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Immobilized *Saccharomyces pastorianus* Cells in Electro-fermentation: A Novel Approach to Bioethanol Production from Mustard Straw

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The dwindling reserves of non-renewable fuels necessitate the exploration of renewable alternatives such as bioethanol. Utilizing and producing bioethanol locally boosts economies, lowers carbon footprints, and encourages self-sufficiency. One such promising lignocellulosic material that serves as an ethanol source is mustard straw. In this study, 3% sulfuric acid was used to hydrolyse

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fresh mustard straw, which included 4.75% moisture, 8.1% ash, 40% cellulose, 14.1% hemicellulose and 3.9% lignin, for 30 minutes at 120°C in an improvised reactor. 470-590 µg/ml of fermentable reducing sugars were produced by this procedure, as shown by glucose standardization with a UV spectrophotometer set at 540 nm. *Saccharomyces pastorianus*, a very effective strain of yeast that produces ethanol, has been isolated and utilized in an electro-fermentation system. In comparison to free cells, immobilizing yeast cells in calcium alginate beads provided benefits like simpler product recovery, cost-effectiveness, stability, and reusability in further fermentation procedures. The electro-fermentation process can be utilized to optimize metabolic processes taking place during fermentation and the immobilization of yeast is used to increase the ecological competence of cultures. In order to produce ethanol, the immobilized yeast cells were employed as the inoculum, with free-cell inoculum acting as the control. In comparison to free-cell cultures, immobilization led to a considerable increase in ethanol generation (15µg/ml) when external voltage of 3V was supplied. The generation of ethanol was seen decreased to14µg/ml when an external voltage of 4V was applied, in comparison to controls. These results show that modest external voltage applications can affect the ethanol production. The ethanol's identity was confirmed by the FTIR spectrum, which displayed the broad bandwidth of O-H stretch of alcohols in 3500-3200 cm^{-1} region, C-H bending at 2000-1650 cm^{-1} and carboxylic acid, C=C stretch around 1400-1395 cm^{-1} and 895-885 cm^{-1} region respectively. Thus, this work emphasizes the potential of electro-fermentation and mustard straw in the production of sustainable bioethanol.

Keywords: Bioethanol; electro-fermentation; mustard straw; yeast immobilization.

1. INTRODUCTION

The accelerated global population, motorization, industrialization and climate change has prompted a significant upsurge in the demand of world energy. With the beginning of industrial revolution in 18th and early 19th century, energy has become essential to sustain high standards and economic prosperity [1]. Currently fossil fuels accounts for 80% of primary energy, with 58% of its proportion consumed by transportation sector playing a major role in emission of greenhouse gases (GHG) [2]. However, these non-renewable fossil fuels are depleting rapidly and are insufficient to meet present-day substantial requirements. The continuously rising demand for fossil fuels influences world economic activity and crude oil pricing [3]. The concern about energy security and greenhouse gas emissions has driven modern society to shift its energy sources towards efficient, renewable, substantial, and economical for a better environment [4].

Bioethanol production with the use of lignocellulosic material from agricultural biomass is considered as a sustainable and value-added product. The lignocellulosic material comprises 80% of polysaccharides and must be treated to produce fermentable sugars [5,6]. In order to degrade the crystalline structures of lignocellulosic material and increase the porosity size, the material has to undergo pretreatment and hydrolysis developments. Acid thermal process using dilute acid is considered as a possible approach, which could be carried out to separate lignin and hemicellulose from cellulose leading to improved hydrolysis [7].

When the biomass is thermochemically pretreated, numerous inhibitory compounds such as furfural, acetic acids and formic acids that interfere with the fermentation process are released reducing the growth rate of the fermenting yeast. Despite the fact that, alcoholic fermentation of sugar is a well-recognized and pervasively used method for ethanol production, numerous advancements need to be made in order to make this process cost-effective. The process of entrapping fermenting yeast in adhesive surfaces, when compared to free cells is a promising approach to confront the challenges of inhibitory compounds, enhance cell stability and viability resulting in improved bioethanol production [8]. To significantly improve the fermentation efficiency, calcium alginate technique is utilized to immobilize cells of Saccharomyces sp. that functions as biocatalyst. Immobilization of yeast cell allows repetitive cycles of ethanol production resulting in increased fermentation efficiency and economic advantages [9].

