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Bioconversion of Rice Husk into Bioethanol Using Deep Eutectic Solvent (DES) Pretreatment

Irawati Ramli [1*], Alfiah Indriastuti [2], Yudith Helene Mustakim[3], Septianus Buntu[4]

[1,2,4] Chemistry Study Program, Sulawesi Institute of Technology
[3]Civil Engineering Study Program, Sulawesi Institute of Technology

e-mail: irawatiramli89@gmail.com

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Abstract

The quantity of waste generated by agriculture is increasing and biomass based renewable energy can be developed based on this waste. Rice husk is one such lignocellulosic biomass that has potential as raw material for the production of bioethanol, but the use is still small because of the complex structure of lignocellulosic biomass, especially the existence of lignin, which is a barrier to cellulose during hydrolysis and fermentation. This study is designed to assess the impact of pretreatment with Choline Chloride – Urea based Deep Eutectic Solvent (DES) on the lignocellulosic properties of rice husks (RH) and its bioethanol production. The research steps comprise of raw material preparation, synthesis of DES, pretreatment process, analysis of the lignocellulosic fractions, enzymatic saccharification process and bioethanol fermentation process. Results from the treatments indicate that the DES treatment changes the lignocellulosic composition by reducing lignin content from 21.77% to 12.87%, hemicellulose content from 18.17% to 11.20% and increasing cellulose content from 35.13% to 52.27%. With the enzymatic saccharification, the glucose concentration obtained was 49.99 g/L and the saccharification efficiency was 86.08%. Later, the maximum concentration of ethanol as 21.40 g/L was obtained on the fifth day with 82.07% fermentation efficiency. The outcome of the experiment shows that the Choline Chloride–Urea DES pretreatment could increase the cellulose accessibility which ultimately enabled the conversion of rice husks to bioethanol.

Keywords: *Bioethanol; Rice husk; Deep Eutectic Solvent; Lignocellulose pretreatment; Fermentation*

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1. Introduction

The increasing demand for energy, coupled with concerns regarding the environmental consequences of fossil fuel consumption, has intensified interest in the development of alternative energy sources derived from renewable resources. Rice husk, a major agricultural residue generated during rice milling, represents a potential feedstock for bioethanol production because of its lignocellulosic composition. Nevertheless, the conversion of rice husk into fermentable sugars remains challenging owing to the structural complexity of lignocellulosic biomass. In particular, the association of cellulose with lignin and hemicellulose can restrict enzymatic accessibility and consequently limit the efficiency of subsequent hydrolysis. Accordingly, pretreatment is an important processing step for modifying biomass structure and facilitating the conversion of structural carbohydrates into fermentable sugar.

Various pretreatment strategies have been investigated to improve the conversion of rice husk into fermentable sugars and bioethanol. Anuradha and Sampath (2023), for example, examined alkaline, acid, and organic-solvent pretreatment approaches for rice husk and evaluated their implications for sugar production. Such studies demonstrate the importance of pretreatment selection and operating conditions in determining biomass conversion performance. However, the effectiveness of a given pretreatment cannot be evaluated solely from the removal of lignin or changes in biomass composition; the availability of fermentable sugars and the performance of subsequent fermentation must also be considered. These considerations support the need for integrated assessments that relate compositional changes after pretreatment to the outcomes of saccharification and fermentation.

Deep eutectic solvents (DES) have attracted research interest as alternative solvent systems for lignocellulosic biomass pretreatment. These solvents are commonly formed through interactions between a hydrogen-bond acceptor and a hydrogen-bond donor. In the choline chloride–urea system, choline chloride functions as the hydrogen-bond acceptor, whereas urea serves as the hydrogen-bond donor. The resulting hydrogen-bonding network contributes to the physicochemical properties of the solvent and may facilitate changes in the distribution or solubilization of biomass components. Pan et al. (2017) investigated the physicochemical transformation of rice straw following pretreatment with choline chloride–urea DES, demonstrating that DES treatment can alter biomass composition and influence subsequent enzymatic hydrolysis. Nevertheless, the effectiveness of such pretreatment depends on the characteristics of the feedstock and the conditions under which the process is conducted; therefore, findings obtained from rice straw cannot automatically be extrapolated to rice husk.

