

# Commentary on the Reuse of Black Liquor for the Enzymatic Hydrolysis and Ethanol Fermentation of Alkali-treated Sugarcane Bagasse

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## Research Article

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### ABSTRACT

To relieve the water consumption and wastewater generation would accelerate the industrialization of the cellulosic ethanol production based on the alkaline pre-treatment. A feasible way to reuse pre-treatment liquid (black liquor) for enzymatic hydrolysis and fermentation was commented. After the black liquor was acidified to pH 4.8 and centrifuged, the supernatant could not be used for ethanol fermentation but cellulolytic hydrolysis, while its diluent was suitable for both ethanol fermentation and cellulolytic hydrolysis. Combined these findings with the current technological development on recycling black liquor and waste washing water, an improving technical flow of cellulosic ethanol production based on alkaline pre-treatment was prospected.

## INTRODUCTION

The Bioconversion of lignocellulosic biomass into ethanol can not only avoid the air pollution arising from the burning of the waste lignocellulose, but also provide the sustainable clean energy to substitute for the petroleum. The application of cellulosic ethanol as the transport fuel can relieve the greenhouse gases emission from the combustion of the fossil fuels to meet the sustainable economic development, and ensure the national energy security [1]. The production of cellulosic ethanol comprises four main steps: pretreatment to overcome the lignocellulosic recalcitrance, enzymatic hydrolysis to depolymerize the carbohydrate into fermentable sugars, fermentation to convert sugars into ethanol, and distillation to extract the ethanol [2]. Pretreatment is the key step to guarantee the high efficiency of the subsequent saccharification. There are various technologies to pretreat lignocellulose, such as milling, ultrasound, acid, alkali, organosolv, hydrothermal processing, and so on [3]. The sodium hydroxide solution (NaOH) pretreatment which is one of the alkaline pretreatments was selected out by the authors to treat sugarcane bagasse (SCB) due to its low energy input and high lignin removal [4]. However, two obvious shortcomings of the NaOH pretreatment prevent its industrial application: a large quantity of water needed to wash the alkali-treated lignocellulose and a plenty of wastewater generation during the pretreatment and washing process. To relieve water consumption and wastewater generation would accelerate the industrialization of cellulosic ethanol based on the alkaline pretreatment.

### Reuse of the pretreatment liquid for enzymatic hydrolysis and fermentation

The pretreatment liquid, namely called black liquor (BL), is generated in the alkaline pretreatment process, and harmful to the environment. It has high pH value and lignin content. The BL generated in the papermaking industry is often combusted, but the combustion is not proper for the BL of alkaline pretreatment due to its high water content. Recently, a few researchers reused the BL to pretreat lignocellulose because of its high pH value [5-10]. Although the BL could be recycled for several times to efficiently treat lignocellulose after being replenished with the alkali [9,10], the enzymatic saccharification efficiency of alkali-treated lignocellulose

decreased with the increasing recycling times of BL [5,9]. In addition, after being recycled for several times, the treatment of the BL generated in the last recycling time has been still a problem. Because the lignin is a useful raw material to produce high value added products, such as plasticizer, dispersant, chelator, and so on [11-13], many technologies like pH adjustment, ultrafiltration, nanofiltration and electrocoagulation have been developed to extract lignin from the BL [14-16]. Nevertheless, the lignin extraction cost is high, and the residual wastewater still need to be treated further.

Due to its high pH value and lignin content, seldom researchers would consider the reuse of BL for enzymatic hydrolysis and fermentation. The enzymatic hydrolysis and fermentation of alkali-treated lignocellulose could be well performed at acid condition (pH 4.8) without lignin-derived inhibitors like p-coumaric acid, ferulic acid, glucuronic acid, etc. [17-19]. However, the pure alkaline lignin which was found to hardly affect the enzymatic hydrolysis of cellulose inspired the investigation on the impact of the BL on the enzymatic hydrolysis of cellulose and ethanol fermentation [20]. The authors firstly used the acetic acid to adjust the pH of BL to 4.8 which is the proper pH value for enzymatic hydrolysis and fermentation. During the pH adjustment process, the lignin was precipitated from the BL. It could not only deposit on the surface of cellulose, but also significantly adsorb the cellulase [20]. Therefore, the precipitated lignin imposed its negative impact on the enzymatic hydrolysis of cellulose through blockage the access of cellulase to cellulose and non-productive adsorption of cellulase [20-22]. It is necessary to separate the precipitated lignin from BL before its application for cellulolytic hydrolysis and fermentation. After isolating lignin from BL through acidification and centrifugation, it was found that the supernatant of BL (BLS) had little inhibition on enzymatic hydrolysis of NaOH-treated SCB, but completely inhibited the ethanol fermentation. Nevertheless, after the BLS was diluted to 4 times with the acetate buffer (pH4.8), it exhibited no inhibition on ethanol fermentation. It meant that the lignin-derived chemicals could exist in the enzymatic hydrolysis buffer, but its concentration should be too low enough to negatively affect the enzymatic hydrolysis and fermentation. This study provided a feasible promising way to accelerate the industrialization of cellulosic ethanol based on the alkaline pretreatment.