A newly developed technique called electro-fermentation which combines electrochemistry with conventional fermentation can be used to improve ethanol output, allowing electrochemical

regulation of the microbial fermentative metabolism. The advantages of this process include its (i) selectivity, (ii) increased efficiency in using sugar (carbon) as fuel, (iii) reduced need for chemicals to maintain pH or redox balance, (iv) improved cell growth, and (v) occasionally improved product recovery [10,11]. To my knowledge at this time, there isn't any information in the literature on using immobilized yeast to produce bioethanol using mustard straw waste in an electro-fermentation system. The present study aims to explore effective strains of Saccharomyces sp. to ferment hexose and pentose sugars that are produced extensively during hydrolysis and to possibly use immobilized yeast in an electro-fermentation system for efficient production.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Mustard Straw Waste

The waste of mustard straw was collected from Bhiwani district in Haryana, India. The straws were cut into small sizes and dried in hot air oven at 70℃ for 24 hours. The dried straws were then ground using laboratory blender and sieved through a 4µm mesh. The powdered straw was stored in a sealed plastic bag at room temperature until further use.

Fig. 1. Preparation of mustard straw

2.2 Raw Material Characterization

2.2.1 Determination of moisture content

The milled mustard straw waste was put on an aluminum foil pre-weighed (W_1), and the weight of straw and aluminum foil was recorded (W_2). Then, the straw was kept in an oven at 100℃ for 8 hours until the sample was completely dried and obtained a constant weight (W_3). Finally, the moisture content was calculated using the formula [12].

$$\text{Moisture}(\%) = \frac{W_2 - W_1}{W_2 - W_3} \times 100$$

2.2.2 Determination of total ash content

10g of grounded straw was taken in as clean dry crucibles and the weight was recorded. The crucible was then transferred to a pre-heated furnace and after 20 min the temperature was increased from 250 to 450℃. 1 hour later the crucible was removed from the furnace, allowed to cool and the weight was recorded [13]. The total ash content was determined by using the equation given below.

$$\text{Total ash content}(\%) = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

W_1 is the weight of empty crucible, W_2 is the weight of crucible and straw and W_3 is the mass of cooled crucible and straw.

2.2.3 Chemical analysis of substrate

To determine the chemical analysis of the substrate, slight modifications were made to [14]. 1g of sample (a) was added to 150ml of distilled water and boiled for 2 hours at 100℃. The filter residue is oven-dried and weighed (b). Followed by this, 150ml of 1N H_2SO_4 was added and kept in a water bath at 100℃ for 1 hour. The filtrate is neutralized, dried, and weighed (c). The dry residue was soaked in 100ml of 72% H_2SO_4 at room temperature. The residue is rinsed with distilled water until neutral pH. The residue is dried until constant weight is attained (d). Using the following formula, the composition was calculated:

$$\text{Lignin level}(\%) = \frac{d-c}{a} \times 100 \qquad \text{Eq. (1)}$$

$$\text{Cellulose level}(\%) = \frac{c-d}{a} \times 100 \qquad \text{Eq. (2)}$$

$$\text{Hemicellulose level}(\%) = \frac{b-c}{a} \times 100 \qquad \text{Eq. (3)}$$

2.3 Acid Thermal Hydrolysis under Optimized Conditions

An overall of 10 experiments were carried out for the hydrolysis of mustard straw powder using an acid thermal process. The hydrolysis was carried out by soaking 10g of straw waste in a 100ml acidic solution followed by treating thermally at 120℃. The parameters selected were different concentrations of H_2SO_4 (1-5%) and hydrolysis

time (20 mins to 30 mins). The optimization of the hydrolysis was done to obtain maximum reducing sugars from the straw [15].

2.4 Estimation of Reducing Sugar Concentration

The reducing sugar analysis of the hydrolysate was done using 3,5 dinitro salicylic acid (DNS) method. DNS reagent was prepared by dissolving 1g of 3,5 dinitro salicylic acid in 20ml of 2N NaOH solution by continuous stirring. 30g of sodium potassium tartrate was added to 50ml of distilled water and the two solutions were mixed and made up to 100ml with distilled water. To analyze the reducing sugars, 0.6 ml of the sample was taken in a test tube and 0.4 ml of distilled water was added to the tube. To this 0.5ml of DNS reagent was added and kept in boiling water bath for 15 minutes. The test tube was allowed to cool and 10ml of distilled water was added to stop the reaction. The absorbance of the sample was measured at 540 nm wavelength against blank. The reading of the absorbance was compared against the standard graph plotted using absorbance reading of glucose concentrations [16].

2.5 Isolation and Identification of Yeast Inoculum

For the isolation of potential yeast strain, spoiled juices of sugarcane, pomegranate, and sweet lime were collected and serially diluted up to 10^{-6} with sterile distilled water on Yeast Extract Peptone Dextrose (YEPD) media. The plates were incubated at 30˚C for 48 hours. The colony morphology of the isolated yeast was identified by observing the cells under 100x light microscope using lactophenol cotton blue and molecular identification was done using 18srRNA sequencing.

