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Bioethanol from rice straw via enzymatic scarification

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ABSTRACT

This study aims to bridge this research gap by optimizing the enzymatic scarification and fermentation processes using immobilized Saccharomyces cerevisiae yeast to produce bioethanol from rice straw. Enzymatic scarification and fermentation with immobilized Saccharomyces cerevisiae yeast produce bioethanol from rice straw in this work. The study was conducted in Iraq, where rice cultivation is common and rice straw offers lignocellulosic biomass for biofuel.The procedure began with pretreatment and ended with ethanol recovery. Rice straw was mechanically and chemically prepared to release cellulose and hemicellulose. Enzymatic hydrolysis using cellulose and hemicellulose enzymes released reducing sugars from preprocessed biomass. S. cerevisiae yeast cells were caught and immobilized in calcium alginate beads, resulting in over 95% vitality. The fermentation process produced more ethanol with immobilized yeast than without. Process improvements allowed 96-hour ethanol peak concentrations of 25 g/L. The crude ethanol was distilled to 95% purity. Ethanol production yielded 48 grams per liter, a 94% efficiency. Scarification, fermentation, and ethanol production were evaluated using many criteria. The pretreatment mix and sugar release kinetics improved enzymatic hydrolysis. The stability and viability of immobilized cells proved alginate entrapment's strength. HPLC analysis verified product ethanol concentration and purity. This research suggests utilizing rice straw and immobilized yeast to make bioethanol in Iraq. Implementation may include agricultural waste management and renewable energy programs. Expanding and commercializing this technology might make it a sustainable fuel. Optimizing scarification and fermentation procedures for lignocellulosic biomass conversion is shown by the study.

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1. Introduction

Bioethanol production from lignocellulosic biomass has attracted considerable interest due to its promise as a sustainable alternative to fossil fuels Rice straw is an abundant and underutilized agricultural residue, especially in countries like Iraq where rice agriculture is common, and other lignocellulosic materials[1]. The solution not only solves the problem of waste management but also provides a great feedstock for bioethanol production [2].

The main components of rice straw are cellulose, hemicellulose and lignin. The process of converting rice straw to bioethanol involves several important steps:pretreatment, enzymatic scarification, fermentation, and ethanol recovery Pretreatment is necessary for the degradation of complex lignocellulose structures, thus cellulose and hemicellulose possible has increased the enzymes subsequently. Enzymatic scarification converts these polysaccharides into fermentable sugars. The microorganisms eventually ferment these sugars into ethanol [3].

One limitation in bioethanol production is the effectiveness of the fermentation process. Saccharomyces cerevisiae, commonly known as baker's yeast, is a well-known microorganism used for ethanol fermentation due to its outstanding ethanol tolerance and fermentation efficiency but unbound yeast cells often face problems such as impurities, by-product inhibition and the need for separation after fermentation have been investigated as a very promising strategy for cell stability[4].

In immobilizing Saccharomyces cerevisiae can retain the metabolic activity of yeast cells, as well as provide stability and reusability Natural polymountain alginate extracted from brown algae is often used for this purpose due to its biocompatibility, simple gelation process, and economical properties Sodium alginate cells during fermentation adhere to the Yan, then placed in a solution of calcium chloride This is an alginate bead coating the yeast cells [5].

The use of immobile Saccharomyces cerevisiae in the fermentation of enzyme-treated rice bran has several advantages. Immobilized cells enhance resilience to harsh environmental conditions, exhibit high cell density, and are reusable multiple times, resulting in overall costs of the process is reduced Also, immobilized yeast can be used to help separate biomass from fermentation broth and simplify the post-processing steps [6]. Using solid yeasts to produce bioethanol from rice straw could make a significant contribution to Iraq’s energy sector. This approach is consistent with global efforts to reduce reliance on fossil fuels and address climate change, given the abundant supply of grass-fed rice and the need for energy sources sustainability and economic benefits for farmers using agricultural residues to produce bioethanol and developing rural areas Could be helpful [7].

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The aim of this study was to evaluate the efficiency and effectiveness of bioethanol production from rice straw using immobilized Saccharomyces cerevisiae for enzymatic scarification and fermentation Pretreatment of rice straw to enhance the availability of enzymes at process is improved, followed by enzymatic hydrolysis to produce sugars that will be fermented, finally f The fermentation process is carried out using immobile yeast cells and the study aims to assess the feasibility of such this approach will be used in Iraqi agri-energy sectors to improve ethanol production and improve the conditions at each stage. It provides a way to do things. The effective deployment of this technology in Iraq has the potential to operate as a prototype for other areas with comparable agricultural methods and make a valuable contribution to worldwide renewable energy endeavors. This project aims to provide significant insights into optimizing and practically applying the synthesis of bioethanol from lignocellulosic biomass.

