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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.

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 cassave 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; Akaracharanya 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 convincible 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 α-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 α-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 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 prehydrolysis 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 KH_2PO_4, 1.0 g/L of $MgSO_4$, 1.0 g/L of $(NH_4)_2SO_4$, 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 power was adjusted to 26.7% (w/w) solids concentration with deionized water and the starch was liquefied for 3 h at 90 °C using α-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 H_2SO_4 at the rate of 0.6 ml/min. All samples were diluted properly and filtered through a 0.22 μm 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% cellulose, 16.73% hemicellulose, 24.07% lignin and ash, and 1.89% starch. All the above data were calculated based on the dry solid matter.

2.7. Calculations

The glucose yield, xylose yield and ethanol yield were calculated using the following equations (Brown and Torget, 1996; Zhang and Bao, 2012):

$$\text{Glucose yield}(\%) = \frac{[\text{Glu}] \times V}{f \times [\text{Biomass}] \times m \times 1.111} \times 100\%$$

$$\text{Xylose yield}(\%) = \frac{[\text{Xyl}] \times V}{f_h \times [\text{Biomass}] \times m \times 1.136} \times 100\%$$

$$\text{Ethanol yield}(\%) = \frac{[\text{Eth}] \times W}{976.9 - 0.804 \times [\text{Eth}]} \cdot \frac{1}{0.511 \times f \times [\text{Biomass}] \times m \times 1.111} \times 100\%$$

where [Glu] and [Xyl] were the glucose and xylose concentration at the end of the hydrolysis (g/L), respectively; [Eth] was the ethanol concentration in the fermentation broth (g/L); V was the final liquid volume of the hydrolysis system or SSF system (L); W was the total water usage during SSF process (g); f was the cellulose content in the cassava residues (g/g); f_h was the hemicellulose content in the cassava residues (g/g); [Biomass] was the solids concentration of cassava residues in the enzymatic hydrolysis or SSF system (%, w/w); m was the total weight of the hydrolysis or SSF systems (g).

2.8. Software

The flowsheet simulation software used was Aspen plus version 11.0 (AspenTech Co., Cambridge, MA, USA). The components used included ethanol and water, and the physical property data used were from the build-in database in the Aspen plus software. The overall thermodynamic method used was NRTL equation.

3. Results and discussion

3.1. Utilization scenarios of cassava cellulose for ethanol production

Cellulose structure in cassava residues had been partially disrupted in the cassava starch fermentation for ethanol production, including short-time liquefication by α-amylase at 95 °C, saccharification by glucoamylase at 55 °C, long time fermentation at 30–37 °C (36–60 h), and distillation at 95–110 °C (Zhang et al., 2003; Yu and Tao, 2009). Therefore, the cellulose in the cassava residues was able to be hydrolyzed easily after these processing similar to the pretreatment of lignocellulose. There are two ways of utilizing the cassava cellulose: (1) direct simultaneous saccharification and ethanol fermentation (SSF) of cassava residues without any further treatment (the direct SSF process); (2) first the pretreatment of cassava residue to further disrupt the cellulose structure in cassava residues, and then the SSF of the pretreated cassava residues (the pretreated SSF process).

Table 1 shows the direct SSF performance of cassava residues under different temperature and pH values at the high cellulose dosage of 45 FPU/ g cellulose. The change of operation parameters did not make a significant difference on the ethanol titer and yield. The ethanol titer and yield increased from 22.7 to 24.1 g/L, and 43.8 % to 46.6%, respectively, when the temperature increased from 30 to 37 °C. When pH changed from 4.5 to 5.0, the ethanol titer and yield were almost the same but decreased from 24.1 to 22.9 g/L and from 46.6% to 44.2%, respectively when pH further increased to 5.5. The result shows that less than half of the cassava cellulose (46.6%) was converted into ethanol in the direct SSF process even at the high cellulase enzyme dosage, indicating the cellulose structure in the original cassava residues was still rigid for cellulase to hydrolysis.

Table 1
Direct SSF processing of cassava residues for ethanol production.

pH (−)	Temperature (°C)	Final ethanol titer (g/L)	Ethanol yield (%, of theoretical)
5.0	30	22.7 ± 1.11	43.8 ± 2.10
5.0	37	24.1 ± 0.89	46.6 ± 1.69
4.5	37	24.1 ± 1.34	46.6 ± 2.54
5.0	37	24.1 ± 0.89	46.6 ± 1.69
5.5	37	22.9 ± 1.10	44.2 ± 2.08

SSF conditions: the pre-hydrolysis stage at 50 °C, 150 rpm lasted for 8 h, and the SSF stage lasted for 72 h at 30 °C, pH 5.0, 37 °C, pH 4.5, 37 °C, pH 5.0, and 37 °C, pH 5.5, respectively. All the experiments were performed twice at 30% (w/w) solids loading in a 5 L helical stirring bioreactor.

Table 2
Dilute acid pretreatment of cassava residues and the enzymatic hydrolysis assay.

