結果の要旨（600～650 字）

植物バイオマスは、エタノールなどの有用物質への変換が期待されている。2000 年代にイオン液体が“植物バイオマスの溶媒”あるいは“効率的なセルロースの加水分解触媒”となることが報告されて以来、様々な基礎検討が行われてきた。しかし、木質系バイオマスをエタノールに変換する上で必要な「前処理」、「加水分解」、「発酵」のプロセスを一貫して連続的に行うことは困難であった。その理由は、イオン液体が発酵性微生物に対して強い毒性を示すためである。

このような背景のもと，学位申請者である Satria 氏は、“溶媒”あるいは“加水分解触媒”としてイオン液体を利用しながら、イオン液体を無毒化することで連続プロセスを構築した。はじめに，イオン液体を無毒化する手法として zwitterion が有効であることを見出した。イオン液体のアニオンとカチオンを共有結合で結んだ zwitterion は、イオン液体と比較して非常に低毒性であることが分かった。加水分解触媒能をもつイオン液体は、硫酸と zwitterion から構成される。そのため、電気透析を行うことで加水分解後の溶液から硫酸を除去し、イオン液体の毒性を回避することに成功した。また、バイオマスを前処理できる新規 zwitterion の開発にも成功し、ワンポットでエタノールを生産できることを実証した。

これらの結果は、新たに高効率なエタノール変換法を構築できることを示しており、その工学的意義は極めて高く，博士（工学）に値するものと判断された。

4．審査結果　(1) 判　　定（いずれかに○印）　(合　格)　・　不合格

(2) 授与学位　　博　士（工学）