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Comparative Study of Bioethanol Production from Cassava Peels by Monoculture and Co-Culture of Yeast

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ABSTRACT

The feasibility of ethanol production from pretreated cassava peels by simultaneous saccharification and fermentation (SSF) with a monoculture of *Saccharomyces diastaticus* 2047 and *S. cerevisiae* 7532 and a co-culture of *S. diastaticus* 2047 and *Candida tropicalis* 5045 was studied. The results indicated that each strain of yeast was able to produce ethanol. From the cassava peels pretreated with distilled water at 135 °C for 30 min under pressure of 1.03 bar, *S. diastaticus* 2047 could produce ethanol yields as high as that of pretreatment with diluted sulfuric acid under the same conditions. The cassava peels pretreated with diluted sulfuric acid and fermented by co-culture of *S. diastaticus* 2047 and *C. tropicalis* 5045 produce greater amounts of ethanol than those fermented by *S. diastaticus* 2047.

Keywords: cassava peels, ethanol, amylolytic yeast

INTRODUCTION

Bioethanol is being considered as a potential liquid fuel due to the limited amount of natural resources. Cellulose biomass is also being investigated as a potential substrate for bioethanol production (Masami *et al*., 2007). In particular, bioethanol produced from non-food lignocellulosic waste products, such as wood chips and straw, or non-food crops, such as willow, could be an environmentally-friendly alternative (Wyman and Goodman, 1993). The carbohydrates, cellulose and hemicellulose are intimately associated with lignin in the plant cell wall. The pentoses might be readily available, but are often found in polymeric chains as xylan, arabinogalactans, arabinans and mannans. Also, the individual sugars might be methylated or acetylated (Biely, 1985) which can affect availability. The hexoses can be fermented to ethanol by yeast, whereas the pentoses can be fermented to ethanol, acetate, lactate, CO_2 and H_2 through the pentose-phosphate pathway with fructose-6-phosphate, glyceraldehyde-3-phosphate and pyruvate as intermediates (Larsen *et al*., 1997). Simultaneous saccharification and fermentation (SSF) has been studied to reduce the time and steps for bioprocessing to produce ethanol from starch and cellulosic biomass. In the SSF process, saccharification involves converting starch to glucose using enzymes and the glucose is catabolized to ethanol by a fermentative microorganism which occurs simultaneously.

Cassava, which is cultivated extensively as a food crop, is the third largest source of carbohydrate for human consumption in the world. Cassava roots play an important role in the diets of many people. In the processing of cassava, the

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roots are normally peeled to rid them of two outer coverings, that is, a thin brown outer covering and a thicker, leathery parenchymatous inner covering. The mature root possesses three distinct regions: a central vascular core, the cortex (flesh) and the phelloderm (peels). The peels are 1˜4 mm thick and may account for 10˜12% of the total dry matter of the root (Nartey, 1979). Analysis of the chemical composition of cassava peels indicates the following chemical composition: dry matter 86.5˜94.5%; or ganic matter 81.9˜93.9%; crude protein 4.1˜6.5%; neutral deter gent fiber 34.4%; and lignin 8.4%. Cassava peels have been evaluated as a feedstuff for animals (Osei *et al*., 1990). The peels constitute about 20˜35% of the weight of the tuber (Ekundayo, 1980). Consequently, a large amount of cassava peel waste is generated annually (Obadina *et al*., 2006). This has led to a new policy of complete utilization of raw materials so that there will be little or no residue left that could pose pollution problems. The agricultural industries generate a significant amount of solid waste that includes peels from cassava, plantains, bananas and oranges, and straw from cereals. Rather than allowing these wastes to become solid municipal wastes, it is necessary to convert them to useful end-products. It is now realized that these wastes may be utilized as cheap raw materials for some industries or as cheap substrates for microbiological processes (Nwabueze and Otunwa, 2006). However, the possibility of using cassava peels for the production of ethanol has not been given much attention. Therefore, this study was initiated to explore the possibility of using cassava peels as a substrate for producing ethanol.

The objective of the study was to assess ethanol production from cassava peels by the SSF process. The ability of dilute-acid, dilute-base and distilled water processes was investigated for pretreatment and hydrolysis of cassava peels that could be converted into ethanol by monoculture of an amylolytic yeast strain of *S. diastaticus* that could ferment starch to ethanol directly and in co-culture with *C. tropicalis* to enhance the ethanol production.

