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Research Article

MADHUCA LONGIFOLIA FLOWERS FOR HIGH YIELDS OF BIO-ETHANOL FEEDSTOCK PRODUCTION

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Abstract

Fermentation process, the production of bio-ethanol from *Madhuca longifolia* (Mahua) flowers by using *Saccharomyces cerevisiae* (Mahua flowers, collected from Eastern Ghats region of Andhra Pradesh, India). The evaluated results showed that, 1000 ml of acidic fermented media at *pH*-4, 5 and 5.7, produced the average quantity of bio-ethanol in test-1 (*S.cerevisiae* + Mahua flowers + Media), test -2 (Mahua flowers + Media) and control (media) ≈ 170.03 ml, ≈ 142.3 ml and ≈ 127.7 ml respectively. The percentage of bio-ethanol was confirmation by using Alcohol meter, the analysis results showed that in test-1, test-2 and control ≈ 46.6 %, ≈ 24.6 % and ≈ 0% respectively. The presence of bio-ethanol were verified by using Spectrophotometer at 204-240 nm.

Keywords: Bio-ethanol; *Madhuca longifolia*; *Saccharomyces cerevisiae*; fermentation

Introduction

Madhuca longifolia is an Indian tropical tree, found largely in the central and north Indian forests. It is commonly known as 'mahua', 'mahwa' or 'Iluppai'. It is a fast-growing tree that grows to ≈ 20 meters in height, possesses evergreen or semi-evergreen foliage, and belongs to the family Sapotaceae (Ramadan *et al.*, 2006). Mahula is a forest tree found in abundance in the tropical rain forests of Asian and Australian continents (Bhagmol and Joshi, 2002). This tree species, however, has been domesticated by tribal people in India and Pakistan for its uses as food (flower), feed (leaves and flower), wood (timber) and alcoholic beverage (fermented flower) locally called 'mahuli' in India (Swain, 2007). The mahuwa flower is edible and a food item for tribals (Priyanka *et al.*, 2012), they are used to make a syrup for medicinal purposes (Gopalkrishnan *et al.*, 2012) and fermented to produce the alcoholic drink mahuwa, a country liquor. In India, various parts of Andhra Pradesh, Maharashtra, Chhattisgarh, some tribal communities cultivate and harvest mahua flowers for alcoholic beverages using traditional methods. Mahuwa is an essential drink for tribal men and women during celebrations (Kirtikar and Basu, 2001; Madhumita and Naik, 2010).

Mahua flowers are rich source of sugars 72.9 %, proteins 4.4%, Fat 0.5%, calcium 150 mg, Iron 15mg/100 gm, magnesium, and vitamins (Ward and Singh, 2002 & 2005; Ward *et al.*, 2006). The fermentation process, bioconversion of glucose/sugar to ethanol consists of four major unit operations are pretreatment, hydrolysis, fermentation, and product separation/distillation. *M. latifolia* flower is a suitable and alternative cheaper carbohydrate source for production of bio-ethanol (Fig.1) (Behera *et al.*, 2010). Ethanol is an ideal fuel to substitute for gasoline (petrol), and the production of ethanol by fermentation has received special attention because the world energy crisis has enhanced the interest in renewable energy sources (Ward and Singh, 2002, 2005).

Fig. 1: *M. longifolia* Flowers

Materials and Methods

Substrate

Mahua flowers (M. longifolia) were obtained from the Eastern Ghats region of Srikakulam district, Andhra Pradesh, India. Flowers were washed with tap water. The

Yeast strain, *S. cerevisiae* was obtained from the Department of Bio-science, Rajiv Gandhi University Knowledge Technologies (RGUKT), Nuzvid, India.

Medium for Seed Culture

Yeast strain, *S. cerevisiae* culture was maintained on the yeast extract, dextrose, peptone at different pH. This nutrient agar medium containing dextrose 20 g/L, yeast extract 10 g/L, peptone 20 g/L. The medium was autoclaved at 15 Kg/Cm^2 for 30 minutes (Benerji *et al.*, 2010).

Fermentation Medium and physic-chemical parameters

Mahua flowers were mixed with water in the ratio of 1:5. This mixture was then autoclaved at pressure 15 Kg/Cm2 for a period of 30 minutes. The fermentation medium was maintained at various optimized parameters and period of 5-7 days, at 65 rpm, temperature at 25-30 ^oC, pH was adjusted by using the NaOH and HCl. The mixture was kept in orbital shaker for proper mixing.

Distillation and Filtration

The slurry was filtered by using the filter paper. The mixture of ethanol were separated by distillation at a temperature of 78 -100 ^oC (Department of chemical Engineering Lab, Rajiv Gandhi University Knowledge Technologies (RGUKT), Nuzvid).

