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OPTIMAL PRETREATMENT CONDITIONS OF INDUSTRIAL HEMP RESIDUES TO GLUCOSE

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ABSTRACT

Hemp (Cannabis sativa L.) is an annual herbaceous plant with excellent agrarian and economic characteristics and has been used since ancient times as a raw material for making fabrics. Industrial hemp, with its high biomass yield per hectare, has great potential for the production of biofuels and valuable products. Regulatory documents in Bulgaria allow the cultivation of cannabis plants only if they are intended for fibres, seeds for animal feed or sowing if they have a content of less than 0.2% tetrahydrocannabinol. The residue of hemp after such processing is characterised by a hardwood-like content of cellulose, hemicellulose and lignin but with a very low bulk density. The specificity of the biomass residues and the woody structure of hemp stalks requires an additional mild, wet beating prior to enzymatic hydrolysis.

The present work aims to investigate the effect of mechanical treatment of steam-exploded hemp residues on glucose yield after enzymatic hydrolysis and to determine the optimal pretreatment conditions. The classic steam and CO_2 steam explosion methods are used as the first pretreatment step in this investigation. It has been found that the mechanical processing of hemp pulp leads to higher glucose yields after enzymatic hydrolysis, and this effect is reduced by increasing the steam explosion temperature.

Key words: hemp, mechanical treatment, enzymatic hydrolysis, glucose.

INTRODUCTION

Industrial hemp (Cannabis sativa L.) is an annual herbaceous plant belonging to the genus Angiosperms and the Hemp family (Cannabaceae). Hemp is rich in fibres and Omega fatty acids, and this is the reason it is valuable as a pharmaceutical product (Tsoumis 1991).

The hemp can be cultivated and grown in different climate conditions, as the plant is sustainable to outside conditions. Because of climate changes and the World energy crisis, industrial hemp is an alternative raw material for biofuels (Kraszkiewicz 2019).

The hemp stalk consists of many layers, which include the epidermis, phloem, xylem and core (Jiang 2018).

Under a microscope investigation of these structures, the outside surface of Cannabis sativa L. can be seen as fibres (Sheedev 2018). Both main stalk fibres – phloem and xylem, differ on the content of cellulose and lignin. In the phloem layer, the average content of cellulose is between 67–78%, and lignin content is 3–13%, while the xylem layer contains 40% cellulose and 17% lignin (Shin 2009).

In recent decades, many different pretreatment methods have been developed, which are separated into 4 main groups: physical (grinding and refining), chemical (with acids and alkalis), physical-chemical (high-temperature hydrolysis, steam-explosion) and biological (fungi, bacteria, and algae) (Kim 2018).

The physical methods used for pretreatment aimed to reduce the size of the raw material particles, which improves the enzyme access by reducing crystallinity and the polymerisation degree of the cellulose (Wawro 2019).

Also, removing lignin and the acetyl groups from the hemicelluloses in the biomass was used the Alkali method because of the high efficiency and low-cost treatment of this method (Łukajtis 2018).

Steam-explosion is a process where the hemp is initially steamed at a high temperature between 190º–230ºC. The shredding of the biomass is due to the momentary opening of the valve, with which the pressure is reduced, and the mass is shredded and blown into a container. Immediate pressure dropping leads to an explosion of plant cell walls, and, respectively, opens the cellulose surface. Steam- explosion is a combined method of chemical modification and mechanical defibrillation. The steam makes the raw material soft, and the fibres can be separated from each other. Therefore, they are formed inhibitors for unwanted enzyme complexes. The main advantages of steam-explosion methods are low impact on the environment and less usage of chemicals (Foston 2018, Ximenes 2018, Kim 2018).

Thibaud Sauvageon proposes steam-explosion treatment for 4 min., T= 190ºC and initial impregnation with NaOH (8%), which conditions lead to 91,2% defibrillation (Sauvageon 2018).

Pakarinen observed that after steam-explosion of the material aт temperature 200º for 5 minutes, glucan content in the biomass from hemp increased from 46% to 69.6%, while hemicelluloses content was reduced from 9.5% to 5.5% (Pakarinen 2012).

Kuglarz`s investigation on steam-explosion by adding H_2SO_4 (0,5–2,0%) at T= 140–180 degrees for 10 min. and had rich that the best results on the sugar yield were received by 1% amount of H_2SO_4 (Kuglarz 2014).

Sipos recommends impregnation with 2% SO2 to reduce high-temperature usage, even though the best sugar yield was observed at T=210°C for 5 min. (Sipos 2010).

The present work aims to investigate the effect of mechanical treatment of steam-exploded hemp residues on glucose yield after enzymatic hydrolysis and to determine the optimal conditions or the pretreatment with CO_2 depending on the temperature and the specificity of the waste hemp pulp used.

