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Optimised Alkaline Extraction of Lignin from Maize Stover Agricultural Waste †

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Abstract

Lignin, an abundant biopolymer in biomass, holds significant potential for environmental applications, particularly in heavy metal adsorption from contaminated water. In Mexico, maize stover, a major agricultural by-product in the Bajío region, is often underutilised, leading to environmental pollution. This study focuses on optimising lignin extraction from maize stover to transform this waste into a value-added material, addressing both waste management and water treatment challenges. Lignin was extracted via alkaline hydrolysis using NaOH under varying conditions: NaOH concentration (10–40% m/V), temperature (25–60 °C), and reaction time (3–72 h). Two particle sizes (20 and 100 mesh) were tested, with mechanical agitation or sonication (40 kHz) as energy sources. The extracted lignin was characterised using FTIR spectroscopy to confirm its structural integrity. Highest lignin yield (11%) was achieved using 40% NaOH at 25 °C with sonication for 25 min, matching the theoretical lignin content in maize stover (11.1%). Traditional mechanical agitation at 60 °C for 72 h yielded only 8%. Ultrasonication not only improved efficiency but also reduced reaction time and energy consumption. Particle size (20 mesh) marginally enhanced yields, though handling larger particles proved more practical. FTIR analysis confirmed the characteristic lignin functional groups, including aromatic rings and hydroxyl groups. NaOH solution was successfully recovered for reuse, enhancing the sustainability of the method. Ultrasonication significantly optimises lignin extraction from maize stover, offering a greener, faster, and more efficient alternative to conventional methods. This approach aligns with circular economy principles by minimising waste and maximising resource efficiency.

Keywords: lignin; maize stover; alkaline hydrolysis; ultrasonication; sustainable extraction

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1. Introduction

Accelerating anthropogenic climate change, driven predominantly by fossil fuel combustion, necessitates an urgent transition towards sustainable energy systems. Devastative impacts of conventional energy resources, including atmospheric CO_2 accumulation, global warming trajectories and associated public health crises underscore

imperative for renewable alternatives. Whitin this framework, biomass emerges as the preeminent bio-based resource for mitigating carbon emissions, owing to this intrinsic role in the biogenic carbon cycle [1].

Biomass is defined as the biodegradable fraction of products, waste or residues of biological origin derived from agricultural activities, forestry or any anthropogenic activity. This includes the biodegradable fraction of municipal and industrial waste. Within the Mexican context, maize stover (*Zea mays*) represents a predominant biomass source, constituting the principal agroindustrial residue. It accounts for approximately 67% of the nation's total agricultural waste output [2]. Bajío region of Guanajuato is a significant contributor, ranking among México's top ten maize-producing areas. Consequently, it generates substantial quantities of associated agricultural residues. Despite partial utilization of this residue as livestock feed within the region, a substantial fraction remains unprocessed. This untreated residue decomposes or is burned in situ, giving rise to pollution issues affecting soils, watercourses and atmospheric emissions through the release of contaminants.

In this context, a significant proportion of this biomass and its derivatives are regarded as low-value waste. One strategy to mitigate the environmental impact associated with this residue is the development of novel and environmentally benign methodologies for valorizing the waste generated within region's agricultural sector. Lignocellulosic biomass is abundant, available, renewable and has served since antiquity as ab energy source. Modern developments have extended biomass use to biocombustibles, chemical industry and advanced materials engineering, with applications from construction to biomedicine and environmental remediation [3]. Agroindustrial residues primarily consist of lignocellulosic biomass, which is structurally a three-dimensional polymer composed of naturally synthesized plant material. A defining characteristic of lignocellulosic material is its role in conferring structural integrity, rigidity and mechanical support to the plant cell wall. This biomass is itself composed of three key polymeric constituents: cellulose, hemicellulose and lignin.

Lignin has attracted particularly extensive and expanding research interest, attributed to its dual significance: as the Earth's second most abundant natural polymer, and due to the emergence of novel valorization pathways that position it as a high-value renewable feedstock. This burgeoning focus is fundamentally aligned with sustainability-driven chemistry [4]. Lignin structure contains numerous hydroxyl groups, which enable the biosorption of heavy metal and serve as reactive sites for chemical modification, thereby enhancing its chemical properties. (Figure 1).