2.6 Immobilization of Yeast Using Calcium Alginate

The yeast cells were immobilized by modifying the procedure of Kumoro et al. [17]. The isolated yeast cells were cultured on YEPD broth for 48 hours at 30℃. 0.4g of sodium alginate was dissolved in 10ml of deionized water and autoclaved at 121℃ for 15 mins. The autoclaved alginate solution was mixed in a beaker containing 48-hour old yeast culture in 1:1 (V/V) and was homogenized using vortex. In another beaker 1.4g of calcium chloride was dissolved in 20ml distilled water and transferred in a sterilized petri plate. The yeast alginate solution was added dropwise to the calcium chloride solution using a sterile syringe with a needle diameter of 1mm. The formed beads were allowed to harden for 10 mins, washed twice with deionized water, filtered, and were dried using tissue paper to remove the water content. The dried beads were stored at 4℃ in falcon tubes until required.

2.7 Viability of Immobilized Yeast Cells Stored at Different Conditions

1g of immobilized yeast cells that were entrapped using calcium alginate were set free by adding in 9ml of 0.2M phosphate buffer. To ensure that the cells were completely released from alginate beads the cells were vortexed for 20 mins. The resulting samples were plated on YEPD agar plates [18].

2.8 Construction of Electro-fermentation System

300ml mustard straw hydrolysate as carbon source and 4% inoculum was poured in a plastic container of 500ml capacity. Copper wire was used as an anode and Aluminum mesh as cathode. To determine the optimum supply of external voltage, the container was supplied with different voltages between (0-5V) constantly. Ethanol production in the presence of voltage was monitored at 12-hours intervals. 300ml of hydrolysate with yeast strain was allowed to produce ethanol by fermenting in the absence of voltage at 30℃ to compare bioethanol production with presence and absence of voltage [19].

2.9 Estimation of Bioethanol Using Chromic Acid Method

Post fermentation the resulting end product was transferred to a 500ml round bottom flask and the ethanol was extracted using fractionating distillation at 78℃ for 4-6 hours. The extracted bioethanol was estimated using chromic acid method [20].

2.10 Specific Gravity Determination of Bioethanol

A pycnometer was used to determine the specific gravity of produced bioethanol. Initially, an empty clean dry pycnometer was weighed (W_0), and then the bottle was filled with bioethanol and reweighed (W_1). Later the ethanol was replaced

with distilled water and reweighed to give (W_2). Specific gravity was measured by using the formula [21],

$$\text{Specific gravity} = \frac{W_! - W_0}{W_2 - W_0}$$

Where:

W_0 is the weight (g) of an empty bottle
W_1 is the weight (g) of bottle + Sample
W_2 is the weight (g) of bottle + Water

2.11 Chromic Acid Oxidation Test of the Distilled Bioethanol

Chromic acid oxidation test was done to identify and confirm that the produced bioethanol is a primary alcohol. 1ml of bioethanol was taken in a clean dry test tube to which 1ml of acetone was added. Later, 2 drops of chromic test reagent were added and the tube was observed for a colour change from orange to blue green [22].

2.12 FTIR Analysis of the Distilled Bioethanol

After distillation the bioethanol produced was examined for the functional groups of alcohol bonds using Fourier- transform infrared (FTIR) spectroscopy. Bruker Spectrum 400 FTIR/FT-FIR Spectrometer with a region of 4000–400 cm−1 was utilized to analyze the chemical structure of bioethanol from mustard straw.

3. RESULTS AND DISCUSSION

The mustard straw sample was examined for its moisture, total ash, cellulose, hemicellulose, and lignin content. The results of the above-mentioned characteristics are given in Table 1. The discoveries of the work revealed that the straw sample has a significant moisture content (4.75%), ash content (8.1%), cellulose (40%), hemicellulose (14.1%) and lignin (3.9%). Similar results were obtained in the study of Nawaz A. [23]. Moisture is a crucial parameter for using biomass as an energy feedstock as it directly influences the conversion and heating efficiency. An ideal fuel should contain low moisture since it escalates the heating value, helping to perform at low temperatures [24]. The ash indicates inorganic residues left behind after ignition or oxidation of the biomass. The cellulose, hemicellulose, and lignin content are equivalent to many agricultural wastes [25].

Table 1. Proximate analysis in mustard straw

Parameters	Value
Moisture content	4.75%
Ash content	8.1%
Cellulose	40%
Hemicellulose	14.1%
Lignin	3.9%

The hydrolysis process converts the complex polysaccharide content of the biomass into monosaccharides by means of acid thermal hydrolysis. Two hydrolysis processes were carried out one to determine the ideal H_2SO_4 concentration and the other to figure out the optimum hydrolysis time. Fig. 2 displays the outcome of the optimized H_2SO_4 concentration and time duration of hydrolysis.

