https://doi.org/10.15407/scine20.06.030

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INFLUENCE OF THE CONDITIONS OF ALKALINE PRETREATMENT OF VEGETABLE RAW MATERIAL BEFORE HYDROLYSIS IN A ROTOR PULSATION DEVICE ON THE CELLULOSE CONVERSION

Introduction. *The growing global energy demand, limited fossil fuel resources, and increasing greenhouse gas emissions highlight the need for expanded use of renewable energy sources, particularly biomass.*

Problem Statement. *A significant drawback in the production of bioethanol is its high cost, primarily due to the presence of hemicellulose and lignin.*

Purpose. *This study aims to determine the effect of treating an alkaline suspension of wheat straw in a rotary pulsation device on the degree of cellulose conversion during enzymatic hydrolysis, a critical step in the production of fuel ethanol.*

Materials and Methods. *The raw material is pre-crushed wheat straw. The experiments have been conducted with the use of a rotary pulsation device in an experimental plant.*

Results. *It has been found that the combined physical effects of the discrete pulse energy input method and reduced straw particle size significantly increase the lignin extraction, from 40.0% at an average particle size of 2—1 mm to 62.0% at an average particle size of 0.4—0.1 mm. Additionally, the treatment of an alkaline suspension of wheat straw at 90 °C for one hour, at alkali concentrations ranging from 1 to 4% (wt./wt.), results in an increase in the cellulose conversion during enzymatic hydrolysis from 38% to 65.8%. Additionally, a corresponding decrease in the lignin content, from 17.1% to 3.16% has been reported.*

Conclusions. *Increasing alkali concentration during the alkaline pretreatment of wheat straw using the discrete-pulse energy input method in a rotary pulsation device enhances the rate of cellulose conversion during subsequent enzymatic hydrolysis.*

Keywords: fuel ethanol, pretreatment, alkali, cellulose conversion, cellulase complex.

Citation: Blume, Ya. B., Obodovych, O. M., and Sydorenko, V. V. (2024). Influence of the Conditions of Alkaline Pretreatment of Vegetable Raw Material Before Hydrolysis in a Rotor Pulsation Device on the Cellulose Conversion. *Sci. innov.*, 20(6), 30—37. https://doi.org/10.15407/scine20.06.030

The global demand for energy continues to rise, particularly in emerging market economies [1]. In 2022, global proven oil reserves were estimated at 1,757 billion barrels. The forecast for global crude oil demand in 2023 is approximately 101.2 million barrels per day, with further increase anticipated [2]. This growing reliance on fossil fuels is contributing to an uptick in greenhouse gas emissions. The World Energy Statistical Review 2023 has reported that carbon dioxide emissions from energy use, industrial processes, flaring, and methane (in carbon dioxide equivalent) increased from 39 Gt CO_2 eq., in 2021, to 39.3 Gt CO_2 eq., in 2022. Specifically, emissions from the energy sector rose from 34 Gt CO_2 eq., in 2021, to 34.3 Gt CO_2 eq., in 2022 [3].

Given these trends, there is a pressing need to expand the use of renewable energy sources. Biomass represents the largest renewable resource utilized globally, exceeding 500 million tons per year. The primary sources of biomass include lignocellulosic materials such as wood and agricultural residues. Estimates suggest that the technical potential of biomass is around 150×10^{18} J/year, with projections indicating potential growth to 200—500×10^{18} J/year by 2050, equivalent to 4—12 Gt oil [4]. Biomass is notably used in the transportation sector as additives to biodiesel and bioethanol fuels [5]. Bioethanol is produced through the fermentation of plant materials [6].

The use of renewable sources, such as biofuels, is associated with lower carbon dioxide emissions and reduced environmental pollution. Additionally, organic waste can be utilized as a raw material for biofuels. Biofuels provide an effective means to mitigate greenhouse gas emissions and counteract the effects of climate change related to transportation [7]. Pure ethanol can reduce greenhouse gas emissions by up to 87%, as compared with gasoline per megajoule of fuel [8]. Lignocellulosic ethanol, in particular, is expected to offer lower life-cycle greenhouse gas emissions, as compared with gasoline and conventional grain-based ethanol [9]. According to the Renewable Fuels Association, in 2023, global bioethanol production reached 29,590 million gallons, representing a 4.6% increase since 2022 [10]. Liquid biofuels now account for approximately 18% of primary energy consumption in the transport sector, with bioethanol holding about 80% of the liquid biofuels market.

