Biblid: 1450-5029, (2024) 28; 1; p 1 - 6
UDK: 633.15
DOI: 10.5937/jpea28-49908

Original Scientific Paper

LIGNIN REMOVAL FROM CORNCOB BY MICROWAVE-COUPLED PEROXIDE TREATMENT

DELIGNIFIKACIJA KUKURUZNOG OKLASKA POMOĆU PEROKSIDNOG PREDTRETMANA POTPOMOGNUTOG MIKROTALASIMA

Dragana MLADENOVIĆ[1], Jovana GRBIĆ[1], Aleksandra ĐUKIĆ-VUKOVIĆ[2], Dušan MIJIN[2], Ljiljana MOJOVIĆ[2]*
[1]Univerzitet u Beogradu, Inovacioni centar Tehnološko-metalurškog fakulteta, 11000 Beograd, Karnegijeva 4, Srbija
[2]Univerzitet u Beogradu, Tehnološko-metalurški fakultet, 11000 Beograd, Karnegijeva 4, Srbija
**Correspondence: dmladenovic@tmf.bg.ac.rs*

ABSTRACT

Lignocellulosic biomass comprises the crop residues, which remain in large quantities from various stages of crop processing. Its main constituents, cellulose, hemicellulose, and lignin, are interlinked by hydrogen and covalent bonds and form a robust and intricate matrix making it resistant to enzymatic degradation. Pretreatment is crucial in lignocellulosic biomass processing, aiming to remove lignin and enhance enzyme access to polysaccharides.

This study examines how microwave-assisted peroxide pretreatment affects the delignification of corncobs, aiming to enhance the efficiency of utilizing this agricultural residue in fermentation processes. Taguchi orthogonal array was used to optimize pretreatment conditions and assess the effects of individual parameters (temperature, H_2O_2 dose, and pretreatment time) on delignification efficiency.

The results showed the significant influence of pretreatment conditions on lignin removal from corncobs, with the highest delignification achieved at an H_2O_2 dose of 500 mg/g, temperature of 100 °C, and pretreatment time of 2 minutes. H_2O_2 dose had the most substantial impact on the delignification, followed by temperature and pretreatment time. The observed 81.6% delignification and 61.9% increase in cellulose content are pivotal for enzymatic hydrolysis efficiency. This improvement suggests enhanced enzyme availability during hydrolysis and reversible binding to polysaccharide active sites, potentially leading to higher sugar yields.

***Keywords**: lignocellulosic biomass; lignin removal; microwave treatment, optimization study.*

REZIME

Lignoceluloznu biomasu čine ostaci poljoprivrednih kultura koji u velikim količinama zaostaju na obradivim površinama ili nastaju u različitim fazama njihove prerade. Glavne komponente lignocelulozne biomase (celuloza, hemiceluloza i lignin) su međusobno povezane vodoničnim i kovalentnim vezama stvarajući čvrstu i kompleksnu matricu koja je otporna na dejstvo enzima. Važan korak u procesu prerade lignocelulozne biomase je predtretman kojim je potrebno ukloniti lignin i tako omogućiti lakšu pristupačnost polisaharidnih frakcija enzimima.

U ovom radu je ispitivan uticaj peroksidnog predtretmana potpomognutog mikrotalasima na delignifikaciju kukuruznog oklaska sa ciljem dobijanja što čistije polisaharidne frakcije čime bi se omogućila efikasnija valorizacija ovog poljoprivrednog otpada u fermentacionim procesima. Tagučijev ortogonalni niz je korišćen za optimizaciju uslova predtretmana i određivanje uticaja pojedinačnih parametara (temperature, doze H2O2 i vremena predtretmana) na efikasnost delignifikacije. Dodatno, optimalni uslovi su primenjeni u eksperimentu sa konvencionalnim zagrevanjem kako bi se uporedili efekti mikrotalasnog i konvencionalnog predtretmana.

Dobijeni rezultati su pokazali da uslovi predtretmana značajno utiču na efikasnost uklanjanja lignina iz kukuruznog oklaska, a najveći stepen delignifikacije je ostvaren pri dozi H2O2 od 500 mg/g, temperaturi od 100 °C i vremenu predtretmana od 2 min. Utvrđeno je da doza H2O2 ima najveći uticaj na proces delignifikacije, a zatim slede temperatura i vreme predtretmana. Delignifikacija od 81.6% i povećanje sadržaja celuloze od 61.9% su značajni za efikasnost enzimske hidrolize u kojoj se može očekivati bolja dostupnost i reverzibilno vezivanje enzima za aktivna mesta na molekulima celuloze i hemiceluloze, a time i bolji prinosi šećera.

