



# Synergistic Enhancement of Lignocellulosic Biomass Saccharification via Ultrasound-Assisted Pretreatment with Magnetic Fe<sub>3</sub>O<sub>4</sub>

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## Abstract

The recalcitrant structure of lignocellulosic biomass necessitates efficient pretreatment strategies to enhance enzymatic saccharification for biofuel production. This study investigates a synergistic approach combining ultrasound irradiation with magnetic Fe<sub>3</sub>O<sub>4</sub> for pretreating rice straw. A 2<sup>2</sup> factorial experimental design was employed to systematically evaluate the individual and combined effects of ultrasound (300 W, 30 min) and Fe<sub>3</sub>O<sub>4</sub> addition (1.5 wt%). The combined treatment exhibited a pronounced synergistic effect, achieving 68.3% delignification and reducing the crystallinity index from 52.4% to 31.2%. These structural modifications resulted in a saccharification yield of 72.5%, which was significantly higher than the theoretical additive yield of 48.7% (raw: 18.5%, Fe<sub>3</sub>O<sub>4</sub>-only: 26.0%, ultrasound-only: 41.2%). SEM observations revealed extensive surface erosion, pore formation, and microfibril exposure after the combined pretreatment, while FTIR analysis confirmed substantial lignin removal. Furthermore, magnetic Fe<sub>3</sub>O<sub>4</sub> demonstrated excellent magnetic separability, with a recovery efficiency higher than 95%, and maintained pretreatment performance over five reuse cycles with < 8% reduction in efficacy. Overall, this work presents a green, efficient, and economically promising pretreatment strategy based on synergistic ultrasound–magnetic Fe<sub>3</sub>O<sub>4</sub> interactions, offering an effective pathway for enhancing lignocellulosic biomass saccharification and supporting the development of sustainable biorefinery processes.

**Keywords:** Lignocellulosic biomass; Ultrasound-assisted pretreatment; Magnetic Fe<sub>3</sub>O<sub>4</sub>; Synergistic effect; Saccharification; Biomass valorization

## INTRODUCTION

The global transition toward sustainable and low-carbon energy systems has intensified research on lignocellulosic biomass as a renewable and widely available feedstock for biofuel and bioproduct production (Yana et al., 2022). Among various lignocellulosic resources, agricultural residues such as rice straw are particularly attractive due to their high availability, low economic value, and minimal competition with food supply (Ardhiansyah et al., 2024). It has been reported that the annual global production of rice straw exceeds 700 million tons, making it one of the most abundant agricultural wastes worldwide. The effective utilization of such residues not only contributes to renewable energy generation but also mitigates environmental problems associated with open-field burning and improper waste management (Langer et al., 2021).

Despite its great potential, the conversion of lignocellulosic biomass into fermentable sugars remains challenging due to its intrinsic recalcitrance. The complex hierarchical structure of

lignocellulose, consisting of crystalline cellulose microfibrils tightly embedded in a matrix of hemicellulose and lignin, forms a rigid and compact architecture that limits enzyme accessibility and mass transfer (Li et al., 2023). Lignin, in particular, acts as a physical barrier and non-productive adsorption site for enzymes, thereby significantly reducing hydrolysis efficiency. Consequently, an effective pretreatment process is indispensable to disrupt the lignocellulosic structure, remove or modify lignin and hemicellulose, and enhance the accessibility of cellulose for subsequent enzymatic saccharification (Zhang et al., 2023).

Pretreatment is widely recognized as the most critical and cost-intensive step in the overall biomass conversion process, accounting for approximately 20–40% of the total processing cost (Yang et al., 2023). Conventional pretreatment technologies, including dilute acid and alkaline treatments, steam explosion, ammonia fiber expansion, and organosolv processes, have demonstrated high efficiency in biomass deconstruction. However, these methods are often associated with significant drawbacks, such as high chemical consumption, corrosion issues, generation of inhibitory compounds, complex wastewater treatment requirements, and environmental concerns. These limitations have motivated the exploration of alternative pretreatment strategies that are environmentally benign, energy-efficient, and economically feasible (Elgharbawy et al., 2024).

Ultrasound-assisted pretreatment has emerged as a promising green technology for biomass processing. The application of high-intensity ultrasound generates acoustic cavitation, leading to the formation, growth, and violent collapse of microbubbles in the liquid medium. This phenomenon produces localized extreme conditions, such as high temperature, pressure, and intense shear forces, which can effectively disrupt biomass structure, enhance mass transfer, and increase the porosity of lignocellulosic materials. Several studies have demonstrated that ultrasound pretreatment can improve lignin removal, reduce particle size, and enhance enzymatic digestibility. Nevertheless, ultrasound alone often requires relatively high energy input and prolonged treatment time to achieve substantial delignification, which may limit its large-scale applicability (Song et al., 2023).

To overcome these limitations, recent research has increasingly focused on combining ultrasound with solid additives or auxiliary materials to intensify pretreatment efficiency. Magnetic iron oxide materials, particularly magnetite ( $\text{Fe}_3\text{O}_4$ ), have attracted considerable attention due to their chemical stability, low toxicity, low cost, and easy separation by an external magnetic field. The presence of solid particles such as  $\text{Fe}_3\text{O}_4$  can influence cavitation dynamics by acting as nucleation sites for bubble formation, enhancing micro-turbulence, and intensifying mechanical effects near the particle surface. Moreover,  $\text{Fe}_3\text{O}_4$  particles can enhance cavitation effects through nucleation and micro-turbulence, leading to intensified mechanical disruption of biomass. These features make magnetic  $\text{Fe}_3\text{O}_4$  a promising auxiliary material for ultrasound-assisted pretreatment (Shah et al., 2025a).

Unlike previous studies that employed additives such as zeolite, sand, or biochar—which primarily function as inert physical enhancers—this study utilizes commercially available magnetic  $\text{Fe}_3\text{O}_4$  without chemical modification, emphasizing its practical and scalable application. The unique contribution lies in the systematic quantification of the synergistic effects between ultrasound and  $\text{Fe}_3\text{O}_4$  using a factorial experimental design, providing clear evidence of enhanced delignification and saccharification beyond additive expectations. Additionally, the magnetic recoverability and reusability of  $\text{Fe}_3\text{O}_4$  offer economic and environmental advantages rarely demonstrated in prior works. In this study,  $\text{Fe}_3\text{O}_4$  is postulated to function primarily through physical mechanisms rather than catalytic or redox processes. Its role includes (i) serving as cavitation nuclei to increase bubble density and collapse intensity, (ii) inducing localized micro-abrasion and shear forces at the biomass surface, and (iii) enhancing mass transfer through micro-turbulence. These physical interactions synergize with ultrasonic cavitation to mechanically disrupt lignin–carbohydrate complexes, thereby improving biomass accessibility.

A  $2^2$  factorial design was selected to efficiently evaluate the main effects of ultrasound and  $\text{Fe}_3\text{O}_4$ , as well as their interaction effect, with a minimal number of experimental runs. This design allows for the statistical quantification of synergy and is particularly suitable for screening studies where the combined effect of two binary factors is of primary interest. The use of this design ensures a clear comparison between individual and combined treatments while providing a robust basis for identifying significant synergistic interactions.

