



Original Research Paper

Enhancing lignocellulosic biorefinery sustainability: mechanisms and optimization of microwave-responsive deep eutectic solvents for rapid delignification

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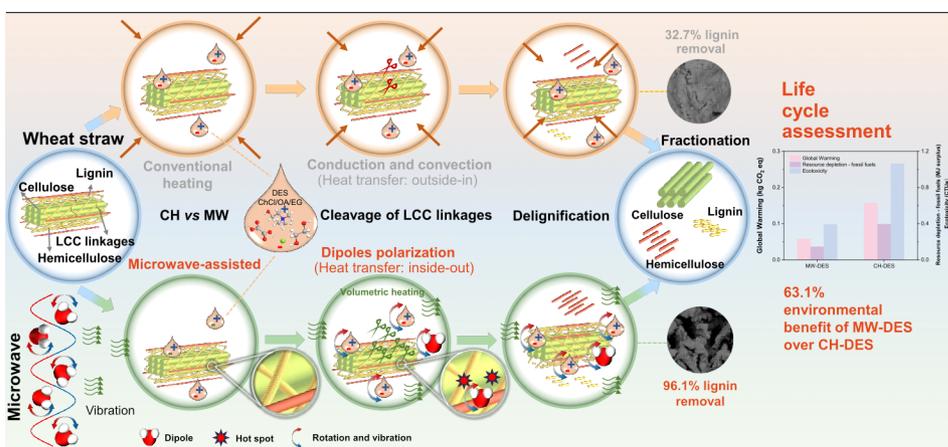
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HIGHLIGHTS

- The correlation between solvent properties and fractionation performance under microwave (MW) irradiation was established.
- MW enhanced the delignification efficiency of DES, particularly with high π^* and α values, leading to a 96.1% lignin removal.
- The optimal CHCl₃/OA/EG (1:2:2) ratio achieved 96.1% lignin removal in just 150 s at 130°C.
- MW-DES pretreatment significantly improved enzymatic hydrolysis, with a 92.4% digestibility, and enabled microbial lipid production of 8.8 g/100 g wheat straw.
- MW-DES reduced environmental impacts by 63.1% and lowered costs by 44.1% compared to CH-DES pretreatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Attaining sustainability and carbon neutrality necessitates a transition towards cleaner biorefinery, while the exploitation of sustainable and eco-friendly pretreatment techniques, as a pivotal stage in lignocellulose biorefinery, represents a challenge. Here, an ultrafast biomass pretreatment strategy enabled by microwave (MW) responsive deep eutectic solvent (DES) is proposed. The solvent properties (Kamlet-Taft parameters) of DES under MW participation are closely correlated with wheat straw fractionation efficiency. The lignin removal exhibits a positive correlation with polarity/polarizability (π^*) and hydrogen-bond-donating ability (α), establishing a strong relationship between the tunable DES properties and MW responsiveness. MW reinforces the delignification efficiency of DES with relatively high π^* and α , as corroborated by comparative analysis with conventional heating (CH) pretreatment. The reinforcement by MW moderates the pretreatment process and enables ultrafast lignocellulose deconstruction (130 °C, 150 s, and 96.1% lignin removal), subsequently with 92.4% enzymatic hydrolysis and 8.8 g microbial lipid/100 g wheat straw at a remarkably low severity factor (R_0). Life cycle assessment manifests the environmental benefits of MW-assisted DES in mitigating impacts by 63.1%, including global warming potential, resource depletion-fossil fuels, and ecotoxicity, in comparison to CH pretreatment. MW-DES exhibits an economic superiority based on life cycle cost analysis, with pretreatment cost 44.1% lower than CH-DES. The mechanistic insights into MW intensification of DES with specific properties provide a viable protocol for tailoring green solvents with enhanced MW responsiveness for efficient and sustainable biorefineries.

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Abbreviations

ANOVA	Analysis of variance
BBD	Box-Behnke design
CH	Conventional heating
CH-DES	Conventional heating pretreatment with DESs
ChCl	Choline chloride
DCW	Dry cell weight
DES	Deep eutectic solvent
EG	Ethylene glycol
HBA	Hydrogen bond donor
HBD	Hydrogen bond acceptor
LCA	Life cycle assessment
LCCA	Life cycle cost assessment
MW	Microwave
MW-DES	Microwave-assisted deep eutectic solvent
NREL	National Renewable Energy Laboratory
OA	Oxalic acid
R ²	Coefficient of determination
R ₀	Severity factor
RED	Relative energy difference
SEM	Scanning electron microscopy
<i>T. cutaneum</i>	<i>Trichosporon cutaneum</i>
α	Hydrogen-bond-donating ability
β	Hydrogen-bond-accepting ability
π*	Polarity/polarizability
δ	Hansen solubility parameter
δ _D	Dispersion parameters
δ _P	Polar parameters
δ _H	Hydrogen bonding parameters

1. Introduction

The sustainable conversion of lignocellulose into value-added chemicals and bioenergy represents a promising technological strategy for reducing fossil fuel dependency (Cai et al., 2023; Xiao et al., 2024). Nevertheless, the

intractable recalcitrance constituted by the cross-linked primary components (i.e., cellulose, hemicellulose, and lignin) of lignocellulose renders its resistance to efficient fractionation and further valorization (Liu et al., 2023a; Wang et al., 2024d). Numerous pretreatment strategies have been employed to overcome this challenge, such as physical (grinding), chemical (acid/alkali, organic solvents), physicochemical (steam explosion), and biological (enzyme, microorganism) pretreatment methods (Chen et al., 2023). Restrictions of the aforementioned traditional methodologies, including elevated energy consumption (physical methods), corrosiveness and toxicity (chemical methods), and extended treatment duration (biological methods), necessitate the investigation of green alternative solvents and sustainable processes (Xie et al., 2021).

Recent innovations in pretreatment, such as hydrothermal treatment, supercritical fluids, steam explosion, and ammonia fiber explosion, have made great achievements, surpassing traditional methods in terms of overall efficiency (Basak et al., 2023). However, high temperatures and pressures challenge equipment, operation, and energy consumption (Chen et al., 2023). Ionic liquids and bio-derived solvents are efficacious in lignocellulose deconstruction, but solvent-induced high costs and poor biocompatibility hamper their industrial viability (Lobato-Rodríguez et al., 2023; Meng et al., 2023). Deep eutectic solvents (DESs) represent an acknowledged and feasible substitute for ionic liquids (Hashemi et al., 2022). Typical DES comprises fairly common constituents such as choline chloride, polyols (ethylene glycol), organic acids (oxalic acid), and amino acids, which are derived from renewable resources and classified as "readily biodegradable" by the OECD (Organization for Economic Co-operation and Development) biodegradability test (No.301) (Azzouz and Hayyan, 2023). As a result, DES is generally both biocompatible and biodegradable. Given the superiority of cost-effective, easy-to-prepare, eco-friendly, recyclable, and tunable nature, DESs can be simply designed from a series of hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs) for lignocellulose pretreatment (Hansen et al., 2021). The malleability of DESs allows for diverse solvent properties, and solvent properties are closely associated with disrupting the intricate resistance of lignocellulose for value-added applications (Hong et al., 2020). Nevertheless, challenges and constraints of DESs in lignocellulose deconstruction remain, particularly in terms of extraction efficiency (Zou et al., 2024). Conventional heating (CH) pretreatment with DESs (CH-DES) suffers from stringent conditions (high temperature of 100–200 °C and prolonged duration of 1–4 h) (Supplementary Information, Table S1), which is typically energy- and time-consuming. Increasing attention is thus placed on exploiting more productive and sustainable pretreatment technologies for lignocellulosic biomass valorization (Chen et al., 2023).

Microwave (MW) enables uniformly and rapidly volumetric heating compared to inefficient and slow heating caused by conduction and convection in the CH process, and the integration of MW technology into

DES pretreatment has gained attention for energy- and time-efficient purposes (Zou et al., 2024). Current MW-assisted DES (MW-DES) investigations through DES screening and pretreatment conditions optimization can obtain moderate to good fractionation results (lignin removal of 41.5%–92.4% and hemicellulose removal of 58.6%–100%) at 80–160 °C for 0.75–30 min, with enzymatic digestibility of pretreated matrix of 52.8%–96.3% (Supplementary Information, Table S2), indicating that MW incorporation can indeed expedite the reaction process. However, the selection of these DESs is decentralized and arbitrary among these investigations, making it challenging to draw robust conclusions regarding the influence of DES properties on lignocellulose deconstruction under MW conditions, which thus cannot guide the design of MW-based DESs.

The characteristics of DESs can be customized through a rational combination of different HBAs and HBDs or varying molar ratios of HBA-HBD within the same DES, thus governing the performance of DESs in lignocellulose fractionation (Hansen et al., 2021). Numerous CH-DES pretreatment studies have been conducted and revealed that solvent properties (e.g., acidity) can modulate the fractionation performance (Hong et al., 2020), but the MW heating mechanism is different from that of CH, and the regulatory rules may change. To reveal the regulatory framework governing the lignocellulose fractionation by the MW-DES process, it is imperative to ascertain the intrinsic solvent properties of DES. Polarity, in particular, is contingent upon the holistic solvation capacity of the solvent, and the high polarity of a solvent is indicative of its potent solubilizing capacity for the majority of substrates to a degree (El Achkar et al., 2021), which can be evaluated by solvatochromic probes (Florindo et al., 2017). Additionally, given that lignin comprises the most influential fraction in lignocellulosic pretreatment, investigating the lignin solubility in the solvent is also key to solvent performance (Zhou et al., 2022). Therefore, correlative analyses of the pretreatment performance and solvent properties can be envisaged to elucidate the principles governing DES-mediated lignocellulose fractionation with MW, contributing to the rational design of MW-responsive DESs.

In this study, a series of DESs with varying solvent properties were synthesized for wheat straw pretreatment by altering the molar ratio. A relationship between solvent properties and fractionation performance under MW conditions was explored, focusing on the correlation between solvent properties (Kamlet-Taft and Hansen solubility parameters) and their effect on wheat straw fractionation. The study aimed to optimize the lignin removal, enzymatic digestibility, and microbial lipid synthesis from both enzymatic hydrolysate and the undetoxified DES waste stream. Life cycle assessment (LCA) and life cycle cost assessment (LCCA) were conducted to evaluate the environmental and economic impacts of the processes. This work provides valuable insights into the potential of MW-based DES pretreatment strategies, contributing to more sustainable lignocellulosic biorefineries.

2. Methodology

2.1. Materials

Wheat straw was supplied by Beijing Sola Biotechnology Co Ltd (China) with cellulose, hemicellulose, and lignin contents of 34.8%, 30.4%, and 23.5% and was dried to constant weight before use. Cellulase (Cellic® CTec2), available from Novozyme, exhibited a measured filter paper activity of 196 FPU/mL. As received, choline chloride (ChCl), oxalic acid (OA), ethylene glycol (EG), and other chemicals of analytical pure were applied.

2.2. Preparation of DES

The preparation of DES was carried out by magnetically stirring ChCl, OA, and EG in the designed ratio at 80 °C for 20 min to achieve transparency and homogeneity, and the resulting mixture was dehydrated overnight and stored in a desiccator (Ceaser et al., 2023).

2.3. MW-DES pretreatment

Wheat straw and DES were mixed and treated under controlled conditions, including the designed temperature, time, MW irradiation

power, and liquid-solid ratio. The resulting hybrid was then vacuum-filtered and washed to neutrality with anhydrous ethanol. The cellulose-rich residue was dried overnight at 60 °C and stored for subsequent enzymatic digestibility analysis. Ethanol in the filtrate was removed via rotary evaporation, and lignin was precipitated using deionized water (3 times the volume of the filtrate) as an anti-solvent. The regenerated lignin was then collected through centrifugation and freeze-drying. The supernatant obtained after centrifugation, containing DES waste and hemicellulosic hydrolysate, was prepared for microbial lipid synthesis (Wang et al., 2024c).

2.4. Statistical analysis

The principal impacts of time (A), temperature (B), and liquid-solid ratio (C) on solid recovery and lignin removal were explored by response surface methodology based on the Box-Behnke design (BBD) method with 3 factors and 3 levels. Statistical design and analyses were conducted using Design Expert 13®, which consisted a total of 17 trials with 5 replications at the central point, with a 95% confidence interval ($p < 0.05$) for analysis of variance (ANOVA).

2.5. Analysis methods

The solvent properties of the prepared DES were mainly accessed by Kamlet-Taft parameters and Hansen solubility parameters. The Kamlet-Taft parameters (π^* , α , and β) were determined by UV-vis spectrophotometry with Nile red and 4-nitroaniline; the detailed procedures have been previously reported by Liu et al. (2019). The Hansen solubility parameter (δ) classifies the cohesive force between solute and solvent into three components: dispersion, polarity, and hydrogen bonding. The solubility of lignin in DES was quantified by the relative energy difference (RED), and it was theorized that decreasing the RED increased the lignin solubility in DES. Specific calculations can be found in the work by Wang et al. (2025).

The constituent determination of the raw and pretreated wheat straw was conducted following the National Renewable Energy Laboratory (NREL) protocol (Sluiter et al., 2012). The morphological changes in pretreated wheat straw were characterized by scanning electron microscopy (SEM, ZEISS SIGMA300) as described by Ma et al. (2022).

2.6. Life cycle assessment

LCA provided a comparative analysis of the environmental impacts of two pretreatment methods for lignin removal from wheat straw: MW-DES (540 W, 130°C) for 150 s and CH-DES (60 °C) for 12 h. The functional unit chosen for this analysis was the removal of 1 g of lignin. The system boundary encompassed all processes from the cultivation and harvesting of wheat straw to the pretreatment stage (Supplementary Information, Fig. S1). The wheat straw dataset was sourced from the Ecoinvent database and represents the cultivation of wheat on 1 ha of land, producing both wheat grain and straw as co-products. The yield of wheat straw was 3,306 kg/ha at a moisture content of 15%. Additionally, this dataset included all machine operations along with the associated infrastructure and storage facilities. During the pretreatment process, the ethanol used for dilution and washing was recovered through rotary evaporation. The DES preparation involved mixing ChCl, OA, and EG at 80 °C for 20 min until a homogeneous state was achieved, which could be recycled 5 times. The production of 1 kg of DES required resource depletion of 1.61 kJ/kg and resulted in CO₂ emission of 0.57 kg/kg. Environmental impact categories assessed included global warming potential, resource depletion-fossil fuel, acidification, ecotoxicity, respiratory effects, photochemical ozone formation, and eutrophication. Data were collected from both experimental trials and literature to model the life cycle stages. All life cycle inventory data were processed using the OpenLCA software, and the TRACI impact assessment method was applied.

