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**RESEARCH ARTICLE**

# BIOETHANOL PRODUCTION FROM CASHEW APPLE JUICE USING *Saccharomyces cerevisiae*

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**ABSTRACT**

*Saccharomyces cerevisiae* was the cheapest strain available for the conversion of substrate. In the present study, it is used for bioethanol production from cashew apple juice. The influencing parameters that affect the production of bioethanol from cashew apple juice were optimized. Parameter optimization was done such as temperature, pH, inoculums size were studied, it was found to be pH of 4.5 temperature of 32°C and inoculum level $10^8$ respectively. Under this optimum operating condition the maximum of yield 6.5 % bioethanol was achieved.



## INTRODUCTION

Nowadays the petroleum products are running out due to unbalanced relation between supply and demand besides air pollution. The hike in petrol cost is mainly due to shortage of resources which leads to search for alternate fuel to replace fossil fuels. An eco-friendly bioethanol is one such alternate fuel that can be used in unmodified petrol engines with current fueling infrastructure and it is easily applicable in present day combustion engine, as mixing with gasoline (Hansen *et al.,* 2005). Combustion of ethanol results in relatively low emission of volatile organic compounds, carbon monoxide and nitrogen oxides. The emission and toxicity of ethanol are lower than those of fossil fuels such as petroleum, diesel etc., (Wyman and Hinman, 1990).

More than a few decades, though there have been several reviews of literature (Beatriz Palmarola *et al.,* 2005; Dale, 1987; Ferrari *et al.,* 1992; Martin *et al.,* 2006; Olsson and Hahn-Hagerdal, 1996) available for the production of bioethanol from various sources. However the *Saccharomyces cerevisiae* is the most important yeast strain widely used for bioethanol production. Moneke *et al*. (2008). Among the different substrates cashew is considered to be an in expensive substrate for ethanol production. However the conditions optimum for ethanol production has not been studied extensively. Hence to present study was undertaken to optimize the conditions suitable for ethanol production using cashew apple juice as substrate.

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## MATERIAL AND METHODS

***Substrate and Pretreatment*:** Waste cashew apples were brought from a factory processing cashew apples to extract the juice. Cashew apples are cut into slices in order to ensure a rapid rate of juice extraction when they are crushed in the juice press. The fruit juice is sterilized in stainless steel pans at a temperature of 85°C in order to eliminate any wild yeast. This juice, which contains high levels of tannins, was clarified by adding gelatin to remove tannins and suspended solids Campos *et al*. (2002). Then the clarified juice was filtered and treated with either sodium or potassium Metabisulphite, to destroy or inhibit the growth of undesirable types of micro-organisms such as acetic acid bacteria, wild yeast and moulds. Juice sample was filled in jars (capacity 2.5 liters) and was preserved at 4°C to prevent any possible degradation or spoilage during storage. This treated juice sample (contains 26.5% of total reducing sugars) was used throughout the experimentation

***Inoculum preparation:*** Inocula were grown aerobically in 250 ml erlenmery flasks containing the above mentioned medium at 30°C in an Environmental shaker (Remi Scientific) at 200rpm for 24h. Active cells were centrifuged in a clinical centrifuge (1200rpm), washed with sterile water, and were used as inoculums. Fermentation for ethanol production was conducted on a shaker at 200rp, in 250 ml flask with 100 ml medium.

***Fermentation:*** Cashew apple juice samples were measured (10ml/flask) and distributed into 250ml Erlenmeyer flasks with the assition of nutrient medium (YPM without glucose) at pH4 followed by sterilization for 15 min at 15 psi (121°C) in an autoclave. Yeast cells at the amount of 2g/l based on the dry weight were inoculated in to the production medium. Flasks were the covered with cotton to allow $CO_2$ produced during fermentation to escape .the flasks were incubated in a rotary shaker (200rpm) at 45°C for 36h.samples were withdrawn periodically, centrifuged in a laboratory desktop centrifuge at 1200rpm, and the supernatants were analyzed for glucose and ethanol concentrations.

***Determination of ethanol concentration:*** Samples were taken periodically and aseptically during fermentation for analysis of the glucose concentrations. The measurement of sugar concentration was done by DNS method (Miller, 1959 and Srinorakutara *et al.,* 2008). Ethanol was estimated using UNCON 5765 Gas chromatography (GC) with a flame ionization detector and propakQ (3mx0.32) column using Nitrogen as the carrier gas at the rate of 35ml/min. the oven temperature was held at 80°C. The injector and detector temperature was maintained at 200°C.