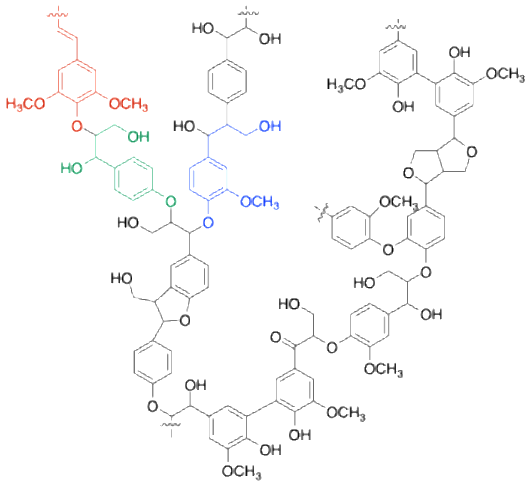

Figure 1. Lignin macromolecular framework (sinapyl alcohol-red, guaiacyl alcohol-blue, *p*-coumaryl alcohol-green) [5].

This study focused on optimizing lignin extraction. Among various methods reported for lignin extraction, alkaline hydrolysis was selected, given that bases such as hydrogen peroxide, calcium hydroxide and sodium hydroxide have proven efficient for alkaline lignin extraction [6]. Specifically, NaOH is used in alkaline pretreatments to promote solubilization and extraction of lignin by disrupting acetyl linkages in hemicellulose and ester linkage between lignin and carbohydrates [7].

2. Methods and Results

2.1. Raw Material Preparation

Maize stover residues were collected from the Bajío region of Guanajuato, México. The biomass was thoroughly washed with distilled water to remove soil and impurities and subsequently dried at 60 °C for 24 h. The dried material was pulverized using a mechanical mill and sieved to obtain two distinct particle sizes: a fine fraction (100 mesh, 0.149 mm) and a coarse fraction (20 mesh, 0.841 mm). The pulverized biomass was stored in a desiccator until use.

2.2. General Lignin Alkaline Extraction with Mechanical Agitation

A mass of 6.0 g of pulverized maize stover (of either 100 or 20 mesh) was placed in a 250 mL round-bottom flask. To this, 100 mL of distilled water was added, and the mixture was hydrated under mechanical stirring (300 rpm) for 30 min at ambient temperature. Subsequently, an amount of NaOH was added to solution to achieve a final concentration within the range of 10–40% mass/Volume (% m/V). Initially alkaline conditions were stablished based on reported literature with different soda concentrations [8].

The reaction mixture was subjected to mechanical agitation for a period ranging from 3 to 72 h. The temperature was controlled a varied between 25 °C and 40 °C using a thermostatically controlled heating mantle. The specific combinations of NaOH concentration, temperature and reaction time were studied systematically, as detailed in Table 1.

Table 1. Optimization of standard reaction conditions for lignin extraction.

Experiment	NaOH (%m/V)	Temperature (°C)	Time	Yield (%)
1	10	25	72 h	Traces
2	10	40	72 h	3
3	40	25	72 h	4
4	40	40	72 h	8
5	40	40	3 h	4
6	40	25	3 h	3

Upon completion of the reaction, the mixture was subjected to gravity filtration to separate the solid residue (cellulose and hemicellulose) from the dark alkaline solution, known as black liquor, which contains the solubilized lignin. The collected black liquor was placed in an ice bath, and dilute H_2SO_4 [1.0 M] was added dropwise under constant stirring until a pH = 2 was attained [9]. This acidification step induced the precipitation of the lignin from the solution. For the lignin recovery, most of the clear supernatant was decanted. The remaining suspension containing the precipitated lignin was transferred to centrifuge tubes and centrifuged at 3000 rpm for 25 min to pellet the solid. The lignin pellet was dried in an oven at 55 °C for 12 h. The resulting dry lignin was weighed, and the extraction yield was calculated using the formula:

$$Yield\ (\%) = \frac{Mass\ of\ dry\ lignin\ (g)}{Mass\ of\ dry\ biomass\ (g)}(100) \quad (1)$$

