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Production of ethanol from tuberous plant (sweet potato) using *Saccharomyces cerevisiae* MTCC-170

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The aim of this work was to research a bioprocess for bioethanol production at laboratory scale from raw sweet potato using *Saccharomyces cerevisiae* MTCC-170. In order to obtain maximum conversion of starch into fermentable sugar, optimum parameters for the liquefaction were determined as 104 to 105°C, 0.15% v/w of α-amylase enzyme solution (300 U/ml) and 30 g dry-weight sweet potato mash/100 ml distilled water, respectively with a 74.38% loss in dry weight during the process. For saccharification process, the optimum dose of amyloglucosidase was 0.25% v/w (300 U/ml) with 16.82% glucose production at pH 5.0 and temperature 60°C after 1 h. The fermentation parameters like inoculum size, temperature, pH and different concentrations of nutrients were also determined. The maximum ethanol concentration, that is, 7.95% (v/v) was obtained with 10% inoculum size at pH 6.0 after 48 h. Furthermore, out of the three nitrogen sources (yeast extract, peptone and ammonium sulphate) tested for ethanol production, peptone at a concentration of 1.5 g/L was found to be best (7.93%). From the present study, it may be concluded that sweet potato can be an attractive feedstock for bioethanol production from both the economic stand points and environment friendly.

Key words: Sweet potato starch, ethanol, liquefaction, saccharification, *Saccharomyces cerevisiae* MTCC-170.

INTRODUCTION

Petroleum is the source of about 170 quads of energy out of the total of more than 460 quads used by the world which is far more than derived from other sources (IPCC, 2007). Besides the negative global warming impact of fossil fuels, volatile oil price and political unstable in oil exporting countries resulted in a significant increase in international interest in alternative fuels and led policy makers in the world to issue ambitious goals for substitution of alternative for conventional fuels (Galbe and Zacchi, 2002; Wyman, 2007). Bioethanol made biologically by fermentation from a variety of biomass sources is widely recognized as a unique transportation fuel and original material of various chemical with powerful economic, environmental and strategic attributes.

According to the US Department of Agriculture, recent experiments note that sweet potatoes yield two to

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three times as much fermentable carbohydrate as field corn (USDA, 2008). Sweet potato (*Ipomea batatas*) has been considered as a promising substrate for alcohol fermentation since it has a higher starch yield per unit land cultivated than grains (Duvernay et al., 2013; Lee et al., 2012; Srichuwong et al., 2009; Ziska et al., 2009). Industrial sweet potatoes are not intended for use as a food crop. They are bred to increase its starch content, significantly reducing its attractiveness as a food crop when compared to other conventional food cultivars (visual aspect, color, taste etc.). Therefore, they offer potentially greater fermentable sugar yields from a sweet potato crop for industrial conversion processes. It has been reported that some industrial sweet potatoes breeding lines developed could produce ethanol yields of 4500 to 6500 L/ha compared to 2800 to 3800 L/ha for corn (Duvernay et al., 2013; Ziska et al., 2009). Sweet potato has several agronomic characteristics that determine its wide adaptation to marginal lands such as drought resistant, high multiplication rate and low degeneration of the propagation material, short grow cycle, low illness incidence and plagues, cover the soil rapidly and therefore protect it from the erosive rains and controlling the weed problem (Cao et al., 2011; Duvernay et al., 2013).

Microorganisms meet their energy demand by converting carbon sources to by-products such as: carbon dioxide, lactic acid, ethanol etc. *Saccharomyces cerevisiae, Zymomonas mobilis, Kluyveromyces* spp. and *Schizosaccharomyces pombe* are microorganisms able to convert sugars to ethanol. Various feedstock and chemically defined media can be used for ethanol fermentation. The most commonly used types of feedstock for ethanol production are corn, sugar cane and wheat (Balat et al., 2008). Sugarcane, sugar beets and molasses are feasible for ethanol fermentation and have been used; however, these carbon sources are high value products as food sources (Nalley and Hudson, 2003; USDA, 2006). In order to meet the low cost requirement, lignocellulosic biomass is another option for ethanol fermentation. However, lignocellulosic biomass is complex and requires expensive pre-treatments. Currently, sweet potatoes are alternative feedstock for ethanol production. Sweet potato like other starchy root crops is a cheaper substrate and can therefore serve as raw material for fermentative production of commodity chemicals. Cultivated in more than 100 countries, sweet potato ranks third of the world root and tuber crops production after potato and cassava (FAO stat, 2010).

The sweet potato (Pusa Lal) used in this work was identified as a sustainable crop for bioethanol production based on both its favourable energy balance and the net greenhouse gas (GHG) emission reduction, evaluated on a life cycle analysis conducted for local conditions in Uruguay (Carrasco-Letelier et al., 2013). It was developed as culture for bioenergy purposes on the basis of its high starch yields. This sweet potato variety has significantly reduced its attractiveness as a food crop when compared to other conventional food cultivars. The main objective of this work was to develop an economical bioprocess technology to produce bioethanol from raw sweet potato at laboratory scales and determined the effect of fermentation temperature, inoculum sizes, pH and effect of different nutrients on fermentation parameters.