**Prospect**

Based on the above findings, an improving and prospective technical flow for cellulosic ethanol production would be developed (Figure 1). The lignocellulosic biomass was firstly treated by NaOH solution under the moderate condition. The BL and alkali-treated solid residue were obtained. The pH value of BL was adjusted to 4.8 with acetic acid. After liquid-solid separation, the lignin could be isolated from the BL and used as the raw material for producing the high value added products. The residual chemicals existing in the BLS should be determined, and their inhibition mechanism on the special functional microorganisms should be explored for breeding inhibitor-tolerant microbial strains. Then the BLS would be not only thoroughly used for enzymatic hydrolysis and fermentation, but also cleansed by microbial strains via aerobic/anaerobic treatment. The alkali-treated solid residue would be washed for several times for the subsequent enzymatic hydrolysis, and a plenty of water would be consumed. The authors developed a new recycled way of the waste washing water for washing alkali-treated lignocellulose [9]. Compared with the traditional washing flow, the new washing flow could save more than 80% water consumption [9]. According to the current development on the alkaline pretreatment, the recycling methods of BL and waste washing water have been developed. The future work for accelerating the industrialization of the prospective technology is to focus on breeding microbial strains which are tolerant to the lignin-derived chemicals. Additionally, a more cost-effective way to recycle BL and waste washing water should be built up.

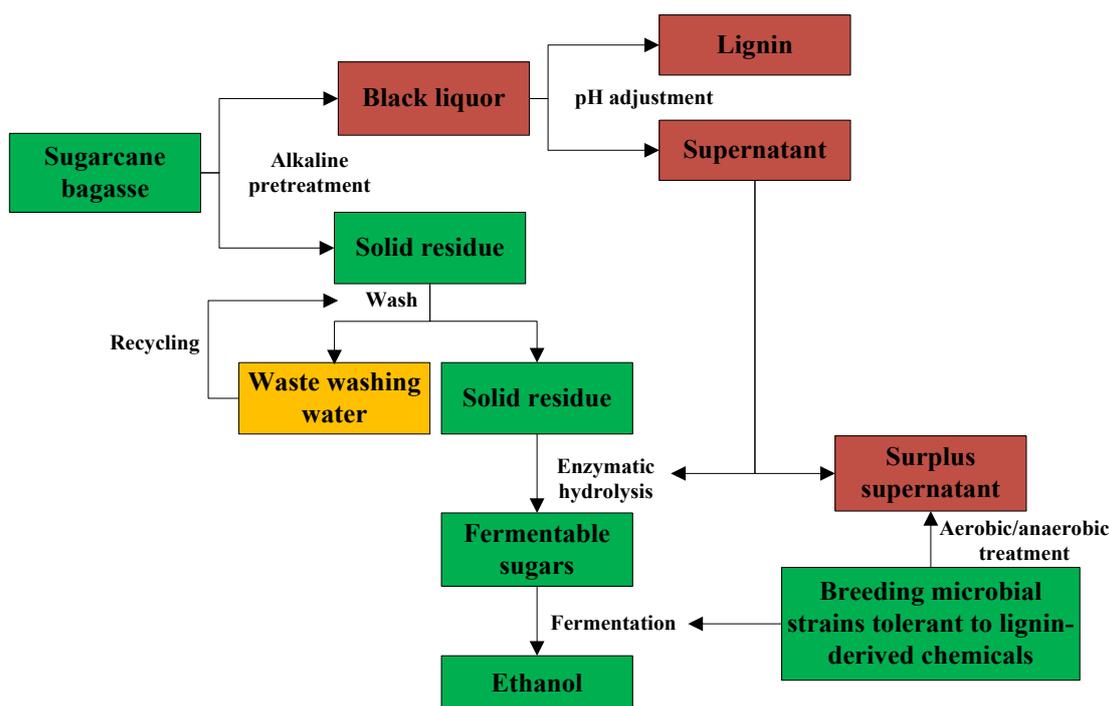


Figure 1. Prospective technical flow for cellulosic ethanol production based on alkaline pretreatment

