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USE OF LIGNOCELLULOSE RAW MATERIALS IN THE PRODUCTION OF BIOETHANOL

Kateryna Danilova*, Sergiy Oliynichuk, Roman Grushetskiy, Irina Grinenko
Institute of Food Recourses of National Academy of Agrarian Sinces of Ukraine, 4A, E. Sverstiyka str., Kyiv, 02002, Ukraine

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Abstract

Wheat straw and corn stalks are considered potential raw materials for bioethanol production as an alternative fuel due to their high cellulose and hemicellulose content, as well as their wide distribution and availability. The aim of the work is to study the pretreatment of wheat straw and post-harvest corn waste by steam explosion and organosolvent delignification methods to obtain the maximum ethanol yield. A combination of steam explosion as a method of pretreatment of straw for the destruction of lignin bonds and organosolvent delignification for the separation of cellulose is proposed. The obtained explosive defibration products and the solid phase after organosolvent delignification were subjected to enzymatic hydrolysis with cellulase and fermented with *Saccharomyces cerevisiae* yeast. For delignification, 50 % ethyl alcohol was used with the addition of sulfuric acid as a catalyst in an amount of 3 %. A study of the quantitative yield of delignification products at each stage of raw material processing was conducted. Analysis of the physicochemical parameters of the obtained explosive defibration products showed an increase in the cellulose content from 42 to 63 %, but the residual lignin content was 12 %. Organosolvent delignification led to almost complete destruction of lignin bonds with the release of cellulose in a form available for enzymatic hydrolysis. Enzymatic hydrolysis of cellulose with a complex of cellulolytic enzymes before fermentation increases the ethanol content in the fermented mashes by 4 times. Improvements in the pretreatment of lignocellulosic raw materials, especially through combinations of different processes, and advances in biotechnology aimed at creating effective enzyme preparations and yeast strains with high enzymatic activity and resistance to inhibitors, will lead to an increase in the economic efficiency of second-generation bioethanol production by 50 %.

Keywords: wheat straw; corn stalks; bioethanol; steam explosion; organosolvent delignification; enzymatic hydrolysis; fermentation.

ЗАСТОСУВАННЯ ЛІГНІНОЦЕЛЮЛОЗНОЇ СИРОВИНИ У ВИРОБНИЦТВІ БІОЕТАНОЛУ

Катерина О. Данілова, Сергій Т. Олійнічук, Роман І. Грушецький, Ірина Г. Гріненко
Інститут продовольчих ресурсів Національної академії аграрних наук України, 4А, вул. Є. Сверстюка, Київ, 02002, Україна

Анотація

Солома пшениці та кукурудзяні відходи вважаються потенційною сировиною для виробництва біоетанолу як альтернативного палива через високий вміст целюлози і геміцелюлози, а також їх широке розповсюдження і доступність. Метою роботи є дослідження попередньої обробки пшеничної соломи і післязбиральних відходів кукурудзи способами парового вибуху й органосольвентної делігніфікації для одержання максимального виходу етанолу. Запропоновано комбінацію парового вибуху як метод попередньої обробки соломи для руйнування зв'язків лігніну та органосольвентної делігніфікації для відділення целюлози. Отримані продукти вибухової дефібрації та тверду фазу після органосольвентної делігніфікації піддавали ферментативному гідролізу целюлазою та зброджували дріжджами *Saccharomyces cerevisiae*. Для делігніфікації використовувати 50 %-й етиловий спирт з додаванням сірчаної кислоти як каталізатора в кількості 3 %. Було проведено дослідження кількісного виходу продуктів делігніфікації на кожному етапі переробки сировини. Аналіз фізико-хімічних показників отриманих продуктів вибухової дефібрації показав збільшення вмісту целюлози з 42 до 63 %, але вміст залишкового лігніну становив 12 %. Органосольвентна делігніфікація призводила до майже повного руйнування лігнінових зв'язків із виділенням целюлози у формі, доступній для ферментативного гідролізу. Гідроліз целюлози комплексом целюлолітичних ферментів перед зброджуванням збільшує вміст етанолу в дозрілій бражці у 4 рази. Удосконалення попередньої обробки лігніноцелюлозної сировини, особливо завдяки комбінаціям різних процесів та прогрес у біотехнології, спрямований на створення ефективних ферментних препаратів та штамів дріжджів з високою ферментативною активністю, стійких до дії інгібіторів, сприяють підвищенню економічної ефективності виробництва біоетанолу другого покоління на 50 %.

Ключові слова: пшенична солома; біоетанол; паровий вибух; органосольвентна делігніфікація; ферментативний гідроліз; зброджування.

**Corresponding author: e-mail: dankoek77@gmail.com*

Introduction

In recent years, there has been a steady trend toward a decrease in crude oil production by OPEC countries and, accordingly, an increase in the cost of gasoline on the world market. That is why there is increasing interest in the use of alternative fuels and chemical products from renewable plant biomass [1]. The USA and Brazil are the two leading countries in the production of ethanol from corn and sugar cane. In 2023, more than 100 billion liters of bioethanol were produced by yeast fermentation of carbohydrates from sugar and starch-containing raw materials.

Of this volume, approximately 28 % of fuel ethanol was produced from Brazilian cane sugar and approximately 53 % from glucose obtained from corn starch, mainly in the USA [2]. However, the production of biofuels from starch and sugar significantly disrupts the global food security of mankind, taking away a share of the food market. The global community attributes a significant increase in the production of ethanol, a decrease in its cost price, and an increase in competitiveness to the use of non-food raw materials, in particular lignin-cellulosic biomass.

This plant biomass is the most available source of renewable raw materials with an estimated annual volume of 1010 million tons in the world [3]. The use of such biomass makes the raw material base for obtaining fuel ethanol practically inexhaustible. Cellulosic biomass is obtained from wood and forest industry waste [4] and non-wood raw materials, which include agricultural waste, household and industrial waste, as well as energy crops such as millet and miscanthus [5]. The main advantages of using non-wood raw materials for the ethanol production are its low cost, availability, reduction of energy costs and chemical reagents for the extraction of cellulose due to the lower content of lignin compared to wood, which contains more lignin and requires greater energy and chemical costs [6].

