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# FUEL OF THE FUTURE : BIOETHANOL FROM *P. FLORIDA* SMS

**A. S. DESHMUKH**

*Matoshree Vimalabai Deshmukh Mahavidyalaya, Amravati, M.S., India*

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**ABSTRACT**

**Wheat (*Triticum aestivum*), Mung (*Phaseolous radiatus*), Tur (*Cajanus cajan*), Soybean (*Glycine max*) and their 15 combinations were used for cultivation of *P. florida* (Sample Code D). The post cultivation substrate, i.e. spent mushroom substrate was sun dried and oven dried. Extraction of fuel bioethanol from SMS as alternative source is the best way for disposal of SMS. 0.2 NaOH was used for pretreatment with heating as I pretreatment and microwave treatment as II pretreatment followed by fermentation using 2%, 4%, 6% baker's yeast for duration of 2 to 8 days. At fermentation level, randomly special supplementation with mushroom alone, mushroom+ jaggary and mushroom + mahua flower in combination in microwave treated (II pretreatment) at 4% 4 days stage was done for augmentation in bioethanol. All the bioethanol samples were estimated using titrimetric as well as spectrophotometric methods. GCMS method for ethanol estimation was used in case of randomly selected samples. Also, the surface changes and cellulose crystallinity were recorded using SEM and XRD respectively. 2.5 fold increase was recorded by addition with [mushroom+ jaggary (15 g each)].**

**KEY WORDS :** Spent mushroom substrate, Bioethanol, Mushroom.

## INTRODUCTION

Declining petroleum reserves has necessitated the demand of alternative fuel source such as bioethanol for both ecological and economical reasons (Bayarakci and Kochar, 2014).

Greener energy replacement for high energy demand is taking place on global level (Li *et al*, 2009). *Pleurotus* mushrooms have the ability to degrade lignocellulose (Patil *et al.*, 2009). Each kilogram of mushroom crop produces about 5 kg of spent mushroom substrate (Sendi *et al.*, 2013). The bioethanol from SMS makes way for second generation ethanol, a need of hour to overcome the food verses fuel debate.

## MATERIALS AND METHODS

### Spent mushroom straw preparation

The spawn of mushroom *Pleurotus florida* was obtained from Agriculture College, Pune and P.K.V., Akola. The different agrowastes *viz.* Wheat *(Triticum aestivum)*, Mung *(Phaseolous radiatus)*, Tur *(Cajanus cajan)*, Soybean *(Glycine max)* were collected from local farms. The substrate straw and beds were prepared according to the procedure described by Bano and Nagarajan (1976) in 15 different combinations. The spent mushroom substrate was collected, mixed. It was sun dried and oven dried for further estimations.

### Alkali Pretreatment

Conventional alkali pretreatment and microwave assisted alkali pretreatment were done with 2% NaOH (Zhu *et al.*, 2006)

### Fermentation

All the above pretreated substrates were fermented at 30 $^{o}$C and 40 $^{o}$C using 2%, 4% and 6% baker's yeast with fermentation duration of 2 to 8 days. The resulting fermentation mixture was distilled out at 72 $^{o}$C and collected the distillate for further estimations. Special additions such as mushroom

(5g, 10g, 15g), Jaggary with mushroom (5g, 10g, 15g), mahuaflower with mushroom (5g, 10g, 15g) were done at the level of fermentation in randomly selected samples for estimating the change in bioethanol content with respect to control sample for better concentration of bioethanol.

**Estimation of Ethanol**

i) **Titrimetric Method :** Estimation of Alcohol content in Wine by Dichromatic Oxidation followed by Redox Titration (Jessica Ferguson, www.sirromet.com)

ii) **Spectrophotometric method :** Colorimetric method for the estimation of ethanol in alcoholic drinks (Sumbhate *et al.*, 2012)

iii) Random sampling was done for GCMS estimation.

