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Production of ethanol from wheat straw

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This study proposes a method for the production of ethanol from wheat straw lignocellulose where the raw material is chemically processed before hydrolysis and fermentation. The usefulness of wheat straw delignification was evaluated with the use of a 4:1 mixture of 95% ethanol and 65% HNO_3 (V). Chemically processed lignocellulose was subjected to enzymatic hydrolysis to produce reducing sugars, which were converted to ethanol in the process of alcoholic fermentation. Chemical processing damages the molecular structure of wheat straw, thus improving ethanol yield. The removal of lignin from straw improves fermentation by eliminating lignin's negative influence on the growth and viability of yeast cells. Straw pretreatment facilitates enzymatic hydrolysis by increasing the content of reducing sugars and ethanol per g in comparison with untreated wheat straw.

Keywords: wheat straw, chemical processing, enzymatic hydrolysis, reducing sugars, ethanol.

INTRODUCTION

Global economic growth contributes to a rapid increase in the consumption of traditional energy sources. According to numerous energy consumption analyses, the progressing depletion of fossil fuels calls for new initiatives on the market of renewable energy. Biomass is one of alternative energy sources[1, 2]. At present, only 5 billion out of 150 billion tons of biomass harvested each year are processed into food. Biomass is not used for energy generation to the extent permitted by the existing technology[3, 4, 5]. Renewable energy sources are becoming increasingly important in the energy balance of the country, and they are a characteristic feature of innovative and forward-looking economies (Kogut et al. 2012)[6]. Energy can be generated from biomass by combustion, gasification, ethanol and methanol fermentation or by using oilseed crops as a source of fuel. According to Nguyen et al. (2013)[7], energy generated from straw by gasification seems to be more environmentally-friendly than that produced by straw combustion. In comparison with natural gas, the heating value of straw is low at $13.5 \div 19.0$ MJ $\cdot$ kg^{-1}, and it is determined by the type of straw and its relative moisture content. The combustion of fossil fuels produces harmful emissions to ambient air, mainly CO_2, which contribute to the greenhouse effect. The use of straw as an alternative source of energy could reduce global warming and the depletion of fossil fuels[7, 9, 10]. The energy value of two tons of wood or straw is equivalent that that of one ton of high-quality hard bituminous coal. Biomass yield per hectare of farmland is estimated at 10–12 tons, i.e. the equivalent of 5–10 tons of coal[11]. One of the methods of generating energy from biomass is alcoholic fermentation. Simple sugars are converted into ethanol by yeasts[12, 13]. Ethanol is dehydrated and used to enhance or substitute petroleum[12, 14, 15, 16, 17].

Biomass-derived products are suitable for human consumption, therefore they constitute an expensive source of energy. Lignocellulosic biomass, including wood, food and agricultural wastes, oilseed crops and other raw materials containing cellulose, pose a less costly alternative[18, 19]. Cellulose resources are abundant in nature. Cellulose does not constitute a human food source, therefore, it is a relatively cheap source of energy and bioethanol[20, 21, 22]. In Brazil, the food processing sector generates 587 million tons of waste per year. New solutions are required for managing valuable plant resources for energy generation purposes[22]. In the United Kingdom, wheat straw is a potential resource for the production of second-generation biofuels[8, 23]. Integrated measures are initiated by the EU countries to encourage the production of biomass fuels and provide farmers with the relevant support. Pursuant to the provisions of Directive 2009/28/EC on the promotion of the use of energy from renewable sources[25], the share of renewable fuels for transport has to reach 10% in every Member State by 2020. The above requirement will lead to a substantial increase in the production of inedible biomass[23, 24]. The aim of the above Directive is to replace bioethanol produced from edible plants with bioethanol obtained from inedible biomass, including plant waste. Biofuels produced from lignocellulose and waste will lower CO_2 emissions. Despite those advantages, the energy inputs and costs associated with biomass conversion to bioethanol are higher for biofuels derived from inedible resources (advanced generation biofuels) than edible crops[26, 27]. Relatively few high-efficiency systems for the conversion of inedible biomass into biofuels have been developed on the industrial scale. The largest industrial system for bioethanol production from straw is operated in Crescentino, Italy[28].