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## REFERENCES

1. Zhu J and Zhuang X. Conceptual net energy output for biofuel production from lignocellulosic biomass through biorefining. *Prog Energy Combust Sci.* 2012;38:583-598.
2. Paulova L, et al. Lignocellulosic ethanol: Technology design and its impact on process efficiency. *Biotechnol Adv.* 2015;33:1091-1107.
3. Silveira MHL, et al. Current pretreatment technologies for the development of cellulosic ethanol and biorefineries. *ChemSusChem.* 2015; 8:3366-3390.
4. Kim JS, et al. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour Technol.* 2016;199:42-48.
5. Cha YL, et al. Alkaline twin-screw extrusion pretreatment of Miscanthus with recycled black liquor at the pilot scale. *Fuel.* 2016;164:322-328.
6. Muryanto, et al. Alkaline delignification of oil palm empty fruit bunch using black liquor from pretreatment. *ISAC 2015.* 2015;16:99-105.
7. Liu H, et al. Comparative study of pretreated corn stover for sugar production using cotton pulping black liquor (CPBL) instead of sodium hydroxide. *Ind Crop Prod.* 2016;84:97-103.
8. Xu J, et al. Pretreatment of corn stover for sugar production with switchgrass-derived black liquor. *Bioresour Technol.* 2012;111:255-260.
9. Wang W, et al. High conversion of sugarcane bagasse into monosaccharides based on sodium hydroxide pretreatment at low water consumption and wastewater generation. *Bioresour Technol.* 2016;218:1230-1236.
10. Rocha GJM, et al. Contributing to the environmental sustainability of the second generation ethanol production: Delignification of sugarcane bagasse with sodium hydroxide recycling. *Ind Crop Prod.* 2014;59:63-68.
11. Kamoun A, et al. Evaluation of the performance of sulfonated esparto grass lignin as a plasticizer-water reducer for cement. *Cem Concr Res.* 2003;33:995-1003.
12. Matsushita Y and Yasuda S. Preparation and evaluation of lignosulfonates as a dispersant for gypsum paste from acid hydrolysis lignin. *Bioresour Technol.* 2005;96:465-470.
13. Sena-Martins G, et al. Eco-friendly new products from enzymatically modified industrial lignins. *Ind Crop Prod.* 2008;27:189-195.
14. Jonsson AS, et al. Concentration and purification of lignin in hardwood kraft pulping liquor by ultrafiltration and nanofiltration. *Chem Eng Res Des.* 2008;86:1271-1280.
15. Ghatak HR. Iron complexed lignin from electrolysis of wheat straw soda black liquor and its characterization. *Ind Crop Prod.* 2013;43:738-744.
16. Minu K, et al. Isolation and purification of lignin and silica from the black liquor generated during the production of bioethanol from rice straw. *Biomass Bioenerg.* 2012;39:210-217.
17. Kim Y, et al. Soluble inhibitors/deactivators of cellulase enzymes from lignocellulosic biomass. *Enzyme Microb Tech.* 2011;48:408-415.
18. Klinke HB, et al. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Appl Biochem Biotechnol.* 2004;66:10-26.
19. Pan X J. Role of functional groups in lignin inhibition of enzymatic hydrolysis of cellulose to glucose. *J Biobased Mater Bioenergy.* 2008;2:25-32.
20. Wang W, et al. Feasibility of reusing the black liquor for enzymatic hydrolysis and ethanol fermentation. *Bioresour Technol.* 2017;228:235-240.
21. Morales LO, et al. Effects of residual lignin and heteropolysaccharides on the bioconversion of softwood lignocellulose nanofibrils obtained by SO<sub>2</sub>-ethanol-water fractionation. *Bioresour Technol.* 2014;161:55-62.
22. Siqueira G, et al. Enhancement of cellulose hydrolysis in sugarcane bagasse by the selective removal of lignin with sodium chlorite. *Appl Energ.* 2013;102:399-402.