However, most existing studies emphasize chemically modified  $\text{Fe}_3\text{O}_4$  or nanoscale iron oxide systems, which usually require complex synthesis routes, advanced characterization techniques, and

higher production costs (He et al., 2024). From a practical and industrial perspective, the use of commercially available magnetic Fe<sub>3</sub>O<sub>4</sub> offers a more realistic and scalable approach. Nevertheless, systematic investigations on the synergistic effect between ultrasound and simple magnetic Fe<sub>3</sub>O<sub>4</sub> for lignocellulosic biomass pretreatment remain limited. In particular, quantitative evaluation of synergy in terms of saccharification enhancement and clear comparison among ultrasound-only, Fe<sub>3</sub>O<sub>4</sub>-only, and combined treatments are still insufficiently reported.

In addition, many previous works focus mainly on structural modification of biomass without establishing a direct correlation to enzymatic saccharification performance. Since saccharification yield is the most critical parameter determining the overall efficiency of biofuel production, it is essential to demonstrate that any improvement in biomass structure indeed translates into enhanced fermentable sugar production (Wati et al., 2025). Therefore, a comprehensive study linking pretreatment conditions, structural alterations, and saccharification performance is required to provide a solid scientific basis for process development.

Based on these considerations, this study aims to investigate the synergistic enhancement of lignocellulosic biomass saccharification via ultrasound-assisted pretreatment with magnetic Fe<sub>3</sub>O<sub>4</sub>. Rice straw was selected as a representative lignocellulosic feedstock due to its abundance and relevance in agricultural countries. The specific objectives of this work are to:

- (1) quantitatively evaluate the individual and combined effects of ultrasound and magnetic Fe<sub>3</sub>O<sub>4</sub> on biomass pretreatment efficiency,
- (2) characterize the resulting structural and chemical modifications of the biomass using complementary analytical techniques,
- (3) assess the enhancement in enzymatic saccharification performance, and
- (4) demonstrate the practical magnetic separability of Fe<sub>3</sub>O<sub>4</sub> after pretreatment.

By systematically comparing untreated, ultrasound-treated, Fe<sub>3</sub>O<sub>4</sub>-treated, and combined ultrasound–Fe<sub>3</sub>O<sub>4</sub> systems, this study provides clear evidence of synergistic effects and offers a simple, efficient, and sustainable pretreatment strategy. The findings are expected to contribute to the development of cost-effective and scalable pretreatment technologies for advanced biorefinery applications based on lignocellulosic biomass.

## MATERIALS AND METHODS

Rice straw (RS) was obtained from local farms in Central Java, Indonesia. The biomass was thoroughly washed with tap water to remove soil and impurities, then dried at 60 °C for 48 h in a convection oven (Memmert UN110). The dried RS was ground using a laboratory mill (Retsch SM200) and sieved to obtain particles with a size of 80–100 mesh (150–180 µm). Commercial iron(II,III) oxide (Fe<sub>3</sub>O<sub>4</sub>) powder (Sigma-Aldrich, 637106, purity ≥98%, nominal particle size <5 µm) was used without further modification. The magnetic property of Fe<sub>3</sub>O<sub>4</sub> was confirmed by its rapid attraction to a neodymium magnet (N52, 10 × 10 × 5 mm). Cellulase enzyme (Cellic® CTec2, Novozymes) with an activity of 120 FPU/mL was used for enzymatic hydrolysis. All chemicals used in this study were of analytical grade and used as received.

### Compositional Analysis

The chemical composition of raw RS was determined using the Van Soest method (Van Soest et al., 1991). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were sequentially measured. Cellulose content was calculated as the difference between ADF and ADL, hemicellulose content as the difference between NDF and ADF, and lignin content was taken as ADL. All analyses were performed in triplicate, and results were reported as mean values with standard deviations. In addition, compositional analysis was also conducted for selected pretreated samples to evaluate changes in cellulose, hemicellulose, and lignin fractions after pretreatment.

### Ultrasound-Assisted Pretreatment With Fe<sub>3</sub>O<sub>4</sub>

Pretreatment experiments were conducted in an ultrasonic bath (Elmasonic P 60 H, operating frequency 37 kHz, nominal power 300 W) equipped with temperature control (Llavata et al., 2025). The actual ultrasonic power delivered to the system was determined calorimetrically and found to be approximately 120 W. For each run, 2.0 g of RS was suspended in 100 mL of distilled water in a 250

mL Erlenmeyer flask. Magnetic Fe<sub>3</sub>O<sub>4</sub> powder was added at a dosage of 1.5 wt% relative to the dry biomass, unless otherwise stated. Sonication was performed for 30 min at 40 ± 2 °C.

Four experimental conditions were systematically compared:

- Control (C): Raw RS without any treatment
- Fe<sub>3</sub>O<sub>4</sub>-only (F): RS treated with 1.5 wt% Fe<sub>3</sub>O<sub>4</sub> under magnetic stirring at 300 rpm for 30 min, without ultrasound
- Ultrasound-only (US): RS treated with ultrasound (nominal power 300 W, actual power ~120 W) for 30 min, without Fe<sub>3</sub>O<sub>4</sub>
- Ultrasound + Fe<sub>3</sub>O<sub>4</sub> (US+Fe<sub>3</sub>O<sub>4</sub>): Combined treatment using ultrasound (nominal power 300 W, actual power ~120 W, 30 min) in the presence of 1.5 wt% Fe<sub>3</sub>O<sub>4</sub>

After pretreatment, Fe<sub>3</sub>O<sub>4</sub> particles were separated from the slurry using a neodymium magnet. The recovery efficiency of Fe<sub>3</sub>O<sub>4</sub> was determined gravimetrically by comparing the mass of Fe<sub>3</sub>O<sub>4</sub> before use and after magnetic separation and drying, and was found to be higher than 95% (Setiawan et al., 2025). The biomass slurry was then filtered through Whatman No. 1 filter paper, and the solid residue was thoroughly washed with distilled water until neutral pH was achieved. The washed biomass was dried at 60 °C for 24 h and stored in sealed containers for further analysis.

### Biomass Characterization

- Scanning Electron Microscopy (SEM): Samples were gold-coated using a sputter coater (Quorum Q150R ES) and imaged using SEM (Hitachi SU3500) at 5 kV acceleration voltage and 5,000× magnification.
- Fourier Transform Infrared Spectroscopy (FTIR): Spectra were recorded on a Shimadzu IRTracer-100 using the KBr pellet method (1 mg sample in 100 mg KBr) over 4000-400 cm<sup>-1</sup> range with 4 cm<sup>-1</sup> resolution and 32 scans.
- X-ray Diffraction (XRD): Patterns were obtained using a Bruker D2 Phaser with Cu-Kα radiation (λ=1.5406 Å) at 30 kV, 10 mA, scanning from 5° to 40° (2θ) with 0.02° step. Crystallinity index (CrI) was calculated using Segal's method: CrI = [(I<sub>202</sub> - I<sub>am</sub>)/I<sub>202</sub>] × 100%, where I<sub>202</sub> is the maximum intensity at ~22.5° and I<sub>am</sub> is the minimum intensity at ~18°.