**Estimation of ethaonol by Titrimetric Method**

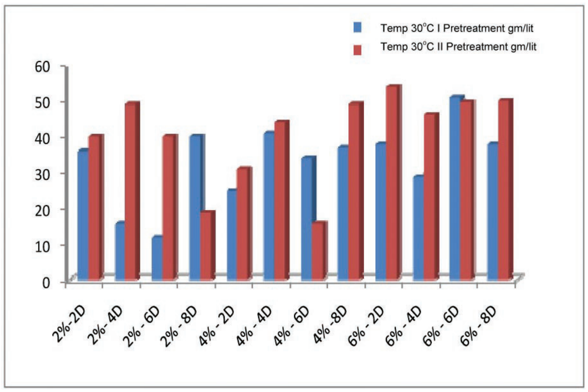



**Fig. 1.** Estimation of Ethanol in *P. florida* SMS.

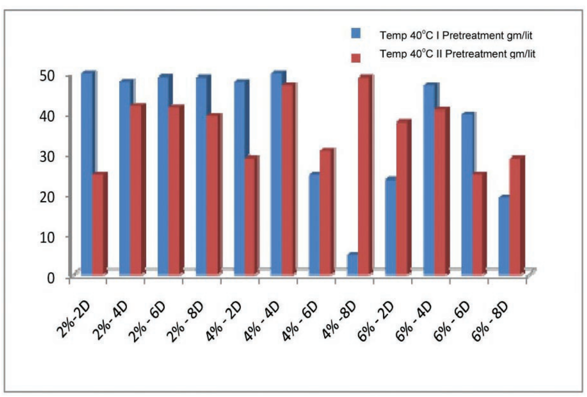



**Fig. 2.** Estimation of Ethanol in *P. florida* SMS.

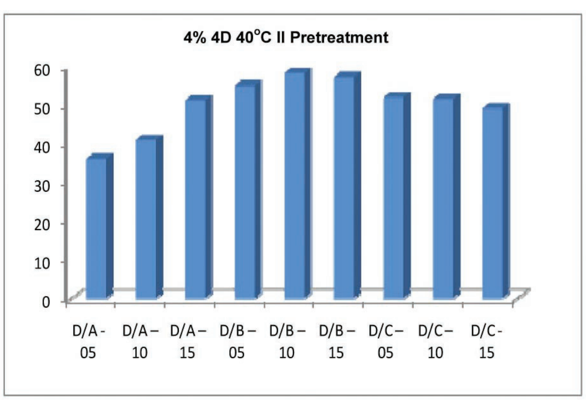



**Fig. 3.** Estimation of Ethanol in *P. florida* SMS.

**Estimation of ethaonol by Spectrophometric Method**

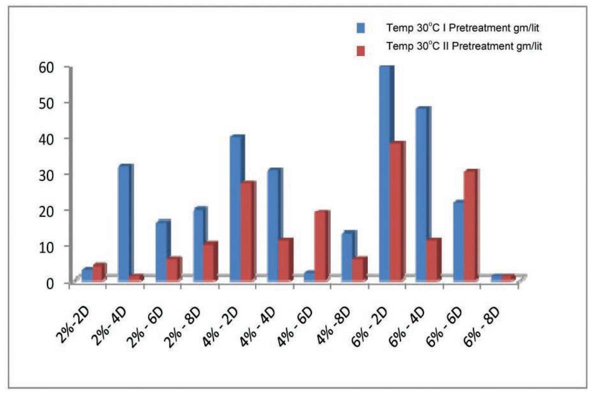



**Fig. 4.** Estimation of Ethanol in *P. florida* SMS.

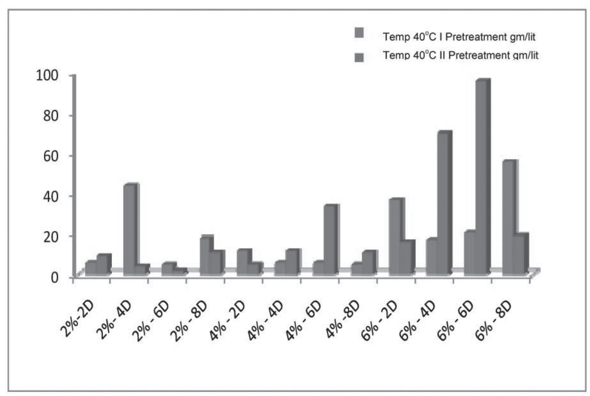



**Fig. 5.** Estimation of Ethanol in *P. florida* SMS.

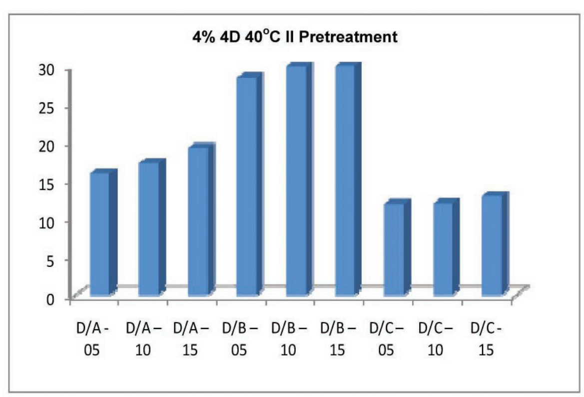



**Fig. 6.** Estimation of Ethanol in *P. florida* SMS.

## RESULTS AND DISCUSSION

In the present study, the bioethanol estimation by titrimetric method recorded, ethanol in the range at 30 °C (12.0 g/L to 51.0 g/L, heating pretreatment), 30 °C (16.0g/L to 54.0 g/L, microwave pretreatment); at 40 °C (4.8 g/L to 50.0 g/L, heating pretreatment) 40 °C (25.0 g/L to 49.0 g/L, microwave pretreatment). Whereas in spectrophotometric method the results are in the