## RESULT AND DISCUSSION

***Optimization of inoculums level:*** The optimum quantity of sugar molasses solution was taken in fermentation flask and the pH and temperature were maintained at 4.0 and 32°C. The various quantities of yeast inoculums level $10^2$, $10^4$, $10^6$, $10^8$ and $10^{10}$ were added and kept for a period of five days and the fermented solution was analyzed at every 12 h intervals. Table 1 show that as the concentration of yeast increases, the yield of bioethanol (6.3%) increase up to $10^8$ and then it starts to decrease.

***Optimization of pH*:** The sample was fermented to different pH values between 2 and 5 to obtain maximum yield of bioethanol by adding lime or Sulphuric acid. The samples were kept in anaerobic condition for a period of four days and the fermented solution was analyzed for every 12 h intervals.

**Table 1. Effect of different levels of inoculum of *Saccharomyces cerevisiae* on bioethanol production from cashew apple juice (CAJ) at different time interval**

| Inoculum Level | Ethanol production (%)[a] | | |
|---|---|---|---|
| | **Fermentation period** | | |
| | **12 h** | **24 h** | **36 h** |
| **$10^2$** | 2.02±0.04$^e$ | 3.90±0.10$^e$ | 4.00±0.04$^e$ |
| **$10^4$** | 3.20±0.10$^d$ | 4.00±0.14$^d$ | 4.12±0.06$^d$ |
| **$10^6$** | 3.92±0.08$^c$ | 4.20±0.02$^c$ | 5.06±0.12$^c$ |
| **$10^8$** | 4.17±0.06$^a$ | 5.00±0.08$^a$ | 6.50±0.08$^a$ |
| **$10^{10}$** | 4.16±0.02$^b$ | 4..90±0.10$^b$ | 5.20±0.10$^b$ |

a - values followed by different letters are significantly differed at 5 % level according to student 't' test.

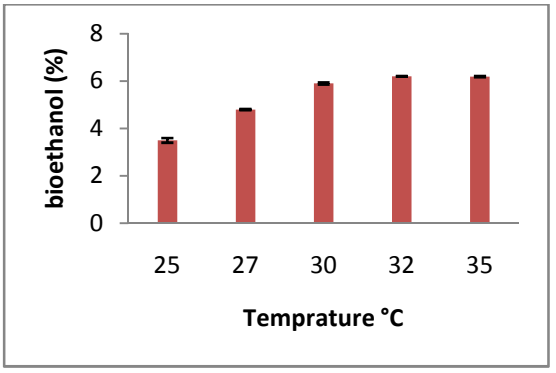


**Fig. 1. Effect of different levels of temperature on bioethanol production of *Saccharomyces cerevisiae* from cashew apple juice (CAJ)**

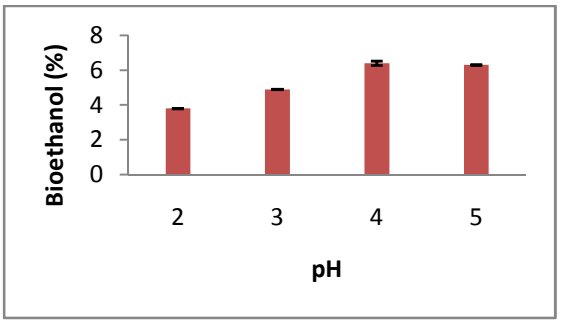


**Fig. 2. Effect of different levels of ph on bioethanol production of *Saccharomyces cerevisiae* (mutant strain scpm-10) from cashew apple juice (CAJ)**

The bioethanol concentration gradually increases along with the increase in pH and reaches a maximum percentage of bioethanol production when pH is equal to 4 and later it starts declining due to the lesser activity of yeast (Fig.1). De Vasconcelos *et al.* (1998) and Nigam, (1999) are also observed the maximum ethanol productivity at pH of 4.2 to 4.5.

***Optimization of Fermentation temperature:*** The sample maintained at an optimum pH 4 was fermented to different temperatures like 26°C, 28°C, 30°C, and 32°C. The samples were kept for fermentation period of four days and the fermented solution was analyzed for every 12 h intervals. Bio-ethanol production increases with the increase in temperature and reaches maximum value at 32°C Figure 2. Further the increasing temperature reduces the percentage of ethanol production and it is mainly due to denature of the yeast cells.

***Productivity of ethanol from cashew apple juice:*** The productivity of bio-ethanol increases along with the increase in fermentation period (Fig. 2.) and the maximum yield was obtained at 32h. The maximum concentration of bioethanol was found to be 8.00% at the temperature of 32 C, pH of 4 and yeast concentration of $10^8$.

## Conclusion

The optimized conditions were found by analyzing the cashew apple juice in the process of fermentation under various parameters like temperature, pH, and inoculum level , to obtain maximum yield of bioethanol. The optimized conditions of cashew apple juice are of temperature 32°C, pH 4.5 and the time 32 h which gives maximum bioethanol yield of 6.5%.

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