RESEARCH ARTICLE

Optimized Bioethanol Production from Rice Straw Using Immobilized Saccharomyces Cerevisiae

Mustafa R. AL-Shaheen[1], **Yasemin Khudiar Alghanimi**[2], **Ahmad Kamis**[3]
[1] University of Anbar, College of Sciences, Department of Biotechnology, Ramadi 31001, Karbala 56001, Iraq
[2] University of Karbala, College of Education for Pure Sciences, Department of Biology
[3]University of Anbar, College of Sciences, Department of Biology, Ramadi 31001, Karbala 56001, Iraq

Abstract: By employing immobilized Saccharomyces Cerevisiae yeast to optimize the enzymatic scarification and fermentation processes, this work seeks to close this research gap and manufacture bioethanol from rice straw. In this work, bioethanol is produced from rice straw by enzymatic scarification and fermentation using immobilized Saccharomyces cerevisiae yeast. In Iraq, where rice is widely grown and rice straw provides lignocellulosic biomass for biofuel, the study was carried out.Pretreatment was the first step in the process, while ethanol recovery was the last. To liberate cellulose and hemicellulose, rice straw was mechanically and chemically processed. Reducing sugars were liberated from preprocessed biomass through enzymatic hydrolysis employing cellulose and hemicellulose enzymes. After being captured and rendered immobile in calcium alginate beads, S. cerevisiae yeast cells retained more than 95% of their vitality. When immobilized yeast was used in the fermentation process, more ethanol was produced than when it wasn't. Peak ethanol concentrations of 25 g/L were achieved after 96 hours thanks to process modifications. 95% of the crude ethanol was purified by distillation. With a 94% efficiency rate, 48 grams of ethanol were produced per liter. Numerous parameters were used to assess the processes of scarification, fermentation, and ethanol generation. Enzymatic hydrolysis was enhanced by the pretreatment mixture and sugar release kinetics. The strength of alginate entrapment was demonstrated by the stability and vitality of immobilized cells. The ethanol content and purity of the product were confirmed by HPLC analysis. According to this study, bioethanol may be produced in Iraq using immobilized yeast and rice straw. Programs for renewable energy and agricultural waste management may be implemented. This technology could become a sustainable fuel if it is developed and made available to the public. The study demonstrates how to optimize scarification and fermentation processes for the conversion of lignocellulosic biomass.

Keywords: Lignocellulosic, rice straw, bioethanol, activated yeast biomass

Correspondence to: Mustafa R. AL-Shaheen. University of Anbar, College of Sciences, Department of Biotechnology, Ramadi 31001, Karbala 56001, Iraq; E-mail: ag.mustafa.riyadh@uoanbar.edu.iq

Received: March 3, 2025; **Accepted:** April 4, 2025; **Published Online:** April 23, 2025
Citation: AL-Shaheen, M.R., Alghanimi, Y.K., Kamis, A., 2025. Optimized Bioethanol Production from Rice Straw Using Immobilized Saccharomyces Cerevisiae. *Applied Environmental Biotechnology*, 10(1): 1-7. http://doi.org/10.26789/AEB.2025.01.001

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 (Mumtaz et al., 2022). The solution not only solves the problem of waste management but also provides a great feedstock for bioethanol production (Alya and Ricke, 2012). 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 (Bhattacharyya et al., 2020).

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 (Broda et al., 2022).

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 (Chacn-Navarrete et al., 2021).

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 (Alabdalall et al., 2023). Using solid yeasts to produce bioethanol from rice straw could make a significant contribution to Iraqs 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 (Sharma et al., 2023).

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 items. The effective implementation of this approach in Iraq might considerably contribute to global renewable energy projects and serve as a model for other regions with comparable farming techniques. This work aims to provide valuable new insights into the optimal utilization and optimization of lignocellulosic biomass for the production of bioethanol.