2.3. Optimization of Standard Reaction Conditions for Lignin Extraction

The initial phase of this study focused on optimizing the key parameters of alkaline hydrolysis using mechanical agitation: NaOH concentration, temperature and reaction time. All experiments start with 6.0 g of biomass. In Experiment 1, employing a low NaOH concentration (10% m/V) at 25 °C for an extended period (72 h) resulted only in trace amounts of lignin. This suggests that the energy barrier for breaking linkages binding lignin to hemicellulose is not surmounted by a diluted alkaline solution at room temperature, even over a prolonged duration. Upon increasing the temperature to 40 °C while maintaining the NaOH concentration at 10% m/V and reaction time at 72 h in Experiment 2, lignin yield improves modestly to approximately 3%, indicating that thermal energy enhances the solvolysis efficiency of the alkaline solution. However, increasing alkali concentration to 40% m/V without temperature elevation in an Experiment 3, yielded a similar lignin amount, indicating that high alkali concentration alone cannot compensate for lack of heating. Experiment 4, show a combined increase in both NaOH concentration to 40% and temperature to 40 °C for 72 h gave the highest yield of 8% (≈480 mg of pure lignin), underscoring the synergistic effect of concentration and temperature in promoting biomass delignification under mechanical stirring. Shortes reaction times (3 h) at 40% NaOH and either 40 °C or 25 °C (Experiments 5 and 6, respectively) resulted in moderated yield reductions to 4% and 3%, confirming the necessity of sustained reaction duration for effective lignin extraction in conventional conditions.

Overall, these experiments demonstrate that efficient lignin recovery via mechanical agitation requires elevated alkali concentration, increased temperature and extended reaction time, to approach but not fully reach the theoretical lignin content (≈11.1%) reported in maize stover [10]. These findings establish a baseline for extraction efficacy and illustrate limitations un conventional hydrolysis methods, motivating exploration of advanced techniques such as ultrasound-assisted reaction, which later data suggest significantly enhance yield and process efficiency [11].

2.4. Optimization of Ultrasound Reaction Conditions for Lignin Extraction

Ultrasound-assisted extraction is an advanced technique for isolation of target compounds from biomass. Process leverages high frequency acoustic energy to disrupt cellular structures and significantly enhance the efficacy of a chosen solvent system. This extraction process has a fundamental mechanism of solid-liquid extraction from biomass occurs in two stages: (1) Solvation and swelling, when solvent initially penetrates tissue plant, hydrating the matrix and solubilizing the desired chemical constituents. (2) Mass transfer: The dissolved compounds then diffuse across the plant tissue and into the bulk solvent, a process governed by concentration gradients and osmotic pressure [12]. The efficacy of ultrasonic irradiation in intensifying this extraction lies in its multi-faceted physical action is attributed to the acoustic cavitation, this phenomenon generates intense localized shear forces and shockwaves in microscopic bubbles within the solvent. These turbulent microenvironments drastically disrupt the biomass facilitating the release of intracellular materials and accelerating their diffusion into the solvent for enhanced mass transfer. Mechanical effects of ultrasound reduce particle size and increase the surface area for interaction. Furthermore, it enhances capillary permeability and solvent access into the plant matrix, overcoming natural hydrophobic barriers. Consequently, ultrasonic irradiation induced a pronounced softening and hydration effect on the rigid plant cell wall, rendering it more pliable. The cumulative mechanical stress from ultrasonic vibration ultimately leads to the physical rupture of cell structures. This synergist breakdown of physical and diffusional barriers results in a marked improvement in extraction yield and kinetics [13].

The comparative analysis of lignin extraction efficiencies under ultrasound irradiation versus conventional mechanical agitation, as summarized in Table 2, elucidated the marked enhancements attributable to sonochemical effects. Bothe methods employed a consistent alkaline concentration of 40% m/V NaOH; however, the source and nature of energy input differed significantly in mode, duration and temperature, providing a robust framework for comparative discussion. Mechanical agitation combined with convection heating at 60 °C for 72 h in Experiment 4, achieved an 8% lignin yield, reflecting the convectional baseline extraction efficiency under optimized alkaline hydrolysis conditions. Notably, when mechanical stirring at ambient temperature was employed for the same duration in Experiment 3, lignin recovery dropped to 4%, underscoring the vital contribution of thermal ene4rgy in facilitating biomass delignification.

