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A Comparative Study on the Alternate Substrate for the Production of Cost Effective Bioethanol using *Saccharomyces Cerevisiae* and *Candida Albicans*

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ABSTRACT

The main objective is to find the alternate substrate for the cheaper production of bioethanol so that even the common people can also produce bioethanol. The bioethanol can be produced by employing waste fruit pulp utilized by micro-organism like *S.cerevisiae and C.albicans*. They are the most prominent organisms for bioethanol production. The waste fruit pulp was subjected to fruit juice extraction and the reducing sugar concentration was estimated by DNSA method. Fermentation was allowed to carried out using fruit juice extract as substrate using *S. cerevisiae* and *C. albicans*. The reducing sugar concentration was decreased gradually in the mixed fruit juices confirming the production of ethanol and ethanol concentration was determined using potassium dichromate method. The residence time was noted to be 6.4 minutes for the ethanol produced by *Candida albicans* by using HPLC and 6.2 minutes for *Saccharomyces cerevisiae*. The yield of bioethanol by *C. albicans* was found to be 84% which was higher than that produced by *S. cerevisiae* (81%).

KEY WORDS: Bioethanol, fruit juice extract, reducing sugar concentration, DNSA method, fermentation, *S. cerevisiae*, *C. albicans*, Potassium dichromate method, HPLC analysis.

1. INTRODUCTION

Due to the increase in population size, there is a greater demand for fuel resources due to the reduction of fossil fuels. Bioethanol, an alternative source of energy has received special attention worldwide due to depletion of fossil fuels. Combustion of fossil fuels have been releasing a lot of Greenhouse Gases (GHGs) which contain high amount of carbon dioxide (CO_2) and methane gas (CH_4). Because of the adverse impacts of fossil fuel and to reduce these kinds of difficulties, an alternative method is the production of biofuel using microorganisms. Currently, there is a growing interest for ecologically sustainable bio-fuels all over the world. Biofuels are combustible materials directly or indirectly derived from biomass for either transport or burning purposes. They can be produced from agricultural and forest products and the biodegradable portion of industrial and municipal waste. There are two main types of biofuels - Bioethanol and biodiesel, which account for more than 90% of global biofuel usage. Biodiesel is made through a chemical process called trans esterification and is used as an alternative for diesel. Bioethanol is a distilled liquid produced by fermenting sugars from sugar plants and cereal crops (sugarcane, corn, beet, cassava, wheat, sorghum) and can be used in pure form in specially adapted vehicles or blended with gasoline.

Bioethanol can be blended with gasoline produces considerably lower emissions of unwanted green gases on combustion than biodiesel and the advantageous of bioethanol over biodiesel is that it only releases the same amount of carbon dioxide as plants bound while growing. Compared to bioethanol, however, total biodiesel production is fairly small. Considered to be the cleanest liquid fuel; bioethanol can be a reliable alternative to fossil fuels[7].There are two biochemical production routes for bioethanol. Due to rapid growth in population and industrialization, worldwide ethanol demand is increasing continuously. Conventional crops such as corn and sugarcane are unable to meet the global demand of bioethanol production due to their primary value of food and feed.

A large amount of renewable biomass is available for conversion to liquid fuel ethanol. Currently, industrial production of ethanol is mainly carried out by using the yeast *S. cerevisiae*. It is used due to its outstanding characteristics of growing at high sugar concentrations and producing ethanol with high yields. Fruit fermentation results from the action of yeast (*S. cerevisiae*) which provides the enzyme zymase that converts the sugar present in the fruit to produce alcohol, carbon dioxide and other by-products. In this study, they were examined and compared to ascertain which microorganism produces higher amount of ethanol.

2. MATERIALS AND METHODS

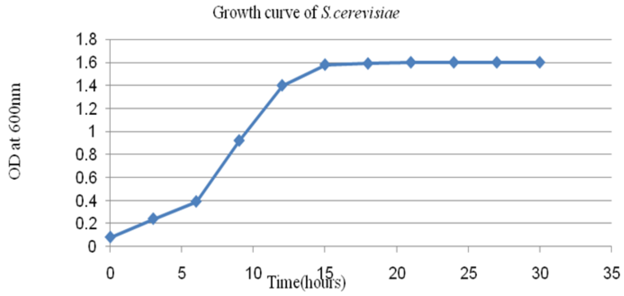
Fungal strain and Collection of substrate: *S.cerevisiae* (Baker's yeast) was collected from the bakery and the culture of *C. albicans* was collected from Bioline laboratory, Coimbatore. These two strains were used for the production of Bioethanol. The waste fruits of Banana, muskmelon, apple, orange, guava and watermelon were collected from nearby fruit shop and it was washed with 5% $KMnO_4$. Then the juice was filtered using muslin cloth and stored in the refrigerator for the further use. The reducing sugar concentration of the fruit juice was determined using DNSA method.