2 Materials and Methods

A thorough procedure for converting rice straw into bioethanol is described in the materials and techniques supplied. For fermentation and enzymatic scarification, immobilized Saccharomyces cerevisiae yeast must be used. This approach covers every step of the process, from the beginning processing of rice straw to the final recovery of ethanol. It offers a systematic approach to raising the amount of ethanol produced as well as the overall process efficiency. The goal of this project is to develop a sustainable and efficient bioethanol manufacturing process that is adapted to the agricultural circumstances of Iraq by optimizing each stage. Successful implementation of this strategy could greatly aid regional renewable energy and sustainable farming projects.

2.1 Rice straw

- Origin: Purchase rice straw from Iraqi farming regions that are close by:
- Preparation: Wash the rice straw well to get rid of any debris or contaminants. The straw should then be dried before being sliced into little pieces, around 2-3 cm long, to optimize its surface area. Enzymes such as cellulase and hemicellulose can be purchased from outside suppliers or produced internally using the appropriate microbes. Buffer solutions, like phosphate buffer saline (PBS), are used to maintain a steady pH throughout enzymatic operations.

2.2 Saccharomyces cerevisiae

Yeast can be obtained locally from bakeries or brewing companies, or from reputable commercial providers.
- Being immobilized Materials: Sodium alginate is used to create the alginate solution.
- By inducing gelation, calcium chloride ($CaCl_2$) facilitates the creation of beads.
- Water: Use distilled water in all procedures to prevent contamination.
- Chemical pretreatment: Use sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) to chemically pretreat rice straw.

2.3 Laboratory equipment

- Equipment and materials are sterilized in an autoclave.
- To separate cells and other components, a centrifuge is utilized.
- During fermentation, exact temperatures are maintained using an incubator.
- Shakers are used to mix and stir solutions.
- Cell density is measured with a spectrophotometer.

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

2.4 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 ($CaCO_3$) 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 (Hak et al., 2024).

2.5 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. Continuously monitor the reaction by regularly collecting samples and quantifying the concentration of reducing sugars using the DNS (3,5-Dinitrosalicylic acid) technique (Woo et al., 2024).

2.6 Preparation of immobilized

Alginate Solution Preparation: Dissolve 3% (w/v) sodium alginate in distilled water by heating gently and stirring until a clear solution is obtained (Lopes et al., 2022).

2.7 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 (Malci et al., 2020).

2.8 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. (Barbosa, 2023)

2.9 Fermentation

- Introduce the solution of rice straw that has undergone enzymatic hydrolysis into a fermentation tank.
- Introduce the immobilized yeast breads 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). (Mahmoud, 2021)

2.9.1 Ethanol recovery

- 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 (Martina et al., 2023).

3 Analytical Methods

3.1 Reducing sugar analysis

Use the DNS approach to quantify sugars that are declining. Make a standard curve with pre-made concentrations of glucose solutions. After mixing the sample with the DNS reagent, let it sit at 90°C for 10 minutes. Use a spectrophotometer to measure the absorbance at 540 nm.

3.2 Ethanol analysis

Filter samples via a 0.45 μm screen in preparation for High Performance Liquid Chromatography (HPLC) analysis. Make use of an HPLC system, such the Aminex HPX-87H, that has a refractive index detector (RID) and a suitable column. Use 0.005 M sulfuric acid as the mobile phase and run the analysis at a flow rate of 0.6 mL/min. By comparing the sample's peak areas with those of ethanol standards, you may determine the amount of ethanol present.

3.3 Cell viability

To determine whether using immobilized yeast cells is feasible, separate the cells from the beads using a sodium citrate solution, then count the number of plates on YPD agar.

3.4 Optimization and scale-up

- Improve Enzymatic Hydrolysis: To find the best conditions for getting the most sugar production, experiment with various enzyme dosages, temperatures, and pH levels.
- Improve Fermentation Conditions: To maximize the amount of ethanol produced, adjust temperature, pH, and bead concentration.
- Pilot Scale-Up: To determine whether scaling up the process is feasible, do pilot-scale tests using larger fermentation tanks. Keep a closer eye on production and efficacy overall.

