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Study on the decomposition of lignocellulosic biomass and subjecting it to alcoholic fermentation
Study on the decomposition of lignocellulosic biomass

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ABSTRACT

The decomposition of lignocellulosic raw material included: mechanical grinding of plant biomass, delignification (removal of lignin – this process was conducted in alkaline environment) and detoxification process (removal of alcoholic fermentation inhibitory compounds).

The study on producing ethanol from corn straw was based on SSF method which involved conducting simultaneous enzymatic hydrolysis of cellulose and fermentation of obtained saccharides.

Based on the study of corn straw alcoholic fermentation it was determined that the way of preparing the raw material in the initial stage of simultaneous saccharification and fermentation, significantly influences the improvement of fermentation yield.

In comparison with an attempt in which biomass detoxification process was not implemented, the attempt with detoxification resulted in gaining higher fermentation yield and in lowering the content of aldehydes, methanol and furfural in the produced spirit.

Moreover, in the attempts in which detoxification of raw material was used, better actual speed, productivity and the yield of alcoholic fermentation of corn straw was noted. The conducted detoxification in the process of lignocellulosic biomass decomposition improved fermentation yield.

1. Introduction

In recent years, the interest in the use of renewable raw materials, including plant biomass (lignocellulosic raw materials), as an inexhaustible source of liquid fuel (second generation biofuel) has been constantly growing.

Moreover, second generation biofuels limit the emission of carbon dioxide to the atmosphere, preventing the greenhouse effect. The plant biomass becomes an alternative energy source for fossil fuels.

Whole plants, straw or agricultural product waste are very suitable for bioethanol production, because they are not related to food supply. They are also available in larger quantities in comparison to e.g. grain, and they are less expensive (by-products laying on the fields, waste from plant production) [1–3].

The lignocellulosic raw materials are built mainly out of three connected compound groups [3–8]:

1) cellulose (comprising 30–40% of floral cell walls) – harder hydrolytic decomposition is connected to its crystalline and amorphous areas,
2) hemicellulose (comprising 30–35% of floral cell walls) – characterized by complex carbohydrate structures (xylose, arabinose mannose, glucose and galactose) that often create branched chains, it is more prone to hydrolysis than cellulose,
3) lignin (comprising 11–25% of floral cell walls) – ensures plants with impermeability and immunity against an attack from micro-organisms and protection from chemical degradation.

The amount of specific compounds of the lignocellulose in floral cell walls is varied due to the floral type, kind and origin. In the case of corn stover is as follows – Table 1.

The purpose of biomass pretreatment is the degradation the cellulosic phibrils, lowering their crystallization and polymerization levels, hemicellulose separation, degradation of cellulose

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Table 1
The amount of lignocellulose components in corn stover [9–14].

Lignocellulosic material	Components of lignocellulose [%]			References
	Cellulose	Hemicellulose	Lignin	
Corn stover	38.90	23.20	19.10	Tian et al., 2013 [9]
	38.14	22.68	23.34	Chu et al., 2013 [10]
	38.70	21.70	19.30	Zhao, Xia 2009 [11]
	36.40	22.60	16.60	Lynd et al., 1999 [12]
	39.00	19.10	15.10	Lee 1997 [13]
	36.80	25.40	16.90	Evans et al., 1988 [14]

Table 2
Fermentation yield from corn straw, alkalization of the environment with the use of $Ca(OH)_2$, $T = 40$ °C, SSF method.

Lignocellulosic raw material	Option type	Fermentation yield [L EtOH/100 kg of raw material]		
		24 h	48 h	**72 h**
Corn straw	without detoxification	10.10 ± 0.03	13.20 ± 0.06	**14.80 ± 0.06**
	Detoxification (activated carbon)	18.60 ± 0.00	23.30 ± 0.00	**24.80 ± 1.91**

The bold values represent the fermentation yield of corn stover after 72h of fermentation. The boldface discriminate fermentation yield of corn stover obtained in the last day of alcoholic fermentation.

complex with lignin and the modification of lignin's structure, delignification, as well as increasing the available area for hydrolytic enzymes activity.

The pretreatment of raw materials has significant influence on the efficiency of the technological process [15].