MATERIALS AND METHODS

The investigation was conducted with two waste hemp residues that underwent different primary processing. The chemical analyses of the hemp samples were performed according to the standard procedures: Pentosan by Tappi – T223 cm-10, Lignin by Tappi – T222, Ash by Tappi – T211 Substances soluble in hot water by Tappi – T207, and cellulose by method's Kurchner-Hofer.

Steam explosion pretreatment was performed in a 2 L stainless steel laboratory installation. The steam explosion was performed under the following conditions: solid-to-liquid ratio of 1:40 (w/v); initial temperature of 20°C; reaction temperatures in the range of 160°C to 200°C; initial pressure of 4 bar by the use of CO_2; heating time 60 min, followed by additional 10 min at the maximum temperature.

The treated biomass was washed with distilled water, and the hydrolysate obtained was filtered and analysed. Before enzymatic treatment, the wet biomass was beaten in a Jokro mill for 5 minutes.

The enzymatic hydrolysis was carried out in polyethene bags in a water bath previously heated to the desired temperature (50°C) with cellulase complexes Cellic® CTec2. The enzyme hydrolysis was performed with biomass consistency of 10%, pH range 5.0–5.6, reaction time from 1 to 72 h, and at 5% enzyme charge.

The residue was washed with distilled water, and the hydrolysate obtained was filtered and subjected to analysis.

The total solubility substances and content of glucose, xylose, furfural, and HMF were determined according to the NREL Technical Report on a Dionex HPLC system by Shodex RI detector. The separation was performed with a Hi-Plex H column, 300 mm x 7.7 mm (mm (Agilent Technologies, USA), at 65°C with ultrapure water (Simplicity® (Merck KGaA, Germany) as the eluent, at a flow rate of 0.5 mL/min. The results were evaluated using the Chromeleon 6.80 software (Dionex Inc., USA).

RESULTS AND DISCUSSION

The chemical analysis on both hemp types showed a higher cellulose content and less lignin and pentosans in the first group of hemp stalks, compared to the second group with relatively small pieces fraction (Table 1). The differences are from 4% to 5%, and the explanation for this is the difference in the long fibres in both groups.

The ash content of both groups is relatively low and corresponds exactly to the literature data. This parameter gives hemp an advantage compared to wheat straw and corn stalks, which have a high ash content.

Table 1: Chemical analysis of waste hemp stalks (% from dry biomass)

Sample type	I group hemp stalks	II group hemp stalks
Moisture [%]	10.38 ± 0.02	12.76 ± 0.02
Substances soluble in hot water [%]	6.25 ± 0.05	1.90 ± 0.05
Pentosanes [%]	21.45 ± 0.05	24.85 ± 0.05
Lignin [%]	16.25 ± 0.05	20.50 ± 0.05
Cellulose [%]	49.00 ± 0.05	45.45 ± 0.05
Ash [%]	1..60 ± 0.01	2.35 ± 0.01

Table 2 shows the results from the experimental work of producing hydrolysate after steam explosion at different temperatures and the addition of CO_2. As can be seen from the results in Table 2, CO_2 addition accelerates the hydrolysis process because of the greater amount of acid in the middle at the beginning, which leads to the full transformation of hemicelluloses into xylan monosaccharides. A transformation of monosaccharides to furfural and HMF was observed, which proved the need for usage at lower temperatures in steam-explosion treatment.

Table 2: Analysis of hydrolysis after different steam-explosion pretreatment.

	Type of steam-explosion			
	without CO_2		with CO_2	
Sample type	**I group hemp stalks**	**II group hemp stalks**	**II group hemp stalks**	
Temperature	**200°C**		**190°C**	**200°C**
Xylose [%]	0.22	0.89	25.05	24.35
Furfural [%]	-	0.27	1.10	5.25
HMF [%]	-	-	-	1.75
Glucose [%]	-	0.50	-	3.17

The specifics of hemp stalks and the content of rough and long fibers before the enzyme hydrolysis impose the need for additional refining on the stock after the steam explosion. Figure 1 shows the impact of the refining on the glucose yield of both groups of raw materials after steam explosion at 200°C.

