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# Bioethanol Production from an Agrowaste, Deoiled Rice Bran by *Saccharomyces cerevisiae* MTCC 4780 via Optimization of Fermentation Parameters

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## Abstract

Rising fuel prices and declining fossil fuel reserves have compelled to search cheap sources of energy as biofuels. Biofuels like bioethanol reduce reliance on imported petroleum and decrease greenhouse gas emissions. Owing to depleting convention fuel reserves, there is global emphasis on ethanol production by microbial fermentation process. To ensure food security, bioethanol production from biomass which does not compete directly with food, is required. In this perspective, Deoiled rice bran (DORB), an agrowaste product represents a renewable and cheap non-food biomass resource. The present work was emphasized on ethanol production from DORB hydrolysate by *Saccharomyces cerevisiae* MTCC 4780 under optimized fermentation condition by specific gravity method. The result revealed maximum ethanol production of 9.68% at temperature 30°C, fermentation duration of 48 h and pH 6. To the best of our knowledge, this is the first report on ethanol production from deoiled rice bran by yeast *Saccharomyces cerevisiae* MTCC 4780.

***Keywords**: Agro waste; Biofuel; Biomass; Fermentation; Deoiled rice bran*

## 1. Introduction

The growing demand of bioethanol as an alternative form of fuel has created considerable interest due to the occurrence of global warming predominantly by the combustion of fossil fuels. Bioethanol, the principal fuel used as a conventional gasoline substitute for road transport is mainly produced by the sugar fermentation process. It is a clear colourless liquid, biodegradable, low toxic and causes lesser environmental pollution upon burning due to its high octane number (Schubert, 2006; Chandel *et al.*, 2007a). Blending of ethanol with gasoline makes the fuel mixture more oxygenated and leads to complete combustion (Wyman, 1996; Chandel *et al.*, 2007b). To meet the current demand of fuel, cheap substrates accompanied with efficient process technology are needed for efficient ethanol production. Current ethanol production processes involves food crops such as sugarcane juice and maize grains for bioethanol production but these substrates are not recommended due to crisis of falling global food stocks and fuel vs food conflict. In this perspective, lignocellulosic feedstock may prove as cheap substrate for ethanol production. Lignocellulosic feedstock such as agro crop residues, fruit and vegetable waste, weeds and forest waste is available in abundance and are cheap sources of fermentable sugars (Lin and Tanaka, 2006; Lynd *et al.*, 2005). Rice is an agricultural product and one of the leading food crops in the world. It is staple food for many Asian countries such as China, India, Indonesia, Bangladesh, Vietnam and Thailand. Rice is mainly processed as milled rice with the global production of 480 million tons per annum (Muthayya *et al*., 2014; Sereewatthanawut *et al*., 2008). Rice grain is composed of three layers: the endosperm, which contains starchy carbohydrates; the germ, which consists of antioxidants; and the outer coating or bran, which is rich in fibre and vitamins. Rice bran contains oil content, which is further processed for the extraction of oil which is known as rice bran oil. The solid residue left after the extraction of oil from rice bran is known as de-oiled rice bran (DORB). DORB is a lignocellulosic agrowaste, which contains 9% protein, 39% cellulose, 28% hemicellulose and 24% lignin (Pogaku *et al*., 2009). De-oiled rice bran (DORB) is a carbohydrate rich agro-industrial by product left over after extraction of oil. DORB represents the low-cost agricultural biomass and can be considered as a potential source for the production of bioethanol. For sustainable production of bioethanol, selection of economically feasible feedstock and suitable fermenting microbe is of utmost importance. A variety of microorganisms including bacteria, fungi and yeast have the ability to degrade the cellulosic biomass. The most widely used yeast for ethanol fermentation from starch based substrates is *Saccharomyces cerevisiae* however it has not been fully explored for conversion of lignocellulosic substrates. Fongsatitkul *et al*. (2018) used an immobilized *Saccharomyces cerevisiae* in beads made from polyvinyl alcohol and palm oil fuel ash to explore the potential to produce ethanol by fermenting a distillery wastewater. A variety of agricultural biomass has been employed but little work has been done to utilize DORB as feedstock for the production of ethanol. The present research investigates production of bioethanol from DORB by *Saccharomyces cerevisiae* via parametric optimization.

## 2. Materials and methods

*2.1 Collection of Deoiled Rice bran sample*

Deoiled Rice Bran (DORB) was collected from Shree Sita Refineries, Durg, Chhattisgarh, India. The sample was sundried and finely grounded to reduce the surface area. Price of DORB varied from 7 Rs/Kg to 14 Rs/Kg depending upon the production, processing of rice and seasonal market value of rice bran.

