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## UNLOCKING THE BIOFUEL POTENTIAL OF RAPESEED STRAW THROUGH ALKALINE PRETREATMENT

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Ukraine, as one of the world's largest agricultural producers, generates substantial volumes of lignocellulosic residues, particularly from rapeseed cultivation. Rapeseed straw, a by-product of harvesting, presents significant potential as a raw material for the production of advanced biofuels, especially bioethanol. This study investigates the efficiency of alkaline pretreatment of rapeseed straw using sodium hydroxide under varying conditions of concentration and treatment time. The aim was to optimize delignification and preserve carbohydrate content for downstream bioethanol conversion. A full factorial design (2<sup>2</sup>) was applied to explore the influence of key parameters, and regression models were developed to describe the response surfaces for product yield and residual lignin content. The results showed that while increasing alkali concentration improves delignification, it also reduces solid yield due to the degradation of valuable polysaccharides. An optimal balance was observed at 4% NaOH and 150 minutes; this condition minimized lignin content to 13.0% but resulted in moderate product yield (29.7%). The results highlight the need for further research on the use of the resulting product as a substrate for efficient biofuel production.

**Keywords:** cellulose product, delignification, lignin, biofuel, bioethanol.

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### Introduction

As the world seeks alternatives to fossil fuels, the production of second-generation biofuels has gained momentum due to their reliance on non-edible plant materials, such as agricultural by-products and lignocellulosic waste [1]. These feedstocks offer an environmentally friendly and sustainable solution that does not compete with food crops, making them particularly attractive for large-scale energy applications [2].

Lignocellulosic biomass encompasses a wide variety of agricultural by-products that are often discarded or underutilized [3]. These include wheat straw, corn stover, sugarcane bagasse, rice husks, barley

straw, cotton stalks, and sunflower husks, among others [4]. The use of such residues not only helps reduce agricultural waste but also adds value to crop production chains [5].

The structural complexity of lignocellulosic biomass, however, poses a significant barrier to its efficient conversion into biofuels [6]. This biomass is mainly composed of tightly bound cellulose, hemicellulose, and lignin, which require disruption before sugars can be effectively released and fermented into liquid fuels [7].

To address this challenge, a wide range of pretreatment strategies have been developed. These include mechanical disruption, thermal processes,

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chemical treatments (such as alkaline or acidic hydrolysis), as well as hybrid approaches that combine multiple techniques [8]. Pretreatment plays a critical role in improving the digestibility of biomass and directly impacts the yield of fermentable sugars in downstream processes [9].

This cellulose-rich fraction is particularly desirable because it contains hexose sugars, primarily glucose, which serve as the main substrate for microbial fermentation into bioethanol. The efficient release and utilization of these hexoses is critical for achieving high ethanol yields and making second-generation biofuel production economically viable [10].

Ukraine, as one of the world's leading agricultural producers, has significant potential in this field [11]. With vast areas dedicated to cultivating cereal grains, oilseeds, and industrial crops, the country generates large volumes of agricultural residues [12]. Notably, rapeseed stems – produced in substantial quantities as a by-product of rapeseed harvesting – represent a valuable lignocellulosic resource [13]. These residues, often underutilized, hold great promise for conversion into advanced biofuels, especially bioethanol [14].

In this study, emphasis is placed on the application of alkaline pretreatment for the processing of rape straw. By adjusting operational parameters like reagent concentration and processing time, the aim is to improve delignification efficiency and unlock higher conversion rates of carbohydrates into bioethanol. The results support ongoing efforts to refine biomass processing methods and strengthen the technological foundation for next-generation biofuel production.

The aim of this study is to investigate the effectiveness of alkaline pretreatment using sodium hydroxide for rapeseed straw, with a focus on efficient delignification conditions to enhance the suitability of the biomass for subsequent bioethanol production. Special attention is given to identifying the influence of reagent concentration and treatment time on product yield and residual lignin content, supported by mathematical modeling for process description.

### **Experimental**

Rapeseed straw collected from agricultural fields in the Vinnytsia region of Ukraine was used as the raw material for this study. To ensure uniformity and enhance the efficiency of subsequent processing steps, the biomass was mechanically chopped into particles ranging from 15 to 20 mm in length. The prepared material was then stored in desiccators to maintain stable moisture content prior to experimental procedures.

