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Ultrasound-Assisted Alkaline Pretreatment of Biomass to Enhance the Extraction Yield of Valuable Chemicals

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Abstract: As a renewable and sustainable resource, lignocellulosic biomass serves as a crucial raw material for the production of biofuels, biochemicals, and various value-added products. This paper aims to develop and optimize a mild alkaline treatment of sawdust assisted by ultrasound, along with enzymatic hydrolysis of the pretreated material. The alkaline sonochemical pretreatment emerged as the optimal approach to enhance the susceptibility of cellulose to subsequent enzymatic hydrolysis to improve the yield of reducing sugars. A comparative study was performed using various ultrasonic applicators (horn and bath) and conventional assisted alkaline pretreatment. The ultrasonic-assisted pretreatment revealed a higher delignification of 68% (horn) and 57% (bath) compared with conventional pretreatment. Processes were optimized using a statistical analysis based on a 2^3 factorial design. The ratios between sawdust and alkaline solution (R_{SL} = 0.5–1.5 g/100 mL), US amplitude (A = 20–60%), and working temperature (t = 30–50 °C) were selected as process factors. The optimal operating conditions to maximize the reducing sugar yield (138.15 mg GE/$g_{substrate}$) were found as follows: a solid/liquid ratio of $R_{SL,opt}$ = 1.25 g/100 mL, US amplitude of A_{opt} = 60%, and pretreatment temperature of t_{opt} = 50 °C. The overall outcomes clearly confirmed the intensification of delignification by ultrasound-assisted alkaline pretreatment.

Keywords: ultrasound; lignin; wood residue; enzymatic hydrolysis

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Citation: Gavrila, A.I.; Vartolomei, A.; Calinescu, I.; Vinatoru, M.; Parvulescu, O.C.; Psenovschi, G.; Chipurici, P.; Trifan, A. Ultrasound-Assisted Alkaline Pretreatment of Biomass to Enhance the Extraction Yield of Valuable Chemicals. *Agronomy* **2024**, *14*, 903. https://doi.org/10.3390/agronomy14050903

Academic Editors: Giuseppe Squillaci and Alessandra Morana

Received: 5 April 2024
Revised: 23 April 2024
Accepted: 24 April 2024
Published: 26 April 2024

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1. Introduction

The depletion of fossil fuels, coupled with climate change necessitates the shift to bio-based chemical sources and biofuels. Also, environmental safety and economic sustainability may be threatened by rising fossil fuel consumption. Biomass is one of the most abundant and sustainable sources of carbon in the world, for the synthesis of compounds with added value and biofuels. Thus, the need for chemical supplies and alternative energy arises simultaneously [1]. Lignocellulosic biomass is the most abundant bioresource to take into account as feedstock for biorefineries to produce fuels, chemicals, and other valuable products. Lignocellulosic biomass contains cellulose (50%), hemicellulose (woods 10–30% or herbaceous biomass 20–40%) and lignin (woods 20–40% or herbaceous biomass 10–40%) [2]. However, proportions of cellulose, hemicellulose, and lignin inside a single plant will change depending on the age, time of harvesting, and culture circumstances [3]. The aim of lignocellulosic biorefinery is to use the main components—lignin, hemicellulose, and cellulose—as a building block platform [4].

Lignocellulose sources include agricultural and forestry waste which includes corn stover, bagasse, wood, grass, forest industrial residues, agro-industrial residues, municipal waste and energy crops, and other materials [5]. The common materials that contain a high

amount of hemicellulose and cellulose are forestry wastes. Statistics show that in furniture manufacturing, more than one-third of a hardwood trunk ends up as waste [6].

In the transformation of lignocellulosic biomass to valuable products, different limiting factors must be overcome. The first problem is the recalcitrance of lignocellulose [7] and the reticular structure of lignin [8]. Consequently, its degradation requires harsh conditions and specific solvents, which makes the process complicated and unsustainable from an ecological and economic point of view. Another issue is due to the various content of components and the approximate chemical constituents in the raw biomass [9]. The most important challenge is the heterogeneity of biomass that leads to a low thermal conductivity that generates a barrier for heat and mass transfer. Conversion and selectivity in biomass transformation tend to be weak due to deficient catalyst–reactant interactions [10]. One of the most challenging aspects involves the extraction of lignin from lignocellulosic biomass, given its complex nature as a phenolic polymer. Despite its complexity, delignification is necessary as lignin impedes the conversion of biomass into valuable chemicals. Applying the saccharification process directly to biomass typically leads to lower sugar yields, whereas combining with delignification makes cellulose more accessible to the medium, which increases the amount of sugar produced [11]. Another limiting factor appears in the cellulose hydrolysis due to its crystallinity, polymerization degree and available surface area. Conversion of lignocellulosic biomass in biorefineries involves three steps: (a) pretreatment of lignocellulosic material to remove the lignin, (b) hydrolysis of cellulose and hemicellulose in fermentable sugars, and (c) transformation of sugars into chemicals and biofuels. In order to reduce the limiting factors prior to biomass conversion, the pretreatment steps present the most important challenge [4,12]. In order to efficiently transform lignocellulosic materials by enzymatic hydrolysis, pretreatment is essential. There are different pretreatment approaches available: biological, chemical, physical, or physicochemical [13]. Typically, the current pretreatment methods have significant limitations in achieving cost-effective, large-scale, environmentally sustainable production. Recently, progress in applied chemistry research has led to the emergence of innovative food processing techniques commercially accessible today. These methods often involve nonconventional and rigorous conditions. Remarkably, in the context of a commercial-scale biorefinery, certain technologies exhibit potential as environmentally friendly approaches for pretreating lignocellulosic biomass, with potential benefits of lower costs and increased productivity [3]. In contrast to other methods, alkali treatment offers several advantages, including a reduced operating time and mild conditions that significantly enhance enzyme hydrolysis [12]. However, alkali pretreatment comes with certain drawbacks, such as long treatment durations and too much alkali usage, which can be addressed by using intensified techniques like ultrasound-assisted methods. Similar to other sound waves, ultrasound travels through a material by inducing a sequence of compression and rarefaction waves in the molecules. When the power level is high enough, the rarefaction cycle can exceed the forces that attract the molecules in the liquid, causing cavitation bubbles to emerge. Small volumes of vapor (or gas) from the medium enter the bubble during its expansion phase and are not completely evacuated after compression. Energy is produced by the collapse of these bubbles during subsequent compression cycles. They induce acoustic cavitation, leading to localized changes in pressure and temperature, causing the generation of highly reactive species such as $HO^{\bullet}$, H atoms, and H_2O_2, which in turn accelerate chemical reactions [14]. Cavitation, along with heat and mechanical actions associated with ultrasound, causes cell wall disruption, particle size reduction, degradation of the plant matrix, and enhances local mass transfer. Ultrasound application can also enhance the efficiency of delignification in thermal and chemical reactions when combined with suitable solvents. Additionally, ultrasound improves the thermal stability of cellulose, with higher breakdown temperatures observed in cellulose extracted using ultrasound-mediated solvent pretreatment compared to techniques without ultrasound [15–17].