The production of bioethanol from lignocellulosic feedstocks, as compared with conventional starch- or sugar-based raw materials, has both advantages and disadvantages [11]. The key advantage is the abundance and low cost of lignocellulosic materials that are typically agricultural or forestry residues. However, the increasing demand for agricultural products raises concerns about deforestation and the conversion of land with high biodiversity value to meet this demand. This expansion also leads to the increased use of freshwater, fertilizers, and pesticides, with negative environmental impacts. Some of these issues can be mitigated by complete processing of lignocellulosic feedstocks (second-generation raw materials) [12]. Nevertheless, a significant drawback is the high cost of bioethanol production, largely because of the complexities associated with converting cellulose into fermentable sugars [13].

The production of ethanol from lignocellulosic materials is particularly challenging due to the presence of hemicellulose and lignin [6]. The primary goal of pretreating lignocellulosic feedstocks for hydrolysis is to break down the heterogeneous matrix, to increase the surface area and porosity of the cellulosic material, and to enhance the accessibility of cellulolytic enzymes to cellulose fibers by removing lignin [14]. The effectiveness of hydrolyzing lignocellulosic biomass depends on the complete conversion of polysaccharides, which is facilitated by pretreatment to improve enzyme accessibility. Ideally, all polysaccharides in lignocellulosic biomass should be hydrolyzed into monosaccharides. However, in practice, not all polysaccharides can be fully converted, leaving some polysaccharide chains, particularly those less accessible, unhydrolyzed. To optimize pretreatment outcomes, there are employed many various methods. They are categorized into chemical, physical, physicochemical, and biological approaches [15].

Each method has its own advantages and limitations. Key factors contributing to the high energy consumption of many pretreatment methods include the use of high temperature and pressure (as in dilute acid pretreatment, alkaline pretreatment, or Hot Water technology), long processing time (as seen in biological methods), high reagent consumption, cellulose degradation (in concentrated acid processing), or low efficiency (in ultrasonic treatment). The exploration of methods that combine several factors to enhance the impact on raw materials remains a promising area of research.

Among the methods of pretreating lignocellulosic raw materials for hydrolysis, alkaline treatment is increasingly regarded as a promising approach due to its high efficiency and environmental friendliness [16]. Alkaline pretreatment of lignocellulosic materials is a subject of ongoing research worldwide, with a growing body of evidence supporting its potential [17—19]. Studies have shown that alkaline pretreatment can positively impact enzymolysis, leading to higher sugar yields compared to acid or hot water treatments [20]. For instance, a study [21] has investigated the effects of various pretreatment methods, including 2% and 4% H_2SO_4 solutions and 2% and 4% NaOH solutions, under autoclave conditions (121 °C) for 1 hour, with a solid-to-liquid ratio of 1 : 10. The solid residue from these pretreatments is subjected to enzymatic hydrolysis at 55 °C for 72 hours. The results have indicated that as NaOH concentration increases from 0.5% to 4%, the cellulose conversion rate rises from 38.1% to 65.8% of its initial content. Furthermore, for a 4% NaOH solution, raising the temperature from 50 °C to 121 °C leads to an increase in the cellulose conversion rate from 55.6% to 65.8%. Post-treatment with NaOH also results in wheat straw samples becoming looser and more porous, indicating enhanced fiber accessibility, as compared with untreated samples. However, the study did not explore has not explored the effects of other factors, such as duration and temperature, beyond those directly related to alkali concentration, on the efficiency of alkaline pretreatment for lignocellulosic hydrolysis.

