

Enhancing fermentable sugar yield from cassava residue using a two-step dilute ultra-low acid pretreatment process

Zhuping Yu^{a,b}, Yangliu Du^{a,b}, Xiaona Shang^{a,b}, Ying Zheng^{a,b}, Jinghong Zhou^{a,b,c,*}

^a College of Light Industry and Food Engineering, Guangxi University, Nanning 530004, China

^b Guangxi Key Laboratory of Clean Pulp & Paper Making and Pollution Control, Nanning 530004, China

^c Collaborative Innovation Center for Sugar Industry, Guangxi, Nanning 530004, China

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ABSTRACT

Cassava residue (CR), a fibrous material, is a cassava processing by-product. The complex structure of the lignocellulosic material in CR limits effective saccharification; therefore, pretreatment is an essential step in biomass fuel production. This study investigated the hydrolysis of CR lignocellulosic components, using amylase to remove the starch from the CR to produce remaining starch-free CR (RSFCR). Ultra-low acid (ULA) CR pretreatments (a one-step ULA using 0.01% w/w H₂SO₄ or a two-step ULA using 0.05% H₂SO₄) were applied for durations of 0 to 50 min and at temperatures of 160 to 200 °C. The one-step ULA pretreatment achieved maximum glucose, xylose, and total sugar recoveries of 7.57%, 39.11%, and 15.14%, while the two-step ULA pretreatment attained 9.82%, 58.62%, and 22.08% recoveries, respectively. ULA RSFCR pretreatment degraded a small percentage of the hemicellulose sugars into the inhibitors and formed xylose-rich liquid prehydrolysate and a cellulose-rich solid. The hydrolytic yields of glucose, xylose, and total sugar from the two-step ULA pretreatment with enzymatic hydrolysis were 7.43, 1.41, and 3.32 times higher, respectively, than those from the two-step ULA pretreatment and 1.02, 1.13, and 1.04 times higher than those from the one-step ULA pretreatment with enzymatic hydrolysis. Fermentation inhibition was not detected in the pretreatment methods. These results indicate the feasibility of using two-step ULA combined with enzymatic hydrolysis for RSFCR pretreatment.

1. Introduction

Bioethanol is a sustainable alternative to gasoline and can alleviate global energy problems caused by the depletion of fossil fuels. In order to avoid competition with food production, large amounts of inedible plants and agricultural/industrial waste are used as feedstock for bioethanol production (Hu et al., 2008). However, the biological conversion of cellulosic biomass into fuels and chemicals is hindered by technological challenges, including, most importantly, the resistance of cellulose to hydrolysis, which necessitates an appropriate pretreatment (Himmel et al., 2007). Of the possible pretreatment procedures, acid pretreatment has always been favored for its hydrolysis efficiency, reaction speed (compared to enzymatic processes), and the low cost of the hydrolyzing agent. Dilute acids at concentrations between 0.2% and 2.5% (w/w) are usually used at temperatures between 130 °C and 210 °C (Balat, 2011; Ge et al., 2011; Sarkar et al., 2012). Acid pretreatment under extreme conditions (i.e., high acid concentrations and temperature), causes greater degradation of hemicellulosic sugars and results in the production of larger amounts of potential fermentation

inhibitors, such as furfural, hydroxymethylfurfural (HMF), and acetic acid. In order to maximize pretreatment efficiency, two-step dilute acid pretreatment procedures have been proposed and applied to numerous substrates (Nguyen et al., 2000; Soderstrom et al., 2005, 2003). The conditions in the first step are generally less severe and serve to hydrolyze the hemicellulose. After the first pretreatment step, the solid and liquid are separated to minimize the degradation of hemicellulosic sugars to furfural and HMF. The solid material recovered from the first step is treated again under more severe conditions that promote enzymatic digestion of cellulose fibers (Soderstrom et al., 2005).

Cassava is an important food source for large amounts of the population in tropical regions in Asia, Africa, and South America (Cereda, 1996; Zhang et al., 2016). Cassava is widely grown throughout southern China, chiefly for cassava starch and bioethanol production, with an annual yield of ca. 9,110,000 tons (Jansson et al., 2009; Leng et al., 2008; Okudoh et al., 2014). As a result, large amounts of cassava residue (CR) are produced by the cassava-based starch and ethanol industries. CR has several characteristics that make it a suitable potential feedstock for fuel ethanol production. In terms of chemical

* Corresponding author at: College of Light Industry and Food Engineering, Guangxi University, Nanning 530004, China.

E-mail address: jzhoudou@gxu.edu.cn (J. Zhou).

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composition, CR contains starch (40–64%), cellulose, and hemicellulose (which can be readily hydrolyzed into fermentable sugars) and lignin in fiber (15–50%) (Klinpratoom et al., 2015; Pooja and Padmaja, 2015; Souto et al., 2017).

In a previous study, our group investigated the composition of hemicellulose in CR and the formation of sugars and inhibitors during hot water pretreatment; the experimental results showed that high-temperature hot water pretreatment can effectively remove hemicellulose from CR (Jia et al., 2017; Lin et al., 2016). Previous studies have shown that CR is rich in starch and sugar, but low in lignin (Jia et al., 2017; Lin et al., 2016). Therefore, it may be feasible to use ultra-low acid (ULA) pretreatment for CR. The low acidity used in the ULA pretreatment process simplifies downstream processing, such as neutralization and waste treatment, and reduces equipment costs. In this study, we systematically investigated the production of fermentable sugars from CR. We compared a one-step ULA pretreatment (i.e., regular hydrolysis) with a two-step ULA pretreatment and investigated enzymatic hydrolysis in the slurry after dilute acid hydrolysis. The effects of temperature and time on the pentose and hexose yields, inhibitor content, and monomer and oligosaccharide ratios in CR were studied. The optimal pretreated solid was enzymatically hydrolyzed to assess enzymatic digestion in the ULA pretreatment. The changes in the total sugar recovery of prehydrolysate and enzymatic hydrolysate were discussed along with crystallinity. This research can be used to inform the use of CR as a substrate in bioenergy production and indicate the suitability of ULA processes for CR pretreatment.