Pretreatment conditions		Recovery yield (% of theoretical)		Inhibitor
Sulfuric acid (%, g/g DM)	Temperature (°C)	Glucose	Xylose	Furfural (mg/g DM)
/	/	42.9 ± 1.4	5.6 ± 0.4	Not detected
0.5	140	59.7 ± 0.5	10.7 ± 0.2	0.18 ± 0.06
0.5	160	61.3 ± 1.5	13.8 ± 0.7	0.38 ± 0.20
0.5	190	61.9 ± 1.7	19.3 ± 0.7	0.81 ± 0.00
1.5	140	57.5 ± 3.7	9.6 ± 0.1	0.16 ± 0.02
1.5	160	58.4 ± 0.1	14.0 ± 1.0	0.46 ± 0.08
1.5	190	63.5 ± 1.2	23.5 ± 0.9	1.66 ± 0.38
2.5	140	56.2 ± 1.2	12.9 ± 0.2	0.23 ± 0.10
2.5	160	58.2 ± 0.4	17.7 ± 0.1	0.64 ± 0.05
2.5	190	63.2 ± 2.4	26.9 ± 2.0	2.08 ± 0.17

Conditions were: cassava residues were presoaked with 0.5–2.5% (w/w) sulfuric acid at solid/liquid ratio of 2 for 12 h and then pretreated at 140–190 °C for 3 min; the enzymatic hydrolysis were performed at 5% solids loading with 10 FPU/g DM in citric acid buffer (0.05 M, pH 4.8) at 50 °C, 150 rpm for 24 h in a water-bath shaker. All the enzymatic hydrolysis experiments were replicated.

Because of the unsatisfactory ethanol production performance of the direct SSF, cassava residues were pretreated for further disruption of cellulose structure before SSF operation (the pretreated SSF process). Table 2 shows the effect of the dry dilute sulfuric acid pretreatment on the hydrolysis of cassava cellulose to sugars (Zhang et al., 2011b). Two important pretreatment parameters, sulfuric acid concentration and pretreatment temperature, were adjusted to obtain an optimal sugar yield and the least inhibitor formation. Table 2 suggests that the glucose yield of cassava cellulose was significantly improved after the pretreatment in all the tests done. The maximum glucose yield was obtained after the pretreatment at 160 °C for 3 min after presoaking with 1.5% (w/w) sulfuric acid. 58.4% of glucose recovery and 14.0% of xylose recovery were obtained while the furfural formed (0.46 g/L) was low enough to be used for direct SSF processing without detoxification step.

Table 3 summarized the ethanol production performance of the direct SSF of cassava residues (Direct SSF) and the SSF of pretreated cassava residues (Pretreated SSF). Different from the virgin lignocellulose (Zhang et al., 2011b), the increase of pretreatment intensity did not result in an improved glucose yield but the inhibitor concentration was increased consistently. The final ethanol titer, yield and productivity using the untreated cassava residues or the pretreated cassava residues at the same cellulase enzyme dosage showed a limited increase: 24.1 g/L, 46.6%, 0.318 g/L/h for the directed SSF and 26.8 g/L, 52.1%, 0.280 g/L/h for the pretreated SSF, respectively. The limited increase of ethanol after the pretreatment

Table 3
Simultaneous saccharification and ethanol fermentation of cassava residues at 30% (w/w) solids loading.

Cassava residues	Ethanol titer (g/L)	Ethanol yield (%)	Ethanol productivity (g/L/h)
Direct SSF process	24.1 ± 0.89	46.6 ± 1.69	0.318
Pretreated SSF process	26.8 ± 0.63	52.1 ± 1.19	0.280

The pretreatment conditions: cassava residues were presoaked with 0.5% sulfuric acid at a solid/liquid ratio of 2 for 12 h, and then pretreated at 160 °C for 3 min; the SSF conditions: prehydrolysis at 30% solids loading with 10 FPU/g DM at 50 °C for 8 h, and then SSF at 37 °C, pH 5.0, 150 rpm for 72 h with non-pretreated cassava residues and 96 h with pretreated cassava residues.

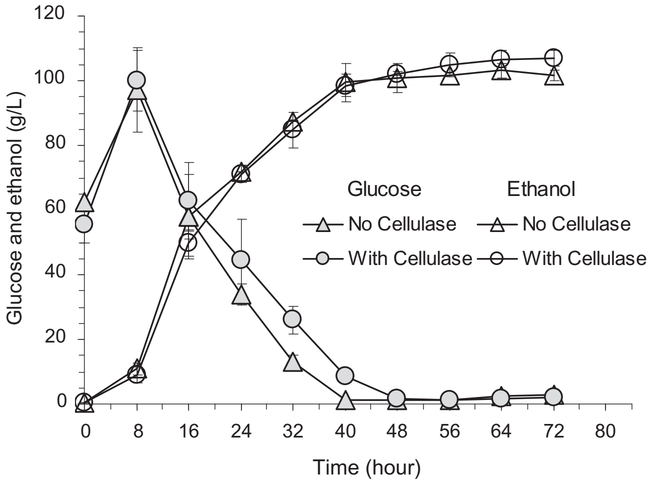

Fig. 1. Simultaneous saccharification and ethanol fermentation of cassava powder with and without cellulase addition. Conditions: 30% (w/w) cassava powder solids loading; cassava powder was liquefied with heat-resistant α-amylase at the dosage of 22 U/g DM at 90 °C for 3 h, then the liquefied mixtures were pre-hydrolyzed at 55 °C for half an hour with glucoamylase at the dosage of 100 U/g DM (or supplement cellulase at the dosage of 15 FPU/g cellulose at the same time), finally, the SSF started with 10% yeast seeds inoculation and lasted for 72 h at 37 °C. All the above processes were maintained at pH 5.0 and 150 rpm in a 5 L bioreactor with a helical stirring impeller.

on cassava residues may not worth the cost of intensive energy input and decreased ethanol productivity.