A vast surplus of straw, a potential source of solid biomass, exists in western Poland. According to estimates, 50 to 70% of that surplus is suitable for industrial processing. In Poland, biomass resources that could be used for energy generation are estimated at more than 10–11 million tons of straw waste[29]. In Poland, only 7% of biomass is used for energy, whereas the average for the EU is 20%[30]. Alternative sources of energy such as cellulosic biomass, in particular wheat straw, limit energy generation from edible crops and ensure the use of sustainable biofuels only[5, 10, 17, 23, 31].

Lignin provides plants with the structural support needed for an erect growth habit. Lignin surrounds cellulose and hemicellulose molecules, making their extraction difficult. Similarly to starch molecules, cellulose molecules are made up of long chains of glucose molecules, but with a different configuration. Due to their specific structural properties, cellulosic materials are much more difficult to hydrolyze than starch[20, 32–36]. The aim of this study was to evaluate the suitability of wheat straw for the production of ethanol fuel and to determine the effect of chemical pretreatment of wheat straw on the content of reducing sugars after hydrolysis and ethanol yield after alcoholic fermentation.

MATERIAL AND METHODS

The experimental materials were: wheat straw harvested in a farm in Święta, municipality of Złotów, Region of Wielkopolska, with the involvement of traditional farming methods. Avicel PH-101 (Sigma Aldrich) powdered microcrystalline straw with 50 μm grain size was used as control. It was dissolved in octane buffer with pH 4.7 and subjected to hydrolysis and fermentation with the use of the same enzymatic preparations and yeasts that were applied to straw wheat samples. Wheat straw (10 g dry matter) was ground in a colloid mill into 1-mm long particles, and it was chemically treated with a 4:1 mixture of 95% ethanol and 65% nitric (V) acid according to the method proposed by Kürschner–Hoffer[37]. The aim of preliminary treatment was to damage lignin structure and increase enzyme accessible space in cellulose. Hydrolysis was carried out using two commercial enzymatic preparations: cellulase containing *Trichoderma reesei* ATCC 26921 (Sigma Aldrich) and cellobiose containing *Aspergillus niger* (Novozym 188). Enzymatic hydrolysis was conducted at 47°C for 72 hours. The hydrolysate was separated from cellulose residues and subjected to alcoholic fermentation. The fermentation process was carried out with the use of *Saccharomyces cerevisiae* Fermentis Ethanol Red (Leaf Technologies), a selected yeast strain for industrial production of ethanol, at a temperature of 37°C for 96 h. At 35°C, the applied yeast strain is capable of concentrating ethanol to 18% v/v. It is also characterized by high viability and resistance to high ethanol concentrations in mash. The ethanol content of the analyzed samples and the viability and count of yeast cells were determined. The ethanol production process was conducted in three replications. Hydrolysate samples were assayed for the content of total reducing sugars and ethanol after fermentation. The content of reducing sugars and ethanol concentrations were expressed as mean values from three replications.

The dry matter content of unprocessed straw was determined in accordance with Polish Standard PN-90/A-75101/03, cellulose content – by the method proposed by Kürschner–Hoffer[37], and the content of Klason lignin – by the method described by Rodrigues[3]. Two replicate determinations were made.

The content of reducing sugars after enzymatic hydrolysis of cellulose was determined quantitatively with the use of 3,5-dinitrosalicylic acid under alkaline conditions. The concentrations of the stained compound were measured in the Helios spectrometer at 540 nm wavelength. In the analyzed samples, glucose levels could be determined quantitatively due to the non-specificity of the applied method where DNS reduction (3,5-dinitrosalicylic acid reduction) was a measure of the sample's general reducing ability. Glucose concentrations were determined by comparing absorbance results with the absorbance profiles of reference solutions[39].

The counts and viability of yeast cells in fermentation solutions were determined directly under a light microscope with a Thoma counting chamber with the use of 0.01% methylene blue solution. Cells were counted in minimum 60 small squares (not less than 700 yeast cells) to improve the reliability of results.