MATERIALS AND METHODS

Monoculture amylolytic yeast strains of *S. diastaticus* 2047 and a standard yeast strain of *S. cerevisiae* 7532 were used in the study. The co-culture was a mix between *S. diastaticus* 2047 and *C. tropicalis* 5045. All cultures were grown on Sabouraud medium at 30 °C. Cassava peels from a factory producing cassava starch were milled to flour in a disc mill (sized 63-425 µm) and dried overnight at 60 °C in a hot-air oven to a moisture content of 10.5%. Then, 1.5% (w/v) cassava peels in 0.1 M sulfuric acid or 6.25 mM sodium hydroxide or distilled water was pretreated for 30 min at 135 °C under pressure of 1.03 bar. The suspension of pretreated cassava peels was neutralized to pH 5.5 for the fermentation process. An enzyme solution (filter-sterilized cellulase 20 FPU per gram substrate, 10 mIU of xylanase and 10 mIU of pectinase solubilized in citrate-phosphate buffer pH 5.0) was used for hydrolysis of the pretreated cassava peels. The pretreated samples were supplemented with additional nutrients to give a base medium composition of 1 g/L yeast extract, 1 g/L $MgSO_4$—$7H_2O$, 2 g/L $(NH_4)_2SO_4$, and 0.5 g/L KH_2PO_4. The enzymatic hydrolysis was undertaken at the same time as the fermentations that were carried out in 125 mL Erlenmeyer flasks containing 50 mL of this medium. The culture was incubated in a rotary shaker at 50 rpm for 48 hr at 30 °C. The amount of released glucose was measured using glucose oxidase/peroxidase assay. The amount of reducing sugar produced was determined using the dintrosalicylic acid (DNS) method. Ethanol concentration was measured by gas chromatography (GC-17A, Shimadzu) using a stainless steel column (2 m length, 3 mm internal diameter) packed with Porapak Q (50˜80 mesh).

The column oven was operated isothermally at 150 °C with a flame ionization detector. At least three parallel samples were used in all analytical determinations and data were presented as the mean of three replicates.

RESULTS AND DISCUSSION

Saccharification of cassava peels

The biomass of the cassava peels was used as feed stock for production of fermentable sugars. The cassava peels were pretreated either with distilled water pH 5.5, 6.25 mM sodium hydroxide or with 0.1M sulfuric acid as described above, and were used as raw material for the saccharification experiments. Table 1 shows the yield of reducing sugars from the cassava peels after 24 h of incubation. The method of pretreatment had a pronounced effect on the yield of reducing sugars. The highest yield of reducing sugar (0.72 g/g dry cassava peels) was obtained from the diluted acid treatment. The value for reducing sugars was calculated from a spectrophotometric determination of sugars and these values may not be directly comparable.

Cassava peels contain sugars in the form of polysaccharides such as starch and holocellulose. They need to be converted to glucose or disaccharides (maltose or cellobiose) for yeast to utilize them efficiently. In the study, amylolytic yeast and the enzyme mixture were used to saccharify the polysaccharides. The enzymes were added at suspension of the cassava peels powder after pretreatment. The total soluble sugar concentrations increased to 8.45, 9.67, and 10.78 g/L by the addition of the enzyme mixture to the cassava peels pretreated with distilled water, 0.025% NaOH and 0.1 M sulfuric acid, respectively. The enzyme mixture produced soluble sugars and worked more effectively than the pretreatment alone. The maximum yield of the sugar concentrations resulted from the pretreatment with 0.1 M sulfuric acid and hydrolysis by the enzyme mixture. Thus, this condition was used to saccharify the cassava peels in the study. The concentration of total soluble sugars was lower than that of total polysaccharides in the cassava peels. Some cellulose with high crystallinity and some hemicellulose would have remained after the enzyme hydrolysis (Kim *et al*., 1995). Most of the soluble sugars were glucose. The reaction mixture might contain xylose, maltose, cellobiose or unknown sugars after the enzymatic saccharification.

The ethanol production results by monoculture and co-culture are summarized in Table 2. The ethanol production by SSF with *S. diastaticus* 2047 was higher than that of *S. cerevisiae* 7532. Fermentation of cassava peels pretreated with 0.1 M sulfuric acid, produced the maximum ethanol yield of 0.418 g/g dry cassava peels (Figure 1A3, Table 2) and substrate pretreated with distilled water (Figure 1A1) produced a higher ethanol yield than that with

Table 1 Yield of reducing sugar from enzymatic hydrolysis of pretreated cassava peels.