MATERIALS AND METHODS

Characterization of raw material: Sweet potato

Raw fresh sweet potato (Pusa Lal) harvested in February 2009 was procured from Ch. Charan Singh Haryana Agriculture University, Hisar and was stored at room temperature (at about 20°C for 30 days). Thoroughly washed peeled sweet potato (1.0 kg) were dried overnight at 70°C and grounded to fine powder. The carbohydrate composition of sweet potato flour was determined by 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Protein concentration was determined by the Kjeldahl method. Ashes were quantified by gravimetric analysis after burning samples at 550°C for 5 h. Moisture content was determined by gravimetric analysis after drying at 105°C to constant weight. The C, H, N was analyzed by standard methods (AOAC, 1990).

Enzyme for liquefaction and saccharification

Commercial α-amylase (Specific activity 300 DUN U/ml) and amyloglucosidase (Specific activity 400 GA U/ml) were obtained from Sigma-Aldrich Pvt. Ltd., India.

Preparation of sweet potato flour slurry

Slurries of various concentrations (10, 15, 20, 25 and 30% w/v) of sweet potato flour starch was prepared in water and treated with liquefying enzyme (0.15% v/w) at 104 to 105°C for 60 min in an autoclave. The slurry prepared by mixing 25 g flour in 100 ml water (1:4) being homogenous, loose, easy to handle was used for further experiments. Liquefaction of sweet potato flour (100 ml slurry) was carried out at 104 to 105°C in an autoclave using varying concentration of enzyme (0.05 to 0.20% v/w) for different time intervals (10 to 240 min). The progress of liquefaction was monitored by employing starch-iodine (1.0 g of iodine and 2.0 g KI in 100 ml water) reaction. Saccharification of liquefied starch was carried out at 60°C for different time intervals using varying concentration (0.05 to 0.45% v/w) of amyloglucosidase. The reaction was monitored by the yield of total reducing sugars estimated by dinitrosalicylic acid method (Miller, 1959).

Yeast strain

A fast fermenting strain of *S. cerevisiae* MTCC-170 was obtained from Microbial Type Culture Collection, Chandigarh (India) and maintained on yeast extract peptone dextrose (YEPD) agar medium containing yeast extract (1%), peptone (2%), dextrose (2%) and agar (2%). Dextrose inoculum medium used for inoculum preparation contained dextrose (6%), peptone (0.5%) and yeast extract (0.5%). Yeast cells pregrown in inoculum medium for 18 h under shaking condition (100 rpm) was used directly as inoculum at 10% (v/v).

Table 1. Composition of starchy raw materials.

Raw material	Source	Chemical composition % (w/w)					
		Starch content		Nitrogen content	Protein content	Phosphorus content	Ash content
		Acid hydrolysis	Enzymatic hydrolysis				
Sweet potato (Pusa Lal)	CCS HAU, Hisar	69.26	70.34	0.75	4.50	0.56	4.10

Optimization of fermentation conditions

Effect of inoculum concentration

The hydrolysate was inoculated with different concentrations of inoculums; that is, 5, 10, 15 and 20% (v/v) and kept for fermentation at 35°C for 48 h.

Effect of temperature

The hydrolysate inoculated with the best combination of nutrients and fermentation was carried out at various temperatures viz. 25, 30, 35 and 40°C. Ethanol content in fermented samples was estimated after 48 h of incubation.

Effect of pH

The pH of hydrolysate was adjusted to different levels and was fermented after supplementation with the best combination of nutrients after inoculating with 10% inoculum (v/v). The fermentation was carried out at 35°C for 48 h.

Effect of nutrient concentration

To 100 ml hydrolysate, different nutrients like ammonium sulphate (0.3%), yeast extract (0.5%) and peptone (0.5%) was added in their single and double concentration. The flasks were inoculated with 10% yeast cells (v/v). The fermentation was carried out at 35°C for 48 h.

Analytical methods

Estimation of reducing sugars

The DNS method given by Miller (1959) was used to estimate reducing sugars of the samples.

Ethanol determination

Ethanol concentration was determined by the method given by Caputi et al. (1968). All reagents were of analytical grade. Concentrations were calculated by means of standard curves relating individual concentration to peak area. Every experiment was conducted in triplicate.

Calculations

The maximum theoretical yield of ethanol from sugar was calculated according to the stoichiometric relation given in Equation (1), that is, 100 g of hexose produce 51.1 g of ethanol and 48.9 g of CO_2. Ethanol yields over total initial sugars (Y_1) and average ethanol productivity rate (Y_2) were calculated according to Equations (2) and (3) as given by Zhang et al. (2011).