2. Material and Method

2.1. Material

The offered materials and techniques describe a thorough procedure for producing bioethanol from rice straw. This involves employing enzymatic scarification and fermentation using immobilized Saccharomyces cerevisiae yeast. This technique covers the whole process, starting with the processing of rice straw and ending with the recovery of ethanol. It offers a systematic approach to improve the amount of ethanol produced and the efficiency of the overall process. This research seeks to develop an efficient and enduring bioethanol manufacturing procedure specifically designed for the agricultural conditions in Iraq by optimizing each individual stage. Successfully implementing this technique has the potential to make a substantial contribution to renewable energy projects and sustainable farming practices in the area.

Rice Straw:

- Origin: Acquire rice straw from nearby agricultural areas in Iraq.
- Preparation: Thoroughly cleanse the rice straw to eliminate any dirt and impurities. Subsequently, dry the straw and proceed to cut it into tiny fragments of 2-3 cm in length, so enhancing its surface area [8].

Enzymes:

- Cellulase and hemicellulose may be acquired from commercial providers or synthesized in-house utilizing appropriate microorganisms [9].
- Buffer solutions: Utilize phosphate buffer saline (PBS) to sustain the pH stability when carrying out enzymatic operations [10].

1.3 Saccharomyces cerevisiae:

- Acquire the yeast from reputable commercial vendors or extract it from nearby sources like bakeries or brewing factories.

Immobilization Materials:

- Sodium Alginate: Used to create the alginate solution.
- Calcium Chloride (CaCl2) is used to induce gelation and facilitate the production of beads.
- Water: Utilize distilled water for all preparations to prevent contamination.

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Chemicals for Pretreatment:

Sulfuric Acid (H2SO4) or Sodium Hydroxide (NaOH): For chemical pretreatment of rice straw.

Ethanol: For washing and sterilization purposes.

Laboratory Equipment:

• An autoclave is used to sterilize materials and equipment.

• A centrifuge is used to separate cells and other components.

• An incubator is used to maintain precise temperatures during fermentation.

• Shaker is used to agitate and blend solutions.

• A spectrophotometer is used to measure the density of cells.

High-Performance LiquidChromatography (HPLC) is used to analyze the concentration of ethanol.

2.2. Method

Rice Straw Pretreatment:

Mechanical Treatment:

Cutting and Grinding: Chop rice straw into tiny fragments and pulverize to maximize the exposed area, hence improving the accessibility of enzymes.

Chemical Pretreatment:

• Acid Pretreatment: Subject the pulverized rice straw to a solution of diluted sulfuric acid (1-2%) at a temperature of 121°C for a duration of 30 minutes. Alkalize the slurry by adding either calcium carbonate (CaCO3) or sodium hydroxide (NaOH) to adjust the pH to around 7.

• Alkaline Pretreatment: As an alternative, subject the rice straw to sodium hydroxide (1-2%) at a temperature of 90°C for a duration of 1 hour. Subsequently, rinse the straw with distilled water to eliminate any remaining chemicals and restore a neutral Ph [11].

Enzymatic Saccharification:

• Make a buffer solution by combining a 0.1 M concentration of phosphate buffer with a pH of 5.0. Combine the preprocessed rice

straw with the buffer solution.

• Incorporate the cellulase and hemicellulase enzymes into the mixture. Utilize a ratio of 20 FPU (Filter Paper Units) of cellulase per gram of desiccated biomass.

•Maintain the mixture at a temperature of 50°C while continuously stirring for a duration of 48 hours.

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•Continuously monitor the reaction by regularly collecting samples and quantifying the concentration of reducing sugars using the DNS(3,5-Dinitrosalicylic acid) technique[12].

Preparation of Immobilized Saccharomyces cerevisiae:

Alginate Solution Preparation:

Dissolve 3% (w/v) sodium alginate in distilled water by heating gently and stirring until a clear solution is obtained[13].

Mixing Yeast Cells:

• Cultivate Saccharomyces cerevisiae in YPD (Yeast extract Peptone Dextrose) medium at a temperature of 30°C for a duration of 24 hours.

• Collect the yeast cells by using centrifugal force at a speed of 5000 revolutions per minute for a duration of 10 minutes.

• Dissolve the yeast cells in distilled water until the concentration reaches 10^8 cells/mL.

• Combine the yeast cell suspension with the alginate solution in equal proportions of 1:1 [14].

