Sustainable Energy Technologies and Assessments 16 (2016) 64–68

Contents lists available at ScienceDirect

Sustainable Energy Technologies and Assessments

journal homepage: www.elsevier.com/locate/seta

Technical Note

Ammonium carbonate as a catalyst for lignocellulose pretreatment and a nitrogen source for fermentation

Muhammad Ajaz Ahmed [a], Ilgook Kim [a], Ga-Yeong Kim [a], Muhammd Saif Ur Rehman [b], Jong-In Han [a,*]

[a] Department of Civil and Environmental Engineering, KAIST, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Department of Chemical Engineering, COMSATS Institute of Information Technology, Lahore, Pakistan

ARTICLE INFO

Article history:
Received 10 September 2015
Revised 4 March 2016
Accepted 3 May 2016

Keywords:
Ammonium carbonate pretreatment
Response surface methodology optimization
Simultaneous saccharification and fermentation
Nitrogen source
Bioethanol

ABSTRACT

In this work, ammonium carbonate (AC), a product released during the regeneration of ammonia-based carbon capture process, was evaluated as an alkaline catalyst for the pretreatment of lignocellulose; and as a nitrogen source in the subsequent fermentation process for bioethanol production. Response surface methodology was employed to attain an optimum pretreatment condition in terms of AC concentration (15–25%), reaction time (5–15 h) and temperature (60–100 °C). The highest enzymatic digestibility of 59.9% was achieved with AC concentration of 20.0% at 79.5 °C of treatment temperature for 9.46 h of reaction time. A fermentation medium containing ammonium ion derived from the liquid hydrolysate after the AC-based pretreatment was found to enhance the final concentration of ethanol produced by *Saccharomyces cerevisiae* from 9.13 g/L to 13.30 g/L. These results indicate that AC can indeed serve as a catalyst option for pretreating lignocellulosic biomass and has an added advantage of being used as a nitrogen source for the fermentation process.

Introduction

Energy security, especially maintained in a way that is sustainable, is one of the greatest challenges that the globe will eventually face. Biofuels have emerged as one likely and desirable option. Among several biofuels candidates, lignocellulosic bioethanol has a distinctive advantage of abundance. The cumulative annual production of lignocellulosic biomass (LCB) amounts to be 1×10^{11} MT worldwide which, in theory, is enough to swap the existing fossil fuels so as to deal with the energy insecurity of the world [1]. Its use as a feedstock for bioethanol production, however, has several technical and economic challenges to overcome. The LCB mainly consists of cellulose, hemicellulose and lignin; each property of these components and arrangement of them make the LCB particularly persistent to the microbial and enzymatic attack [2–4]. To facilitate the subsequent bioprocessing steps such as hydrolysis and fermentation, therefore, pretreatment is of absolute necessity [5]. Most commonly practiced or investigated pretreatment methods include acidic, alkaline and biological treatments, wet oxidation and steam explosion [2,6], each of which has both advantages and disadvantages.

Alkaline treatment based on ammonia is a rather well-established pretreatment method. Ammonia is not very toxic and corrosive, and also is easily recyclable due to high volatility. It is known to enhance the bioavailability of cellulose by way of altering crystallinity index and morphology of LCB.

Ammonia fiber explosion is the representative technology; and soaking in aqueous ammonia and ammonia recycle percolation are also somewhat well studied. Recently a related chemical, i.e., ammonium carbonate was proposed as a novel alkaline catalyst [6]. It rendered enzymatic digestibility escalated to 72.2% through the same mechanism of ammonia, namely the surface modification of LCB. In fact, one mole of ammonium carbonate yields two moles of ammonia with low energy consumption of 24.1 kcal [7,8].

$$(NH_4)_2CO_3\ (aq) \leftrightarrow 2NH_3\ (aq) + H_2O\ (l) + CO_2\ (g)\ \Delta H = 24.1\ \text{kcal}$$

In addition to the distinct catalytic activity, ammonium carbonate can also serve as a nitrogen source in the ensuing fermentation. These potential advantages would lead to the reduction of the overall cost of bioethanol production from LCB.

In the present work, therefore, we employed the response surface methodology (RSM) to optimize the critical pretreatment parameters aiming at highest enzymatic digestibility. Besides, simultaneous saccharification and fermentation (SSF) of ammonium carbonate treated corn stover was performed to evaluate

* Corresponding author. Tel.: +82 42 350 3629; fax: +82 42 350 3610.
E-mail address: hanj2@kaist.ac.kr (J.-I. Han).

http://dx.doi.org/10.1016/j.seta.2016.05.002
2213-1388/

Table 1
Solid recovery, glucan recovery, lignin removal and enzymatic hydrolysis.

