Cascading Biorefinery of Chickpea Using Deep Eutectic Solvents for Protein Recovery and Bioethanol Production

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Abstract. A cascading biorefinery strategy using deep eutectic solvents (DES) was developed for protein extraction from chickpea biomass and subsequent bioethanol production from the residual lignocellulosic material. Protein was first extracted using DES, after which the remaining biomass was further pretreated with a choline chloride:oxalic acid:glycerol (ChCl:OA:G, 1:1:2) system to enhance enzymatic hydrolysis. Pretreatment parameters were optimized using Response Surface Methodology (RSM), and the optimal conditions were identified as 6% solid loading, 122 °C, and 200 min. Under this optimal condition, the reducing sugar concentration reached 14.56 mg/mL. Fermentation of the hydrolysate using Saccharomyces cerevisiae resulted in an ethanol concentration of 5.92 g/L. The results showed that DES pretreatment significantly increased sugar and ethanol concentrations due to improved structural disruption and enzyme accessibility. This study demonstrates the potential of DES as a sustainable pretreatment solvent for integrated food and bioenergy production within a circular economy framework.

1 Introduction

Sustainable development in the context of environment and renewable energy requires a fundamental transition away from fossil fuel–based systems toward low-carbon, resource-efficient technologies. Continued reliance on fossil fuels remains a major contributor to global CO_2 emissions and climate change, driving the urgent need for renewable energy solutions that are environmentally benign and compatible with circular economy principles. In this regard, integrated biomass utilization has emerged as a promising strategy to simultaneously address energy security, waste reduction, and environmental sustainability [1].

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Bioethanol is widely recognized as a renewable energy carrier with significant potential to reduce greenhouse gas emissions due to its renewability, biodegradability, and compatibility with existing fuel infrastructure. In particular, second-generation bioethanol derived from lignocellulosic biomass offers a sustainable alternative to fossil-derived fuels by utilizing agricultural residues rather than food-based feedstocks, thereby mitigating food–energy competition and enhancing overall resource efficiency [2]. Despite these advantages, large-scale deployment of lignocellulosic bioethanol remains constrained by the intrinsic recalcitrance of biomass, especially during pretreatment and enzymatic hydrolysis stages. The biorefinery concept provides a holistic framework for renewable energy production by enabling the conversion of biomass into multiple energy carriers and value-added co-products. Within this framework, agricultural crops and residues can be integrated into circular production systems that maximize energy recovery while minimizing waste generation and environmental impacts [3].

Chickpea (*Cicer arietinum*), a widely cultivated legume, represents a suitable biomass resource not only for food production but also as a renewable energy feedstock. After protein extraction, the residual chickpea biomass contains significant lignocellulosic fractions that can be valorized for bioethanol production, supporting a cascading and energy-oriented biorefinery approach. Efficient conversion of lignocellulosic biomass into fermentable sugars is a critical challenge in renewable bioethanol production. Conventional pretreatment technologies often require harsh chemicals and high energy inputs, leading to the formation of inhibitory compounds and increased environmental burdens. Consequently, the development of green pretreatment technologies that enhance biomass digestibility while reducing chemical consumption and emissions is essential for advancing sustainable bioenergy systems.

Deep eutectic solvents (DESs) have emerged as environmentally friendly alternatives to conventional solvents, characterized by low toxicity, biodegradability, non-flammability, and tunable physicochemical properties. DESs have demonstrated strong potential for lignocellulosic biomass pretreatment by selectively disrupting lignin and hemicellulose structures and improving cellulose accessibility, thereby enhancing enzymatic hydrolysis and fermentable sugar release [4]. In addition, DESs enable the recovery of biomacromolecules, e.g., protein, chitin as a co-product, contributing to improved energy efficiency and overall sustainability of the biorefinery system [5]. This study investigates an integrated DES-based bioprocessing strategy for renewable energy production from chickpea biomass. Four DES formulations were evaluated for protein extraction, while the ternary DES system choline chloride:oxalic acid:glycerol (1:1:2) was applied to pretreat residual biomass to optimize sugar release for subsequent bioethanol fermentation. By integrating protein recovery with bioethanol production, this approach supports circular bioenergy systems and aligns with the objectives of environmental protection, greenhouse gas reduction, and sustainable renewable energy development. The findings highlight the potential of DES-enabled green biorefineries as a viable pathway toward environmentally responsible and energy-efficient utilization of agricultural biomass.

