Enhancing enzymolysis and fermentation efficiency of sugarcane bagasse by synergistic pretreatment of Fenton reaction and sodium hydroxide extraction

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**Highlights**
- Fenton reaction combined with NaOH extraction was employed to pretreat SCB.
- The process parameters of Fenton reaction were optimized.
- The pretreatment performance was related to the combinational sequence.
- The combinational pretreatment had an excellent SSF performance.

**Abstract**
A study on the synergistic pretreatment of sugarcane bagasse (SCB) using Fenton reaction and NaOH extraction was conducted. The optimized process conditions for Fenton pretreatment were 10% (w/w) of H\textsubscript{2}O\textsubscript{2}, 20 mM of Fe\textsuperscript{2+}, pH 2.5, pretreatment time 6 h, and pretreatment temperature 55 °C. Sequential pretreatments were performed in combination with NaOH extraction (NaOH 1% (w/w), 80 °C, 5% of solid loading, 1 h). Among all the pretreatments, Fenton pretreatment followed by NaOH extraction had the highest efficiency of 64.7% and 108.3% for enzymolysis and simultaneous saccharification fermentation (SSF) with an ethanol concentration of 17.44 g/L. The analyses by the scanning electron microscopy, X-ray diffraction and confocal laser scanning microscopy revealed that Fenton pretreatment disrupts the structure of SCB to facilitate the degradation of lignin by NaOH. The overall data suggest that this combinatorial strategy is a promising process for SCB pretreatment.

**1. Introduction**

With the gradual exhaustion of the fossil fuels like coal, petroleum and natural gas on the earth, people become more anxious about the energy crisis (Farrell et al., 2006). In order to solve the problem, many attempts have been made by researchers and biofuels are found to be a promising solution. Biofuels, such as bioethanol and biobutanol, can be produced in large scale from lignocellulose due to its massive amount on the planet (Demirbas, 2008). Lignocellulose is mainly composed of cellulose, hemicellulose and lignin, and the first two can be converted into reducing sugars, and then ethanol or other kinds of biofuels by enzymes addition or microbial fermentation (Galbe and Zacchi, 2007). However, the degradation of lignocellulose is very difficult owing to the compact structure and the existence of lignin. Therefore, pretreatment of the lignocellulose is necessarily required (Jørgensen et al., 2007). Pretreatment can interrupt the dense structure and help to remove the lignin, thus facilitating the exposure of the embedded cellulose to enzymes or microbes.

Pretreatment methods are generally divided into four kinds: chemical method, physical method, physical–chemical method and biological method (Mosier et al., 2005). Nevertheless, these methods do have the intrinsic weaknesses (Agbor et al., 2011). For the chemical method, the chemicals used are either too acid or alkaline, or toxic and are highly demanding for the apparatus and operation. Besides, they can also cause environmental problems. For the physical method, harsh processing parameters such as high temperature and pressure are usually demanded.

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as high pressure or temperature are conventionally needed in the pretreatment, suggesting the high cost and energy consumption in production. The physical–chemical method seems to seek a balance between the two, but it often inherits the demerits of the two methods despite higher efficiency. As for the last one, it would be much time-consuming to yield a satisfying performance, because the propagation and processing of microbes like fungi usually take time. Hence, more efforts should be made to overcome these problems without sacrificing the performance. Additionally, it seems that any single pretreatment method cannot achieve a perfect performance in both efficiency and cost.

In recent years, the application of Fenton reaction to lignocellulose pretreatment has been attempted, which is naturally present in fungi to degrade wood (Arantes et al., 2012). Moreover, Fenton reaction pretreatment has been widely used to degrade lignocellulose and improve the degradation of lignin (Arantes et al., 2011). It has been reported that an enzymatic saccharification of Fenton pretreated biomass showed an average 20.1% increase relative to untreated control among all the four feedstocks (miscanthus, switchgrass, cornstover and wheat straw) (Kato et al., 2014). When used to pretreat rice straw, an enzymatic digestibility of 93.2% of the theoretical glucose yield was obtained (Jung et al., 2015). Fenton reaction is convenient and cost-effective, so it can be a prospective alternative to pretreat the lignocellulose.