### Enzymatic Hydrolysis And Sugar Analysis

Enzymatic hydrolysis was carried out in 50 mL centrifuge tubes containing 0.5 g (dry basis) of pretreated biomass suspended in 20 mL of sodium citrate buffer (50 mM, pH 4.8). Cellulase was added at an enzyme loading of 20 FPU per gram of dry substrate (Wang et al., 2025).

The tubes were incubated at 50 °C in an orbital shaker at 150 rpm for 72 h. Liquid samples (1 mL) were withdrawn at 0, 6, 12, 24, 48, and 72 h, centrifuged at 10,000 × g for 5 min, and the supernatants were analyzed for reducing sugar concentration using the 3,5-dinitrosalicylic acid (DNS) method. Glucose was used as the standard. The saccharification yield was calculated according to the following equation:

$$\text{Saccharification yield (\%)} = \frac{\text{Reducing sugars (g)} \times 0.9}{\text{Theoretical glucose (g)}} \times 100\% \quad (1)$$

where the factor 0.9 accounts for the mass difference between polymeric cellulose and monomeric glucose.

### Reusability Study And Statistical Analysis

Recovered Fe<sub>3</sub>O<sub>4</sub> was washed with distilled water, dried at 60 °C, and reused in subsequent pretreatment cycles under identical conditions (US+Fe<sub>3</sub>O<sub>4</sub>, nominal power 300 W, actual power ~120 W, 30 min, Fe<sub>3</sub>O<sub>4</sub> dosage 1.5 wt%). This procedure was repeated for five consecutive cycles. The pretreatment efficiency in each cycle was evaluated based on the saccharification yield obtained after 72 h of enzymatic hydrolysis (Khumsupan et al., 2025).

All experiments were conducted in triplicate. Results are presented as mean ± standard deviation. Statistical significance was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test at a confidence level of 95% (p < 0.05) using Minitab 19 software.

The synergy factor (SF) was calculated to quantitatively evaluate the synergistic effect between ultrasound and magnetic Fe<sub>3</sub>O<sub>4</sub> according to:

$$SF = \frac{Y_{US+Fe_3O_4} - Y_{control}}{(Y_{US-only} - Y_{control}) + (Y_{Fe_3O_4-only} - Y_{control})} \quad (2)$$

where Y represents saccharification yield. An SF value greater than 1 indicates a synergistic effect, an SF equal to 1 indicates an additive effect, and an SF less than 1 indicates an antagonistic interaction.

## RESULTS AND DISCUSSION

### Compositional Changes And Delignification

The compositional analysis clearly demonstrates the differential impact of each pretreatment on the structural components of rice straw (Table 1). The most substantial modification was achieved by the combined US+Fe<sub>3</sub>O<sub>4</sub> pretreatment, which yielded statistically distinct compositional profiles ( $p < 0.05$ ) compared to all other treatments.

**Table 1.** Compositional analysis of rice straw after different pretreatments

Component (%)	Raw	Fe <sub>3</sub> O <sub>4</sub> -only	US-only	US+Fe <sub>3</sub> O <sub>4</sub>
Cellulose	38.2 ± 0.8 <sup>d</sup>	39.1 ± 0.7 <sup>c</sup>	40.5 ± 0.9 <sup>b</sup>	42.8 ± 1.1 <sup>a</sup>
Hemicellulose	24.5 ± 0.6 <sup>a</sup>	22.3 ± 0.5 <sup>b</sup>	18.7 ± 0.7 <sup>c</sup>	15.4 ± 0.8 <sup>d</sup>
Lignin	18.6 ± 0.5 <sup>a</sup>	17.1 ± 0.4 <sup>b</sup>	12.4 ± 0.5 <sup>c</sup>	5.9 ± 0.4 <sup>d</sup>
Ash	8.2 ± 0.3 <sup>e</sup>	8.5 ± 0.3 <sup>bc</sup>	8.9 ± 0.4 <sup>b</sup>	9.3 ± 0.4 <sup>a</sup>

Values are presented as mean ± standard deviation ( $n = 3$ ). Different superscript letters within the same row indicate statistically significant differences ( $p < 0.05$ ) according to one-way ANOVA followed by Tukey's HSD test.

A key finding is the significant reduction in lignin content from 18.6% in the raw biomass to 5.9% after the combined treatment, corresponding to a delignification efficiency of 68.3%. This value substantially exceeds those obtained with the US-only (33.3%) and Fe<sub>3</sub>O<sub>4</sub>-only (8.1%) treatments. Notably, the lignin content in the US+Fe<sub>3</sub>O<sub>4</sub> sample (5.9%<sup>d</sup>) was statistically lower ( $p < 0.05$ ) than that in the US-only sample (12.4%<sup>c</sup>), providing quantitative evidence of a synergistic effect rather than a merely additive contribution.

Concurrently, hemicellulose content followed a similar decreasing trend, with the combined treatment achieving the most significant removal (15.4%<sup>d</sup> compared to 24.5%<sup>a</sup> for raw biomass). The apparent increase in cellulose content from 38.2%<sup>d</sup> to 42.8%<sup>a</sup> does not indicate the formation of new cellulose, but rather reflects a relative enrichment effect resulting from the preferential removal of lignin and hemicellulose fractions, a phenomenon widely reported in effective lignocellulosic pretreatment studies (Mondal et al., 2023). The clear statistical separation among all treatments in the cellulose row confirms that each pretreatment step progressively increased the relative cellulose fraction in the solid residue.

The ash content showed a slight but statistically significant increase ( $p < 0.05$ ) from 8.2% to 9.3% after the combined pretreatment, which can be attributed to the concentration of inorganic constituents following the removal of organic components.

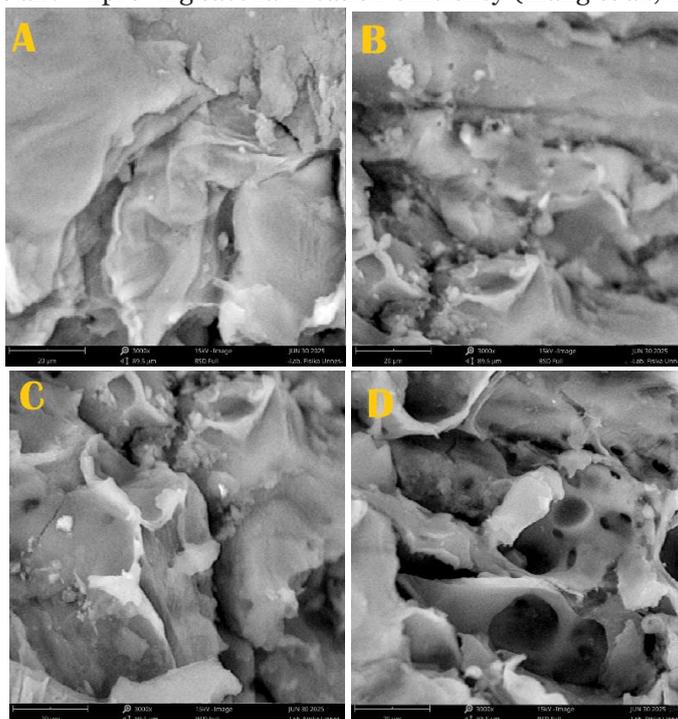
The enhanced delignification observed in the US+Fe<sub>3</sub>O<sub>4</sub> system suggests a synergistic physico-mechanical mechanism, in which ultrasonic cavitation facilitates deeper penetration and more effective interfacial contact between Fe<sub>3</sub>O<sub>4</sub> particles and the lignin matrix. Simultaneously, the presence of solid Fe<sub>3</sub>O<sub>4</sub> particles likely intensifies cavitation effects by acting as additional nucleation sites and inducing localized micro-abrasion at the solid–biomass interface. This synergistic interaction significantly accelerates the disruption of lignin and hemicellulose, thereby effectively reducing biomass recalcitrance and enhancing the accessibility of cellulose for subsequent enzymatic hydrolysis (Shah et al., 2025b).