The chemical composition of the raw biomass was analyzed using standard analytical protocols

commonly employed for lignocellulosic materials. A detailed breakdown of the chemical composition of rapeseed straw is as follows: 42.1% cellulose, 75.9% holocellulose, 21.4% lignin, 3.0% resins, fats, and waxes, and 2.3% ash.

To evaluate the potential for hexose recovery, the rapeseed straw biomass was subjected to alkaline treatment using sodium hydroxide (NaOH) solutions of varying concentrations. The liquid-to-solid ratio was maintained at 5:1, and the treatment was carried out at a constant temperature of 120°C. The experiments were carried out under laboratory conditions using stainless steel acid-resistant autoclaves, which were heated in a glycerin bath. The treated substrate was thoroughly washed with distilled water and then dried to a final moisture content of approximately 6%. The efficiency of the pretreatment process was assessed based on the yield of the resulting solid fraction and the residual lignin content within the substrate.

The chemical reactions between the alkaline and lignocelluloses mainly include lignin reactions and carbohydrates (i.e., cellulose and hemicellulose) reactions (Figs. 1 and 2). Lignin reactions lead to the degradation and dissolution of lignin, which benefits the subsequent enzymatic hydrolysis, while cellulose and hemicellulose reactions result in sugar loss, which should be diminished as much as possible. Different chemicals used in pretreatment may bring different degradation reactions of lignocellulosic components, despite the main mechanism of alkali-based pretreatment being to efficiently break the ester bonds cross-linking lignin and xylan through solvation and saponification [15] (Figs. 1 and 2).

To reduce the number of experimental runs and identify the optimal conditions for substrate production, a full factorial design of the 2<sup>2</sup> type was employed. During the study, two independent variables (factors) were selected:  $x_1$  – alkali concentration (%),  $x_2$  – treatment time (min). The dependent variables  $y_i$  used to evaluate the effectiveness of pretreatment were  $y_1$  – substrate yield (%) and  $y_2$  – lignin content (%).

The initial values used to construct the experimental matrix, including the variation levels and coding intervals of the factors, are presented in Table 1.

To mathematically describe the process, second-order polynomial regression models were developed. Modeling, identification, and statistical validation of the adequacy of the models were performed using specialized software tools. The general form of the second-order polynomial regression equation for two independent variables is expressed as follows:

Table 1

Coded levels and variation ranges of factors  $x_i$ 

| Factor $x_i$ | Variation levels |      |     | Variation interval<br>( $\Delta x_i$ ) |
|--------------|------------------|------|-----|--|
|              | (+1)             | (-1) | (0) |  |
| $x_1$        | 6                | 2    | 4   | 2                                      |
| $x_2$        | 150              | 30   | 90  | 30                                     |