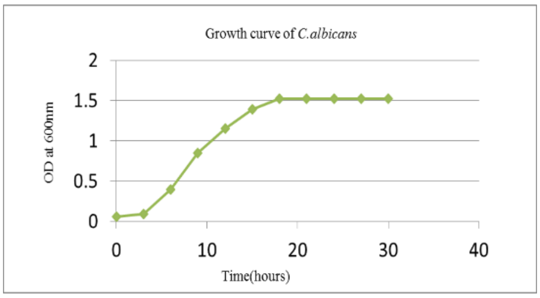
Growth characteristics of *S. cerevisiae* and *C. albicans*: *S. cerevisiae* and *C. albicans* was sub cultured in Potato Dextrose broth and Sabourad broth and the growth was determined at regular intervals of time using UV-Visible spectrophotometer at 600nm.

Preparation of fermentation medium: A set of experiments was conducted by adding 0.05 g of Ammonium sulphate, 0.5 g of glucose was kept as control and the mixture of fruit juice was added in the another set of experiments instead of glucose as the carbon source. In both the set of experiments, the medium was inoculated with *S. cerevisiae* and *C. albicans* and subjected to 48 hours of fermentation. During the course of fermentation, 10.0 mL of inoculum was taken and reducing sugar concentration was determined at different intervals of time. The concentration of the bioethanol obtained was estimated using potassium dichromate method. The supernatant was subjected to distillation process and the distillate collected was analysed using High Performance Liquid Chromatography.

3. RESULT AND DISCUSSION

Growth characteristics of *S. cerevisiae* and *C. albicans*: The growth characteristic of *S. cerevisiae* and *C. albicans* was studied by observing the absorbance at 600nm at every 3 hours time interval. The objective of the current study is to determine the logarithmic phase of growth since bioethanol is a growth associated product. This study would be very much useful for the isolation and extraction of the product. From the growth curve, it is clear that *S. cerevisiae* shows logarithmic phase during 12-15 hours after incubation and stationary phase starts at 16th hour after incubation. Meanwhile, in case of *C. albicans*, the logarithmic phase starts at 13th hour and lasts up to 5 hours and stationary phase extends from 19th hour to 30 hours of incubation.

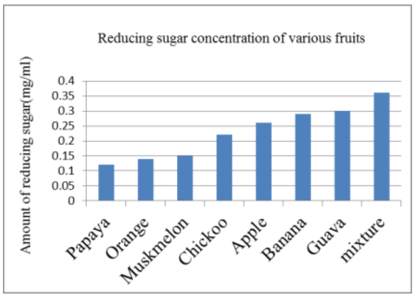


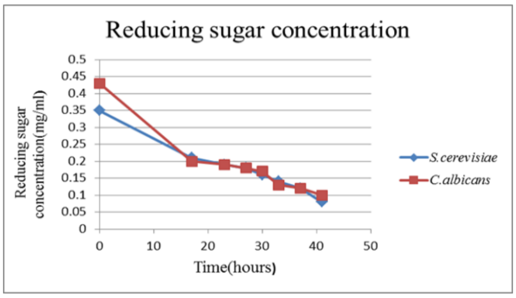


Graph.1. Growth curve of *S.cervisiae* **Graph.2. Growth curve of *C.albicans***

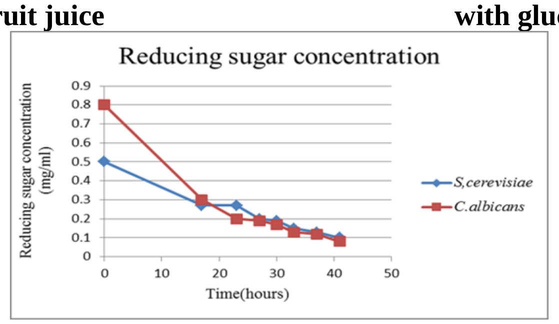
Reducing sugar concentration during the course of fermentation: The reducing sugar concentration is the term coined to study the influence of carbon sources during the course of fermentation since it seems to be the primary component for the growth of any living organisms. The control was kept as the medium containing glucose, ammonium sulphate and test was kept as fruit juice as carbon source and ammonium sulphate. This has been carried out for both *S. cerevisiae* and *C. albicans.*

Reducing sugar concentration of each fruit juice: Amount of reducing sugar was calculated from the calibration curve and concentration of reducing sugar of each and mixture of fruit juice was calibrated and graph was obtained as shown below.