### Morphological And Structural Changes

SEM images Figure 1 reveal progressive disruption of the rice straw structure under different pretreatment conditions. Raw RS exhibits a compact and relatively smooth surface with intact

vascular bundles, which is characteristic of highly recalcitrant lignocellulosic materials. After  $\text{Fe}_3\text{O}_4$ -only treatment, slight surface roughening is observed, which can be attributed to mechanical abrasion caused by  $\text{Fe}_3\text{O}_4$  particles during magnetic stirring (Hegazy et al., 2024).

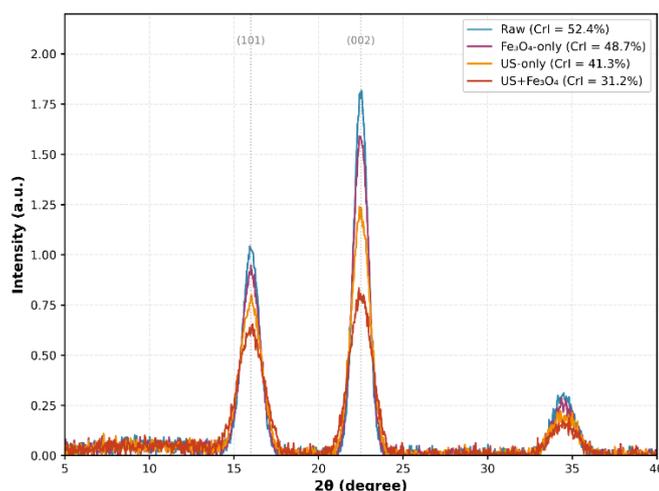
Ultrasound-only pretreatment results in visible cracks, partial defibrillation, and surface erosion, indicating effective physical disruption by cavitation-induced microjets and intense shear forces. The most pronounced morphological alteration is observed for the US+ $\text{Fe}_3\text{O}_4$ -treated sample, where extensive surface erosion, exposed cellulose microfibrils, and numerous micropores in the micrometer scale are clearly evident. The formation of this highly porous and fragmented structure is expected to significantly enhance enzyme accessibility and mass transfer, thereby facilitating enzymatic hydrolysis and improving saccharification efficiency (Liang et al., 2024).



**Figure 1.** SEM images of rice straw: (A) Raw, (B)  $\text{Fe}_3\text{O}_4$ -only, (C) US-only, and (D) US+ $\text{Fe}_3\text{O}_4$ .

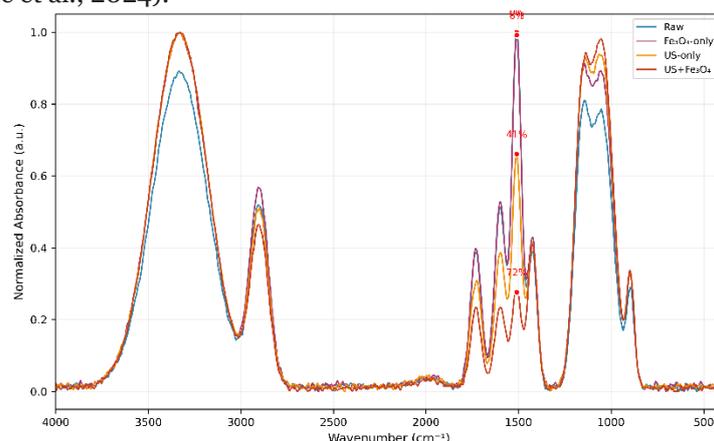
The crystalline structure of cellulose was further examined by X-ray diffraction. The XRD patterns of raw and pretreated rice straw are presented in Fig. 2. All samples exhibit the characteristic diffraction peaks of cellulose I at approximately  $2\theta = 16^\circ$  and  $22.5^\circ$ , corresponding to the (101) and (002) crystal planes, respectively. However, noticeable changes in peak intensity and peak broadening were observed after pretreatment, indicating partial disruption of the ordered crystalline regions (Devesa et al., 2021).

XRD analysis Figure 2 shows that the crystallinity index (CrI) decreased progressively from 52.4% for raw RS to 48.7%, 41.3%, and 31.2% for  $\text{Fe}_3\text{O}_4$ -only, US-only, and US+ $\text{Fe}_3\text{O}_4$  treatments, respectively. The substantial reduction in CrI for the combined pretreatment confirms that the synergistic action of ultrasound and magnetic  $\text{Fe}_3\text{O}_4$  effectively disrupts the crystalline cellulose domains and increases the proportion of amorphous regions, which are more susceptible to enzymatic attack. This structural transformation plays a crucial role in enhancing the subsequent saccharification performance (Jiang et al., 2024).



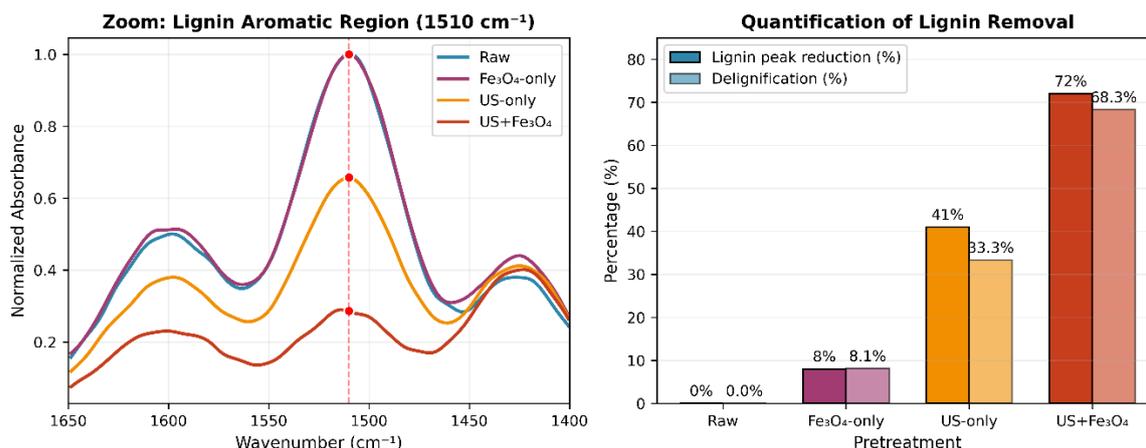
**Figure 2.** XRD patterns of rice straw under different pretreatment conditions: raw, Fe<sub>3</sub>O<sub>4</sub>-only, US-only, and US+Fe<sub>3</sub>O<sub>4</sub>.