The results show that 4% H_2SO_4 resulted the maximum glucose concentration (584µg/ml) at 20 mins and at 30 mins hydrolysis 3% H_2SO_4 showed the highest glucose concentration (591µg/ml). The findings show that the optimum time for hydrolysis is 30 mins at a concentration of 3% H_2SO_4. The outcomes of Zainal et al. [26] revealed that an increase in hydrolysis time and H_2SO_4 concentration increases the glucose concentration.

The physical and morphological characteristics taking place in cell wall of treated (Mustard straw) are exposed due to hydrolysis. The pretreated biomass revealed removal of inhibitory hydrocarbons, and enlarged porosity resulting in augmentation of exposed area of cellulosic biomass [27].

The isolated yeast strain exhibited good growth on YEPD media with cream white colonies with smooth texture and based on the morphological characteristics the isolate was assumed to be *Saccharomyces* and was selected for molecular identification.

The Molecular identification is a more reliable method for identifying microorganisms than the morphological characterization. Therefore, the selected isolate was subjected to molecular identification using 18srRNA sequencing. Subsequently, nucleotide sequences were compared using the NCBI-BLAST algorithm. The nucleotides of the isolate were deposited in NCBI database with Accession number: SUB12515552 and matched with already existing sequences to construct a phylogenetic tree. The screened yeast isolate revealed 100% similarity with the sequence of *Saccharomyces pastorianus*.

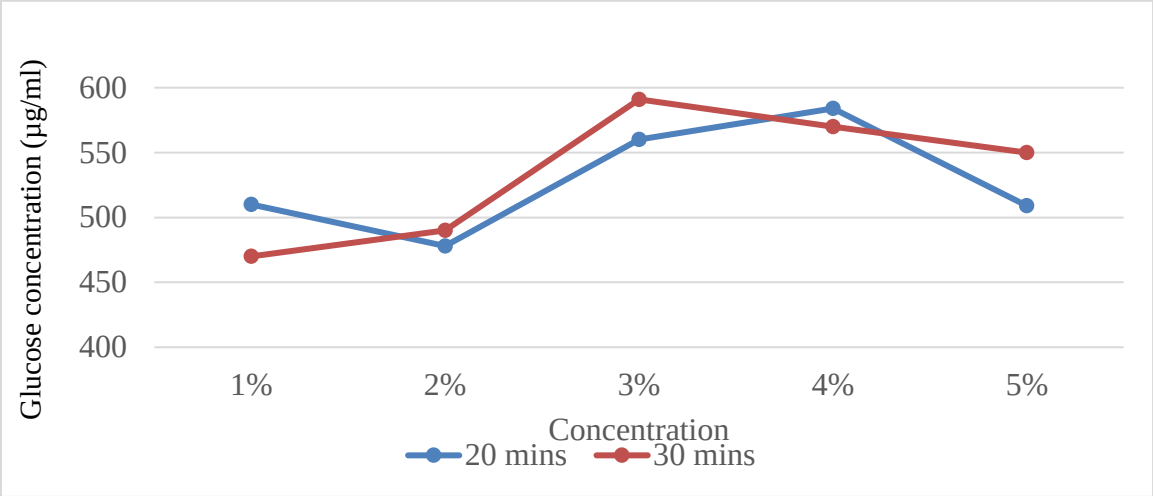

Fig. 2. Impact of acid thermal hydrolysis at different acid concentration and time