In the present study, the feasibility and efficiency of Fenton’s reagents in pretreating sugarcane bagasse (SCB) were evaluated. Additionally, a combination of Fenton reaction with NaOH extraction for SCB pretreatment was also investigated. Finally, the mechanism of the pretreatments was explored using scanning electron microscopy (SEM), X-ray powder diffraction (XRD), and confocal laser scanning microscopy (CLSM).

2. Materials and methods

2.1. Materials

The SCB was provided by the Guangzhou Sugarcane Industry Research Institute (Guangdong Province, China). The SCB was ground to obtain particles with dimensions of <0.1 mm. Fenton’s reagents including FeCl$_2$·4H$_2$O and H$_2$O$_2$ (30%, w/w) were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). Commercial cellulase (Celluclast® 1.5L) and β-glucosidase (Novozyme 188, Denmark) were purchased from Sigma (St. Louis, MO, USA).

2.2. Fenton/NaOH pretreatment

Fenton pretreatment was carried out in a 250 ml Erlenmeyer flask with a solid–liquid ratio of 5%. Briefly, the SCB (5 g) was added to the flask, followed by supplementation of 50 ml of the desired concentration of Fe$^{2+}$, and keeping the flask in a shaker for 30 min to totally soak the SCB in the solution. The Fenton reaction was initiated by adding 50 ml of desired concentration of H$_2$O$_2$, followed by sealing the bottleneck with plastic thin-film to avoid H$_2$O$_2$ volatilization, and placing the flask in the shaker for desired time. After the pretreatment, the suspension was quickly filtrated through the absorbent cotton gauze; the residue was repeatedly washed until the neutral pH, and then dried in an oven at 60 °C overnight. Finally, the dried SCB was ground to the size of less than 0.1 mm. NaOH pretreatment was performed in a 100 ml beaker, with a desired amount of SCB soaked in 1% (w/w) NaOH solution and incubated for 1 h in a water bath cauldron at 80 °C with a liquid to solid rate of 20:1 (w/w). After the pretreatment, the suspension was filtrated, the obtained residue was washed to the neutral pH, then dried and ground to the size of less than 0.1 mm.

2.3. Enzymatic hydrolysis

The experiment was done in a 10 ml penicillin bottle with a working volume of 5 ml. Briefly, 0.1 g of pretreated SCB was added to the bottle, which was supplemented with 5 ml of sodium citrate buffer (pH 4.8). The cellulase was added at a loading of 35 FPU/g of pretreated SCB. Finally, the mouth of the bottle was sealed by a rubber stopper with an aluminum lid crimped in case of leaking. The enzymatic mixtures were incubated at 55 °C and 150 rpm for 48 h in a shaking incubator. The hydrolysates were withdrawn periodically for sugar analysis. The reducing sugars were measured by the 3,5-dinitrosalicylic acid (DNS) assay. The enzymolysis efficiency was calculated using Eq. (1):

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\text{Enzymolysis efficiency} = \frac{\text{Reducing sugars concentration, g/L}}{\text{Solid loadings} \times \text{glucan}\% \times 1.11 + \text{Solid loadings} \times \text{xylan}\% \times 1.14} \times 100\% \tag{1}
\]

where 1.11 is the coefficient of glucose obtained from glucan, and 1.14 is the coefficient of xylose obtained from xylan.

2.4. Simultaneous saccharification fermentation (SSF)

2.4.1. Microorganism strains

SHY07-1 yeast, which was an intergeneric protoplast fusant between Saccharomyces cerevisiae (a gift from the Sanhe ethanol factory, Zhanjiang, China) and Pichia stipitis (Guangzhou Sugarcane Industry Research Institute, Guangzhou, China), could convert glucose and xylose into ethanol (Zhu et al., 2012).

2.4.2. Inoculum

A glycerol stock (1.5 ml) from −80 °C freezer was thawed quickly and inoculated to the seed medium. It was revitalized as a shake culture for 16 h at 30 °C and 150 rpm. After that, the culture was transferred to the fermentation bottle. The inoculation ratio was 10% (v/v).

2.4.3. Culture media

The seed medium was YPX medium consisting of the yeast extraction (10 g/L), peptone (20 g/L) and xylose (20 g/L). The growth medium was composed of (NH$_4$)$_2$SO$_4$ 2 g/L, and the yeast extraction 5 g/L, KH$_2$PO$_4$ 5 g/L, MgSO$_4$·7H$_2$O 0.5 g/L. The pH was of the natural pH (5.45).
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