GC-MS spectra of (sample code D) *P. florida* SMS

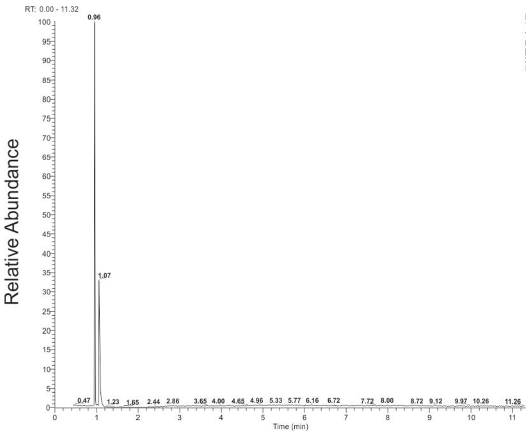



**Fig. 1.** GC-MS spectra of sample D-30-II (*P. florida* 2% 2D).

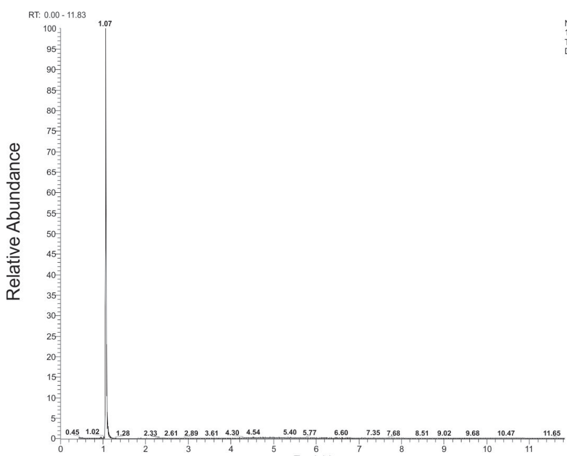



**Fig. 2.** GC-MS spectra of sample D 40 I (*P. florida* 4% 4D).

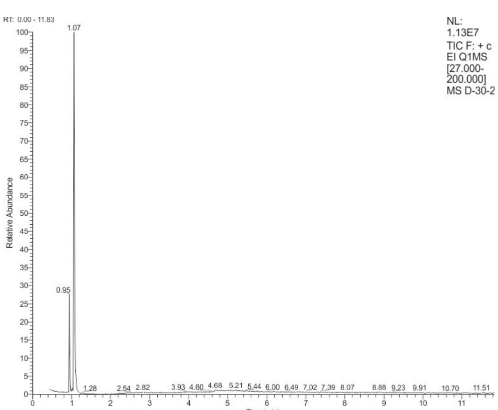



**Fig. 3.** GC-MS spectra of sample D/40I (*P. florida* 6% 2D).

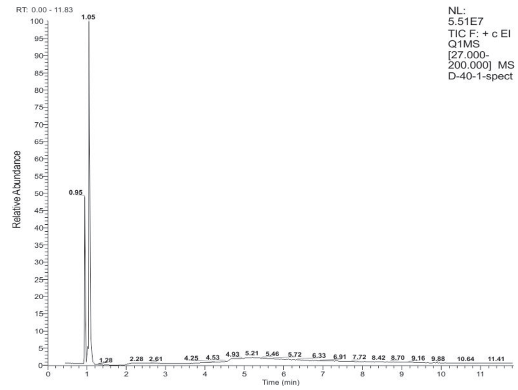



**Fig. 4.** GC-MS spectra of sample code D-40-I (*P. florida* 6% 6D).

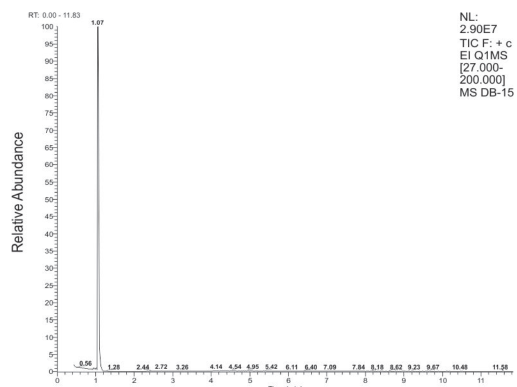



**Fig. 5.** GC-MS spectra of sample D 40II *P. florida* 4% 4D.

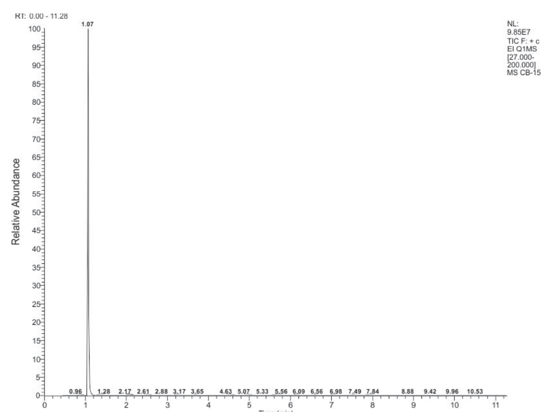



**Fig. 6.** GC-MS spectra of sample DB/10 40II (*P. florida* 4% 4D with additives 10g Mushroom + 10g Jaggary).

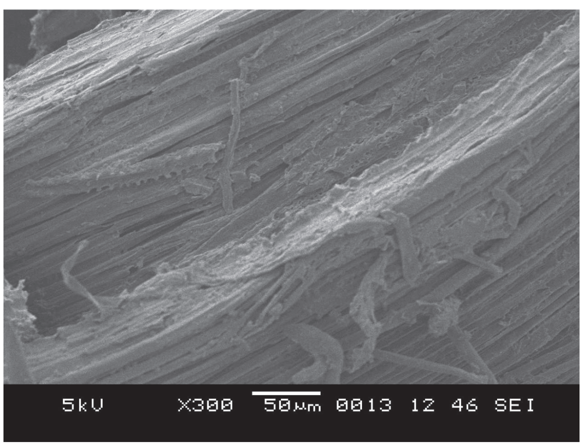
range at 30 °C (1.0 g/L to 60.0 g/L, heating pretreatment), 30 °C (1.0 g/L to 38.40 g/L, microwave pretreatment); at 40 °C (5.0 g/L to 56.0 g/L, heating pretreatment) 40 °C (2.0 g/L to 96.0 g/L, microwave pretreatment)

GCMS estimation for random sampling gave similar results as in spectrophotometric analysis. Thus, confirming the later. Special additives were added at temperature 40 °C, microwave pretreatment, 4% 4 day sample. In spectrophotometric analysis the higher values for bioethanol concentration were observed in case with addition [Mushroom + Jaggary (15 g each), 30 g/L]. The higher values for bioethanol concentration were seen in case with addition [Mushroom + Jaggary (10 g each), 29.90 g/l], [Mushroom + Jaggary (5 g each), 28.40 g/L] from the basic value of 12.0 g/L. Thus, the effectiveness of special additions of mushroom + jaggary was proved with nearly 2.5 fold augmentation of ethanol in *P. florida* SMS. The difference of results in estimation by titration method may be due to already much biodegraded lignin content in SMS (Patil *et al.*, 2009) and interference of phenolic compounds in the mixture.