The application of choline chloride–urea DES to rice husk has also been investigated directly. Anuradha and Sampath (2024) examined the optimization of pretreatment conditions for reducing-sugar production from rice husk, demonstrating that this solvent system and its process variables have already received specific research attention. Furthermore, Sawhney et al. (2023) investigated

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the conversion of rice straw into bioethanol through an integrated pretreatment approach involving DES, microwave irradiation, and laccase, followed by enzymatic saccharification and fermentation. Collectively, these studies indicate that DES-based pretreatment, process optimization, and the integration of biomass conversion stages have been explored in previous research. Consequently, neither the use of DES for lignocellulosic pretreatment nor its application to rice-derived biomass can, by itself, be considered the principal novelty of the present study.

Against this background, further experimental characterization of rice husk conversion under specified pretreatment conditions remains relevant. The present study investigates rice husk pretreated with choline chloride–urea DES at 100 °C for 120 min, followed by enzymatic saccharification and fermentation. The investigation focuses on changes in cellulose, lignin, and hemicellulose contents following pretreatment, the reducing-sugar concentration obtained after saccharification, and ethanol concentrations measured at fermentation intervals of 1, 3, and 5 days.

The principal contribution of this study is the characterization of these outcomes within the specified experimental sequence and operating conditions. Unlike studies designed to optimize pretreatment variables or compare multiple pretreatment technologies, the present investigation evaluates a single pretreatment condition and does not establish an optimum operating condition or demonstrate superior performance relative to conventional methods. In addition, DES recovery and reuse, energy consumption, and waste management were not evaluated; therefore, conclusions regarding the overall environmental sustainability of the process would be premature.

Accordingly, this study aims to characterize the effects of choline chloride–urea DES pretreatment on rice-husk composition and its subsequent conversion through enzymatic saccharification and fermentation. Specifically, it seeks to determine changes in cellulose, lignin, and hemicellulose contents after pretreatment, quantify the reducing-sugar concentration following saccharification, and measure ethanol concentrations at selected fermentation times. The study addresses the following research question: How does the investigated choline chloride–urea DES pretreatment condition affect the compositional characteristics of rice husk, reducing-sugar concentration after saccharification, and ethanol concentration during fermentation? By addressing this question, the study provides a condition-specific experimental assessment of rice-husk conversion while identifying the need for further validation of ethanol yield calculations and evaluation of solvent recovery and process sustainability.

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2. Methods

The research was carried out in several stages based on the research time table. The stages that have been implemented are:

1. Preparation for the research
2. Collection of equipment and materials
3. Rice husk treatment / rice husk sample preparation
4. Analysis of initial biomass composition (lignin, cellulose, and hemicellulose content)
5. Analysis after DES pretreatment (lignin reduction, residual cellulose and hemicellulose content)
6. Enzymatic saccharification analysis (glucose content, saccharification efficiency, hemicellulose hydrolysis yield)
7. Identification test

2.1 Equipment and Materials

The equipment used included: a hotplate, batch reactor, oven, desiccator, centrifuge, pH meter, conductometer, viscometer, incubator shaker, analytical balance, autoclave, and UV-Vis spectrophotometer.
Materials: rice husks, distilled water, sodium acetate buffer, reagents, urea, choline chloride, technical-grade ethanol, DNS reagent, NaOH, and HCl.

2.2 Biomass Preparation

Rice husk was cleaned to remove visible impurities, dust, and other foreign materials. The cleaned rice husk was dried in an oven at 105 °C for 3 h. The dried material was subsequently milled and sieved to obtain a particle size of approximately 60 mesh. The prepared biomass was characterized to determine its initial lignocellulosic composition, including cellulose, hemicellulose, lignin, and ash contents, as well as its moisture content.
characterized to determine its initial lignocellulosic composition, including cellulose, hemicellulose, lignin, and ash contents, as well as its moisture content.

2.3 Preparation of Deep Eutectic Solvent (DES)

The deep eutectic solvent (DES) was synthesized using choline chloride as the hydrogen bond acceptor (HBA) and urea as the hydrogen bond donor (HBD) at a molar ratio of 1:2. Both components were weighed according to the specified molar ratio and mixed in a heat-resistant container. The mixture was heated at 80–100 °C for 2 h under continuous stirring until a homogeneous liquid was obtained. After heating, the synthesized DES was allowed to cool to room temperature before being used for the rice husk pretreatment process.