The amount of ethanol produced during decomposition of wheat straw cellulose was determined with the use of the ROCHE[40] kit (Enzymatic BioAnalysis/Food Analysis) that relies on UV radiation to measure ethanol concentrations in food products.

RESULTS

Straw is a lignocellulosic material and an agricultural by-product. Its main components are cellulose, hemicellulose, lignin, nitrogen compounds and ash. The exact composition of straw is determined by its type and variety[41]. On average, straw contains 35–50% cellulose, 15–30% hemicellulose, 20–30% lignin and smaller amounts of ash and other compounds[41, 42].

The dry matter content of straw was determined at 91.5%. The content of Klason lignin reached 28.4%. Klason lignin is the lignin fraction remaining after hydrolysis of lignocellulosic material with sulfuric (VI) acid. Klason lignin and lignin dissolved in sulfur acid make up the total lignin content of lignocellulosic materials[38]. The analyzed wheat straw contained 39.5% cellulose. An image of untreated and treated straw samples is presented in Figure 1.

The objective of this study was to determine the effect of wheat straw pretreatment on enzymatic hydrolysis and the production of reducing sugars, which are converted into ethanol by *S. cerevisiae* yeasts during the fermentation process.

Lignin is one of the key factors limiting straw's potential for bioethanol production. Cellulose forms complexes with lignin, and in straw with high lignin content, cellulose is difficult to extract by hydrolysis. In this experiment, lignin was removed from wheat straw by a 4:1 mixture of nitric acid and ethanol. Preliminary processing of wheat straw increased the content of reducing sugars after hydrolysis and the content of ethanol after fermentation. Similar results were reported by Ruiz et al. (2011)[43] who also removed lignin from wheat straw. The cited authors attributed the observed increase in the content of reducing sugars to lignin separation from cellulose and an increase in enzyme accessible space.

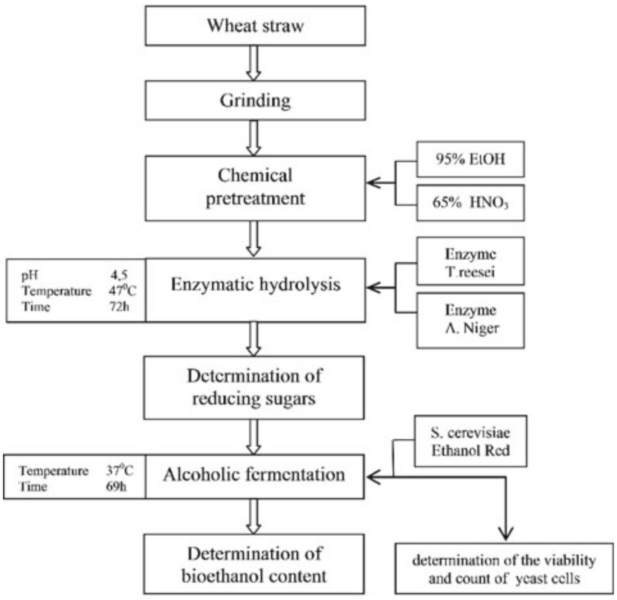

Figure 1. Flow diagram of bioethanol production from wheat straw

Delignification produced microcrystalline cellulose that was dried and hydrolyzed by *T. reesei* and *A. niger* cellulolytic enzymes. Both fungi produce large quantities of extracellular cellulases for decomposing microcrystalline cellulose, and they are popularly used in the food industry[44, 45].

Traditional ethanol production methods were based on conventional techniques of enzymatic hydrolysis and fermentation of sugars from starch decomposition, with the use of *S. cerevisiae* yeasts. Fermentation took place inside cells which produce fermentation enzymes –decarboxylase and alcohol dehydrogenase[46].

In this experiment, all samples (processed wheat straw, unprocessed wheat straw, microcrystalline cellulose – control) were incubated at 47°C for 72 h. Specimens for analysis were collected every hour for 12 hours, and then every 12 hours for three days. Changes in glucose levels during enzymatic hydrolysis are presented in Figures 2 and 3.