Verification of Bio-ethanol

The composite of bio-ethanol were confirmed by using Spectrophotometer at 204-240 nm, and the percentage of bio-ethanol were confirmed by using Alcohol meter.

Result

The evaluated results showed that, 1000 ml of fermented media, test-1 (*S.cerevisiae* + Mahua flowers + Media), test -2 (Mahua flowers + Media) and control (media) at *pH*-4 and slurry composition ratio1:5, the produced bio-ethanol ≈ 165 ml, ≈ 135.3 ml and ≈ 101.5 ml (Fig. 2, Table 1), and the percentage of bio-ethanol were confirmed by using Alcohol meter analysis ≈ 45.6 %, ≈ 24.3% and ≈ 0% respectively (Fig. 3, Table 1). The verified results showed that at *pH*-5 and *pH*-5.7 in a slurry composition ration 1:5, the produced bio-ethanol in a test-1 (*S. cerevisiae* + Mahua flowers + Media), test-2 (Mahua flowers + Media) and control (media) samples ≈ 175 ml, ≈ 50.3 ml and ≈ 145.5 ml and 170.1 ml, ≈ 140.5 ml and ≈ 125.2 ml respectively (Fig. 2, Table 1). The percentage of bio-ethanol were confirmed by using Alcohol meter analysis ≈ 48.2 %, ≈ 26.4%, ≈ 0% and ≈ 46.2 %, ≈ 23.1% and ≈ 0% respectively (Fig. 3, Table 1). The composite of bio-ethanol peaks were observed in a test-1 and test-2 samples by using Spectrophotometer analysis at 204-240 nm (Fig. 4, 5), this result had been matched with the peak values of ethanol (Fig.4,5) (http://www.kayelaby.npl.co.uk/chemistry).whereas in control sample peaks were absent at 204-240nm (Fig. 6).

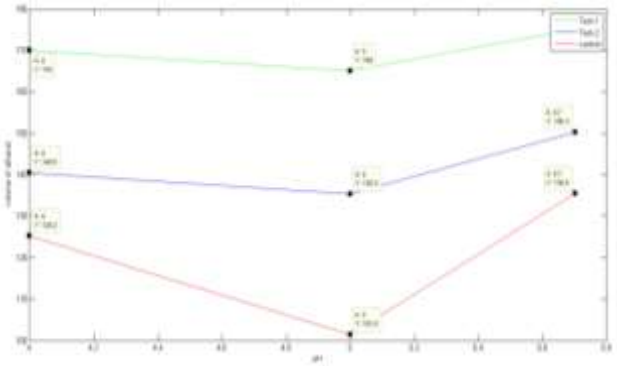

Fig. 2: The produced volume of Bio-ethanol from *M. longifolia* Flowers at pH-5; Green, Blue and Red lines indicates test-1, test-2 and control of 170 ml, 140ml and 125 ml respectively.

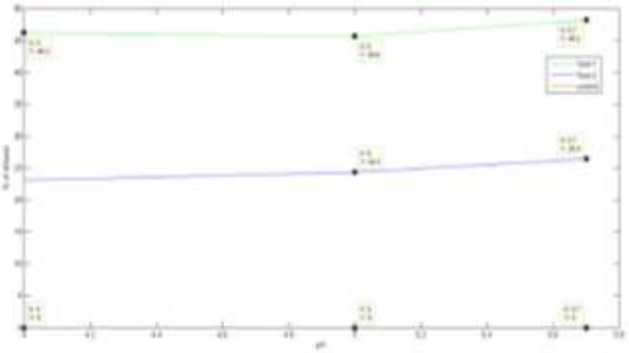

Fig. 3: The percentage of Bio-ethanol from *M. longifolia* Flowers at pH-5, confirmed by Alcohol meter. Green, Blue and black lines indicates test-1, test-2 and control of 46.6%, 24.6 % and 0 % respectively.

Table: 1 Percentage of ethanol obtained in 1000mL of fermentation media

S.N.	Test	Slurry (flowers: water ratio)	pH	% of Ethanol	Average volume (ml)
1	Test(Flower + *S. cerevisiae* + media)	1:5	5.7	46.2	170
	Control-1(Flower + media)	1:5	5.7	23.1	140.5
	Control-2(media)	1:5	5.7	0	125.2
2	Test(Flower+ *S. cerevisiae* + media)	1:5	4	45.6	165
	Control-1(Flower + media)	1:5	4	24.3	135.3
	Control-2(media)	1:5	4	0	101.5
3	Test(Flower + *S. cerevisiae* + media)	1:5	5	48.2	175.1
	Control-1(Flower + media)	1:5	5	26.4	150.3
	Control-2(media)	1:5	5	0	135.5