The process of converting pretreated lignocellulose into fermentable sugars through enzymatic hydrolysis has long been researched as a means of depolymerizing biomass

in order to produce biofuels and biochemicals [18]. The goal of enzymatic hydrolysis is to liberate simple sugars from hemicellulose and crystalline cellulose. After that, the monosugars are fermented. Therefore, the effective conversion of biomass to the wanted valuable products depends on the efficiency of enzymatic hydrolysis. The enzymatic hydrolysis of lignocellulosic material is more efficient and economical as a result of the pretreatment step [19].

The aim of this paper is the development and optimization of an ultrasound-assisted mild alkaline treatment of lignocellulosic biomass. The pretreated material will be tested in an enzymatic hydrolysis reaction using mild alkaline solutions, low temperatures, and shorter pretreatment times. The raw material consists of sawdust waste from furniture manufactured with equal amounts of softwood (fir) and hardwood (hardwood, namely beech). In this research, the influence of the alkaline pretreatment on lignin separation from extracted lignocellulosic material using different ultrasound equipment (Vibracell VCX probe, Hilscher probe, and ultrasonic bath) was studied. For the optimization of significant parameters, a 2^3 factorial design was used in order to achieve more fermentable sugars with less energy consumption and reduced cost. To the best of our knowledge, the optimization of ultrasound-assisted alkaline pretreatment of sawdust to maximize the reducing sugars yielded during enzymatic hydrolysis was used for the first time in this study.

2. Materials and Methods

2.1. Materials

Wood-chip residue (sawdust) from a furniture factory (Bucharest, Romania), which contains equal proportions of hardwood (beech) and softwood (fir), was used for the experiments. The wood residue was ground and screened to a particle size between 0.315 and 0.5 mm. Before the alkaline pretreatment combined with ultrasounds, the lignocellulosic material was successively extracted with a mixture of toluene:ethanol = 1:2 (for 6 h) and then with water (for 6 h) to remove waxes and extractives, as they have an adverse effect on the degree of enzymatic degradation of lignocellulosic biomass [20].

The standards used for lignin and sugar determination were Kraft lignin with low sulfur content and glucose, respectively, and were purchased from Sigma-Aldrich Co, Bucharest, Romania. The cellulase enzyme used for the enzymatic hydrolysis was the commercial Celluclast 1.5 L (enzyme activity of 700 endoglucanase units per gram) and was purchased from Novozyme, Denmark.

2.2. Procedure of Ultrasound-Assisted Alkaline Pretreatment

The alkaline pretreatment of the extracted sawdust was carried out in a special thermostat-jacketed glass reactor using an ultrasound bath (ES375H Bench Top Ultrasonic Tank, Hilsonic Ultrasonic Cleaners, Birkenhead, UK) with a power of 120 W, a volume of 3 L, a frequency of 40 kHz, and two ultrasonic processors: the Vibracell VCX750 with a titanium probe—Φ = 12 mm (Sonics & Materials, Inc., Newtown, CT, USA) and the Hielscher UP 200St with a glass probe—Φ = 25 mm (Hielscher Ultrasonics GmbH, Teltow, Germany). The reactor was loaded with extracted sawdust and sodium hydroxide solution with a concentration of 40 g/L in ratios of 1:50–1:150 (*w*/*w*) and temperatures of 30–50 °C. These conditions were chosen based on our previous work [21]. The pretreatment time was 5 h, and samples were taken at 1 h intervals. The determination of the lignin content in the resulting supernatant was performed after centrifuging the samples for 10 min at 3500 rpm. The pretreated sawdust was filtered and washed until the pH of the washing water was neutral and dried at 50 °C to a constant weight. The dry pretreated sawdust was stored for further use in enzymatic hydrolysis. To compare the effect of ultrasound in the alkaline pretreatment process, sawdust was also subjected to alkaline pretreatment by the conventional method, without the use of ultrasound, using the same pretreatment conditions. The detailed diagram of the general stages of conversion of sawdust to reducing sugars is shown in Figure 1. All experiments were performed in triplicate.