The objective of this study is to develop a sustainable cascading biorefinery process for chickpea biomass using deep eutectic solvents (DES) by integrating plant protein extraction with DES-based pretreatment of residual biomass to enhance enzymatic hydrolysis and bioethanol production. Process parameters were optimized using Response Surface Methodology (RSM) to maximize reducing sugar and ethanol yields, in alignment with circular economy and green chemistry principles.

2 Materials and Methods

2.1 Preparation of Chickpea Samples and Synthesis of Deep Eutectic Solvents

Raw chickpea (*Cicer arietinum*) samples obtained from Baiya Company were ground into a fine powder using a household blender and sieved through a 2-mm mesh to obtain a uniform particle size. The processed chickpea powder was stored at –20 °C until further use. The experimental workflow was designed based on a cascading biorefinery process for protein recovery and renewable bioethanol production from chickpea biomass. (Fig. 1.)

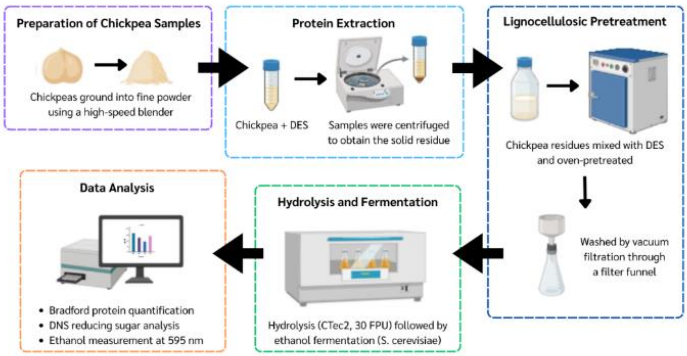

Fig. 1. Overview of the experimental procedure.

Four choline chloride–based deep eutectic solvents (DESs) were prepared according to their respective molar ratios. All mixtures were placed in round bottles and heated with a stirring mantle at 80 °C for 1 h. When the mixtures become clear, transparent solutions, they are kept in a desiccator until used. The prepared DESs were subsequently characterized in terms of pH, viscosity, and density to evaluate their suitability for protein extraction and lignocellulosic biomass pretreatment. As summarized in Table 1, the DESs exhibited distinct physicochemical properties, with choline chloride–urea (1:2) showing the highest viscosity, while choline chloride–oxalic acid–ethylene glycol (1:1:2) exhibited the lowest viscosity. The oxalic acid–based DESs displayed strongly acidic pH values, which are favorable for lignocellulosic pretreatment due to enhanced solvent–biomass interactions.

Table 1. Viscosity measurements of the Deep Eutectic Solvent (DES).

Deep Eutectic Solvent (DES)	pH	Viscosity(mpa.s)	Density(g/cm^3)
ChCl:Urea (1:2)	5	560	1.03
ChCl:Oxalic Acid (2:1)	0	220	1.20
ChCl:Oxalic acid:Glycerol (1:1:2)	0	175	1.17
ChCl:Oxalic acid:Ethylene Glycol (1:1:2)	0	50	1.20

2.2 Protein Extraction Using Deep Eutectic Solvent (DES)

Chickpea powder was mixed with the prepared DES at solid-to-solvent ratios of 5.0, 12.5, and 20.0% (w/w), with a total mixture mass of 10.0 g. Protein extraction was conducted at different temperatures (30, 45, and 60 °C) and extraction times (1, 2, and 3 h) using an incubator shaker. The experimental design was generated using response surface

methodology (RSM) with Design-Expert software. A control experiment was performed under identical conditions using distilled water instead of DES. After extraction, the mixtures were centrifuged at 8,000 rpm and 10 °C for 15 min to separate the liquid and solid phases. The supernatants were collected for protein quantification using the Bradford assay. The solid residues were washed three times with 10 mL of distilled water and dried at 60 °C to constant weight before further experiments.

The combination of solid-to-solvent ratio, temperature, and extraction time that resulted in the highest protein yield was selected as the optimal condition and subsequently repeated for comparison with the control.