*2.2 Microorganism and maintenance*

*Saccharomyces cerevisiae* MTCC 4780 was procured from the Microbial Type culture Collection (MTCC), Chandigarh, India and maintained on Yeast Peptone Glucose (YPG) Agar medium, stored at 4°C. Lacto phenol cotton blue mount of the yeast was prepared to view the microscopic structure (Prescott, 2002).

*2.3 Fermentation medium and inoculum preparation*

*Saccharomyces cerevisiae* MTCC 4780 broth culture in YPG was used as inoculum and Deoiled Rice Bran (DORB) medium was prepared by autoclaving DORB with Distilled water in the ratio of 1:10 in 250 ml Erlenmeyer flask.

*2.4 Ethanol production from DORB hydrolysate*

24 hours old *Saccharomyces cerevisiae* MTCC 4780 broth culture was inoculated (inoculum size 2% v/v) in Deoiled Rice Bran (DORB) medium (1:10 ratio of DORB: Distilled water) and incubated under unoptimized condition in incubator at 27±2 °C for fermentation period of 72 h for fermentation and thereafter flasks were removed from incubator and fermented hydrolysate was collected.

*2.5 Parametric optimization*

Optimization of various physical factors viz. fermentation duration, temperature, pH were studied to determine the best suitable conditions for maximum ethanol production from DORB hydrolysate. These parameters include: fermentation duration (24-96 h), pH (4.0-7.0), temperature (20-35°C). All the experiments were carried out in triplicates.

*2.6 Analytical methods*

*2.6.1. Qualitative test of Ethanol*

Jones test was used for qualitative estimation of ethanol in crude fermented sample

(Jones, 1953). Crude DORB fermented hydrolysate, 2% $K_2Cr_2O_7$, conc. $H_2SO_4$ in the of 1:2:1 ratio was taken in a test tube, appearance of blue green colour within seconds indicates presence of ethanol in fermented sample (Caputi *et al*., 1968). After confirmation of the presence of ethanol in fermented sample, the fermented DORB was distilled through simple distillation assembly and quantitative estimation of ethanol was done.

*2.6.2. Quantitative estimation of Ethanol*

The fermented DORB filtrate was finally distilled through simple distillation assembly to collect the distillate and quantify the ethanol concentration of the fermented sample. Ethanol concentration of the distillate was calculated by determining the specific gravity according to protocol of Pharmacopoeia of India (1985). Specific gravity method (Yadav, 2003) was used to determine the ethanol percentage (in v/v) from the standard table correlating the percentage volume of ethanol with specific gravity at 25˚C.

*2.7. Statistical analysis*

All the experiments were performed in triplicate and data were analyzed by one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used for tests of significance at 0.05 levels with SPSS version 10.0.

## 3. Results and discussion

Effect of various physical factors on ethanol production from *S. cerevisiae* using DORB

*3.1. Effect of fermentation duration*

Deoiled rice bran hydrolysate was kept for fermentation for different duration ranging from 24 h to 96 h. The ethanol production achieved after incubation period of 24 h, 48 h, 72 h, 96 h was 8.49%, 9.03%, 7.85%, 7.76% respectively. Ethanol concentration increased with increase in fermentation time up to 48 h and thereafter decreased. Maximum ethanol production of 9.03% was achieved after 48 h of fermentation (Figure1a).When fermentation was carried out for 72 and 96 h, there was decrement in ethanol concentration i.e. 7.85% & 7.76% respectively. Ado *et al*. (2009) obtained maximum ethanol concentration of 4.30 g/100 ml on the 4th day of fermentation using cassava starch as substrate by co-cultures of *Aspergillus niger* and *S. cerevisiae* in a simultaneous saccharification and fermentation process. Beliya *et al*. (2013) obtained maximum bioethanol production of 10.5% from deoiled rice bran by on 3rd day of fermentation by yeast *Pichia kudriavzevii* RCEF 4907.

*3.2. Effect of Temperature*

Optimization of fermentation temperature is an important parameter in any bioethanol production process. Incubation temperature varying from 20 ºC to 35 ºC was taken for ethanol production from DORB hydrolysate. Ethanol concentration of 8.31%, 7.85%. 9.68%, 8.94% was obtained at fermentation temperature of 20 ºC, 25 ºC, 30 ºC & 35 ºC respectively. Maximum ethanol production of 9.68% was obtained at 30ºC (Figure 1b). Similar result was observed by Chandel *et al*. (2009) as they have also reported 30ºC as optimum temperature for maximal ethanol yield (0.44±0.011g/g) from deoiled rice bran acid hydrolysate using *P. stipitis* NCIM 3499. Beliya *et al*. (2013) observed that 25ºC as optimum temperature for bioethanol production from deoiled rice bran. Tahir *et al*. (2010) investigated effect of cultural conditions on ethanol production by locally isolated *S. cerevisiae* Bio-07 and observed that 30ºC was optimum temperature for ethanol production.