2.7. Enzymatic digestibility and lipid synthesis

The enzymatic digestibility of substrates (raw/pretreated wheat straw) was conducted based on previous literature (Wang et al., 2024c). Microbial lipid was synthesized from enzymatic hydrolysates of cellulose-rich residue and DES waste solution (containing hemicellulose hydrolysate) using

Trichosporon cutaneum ACCC 20119 (Agricultural Culture Collection of China). Detailed procedures could be found in a previous study by Di Fidio et al. (2021). A suitable quantity of cell suspension was inoculated to guarantee an initially uniform dry cell weight (DCW) of 1.7 g/L (Di Fidio et al., 2020).

3. Results and Discussion

3.1. Optimization of solvent properties and fractionation performance correlations under MW irradiation

The flexible tunability of DES allows for a range of solvent properties, which significantly influence the pretreatment efficiency of lignocellulose (Ji et al., 2020). Solvatochromic parameters represent a robust instrument for quantifying the physicochemical properties of solvents and are closely associated with solvent performance (e.g., fractionation effect, solubility) (Zhou et al., 2022). The Kamlet-Taft parameters have been commonly applied to quantify the solvent characteristics (Liu et al., 2019). Additionally, Hansen solubility parameters have been employed to evaluate lignin solubility (Na Wang et al., 2022), which quantify solubility properties by considering the solute-solvent interactions at the molecular level and dividing the cohesion energy into three components: dispersion, polarity, and hydrogen bonding (Hansen, 2007). Solvent (e.g., DES) is essential for conquering the intermolecular forces and facilitating solute (e.g., lignin) dissolution; hence, the solubility parameters of solute and solvent are therefore analogous, following the principle of "like seeks like" (Hansen, 2007). Here, a series of DESs with varying solvent properties were synthesized for wheat straw fractionation by altering the molar ratios, and Pearson correlation analysis of the solvent properties and fractionation performance of different DESs was performed.

The principal parameters of the MW-DES pretreatment process were optimized initially, as illustrated in Figure 1a-d. Elevating temperature (100–120 °C) increased the removal of hemicellulose (43.7%–87.4%) and lignin (57.6%–88.2%), while the cellulose recovery exhibited a significant decline (85.3%–35.7%) as the temperature continued to rise (120–160 °C). With the extension of time (30 s–180 s), hemicellulose and lignin removals increased from 41.1% to 91.7% and 49.2% to 91.8%, respectively. However, when the duration was extended from 90 s to 180 s, the cellulose recovery decreased from 85.3% to 74.0%. Excessive temperature and time can lead to cellulose degradation, which agrees with previous research (Wang et al., 2024b). With an increase in MW irradiation power from 360 W to 900 W, the lignin removal was increased from 78.4% to 91.6%, but cellulose recovery decreased (85.3%–75.6%) with the MW irradiation power rose from 540 W to 900 W. This result is consistent with previous studies that increasing MW irradiation power aggravates cellulose degradation (Fan et al., 2023). With increasing the liquid-solid ratio from 10 to 20, the removal of hemicellulose (61.7%–87.4%) and lignin (75.1%–88.2%) was improved accordingly. Cellulose recovery decreased from 85.3% to 79.8% as the liquid-solid ratio further increased to 30. A low liquid-solid ratio would result in incomplete saturation of wheat straw by DES, resulting in insufficient solubilization and lignin/hemicellulose removal, while a high liquid-solid ratio might lead to over-degradation of cellulose (Xu et al., 2020).

The fractionation performance of DESs with different solvent properties was evaluated under the aforementioned optimized conditions, namely 120 °C for 90 s at MW irradiation power of 540 W with a liquid-solid ratio of 20 (Fig. 1e). The DES (ChCl: OA: EG) with a molar ratio of 1:2:2 could produce superior hemicellulose and lignin removal while preserving cellulose for wheat straw pretreatment. The alteration of molar ratios modulated DES solvent properties and subsequently affected the fractionation performance (Supplementary Information, Table S3), agreeing with the previous literature (Okur and Eslek Koyuncu, 2020). In several previous CH-DES pretreatments, the α and β parameters of DES showed a stronger correlation with lignin removal compared to π^* (Liu et al., 2019; Hong et al., 2020). Additionally, solvents with Hansen solubility parameters similar to those of lignin have been found to enhance lignin removal (Mohan et al., 2022; Na Wang et al., 2022). With the MW-DES methods, lignin removal correlated positively ($p < 0.01$) with π^* , α , and α - β and was negatively correlated ($p < 0.01$) with β of DES (Fig. 1f). The π^* offers a quantitative measurement of the intermolecular interactions in

solvent-solute and stabilization charges/dipoles by dielectric effects, providing an evaluation of the solvent's polarity/polarizability (Fernandes et al., 2023). The direct interaction of MW with polar molecules in DES causes rapid molecular rotation, transferring energy to the solvent (Zou et al., 2024). This enhances the contribution of π^* to delignification in MW-DES, in contrast to the stronger influence of α and β on lignin removal in CH-DES pretreatment.

The parameter α is linked to solvent acidity and its ability to donate protons (Fernandes et al., 2023). The strong positive correlation between α and lignin removal suggests that proton-catalyzed bond cleavage, which is prominent in CH-DES pretreatment, also plays a key role in the MW-DES process. This involves proton-driven catalytic breakdown of ether/ester bonds in lignin-carbohydrate complexes and glycosidic bonds in carbohydrates, leading to the simultaneous extraction of hemicellulose and lignin (Yu et al., 2022). The elevated α - β value of DES signifies its considerable net hydrogen bond donor capacity, thereby facilitating robust hydrogen bond formation with lignin and consequently extracting lignin. This finding follows the observed positive correlation between α - β and lignin dissolvability in CH-DES (Zhou et al., 2022). Figure 1f illustrates a notable positive correlation between hemicellulose and lignin removal; however, both hemicellulose and lignin removal are negatively correlated with solid recovery. It is suggested that both hemicellulose and lignin are removed concurrently during MW-DES pretreatment, thereby reducing solid recovery. These results reconfirm the proton-catalyzed bond-breaking role in the MW-DES process.

The Hansen solubility parameters of DESs (ChCl/OA/EG) ($\delta = 33.61$ – 35.63) closely resemble those of lignin ($\delta = 31.05$), and the low relative energy difference ($RED \leq 0.50$) suggests that DESs may have potential for lignin extraction. This hypothesis was further supported by the results, which showed lignin removal ranging from 73.0% to 95.7%. However, the Hansen solubility parameters of DESs did not show a correlation with wheat straw fractionation under MW irradiation (Fig. 1f). MW enhances the vibrational movement of dipoles in DESs, promoting the rearrangement of polar molecules within the radiation field and disrupting intermolecular forces. This results in a weakened positive correlation between Hansen solubility parameters and lignin removal (Haldar and Purkait, 2021). These findings clarify the relationship between solvent properties and fractionation performance in MW-DES pretreatment. Specifically, DESs with relatively high π^* and α are effective for fractionation under MW conditions.

3.2. Optimization of MW-DES pretreatment through response surface methodology

For satisfactory solid recovery and delignification, 17 experiments were conducted based on BBD, considering reaction time, temperature, and solid-liquid ratio as factors to optimize the MW-DES process (Table 1). The ANOVA can evaluate the significance of the quadratic regression model and its terms (A, B, and C). The p-value less than 0.05 indicates a statistical significance. The coefficient of determination (R^2) value means the fitness of the model to experimental data, with a value closer to 1 signifying a superior fit. The ANOVA for solid recovery (Table S4) showed that the model was statistically significant ($F = 55.05$, $p < 0.0001$) with a non-significant lack of fit ($p = 0.8094$). The R^2 value was 0.9861, and the difference between the adjusted R^2 (0.9682) and predicted R^2 (0.9389) was within 0.2. Similarly, the ANOVA for lignin removal (Table S5) indicated statistical significance ($F = 55.11$, $p < 0.0001$) for the model, with a non-significant lack of fit ($p = 0.1096$). The R^2 value was 0.9861, and the difference between the adjusted R^2 (0.9682) and predicted R^2 (0.8282) was less than 0.2. The model-predicted solid recovery and lignin removal showed a nearly linear correlation with the actual results (Supplementary Information, Figs. S2a-b), demonstrating that the quadratic models were fitted with high accuracy and had satisfactory predictive ability. The second-order polynomial models presented below were derived through multivariate regression analysis of the experimental data set (Eqs. 1 and 2).

$$\begin{aligned} \text{Solid recovery (\%)} = & 319.3690 - 1.3221A - 2.2481B - 2.0947C \\ & + 0.0033AB + 0.0022AC + 0.0177BC \\ & + 0.0028A^2 + 0.0045B^2 - 0.0190C^2 \end{aligned} \quad \text{Eq. 1}$$

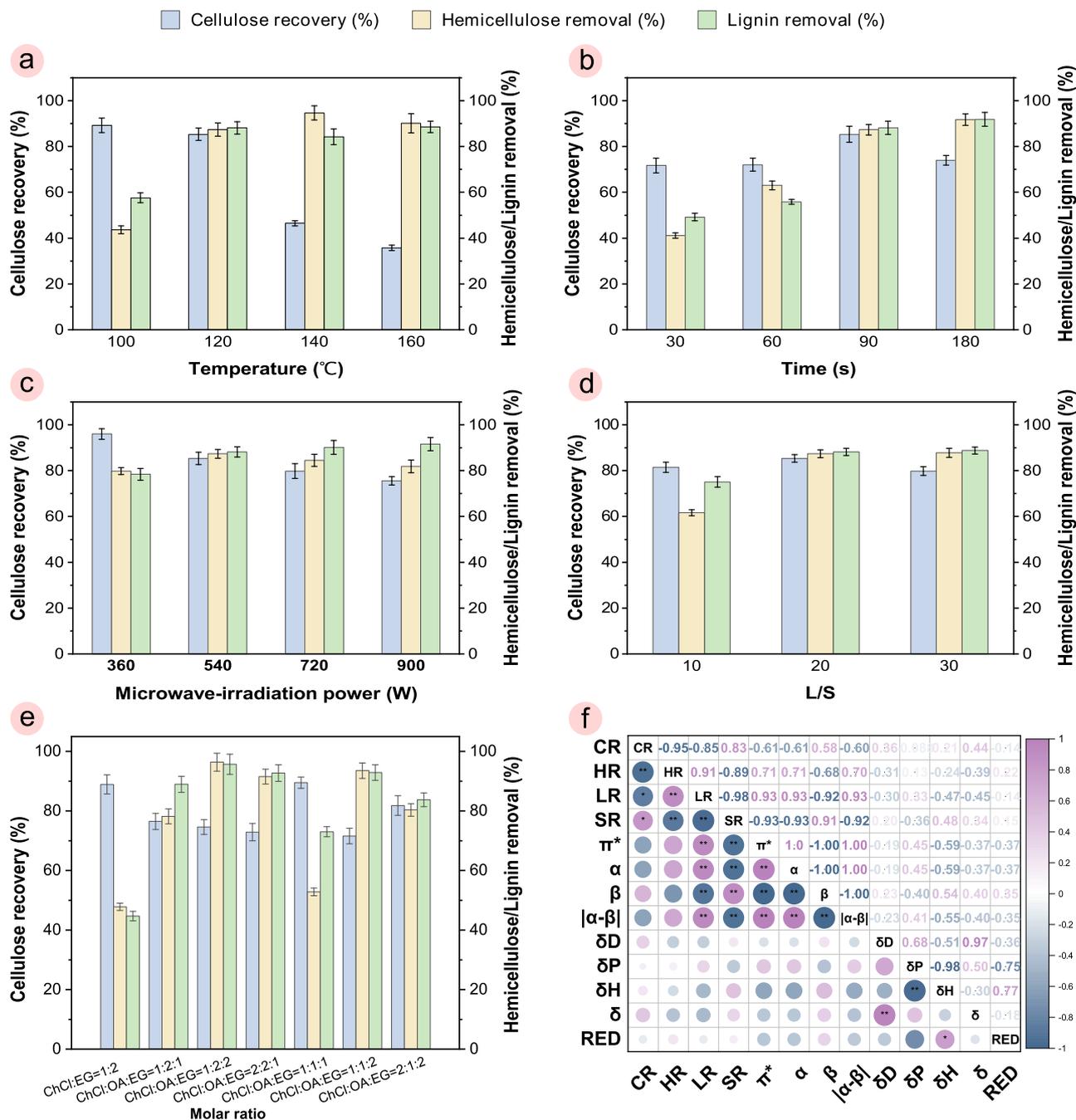


Fig. 1. Cellulose recovery, hemicellulose, and lignin removal for MW-DES (ChCl/OA/EG) pretreatment under different (a) temperatures, (b) times, (c) MW irradiation powers, (d) liquid-solid ratios, and (e) molar ratios; (f) Pearson correlation heatmap of solvent properties of DES with cellulose recovery (CR), hemicellulose removal (HR), lignin removal (LR) and solid recovery (SR) under MW-DES pretreatment (* $p \leq 0.05$, ** $p \leq 0.01$) (Kamlet-Taft parameters: polarity/polarizability (π^*), hydrogen-bond-donating ability (α), and hydrogen-bond-accepting ability (β); Hansen solubility parameters: solubility parameter (δ), dispersion (δ_D), polar (δ_P), hydrogen bonding (δ_H) parameters, and relative energy difference (RED)).

$$\begin{aligned} \text{Lignin removal (\%)} = & -569.5110 + 0.9781A + 9.2278B + 1.0041C \\ & - 0.0007AB + 0.0040AC + 0.0061BC \\ & - 0.0031A^2 - 0.0365B^2 - 0.06492C^2 \end{aligned} \quad \text{Eq. 2}$$

where, A, B, and C represent the time, temperature, and liquid-solid ratio, respectively.

The ANOVA results (Tables S4 and S5) and 3D surface plots (Fig. 2) demonstrate that linear (A, B, and C) and interaction/quadratic (AB, BC, and A²) model terms were statistically significant ($p < 0.05$) for solid recovery, and that linear (A, B, and C) and quadratic (A², B², and C²) were significantly modeled terms for lignin removal ($p < 0.05$). From the F-values, time was found to be the most significant for solid recovery and lignin removal, followed by temperature, and liquid-solid ratio. The result

Table 1.
The Box-Behnke design for modeling solid recovery and lignin removal rates.