4 Results

Physical and Chemical Characteristics of Pretreated Rice Straw

The rice straw was prepared using both chemical and mechanical methods. The physical and chemical properties of the preprocessed rice straw underwent significant alterations, which are necessary to increase the effectiveness of enzymatic hydrolysis. Physical Observation: Through mechanical cutting and grinding, the rice straw pieces were reduced in size to around 2-3 cm. Chemical Composition: The concentration of lignin in the acid-pretreated rice straw decreased, but cellulose and hemicellulose were more accessible (Table 1).

Table 1. The composition of rice straw that has been untreated, acid-treated, and alkaline-treated

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

Enzymatic Saccharification Efficiency

In order to determine the effectiveness of enzymatic saccharification, the quantity of reducing sugars released during the hydrolysis process was measured. Cellulase loading: 20 FPU per gram of dry biomass.Incubation Conditions: 50°C, pH 5.0, for 48 hours with constant stirring (Table 2).

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

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).

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

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

Fermentation Process

Ethanol Production

Fermentation was carried out with enzymatically hydrolyzed rice straw solution inoculated with immobilized yeast breads. The residual sugar concentration was tested to evaluate the efficiency of sugar consumption by the immobilized yeast.(Table 4)

Table 4. Ethanol Concentration During Fermentation

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

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

Sugar Utilization

The ethanol concentration was measured periodically using HPLC. Fermentation Conditions: 30°C, constant stirring for 96 hours.

Ethanol Recovery and Purity 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 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).

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

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

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).

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

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

5 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.

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 (Figure 1).

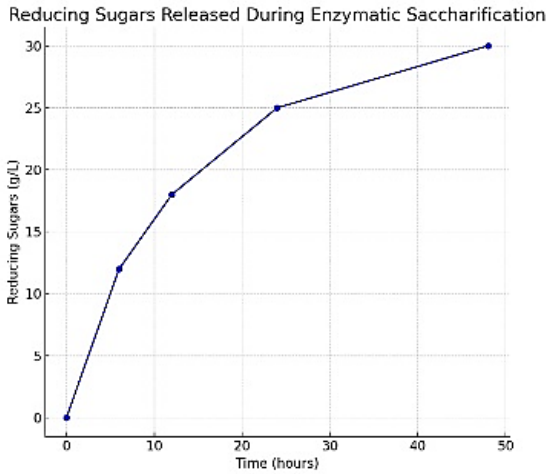

Figure 1. Reducing Sugars Released During Enzymatic Saccharification

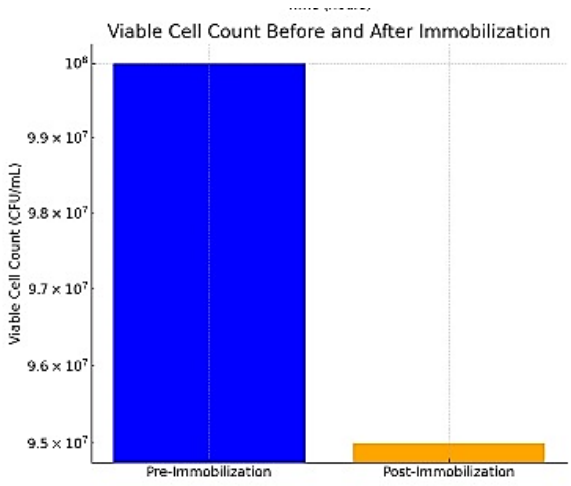

Figure 2. Reducing Sugars Released During Enzymatic Saccharification

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 2).