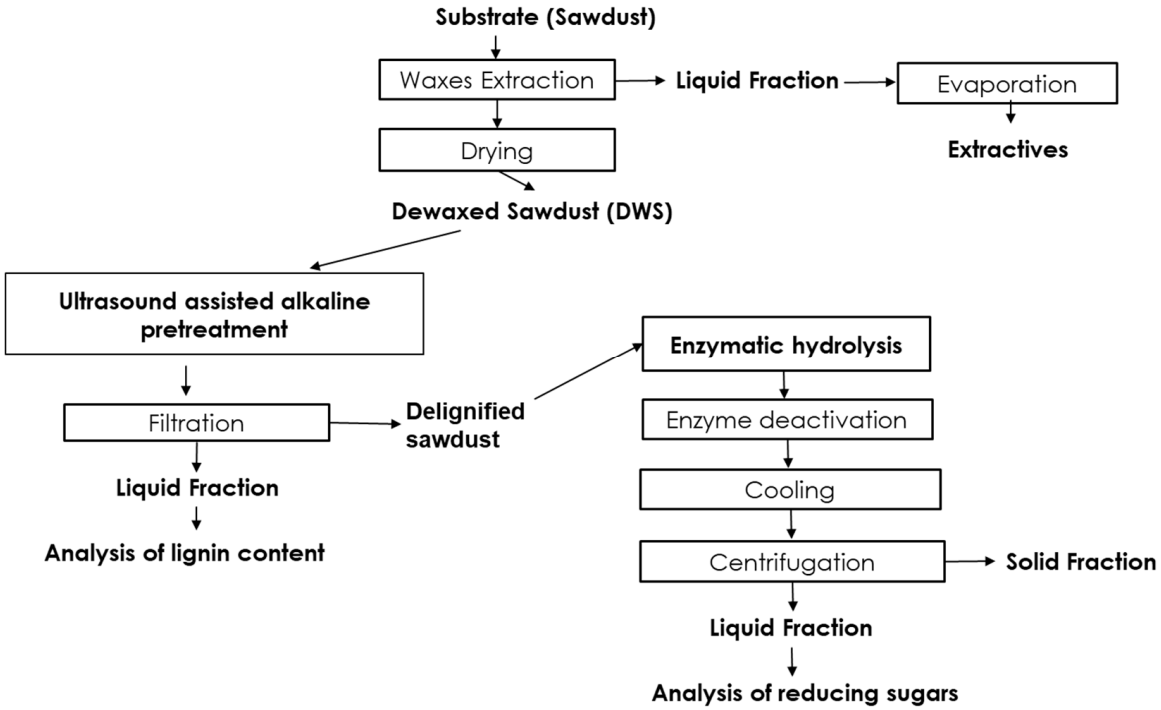

Figure 1. Schematic diagram of the general stages of conversion of sawdust to reducing sugars.

2.3. Analysis of the Soluble Lignin Content

The soluble lignin content was determined according to the Technical Report NREL/TP-510-42618 with minor modifications [22]. The soluble lignin content was expressed as milligrams of lignin per 1 g of dry substrate (mg lignin/$g_{substrate}$), and it was represented using a standard curve that corresponded to 7–200 mg/L of Kraft lignin with low sulfur content solution. The absorbance of the diluted extracts was measured at 320 nm using a Shimadzu UV mini-1240 UV/Visible Scanning Spectrophotometer, 115 VAC (Shimadzu Deutschland GmbH; Duisburg, Germany).

2.4. Enzymatic Hydrolysis Procedure

The treated lignocellulosic material obtained after the alkaline treatment was washed with distilled water until a neutral pH and subjected to enzymatic hydrolysis at a pH value of 5 for 48 h. Enzymatic hydrolysis was performed at 50 °C, in a reciprocating shaking water bath (120 rpm). Hydrolysis processes were carried out in 100 mL reactors containing a mixture of buffer solution at pH = 5 (citric acid/Na_2HPO_4) and treated sawdust in a ratio of 1:25 (*w*/*v* solid/liquid). Enzyme—Celluclast 1.5 L (0.6 mL per gram of substrate) is added to this mixture (the enzyme amount was selected based on preliminary tests that were part of a research project by [21]). During the reaction, samples were taken at 24 and 48 h. In order to stop the hydrolysis process, the samples were heated at 80 °C to deactivate the enzymes, and then, the samples were immersed in iced water. Further, the reaction mixture was centrifuged at 3500 rpm for 10 min to separate the solid residue, and the supernatants were analyzed to determine the reducing sugar concentrations.

2.5. Analysis of Reducing Sugars Content

The concentration of reducing sugars resulting from the enzymatic hydrolysis of lignocellulosic materials was determined following a modified 3,5-dinitrosalicylic acid assay [23,24]. The absorbance was measured at 575 nm using a Jasco V-550 UV/Vis Spectrophotometer. The reducing sugars of the samples were quantified as milligrams of glucose equivalents per 1 g of dry substrate (mg GE/$g_{substrate}$) using a standard curve corresponding to 0.24–2 g/L of glucose solution.