Cellulosic raw materials contain polysaccharide cellulose (23-50%), hemicellulose (12–29 %) and aromatic polymer lignin (13–31 %) [7]. Lignins cross-link cellulose and hemicellulose, which makes the structure of lignin-cellulosic raw materials stronger and firmer. That is why, during the processing of this raw material, preliminary treatment is required to remove lignin (delignification), increase the porosity of the fibers and increase hydrolysis in the process of processing biomass into ethanol. The review [3] states that pre-treatment of biomass by physical, chemical and biological methods is necessary to dissolve the structure of lignocellulosic biomass, and their combination leads to more effective destruction and makes the biomass available for further enzymatic hydrolysis and fermentation. Methods of steam explosion and biological treatment with microorganisms and enzymes are considered to be promising and environmentally friendly tendency [8; 9].

Wheat stalks are considered a potential raw material for ethanol production due to their high content of cellulose and hemicellulose, as well as their wide distribution and availability. In studies [10; 11], it is recommended to moisten raw materials with water before steam explosion to increase the yield of sugars during the subsequent enzymatic hydrolysis of cellulosic biomass.

In works [12; 13] it is shown that the steam explosion provides a higher conversion of cellulose due to the removal of lignin, an increase in the surface area of the processed biomass by 3.1 times. The results showed that different conditions of pretreatment with steam explosion affect both the biomass structure and the yield of enzymatic hydrolysis products [14]. At the same time, the temperature of the steam explosion had a greater influence on the formation of reducing sugars than the residence time of the raw materials [15; 16]. Thus, among the methods of pretreatment of lignocellulosic biomass, steam explosion is an environmentally friendly, cheap and effective approach to biomass delignification, which significantly increases the efficiency of the ethanol production process and increases its yield. But for the implementation of this method on an industrial scale, more in-depth research is needed [17].

Another method of pre-treatment of lignocellulosic biomass is the use of organic solvents, such as ethanol, glycerin, acetone, methanol, etc., which are added to biomass in the presence of catalysts at a certain temperature and pressure. Catalysts can be sulfuric acid, sodium hydroxide or magnesium sulfate [18–21].

In our opinion, the most economical and ecological will be the use of ethyl alcohol as an organic solvent for organosolvent delignification, since it is the final product of lignocellulosic biomass processing. We used dilute sulfuric acid as a catalyst. Taking into account the peculiarities of ethanol production, the study of the influence of processing parameters and solvent composition on the course of the process of delignification of lignocellulosic biomass and the formation of ethyl alcohol is relevant.

The current available technology, which is based on dilute acid hydrolysis, has about 35 %

efficiency (HHV) from biomass to ethanol. The overall efficiency, with electricity co-produced from the not fermentable lignin, is about 60 %. Improvements in pre-treatment and advances in biotechnology, especially through process combinations can bring the ethanol efficiency to 48 % and the overall process efficiency to 68 % [22].

The feedstock for biofuel production has a significant impact on the economic efficiency of the process. The production costs of biofuels produced from starch-containing feedstocks (first-generation biofuels) are significantly lower than those produced from lignocellulosic biomass and waste (second-generation biofuels). In 2018, the price of bioethanol produced from sugarcane was 0.56 USD/L, and from wheat was 0.25 USD/L. The minimum price of bioethanol produced from wheat straw and corn waste was 0.54–0.57 USD/L) [23]. The current costs of cellulosic ethanol production in Europe are 2.3 times higher than in the USA. Reducing these costs consists in improving the technological stages of production, large-scale process integration, reducing the cost of enzymes and using lignin residues for electricity generation. The environmental and economic feasibility of first-generation biofuels is questionable, as they depend on crops used for food production and require arable land, fertilizer, and water to grow. In addition, increasing demand for raw materials leads to land use change and deforestation, accelerating climate change and biodiversity loss. Bioethanol production still relies heavily on subsidies and other market forces to compete economically with fossil fuels. Despite this, second-generation biofuels have greater potential to reduce greenhouse gas emissions than first-generation biofuels.

The purpose of the work is to study the pretreatment of wheat straw and corn stolks by the methods of steam explosion and organosolvent delignification to obtain the maximum yield of ethanol.

Materials and methods

Materials

1. The wheat straw (WS) and corn stalks (CS) was obtained from local farmers. Relative humidity was 6.4 %. Before the steam explosion, the straw and post-harvest corn waste was crushed to a particle size of 2–3 cm using an electric shredder. The composition of wheat straw and corn stalks is shown in Table 1.

Table 1

The composition of wheat straw and corn stolks

Content	Indicator	
	Wheat straw	Corn stalks
Moisture, %	(6.43±0.9)^{a}	(6.9±0.9)^{a}
Ash, %	(6.51±0.9)^{a}	(2.3±0.1)^{a}
Cellulose, %	(42.56±1.5)^{b}	(30.5±1.5)^{a}
Hemicellulose, %	(20.71±1.5)$^{b.}$	(31.9±1.5)^{a}
Lignine, %	(24.47±1.5)^{b}	(24,0±1.5)^{a}

Data are mean value±S.D., N=3. Different letters in a row indicate significant differences at $p<0.05$ level

The cellulose content of wheat straw was higher than that reported in [21], but consistent with the value determined in [24]. The hemicellulose content was lower than that reported in [21], but consistent with that determined in [24; 25]. The lignin content was consistent with that determined in [24; 25].

The corn waste, compared to wheat straw, is characterized by a lower cellulose content and a higher hemicellulose content, which is easily hydrolyzed to form reducing sugars.

2. Cellulase, enzyme *Cellic CTec2*, Novonesis, Denmark.

3. The dry yeast *Sacharomyces cerevisiae* strain Ethanol Red used in this study was gratefully supplied by company "Enzym", Lviv, Ukraine.

