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Bioethanol Production from Sweet Potato (*Ipomoea batatas* L.) by Enzymatic Liquefaction and Simultaneous Saccharification and Fermentation (SSF) Process

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ABSTRACT

The starch content in sweet potato (*Ipomoea batatas* var. Sankar) flour and fresh tubers was liquefied by treatment with 0.08% (v/w) commercial thermostable α-amylase (Termamyl®, Novozyme, Denmark) and 0.33% (v/w) amyloglucosidase (AMG®, Novozyme, Denmark). The hydrolysate was subsequently fermented with a thermotolerant strain of *Saccharomyces cerevisiae* into ethanol. The result showed that the final ethanol yield of 240 and 235 $g.kg^{-1}$ (flour) and 96 and 93 $g.kg^{-1}$ (tuber), was little influenced by liquefaction conditions i.e. 80°C for 2 h and 90°C for 1 h, respectively in conjunction with treatment of 0.33% (v/w) AMG (45°C for 24 h). Similarly, the treatment of AMG for 45°C for 48h and 45°C for 24 h had little effect on the ultimate ethanol yield, i.e. 242 and 238 $g.kg^{-1}$ (flour) and 94 and 93 $g.kg^{-1}$ (tuber), respectively after liquefaction (90°C for 1 h) treatment. Another experiment was designed to study simultaneous saccharification and fermentation by saccharifying the liquefied (90°C for 1 h) hydrolysate and co-fermenting with *S. cerevisiae* at 40°C for 96 h. The ethanol yield was 258 $g.kg^{-1}$ for flour and 95 $g.kg^{-1}$ for tuber, respectively.

Keywords: amyloglucosidase, feedstocks, *Saccharomyces cerevisiae*, termamyl
Abbreviations: AMG, amyloglucosidase; **CTCRI**, Central Tuber Crops Research Institute; **PDA**, potato dextrose agar; **SSF**, simultaneous saccharification and fermentation; **YENA**, yeast extract-nutrient broth

INTRODUCTION

The natural energy resources such as fossil fuels (petroleum and coal) are being utilized in a rapid rate and these resources have been estimated to last only for few more years (Chandel *et al*. 2007). From various alternative energy resources that can susbstitute natural energy resources, bioethanol is the most promising because it is of biological and renewable origin, normally derived from energy crops such as maize, sugarcane, cassava and sweet potato and by-products of agriculture and forestry (Ward *et al.* 2002). Bioethanol is an alcohol produced by fermenting and distilling various feedstocks (sugar, starch and cellulose), which have been converted to simple sugars by enzymatic or acid hydrolysis (Larssen *et al.* 2003). Sweet potato (*Ipomoea batatas* L.) is one of the important starchy crops having a short growth cycle (90-120 days) and capable of growing in various agro-climatic conditions (Ray and Ravi 2005).

The most important process development made for enzymatic hydrolysis of various starch-containing crops and biomass is the introduction of simultaneous saccharification and fermentation (SSF) process (Ward *et al.* 2006). This process employs thermotolerant yeast strains to reduce the number of reactors involved by eliminating the separate (saccharification) reactor and more importantly, avoiding the problem of product elimination associated with enzymes such as build up of cellobiase and AMG which shut down starch degradation (Chandel *et al.* 2007). However, in SSF, both saccharifying enzyme (AMG) and fermenting microbes are applied simultaneously. As the conversion of starch into sugars is processed by AMG, the fermentative organisms convert them into ethanol (Ward *et al.* 2002).

In this paper, enzymatic liquefaction and saccharification processes of sweet potato (flour and tubers) was studied in relation to time factor (incubation). Further emphasis was laid on the application of SSF in the production of ethanol from liquefied sweet potato starchy biomass.

METHODOLOGY

Sweet potato

Fresh, unbruised and uninfected sweet potato tubers (var. Sankar) having 14% extractable starch and 3% free sugar (on a fresh weight basis) were collected from the field of the regional Centre, CTCRI, Bhubaneswar, India. The tubers were washed properly under tap water to remove soil and dirt. For flour preparation, the fresh tubers were sun-dried in open air for 4-5 days and then dried in oven at 80°C for 5 days to reduce moisture content to 8-10%. Then, these were ground in a laboratory mill (Pelican Instruments, Chennai, India) to prepare flour for experimental purposes. Both fresh tubers and flour prepared as above were used for ethanol fermentation.