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2
	A: Time (s)	B: Temperature (°C)	C: Liquid-Solid ratio (w/w)	Solid Recovery (%)	Lignin Removal (%)
1	180	100	20	45.4	76.1
2	120	120	20	40.8	96.0
3	60	120	30	55.3	56.8
4	120	140	10	34.2	85.4
5	60	140	20	54.2	62.1
6	60	100	20	79.3	42.2
7	120	120	20	41.1	92.6
8	120	120	20	44.1	95.6
9	120	100	10	58.4	65.5
10	120	100	30	42.0	57.7
11	120	120	20	38.1	93.9
12	60	120	10	67.1	71.2
13	180	120	10	42.2	91.1
14	180	120	30	35.7	86.3
15	180	140	20	36.1	92.6
16	120	120	20	44.7	91.3
17	120	140	30	32.0	82.5

from the perturbation plots (Supplementary Information, Fig. S2c-d) aligned with the F-value analysis, showing that factors with steeper slopes had a greater impact on solid recovery and lignin removal than those with flatter slopes. Specifically, time, temperature, and liquid-solid ratio exhibited high to moderate significance in influencing these outcomes. Optimal conditions were predicted by BBD for MW-DES pretreatment toward superior lignin removal, with predicted and experimental values of 33.6% and 34.5% for solid recovery, and 96.0% and 96.1% for lignin removal under 130 °C, 150 s, and 25 (liquid-solid ratio). The validity of the regression model is further demonstrated.

According to the aforementioned analysis, the ideal pretreatment parameters for MW-DES were 130 °C for 150 s and a liquid-solid ratio of 25. Under these conditions, the solid recovery was recorded at 34.5%, while the lignin removal reached 96.1%.

3.3. Reinforcement effect of microwave

To demonstrate the reinforcing effect of the MW-assisted approach for lignocellulose pretreatment—specifically, the reduction of reaction severity (by lowering temperature and/or shortening reaction time)—the process severity was quantified using a severity factor (R_0) (Eq. 3) (Wang et al., 2024c).

$$R_0 = t \times e^{\left(\frac{T-100}{14.75}\right)} \quad \text{Eq. 3}$$

where t and T are the reaction time (min) and the temperature (°C), respectively. An elevated R_0 value is indicative of more stringent conditions. In the majority of previous CH-DES pretreatment methods, high delignification with harsh reaction conditions or low delignification with

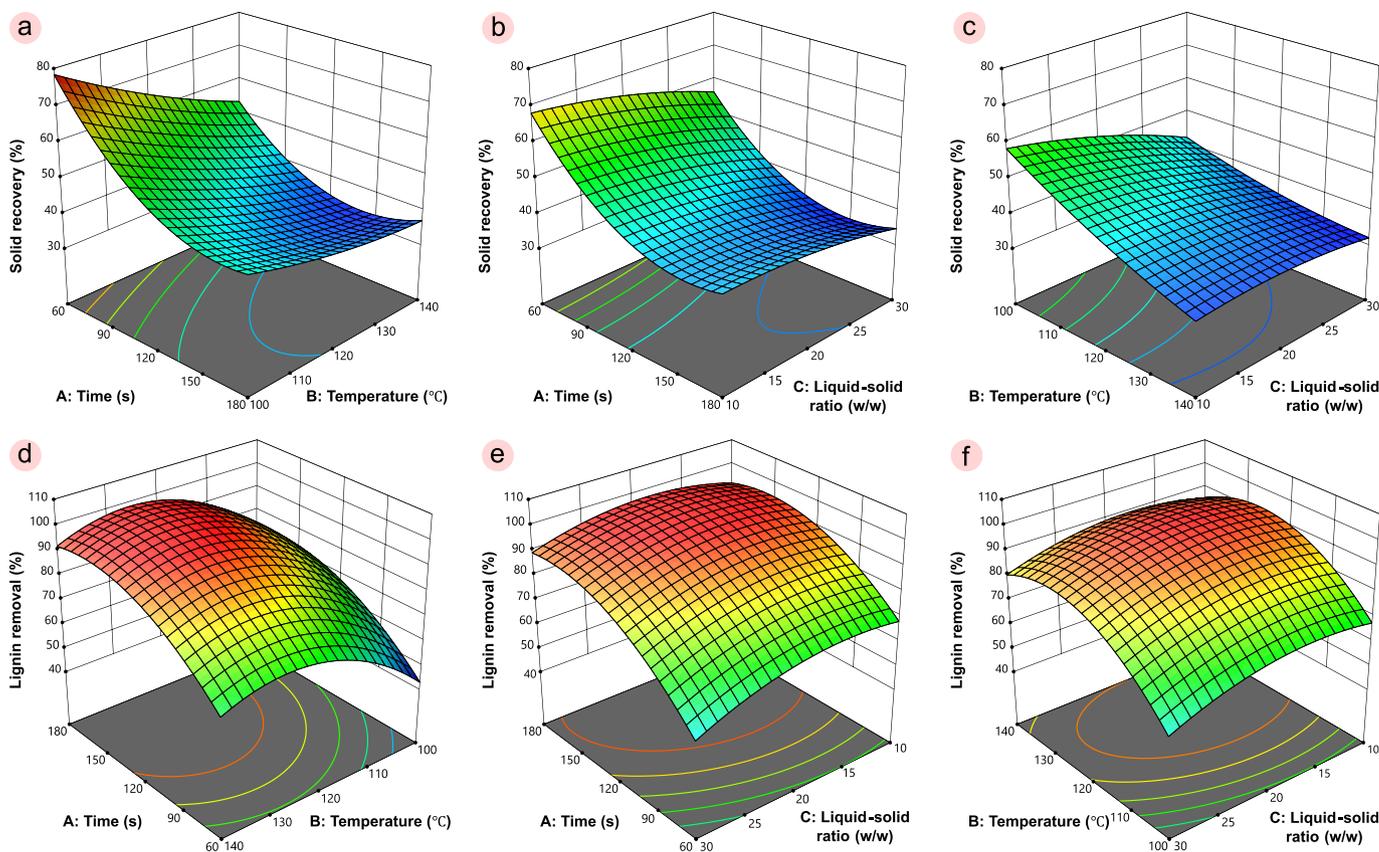


Fig. 2. Response surface plots of solid recovery with (a) time vs. temperature, (b) time vs. liquid-solid ratio, (c) temperature vs. liquid-solid ratio; lignin removal with (d) time vs. temperature, (e) time vs. liquid-solid ratio, and (f) temperature vs. liquid-solid ratio.

moderate conditions is demonstrated (Fig. 3). For MW-DES pretreatment, however, a notable reduction in R_0 was observed, accompanied by exceptional lignin removal. This study presented a comparison of the pretreatment effects (lignin removal and R_0) of MW-DES (130 °C, 150 s) and CH-DES (60 °C, 12 h) pretreatment processes using the same DES (ChCl: OA: EG = 1:2:2) and substrate (wheat straw), along with previous research on MW-DES and CH-DES pretreatment (Fig. 3). Compared with CH-DES, MW-DES exhibited superior delignification (96.1% vs. 32.7%), hemicellulose removal (93.0% vs. 31.5%), higher cellulose content (76.3% vs. 40.2%), and lower R_0 (1.9×10^{-3} vs. 4.8×10^{-3}), illustrating that MW facilitated the lignocellulose fractionation more effectively. A comparison of MW-DES pretreatment with other emerging green pretreatment technologies reveals notable advantages in terms of time (150 s vs. 35 min to 96.25 h) and lignin removal (96.1% vs. no change to 79.1%) (Table S6).

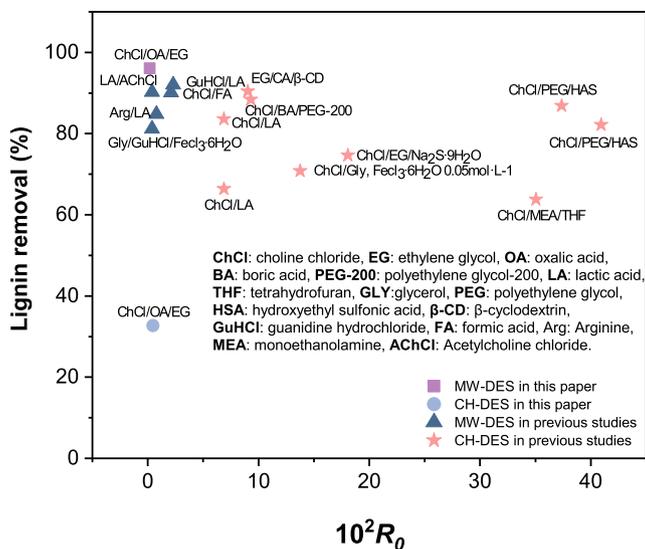


Fig. 3. Comparison of lignin removal and severity factor (R_0) between the present work and previous related studies (Ci et al., 2020; Lin et al., 2020; Su et al., 2021; Huang et al., 2022; Shen et al., 2022; Zhong et al., 2022; Ceaser et al., 2023; Raikwar et al., 2023; Tan et al., 2023; Xie et al., 2023; Liu et al., 2023b; Yang et al., 2024).

Exploring the mechanistic insights of MW-DES pretreatment revealed that the strong response of DES to MW reinforced its fractionation effect on lignocellulose (Fig. 4). CH methods rely on convective and conductive heat transfer to the surface of the target material, followed by non-selective heating of the interior, making the process time-consuming and energy-intensive (Haldar and Purkait, 2021). MW heating is volumetric and energy-efficient, relying on dipole polarization and ionic conduction mechanisms (Sun et al., 2023; Zou et al., 2024). Polar groups (e.g., hydroxyl groups) in lignocellulose are considered MW-responsive, and the MW energy causes these groups to vibrate intensely, generating hot spots that induce localized structural rupture (Aguilar-Reynosa et al., 2017). This is evidenced by the loss of cellulose and the easier removal of hemicellulose, as shown in Figure 1.

Therefore, the strong interaction between MW and lignocellulose can be a partial explanation for the intensification of the pretreatment effect by MW. The π^* (polarity/polarizability) of DES is significantly and positively correlated with the delignification in the MW-DES process. With the high-frequency electric field (MW component), the polarized DES molecules begin to rotate rapidly in response to the swift alteration in field direction, resulting in molecular motion, which leads to collisions, friction, and heat generation (Zou et al., 2024). Volumetric heating enables the internal generation of heat in the substrate, which provides uniform mass and energy transfer direction throughout the substrate, thus significantly increasing pretreatment efficiency and reducing pretreatment time (Mao et al., 2023).

Highly dipolar DES leads to more dielectric losses and, therefore, more energy generation, which can improve the pretreatment efficiency (Aguilar-Reynosa et al., 2017).

The significant positive correlation observed between α and α - β with lignin removal suggests that a proton-catalyzed bond-breaking mechanism is at play in the MW-DES process. By optimizing the ionic characteristics and polarity of DES, MW enables the polarized/dissociated DES ions/molecules to function as highly effective "spinning cutters", thereby enhancing the capacity to deconstruct lignocellulose (Duan et al., 2023). By keeping the substrate and reaction conditions constant, the delignification rate was significantly improved from 73.0% to 95.7% by adjusting the molar ratio of DES (ChCl: OA: EG) from 1:1:1 to 1:2:2. This indicates that the interaction with DES is the key factor influencing the performance of MW-enhanced fractionation. The synergistic mechanisms lead to ultrafast lignin removal from MW-DES pretreated lignocellulose, which may facilitate subsequent enzymatic hydrolysis and value-added utilization. González-Rivera et al. (2021) suggested that the polarizability of DES is linked to its MW absorption properties, with DESs exhibiting high polarizability, showing superior performance in polyphenol recovery and delignification of chestnut shell waste. Similarly, Wang et al. (2024c) proposed a synergistic interaction between MW and DES, which facilitates the effective deconstruction of lignocellulose, achieving a lignin removal rate of 89.99%.

The methodology outlined by Wang et al. (2013) was employed to make a rough comparison of the energy (Q) demands of two pretreatment methods for lignin removal from wheat straw: MW-DES (540 W, 130 °C) for 150 s and CH-DES (60 °C) for 12 h. The energy required for the MW-DES approach was calculated as follows: $Q_{MW} = 540 \text{ (power, W)} \times 150 \text{ (time, s)} = 8.1 \times 10^4 \text{ J}$. For the CH-DES pathway, a magnetic stirring heater with a power-to-temperature ratio of 1.6 (550 W/340 °C) was employed for energy calculation: $Q_{CH} = 60 \times (550/340) \text{ (power, W)} \times 43200 \text{ (time, s)} = 4.2 \times 10^6 \text{ J}$. The MW-DES pathway required only 1.9% of the energy needed for CH-DES, demonstrating a significant improvement in energy efficiency compared to CH-DES methods.

3.4. Life cycle assessment

Despite the benefits of DES in lignocellulosic pretreatment, little is known about its environmental impacts (Hessel et al., 2022). Life cycle assessment (LCA) is a systematic and powerful methodology for quantifying the environmental impacts of technological processes (Gheewala, 2023; Song et al., 2023). To the best of our knowledge, no LCA studies have been conducted on MW-DES. Evaluating whether combining MW technology with DES can result in a more sustainable biorefinery process through LCA is particularly important, as DES pretreatment is still in its emerging phase.

The LCA demonstrated that MW-assisted technology presented a notable environmental benefit compared to CH approaches in lignocellulose pretreatment for lignin removal (Fig. 5, Table 2). In comparison to MW-DES, the CH-DES approach exhibited a 2.7-fold higher global warming potential, emphasizing the energy-intensive nature of prolonged heating and its corresponding contribution to greenhouse gas emissions, making the CH-DES process unfavorable in terms of its carbon footprint. In terms of resource depletion-fossil fuel, the MW-DES showed a considerable reduction, with a resource depletion indicator of only 36.9% of the CH method. In brief, the MW-DES pathway was energy-efficient and also reduced the consumption of non-renewable resources, contributing to technology feasibility and sustainability. The MW-DES method was also found to yield favorable outcomes concerning other impact categories, namely acidification, eutrophication, and ecotoxicity. This suggested that the MW pathway exerted less environmental stress, presumably due to reduced emissions and by-products in an ultrafast and efficient process. The lower impacts of the MW-DES method on acidification and eutrophication suggest that the reduction of SO_2 and nitrogen oxide (NO_x) emissions—major contributors to these environmental issues—was a significant benefit of the method. Overall, the substantial reductions in environmental impacts render the MW-assisted pathway an attractive alternative, both from an environmental and industrial perspective.

