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BIOCONVERSION STUDY OF DEOILED RICE BRAN FOR BIOETHANOL PRODUCTION

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ABSTRACT

Bioethanol is the most promising alternative fuel for transportation. It is obtained from lignocellulosic biomass through microbial conversion leads towards the development of second generation biofuel technology. In the present work, Deoiled rice bran (DORB) a lignocellulosic by-product was used as a substrate for bioethanol production by *Pichia stipitis* NCIM 3497 and also optimizes its fermentation conditions; inoculum size, pH, temperature and fermentation period. The effect of nutrient supplementation on bioethanol production was also analyzed. The results of this study revealed that maximum bioethanol 9.31±0.08 g/L was produced at 30ºC temperature, pH 6 after 48 h of fermentation period using *Pichia stipitis* NCIM 3497 of inoculum size 1.5% v/v whereas on addition of ammonium sulphate at 2mM concentration gave 12.56% more bioethanol compared to control.

KEYWORDS: Deoiled Rice Bran, Bioethanol, Bioconversion, *Pichia stipitis* NCIM 3497

Bioethanol is an eco-friendly fuel that replaces additive methyl tertiary butyl ether (MTBE) from gasoline (Sun and Cheng; 2002). The environmental advantage of an oxygenated compound ethanol over gasoline, as it provides more oxygen on combustion and has better combustion efficiency. Thus the utilization of ethanol helps in maintaining level of greenhouse gases as well as decreases dependency on fossil fuels. Biofuel can be categorized on the basis of feedstock used for its production. Second generation biofuel can be produced from non-food crops such as lignocellulosic biomass, industrial wastes and agricultural residue etc (Bhatia and Johri; 2015). In order to maintain food security, second generation biofuel grasp more attention by researcher's.

Many agro-industrial by-products can be used as a carbon source for bioconversion in alcohol distilleries. Deoiled rice bran (DORB), an agro-industrial lignocellulosic residue left after the extraction of oil from rice bran, rich in carbohydrate; cellulose 39%, hemicellulose 31%, lignin less than 4% with crude fibers and ashes (Chandel et al. 2009). Several microorganisms have a capability to ferment glucose and xylose after complete hydrolysis of cellulose and hemicelluloses respectively into ethanol (Behera et al. 2014). *Pichia stipitis* are naturally occurring yeasts which has a potential for fermenting glucose and xylose into ethanol with a high yield (Chandel et al. 2009; Bhatia and Johri; 2015). Each micro-organism has its own optimum conditions for fermentation therefore the present study firstly deals to optimize fermentation conditions and then the nutrient optimization for bioethanol production from DORB by *Pichia stipitis* NCIM 3497.

MATERIALS AND METHODS

Substrate and Microbial Culture

Deoiled rice bran (DORB), was collected from Shree Sita Agro Food Private Limited, Dhamdha Naka, Durg, Chhattisgarh, India. *Pichia stipitis* NCIM 3497 used as a fermentative microorganism, procured from School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur Chhattisgarh, India. It was cultured and maintained in MGYP broth (w/v) [0.5 % Malt extract, 0.3 % Yeast extract, 0.5% Peptone and 1% Glucose] at pH 6.5 and 30°C for 12 h.

Fermentation Process and Optimizes its Parameters

For fermentation, 15 g of DORB was added in 150 ml of distilled water (1:10, w/v) in 250 conical flasks then autoclaved to obtain DORB hydrolysate. This DORB hydrolysate was used as fermentation media and inoculated with 1% v/v inoculum size of *Pichia stipitis* NCIM 3497 aseptically then incubated at 30±2ºC for fermentation to produce bioethanol. Different parameters of fermentation condition were optimized for bioethanol production like, inoculum size, pH, temperature and fermentation period. Initially inoculum sizes of *Pichia stipitis* NCIM 3497 were used to optimize in the range of 0.5-2% (v/v), then pH ranges (5-7), temperatures (20-40ºC) after that different fermentation periods of 24, 48, 72, 96, 120 and 144 h (hours) were performed for higher amount of bioethanol.

Nutrient Supplementation

The presence of nutrients and its concentration both are played an essential role in metabolic pathway of micro-organisms. Therefore, inorganic nutrient salts

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$ZnSO_4$, $MgSO_4.7H_2O$, $(NH_4)_2SO_4$ and $FeSO_4.7H_2O$ were used as a source of zinc, magnesium, nitrogen and iron for optimization, each salts was added individually at different concentrations of 1mM, 2 mM and 3 mM (v/v) separately before autoclave and then inoculated with *Pichia stipitis* NCIM 3497 for fermentation to optimize nutrient salts.

METHODS

Fermented broth was removed and distilled in distillation unit and amount of bioethanol was calculated by specific gravity method as given in Pharmacopoeia of India (Ministry of Health and Family Welfare, 1985). The quantity of ethanol was calculated by using formula given below, (Yadav, 2003)

$$\rho t0 = \frac{W3 - W1}{W2 - W1} \times \text{Density of water at t℃}$$

where; ρ^{t0} = specific gravity, W1 = weight of empty specific gravity bottle, W2 = weight of empty bottle + distilled water, W3 = weight of empty bottle +fermented liquid.