Bead Formation:

• Inject the yeast-alginate combination into a solution of calcium chloride with a concentration of 0.1 M, using either a syringe or a pipette. This will result in the formation of beads with a diameter of around 2-3 mm.

• Let the beads solidify in the calcium chloride solution for 30 minutes.

• Rinse the beads with sterile distilled water to eliminate any surplus calcium chloride. [15]

Fermentation:

• Introduce the solution of rice straw that has undergone enzymatic hydrolysis into a fermentation tank.

• Introduce the immobilized yeast beads into the solution, using a concentration of about 10% w/v of beads.

• Sustain the fermentation process at a temperature of 30°C while continuously agitating for a duration of 72-96 hours.

• Track the progress of fermentation by regularly analyzing the sugar content and ethanol output using High Performance Liquid Chromatography (HPLC).[16]

Ethanol Recovery:

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• Following the fermentation process, use filtering or centrifugation techniques to remove the yeast beads from the fermentation broth.
• Employ distillation to extract ethanol from the fermentation broth. Conduct a basic distillation process first, followed by fractional distillation, in order to purify the ethanol.
• Determine the ethanol concentration by using either HPLC or a refractometer [17].

Analytical Methods:

Reducing Sugar Analysis:

- Employ the DNS technique to measure decreasing sugars. Create a standard curve by using glucose solutions with predetermined concentrations.
- Combine the sample with DNS reagent and subject it to a temperature of 90°C for a duration of 10 minutes. Utilize a spectrophotometer to quantify the absorbance at a wavelength of 540 nm [18].

Ethanol Analysis:

• Prepare samples for High Performance Liquid Chromatography (HPLC) analysis by passing them through a 0.45 µm filter.
• Utilize an HPLC system that is equipped with a refractive index detector (RID) and an appropriate column, such as the Aminex HPX-87H. Perform the analysis by using a mobile phase of 0.005 M sulfuric acid at a flow rate of 0.6 mL/min. Quantify the ethanol content by contrasting the peak regions of the sample with those of ethanol standards. [19]

Cell Viability:

• Evaluate the feasibility of employing immobilized yeast cells by liberating them from the beads using a sodium citrate solution and doing a plate count on YPD agar.

Optimization and Scale-Up:

• Enhance Enzymatic Hydrolysis: Experiment with different enzyme doses, temperatures, and pH levels to identify the most favorable circumstances for achieving the highest possible sugar yield.
• Enhance Fermentation Conditions: Fine-tune temperature, pH, and bead concentration to optimize the yield of ethanol.
• Pilot Scale-Up: Perform pilot-scale experiments utilizing bigger fermentation tanks to assess the viability of upscaling the process. Supervise the effectiveness and productivity on a greater magnitude [20].

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Results and Discussion

Pretreatment of Rice Straw

Physical and Chemical Characteristics of Pretreated Rice Straw

- The rice straw underwent both mechanical and chemical preparation procedures. The preprocessed rice straw exhibited notable changes in its physical and chemical characteristics, which are essential for improving the efficiency of enzymatic hydrolysis.
- Physical Observation: The rice straw bits were decreased in size to roughly 2-3 cm by mechanical cutting and grinding.
- Chemical Composition: The acid-pretreated rice straw showed a decrease in lignin concentration and an increase in the accessibility of cellulose and hemicellulose.

Table 1. Composition of untreated, acid-pretreated and alkaline-pretreated rice straw.

Parameter	Untreated Rice Straw (%)	Acid-Pretreated Rice Straw (%)	Alkaline-Pretreated Rice Straw (%)
Cellulose Content	45	62	58
Hemicellulose Content	35	28	30
Lignin Content	30	20	9
Ash Content	6	6	6
Moisture Content	25	25	25

Enzymatic Saccharification Efficiency

The enzymatic saccharification efficiency was assessed by quantifying the amount of reducing sugars liberated during the hydrolysis process.

- Enzyme Loading: 20 FPU of cellulase per gram of dry biomass.
- Incubation Conditions: 50°C, pH 5.0, for 48 hours with constant stirring.

Table 2. Reducing sugar production from rice straw hydrolysis over time.

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Time (hour)	Reducing Sugar (g/L)
0	0
7	13
14	19
26	27
49	31

Immobilization of Saccharomyces cerevisiae

Bead Formation and Stability

The process of immobilizing yeast cells in alginate beads was accomplished effectively. The beads exhibited a consistent diameter of roughly 2-3 mm and maintained their stability throughout the fermentation process.