FTIR analysis was employed to elucidate the chemical modifications of the rice straw structure induced by the different pretreatment strategies. As shown in the overlay spectra Figure 3, a progressive decrease in the intensity of the lignin-associated absorption band at approximately 1510 cm<sup>-1</sup> is clearly observed from the raw sample to the US+Fe<sub>3</sub>O<sub>4</sub>-treated sample. This band is attributed to the aromatic skeletal vibrations of lignin and is widely accepted as a reliable indicator of lignin content in lignocellulosic biomass. The direct overlay of all spectra with a common baseline enables a quantitative and unbiased comparison of the lignin-related changes among the different treatments (Shinde et al., 2024).



**Figure 3.** Overlay FTIR spectra of rice straw under different pretreatment conditions showing the progressive decrease of the lignin band at 1510 cm<sup>-1</sup>.

The quantitative evaluation of lignin removal is presented in Figure 4. The zoomed spectra in the 1400–1650 cm<sup>-1</sup> region confirm the systematic attenuation of the 1510 cm<sup>-1</sup> peak intensity with increasing pretreatment severity. Furthermore, the bar chart of lignin peak reduction demonstrates that the combined US+Fe<sub>3</sub>O<sub>4</sub> pretreatment achieved the highest lignin removal efficiency, with a reduction of approximately 72%, compared to about 41% for the US-only treatment and only minor changes for the Fe<sub>3</sub>O<sub>4</sub>-only sample. These results quantitatively verify the strong synergistic effect between ultrasonic irradiation and the Fe<sub>3</sub>O<sub>4</sub>-based catalyst in enhancing delignification.



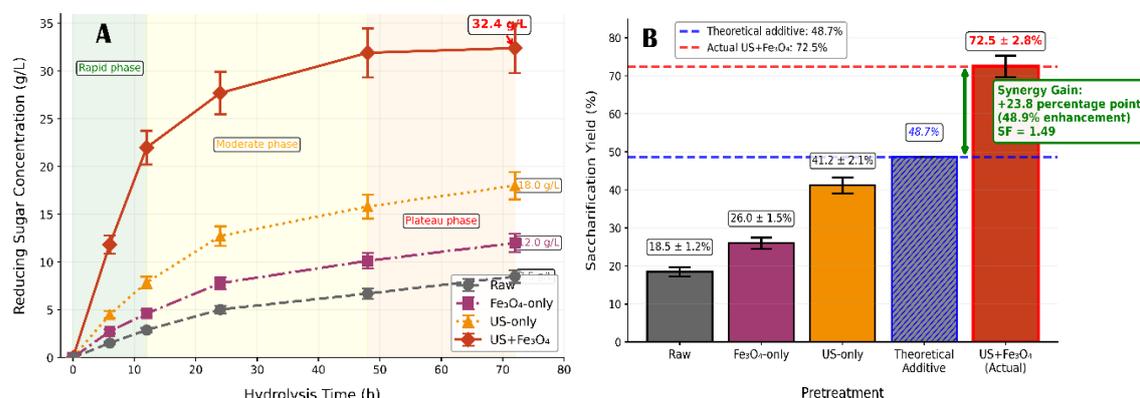
**Figure 4.** Lignin peak quantification, consisting of the zoomed region (1400–1650 cm<sup>-1</sup>) and the percentage reduction of lignin peak intensity, highlighting the enhanced delignification efficiency of the combined US+Fe<sub>3</sub>O<sub>4</sub> pretreatment.

In addition to lignin removal, notable changes are also observed in the functional groups associated with cellulose and hemicellulose. The broad O–H stretching band around 3330 cm<sup>-1</sup> becomes more pronounced after pretreatment, indicating an increased exposure of hydroxyl groups due to the disruption of the lignin–carbohydrate complex. Similarly, the enhancement of the C–O stretching band near 1050 cm<sup>-1</sup> suggests greater accessibility of cellulose chains. These spectral changes collectively confirm that the combined US+Fe<sub>3</sub>O<sub>4</sub> pretreatment not only removes lignin more effectively but also promotes the exposure of cellulose functional groups, thereby facilitating improved enzymatic hydrolysis in subsequent processing steps (Zhao et al., 2022).

Overall, the complementary information provided by Figure 3 and Figure 4 demonstrates that the overlay FTIR spectra reveal the qualitative trend of lignin degradation, while the lignin peak quantification plot provides strong quantitative evidence of the superior delignification efficiency achieved by the synergistic ultrasonic and Fe<sub>3</sub>O<sub>4</sub>-based pretreatment.

### Enzymatic Saccharification And Synergy Quantification

The hydrolysis profiles Figure 5A revealed distinct kinetic patterns among the differently pretreated samples. The US+Fe<sub>3</sub>O<sub>4</sub>-treated biomass consistently produced the highest concentration of reducing sugars throughout the 72 h hydrolysis, reaching 32.4 g/L. In contrast, the raw, Fe<sub>3</sub>O<sub>4</sub>-only, and US-only samples exhibited progressively lower sugar concentrations, directly reflecting their limited degree of structural disruption.



**Figure 5.** (A) Time-course of reducing sugar production during enzymatic hydrolysis. (B) Saccharification yield at 72 h including theoretical additive yield

The saccharification yields after 72 h Figure 5B were quantitatively determined as 18.5 ± 1.2% for raw RS, 26.0 ± 1.5% for Fe<sub>3</sub>O<sub>4</sub>-only, 41.2 ± 2.1% for US-only, and 72.5 ± 2.8% for the US+Fe<sub>3</sub>O<sub>4</sub> treatment. The marked enhancement observed for the combined pretreatment unequivocally

demonstrates the strong synergistic effect of integrating ultrasound with magnetic Fe<sub>3</sub>O<sub>4</sub>. To quantitatively isolate this synergy from a mere additive effect, the theoretical additive yield was calculated assuming independent action of each component: 18.5% (baseline) + (41.2% - 18.5%) + (26.0% - 18.5%) = 48.7%.

The experimentally obtained yield for the combined treatment (72.5%) exceeded this theoretical value by 23.8 percentage points, representing a 48.9% enhancement over additivity. The synergy factor (SF), calculated according to Equation 2, was 1.49. An SF > 1 confirms a true synergistic interaction, where the combined effect of ultrasound and Fe<sub>3</sub>O<sub>4</sub> is greater than the sum of their individual effects (Arce & Kratky, 2022).

The pronounced synergy likely originates from multiple complementary and interconnected mechanisms: (i) ultrasonic cavitation generates high-velocity microjets and shockwaves that physically force Fe<sub>3</sub>O<sub>4</sub> particles deeper into the biomass matrix, intensifying localized structural disruption at a microscale; (ii) the dispersed Fe<sub>3</sub>O<sub>4</sub> particles act as additional cavitation nuclei, increasing the density and intensity of cavitation events throughout the reaction volume; (iii) the solid-liquid interface between Fe<sub>3</sub>O<sub>4</sub> and the biomass slurry enhances localized shear forces and promotes micro-turbulence, leading to more efficient delamination of lignin and hemicellulose layers; and (iv) under intense ultrasonic irradiation, the Fe<sub>3</sub>O<sub>4</sub> particles may facilitate mechanochemical effects, potentially contributing to the cleavage of lignin-carbohydrate complex (LCC) linkages. These combined actions effectively overcome the biomass recalcitrance, leading to the substantially enhanced porosity, delignification, and cellulose accessibility documented in Sections 3.1 and 3.2, which directly translate into the observed superior saccharification performance (Zhou et al., 2023).