4. Antiseptic Bactrilon, company «Tristan», Kyiv, Ukraine

Ways of processing wheat straw and corn stalks:

1. Steam explosion.

The steam explosion was carried out on the equipment of the biogas plant of the Teofipol Energy Company (Ukraine). Dry straw was mixed with water. Straw was fed in portions to a steam reactor, where it was heated to a temperature of 150–200 °C, at which the destruction of lignin bonds with the formation of cellulose and hemicellulose occurred. During the preparation process at high temperature and pressure, the raw materials were completely soaked with water. Then the pressure was reduced to atmospheric in a second. As a result, the water turned into steam, creating an explosion effect that ruptured the straw fibers. Conditions for steam explosion:

Mode 1 – pressure 0.5 MPa, temperature 150 °C, treatment duration 20 minutes;

Mode 2 – pressure 0.5 MPa, temperature 180 °C, treatment duration 20 minutes.

Mode 3 – pressure 0.8 MPa, temperature 200 °C, treatment duration 10 minutes

The resulting products of explosive defibration were subjected to organosolvent delignification to free them from residual lignin.

2. Organosolvent delignification**.**

The plant mass was mixed with a solvent in a ratio of 1 : 4, the composition of which was 50 % ethyl alcohol, 47 % water and 1–4 % sulfuric acid, delignification was carried out in a hermetic autoclave at a temperature of 120–140 °C and an excess pressure of 0.2–0.4 MPa for 2 hours.

The procedure for conducting research. The resulting reaction mixture was separated by filtration. The solid phase freed from lignin, containing mainly cellulose, was used for enzymatic hydrolysis to monosugars by cellulolytic enzymes, and the solvent was removed from the liquid fraction by distillation. Under such conditions, lignin precipitated and after drying was used as a finished product**.**

3. Enzymatic hydrolysis.

The product of explosive defibration and the solid phase after delignification were subjected to enzymatic hydrolysis with a complex of cellulolytic enzymes Cellic Tec2 at a concentration of 42 units/g at a temperature of 50–55 °C at a pH of 5.0–5.3 for 24 hours.

4. Fermentation.

For the obtaine of ethanol, fermentation was carried out with dry yeast *Saccharomyces cerevisiae* (Ethanol Red) at a temperature of 30 °C for 48 hours according to the following options:

Option 1–30 g of explosive defibration product with a moisture content of 12 % + 310 cm^3 of water + 1 g of dry yeast + Bactrilon (1 cm^3) for fermentation for 48 hours;

Option 2–30 g of explosive defibration product with a moisture content of 12% + 310 cm^3 of water + 7 cm^3 of the *Cellic Tec2* enzyme preparation for 24 hours of enzymatic hydrolysis + 1 g of dry yeast + Bactrilon (1 cm^3) for fermentation for 48 hours;

Option 3–30 g of explosive defibration product with a moisture content of 12 % for organosolvent delignification for 2 hours, a solid fraction of cellulose (70.2 g) with a moisture content of 90 % + 180 cm^3 of water + 7 cm^3 of the enzyme preparation *Cellic Tec2* for 24 hours of enzymatic hydrolysis + 1 g dry yeast + Bactrilon (1 cm^3) for fermentation for 48 hours.

Research Methods

The moisture content of cellulosic raw materials was determined by drying to constant weight at a temperature of 100–105 °C. Lignin content – by direct method by hydrolysis with a mixture of concentrated hydrochloric acid and 72 % sulfuric acid [26]. The amount of polysaccharides, easily and difficultly hydrolyzed, was determined by the number of monosaccharides by reducing ability determined by the Maquenne-Schoorl method [27].

Alcohol content in fermented distillates was determined hydrometrically [28], the mass concentration of unfermented carbohydrates – by the photoelectric colourimetric anthrone method [29].

Statistical analysis

The results are presented as means and standard deviation. Data were subjected to ANOVA and significant differences between means were revealed by post – hoc Duncan's multiple range test ($p < 0.05$), using IBM SPPSS Statistics 20.0 software.

Results and discussion

Delignification of wheat straw and corn stalks by steam explosion.

We studied samples of wheat straw and corn stalks after preliminary treatment by the method of steam explosion under a pressure of 0.5–0.8 MPa according to the decompression principle, obtained under different conditions. The results are shown in Table 2.

Table 2

Physico-chemical composition of semi-products of steam explosive

Content	Processing conditions					
	Mode 1		Mode 2		Mode 3	
	WS	CS	WS	CS	WS	CS
Dry matter content, %	(33.2±1.5)^a	(40.4±1.5)^a	(8.83±0.9)^a	(11.3±0.9)^a	(9.34±0.9)^a	(12.5±0.9)^a
Humidity, %	(66.8±2.8)^b	(69.1±2.8)^b	(91.17±2.8)^b	(93.7±2.8)^b	(90.66±2.8)^b	(94.82±2.8)^b
pH	(4.36±0.5)^c	4.5±0.5)^c	(4.82±0.5)^c	(5.1±0.5)^c	(5.19±0.5)^c	(5.4±0.5)^c
The content of easily hydrolyzable polysaccharides (hemicelluloses), %	(15.6±1.5)^d	(24,5±1.5)^d	(11.2±1.5)^d	(22.3±1.5)^d	(10.7±1.5)^d	(18.6±1.5)^d
The mass fraction of reducing substances in the hydrolyzate of easily hydrolyzable polysaccharides, %	(0.03±0.005)^e	(0.02±0.005)^e	(0.16±0.02)^e	(0.14±0.02)^e	(0.18±0.02)^e	(0.17±0.02)^e

Continued from the Table 2

The content of polysaccharides that are difficult to hydrolyze (cellulose), %	$(42.2\pm1.5)^{f}$	$(38.7\pm1.5)^{f}$	$(51.4\pm1.5)^{f}$	$(47.4\pm1.5)^{f}$	$(63.3\pm1.5)^{f}$	$(57.2\pm1.5)^{f}$
The mass fraction of reducing substances in the hydrolyzate of polysaccharides that are difficult to hydrolyze, %	$(0.12\pm0.02)^{g}$	$(0.11\pm0.02)^{g}$	$(0.15\pm0.02)^{g}$	$(0.14\pm0.02)^{g}$	$(0.17\pm0.02)^{g}$	$(0.16\pm0.02)^{g}$
The content of residual lignin by the direct method, %	$(22{,}71\pm1.5)^{h}$	$(20.8\pm1.5)^{h}$	$(18.4\pm1.5)^{h}$	$(19.6\pm1.5)^{h}$	$(12.1\pm1.5)^{h}$	$(15.7\pm1.5)^{h}$