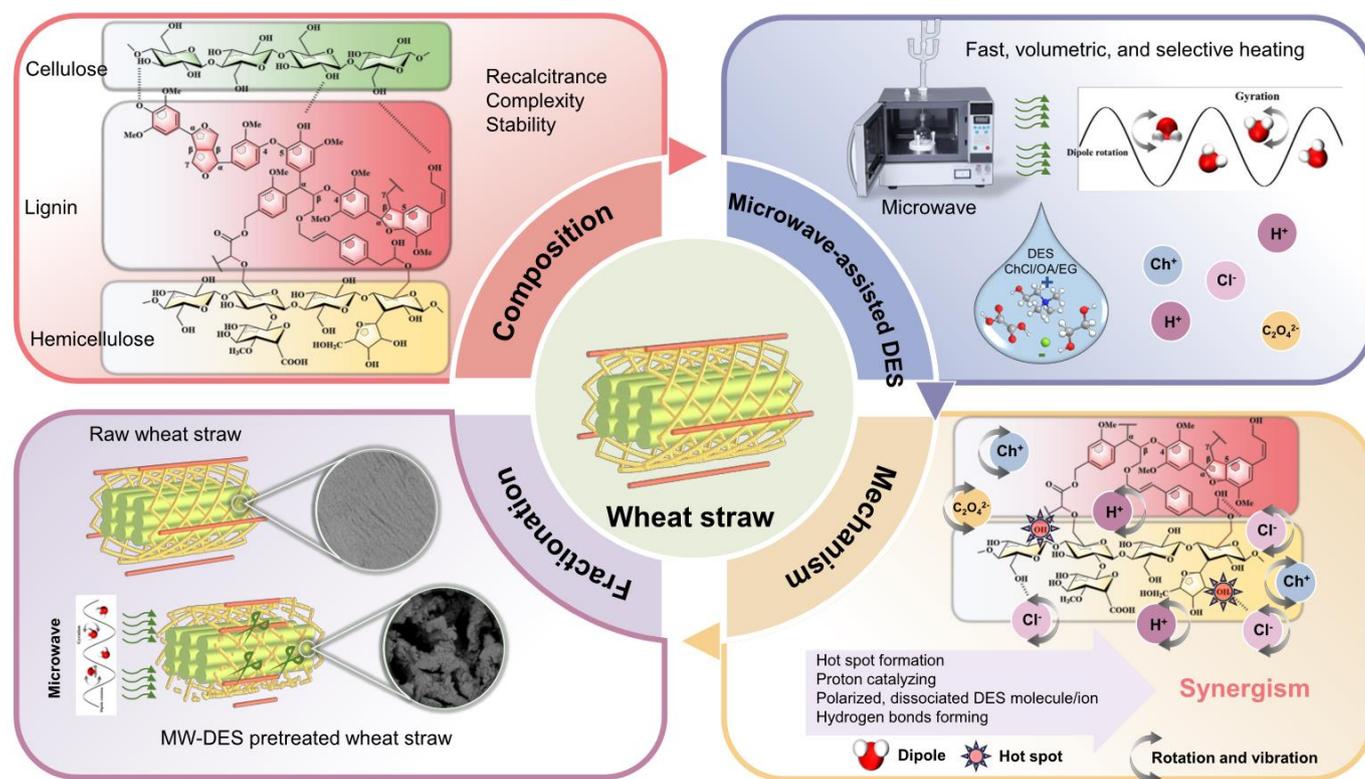


Fig. 4. The reinforcement mechanism of the developed MW-DES (ChCl/OA/EG) pretreatment strategy.

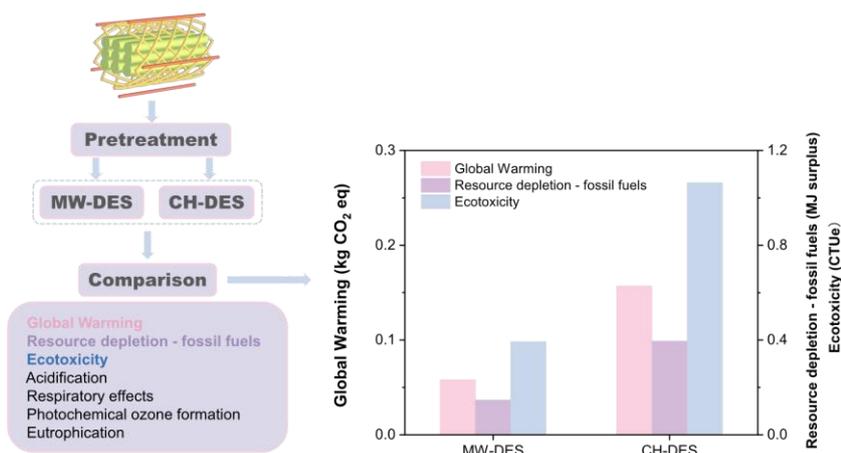


Fig. 5. Life cycle assessment of MW-DES and CH-DES pretreatment methods.

3.5 Enzymatic hydrolysis and microbial lipid synthesis

3.5.1. Enzymatic hydrolysis

Raw wheat straw and cellulose-rich residues obtained by MW-DES and CH-DES pretreatment were subjected to enzymatic digestibility for pretreatment efficiency evaluation (Ji et al., 2020). After a 48-h incubation period, the enzymatic hydrolysis of raw wheat straw was only 17.9% (Fig. 6a). This is attributed to the inherent toughness of raw wheat straw, which has a dense and rigid morphology (Fig. 6b), hindering cellulase

accessibility, consistent with findings from a previous study (Tan et al., 2023). The modest improvement in enzymatic digestibility of CH-DES-treated wheat straw, amounting to 46.0%, can be ascribed to the inadequate removal of lignin (32.7%) and hemicellulose (31.5%). This inadequacy leads to diminished cellulase accessibility and unproductive adhesion of lignin to cellulase, which in turn renders the saccharification enhancement ineffective (Cai et al., 2023). The MW-DES pretreated wheat straw exhibited an enzyme digestibility of 92.4%, which was 5.2 times higher than that of the raw wheat straw (17.9%) and 2 times higher than that of the CH-DES pretreated substrate (46.0%).

Table 2. Environmental impacts for removal of 1 g lignin by MW-DES and CH-DES pretreatment methods.

Impacts	Unit	MW-DES	CH-DES
Global Warming potential	kg CO ₂ eq	0.0580	0.1570
Resource depletion - fossil fuels	MJ surplus	0.1462	0.3959
Acidification	kg SO ₂ eq	2.6253×10 ⁻⁴	7.1067×10 ⁻⁴
Ecotoxicity	CTUe	0.3931	1.0642
Respiratory effects	kg PM 2.5 eq	4.4888×10 ⁻⁵	1.2151×10 ⁻⁴
Photochemical ozone formation	kg O ₃ eq	0.0031	0.0084
Eutrophication	kg N eq	1.6391×10 ⁻⁴	4.4371×10 ⁻⁴

This can be attributed to the significant lignin removal (96.1%) achieved by MW-DES, which alleviated the hindrance to cellulase. Additionally, the incorporation of EG stabilized the lignin structure and reduced the deposition of condensed lignin on the cellulose surface, resulting in

outstanding enzymatic efficacy (Liu et al., 2021; Yuan et al., 2024). Furthermore, the high solubility of this DES with lignin (RED = 0.5) facilitated the removal of most lignin while minimizing its redeposition on the cellulose-rich residue surface, further enhancing enzymatic digestibility, consistent with previous studies (Mohan et al., 2022; Yang et al., 2024).

The morphological changes in lignocellulose by MW-DES and CH-DES pretreatment were investigated using SEM. As illustrated in Figure 6b-e, the pretreatment induced variations in surface morphology. The raw wheat straw exhibited a smooth surface and a dense structure (Fig. 6b). Following CH-DES, a sparsely fractured surface structure was observed (Fig. 6c). However, a substantial portion of the lignocellulose remained visibly intact, also elucidating the low enzymatic digestibility of raw and CH-DES-treated wheat straw. The MW-DES treatment significantly disrupted the lignocellulose structure (Figs. 6d-e), with a considerable amount of lignin and hemicellulose removed. This enhances cellulose accessibility, thereby facilitating enzymatic saccharification, a result consistent with enzymatic digestibility.

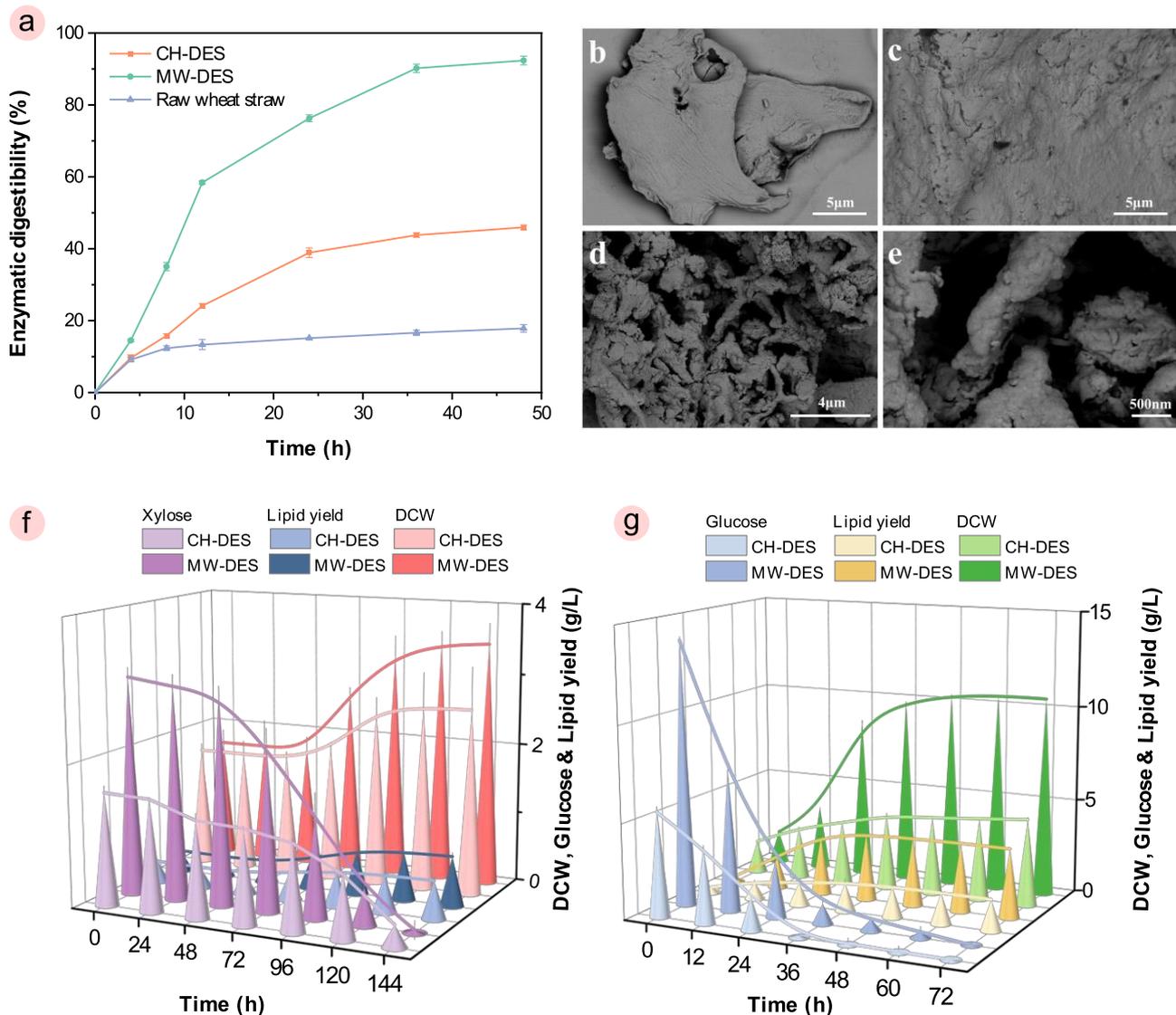


Fig. 6. (a) Enzymatic digestibility of raw and pretreated wheat straw; Scanning electron microscopy (SEM) images of (b) raw wheat straw, (c) CH-DES pretreated wheat straw, and (d and e) MW-DES pretreated wheat straw. Plots of glucose, xylose, lipid concentration, and dry cell weight (DCW) during fermentation with *T. cutaneum* for different (f) hemicellulose hydrolysates, and (g) cellulose-rich residue obtained by MW-DES, CH-DES pretreatment methods.

3.5.2. Microbial lipid synthesis

T. cutaneum is regarded as an ideal strain to produce microbial lipid from lignocellulose, owing to its capacity to consume both hexoses and pentoses for efficient lipid accumulation and its notable adaptability to inhibitors such as furans and aromatic compounds in lignocellulosic hydrolysates (Hu et al., 2011; Zhao et al., 2018). The process of lipid synthesis can be categorized into four distinct stages (Fig. S3): the initial synthesis of acetyl CoA, the subsequent fatty acid synthesis, the extension and desaturation of fatty acid carbon chains, and the final synthesis of triglycerides. The synthesis of lipids composed of 80-90% triglycerides is achieved through the utilization of multiple substrate carbon sources (Wang et al., 2016; Ji et al., 2024).

Hemicellulose, a heteropolysaccharide, is readily hydrolyzed to oligosaccharides, monosaccharides, and their degradation byproducts through chemical processes (Rao et al., 2023). Moreover, the complexity of hemicellulosic hydrolysates arising from the dissolution of hydrophilic lignin and/or its derived products during pretreatment renders them inaccessible to microorganisms or necessitates detoxification procedures for further biotransformation (Amiri and Karimi, 2018). The utilization of the non-detoxified hemicellulose fraction has the potential to enhance the industrial feasibility of biofuels. Here, the superiority of MW-DES technology permitted the utilization of the DES waste stream, rich in hemicellulose hydrolysates, as carbon sources directly for lipid synthesis by *T. cutaneum* (Fig. 6f). Owing to the complication of hemicellulose hydrolysate, the microorganisms had a noticeable lag phase, with no significant growth during the initial 72 h, consistent with earlier literature (Di Fidio et al., 2021). After 144 h of fermentation, *T. cutaneum* obtained net dry cell weight (DCW) of 1.8 g/L and 0.9 g/L and net lipid concentrations of 0.4 g/L and 0.2 g/L from MW-DES and CH-DES pretreated hemicellulose hydrolysates, respectively. MW-DES pretreatment facilitated hemicellulose biodegradation to form more xylose, while lower pretreatment severity might have also diminished the formation of inhibitors (Zhang et al., 2023). Hence, the effective application of *T. cutaneum* for the synthesis of microbial lipids from the DES waste stream of hemicellulose-rich hydrolysate as a carbon source is demonstrated. Notably, the hemicellulose hydrolysate-rich DES waste liquid used as substrate did not require detoxification, which simplified the fermentation process.