Statistical Analysis

All the data were given as means ± standard error (SE) of triplicate values and analyzed by using ANOVA (one-way analysis of variance) with its significant difference level at 5% with Duncan's Multiple Range Test using the Statistical Package for Social Science Research (SPSS) version16.

RESULTS AND DISCUSSION

In this study, DORB hydrolysate was solely used as a carbon source for bioethanol production by yeast *Pichia stipitis* NCIM 3497. The inoculum size used for fermentation is a key factor for providing a better product formation. Hence it is essential to optimizes inoculum size of *Pichia stipitis* NCIM 3497 and found that 1.5 % of inoculum size showed higher amount of bioethanol from DORB hydrolysate.

pH, temperature and fermentation period are essential parameters to be optimized for higher bioethanol production. After optimizing the inoculum size, pH was optimized it was found that after autoclave pH of DORB hydrolysate was 6 and then after fermentation its pH falls to 4.8, this confirms the formation of acid during fermentation. The result of pH showed that pH 6 gave maximum production of bioethanol.

The increased amount of bioethanol was produced at 20°C, 25°C and 30° C but the maximum bioethanol was obtained at 30°C. Then its production decreases temperature at 35°C and 40°C. At optimized pH and temperature, fermentation period was analyzed in hours (h). The bioethanol produced at 24 h and 48 h of fermentation period was increases. It was found that its production decreases gradually on further increases the fermentation period from 72 h to 144 h (Table-1). The result was clearly shown that maximum amount of bioethanol was obtained at 48 h of fermentation period. Hence the highest bioethanol 9.31±0.08 g/L was produced at pH 6, temperature 30ºC and fermentation period of 48 h by *Pichia stipitis* NCIM 3497 under anaerobic conditions.

Table 1: Optimization of different parameters for bioethanol production

Inoculum size		pH		Temperature		Fermentation period	
% (v/v)	Bioethanol (g/L)	pH	Bioethanol (g/L)	°C	Bioethanol (g/L)	Hours	Bioethanol (g/L)
0.5	6.81±0.23^{c}	5	6.60±0.2^{b}	20	5.15 ± 0.08^{d}	24	8.69 ± 0.08^{b}
1	7.50±0.03^{b}	**6**	**8.67 ± 0.07^{a}**	25	7.20 ± 0.05^{b}	**48**	**9.31±0.08^{a}**
1.5	**8.36±0.06^{a}**	**7**	5.33± 0.2^{c}	**30**	**8.69 ±0.08^{a}**	72	8.05 ± 0.03^{c}
2	7.21±0.05^{b}			35	6.30 ± 0.07^{c}	96	7.27± 0.07^{d}
3	6.38±0.06^{d}			40	4.63 ± 0.22^{e}	120	7.05 ±0.03^{e}
						144	6.15 ± 0.08^{f}

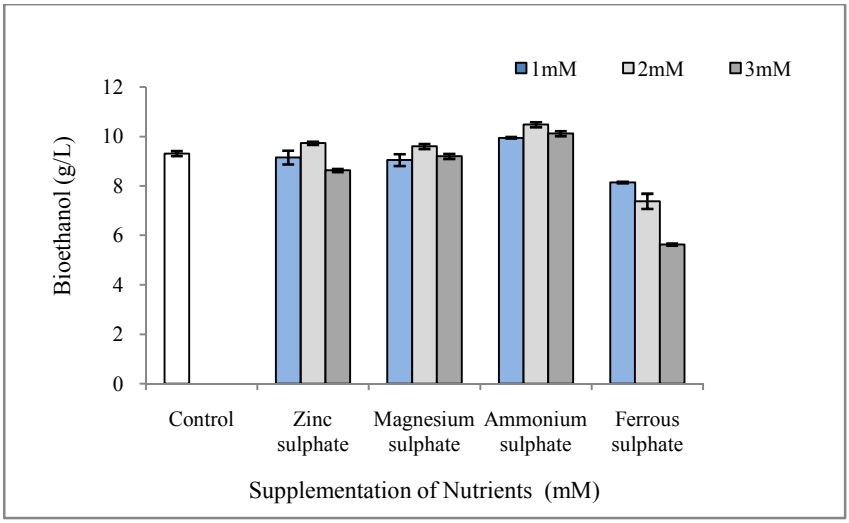
ANOVA of- Inoculum size - F= 41.85, p<0.000; pH - F= 109.99, p<0.000; Temperature- F= 188.56, p<0.000; Fermentation period- F= 267.92, p< 0.000. Means followed by similar superscript letters of each parameter did not differ significantly at the 5% level.

P.stipitis is the most promising fermenting yeast that metabolize xylose by xylose reductase and capable to use both NADPH and NADH as a cofactor (Bhatia and Johri; 2015). *Pichia stipitis* NCIM 3498 was optimized for ethanol production and found maximum ethanol 10.9 g/l at 2% inoculum level from lignocellulosic feedstock of

Ananas cosmosus (L.) Merr. at 30º C, in anaerobic batch fermentation media (Bhatia and Johri; 2015).