***Ključne reči**: lignocelulozna biomasa, delignifikacija, mikrotalasni tretman, optimizacija.*

INTRODUCTION

As the global population expands, there is a growing urgency for environmentally sustainable methods to produce essential resources like food, fuel, and other necessities. Biomass offers a promising opportunity to meet these expanding needs in a sustainable manner. However, this potential is often hindered by the diversion of valuable food crops like corn towards fuel production, rather than fulfilling immediate food requirements *(Milašinović-Šeremešić et al., 2018)*. This practice, particularly noticeable in developed countries, contrasts with the ongoing challenges of food scarcity and malnutrition that persist in the developing world. The arguable issue of using edible crops for fuel, known as the "food vs. fuel" debate, intensifies when food supplies are redirected for fuel production *(Thompson P.B., 2012)*. An effective strategy for alleviating this problem involves converting agricultural residues such as corn stover (the leaves and stalks of corn) and corncobs into biofuels and other valuable bioproducts.

The complex lignocellulosic structure and composition of agricultural residues present a substantial challenge to their effective utilization in biorefinery processes. These residues consist of primary structural components such as lignin, cellulose, and hemicellulose, which are tightly bound together through covalent and hydrogen bonds. Lignin plays a crucial role in biomass recalcitrance, significantly limiting enzyme access to polysaccharides and impeding their conversion into fermentable sugars *(Jović et al., 2021)*. According to the principles of a biorefinery, for maximizing the value of bioproducts it is essential to fully utilize all components of raw materials. To achieve this objective, an efficient pretreatment process that effectively removes lignin and fractionates biomass is essential, ensuring none of the valuable components are sacrificed in the process.

Oxidative processes are gathering significant attention among the various pretreatment methods studied for biomass fractionation, owing to their numerous advantages. Usually conducted under mild conditions, oxidative pretreatments minimize the formation of inhibitory compounds like furfural *(Grbić et al., 2022; Xia et al., 2022)*. These methods can effectively enhance enzymatic hydrolysis by primarily targeting lignin removal with the aid of oxidants, leaving the majority of cellulose intact in the solid fraction. Alkaline hydrogen peroxide pretreatment is a type of conventional oxidative method that has been applied to the pretreatment of various lignocellulosic biomass like rice hulls and straw, corn stover, bamboo, etc. *(Cabrera, et al., 2014; Mittal et al. 2017; Huang et al., 2020)*, leading to significant improvement of cellulose digestibility, owing to its ability to remove hemicelluloses and lignin. This pretreatment is effective under basic conditions when hydroxyl (OH·) and superoxide (O_2·) radicals are generated by H_2O_2 decomposition playing an important role in the oxidative delignification of biomass *(Zhou et al., 2023)*. The efficacy of alkaline hydrogen peroxide pretreatment is greatly affected by H_2O_2 concentration, alkali loading, biomass loading, pretreatment time and temperature *(Dutra et al., 2018)*. Therefore, it is crucial to optimize process parameters to achieve a balance between lignin removal and preservation of polysaccharide fractions. This equilibrium is vital for the efficient conversion of biomass into biofuels and biochemical. Over the past few decades, microwave pretreatment has emerged as a promising thermal process in biorefinery applications. This method offers several advantages, such as non-contact and volumetric heating, reduced reaction times, lower solvent consumption, minimized side reactions, and ease of parameter control *(Bichot et al., 2020; Aguilar-Reynosa et al., 2017)*. Combining alkaline hydrogen peroxide with microwave treatment could result in decreased processing time and energy consumption, thus reducing the operating costs of the pretreatment process.

The objective of this study was to enhance alkaline hydrogen peroxide pretreatment by integrating microwave irradiation and determining the optimal combination of process parameters for lignin removal from corncob. Microwave-coupled peroxide treatment was performed under different temperature regimes, while the optimal conditions were challenged in conventional heating pretreatment to compare the efficiency of these two heating sources.

