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Green and Efficient Pretreatment of Lignocellulosic Biomass for Bioethanol Production Using Deep Eutectic Solvent Synthesized from Choline Chloride, Zinc and Urea

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Abstract: Organic *solvents have been the focus of numerous restrictions and legislation recently because of their detrimental impact on the environment and toxicity to human health. This research offers alternatives to the ionic liquids (ILs) as they pose danger to the environment with vehemently socioeconomic conflicts inherently in first-generation biofuels convertion. This study synthesized and evaluated two bio-based DES systems:Choline Chloride-Urea (ChCl:Urea) and Zinc Chloride-Urea (ZnCl:Urea) at a 1:2 molar ratio for the pretreatment of agricultural residues, specifically maize, rice, and corn straws. The physicochemical analysis confirmed the formation of stable, homogeneous solvent systems. The ChCl:Urea system exhibited a lower viscosity (0.94 kg/m·s) and higher conductivity (3.12 mS/cm) compared to the ZnCl:Urea system (1.15 kg/m·s and 2.61 mS/cm, respectively), suggesting superior mass transfer capabilities. Experimental results from this research work reveal that biomass pretreated with ChCl:Urea consistently achieved higher bioethanol yields across all substrates with the highest yield being observed in corn straw at 48.94%, followed by maize straw (39.24%) and rice straw (36.63%). Subsequently, the pH values of 8.95–9.73 indicate the basic nature of these and that helps facilitates the deprotonation of phenolic groups in lignin, thereby enhancing cellulose accessibility. The study also concludes that DES pretreatment, particularly using bio-based organic cations, effectively mitigates the recalcitrance of lignocellulosic matrices, providing a scalable and environmentally benign pathway for commercially viable bioethanol production.*

***Keywords**: Bioethanol; Deep Eutectic Solvents; organic solvents; Lignocellulosic Biomass; Pretreatment; Sustainable Energy; Ionic liquid*

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1.0 Introduction

The global transition toward a circular bioeconomy is driven by the depletion of fossil reserves, fluctuating energy security, and the urgent need. As energy demand surges, lignocellulosic biomass has emerged as the most abundant renewable feedstock, with an annual production rate of approximately 170–200 billion tons (Iqbal *et al.,* 2013). Unlike first-generation feedstocks, lignocellulosic residues do not compete with food security, offering a sustainable pathway for the production of second-generation biofuels and platform chemicals (Clark and Deswarte, 2015).

Among various biomass sources, cereal crop residues, specifically maize straw, corn straw, and rice straw, represent a massive, underutilized resource. In agrarian economies, these residues are often managed through open-field burning or left to decompose, leading to environmental degradation and the loss of energy potential. Efficiently converting these residues into value-added products could address environmental concerns while providing a robust alternative to crude oil-based derivatives (Clark and Deswarte, 2015).

Fossil fuels, the backbone of industrial progress, have caused significant environmental and public health consequences. The combustion of coal, oil, and natural gas releases pollutants contributing to climate change and respiratory diseases (Perera, 2018). Extraction processes contaminate groundwater and degrade ecosystem (Betrand, 2021). Rising atmospheric carbon dioxide levels continue to intensify extreme weather events (Natural Resources Defense Council, 2025). These challenges highlight the urgent need to transition toward sustainable energy systems. These environmental and health concerns underscore the necessity for cleaner and more sustainable energy systems.

The urgency for sustainable alternatives is intensified by accelerating climate change. Global emissions must decline substantially to limit warming to 1.5 °C (United Nations, 2025). Renewable energy technologies provide viable solutions (International Energy Agency, 2025). Liquid biofuels such as bioethanol are particularly important for decarbonizing transport. Bioethanol derived from biomass can reduce greenhouse gas emissions significantly compared to gasoline (Devi *et al.,* 2022). However, first-generation bioethanol produced from food crops raises food-versus-fuel concerns and affects commodity prices (Filip and Janda*.,* 2019). Additionally, monoculture practices associated with these crops contribute to soil degradation and biodiversity loss (Devi *et al.,* 2022). (Devi *et al.,* 2022).

Consequently, the socioeconomic implications of crop-based ethanol further complicate its sustainability. (Azadi *et al.,* 2017), while policy-driven production systems have shown economic limitations (Filip and Janda*,* 2019). These issues have redirected attention toward second-generation bioethanol. Cellulosic substrates such as corn stover and agricultural residues offer a promising alternative (Devi *et al.,* 2022). Utilizing such residues avoids competition with food systems and provides a sustainable pathway for second-generation bioethanol production (Zabed *et al.,* 2017).