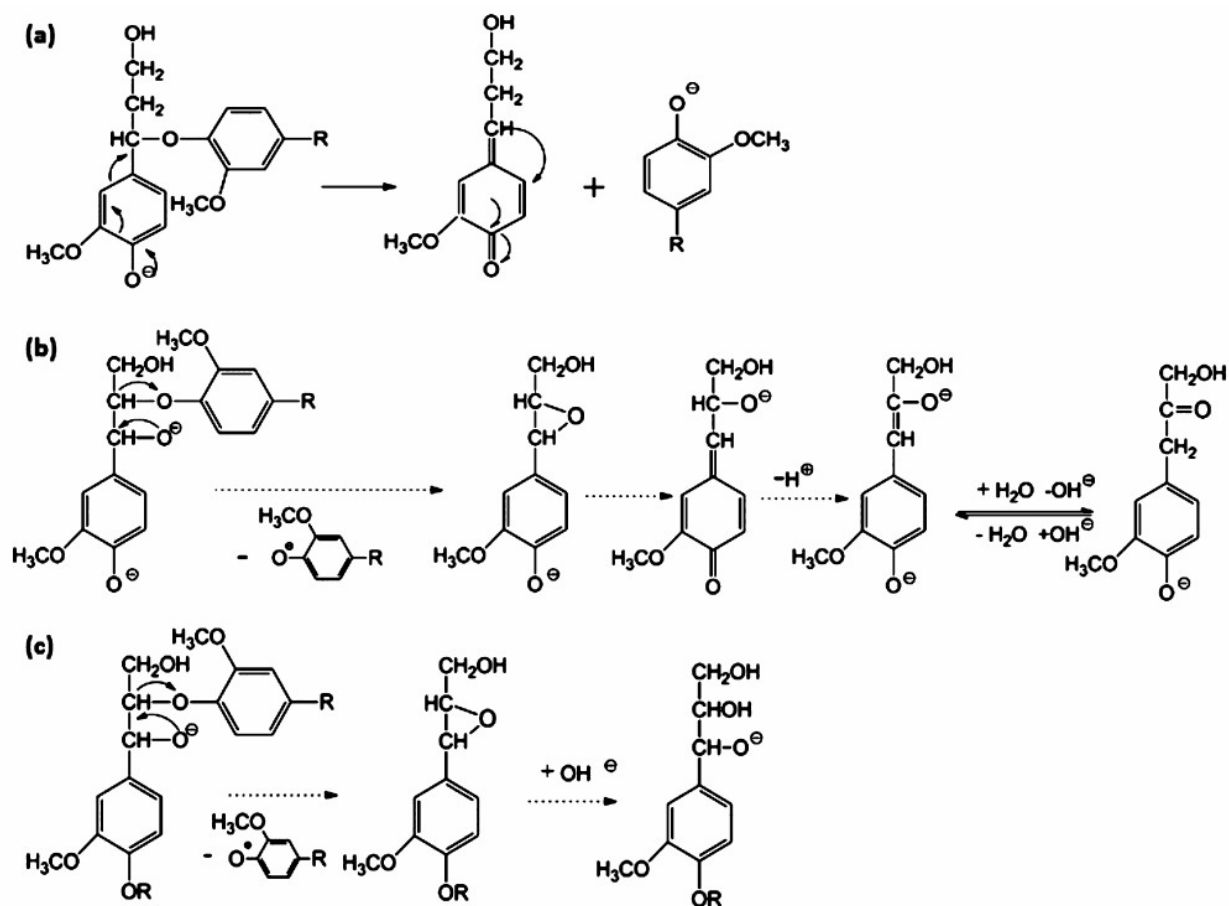


Fig. 1. Typical lignin reactions in alkaline conditions: (a) the fracture of the phenol-type  $\alpha$ -aryl ethers; (b) the cleavage of the phenol-type  $\beta$ -aryl ethers; (c) the fracture of the non-phenol-type  $\beta$ -aryl ethers [15]

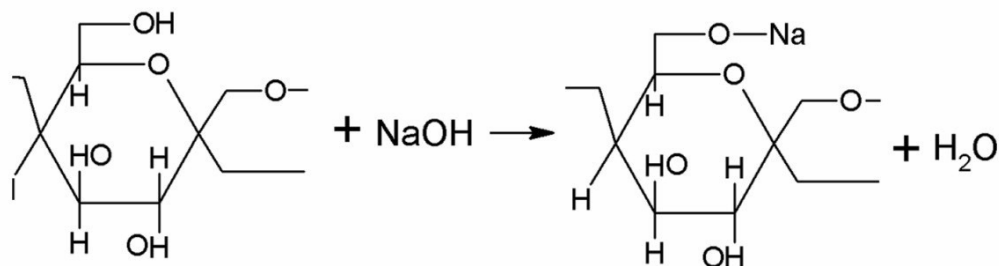


Fig. 2. Reaction of cellulose fiber with NaOH

$$y_i = b_0 + b_1x_1 + b_2x_2 + b_3x_1x_2 + b_4x_1^2 + b_5x_2^2, \quad (1)$$

where  $y_i$  is the dependent variable,  $x_1$  and  $x_2$  are the independent variables,  $b_0$ ,  $b_1$ ,  $b_2$ ,  $b_3$ ,  $b_4$  and  $b_5$  are the regression coefficients representing the contribution of each corresponding term in the model.

### Results and discussion

The design of the experiment and the corresponding results for the two independent variables are summarized in Table 2. Each value of the response variable  $y_i$  represents the mean of three parallel measurements, enhancing the credibility of the results.

Analysis of the experimental data revealed a direct correlation between the concentration of the alkaline solution and the degree of delignification. An increase in the variable  $x_1$  from 2% to 4% with  $x_2$  ranging from 30 to 150 minutes significantly intensified the removal of the aromatic fraction. This effect is confirmed by a decrease in lignin content in the final product, indicating the high efficiency of disrupting the aromatic matrix of lignocellulosic biomass.