Another alternative for cassava cellulose utilization is to hydrolyze cellulose into glucose concomitantly during the period of cassava starch ethanol fermentation, instead of cassava residues processing after cassava starch fermentation. Cellulase is added into the cassava fermentation slurry together with glucoamylase after the cassava starch was liquefied by α-amylase. Fig. 1 shows the time course of the simultaneous saccharification of cassava starch and cellulose, and ethanol fermentation (Co-SSF) with and without cellulase addition after the starch portion of cassava tubers were liquefied by α-amylase at the dosage of 22 U/g dry cassava mass (DM). The cellulase addition was 15 FPU/g cellulose, about one third of the enzyme dosage used in the cassava residue SSF process. The results indicate that when cellulase was added, the ethanol formation was 107.1 g/L after 72 h' SSF, almost 5.5 g/L higher than that of no cellulase addition. At the same time, the glucose consumption rate decreased slightly with cellulase addition, because of glucose release from cellulose degradation. The water insoluble solids content in the final fermentation broth were 7.35% and 8.04%, with and without cellulase supplementation, respectively, confirmed that the insoluble cellulose degradation in the cassava fermentation was enhanced by cellulase addition.

The ethanol increase with cellulase addition in the Co-SSF process may be generated either from cassava cellulose hydrolysis, or from the hydrolysis of residual cassava starch. A simple calculation may clarify where the increased ethanol came from. The starch content in cassava residues after starch-ethanol fermentation

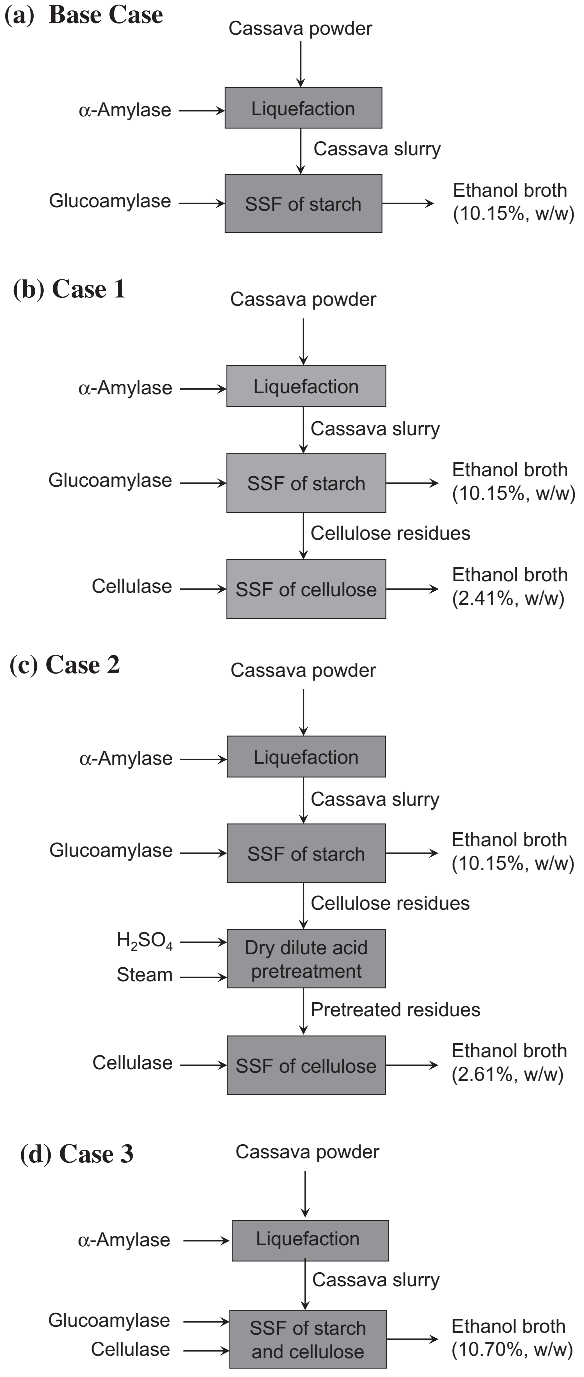

Fig. 2. Cassava ethanol production process: Schematic flowsheet. (a) Base case: cassava starch fermentation only without cellulose utilization; (b) Case 1: direct SSF of cassava cellulose residues; (c) Case 2: SSF of the pretreated cassava cellulose residues; (d) Case 3: Co-SSF of cassava starch and cassava cellulose.

Table 4
List of components in flowsheet simulation model on Aspen plus platform.

Number	ID	Type	Formula	Report name
1	Ethanol	C	$C_2H_6O_2^-$	Ethanol
2	Water	C	H_2O	Water
3	Glucose	C	$C_6H_{12}O_6$	Glucose
5	Cellulose	C	$C_6H_{10}O_5$	Cellulos
6	Xylan	C	$C_5H_8O_4$	Xylan
7	Lignin	C	$C_XH_XO_X$	Lignin
8	Extractive	C	$C_{18}H_{36}O_2$	Extracti
9	CO_2	C	CO_2	CO_2
10	O_2	C	O_2	O_2

was only 1.89% (w/w) of the dry cassava residues weight as shown in the Materials and Methods section, and the insoluble solids content was 8.04% (w/w) in the slurry, then the total residual starch was equivalent to 1.52 g/L (starch content in cassava residues 1.89% multiplied the insoluble solids content in the slurry 8.04%). Given the total starch in the cassava residues was converted into ethanol according to the theoretical yield (0.511 g ethanol/g starch) during the Co-SSF process, the theoretical ethanol increase was only 0.77 g/L, far less than the total ethanol increase 5.5 g/L with cellulase addition. Therefore, it is reasonable to conclude that most of the ethanol increased after cellulase addition was from the glucose released from cellulose. The ethanol increased was attributed to the cassava cellulose conversion was reasonable and viable.