After completed pretreatment, the most effective and prospective method of cellulose hydrolysis is the enzymatic method with the help of cellulase and hemicellulose [16,17].

After the pretreatment of lignocellulosic raw material, the obtained monosaccharides are subjected to alcoholic fermentation, using yeast.

It is very important in alcoholic fermentation process to use heat resistant yeast breeds due to the temperature in which the enzymatic hydrolysis is conducted (optimal temperature of cellulases' complex working is 40–50 °C) [18].

The aim of the research was to conduct the decomposition of lignocellulosic biomass in conditions appropriate for obtaining the highest ethanol yield and beneficial chemical composition of the spirit, attesting to its quality.

When taking into account the overview of current literature, there are many papers on various aspects of cellulose decomposition, however they do not contain information about the quality of the obtained spirit. The spirit quality, i.e. its purity, indicates the possibilities of its application, and the profitability of its production.

2. Materials and methods

2.1. Material

The study was conducted using corn straw (leaves and stems). The material was obtained from a farm in Wojnowo. The corn straw contained 6.33% of humidity and 93.67% of dry mass.

2.2. Enzymatic preparation

The following preparations were used for enzymatic hydrolysis (decomposition of cellulose into glucose, cellobiose and higher glucose polymers):

- **Celluclast 1.5L** (Novozymes Company, Denmark, Bagsvaerd) – preparation obtained from *Trichoderma reesei* fungus cultivation, which catalyzes the decomposition of cellulose into glucose, cellobiose and higher glucose polymers. Optimal conditions: temperature of 25–55 °C and pH of 4.0–6.0.
- **Novozyme 188** (Novozymes Company, Denmark, Bagsvaerd) – enzyme obtained from *Aspergillus niger* fungus cultivation, which catalyzes the decomposition of cellobiose into glucose. This preparation is a biocatalyst that supports the effect of the Celluclast 1.5 L enzyme. Optimal conditions: temperature of 25–55 °C and pH of 4.0–5.5.

2.3. Microorganisms

The yeast strain D-2 is *Saccharomyces cerevisiae*, obtained from a collection of pure cultures from IBPRS (Institute of Agricultural and Food Biotechnology) Distillery Technology and Renewable Energy Division in Bydgoszcz. D-2 strain is characterized by: alcohol resistance (above 95 g/L EtOH), osmophily (22-24°Blg), thermophily (38–40 °C) and acidophily (pH ok.3).

2.4. Processing of lignocellulosic raw material

The degradation of fiber structure of lignocellulosic corn straw was conducted by:

- Mechanical grinding of plant biomass into particles with 0.12 ÷ 0.43 mm diameter,
- Raw material processing in alkaline environment: corn straw (10 g) was subjected to a solution of calcium hydroxide (5 g $Ca(OH)_2$ + 130 mL of distilled H_2O), and then to pressure (0.15 MPa) and thermal (135 °C) treatment in 1 h time,
- Detoxification process (removal of alcoholic fermentation inhibiting compounds). Detoxification was performed with the use of activated carbon (20 g/100 g of the material) in the temperature of 80 °C in 2 h time (with simultaneous shaking of 150 rpm). Then the lignocellulosic substrate was rinsed with

Table 3
Parameters describing alcoholic fermentation of corn straw – processing with the use of $Ca(OH)_2$, with detoxification, $T = 40$ °C, SSF method.

Option	Apparent extract [°Blg]				Concentration of ethanol [g/L]			Actual extract [°Blg]	
	0 h	24 h	48 h	72 h	24 h	48 h	72 h	0 h	24 h
Without detoxification	7.80 ± 0.00	6.30 ± 0.00	6.00 ± 0.07	5.70 ± 0.07	8.00 ± 0.03	10.50 ± 0.06	11.80 ± 0.06	7.80 ± 0.00	6.40 ± 0.00
Detoxification (activated carbon)	5.50 ± 0.00	4.20 ± 0.00	4.10 ± 0.00	4.00 ± 0.00	14.70 ± 0.00	18.50 ± 0.00	19.60 ± 0.19	5.50 ± 0.00	4.40 ± 0.00

Notes: alcohol concentration – the results for 0 h are missing because at that time the samples were not subjected to the fermentation process.