2.3 Optimization of Pretreatment Conditions for Chickpea Residues Using Deep Eutectic Solvent (DES)

Chickpea residues obtained after protein extraction were mixed with DES at solid-to-solvent ratios of 5.0%, 12.5%, and 20.0% (w/w) to obtain a total mixture mass of 10 g. Pretreatment was performed at temperatures of 70 °C, 100 °C, and 130 °C, with reaction times of 40, 120, and 200 minutes. The experimental conditions were designed using Response Surface Methodology (RSM) generated by Design Expert software (Table 2). A control experiment was conducted under the same conditions using distilled water instead of DES. After pretreatment, the samples were washed with 20 mL of distilled water until the pH was neutral. The mixtures were then centrifuged at 6,000 rpm for 10 minutes at room temperature to separate the solid fraction from the washing solution. The solid residues were subsequently dried at 60 °C until a constant weight was achieved before being used for further experiments.

Table 2. Optimization of Pretreatment Conditions for Chickpea Residues Using Deep Eutectic Solvent (DES).

Run	Factor 1: Temp (°C)	Factor 2: Time (h)	Factor 3: Solid loading (%)
1	130	200	12.5
2	130	120	5
3	100	200	20
4	100	120	12.5
5	100	200	5
6	100	120	12.5
7	70	40	12.5
8	100	120	12.5
9	70	200	12.5
10	70	120	20
11	100	120	12.5
12	130	40	12.5
13	100	40	20

14	70	120	5
15	100	120	12.5
16	100	40	5
17	130	120	20

2.4 Enzymatic Hydrolysis and Fermentation

The pretreated chickpea biomass obtained under the optimized conditions was subjected to enzymatic hydrolysis using CTec2. A total of 0.1 g of dried residue was suspended in 4 mL of 0.05 M citrate buffer, followed by the addition of 40 µL of sodium azide to prevent microbial contamination. CTec2 was added at a dosage of 30 FPU/g, and the mixture was incubated at 50 °C with continuous shaking for 72 hours. The released reducing sugars were quantified using the DNS method, and the hydrolysis data were analyzed using Design Expert software to determine the optimal pretreatment parameters. The hydrolysate produced under these optimal conditions was subsequently used for ethanol fermentation. *Saccharomyces cerevisiae* was inoculated into the hydrolysate supplemented with 1% yeast extract and 1% glucose to support yeast acclimatization, and fermentation was carried out at 30 °C for 48 hours. Ethanol concentration in the fermentation broth was then quantified by spectrophotometry assay [6].

3 Result and Discussion

3.1 Protein Extraction from Chickpea Using Deep Eutectic Solvents (DES)

Protein extraction from chickpea (*Cicer arietinum*) was investigated using four deep eutectic solvents (DESs): choline chloride–urea (1:2) [ChCl:U], choline chloride–oxalic acid (2:1) [ChCl:OA], choline chloride–oxalic acid–glycerol (1:1:2) [ChCl:OA:G], and choline chloride–oxalic acid–ethylene glycol (1:1:2) [ChCl:OA:EG]. The effects of solid-to-liquid ratio, extraction temperature, and extraction time on protein yield were evaluated using response surface methodology (RSM), as described in Section 2.2. The optimal extraction conditions varied depending on the DES system. For ChCl:U (1:2), the maximum protein yield of 97.83 mg/mL was achieved at a 20% solid-to-liquid ratio, 60 °C, and an extraction time of 3 h. ChCl:OA (2:1) produced the highest protein yield of 221.92 mg/mL under a 20% ratio at 45 °C for 3 h. For ChCl:OA:G (1:1:2), the optimal conditions were a 20% ratio, 30 °C, and 2 h, resulting in a protein yield of 204.52 mg/mL. In contrast, ChCl:OA:EG (1:1:2) achieved a maximum yield of 132.38 mg/mL at a 20% ratio, 30 °C, and 1 h.

