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EFFECT OF NON-THERMAL PLASMA ON CELLULOSE CRYSTALLINITY AND LIGNIN CONTENT IN CORN STALKS

UTICAJ TRETMANA NETERMALNOM PLAZMOM NA KRISTALINIČNOST CELULOZE I SADRŽAJ LIGNINA U KUKURUZNOJ STABLJICI

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ABSTRACT

Lignocellulosic biomass is a cheap raw material that, thanks to its high carbohydrate content, can be used in fermentation to produce biofuels, biogas and other compounds. Its complex structure, including cellulose, hemicellulose and lignin, requires prior treatment of the biomass to facilitate hydrolysis to simple sugars. Today, biomass is only partially utilized and generates about 14% of the world´s energy. This is because the most commonly used physical, chemical and physicochemical treatments are not sustainable. They are energy-consuming but still low in productivity and toxic inhibitors formed during these treatments could hinder later steps of fermentation.Biomass treatment with advanced oxidation techniques has great potential as an environmentally friendly, so-called "green" treatment. These processes generate reactive species (radicals, electrons, ions and peroxides) that attack cellulose, hemicellulose, and lignin components. In this work, the effects of non-thermal plasma, the Fenton process, and the combined treatment of corn stalks with non-thermal plasma/Fenton were compared. Grounded biomass of corn stalks was mixed with Fenton reagent and hydrogen peroxide at different ratios and subjected to non-thermal plasma treatment. Carbohydrate content was decreased in non-thermal plasma treated samples both with and without Fe^{2+}. However, a specific biomass: Fe^{2+}:H_2O_2 ratio was required to achieve the highest rate of lignocellulose decomposition. The cellulose and hemicellulose fractions were affected and reduced by the treatments studied but resulted in almost no changes in the cellulose crystallinity index. The lower lignin content and cellulose crystallinity allow for more efficient enzyme hydrolysis of the treated lignocellulose and new options for valorization in fermentations.

***Keywords**: lignocellulose, non-thermal plasma, oxidation, biorefinery, degradation, Fenton process.*

REZIME

Lignocelulozna biomasa predstavlja jeftinu sirovinu koja se može koristiti u fermentacionim procesima za dobijanje biogoriva, biogasa i drugih jedinjenja, zahvaljujući visokom sadržaju ugljenih hidrata. Složena struktura, koja uključuje celulozu, hemicelulozu i lignin, zahteva prethodni tretman biomase kojim se olakšava hidroliza do prostih šećera. Danas se biomasa samo delimično eksploatiše i generiše oko 14% energije na svetskom nivou. To je prevashodno zbog male održivosti najčešće korišćenih fizičkih, hemijskih i fizičko-hemijskih tretmana. Ovi procesi troše veliku količinu energije, imaju malu produktivnost, a toksični sporedni proizvodi koji nastaju tokom tretmana mogu ometati kasnije korake fermentacije. Tretman biomase naprednim oksidacionim procesima ima veliki potencijal kao ekološki prihvatljiv, tzv. „zeleni“ tretman. Tokom ovog procesa dolazi do stvaranja reaktivnih vrsta (radikala, elektrona, jona i peroksida), koje napadaju celulozu, hemicelulozu i lignin. U ovom radu upoređeni su efekti tretmana kukuruzne stabljike netermalnom plazmom, Fentonovim reagensom i kombinovanog tretmana netermalnom plazmom/Fenton reagensom. Samlevena biomasa kukuruzne stabljike pomešana je sa Fentonovim reagensom i vodonik peroksidom u različitim odnosima, a zatim je podvrgnuta tretmanu netermalnom plazmom. Sadržaj celuloze i hemiceluloze je značajno smanjen u uzorcima tretiranim netermalnom plazmom i u prisustvu i u odsustvu Fe^{2+}. Ipak, najveći stepen redukcije lignoceluloze je postignut pri određenom odnosu biomasa:Fe^{2+}:vodonik peroksid. Primenjeni tretmani su uticali i na hemiceluloznu frakciju, ostavljajući indeks kristaličnosti celuloze skoro nepromenjenim. Niži sadržaj lignina i manji indeks kristaličnosti celuloze omogućavaju efikasniju enzimsku hidrolizu tretirane lignoceluloze i nove načine za valorizaciju u fermentacionim procesima.