MATERIAL AND METHOD

Preparation and pretreatment of corncob

Dried corncob was kindly provided by the farm located in South Banat, Serbia. The biomass is chopped and ground to obtain a particle fraction between 0.5 mm and 1 mm. This fraction was first extracted with 95% ethanol at 100 °C for 30 min to remove extractives that could interfere with the analysis of biomass composition. It was then dried to a constant weight and stored in a desiccator at room temperature until further use.

Pretreatment was carried out in the MonowaveTM 300 microwave reactor (Anton Paar, Austria) using glass vials with a total filling volume of 30 ml. The glass vials were charged with 1 g of corncob biomass and 10 ml of alkaline hydrogen-peroxide solution. Alkaline hydrogen peroxide was freshly made by diluting the commercial 30% solution to the required concentration and correcting the pH to 11.5 with 20% (w/w) NaOH. During pretreatment, the sample in a glass vial was stirred at 400 rpm and the temperature was constantly controlled with an infrared sensor. The pretreatment was carried out at the conditions stated in Table 1, after which the pretreated biomass was vacuum-filtered, thoroughly washed with distilled water and then dried at 50 °C to a constant weight. Dried pretreated biomass was used to analyse solid recovery rate and lignin content. The solid recovery rate was calculated as per the following equation:

$$\text{Solid recovery rate} = \frac{\text{Mass of corncob after pretreatment (dry mass)}}{\text{Mass of untreated corncob (dry mass)}} \times 100\% \quad (1)$$

To compare the efficiency of microwave heating with the conventional heating method, the pretreatment of corncob was performed in an oil bath. A 30 ml glass vials equipped with a stir bar were loaded with 1 g of corncob biomass and 10 ml of alkaline hydrogen peroxide solution. The mixtures were placed into a preheated oil bath heated by a hot plate with magnetic stirring. Pretreatment was carried out at the temperature and time determined to be optimal in the microwave-coupled peroxide experiment. Temperature control was achieved using an infrared sensor, and the pretreatment time was recorded from the moment the target temperature was reached. After pretreatment, biomass was separated from liquid fraction and subjected to the same procedure of washing and drying as previously described.

Pretreatment optimisation by taguchi method

Design of experiment (DOE) was applied to determine the most important factors of microwave-coupled peroxide treatment that affect lignin removal from corncob. Taguchi method is a statistical approach widely used for the optimization of manufacturing and engineering practices because it efficiently improves the quality of industrial products and processes while minimizing variation and cost. Taguchi's design allows for analysing a broad variety of factors and levels with a minimum number of experiments. This optimization methodology uses the signal-to-noise (S/N) ratio to identify the combination of factors and their levels that maximizes the desired outcome while reducing the effects of noise or variability *(Roy, R. K., 2010)*. Taguchi design L9 (3^3) was applied varying three factors (temperature, hydrogen peroxide dosage and pretreatment time) at three levels each. In total, nine experimental runs representing different combinations of factor levels were generated by Minitab® statistical software (Table 1). The lignin content in pretreated biomass was analyzed as a process response and corresponding means and S/N ratios were determined. The goal of this study was to remove the lignin i.e. to minimize the response, so the S/N ratio was calculated by applying smaller is better criteria according to the following equation:

$$\mathrm{S/N} = -10 \times \log\left(\sum \frac{\mathrm{Y}^2}{\mathrm{n}}\right) \quad (2)$$

Where n represents how many times the experiment was repeated under the same conditions, and Y is the estimated value of process response.

After determining the optimal pretreatment conditions, the expected value of process response, as well as the S/N ratio were predicted, and the results were verified by conducting a confirmation experiment. The effect of three factors on the process response was studied using analysis of variance (ANOVA). Each experimental run, including the confirmation experiment, was repeated twice, and all results were analyzed using Minitab® 18 statistical software.