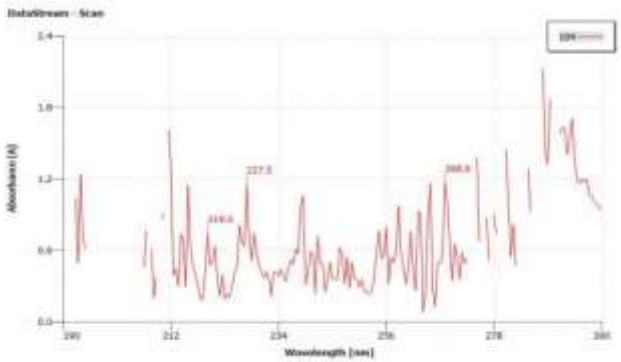

Fig. 4: The composite of Bio-ethanol peaks were observed in test-1 sample by using Spectrophotometer analysis at 204-240 nm.

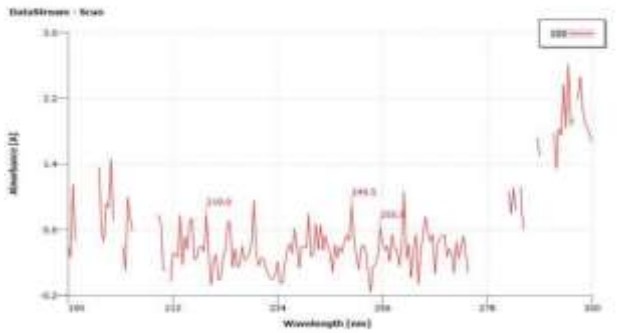

Fig. 5: The composite of Bio-ethanol peaks were observed in test-2 sample by using Spectrophotometer analysis at 204-240 nm.

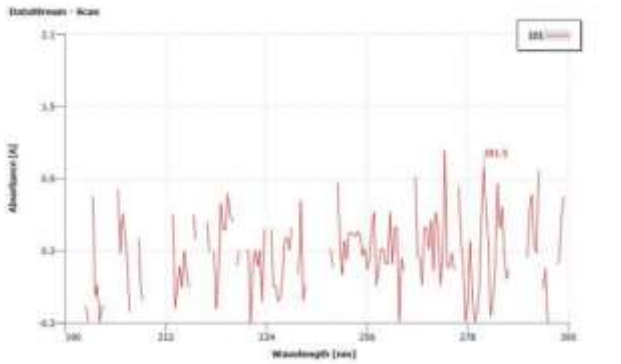

Fig. 6: The composite of Bio-ethanol absence of peaks were observed in control sample by using Spectrophotometer analysis at 204-240 nm.

Discussion

The production of bio-ethanol were verified at different *pH*, the range of pH (4, 5 and 5.7) and slurry compositions ratio 1: 5, and an incubation period was 4-7 days. The produced volume and percentage of bio-ethanol were high at *pH*-5, when compared to *pH*- 4 and *pH*-5.7 (Fig.2, Table1). The verified results of test-1 (*S. cerevisiae* + Mahua flowers + Media), test-2 (Mahua flowers + Media) and control (media) showed that at *pH* -5, the produced ethanol volume in the process of distillation ≈ 170 ml, ≈ 150 ml and ≈ 155 ml and ≈ 48.2 %, ≈ 26.4%, ≈ 0% respectively (Fig.2, 3 ; Table1). This result could saying that the yeast cells played an important role for the enhancement of the conversion of sugar molecules into bio-ethanol at *pH*-5. The yeast cells grow and actively work at the optimum *pH* of 4-5 for an incubation period of 4-7 days, if the incubation period was extended more than the 7 days the yeast cells again convert the alcohol into toxic substances, and the slurry composition also decide the percentage of the alcohol obtained (Fig.2, Table1).

Conclusion

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 (Ward and Singh, 2002 & 2005; Ward *et al.*, 2006). *M. longifolia* flowers containing 1000 ml of acidic media at *pH*-5 produced at ≈ 170.03 ml of ethanol in test-1 (*S. cerevisiae* + Mahua flowers + Media), test -2 (Mahua flowers + Media) ≈ 142.3 ml and control (media) ≈ 127.7 ml(Fig.2, Table 1), and the purity of bio-ethanol percentage was confirmation by using Alcohol meter ≈ 46.6 %, ≈ 24.6 % and ≈ 0% respectively(Fig.3, Table1). The results suggested that *M. longifolia* flowers favour for high yields of bio-ethanol production at *pH* -5 (Fig.2, 3 and Table1).

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