A promising approach to intensifying chemical-technological processes is the method of discrete-pulse energy input, particularly as implemented in rotary pulsation devices [22]. The impact of complex thermophysical phenomena, including high shear stress gradients, hydraulic shock, and cavitation, on liquid heterogeneous media (such as dispersions and emulsions) enhances such processes as dispersion, homogenization, and mixing, while also accelerating chemical reactions at the phase contact zones. Given the multifaceted effects of discrete-pulse energy input on heterogeneous liquid media, it is crucial to investigate how this method, when combined with the primary factors of alkaline pretreatment, affects the degree of cellulose conversion during enzymatic hydrolysis.

The objective of this study is to evaluate the effect of discrete-pulse energy input treatment on an alkaline suspension of wheat straw using a rotary pulsation device, specifically focusing on the degree of cellulose conversion during enzymatic hydrolysis in the production of fuel ethanol.

The raw material consists of wheat straw harvested in August 2020 from Kyiv Oblast of Ukraine, with the following composition by weight: 45.6% cellulose, 17.1% lignin, 5.4% extractives, 25.8% hemicelluloses, and 4.2% ash. Initially, the straw is cut to a size of 10—50 mm by a *Rozumnytsia* straw cutter, followed by a single pass through a disintegrator. The particle size distribution after disintegration is presented in Fig. 1. The processed raw materials are stored in airtight polyethylene bags to preserve their integrity.

The alkaline suspension of wheat straw is processed with the use of an experimental plant equipped with a mixing reactor, specifically a rotary pulsation device (Fig. 2). The design and operational principles of this experimental plant are detailed in [23].

The procedure for raw material processing is as follows. The straw is soaked in water until a homogeneous suspension is formed. The remaining water is poured into the receiving vessel of the installation, mixed with sodium hydroxide in the ratio specified by the experimental conditions, and heated to the required temperature. Throughout the entire series of studies, the straw concentration in the suspension is maintained at 10% wt. Once the engine starts, the frequency converter is used to set the rotor speed at 47.75 rpm for all experiments.

The samples obtained after the pretreatment are neutralized, filtered, washed, and dried before undergoing enzymatic hydrolysis. The hydrolysis procedure is as follows: 1 g pretreated straw is immersed in 30 ml of 50 mM sodium citrate buffer, with a pH of 4.8. For the hydrolysis of lignocellulosic biomass, we add 35 FPU/g cellulase from *Trichoderma reesei* (*Celluclast*® 1.5) and 61.5 FPU/g cellulase from *Aspergillus niger* (*Novozym* 188), both from *Novozymes* (Denmark), are added. To prevent microbial contamination, sodium azide (0.3% w/v) is also added to the solution. The lignocellulosic biomass is incubated at 55 °C with dynamic agitation (150 rpm) for 72 h [24]. The target components are determined based on the biomass analysis standards of the National Renewable Energy Laboratory of the U.S. Department of Energy. Each experimental series is measured in triplicate, with an error margin not exceeding 5%.

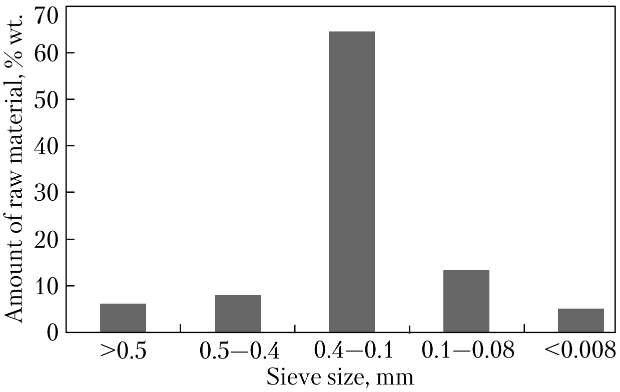

Fig. 1. Distribution of wheat straw particles on the sieves after the treatment in disintegrator