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (1)$$

$$Y_1 = \frac{\text{ethanol produced in fermentation}}{\text{ethanol produced in theoretical}} \times 100 \quad (2)$$

$$Y_2 = \text{final ethanol concentration/fermentation time} \quad (3)$$

Statistical analysis

All experiments were carried out in a completely randomized design. The results were subjected to analysis of variance (one-way ANOVA), and the treatment means were compared using the least significant difference (LSD) values at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Composition of sweet potato

Carbohydrate composition of sweet potato flour was analyzed by hydrolyzing the sweet potato flour by 54% concentrated perchloric acid at high temperature of 100°C for 2 h. Total glucose derived from starch, cellulose and soluble portion occupied approximately 91% of dry matter.

The total carbohydrates concentration of sweet potato tuber was 76.34% (v/v) and contained 63.30% (v/v) of starch, 70.34% (v/v) of glucose. Nitrogen contents, ashes, protein contents and phosphorus contents of sweet potato mash were approximately 0.75% (w/v), 4.10% (w/v), 4.50% (v/v) and 0.56% (v/v), respectively (Table 1).

Optimization of condition for liquefaction process

The optimum combination of temperature, dose of enzyme (α-amylase) and amount of sweet potato flour slurry was determined as 104 to 105°C, 0.10% v/w of α-amylase enzyme solution (300 U/ml) and 30 g dry-weight sweet potato mash/100 ml distilled water, respectively

Table 2. Summary of liquefaction.

Raw material	Slurry % (w/v)	Enzymes % (v/w)	pH	Temperature (°C)	Time (h)	Ca^{++} (mM)	K^{+} (mM)
Sweet potato	25	0.10	6.2-7.0	104-105	1	0.36	0.30
Pusa Lal	30	0.10	6.2-7.0	104-105	1	0.72	0.30

Table 3. Summary of saccharification.

Raw material	Slurry %(w/v)	Enzymes % (v/w)	pH	Temperature (°C)	Time (h)	Sugar production %(w/v)
Sweet potato	25	0.25	5.0	60	1	14.58
Pusa Lal	30	0.25	5.0	60	1	16.62

with a 68.86% loss in dry weight during the process (Table 2). Kumar et al. (2013) used different concentrations (15 to 45% dry weight/volume) of potato powder for maximum liquefaction, which was carried out using steam under pressure (0.3 to 0.4 lbs, 104 to 105^{o}C) to liquefy the slurry in 1 h. Slurry having 25 and 30% substrate concentration was found to be the best. Alpha-amylase dose optimized for the liquefaction process was 0.15% v/w.

Optimization of saccharification

Dose of enzyme, temperature and saccharification time were optimized for the saccharification process. The optimum dose of amyloglucosidase was 0.25% v/w (300 U/ml) with 16.62 g/100ml glucose production after 1h at 60°C for sweet potato (Table 3). Kumar et al. (2013) observed that optimum temperature for saccharification was found to be 60°C, pH was 5.0 and dose of amyloglucosidase was 0.35% (v/w) when potato was used as a substrate.

Optimization of fermentation conditions

Optimization of inoculum size for ethanol production

In the present study, initial total carbohydrate concentration was 168.1 g/L. In order to determine the economic inoculums size, the inoculums sizes ranging from 5 to 20% were tested for ethanol production using SSF of sweet potato flour with *S. cerevisiae* MTCC-170. As shown in Figure 1, there was significant difference among the inoculum sizes (5, 10, 15 and 20%) tested w.r.t. kinetic parameters in ethanol production. The maximum ethanol concentration (6.73% v/v), sugar utilization (78.19%) and ethanol yield (74.70%) were obtained with an initial inoculum of 10%, which is economic and environment friendly. The shortening of fermentation time with the raise in the inoculum size was due to the fast cell growth within the reactor and most of the substrate was immediately converted to ethanol. According to the study of Fadel (2000), the maximum alcohol production (12.9%) was obtained when inoculated with 10% culture of *S. cerevisiae.* Similarly, Afifi et al. (2011) also observed maximum ethanol production from industrial solid potato wastes when inoculated with 10% (v/w) inoculums size of *S. cerevisiae.* Different inoculum sizes (2, 4, 6, 8 and 10% v/v) were tested for a period of 24 h and observed that the maximum ethanol concentration that is, 8.8% was obtained at 10% inoculum size (Neelakandan and Usharan, 2009). Turhan et al. (2008) reported that maximum ethanol concentration, ethanol productivity and ethanol yield were 42.90 g/L, 3.7 g/L/h and 45%, respectively, obtained with an initial inoculum of 3% when carob extract used as a substrate by using *S. cerevisiae*.

