

# PRODUCTION OF ETHANOL FROM MAHUA FLOWER (MADHUCA LATIFOLIA L.) USING SACCHAROMYCES CEREVISIAE – 3044 AND STUDY OF PARAMETERS WHILE FERMENTATION

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## ABSTRACT

*This study presents production of ethanol from mahua flower (Madhuca latifolia L.) in submerged fermentation (SmF) using immobilized cells of Saccharomyces cerevisiae-3044. The change in certain parameters during the course of fermentation and maintaining them while production of ethanol has been studied. The substrate mahua flower contains 72 – 74% of total sugar. Yeast strain S.cerevisiae-3044 was obtained from NCL laboratories Pune. Maximum production of ethanol is obtained at different optimized parameters such as slurry composition as 1:5, pH at 5 to 5.5, inoculum level at 1.5g/100mL, inoculum age at 48 hours, temperature 30–32°C, nitrogen source 0.05-0.06%, sodium potassium tartarate 1.2 g/L and fermentation period is 2-4 days is 338 ml for 1:5 slurry composition. Maximum production of ethanol is obtained by use of sodium potassium tartarate and urea. The method and design can be used for ethanol yield for large scale production.*

**Keywords:** Madhuca latifolia L. ethanol, urea, Saccharomyces cerevisiae–3044

## INTRODUCTION

Ethanol production by fermentation has received special attention to solve world energy crisis. (Ward O.P. and Singh A. 2006) Mahua flowers are found in abundance in tropical rain forest of Asian continents. (Behera S., Kar S., Mohanty R.C. and Ray R.C. 2010) Mahua flowers are rich source of sugars, contains 72.9 %, proteins 4.4%, Fat 0.5%, calcium 150 mg, Iron 15mg/100 gm, magnesium, and vitamins.(The Wealth of India, CSIR, 1962) In India, various parts of Andhra Pradesh, Maharashtra, Chhatissgrah, some tribal communities cultivate and harvest mahua flowers for alcoholic beverages using traditional methods.(Yadav P., Garg N., Diwedi D.H. 2009) Mahua flowers were used for extraction of sugar where 1 kilogram of mahua flowers autoclaved with 2 liters of water at 1 steam pressure for 15 minutes.(Rao R.T.N., Dwaraknath C.T., Johar D.S. 1961) For the production

of vinegar mahua flowers were used and alcohol production is likely to continue where mahua flowers are available at low cost and where alcohol intended for used as potable spirits. (Wealth of India CSIR 1964) Sarkar et al. studied structural feature of polysaccharides of mahua flowers.(Sarkar N. and Chatterjee P.B. 1984) Preparation of sugar and analysis of flowers from various districts have been studied, the analysis revealed that mahua contains 65–70% sugars, 48–55% reducing sugars, 14–18% invert sugars, 4.0–6.5% crude protein, 21–48% ferrous, 0.9–1.3 fat, 2.5–5.2% ash, 177–266% calcium.(Sutaria P.B. and Magar N.G. 1955) Ethanol tolerance yeast isolated from fermenting cashew juice.(Osho A. 2005)

Mahua flower (*Madhuca latifolia L.*) is abundant in the forests of Chandrapur and Gadchiroli districts, Maharashtra, India and it is having good keeping qualities. If the mahua flowers are utilized as a substrate for the production of ethanol through submerged fermentation, it will become a great economic advantage in the Indian context. (Benerji D.S.N. et. Al. 2010) Due to the above advantages present study is based on the usage of mahua flower as a substrate in production of ethanol.

## **MATERIALS AND METHODS**

### **Substrate**

Mahua flowers (*Madhuca latifolia L.*) are obtained from the forests of Chandrapur and Gadchiroli districts, Maharashtra, India. At Chemistry Lab, S.P. College, Chandrapur flowers were washed in tap water and sun-dried for 2 days to reduce moisture content to 16 – 18 %. Yeast strain *S.cerevisiae*–3044 was obtained National Collection of Industrial Microorganisms (NCIM) National Chemical Laboratories, Pune (Maharashtra).

### **Sterilization**

After washing and sun drying the mahua flowers are sterilized by autoclaving at pressure 10 lb/inch<sup>2</sup> for a period of 20 minutes.

### **Estimation of Sugar**

Total sugar of mahua flower is estimated by anthrone method and found to be 72 – 74%.

### **(Micro-organisms) Fungii**

Yeast strain *Saccharomyces cerevisiae*–3044 was obtained from National Collection of Industrial Microorganisms (NICM), National Chemical Laboratories (NCL), Pune (M.S.).

### **Medium for Seed Culture**

Yeast strain *S. cerevisiae* culture is maintained on the yeast extract, glucose, malt extract, peptone at pH 5. This nutrient agar medium containing malt extract 15 g/L, glucose 50 g/L, yeast extract 15 g/L peptone 25 g/L and seed culture for fermentation is prepared as 3-4% of fermentation slurry and the medium is autoclaved at 15 lbs pressure, 2-3 loops of original culture were transferred and incubated at 30<sup>0</sup>C for 48 hours. (Benerji D.S.N. et. Al. 2010)

### **Estimation of Ethanol**

Ethanol is estimated by potassium dichromate oxidation method and followed by spectrophotometric method.