At the same time, this intensification of delignification was accompanied by a notable reduction (17.7%) in the total mass of the solid residue. This decrease is likely due not only to lignin removal but also to the co-extraction of other components such as extractives and mineral impurities, which are also soluble in alkaline media.

Further increasing the sodium hydroxide concentration to 6% under the same holding times (30–150 min) led to an additional 5.6% reduction in lignin content. However, this was also accompanied by a 6.5% further decrease in solid yield. These results suggest that at higher alkali concentrations, in addition to the target delignification, more intensive hydrolysis of low-molecular-weight polysaccharides occurs, particularly hemicelluloses and partially cellulose. This excessive degradation of polysaccharides negatively affects the final mass of the residue containing valuable

carbohydrate components.

The data processing and construction of mathematical models of biomass treatment with alkaline solution were performed using the least squares method, which allowed determining the regression coefficients to describe the relationship between factors and response functions. The adequacy of the models was confirmed by Cochran's and Fisher's criteria, indicating their suitability for further construction of 3D surfaces of the properties of alkaline-treated rapeseed straw products.

The following regression equations were obtained:

$$y_1 = 58.394 - 4.16x_1 - 0.138x_2 + 0.018x_1x_2 - 0.044x_1^2 - 0.00006x_2^2,$$

$$y_2 = 26.896 - 2.556x_1 - 0.102x_2 + 0.0015x_1x_2 + 0.252x_1^2 - 0.0003x_2^2.$$

The presented regression models describe the dependence of two key process indicators –  $y_1$  (product yield) and  $y_2$  (residual lignin content) – on two factors:  $x_1$  (alkali concentration) and  $x_2$  (treatment duration). Comparing the coefficients of these models allows for identifying the differentiated impact of each factor on the response variables. In both models, a negative linear effect of both factors ( $x_1$  and  $x_2$ ) on the dependent variables is observed. This indicates that increasing either the alkali concentration ( $x_1$ ) or the treatment duration ( $x_2$ ) leads to a decrease in both  $y_1$  and  $y_2$ .

However, the degree of this influence varies significantly. For both  $y_1$  and  $y_2$ , factor  $x_1$  shows a much stronger linear impact than factor  $x_2$ . In the model for  $y_1$ , the coefficient for  $x_1$  is  $-4.16$ , while for  $x_2$  it is only  $-0.138$ . Similarly, in the model for  $y_2$ , these values are  $-2.556$  and  $-0.102$ , respectively. This suggests that changes in reagent concentration have a more pronounced direct effect on the final product characteristics and delignification efficiency than changes in treatment time within the studied range.

Significant differences are also evident in the quadratic terms, which reflect the nonlinear nature of the relationships. For  $y_1$ , the coefficients for the quadratic terms ( $x_1^2 = -0.044$ ;  $x_2^2 = -0.00006$ ) are negative. This indicates that both factors tend to reduce  $y_1$  after reaching a certain maximum (if it lies within the studied range). At the same time, the nonlinear effect of  $x_1$  is more pronounced than that of  $x_2$ , whose quadratic coefficient is extremely small. This implies that further increases in  $x_1$  beyond a certain threshold may lead to an undesirable decrease in  $y_1$ , likely due to the degradation of the target polysaccharide component.

Table 2

Design matrix of the 2<sup>2</sup> full factorial experiment for two independent variables

| Parameter |       | Value |       |
|-----------|-------|-------|-------|
| $X_1$     | $X_2$ | $Y_1$ | $Y_2$ |
| -1        | -1    | 46.7  | 20.0  |
|           | 0     | 40.4  | 16.3  |
|           | 1     | 33.0  | 14.9  |
| 0         | -1    | 38.8  | 18.3  |
|           | 0     | 34.9  | 14.5  |
|           | 1     | 29.7  | 13.0  |
| 1         | -1    | 31.2  | 18.0  |
|           | 0     | 28.2  | 14.8  |
|           | 1     | 26.1  | 13.6  |

In contrast, for  $y_2$ , the coefficient for  $x_1^2$  is positive (+0.252), while that for  $x_2^2$  is negative (−0.0003). The positive quadratic coefficient for  $x_1^2$  suggests that the effect of  $x_1$  on  $y_2$  may exhibit a minimum, that is, after a certain point, a further increase in  $x_1$  results in rising  $y_2$  values. This is undesirable, as the goal is to minimize lignin content. However, it may also indicate a trade-off, where excessive  $x_1$  concentration no longer contributes to effective lignin removal or leads to other unwanted reactions. The quadratic effect of  $x_2$  on  $y_2$  is extremely small and practically negligible.