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2.4 DES Pretreatment of Rice Husk

A total of 10 g of dry rice husk was mixed with 100 mL of the synthesized DES, resulting in a biomass-to-solvent ratio of 1:10 (w/v). The mixture was heated at 100 °C for 120 minutes under continuous stirring at 200 rpm. Following the pretreatment, the solid biomass was separated from the DES via vacuum filtration and washed with water until a neutral pH was reached. The resulting solid fraction was then dried in an oven at 105 °C for 3 hours, after which it was used for lignocellulose characterization and enzymatic saccharification. The pretreated rice husk was analyzed to determine its cellulose, hemicellulose, lignin, and ash contents using. The results were compared with the initial composition of untreated rice husk to evaluate changes in biomass composition following DES pretreatment. Changes in the relative composition of the biomass were interpreted separately from changes in the absolute mass of each component.

2.5 Enzymatic Saccharification

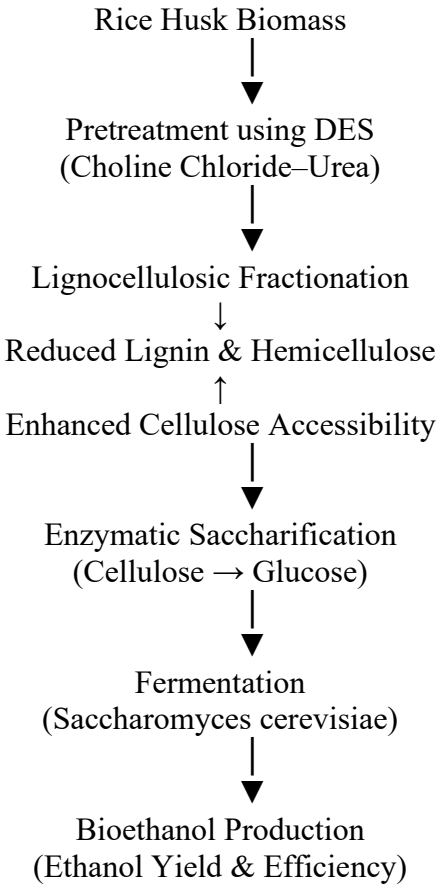
Enzymatic saccharification was performed using pretreated rice husks. A 5 g portion of the pretreated biomass was mixed with 50 mL of citrate buffer (pH 4.8), followed by the addition of 5 g of enzyme. The mixture was incubated at 50 °C for 72 hours with agitation at 150 rpm. Subsequently, the glucose concentration and saccharification efficiency were analyzed

2.6 Fermentation Process

A 50 mL aliquot of the saccharification hydrolysate was transferred into a fermentation vessel, followed by the addition of 5 mL of a *Saccharomyces cerevisiae* culture. Fermentation was carried out, with sampling performed on days 1, 3, and 5. Samples were collected on these days to evaluate changes in ethanol concentration and the remaining level of reducing sugars. Fermentation conditions and sampling procedures were kept consistent throughout the sampling period.Ethanol concentration in the fermentation samples was determined using gas chromatography–mass spectrometry (GC–MS).

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2.7 flow diagram



3. Result and Discussion

3.1 Result

The data obtained during the research are as follows:

Table 1. *Changes in Lignocellulosic Composition of Rice Husk Before and After DES Pretreatment*

Parameter	Without Pretreatment	After DES Pretreatment	Change/Effectiveness
Hemiselulosa (%)	18.17	11.20	Decrease of 38.35 %
Cellulose (%)	35.13	52.27	Relative increase of 48.77%
Lignin (%)	21.77	12.87	Decrease of 40 %
Ash (%)	15.00	13.87	Decrease of 7.53 %

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Table 2. *Saccharification Analysis Test Results*

Sample condition	Glucose (g/L)	Saccharification efficiency
Without pretreatment	21,80	55,87%
After DES pretreatment	49,99	86,08%

Table 3. *Effect of Fermentation Time on Sugar Reduction, Ethanol Formation, and Bioethanol Fermentation Efficiency*

Fermentation Time	**Initial Glucose (g/L)**	**Initial Glucose (g/L)**	**Ethanol (g/L)**	**Efficiency (%)**
Day 1	49,99	32,45	8,15	31,25
Day 3	49,99	18,20	16,85	64,63
Day 5	49,99	12,60	21,40	82,07

3.2 Discussion

Figure 1. Change in Hemicellulose, Cellulose, Lignin, and Ash Contents of Rice Husk Before and After Choline Chloride–Urea DES Pretreatment.