Microorganisms and culture conditions

A thermotolerant yeast strain, *Saccharomyces cerevisiae* CTCRI 10 (temperature tolerance <40°C), used in alcohol fermentation was adopted as the experimental strain and maintained in the laboratory on potato dextrose agar (PDA) slants. The yeast was grown first in 250 ml Erlenmeyer flasks containing 100 ml sterilized medium (yeast extract-nutrient broth, YENA) with sugar level of 12% (w/w) and pH was adjusted to 5.5 by dilute (0.1 N) HCl. Then it was cultured for 48h at 30°C in an incubator. This served as starter culture for bioethanol production.

Fermentation medium

The fermentation medium used in these studies was sweet potato flour and tuber hydrolysate containing 51-52 and 17-20% starch,

Received: 24 July, 2008. Accepted: 21 August, 2008.

Short Communication

respectively. The sweet potato slurry was prepared by mixing corresponding flour or mashed tuber with water in 1: 6 (w/w) proportion. The slurry was liquefied with 0.08% commercial thermostable α-amylase (Termamy®, Novozyme, Denmark) at 80°C for 2 h or 90°C for 1 h and subsequently saccharified by treatment with 0.33% amyloglucosidase (AMG®, Novozyme, Denmark) at 45°C for 48 h or 45°C for 24 h. When the saccharification process was over, the pH of the hydrolysate was monitored and adjusted to between 5.5-6.0 by the addition of 0.1 N HCl or 1 N NaOH.

Batch fermentation

Batch fermentation was carried out in 1000 ml Erlenmeyer flasks with 360 ml of hydrolysate on a laboratory bench at room temperature (30 ± 2°C) for 96 h. For fermentation, starter culture equivalent to 10% (v/v) of the fermentation medium was used as inoculum. Then, 0.6 g of urea was added as a nitrogen source to the fermentation medium. The initial pH of the hydrolysate was set at between 5.5-6.0 by the addition of 0.1 N HCl or 1 N NaOH. Three replicates were established for each treatment.

SSF

SSF using *S. cerevisiae* was carried out by following similar steps up to liquefaction treatment as in batch fermentation. Then, both saccharification and fermentation processes were carried out simultaneously at 40°C by adding 0.33% AMG and starter culture equivalent to 10% (v/v) of the fermentation medium. Urea (0. 6g) was also added to the sweet potato slurry prepared either from flour or mashed tubers, as a nitrogen source. The initial pH of the hydrolysate was set at 5.5-6.0 by the addition of 0.1 N HCl or 1 N NaOH and fermentation was continued until 96 h at 40°C. Three replicates were established for this experiment.

Analytical methods

After the fermentation period was over, the alcohol was extracted by distillation and the concentration of the extracted alcohol was determined by measuring through specific gravity method (Amerine and Ough 1989). Apparent total sugar concentrations were tested using a Brix Refractometer (Sipcon, Jallandhar, India). The pH was measured by a pH meter (Systronics, Ahmadabad, India) using a glass electrode. The yeast cell population was counted using a hemocytometer. The yeast biomass was determined by reading the absorbance at 550 nm against a suitable blank in a UV-Vis Spectrophotometer (Cecil Instruments, UK). The corresponding dry weight was obtained from a standard curve of absorbance versus dry weights (Swain *et al.* 2007). Fermentation kinetics was studied as per the formulae given by Bailey and Ollis (1986).

RESULTS AND DISCUSSION

Sweet potato flour and tuber were hydrolyzed by using commercial thermostable α-amylase and AMG, and subsequently fermented by *S. cerevisiae* into bioethanol as per the conventional batch fermentation method developed at CTCRI, Thiruvanathapuram, India (Vijayagopal and Balagopalan 1989). In the two-step hydrolysis treatment (liquefaction-saccharification), comparison studies were carried out for obtaining the optimal temperature versus time of incubation for maximum conversion of starch to fermentable sugar with concomitant production of ethanol in batch fermentation. For liquefaction, sweet potato hydrolysate was treated with 0.08% Termamyl to convert the starch present into limited dextrin form by incubating either at 80°C for 2 h or 90°C for 1 h. Subsequently, the partially hydrolysed starch was treated with AMG (45°C for 24 h) for complete conversion into sugar, which was subjected to fermentation by *S. cerevisiae*. There was no significant difference (Fisher's LSD test, $p < 0.05\%$) on ethanol yield between treatment at 80°C for 2 h or 90°C for 1 h (**Table 1**). It has been reported that the percentage conversion of cassava starch to dextrin, i.e. liquefaction process in high fructose syrup production was better at 90°C for 1 h treatment than at 80°C for 2 h (Paolucci *et al.* 2000; Johnson *et al.* 2004). In another report, it was found that temperature of 85-95°C and average incubation time of 15-30 min were significant to cause complete conversion of cassava tuber slurry by α-amylase enzyme (Berghofer and Sarhaddar 1986).