Determination of process response

As a process response, the lignin content in pretreated corncob was estimated following the procedure proposed by *Fuku-*

shima and Kerley (2011). Briefly, the solid sample (5 mg) was mixed with a freshly prepared 25% (w/w) solution of acetyl bromide in glacial acetic acid (0.5 ml). The reaction mixture was incubated at 50 °C for 2 hours and then centrifuged at 10.000×g for 10 minutes. Obtained supernatant (0.1 ml) was combined with glacial acetic acid (1.8 ml), 0.3 M NaOH (0.4 ml), and 0.5 M hydroxylamine hydrochloride (0.2 ml). After mixing well on a vortex mixer, 0.2 ml of this solution was transferred to a 96-well plate and the absorbance was measured at 280 nm using an EpochTM microplate spectrophotometer (BioTek®, USA). A blank prepared in the same way but without biomass was used for absorbance correction. The lignin content was calculated according to the following equation (Fukushima and Kerley, 2011):

$$\text{ABSL}\ (\%) = \frac{A_{280nm}}{\varepsilon \times L} \times \frac{V \times D}{m} \qquad (3)$$

Where ABSL (%) refers to acetyl-bromide lignin, ε is the average extinction coefficient (23.077 l/g cm), L (cm) is pathlength (0.543 in this work), V (ml) is the volume of 25% (w/w) acetyl bromide in glacial acetic acid used for sample digestion, D is the dilution factor from the digested sample (25 in this work), m (mg) is sample weight.

Compositional analysis of corncob biomass

The pretreated corncob underwent additional characterization to determine its cellulose, hemicellulose, and lignin content, following the analytical procedure outlined by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008). Cellulose and hemicellulose content were quantified based on glucose and xylose concentrations, respectively, using a Dionex Ultimate 3000 HPLC system (Thermo Scientific, Waltham, MA, USA) equipped with a carbohydrate column (Agilent, Santa Clara, CA, USA). The analysis was conducted at 80 °C with deionized water as the mobile phase, at an elution rate of 0.6 ml/min. Detection of sugars was carried out using an RI detector (RefractoMax 520, ERC GmbH, Riemerling, Germany) heated to 40 °C, with data acquisition and processing performed using Chromeleon 7.2."

RESULTS AND DISCUSSION

Optimization of process parameters for lignin removal by microwave-coupled peroxide treatment

In this study, the Taguchi method was employed to identify the optimal conditions for microwave-coupled peroxide treatment, aiming to achieve the most effective lignin removal from corncob. A series of nine experimental runs was designed, varying temperature, peroxide dosage, and treatment time as process factors, with each factor set at three levels (Table 1). The Taguchi method utilizes the S/N ratio to identify the combination of factors and their levels that optimize the response while minimizing the influence of noise or variability. The experimental results for lignin content and their corresponding S/N ratios are provided in Table 1.

Table 1. Taguchi L9 orthogonal array, experimental results for lignin content and calculated S/N ratios.

Run	Factors			Results	
	Temperature (°C)	Peroxide dosage (mg/g)	Pretreatment time (min)	Lignin content (%)	S/N ratio
1	60	100	1	21.90±0.27	-26.81
2	60	300	2	17.19±0.83	-24.71
3	60	500	4	11.06±0.42	-20.88
4	80	100	2	18.64±0.85	-25.41
5	80	300	4	12.87±1.41	-22.22
6	80	500	1	7.44±0.47	-17.43
7	100	100	4	13.93±1.74	-22.91
8	100	300	1	8.11±0.41	-18.18
9	100	500	2	4.03±0.47	-12.13

Figure 1 shows the mean value of the S/N ratio of lignin content for each factor and all levels. The optimum level of each factor was determined from the highest S/N ratio. As shown in Fig 1, the optimum level setting for the best lignin removal from corncob was obtained to be 100 °C temperature (level 3), 500 mg/g of peroxide dosage (level 3) and 2 min of pretreatment time (level 2).