**Experimental**

**Fermentation Medium and physic-chemical parameters**

Sterile Mahua flowers were grinded with water (mahua:water 1:5) in mixer grinder to make slurry. This slurry was then autoclaved at pressure 10 lb/inch<sup>2</sup> for a period of 30 minutes. The fermentation medium is maintained at various optimized parameters such as substrate concentration 25-30%, Inoculum level 2-3%, Inoculum age is 48 hours to get best results. Agitation is maintained at 80-100 rpm, pH is kept around 5-5.5, temperature at 30 – 32<sup>0</sup>C. Nitrogen sources urea and ammonium sulfate 0.06% and 0.05% is used, chelating agents such as sodium potassium tartarate 1.2 g/L gives good results.

**Distillation and Filtration**

Fermented broths were removed at 48 hours of interval and contents were analyzed for total sugar and ethanol. The mixture of ethanol and hot water were separated by simple distillation unit at a temperature of 78 – 96<sup>0</sup>C. In this method 80% of pure ethanol is obtained, which rectified by using rectifier units to obtain 99.2% pure ethanol.

**RESULT AND DISCUSSION**

**Effect of Slurry Composition**

Effect of sterile mahua flowers slurry composition on the production ethanol using *s. crevisiae-3044* was carried out by varying slurry composition of mahua and water in 1:1 to 1: 8 ratios (w/v). Other parameters such as inoculum age 72 hours, inoculums level 1.5 g/100 mL, agitation 50-70 rpm, nitrogen sources i.e. urea 0.32 g w/v, and pH at 5 – 5.5 at room temperature was maintained. (Benerji D.S.N. et. Al. 2010)

It can be seen from table–1 that the maximum yield of ethanol is obtained at 1:5 (kg/L) ratio of slurry and the fermentation period for first step was kept for 48 hours. It was observed that the percentage of ethanol production decreases further with increase in slurry concentration ratio. Also ethanol production decreases during second slot of 48 hours with the decrease of sugar concentration level as only the remnant of the first slot is used.

**Table 1.** Production of Ethanol for Various Slurry Compositions

Composition of Slurry Mahua flower : water in litres	Production of Ethanol
1:1	138 mL
1:2	187 mL
1:3	242 mL
1:4	296 mL
1:5	338 mL
1:6	302 mL
1:7	227 mL
1:8	146 mL

**Effect of pH during the Period of Fermentation**

The value of pH during the fermentation period gradually changes from 5.0 – 4.4. This change in pH was adjusted by addition of NH<sub>4</sub>OH and dil. H<sub>2</sub>SO<sub>4</sub> after every 6 hours interval till the fermentation time reaches the first slot of 48 hours. It is clear from the table -2 that if the fermentation time is kept for first slot of 48 hours and then the second slot of 48 hours and the pH values was maintained between 5 – 5.5 then the production of ethanol was more as compared if the pH value is kept 4.4. It is to be noted that in the second slot of fermentation the raw used is the remnant of the first slot after filtration.

**Table 2.** Fermentation Period with Corresponding pH Values

Hours of Fermentation	pH
0	5.0
6	4.9
12	4.8
18	4.8
24	4.7
30	4.6
36	4.5
42	4.5
48	4.4
54	4.4
60	4.3

**Effect of Temperature during the Period of Fermentation**

According, to the previously reported studies<sup>10</sup> fermentation temperature is an important parameter. The optimum temperature of fermentation medium should be 30<sup>0</sup>C, to get maximum ethanol yield. It is observed in the present study that the temperature rises gradually in the period of 12 hours to 48 hours and affects the production of ethanol. This change in temperature is controlled by adjustable cooling jackets.

If the temperature is allowed to rise, the microbial activity (metabolism) changes due to change in the environment, which decreases the percentage production of ethanol. The decrease in production of ethanol was found to be about 50 % at a temperature range of 32 – 45<sup>0</sup>C.

**Effect of Inoculum Age**

Inoculum age is investigated to determine the potentiality of the *s. cerevisiae* –3044. The maximum growth can be obtained at 48 hours age of yeast.

**Effect of Inoculum Level**

Inoculum level was studied to determine the wet weight of *s. cerevisiae* –3044 cells. The maximum ethanol yield was obtained at 2-3% of inoculums level.

**Effect of Nitrogen Source**

*S. cerevisiae*-3044 utilizes nitrogen to metabolize nitrogenous substances for the growth and their activity. Urea and ammonium sulfate were used as nitrogen sources. It is easily beak-

down by micro-organisms. It was observed that maximum production was obtained at 0.06 g/L concentration of urea, indicating that urea has significant effect on the rate of production of ethanol.

## CONCLUSION

Studies including physico- chemical parameters for the production of ethanol were done in simplified way. The results shows that mahua flowers proved to be cheap and suitable substrate, since it consists high sugars 72-74%, which can be obtained from any part of India. All these features of mahua flowers can be used as suitable raw material for industrial production of ethanol. The change in the fermentation method by separating the liquid portion after 48 hours gives more percentage yield of ethanol. Also it can be concluded that maximum production is obtained at 0.06 g/L concentration of urea.

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