Effect of temperature on ethanol production

Effect of temperature on growth and ethanol production of *S. cerevisiae* MTCC- 170 was also studied. Different temperatures (30, 35 and 40°C) were tested to check the thermal tolerance of *S. cerevisiae* MTCC-170 in sweet potato flour media. From the present study, it was observed that 35°C was the most appropriate temperature for yeast growth. Production of ethanol by *S. cerevisiae* MTCC-170 was also favoured by this temperature and reached its maximum (7.99% v/v) after 48 h. At 45°C, ethanol production was reduced to 2.26% v/v. Both low and high temperature (30 and 40°C) had detrimental effect on ethanol production and reduced it to 7.50 and 4.30% v/v, respectively (Figure 2). Thermostability of a yeast strain is more likely genetically controlled.

Variation in thermal requirements for biomass and ethanol production stimulates the suggestion that enzymes involved in ethanol fermentation, vary in their thermal optima than those that are involved in biomass synthesis. Hashem and Darwish (2010) observed that production of ethanol by *S. cerevisiae* y-1646 was

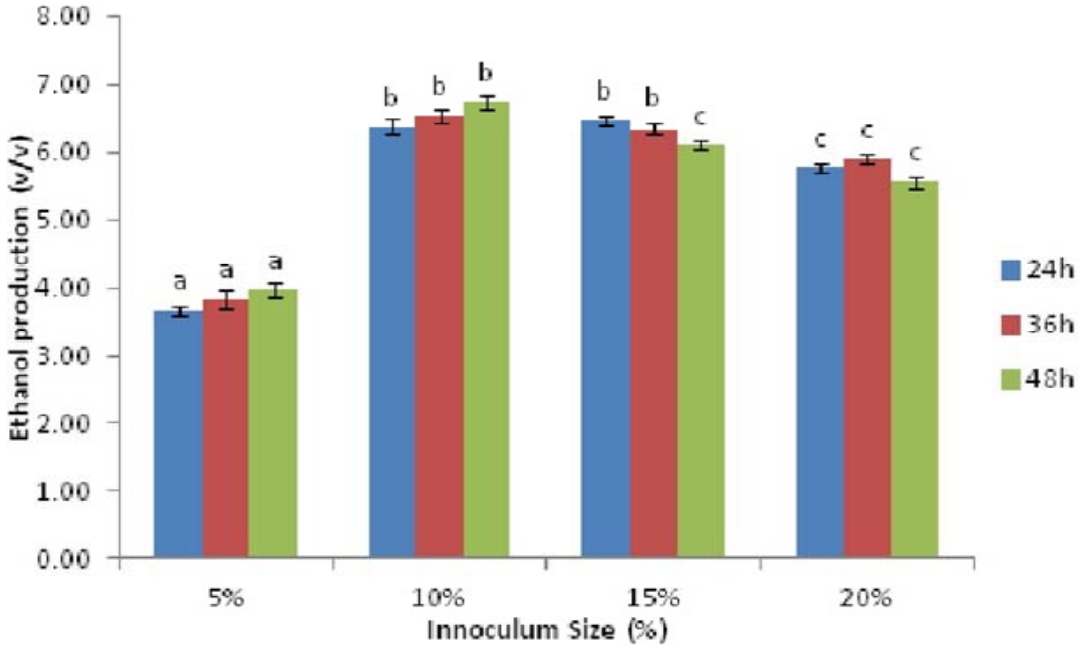

Figure 1. Effect of inoculums size on ethanol production from supplemented sweet potato flour hydrolysate. Values followed by the same letter are not significant at 5% level.