Data are mean value±S.D., N=3. Different letters in a row indicate significant differences at $p<0.05$ level

The obtained results show that the steam explosion changed the structure of wheat straw and corn stolks. While the raw material had an organized and compact structure, the surface of the straw and stolks after the steam explosion became loose and porous due to the destruction of the lignin-cellulose-hemicellulose complex. The conditions of the steam explosion determined the composition of the semi-finished products obtained. As the processing temperature increased from 150 to 200 °C, the cellulose content increased from 42 to 63 % for wheat straw and from 39 to 57 % for corn stalks, which indicates the release of lignin from the structure. This is also confirmed by the decrease in the content of residual lignin in the product by more than 2 times due to the destruction of lignin bonds. However, the content of residual lignin in the intermediate product of explosive defibrillation remains at the level of 12–15 % for all types of raw materials, which indicates the incomplete destruction of lignin bonds in the molecular structure, which will interfere with enzymatic hydrolysis and reduce the yield of ethanol.

The obtained data correlate with the results given in the literature [30; 31]. As a result of the treatment of corn stalks with alkali and steam explosion, approximately 71.58–79.59 % of hemicellulose and 64.32–71.83 % of lignin were removed [30], in our case, as a result of steam explosion, the hemicellulose content decreased by 58 %, and the lignin content by 49 %. This indicates the insufficiency of delignification by only one method. Additional processing is necessary to remove lignin and increase the cellulose content.

It is known that increasing the processing difficulty factor (temperature, pressure, and duration of processing) contributed to a more complete destruction of the structure of straw, but increased the amount of formation of acetic acid, furfural, and formic acid, which are inhibitors of enzymatic hydrolysis [32]. Therefore, for further research, we adopted the optimal conditions for conducting a steam explosion - a temperature of 200 °C and a treatment duration of 10 minutes, when the formation of inhibitors was minimal.

The resulting semi-product of steam explosive was subjected to additional hydrolysis with the help of a solvent containing 50 % ethyl alcohol and 1–4 % one-normal sulfuric acid to remove residual lignin from it. Conditions of organosolvent hydrolysis – pressure 0.3 MPa, temperature 135 °C, treatment duration 2 hours.

Effect of solvent composition on the yield of lignin and reducing sugars

In the technology of organosolvent delignification of vegetable raw materials, the composition of the solvent is an important factor that affects the intensity, completeness and course of lignin dissolution. As a result of experimental studies, it was observed that the ratio of solvent components, in particular the sulfuric acid content, has a certain effect on the release of lignin. In [21], experiments were conducted with organosolv (50 % ethanol) at low temperature (140 °C) without adding a catalyst and with the addition of acetic (50 mM) or sulfuric acids (50 mM). Sulfuric acid at 140 °C promoted the hydrolysis of xylan to 71 % and the delignification yield to 72 % for wheat straw. Acetic acid as a catalyst did not promote hydrolysis, and delignification was very low. There are few studies with organosolv at low temperatures, but the addition of sulfuric acid at 140 °C undoubtedly promotes xylan hydrolysis and chemical depolymerization of lignin [33].

We carried out organosolvent treatment of wheat straw with 50 % ethyl alcohol with the addition of sulfuric acid in an amount of 1 to 3 % of the solvent volume (Fig. 1). Increasing the sulfuric acid concentration from 1 to 3 % contributed to a decrease in the amount of residual lignin in the product from 80 % to 62 % and the transition of easily hydrolyzed polysaccharides into solution.

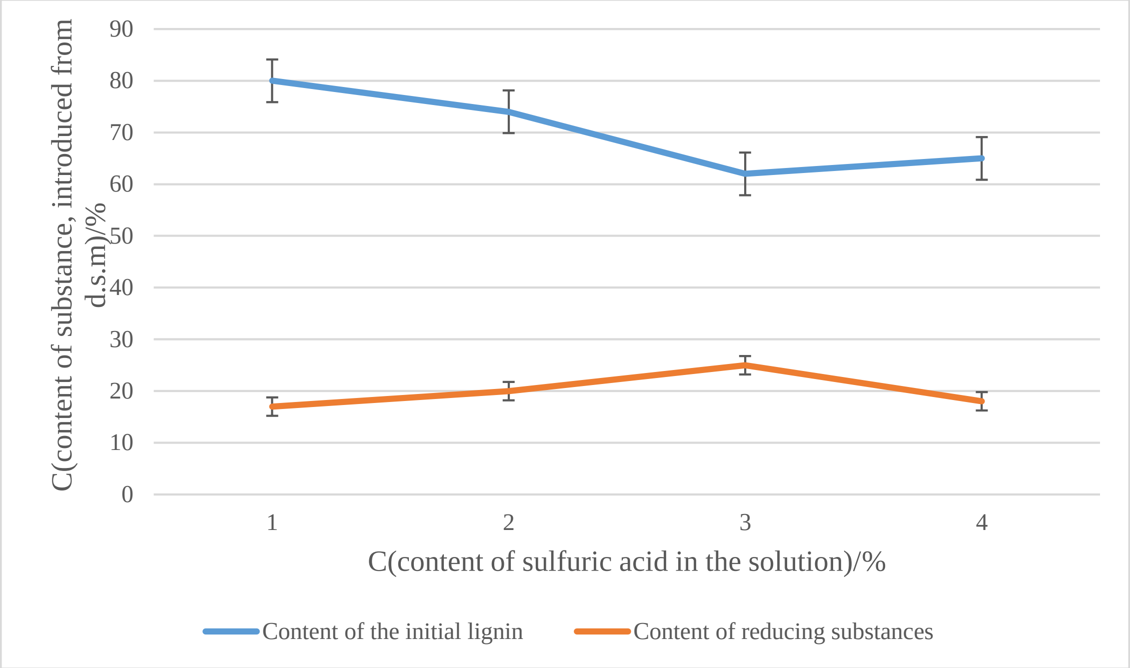

Fig.1. Effect of acid concentration in the solvent on the yield of lignin and reducing substances

The number of reducing substances in the filtrate also increased with increasing acid content and was 32 % higher at 3% compared to 1 % acid content. This is explained by the fact that sulfuric acid is a catalyst for the hydrolysis reaction of polysaccharides and in aqueous solutions dissociates into the hydroxonium ion (H_3O^+), which protonates the oxygen of the glycosidic bond. As a result, the acetal oxygen changes to a 4-valent state, the stability of the glycosidic bond decreases, and it splits with the formation of two parts of macromolecules. The final product of this reaction is monosaccharides [34].