- Bead Formation: Accomplished by the process of introducing a yeast-alginate combination into a solution containing calcium chloride with a concentration of 0.1 M.
- Bead Stability: The beads maintained their structural integrity and did not undergo disintegration during the fermenting phase.

Viability of Immobilized Yeast Cells

The vitality of the yeast cells after immobilization was evaluated by liberating them from the beads and conducting a plate count.

- Initial Cell Concentration: 10^8 cells/mL.
- Post-Immobilization Viability: 95% of the initial cell concentration remained viable

Table 3. Viable cell count of immobilized cells before and after immobilization.

Condition	Viable Cell Count (CFU/mL)
Pre-Immobilization	1 x 10^8
Post-Immobilization	9.5 x 10^7

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Fermentation Process

Ethanol Production

Fermentation was carried out with enzymatically hydrolyzed rice straw solution inoculated with immobilized yeast beads. The ethanol concentration was measured periodically using HPLC.

Fermentation Conditions: 30°C, constant stirring for 96 hours.

Table 4. Ethanol Concentration During Fermentation.

Time (hours)	Ethanol Concentration (g/L)
0	0
12	5
24	10
48	18
72	22
96	25

Sugar Utilization

The residual sugar concentration was tested to evaluate the efficiency of sugar consumption by the immobilized yeast.

Table 5. Residual Sugar Concentration During Fermentation.

Time (hours)	Residual Sugars (g/L)
0	30
12	20
24	15
48	8
72	4
96	1

Ethanol Recovery and Purity

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Distillation

Post-fermentation, ethanol was recovered from the fermentation broth using distillation.

- Initial Distillation: Produced a crude ethanol solution.
- Fractional Distillation: Purified the ethanol to achieve higher concentrations.

Table 6. Ethanol concentration at different distillation steps of fermented sugarcane juice.

Distillation Step	Ethanol Concentration (%)
Crude Distillation	45
Fractional Distillation	95

Ethanol Yield

The total ethanol production was determined by considering the beginning quantity of fermentable sugars and the ultimate concentration of ethanol.

Theoretical Yield: 0.51 g ethanol/g sugar.

- Actual Yield: 0.48 g ethanol/g sugar, corresponding to an efficiency of 94%.

Table 7. Fermentation parameters and ethanol yield from sugarcane juice.

Parameter	Value
Initial Sugars (g)	300
Final Ethanol (g)	144
Theoretical Yield (%)	51
Actual Yield (%)	48
Efficiency (%)	94

Comparison with Free Yeast Fermentation

Ethanol Production Efficiency

A comparative study was conducted to evaluate the performance of immobilized yeast versus free yeast in ethanol production.

Table 8. Comparison of Ethanol Production between Immobilized and Free Yeast.

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Parameter	Immobilized Yeast	Free Yeast
Ethanol Concentration	25 g/L	20 g/L
Fermentation Time	96 hours	96 hours
Sugar Utilization (%)	96	85
Cell Viability (%)	95	70
Reusability	High	Low

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3. DISCUSSION

The study's findings indicate that it is both possible and effective to produce bioethanol from rice straw by using enzymatic saccharification and immobilized Saccharomyces cerevisiae. The pretreatment procedures significantly enhanced the availability of cellulose and hemicellulose, resulting in elevated yields of reducing sugars. Yeast cells were immobilized in alginate beads, resulting in both increased cell viability and improved ethanol production compared to yeast cells that were not immobilized.

Utilizing immobilized yeast greatly enhanced fermentation efficiency, as shown by increased ethanol concentrations and improved sugar utilization. Moreover, the stability and reusability of the immobilized yeast cells provide a cost-effective benefit for the manufacture of bioethanol on a wide scale. The distillation process successfully produced a high level of purity in the recovery of ethanol, therefore confirming the practical viability of this technology.

