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Ethanol production from potato flour by *Saccharomyces cerevisiae*

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Abstract

Flour prepared from potato tubers (*Solanum tuberosum*) after cooking and drying at 70°C was used for ethanol production. Homogenous slurry of potato flour was prepared in water at solid liquid ratio 1:4. Liquefaction of potato flour slurry with α-amylase (2.05 DUN U/g starch) at 80°C for 30 min followed by saccharification with glucoamylase (20.5 GA U/g starch) at 60°C for 2 h generated 15.2% total reducing sugars in the hydrolysate. Fermentation of hydrolysate with *Saccharomyces cerevisiae* HAU-1 at 30°C for 48 h resulted in production of 56.8 gl^{-1} ethanol. Supplementation of nitrogen sources to potato flour did not contribute significantly to ethanol yield. Simultaneous saccharification and fermentation of hydrolysate was as effective as separate hydrolysis and fermentation.

Keywords: Ethanol, fermentation, liquefaction, potato flour, saccharification, *S. cerevisiae.*

Introduction

Bioethanol as an alternative source of energy has received special attention world over due to depletion of fossil fuels. According to United States department of energy, for every unit of energy put towards ethanol production 1.3 units are returned (Hill *et al*., 2006). In India sugarcane molasses is the main raw material for ethanol production but now the short supply and increased cost is the main hindrance for its use. There are about 342 distilleries in the country with an installed capacity of over 3 billion litres of ethanol annually (Narde, 2009) which is short of requirement and is met through imports. The efficiency of ethanol production largely depends on the availability of suitable substrate, yeast strain and method employed.

The sugary substrates available are comparatively expensive than molasses but can be easily used for ethanol production with some modification in the process. On the other hand cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and is expensive. The starchy substrates are promising due to their economic viability and availability. Starchy crops like corn, barley, wheat, rice and tuber crops viz. potato, sweet potato are being exploited for the production of bioethanol world wide (Szambelan *et al*., 2004; Shigechi *et al*., 2004). The world over production of potatoes in 2007 was 325.3 million tones while in India it was approximately 26 million tons (Rani *et al*., 2009) showing this as a promising crop but is being used for production of ethanol in some countries (Kobayashi *et al*., 1998). Moreover, potatoes are rich in starch, which makes it a cheap substrate for ethanol production. The problems associated with its processing will also be less than in other grains. It is also semi-perishable food which can be stored for considerable period without spoilage. Good quality alcohol can be produced from potato which can be used for both fuel as well as potable purpose. Therefore there is a need to explore the possibility of ethanol production from potato after suitable processing and this study was planned to develop a suitable technology for conversion of potato starch into ethanol.

Materials and methods

Substrate: Potato tubers were procured from university farm and analyzed for different components by standard methods (AOAC, 1990). Thoroughly washed unpeeled potatoes (1 kg) were cooked in a pressure cooker in one litre water containing 0.5% potassium metabisulphite. Boiled potatoes were mashed, dried overnight at 70°C and ground to fine powder (210 µm).

Enzyme for liquefaction and saccharification: Commercial α-amylase (Termamyl-100, specific activity 30 DUN U/ml) and amyloglucosidase (Amylo 300, Specific activity 400 GA U/ml) were received from Ms. Jagatjeet Industry Ltd., Jalandhar (Punjab).

Preparation of potato flour slurry: Slurries were prepared from potato flour mixed with water at different solid liquid ratio (1:1-1:10) and treated with liquefying enzyme (1000 µl/100 ml) at 80°C for 30 min under shaking conditions. The slurry prepared by mixing 20 g flour in 80 ml water (1:4) being homogenous, loose, easy to handle was used for further experiments. Liquefaction of potato flour (100 ml slurry) was carried out at 80°C in a shaking water bath using varying concentration of enzyme (100-2000 µl) at different incubation temperatures (60-90°C) for different time intervals (10-40 min). The progress of liquefaction was monitored by employing starch-iodine (1 g of Iodine & 2 g KI in 100 ml water) reaction.