The optimized conditions for each DES system were subsequently selected for replication and comparison with the control to determine the maximum extractable protein content. Based on the comparative results, the DES providing the highest protein recovery was selected for use in subsequent biomass pretreatment and bioethanol production experiments. The protein content extracted from chickpea using four Deep Eutectic Solvents (DES) was quantified using the Bradford protein assay, as summarized in Figure 2. Extraction with ChCl:U (1:2) yielded 97.53 mg/mL of protein, compared to 63.23 mg/mL obtained from the control, representing a 54.24% increase. Similarly, ChCl:OA (2:1) produced a protein concentration of 177.97 mg/mL, which was 65.33% higher than the control (107.64 mg/mL). Extraction with ChCl:OA:G (1:1:2) resulted in a protein yield of 203.92 mg/mL, exceeding the control value of 152.26 mg/mL by 33.93%. In the case of ChCl:OA:EG (1:1:2), the

extracted protein concentration was 147.23 mg/mL, showing a modest increase of 3.19% compared to the control (142.67 mg/mL). Among all DES formulations tested, ChCl:OA:G (1:1:2) provided the highest protein yield (203.92 mg/mL), demonstrating superior extraction efficiency over the other DES types.

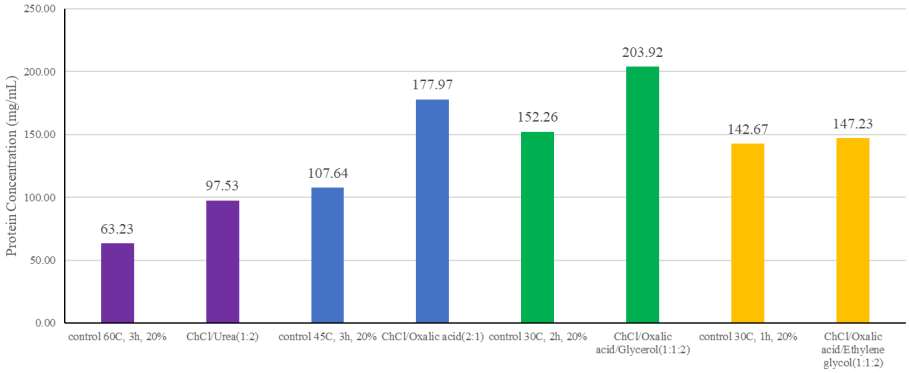

Fig. 2. Protein concentration (mg/mL) extracted from chickpea using four Deep Eutectic Solvents (DES), compared with the control.

3.2 Optimization for Maximum Reducing Sugar Yield Using ChCl:Oxalic Acid:Glycerol Deep Eutectic Solvent

The pretreatment of chickpea biomass was performed after protein extraction using the ChCl:OA:G. Pretreatment was carried out under varying solid-to-liquid ratios, temperatures, and times as shown in Table 2. The reducing sugar concentrations obtained from enzymatic hydrolysis were subsequently input into the RSM design matrix to determine the optimal conditions for maximum reducing sugar yield.

The response surface plots in Figure 3 demonstrated the combined effects of temperature, pretreatment time, and solid-to-liquid ratio on reducing sugar release from the pretreated chickpea biomass with ChCl:OA:G. The RSM model indicated that higher temperatures and longer residence times significantly increased sugar yield, which aligned with recent findings showing that elevated thermal energy promotes disruption of lignocellulosic hydrogen-bond networks and enhances cellulose accessibility during DES pretreatment [9]. Statistically, the model was highly significant (p-value < 0.0001) with a strong goodness of fit (R^2 = 0.9349, adjusted R^2 = 0.8958), indicating that it explained approximately 93.5% of the variability in sugar yield. Temperature, time, and solid loading were all significant main effects (p-value ≤ 0.05), and their interaction terms (temperature×time, temperature×load, and time×load) were also significant (p-value ≤ 0.05), revealing synergistic influences among process variables. Furthermore, the lack of fit was not significant (p-value = 0.1772), confirming the adequacy and reliability of the proposed model for describing sugar release behavior under the studied conditions.