Introduction of ultrasound at 40 kHz fundamentally transformed the extraction kinetics and yield outcomes. Ultrasound-assisted extraction performed at 60 °C but for a dramatically reduced total irradiation time of only 25 min in Experiment 7, elevated the yield to 10.5%, surpassing the mechanical baseline despite a reaction time reduction. Furthermore, remarkably comparable yields of approximately 11% were attained when ultrasonication was applied at ambient temperature for 25 min (Experiment 8), matching the ≈11% lignin content reported for maize stover [10]. This indicates that ultrasound compensates for the absence of external heating through localized cavitational microenvironments inducing intense shear forces, microjets and shockwaves at the biomass-liquid interface, thereby enhancing solvent penetration, breaking cellular structures an accelerating mass transfer.

Table 2. Reaction conditions for lignin extraction via ultrasound irradiation vs. mechanical agitation and conventional heating.

Experiment	Energy Source	NaOH (%m/V)	T (°C)	Time	Yield (%)
3	Mechanical/ambient	40	25	72 h	4
4	Mechanical/convection	40	60	72 h	8
7	Ultrasound/convection	40	60	25 min [1]	11
8	Ultrasound/ambient	40	25	25 min [1]	11

[1] Ultrasound at 40 kHz, 5 cycles of 5 min each, 25 °C.

The substancial reduction in reaction time from multiple hours to minutes, alongside decreased or negligible heating requirements, positions ultrasounds-assisted alkaline hydrolysis as a highly atrtractive, energy-efficient alternative. This synergy between ultrasound and alkali chemistry not only optimises lignin solubilisation but also aligns with green chemistry principles by minimizing energy consumption and operational duration.

2.5. Lignin Characterisation

Extracted lignin was characterized by ATR-FTIR spectroscopy (Perkin-Elmer Spectrum Two, Shelton, CT, USA).The FTIR spectrum of the commercial lignin (Sigma Aldrich, St. Louis, MO, USA) and our spectra lignin was compared and were similar. Spectra were analyzed to confirm the presence of key functional group and structural features indicative of the lignin polymer. Signals at 3400–3100 cm^{-1} correspond to O-H stretching of hydroxyl groups and may include absorbed water; 2980–2800 cm^{-1} bands correspond to C-H stretching vibration, 1560 cm^{-1} to aromatic ring stretching and bands near 1000cm^{-1} are consistent with ring vibrations overlapping with C-OH and C-O-C stretches [14,15].

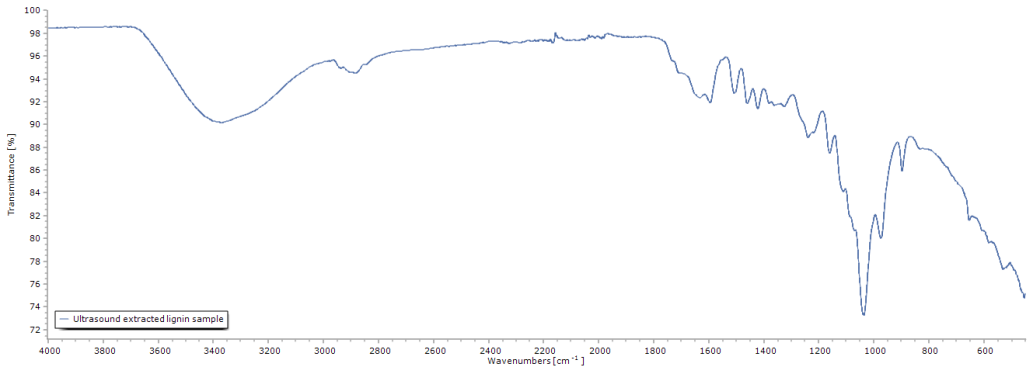

Figure 2. Atenuated total reflection Fourier-transform infrared (ATR-FTIR) spectrum of lignin extracted from maize stover. (Generated using eFTIR [16]).

3. Conclusions

Application of ultrasound significantly accelerates the reaction and enhances lignin solubilization in aqueous phase, leading to yields comparable to those reported in the literature without inducing significant alterations to their structure. This method provides key advantages of operating at ambient temperatures and greatly reducing reaction times. Overall, ultrasonication use represents a substantial optimization of lignin extraction process, presenting a more environmentally benign, fast and efficient alternative to traditional approaches. Furthermore, this technique is consistent with the principles of the circular economy, as it promotes resource efficiency and minimizes waste generation.

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