3.2. Flowsheet simulation of ethanol production process from cassava cellulose on Aspen plus platform

The cellulase cost accounts for nearly 25–50% of the total lignocellulose bioprocessing cost (Wingren et al., 2003; Zhuang et al., 2004). Therefore, the cost of these three cassava cellulose utilization scenarios, including the SSF of the untreated cassava residue (Case 1, the Direct SSF) and the pretreated cassava residue (Case 2, the Pretreated SSF), as well as the simultaneous co-saccharification of cassava starch/cellulose and ethanol fermentation (Case 3, the Co-SSF) was analyzed in terms of cellulase enzyme usage and steam energy cost per unit ethanol produced. Fig. 2 shows the schematic flowsheet of the three scenarios: Base case is the ethanol fermentation from cassava starch only (data cited from Fig. 1), Case 1 is the directed SSF, Case 2 is the pretreated SSF, and Case 3 is the co-SSF. The data cited here were from Tables 1 and 3, and Fig. 1.

To analyze the cost of cellulase enzyme usage and steam energy in commercial scale of cassava ethanol production for the three scenarios of cassava cellulose utilization, a simplified flowsheet simulation model was established on Aspen plus platform. The production capacity of fuel ethanol (99.5%, w/w) was 200,000 t/a using cassava as feedstock. Ten components were included in the model including ethanol, water, glucose, cellulose, xylan, lignin, extractive, CO_2, and O_2 as shown in Table 4. The physical property data were either from built-in database of Aspen plus system, or from the published NREL data (Wooley and Putsche, 1996). The NRTL equation was selected as the base thermodynamic method.

The major assumptions of the model include:

(1) Process operated at continuous mode without consideration of plant start-up and shut-down;
(2) Starch, cellulose and xylan were hydrolyzed into glucose and xylose, but only glucose participated ethanol fermentation;
(3) Lignin and extractives components in the cassava residues did not involve the hydrolysis and metabolism of ethanol fermentation;
(4) Mass balance were established on the major components as listed in Table 4 and the minor components such as enzymes, yeast cells, inorganic salts, and nutrition substances were neglected form the mass balance;
(5) Heat dissipation of the equipment was neglected;

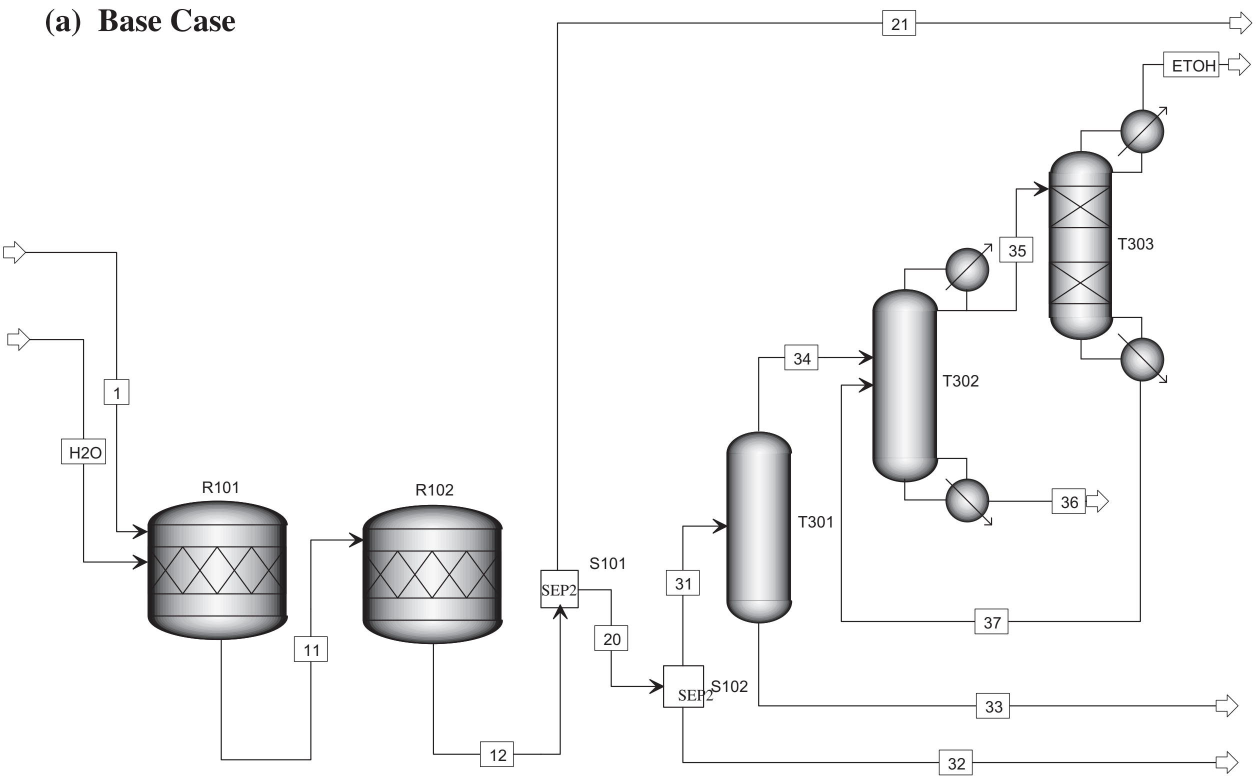
(a) Base Case

Fig. 3. Cassava ethanol production process: Simulation flowsheet on Aspen plus platform. (a) Base case: cassava starch fermentation only without cellulose utilization; (b) Case 1: direct SSF of cassava cellulose residues; (c) Case 2: SSF of the pretreated cassava cellulose residues; (d) Case 3: Co-SSF of cassava starch and cassava cellulose.