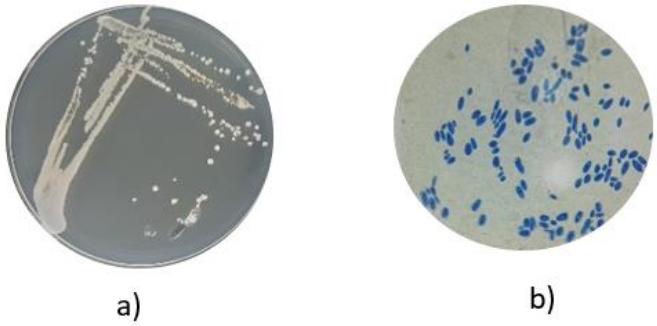

Fig. 3. a) yeast colonies on YEPD plate b) Budding cells

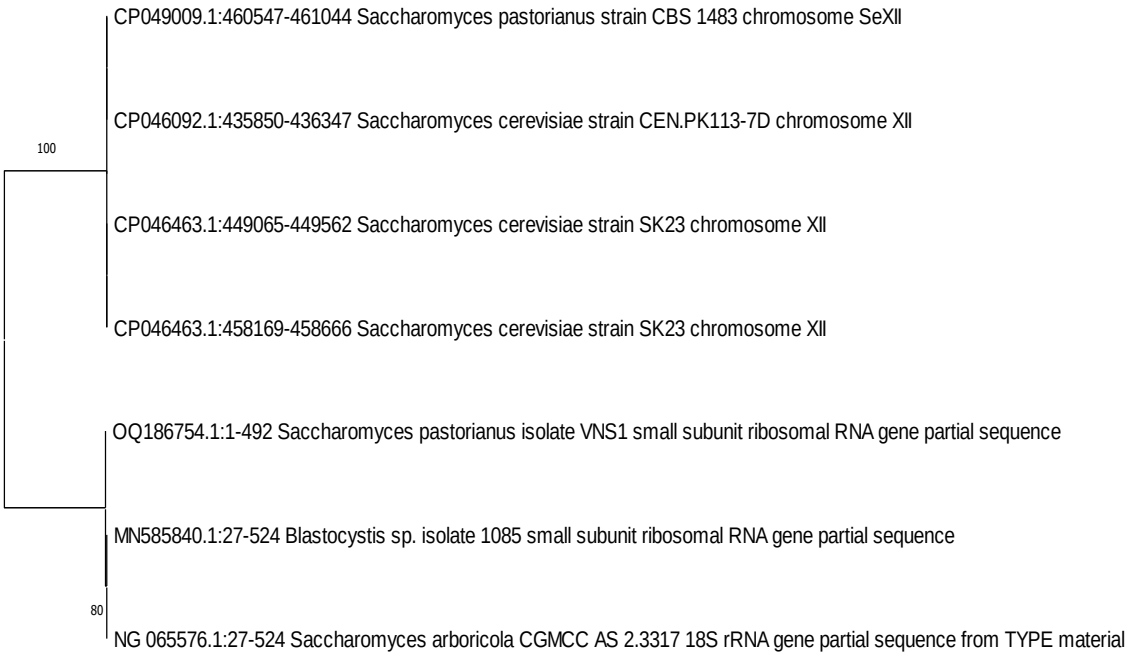

Fig. 4. Phylogenetic tree of yeast isolate

The immobilization of yeast strains relies on the properties of cell adhesion and the bioconversion conditions [28]. Stable active beads of isolated yeast strain were produced using calcium alginate (Fig. 5). The production of bioethanol was higher by immobilized cells after 72 hours. The experiment conducted using free cells in batch fermentation resulted low ethanol yield. The immobilized cells retained their shape and were not degraded during fermentation process. The findings of Apinya S. et. al. [29] reported that the immobilized cells exhibited higher ethanol yield in five consecutive batch fermentation without any cell adaptation.

Fig. 5. Immobilized calcium alginate beads of yeast

The fermentation was performed under optimized conditions in electro-fermentation system using immobilized yeast cells in different voltage supply (Fig. 6). The results depicted that maximum ethanol yield obtained with external supply of 3V (Fig. 7). The obtained results correlate with findings of Jarina et al. [10].

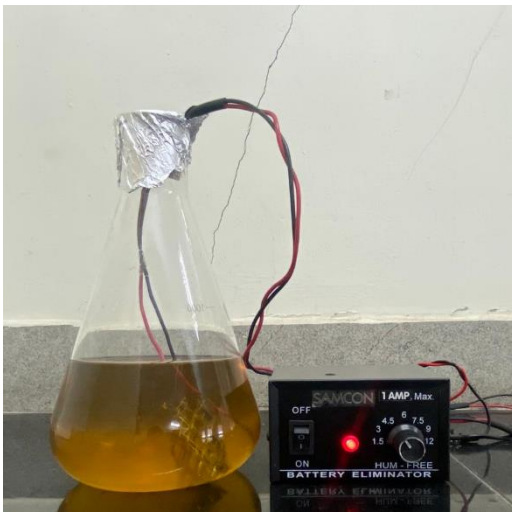

Fig. 6. Construction of Electro-fermentation system

The ratio of fuel density to water density at the same temperature is known as specific gravity (SG). The density of the fuel and water in this study are calculated at a temperature of 30ºC. The specific gravity of the bioethanol produced is 0.8634, which is close to the specific gravity of alcohol, at a distillation temperature of 70°C with a tolerance of ±5°C. This is due to the fact that high temperatures during the distillation process will cause more water to accompany the alcohol and vice versa, increasing the distillate's density [30].