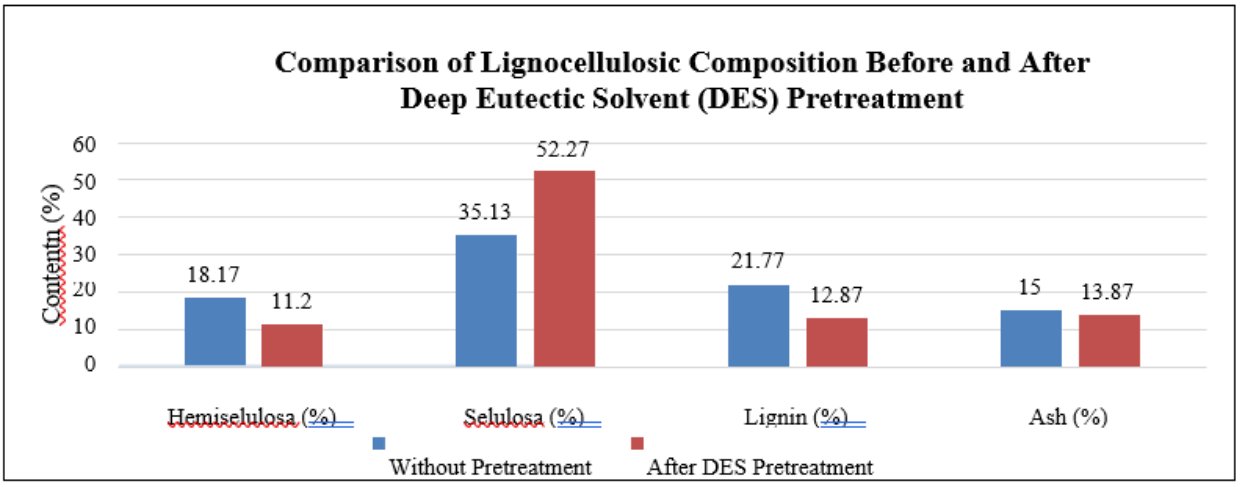
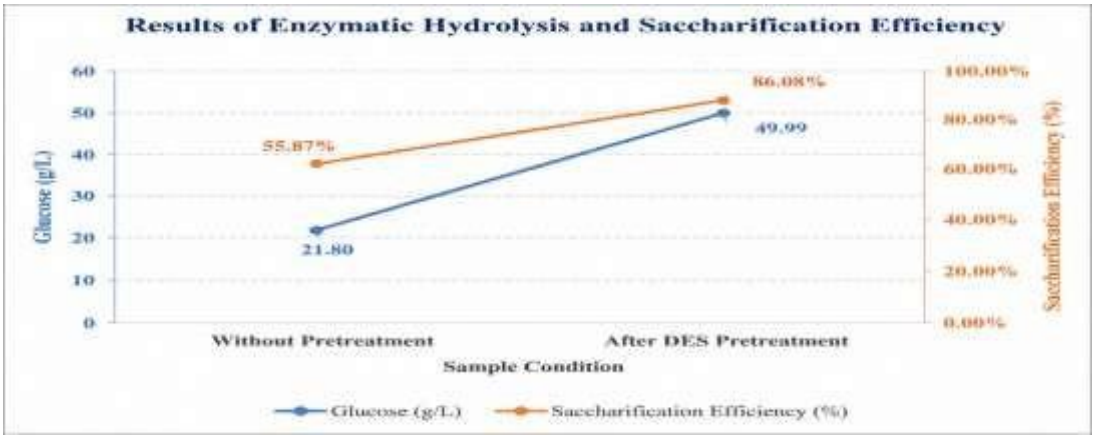


Figure 1 shows that pretreatment with DES alters the relative composition of rice husks. The cellulose content increases from 35.13% to 52.27%, while the lignin content decreases from 21.77% to 12.87%, and the hemicellulose content decreases from 18.17% to 11.20%. This increase in the proportion of cellulose is attributed to the partial removal of lignin and hemicellulose during the pretreatment, which leads to a relative enrichment of cellulose in the recovered solids. Such changes can enhance the accessibility of cellulose to enzymes during the subsequent