Analyzing the studied parameters, it can be concluded that the most effective way to delignify wheat straw is to use a water-alcohol oxidant, which contains 3 % (by volume) sulfuric acid as a catalyst.

When delignifying corn stolks by an organosolvent method, with practically the same lignin content in wheat straw and corn stalks, the degree of lignin conversion for CS was approximately 30 % lower compared to wheat straw, regardless of the delignification conditions. This can be explained by the fact that the cellulose of the vegetative parts of corn has a lower crystallinity index compared to wheat straw. The hydrolysate from corn stalks contains a larger amount of amorphous polysaccharide fraction, which, due to its high surface area, fixes a larger amount of lignin released during the delignification process. Therefore, pre-treatment of corn stolks by steam explosion increases the crystallinity of CS and increases the yield of lignin.

The resulting products of explosive defibration and the solid phase after organosolvent delignification containing cellulose were subjected to enzymatic hydrolysis with the *Cellic Tec2* cellulolytic enzyme. During enzymatic hydrolysis, reducing sugars in the form of glucose were mainly formed. Table 3 shows the content of the main components after each stage of delignification.

Table 3

The content of the main components after each stage of the process

Indicator	After steam explosive		After organosolvent delignification	
	Wheat straw	Corn stalks	Wheat straw	Corn stalks
The content of polysaccharides that are difficult to hydrolyze (cellulose), %	(63.3±2.8)^{a}	(57.2±2.8)^{a}	(65,6±2.8)^{a}	(58.7±2.8)^{a}
The content of easily hydrolyzable polysaccharides (hemicelluloses), %	(10.8±1.5)^{b}	(18,6±1.5)^{b}	(3.2±0.5)^{b}	(7.4±0.5)^{b}
The content of residual lignin by the direct method, %	(12.1±1.2)^{c}	(15.7±1.5)^{c}	(2.1±0.5)^{c}	(2.8±0.5)^{c}
Content of reducing sugars, g/100 cm^3	(0.24±0.01)^{d}	(3.15±0.5)^{d}	(1.08±0.02)^{d}	(3.76±0.5)^{d}

Data are mean value±S.D., N=3. Different letters in a row indicate significant differences at $p<0.05$ level

As it can be seen from the table, organosolvent delignification promoted hemicellulose hydrolysis by 70 % for wheat straw and by 60 % for corn stalk, and almost complete destruction of lignin bonds was observed. The content of residual lignin after organosolvent delignification in the solid fraction was 2.1 and 2.8 % for WS and CS, respectively. According to literature data, to achieve the best ethanol yield, the content of residual lignin in the product can be in the range from 0.35 % to 3.5 % [35].

Thus, organosolvent delignification using a small amount of sulfuric acid as a catalyst promotes the hydrolysis of hemicelluloses and increases the delignification of vegetable raw materials by 87 % for wheat straw and 82 % for corn stalk, which is consistent with the data presented in [21; 33]. From the liquid fraction after organosolvent delignification, ethyl alcohol was removed by distillation, resulting in lignin precipitation, which was filtered and dried. The resulting lignin can be used to produce electricity or other valuable products.

The solid fraction after organosolvent delignification was subjected to enzymatic hydrolysis with the cellulolytic enzyme preparation Cellic Tec2 at pH 5.2 and temperature 50–53 °C. After 24 and 48 hours, the content of reducing sugars was determined (Fig. 2). During enzymatic hydrolysis, reducing sugars were mainly formed in the form of glucose. As it can be seen from the data in Fig. 2, their content after 24 hours increased by 6 times for wheat straw and by 4.65 times for corn stolks. After 48 hours, the content of reducing sugars increased by another 1.3 and 1.1 times, respectively. The results obtained are supported by the findings in [21; 37], which indicated that in organosolvent delignification without the addition of a catalyst, the saccharification yield was only slightly higher than that obtained directly in untreated samples of the starting material. At 140 °C, only sulfuric acid was effective in promoting cellulose hydrolysis, and the saccharification value was 82.6 % for wheat straw and 54.4 % for eucalyptus residues.

