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METHODICAL OPTIMISATION AND PRODUCTION OF ALCOHOL FROM FRUIT WASTE USING *SACCHAROMYCES CEREVISIAE*

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Abstract: **The treatment and disposal of wastes of all kinds has been the problem of concern. Waste could be treated by reducing its bulk or by recovering and reprocessing it into useful substances. Waste materials from domestic and commercial establishments like fruit juice stalls are chief sources for the production of Alcohol. In the present study, waste from fruits was subjected for fermentative production of alcohol using *Saccharomyces cerevisiae*. Fermentation of fruit waste was studied for the reducing sugar concentration and the alcohol yield at every 72 hours interval. The results showed that after 9 days of fermentation, the production of alcohol was 8.5%.The study could be focused towards industrial scale on the methods which are economically reliable and environmental friendly.**

Keywords: **Fruit waste, Alcohol, Bio fuel, *Saccharomyces cerevisiae*, fermentation, Glucose concentration.**

I. INTRODUCTION

The field survey in Davangere city (total area 77 km^2) carried out at prominent and major commercial areas was found to be generating nearly one tone fruit waste in juice stalls alone. This could be estimated to the tune of about 350 tonnes per year which can potentially be tapped for the fermentative production of alcohol. *Saccharomyces cerevisiae* the most important fungus converts glucose into energy by virtue of its ability to metabolize its food through both aerobic respiration and anaerobic respiration. In the absence of oxygen it produces two byproducts; carbon dioxide and alcohol. The objective of the study was to identify the suitability for the *Saccharomyces cerevisiae* for the production of alcohol from fruit waste juice in controlled fermentation parameters. The optimized fermentative conditions for the production of alcohol economically would yield more product and also check the disposal of fruit waste into the environment that could pose health risks to humans. With increase in the demand of petroleum products and burning them are costing dear to the health of the environment. Hence promising alternative sources of energy are being considered worldwide. Till now the research community in India and abroad is trying to produce bio fuels from sources such as Jatrophacurcas, Pongamiapinnata, Azadirachtaindica, Madhucaindica, corn etc.

II. RESEARCH DESCRIPTION AND METHODS

a. Culturing:

***Saccharomyces cerevisiae*:** Using Sabouraud dextrose agar and Sabouraud dextrose broth, *Saccharomyces cerevisiae* was plate and broth cultured by incubating at 37^oC for 24 hours. A 25ml *S. cerevisiae* suspension was used as inoculums for the anaerobic fermentation process. The dry weight of yeast was estimated to be 2g after subjecting 25ml yeast suspension to centrifugation at 3000 rpm for 10 minutes and the sediment of yeast was freeze dried. However the top fermenting organisms are known to have higher growth rate in the temperature range between 38^oC to 42^oC.

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b. Proceeding With Fermentation:

In the sterilized fermentor vessel 3 liters of fruit waste juice extract was taken and the experimental parameters like pH at 4.0 and temperature at 38^{o}C for first 72 hours (3 Days), 40^{o}C for second 72 hours (6 days) and 42^{o}C for the third 72 hours (9 days) were set in an fully automated fermentor. A 25 ml batch of *Saccharomyces cerevisiae* was inoculated after biochemical estimation of glucose concentration in the fruit juice extract. The process was begun initially by providing aeration for 30 min in an intention to avoid sudden choking for organism and to acclimatize with changing experimental conditions. Ceasing aeration, the agitation of 80 rpm was started so that surface area for the organism to breakdown available glucose be increased as much as possible.

In nine day and night long fermentation process, for every 72 hours of interval the biochemical estimation of glucose concentration and the amount of alcohol produced was estimated in percentage by sampling the fermented broth. After the sampling, a fresh batch of 25 ml suspension of *Saccharomyces cerevisiae* was supplemented along with 3g of glucose with 30 minutes of aeration for gradual acclimatization. The depreciation of sugar concentration by the activity of organism was compensated by the addition of Glucose in place of Sucrose that minimizes the expense of metabolic energy by the fresh batch of organism to adapt to the changing experimental parameters. Further, the process of fermentation be economized in terms of energy needs to run the fermentor.

c. Biochemical Estimation of Glucose Concentration:

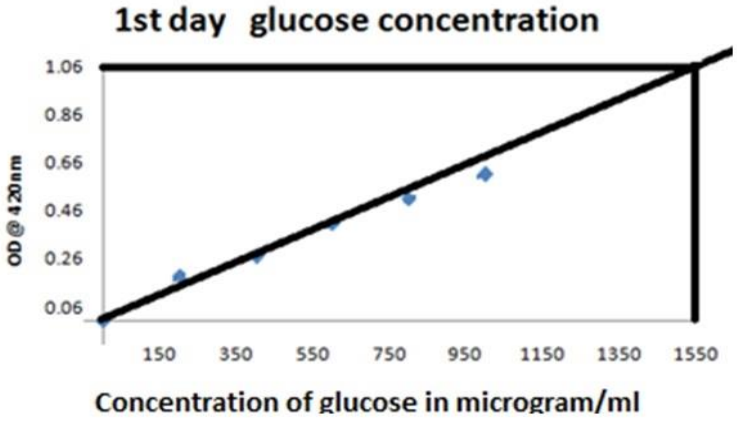
The Folin wu method of estimating glucose concentration was conducted for each sampling taken respectively after third, sixth and ninth day before inoculating fresh batch of organism and before the addition of 3g of glucose. The reagents alkaline copper solution and phosphomolybdic acid were used for the colorimetric analysis of glucose concentration. The finding was absolute in a sense that there was a successive depreciation of glucose concentration from 1250µg/ml to 810µg/ml to 380µg/ml respectively for third, sixth and ninth day samplings.

d. Spectrophotometric Estimation For Alcohol By Potassium Dichromate Method:

The usual methods for estimation of alcohol are relying on distillation of sample and the subsequent determination of specific weight of distillate collected. These methods are laborious and prolonged. Hence a sensitive spectrophotometric method for the determination of alcohol with potassium dichromate was deemed significant. The chemical oxidation reaction is complete in about 20 minutes at room temperature giving chromium and acetic acid per dichromate consumed. Moreover the sensitive spectrophotometric character of chromium enables the analysis of the sample in very small volume without a previous distillation. The method as customized provided satisfactory rate, exactness, and correctness for analysis of alcohol at 560nm. The method was followed for the 3 fermented broth samplings of third, sixth and ninth day before adding a fresh batch of *Saccharomyces cerevisiae*. The results shown that the alcohol percent in each of the fermented broth samples from third, sixth and ninth day analyzed has substantial increase in compliance with the respective change in temperature from 38^{0}C, 40^{0}C and 42^{0}C.

III. TABLES AND GRAPHS

1. Fruit waste showing Glucose concentration in an unfermented broth was 1550 µg/ml.



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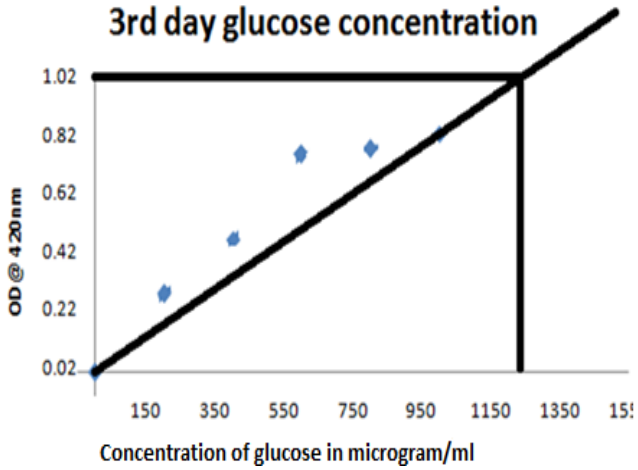
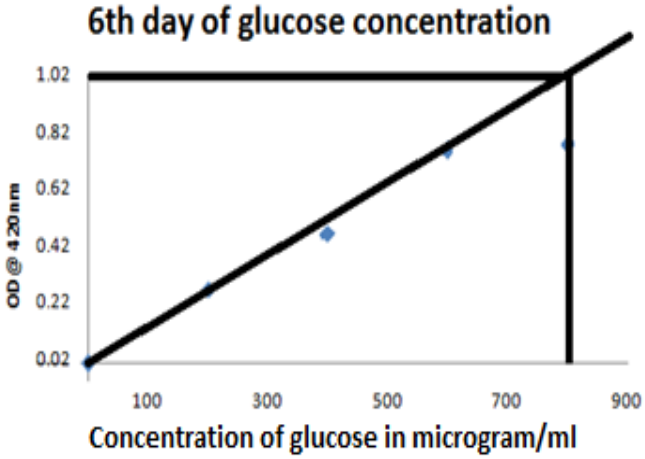
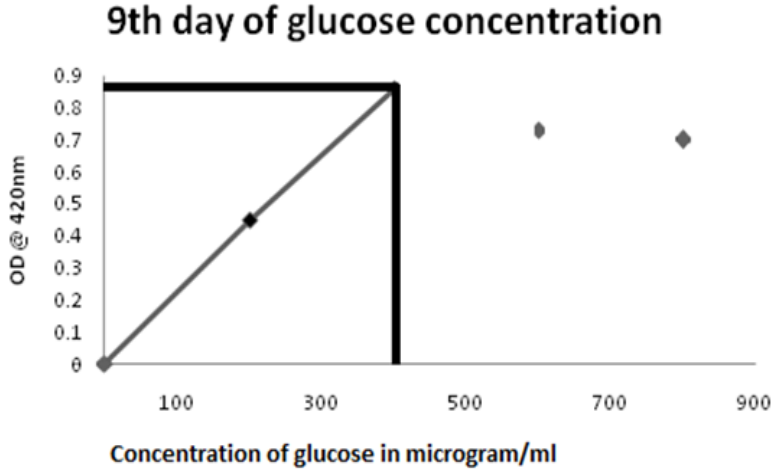
2. The concentration of Glucose in a fermented broth sampled on third day of fermentation process at 38^0C was found to be 1250 µg/ml.