T. cutaneum can also utilize enzymatic hydrolysates of cellulose-rich residue as carbon sources for lipid production. The enzymatic hydrolysate, primarily glucose with minimal inhibitors due to moderate cellulase-mediated hydrolysis, enabled immediate glucose metabolism and yeast growth without a significant lag phase. The lipid concentration paralleled cell growth (Fig. 6g), contrasting with the use of hemicellulose hydrolysate for lipid synthesis. After 48 h of fermentation, glucose concentration decreased from 13.9 g/L to 0.6 g/L, indicating that selective enzymatic hydrolysis produced high-quality hydrolysates, with glucose serving as a superior carbon source (Masri et al., 2019). The net DCW and lipid concentrations for MW-DES pretreated wheat straw were 8.8 g/L and 3.3 g/L, respectively, significantly higher than those from CH-DES pretreated wheat straw (2.9 g/L and 1.3 g/L). This is mainly due to the enhanced cellulose enrichment in MW-DES compared to CH-DES pretreatment (96.1% vs. 32.7% lignin removal and 93.0% vs. 31.5% hemicellulose removal), which facilitates the thorough release of fermentable sugars from cellulose. In summary, the efficient delignification achieved by MW-DES has significantly improved the feasibility of converting lignocellulose into microbial lipids.

3.6. Scalability and life cycle cost assessment (LCCA)

Lab-scale MW reactors typically handle 1-10 g of feedstock and 1-100 mL of solvent, necessitating the scale-up capacity for commercial manufacturing (Goswami et al., 2022). Presently, two categories of MW reactors exist: multi- and mono-mode. Multi-mode reactors are commonly applied because of their ease of operation, simplicity of construction, and large reaction cavity, while the reproducibility of heating inhomogeneity has been questioned (Aguilar-Reynosa et al., 2017). Mono-mode reactors utilizing waveguides achieve notable reaction outcomes due to their uniform and reproducible heating characteristics. However, due to the limitations imposed by the waveguide geometry, mono-mode reactors have a constrained processing capacity (Wang et al., 2022b). Continuous reactors

enable the continuous pumping of the reaction mixture while being heated by spirals in multi-, mono-mode reaction reactor cavities, thereby providing a scalable alternative for industrial applications in MW technology (Ocreto et al., 2021). The expansion of production capacity may present challenges related to penetration depth, precise temperature monitoring and controlling, and proper reactor construction (Khan and Rathod, 2018). From an industrial perspective, scaling up MW technology does not require large vessels; instead, it enables industrial-scale productivity through continuous or batch reactors operating at maximum throughput capacity (Ocreto et al., 2021). Xu et al. (2023) designed a continuous MW bioreactor for biodiesel production using a leaky waveguide, ensuring uniform MW energy distribution both axially and longitudinally. This design improved thermal consistency and energy efficiency, achieving an MW efficiency of 95.39% at an initial flow rate of 30 mL/s (Xu et al., 2023). A pilot-scale continuous MW reactor was developed for lignocellulose pretreatment, exhibiting a productivity of 0.28 kg/h and an ethanol yield of 31.29 g/100 g corn stover (Peng et al., 2014). A pilot-scale MW fractionation system (1000 L) was recently developed for bamboo pretreatment, achieving lignin and hemicellulose removal rates of 93.2% and 84.7%, respectively, while retaining 89.1% of the cellulose, marking a significant advancement in MW reactor technology (Zhang et al., 2022). These advancements provide a strong foundation for the industrial implementation of MW processes to facilitate the value-added utilization of lignocellulose. Additionally, enhanced oversight of MW leakage, particularly in high-power equipment, is critical to ensure environmental safety and operator well-being (Zou et al., 2024). Proper cavity structure, optimized electromagnetic field distribution, and continuous process design are essential to prevent microwave leakage (Wang et al., 2024a). The use of numerical simulations to visualize electromagnetic waves serves as a valuable tool in guiding equipment design and ensuring safe operation (Zhang et al., 2021).

Life cycle cost assessment (LCCA) is the evaluation of the financial impact associated with a procedure or product throughout its life cycle, covering raw materials, manufacturing approaches, and recyclability (Das et al., 2024). The viability of lignocellulosic biorefineries from an industrial and commercial standpoint is contingent on the productivity and cost-effectiveness of pretreatment (Chen et al., 2023). A brief LCCA was conducted, from wheat straw to microbial lipid synthesis (Tables S7 and S8), to evaluate the financial feasibility of the MW-DES pretreatment strategy. It was estimated that the cost of pretreatment methods involving MW-DES and CH-DES from wheat straw to microbial lipids would be USD1.077 and USD1.925, respectively, based on 100 g of wheat straw. Although the present economic evaluation may not precisely correspond to actual industrial production, it provides an approximate cost that paves the way for the industrialization of MW-DES application for lignocellulosic biorefinery.

On the whole, the MW-DES process was found technically, environmentally, and economically superior. Briefly, the MW-DES approach could obtain 42.0% higher microbial lipids (8.8 g vs. 5.1 g) than CH-DES for 100 g wheat straw, reduced the energy consumption by 98.1% (8.1×10^4 J vs. 4.2×10^6 J), and decreased the cost by 44.1% (USD1.077 vs. USD1.925).

4. Conclusions

In summary, the reinforcement mechanism of MW-DES pretreatment was explored by constructing relationships between solvent properties and fractionation performance, providing a foundation for sustainable and eco-friendly pretreatment processes. The proactive contributions of π^* and α in DES were key to the ultrafast and efficient lignin removal by MW-DES under mild conditions. The enhanced responsiveness of DES with high π^* and α to MW pretreatment was demonstrated by comparing the severity factor (R_0) and delignification rates with CH pretreatment. Thanks to this MW enhancement, 96.1% lignin removal was achieved at a relatively low R_0 (1.9×10^{-3}), along with 92.4% enzymatic digestibility and 8.8 g microbial lipid/100 g wheat straw. The LCA and LCCA revealed that MW-DES resulted in a 63.1% reduction in environmental impacts and a 44.1% cost reduction compared to CH-DES pretreatment, offering significant environmental and economic benefits. A detailed analysis of solvent properties and fractionation performance correlations under MW irradiation

provided novel insights for designing efficient and sustainable pretreatment processes.

Acknowledgments

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References

- [1] Aguilar-Reynosa, A., Román, A., Ma, Rodríguez-Jasso, R., Aguilar, C.N., Garrote, G., Ruiz, H.A., 2017. Microwave heating processing as alternative of pretreatment in second-generation biorefinery: an overview. *Energy Convers. Manage.* 136, 50-65.
- [2] Amiri, H., Karimi, K., 2018. Pretreatment and hydrolysis of lignocellulosic wastes for butanol production: challenges and perspectives. *Bioresour. Technol.* 270, 702-721.
- [3] Azzouz, A., Hayyan, M., 2023. Are deep eutectic solvents biodegradable?. *Process Saf. Environ. Prot.* 176, 1021-1025.
- [4] Basak, B., Kumar, R., Bharadwaj, A.V.S.L.S., Kim, T.H., Kim, J.R., Jang, M., Oh, S.E., Roh, H.S., Jeon, B.H., 2023. Advances in physicochemical pretreatment strategies for lignocellulose biomass and their effectiveness in bioconversion for biofuel production. *Bioresour. Technol.* 369, 128413.
- [5] Cai, C., Zhang, C., Li, N., Liu, H., Xie, J., Lou, H., Pan, X., Zhu, J.Y., Wang, F., 2023. Changing the role of lignin in enzymatic hydrolysis for a sustainable and efficient sugar platform. *Renew. Sust. Energy Rev.* 183, 113445.
- [6] Ceaser, R., Rosa, S., Montané, D., Constantí, M., Medina, F., 2023. Optimization of softwood pretreatment by microwave-assisted deep eutectic solvents at high solids loading. *Bioresour. Technol.* 369, 128470.
- [7] Chen, Z., Chen, L., Khoo, K.S., Gupta, V.K., Sharma, M., Show, P.L., Yap, P.S., 2023. Exploitation of lignocellulosic-based biomass biorefinery: a critical review of renewable bioresource, sustainability and economic views. *Biotechnol. Adv.* 69, 108265.
- [8] Ci, Y., Yu, F., Zhou, C., Mo, H., Li, Z., Ma, Y., Zang, L., 2020. New ternary deep eutectic solvents for effective wheat straw deconstruction into its high-value utilization under near-neutral conditions. *Green Chem.* 22(24), 8713-8720.
- [9] Das, A., Guchhait, C., Adhikari, B., Saikia, K., Shi, D., Li, H., Rokhum, S.L., 2024. A Spherical superhydrophobic activated carbon catalyst for biodiesel production -exploring process efficiency, kinetics, thermodynamics and life cycle cost analysis. *Adv. Funct. Mater.* 34(41), 2406262.
- [10] Di Fidio, N., Dragoni, F., Antonetti, C., De Bari, I., Raspolli Galletti, A.M., Ragolini, G., 2020. From paper mill waste to single cell oil: enzymatic hydrolysis to sugars and their fermentation into microbial oil by the yeast *Lipomyces starkeyi*. *Bioresour. Technol.* 315, 123790.
- [11] Di Fidio, N., Ragolini, G., Dragoni, F., Antonetti, C., Raspolli Galletti, A.M., 2021. Integrated cascade biorefinery processes for the production of single cell oil by *Lipomyces starkeyi* from *Arundo donax* L. hydrolysates. *Bioresour. Technol.* 325, 124635.
- [12] Duan, C., Tian, C., Feng, X., Tian, G., Liu, X., Ni, Y., 2023. Ultrafast process of microwave-assisted deep eutectic solvent to improve properties of bamboo dissolving pulp. *Bioresour. Technol.* 370, 128543.
- [13] El Achkar, T., Greige-Gerges, H., Fourmentin, S., 2021. Basics and properties of deep eutectic solvents: a review. *Environ. Chem. Lett.* 19(4), 3397-3408.
- [14] Fan, D., Yang, J., Xie, X., Cao, M., Xu, L., Qiu, X., Liu, Q., Ouyang, X., 2023. Microwave-assisted fractionation of poplar sawdust into high-yield noncondensed lignin and carbohydrates in methanol/*p*-toluenesulfonic acid. *Chem. Eng. J.* 454, 140237.
- [15] Fernandes, C.C., Paiva, A., Haghbakhsh, R., Rita C. Duarte, A., 2023. Is it possible to correlate various physicochemical properties of Natural Deep eutectic systems in order to predict their behaviours as solvents?. *J. Mol. Liq.* 384, 122280.
- [16] Florindo, C., McIntosh, A.J.S., Welton, T., Branco, L.C., Marrucho, I.M., 2017. A closer look into deep eutectic solvents: exploring intermolecular interactions using solvatochromic probes. *Phys. Chem. Chem. Phys.* 20(1), 206-213.
- [17] Gheewala, S.H., 2023. Life cycle assessment for sustainability assessment of biofuels and bioproducts. *Biofuel Res. J.* 10(1), 1810-1815.
- [18] González-Rivera, J., Mero, A., Husanu, E., Mezzetta, A., Ferrari, C., D'Andrea, F., Bramanti, E., Pomelli, C.S., Guazzelli, L., 2021. Combining acid-based deep eutectic solvents and microwave irradiation for improved chestnut shell waste valorization. *Green Chem.* 23(24), 10101-10115.
- [19] Goswami, L., Kayalvizhi, R., Dikshit, P.K., Sherpa, K.C., Roy, S., Kushwaha, A., Kim, B.S., Banerjee, R., Jacob, S., Rajak, R.C., 2022. A critical review on prospects of bio-refinery products from second and third generation biomasses. *Chem. Eng. J.* 448, 137677.
- [20] Haldar, D., Purkait, M.K., 2021. A review on the environment-friendly emerging techniques for pretreatment of lignocellulosic biomass: mechanistic insight and advancements. *Chemosphere.* 264, 128523.
- [21] Hansen, B.B., Spittle, S., Chen, B., Poe, D., Zhang, Y., Klein, J.M., Horton, A., Adhikari, L., Zelovich, T., Doherty, B.W., Gurkan, B., Maginn, E.J., Ragauskas, A., Dadmun, M., Zawodzinski, T.A., Baker, G.A., Tuckerman, M.E., Savinell, R.F., Sangoro, J.R., 2021. Deep eutectic solvents: a review of fundamentals and applications. *Chem. Rev.* 121(3), 1232-1285.
- [22] Hansen, C.M., 2007. Hansen solubility parameters: a user's handbook, 2nd ed. ed. CRC Press, Boca Raton.
- [23] Hashemi, B., Shiri, F., Švec, F., Nováková, L., 2022. Green solvents and approaches recently applied for extraction of natural bioactive compounds. *TrAC, Trends Anal. Chem.* 157, 116732.
- [24] Hessel, V., Tran, N.N., Asrami, M.R., Tran, Q.D., Long, N.V.D., Escribà-Gelonch, M., Tejada, J.O., Linke, S., Sundmacher, K., 2022. Sustainability of green solvents-review and perspective. *Green Chem.* 24(2), 410-437.
- [25] Hong, S., Shen, X., Xue, Z., Sun, Z., Yuan, T., 2020. Structure-function relationships of deep eutectic solvents for lignin extraction and chemical transformation. *Green Chem.* 22(21), 7219-7232.
- [26] Hu, C., Wu, S., Wang, Q., Jin, G., Shen, H., Zhao, Z.K., 2011. Simultaneous utilization of glucose and xylose for lipid production by *Trichosporon cutaneum*. *Biotechnol. Biofuels.* 4(1), 25.
- [27] Huang, Y., Xu, Y., Zhu, Y., Huang, R., Kuang, Y., Wang, J., Xiao, W., Lin, J., Liu, Z., 2022. Improved glucose yield and concentration of sugarcane bagasse by the pretreatment with ternary deep eutectic solvents and recovery of the pretreated liquid. *Bioresour. Technol.* 366, 128186.
- [28] Ji, Q., Yu, X., Yagoub, A.E.G.A., Chen, L., Zhou, C., 2020. Efficient removal of lignin from vegetable wastes by ultrasonic and microwave-assisted treatment with ternary deep eutectic solvent. *Ind. Crop. Prod.* 149, 112357.
- [29] Ji, X., Chen, L., Yang, G., Tang, C., Zhou, W., Liu, T., Lu, X., 2024. Mutagenesis and fluorescence-activated cell sorting of oleaginous *Saccharomyces cerevisiae* and the multi-omics analysis of its high lipid accumulation mechanisms. *Bioresour. Technol.* 406, 131062.
- [30] Khan, N.R., Rathod, V.K., 2018. Microwave assisted enzymatic synthesis of speciality esters: a mini-review. *Process Biochem.* 75, 89-98.
- [31] Lin, W., Xing, S., Jin, Y., Lu, X., Huang, C., Yong, Q., 2020. Insight into understanding the performance of deep eutectic solvent pretreatment on improving enzymatic digestibility of bamboo residues. *Bioresour. Technol.* 306, 123163.
- [32] Liu, Q., Zhao, X., Yu, D., Yu, H., Zhang, Y., Xue, Z., Mu, T., 2019. Novel deep eutectic solvents with different functional groups towards highly efficient dissolution of lignin. *Green Chem.* 21(19), 5291-5297.
- [33] Liu, T., Huang, J., Li, J., Wang, K., Guo, Z., Wu, H., Yang, S., Li, H., 2023a. Heterogeneous photocatalysis for biomass valorization to organic acids. *Green Chem.* 25(24), 10338-10365.