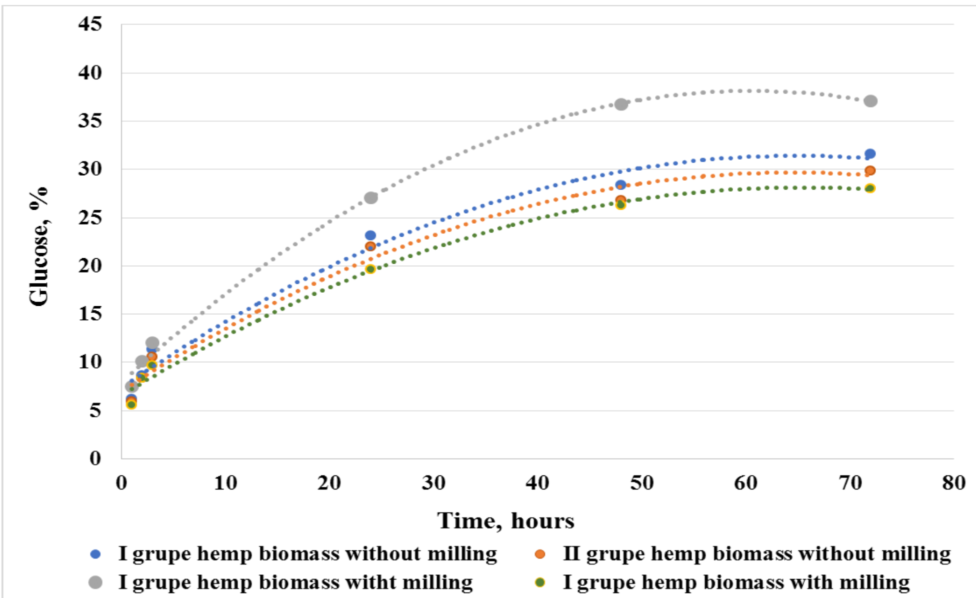

Figure 1: Kinetic curve of the process of enzyme hydrolyses after steam-explosion treatment at 200° without CO_2 depending on the additional mechanical grinding and the specifics of the waste hemp mass.

The obtained kinetic curves after the refining show that the glucose yield increased by 9% in the first group of hemp, which has higher cellulose content and, respectively, higher content of rough fibres. The total yield of glucose is higher than wheat straw. During the work of the Jokro lab, the refiner is observed splintering of fibres, which effect can create a technology problem.In the second group (small-sized particles), there was no problem with refining, and the increase of the glucose yield was under 2% on the refined stock.

The impact of temperature on steam explosion with CO2 and the additional refining of glucose yield was researched for the second group and presented in Figure 2. The results show that the temperature can be decreased to 180°C, but it is necessary to refine in mild conditions before the enzyme hydrolysis. This is valid for the second sample. The steam explosion cannot lead to under 170ºC without refining the stock before the enzyme hydrolysis.

Industrial hemp fibres contain long and rough fibres, which cannot be defibrillated only by steam explosion. The additional refining of the raw material helps increase access of the enzymes to the cellulose chains, which will lead to a significant increase in the yield of glucose.

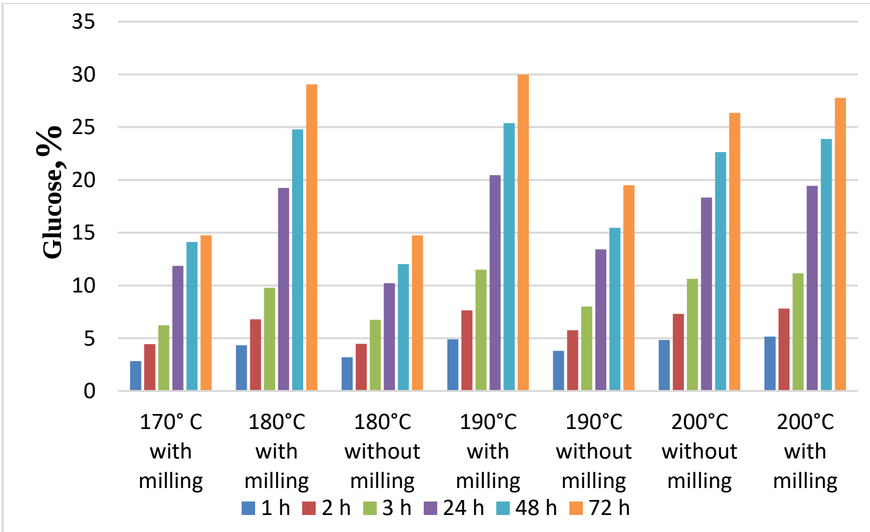

Figure 2: The influence of the temperature of the steam-explosion treatment with CO_2 and grinding of the amount of glucose after enzyme hydrolysis in the second hemp group.

CONCLUSIONS

It is established that the specificity of the hemp residues and the content of long and coarse fibers predetermine the need to carry out mechanical treatment of the pulp after the steam-explosive treatment and before enzymatic hydrolysis.

The refining of hemp pulp leads to fibrillation of the coarse hemp fibres. It increases the accessibility of the enzymes to the cellulose chains, whereby the glucose yield can be increased to values exceeding those for wheat straw.

Using the hemp stalks with lower long fibre content and adding CO_2 in the steam-explosion treatment results in mild refining before the enzyme stage or a reduced steam explosion temperature of up to 20 degrees.

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CONTENTS