



# Cost analysis of cassava cellulose utilization scenarios for ethanol production on flowsheet simulation platform



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## HIGHLIGHTS

- ▶ Three scenarios of cassava cellulose utilization were experimentally tested.
- ▶ Flowsheet simulation of cassava cellulose utilization was done on Aspen plus.
- ▶ Co-hydrolysis of cassava starch/cellulose for ethanol production is cost effective.

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## ABSTRACT

Cassava cellulose accounts for one quarter of cassava residues and its utilization is important for improving the efficiency and profit in commercial scale cassava ethanol industry. In this study, three scenarios of cassava cellulose utilization for ethanol production were experimentally tested under same conditions and equipment. Based on the experimental results, a rigorous flowsheet simulation model was established on Aspen plus platform and the cost of cellulase enzyme and steam energy in the three cases was calculated. The results show that the simultaneous co-saccharification of cassava starch/cellulose and ethanol fermentation process (Co-SSF) provided a cost effective option of cassava cellulose utilization for ethanol production, while the utilization of cassava cellulose from cassava ethanol fermentation residues was not economically sound. Comparing to the current fuel ethanol selling price, the Co-SSF process may provide an important choice for enhancing cassava ethanol production efficiency and profit in commercial scale.

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## 1. Introduction

Cassava tuber contains 70% starch of its dry weight and has been used as the promising feedstock for fuel ethanol industry (Zhang et al., 2003; Yu and Tao, 2009; Huang et al., 2010). As the outcome, massive amount of cassava residues were also produced as the by-products of ethanol production, nearly half ton of cassava residues for producing one ton of ethanol (Zhang et al., 2011a). Because of the high lignocellulose content in the cassava residues, its nutritional value was low to be used as the distillers dried grains with solubles (DDGS) as in the corn ethanol fermentation. Thus the cassava residues were usually discarded as a solid waste in the cassava ethanol industry. A reasonable utilization of the cassava residues for upgrading the cassava fuel ethanol value becomes an important topic.

Cassava cellulose accounts for nearly one quarter of the dry cassava residues weight, thus the conversion of cassava cellulose into

ethanol along with the cassava starch is the most direct way of its utilization considering the handy ethanol fermentation equipments. Three scenarios of cassava cellulose utilization for ethanol production had been investigated. The first one is the direct use of cassava residues for simultaneous saccharification and ethanol fermentation (SSF) (Direct SSF). However, the rigid cellulose in the cassava residues seems not yet to be degraded in the cassava ethanol fermentation process and the cellulose ethanol yield was low. The second method is to pretreat the cassava residues using various pretreatment methods, followed by the SSF processing (pretreated SSF). The ethanol yield was significantly increased after the energy-intensive pretreatment was processed on the cassava residues (Zhang et al., 2011a; Divya Nair et al., 2011; Akaracharany et al., 2011). The third method is to add glucoamylase and cellulase enzymes into the cassava saccharification step and make the hydrolysis of cassava starch and cassava cellulose occur simultaneously, accompanied by the ethanol fermentation of the sugars released from both starch and cellulose (Co-SSF). A trial by Rattanachomsri et al. (2009) showed that the SSF of cassava pulp, composed of cassava starch and cassava cellulose components,

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produced more ethanol when the cellulase, pectinase and hemicellulase enzymes were added into the cassava pulp slurry in addition to the glucoamylase.

All these three cassava cellulose bioprocessing scenarios worked for increasing the ethanol yield from the cassava residues. The question is to select the most proper method from the viewpoint of process economy and easy technical handling: could the cellulose ethanol produced be able to balance the increased cost of energy and enzyme usage? However, the previous studies were carried out in individual cases with different cassava residue feedstocks, different enzymes and fermentation strains used, and even in different fermentation and analysis methods. Therefore, these results could not be used for evaluation of cassava cellulose utilization performance and give a convincing conclusion for lack of same comparisons basis.

In this study, three scenarios of cassava cellulose utilization for ethanol production were experimentally tested under same conditions and equipments. Based on the experimental results, a rigorous flowsheet simulation model was established on Aspen plus platform and the cost of cellulase enzyme and steam energy in the three cases was calculated. The results show that the simultaneous co-saccharification of cassava starch/cellulose and ethanol fermentation process (Co-SSF) provided a cost effective option of cassava cellulose utilization for ethanol production, while the utilization of cassava cellulose from cassava ethanol fermentation residues was not economically sound. Comparing to the current fuel ethanol selling price, the Co-SSF process may provide an important choice for enhancing cassava ethanol production efficiency and profit in commercial scale.

## 2. Methods

### 2.1. Materials and strain

Cassava tubers and cassava residues were obtained from Guangxi Cofco Bio-Energy Co. (Beihai, China). The tubers were milled and screened through a 5 mm diameter mesh, while the cassava residues were used at its original size. The materials were dried at 105 °C to a constant weight and then sealed in plastic bags until use.