However, the recalcitrant structure of lignocellulosic biomass, composed mainly of cellulose, hemicellulose, and lignin, limits enzyme accessibility and reduces direct hydrolysis efficiency (Baruah *et al.,* 2018). Integrated bioconversion processes such as simultaneous saccharification and fermentation (SSF) have been developed to improve the efficiency and economic feasibility of lignocellulosic bioethanol production by reducing enzyme inhibition and processing time (Sarkar et al., 2012). A general pretreatment pathway is illustrated in Fig. 1 (Ji *et al.,* 2012). To overcome these limitations, more environmentally benign and efficient solvent systems are being explored.

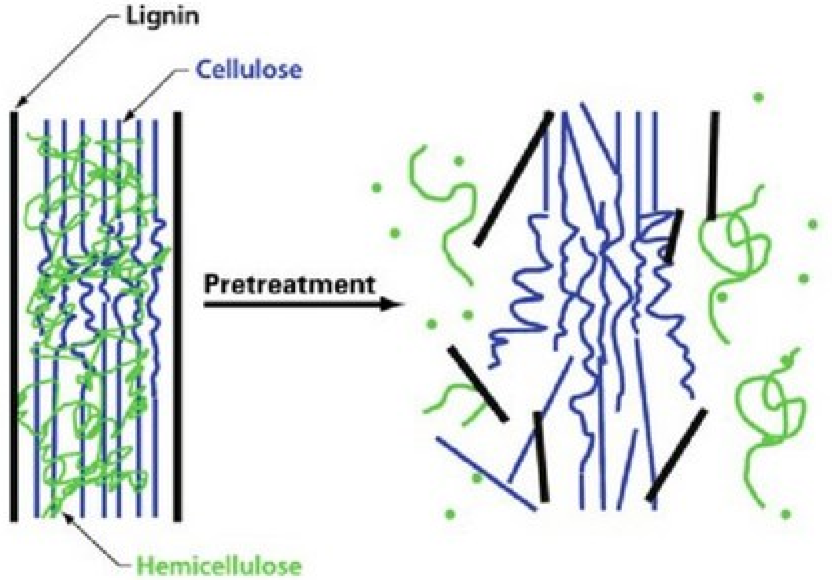

Fig. 1: A diagram of pretreatment of lignocellulosic biomass.

Conventional pretreatment methods, including dilute acid hydrolysis, often generate fermentation inhibitors and suffer from limited sugar recovery (Broda *et al.,* 2022). Although ionic liquids possess favorable physicochemical properties, they are not inherently green and present economic and environmental challenges (Goncalves *et al.,* 2021). Deep eutectic solvents (DESs) have recently emerged as promising, sustainable alternatives due to their low toxicity, biodegradability, and ease of synthesis (Florindo *et al.,* 2014). Improved preparation techniques enhance solvent performance (Dai *et al.,* 2013). DES systems formed from bio-based components such as choline chloride and hydrogen bond donors represent an environmentally benign breakthrough in biomass pretreatment. They can significantly enhance sugar recovery and improve ethanol yields (Contreras-Gamez *et al.,* 2022). Furthermore, advances in DES recycling and process optimization are improving economic feasibility (Contreras-Gamez *et al.,* 2022). Their tunable chemistry and selective delignification properties make them highly attractive for green biorefinery applications (Abbott *et al.,* 2004; Smith *et al.,* 2014).

Despite significant progress in the application of deep eutectic solvents for biomass pretreatment, there remains a lack of systematic comparison between bio-based DESs and metal-containing DESs under similar operating conditions. In addition, the relationship between the physicochemical properties of DESs such as viscosity, conductivity, and pH and their effectiveness in enhancing fermentable sugar recovery and bioethanol yield is not yet fully understood.

In this study, two different DESs were synthesized from mixtures of choline chloride–urea and zinc chloride–urea at a molar ratio of 1:2, respectively. The physicochemical parameters of the synthesized solvents were determined and characterized using Fourier Transform Infrared (FT-IR) spectroscopy. The synthesized solvents were subsequently employed in the pretreatment of cellulosic agricultural waste for enhanced fermentable sugar recovery and bioethanol production. This study aims to synthesize and characterize choline chloride–urea and zinc chloride–urea deep eutectic solvents and evaluate their effectiveness in the pretreatment of lignocellulosic agricultural residues for enhanced fermentable sugar recovery and bioethanol production.