Saccharification of liquefied starch was carried out at 60 °C for different time intervals using varying

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concentration (100-1000 µl/100 ml) of Amylo-300 containing amyloglucosidase. The reaction was monitored by the yield of total reducing sugars estimated by dinitrosalicyclic acid method (Miller, 1959).

Yeast culture: A fast fermenting strain of *Saccharomyces cerevisiae* HAU-1 was obtained from culture collection Dept. of Microbiology, CCS Haryana Agricultural University, Hisar (Haryana) and maintained on yeast extract peptone dextrose (YEPD) agar medium containing yeast extract (1%), peptone (2%), dextrose (2%) and agar (2%). Dextrose inoculum medium (IM) used for inoculum preparation contained dextrose (6%), peptone (0.5%), yeast extract (0.5%). Yeast cells pre grown in inoculum medium for 18 h under shaking condition (100 rpm) were centrifuged at 8000 rpm for 15 min and inoculated into hydrolysate at a concentration of 0.5% (w/v) wet weight basis. Flasks were incubated at 30±2°C under stationary conditions and ethanol content was measured at an interval of 24 h by gas liquid chromatography (Systronics, GC 8200) using capillary column and flame ionization detector.

Table 1. Composition of potato and potato flour

Parameters	Composition (%)	
	Potato	Potato flour
Moisture	80.28	8.12
Starch	20.0	73.0
Total protein	2.19	10.86
Crude fiber	0.85	1.65
Ash content	0.65	2.15
Total sugars	0.41	0.91
Total lipids	0.12	1.00

Results and discussion

Potato tubers contained 80.3% moisture, 20% starch, 2.19% proteins and 0.41% total sugars while the respective value for potato flour were 8.12, 73.0, 10.86 and 0.91% (Table 1).

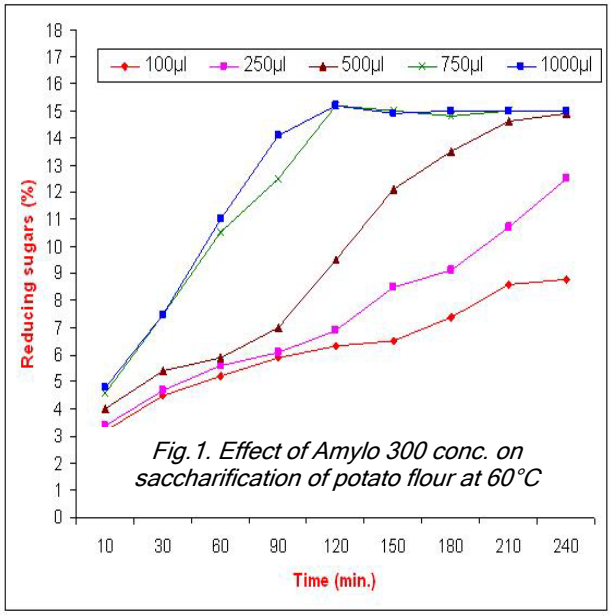


Fig. 1. Effect of Amylo 300 conc. on saccharification of potato flour at 60°C

Fig. 2. Ethanol production from potato flour by simultaneous saccharification and fermentation