2.6. Statistical Analysis

Data were reported as mean value ± SD (standard deviation) for triplicate measurements (n = 3). Univariate analysis of variance (ANOVA) was employed to assess the dissimilarities of the obtained data. For p-value less than 0.05, the differences were considered statistically significant. XLSTAT Version 2019.1 (Addinsoft, New York, NY, USA) was used for statistical analysis. Statistical analysis and process factor optimization were performed using STATISTICA version 10.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

3.1. Influence of Ultrasound Equipment on the Assisted Alkaline Pretreatment

The main impediment of complete lignocellulosic biomass hydrolysis is the low availability of cellulose due to the "shielding" by lignin. Due to its complex phenolic polymer structure, lignin hinders the conversion of biomass into valuable chemicals. Directly subjecting biomass to the saccharification process typically leads to lower sugar yields. To increase the availability of cellulose for enzymatic action, new strategies and techniques are therefore required. Therefore, incorporating a delignification pretreatment enhances the accessibility of cellulose to the medium, resulting in increased sugar production [11].

In the first stage, the alkaline pretreatment was performed using three different types of ultrasonic applicators: an ultrasound bath (ES375H Bench Top Ultrasonic Tank, Hilsonic Ultrasonic Cleaners, Birkenhead, UK) and two ultrasonic processors (Vibracell VCX-750, Sonics & Materials, Inc., Newtown, CT, USA, and Hielscher UP 200St, Hielscher Ultrasonics GmbH, Teltow, Germany). It can be noticed that the ultrasound pre-treatment of the sawdust leads to significantly higher soluble lignin content compared with the experiments with conventional pre-treatment ($p < 0.05$) (Figure 2A). The highest content of the soluble lignin was obtained for the Vibracell applicator (approximatively 68% higher than conventional pretreatment), followed by the ultrasonic bath and Hielscher horn (approximatively 57% and 35% higher, respectively). This may be due to the cavitation phenomenon, which can accelerate the rate of mass transfer by causing cell wall disruption. The distribution of ultrasound energy in the bath is uneven, and its intensity is low. However, the horn exhibits a high localized intensity of cavitation and, implicitly, a more efficient sonication process [25]. Therefore, the significantly higher soluble lignin content achieved using the Vibracell equipment (an increase of approximately 7% more than the ultrasonic bath and 25% more than Hielscher horn, $p < 0.05$) could be due to these differences between the ultrasonic bath and horns. Therefore, the following ultrasound-assisted alkaline pretreatments of sawdust were performed using the Vibracell ultrasonic processor.

The ultrasound-assisted pretreatment efficiency was also highlighted by the enzymatic hydrolysis of pretreated sawdust in order to determine the production of reducing sugars (Figure 2B). It can be noticed that ultrasonically pretreated sawdust used as the substrate for enzymatic hydrolysis led to a significant increase in sugar yields ($p < 0.05$). The highest content of reducing sugar was reached for ultrasonic pretreated sawdust using the Vibracell horn 56.45 ± 0.81 mg GE/gsubstrate after 48 h enzymatic hydrolysis. This result justified the conclusion that ultrasound-assisted alkaline pretreatment could enhance the enzymatic hydrolysis of sawdust by removing more lignin and increasing its accessibility to hydrolytic enzymes.