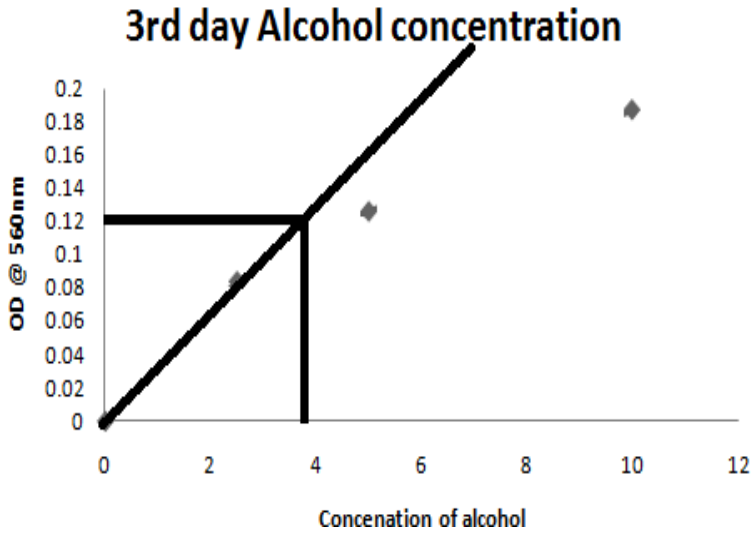
3. The concentration of Glucose in a fermented broth sampled on third day of fermentation process at 38^0C was found to be 810µg/ml.



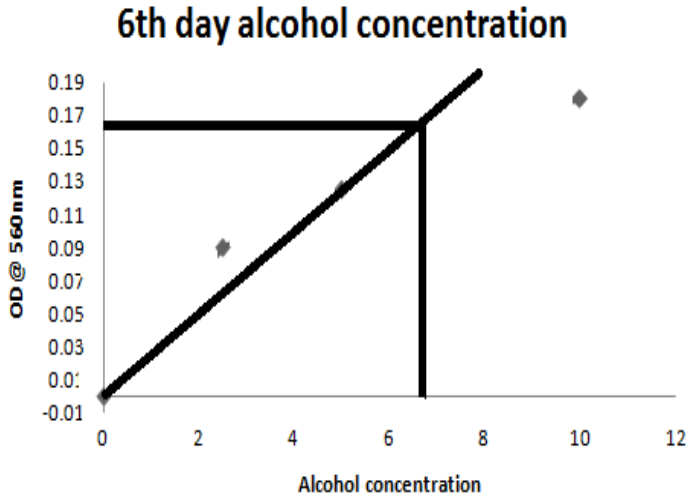
4. The concentration of Glucose in a fermented broth sampled on third day of fermentation process at 38^0C was found to be 810µg/ml.