The  $\alpha$ -amylase HTAA, the glucoamylase GA-L NEW, and the cellulase Accellerase 1000 were purchased from Genencor International (Rochester, NY, USA). The cellulase Youtell #6 was kindly provided by Hunan Youtell Biochemical Co. (Yueyang, China). The activity of  $\alpha$ -amylase HTAA was 22,000 U/ml; the activity of glucoamylase GA-L NEW was 100,000 U/ml. The activity of Accellerase 1000 was 55.0 FPU/ml in the filter paper unit (FPU) and 152.0 IU/ml in the cellobiase unit (IU). The activity of Youtell #6 was 145.0 FPU/g in the filter paper unit (FPU) and 344.0 IU/g in the cellobiase unit (IU).

The ethanol fermentation strain *Saccharomyces cerevisiae* DQ1 was obtained in our laboratory and stored in China General Microbial Collection Center (CGMCC) with the register number of 2528. The detailed procedure for adaptation of *S. cerevisiae* DQ1 in the hydrolysate of cassava residues was discussed in Zhang et al. (2010).

### 2.2. Dry dilute sulfuric acid pretreatment of cassava residues

Cassava residues were pretreated using the dry dilute sulfuric acid pretreatment proposed by Zhang et al. (2011b). Briefly, the dried cassava residues were presoaked with dilute sulfuric acid (0.5–2.5% sulfuric acid concentration) at a solid/liquid ratio of 2:1 for 12 h. The materials were put into the pretreatment reactor and the hot steam was jetted into the reactor to heat the materials

to 190 °C for about 3 min. After the pretreatment, the pressure was released and the pretreated cassava residues were removed from the reactor.

The enzymatic hydrolysis assay of the cassava residues were conducted at 5.0% (w/w) solids content with 10.0 FPU/g DM (dry solid matter) cellulase dosage in 0.05 M citric acid buffer (pH 4.8), 50 °C and 150 rpm in a water-bath shaker for 24 h (Brown and Torget, 1996). Samples were periodically taken and centrifuged at 13,000 rpm for 5 min, then the supernatant were analyzed on HPLC. All the enzymatic hydrolysis experiments were performed twice and the average data were used.

### 2.3. Simultaneous saccharification and ethanol fermentation (SSF) of cassava residues

The SSF operations of cassava residues (either the original or the pretreated) were conducted in a helical stirring bioreactor at the 30% (w/w) cassava residues solids loading. The details of the operation were in described in Zhang et al. (2010). The first 8-h pre-hydrolysis stage at 50 °C was to partially convert the cassava cellulose into glucose by fed-batch addition of the cassava residues until reached 30% solids loading. The yeast seeds were inoculated at 10% (v/v) ratio when the temperature was switched to 37 °C and the SSF stage started. The nutrition in the SSF slurry included 2.0 g/L of  $\text{KH}_2\text{PO}_4$ , 1.0 g/L of  $\text{MgSO}_4$ , 1.0 g/L of  $(\text{NH}_4)_2\text{SO}_4$ , and 1.0 g/L of yeast extract. The pH in the pre-hydrolysis stage was maintained at 5.0 and the SSF stage was maintained at 4.5, 5.0 or 5.5, respectively with 5 M NaOH. Samples were periodically taken and centrifuged at 13,000 rpm for 5 min. The samples were stored at –20 °C before analysis on HPLC. All experiments were repeated for three times and the error ranges were given in the tables and figures.

### 2.4. SSF of cassava starch and cellulose concurrently (Co-SSF)

SSF of cassava starch and cellulose in cassava powder concurrently could be divided into three stages according to the different temperature used. First, dry cassava powder was adjusted to 26.7% (w/w) solids concentration with deionized water and the starch was liquefied for 3 h at 90 °C using  $\alpha$ -amylase at 22 U/g DM. Then the pre-hydrolysis was processed for 30 min at the decreased temperature of 55 °C using the glucoamylase at 100 U/g DM and the cellulase at the cellulase dosage of 15.0 FPU/g dry cellulose. Finally, the SSF stage started with the yeast seeds inoculation at 37 °C and the pH of 5.0. All experiments were repeated for three times and the error ranges were given in the tables and figures.

### 2.5. Analysis of sugars, ethanol and inhibitors on HPLC

Glucose, xylose, ethanol, and inhibitory compounds, such as furfural and HMF were determined using high-performance liquid chromatography (LC-20AD, refractive index detector RID-10A, Shimadzu, Japan) with a Bio-rad Aminex HPX-87H column at the column temperature of 65 °C. The mobile phase was 0.005 M  $\text{H}_2\text{SO}_4$  at the rate of 0.6 ml/min. All samples were diluted properly and filtered through a 0.22  $\mu\text{m}$  filter before analysis.

### 2.6. Analysis of the compositions of cassava and cassava residues

The compositions of cassava powder and cassava residues were analyzed using ANKOM 220 Cellulose Analyzer (ANKOM Technology, Macedon, NY, USA) (Zhao et al., 2012). The cassava powder contained 75.44% starch, 4.67% cellulose, 4.09% hemicellulose, 3.07% lignin and ash. The cassava residues contained 22.34% cellu-

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