(b) Case 1

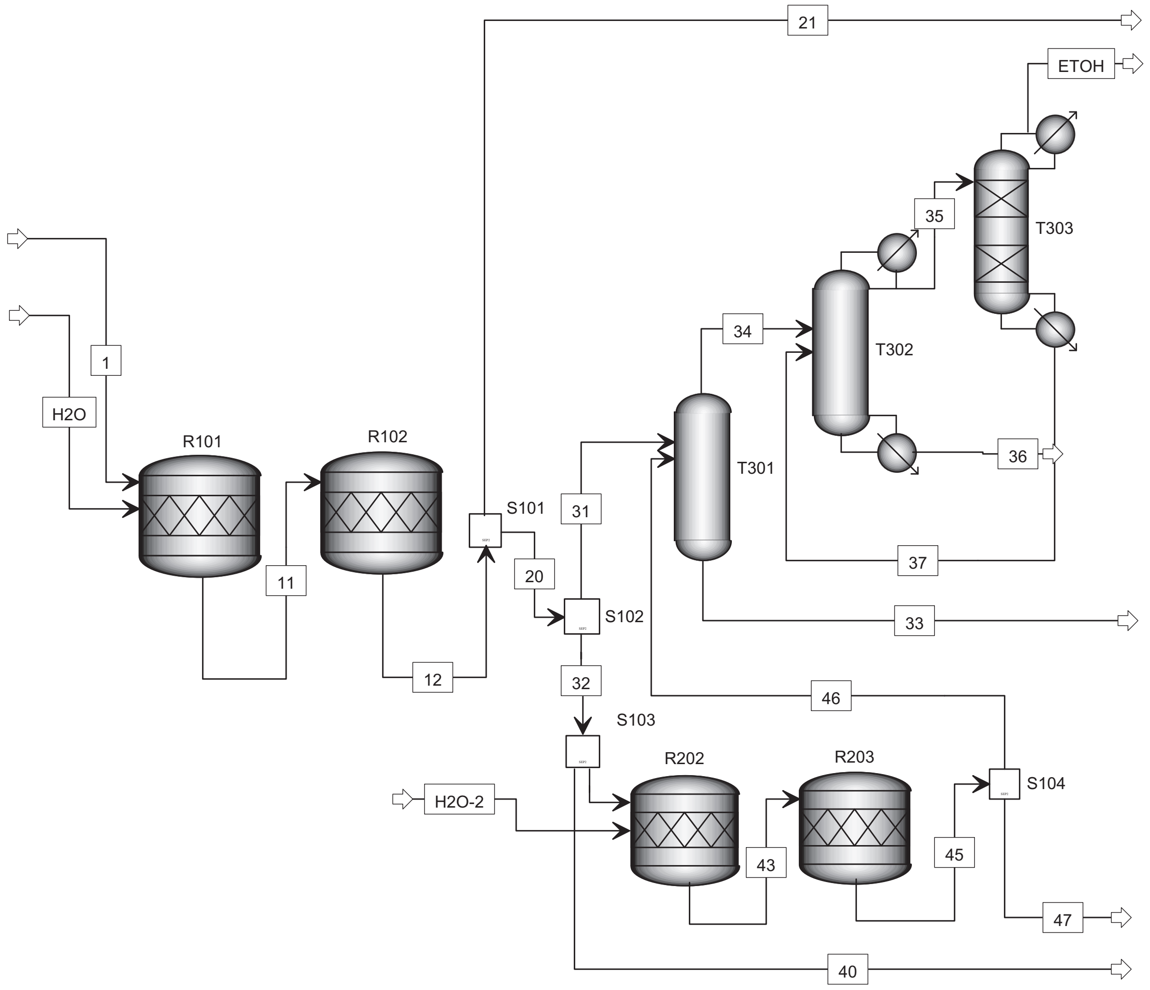

Fig. 3. (*continued*)

The simulation flowsheet chart and the operation parameters were shown in Fig. 3 and Table 5. The operational conditions and results were assumed to follow the results of this work (Tables 1–3, and Fig.1). Fig. 3(a) indicates the process flowsheet of Base case. The process started from liquefaction of cassava feedstock stream 1 (80 ton/h, including 55.831 ton/h of starch and 3.456 ton/h of cellulose) in the reactor R101 converting starch into liquid sugar oligomers by α-amylase; then the cassava slurry was fed into the SSF reactor R102 converting sugar oligomers into glucose by glucoamylase, then the glucose was fermented into ethanol. The CO_2 stream 21 and the cassava residues stream 32 were separated from the ethanol fermentation broth stream 12 to give a ethanol broth stream 31 (245.117 ton/h, 10.15% of ethanol in w/w), and it was fed into the stripping column T301 to elevate the ethanol from 10.15% (w/w) to 30% (%) in the distillate 34; then stream 34 was fed into the rectification column T302 to distillate the stream to close its azeotropic composition of 92% (w/w). The final step was the dehydration of ethanol stream 35 to the fuel ethanol concentration of 99.5% (w/w) in the molecular sieve column T303. The fuel ethanol product stream ETOH was 25 ton/h, equivalent to 200,000 ton/a (8,000 h operation time annually).

Fig 3(b) indicates the process flowsheet of Case 1 for simulation of the direct SSF process. The cassava residues stream 32 was fed into the saccharification reactor R202 converting cellulose into glucose by cellulase, then fed into the fermentation reactor R203 converting glucose into ethanol. Then the ethanol broth 46 (28.140 ton/h, 2.39% of ethanol in w/w) was fed into the stripping column T301 with the starch ethanol stream 31. The fuel ethanol product stream ETOH was 25.676 ton/h, equivalent to 205,408 ton/a.