Pretreatment	Initial reducing sugar concentration (g/L)	Reducing sugars concentration (g/L)	Reducing sugar yield (wt g%)
Distilled water	1.06 ± 0.04^{a}	8.45 ± 0.42^{a}	56.33 ± 2.71^{a}
6.25 mM NaOH	4.04 ± 0.17^{b}	9.67 ± 0.36^{b}	64.47 ± 1.63^{b}
0.1 M sulfuric acid	5.17 ± 0.34^{c}	10.78 ± 0.51^{c}	72.87 ± 2.82^{c}
0.1 M sulfuric acid*	5.23 ± 0.29^{c}	4.86 ± 0.22^{d}	32.40 ± 0.67^{d}

* Without enzymatic hydrolysis.

Values in columns with different superscripts differ significantly ($P < 0.05$).

Values are shown as mean ± SD for triplicate measurements.

0.025% sodium hydroxide (Figure 1A2). The lowest ethanol yield (0.177 g/g dry cassava peels) was produced by *S. cerevisiae* 7532 from cassava peels pretreated with distilled water (Figure 1B1). The ethanol yield by co-culture indicated that cassava peels pretreated with 0.1 M sulfuric acid (0.441 g/g dry cassava peels) produced more than that of cassava peels pretreated with distilled water (Figures 2C2 and 2C1, respectively) and represented the highest ethanol yield production. Thus, *S. diastaticus* 2047 and *C. tropicalis* 5045 were appropriate for SSF with co-culturing to enhance the productivity of ethanol. The study showed that an increase of released fermentable sugars by the enzyme increased the ethanol production, though the yields of ethanol were slightly affected.

Fermentation of cassava peel by monoculture and co-culture

The results of cassava peel utilization for ethanol production by monoculture are given in Figure 1. Ethanol production using a monoculture was comparatively higher with the strain *S. diastaticus* 2047 (22.4 g/100 g), than with the standard strain 7352 (19.2 g/100 g). Fewer remaining reducing sugars and glucose were also observed at the end of the fermentation with *S. diastaticus* 2047. Among the different chemical pretreatments, 0.1 M sulfuric acid was found to be optimum for both strains of *Saccharomyces*, producing maximum ethanol in 18 and 36 h for *S. diastaticus* 2047 and the standard strain 7352, respectively. Consequently, the cassava peel substrate used was more suitable with the local strain of S. *diastaticus* 2047 than with the standard strain. An ethanol yield of 103.7% of the theoretical maximum was obtained with the S. *diastaticus* 2047 strain, whereas the yield was 67.3% with the standard strain, with cassava peel substrate in monoculture. The results of cassava peel utilization for ethanol production in monoculture are shown in Table 2. More ethanol was produced by the co-culture strain of S. *diastaticus* 2047 and *C. tropicalis* 5045 using cassava peels pretreated with 0.1M sulfuric acid than with the monoculture of S. *diastaticus* 2047 *and* the co-culture of S. *diastaticus* 2047 and *C. tropicalis* 5045 using cassava peels pretreated with water, as shown in Figure 2 and Table 2. Pretreatment by diluted acid produced more ethanol than water pretreatment due to diluted acid damage to the substrate structure that assisted hydrolysis by the enzymes more than the water pretreatment did. Consequently, fewer reducing sugars were observed with S. *diastaticus* 2047 than with the standard strain 7352. A 1.5% concentration of the substrate was found to be optimum for both *S. cerevisiae* strains, producing maximum ethanol in 16 h. S. *diastaticus* 2047 was

Table 2 Comparison of ethanol production in culture with monoculture of *S. diastaticus* 2047 and co-cultures of S. *diastaticus* 2047 and *C. tropicalis* 5045 with different pretreatments of the cassava peels.

Process	Ethanol yield (cassava peels) pretreated by		
	Distilled water	6.25 mM NaOH	0.1 M sulfuric acid
SSF with monoculture			
S. diastaticus 2047	37.41 ± 0.86^{a}	36.52 ± 0.74^{a}	41.82 ± 0.34^{a}
S. cerevisiae 7532	17.73 ± 1.26^{b}	17.86 ± 0.62^{b}	28.07 ± 1.37^{b}
SSF with co-culture			
S. diastaticus 2047 and *C. tropicalis* 5045	36.46 ± 0.42^{a}	-	44.16 ± 0.43^{c}

Values in columns with different superscripts differ significantly ($P < 0.05$).
Values are shown as mean ± SD for triplicate measurements.