- [34] Liu, X., Cheng, J., Huang, C., Wang, J., Fang, G., Shen, K., Meng, X., Ragauskas, A.J., 2023b. Alkali-facilitated deep eutectic solvent for effective bamboo saccharification. *Bioresour. Technol.* 367, 128297.
- [35] Liu, Y., Deak, N., Wang, Z., Yu, H., Hameleers, L., Jurak, E., Deuss, P.J., Barta, K., 2021. Tunable and functional deep eutectic solvents for lignocellulose valorization. *Nat. Commun.* 12(1), 5424.
- [36] Lobato-Rodríguez, Á., Gullón, B., Romani, A., Ferreira-Santos, P., Garrote, G., Del-Río, P.G., 2023. Recent advances in biorefineries based on lignin extraction using deep eutectic solvents: a review. *Bioresour. Technol.* 388, 129744.
- [37] Ma, C.Y., Wang, H.M., Wen, J.L., Shi, Q., Wang, S.F., Yuan, T.Q., Sun, R.C., 2020. Structural elucidation of lignin macromolecule from abaca during alkaline hydrogen peroxide delignification. *Int. J. Biol. Macromol.* 144, 596-602.
- [38] Mao, Y., Gerrow, A., Ray, E., Perez, N.D., Edler, K., Wolf, B., Binner, E., 2023. Lignin recovery from cocoa bean shell using microwave-assisted extraction and deep eutectic solvents. *Bioresour. Technol.* 372, 128680.
- [39] Masri, M.A., Garbe, D., Mehlmer, N., Brück, T.B., 2019. A sustainable, high-performance process for the economic production of waste-free microbial oils that can replace plant-based equivalents. *Energy Environ. Sci.* 12(9), 2717-2732.
- [40] Meng, X., Wang, Y., Conte, A.J., Zhang, S., Ryu, J., Wie, J.J., Pu, Y., Davison, B.H., Yoo, C.G., Ragauskas, A.J., 2023. Applications of biomass-derived solvents in biomass pretreatment-strategies, challenges, and prospects. *Bioresour. Technol.* 368, 128280.
- [41] Mohan, M., Huang, K., Pidatala, V.R., Simmons, B.A., Singh, S., Sale, K.L., Gladden, J.M., 2022. Prediction of solubility parameters of lignin and ionic liquids using multi-resolution simulation approaches. *Green Chem.* 24(3), 1165-1176.
- [42] Ocreto, J.B., Chen, W.H., Ubando, A.T., Park, Y.K., Sharma, A.K., Ashokkumar, V., Ok, Y.S., Kwon, E.E., Rollon, A.P., De Luna, M.D.G., 2021. A critical review on second- and third-generation bioethanol production using microwave-assisted heating (MAH) pretreatment. *Renew. Sust. Energy Rev.* 152, 111679.
- [43] Okur, M., Eslek Koyuncu, D.D., 2020. Investigation of pretreatment parameters in the delignification of paddy husks with deep eutectic solvents. *Biomass Bioenergy.* 142, 105811.
- [44] Peng, H., Luo, H., Jin, S., Li, H., Xu, J., 2014. Improved bioethanol production from corn stover by alkali pretreatment with a novel pilot-scale continuous microwave irradiation reactor. *Biotechnol. Bioprocess Eng.* 19(3), 493-502.
- [45] Raikwar, D., Van Aelst, K., Vangeel, T., Corderi, S., Van Aelst, J., Van den Bosch, S., Servaes, K., Vanbroekhoven, K., Elst, K., Sels, B.F., 2023. Elucidating the effect of the physicochemical properties of organosolv lignins on its solubility and reductive catalytic depolymerization. *Chem. Eng. J.* 461, 141999.
- [46] Rao, J., Lv, Z., Chen, G., Peng, F., 2023. Hemicellulose: structure, chemical modification, and application. *Prog. Polym. Sci.* 140, 101675.
- [47] Shen, Feiyue, Wu, S., Huang, M., Zhao, L., He, J., Zhang, Y., Deng, S., Hu, J., Tian, D., Shen, Fei, 2022. Integration of lignin microcapsulated pesticide production into lignocellulose biorefineries through FeCl₃-mediated deep eutectic solvent pretreatment. *Green Chem.* 24(13), 5242-5254.
- [48] Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2012. Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure (LAP). Technical Report NREL/TP-510-42618. National Renewable Energy Laboratory.
- [49] Song, W., He, Y., Huang, R., Li, J., Yu, Y., Xia, P., 2023. Life cycle assessment of deep-eutectic-solvent-assisted hydrothermal disintegration of microalgae for biodiesel and biogas co-production. *Appl. Energy.* 335, 120758.
- [50] Su, Y., Huang, C., Lai, C., Yong, Q., 2021. Green solvent pretreatment for enhanced production of sugars and antioxidative lignin from poplar. *Bioresour. Technol.* 321, 124471.
- [51] Sun, L., Yue, Z., Sun, Shaochao, Li, Y., Cao, X., Sun, Shaoni, 2023. Microwave-assisted choline chloride/1,2-propanediol/methyl isobutyl ketone biphasic system for one-pot fractionation and valorization of Eucalyptus biomass. *Bioresour. Technol.* 369, 128392.
- [52] Tan, J., Huang, J., Yuan, J., Chen, J., Pei, Z., Li, H., Yang, S., 2023. Novel supramolecular deep eutectic solvent-enabled in-situ lignin protection for full valorization of all components of wheat straw. *Bioresour. Technol.* 388, 129722.
- [53] Wang, H., Chen, J., Pei, Z., Fang, Z., Yang, S., Li, H., 2025. Bio-based deep eutectic solvent of enhanced lignin solubility for wheat straw fractionation and full-component utilization. *Ind. Crop. Prod.* 223, 120054.
- [54] Wang, H., Maxim, M.L., Gurau, G., Rogers, R.D., 2013. Microwave-assisted dissolution and delignification of wood in 1-ethyl-3-methylimidazolium acetate. *Bioresour. Technol.* 136, 739-742.
- [55] Wang, J., Gao, Q., Bao, J., 2016. Genome sequence of *Trichosporon cutaneum* ACCC 20271: an oleaginous yeast with excellent lignocellulose derived inhibitor tolerance. *J. Biotechnol.* 228, 50-51.
- [56] Wang, N., Liu, K., Hou, Z., Zhao, Z., Li, H., Gao, X., 2024a. The comparative techno-economic and life cycle assessment for multi-product biorefinery based on microwave and conventional hydrothermal biomass pretreatment. *J. Clean. Product.* 474, 143562.
- [57] Wang, N., Liu, K., Xu, A., Li, H., Zhao, Z., Fan, X., Gao, X., 2024b. An efficient microwave-assisted method using ethyl lactate/water cosolvent for lignocellulosic biomass fractionation. *Chem. Eng. J.* 494, 152724.
- [58] Wang, N., Xu, A., Liu, K., Zhao, Z., Li, H., Gao, X., 2024c. Performance of green solvents in microwave-assisted pretreatment of lignocellulose. *Chem. Eng. J.* 482, 148786.
- [59] Wang, K., Li, Z., Guo, Z., Huang, J., Liu, T., Zhou, M., Hu, J., Li, H., 2024d. Electroreductive upgradation of biomass into high-value chemicals and energy-intensive biofuels. *Green Chem.* 26(5), 2454-2475.
- [60] Wang, Na, Xu, B., Wang, X., Lang, J., Zhang, H., 2022a. Insights into the mechanism of lignin dissolution via deep eutectic solvents by using Hansen solubility theory. *J. Mol. Liq.* 366, 120294.
- [61] Wang, Nannan, Zou, W., Li, X., Liang, Y., Wang, P., 2022b. Study and application status of the nonthermal effects of microwaves in chemistry and materials science-a brief review. *RSC Adv.* 12(27), 17158-17181.
- [62] Xiao, T., Hou, M., Guo, X., Cao, X., Li, C., Zhang, Q., Jia, W., Sun, Y., Guo, Y., Shi, H., 2024. Recent progress in deep eutectic solvent (DES) fractionation of lignocellulosic components: a review. *Renew. Sust. Energy Rev.* 192, 114243.
- [63] Xie, J., Chen, J., Cheng, Z., Zhu, S., Xu, J., 2021. Pretreatment of pine lignocelluloses by recyclable deep eutectic solvent for elevated enzymatic saccharification and lignin nanoparticles extraction. *Carbohydr. Polym.* 269, 118321.
- [64] Xie, Y., Zhao, J., Wang, P., Ling, Z., Yong, Q., 2023. Natural arginine-based deep eutectic solvents for rapid microwave-assisted pretreatment on crystalline bamboo cellulose with boosting enzymatic conversion performance. *Bioresour. Technol.* 385, 129438.
- [65] Xu, C., Lan, J., Ye, J., Yang, Y., Huang, K., Zhu, H., 2023. Design of continuous-flow microwave reactor based on a leaky waveguide. *Chem. Eng. J.* 452, 139690.
- [66] Xu, H., Peng, J., Kong, Y., Liu, Y., Su, Z., Li, B., Song, X., Liu, S., Tian, W., 2020. Key process parameters for deep eutectic solvents pretreatment of lignocellulosic biomass materials: a review. *Bioresour. Technol.* 310, 123416.
- [67] Yang, H., Chai, M., Geun Yoo, C., Yuan, J., Meng, X., Yao, L., 2024. Role of lignin in synergistic digestibility improvement of wheat straw by novel alkaline deep eutectic solvent and tetrahydrofuran pretreatment. *Bioresour. Technol.* 397, 130460.
- [68] Yu, H., Xue, Z., Shi, R., Zhou, F., Mu, T., 2022. Lignin dissolution and lignocellulose pretreatment by carboxylic acid based deep eutectic solvents. *Ind. Crop. Prod.* 184, 115049.
- [69] Yuan, J., Huang, J., Ruatpuia, J.VL., Chen, J., Wang, H., Rokhum, S.L., Li, H., 2024. Electro-driven deep eutectic solvent pretreatment of wheat straw with enhance component fractionation and hydrogen evolution at room temperature. *Green Chem Eng.*
- [70] Zhang, Q., Dai, C., Tan, X., He, X., Zhang, K., Xu, X., Zhuang, X., 2023. Biphasic fractionation of lignocellulosic biomass based on the

combined action of pretreatment severity and solvent effects on delignification. *Bioresour. Technol.* 369, 128477.

- [71] Zhang, Y., Eberhardt, T.L., Cai, B., Wu, M., Xu, X., Feng, J., Pan, H., 2022. Organosolv fractionation of a lignocellulosic biomass feedstock using a pilot scale microwave-heating reactor. *Ind. Crop. Prod.* 180, 114700.
- [72] Zhang, Y., Zhao, Z., Li, H., Li, X., Gao, X., 2021. Numerical modeling and optimal design of microwave-heating falling film evaporation. *Chemical Eng. Sci.* 240, 116681.
- [73] Zhao, C., Xie, B., Zhao, R., Chen, S., Fang, H., 2018. Intracellular amino and nonamino organic acids profiling of *Trichosporon cutaneum* on rich and limited nitrogen conditions for lipid production. *Biomass Bioenergy.* 118, 84-92.

- [74] Zhong, L., Wang, C., Yang, G., Chen, J., Xu, F., Yoo, C.G., Lyu, G., 2022. Rapid and efficient microwave-assisted guanidine hydrochloride deep eutectic solvent pretreatment for biological conversion of castor stalk. *Bioresour. Technol.* 343, 126022.
- [75] Zhou, M., Fakayode, O.A., Ahmed Yagoub, A.E., Ji, Q., Zhou, C., 2022. Lignin fractionation from lignocellulosic biomass using deep eutectic solvents and its valorization. *Renew. Sust. Energy Rev.* 156, 111986.
- [76] Zou, R., Zhou, X., Qian, M., Wang, C., Boldor, D., Lei, H., Zhang, X., 2024. Advancements and applications of microwave-assisted deep eutectic solvent (MW-DES) lignin extraction: a comprehensive review. *Green Chem.* 26(3), 1153-1169.



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Supplementary Information

S1. DES recycling

The re-utilization of DES offers financial benefits in industrial operations. The recyclability of DES has been documented in previous literature on DES pretreatment of lignocellulose. Yan et al. (2022) proposed that ChCl-OA exhibited no evident reduction in pretreatment capacity after 10 cycles by supplementation with oxalic acid and fresh DES. In the MW-DES pretreatment of bamboo dissolving pulp, 91.5% of DES could be recovered after 5 cycles, and no substantial deterioration in pretreatment performance was observed in conjunction with fresh DES supplementation (Duan et al., 2023b). Liu et al. (2021) reported that the pretreatment capacity of DES could be ensured by replenishing the acid and DES that were lost during the pretreatment process. Supplementation of DES fractions is an important factor to ensure its pretreatment capacity during the recycling process. However, none of the above literature utilized the hemicellulose fraction of DES, and only cellulose and lignin were isolated. In this work, *Trichosporon cutaneum* was used to synthesize microbial lipids from hemicellulose hydrolysates in DES (without detoxification procedures). The investigation into the recyclability and reusability of DES was conducted with the consideration of pretreatment performance and cost to accurately assess the impact of DES recycling on the industrial scale-up process.

The recycling of DES has been discussed in previous literature (Liu et al., 2021; Yan et al., 2022). After microbial lipid production, the supernatant was rotarily evaporated to remove water, supplemented with oxalic acid and fresh DES to maintain the original composition and weight of the DES, and reused for wheat straw pretreatment (MW-DES). Specifically, 25 g of DES was supplemented with 10.1 g of oxalic acid and 4.8 g of fresh DES, resulting in regenerated DES. Based on the assumption of 5 cycles of DES for LCCA, the cost of using fresh DES was USD0.807 (Table S7, cost of ME-DES pretreatment (CMDP)), and the cost of recycled DES was USD0.737, a cost reduction of only 8.7% was observed, using 100 g of pretreated wheat straw as substrate. Regarding pretreatment effectiveness, the DES from the initial cycle proved to be non-treatable, exhibiting no alteration in lignin content and achieving 97.2% solids recovery. Consequently, the recycling of DES after microbial fermentation is not a viable option. Additionally, the recycling of DES fractions that require supplemental DES is deemed to be uneconomical.