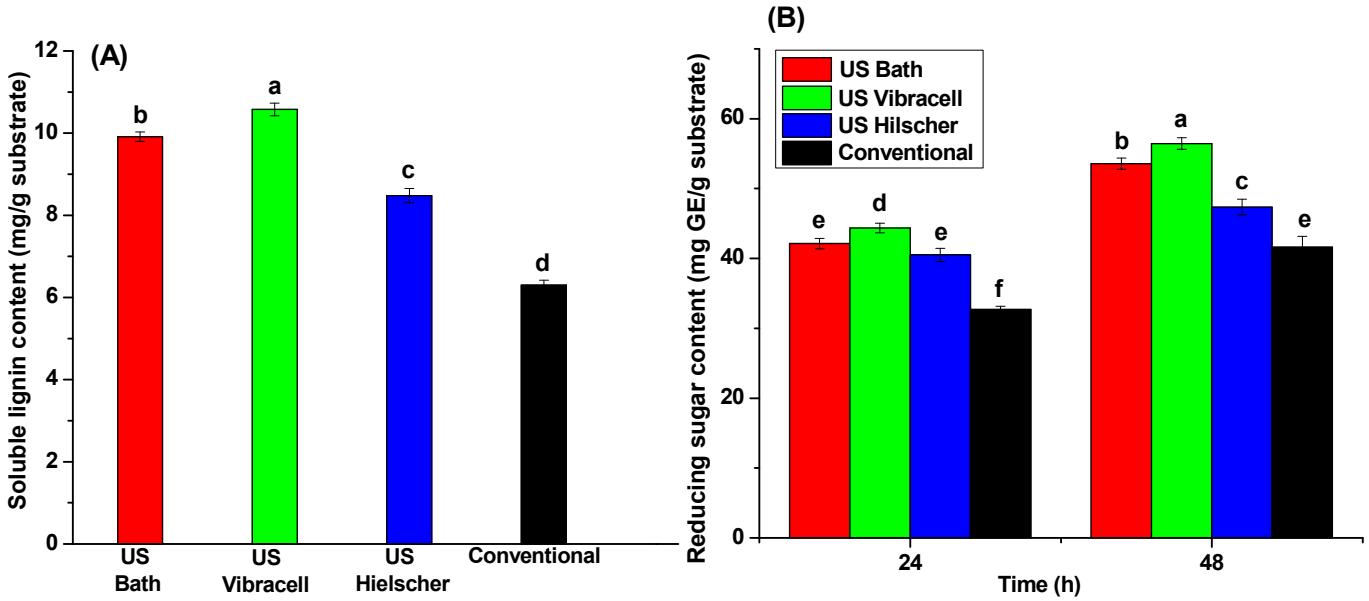

Figure 2. Effect of ultrasound applicators on the lignin and sugar content. (**A**) Ultrasound-assisted alkaline pretreatment (temperature 30 °C, hydrolysis time 1 h, sawdust/solvent ratio = 1/100 (*w*/*v*)). (**B**) Enzymatic hydrolysis of pretreated sawdust (temperature 50 °C, hydrolysis time 48 h, pretreated sawdust/solvent ratio = 1/25 (*w*/*v*), enzyme: Celluclast 1.5 L, 0.5 mL/gsubstrate). The significant differences between groups ($p < 0.05$, ANOVA) are highlighted by different letters (a–f).

3.2. The Influence of Ultrasonic Power on the Lignin Extraction and Sugars Yield

Ultrasound intensity and duty cycle are important factors for the efficiency of the sonochemical processes. To assess the impact of ultrasound amplitude on the ultrasound-assisted alkaline pretreatment, two ultrasound amplitudes of 20 to 40% were chosen. The duty cycle's influence was examined by adjusting the on–off time of ultrasonic irradiation. To determine the influence of the pulse frequency on the ultrasound-assisted alkaline pretreatment, the experiments were performed by changing the sonication time (pulses 3 s on/3 s off—50%; continuous—100%). The ultrasonic power corresponding to each duty cycle was recorded by the Vibracell device. Figure 3 presents the effect of ultrasound amplitude and duty cycle on the soluble lignin content and their effect on enzymatic hydrolysis (Figure 3A,B).

Compared with the use of the conventional process, all the ultrasonic-assisted pretreatments showed better results (Figure 3A). It was observed that the delignification increased with the increase in ultrasound power and in the continuous application mode. As the power increases up to an optimum level, cavitational activity intensifies, leading to a greater extent of delignification. This is attributed to effects such as improved solvent penetration or increased rates of mass transfer [26]. When the actual power dissipation exceeds the optimal power, a large amplitude of ultrasound across the solvent medium creates an excessive number of cavities. This cushioned collapse of the cavities results in a decreased intensity of cavitation, which in turn limits the extent of delignification [27]. Figure 3B presents the effects of ultrasound pretreatment duty cycle on the reducing sugar yield of pretreated sawdust. The reducing sugar yield significantly increased for the continuous ultrasound pretreated samples; the highest sugar content was achieved for an amplitude of 40% (89.2.45 $\pm$ 3.9 mg GE/$g_{substrate}$). The optimal ultrasound amplitude for alkaline pretreatment, with maximization of sugars yield, will be established by the following statistical model.

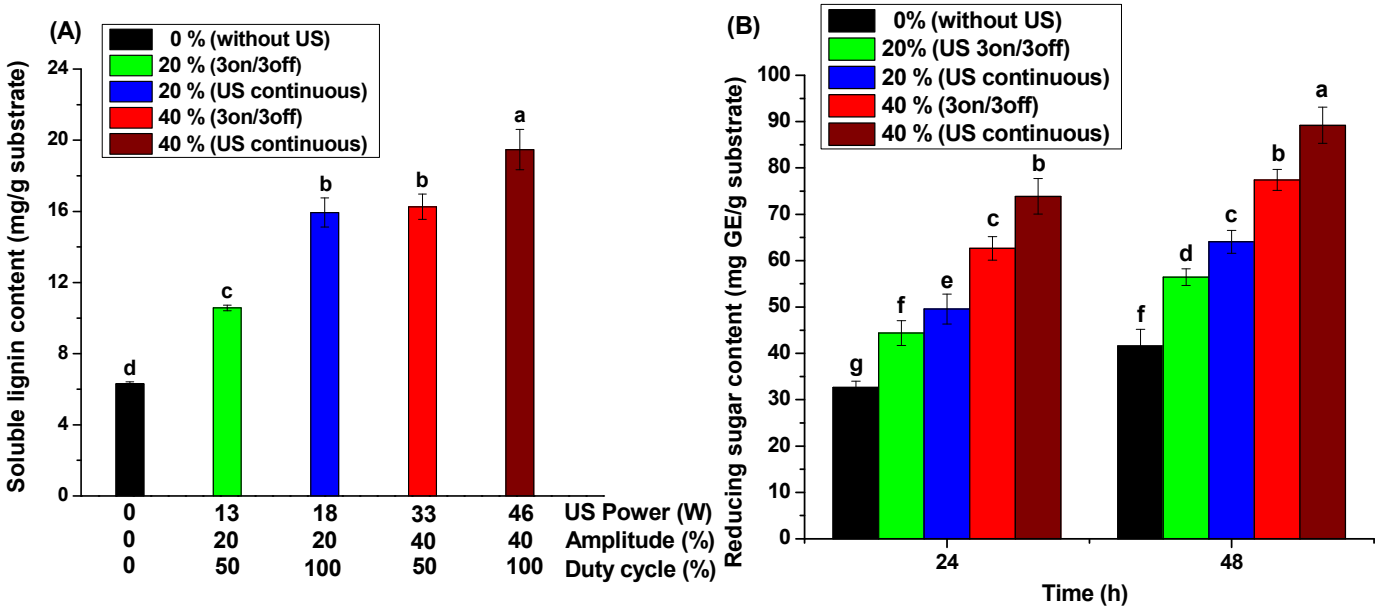

Figure 3. Effect of ultrasonic power on the lignin and sugar content. (**A**) Ultrasound-assisted alkaline pretreatment (temperature 30 °C, hydrolysis time 1 h, sawdust/solvent ratio = 1/100 (*w*/*v*)). (**B**) Enzymatic hydrolysis of sawdust subjected to alkaline pre-treatment combined with ultrasound (temperature 50 °C, hydrolysis time 48 h, pretreated sawdust/solvent ratio = 1/25 (*w*/*v*), enzyme: Celluclast 1.5 L, 0.5 mL/gsubstrate). The significant difference between groups ($p < 0.05$, ANOVA) are highlighted by different letters (a–g).