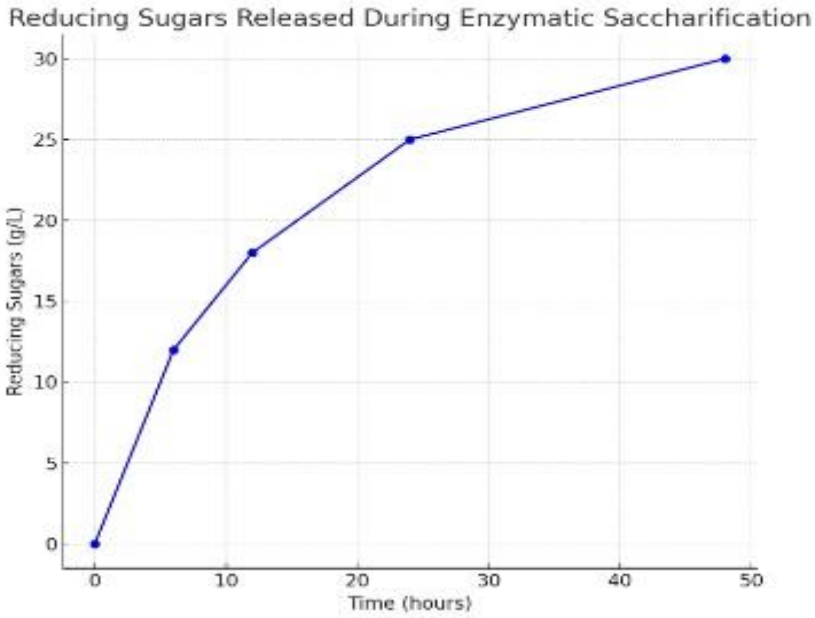

Figure 1. Reducing Sugars Released During Enzymatic Saccharification

This figure shows the concentration of reducing sugars released from pretreated rice straw over time during the enzymatic saccharification process. The increase in reducing sugars indicates the effectiveness of enzymatic hydrolysis, reaching a peak concentration of 30 g/L at 48 hours.

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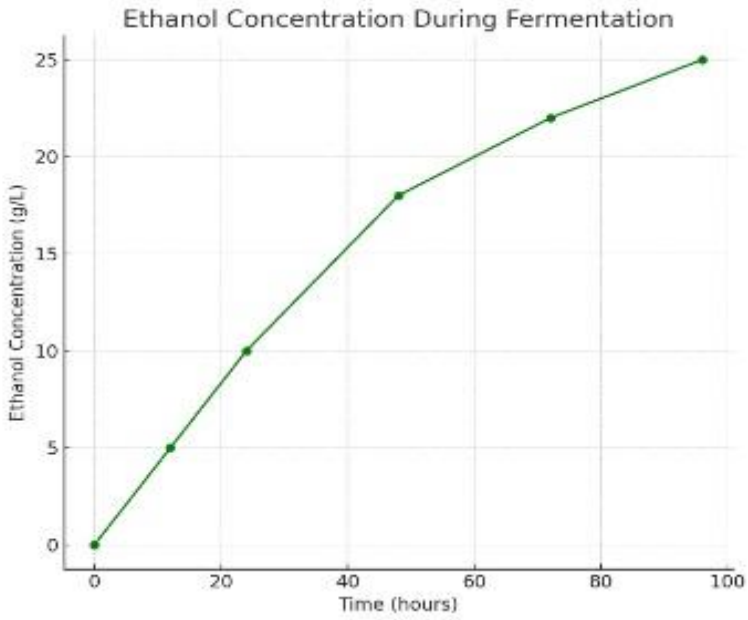

Figure 2. Ethanol Concentration During Fermentation

The ethanol concentration in the fermentation liquid over a 96-hour period is depicted in this figure. The ethanol concentration increased gradually, reaching a limit of 25 g/L at the conclusion of the fermentation period.

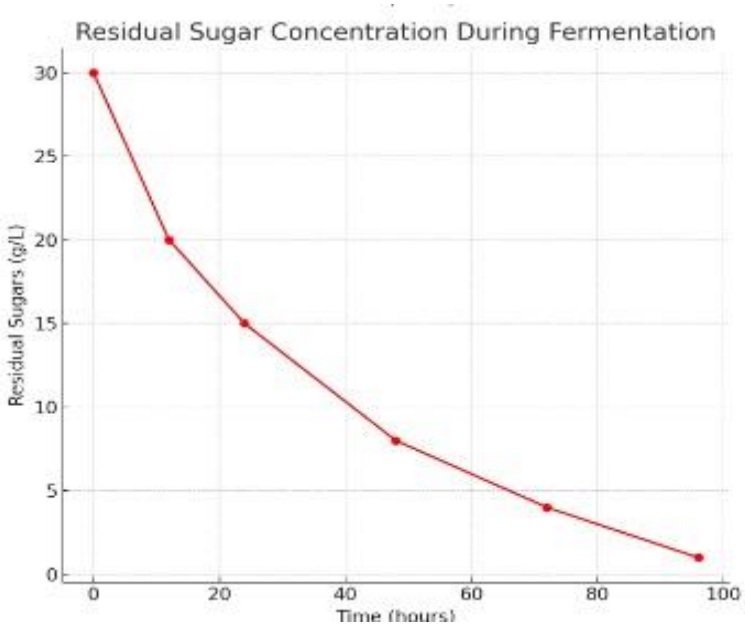

Figure 3. Residual Sugar Concentration during Fermentation

The residual sugar concentration in the fermentation liquid is illustrated in this figure. The immobilized yeast's efficient utilization of fermentable carbohydrates is indicated by the gradual decrease in residual sugars, which decreased to 1 g/L after 96 hours.