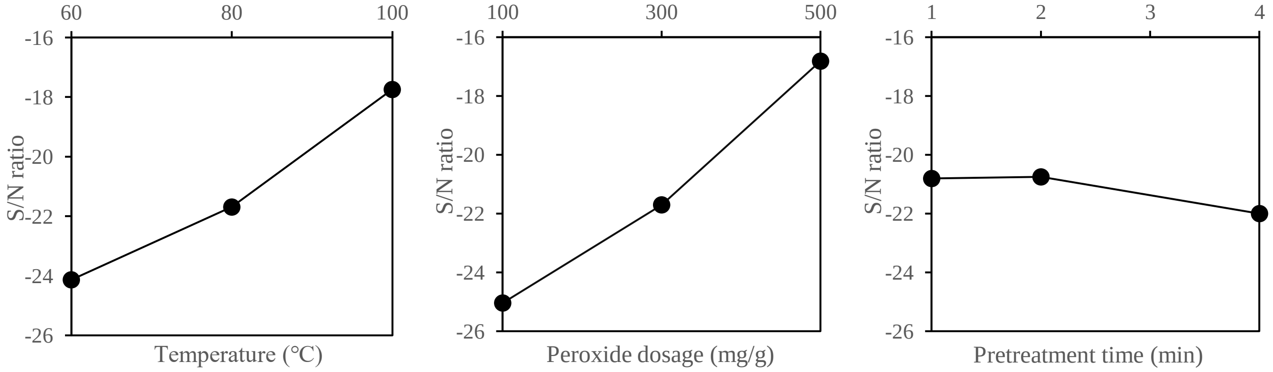

Fig. 1. The effect of temperature, peroxide dosage and pretreatment time on lignin content in corncob

After identifying the optimal pretreatment conditions, Minitab® statistical software was used to predict the optimal response value. The predicted lignin content in corncob, achieved by applying these optimal conditions, was 3.89%. The next step in the Design of Experiments (DOE) methodology involves conducting confirmation experiments for these optimal conditions.

As previously mentioned, the most effective lignin removal was achieved with a temperature of 100 °C, a peroxide dosage of 500 mg/g, and a pretreatment time of 2 minutes. This combination of factors was part of the nine experimental runs conducted using the Taguchi design (Table 1) and the average lignin content in corncob pretreated under these conditions was estimated to be 4.03%, which closely aligns with the predicted value of 3.89%.

Table 2. ANOVA of S/N ratio for the lignin content.

Source	DF	Seq SS	Adj SS	Adj MS	SS'	F	P	Contribution (%)
Temperature (°C)	2	62.356	62.356	31.178	59.416	21.21	0.045	34.73
Peroxide dosage (mg/g)	2	102.814	102.814	51.407	99.874	34.97	0.028	58.37
Pretreatment time (min.)	2	2.990	2.990	1.495	0.05	1.02	0.496	0.03
Residual error	2	2.940	2.940	1.470	11.760			6.87
Total	8	171.101						100
DF-degrees of freedom; Seq SS-Sequential sum of squares, Adj SS-Adjusted sum of squares, Adj MS-Adjusted mean squares, SS'-Pure sum of squares, F-Fisher value, P-Probability value								

The ANOVA statistical tool was employed to assess the impact of individual factors on lignin removal efficiency. Table 2 shows the calculated ANOVA values for the S/N ratio. The *P*-test was utilized to identify the process factors that significantly affect lignin removal from corncob. According to the ANOVA analysis, the peroxide dosage exhibited the most substantial effect on lignin removal, followed by temperature and pretreatment time. Furthermore, the *P*-values for temperature and peroxide dosage were found to be less than 0.05 indicating that there was a statistically significant association between lignin removal and these two factors. The percentage contribution shown in Table 2 reveals a factor's corresponding power to reduce variation. This means that a small change in a factor with a high percentage contribution will have a significant impact on lignin removal efficiency. The percentage of contribution is a function of the sum of squares for each significant factor and can be calculated as follows *(Roy, R. K., 2010)*:

$$F_i = \frac{SS'_i}{Seq\ SS_{Total}} \qquad (4)$$

Where F_i refers to the contribution of factor i, SS'_i is the pure sum of squares for factor i, and Seq SS_{Total} refers to the total sequential sum of squares.

Based on the results presented in Table 2, peroxide dosage was the most influential factor with a 58% contribution, followed by temperature (34%). In addition to measuring lignin content, the total solid recovery was determined for each experimental run, which included the pretreatment conducted in an oil bath. These results were then correlated to gain insight into the effects of various pretreatment conditions on both lignin removal and solid recovery. As shown in Figure 2, the lignin content of pretreated biomass and the total solid recovery exhibited a positive correlation, with a Pearson's correlation coefficient of 0.95. This indicates that when the total solid recovery was high, the corncob biomass retained a high lignin content, suggesting that the applied pretreatment conditions were not effective in removing lignin. The strong correlation suggests that as more lignin is removed, there is a tendency for less solid material to be recovered after the pretreatment process. This emphasizes the importance of finding a balance between delignification and preserving solid material, particularly cellulose, allowing for efficient biomass processing, particularly in industries focused on biofuels and biochemical production.