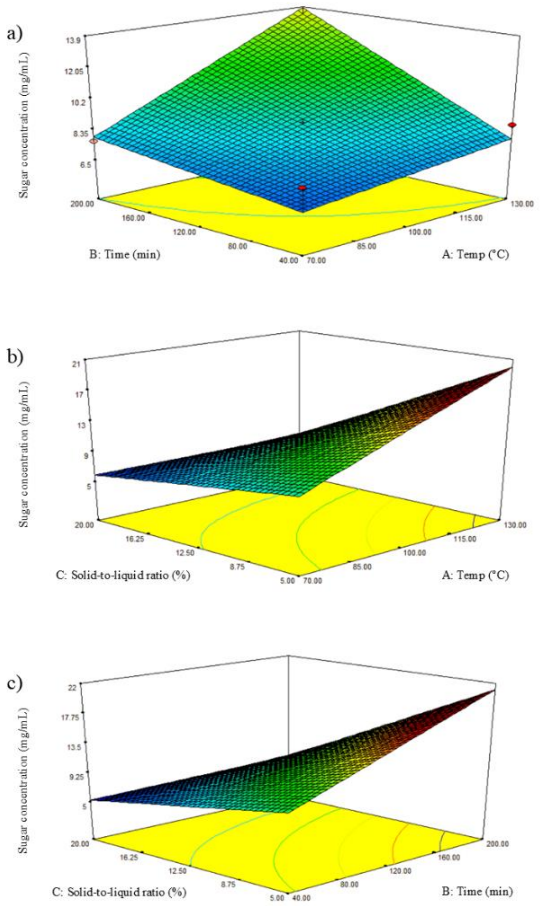

Fig. 3. Response Surface Methodology (RSM) analysis identifying the optimal pretreatment conditions for maximizing reducing sugar yield using the Choline Chloride:Oxalic Acid:Glycerol (1:1:2).

The optimized pretreatment conditions (6% solid loading, 122 ºC, and 200 min) produced the highest reducing sugar concentration. Organic-acid-based DES such as ChCl:OA:G exhibit strong depolymerizing effects on hemicellulose and effectively swell amorphous cellulose regions, improving enzymatic hydrolysis efficiency [7]. Recent studies have shown that acidic DES containing oxalic acid can promote proton-catalyzed cleavage of key structural bonds within lignocellulosic biomass, including glycosidic and ether linkages, leading to partial depolymerization and loosening of plant cell-wall architecture [8]. In ternary DES systems incorporating glycerol as a hydrogen-bond donor, reduced viscosity and improved solvent mobility have been reported, which enhance solvent penetration into biomass and facilitate lignin and hemicellulose solubilization, consistent with observations in glycerol-based DES pretreatments [9].

The interactive plots also reveal that reducing sugar release increases markedly at low biomass loadings (Figure 3b–c), likely due to enhanced mass transfer and greater DES accessibility. This behavior is widely reported in pretreatment systems where low substrate concentrations promote more uniform solvent penetration and greater disruption of recalcitrant structure [10].

3.3 Reducing Sugar Quantification

The effect of biomass pretreatment using the ternary DES ChCl:OA:G (1:1:2) under the optimized conditions (solid-to-liquid ratio of 6%, temperature of 122 °C, and pretreatment time of 200 minutes) was evaluated based on the reducing sugar released after enzymatic hydrolysis. The results showed that the pretreated biomass produced a maximum reducing sugar concentration of 14.56 mg/mL. In comparison, the unpretreated sample generated only 3.12 mg/mL of reducing sugar (Figure 4). This suggested that DES pretreatment is a critical step to enhance enzymatic saccharification under optimal conditions, as sugar concentration increased by 4.67 times compared to the untreated sample. This indicated the effectiveness of DES in disrupting lignocellulosic structures and improving enzyme accessibility to polysaccharides. However, during DES pretreatment, the partial degradation or loss of certain biomass components, such as hemicellulose or water-soluble monosaccharides, may occur [11]. Such losses reduce the remaining dry biomass mass, resulting in a lower calculated yield despite the higher sugar concentration in the hydrolysate.

Previous studies have reported that DES formulations containing organic acids, such as oxalic acid, can disrupt the crystalline structure of cellulose and solubilize portions of lignin, thereby enhancing sugar release after hydrolysis [12]. However, biomass loss during pretreatment has also been identified as a major factor contributing to lower yield values when calculations are based on the original dry mass. Therefore, the outcomes of this study are consistent with these observations.