***Ključne reči**: lignoceluloza, netermalna plazma, oksidacija, biorafinerija, degradacija, Fenton process.*

INTRODUCTION

Corn or maize (*Zea mays* L.) is one of the most grown cereals worldwide. According to the produced quantities and areas used for its cultivation, it is a major field crop in the Republic of Serbia (*Milašinović Šeremešić et al., 2017*). The annual yield of corn is about 5.76 t/ha worldwide (*USDA, 2021*). The corn stalk is used in domestic animal feeding, as compost, or as a source of energy in households. The major constituents of corn stalk are lignin, cellulose and hemicellulose. Thanks to its high content of carbohydrates, corn stalks can be used as an alternative source of sugar in the production of biogas, biofuels and biofertilizers, as substrates for the production of secondary metabolites, vitamins, enzymes, organic acids and oligosaccharides, as forage, a substrate for mushroom growth and as media in numerous fermentation processes (*Sadh et al., 2018; Sánchez, 2009*). The usage of residual lignocellulosic biomass such as corn stalks compared to other carbohydrate materials is more profitable, since the residual lignocellulosic biomass is not consumed in human nutrition and does not require additional land for production.

The world´s current biomass reserves are about 1.8 trillion tons, which is enough to produce about 33,000 EJ of energy (80 times more than annual consumption). However, biomass is only partially exploited and generates about 14% of the world´s energy (*WBA, 2021*). According to the United Nations Development Program (UNDP), biomass has the highest available potential of all renewable energy sources in the Republic of Serbia, with a share of 61%. Most of the biomass reserves come from agriculture (residues in arable farming, fruit growing, viticulture, primary fruit processing and livestock). Its potential is about 1.67 million tons of oil equivalent, but the degree of utilization does not exceed 2% (*Final Conference UNDP, 2019*). The main reason for this is the complex structure of biomass, especially lignocellulosic biomass, which requires prior treatment to facilitate hydrolysis to simple sugars.

Conventional methods used for biomass treatment can break down the components of lignocellulose into smaller fragments, but they are energy-consuming. Also, various by-products that are formed may have an inhibitory effect on microorganisms that ferment sugars in further steps of biomass processing. The reagents used in chemical methods, such as different acids, alkali, and organic or ionic solvents, can affect the environment and their proper treatment and disposal after the process may require additional energy and financial investments. In recent years, more attention is being paid to the so-called ″green″ treatments that are environmentally justified.

Advanced oxidation processes (AOP) are methods that involve the *in-situ* generation of highly reactive oxygen species (ROS), such as hydroxyl radicals, hydrogen peroxide, ozone and superoxide anion radicals, or reactive nitrogen species (RNS), such as nitrates, nitrites and different nitrogen oxides (*Kanakaraju et al., 2018*). Among these methods are the Fenton process and non-thermal plasma treatment. The Fenton process is based on the utilization of a Fenton reagent, consisting of hydrogen peroxide and ferrous or ferric ions (*Popović et al., 2019*). These ions can decompose hydrogen peroxide into hydroxyl radicals, attacking and degrading lignin and hemicellulose, making cellulose more accessible to enzymes (*Arantes et al., 2012*). Fenton process has the advantages of low energy requirement and mild reaction conditions, but it is very time-consuming, mostly due to the low concentration of hydroxyl radicals generated during the process. This is a competitive reaction system because both lignocellulose and Fenton reagent can react with hydroxyl radicals (*Gan et al., 2018*). During non-thermal plasma treatment, numerous reactive species are generated due to the interaction between ions from the plasma source and treated material (*Đukić-Vuković et al., 2017*). Like hydroxyl radicals, other oxygen radicals (H_2O_2, O_3, O_2^-) may contribute to the degradation of lignocellulose. Which radicals will be formed depends on various factors, such as feed gas and media composition, duration of the treatment, the distance of the discharge source from the treated material, the type of electrodes used, as well as the type of plasma source (*Lukes et al., 2014*). Non-thermal plasma treatment can be combined with homogeneous or heterogeneous catalysts to make this process more efficient (*Shang et al., 2019*). Hence, non-thermal plasma treatment combined with the Fenton process could increase the amount of generated reactive species and therefore improve biomass decomposition.

The aim of this work is to compare the effects of various advanced oxidation treatments on corn stalk's composition. Furthermore, the possibility of enhancing the lignocellulose degradation by combining non-thermal plasma treatment and the Fenton process was investigated.

MATERIAL AND METHOD

Substrate preparation

Corn stalks used in this study were obtained after harvesting in the municipality Kovin, South Banat, Serbia. Dried samples were grounded to powder in a mill (Mixer Mill, Retsch MM400, Germany). Substrates were prepared by mixing 50 mg of grounded biomass with 10 mL of a solution containing water and 30 wt% hydrogen peroxide in a different ratio as given in Table 1. All chemicals used in work were p.a. purity.