To gain insight into the compositional changes of pretreated corncob, a sample obtained under the optimal pretreatment conditions of microwave-coupled peroxide treatment was analyzed for cellulose and hemicellulose content. The results of this analysis are discussed in the following section. Additionally, the sample obtained from the oil bath experiment underwent the same analysis to allow for a comparison between conventional and microwave heating sources.

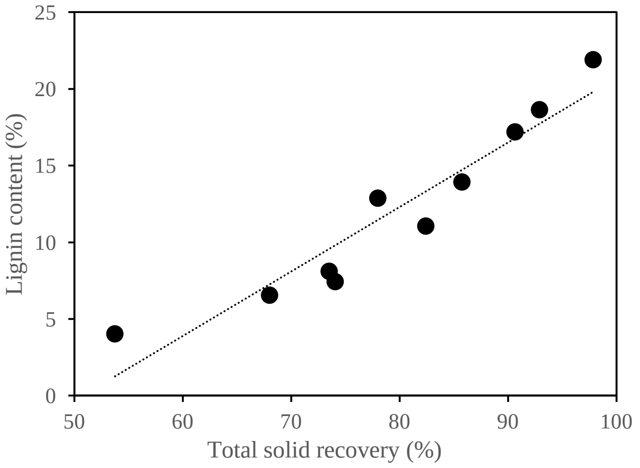

Fig. 2. The correlation between lignin content and the total solid recovery rate in microwave heating pretreatment and conventional heating pretreatment

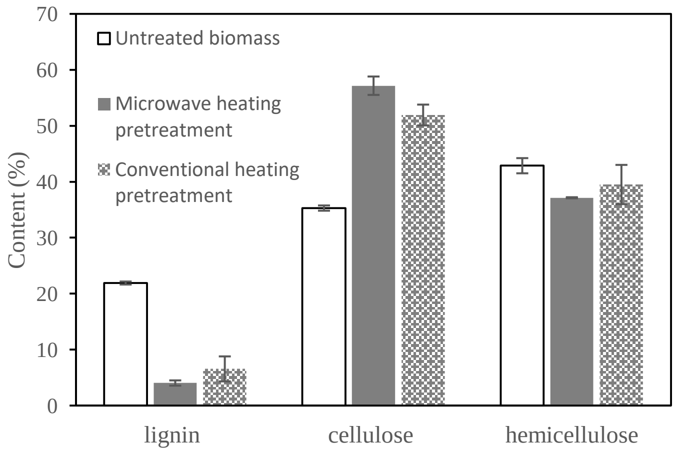

Fig. 3. Content of lignin, cellulose and hemicellulose in untreated and pretreated corncob

Compositional analysis of pretreated and untreated corncob

The analysis of cellulose, hemicellulose, and lignin content in both pretreated and untreated (control) biomass, determined using the NREL analytical method, is shown in Figure 3. It can be seen that the content of structural polysaccharides and lignin in corncob underwent significant changes caused by microwave-coupled peroxide treatment. Upon comparing the microwave-pretreated and untreated biomass, there was a significant reduction in lignin and hemicellulose content by 81.6% and 13.4%, respectively. In contrast, the cellulose content showed a substantial increase of 61.9% compared to the untreated biomass. This compositional analysis of corncob suggests that the optimized microwave pretreatment predominantly impacted lignin and hemicellulose molecules while preserving cellulose, resulting in a substantial increase in cellulose concentration in the pretreated biomass. Comparing the biomass pretreated in an oil bath with the untreated sample, lignin and hemicellulose content decreased by 70.1% and 7.8%, respectively, while cellulose content increased by 47.1%. The conventional heating pretreatment exhibited a similar pattern in biomass decomposition, primarily affecting lignin and hemicellulose. However, the changes in lignin, cellulose, and hemicellulose content were less pronounced compared to microwave heating pretreatment.

While microwave heating showed a slight superiority over conventional heating pretreatment in this study, it is important to note the challenges associated with ensuring identical conditions for both heating methods, particularly in achieving similar heating rates. Conventional heating methods typically require more time to reach the target temperature compared to microwave heating. The efficiency of microwave heating lies in its ability to directly transfer energy to the molecules in the material, resulting in rapid and uniform heating throughout the sample. However, achieving the exact target temperature poses a challenge, especially in short-term processes lasting one to two minutes, where the time difference between microwave and conventional heating can be quite noticeable. Microwave heating offers a significant advantage in providing rapid and efficient heating within short-duration processes such as pretreatment studied in this work. Since disparity in heating rates complicates the comparison of pretreatment efficiency achieved by these two methods, additional experimental setups and analyses are necessary for more definitive conclusions and confirmation of possible non-thermal effects of microwave pretreatment.