Fermentation parameter was also optimized by other researchers. The maximum ethanol production from xylose fermentation by *P. stipitis* Y-7124 cells was also reported at 30°C temperature (Lee et al. 2000). Bioethanol produced from cashew apple juice fermented by immobilized yeast and also found maximum production at pH 6 (Neelakandan and Usharani; 2009). Ethanol from acid pretreated DORB hydrolysate was reported 12.47±0.26 g/L by *P. stipitis* NCIM3499 at 30ºC, pH 5.5 after 72 h of incubation period (Chandel et al. 2009). But in our work maximum bioethanol was produced by *P. stipitis* NCIM 3497 from DORB without any pretreatment step.

In nutrient supplementation, addition of $ZnSO_4$ and $MgSO_4$ salts individually as a source of zinc and magnesium in fermentation media. At 2mM concentration both salts were increases bioethanol as compared with control but failed to increase its production at 1mM and 3mM concentration. The increases amount of bioethanol was produced on addition of all concentrations of ammonium sulphate (nitrogen source) but maximum was obtained at 2mM concentration. The ferrous sulphate was added in fermentation media as a source of iron and found that production of bioethanol decreases in all concentrations, even less than the control as clearly shown in Figure 1.



ANOVA of Bioethanol - 1mM- F= 18.92, p< 0.000; 2mM- F=55.39, p<0.000; 3mM- F=102.673, p<0.000

Figure 1: Production of bioethanol on supplementation of different nutrient salts

Some other researchers were also found similar results with our findings of nutrients supplementation. The maximum ethanol 10.9 g/l was obtained from *Ananas cosmosus* (L.) Merr. on addition of ammonium sulphate by *Pichia stipitis* NCIM 3498 in anaerobic batch fermentation media that supports the finding of our work (Bhatia and Johri; 2015). The highest ethanol concentration was produced by *Saccharomyces cerevisiae* on addition of zinc sulphate (0.05 g/L) in synthetic media at 30°C (Zhao et al. 2009). *Saccharomyces cerevisiae* was reported, maximum ethanol 18.6 ± 0.5% (v/v) from low cost basal medium on supplementation of 3.8 g/l $MgSO_4$ (Pereira et al. 2010). Similarly, decreases amount of bioethanol was also reported by *Saccharomyces cerevisiae* in presence of ferrous ions in fermentation media and showed the negative effect on ethanol production (Pereira et al. 2010).

CONCLUSION

Results from this study also concluded that optimum fermentation parameters as well as nutrient supplementation have a great impact on bioconversion of DORB for bioethanol production. This work would help in the development of environment friendly and cost effective technique for bioethanol production from a lignocellulosic biomass.

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REFERENCES

Behera S., Arora R., Sharma N.K. and Kumar S., 2014. Fermentation of Glucose and Xylose sugar for the production of ethanol and xylitol by the newly isolated NIRE-GX1yeast. Recent Advances in Bioenergy Research, **3**:176-182.

Bhatia L. and Johri S., 2015. Biovalorization potential of peels of Ananas cosmosus (L.) Merr. For ethanol production by *Pichia stipitis* NCIM 3498 & *Pachysolen tannophilus* MTCC 1077. Indian J. Exp. Biol., **53**:819-827.

Chandel A.K., Narasu M.L., Rudravaram R., Pogaku R. and Rao L.V., 2009. Bioconversion of de-oiled rice bran (DORB) hemicellulosic hydrolysate into ethanol by *Pichia stipitis* NCIM 3499 under optimized conditions. Int. J. Food Eng., **5**:1-13.

Lee T.Y., Kim M.D., Kim K.Y., Park Y., Ryu Y.W. and Seo J.H., 2000. A parametric study on ethanol production from xylose by *P. stipitis*. Biotechnol. Bioprocess Eng., **5**: 27-31.

Ministry of Health and Family Welfare; 1985. The Indian Phamacopoeia. The controller of publications, Delhi, India:113-115.

Neelakandan T. and Usharani G., 2009. Optimization and production of bioethanol from cashew apple juice using immobilized yeast cells by *S. cerevisiae*. Am. Euras. J. Sci. Res., **4**:85–88.

Pereira F.B., Guimarães P.M.R., Teixeira J.A. and Domingues L., 2010. Optimization of low-cost medium for very high gravity ethanol fermentations by *Saccharomyces cerevisiae* using statistical experimental designs. Bioresource Technol., **101**:7856–7863.

Sun Y. and Cheng J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technol., **83**:1–11.

Yadav J.B., 2003. Advanced practical, physical chemistry. Goel publication house. Krishna Prakashan Media (P) Ltd, Meerut, India: 52-56.

Zhao X.Q., Xue C., Ge X.M., Yuan W.J., Wang J.Y., and Bai F.W., 2009. Impact of zinc supplementation on the improvement of ethanol tolerance and yield of self-flocculating yeast in continuous ethanol fermentation. J. Biotechnol., **139**: 55–60.