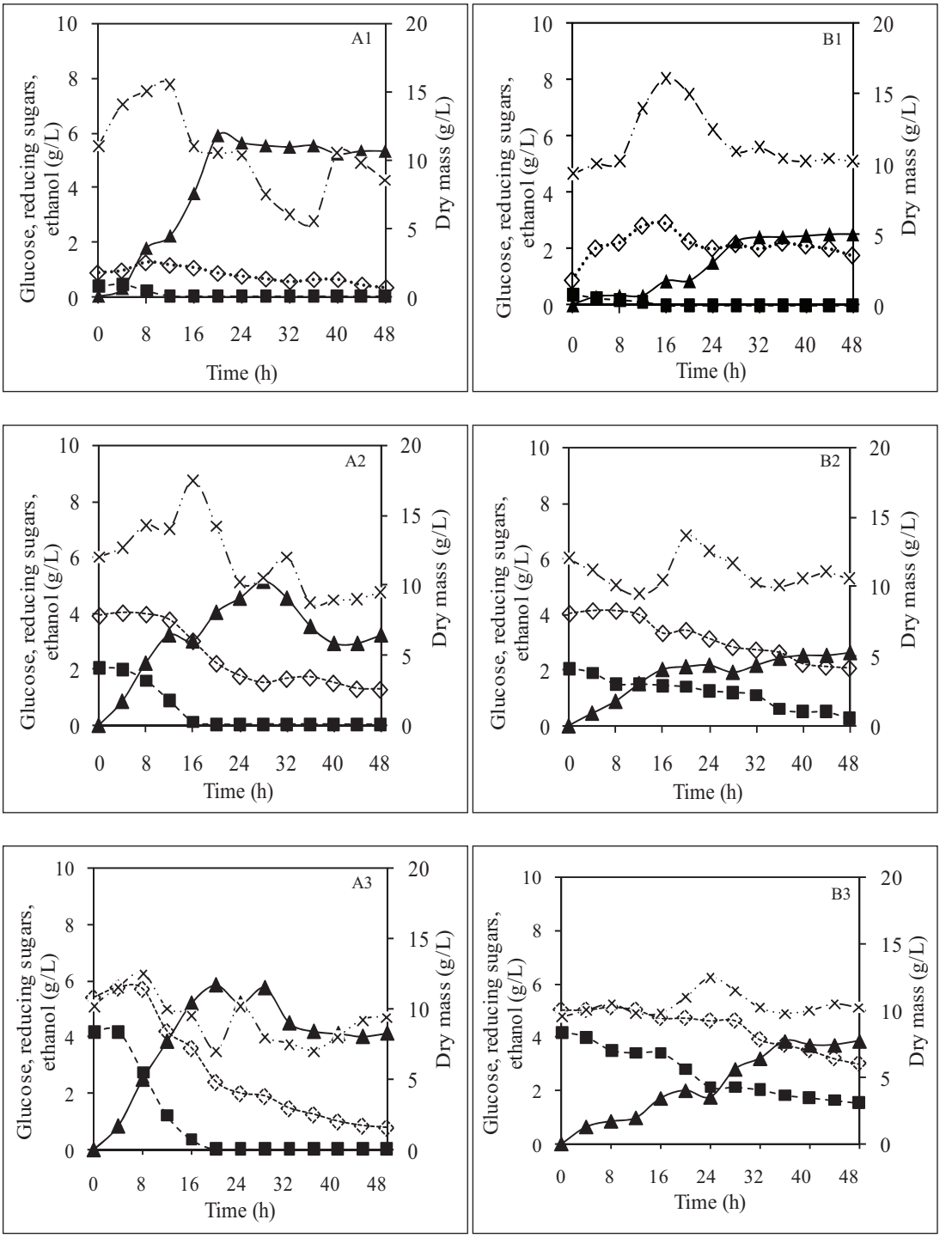

Figure 1 Simultaneous saccharification and fermentation of cassava peels for ethanol production by *S. diastaticus* 2047 (A) and *S. cerevisiae*7532 (B) with distilled water (1) 0.025% sodium hydroxide (2) and 0.1 M sulfuric acid (3). Symbols: × = dry mass; ▲ = ethanol; ◊ = reducing sugar; ■ = glucose.

more suitable for cassava peel utilization than was the standard strain of 7352. The diluted acid pretreated cassava peels produced more ethanol than did the water pretreated cassava peels with both strains of S. *diastaticus* 2047 in co-culture with *C. tropicalis* and comparatively more left-over sugars were accumulated after the fermentation with the diluted acid pretreated cassava peel substrate. This might have been due to the damaged cassava peel substrate being susceptible to enzymatic hydrolysis and suitable for yeast growth in the diluted acid pretreated cassava peel substrate.