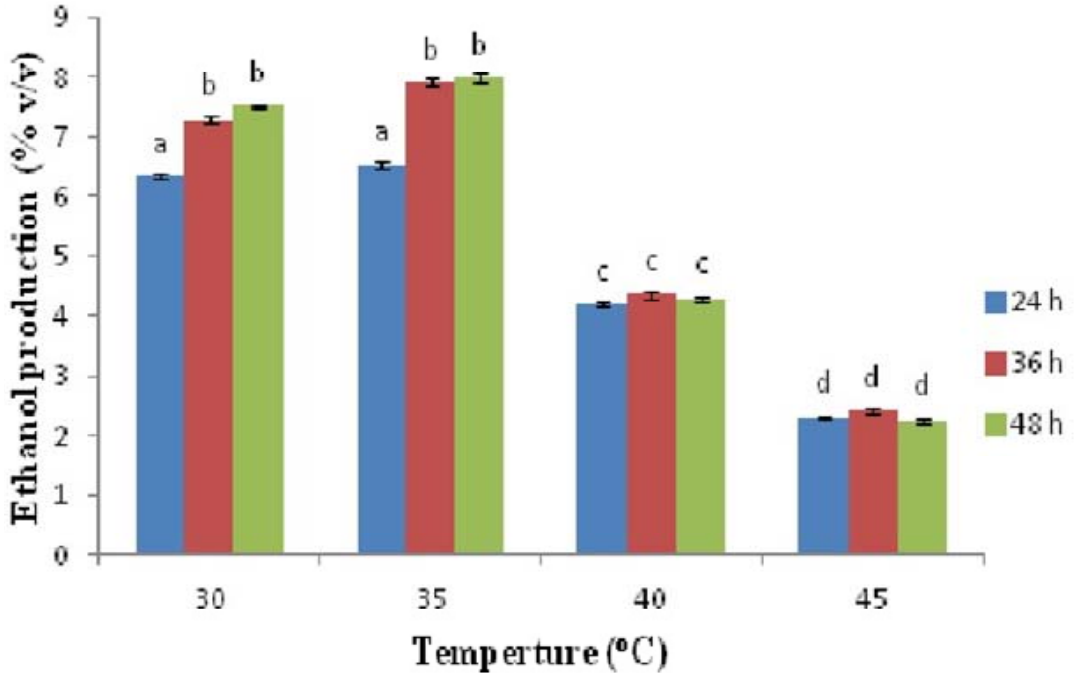

Figure 2. Effect of temperature on ethanol production from supplemented sweet potato flour hydrolysate. Values followed by the same letter are not significant at 5% level.

favored at 35°C temperature and reached its maximum value (5.29 g/L) after 36 h. At 37°C, ethanol production was reduced to 4.38 g/l. Rivera et al. (2006) considered the temperature as the variable to evaluate the optimum expected parameter of ethanol fermentation. Based on experimental data, maximum ethanol production is achieved at 28 to 31°C.

Rani et al. (2010) observed that maximum ethanol

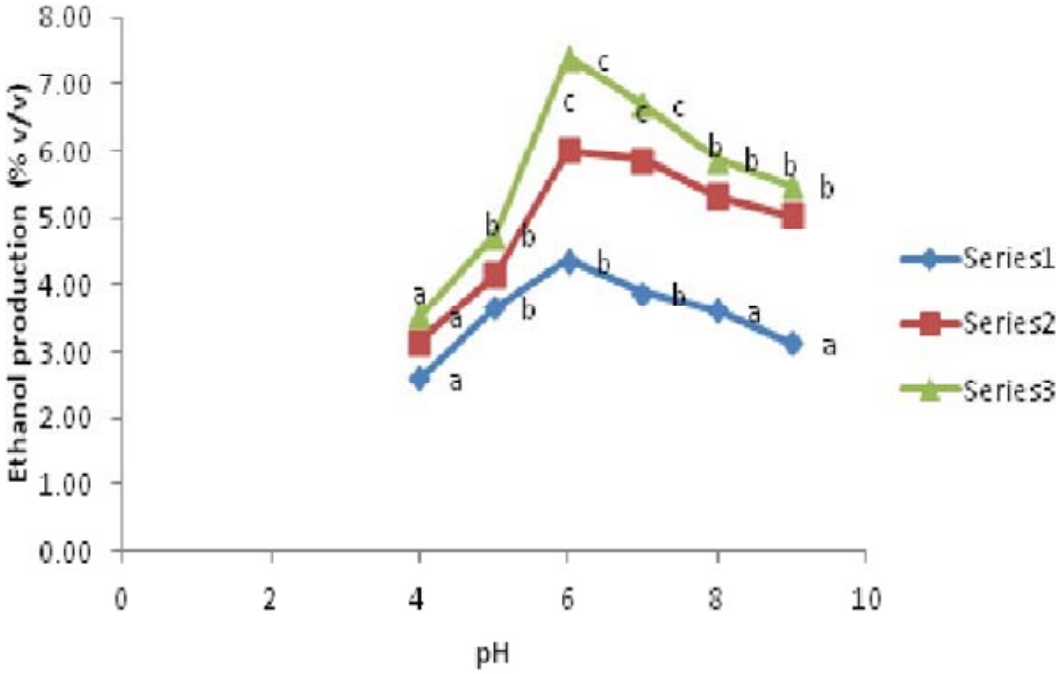

Figure 3. Effect of pH on ethanol production from supplemented sweet potato flour hydrolysate. Values followed by the same letter are not significant at 5% level. Series 1, 2 and 3 represent 24, 36 and 48 h fermentation, respectively.

content of 56.8 g/L was recorded after 48 h of fermentation at 30°C. However at temperatures 35, 37 and 40°C, the corresponding values were 53.6, 50.0 and 46.0 g/L, respectively showing a decline with increase in temperature of fermentation. Duhan et al. (2013) observed bio-ethanol production increases with increase in temperature and reaches its maximum value at 35°C. Asli (2010) observed best ethanol production rate at 32°C temperature. Osman et al. (2011) obtained maximum ethanol production and biomass from sugar cane bagasse at 28 to 30°C.