3.3. Statistical Models

Relevant variables of alkaline pretreatment in terms of the solid/liquid ratio (R_{SL} = 0.5–1.5 g/100 mL), US amplitude (A = 20–60%), and working temperature (t = 30–50 °C) were selected as independent variables (factors). The effects of these process factors on dependent variables (responses), i.e., the yields of lignin (Y_L) and sugars (Y_S), were quantified using statistical models based on a 2^3 factorial design [28]. According to a 2^3 factorial design, eight experimental runs were performed at two levels (minimum and maximum) of process factors (runs 1–8 in Table 1).

Dimensionless values of process factors (x_1, x_2, and x_3) are given by Equations (1)–(3), where $R_{SL,cp}$ = 1 g/100 mL, A_{cp} = 40%, and t_{cp} = 40 °C are center points. In addition, four center-point experimental runs were performed (runs 9–12 in Table 1).

$$x_1 = \frac{R_{SL} - \frac{R_{SL,\max} + R_{SL,\min}}{2}}{\frac{R_{SL,\max} - R_{SL,\min}}{2}} = \frac{R_{SL} - 1}{0.5} \quad (1)$$

$$x_2 = \frac{A - \frac{A_{\max} + A_{\min}}{2}}{\frac{A_{\max} - A_{\min}}{2}} = \frac{A - 40}{20} \quad (2)$$

$$x_3 = \frac{t - \frac{t_{\max} + t_{\min}}{2}}{\frac{t_{\max} - t_{\min}}{2}} = \frac{t - 40}{10} \quad (3)$$

Statistical models described by Equation (4) link the process dimensionless factors x_j (j = 1,2,3) and their interactions to predicted process responses $y_{i,pred}$ (i = 1,2), i.e., $y_{1,pred} = Y_{L,pred}$ and $y_{2,pred} = Y_{S,pred}$, where regression coefficients β_{ki} (k = 1, 2, …, 8, i = 1, 2, 3) were identified from the experimental data specified in Table 1. Statistical analysis highlighted that only β_{1i}, β_{2i}, β_{3i}, and β_{4i} (i = 1,2) in Equation (4) were statistically significant. Consequently, the process responses can be predicted depending on dimension-

less factors using Equations (5) and (6), obtained after removing statistically non-significant terms in Equation (4).

$$y_{i,pred} = \beta_{1i} + \beta_{2i}x_1 + \beta_{3i}x_2 + \beta_{4i}x_3 + \beta_{5i}x_1x_2 + \beta_{6i}x_1x_3 + \beta_{7i}x_2x_3 + \beta_{8i}x_1x_2x_3 \quad (4)$$

$$y_{1,pred} = Y_{L,pred} = 16.58 - 2.073x_1 + 3.899x_2 + 1.343x_3 \quad (5)$$

$$y_{2,pred} = Y_{S,pred} = 114.9 + 9.332x_1 + 10.47x_2 + 8.090x_3 \quad (6)$$

Table 1. Dimensional and adimensional process factors, experimental and predicted process responses (2^3 factorial design).

Run	R_{SL} (g/100 mL)	A (%)	t (°C)	x_1	x_2	x_3	Y_L (mg/g)	Y_S (mg GE/g)	$Y_{L,pred}$ (mg/g)	$Y_{S,pred}$ (mg GE/g)
1	0.5	20	30	−1	−1	−1	11.55	95.08	13.42	87.04
2	1.5	20	30	1	−1	−1	10.17	97.47	9.27	105.70
3	0.5	60	30	−1	1	−1	22.57	101.79	21.21	107.97
4	1.5	60	30	1	1	−1	17.17	132.58	17.07	126.64
5	0.5	20	50	−1	−1	1	15.47	98.47	16.10	103.22
6	1.5	20	50	1	−1	1	14.04	126.39	11.96	121.88
7	0.5	60	50	−1	1	1	25.53	126.61	23.90	124.15
8	1.5	60	50	1	1	1	17.17	140.17	19.75	142.82
9	1	40	40	0	0	0	15.96	106.31	16.58	114.93
10	1	40	40	0	0	0	16.15	129.30	16.58	114.93
11	1	40	40	0	0	0	16.29	118.66	16.58	114.93
12	1	40	40	0	0	0	16.94	106.31	16.58	114.93
13	1.25	60	50	0.5	1	1	18.73	131.56	20.79	138.15
14	1.25	60	50	0.5	1	1	20.16	136.84	20.79	138.15
15	1.25	60	50	0.5	1	1	22.45	140.18	20.79	138.15