The study of *Mittal et al. (2017)* reported pretreatment of corn stover using an alkaline peroxide loading of 500 mg/g at a temperature of 50 °C. The study required a 3-hour treatment to achieve a comparable lignin removal rate of 81% and a more modest hemicellulose solubilization of 8%, in contrast to the two-minute treatment duration used in this work. In another study, *Li et al. (2018)* found an even longer 24-hour alkaline peroxide treatment of corn stover necessary to achieve a reduction in initial lignin content by 91.5% and hemicellulose by 55.7% at a peroxide loading of 500 mg/g and a temperature of 30 °C. Ethanol-assisted alkaline peroxide treatment of bamboo resulted in a comparable lignin removal rate of 80% at 100 °C for 60 min. This percentage was higher than the 74.9% achieved in the pretreatment of bamboo without ethanol assistance *(Huang et al., 2020).*

Comparing our results with previously published data, the substantial lignin removal rate achieved for corncob through a short-term two-minute microwave-coupled peroxide treatment could have significant implications for overall process sustainability. The shortened treatment time facilitated by microwave irradiation not only improves throughput but also allows for more treatment cycles or batches to be completed within a defined timeframe. Microwave heating is known for its potential energy efficiency in short processes compared to conventional methods, thanks to its rapid and direct energy transfer, quick heating times, uniform heating, and reduced energy consumption. These characteristics make microwave irradiation particularly advantageous for rapid processes where efficient and swift temperature attainment is crucial. Moreover, the high lignin removal rate achieved under optimal conditions of microwave-coupled peroxide treatment in this work is particularly noteworthy for enzymatic hydrolysis efficiency. The reduced lignin content in pretreated corncob enhances the availability and reversible binding of enzymes to active sites on cellulose and hemicellulose molecules *(Huang et al., 2022)*, thereby ensuring higher sugar yields and improving the overall efficiency of corncob processing into value-added products.

CONCLUSION

The Taguchi orthogonal design showed that the process conditions of the microwave-coupled peroxide treatment had a significant role in the lignin removal efficiency. The peroxide dosage was found to be the most influential process parameter, followed by temperature and treatment time, while the prediction of the Taguchi design was in good agreement with the experimental results. The microwave-coupled peroxide treatment resulted in notable lignin removal from corncob, with the highest removal reaching 81.6% at 100 °C, 500 mg/g of peroxide dosage and 2 min of treatment time. This was higher than the 70.1% achieved under the same conditions with conventional heating. The high delignification rate achieved in this study plays a crucial role in enhancing hydrolysis efficiency for the subsequent stage of processing corncobs into biofuels or biochemical. It enhances polysaccharide accessibility and promotes reversible adsorption of hydrolytic enzymes on the treated biomass, thereby increasing the overall effectiveness of processing corncob into value-added products.

ACKNOWLEDGMENT: This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-66/2024-03/200287 and 451-03-65/2024-03/200135) and by the Alliance of International Science Organizations, project SparkGREEN (ANSO-CR-PP 2022-08).

REFERENCES

Aguilar-Reynosa, A., Romaní, A., Rodríguez-Jasso, R. M., Aguilar, C. N., Garrote, G., & Ruiz, H. A. (2017). Microwave heating processing as alternative of pretreatment in second-generation biorefinery: An overview. Energy Conversion and Management, 136, 50-65. doi.org/10.1016/j.enconman.2017.01.004

Bichot, A., Lerosty, M., Radoiu, M., Méchin, V., Bernet, N., Delgenès, J. P., & García-Bernet, D. (2020). Decoupling thermal and non-thermal effects of the microwaves for lignocellulosic biomass pretreatment. Energy conversion and management, 203, 112220. doi.org/10.1016/j.enconman.2019.112220

Cabrera, E., Muñoz, M. J., Martín, R., Caro, I., Curbelo, C., & Díaz, A. B. (2014). Alkaline and alkaline peroxide pretreatments at mild temperature to enhance enzymatic hydrolysis of rice hulls and straw. Bioresource Technology, 167, 1-7. doi.org/10.1016/j.biortech.2014.05.103