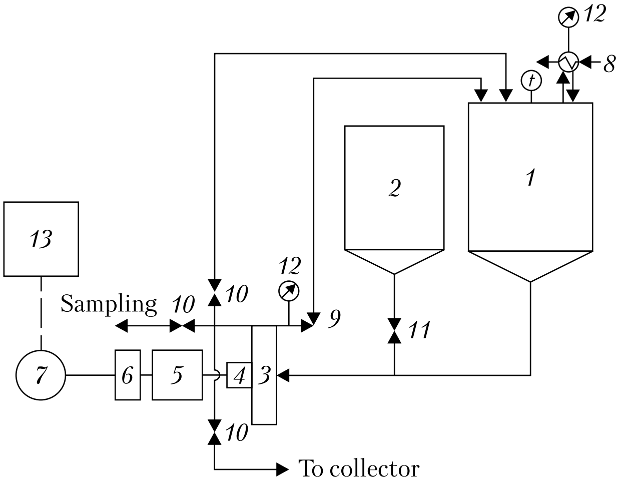

Fig. 2. Hydraulic diagram of the experimental plant [23]: *1* — receiving tank; *2* — raw material container; *3* — reactor-mixer; *4* — gland assembly; *5* — bearing assembly; *6* — coupling; *7* — electric motor; *8* — dephlegmator; *9* — angled ball valve; *10* — ½″ ball valve; *11* — 1″ ball valve; *12* — manometer; *13* — control unit

Among the key parameters influencing the delignification of lignocellulosic raw materials during their pretreatment for hydrolysis are the temperature of the process, the concentration of alkali, the duration of the process, and the size of the raw material particles. Several studies have shown that reducing the average particle size leads to an increase in sugar yield [25—27]. Based on this, the initial stages of the research focus on determining the impact of the combined physical effects of the discrete pulse energy input method and particle size on the degree of lignin extraction during the alkaline pretreatment of wheat straw in a rotary pulsation device. The experiments are conducted under the following conditions: a temperature of 90 °C, a lye concentration of 4% wt., and a duration of 1 h. The effect of particle size on lignin removal is presented in Fig. 3. The data indicate that increasing the dispersion of raw materials leads to a higher degree of lignin extraction, rising from 40% with an average particle size of 2—1 mm to 62% with an average particle size of 0.4—0.1 mm.

According to some authors, the concentration of alkali in the process of alkaline pretreat-

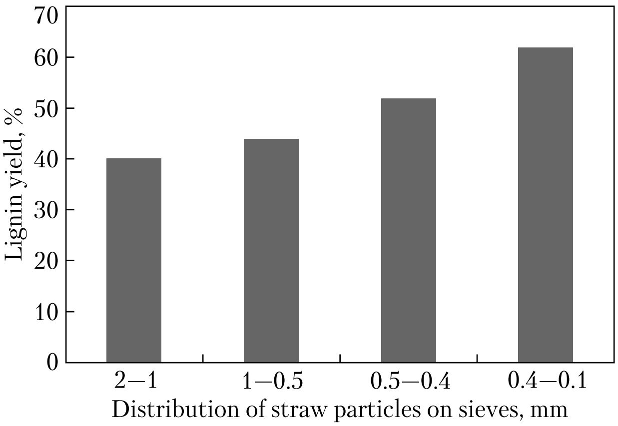

Fig. 3. Dependence of the lignin yield on the size of the solid phase particles during the processing of an alkaline suspension of wheat straw in a rotary pulsation device

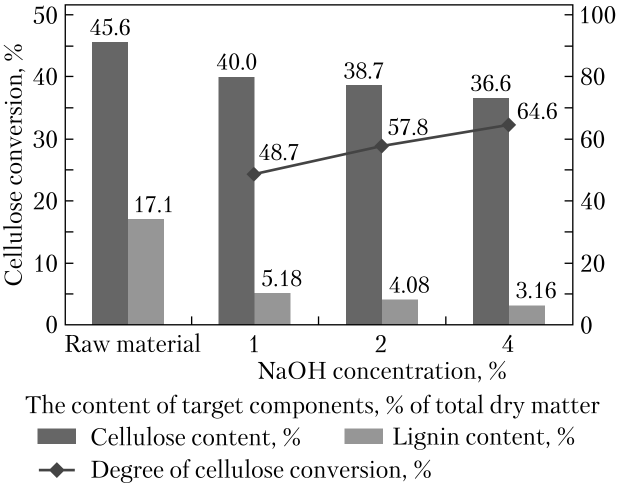

Fig. 4. Dependence of cellulose conversion during enzymatic hydrolysis in a rotary pulsation device at alkali concentrations of 1%, 2%, and 4% wt