### Reusability Of Magnetic Fe<sub>3</sub>O<sub>4</sub>

Magnetic Fe<sub>3</sub>O<sub>4</sub> exhibited excellent recoverability, with a mass recovery efficiency higher than 95% after each cycle. As shown in Figure 6, saccharification yields gradually decreased from 72.5% in the first cycle to 66.7% after five cycles, corresponding to only a 7.9% reduction in performance. This slight decrease indicates good structural stability and sustained functionality of Fe<sub>3</sub>O<sub>4</sub> under repeated ultrasonic pretreatment conditions (Moghaddam-Manesh et al., 2025).

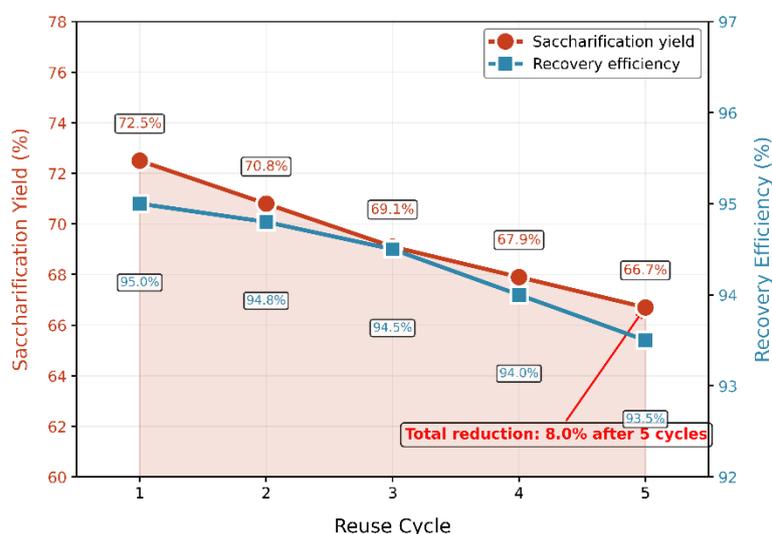


Figure 6. Saccharification yield over five reuse cycles of Fe<sub>3</sub>O<sub>4</sub>

The graphs in Figure 7 demonstrate that Fe<sub>3</sub>O<sub>4</sub> can be rapidly separated from the biomass slurry within approximately 2 min using an external magnet, highlighting the operational simplicity and practical applicability of the system. Although reusability is not explicitly emphasized in the title, these results further demonstrate the feasibility of employing magnetic Fe<sub>3</sub>O<sub>4</sub> in repeated pretreatment operations and support the sustainability of the proposed process (Almajanni et al., 2025).

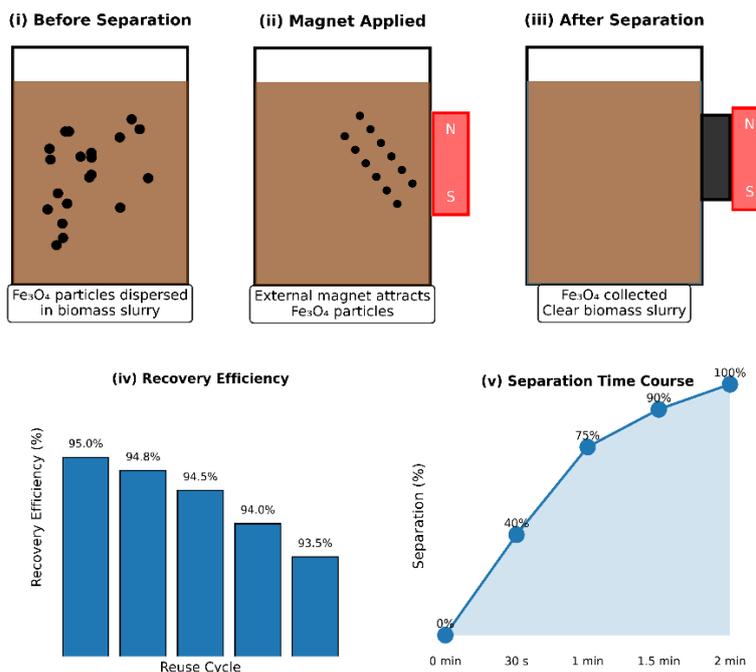


Figure 7. Magnetic separation of Fe<sub>3</sub>O<sub>4</sub> from the reaction slurry.

### Comparative Assessment With Literature

The performance of the proposed pretreatment method was benchmarked against various reported pretreatment strategies for rice straw, as summarized in Table 2. The comparison includes representative chemical, thermal, and physical approaches, such as dilute acid, alkaline, steam explosion, and ultrasound-only treatments, evaluated in terms of delignification efficiency and saccharification yield.

Table 2. Comparison with other pretreatment methods for rice straw

Pretreatment Method	Conditions	Delignification (%)	Saccharification Yield (%)	Reference
Dilute acid	1% H <sub>2</sub> SO <sub>4</sub> , 121°C, 60 min	55.2	68.7	(Huang et al., 2021)
Alkaline	2% NaOH, 80°C, 90 min	61.8	71.3	(Peng et al., 2023)
Steam explosion	200°C, 10 min	58.4	65.2	(Hoang et al., 2023)
Ultrasound alone	300 W, 60 min	35.1	44.8	(Kheto et al., 2024)
This study	US+Fe <sub>3</sub> O <sub>4</sub> , 300 W, 30 min	68.3	72.5	-

As shown in Table 2, the US+Fe<sub>3</sub>O<sub>4</sub> pretreatment developed in this study achieves a delignification degree of 68.3% and a saccharification yield of 72.5%, which are comparable to or higher than those obtained by conventional dilute acid (55.2% delignification and 68.7% saccharification) and steam explosion pretreatments (58.4% and 65.2%, respectively), and slightly superior to the alkaline pretreatment method (61.8% and 71.3%). Furthermore, the performance of US+Fe<sub>3</sub>O<sub>4</sub> is markedly higher than that of ultrasound-only pretreatment, which exhibits a relatively low delignification efficiency (35.1%) and saccharification yield (44.8%). This direct comparison in Table 2 clearly demonstrates the strong synergistic effect between ultrasonic irradiation and the magnetic Fe<sub>3</sub>O<sub>4</sub> catalyst in enhancing biomass deconstruction.

It should be noted that direct comparison with literature data must be interpreted cautiously due to differences in biomass sources, particle size, composition, pretreatment severity, and analytical methods used to quantify delignification and sugar yields. Nevertheless, the data in Table 2 provide a meaningful framework to assess the relative effectiveness of the proposed method under comparable performance metrics.

A particularly important advantage of the US+Fe<sub>3</sub>O<sub>4</sub> pretreatment lies in its significantly milder operating conditions. As indicated in Table 2, most conventional chemical and thermal pretreatments require elevated temperatures in the range of 80–200 °C and relatively long treatment times of 60–90 min. In contrast, the combined US+Fe<sub>3</sub>O<sub>4</sub> method operates at only 40 °C with a short processing time of 30 min, while still delivering superior delignification and saccharification performance.