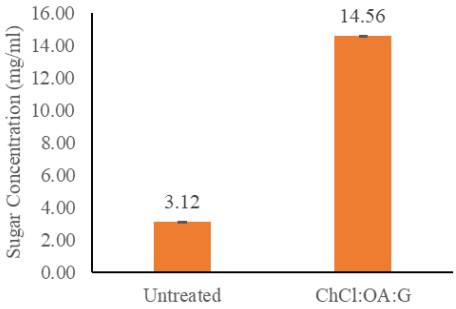

Fig. 4. Reducing sugar concentration (mg/mL) after hydrolysis of biomass pretreated with ChCl:OA:G (1:1:2) under optimal conditions, compared with the untreated sample.

3.4 Ethanol Results

Fermentation of the hydrolysate obtained from biomass pretreated with ChCl:OA:G (1:1:2) under the optimal conditions (solid-to-liquid ratio of 6%, 122 °C, 200 min) for 48 hours resulted in a maximum ethanol concentration of 0.75%, corresponding to an ethanol yield of 10.11%, which was higher than untreated sample for 1.56 times (Figures 5). These results highlight the correlations between ethanol concentration in the hydrolysate and the sugar yield. Both sugar and ethanol products were influenced by changes in biomass structure during pretreatment. While the fermentation results indicate that hydrolysate pretreated with ChCl:OA:G (1:1:2) generated a higher ethanol concentration than the control, demonstrating the potential of DES to enhance enzymatic accessibility and the release of fermentable sugars. This trend aligns with observations from the hydrolysis step, where DES pretreatment was found to increase reducing sugar concentration but also caused a reduction in overall biomass mass. The mass loss may result from the solubilization of hemicellulose and partial disruption of the cellulose matrix during chemical pretreatment [13].

Previous studies support this pattern, reporting that DES pretreatment—particularly those containing organic acids like oxalic acid—can improve ethanol production by enhancing lignocellulosic disruption and reducing lignin content, thereby promoting enzymatic conversion [14]. However, these studies also note a key limitation that DES can solubilize certain carbohydrate fractions, leading to decreased overall yield despite increased product concentration [15].

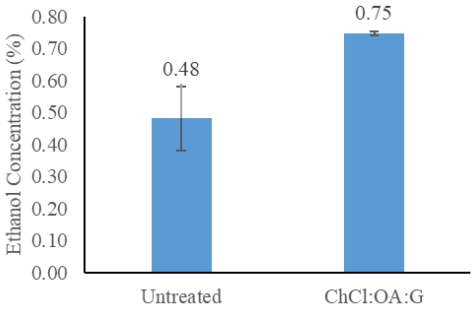

Fig. 5. Ethanol concentration obtained after fermentation of the hydrolysates derived from biomass pretreated with ChCl:OA:G (1:1:2) under optimal conditions, compared with the untreated control.

4 Conclusion

This study demonstrates the development of an integrated, sustainable bioprocessing approach for chickpea biomass using deep eutectic solvents (DES) to extract proteins and valorize the remaining residues for bioethanol production. Among the four DES formulations tested, Choline Chloride:Oxalic Acid:Glycerol (1:1:2) exhibited the highest efficiency in protein extraction, achieving 203.92 mg/mL, significantly outperforming the corresponding water-based controls. Following protein extraction, the application of the same ternary DES to chickpea residues effectively enhanced biomass deconstruction, enabling improved enzymatic accessibility and the release of higher reducing sugar concentrations under optimized pretreatment conditions.

Although the DES-treated biomass produced higher concentrations of reducing sugars and ethanol during hydrolysis and fermentation, the corresponding yields were lower than the control due to partial biomass loss during pretreatment—an inherent challenge associated with acidic DES systems. Nevertheless, the findings confirm that DES, particularly organic-acid-based formulations, offer substantial advantages in fractionating lignocellulosic biomass and facilitating downstream bioconversion. Overall, this work highlights the potential of DES-based green solvents as promising alternatives to conventional chemical methods for protein extraction and biomass pretreatment. The integrated process aligns with circular biorefinery principles and the Bio-Circular-Green (BCG) economy framework, maximizing resource utilization and promoting sustainable production of plant-based proteins and renewable bioethanol from chickpeas. Further optimization of DES recycling, improvement of biomass recovery efficiency, and evaluation of process scalability are necessary to enhance the practicality of this green bioprocess and support its future industrial implementation.