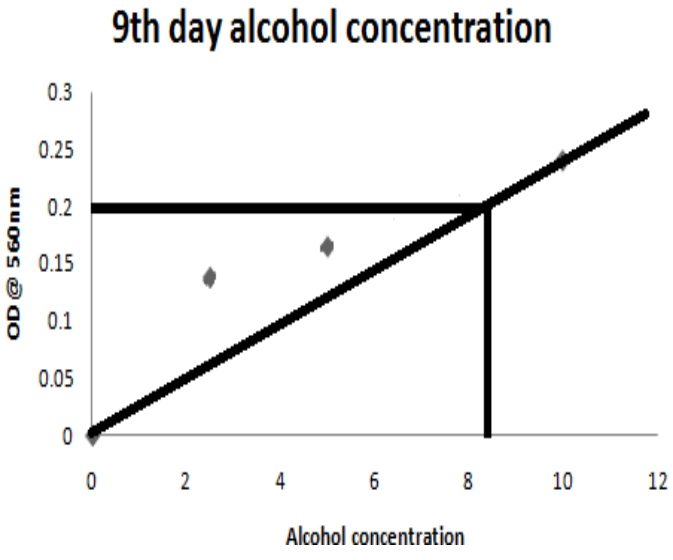
5. The alcohol estimation by potassium dichromate method for third day fermented broth sample was found to be 3.8%.



6. The alcohol estimation by potassium dichromate method for sixth day fermented broth sample was found to be 6.8%.



7. The alcohol estimation by potassium dichromate method for ninth day fermented broth sample was found to be **8.5%**.



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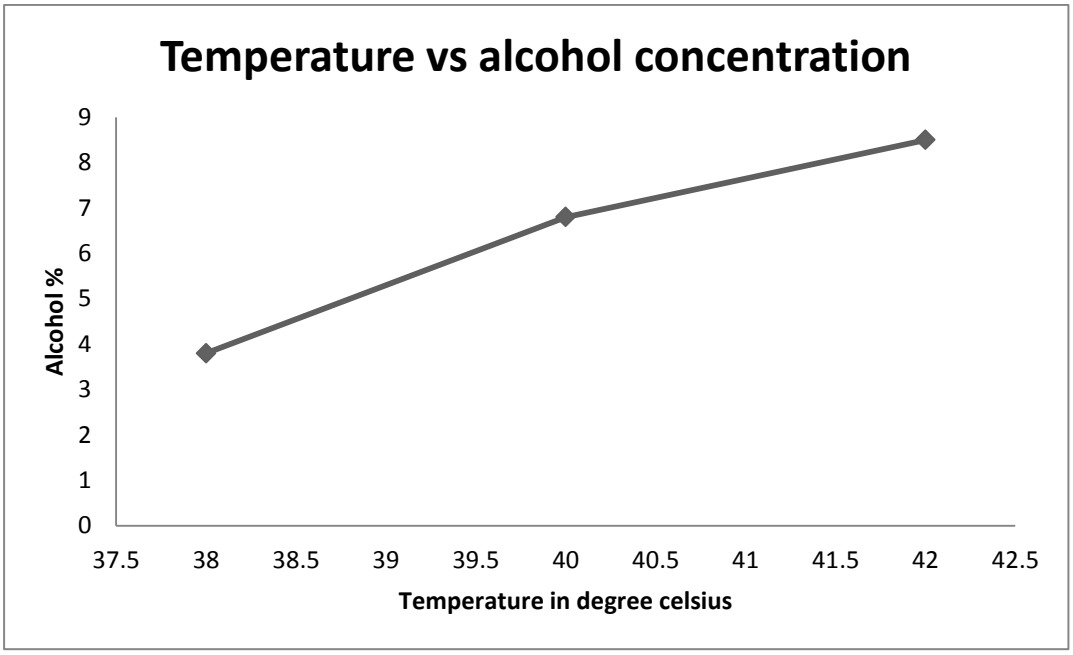
8. The substantive increase in percentage of alcohol yielded as a function of temperature gradient from 38^0C, 40^0C and 42^0C is depicted as under.

Temperature ^{0}C	% of Alcohol
38	3.8
40	6.8
42	8.5



IV. CONCLUSION

The study lead to the production of alcohol in significant percentage sufficient enough to think in the lines to scale it up industrially and to meet all the objectives of economical way of fermentation to environmental friendly protocols and also help reducing human health risks if fruit wastes gone unprocessed. The study has lot of scope in terms of molecular tailoring for the ability of *Saccharomyces cerevisiae* to enhance its productivity of alcohol by altering parameters with regard to pH, temperature and by employing different methods of fermentation. Scale up processes of fermentative alcohol production from fruit waste is very promising in answering many challenges of current energy needs and also lighten the dependency of fossil fuels. Furthermore, the alcohol produced has number of applications in vast areas like Perfumes to explosives, automotive fuel industries, paints and is useful for processing antibiotics, vaccines, tablets, pills, vitamins etc, and also used as some liquid animal feed products as an energy source.

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