Figure 3 presents the 3D surfaces of the properties of products obtained from the treatment of rapeseed straw.

The analysis of residual lignin content data revealed a more complex relationship, particularly concerning factor  $x_1$ . In general, an increase in both  $x_1$  and  $x_2$  contributes to a reduction in lignin content, which is a desirable effect of delignification. For

example, at 2% alkali concentration, increasing time from 30 to 150 minutes reduced lignin content from 20.0% to 14.9%. However, the influence of  $x_1$  exhibits a nonlinear pattern. At 30 minutes, increasing the concentration from 2% to 4% reduced  $y_2$  from 20.0% to 18.3%, while a further increase to 6% resulted in only a slight decrease to 18.0%. This trend becomes even more evident at 90 minutes, where  $y_2$  decreased from 16.3% to 14.5%, but then slightly increased to 14.8% at 6%. This indicates that there is an optimal alkali concentration for lignin minimization, beyond which further increases may be ineffective or even counterproductive due to lignin recondensation or other secondary reactions that affect the measured lignin content.

Careful understanding of process parameters is essential to achieve a balance between lignin removal and preservation of cellulose-rich fractions. The lowest residual lignin content was achieved under conditions of 4% and 150 minutes.

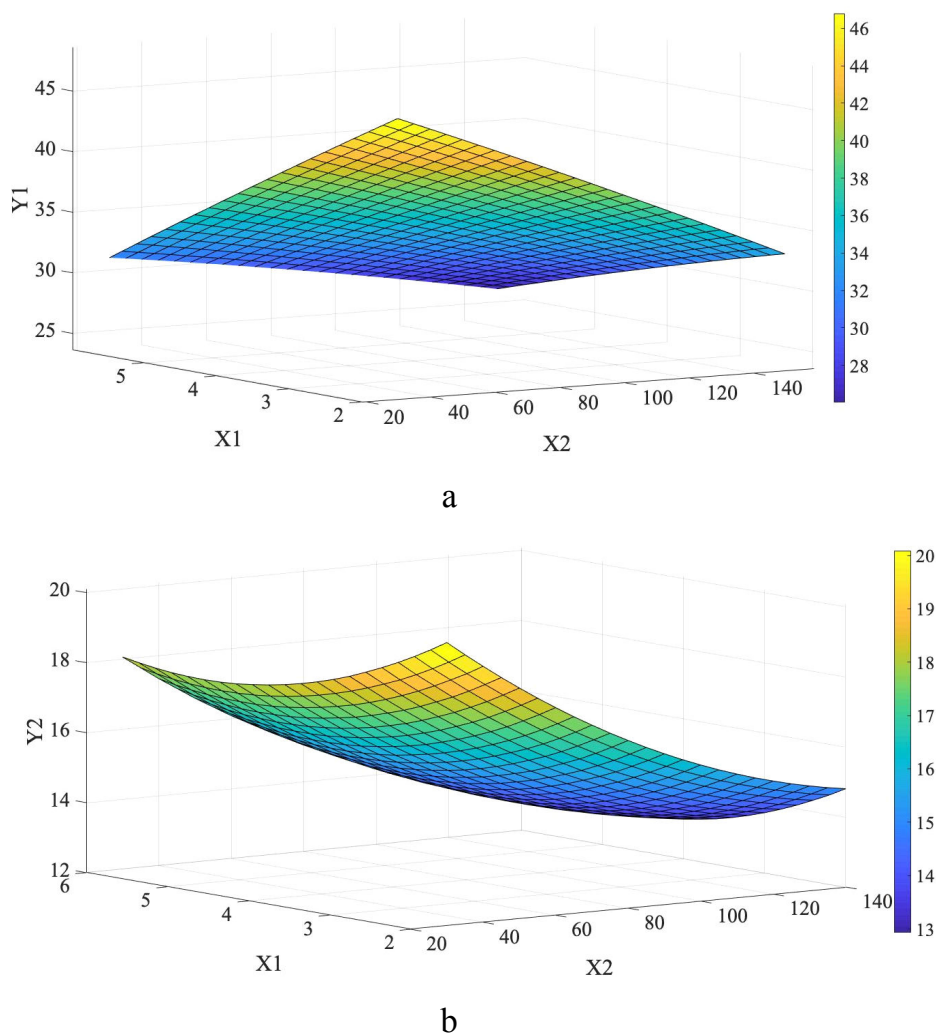


Fig. 3. Response surface plots of product yield (a) and residual lignin content (b) after alkaline treatment of rapeseed straw

The experimental results demonstrate an inverse relationship between the two key indicators. Conditions that most effectively promote delignification often result in significant losses of the target product.