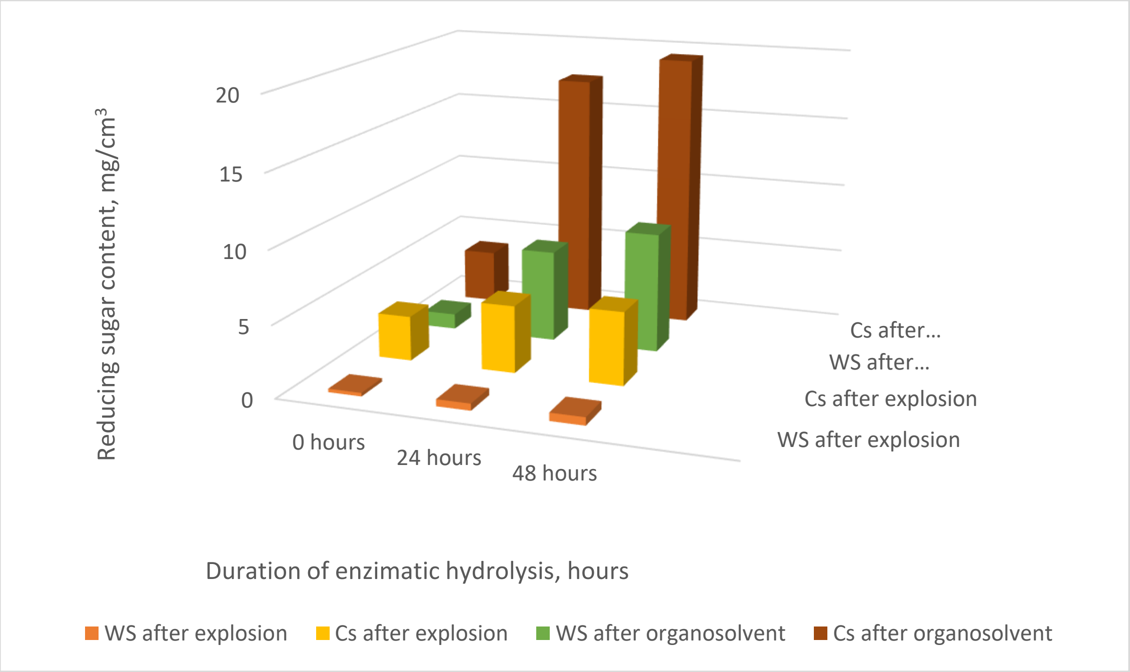

Fig. 2. Content of reducing sugars after enzymatic hydrolysis of delignification products

To determine the ethanol content, the resulting delignification products were fermented with *Saccharomyces cerevisiae* yeast strain Ethanol Red, which is currently used in second-generation ethanol plants [35] (Table 4).

Table 4

Indicators of alcoholic fermentation of wheat straw and corn stalks delignification products

Fermented product	pH		Mass of CO_2, g		Ethanol content, % vol.		Content of unfermented sugars, g/100 cm^3	
	WS	CS	WS	CS	WS	CS	WS	CS
Option 1	$(4.0\pm0.5)^a$	$(4.1\pm0.5)^a$	$(0.15\pm0.05)^a$	$(0.20\pm0.05)^a$	-	-	$(0.24\pm0.05)^a$	$(0.31\pm0.05)^a$
Option 2	$(4.0\pm0.5)^b$	$(4.1\pm0.5)^a$	$(2.0\pm0.1)^b$	$(2.6\pm0.1)^b$	$(1.5\pm0.5)^b$	$(2.1\pm0.5)^b$	$(0.068\pm0.01)^b$	$(0.075\pm0.01)^b$
Option 3	$(4.3\pm0.5)^c$	$(4.5\pm0.5)^c$	$(12.3\pm0.5)^c$	$(14.1\pm0.5)^c$	$(6.1\pm0.5)^c$	$(7.5\pm0.5)^c$	$(0.029\pm0.01)^c$	$(0.034\pm0.01)^c$

Data are mean value±S.D., N=3. Different letters in a row indicate significant differences at p<0.05 level

As it can be seen from the data in Table 4, after fermentation of the explosive defibration product, no alcohol was formed, but after enzymatic hydrolysis with a complex of cellulolytic enzymes, the ethanol content as a result of alcoholic fermentation was 1.5 % vol for wheat straw and 2.1 % for corn waste. Organosolvent delignification led to almost complete destruction of lignin bonds with the release of cellulose in a form accessible to enzymatic hydrolysis. Therefore, further fermentation of the hydrolysis products contributed to an increase in the amount of ethyl alcohol by 4 times. Accordingly, the content of unfermented sugars in the mash decreased by almost 2.3 times. The ethanol yield was 0.24 g/g biomass for wheat straw and 0.29 g/g biomass for corn waste. This correlates with [38], where the ethanol yield from lignocellulosic plant feedstock was 0.24 g/g, but is lower than that reported by Tran et al. [39], where simultaneous co-fermentation of lignocellulosic hydrolysates, composed of 39.6 g/L glucose and 23.1 g/L xylose, within 24 h yielding 0.48 g/g ethanol (94 % theoretical maximum). Such high ethanol yield is attributed by shuffling the *Saccharomyces cerevisiae* genome to create even more efficient yeast strains [40].

Thus, lignocellulosic biomass is a promising renewable resource for biofuel production, but its complex structure and resistance to enzymatic degradation create problems for its effective use and require additional operations for the destruction of lignocellulosic bonds, the release of cellulose and its enzymatic hydrolysis to reducing sugars, which can be fermented by yeast to ethanol. We recommend a 2-stage processing of vegetable raw materials – steam explosion and organosolvent delignification. In this case, almost pure cellulose is formed, after its saccharification by cellulolytic enzymes, glucose is formed, which is fermented by yeast into ethyl alcohol. At the same time, the residual lignin also decreased to 2.06 % for wheat straw and 2.83 % for corn stalks. Pretreatment of lignocellulosic biomass with only organosolv with the addition of sulfuric acid as catalyst reduced the lignin content to 10 % of the initial lignin, which indicates incomplete delignification of the raw material and interferes with further enzymatic hydrolysis of cellulose [21].

Conclusions

Second-generation bioethanol production is the most promising source of energy from renewable vegetable raw materials. Pretreatment of lignocellulosic raw materials is an important and necessary step for biomass delignification, that is, the destruction of lignin bonds to ensure the availability of cellulose and hemicellulose in subsequent processes of enzymatic hydrolysis and fermentation to obtain ethanol. Steam explosion as a method of preliminary treatment of lignocellulosic raw materials contributes to changing the structure of wheat straw and corn stalks, which became loose and porous due to the destruction of the lignin-cellulose-hemicellulose complex. The cellulose content increased from 42 to 63 % for wheat straw and from 30 to 57 % for corn stalks, however, the residual lignin content in the product remained at 12 %, indicating incomplete destruction of lignin bonds in the molecular structure, which will interfere with enzymatic hydrolysis. Conducting organosolvent delignification with a solvent based on 50 % ethyl alcohol and 3 % sulfuric acid caused almost complete destruction of lignin bonds with the formation of cellulose in a form accessible to enzymatic hydrolysis, the final lignin content was 2.06 and 2.83 % for wheat straw and corn stalks, respectively. Hydrolysis by a complex of cellulolytic enzymes and fermentation with *Saccharomyces cerevisiae* yeast produced 4 times more ethanol compared to fermentation of the product without organosolvent delignification. Improvement of pretreatment of lignocellulosic raw materials, especially through combinations of different processes and progress in biotechnology aimed at creating effective enzyme preparations and yeast strains with high enzymatic activity, resistant to inhibitors, can increase the economic efficiency of second-generation bioethanol production by 50 %.