Effect of pH on ethanol production

The pH is one of the most important factors for any fermentation process and depends upon microorganisms because each microorganism possesses pH range for its growth and activity. Increase or decrease in pH on either side of the optimum value resulted in decrease in growth and activity of microorganisms. Ethanol fermentation was evaluated at different pH profiles (ranges) to determine the effect of pH on ethanol production. As shown in Figure 3, the ethanol concentration was increased from pH 4.0 to 6.0 and decreased marginally above this value. The maximum ethanol concentration 7.41% was obtained when culture (*S. cerevisiae* MTCC-170) was grown at pH 6.0. Fadel (2000) reported that high ethanol production was obtained by using initial pH 5.0 to 6.0. Graves et al. (2006) observed that no ethanol production exists lower than pH 4.0. Turhan et al. (2008) also reported that maximum ethanol yield, growth rate and biomass concentration were obtained at pH 5.5 on carob as a medium for ethanol production. Osman et al. (2011) tested wide initial pH range and found that at pH 3.0, no growth was observed and no ethanol was produced, while pH 6.0 was the optimum for both biomass and ethanol production. Mohanty et al. (2009) reported that pH 6.0 was optimum for bioethanol production from mahula (*Madhuca latifolia* L.) flowers by solid-state fermentation. Togarepi et al. (2012) also obtained maximum ethanol production at pH 6.0 when *Ziziphus mauritiana* fruit pulp was used as a substrate. Afifi et al. (2011) optimized pH; that is, 3.5 for maximum ethanol production from industrial solid potato wastes. Kundiyana et al. (2010) studied the fermentation of sweet sorghum juice at different pH levels and observed that the highest ethanol yield could be obtained at a pH of 4.3.

Effect of nutrients on ethanol production

Effect of ammonium sulfate (nitrogen source) on ethanol production

The effects of three nutrients (ammonium sulfate, dipotassium hydrogen phosphate and yeast extract) on the ethanol yield from sweet potato were investigated.

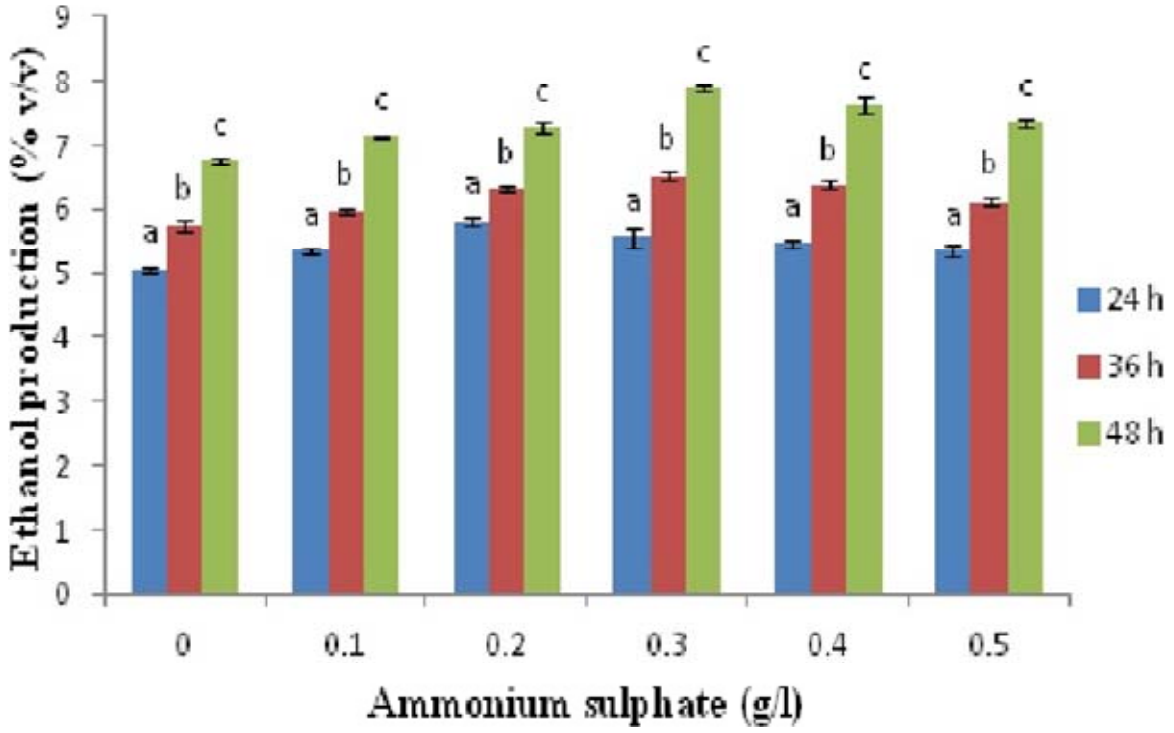

Figure 4. Effect of ammonium sulphate on ethanol production from supplemented sweet potato flour hydrolysate. Values followed by the same letter are not significant at 5% level.