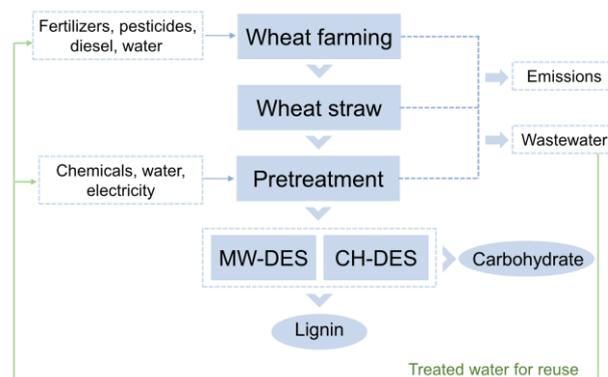


Fig. S1. The system boundary of LCA for MW-DES and CH-DES pretreatment.

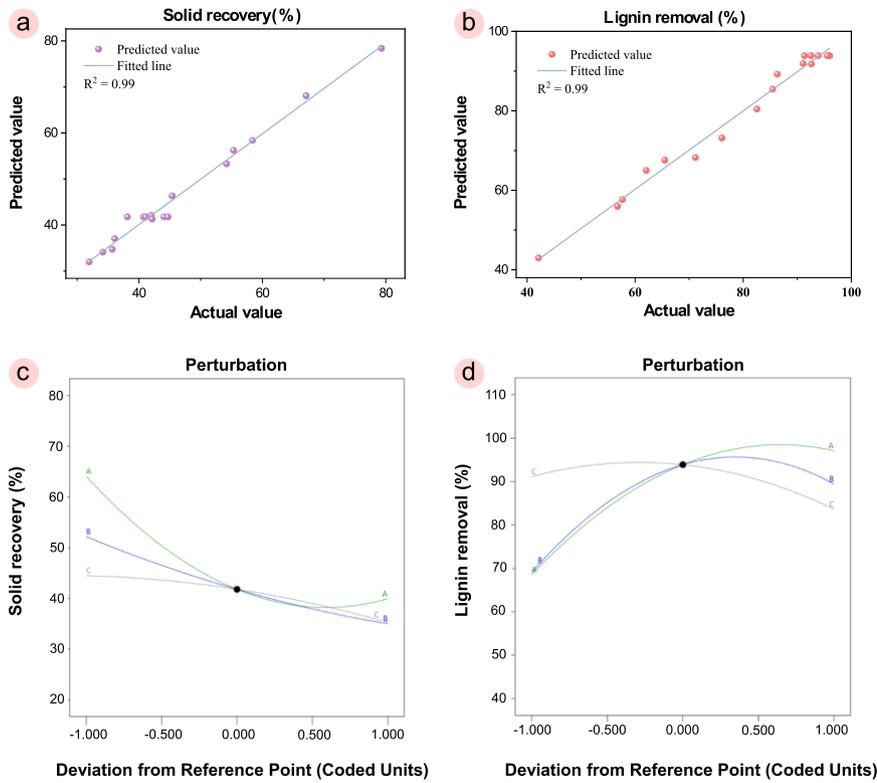


Fig. S2. Correlation of predicted and experimental values of (a) solid recovery and (b) lignin removal; Perturbation plots showing significant variables influencing (c) solid recovery, and (d) lignin removal.

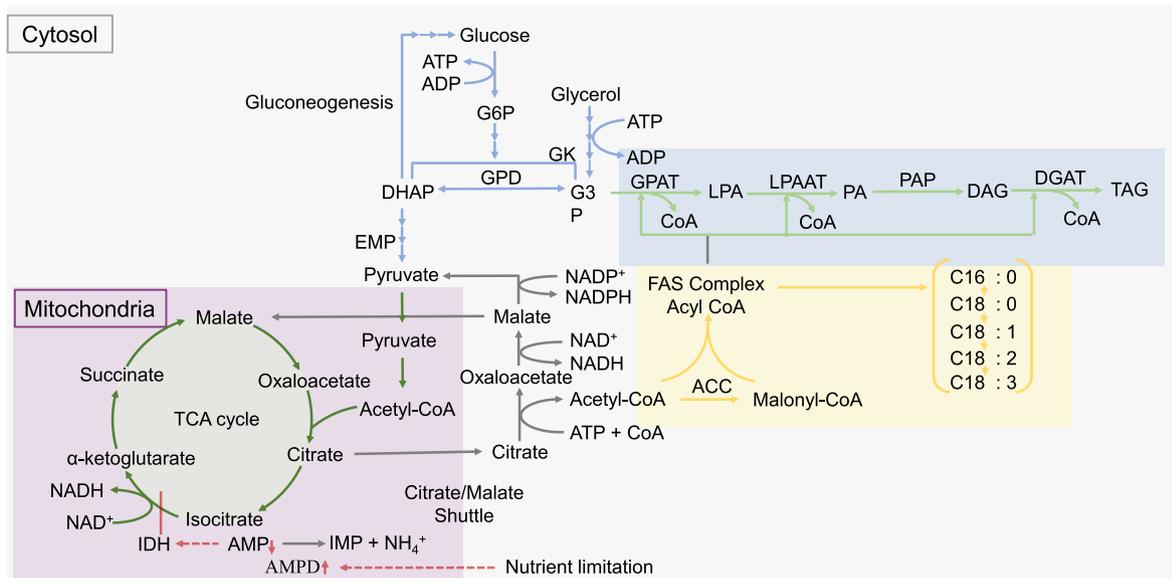


Fig. S3. Lipid biosynthesis pathway in oleaginous yeasts. The citrate/malate pathways are represented by purple, the process of fatty acid synthesis is represented by yellow, and the synthesis of triacylglyceride (TAG) is represented by blue. DHAP, Dihydroxyacetone phosphate; EMP, Embden-Meyerhoff-Parnas Pathway; AMPD, adenosine monophosphate deaminase; IDH, isocitrate dehydrogenase; IMP, inosine monophosphate; GPD, glycerol-3-phosphate dehydrogenase; ACC, acetyl-CoA carboxylase; GK, glycerol kinase; GPAT, glycerol-3-phosphate acyltransferase; LPAAT, lysophosphatidic acid acyltransferase (or AGPAT, acylglycerophosphate acyltransferase); PAP, phosphatidate phosphatase; DGAT, diacylglycerol acyltransferase. Figure adapted from Dey and Maiti (2013), Zeng et al. (2018), and Chattopadhyay et al. (2021).

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Table S1.
Performance comparison of different DES pretreatment strategies for lignocellulose fractionation.

Feedstock	DES Type and Pretreatment Conditions	Cellulose Content (%)	Lignin Removal (%)	Hemicellulose Removal (%)	Enzymatic Digestibility (%)/Time (h)	Ref.
Wheat straw	Choline Chloride (ChCl): monoethanolamine: tetrahydrofuran = 1:6:1, 160 °C, 60 min	46.89	63.77	46.83	81.61/72	Yang et al. (2024)
<i>Eucommia ulmoides</i> seed shells	ChCl: oxalic acid: ethylene glycol = 1:1:2, 100 °C, 180 min	-	65.6	79.7	69.1/108	Duan et al. (2023a)
Bamboo	ChCl: lactic acid = 1:4, 130 °C, 90 min	71.8	83.6	75.7	76.9/72	Lin et al. (2020)
Bamboo powder	ChCl: glycerol = 1:2, FeCl ₃ ·6H ₂ O loading 0.05mol·L ⁻¹ , 130 °C, 180 min	68.28	70.8	76.3	81.6/96	Shen et al. (2022)
Poplar sawdust	ChCl: lactic acid = 1:2, 130 °C, 90 min	67.9	66.4	64.3	75.8/108	Su et al. (2021)
Rape straw	Cetyltrimethylammonium bromide and glycerol = 1:6, 200 °C, 90 min	49.6	46.1	52.7	67.9/72	Tang et al. (2023)
Eucalyptus wood chips	Lactic acid: betaine hydrochloride: AlCl ₃ = 14.5:1:0.1, 105 °C, 145 min	71	86	95	96/72	Huo et al. (2023)
Sugarcane bagasse	ChCl: PEG: hydroxyethyl sulfonic acid = 1:4:1.34%	85.72	86.89	100.00	65.04/72	Huang et al. (2022)
Wheat straw	ChCl: boric acid: polyethylene glycol-200 = 1:1:1.5, 120 °C, 240 min	-	88.39	84.38	59.3/120	Ci et al. (2020)
Corn stover	Tetramethyl ammonium chloride: lactic acid = 1:2, 130 °C, 120 min	54.61	78.66	62.57	73.30/48	Liang et al. (2021)

Table S2.
Performance comparison of different microwave-assisted DES pretreatment strategies for lignocellulose fractionation.

Feedstock	DES Type and Pretreatment Conditions	Cellulose Content (%)	Lignin Removal (%)	Hemicellulose Removal (%)	Enzymatic Digestibility (%)/Time (h)	Ref.
Stalk	Guanidine hydrochloride: lactic acid = 1:2, 130 °C, 30 min, 400 W	73.39	92.02	77.56	96.3/72	Zhong et al. (2022)
Moso bamboo	Glycerol: guanidine hydrochloride: FeCl ₃ ·6H ₂ O = 2:1:0.2, 120 °C, 10 min	74.37	81.17	85.42	90.15/72	Feng et al. (2024)
Moso bamboo	Lactic acid: acetylcholine chloride = 1:2, 120 °C, 10 min, 600 W	89.9	90.2	100	84.4/96	Ling et al. (2023)
Moso bamboo	Arginine: lactic acid = 1:7, 120 °C, 20 min, 600 W	72.1	84.8	76.9	81.9/72	Xie et al. (2023)
Rice straw	ChCl: p-toluene sulfonic acid: ethylene glycol = 1:1:9, 80 °C, 22.5 min	50.1	61.6	-	93.4/72	Poy et al. (2023)
Milled softwood mixture	ChCl: formic acid = 1:4, 140°C, 14 min, 800 W	89.2	90.1	96.2	-	Ceaser et al. (2023)
Switchgrass	ChCl: lactic acid = 1:4, 45 s, 800 W	64.50	72.23	83.65	79.14/72	Chen and Wan (2018)
Corn stover		65.78	79.60	90.06	78.50/72	
	Oxalic acid anhydrate: ChCl = 2:1, 160 °C, 10 min, 600 W	48.7	48.2	58.6	58.5/72	
	Malonic acid: ChCl = 2:1, 160 °C, 10 min, 600 W	61.3	44.5	69.4	57.6/72	
Moso bamboo	Succinic acid: ChCl = 2:1, 160 °C, 10 min, 600 W	64.5	44.8	86.4	52.8/72	Ling et al. (2021)
	L-malic acid: ChCl = 2:1, 160 °C, 10 min, 600 W	55.3	43.9	71.0	59.0/72	
	L-tartaric acid: ChCl = 2:1, 160 °C, 10 min, 600 W	46.9	41.5	67.6	63.5/72	
<i>Eucalyptus grandis</i>	ChCl: 1,2-propanediol = 1:2, DES/methyl isobutyl ketone = 1:2 (w/v), 140 °C, 15 min, 400 W, 0.075M AlCl ₃ ·6H ₂ O, 0.05M HCl	80.8	92.4	-	90.3/72	Sun et al. (2023)
Wheat straw	ChCl: ethylene glycol: oxalic acid = 1:2:2, 130 °C, 150 s, 540 W	76.3	96.1	93.0	92.4/48	This Study

Table S3. Kamlet-Taft parameters, Hansen solubility parameters, and pretreatment effects of different DESs.

Molar Ratio	π^*	α	β	$ \alpha-\beta $	δ	RED
ChCl: EG=1:2	0.71	0.95	1.08	0.13	35.27	0.49
ChCl: OA: EG=1:2:1	1.41	1.80	0.26	1.55	33.91	0.40
ChCl: OA: EG=1:2:2	1.43	1.83	0.19	1.64	33.61	0.50
ChCl: OA: EG=2:2:1	1.40	1.79	0.29	1.50	35.42	0.42
ChCl: OA: EG=1:1:1	1.37	1.75	0.25	1.50	34.85	0.39
ChCl: OA: EG=1:1:2	1.40	1.80	0.21	1.58	34.22	0.47
ChCl: OA: EG=2:1:2	1.37	1.76	0.28	1.48	35.63	0.45
Lignin (Hansen, 2007)					31.05	

Kamlet-Taft parameters: π^* (polarity/polarizability), α (hydrogen bonding donating ability), and β (hydrogen bonding accepting ability); δ (Hansen solubility parameter), RED (relative energy difference).

Table S4. Analysis of variance (ANOVA) for the quadratic model of solid recovery by microwave-assisted DES pretreatments based on Box-Behnke design (BBD).

Source	Sum of Squares	df	Mean Square	F-Value	p-Value
Model	2515.20	9	279.47	55.05	< 0.0001*
A-Time	1166.44	1	1166.44	229.77	< 0.0001*
B-Temperature	590.13	1	590.13	116.25	< 0.0001*
C- Liquid-solid ratio	169.92	1	169.92	33.47	0.0007*
AB	62.57	1	62.57	12.32	0.0099*
AC	7.02	1	7.02	1.38	0.2780
BC	50.34	1	50.34	9.92	0.0162*
A ²	439.63	1	439.63	86.60	< 0.0001*
B ²	13.35	1	13.35	2.63	0.1489
C ²	15.19	1	15.19	2.99	0.1273
Residual	35.54	7	5.08		
Lack of Fit	6.94	3	2.31	0.3238	0.8094
Pure Error	28.59	4	7.15		
Cor Total	2550.73	16			
R ²	0.9861				
Adjusted R ²	0.9682				
Predicted R ²	0.9389				
Adeq Precision	26.8429				

* Significant at a 95% confidence level ($p < 0.05$).
df: degree of freedom.

Table S6. Comparison of the MW-DES pretreatment strategy proposed in this paper with other emerging green pretreatment methods.

Pretreatment Methods	Lignocellulose	Conditions	Lignin Removal (%)	Advantages	Disadvantage	Ref.
Supercritical CO ₂ explosion	Green coconut fiber	25-45 Mpa, 80 °C, 60 min,	No change	Green and fast mass transfer, increasing the porosity	Action cellulose only, high-pressure CO ₂ requirement	Putrino et al. (2020)
Hydrothermal pretreatment	Sunflower stalk	180°C, 120 min	33.5	No chemical utilization	High energy and water consumption, long duration, low lignin removal	Yang et al. (2023)
Integrated Hydrothermal-DES	Bamboo	Hydrothermal 180 °C, 35 min DES (ChCl/LA) 140 °C, 120 min	79.1	Higher lignin removal than hydrothermal pretreatment alone	High energy and water consumption, long-duration	Chang et al. (2023)
Ammonia fiber expansion assisted DES	Wheat straw	Liquid ammonia with DES (ChCl/LA) 120 °C, 90 min	56.1	Reduced time compared to DES pretreatment alone	Inefficient and high-pressure requirement	Hu et al. (2023)
Ammonia fiber expansion combined with white-rot	<i>Pennisetum sinense</i>	Lignocellulose with liquid ammonia 130°C, high pressure, 15 min and then incubated with white rot bacteria, 96h	51.12	Increased lignocellulose-specific surface area and reduced pretreatment time compared to biological pretreatment	High pressure and overall long retention time	Hu et al. (2024)
	<i>Salix</i> chips		70.23			
	Pine chips		64.60			

Table S5. Analysis of variance (ANOVA) for the quadratic model of lignin removal by microwave-assisted DES pretreatments based on Box-Behnke design (BBD).