The findings of this study are expected to contribute to the development of environmentally benign and cost-effective pretreatment technologies, thereby advancing sustainable biorefinery processes and supporting large-scale production of second-generation bioethanol, particularly in regions with abundant agricultural residues.

2.0 Materials and Methods

2.1 Materials

All reagents used in this study were of analytical grade and used without further purification. The equipment employed included a water bath (XMTE-205), hot plate with magnetic stirrer (JB-4A), fume hood, thermometer, analytical balance (JA303P), Nicolet 380 Thermo Fisher-Scientific FTIR spectrometer, CAP1000+ viscometer, conductivity meter (Model 470), pH meter (HI98107), distillation apparatus, melting point apparatus, and mortar and pestle.

2.2 DES Synthesis

Two different deep eutectic solvents (DESs) were synthesized by mixing zinc chloride with urea and choline chloride with urea at a molar ratio of 1:2, respectively. Each mixture was heated on a hot plate at 80 °C under constant stirring at 600 rpm until a clear and homogeneous liquid was obtained. The resulting DESs were allowed to stand at room temperature for 24 h, during which no crystallization or phase separation was observed, confirming their stability. The synthesized solvents were subsequently stored in airtight containers for further analysis.

2.3 Characterization of DES

2.3 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the synthesized DESs and their individual components were recorded using a Nicolet 380 Therma Fisher-Scientific FTIR spectrometer. Spectra were acquired over the range of 4000–650 cm^{-1} at a resolution of 4 cm^{-1} and processed using EZOMNIC software. The spectra were interpreted based on standard functional group assignments (Figure 2).

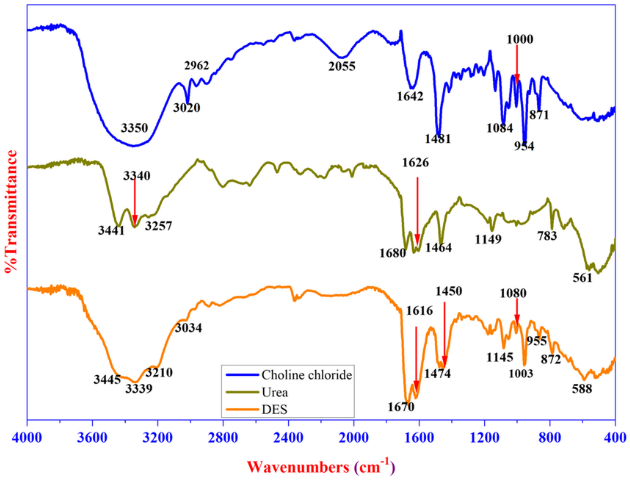

Fig. 2: FTIR spectra of synthesized deep eutectic solvents and their components.

The pH of the synthesized DESs was determined by immersing the electrode of a calibrated pH meter into each sample, and the readings were recorded.

2.3.2 Density

The density of the DESs was determined using the gravimetric method. The mass of an empty 25.00 mL density bottle was first recorded using an analytical balance. The bottle was then filled with the DES sample, and the total mass was measured. This procedure was repeated in triplicate, and the temperature was recorded using a thermometer. The density was calculated using equation 1, and the average value was reported.

$$Density = \frac{weight\ of\ DES}{Volume} \qquad (1)$$

2.3.3 Specific Gravity

The specific gravity of the DESs was determined using a gravimetric approach. The mass of a 25.00 mL density bottle filled with DES was recorded and compared with the mass of the same bottle filled with distilled water. The specific gravity was calculated using equation 2

$$Specific\ Gravity = \frac{weight\ of\ DES}{weight\ of\ water} \qquad (2)$$

2.3.4 Viscosity

The viscosity of the DESs was measured using a viscometer. The instrument was cleaned and filled with the sample up to the calibration mark, then placed vertically in a thermostatically controlled water bath maintained at 25 °C and allowed to equilibrate for 10 min. The time required for the liquid to flow between two marked points was recorded. The viscosity was calculated using equation 3

$$Viscosity = k\rho t \qquad (3)$$

where: k is the viscometer constant; ρ is the density of the sample (DES); and t is the time taken for the sample to flow between the calibration marks on the viscometer.