Experimental set up for Fenton process and non-thermal plasma treatment

To investigate the effect of non-thermal plasma treatment on the decomposition of lignocellulose, one set of samples was treated under the non-thermal plasma needle for 10 minutes. Our plasma needle is custom-made. The body is made of Teflon with a central electrode made of copper axially positioned in a glass tube. Discharge is generated at the tip of the electrode which can be kept above the sample at distances up to 5 cm (or if needed, immersed into a liquid sample). The power supply operates in kHz and kV range. Comprehensive electrical characterization of the same plasma device has been performed earlier (*Zaplotnik et al., 2014*). For all our treatments, we have used 0.5 slm of Argon as a feed gas. The distance between the tip of the plasma needle and our samples was 2 cm.

After the treatment, $FeSO_4 \cdot 7H_2O$ was added in both, treated and untreated samples and the ratio of Fe^{2+}- salt to hydrogen peroxide remained 1:5 in all samples (Table 1). These suspensions were kept at 30°C for 24 hours to allow for the Fenton reaction to occur. The resulting suspensions were filtered, and the residues obtained were successively rinsed with distilled water, 5 wt% oxalic acid solution and distilled water, and dried at 105°C for 24 hours.

Table 1. Composition of suspensions

Sampl	Biomass, m_B	H_2O, m_W	H_2O_2 (30 wt%), m_P	$FeSO_4 \cdot 7H_2O$, m_S
C	50	10.000	-	-
H-1	50	9.975	0.025	-
H-2	50	9.950	0.050	-
H-3	50	9.900	0.100	-
F-1	50	9.975	0.025	1.85
F-2	50	9.950	0.050	4.35
F-3	50	9.900	0.100	9.23

The control sample, samples containing only hydrogen peroxide and samples containing Fenton reagent were denoted as C, H and F, respectively. Additionally, samples treated with non-thermal plasma were labeled with a subscript p.

Lignin content determination

The level of delignification of corn stalk was determined by measuring the content of acetyl bromide soluble lignin using the modified method of Fukushima and Hatfield (*Fukushima and Hatfield, 2001*). After drying, 5 mg of each sample was weighed into a 2-ml Eppendorf tube. Samples were digested with 500 µL of 25% (v/v) acetyl bromide in glacial acetic acid for 2 h at 50°C with constant shaking (800 rpm, Thermo shaker LLG-uniTHERMIX 1 pro). After digestion, samples were centrifuged at 12,000 rpm for 10 min and 100 µL aliquots of the solution were transferred to new tubes and mixed with 400 µL 0.3 M NaOH and 200 µL 0.5 M hydroxylamine hydrochloride. The samples were then diluted to 2.0 mL with glacial acetic acid. Finally, 200 µl of the solutions were pipetted into a UV-specific

96-well plate and read in a microplate reader (Epoch Microplate Spectrophotometer, US) at 280 nm against a blank containing all chemical reagents, but no sample material. A standard curve was constructed using alkali lignin. The percentage of acetyl bromide soluble lignin (% ABSL) was determined using the following formula:

$$\% ABSL = (0{,}11452 \cdot A + 0{,}0008) \cdot 10 \cdot \frac{R}{m} \cdot 100(\%) \quad (1)$$

where A is the absorbance at 280 nm, R is the dilution factor and m is the mass (mg) of weighed samples.

Cellulose crystallinity index determination

Changes in crystallinity of cellulose were determined using the Schwertassek method (*Nikolić et al., 2011*). The cellulose crystallinity index was defined indirectly, by measuring the iodine number for each sample. The determination of iodine number is based on determining the amount of bound iodine in amorphous parts of cellulose. Iodine remaining in the solution is quantified by titration, using 0,2 M sodium thiosulfate and 1 wt% solution of starch as an indicator. The iodine sorption values (*ISV*), or iodine numbers, were calculated as follows:

$$ISV = \frac{(V_b - V_s) \cdot 2{,}04 \cdot 2{,}54}{m} \quad (2)$$

where V_b is the volume (ml) of $Na_2S_2O_3$ solution for blank titration, V_s is the volume (ml) of $Na_2S_2O_3$ solution for the titration of sample solution and m is the weight (g) of absolute dry samples.

The cellulose crystallinity index (Cr_I) was calculated using the following equation:

$$\% C_{r_I} = 100 - \left(\frac{ISV}{412} \cdot 100\right) \quad (3)$$

Statistical analysis of data

Statistical analysis was performed in OriginPro 9 software using a one-way ANOVA analysis of variance with Fisher´s LSD (Least Significance Difference) test. Differences between the means with probability $p<0.05$ were accepted as statistically significant. All measurements were done in duplicate and repeated independently on three different days. Obtained results were presented as mean values along with standard deviation as error bars.

RESULTS AND DISCUSSION

Lignin content

The acetyl bromide-soluble lignin content for each sample is shown in Figure 1. On average, the lignin content in the untreated corn stalks used in this study was about 26%. According to the literature, the expected content of acetyl bromide-soluble lignin is between 20 and 50% by weight of the treated samples (*Foster et al., 2010*).