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References

1. K. L. Hefferon, Alternative protein innovations and challenges for industry and consumer: an initial overview, Front. Sustain. Food Syst. **7** (2023).
2. K. Malik, P. Sharma, Y. Yang, P. Zhang, L. Zhang, X. Xing, J. Yue, Z. Song, L. Nan, Y. Sun, M. M. El-Dalatony, Lignocellulosic biomass for bioethanol: Insight into the advanced pretreatment and fermentation approaches, Ind. Crops Prod. **188**, 115569 (2022).
3. A. Saravanan, P. S. Kumar, S. Jeevanantham, S. Karishma, D. V. N. Vo, Recent advances and sustainable development of biofuels production from lignocellulosic biomass, Bioresour. Technol. **344**, 126203 (2022).
4. Z. Yao, G. Chong, H. Guo, Deep eutectic solvent pretreatment and green separation of lignocellulose, Appl. Sci. **14**, 7662 (2024).
5. T. C. Hai, N. T. Dat, Q. L. A. Tuan, H. L. T. Ngan, N. D. T. Tu, N. T. Thinh, D. N. Quang, L. T. H. Anh, P. V. Man, Extraction of chitin from giant tiger prawn (Penaeus monodon) shrimp shell using deep eutectic solvents and citric acid, Appl. Sci. Eng. Prog. **17**, 7177 (2024).
6. M. Sriariyanun, M. P. Gundupalli, V. Phakeenuya, T. Phusamtisampan, Y.-S. Cheng, P. Venkatachalam, Biorefinery approaches for production of cellulosic ethanol fuel using recombinant engineered microorganisms, J. Appl. Sci. Eng. **27**, 1985–2005 (2023).
7. N. Arshad, E. J. Panakkal, A. Chantarasiri, P. Pornwongthong, H. El Bari, W. Fatriasari, P. Tantayotai, M. Sriariyanun, Deep eutectic solvents (DESs) in lignocellulosic biomass pretreatment: Mechanisms and process optimization, Bioresour. Technol. Rep. **20**, 102190 (2025).
8. Y. Liu, X. Zhang, W. Chen, J. Wang, Exploring carbohydrate extraction from biomass using acidic deep eutectic solvents, iScience **26** (2023).
9. Y. Yang, H. Zhang, S. Li, Z. Wang, Effect of ternary deep eutectic solvents on bagasse cellulose and lignin structure in low-temperature pretreatment, Processes **10**, 778 (2022).
10. N. Arshad, E. J. Panakkal, P. B. Kalivarathan, A. Tawai, S. Chuetor, W. Wanmolee, S. Kirdponpattara, A. Chantarasiri, S. Rakesh, A. A. Septevani, P. Venkatachalam, Emerging technologies in pretreatment and hydrolysis for high-solid-loading bioethanol production from lignocellulosic biomass, Fermentation **11**, 613 (2025).
11. S. Areeya, E. J. Panakkal, M. Sriariyanun, T. Kangsadan, A. Tawai, S. Amornraksa, U. W. Hartley, P. Yasurin, A review on chemical pretreatment of lignocellulosic biomass for the production of bioproducts: mechanisms, challenges and applications, Appl. Sci. Eng. Prog. **16**, 6767 (2023).
12. X. D. Hou, A. L. Li, K. P. Lin, Y. Y. Wang, Z. Y. Kuang, Insight into the structure–property relationships of deep eutectic solvents for sustainable lignocellulose fractionation, Green Chem. **20**, 4428–4443 (2018).
13. L. Yao, P. Cui, X. Chen, C. G. Yoo, Q. Liu, X. Meng, L. Xiong, A. J. Ragauskas, H. Yang, A combination of deep eutectic solvent and ethanol pretreatment for synergistic delignification and enhanced enzymatic hydrolysis for biorefinery process, Bioresour. Technol. **350**, 126885 (2022).
14. Y. Sun, H. Chen, H. Li, L. Wang, Pretreatment of lignocellulosic biomass with deep eutectic solvents for bioethanol production: A review, Bioresour. Technol. **339**, 125606 (2021).
15. M. Zdanowicz, K. Wilpiszewska, T. Spychaj, Deep eutectic solvents for polysaccharides processing, Carbohydr. Polym. **200**, 361–380 (2018).