2.3.5 Conductivity

The conductivity of the DES samples was measured using a calibrated conductivity meter. The instrument was standardized using appropriate electrolyte solutions prior to measurement. The probe was rinsed with distilled water and immersed in the sample, and the conductivity values were recorded. Measurements were carried out in triplicate, and the average value was reported.

2.4 Bioethanol Production from Lignocellulosic Biomass

Bioethanol production from lignocellulosic biomass was carried out through four main stages: sample collection and preparation, DES pretreatment, hydrolysis and fermentation, and distillation.

2.5 Sample collection and preparation

A total of 100 g each of maize, rice, and corn straws were collected from farmland within Bauchi Local Government Area. The samples were air-dried, ground using a mortar and pestle, and stored in airtight containers prior to use.

2.6 DES pretreatment

The prepared biomass samples were soaked in 50.00 cm³ of DES and heated at 120 °C for 6 h. After pretreatment, the solid residues were washed thoroughly with hot distilled water to remove residual DES and then dried at room temperature for 24 h.

2.7 Hydrolysis and Fermentation

The pretreated biomass samples were subjected to hydrolysis by adding distilled water in separate reaction vessels to convert the polysaccharides into fermentable sugars. The resulting hydrolysates were transferred into fermentation vessels and sterilized by autoclaving at 121 °C. After cooling, zymase enzyme was added, and the pH was adjusted to 4.5. Fermentation was carried out at 30 °C for 72 h.

2.8 Distillation

The fermented mixtures were subjected to distillation using a standard distillation setup. Each sample was transferred into a round-bottom flask and heated, and the distillate was collected at 78 °C, corresponding to the boiling point of ethanol.

2.9 Percentage Bioethanol yield

The percentage bioethanol yield was calculated by dividing the mass of ethanol obtained after distillation by the initial mass of biomass used for fermentation, as shown in equation 4

$$Percentage\ yield = \frac{mass\ of\ bioethanol}{initial\ mass\ of\ sample\ used} \times 100 \quad (4)$$

3.0 Results and Discussion

Table 1 summarizes the physicochemical properties of the synthesized deep eutectic solvents (DESs), choline chloride–urea (ChCl:Urea) and zinc chloride–urea ($ZnCl_2$: Urea), at 298 K. Both solvents, prepared at a 1:2 molar ratio, formed stable and homogeneous liquids, confirming successful DES formation. The atom economy of DESs is effectively 100%, as their synthesis involves simple mixing without by-product formation (Abbott *et al.,* 2004; Coutinho *et al.,* 2017).

Table 1: Physicochemical properties of synthesized deep eutectic solvents at 298 K

Property	**ChCl:Urea**	**$ZnCl_2$:Urea**
pH	**8.95**	**9.73**
Density (g/cm³)	**1.14**	**1.31**
Specific gravity	**1.12**	**1.30**
Viscosity ($kg \cdot m^{-1} \cdot s^{-1}$)	**0.94**	**1.15**
Conductivity (mS/cm)	**3.12**	**2.61**
Melting point (°C)	**14**	**23**

The structural characteristics of the synthesized DESs were further examined using FTIR spectroscopy. FTIR spectra of the synthesized DESs were recorded over the range 4000–650 cm^{-1}. The observed shifts and broadening of O–H and N–H stretching vibrations (3100–3500 cm^{-1}), relative to the pure components, indicate strong hydrogen bonding interactions between urea and choline chloride or $ZnCl_2$. The disappearance and shifting of characteristic peaks from the individual precursors confirm the formation of a single-phase DES with reduced lattice energy and a lower melting point than the parent compounds. In the $ZnCl_2$:Urea system, shifts in the C=O stretching vibration of urea suggest coordination between zinc ions and oxygen atoms, consistent with reported behavior of metal-based DESs (Smith *et al.,* 2014).