Source	Sum of Squares	df	Mean Square	F-Value	p-Value
Model	4343.95	9	482.66	55.11	< 0.0001*
A-time	1621.65	1	1621.65	185.17	< 0.0001*
B-temperature	826.01	1	826.01	94.32	< 0.0001*
C- Liquid-solid ratio	111.98	1	111.98	12.79	0.0090*
AB	2.86	1	2.86	0.3261	0.5858
AC	23.23	1	23.23	2.65	0.1474
BC	6.03	1	6.03	0.6882	0.4341
A ²	514.05	1	514.05	58.7	0.0001*
B ²	897.12	1	897.12	102.44	< 0.0001*
C ²	177.44	1	177.44	20.26	0.0028*
Residual	61.3	7	8.76		
Lack of Fit	45.77	3	15.26	3.93	0.1096
Pure Error	15.53	4	3.88		
Cor Total	4405.25	16			
R ²	0.9861				
Adjusted R ²	0.9682				
Predicted R ²	0.8282				
Adeq Precision	22.4215				

* Significant at 95% confidence level.
df: degree of freedom

Table S6.
continued.

Pretreatment Methods	Lignocellulose	Conditions	Lignin Removal (%)	Advantages	Disadvantage	Ref.
Bio-derived solvents Cyrene	Populus	Cyrene/H ₂ O, H ₂ SO ₄ (75mM) 60 min	68	Close-loop biorefinery lignin-target	Solvent-induced high cost and potentially modifying the cellulosic structure by grafting	Meng et al. (2020)
MW-DES	Wheat straw	130 °C, 150 s, 540W DES (ChCl/OA/EG)	96.1	Green, efficient, and rapid lignin removal, moderate conditions, low energy consumption	Large-scale equipment needs to be developed for industrialization	This Study

Table S7.

Life cycle cost assessment of microbial lipids synthesized from MW-DES pretreated wheat straw.

Step	Description	Amount (USD)
Cost of 100 g dry wheat straw (CDWS)	100 g × USD0.001/g = 0.100	0.100
Cost of MW-DES pretreatment (CMDP)	Cost of DES (choline chloride, oxalic acid, ethylene glycol) + Prepare time (h) × units consumed × cost per unit + Pretreatment time (h) × units consumed × cost per unit = 0.789 + 0.004 + 0.014 = USD0.807	0.807
Cost of washing (CW)	Cost of water + cost of ethanol = 0.002 + 0.009 = USD0.011	0.011
Cost of drying (CD)	Time (h) × units consumed × cost per unit = USD0.013	0.013
Cost of enzymatic digestibility (CED)	Cost of enzymes and buffer solution = USD0.061	0.061
Cost of fermentation for lipid synthesis (CFLS)	Cost of preculture medium + Cost of fermentation medium = 0.005 + 0.063 = USD0.068	0.068
Cost of freezing (CF)	Time (h) × units consumed × cost per unit = USD0.002	0.002
Cost of lipid extraction (CLX)	Cost of reagents = USD0.015	0.015
The total cost of obtaining 8.8 g microbial lipids from pretreating 100 g wheat straw	CDWS + CMDP + CW + CD + CED + CFLS + CF + CLX = 0.100 + 0.807 + 0.011 + 0.013 + 0.061 + 0.068 + 0.002 + 0.015 = USD1.077	1.077

Table S8.

Life cycle cost assessment of microbial lipids synthesized from CH-DES pretreated wheat straw.

Step	Description	Amount (USD)
Cost of 100 g dry wheat straw (CDWS)	100 g × USD0.001/g = USD 0.100	0.100
Cost of MW-DES pretreatment (CMDP)	Cost of DES (choline chloride, oxalic acid, ethylene glycol) + Prepare time (h) × units consumed × cost per unit + Pretreatment time (h) × units consumed × cost per unit = 0.789 + 0.004 + 0.723 = USD1.516	1.516
Cost of washing (CW)	Cost of water + cost of ethanol = 0.004 + 0.015 = USD0.019	0.019
Cost of drying (CD)	Time (h) × units consumed × cost per unit = USD0.013	0.013
Cost of enzymatic digestibility (CED)	Cost of enzymes and buffer solution = USD0.137	0.137
Cost of fermentation for lipid synthesis (CFLS)	Cost of preculture medium + Cost of fermentation medium = 0.126 + 0.007 = USD0.133	0.133
Cost of freezing (CF)	Time (h) × units consumed × cost per unit = USD0.001	0.001
Cost of lipid extraction (CLX)	Cost of reagents = USD0.006	0.006
The total cost of obtaining 5.1 g microbial lipids from pretreating 100 g wheat straw	CDWS + CMDP + CW + CD + CED + CFLS + CF + CLX = 0.100 + 1.516 + 0.019 + 0.013 + 0.137 + 0.133 + 0.001 + 0.006 = USD1.925	1.925

References

- [1] Ceaser, R., Rosa, S., Montané, D., Constantí, M., Medina, F., 2023. Optimization of softwood pretreatment by microwave-assisted deep eutectic solvents at high solids loading. *Bioresour. Technol.* 369, 128470.
- [2] Chang, L., Ye, R., Song, J., Xie, Y., Chen, Q., Yan, S., Sun, K., Gan, L., 2023. Efficient fractionation of green bamboo using an integrated hydrothermal-deep eutectic solvent pretreatment for its valorization. *Appl. Sci.* 13(4), 2429.
- [3] Chattopadhyay, A., Mitra, M., Maiti, M.K., 2021. Recent advances in lipid metabolic engineering of oleaginous yeasts. *Biotechnol. Adv.* 53, 107722.
- [4] Chen, Z., Wan, C., 2018. Ultrafast fractionation of lignocellulosic biomass by microwave-assisted deep eutectic solvent pretreatment. *Bioresour. Technol.* 250, 532-537.
- [5] Ci, Y., Yu, F., Zhou, C., Mo, H., Li, Z., Ma, Y., Zang, L., 2020. New ternary deep eutectic solvents for effective wheat straw deconstruction into its high-value utilization under near-neutral conditions. *Green Chem.* 22(24), 8713-8720.
- [6] Dey, P., Maiti, M.K., 2013. Molecular characterization of a novel isolate of *Candida tropicalis* for enhanced lipid production. *J. Appl. Microbiol.* 114(5), 1357-1368.
- [7] Duan, C.J., Han, X., Chang, Y., Xu, J., Yue, G., Zhang, Y., Fu, Y., 2023a. A novel ternary deep eutectic solvent pretreatment for the efficient separation and conversion of high-quality gutta-percha,

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- value-added lignin and monosaccharide from *Eucommia ulmoides* seed shells. *Bioresour. Technol.* 370, 128570.
- [8] Duan, C., Tian, C., Feng, X., Tian, G., Liu, X., Ni, Y., 2023b. Ultrafast process of microwave-assisted deep eutectic solvent to improve properties of bamboo dissolving pulp. *Bioresour. Technol.* 370, 128543.
- [9] Feng, Y., Eberhardt, T.L., Meng, F., Xu, C., Pan, H., 2024. Efficient extraction of lignin from moso bamboo by microwave-assisted ternary deep eutectic solvent pretreatment for enhanced enzymatic hydrolysis. *Bioresour. Technol.* 400, 130666.
- [10] Hansen, C.M., 2007. Hansen solubility parameters: a user's handbook, 2nd ed. ed. CRC Press, Boca Raton.
- [11] Hu, M., Cai, Z., Zhang, J., Yuan, L., Fu, Q., Ji, D., 2024. Ammonia fiber expansion (AFEX) combined with white-rot fungi pretreatment to improve enzymatic hydrolysis of lignocellulose. *Biomass Conv. Bioref.* 14(9), 10085-10099.
- [12] Hu, M., Yuan, L., Cai, Z., Zhang, W., Fu, Q., Ji, D., 2023. Ammonia fiber expansion-assisted deep eutectic solvent treatment for wheat straw fraction separation and bioconversion. *Bioresour. Technol.* 367, 128242.
- [13] Huang, Y., Xu, Y., Zhu, Y., Huang, R., Kuang, Y., Wang, J., Xiao, W., Lin, J., Liu, Z., 2022. Improved glucose yield and concentration of sugarcane bagasse by the pretreatment with ternary deep eutectic solvents and recovery of the pretreated liquid. *Bioresour. Technol.* 366, 128186.
- [14] Huo, D., Sun, Y., Yang, Q., Zhang, F., Fang, G., Zhu, H., Liu, Y., 2023. Selective degradation of hemicellulose and lignin for improving enzymolysis efficiency via pretreatment using deep eutectic solvents. *Bioresour. Technol.* 376, 128937.
- [15] Liang, X., Zhu, Y., Qi, B., Li, S., Luo, J., Wan, Y., 2021. Structure-property-performance relationships of lactic acid-based deep eutectic solvents with different hydrogen bond acceptors for corn stover pretreatment. *Bioresour. Technol.* 336, 125312.
- [16] Lin, W., Xing, S., Jin, Y., Lu, X., Huang, C., Yong, Q., 2020. Insight into understanding the performance of deep eutectic solvent pretreatment on improving enzymatic digestibility of bamboo residues. *Bioresour. Technol.* 306, 123163.
- [17] Ling, Z., Tan, Y., Li, X., Xie, Y., Wang, P., Su, Y., Yong, Q., 2023. Elucidating multi-scale deconstruction of bamboo crystalline cellulose by novel acetylcholine chloride based deep eutectic solvents for enhanced enzymatic hydrolysis. *Ind. Crop. Prod.* 203, 117156.
- [18] Ling, Z., Tang, W., Su, Y., Shao, L., Wang, P., Ren, Y., Huang, C., Lai, C., Yong, Q., 2021. Promoting enzymatic hydrolysis of aggregated bamboo crystalline cellulose by fast microwave-assisted dicarboxylic acid deep eutectic solvents pretreatments. *Bioresour. Technol.* 333, 125122.
- [19] Liu, Y., Deak, N., Wang, Z., Yu, H., Hameleers, L., Jurak, E., Deuss, P.J., Barta, K., 2021. Tunable and functional deep eutectic solvents for lignocellulose valorization. *Nat. Commun.* 12(1), 5424.
- [20] Meng, X., Pu, Y., Li, M., Ragauskas, A.J., 2020. A biomass pretreatment using cellulose-derived solvent Cyrene. *Green Chem.* 22(9), 2862-2872.
- [21] Poy, H., Lladosa, E., Arcés, A., Gabaldón, C., Loras, S., 2023. Microwave-assisted ternary deep eutectic solvent pretreatment for improved rice straw saccharification under mild pretreatment conditions. *Ind. Crop. Prod.* 206, 117639.
- [22] Putrino, F.M., Tedesco, M., Bodini, R.B., Oliveira, A.L. de, 2020. Study of supercritical carbon dioxide pretreatment processes on green coconut fiber to enhance enzymatic hydrolysis of cellulose. *Bioresour. Technol.* 309, 123387.
- [23] Shen, F., Wu, S., Huang, M., Zhao, L., He, J., Zhang, Y., Deng, S., Hu, J., Tian, D., Shen, Fei, 2022. Integration of lignin microcapsulated pesticide production into lignocellulose biorefineries through FeCl₃-mediated deep eutectic solvent pretreatment. *Green Chem.* 24(13), 5242-5254.
- [24] Shuai, L., Questell-Santiago, Y.M., Luterbacher, J.S., 2016. A mild biomass pretreatment using γ -valerolactone for concentrated sugar production. *Green Chem.* 18(4), 937-943.
- [25] Su, Y., Huang, C., Lai, C., Yong, Q., 2021. Green solvent pretreatment for enhanced production of sugars and antioxidative lignin from poplar. *Bioresour. Technol.* 321, 124471.
- [26] Sun, L., Yue, Z., Sun, Shaochao, Li, Y., Cao, X., Sun, Shaoni, 2023. Microwave-assisted choline chloride/1,2-propanediol/methyl isobutyl ketone biphasic system for one-pot fractionation and valorization of Eucalyptus biomass. *Bioresour. Technol.* 369, 128392.
- [27] Tang, W., Fan, B., Wang, X., Huang, C., Tang, Z., He, Y., 2023. Facilitating enzymatic hydrolysis efficiency of rape straw by pretreatment with a novel glycerol-based deep eutectic solvent. *Ind. Crop. Prod.* 206, 117587.
- [28] Xie, Y., Zhao, J., Wang, P., Ling, Z., Yong, Q., 2023. Natural arginine-based deep eutectic solvents for rapid microwave-assisted pretreatment on crystalline bamboo cellulose with boosting enzymatic conversion performance. *Bioresour. Technol.* 385, 129438.
- [29] Yan, G., Zhou, Y., Zhao, L., Wang, W., Yang, Y., Zhao, X., Chen, Y., Yao, X., 2022. Recycling of deep eutectic solvent for sustainable and efficient pretreatment of corncob. *Ind. Crop. Prod.* 183, 115005.
- [30] Yang, H., Chai, M., Geun Yoo, C., Yuan, J., Meng, X., Yao, L., 2024. Role of lignin in synergistic digestibility improvement of wheat straw by novel alkaline deep eutectic solvent and tetrahydrofuran pretreatment. *Bioresour. Technol.* 397, 130460.
- [31] Yang, Q., Tang, W., Li, L., Huang, M., Ma, C., He, Y.C., 2023. Enhancing enzymatic hydrolysis of waste sunflower straw by clean hydrothermal pretreatment. *Bioresour. Technol.* 383, 129236.
- [32] Zeng, S.-Y., Liu, H.-H., Shi, T.Q., Song, P., Ren, L.J., Huang, H., Ji, X.J., 2018. Recent advances in metabolic engineering of *Yarrowia lipolytica* for lipid overproduction. *Europ. J. Lipid Sci. Technol.* 120(3), 1700352.
- [33] Zhong, L., Wang, C., Yang, G., Chen, J., Xu, F., Yoo, C.G., Lyu, G., 2022. Rapid and efficient microwave-assisted guanidine hydrochloride deep eutectic solvent pretreatment for biological conversion of castor stalk. *Bioresour. Technol.* 343, 126022.