Fig 3(c) indicates the process flowsheet of Case 2 for simulation of the pretreated SSF process. The cassava residues stream 32 was pretreated in the pretreatment reactor R201, then fed into the saccharification reactor R202 and the fermentation reactor R203 to yield the ethanol broth stream 46 (28.226 ton/h, 2.69% of ethanol in w/w) was fed into the stripping column T301 with the starch ethanol stream 31. The fuel ethanol product stream ETOH was 25.765 ton/h, equivalent to 206,120 ton/a.

Fig 3(d) indicates the process flowsheet of Case 3 for simulation of the co-SSF process, and flowsheet was basically the same with the Base case in Fig. 1(a). The only difference in the SSF reactor R102 with Fig. 3(a) was that both starch and cellulose in cassava feedstock were saccharified into glucose by glucoamylase and cellulase, respectively. The liquefied cassava slurry stream 11 was fed into the SSF reactor R102, then the glucose from the hydrolysis of starch and cellulose was fermented into ethanol to give the ethanol broth stream 32 (246.560 ton/h, 10.70% of ethanol in w/w). The fuel ethanol product stream ETOH was 26.510 ton/h, equivalent to 212.080 ton/a (8000 h operation period).

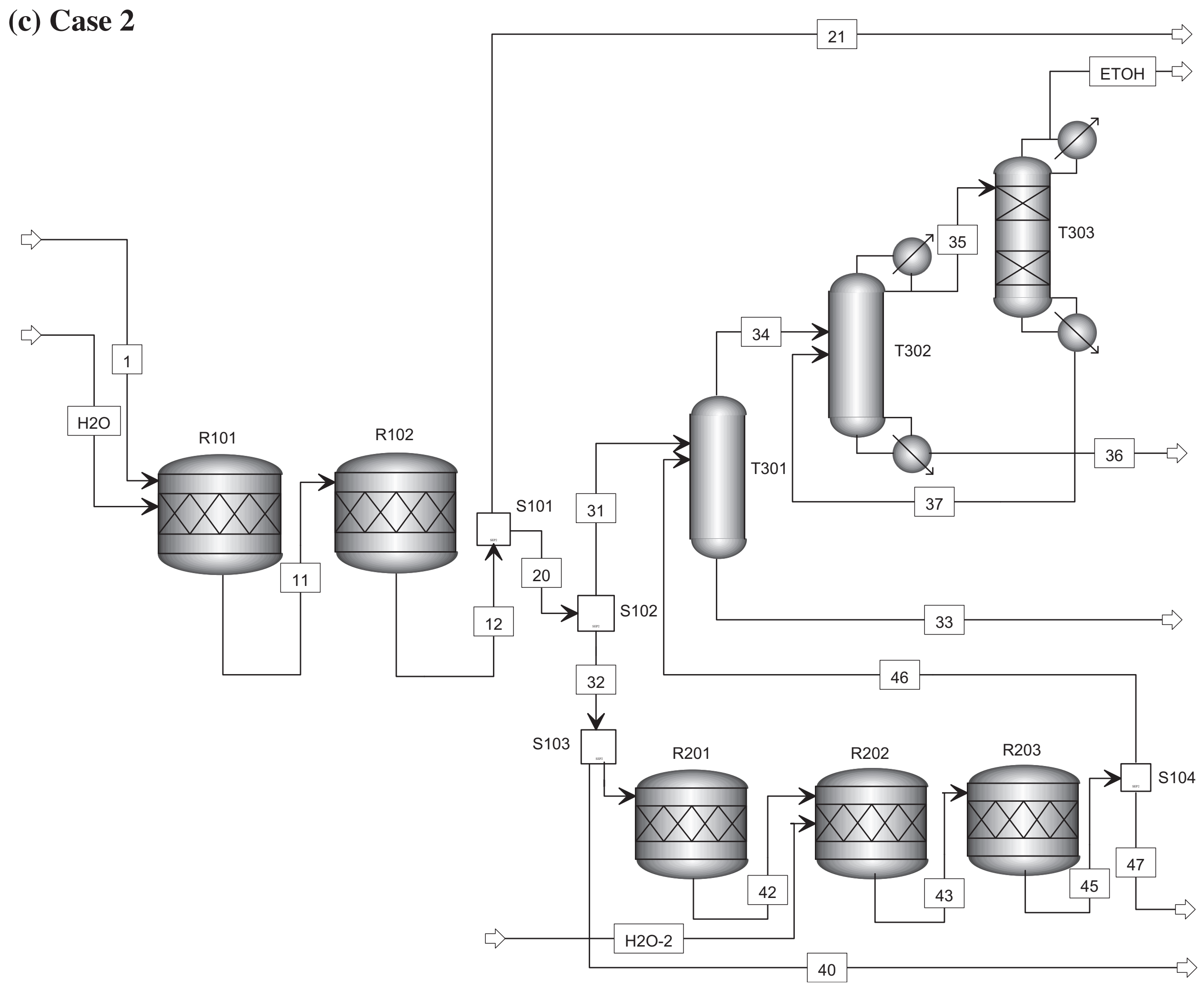
(c) Case 2

Fig. 3. (*continued*)

3.3. Cost calculation of enzyme and steam used in the cassava cellulose utilization scenarios

The Aspen plus model simulation results in Table 6 shows that, starting from the 80 ton/h of cassava feedstock, the increment of fuel ethanol product in the stream ETOH were 0.676, 0.765, and 1.510 ton/h for Case 1, Case 2, and Case 3, respectively. To give a preliminary analysis on the cost of cassava cellulose utilization, the two aspects of the cellulose utilization were calculated based on the Aspen plus flowsheet simulation model: (1) the cellulase enzyme cost used for hydrolyzing cassava cellulose; and (2) the steam energy cost used for distillating the increased ethanol.