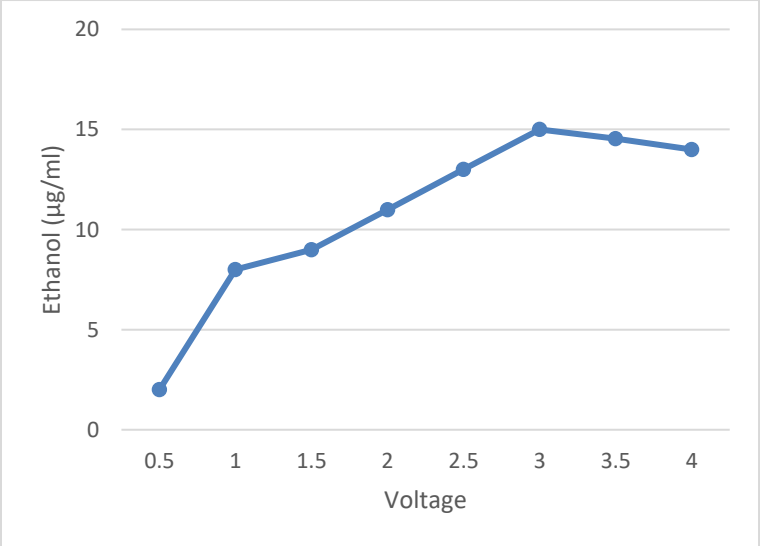

Fig. 7. Bioethanol yield at different external voltage supply

Table 2. Specific gravity of bioethanol

Description	Symbol	Weight(g)		
		1st run	2nd run	3rd run
Empty pycnometer (EP)	W_o	50.0000	50.0000	50.0000
EP + Ethanol	W_1	71.5862	71.5862	71.5862
EP + Water	W_2	75.0000	75.0000	75.0000
Specific gravity (g/ml)		0.8634	0.8634	0.8634
Average specific gravity (g/ml)		0.8634		

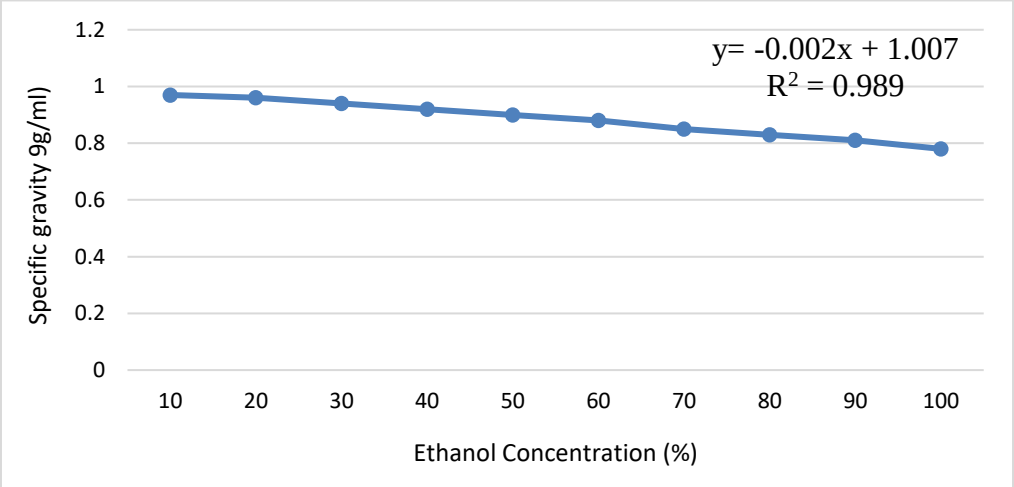

Fig. 8. Specific gravity of ethanol-water mixture at various temperature

Fig. 9. Positive test for chromic acid oxidation test

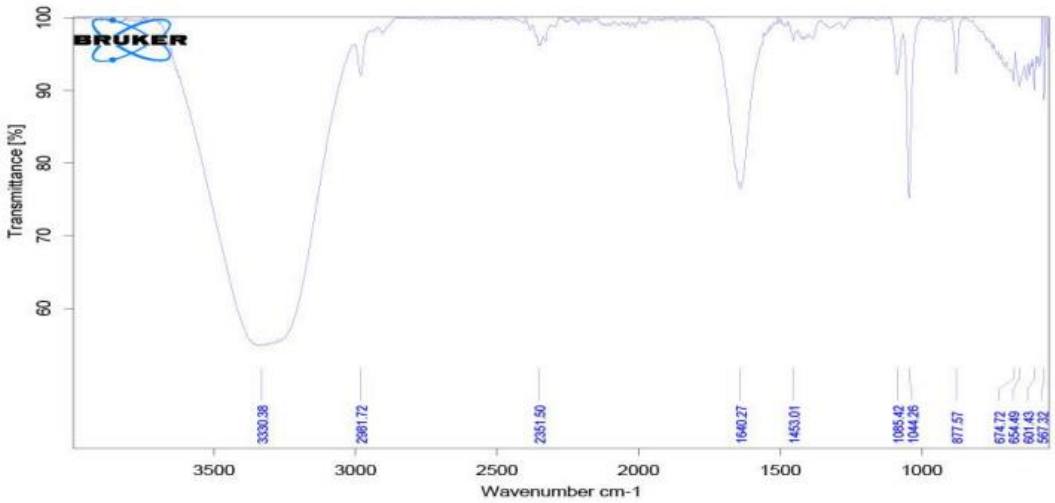

Fig. 10. FTIR spectrum of produced bioethanol

The value specific gravity (0.8634) obtained in this study was very close to ASTM set limit (0.87) for bioethanol [31]. The obtained result was almost nearer to the specific gravity value (0.9) reported in [32] for bioethanol produced. The chromic acid oxidation test confirms the presence of primary alcohol by oxidizing aldehydes and alcohols and reducing chromic acid, indicated by colour change.