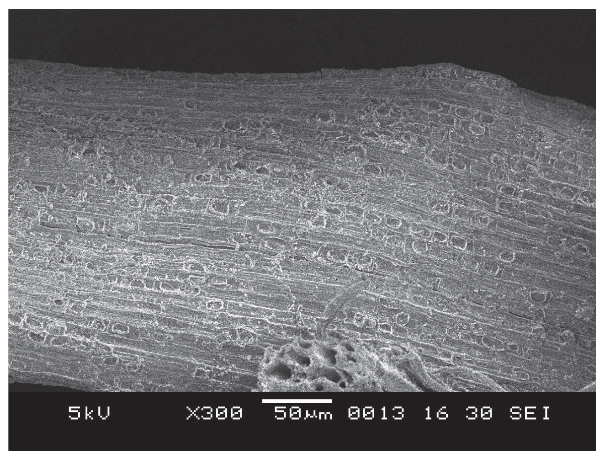
The SEM study of plain agrowaste compared with SMS and fermented samples showed that the surface of untreated (control) sample is rough while the surface of fermented sample is relatively smooth. The comparison of cellulose crystallinity in XRD study showed a decrease in crystallinity in fermented samples.

Among all the tested steam pretreated substrates by fermentation with *S. cervisae* using commercial cellulose as enzyme for hydrolysis, the resulted bioethanol was recorded as in sugarcane baggase (77 g/L), rice straw (62 g/L), wheat straw (44g/L) (Irfan *et al.*, 2014). Bioethanol content of 6.48 g/L was observed in spent paddy straw by using *P. eous* mushroom (Koshy and Nambison, 2012). In our experiment the additives Mushroom + Jaggary proves to be better in giving higher ethanol concentration.

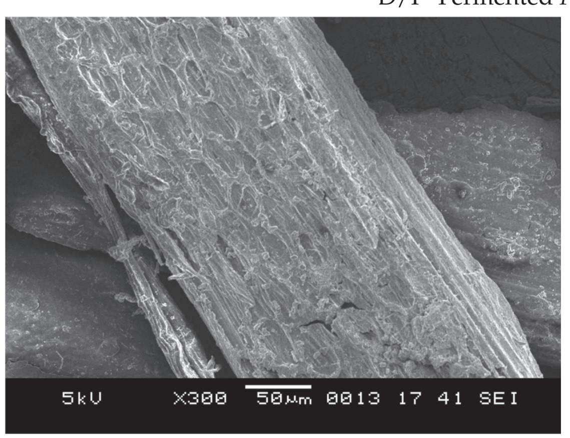
**Scanning Micrographs**



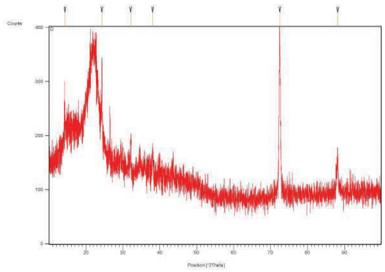

D-*P. florida* SMS



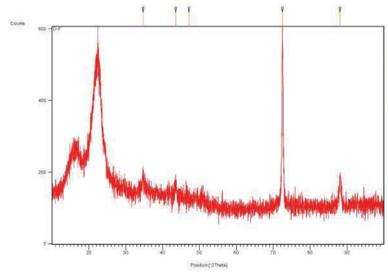

D/F- Fermented *P. florida* SMS (40°C II 4%4D)



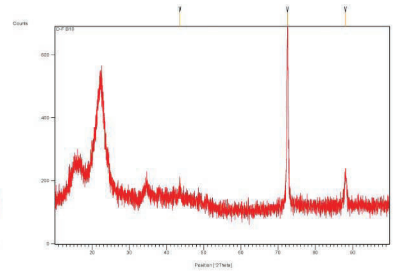

P-Agrowaste



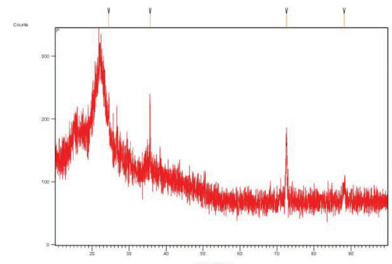
D-*P. florida* SMS



D/F- Fermented *P. florida* SMS (30$^{o}$C II 6%2D)



D/F- B10 Fermented *P. florida* SMS with additives (40$^{o}$C II 4%4D)



P-Agrowaste

## CONCLUSION

Finally we can conclude that *P. florida* spent mushroom substrate which is zero value renewable lignocellulosic biomass can be used as potential source of bioethanol production. In our research, the experimental set up without addition of costly commercial external enzyme, the hike of ethanol concentration by 2.5 fold was observed with addition of [Mushroom + Jaggary 5g, 10g,15g (each)] for low cost bioethanol production.

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