The alkaline nature of the DESs, as indicated by pH values of 8.95 (ChCl:Urea) and 9.73 ($ZnCl_2$:Urea), enhances lignin dissolution through deprotonation of phenolic hydroxyl groups, thereby improving cellulose accessibility during enzymatic hydrolysis (Abbott *et al.,* 2004; Contreras-Gamez *et al.,* 2022). The higher density and specific gravity observed for ZnCl$_2$:Urea can be attributed to the presence of the transition metal ion, which promotes stronger intermolecular interactions and tighter molecular packing. An inverse relationship between viscosity and conductivity was observed: ZnCl$_2$: Urea exhibited higher viscosity (1.15 $\mathrm{kg \cdot m^{-1} \cdot s^{-1}}$) and lower conductivity (2.61 mS/cm), while ChCl:Urea showed lower viscosity (0.94 $\mathrm{kg \cdot m^{-1} \cdot s^{-1}}$) and higher conductivity (3.12 mS/cm). This suggests reduced ionic mobility in ZnCl$_2$:Urea and improved mass transfer characteristics in ChCl:Urea (Abbott *et al.,* 2004). The effect of DES pretreatment on bioethanol yield is presented in Table 2.

Table 2: Bioethanol yield from lignocellulosic biomass pretreated with deep eutectic solvents

Biomass type	ChCl:Urea (%)	ZnCl$_2$:Urea (%)
Maize straw	**39.24**	**35.81**
Rice straw	**36.63**	**32.39**
Corn straw	**48.94**	**47.22**

Pretreatment with ChCl:Urea consistently resulted in higher bioethanol yields across all biomass types compared to ZnCl$_2$:Urea, in agreement with previous studies (Zhang *et al.,* 2023; Contreras-Gamez *et al.,* 2022). Corn straw exhibited the highest yield (48.94%), likely due to its relatively higher cellulose content and lower lignin and hemicellulose fractions, which enhance susceptibility to DES-mediated fractionation. Bio-based DESs facilitate selective lignin removal while minimizing the formation of inhibitory by-products such as furfural, thereby improving fermentable sugar recovery and ethanol yield (Abbott *et al.,* 2004; Contreras-Gamez *et al.,* 2022).

Overall, both the nature of the DES and the type of lignocellulosic substrate significantly influenced bioethanol yield. The superior performance of ChCl:Urea can be attributed to its lower viscosity, higher conductivity, and moderate alkalinity, which collectively enhance mass transfer, lignin solubilization, and enzymatic accessibility. These findings highlight the potential of bio-based DESs as environmentally benign and efficient pretreatment solvents for sustainable second-generation bioethanol production (Smith *et al.,* 2014; Contreras-Gamez *et al.,* 2022).

4.0 Conclusion

This study highlights the growing interest in Deep Eutectic Solvents (DESs) as sustainable "green" solvents, particularly for the pretreatment of lignocellulosic biomass. Lignocellulosic biomass is an abundant, low-cost, and renewable resource composed primarily of cellulose and hemicellulose (50–65%) and lignin (10–30%), making it an attractive feedstock for the production of biofuels, biochemicals, and other value-added products. Among the pathways for lignocellulosic biomass utilization, the fermentation of cellulose and hemicellulose into bioethanol remains the most commercially viable. However, effective pretreatment is essential to reduce biomass recalcitrance and enhance enzymatic hydrolysis efficiency. Traditional pretreatment technologies are often limited by high costs, toxicity, and environmental concerns.

DESs offer a promising alternative due to their unique physicochemical properties, ease of synthesis, low cost, stability, and environmental friendliness. Formed by combining hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs), DESs exhibit performance comparable to conventional ionic liquids while avoiding their associated drawbacks. In this study, DESs effectively dissolved lignin under mild conditions, improving carbohydrate accessibility and enhancing bioethanol yield. While DESs have been explored in other industries, including metal finishing, biocompound extraction, and synthetic applications, their use in biomass pretreatment remains relatively underexplored. Challenges such as the presence of minerals and impurities in biomass, thermal stability, and solvent recyclability must be addressed to scale up DES-based pretreatment industrially.

Overall, this research demonstrates that DESs are a sustainable, efficient, and eco-friendly alternative to traditional pretreatment agents. Their ability to enhance sugar recovery from lignocellulosic biomass under mild conditions paves the way for the commercial production of cellulosic bioethanol, contributing to a greener and more sustainable bioeconomy.

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Consent for publication

Not Applicable

Availability of data and materials

The publisher has the right to make the data public

Conflict of Interest

The authors declared no conflict of interest

Ethical Considerations

Not applicable

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Authors' Contribution

DM, BL, and AEO conducted the experimental work, data collection, and laboratory analyses. OOA, AHI, and MM supervised the study design and interpretation of results. YST, HA, WMI, and JIS contributed to biomass pretreatment, characterization, and manuscript drafting. HAG and JD assisted in data validation and literature review, while MM coordinated the research, revised the manuscript, and approved the final version.