Furthermore, the results of FTIR analysis of produced bioethanol displayed the O-H stretch of alcohols with an extremely broad bandwidth by the region 3500-3200 cm^{-1}, while the C-H bending is confirmed by the region 2000-1650 cm^{-1}. The bands at around 1400-1395 cm^{-1} were assigned as carboxylic acids. The C=C bending is confirmed with stretch at 895-885 cm^{-1} region. This confirms that the obtained product from the mustard straw is ethanol and is confirmed by the regions shown in Fig. 10.

4. CONCLUSION

The study concludes that the mustard straw waste can be used as a potential substrate and could be utilized as an energy feedstock post undergoing hydrolysis treatment. Acid thermal hydrolysis is a promising approach for the extraction of lignin and recycling the used of solvents. The encapsulation of *Sccharomyces pastorianus* yeast isolate in calcium alginate is the most reliable and competent method of immobilizing yeast cells. Higher ethanol yield is obtained with immobilized yeast cells in comparison to free cells. Use of immobilized cells in an electro-fermentation system proficiently utilize reducing sugars and ethanol production can be boosted by supplying low level voltage externally. Moreover, beads of *S. pastorianus* relatively displayed good storage stability confirming its applicability for commercial production. Finally, this study could be extended to produce various industrial metabolites from renewable sources, such as butanol, organic acids, biodiesel and biopolymers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Broda M, Yelle DJ, Serwańska K. Bioethanol production from lignocellulosic biomass-challenges and solutions. Molecules. 2022;27(24):8717.
2. Kasibhatta S. Alcohol fuels as an alternative fuels - bringing new heights in sustainability. Alcohol Fuels - Current Technologies and Future Prospect. IntechOpen; 2020.
3. Malik K, Capareda SC, Kamboj BR, Malik S, Singh K, Arya S, Bishnoi DK. Biofuels production: A review on sustainable alternatives to traditional fuels and energy sources. Fuels. 2024;5(2):157-175.
4. Ekpan FM, Ori MO, Samuel HS, Egwuatu OP. Emerging technologies for Eco-friendly production of Bioethanol from Lignocellulosic Waste Materials. Eurasian J. Sci. Technol. 2024;4(3):179-194.
5. Guojie Song, Chihe Sun, Yun Hu, Chen Wang, Changlei Xia, Maobing Tu, Ezhen Zhang, Pau-Loke Show, Fubao Sun. Construction of anhydrous two-step organosolv pretreatment of lignocellulosic biomass for efficient lignin membrane-extraction and solvent recovery. J. Phys. Energy. 2023;5.
6. Beluhan S, Mihajlovski K, Šantek B, Ivančić Šantek M. The production of bioethanol from lignocellulosic biomass: Pretreatment methods, fermentation, and downstream processing. Energies. 2023;16(19):7003.
7. Das N, Jena PK, Padhi D, Kumar Mohanty M, Sahoo G. A comprehensive review of characterization, pretreatment and its applications on different lignocellulosic biomass for bioethanol production. Biomass Conv. Bioref. 2021;82:1–25.
8. Ifeanyi A Ndubuisi, Qijian Qin, Guiyan Liao, Bin Wang, Anene N Moneke, James C Ogbonna, Cheng Jin, Wenxia Fang. Effects of various inhibitory substances and immobilization on ethanol production efficiency of a thermotolerant Pichia kudriavzevii. Biotechnol Biofuels. 2020;13:91.