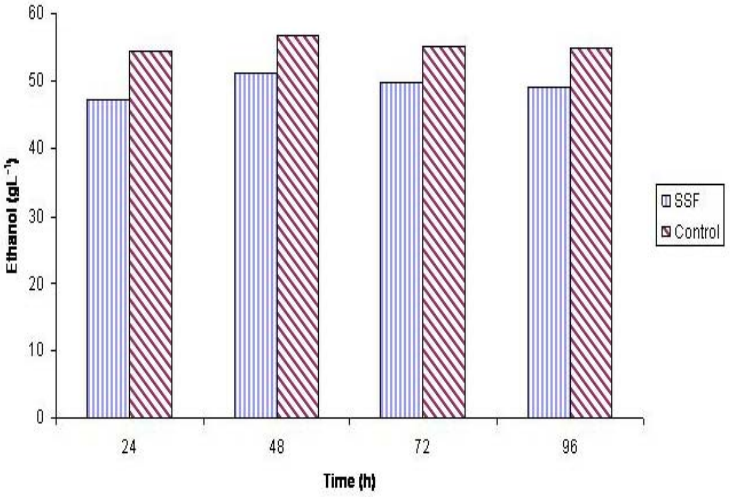


Fig. 3. Effect of temperature on ethanol production from potato flour

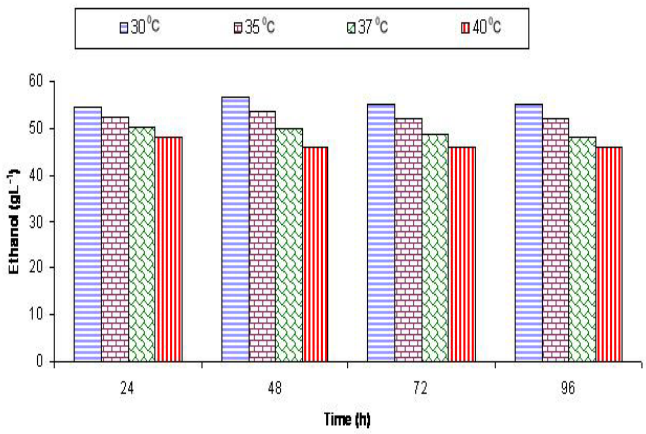
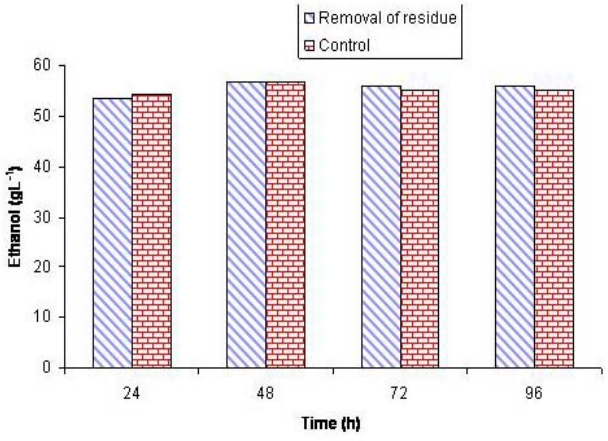


Fig. 4. Ethanol production from potato flour after removal of residue



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Hydrolysis of potato flour: 100 ml slurry of potato flour (20%) was liquefied with 1000 µl Termamyl-100 at 80°C within 30 min. The liquefied potato flour saccharified with different doses of Amylo 300 (100-1000 µl) at 60°C released 15.2% total reducing sugars at enzyme concentration of 750 µl after 2 h, however further increase in reaction time and temperature and enzyme concentration did not have appreciable effect (Fig.1).

Ethanol production from hydrolysate of potato flour: The hydrolysate from potato flour on inoculation with *Saccharomyces cerevisiae* at 0.5% concentration (w/v) generated 56.8 gl^{-1} ethanol after 48 h fermentation at 30±2°C. On supplementation of various nitrogen sources viz. urea, ammonium sulphate, peptone and yeast extract at 0.1-0.5% (w/v) maximum 59.9 gl^{-1} ethanol was obtained with supplementation of peptone (0.2%) or yeast extract (0.2%) under similar conditions (Table 2).

A maximum of 51.2 gl^{-1} ethanol was produced in 48 h as compared to 56.8 gl^{-1} ethanol in 48 h when potato starch was saccharified simultaneously during fermentation suggesting that simultaneous saccharification and fermentation is equally effective as separate hydrolysis and fermentation (Fig. 2).

