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Production of Ethanol from *Ipomoea batatas* using *Saccharomyces cerevisiae*

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A study was carried out on yeast fermentation of sweet potato (*Ipomoea batatas*) using baker's yeast (*Sacchromyces cerevisiae*). The fermented sugar syrup (broth) was analyzed for yeast growth, alcohol content, reducing sugar, pH, soluble solids, volatile acids and total acidity using standard protocols. Yeast growth was also monitored. Results showed that pH values decreased with increased total acidity with concomitant increase in yeast growth (biomass) and alcohol contents of the fermenting sugar syrup. There were decreases in soluble solid contents, refractive indices of the fermenting medium. The reducing sugar in the *Ipomoea batatas* was lowest after 48 h of saccharification using *Sacchromyces cerevisiae* .The value recorded was 132 to 87 mg/100ml. Volatile acids (as acetic acids), increased with alcoholic fermentation. Fermentation of sugar syrup from *Ipomoea batatas* is associated with physical and chemical changes that occur in other form of fermentation alongside increased in biomass. The fermented *Ipomoea batatas* yielded ethanol contents of 11.5 to 53.0% (v/v).

Key words : *Ipomoea batatas*, Fermentation, *Saccharomyces cerevisiae*, Alcohol, Sugar syrup, Biomass.

INTRODUCTION

With the increasing value of petrochemical feedstocks, ethanol fermentation is bound to receive more attention (Ameh *et al*., 1988). The use of ethanol as an alternative motor fuel has been steadily increasing around the global scenario. It can be made synthetically from petroleum or by microbial conversion of biomass through fermentation and is used in the manufacture of drugs, plastics, polishes, plasticizers, perfumes, cosmetics, rubbers, accelerators and cellulose nitrate. Fermentation is one of the oldest processes known to man, and it is used in making a variety of products including foods, flavorings, beverages, pharmaceuticals, and chemicals (Gordon and Michael, 1979).

Ethanol is made from a variety of products such as grain, molasses, fruit, cobs, and shell; its production, excluding that of beverages, has been declining since the 1930s because of the low cost (Othman, 1981). It is one of the important industrial chemicals, can be produced extensively from *Ipomoea batatas*. The main constituents of this class of crop are carbohydrates (Chang *et al*., 1981; Cowling, 1976) that can be excellent energy source. This root vegetable qualified as an excellent source of vitamin A, C, B6, manganese, copper, dietary fiber, potassium and iron. It contains unique root storage proteins which had about one-third of internally produced antioxidant activity of *glutathione* (Hou *et al*., 2001). In this present study, *Ipomoea batatas*, which are readily available, were used for ethanol production.

MATERIALS AND METHODS

Isolation of yeast:

The *S.cerevisiae* was isolated from fermented grapes using yeast peptone glucose medium.

Formulation of fermentation medium:

The substrate namely sweet potato (*Ipomoea batatas*) was procured in a local market and the skin was removed. Then it was processed to formulate the medium through pasteurization followed by filtration of the syrup.

Fermentation:

In batch fermentation, the sterile syrup (200ml) was inoculated with 2% inoculum of *S. cerevisiae* in 500ml Erlenmeyer flask. The pH was adjusted to 4.5 and incubated in shaker at 110 rpm for 6 days at 30°C with intermittent collection of broth followed by biochemical analysis. At the end of fermentation, the alcohol was recovered by distillation and the content was determined by specific gravity method (AOAC, 1970).

Physical and chemical analysis:

pH was measured with a TOA pH meter (HM-3OS, TOA Electronics Limited, Tokyo, Japan). Total and volatile acidity were determined by the methods described in ISI Handbook of Food Analysis, Part X (ISI, 1984). Soluble solids and refractive index were measured using an Abbé refractometer (Mouri Industries Company Limited, Japan). Sugars were determined using AOAC methods 923.09 and 930.45 (AOAC, 1990). Alcohol content was determined using AOAC methods 920.57 and 913.02 (AOAC, 1990).

Total cell count:

100µl of sample from fermented broth taken at regular intervals was subjected to total cell count (TCC) using haemocytometer.

$$\text{Cell count} = \frac{X \times 400 \times 10}{16 \times 5} \times 10^3 \text{ cells/cm}^3$$

Estimation of reducing sugar:

The reducing sugar (glucose) content of the medium was determined by dinitrosalicyclic (DNS) method (Miller, 1959). The pH of the syrup was determined and adjusted to 4.5 with 0.5 M NaOH.