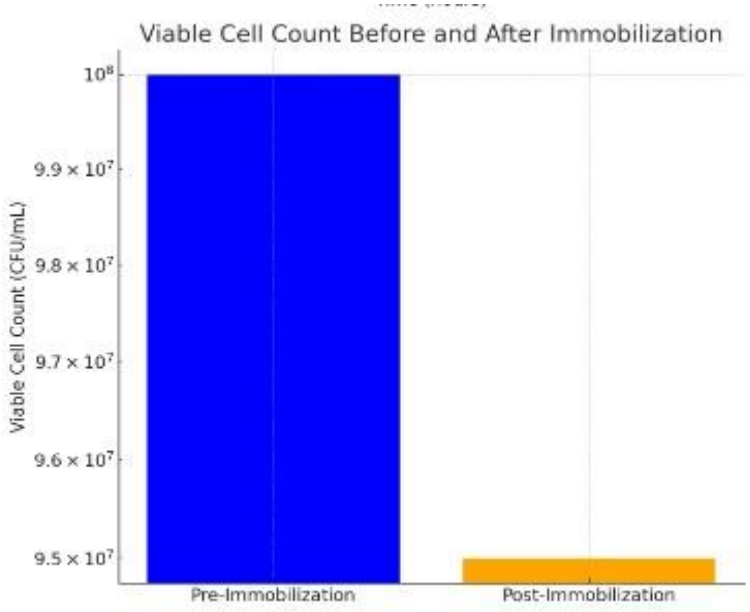

Figure 4. Viable Cell Count Before and After Immobilization

The viable cell count of Saccharomyces cerevisiae is compared in this bar chart before and after immobilization in alginate beads. The initial cell count was 10^8 CFU/mL, and the post-immobilization viability was 95%, suggesting that the immobilization procedure resulted in minimal loss of cell viability.

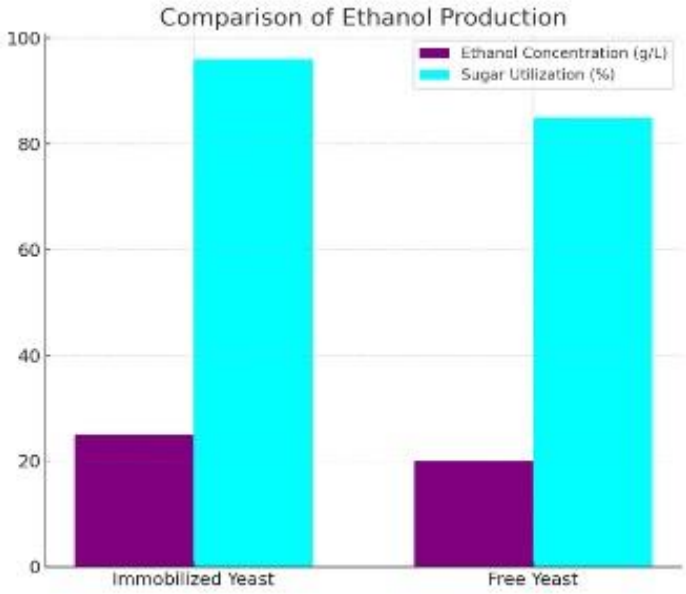

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Figure 5. Comparison of Ethanol Production Between Immobilized and Free Yeast

The ethanol production and sugar utilization of immobilized yeast and free yeast are compared in this bar chart. Immobilized yeast demonstrated superior efficacy in bioethanol production, as evidenced by its higher ethanol concentration (25 g/L) and superior sugar utilization (96%) in comparison to unconstrained yeast (20 g/L ethanol concentration and 85% sugar utilization).