Table 2.Ethanol production from potato flour after supplementation with various nitrogen sources

Nitrogen source conc. (% w/v)		Ethanol (gl^{-1})		
		24 h	48 h	72 h
Ammonium sulphate	0.1	56.0	56.8	56.4
	0.2	56.8	58.3	58.3
	0.3	55.2	56.0	56.0
Peptone	0.1	56.8	57.5	57.5
	0.2	58.3	59.9	59.1
	0.3	57.5	57.5	56.8
Urea	0.1	56.0	56.0	55.2
	0.2	57.5	59.1	56.8
	0.3	55.2	56.0	55.2
Yeast extract	0.1	56.0	58.3	58.3
	0.2	58.3	59.9	59.1
	0.3	56.0	57.5	57.5
Control	No nitrogen source	54.4	56.8	55.2

Effect of temperature on ethanol production: During summer season there is no strict control over temperature of fermentation vats which ever goes to 40°C affecting the rate of ethanol production (Cazetta *et al*., 2007). Fermentation of 20% slurry of potato flour was carried out at different temperatures (30, 35, 37 & 40°C) under stationary conditions up to 96 h. A maximum ethanol content of 56.8 gl^{-1} was recorded after 48 h of fermentation at 30°C (Fig. 3). However at temperature 35, 37 and 40°C, the corresponding values were 53.6, 50.0 and 46.0 gl^{-1} respectively showing a decline with increased temperature of fermentation.

Ethanol production after removal of residue from hydrolysate: The residual suspended matter after liquefaction and saccharification of starch in potato slurry may interfere in the handling of slurry during large scale fermentation. Thus the sediment was removed by centrifugation at 4000 rpm and the supernatant with soluble sugars was used for production of ethanol. Ethanol level of 53.6 gl^{-1} was produced in supernatant after 24 h which further increased to 56.8 gl^{-1} after 48 h (Fig. 4). This suggests that the supernatant after liquefaction and saccharification of starch present in potato flour can be used as effectively as the potato flour.

Discussion

Potatoes can be an appropriate alternate substrate for bioethanol production due to their plenty availability, low cost and easy processing. For better penetration of enzyme into substrate size reduction is must. Potato flour prepared by cooking, mashing and grinding was used for ethanol production by *Saccharomyces cerevisiae*. Smooth slurry is prerequisite for fermentation that was prepared by liquefaction of potato flour with amylase at 80°C for 30 min and it took 2 h for saccharification of liquefied slurry by glucoamylase at 60°C.

Addition of nutrients such as ammonium sulphate, urea, yeast extract and peptone have been reported to play a vital role in boosting ethanol production and its rate (Fundora *et al*., 2000). Wang *et al.* (2007) found that addition of 1.5% peptone in the medium increased the final ethanol titre from 14.2%-17.0% in 48 h. The response of added nutrients in potato flour was low for ethanol production suggesting that potato as such may be a good substrate for fermentation was an interesting observation. Because of the long period (2 h) needed to achieve the saccharification it was necessary to explore the possibilities to carryout simultaneous saccharification and fermentation (SSF) after liquefaction that was found equally effective as separate hydrolysis and fermentation. Chen *et al.* (2007) found that ethanol concentration in solid state SSF using temperature cycling (10 h at 37°C with 15 min at 42°C) was 2 times more effective than using constant 37°C. Montesinos and Navaroo (2000) also reported reduction in fermentation time in SSF of wheat flour giving ethanol concentration of 67 gl^{-1} .

Conclusion

Potato flour prepared by cooking, mashing and grinding was used for ethanol fermentation by *Saccharomyces cerevisiae.* Homogenised (1:4) slurry was obtained on treatment with α-amylase (2.05 DUN U/g starch) at 80°C for 30 min which was saccharified with glucoamylase (20.5 GA U/g starch) at 60°C for 2 h. The addition of nitrogen sources did not affect ethanol production. The other conditions were also standardized for optimization of ethanol production. The interesting observation was the feasibility of ethanol production with

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simultaneous saccharification and fermentation (SSF) which is being scaled up at pilot scale.

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