2. Methods

The CR sugar recovery reaction pathways in various treatments are shown in Fig. 1. First, CR is treated with amylase to obtain the remaining starch-free CR (RSFCR). Then, the solid (step1-PR) and liquid (Pre-step1) fractions are separated. Step1-PR is used in enzymatic hydrolysis (step1-PR-ER + Enz-step1) and the second ULA hydrolysis (step2-PR + Pre-step2). Finally, step2-PR undergoes enzymatic hydrolysis and is separated into solid (step2-PR-ER) and liquid (Enz-step2) fractions.

All experiments were repeated three times, and the mean values \pm standard deviation data are reported herein. The RSFCR and hydrolyzed residues compositions are expressed on a 100% dry matter basis throughout this work.

2.1. Raw materials

CR was obtained from the Guangxi Mingyang Starch Factory (Nanning, China), dried under sunlight, filtered using an 18-mesh sieve, and then stored in a polyethylene container at room temperature. To study the degradation of cellulose and hemicellulose in CR, we first removed the starch from the CR using amylase. The major components of the CR, RSFCR, step1-PR, and step2-PR were analyzed using the standard laboratory procedures for biomass analysis provided by the National Renewable Energy Laboratory (Sluiter et al., 2010). The chemical composition of the CR was 1.45% alcohol-benzene (2:1v/v) extract, 1.30% arabinose, 1.13% galactose, 61.23% glucose, 1.13% mannose, 8.15% xylose, 7.33% acid-insoluble lignin, and 18.28% other.

2.2. ULA pretreatment

ULA pretreatment was performed in a 1.13 L reactor (TOP120, TOP INDUSTRIE) with an electric heater and agitation. The solid-liquid ratio during the pretreatment was 1:20 (w/v). For the step-1 acid pretreatment, RSFCR was mixed with 0.01% (w/w) H_2SO_4 (solid/liquid: 1:20 w/v) via stirring at 500 rpm. The pretreatment temperatures and reaction durations ranged from 160 to 200 °C and 0 to 50 min, respectively. First, the RSFCR was pretreated in the reactor. After the pretreatment was completed, the reactor was immediately cooled to below 70 °C using cooling water. Then, the solid and liquid components were separated by filtration. Sugar (arabinose, galactose, glucose, mannose, and xylose) and inhibitor (formic acid, acetic acid, furfural, and 5-HMF) contents in the prehydrolysate were determined by high-pressure liquid chromatography (HPLC) and ion chromatography (IC), respectively. The solid fraction was air-dried and stored before step-2 acid pretreatment, in which 0.05% (w/w) H_2SO_4 was used while the other conditions remained the same as those used in the step-1 treatment. The step-2 hydrolysate was collected and analyzed in the same manner as the step-1 hydrolysate. Then, the solid fraction was air-dried and stored for enzymatic hydrolysis and X-ray diffraction (XRD) analysis.

2.3. Enzymatic hydrolysis

Amylase hydrolysis: Enzymatic hydrolysis was performed in 1000 mL flasks using 5% dry matter (w/w) at 60 °C in a shaker at 150 rpm for 12 h using 0.5 mL of amylase. The amylase was purchased from Aladdin Reagent Inc. (Shanghai, China) had an enzymatic activity

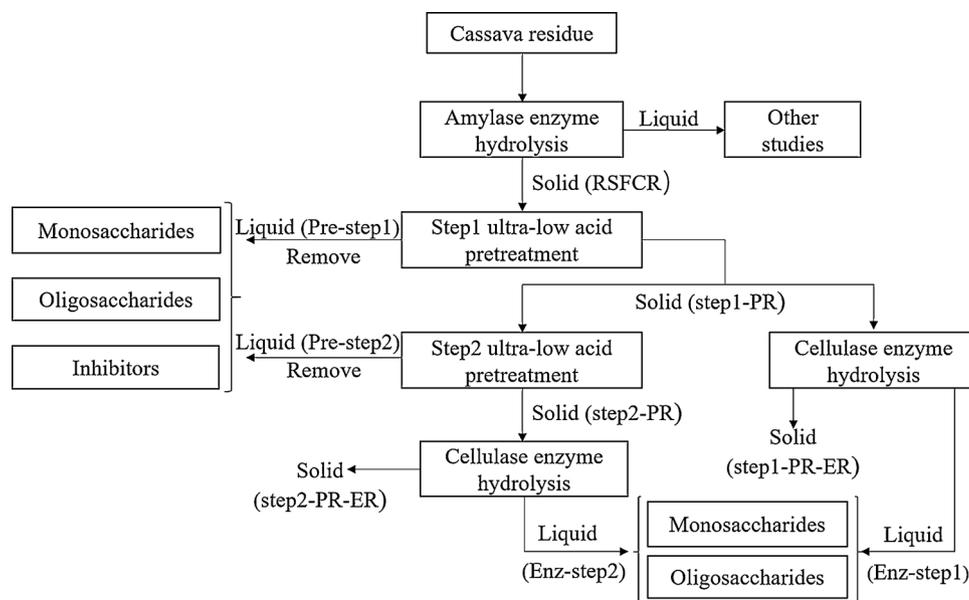


Fig. 1. Flow chart of fermentable sugar production from cassava residue.

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