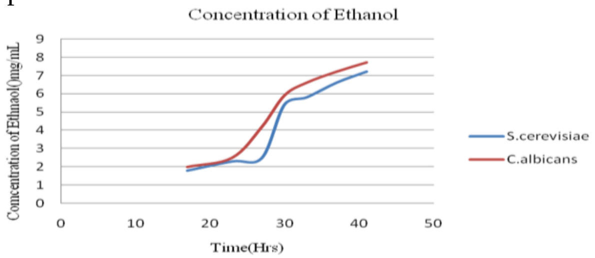


Graph.3. Reducing sugar Concentration of each and mixture of fruit juice **Graph.4. Concentration of reducing sugar with glucose as a carbon source**

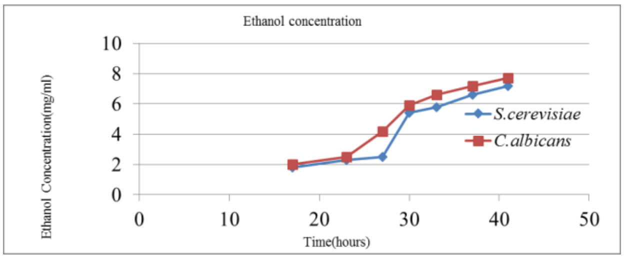


Graph.5. Concentration of reducing sugar without glucose as a carbon source

Ethanol concentration during the course of fermentation: The bioethanol content of fermentation medium utilizing glucose as a carbon source in addition to fruit juice and fermentation medium utilizing fruit juice as the only substrate was estimated and concentration of bioethanol content are provided respectively. The graph was plotted between concentration of bioethanol versus Time was provided in Graph 6 and 7.



Graph.6. Concentration of ethanol with glucose as a carbon source



Graph.7. Concentration of Bioethanol with fruit juice as a carbon source

HPLC Analysis:

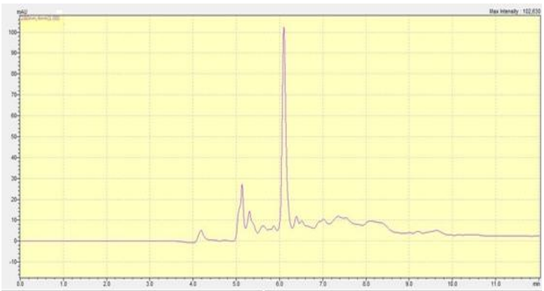


Figure.1. HPLC Chromatogram of Bioethanol obtained from *S.cerevisiae*

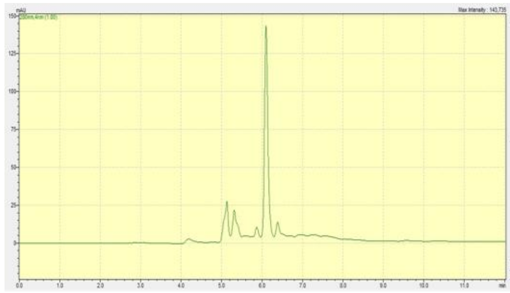


Figure.2. HPLC Chromatogram of Bioethanol obtained from *C. albicans*

DISCUSSION

The reducing sugar concentration of each fruit and mixture of fruit juice were estimated. Mixture of fruit juice containing high amount of reducing sugar (0.36 mg/mL).The growth of *S.cerevisiae* and *C.albicans* was studied. *S.cerevisiae* reached the stationary phase at 15 hours whereas *C.albicans* reached at 18 hours. Ethanol and reducing sugar concentration was estimated at different time interval using Potassium dichromate method and DNSA method. From the graph 4 & 5, the reducing sugar concentration was found to be decreased gradually which indicates increase in the level of ethanol production. The reducing sugar concentration was inversely proportional to the ethanol concentration.

C. albicans was found to be produced high amount of bioethanol compared to *S. cerevisiae*. The product was analyzed using HPLC. The retention time of standard ethanol is 8.2 min with respect to Rachita (2012). The bioethanol produced from *C. albicans* is found to be 6.4 min and for the *S. cerevisiae* it is found to be 6.2 min. It is observed that there is a minor difference in retention time of standard ethanol to bioethanol from *C. albicans* and *S. cerevisiae* which confirmed that the produced product was ethanol. The difference in retention time is due to presence of impurities. It can be reduced by re-distillation which increases the concentration of ethanol.

The %yield for *S. cerevisiae* and *C. albicans* is given in the table.1.

Table.1. Effect of glucose and without glucose utilized by *S.cerevisiae* and *C.albicans* for the production of Bioethanol

%yield	With glucose	Without glucose
S. cerevisiae	78.125	81.38
C. albicans	83.550	84.635

The product yield was calculated using the formula,

Theoretical yield =amount of glucose x 1mole of glucose/ molecular weight of glucose (180) x 2mol of ethanol x molecular weight of ethanol (46.08).

%yield = actual yield/theoretical yield x 100

4. CONCLUSION

In our present study, it was tried to obtain a higher concentration (>90%) of Bioethanol using mixture of fruit juice by fermenting them with the help of microorganisms. *Candida albicans* produces a yield of 84.63 % whereas *Saccharomyces cerevisiae* produces a yield of 81.38 % which is lower than the first case. Bioethanol obtained after distillation was subjected to HPLC analysis and the chromatogram was compared with standard ethanol chromatogram as per Rachita (2012). Hence, it can be concluded that the higher concentration of bioethanol obtained from *Candida albicans* can be used as biofuel whereas Bioethanol from *Saccharomyces cerevisiae* need further distillation for the better product. As this process is cost effective and does not involve with any toxic residues. Hence even a common man can develop this technique and it would be also possible to produce in large scale through industries.

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