Effect of ammonium sulphate as a nitrogen source was studied by varying its concentration between 1.0 to 5.0 g/L keeping rest of the parameters at their optimal conditions. Data from the Figure 4 shows that as the concentration of ammonium sulphate increased from 1 to 3 g/L, ethanol production also increased from 6.98 to 7.28% for *S. cerevisiae* MTCC-170, above this concentration, ethanol production decreases when sweet potato was used as substrates. Beltran et al. (2007) studied the effect of ammonium sulphate with different concentrations ranging from 0.01 to 0.09 g/L and observed that maximum production was obtained at 0.06 g/L concentration of ammonium sulphate.

Similarly, Amutha and Gunashekaran (2000) also observed that ethanol yield increase from 44.2 and 54.9 g/L, by supplementation of liquefied cassava starch with ammonium sulphate (1 g/L). Srichuwong et al. (2009) studied the saccharification simultaneous fermentation of very high gravity (VHG) potato mash for the production of ethanol and obtained 2.0 to 2.5% more ethanol concentration as compared to control when supplemented with ammonium sulphate. Anupama et al. (2010) obtained optimum ethanol yield of 5.6% with 3 g/L concentration of ammonium sulfate used as a nitrogen source. Slight increase in growth and ethanol production by *S. cerevisiae* y-1646 was observed after addition of NH_4NO_3 (4 g/L) as a source of nitrogen (Hashem and Darwish, 2010).

Effect of yeast extract on ethanol production

Yeast extract proved to be very efficient for increasing fermentation rate, but yeast extract is an expensive additive, which should at least be added in smallest possible amounts in order to make the process economical viable. Effect of yeast extract was studied by varying its concentration from 1.0 to 3.0 g/L, keeping rest of the parameters at their optimal conditions. Data from the Figure 5 shows that as the concentration of yeast extract increased from 1.0 to 2.0 g/L, ethanol production also increased from 6.55 to 7.11% with *S. cerevisiae*, above this concentration, ethanol production was decreased when sweet potato was used as substrates. Likewise, Nuanpeng et al. (2012) studied the effect of yeast extract concentrations on sugar consumption, ethanol production and yeast cell viability during very high gravity batch fermentation of *S. cerevisiae* NP 01 from sweet sorghum juice and observed the highest ethanol concentration in the EP medium containing 9.0 g/L of yeast extract. Thomas and Ingledew (1992) observed that 1% yeast extract supplementation stimulate VHG fermentation of wheat mash to yield 21% (v/v) of ethanol within 4 days. Similarly, Duhan et al. (2013) showed that maximum ethanol production that is, 7.11% was obtained at 2.0 g/L yeast extract for *S. cerevisiae* when potato (Kufri Bahar) was used as substrates.

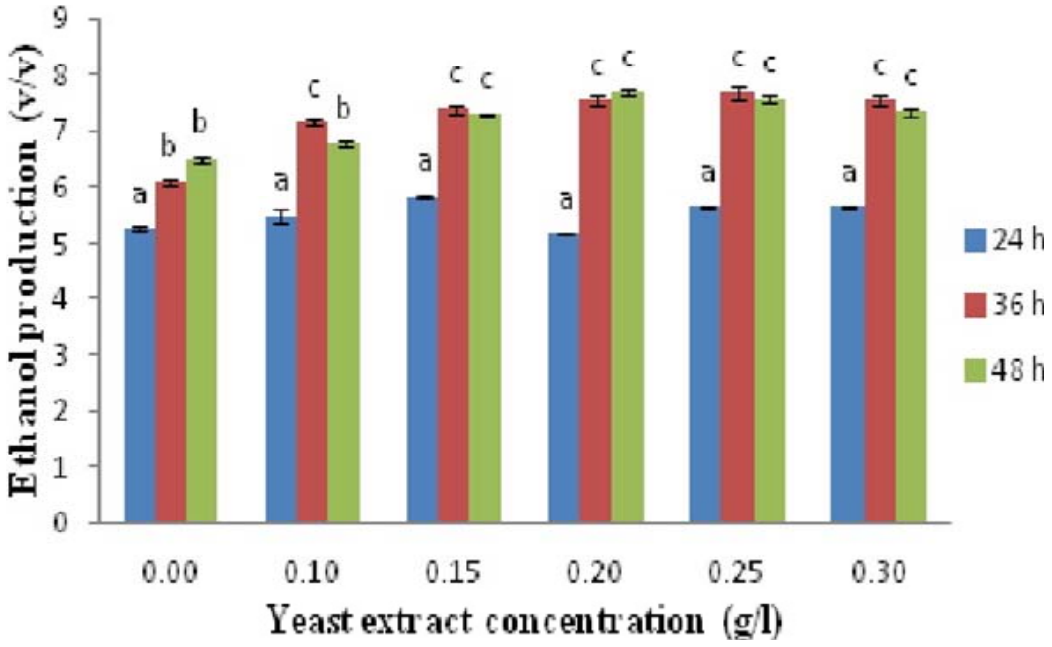

Figure 5. Effect of yeast extract on ethanol production from supplemented sweet potato flour hydrolysate. Values followed by the same letter are not significant at 5% level.