The cellulase cost for the increased ethanol was calculated based on the dosage of 45, 45, and 15 FPU/g cellulose for Case 1, Case 2, and Case 3, respectively. The cellulase dosage for one ton of the increased ethanol was 2.30×10^8, 2.03×10^8, and 3.43×10^7 FPU for Case 1, Case 2, and Case 3, respectively. According to the cellulase cost estimations by Dr. Blanch's group of Berkeley, the baseline production cost was $10.14 per kg of cellulase protein (Klein-Marcuschamer et al., 2012). The typical commercial cellulase enzyme solution contained approximately 15% protein (Roche et al., 2009; Gusakov, 2011). Therefore, the estimated baseline production cost was $1.52 per kg of commercial cellulase solution. This estimation was in agreement with the minimum selling price of Youtell #6 provided Shanghai Youtell Biotech Co. (www.youtellbio.com). The minimum cost for one kg of Youtell #6 cellulase used in this study was estimated to be approximately 10 Yuan of Chinese RMB, approximately $1.60 per kg of cellulase, although this price was not tagged to cellulase product and all cellulase products were sold under much higher prices. Based on this estimation, the cellulase cost was calculated to be $2517.79, $2222.22, and $375.48 per ton of increased ethanol product. Clearly, the Co-SSF process in Case 3, that is the simultaneous co-saccharification of cassava starch/cellulose and ethanol fermentation process, demonstrated great advantage in cutting the cellulase cost. On the other hand, the cellulase costs in Case 1 and Case 2 were much more expensive than the fuel ethanol selling price (approximately $1,000 per ton of fuel ethanol) thus the profit from the two cases were negative.

The steam energy cost for the increased ethanol was calculated based on the heat duty of the first two columns, the stripping column T301 and rectification column T302, because the steam was mainly consumed on these two distillation columns (Galbe et al., 2007). The increments of the heat duty in T301 and T302 in the cassava utilization cases were obtained by subtracting the heat duty in the same columns in Base Case. The heat duty increments of T301 and T302 were 16,703, 16,701, and 16,726 MJ per ton of fuel ethanol. According to Chinese National Standard GB/T 2589-2008 ("Transformation coefficients from different energy forms to standard coal"), the LHV (low heat value) value for the low pressure steam used for ethanol distillation is 3763 MJ/ton of steam. The steam usage was 4.438, 4.438, and 4.445 ton for producing

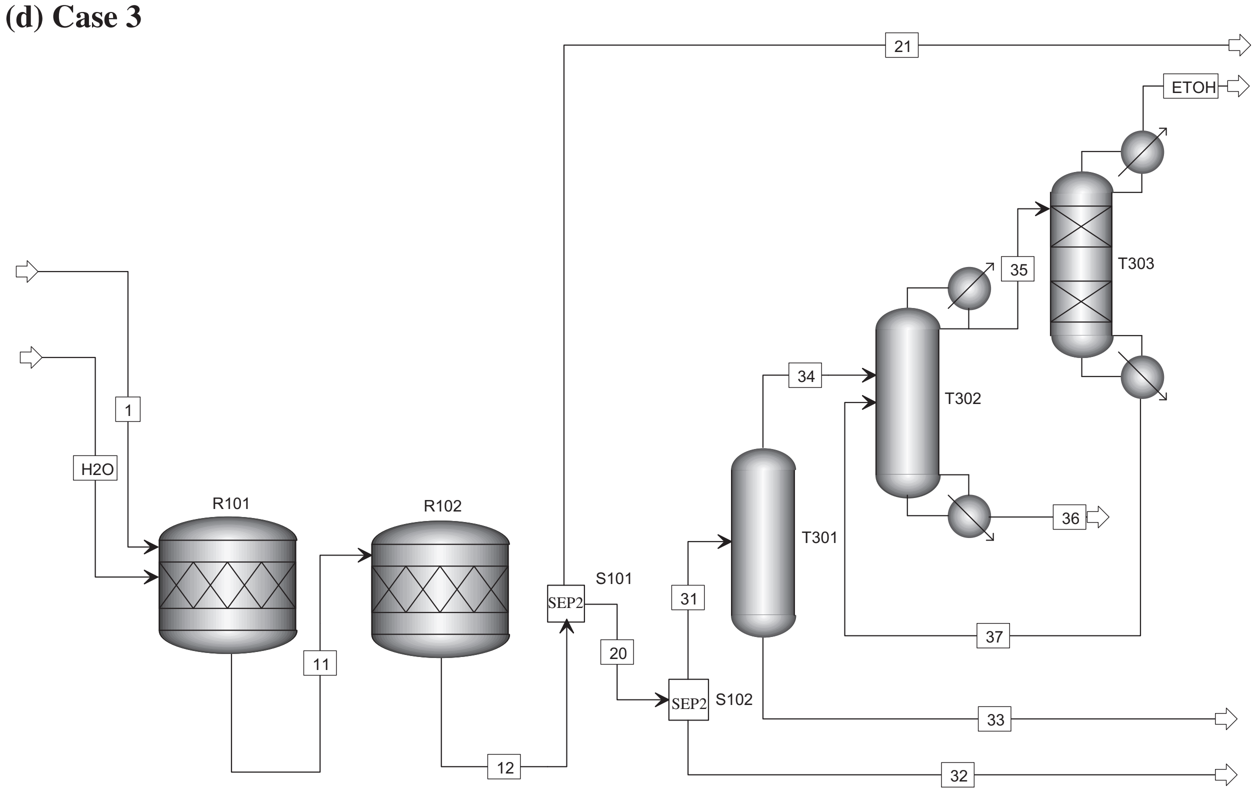

Fig. 3. (*continued*)

Table 5
Operation parameters of cassava cellulose utilization process in Aspen plus simulation model.