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saccharification process. A deep eutectic solvent (DES) based on choline chloride–urea is characterized by a network of hydrogen bonds among its constituent components. Choline chloride acts as a hydrogen bond acceptor (HBA), while urea serves as a hydrogen bond donor (HBD). These intermolecular interactions contribute to the physicochemical properties of the DES and can facilitate interactions with lignocellulosic components during pretreatment (Hunt et al., 2016). The ash content decreases from 15.00% to 13.87% following the pretreatment. Rice husk ash is generally rich in silica, although it also contains other inorganic constituents. The observed reduction reflects the partial removal of inorganic materials during the pretreatment and washing processes. However, since the total ash content does not specifically measure silica levels, further elemental or mineralogical analysis is required to determine whether the silica content has also changed. Overall, these results indicate a shift in the relative composition of rice husks following pretreatment with DES.

Figure 2. Effect of DES Pretreatment on Enzymatic Hydrolysis Performance and Saccharification Efficiency of Rice Husk Biomass



As shown in Figure 2, the enzymatic hydrolysis yield and the saccharification efficiency are improved after using the Choline Chloride–Urea Deep Eutectic Solvent (DES) for pretreatment. Concentration of glucose increased from 21.80g/L to 49.99g/L suggesting that DES treatment made cellulose more available for conversion to simple sugars. In untreated samples, the lignin, hemicellulose and cellulose still form a complex structure, making the hydrolytic enzymes ineffective. DES pretreatment has an effect on biomass structure, partially dissolving the lignin and hemicellulose to make the cellulose more accessible to the enzymes. The hydrogen bond donor (HBD) – hydrogen bond acceptor (HBA) of the reaction between Choline Chloride (HBA) and urea (HBD) is one of the most important factors in reducing the bond strength between the lignocellulosic components. These conditions improve the efficiency of enzymatic hydrolysis and yield more glucose which can be used as feedstock for fermentation of bioethanol.

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Figure 3. Effect of Fermentation Duration on Sugar Consumption, Ethanol Production, and Fermentation Efficiency of Rice Husk Hydrolysate

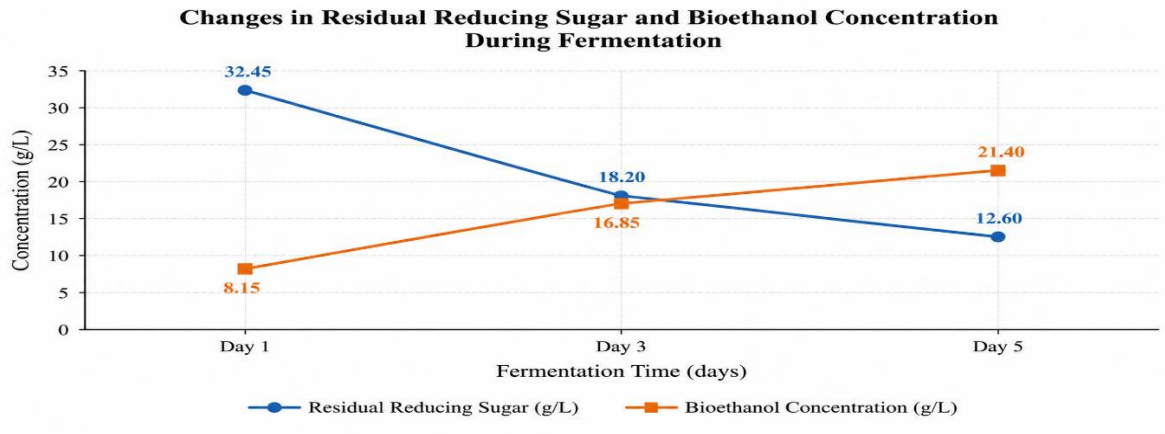
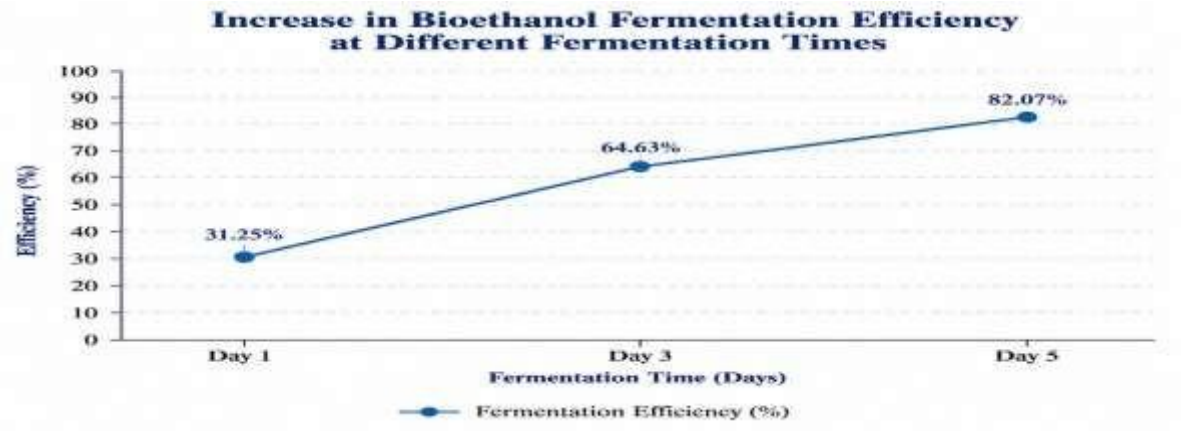