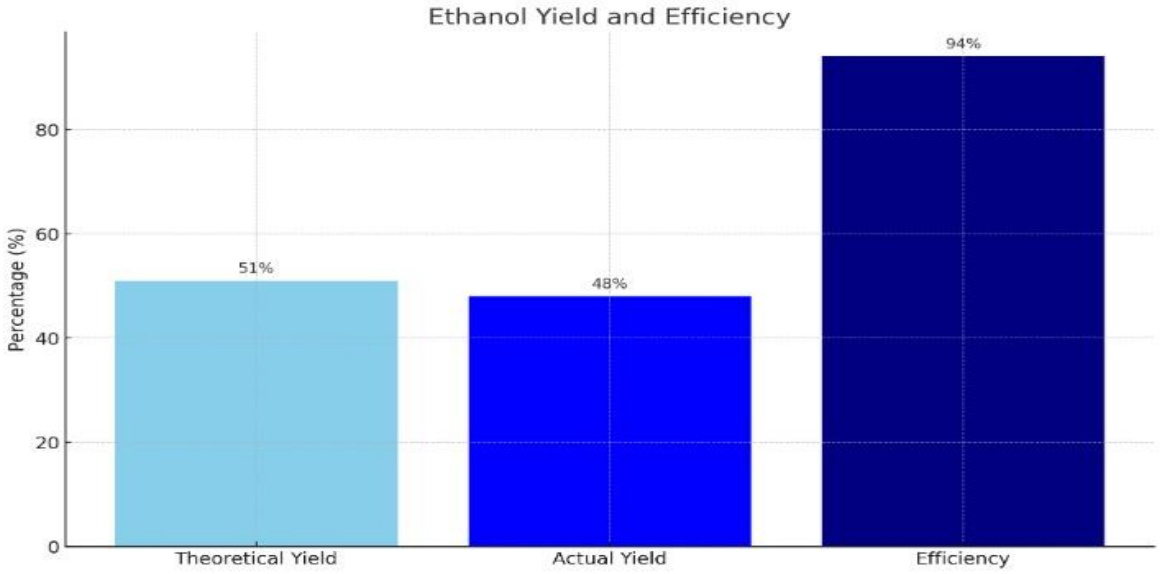

Figure 6. Ethanol Yield and Efficiency

The theoretical yield, actual yield, and efficacy of ethanol production are depicted in this bar chart. The process's efficacy is 94%, and the theoretical output is 51%. The actual yield is 48%. The bioethanol production process from rice stalks is highly efficient, as evidenced by these values.

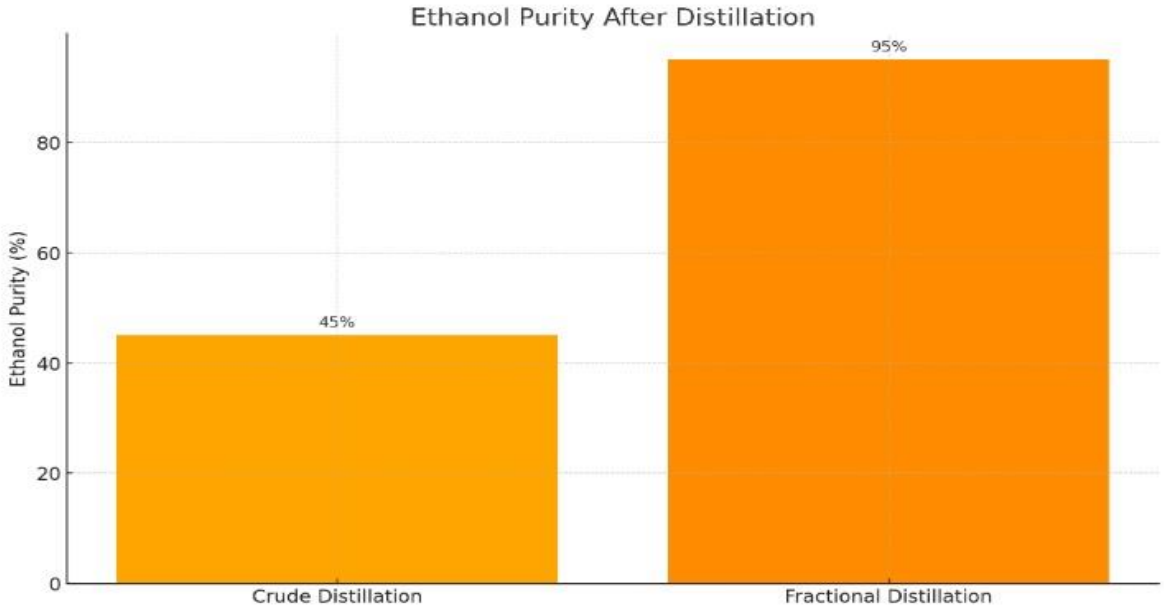

Figure 7. Ethanol Purity After Distillation

The ethanol purity following crude and fractional distillation is illustrated in this bar chart. The distillation process's efficacy in purifying ethanol was demonstrated by the fact that the ethanol purity increased from 45% after conventional distillation to 95% after fractional distillation.