Dutra, E. D., Santos, F. A., Alencar, B. R. A., Reis, A. L. S., de Souza, R. D. F. R., Aquino, K. A. D. S., Morais Jr., M. A., & Menezes, R. S. C. (2018). Alkaline hydrogen peroxide pretreatment of lignocellulosic biomass: status and perspectives. Biomass Conversion and Biorefinery, 8, 225-234. doi.org/10.1007/s13399-017-0277-3

Fukushima, R. S., & Kerley, M. S. (2011). Use of lignin extracted from different plant sources as standards in the spectrophotometric acetyl bromide lignin method. Journal of Agricultural and Food Chemistry, 59(8), 3505-3509. doi.org/10.1021/jf104826n

Grbić, J., Đukić-Vuković, A., Mladenović, D., Lazović, S., & Mojović, L. (2022). Effect of non-thermal plasma on cellulose crystallinity and lignin content in corn stalks. Journal on Processing and Energy in Agriculture, 26(2). doi.org/10.5937/jpea26-36871

Huang, C., Fang, G., Yu, L., Zhou, Y., Meng, X., Deng, Y., Shen, K., & Ragauskas, A. J. (2020). Maximizing enzymatic hydrolysis efficiency of bamboo with a mild ethanol-assistant alkaline peroxide pretreatment. Bioresource technology, 299, 122568. doi.org/10.1016/j.biortech.2019.122568

Huang, C., Jiang, X., Shen, X., Hu, J., Tang, W., Wu, X., Ragauskas, A., Jameel, H., Meng, X., & Yong, Q. (2022). Lignin-enzyme interaction: A roadblock for efficient enzymatic hydrolysis of lignocellulosics. Renewable and Sustainable Energy Reviews, 154, 111822. doi.org/10.1016/j.rser.2021.111822

Jović, J., Kocić-Tanackov, S., & Mojović, L. (2021). Pretreatment of lignocellulosic biomass with autochthonous sfungi from serbia. Journal on Processing and Energy in Agriculture, 25(2), 74-77. doi.org/10.5937/jpea25-31108

Li, J., Lu, M., Guo, X., Zhang, H., Li, Y., & Han, L. (2018). Insights into the improvement of alkaline hydrogen peroxide (AHP) pretreatment on the enzymatic hydrolysis of corn stover: chemical and microstructural analyses. Bioresource technology, 265, 1-7. doi.org/10.1016/j.biortech.2018.05.082

Milašinović-Šeremešić, M., Radosavljević, M., Terzić, D., & Nikolić, V. (2018). Maize processing and utilisation technology: Achievements and prospects. Journal on Processing and Energy in Agriculture, 22(3), 113-116. doi.org/10.5937/JPEA1803113M

Mittal, A., Katahira, R., Donohoe, B. S., Black, B. A., Pattathil, S., Stringer, J. M., & Beckham, G. T. (2017). Alkaline peroxide delignification of corn stover. ACS Sustainable Chemistry & Engineering, 5(7), 6310-6321. doi.org/10.1021/acssuschemeng.7b01424

Roy, R. K. (2010). A primer on the Taguchi method. Society of manufacturing engineers. ISBN: 087263468X, 9780872634688.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. L. A. P. (2008). Determination of structural carbohydrates and lignin in biomass. Laboratory analytical procedure, 1617(1), 1-16.

Thompson, P. B. (2012). The agricultural ethics of biofuels: the food vs. fuel debate. Agriculture, 2(4), 339-358. doi.org/10.3390/agriculture2040339

Xia, Y., Liu, Q., Hu, X., Li, X., Huang, Y., Li, W., & Ma, L. (2022). Structural evolution during corn stalk acidic and alkaline hydrogen peroxide pretreatment. Industrial Crops and Products, 176, 114386. doi.org/10.1016/j.indcrop.2021.114386

Zhou, Z., Ouyang, D., Liu, D., & Zhao, X. (2023). Oxidative pretreatment of lignocellulosic biomass for enzymatic hydrolysis: Progress and challenges. Bioresource Technology, 367, 128208. doi.org/10.1016/j.biortech.2022.128208

Received: 18. 03. 2024. Accepted: 28. 03. 2024.