ment is the most significant factor affecting the conversion of cellulose during the subsequent enzymatic hydrolysis [28]. Figure 4 shows the influence the physical factors involved in the process due to the application of the method of discrete pulse energy input and alkali concentration on the content of target components and the degree of cellulose conversion as a result of enzymatic hydrolysis during the processing of an alkaline suspension of wheat straw in a rotary pulsation device at a temperature of 90 °C during 1 h.

Increasing the alkali concentration in the alkaline suspension of wheat straw from 1 to 4% (wt./wt.) leads to a decrease in the lignin content in the samples from 17.1 to 3.14% relative to the total content of components in the raw material. The effect of alkali as a result of treatment manifests itself in a slight decrease in the cellulose content in the samples from 48.7% for a 1% solution of NaOH to 36.6% for a 4% solution of this alkali. Increasing the concentration of NaOH in the studied straw solution together with the complex of physical effects of the method of discrete-pulse energy input leads to an increase in the degree of cellulose conversion during the next enzymatic hydrolysis from 48.7% to 64.6% of the total cellulose content after pretreatment.

The results from the pretreatment of wheat straw for hydrolysis for one hour using the discrete pulse energy input method in a rotary pulsation device at a treatment temperature of 90 °C have been compared with those obtained through autoclaving at 121 °C and 1 atm pressure, is presented in Fig. 5. These conditions aim to optimize the preparation process while avoiding excessive cellulose loss and lignin re-precipitation. The data in this figure indicate that the discrete-pulse energy input method enhances the degree of cellulose conversion across the entire range of alkali concentrations.

Increasing the sodium hydroxide concentration from 1% to 4% during the pretreatment of wheat straw in an autoclave results in an increase in the cellulose conversion during subsequent enzymatic hydrolysis, from 54.6% to 73.59%. Under the conditions of discrete pulse energy input in a rotary pulsation device, the same increase in sodium hydroxide concentration leads to a growth in the cellulose conversion from 48.7% to 64.6%.

Figure 5 also illustrates the residual lignin content in the samples after pretreatment. When the sodium hydroxide concentration increases from 1% to 4% during autoclave preparation, the lig-

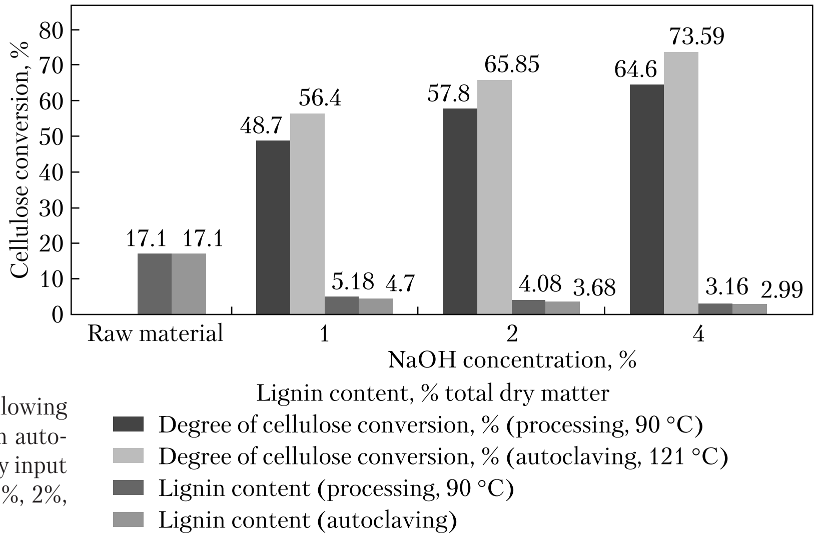

Fig. 5. Dependence of cellulose conversion following enzymatic hydrolysis after pretreatment in an autoclave (121 °C) and by the discrete pulse energy input method (90 °C), at alkali concentrations of 1%, 2%, and 4% wt

nin content decreases from 100% to 27.48%, and further to 17.48%. Similarly, during the pretreatment of wheat straw using the discrete pulse energy input method in a rotary pulsation device, the lignin content decreases from 100% to 30.29% and further to 18.48%.

