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Simultaneous Saccharification and Fermentation of Overnight Soaked Sweet Potato for Ethyl Alcohol Fermentation

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Abstract: The aim of the study is to develop an efficient method of production of ethyl alcohol by fermentation of sweet potato powder. The method emphasized on enhancement of α-amylase accessibility by overnight soaking of sweet potato powder and decreasing unwanted bacterial fermentation by utilizing simultaneous saccharification and fermentation. The crystalline structure of starch limits the accessibility of α-amylase to starch during enzymatic hydrolysis and thus reduces hydrolysis rate. This might be the reason for longer hydrolysis time. Effect of overnight soaking in water on eight variety of sweet potato was investigated followed by Simultaneous Saccharification and Fermentation (SSF). The result reflected the advantage of overnight soaking on α-amylase accessibility to starch, which favor higher hydrolysis rate. Implementing SSF, the fermentation process become efficient due to less free sugar at an instant and maximum efficiency of 96.7% was achieved in a 64.65% starch containing substrate.

Key words: Amylase accessibility, efficiency, fermentation time, overnight soaking, SSF, sweet potato

INTRODUCTION

In accordance to current energy and environmental status, ethanol has great importance as energy source, with environmental sustainability aspects. Ethanol combustion as fuel emits low CO, low unburned hydrocarbon and SO_2 free exhaust. Along with this, ethanol has rich octane number of 120 as compared to 87-98 in case of gasoline. Another important reason behind use of ethanol as biofuel is its zero CO_2 emission. When ethanol burns, it emits CO_2 which is further assimilated by plant for carbohydrate production during photosynthesis. This carbohydrate is the precursor for ethanol, in fermentation process. Because of such advantages, ethanol is blended in gasoline in a range of 5-20%. Use of pure ethanol is discouraged due to its acetaldehyde emission rate, which is 2-4 times lower in case of gasoline and this aldehyde has adverse health effect on respiratory system of inhalers (Levin *et al.*, 2008). Therefore blending is required. But commercialization of this blending programme demands ethanol production to be economic.

Generally tubers contain fair amount of starch, which furnish them as a raw material for ethanol production. Among tubers sweet potato might be the better option because it is cheap and generally not used for valuable product formation. Recent studies on sweet potato revealed that, simultaneous saccharification and fermentation yields 91.4% ethyl alcohol on its starch content (Zhang *et al*., 2011), where simultaneous saccharification and fermentation of high gravity slurry of 304 gm/L was shown to display fermentation efficiency of 89.7% (Srichuwong *et al.*, 2009). Generally native starch granules are semi-crystalline, round to oval in shape and have smooth surface. Native starch generally resists α-amylase activity. However, they are readily hydrolyzed when get gelatinized (Tester and Karkalas, 2006; Gunaratne and Hoover, 2002). Enzymatic hydrolysis of starch shown to have better hydrolysis rate as compared to acid hydrolysis. Acid hydrolysis of starch with 1.2% (w/w), 1M HCL yields 94% dextrose with 0.04% hydroxymethylefurfural (HMF). This HMF acts as inhibitor during fermentation. Subsequent fermentation of acid hydrolyzed starch, shown to yield 31 gm/L ethanol (Marija *et al*., 2008). Again Phosphorous and amylose content influence the resistance to hydrolysis and rate of hydrolysis (Absara *et al*., 2009; Nodaa *et al.*, 2008). Apart from yeast, Zymomonas mobilis was also used for ethanol fermentation, with pH-4, 20% substrate loading, 7.5% inoculums and fermentation time of 24 h, which results in 66.4 g/L ethanol (Zhang and Feng, 2010).The major objective of the present investigation is to assess the effect of overnight soaking of sweet potato on its enzymatic hydrolysis (liquefaction) followed by Simultaneous Saccharification and Fermentation.

MATERIALS AND METHODS

The experimental set up was designed and the investigation was carried out in the Food Science Laboratory of Centre for Food Science and Technology, Sambalpur University, during the year 2011. Sweet potato

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samples were obtained from local market The Sweet Potato samples were shredded dried at 105ºC and ground to powder. The powder was made to slurry of 25% total solid with distilled water and subsequently hydrolyzed by appropriate enzyme followed by fermentation with Saccharomyces cerevisiae, which are describe below. Before the powder was used to make slurry, moisture content was analyzed after keeping the sample at 105ºC for 6 h.

Starch analysis: Starch estimation of above sweet potatoes was done by acid hydrolysis method (BSI, 1978). A sample of 0.5 gm was accurately weighed and kept on a filter paper. After thorough wash with 10 mL of diethyl ether for five times followed by 150 mL of 10% alcohol. Then the powder was poured into a volumetric flask containing 220 mL of 2.5% HCL. The solution was refluxed for 2.5 h followed by neutralization with 20% $CaCO_3$ (w/v) using phenolphthalein as indicator. The final volume was made up to 250 mL. Then the solution was titrated against Fehling solution A and B and the sugar% was determined by the formula:

$$\text{Sugar\%} = (FF \times 100 \times 500) / \text{volume consumed}$$

From above sugar %, the starch % can be calculated as follows:

$$\text{Starch \%} = \text{sugar \%} \times 0.93$$

Enzymatic hydrolysis: Sweet potato samples were grouped into two, each with four sub sample (A_1-A_4 and B_1-B_4). 62.5 g of sub samples A_1-A_4 were subjected to 24 h soaking with 250 mL distilled water followed by liquefaction and SSF. However, in case of subsample (B_1-B_4), the powder was made to slurry with 250 mL of distilled water and immediately processed for liquefaction, saccharification and fermentation separately.