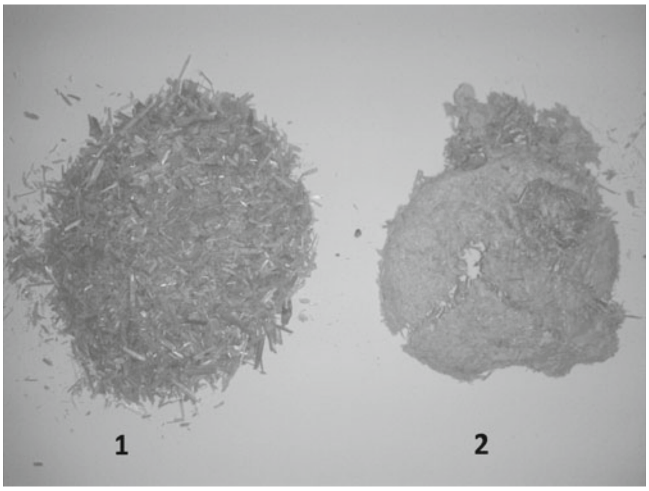

Figure 2. Wheat straw: 1 – untreated, 2 – chemically treated

Raw wheat straw cannot be degraded by hydrolysis, and it was processed to make it susceptible to hydrolytic enzymes. The highest glucose concentration of 82.67 g · dm^{-3} hydrolysate was observed in processed wheat straw after 48 hours. Hydrolysis results for untreated straw and control straw were nearly identical, i.e. less than 20 g · dm^{-3} reducing sugars was released. The content of reducing sugars in delignified straw was more than four-fold higher than in untreated straw.

Saha and Cotta (2007)[47] hydrolyzed lime-treated wheat straw and observed that the content of glucose and total reducing sugars increased with a rise in calcium hydroxide [$Ca(OH)_2$] concentrations during preliminary treatment. The influence of the $Ca(OH)_2$ dose was always much greater than that of treatment time. Total sugar content increased from 247 ±6 mg to 451 ±3 mg (83% increase in sugar release) when the lime dose was increased from 25 to 100 mg per g of straw. Total sugar content increased from 410 ±4 mg to 451 ±3 mg (by 10%) when pretreatment time was increased from 6 minutes to 1 hour. The highest total sugar content (451 ±3 mg · g^{-1} straw, 252 ±6 mg of glucose, 173 ±3 mg of xylose, 27 ±2 mg of arabinose; 65% conversion) was