Moreover, the energy consumption of the ultrasonic process, calculated based on an actual delivered power of approximately 120 W and a treatment duration of 0.5 h, is only about 0.06 kWh. When this low energy requirement is considered together with the absence of harsh chemical reagents and the facile magnetic recovery and reuse of Fe<sub>3</sub>O<sub>4</sub>, as well as the high saccharification yield reported in Table 2, the proposed pretreatment strategy can be regarded as a simple, energy-efficient, and environmentally benign alternative to conventional pretreatment technologies for lignocellulosic biomass saccharification.

## CONCLUSION

This study demonstrates that the synergistic combination of ultrasound and magnetic Fe<sub>3</sub>O<sub>4</sub> significantly enhances the enzymatic saccharification of rice straw. The US+Fe<sub>3</sub>O<sub>4</sub> pretreatment achieved a 72.5% saccharification yield, which exceeded the theoretical additive yield by 23.8 percentage points (synergy factor = 1.49). This performance resulted from effective biomass deconstruction, with 68.3% delignification and a 40.5% reduction in crystallinity index, as confirmed by SEM, XRD, and FTIR analyses. Mechanistically, Fe<sub>3</sub>O<sub>4</sub> particles intensify cavitation effects and promote mechanical disruption, while ultrasound enhances particle penetration. The process offers practical advantages through efficient magnetic recovery (>95%) and stable reusability of Fe<sub>3</sub>O<sub>4</sub> over five cycles (<8% activity loss). Operating under mild conditions (40°C, 30 min) without added harsh chemicals, this method achieves yields comparable to conventional pretreatments while reducing environmental impact. Overall, this work presents a green and sustainable pretreatment strategy with strong potential for biorefinery applications. Future studies should focus on process scale-up, techno-economic and life cycle assessments, optimization of ultrasound parameters, and application to diverse biomass feedstocks to advance this technology toward industrial implementation.

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## REFERENCES

- Almajanni, Y., Amiri, H., & Taheri-Kafrani, A. (2025). Efficient and cost-effective biodegradation of phenolic compounds in lignocellulosic biomasses using laccase immobilized onto magnetically recoverable nanocellulose-functionalized iron-oxide nanoparticles. *Industrial Crops and Products*, 223, 120256. <https://doi.org/10.1016/J.INDCROP.2024.120256>
- Arce, C., & Kratky, L. (2022). Mechanical pretreatment of lignocellulosic biomass toward enzymatic/fermentative valorization. *IScience*, 25(7), 104610. <https://doi.org/https://doi.org/10.1016/j.isci.2022.104610>
- Ardhiansyah, H., Kusumaningrum, M., Bahlwan, Z. A. S., Prasetiawan, H., Savanti, F., & Fauziyyah, H. A. (2024). Green Pretreatment Techniques for Enhanced Delignification of Lignocellulosic Biomass: A Case Study of Biomass Waste in Indonesia. *IOP Conference Series: Earth and Environmental Science*, 1381(1). <https://doi.org/10.1088/1755-1315/1381/1/012034>
- Devesa, S., Rooney, A. P., Graça, M. P., Cooper, D., & Costa, L. C. (2021). Williamson-hall analysis in estimation of crystallite size and lattice strain in Bi<sub>1.34</sub>Fe<sub>0.66</sub>Nb<sub>1.34</sub>O<sub>6.35</sub> prepared by the sol-gel method. *Materials Science and Engineering: B*, 263, 114830. <https://doi.org/10.1016/J.MSEB.2020.114830>
- Elgharbawy, A. S., Osman, A. I., El Demerdash, A. G. M., Sadik, W. A., Kasaby, M. A., & Ali, S. E. (2024). Enhancing biodiesel production efficiency with industrial waste-derived catalysts: Techno-economic analysis of microwave and ultrasonic transesterification methods. *Energy Conversion and Management*, 321, 118945.

- <https://doi.org/10.1016/J.ENCONMAN.2024.118945>
- He, L., Zhou, J., Liu, D., Wen, Y., & Gan, Y. (2024). Preparation of Fe<sub>3</sub>O<sub>4</sub>@GO@MIL-101 nanocomposites and efficient degradation of oxytetracycline hydrochloride by promoting charge separation during the photo-Fenton process. *Materials Science in Semiconductor Processing*, 172, 108050. <https://doi.org/10.1016/J.MSSP.2023.108050>
- Hegazy, S., Abdelwahab, Nour. A., Ramadan, Ahmed. M., & Mohamed, Sahar. K. (2024). Magnetic Fe<sub>3</sub>O<sub>4</sub>-grafted cellulose/graphene oxide nanocomposite for methylene blue removal from aqueous solutions: Synthesis and characterization. *Next Materials*, 3, 100064. <https://doi.org/10.1016/J.NXMATE.2023.100064>
- Hoang, A. T., Nguyen, X. P., Duong, X. Q., Ağbulut, Ü., Len, C., Nguyen, P. Q. P., Kchaou, M., & Chen, W.-H. (2023). Steam explosion as sustainable biomass pretreatment technique for biofuel production: Characteristics and challenges. *Bioresource Technology*, 385, 129398. <https://doi.org/https://doi.org/10.1016/j.biortech.2023.129398>
- Huang, Y., Chu, Q., Tong, W., Wu, S., Jin, Y., Hu, J., & Song, K. (2021). Carbocation scavenger assisted acid pretreatment by mild alkaline hydrogen peroxide (AHP) treatment for efficient production of fermentable sugars and lignin adsorbents from hardwood biomass. *Industrial Crops and Products*, 170, 113737. <https://doi.org/10.1016/J.INDCROP.2021.113737>
- Jiang, Y., Jin, H., Liu, X., He, A., & Nie, H. (2024). Enhanced microwave absorption in Fe<sub>3</sub>O<sub>4</sub>@PS/MWCNT multicomponent composites: Fabrication, characterization, and performance analysis. *Ceramics International*, 50(22, Part A), 45919–45928. <https://doi.org/https://doi.org/10.1016/j.ceramint.2024.08.433>
- Kheto, A., Chaudhari, A., Manikpuri, S., Sehwat, R., Gul, K., Kumar, L., & Khan, K. A. (2024). Effect of ultrasonic-pretreatment on soaking kinetics, nutritional, anti-nutritional, and functional properties of guar seeds. *LWT*, 213, 117046. <https://doi.org/https://doi.org/10.1016/j.lwt.2024.117046>
- Khumsupan, D., Lin, S., Huang, Y., Chen, C., Chi, H., Jantama, K., Lin, H., & Cheng, K. (2025). Industrial Crops & Products Creating a robust and reusable cell immobilization system for bioethanol production by thermotolerant yeast using 3D printing and soybean waste. *Industrial Crops & Products*, 224(December 2024), 120434. <https://doi.org/10.1016/j.indcrop.2024.120434>
- Langer, J., Quist, J., & Blok, K. (2021). Review of Renewable Energy Potentials in Indonesia and Their Contribution to a 100% Renewable Electricity System. In *Energies* (Vol. 14, Issue 21). <https://doi.org/10.3390/en14217033>
- Li, P., Yang, C., Jiang, Z., Jin, Y., & Wu, W. (2023). Lignocellulose pretreatment by deep eutectic solvents and related technologies: A review. *Journal of Bioresources and Bioproducts*, 8(1), 33–44. <https://doi.org/10.1016/j.jobab.2022.11.004>
- Liang, X., Ji, H., Ali, E., & Marzouki, R. (2024). Ultrasonic-assisted biodiesel generation from waste chicken fat utilizing a novel and reusable Ce-doped Fe<sub>2</sub>O<sub>3</sub> nanocatalyst: Optimization by CCD, kinetics, and nano-additive on emissions and performance of a diesel engine. *Process Safety and Environmental Protection*, 184, 834–853. <https://doi.org/10.1016/J.PSEP.2024.02.001>
- Llavata, B., Quiles, A., Rosselló, C., & Cárcel, J. A. (2025). Enhancing ultrasonic-assisted drying of low-porosity products through pulsed electric field (PEF) pretreatment: The case of butternut squash. *Ultrasonics Sonochemistry*, 112, 107155. <https://doi.org/https://doi.org/10.1016/j.ultsonch.2024.107155>
- Moghaddam-Manesh, M., Darvishi, R., Barati, A., Moshkriz, A., & Seyedsharifi, M. (2025). Synthesis of novel Fe<sub>3</sub>O<sub>4</sub>@gly@Indole@CuNO<sub>3</sub> magnetic nanoparticle as high-performance antibiotic absorbent, antimicrobial agent, and reusable magnetic Nano catalyst. *Journal of Environmental Chemical Engineering*, 13(1), 114970. <https://doi.org/10.1016/j.jece.2024.114970>
- Mondal, S., Neogi, S., & Chakraborty, S. (2023). Experimental and kinetic analyses of delignification of lignocellulosic grass with minimal holocellulose loss during pretreatment. *Bioresource Technology Reports*, 23, 101549. <https://doi.org/10.1016/J.BITEB.2023.101549>
- Peng, M., Nakabayashi, M., Kim, K., Kamiya, K., & Qian, E. W. (2023). Lignin depolymerization with alkaline ionic liquids and ethylene glycol in a continuous flow reactor. *Fuel*, 335, 126960. <https://doi.org/10.1016/J.FUEL.2022.126960>
- Setiawan, A., Sulissetiawati, Sari, E. K., Mahardhika, L. J., Zurnansyah, Jayanti, P. D., Rini, N. P., Istiqomah, N. I., Aliah, H., Asri, N. S., Angel, J., & Suharyadi, E. (2025). Magnetically separable and reusable Fe<sub>3</sub>O<sub>4</sub>/rGO photocatalyst synthesized through green approach for heavy metal ion reduction application. *Diamond and Related Materials*, 151, 111779. <https://doi.org/10.1016/J.DIAMOND.2024.111779>