The obtained results therefore underscore the pressing need for further research focused on the comprehensive utilization of these processed products, specifically as a substrate for efficient second-generation biofuel production. Future work should particularly explore the enzymatic digestibility of the obtained polysaccharide-rich fraction, optimize fermentation processes to convert the liberated sugars into bioethanol, and investigate the potential for integrated biorefinery concepts that maximize the value of all fractions, including the valorization of recovered lignin. Such holistic research is essential to fully unlock the potential of agricultural residues as a sustainable source of energy and biochemical, contributing to energy security and environmental sustainability.

### Conclusions

Rapeseed straw is a promising lignocellulosic feedstock for the production of second-generation biofuels, owing to its high cellulose content and availability in Ukraine. Alkaline pretreatment using sodium hydroxide effectively reduces lignin content in the biomass, with a clear influence of reagent concentration and treatment time on both delignification and solid yield. Regression analysis and response surface modeling revealed a nonlinear relationship between the process variables and the key performance indicators (product yield and lignin content), highlighting the existence of optimal operational conditions. The optimal conditions for minimal residual lignin (13.0%) were determined to be 4% NaOH and 150 minutes of treatment. The developed mathematical models provide a valuable tool for process optimization and can inform the design of efficient pretreatment strategies for lignocellulosic biomass in next-generation biorefineries.

The conducted research clearly demonstrates that alkaline pretreatment is a highly effective method for the preliminary processing of lignocellulosic biomass, such as rape straw, for subsequent biotechnological applications. A key advantage of this method is its ability to achieve efficient delignification, which, as shown, significantly enhances the accessibility of the polysaccharide fraction. This fraction serves as the foundation for enzymatic hydrolysis and subsequent fermentation into bioethanol. Thus, the obtained data strongly underscore the feasibility and promise of employing alkaline pretreatment as a crucial step in the production of second-generation biofuels from domestic agricultural residues. This approach enables the necessary structural modification of biomass, which

is critically important for maximizing sugar yield and, consequently, the overall efficiency of the bioconversion process.

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## РОЗКРИТТЯ БІОПАЛИВНОГО ПОТЕНЦІАЛУ РІПАКОВОЇ СОЛОМИ ШЛЯХОМ ЛУЖНОЇ ПОПЕРЕДНЬОЇ ОБРОБКИ

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Україна, як один з найбільших сільськогосподарських виробників у світі, створює значні обсяги лігноцелюлозних залишків, зокрема від вирощування ріпаку. Ріпакова солома, побічний продукт збирання врожаю, має значний потенціал як сировина для виробництва біопалива, зокрема, біоетанолу. Дане дослідження показує ефективність лужної попередньої обробки ріпакової соломи з використанням натрій гідроксиду за різних умов концентрації та часу обробки. Метою було оптимізувати делігніфікацію та зберегти вміст вуглеводів для подальшого перетворення біоетанолу. Для дослідження впливу ключових параметрів було застосовано повний факторіальний план (2<sup>2</sup>), а також розроблено регресійні моделі для опису поверхонь відгуку для виходу продукту та вмісту залишкового лігніну. Результати показали, що хоча збільшення концентрації лугу покращує делігніфікацію, воно також знижує вихід твердої речовини через деградацію цінних полісахаридів. Оптиміальний баланс спостерігався при 4% NaOH та 150 хвилинах, що мінімізувало вміст лігніну до 13,0%, але призвело до зниження виходу продукту (29,7%). Отримані результати підкреслюють необхідність подальших досліджень щодо використання отриманих продуктів як сировини для ефективного виробництва біопалива.

**Ключові слова:** целюлозний продукт, делігніфікація, лігнін, біопаливо, біоетанол.

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