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Effect of antioxidant extraction on the enzymatic hydrolysis and bioethanol production of the extracted steam-exploded sugarcane bagasse

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ABSTRACT

Ethyl acetate extraction (EAE) of the steam exploded sugarcane bagasse may be an effective and economic way to extract antioxidants as well as enhance the enzymatic hydrolysis and bioethanol yield from the extracted residue. Therefore, the effects of EAE on steam-exploded sugarcane bagasse (SESB) were studied. Under boiling solvent extraction (BSE), the efficiency of EAE for obtaining phenolics from SESB was approximately 20%. EA extracts obtained under BSE showed an H_2O_2 scavenging activity (210 μL) of 99%. The IC_{50} values for 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and reducing power of BSE40 were 50.89 and 256.38 μg/mL, respectively, while those of vitamin C were 24 and 112 μg/mL, respectively. EAE improved the glucose yield by 30% but had no significant effect on the xylose yield during the enzymatic hydrolysis obtained using Celluclast 1.5L and Novozym 188. EAE also increased the ethanol yield by 8.78% by employing simultaneous saccharification and fermentation. The present study may be of great importance in industrial bioethanol production from steam-exploded biomass environmentally friendly and economically.

1. Introduction

Steam explosion has been used as one of the methods to release antioxidative compounds effectively [1–3]. The released compounds from lignocellulose are mainly phenolics which are always extracted by ethyl acetate [4–8]. Phenolics from lignocellulose, such as hydroxytyrosol, 3,4-dihydroxybenzaldehyde, tyrosol, vanillin, and syringaldehyde, show good solubility in ethyl acetate [9]. Additionally, the resultant extracts show potent antioxidant activities [4,6,7,9–12].

Meanwhile, steam explosion is one of the most widely implemented pretreatment methods that can degrade hemicellulose and transform lignin to increase the potential digestibility of cellulose [13] prior to bioethanol production. Water soluble compounds, such as acetic acid, formic acid, sugar-derived by-products, and lignin degradation products, are formed during steam explosion [14]. Some of these by-products, such as phenols, formic acid, and levulinic acid, are strong inhibitors to cellulases [15,16]. Some degraded by-products, such as furfural, acetic acid, hydroxymethyl furfural (HMF), phenols, and inorganic salts, show inhibitory effects to fermentation [17], meaning that cellulases and microorganisms, the most important components in simultaneous saccharification and fermentation (SSF), are both inhibited by the degradation by-products during steam explosion pretreatment.

Based on the above, steam explosion is not only an effective way to release antioxidative compounds but also a good pretreatment method to improve the digestibility of the lignocellulosic biomass by cellulase. However, those compounds released during the steam explosion inhibit cellulase and yeast fermentation. Li et al. [20] reported that antioxidants extraction from steam-exploded sugarcane bagasse (SESB) by using anhydrous ethanol and deionized water could enhance the reducing sugar production from the solid fractions. However, the results also showed that more sugar was removed from SESB by using anhydrous ethanol and

Abbreviations: EA, ethyl acetate; EAE, ethyl acetate extraction; SESB, steam-exploded sugarcane bagasse; ESESB, extracted steam-exploded sugarcane bagasse; SSF, simultaneous saccharification and fermentation; RTE, room temperature extraction; BSE, boiling solvent extraction; RTE1, 24, room temperature extraction for 1 and 24 h, respectively; BSE20, 40, 60, boiling solvent extraction for 20, 40, and 60 min, respectively; LSR, liquid/solid ratio; DCW, dry cell weight; FPU, filter paper unit; IU, international unit; DMSO, dimethylsulfoxide; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; Sa, DPPH scavenging activity; IC_{50}, 50% inhibitory concentration for Sa; IC_{50rp}, the effective concentration at which the reducing power was 0.5; HPIA, hydrogen peroxide inhibition ability.

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deionized water extraction than that using ethyl acetate. Therefore, in this study, we selected ethyl acetate as the solvent to investigate the efficiency of ethyl acetate extraction (EAE) and evaluate the antioxidant activities of the extracts using various methods. The monosaccharide production by using cellulase and ethanol production by adopting SSF from SESB and extracted steam-exploded sugarcane bagasse (ESESB) were also studied.

2. Materials and methods

2.1. Preparation of SESB and ethyl acetate extracts

SCB was kindly provided by the Qianwu sugar refinery plant in Zhuhai, Guangdong, China. It was air-dried and maintained at room temperature in woven bags. Steam explosion was performed in equipment designed by the Gentle Science & Technology Co. Ltd. (Henan, China). The parameters of the steam explosion treatment were as follows: 220 °C, liquid/solid ratio (LSR) of 1:1 (v/w), and residence time of 5 min. SESB was stored in plastic bags at 4 °C until use. SESB was oven-dried at 85 °C, milled to pass through a 0.15 mm screen (Model F2102, Taisite Instrument Co. Ltd., Tianjin, China), and homogenized before extraction. The extractions of SESB using ethyl acetate (EA) were carried out under different conditions with a LSR of 20:1. The extraction was conducted at room temperature in a shaker at 150 rpm for 1 and 24 h for room temperature extractions (RTE). The extractions were carried out in a Soxhlet extractor at the boiling point of EA for 20, 40 and 60 min for boiling solvent extractions (BSE). The liquid and solid phases were separated by filtering through filter paper (Shuangquan 102, Xinhua Paper Industry Co., Ltd., Hangzhou, China). The liquid fractions were collected as EA extracts for determining the antioxidant activities. The solid fractions were washed with milliliters of deionized water and then oven dried at 80 °C overnight for enzymatic hydrolysis and fermentation experiments.