It was reported in a previous study by Ryn *et al*. (1994) that 64.3 g/L of ethanol was produced, utilizing 94% of 150 g/L soluble starch, with a mixed culture of mutant M-6 *Schwannimnyces castelli* and S. *cerevisiae.* The starch content in the damaged grains used was lower by 30% and 40% when compared with fresh grains of sorghum (Rehm and Reed, 1996) and rice (Gopalan *et al.,* 1996*),* respectively (Table 1). However, as cassava peels are cheaper than fresh grains, it would still be cheaper to utilize the peels for ethanol production in co-culture. Nonetheless, efforts are being made to improve the strain of S. *diastaticus* 2047 to utilize more than 1.5% substrate concentration and to reduce the duration of fermentation. The results from the present study indicated that simultaneous saccharification and fermentation of cassava peel starch to ethanol can be conducted efficiently using a co-culture of amylolytic yeast, S. *diastaticus* 2047, and a non-amylolytic sugar fermenter, *C. tropicalis* 5045.

In this study, ethanol production from cassava peels using the SSF process was carried out either with a monoculture of *S. diastaticus* or in a co-culture with *C. tropicalis*. The ethanol concentration and productivity by the monoculture of *S. diastaticus* 2047 were approximately twice as high as those of the monoculture of *S. cerevisiae* 7532. The co-culture of *S. diastaticus* 2047 and *C. tropicalis* 5045 produced the highest ethanol yield from the cassava peels pretreated with diluted sulfuric acid. Pretreatment with diluted sulfuric acid had produced some of the sugar prior to the enzymatic hydrolysate reaction. In the present study, the maximum ethanol yield was 0.441 g/g dry cassava peels. The study showed that cassava peels could be used in the fermentation process

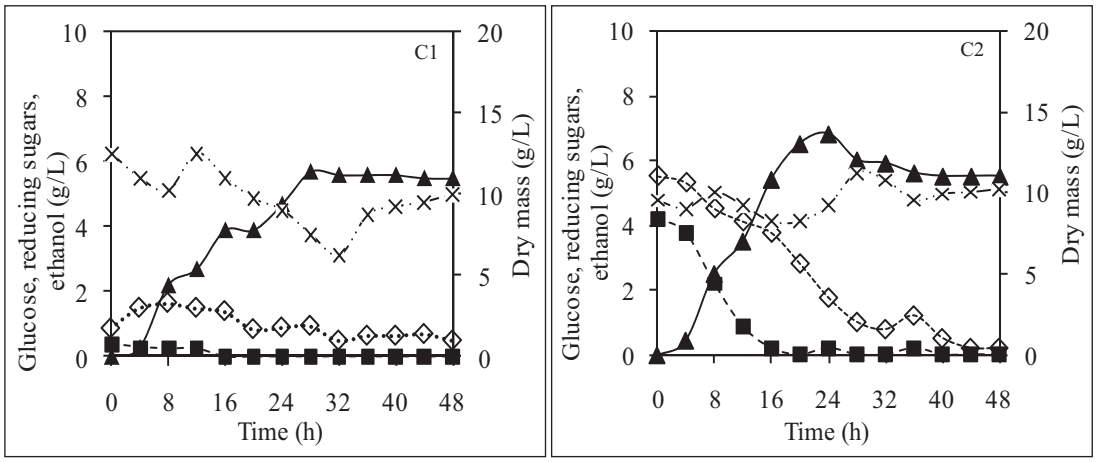

Figure 2 Simultaneous saccharification and fermentation of cassava peels for ethanol production by simultaneous co-culture of *S. diastaticus* 2047 and *C. tropicalis* 5045 (C) with distilled water (1) and 0.1 M sulfuric acid (2). Symbols: × = dry mass; ▲ = ethanol; ◊ = reducing sugar; ■ = glucose.

and the peels could produce a high ethanol yield, so it is possible to use them as an alternative substrate for yeast fermentation in ethanol production. These co-cultures may have several industrial advantages, which can result in the conversion of glucose/xylose into ethanol. Effective ethanol production demands the selection of suitable fermenting strains from among diverse microorganisms, depending on the biomass feedstock chemical composition.

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