Figure 3 illustrates the changes in residual reducing sugar and bioethanol concentrations during fermentation on days 1, 3, and 5. The residual reducing sugar concentration decreased from 32.45 g/L on day 1 to 18.20 g/L on day 3 and 12.60 g/L on day 5. In contrast, the bioethanol concentration increased from 8.15 g/L to 16.85 g/L and 21.40 g/L over the same period. The concurrent decrease in residual reducing sugar and increase in bioethanol concentration are consistent with substrate utilization and product accumulation during fermentation. The largest observed ethanol concentration occurred on day 5, while the residual reducing sugar concentration reached its lowest measured value. The reduction in residual reducing sugar suggests that fermentable carbohydrates were progressively utilized by *Saccharomyces cerevisiae*. However, the DNS assay measures total reducing sugars rather than glucose specifically; therefore, the observed decrease cannot be attributed exclusively to glucose consumption (Miller, 1959). In lignocellulosic hydrolysates, the composition of the sugar mixture may influence fermentation performance, and the individual contributions of glucose and other reducing sugars cannot be established from the available measurements.

Accordingly, the trend in Figure 3 supports an association between decreasing residual reducing sugar concentration and increasing ethanol concentration,Overall, day 5 showed the highest measured bioethanol concentration (21.40 g/L) and the lowest residual reducing sugar concentration (12.60 g/L) among the three sampling points. This observation indicates that ethanol accumulation continued throughout the fermentation period

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Figure 4. Effect of Fermentation Duration on Bioethanol Fermentation Efficiency from Rice Husk Hydrolysate.



From Figure 4, it is seen that the fermentation efficiency improved with the increase in the fermentation time. The efficiency of the first day was 31.25%, which means that the glucose utilization process by Saccharomyces cerevisiae was still in its initial stage. The efficiency level increased to 64.63% after three days of fermentation period indicating that the microorganisms had started to actively convert fermentable sugars into ethanol. The best efficiency was obtained on the fifth day with a value of 82.07%. This increase evidences that most of the glucose produced from hydrolysis was consumed in fermentation process. The results showed that the optimum condition for bioethanol production using hydrolysate rice husk after DES treatment is 5 days fermentation process.

4. Conclusion

Choline chloride–urea deep eutectic solvent (DES) pretreatment altered the lignocellulosic composition of rice husk, increasing the cellulose content from 35.13% to 52.27% while decreasing the lignin and hemicellulose contents from 21.77% to 12.87% and from 18.17% to 11.20%, respectively. Following enzymatic saccharification, the reported reducing-sugar concentration of the pretreated biomass reached 49.99 g/L, compared with 21.80 g/L for the untreated biomass, with a reported saccharification efficiency of 86.08%. During fermentation, the ethanol concentration increased from 8.15 g/L on day 1 to 16.85 g/L on day 3 and 21.40 g/L on day 5. The highest measured ethanol concentration was therefore recorded on day 5, the final sampling point. These findings indicate that the investigated DES pretreatment condition has potential for modifying rice-husk composition and supporting subsequent saccharification and fermentation. Nevertheless, the reported fermentation-efficiency calculation requires further verification, and the results do not establish an optimum fermentation condition or demonstrate superiority over alternative pretreatment methods. Further studies should validate ethanol yield calculations and evaluate DES

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recovery and reuse, energy consumption, and waste management before the overall process efficiency and environmental sustainability can be established.

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