Liquifection of starch: The samples, after soaking (A_1-A_4) and after slurry preparation (B_1-B_4) were adjusted to pH 5.6 using dilute orthophosphoric acid. Thermostable α-amylase (novozyme make) was added at loading of 10 U/g substrate. Then the slurry were liquefied at 95ºC for 3 h in a shaking water bath. During liquefaction iodine test was done at 1 h interval to observe the rate of starch hydrolysis.

(B) Saccharification: This step was carried out only for subsample B1-B4 with α-glucosidase loading of 10 U/gm substrate at 65ºC for 3 h at pH 4.5. This was followed by addition of 12% of inoculum with 5% solution of Urea, $ZnSO_4$ and $MgSO_4$ of 5, 2.5 and 2.5 mL respectively. Then its final volume was made up to 500 mL it was fermented at 32ºC-35ºC for 24 h.

Simultaneous saccharification and fermentation: Three hundred mL liquefied slurry of A1-A4 subsample were taken and 10 U/g substrate of α-glucosidase was added along with 12% inoculums, with 5% solution of Urea, $ZnSO_4$ and $MgSO_4$ of 5, 2.5 and 2.5 mL respectively. The final volume was made up to 500 mL and followed by fermentation at 32ºC-35ºC for 24 h.

Alcohol estimation: Fermented wash and distilled water was mixed at 1:1 proportion and distillated on a lab scale distillation unit. The distillate was collected and volume was made up to 200 mL. Then its specific gravity and temperature was determined and alcohol percentage was calculated using alcohol %, specific gravity and temperature chart. The calculated alcohol % value was corrected using standard graph made by taking known alcohol % solution and alcohol % shown by hydrometer (Wright, 1907).

RESULTS AND DISCUSSION

Starch content, liquifection and effect of overnight soaking on enzymatic hydrolysis time: Table 1 presents starch content and hydrolysis rate of sweet potato. Starch content analysis of sweet potato showed a variation from 53.3 to 64.65%. The 3 h liquefaction was also studied

Table 1: Sweet potato powder analysis and liquifection

			Liquefactio (hydrolysis rate)			
Samples	Moistureg (%)	Starchg (%)	0 HR	1 HR	2 HR	3 HR
A1	9	64.65±0.095	++++	----	----	----
A2	10	61.8±0.26	++++	+---	----	----
A3	7	62.13±0.15	++++	+---	----	----
A4	7	53.5±0.20	++++	+---	----	----
B1	9	64.5±0.30	++++	++--	++--	+---
B2	5	61.5±0.40	++++	++--	++--	+---
B3	5	59.9±0.30	++++	++--	++--	+---
B4	7	55.3±0.55	++++	++--	++--	+---
		Grand mean = 60.4				

++++: Complete unhydrolyzed state; ----: Complete hydrolyzed state

Table 2: Fermentation of sweet potato

Samples	Alcohol (%)	Efficiency (%)
A_1	4.8	96.7
A_2	4.2	93.3
A_3	4.6	94.35
A_4	4.0	96.0
B_1	4.2	84.8
B_2	4.0	81.0
B_3	4.0	83.3
B_4	3.3	76.0

which showed complete liquefaction incase of subsample A_1-A_4, where as partial unhydrolyzed state was observed in subsample B_1-B_4. Samples A1-A4 were soaked overnight and soaking might have cause swelling and make it porous, which enhances its α-amylase accessibility (Arnates and Sadder, 2011). As soaked samples are completely liquefied they are more suitable for SSF as compared to non-soaked.

Simultaneous saccharifection and fermentation: Table 2 reveals the alcohol content and the alcohol production efficiency. The Simultaneous Saccharification and fermentation gives maximum of 96.7% alcohol production efficiency in case sample A_1-A_4, where as sample B_1-B_4, where saccharification and fermentation were done separately, had an maximum efficiency of 84.4% .The difference in efficiency between SSF processed and traditionally processed sample is significantly different ($t = 3.71$, $p<0.05$). The key point of this study is gradual release of free monosaccharide from starch and its simultaneous uptake by S.cerevesiae for ethyl alcohol production. Though the sugar releasing process is gradual and instant free sugar is less, unwanted bacterial contamination is decreases and alcohol yield increases.

Studies made by Shariff *et al.* (2009) on the enzymatic hydrolysis of sweet potato showed significant increase in dextrose equivalent, below its gelatinization temperature. Correlating the structural attributes of sweet potato starch with susceptibility to hydrolysis confers the role of amylose, amylopectin, and granule size and gelatinization temperature. High amylopectin content of sweet potato starch showed high gelatinization temperature and less susceptibility to α-amylase attack (Zhang and Oates, 1999). In this study data of 3 h liquefaction showed complete liquefied state in case of overnight soaked sample, which might be due to better accessibility of amylase to starch. Fermentation of sweet potato starch, after cold enzyme hydrolysis showed 92.5 to 94.64% of fermentation efficiency (Yingling *et al*., 2011). Similar study on sweet potato fermentation, which involves simultaneous saccharification and fermentation showed 91.4% fermentation efficiency (Zhang *et al*., 2011). In this current study maximum fermentation efficiency of 96.4% was achieved through SSF and by soaking the raw mass over night in water.

CONCLUSION

From above study we can infer that overnight soaking along with simultaneous saccharification and fermentation gives greater efficiency and higher ethanol yield.

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