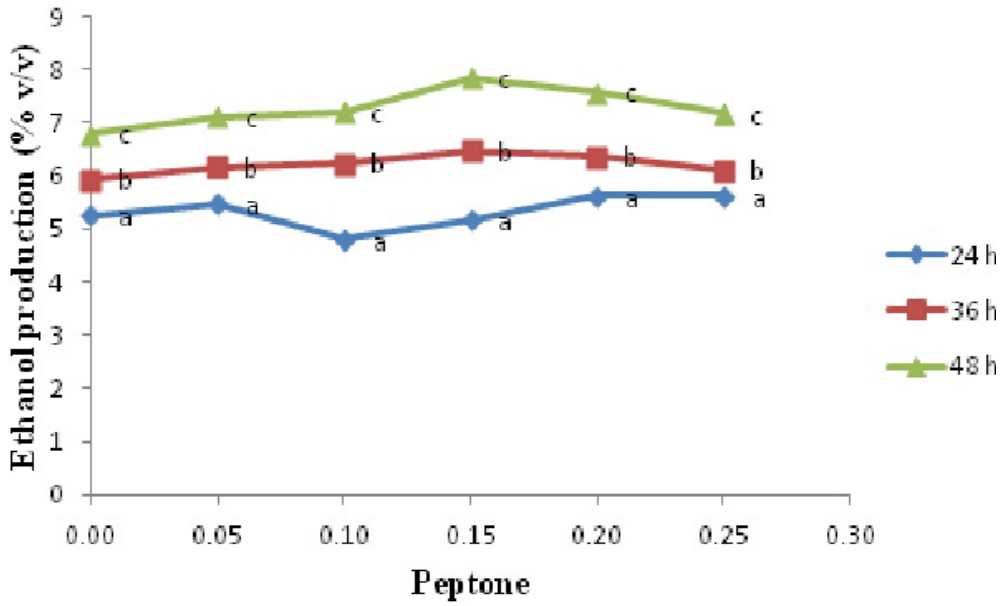

Figure 6. Effect of peptone on ethanol production from supplemented sweet potato flour hydrolysate. Values followed by the same letter are not significant at 5% level.

Effect of peptone on ethanol production

To study the effect of peptone on ethanol production on various concentrations ranging from 0.5 to 2.5 g/L were used. Data in the Figure 6 shows that as the concentration of peptone increased from 0.5 to 1.5 g/L, ethanol production also increases from 5.17 to 7.86% with *S. cerevisiae* and above 1.5 g/L concentration ethanol production was decreased when sweet potato was used as substrate. Wang et al. (2007) studied that 1.5% (w/v) peptone in the medium increased the final ethanol titre from 14.2% (v/v) to 17% (v/v) in 48 h.

Addition of peptone at a concentration of 1% reported to play a very important role in increasing the ethanol yield and the rate of fermentation (Fundora et al., 2000). Dake et al. (2010) obtained maximum ethanol concentration at 0.5% (w/v) of peptone concentration.

Conclusion

Sweet potato flour prepared by oven drying, mashing and grinding was used for ethanol fermentation by *S. cerevisiae* MTCC-170. According to the results in terms of liquefaction, the process conducted at 105°C using 30 g flour of sweet potato and 0.10% v/w α-amylase for 1 h was found to be the most suitable, considering higher liquefaction yield, and when saccharified with gluco-amylase (20.5 GA U/g starch) at 60°C for 2 h the maximum amount of fermentable sugar was released from sweet potato flour that is, 16.84 g/100 ml. In the present study, experimental conditions were tested for liquefaction and saccharification, revealing the higher performance of α-amylase and amyloglucosidase. The addition of nitrogen sources in the fermentable medium increase the ethanol production. The other conditions were also standardized as temperature 35°C, pH 6.0, fermentation medium containing 168.1 g/L reducing sugars supplemented with 1% ammonium sulphate as nitrogen source, inoculum size of 10% of 24 h yeast culture (0.01 at 600 nm) and shaking rate 120 rpm for maximum ethanol production. Finally, 88.1 g/L ethanol was detected under these optimum conditions by batch fermentation. According to the results, it could be concluded that sweet potato is an attractive feedstock for the bioethanol production, since it provided the necessary nutrient element and the appropriate hydrogen balance for the fermentation.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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