	Unit/stream ID	Base Case	Case 1	Case 2	Case 3
Cassava input (t/h)	1	80.0	80.0	80.0	80.0
Cellulose input (t/h)	1	3.456	3.456	3.456	3.456
1st Distillation feedstock	31				
Flowrate (t/h)		245.117	245.117	245.117	246.560
Ethanol (%, w/w)		10.15	10.15	10.15	10.70
2nd Distillation feedstock	46				
Flowrate (t/h)		/	28.140	28.226	/
Ethanol (%, w/w)		/	2.39	2.69	/
Ethanol product	ETOH				
Flowrate (t/h)		25.000	25.676	25.765	26.510
Ethanol (%, w/w)		99.5	99.5	99.5	99.5
Heat duty (MJ/h)					
1st Distillation column	T301	314159	322642	323757	333134
2nd Distillation column	T302	104016	106824	107194	110298

one ton of ethanol in Case 1, Case 2, and Case 3, respectively. According to the same Chinese National Standard (GB/T 2589-2008), 0.1286 ton of standard coal is required for generating one ton of low pressure steam, and 0.7143 ton of standard coal is equivalent to one ton of steam coal in its LHV values. Thus, the steam coal usage was 0.7990, 0.7989, and 0.8001 ton for producing one ton of fuel ethanol in Case 1, Case 2, and Case 3, respectively. According to the latest coal price information at Qinghuangdao Harbor, China, the largest harbor in Northern China, at December 31, 2012 (China Coal Information Net website, www.meitaninfor.com.cn), one ton of steam coal was 635 Yuan RMB, approximately equivalent to $100 per ton of steam coal. Therefore, the steam energy cost for one ton of the increased ethanol was $79.90, $79.89, and $80.01 in Case 1, Case 2, and Case 3, respectively. These results indicate that the steam energy cost in the three cassava cellulose utilization cases was almost equal and the cost percentage in the overall fuel ethanol selling price was only 8%.

The calculations based on Aspen plus modeling show that the cost of cellulase enzyme and steam energy was $2597.69, $2302.11, and $455.49 for producing one ton of increased ethanol from cassava cellulose in Case 1, Case 2, and Case 3, respectively. The result suggests that the process options of Case 1 and Case 2 should be deleted from the practical consideration because of the high cost. In Case 3, the two major costs of cellulase enzyme and steam energy only cover less than 50% of the commercial fuel ethanol selling price (about $1,000 per ton of fuel ethanol). In Case 3, besides the two major costs, the cost of cellulosic ethanol production may also include cellulase enzymes storage, heat removal from the fermentors, and steam usage for molecular sieve dehydration (T303) and so on, but these costs are minor. It is highly possible that in Case 3, 50% ($500) of the profit could be achieved for

Table 6
Cost calculation for increased ethanol from cassava residues based on the Aspen plus modeling.

	Case 1	Case 2	Case 3
Increased cellulosic ethanol (t/h)	0.676	0.765	1.510
Increased cellulase cost[1,2]			
Dosage per cellulose (FPU/t cellulose)	4.50×10^{7}	4.50×10^{7}	1.50×10^{7}
Total dosage used (FPU)	1.56×10^{8}	1.56×10^{8}	5.18×10^{7}
Dosage per ethanol (FPU/t ethanol)	2.30×10^{8}	2.03×10^{8}	3.43×10^{7}
Cellulase cost ($/t ethanol)	2517.79	2222.22	375.48
Increased steam energy cost[2,3,4,5,6]			
Steam energy usuage (MJ/t ethanol)	16,703	16,701	16,726
Steam usage (t/t ethanol)	4.4387	4.4382	4.4448
Steam cost ($/t ethanol)	79.90	79.89	80.01

Information sources:

[1] The exchange rate of Chinese Yuan (RMB) against the US dollar was approximately 6.3 Yuan RMB to one US dollar on December 31, 2012 (Bank of China website, www.boc.cn, December 2012).

[2] The minimum cost for one kg of cellulase with the total FPU value of 50,000 per kg is approximately 10 Yuan RMB, approximately $1.60 per kg of cellulase (personal communication, Dr. Xinliang Li, Shanghai Youtell Biotech Co., www.youtellbio.com).

[3] The LHV (low heat value) value for the low pressure steam used for ethanol distillation is 3763 MJ/ton of steam (Chinese National Standard GB/T 2589-2008: Transformation coefficients from different energy forms to standard coal).

[4] The LHV for one ton of low pressure steam is equivalent to that for 0.1286 ton of standard coal (Chinese National Standard GB/T 2589-2008).

[5] The LHV value for one ton of steam coal is equivalent to that for 0.7143 ton of standard coal (Chinese National Standard GB/T 2589-2008).

[6] The price per ton of steam coal was 635 Yuan RMB at Qinghuangdao Harbor, China, the largest harbor in Northern China, at December 31, 2012 (China Coal Information Net website, www.meitaninfor.com.cn), approximately equivalent to $100 per of steam coal.

producing one ton of fuel ethanol from cassava cellulose. Take a 200,000 t/a fuel cassava ethanol plant such as in Cofco Bioenergy Co, Beihai, China as an example: the deployment of Case 3 will result in approximately 12,080 ton of cellulosic fuel ethanol production per year, and a net annual profit of $6.04 million will be achieved. Besides, the feedstock handling and residue disposal of Base Case and Case 3 are exactly the same, perhaps the disposal cost of residues could be reduced because the removal of cellulose from cassava tubers caused the reduction of residue volume.

4. Conclusion

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 in commercial scale, while the utilization of cassava cellulose from cassava ethanol fermentation residues was not economically sound.

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