References

[1] Tran, T., Le, P., Mai, P.T., Nguyen, Q.D. (2019). *Bioethanol Production from Lignocellulosic Biomass*. In: Edited by Yongseung Yun. Alcohol Fuels: Current Technologies and Future Prospect, 4–11. http://dx.doi.org/10.5772/intechopen. 86437

[2] (2023). RFA analysis of public and private data sources of Annual World Fuel Ethanol Production. USA: Renewable Fuels Association. https://ethanolrfa.org/markets-and-statistics/annual-ethanol-production.

[3] Shukla, A., Kumar, D., Girdhar, M., Kumar, A., Goyal, A., Malik, T., Mohan, A. (2023). Strategies of pretreatment of feedstocks for optimized bioethanol production: distinct and integrated approaches. *Biotechnol Biofuels*, *16*, 44. https://doi.org/10.1186/s13068-023-02295-2

[4] Konstantinavičienė, J., Vitunskienė, V. (2023). Definition and classification of potential of forest wood biomass in

terms of sustainable development: A review. *Sustainability*, *15*(12), 11–93. https://doi.org.10.3390/SU15129311

[5] Owonubi, S.J., Agwuncha, S.C., Malima, N.M., Shombe, G.B., Makhatha, E.M., Revaprasadu, N. (2021). Non-woody biomass as sources of nanocellulose particles: A review of extraction procedures. *Frontiers in Energy Research*., *9*, 608-25. https://doi.org/10.3389/FENRG.2021.608825/BIBTEX

[6] Bajpai, P. (2018). *Biotechnology for Pulp and Paper Processing*. DORDRECHT, Netherlands: Springer Science+Business Media. https://doi.org/10.1007/978-981-10-7853-8

[7] Abolore, R.S., Jaiswal, S., Amit, K., Jaiswal, A.K. (2024). Green and sustainable pretreatment methods for cellulose extraction from lignocellulosic biomass and its applications: A review. *Carbohydrate Polymer Technologies and Applications*, *7*(1), 28. https://doi.org/10.1016/j.carpta.2023.100396

[8] Danilova, K. (2024). [Comparative assessment of the delignification methods of lignocellulose biomass for the second generation bioethanol production (overview)]. *Food Resources*, *12*(22), 61–73 (in Ukrainian) https://doi.org/10.31073/foodresources2024-22-07.

[9] Shen, F., Zhong, B., Wang, Y., Xia, X., Zhai, Z., Zhang, Q. (2019). Cellulolytic microflora pretreatment increases the efficiency of anaerobic co-digestion of rice straw and pig manure. *BioEnergy Research*., 12, 703–13. https://doi.org/10.1007/s12155-019-10013-w

[10] Kumar, R., Prakash, O. (2023). Experimental investigation on effect of season on the production of bioethanol from wheat-stalk (WS) using simultaneous saccharification and fermentation (SSF) method. *Fuel*., *351*, 128958. https://doi.org/10.1016/j.fuel.2023.128958

[11] Sulzenbacher, D., Atzmuller, D., Hawe, F., Richter, M., Cristobal-Sarramian, A., Zwirzitz, A. (2023). Optimization of steam explosion parameters for improved biotechnological use of wheat straw. *Biomass Conversion and Biorefinery*, 13 (2), 1035-1046. https://doi.org/10.1007/s13399-020-01266-z.

[12] Ibarra, D., Eugenio, M.E., Alvira, P., Ballesteros, I., Ballesteros, M., Negro, M.J. (2023). Effect of Laccase Detoxification on Bioethanol Production from Liquid Fraction of Steam-Pretreated Olive Tree Pruning. *Fermentation., 9*(3), 214. https://doi.org/10.3390/fermentation9030214

[13] Selvaraj, A., Sriramulu, G. (2020). Physicochemical Characterization of Native and Steam Explosion Pretreated Wild Sugarcane (*Saccharum spontaneum*). *International Journal of Renewable Energy Development, 9*(3), 353–359. https://doi.org/ 10.14710/ ijred.2020.30240.

[14] Rocha, G.J.M,, Gonçalves, AR,, Nakanishi, S.C., Nascimento, V.M., Silva, V.F.N. (2015). Pilot scale steam explosion and diluted sulfuric acid pretreatments: Comparative study aiming the sugarcane bagasse saccharification. *Industrial Crops and Products,* 74, 810-816. https://doi.org/10.1016/j.indcrop.2015.05.074

[15] Zhao, G.Z., Kuang, G.L., Wanga, Y., Yaoa, Y., Zhangb, J., Pan, Z.H. (2020). Effect of steam explosion on physicochemical properties and fermentation characteristics of sorghum (*Sorghum bicolor* (L.) Moench). *LWT - Food Sci Techn., 129*(4), e109579. https://doi.org/10.1016/j.lwt.2020.109579

[16] Bonfiglio, F., Cagno, M., Rey, F., Torres, M., Bothig, S., Menendez, P., Mussatto, S.I. (2019). Pretreatment of switchgrass by steam explosion in a semi-continuous pre-pilot reactor. *Biomass Bioenerg*., *121*, 41-47. https://doi.org/10.1016/j.biombioe.2018.12.013

[17] Hoang, A.T., Nguyen, X.P., Duong, X.Q., Ağbulut, U., Len, C,, Nguyen, P.Q.P. et *al.* (2023). Steam explosion as sustainable biomass pretreatment technique for biofuel production: Characteristics and challenges. *Bioresource Technol*., 385, e129398. https://doi.org/10.1016/j.biortech.2023.129398

[18] Milford, H., Gerald, B., Vesselin, M. (2001). *Production of microcrystalline cellulose by reactive extrusion.* Patent USA US 6228213B1.

[19] Koo, B.W., Kim, H.Y., Park, N., Lee, S.M., Yeo, H., Choi, I.G. (2011). Organosolv pretreatment of Liriodendron tulipifera and simultaneous saccharification and fermentation for bioethanol production. *Biomass Bioenerg*., 35, 1833–40. https://doi.org/10.1016/j.biomb ioe.2011.01.014.