Statistically significant regression coefficients and related standard errors (SE_{ki}), t statistics (t_{ki}), and p-values (p_{ki}) are summarized in Table 2. Table 2 also contains the values of the multiple determination coefficient (R^2), adjusted R^2 (R^2_{adj}), standard error of estimate (SEE), F statistic (F), and significance F (p-value for F). Statistical models expressed by Equations (5) and (6) along with corresponding results of multiple regression analysis (Table 2) reveal the following: (i) $Y_{L,pred}$ increases with a decrease in x_1 and an increase in x_2 and x_3; (ii) $Y_{S,pred}$ increases with an increase in all process factors; (iii) there is a reasonable agreement between experimental and predicted values of process responses and both statistical models are statistically significant ($R^2 \geq 0.769$, $R^2_{adj} \geq 0.682$, $SEE \leq 8.882$, $F \geq 8.859$, $p \leq 6.4 \times 10^{-3}$). Accordingly, statistical models expressed by Equations (5) and (6) can be applied to estimate the process performance for factor levels within the ranges considered in the experimental study.

The desirability function approach was applied to identify the optimal factor levels to maximize the process responses [29]. Profiles of predicted values of process responses and desirability function (d) at different levels of dimensionless factors, which are shown in Figure 4, highlight the following optimal levels of process factors: $x_{1,opt} = 0.5$ ($R_{SL,opt} = 1.25$ g/100 mL), $x_{2,opt} = 1$ ($A_{opt} = 60\%$), and $x_{3,opt} = 1$ ($t_{opt} = 50$ °C). Under these optimal conditions, the predicted values of process responses were $Y_{L,pred,opt} = 20.79$ mg/g and $Y_{S,pred,opt} = 138.15$ mg GE/g, while the value of desirability function was $d_{opt} = 0.813$.

Table 2. Results of multiple regression analysis for $y_{1,pred} = Y_{L,pred}$ and $y_{2,pred} = Y_{S,pred}$ expressed by Equations (5) and (6).

Regressor	k	β_{k1}	SE_{k1}	t_{k1}	p_{k1}	β_{k2}	SE_{k2}	t_{k2}	p_{k2}
Intercept	1	16.58	0.468	35.46	0.0000	114.9	2.564	44.82	0.0000
x_1	2	−2.073	0.573	−3.619	0.0068	9.332	3.140	2.972	0.0178
x_2	3	3.899	0.573	6.806	0.0001	10.47	3.140	3.333	0.0103
x_3	4	1.343	0.573	2.345	0.0471	8.090	3.140	2.576	0.0328
R^2			0.890				0.769		
R^2_{adj}			0.849				0.682		
SEE			0.044				8.882		
F			21.64				8.859		
p (significance F)			3.4×10^{-4}				6.4×10^{-3}		