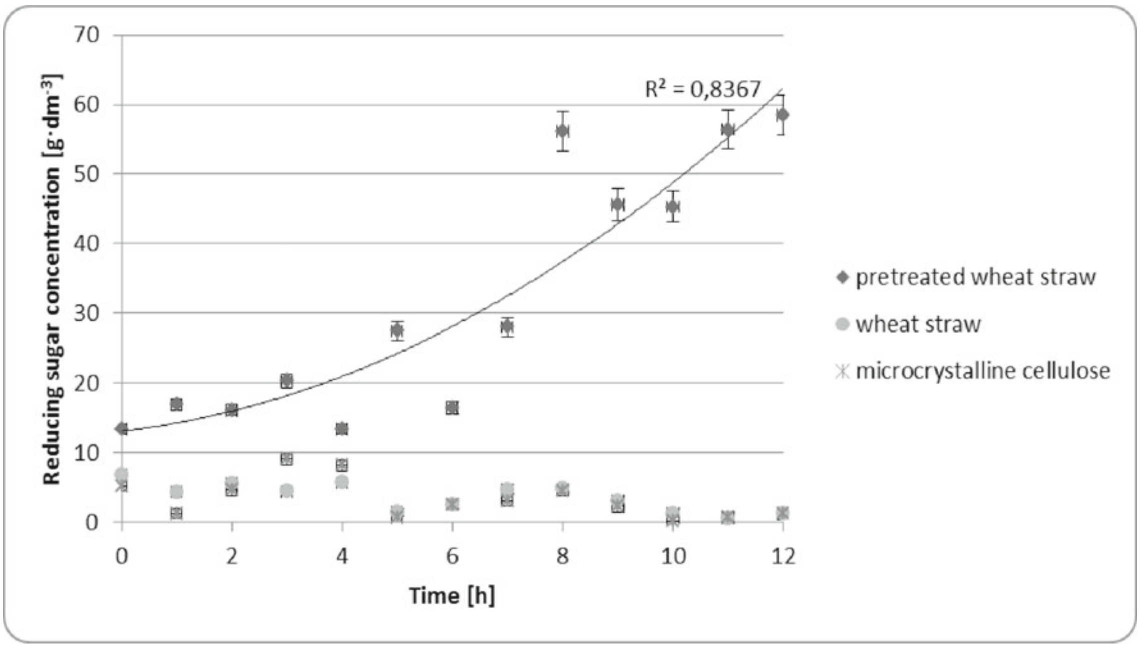

Figure 3. Changes in the content of reducing sugars during 12 hours of enzymatic hydrolysis

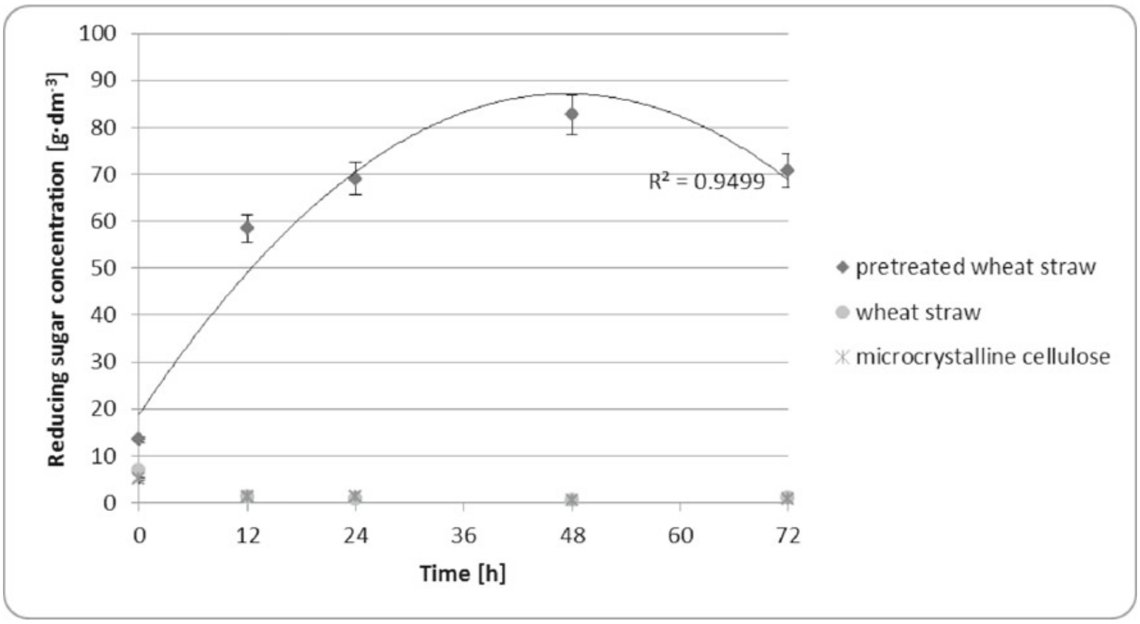

Figure 4. Changes in the content of reducing sugars during 72 hours of enzymatic hydrolysis

achieved at the $Ca(OH)_2$ dose of 100 mg and 1 hour of pretreatment.

Szczodrak (1998)[48] hydrolyzed wheat straw under alkaline conditions to obtain 2.4% (w/v) ethanol from 10% (w/v) chemically processed straw in 48 hours. When, in addition to the enzyme extracted from *T. reesei*, β-glucosidase from *A. niger* was included in the hydrolysis process, ethanol concentration increased to 3%, and treatment time was reduced to 24 hours. According to Han et al. (2012)[49] and Silva et al. (2012)[50], the efficiency of enzymatic hydrolysis of cellulosic biomass can be increased by grinding and pretreating raw material under alkaline conditions. In the cited studies, the efficiency of enzymatic hydrolysis increased with a rise in NaOH concentrations, and the highest content of reducing sugars was noted at 1% NaOH. Alkaline pretreatment is generally more effective in facilitating the hydrolysis of agricultural waste and herbaceous plants than woody plants[51].