Acidity and volatile test:

The sample was taken and titrated against 0.1N NaOH using phenolphthalein as an indicator to measure the acidity (Aneja, 2003). Appearance of pale pink color serves the end point. Amount of alkali consumed was noted and the titration was repeated for concordant values.

$$\text{Total Acidity} = \frac{\text{ml of alkali added} \times 7.5 \times N}{\text{Amount of sample}}$$

$$\text{Volatile Acidity} = \frac{\text{ml of alkali} \times 6 \times N}{\text{Amount of sample}}$$

Estimation of alcohol (Caputi et al., 1968):

At 0th, 2nd, 4th, 6th day intervals the samples were collected and centrifuged at 10,000 rpm for 15 mins. The supernatant was taken and added with 25ml of chromic acid (potassium dichromate reagent) followed by incubation in a water bath at 80° C for 15 mins. After incubation 1ml of 40% sodium potassium tartarate was added and the OD was measured in a spectrophotometer at 600 nm. A standard graph was plotted by taking the different concentration of absolute alcohol (10 to 50%) on X-axis and OD on Y-axis, to compute the concentration of alcohol in test sample.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below:

Physical parameters:

Physical parameters like odour, color and clarity of the sugar syrup was examined at regular intervals (Table 1). The odour was sweet and fruity at the initial days, later that were in slight alcoholic smell and the color turned from light brown to dark brown. The sample was clear on the 0th day and slight turbidity was observed from 3-6th day, owing to growth of yeast.

Table 1: Physical parameters of the fermentation medium

Day intervals	Odour	Clarity	Color
0	Fruity	Clear	Light brown
2nd	Fruity	Turbid	Brown
4th	Alcoholic	Turbid	Brown
6th	Alcoholic	Turbid	Dark brown

Yeast biomass, reducing sugar, and alcohol content:

There was a rapid decrease in reducing sugar (132 to 87 mg/100ml) with concomitant increase in the alcohol content (11.5 to 53.0 %, v/v) over the fermentation period (Table 2). According to Webb (1964), it is evident that as the alcohol content of the broth increases, the sugar content decreases by a proportionate amount. The initial rapid decrease in the reducing sugar could correlate with rapid proliferation and catabolism of yeast cells (Nester *et al.*, 1995). The conversion of reducing sugars cease with 3rd day of fermentation, with the maximum alcohol accumulation (Stark, 1954; Ueda *et al.*, 1981; Ocloo and Ayernor, 2008).

Table 2 : Factors Influencing the Process of Saccharification

Period of saccharification (days)	Yeast biomass (cells/cm3)	Reducing sugar content (mg/100ml)	Ethanol yield (% v/v)
0	$400x10^3$	132 ±0.02	11.5± 0.12
2nd	$1250x10^3$	109 ±0.28	29.5± 0.03
4th	$1850x10^3$	99 ±0.49	41.5± 0.04
6th	$2100x10^3$	87± 0.12	53.0± 0.16

Note: ± values are means of duplicate experiments

The total number of cells was increased with increase in incubation period that showed *I. batatas* was a rich source of sugar and other minerals required for the growth of yeast cells. The percentage of alcohol production was

increased when concentration of the yeast was increased (Akin-Osanaiye *et al.*, 2005).

pH and total acidity:

A rapid decrease in pH of the syrup was observed until 4th day of fermentation followed by constant values (4.24). Consequently, there was a corresponding steady and rapid increase in the total acidity of the medium till the day 6 (Table 3). This phenomenon is parallel to the mechanism that the unionized weak acids such as acetic acid, which cause buffering action in the fermenting medium (Mark *et al.*, 1963). The change in pH could also attribute to the various intermediates produced during fermentation (Ashok *et al.*, 1999). The rise in the total acidity also corresponds to the fall in the reducing sugar and increase in alcohol concentration (Ocloo and Ayernor, 2008).

Table 3 : Changes in the and total acidity of the fermentation medium

Period of saccharification (days)	pH	Total acidity (g/100mL)
0	4.61	0.13
2nd	4.42	0.16
4th	4.24	0.45
6th	4.24	0.90

Soluble solids, refractive index and volatile acidity:

Soluble solids decreased from 26 to 13% after the 4th day of fermentation and then remained constant throughout the period. The decrease in soluble solids was in line with decreases in refractive index of the sugar syrup. The volatile acid of the medium increased during the fermentation (Table 4), which correlates with the results of Ocloo and Ayernor (2008).

Table 4 : Physiochemical properties of sugar syrup from sweet potato

Period of saccharification (Days)	Soluble solids (%)	Refractive index	Volatile acids as acetic acid (g/100mL)
0	26.0±0.56	1.373±0.46	0.10±0.96
2nd	15.0±0.25	1.361±0.72	0.13±0.38
4th	13.0±0.86	1.357±0.42	0.36±0.94
6th	13.0±0.75	1.357±0.65	0.72±0.37

Note: ± values are means of duplicate experiments

Conclusion:

The results obtained from the experiment reveal that glucose is present in a reasonable amount in *I. batatas*. If the product (glucose) is fermented under the stipulated experiment conditions with *Saccaharomyces cerevisiae* (baker's yeast), a substantial amount of ethanol, which is used as a chemical feedstock, will be produced. Thus, the importation of ethanol can be reduced if substantial energy is devoted to the production of ethanol from biomass. This will also have a multiplier effect such as jobs for the unemployed.

It is concluded from the investigation that *I. batatas*, a suitable substrate for the efficient production of biofuel ethanol using *S. cerevisiae*. Since the technology is cost effective and easy to operate, it is a boon to developing countries like India to solve the fuel needs, the alarming challenge of the nation. Future work in this scenario using enzymes and acid hydrolysis could ravel innovative approaches to excel in this regard.

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