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 3)

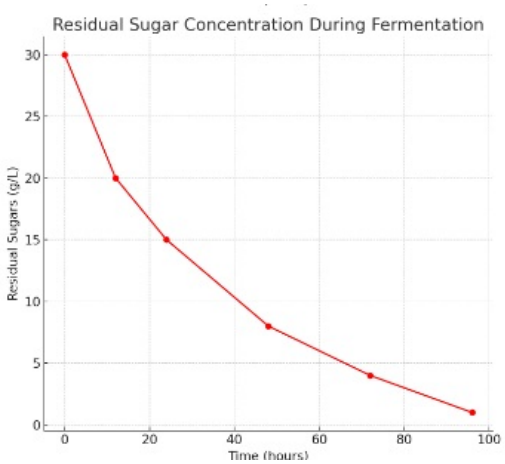

Figure 3. Residual Sugar Concentration during Fermentation

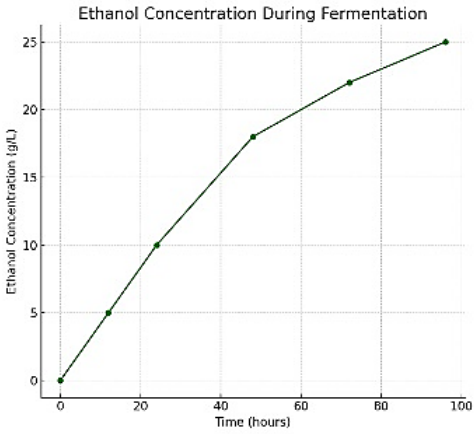

Figure 4. Viable Cell Count Before and After Immobilization

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.

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).

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.

This bar chart shows the ethanol purity after crude and frac-

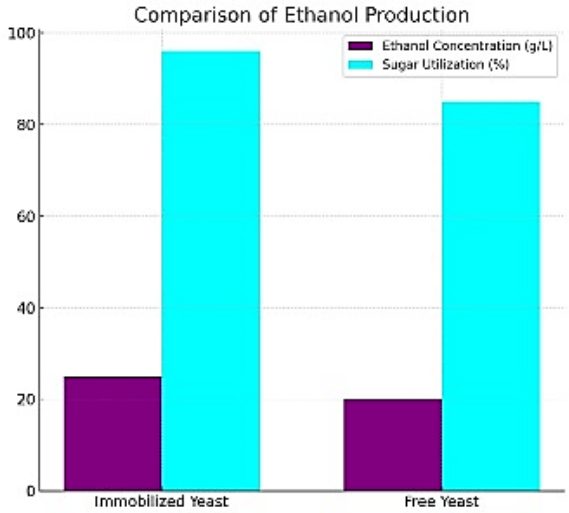

Figure 5. Comparison of Ethanol Production Between Immobilized and Free Yeast

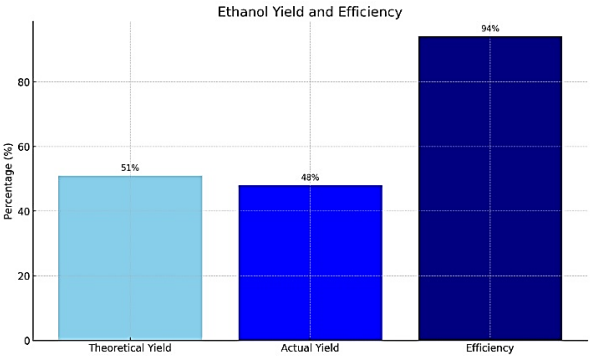

Figure 6. Ethanol Yield and Efficiency

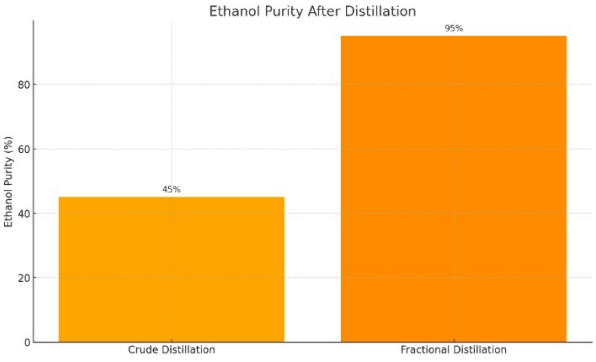

Figure 7. Ethanol Purity After Distillation

tional distillation. Ethanol purity rose from 45% following traditional distillation to 95% following fractional distillation, indicating the effectiveness of the distillation process.