The combination of the physical effects provided by the discrete pulse energy input method, along with appropriate alkali concentrations during the pretreatment of lignocellulosic raw materials, facilitates an accelerated conversion of cellulose during subsequent enzymatic hydrolysis. This enhancement can be attributed to the more effective removal of lignin into the solution during the pretreatment stage, the prevention of lignin re-deposition, and an increased contact area between cellulose fibers and cellulolytic enzymes.

The research has demonstrated that incorporating the physical effects of the discrete pulse energy input method into the primary factors of alkaline pretreatment (specifically, alkali concentration) leads to a significant increase in the cellulose conversion during enzymatic hydrolysis — from 48.7% to 64.6%. The results of treatment at temperature below 100 °C have been found to be comparable with those obtained through autoclaving at higher temperature and pressure. This implies that lower temperature treatment results in reduced cellulose loss and less lignin re-deposition, thereby enhancing the efficiency of hydrolysis.

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Received 22.08.2023
Revised 10.05.2024
Accepted 31.05.2024

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ВПЛИВ УМОВ ПОПЕРЕДНЬОЇ ЛУЖНОЇ ОБРОБКИ РОСЛИННОЇ СИРОВИНИ ДО ГІДРОЛІЗУ В РОТОРНО-ПУЛЬСАЦІЙНОМУ АПАРАТІ НА СТУПІНЬ КОНВЕРСІЇ ЦЕЛЮЛОЗИ

Вступ. Виходячи зі зростання глобального попиту на енергію, вичерпність викопних джерел енергії та збільшення емісії парникових газів, виникає потреба ширшого впровадження відновлюваних джерел енергії, зокрема біомаси.

Проблематика. Основним недоліком виробництва біоетанолу з лігноцелюлозної сировини є висока вартість, що зумовлено наявністю в сировині геміцелюлоз та лігніну.

Мета. Визначення впливу обробки лужної суспензії соломи пшеничної методом дискретно-імпульсного введення енергії в роторно-пульсаційному апараті на ступінь конверсії целюлози ферментативним гідролізом у технології виробництва паливного етанолу.

Матеріали і методи. Сировиною слугувала попередньо грубо подрібнена солома пшенична. Дослідження виконано на установці з роторно-пульсаційним апаратом.

Результати. Визначено, що одночасний вплив комплексу фізичних ефектів методу дискретно-імпульсного введення енергії та зменшення розміру частинок соломи призводить до підвищення ступеня вилучення лігніну з 40,0 % при середньому розмірі частинок 2—1 мм до 62,0 % при середньому розмірі частинок 0,4—0,1 мм. Також визначено, що вплив комплексу фізичних ефектів методу дискретно-імпульсного введення енергії та концентрації лугу на ступінь конверсії целюлози в результаті ферментативного гідролізу при обробці лужної суспензії соломи пшеничної за температури 90 °C протягом години з 1 до 4 % мас/мас призводить до збільшення ступеня конверсії целюлози протягом ферментативного гідролізу з 38 до 65,8 %. Також відбувається відповідне зменшення вмісту лігніну — з 17,1 до 3,16 %.

Висновки. Додавання комплексу фізичних ефектів методу дискретно-імпульсного введення енергії до основних чинників лужної попередньої підготовки соломи пшеничної (зокрема концентрації лугу) призводить до збільшення ступеня конверсії целюлози при наступному ферментативному гідролізі. Результати обробки за температури нижче 100 °C виявились сумірними з результатами автоклавування за підвищеної температури й тиску.

Ключові слова: паливний етанол, попередня обробка, луг, конверсія целюлози, целюлазний комплекс.