*3.3. Effect of pH*

pH of fermentation medium is an important physiological parameter in bioethanol production by microorganisms. Natural pH of DORB hydrolysate is 6, hence pH above and below this was used to study the effect on ethanol production. Decrease or increase in pH may affect the metabolism of the fermenting microorganism and thus the yield of ethanol. Ethanol concentration of 8.67%, 7.58%, 9.68% and 7.67% was obtained at pH of 4, 5, 6 and 7 respectively. However, the ethanol yield was lesser than that obtained at the natural pH of DORB hydrolysate i.e pH 6 (Figure 1c). Das *et al*. (2013) observed optimum production of ethanol (40.1 g $L^{-1}$) at medium pH of 5.9 by mixed fermentation of rice straw by *Saccharomyces cerevisiae* MTCC 173 and *Zymomonas mobilis* MTCC 2428. Ado *et al*. (2009) studied bioconversion of cassava starch into ethanol and found maximum yield of ethanol at pH 5 by simultaneous saccharification and fermentation by *Aspergillus niger* and *S. cerevisiae*. Asli (2010) recorded best ethanol production at pH 4.5 by *S. cerevisiae* SC1 from grapes pomace. Beliya *et al*. (2013) reported pH of 6.5 for maximum bioethanol production from Deoiled rice bran by *Pichia kudriavzevii*.

Upon parametric optimization, fermentation duration 48 hours, temperature 30 °C and unaltered pH i.e. 6 of the DORB hydrolysate were found optimum for the production of bioethanol by *S. cerevisiae*. Deoiled Rice bran medium inoculated with *S. cerevisiae* MTCC 92 gave an appreciably higher ethanol production of 9.68% under optimized fermentation conditions.

## 4. Conclusions

Deoiled Rice bran medium fermented by *Saccharomyces cerevisiae* MTCC 4780 gave an appreciable ethanol production under optimized fermentation conditions. It may be concluded that *S. cerevisiae* was capable of hydrolysing sugar present in DORB hydrolysate and convert it into ethanol efficiently. This study showed that DORB can be used as a potential biomass for ethanol production by *Saccharomyces cerevisiae* and can be scaled upto industrial ethanol production level in a cost efficient manner.

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## References

Ado SA, Olukotun GB, Ameh JB, Yabaya A. Bioconversion of cassava starch to ethanol

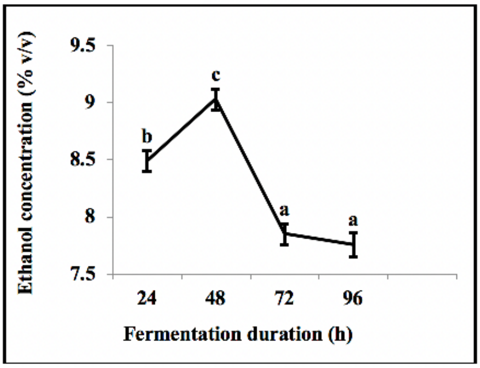


**Figure1a.** Effect of fermentation duration on bioethanol production from DORB by *Saccharomyces cerevisiae* MTCC 4780 at 27 °C and pH 6.0 (df=3 F=38.747 p=0.000) Mean values not sharing a common alphabets differ significantly at 0.05 levels by DMRT (Duncan's Multiple Range Test)

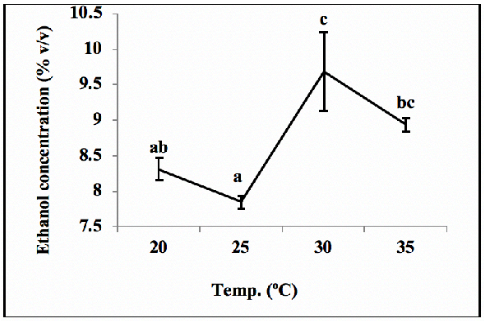


**Figure1b.** Effect of temperature on fermentation for bioethanol production from DORB by *Saccharomyces cerevisiae* MTCC 4780 for 48 h at pH 6.0 (df=3 F=7.093 p=0.012) Mean values not sharing a common alphabets differ significantly at 0.05 levels by DMRT (Duncan's Multiple Range Test)

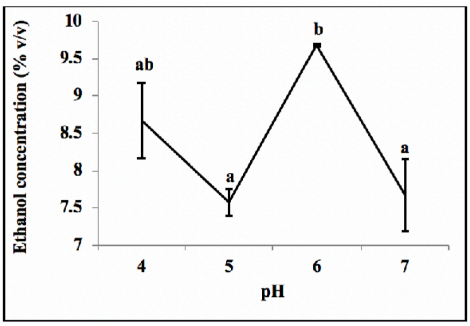


**Figure 1c.** Effect of pH on fermentation for bioethanol production from DORB by *Saccharomyces cerevisiae* MTCC 4780 for 48 h at 30°C. (df=3 F=7.470 p=0.100). Mean values not sharing a common alphabets differ significantly at 0.05 levels by DMRT (Duncan's Multiple Range Test)

in a simultaneous saccharification and fermentationprocess by co-cultures of *A. niger* and *S. cerevisiae*. Science World Journal 2009; 4(1):19–22.