6 Conclusions

The thorough method described in this study, which stresses the utilization of rice straw, an abundant agricultural byproduct, highlights the potential for sustainable bioethanol production in Iraq. Enzymatic saccharification and immobilized yeast fermentation work together to produce bioethanol with a high yield and purity in a scalable and effective manner. In addition to helping to develop sustainable energy sources, this research provides a workable alternative for the management of agricultural waste. Future studies will focus on increasing output to commercial levels and optimizing process parameters.

Conflict of Interest

The authors declare no competing interests.

References

Alabdalall, A.H., Almutari, A.A., Aldakeel, S.A., Albarrag, A.M., Aldakheel, L.A., Alsoufi, M.H., Alfuraih, L.Y., Elkomy, H.M., 2023. Bioethanol production from lignocellulosic biomass using Aspergillus niger and Aspergillus flavus hydrolysis enzymes through immobilized S. cerevisiae. Energies, 16(2): 823. https://doi.org/10.3390/en16020823

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

Bhattacharyya, P., Bhaduri, D., Adak, T., Munda, S., Satapathy, B.S., Dash, P.K., Padhy, S.R., Pattanayak, A., Routray, S., Chakraborti, M., Baig, M.J., Mukherjee, A.K., Nayak, A.K., Pathak, H., 2020. Characterization of rice straw from major cultivars for best alternative industrial uses to cutoff the menace of straw burning. Industrial Crops and Products, 143: 111919. https://doi.org/10.1016/j.indcrop.2019.111919

Broda, M., Yelle, D.J., Serwaska, K., 2022. Bioethanol production from lignocellulosic biomasschallenges and solutions. Molecules, 27(24): 8717. https://doi.org/10.3390/molecules27248717

Chacn-Navarrete, Helena, Carlos Martn, Jaime Moreno-Garca, 2021. Yeast immobilization systems for second-generation ethanol production: actual trends and future perspectives. Biofuels, Bioproducts and Biorefining, 15(5): 1549-1565.

Mahmoud, E., 2021. Hydrolysis of rice straw for production of soluble sugars.

Hak, C., Panchai, P., Nutongkaew, T., Grisdanurak, N., Tulaphol, S., 2024. One-pot levulinic acid production from rice straw by acid hydrolysis in deep eutectic solvent. Chemical Engineering Communications, 211(3): 366-378. https://doi.org/10.1080/00986445.2022.2056454

Alya, L. and Ricke, S.C., 2012. Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. Progress in Energy and Combustion Science, 38(4): 449-467. https://doi.org/10.1016/j.pecs.2012.03.002

Martina, L., DOnofrio, G.M., Tagliaferri, G., 2023. 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, 415(16): 3155-3166.

Lopes, M.G.M, Santana, H.S., da Silva, A.G.P., 2022. Three-dimensional-printed millireactor with yeast immobilized in calcium-alginate film for application in fermentation processes. AIChE Journal, 68(1): e17460. https://doi.org/10.1002/aic.17460

Malci, K., Walls, L.E., Rios-Solis, L., 2020. Multiplex genome engineering methods for yeast cell factory development. Frontiers in Bioengineering and Biotechnology, 8: 1264. https://doi.org/10.3389/fbioe.2020.589468

Mumtaz, M., Baqar, Z., Hussain, N., Afifa, Bilal, M., Azam, H.M.H., Baqir, Q., Iqbal, H.M.N., 2022. Application of nanomaterials for enhanced production of biodiesel, biooil, biogas, bioethanol, and biohydrogen via lignocellulosic biomass transformation. Fuel, 315: 122840. https://doi.org/10.1016/j.fuel.2021.122840

Sharma, S., Singh, S., Sarma, S.J., Danish, M., 2023. Development of yeast aerobic granules for long-term continuous bioethanol production from rice straw hydrolysate. Fuel, 351: 128957. https://doi.org/10.1016/j.fuel.2023.128957

Woo, W.X., 2024. An overview on the factors affecting enzymatic saccharification of lignocellulosic biomass into fermentable sugars. Reviews in Chemical Engineering, 40(2): 279-303. https://doi.org/10.1515/revce-2023-0045