2.2. Microorganism strain, media, and inoculum preparation

The *Saccharomyces cerevisiae* W303-1A strain (*ade2-1°, his3-11, leu2-3, 112trp1-1a, ura3-1, can100°*), referred to as wild type (ATCC 200060), was used in the present study. This strain was provided as a gift by Dr. Liang Zhang of Jiangnan University. The strain was grown aerobically at 30 °C in a rotary shaker at 200 rpm in a yeast extract peptone dextrose medium (YPD) containing 20 g/L of glucose, 10 g/L of yeast extract, and 5 g/L of peptone. The medium was sterilized by steam autoclaving at 121 °C for 20 min. The seed culture for the fermentation inoculum was prepared from the culture of YPD medium in sterile test tubes and incubated at 30 °C at 200 rpm for 24 h in a shaker. Then, 20 mL of activated cells was aseptically transferred to 200 mL of sterile YPD medium in a 500-mL Erlenmeyer flask. The flask was incubated at 30 °C at 200 rpm for 48 h. The cells were harvested by centrifugation in 50-mL sterilized centrifuge tubes for 10 min at 5000 rpm at 4 °C using a centrifuge (Shanghai Anke, Shanghai, China). The cell pellets were washed twice with sterile deionized water. The cells were then resuspended by sterile deionized water and used to initiate fermentation. The entire process was completed within 2 h to ensure the activity of the cells.

2.3. Antioxidant and free radical scavenging activity

2.3.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH (Tokyo Kasei Kogyo Co., Ltd.) radical scavenging activity (Sa) was determined according to [18]. Ascorbic acid was used as a positive control. Samples with different volumes were tested after dilution to 2 mL.

2.3.2. Reducing power

The reducing power of the extracts was determined according to [19]. Ascorbic acid was used as a positive control. Samples with different volumes were tested after dilution to 1 mL. Then, 2.5 mL of 0.2 mol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide were added. The mixture was placed in a water bath at 50 °C for 20 min. After it, 2.5 mL of 10% (w/v) trichloroacetic acid was added and another 10 min was remained. Finally, a 2.5 mL aliquot of the mixture was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride. Absorbance was recorded at 700 nm using a UV/vis spectrophotometer (UNICO, Shanghai).

2.3.3. Hydrogen peroxide inhibition ability

The ability of different extracts to scavenge hydrogen peroxide was determined following the method of [20] with some modifications. Briefly, distilled water was replaced by ethanol to enhance the solubility of the EA extract.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis of ESESB and SESB was conducted with 2% (w/v) of substrate in 50 mL of 0.1 M citrate buffer (pH 4.8) supplemented with 80 μg/mL tetracycline and 60 μg/mL nystatin (dissolved in DMSO) to prevent microbial contamination. The mixture was incubated at 45 °C in a rotary shaker at 160 rpm. The enzyme activity of Celluclast 1.5 L and Novozym 188 (both kindly provided by Novozymes (China) Investment Co. Ltd.) was 83.43 FPU/mL and 748 IU/mL, respectively, as measured using the methods of [21,22]. A mixture of Celluclast 1.5 L and Novozym 188 with loadings of 10 FPU/g and 40 IU/g substrate, respectively, was added to initiate the enzymatic digestibility after acclimation overnight. Samples (0.8 mL) were collected from the reaction mixture at different time intervals. Each sample from the hydrolysate was heated in boiling water for 10 min to deactivate the enzymes and then centrifuged for 2 min at 12,000 rpm. The supernatant was used to determine the glucose and xylose by HPLC.

2.5. SSF

SSF was conducted in 100 mL serum bottles with rubber stoppers. The composition of the fermentation medium was 50, 0.5, 0.025, and 1.0 g/L of biomass, $(NH_4)_2HPO_4$, $MgSO_4 \cdot 7H_2O$, and yeast extracts dissolved in 0.1 M citrate buffer (pH 4.8), respectively [23]. Each serum bottle contained 50 mL of fermentation medium and was sterilized in an autoclave at 121 °C for 15 min. Celluclast 1.5 L and Novozym 188 were filtered through a 0.22 μm sterilized membrane (Millipore Corp. Carrigtwohill, Co. Cork, Ireland) before being added to the media. A mixture of Celluclast 1.5 L and Novozym 188 with loadings of 10 FPU/g and 40 IU/g substrate, respectively, was added to the cooled media. The yeast cell suspension was added at concentrations of 0.1 g dry yeast cells/L (DCW/L) to initiate fermentation. Subsequently, the bottles were sealed with rubber stoppers equipped with a water trap, which permitted CO_2 removal without air injection. The bottles were placed on a shaker at 200 rpm at 30 °C. Samples were periodically collected under aseptic conditions for determining the glucose, xylose, and ethanol concentrations.

2.6. Analytical methods

The total phenolic content was assayed as described previously [20].