Asli MS. A study on some efficient parameters in batch fermentation of ethanol using *Saccharomyces cerevesiae* SC1 extracted from fermented siahe sardasht pomace. African Journal of Biotechnology 2010; 9(20): 2906–2912.

Beliya E, Tiwari S, Jadhav SK, Tiwari KL. Deoiled rice bran as a source of bioethanol. Energy Exploration & Exploitation 2013; 31(5): 771–782.

Caputi AJr, Ueda M, Brown T. Spectrophotometric determination of ethanol in wine. American Journal of Enolnology and Viticulture 1968; 19(3): 160–165.

Chandel AK, Narasu ML, Rudravaram R, Pogaku R, Rao LV. Bioconversion of de-oiled rice bran (DORB) hemicellulosic hydrolysate into ethanol by *Pichia stipitis* NCM3499 under optimized conditions. International Journal of Food Engineering 2009; 2(4): 1-12.

Chandel AK, Chan EC, Rudravaram R, Narasu ML, Rao LV, Ravindra P. Economics and environmental impact of bioethanol production technologies: an appraisal. Biotechnology and Molecular Biology Reviews 2007a; 2: 14-32.

Chandel AK, Kapoor RK, Narasu ML, Viswadevan V, Kumaran SSG, Ravinder R, Rao LV, Tripathi KK, Lal B, Kuhad RC. Economic evaluation and environmental benefits of biofuel: an Indian perspective. International Journal of Global Energy Issues 2007b; 28: 357-381.

Das A, Paul T, Jana A, Halder SK, Ghosh K, Maity C, Mohapatra PKD, Pati BK, Mondal KC. Bioconversion of rice straw to sugar using multizyme complex of fungal origin and subsequent production of bioethanol by mixed fermentation of *Saccharomyces cerevisiae* MTCC 173 and *Zymomonas mobilis* MTCC 2428. Industrial Crops and Products 2013; 46: 217-225.

Fongsatitkul P, Khongkhaem P, Elefsiniotis P, Supawadee P. Use of Immobilized *S. cerevisiae* in Beads Made from Polyvinyl Alcohol and Palm Oil Fuel Ash to Enhance Ethanol Production from a Distillery Wastewater. EnvironmentAsia 2018; 11(1): 61-79.

Jones ER. Jones reagent. Journal of the Chemical Society 1953; 457: 2548-3019.

Lin Y, Tanaka S. Ethanol fermentation from biomass resources: Current state and prospects. Applied Microbiology and Biotechnology 2006; 69: 627-642.

Lynd LR, Van Zyl WH, McBride JE Laser M. Consolidated bioprocessing of cellulosic biomass: an update. Current Opinion in Biotechnology 2005; 16:577–583.

Muthayya S, Sugimoto JD, Montgomery S, Maberly GF. An overview of global rice production, supply, trade, and consumption. Annals of the New York Academy of Sciences 2014; 1324: 7-14.

Pharmacopoeia of India. The Indian Pharmacopoeia. Controller of publications, India. 1985.

Pogaku R, Rudravaram R, Chandel AK, Linga VR., Yim ZH. The effect of de-oiled rice bran for single cell protein production using fungal cultures under solid state fermentation. International Journal of Food Engineering 2009; 5(2):1-8.

Prescott H. Laboratory exercises in microbiology. 5th ed. Mac Graw Hill, Newyork. 2002.

Schubert C. Can biofuels finally take center stage. Nature Biotechnology 2006; 24:777-784.

Sereewatthanawut I, Prapintip S, Watchiraruji K, Goto M, Sasaki M, Shotipruk A. Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. Bioresource Technology 2008; 99: 555-61.

Tahir A, Aftab M, Farasat T. Effect of culture conditions on ethanol production by locally isolated *S. cerevisiae.* Journal of Applied Pharmaceutical Science 2010; 3(2): 72–78.

Wyman CE. Ethanol production from lignocellulosic biomass: overview In C: E: Wyman (Eds.), Handbook on bioethanol: production and utilization. Taylor and Francis, Washington, DC.1996.

Yadav JB. Advanced practical physical chemistry. Goel publishing house unit of Krishna prakashan media, India. 2003.