Detroy et al. (1981)[52] converted wheat straw to ethanol and demonstrated that raw straw pretreated with 2% NaOH for 4 hours and subjected to enzymatic hydrolysis was responsible for 76% cellulose conversion, whereas straw pretreated under acid/alkaline conditions supported only 43% conversion. Hemicellulose, a polymer composed of pentoses, hexoses and sugar acids, can be easily converted to monomeric sugars by applying diluted H_2SO_4 at higher temperatures[53] and intensifying the process with the use of supercritical CO_2 and steam[18]. Research into cellulose processing revealed that pretreatment costs can be reduced by recycling the solvent.

The results of our study indicate that lignin removal during the pretreatment of wheat straw significantly increases ethanol yield. Pretreated wheat straw was characterized by a significantly higher content of reducing sugars (Figs. 2 and 3) and ethanol (Fig. 4) after fermentation (4.09 g · dm^{-3}) than unprocessed, lignin-containing straw (1.23 g · dm^{-3}).

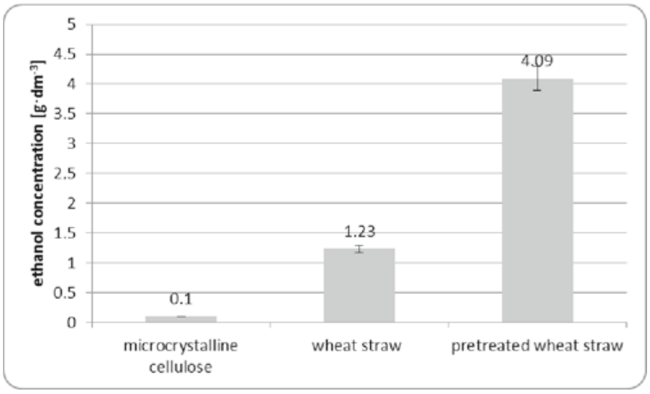

Figure 5. Ethanol concentrations in samples after fermentation

The observed yeast cell counts (Table 1) indicate that fermentation was not adversely influenced by delignification. Differences in yeast viability were observed between samples of processed and unprocessed straw. Straw pretreatment increased the viability of cultured yeast cells due to a higher content of sugars fermenting in the hydrolysate.

Despite differences in yeast cell counts between samples of processed and unprocessed straw, the total number of viable cells was too low for effective bioethanol production. The above could be attributed to insufficient access to nitrogen sources or the presence of residues from chemical pretreatment. The problem could be addressed by using a yeast growth medium, which would enhance the viability of yeast cells and increase ethanol yield per g of wheat straw.

Table 1. Counts and viability of yeast cells in the analyzed samples after fermentation

Sample	Cell viability [%]	Cell count [cfu · cm^{-3}]
Microcrystalline cellulose	60 ±1.1	$4.0x10^6 ±1.5x10^5$
Pretreated wheat straw	72 ±0.9	$8.4x10^5 ±2.5x10^4$
Wheat straw	60 ±1.9	$5.6x10^5 ±2.5x10^4$

Cell count [cfu · cm^{-3}] – Number of microorganisms expressed as the number of colony forming units per cm^3

CONCLUSIONS

The following conclusions can be formulated based on the results of this study:

– Delignification of wheat straw increases the efficiency of enzymatic hydrolysis and increases glucose concentrations nearly four-fold in comparison with unprocessed straw.

– Ethanol concentrations reached 0.4 g per 1 g (dry matter) of pretreated wheat straw, but only 0.1 g per 1 g (dry matter) of untreated wheat straw.

– Chemical pretreatment of wheat straw increased ethanol yield three-fold.

– Delignification does not inhibit the growth of yeast cells and has no adverse effects on yeast viability.

– Chemical pretreatment of wheat straw does not inactivate cellulolytic enzymes secreted by *Trichoderma reesei* and *Aspergillus niger*.

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