[20] Joy, S.P., Kumar, A.A., Gorthy, S., Jaganathan, J., Kunappareddy, A., Gaddameedi, A. et *al*. (2021). Variations in structure and saccharification efficiency of biomass of different sorghum varieties subjected to aqueous ammonia and glycerol pretreatments. *Ind Crops Prod*., 159, e113072 https://doi.org/10.1016/j.indcrop.2020.113072.

[21] Carvalheiro, F., Duarte, L.C., Van-Dúnem, V., Pires, F. (2022). Effective Mild Ethanol-Based Organosolv Pre-Treatment for the Selective Valorization of Polysaccharides and Lignin from Agricultural and Forestry Residues. *Energies.,* 15(15), 5654. https://doi.org/10.3390/en15155654

[22] Hamelinck, C.N., Hooijdonk, G., Faaij, A. (2005). Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass and Bioenergy*., 28(4), 384-410. https://doi.org/10.1016/j.biombioe.2004.09.002

[23] Sadhukhan, J., Martinez-Hernandez, E., Amezcua-Allieri, M.A., Aburto, J., Honorato, J.A. (2019). Economic and environmental impact evaluation of various biomass feedstock for bioethanol production and correlations to lignocellulosic composition. *Bioresource Technology Reports*., 7, 100230. https://doi.org/10.1016/j.biteb.2019.100230

[24] Carvalheiro, F., Silva-Fernandes, T., Duarte, L.C., Girio, F.M. (2009). Wheat straw autohydrolysis: Process optimization and products characterization. *Appl. Biochem. Biotechnol*., *153*, 84–93. https://doi.org/10.1007/s12010-008-8448-0

[25] Wildschut, J., Smit, A.T., Reith, J.H., Huijgen, W.J. (2013). Ethanol-based organosolv fractionation of wheat straw for the production of lignin and enzymatically digestible cellulose. *Bioresour. Technol.,* 135, 58–66. https://doi.org/10.1016/j.biortech.2012.10.050

[26] Linskens, H.F., Tracey, M.V., Beiss, U., Bendall, F. (2013). *Modern Methods of Plant Analysis*. Springer-Verlag.

[27] (2009) Official Method GS 1-5. Raw Sugar Methods - *The Determination of Reducing Sugars in Cane Raw Sugar by the Luff-Schoorl Procedure*. ICUMSA.

[28] Marcos, A.S., Brasil, L.H., Gomes, M.Y., Kamogawa, L.C.B. (2020). Ethanol determination in fermented sugarcane substrates by a diffusive micro-distillation device. *J Microbiol Biotechn*., 178, e106085. https://doi.org/10.1016/j.mimet.2020.106085

[29] Albalasmeh, A.A., Berhe, A.A., Ghezzehei, T.A. (2013). A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydrate Polymers*, 97(2),

253–261 https://dx.doi.org/10.1016/j.carbpol.2013.04.072

[30] Sun, Y.G., Ma, Y.L., Wang, L.Q., Wang, F.Z., Wu. Q.Q. (2015). Physicochemical properties of corn stalk after treatment using steam explosion coupled with acid or alkali. *Carbohydrate Polymers,* 117(2), 486-93. https://doi.org/10.1016/j.carbpol. 2014.09.066

[31] Wang, F., Yin, S., Xie, H., Ren, T. (2012). Effects of pretreatments on steam exposition efficiency of corn stalk. *Nongye Gongcheng Xuebao/Transactions of the Chinese Society of Agricultural Engineering, 28*(12), 273–280. https://doi.org/10.3969/j.issn.1002-6819.2012.12.044

[32] Sulzenbacher, D., Atzmuller, D., Hawe, F., Richter, M., Cristobal-Sarramian, A., Zwirzitz, A. (2013). Optimization of steam explosion parameters for improved biotechnological use of wheat straw. *Biomass Conversion and Biorefinery*. 13(2), 1035-46. https://doi.org/10.1007/s13399-020-01266-z

[33] Smit, A.T., Huijgen, W.J.J., Grisel, R.J.H. (2014). *Process for the Treatment of Lignocellulosic Biomass.* Patent WO 2014/126471.

[34] Tardy, B.L., Mattos, B.D., Otoni, C.G., Beaumont, M., Majoinen, J., Kämäräinen, T., Orlando, J., Rojas, O.J. (2021). Deconstruction and Reassembly of Renewable Polymers and Biocolloids into Next Generation Structured Materials. *Chemical Reviews,* 121(22), 14088–14188. https://doi.org/10.1021/acs.chemrev.0c01333

[35] Delmas, M., Benjelloun, M.B. (2008). *Process for pretreating a lignocellulosic material with a view to producing bioethanol, and bioethanol production process.* WO2009092749A1.

[36] Cagnin, L., Gronchi, N., Basaglia, M., Favaro, L., Casella, S. (2021). Selection of Superior Yeast Strains for the Fermentation of Lignocellulosic Steam-Exploded Residues. *Front Microbiology,* 12, 756032. https://doi.org/10.3389/fmicb.2021.756032

[37] Rumpf, J., Do, X.T., Burger, R., Monakhova, Y.B., Schulze, M. (2020). Extraction of high-purity lignins via catalyst-free organosolv pulping from low-input crops. *Biomacromolecules, 21*(5), 1929–1942. https://doi.org/10.1021/acs.biomac.0c00123

[38] Mlayah, B.B., Delmas, M. (2014). *Process for the production, in particular of ethanol, by sequent enzymatic hydrolysis of cellulose and hemicelluloses of lignocellulosic raw material.* Patent FR3021975B1.

[39] Tran, P.H.N., Ko, J.K., Gong, G., Um, Y., Lee, S.M. (2020). Improved simultaneous co-fermentation of glucose and xylose by *Saccharomyces cerevisiae* for efficient lignocellulosic biorefinery. *Biotechnol. Biofuels, 13*(12), 1–14. https://doi.org/10.1186/s13068-019-1641-2

[40] Tsegaye, K.N., Alemnew, M., Berhane, N. (2024). *Saccharomyces cerevisiae* for lignocellulosic ethanol production: a look at key attributes and genome shuffling. *Front Bioeng Biotechnol*., 12, 1466644. https://doi.org/10.3389/fbioe.2024.1466644