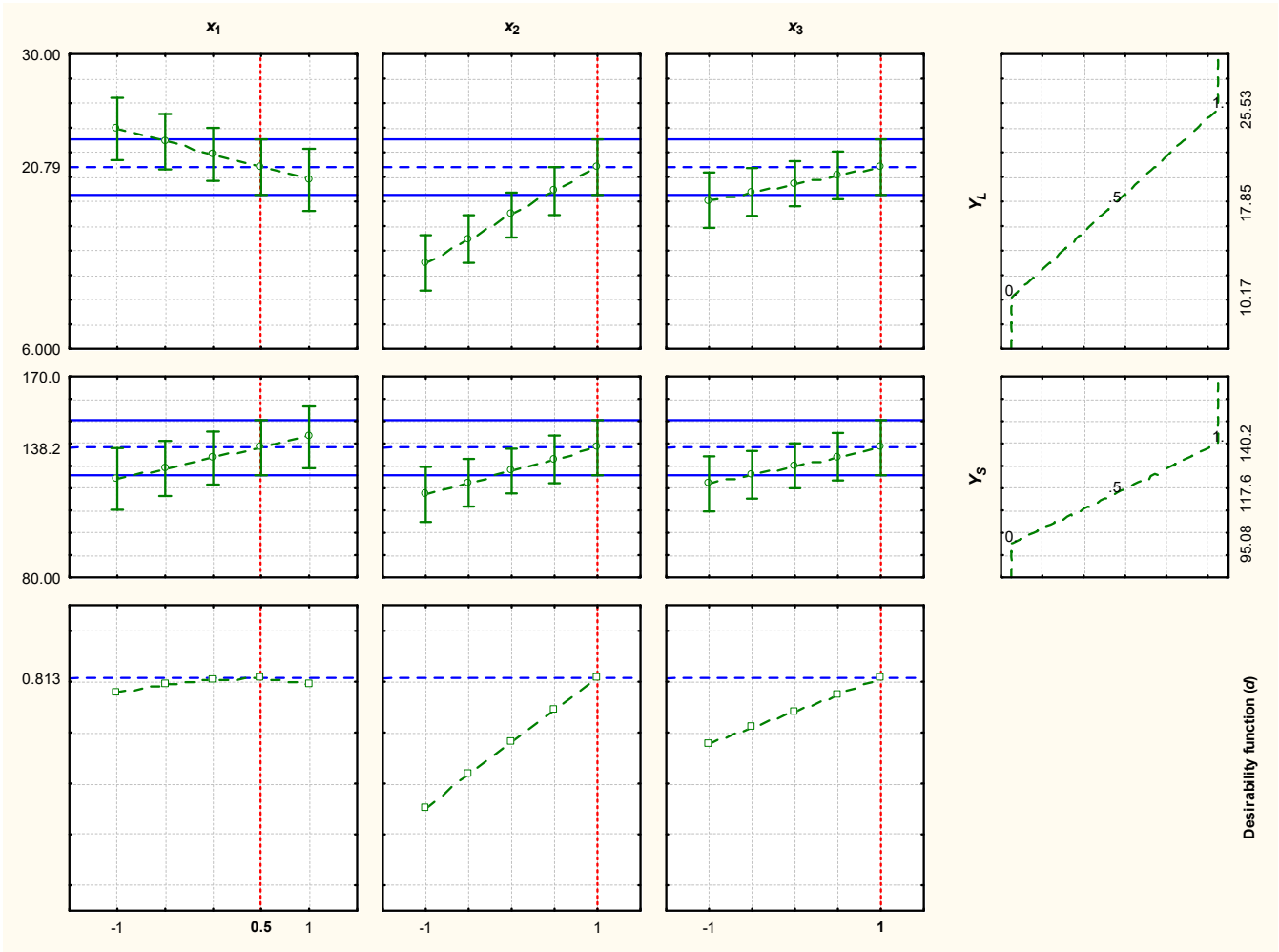

Figure 4. Profiles of predicted values of process responses and desirability function.

To validate the statistical models described by Equations (5) and (6), three experimental runs were performed at optimal levels of process factors (runs 13–15 in Table 1). According to the results of the *t*-test for two samples assuming unequal variances, the mean experimental values of process responses for these replicates ($Y_{L,m,opt}$ = 20.45 mg/g and $Y_{S,m,opt}$ = 136.19 mg GE/g) and related predicted values ($Y_{L,pred,opt}$ = 20.79 mg/g and

$Y_{S,pred,opt}$ = 138.15 mg GE/g) were not significantly different ($p \geq 0.26$), which proves the validity of both statistical models.

4. Conclusions

This study is focused on improving the enzymatic hydrolysis of the wood residue (sawdust) from furniture manufacturing. The influence of the alkaline pretreatment combined with ultrasound on the separation of lignin from the lignocellulosic material (extracted sawdust) was studied. This partial delignification was carried out to improve the availability of the pretreated material for enzymatic hydrolysis in order to obtain a greater yield of reducing sugars. The ultrasound-assisted pretreatment resulted in significant delignification (68%) compared with the conventional pretreatment. A pretreatment with ultrasounds changes the chemical composition of lignocellulose substrates, leading to more effective enzyme accessibility to biomass, improving enzymatic hydrolysis.

A 2^3 factorial design was used to optimize the ultrasound-assisted alkaline pretreatment conditions in order to maximize the soluble lignin content and the concentration of reducing sugars obtained from enzymatic hydrolysis. The optimal operating conditions were found as follows: a solid/liquid ratio of $R_{SL,opt}$ = 1.25 g/100 mL, US amplitude of A_{opt} = 60%, and pretreatment temperature of t_{opt} = 50 °C. Under these conditions, the maximum reducing sugar yield reached 138.15 mg GE/$g_{substrate}$.

These results showed that ultrasound-assisted alkaline pretreatment of lignocellulosic biomass is an efficient and promising alternative to the conventional process. The current pretreatment technique for the processing of the abundant lignocellulosic waste (sawdust) may also be employed as a viable strategy for producing bioethanol. The fermentation settings can be statistically optimized to further improve this. However, in order to adopt a large-scale ultrasounds-based pretreatment process, energy input and cost saving must be evaluated in order to maintain the ideal pretreatment conditions. The full potential benefits of ultrasound pretreatment will only be realized with further effort to scale-up system designs to large batch or continuous processes. However, it should be highlighted that because the initial capital investment and operating expenses of ultrasound applications are high, a critical evaluation of the costs and benefit analysis is required. Based on practical experiences and ultrasonic power, a reasonable estimate of the capital cost might also be determined. These cost estimates serve as a guide when assessing whether treating lignocellulosic materials using ultrasounds is feasible.

Author Contributions: Conceptualization, I.C., M.V. and A.I.G.; methodology, A.T.; software, O.C.P.; validation, I.C., M.V. and O.C.P.; formal analysis, A.I.G. and P.C.; investigation, A.V., P.C. and G.P.; resources, A.I.G.; data curation, A.I.G., A.T. and G.P.; writing—original draft preparation, A.V., A.I.G. and O.C.P.; writing—review and editing, A.I.G. and O.C.P.; supervision, I.C.; project administration, A.I.G., M.V. and I.C.; funding acquisition, A.I.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from the National Program for Research of the National Association of Technical Universities—GNAC ARUT 2023, project: "Encapsulated phytoextracts from autochthonous plants with neuroprotective properties—FitoNeuroPro", code 179, no. 4/06.10.2023.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors acknowledge the financial support received from the Ministry of Research, Innovation, and Digitization, CNCS/CCCDI—UEFISCDI, project "A new hybrid technology for bio-refineries: Microwaves (MW) and Ultrasound (US) in one Apparatus", project number PN-III-P4-ID-PCE-2020-1286. Some pretreatment procedures were possible due to European Regional Development Fund through Competitiveness Operational Program 2014–2020, Priority axis 1, Project No. P_36_611, MySMIS code 107066, Innovative Technologies for Materials Quality Assurance in Health, Energy and Environmental—Center for Innovative Manufacturing Solutions of Smart Biomaterials and Biomedical Surfaces—INOVABIOMED.

Conflicts of Interest: The author Anamaria Vartolomei is an employee of MDPI; however, she did not work for the journal *Agronomy* at the time of submission and publication. The authors declare no conflicts of interest.

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