Serial No.	Temperature (°C)	Time (h)	AC conc. (%)	Solid recovery (%)	Glucan recovery (%)	Lignin removal (%)	Enzymatic hydrolysis (%)
1	60	5	20	52.51	64.43	51.42	38.82
2	60	10	15	59.6	81.32	43.60	35.33
3	60	10	25	53.08	64.57	55.56	45.53
4	60	15	20	53.91	75.11	52.23	46.27
5	80	5	15	52.84	62.93	48.03	45.12
6	80	5	25	55.1	73.86	51.07	53.94
7	80	10	20	51.31	62.49	54.53	59.87
8	80	10	20	48.85	65.69	62.27	59.87
9	80	10	20	51.08	73.44	58.99	59.10
10	80	15	15	49.05	70.12	54.90	50.61
11	80	15	25	49.38	69.88	51.15	54.10
12	100	5	20	55.77	100.88	46.48	41.95
13	100	10	15	57.58	79.38	46.03	44.39
14	100	10	25	54.45	82.97	46.38	58.54
15	100	15	20	51.67	73.35	53.82	55.49

the effect of ammonium as a nitrogen source on the following fermentation by *Saccharomyces cerevisiae.*

Materials and methods

Feedstock preparation

Corn stover was collected from the city of Changwon, South Korea. The biomass was air-dried, chopped, milled and ground using a laboratory mortar grinder. The ground biomass was sieved using a sieve of 200 μm to a size of less than 0.2 cm. Compositional analysis was performed according to national renewable energy protocol [9]. It was found that the corn stover has a glucan 38.0%, xylan 18.6% and lignin 22.3%.

Ammonium carbonate (AC)-based pretreatment

A stainless steel batch reactor was used to perform pretreatment. Corn stover and ammonium carbonate solutions of 15%, 20% and 25% concentrations were mixed together in the reactor keeping a solid to liquid ratio of 1:10. The slurry was heated in an oil bath for different temperatures of 60 °C, 80 °C and 100 °C and for time span of 5, 10 and 15 h. The treated slurry was then washed, filtered and kept in an oven at a temperature of 50 °C for subsequent processing. All the experiments were performed in triplicates.

Enzymatic hydrolysis

Enzymatic hydrolysis was carried out in an Erlenmeyer flask with 50 mM of sodium citrate buffer solution (pH = 4.8–5.0) keeping a solid loading of 10%. The buffer solution was prepared by dissolving 4.83 g of citric acid along with 7.94 g of sodium citrate in 1000 ml of deionized water. Cellic C-Tec derived from *Trichoderma reesei* (Novozymes, Denmark) was loaded to the pretreated biomass with an activity of 30 FPU/g (filter paper unit per gram) of cellulose. This flask was subjected to hydrolysis at a temperature of 50 °C and 170 rpm for a period of 3 days in a shaking incubator. The samples were taken periodically for sugar analysis.

Fermentation

S. cerevisiae was used for fermentation. The yeast cells were first grown on Yeast Malt agar plates consisting of glucose 10 g/L, yeast extract 3 g/L, malt extract 3 g/L, agar 20 g/L and peptone 5 g/L. Some colonies were then transferred into an inoculation medium, containing 3 g of yeast extract, 5 g of peptone and 30 g of glucose per liter of solution, and grown at 37 °C and 200 rpm for 48 h. Actively growing cells were harvested and used as an inoculum. Fermentation was carried out in a sterile 250 mL glass Erlenmeyer flask with a special arrangement of cap with a needle hole to remove CO_2 from the system at 30 °C and 170 rpm in a shaking incubator for 72 h.

The fermentation flask was filled with 90% volume of the fermentation medium and with 10% of the inoculum. The fermentation medium was provided with 4 g/L $MgSO_4$ and 5 g/L each of KH_2PO_4, peptone and yeast extract. Sodium citrate and citric acid were used as a buffer to maintain pH in a range of 4.8–5.1. Small amount of trace elements in mg/L was also added, consisting of 270 mg $ZnCl_2$, 1.5 mg $FeCl_2{\cdot}4H_2O$, 36 mg H_3BO_3, 100 mg $MnCl_2{\cdot}4H_2O$, 2 mg $CuCl_2{\cdot}2H_2O$, 190 mg $CoCl_2{\cdot}6H_2O$, 36 mg $Na_2MoO_4{\cdot}2H_2O$ and 240 mg $NiCl_2{\cdot}6H_2O$. One stream of the AC-treated filtrate was used as a nitrogen source for the fermentation. In order to compare the efficiency, two controls were also

Table 2
ANOVA.

Source	Sum of squares	df	Mean square	*F* value	*P* value prob > *F*	
Model	847.79	9	94.20	7.88	0.0175	Significant
X1-temp	148.09	1	148.09	12.39	0.0169	
X2-time	88.71	1	88.71	7.42	0.0415	
X3-conc.	167.99	1	167.99	14.06	0.0133	
X1X2	9.27	1	9.27	0.78	0.4187	
X1X3	3.90	1	3.90	0.33	0.5925	
X2X3	7.10	1	7.10	0.59	0.4755	
X11	332.38	1	332.38	27.82	0.0033	
X22	74.53	1	74.53	6.24	0.0546	
X33	64.45	1	64.45	5.39	0.0678	
Residual	59.74	5	11.95			
Lack of fit	59.34	3	19.78	100.09	0.0099	Significant
Pure error	0.40	2	0.20			