9. Helena CN, Crlos M, Jaime MG. Yeast immobilization system for second generation ethanol production: Actual trends and future perspectives. Biofuels, Bioproducts & Biorefining. 2021;15(5): 1549-1565.
10. Wang C, Chang D, Zhang Q, Yu Z. Enhanced bioethanol production by evolved *Escherichia coli* LGE2-H in a microbial electrolysis cell system. Bioresources and Bioprocessing. 2024; 11(1):1-10.
11. Sharma J, Kumar V, Prasad R. Engineering of *Saccharomyces cerevisiae* as a consolidated bioprocessing host to produce cellulosic ethanol: Recent advancements and current challenges. 2022;56.
12. Luiza Astolf A, Alan R, Vitor AFC, Maycon A, Kricelle MD, Lucian MC, Jorge AVC. Simultaneous saccharifIcation and fermentation of Spirulina sp. and corn starch for the production of bioethanol and obtaining biopeptides with high antioxidant activity. Bioresour Technol. 2020; 301:122698.
13. Getachew AT, Kumsa DK, Gadissa TG. Optimization of fermentation condition in bioethanol production from waste potato and product characterization. Biomass Conversion and Biorefinery. 2022;10: 1007.
14. Palupi B, Fachri BA, Rahmawati I, Rizkiana MF, Amini HW. Pretreatment of tobacco stems as bioethanol raw material: The effect of temperature and time using chemical method. In AIP Conference Proceedings. 2020;2278(1).
15. Braide W, Kanu IA, Oranusi S, Samuel AA. Production of bioethanol from agricultural waste. Journal of Fundamental and Applied Sciences. 2016;8(2):372-386.
16. Kasegn MM, Simachew A, Redda YT. et al. Evaluation of sugar content and bioethanol production of Ethiopian local varieties "*Nech Tinkish*" and "*Hawaye*" sweet sorghum (*Sorghum bicolor* (L.)). Biotechnol Environ. 2024;1(2).
17. Kumoro AC, Damayanti A, Bahlawan ZA, Melina M, Puspawati H. Bioethanol production from oil palm empty fruit bunches using Saccharomyces cerevisiae immobilized on sodium alginate beads. Periodica Polytechnica Chemical Engineering. 2021;65(4):493–504.
18. Kailasapathy K. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. LWT 2005;39:1221–1227.
19. Jarina J, Pradip D, Bikram P, Rocky M, Pranita P, Mukesh Y, Milan M, Amar PY, Tribikram B, Lakshmaiah S. Enhancement of ethanol production in electrochemical cell by *Saccharomyces cerevisiae* (CDBT2) and *Wickerhamomyces anomalus* (CDBT7). Frontiers in Energy Research. 2019;7:70.
20. Caputi A, Ueda MT, Brown T. Spectrophotometric determination of ethanol in wine, American Journal of Enol. Vitic. 1968;19:160-165.
21. Aleme HG, Costa LM, Barbeira PJ. Determination of ethanol and specific gravity in gasoline by distillation curves and multivariate analysis. Talanta. 2009;78(4-5):1422-1428.
22. Sani U, Abubakar S, Suleiman F, Olabode HO. Proximate analysis nd production of bio-ethanol from sweet potato (*Ipomoea batatas*) white cultivars obtained from Samaru Zaria. Bayero Journal of Pure and Applied Sciences. 2021;14(2): 157–168.
23. Ahmad Nawaz, Pradeep Kumar. Pyrolysis of mustard straw: Evaluation of optimum process parameters, kinetic and thermodynamic study. Bioresource Technology. 2021;340:125722.
24. Koci J, Korecky T, Cerny R. Identification of moisture diffusivity of autoclaved aerated concrete in the form of smooth two-variable function. Energy Procedia. 2017;132:219–224.
25. Lopa Pattanaik, Falguni Pattnaik, Devesh Kumar Saxena, Satya Narayan Nai. Biofuels from agricultural waste. Second and third generation of feedstock: the evolution of biofuels. 2019;103-142.
26. Zainal A, Ellena S, Tadjuddin N. Bioethanol production from waste of the cassava peel (*Manihot esculenta*) by acid hydrolysis and fermentation process. International Journal of PharmTech Research. 2014;6(4):1209-1212.
27. Bharathiraja B, Sudharasnaa T, Bharghavi A, Sri Sowmeya G, Gopika B. Insights on Lignocellulosic pretreatments for Biofuel production - SEM and Reduction of Lignin analysis. International journal of Chem Tech Research. 2014;6(9): 4334–4444.
28. Helena CN, Carlos M, Jaime MG. Yeast immobilization systems for second-generation ethanol production: actual

trends and future perspectives. Bio-fuels, Bioproducts and Biorefining. 2021; 15(5):1549-1565.
29. Sowatad A, Todhanakasem T. Bioethanol production by repeated batch using immobilized yeast cells on sugarcane bagasse. Waste Biomass Valor. 2020;11:2009–2016.
30. Tira HS. Analysis of the calorific value and specific gravity of breadfruit peel bioalcohol (*Artocarpus altilis*) as an alternative fuel at various distillation temperatures. Journal Penelitian Pendidikan IPA. 2023;5163-5169.
31. Harrison OE. Production and Characterization of bioethanol from acid catalysed hydrolysis of Cellulosic biomass (Maize Cob). *J.* Energy Environ. Chem. Eng. 2022;66-70.
32. Tenkolu GA. Optimization of fermentation condition in bioethanol production from waste potato and product characterization. Biomass Conversion and Biorefinery. 2024;5205-5223.

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