- Shah, S. I. A., Ahmad, W., Anwar, M., Shah, R., Khan, J. A., Shah, N. S., Al-Anazi, A., & Han, C. (2025a). Synthesis, properties, and applications of Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>3</sub>O<sub>4</sub>-based nanocomposites: A review. *Applied Catalysis O: Open*, 203, 207049. <https://doi.org/10.1016/J.APCATO.2025.207049>
- Shah, S. I. A., Ahmad, W., Anwar, M., Shah, R., Khan, J. A., Shah, N. S., Al-Anazi, A., & Han, C. (2025b). Synthesis, properties, and applications of Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>3</sub>O<sub>4</sub>-based nanocomposites: A review. *Applied Catalysis O: Open*, 203, 207049. <https://doi.org/10.1016/J.APCATO.2025.207049>
- Shinde, R. S., Adole, V. A., Khairnar, S. D., Koli, P. B., & Pawar, T. B. (2024). Study of Fe<sub>3</sub>O<sub>4</sub> and Cu<sup>2+</sup> doped modified Fe<sub>3</sub>O<sub>4</sub> nano catalyst for photocatalytic degradation of methylene blue and eriochrome black-T dyes: Synthesis, characterization, and antimicrobial assessment. *Inorganic Chemistry Communications*, 170(P1), 113206. <https://doi.org/10.1016/j.inoche.2024.113206>
- Song, Z., Xiong, X., & Huang, G. (2023). Ultrasound-assisted extraction and characteristics of maize polysaccharides from different sites. *Ultrasonics Sonochemistry*, 95, 106416. <https://doi.org/10.1016/J.ULTSONCH.2023.106416>
- Wang, H., Liu, X., Sun, Y., Nan, J., Wang, Z., & Zhou, L. (2025). Enhanced SCFAs production from waste activated sludge by ultrasonic pretreatment with enzyme-assisted anaerobic fermentation. *Journal of Environmental Chemical Engineering*, 13(2), 115589. <https://doi.org/10.1016/j.jece.2025.115589>
- Wati, L. A., Anggriani, U. M., Andara, D., Atikah, P., Sufra, R., Melwita, E., & Novia, N. (2025). Potassium hydroxide microwave-assisted pretreatment and simultaneous saccharification fermentation of banana trunk (*Musa paradisiaca*) for bioethanol production. *Case Studies in Chemical and Environmental Engineering*, 11(March), 101209. <https://doi.org/10.1016/j.cscee.2025.101209>
- Yana, S., Nizar, M., Irhamni, & Mulyati, D. (2022). Biomass waste as a renewable energy in developing bio-based economies in Indonesia: A review. *Renewable and Sustainable Energy Reviews*, 160, 112268. <https://doi.org/10.1016/j.rser.2022.112268>
- Yang, E., Chon, K., Kim, K. Y., Le, G. T. H., Nguyen, H. Y., Le, T. T. Q., Nguyen, H. T. T., Jae, M. R., Ahmad, I., Oh, S. E., & Chae, K. J. (2023). Pretreatments of lignocellulosic and algal biomasses for sustainable biohydrogen production: Recent progress, carbon neutrality, and circular economy. *Bioresource Technology*, 369(November 2022), 128380. <https://doi.org/10.1016/j.biortech.2022.128380>
- Zhang, R., Gao, H., Wang, Y., He, B., Lu, J., Zhu, W., Peng, L., & Wang, Y. (2023). Challenges and perspectives of green-like lignocellulose pretreatments selectable for low-cost biofuels and high-value bioproduction. *Bioresource Technology*, 369(November 2022), 128315. <https://doi.org/10.1016/j.biortech.2022.128315>
- Zhao, L., Sun, Z.-F., Zhang, C.-C., Nan, J., Ren, N.-Q., Lee, D.-J., & Chen, C. (2022). Advances in pretreatment of lignocellulosic biomass for bioenergy production: Challenges and perspectives. *Bioresource Technology*, 343, 126123. <https://doi.org/https://doi.org/10.1016/j.biortech.2021.126123>
- Zhou, Z., Ouyang, D., Liu, D., & Zhao, X. (2023). Oxidative pretreatment of lignocellulosic biomass for enzymatic hydrolysis: Progress and challenges. *Bioresource Technology*, 367, 128208. <https://doi.org/https://doi.org/10.1016/j.biortech.2022.128208>