In the next step, saccharification process (temperature versus time of incubation) was standardized for subsequent bioconversion into ethanol. The liquefied (90°C for 1 h) sweet potato hydrolysate was treated at two different saccharifying conditions (45°C for 48 h or 45°C for 24 h). The results showed that there was no significant difference (Fisher's LSD test, $p < 0.05\%$) on ethanol yield (**Table 2**).

In the SSF process, the saccharification step was carried out at 40°C for 96h along with simultaneous incubation with yeast culture (the organism could grow and produce ethanol up to 40°C, pre-determined by experiment) and then the fermented mash was distilled for ethanol. The conversion rate of starch present in sweet potato flour and tuber (hydrolysate sample) to fermentable sugar and then to ethanol showed somewhat higher yield, i.e. 258g kg^{-1} (flour) and 95 g kg^{-1} (tuber) (**Table 3**). The growth and fermentation kinetics of *S. cerevisiae* cells were also studied (**Table 4**). Except final ethanol (p, g l^{-1}) and % conversion rate, there was no significant (Fisher's LSD test, $p < 0.05\%$) variation in other kinetics parameters.

Table 1 Ethanol yield from sweet potato on fermentation of liquefied (80°C for 2 h and 90°C for 1 h) and saccharified (45°C for 24 h) slurry with *S. cerevisiae*.

Sample	Liquefaction treatment			
	80°C		90°C	
	Ethanol yield (g kg^{-1})	Sugar conversion rate (%)	Ethanol yield (g kg^{-1})	Sugar conversion rate (%)
SP flour	240	84	235	82
SP tuber	96	96	93	93

Table 2 Ethanol yield from sweet potato on fermentation of liquefied (90°C for 1 h) and saccharified (45°C for 24 h) slurry with *S. cerevisiae*.

Sample	Saccharification treatment			
	45°C for 48 h		45°C for 24 h	
	Ethanol yield (g/kg)	Sugar conversion rate (%)	Ethanol yield (g/kg)	Sugar conversion rate (%)
SP flour	240	84	235	82
SP flour	96	96	93	93

Table 3 Ethanol production by simultaneous saccharification and fermentation (SSF) technology.

Sample	SSF process	
	Ethanol yield (g kg^{-1})	Sugar conversion rate (%)
SP flour	258	91
SP flour	95	95

Table 4 Growth and fermentation kinetics of *S. cerevisiae* for ethanol production from sweet potato by SSF.

	Flour	Tuber
Final ethanol (p, g.l^{-1})	40.26	15.67
Final biomass concentration (x, g.l^{-1})	4.35	4.32
Specific growth rate (μ)h^{-1}	0.098	0.098
Cell yield (g.g^{-1}) Yx/s	0.026	0.032
Ethanol yield (Yp/s, g.g^{-1})	0.43	0.48
Volumetric substrate uptake (Qs, g.l^{-1}.h^{-1})	0.538	0.542
Volumetric product productivity (Qp, g.l^{-1}.h^{-1})	0.42	0.16

$Y_{P/S} = \frac{\text{Mass of product (ethanol) formed}}{\text{Mass of substrate (glucose) consumed}}$

$Y_{X/S} = \frac{\text{1 g of biomass (yeast cell) formed}}{\text{Mass of substrate (glucose) consumed}}$

Q_S = Substrate (glucose) uptake (g) per liter of hydrolysate per hour.

Q_P = Product formed (g) per liter of hydrolysate per hour.

[a]μ(h^{-1}) = Standardized value (0.098) for specific microorganism (yeast: *S. cerevisiae*) under specific substrate (glucose) consumption (Bailey and Ollis 1986).

It has been reported that SSF is a more efficient process, which can minimize the production cost and time period than batch fermentation by using cassava fibrous residues, various cellulosic feed stocks and agricultural residues for ethanol production (Ward and Singh 2006). Also, SSF with co-culturing technology (simultaneously culturing hexose and pentose fermenting yeasts) is being used under immobilized conditions for formulating low cost ethanol production (Fujii *et al*. 2001).

ACKNOWLEDGEMENTS

The authors thank the Director, CTCRI, Thiruvanathapuram, for facilities.

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