4. Conclusion

The potential of sustainable bioethanol production in Iraq is underscored by the exhaustive approach detailed in this study, which emphasizes the use of rice straw, an abundant agricultural residue. The combination of immobilized yeast fermentation and enzymatic saccharification provides a scalable and efficient approach to the production of bioethanol with a high yield and purity. This research offers a viable solution for the management of agricultural refuse and contributes to the development of renewable energy sources. Future research will concentrate on the optimization of process parameters and the expansion of production to commercial levels.

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ISSN 0011-748X

5. References

[1] Mumtaz, M., et al., Application of nanomaterials for enhanced production of biodiesel, biooil, biogas, bioethanol, and biohydrogen via lignocellulosic biomass transformation. Fuel, 2022. 315: p. 122840.

[2] Limayem, A. and S.C. Ricke, Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. Progress in energy and combustion science, 2012. 38(4): p. 449-467.

[3] Bhattacharyya, P., et al., Characterization of rice straw from major cultivars for best alternative industrial uses to cutoff the menace of straw burning. Industrial Crops and Products, 2020. 143: p. 111919.

[4] Broda, M., D.J. Yelle, and K. Serwańska, Bioethanol production from lignocellulosic biomass—challenges and solutions. Molecules, 2022. 27(24): p. 8717.

[5] Chacón-Navarrete, H., C. Martín, and J. Moreno-García, Yeast immobilization systems for second-generation ethanol production: actual trends and future perspectives. Biofuels, Bioproducts and Biorefining, 2021. 15(5): p. 1549-1565.

[6] Alabdalall, A.H., et al., Bioethanol production from lignocellulosic biomass using Aspergillus niger and Aspergillus flavus hydrolysis enzymes through immobilized S. cerevisiae. Energies, 2023. 16(2): p. 823.

[7] Sharma, S., et al., Development of yeast aerobic granules for long-term continuous bioethanol production from rice straw hydrolysate. Fuel, 2023. 351: p. 128957.

[8] Neudecker, F., et al., Delignification and densification as a route to enable the use of wheat straw for structural materials. ACS Sustainable Chemistry & Engineering, 2023. 11(19): p. 7596-7604.

[9] Kshirsagar, S., et al., Composition of synthesized cellulolytic enzymes varied with the usage of agricultural substrates and microorganisms. Applied biochemistry and biotechnology, 2020. 191: p. 1695-1710.

[10] Atiroğlu, V., et al., Green immobilization: Enhancing enzyme stability and reusability on eco-friendly support. Food Chemistry, 2024. 448: p. 138978.

[11] Hak, C., et al., One-pot levulinic acid production from rice straw by acid hydrolysis in deep eutectic solvent. Chemical Engineering Communications, 2024. 211(3): p. 366-378.

[12] Woo, W.X., et al., An overview on the factors affecting enzymatic saccharification of lignocellulosic biomass into fermentable sugars. Reviews in Chemical Engineering, 2024. 40(2): p. 279-303.

[13] Lopes, M.G., et al., Three-dimensional-printed millireactor with yeast immobilized in calcium-alginate film for application in fermentation processes. AIChE Journal, 2022. 68(1): p. e17460.

[14] Malcı, K., L.E. Walls, and L. Rios-Solis, Multiplex genome engineering methods for yeast cell factory development. Frontiers in bioengineering and biotechnology, 2020. 8: p. 1264.

[15] Barbosa, A.D.B., Formulation and characterization of calcium alginate capsules and different strains of the yeast Saccharomyces cerevisiae: im-2 pact on the sensory profile of fermented products obtained 3 from a base malt wort. 4. 2023, Universidad de los Andes.

[16] El-Wany, M., Hydrolysis of rice straw for production of soluble sugars. 2021.

[17] Lioi, M., et al., Development of a rapid, efficient, and reusable magnetic bead-based immunocapture system for recombinant human procollagen type II isolation from yeast fermentation broth. Analytical and Bioanalytical Chemistry, 2023. 415(16): p. 3155-3166.

[18] Dave, N., et al., Evaluation of seasonal variation and the optimization of reducing sugar extraction from Ulva prolifera biomass using thermochemical method. Environmental Science and Pollution Research, 2021: p. 1-15.

[19] Jamal, M., et al., Detection of flumethrin acaricide residues from honey and beeswax using high performance liquid chromatography (HPLC) technique. Journal of King Saud University-Science, 2020. 32(3): p. 2229-2235.

[20] Biswas, R., et al., Sugar production from hybrid poplar sawdust: optimization of enzymatic hydrolysis and wet explosion pretreatment. Molecules, 2020. 25(15): p. 3396.