This study aimed to compare different advanced oxidation techniques' effects on lignocellulose structure. When it comes to plasma treatment, the obtained results showed higher lignin content in samples treated with non-thermal plasma, containing either Fenton reagent or just hydrogen peroxide, compared to the control sample. This is probably due to cellulose and/or hemicellulose decomposition under conditions applied for the treatment of mentioned samples. Higher lignin content means more lignin present in treated samples and, therefore, reduced cellulose and hemicellulose content. Likewise, higher lignin content in the sample containing 0.5% (v/v) hydrogen peroxide and $FeSO_4 \cdot 7H_2O$ after non-thermal plasma treatment (F-2p) compared to the sample containing only 0.5% (v/v) hydrogen peroxide without plasma treatment (H-2) could be explained by the fact that combined non-thermal plasma/Fenton reagent treatment promotes the degradation of carbohydrates in lignocellulose. Treating samples only with hydrogen peroxide didn't significantly affect the sample's composition. However, a combination of hydrogen peroxide treatment with non-thermal plasma showed better results in favor of carbohydrate degradation. During plasma treatment, more oxygen radicals are being generated in the samples containing hydrogen peroxide (H-2p, H-3p) than in the sample containing only water (control sample). These radicals contributed to carbohydrate degradation and led to slightly higher lignin content in the mentioned samples.

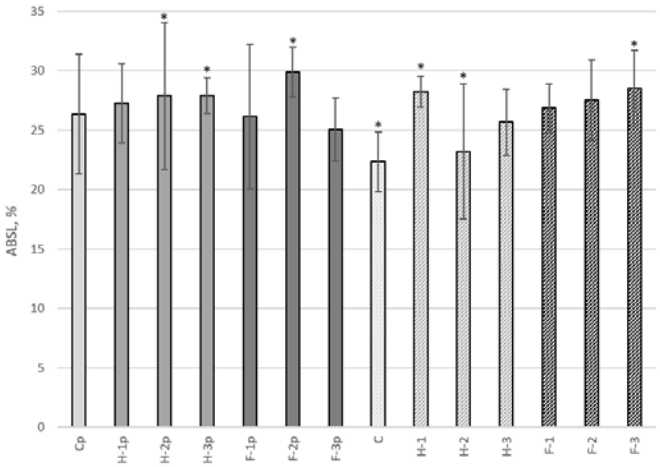

*Fig. 1. Acetyl bromide-soluble lignin content of the control sample (C), samples containing hydrogen peroxide (H 1-3) and samples containing Fenton reagent (F 1-3), both plasma treated (p) and non-treated. Samples labeled with * showed statistically significant differences at the 0,05 level.*

Jeong and Lee (2020) confirmed the presence of xylose and acetic acid in the liquid fraction after the Fenton reaction as a result of cellulose and hemicellulose decomposition during the Fenton process. There are reports in the literature that when the Fenton process is combined with longer non-thermal plasma treatment and in presence of ethanol, the lignin fraction is more affected (*Zhou et al, 2020*). *Zhou et al.* (2020) reported a very efficient degradation of alkali lignin, reaching around 66.0% when the Fenton reagent consisted of Fe_2O_3 and hydrogen peroxide in a 1:6 ratio. The recommended iron: hydrogen peroxide ratio varies from 1:5 to 1:25 (*Bishop, 1968; Walling, 1975*). for standard Fenton processing of lignocellulose. However, Fenton oxidation of corn stalk is usually performed with $FeSO_4 \cdot 7H_2O$ and H_2O_2 in the molar ratio of 1:25 (w/w) (*Jeong and Lee, 2020; Yu et al., 2017*) and it significantly affects the degradation of carbohydrates. The ratio in this study was set to 1:5 in order to minimize the media supplementation with peroxide since some amount of H_2O_2 is generated during the plasma treatment also. It is observed that the plasma-generated peroxide in the water per se does not affect significantly substrate composition, so supplementation with peroxide and iron salts was needed.

The role of different iron: hydrogen peroxide ratios and the type of iron salts could be examined in the future in combination with non-thermal plasma since there were no references on these aspects in the currently available literature. In the standard Fenton process, $FeSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$ and F_2O_3 are some of the most efficient iron sources used for lignocellulose treatment

(*Gan et al., 2018*). *Ravindran et al.* (2017) subjected spent coffee waste (SCW) which is a lignocellulosic substrate to similar treatment, using DBD plasma configuration and Fenton reagent containing $FeCl_3$. Considering five parameters of experiments, this combination of treatments revealed substantial amounts of lignin removal (*Ravindran et al., 2017*).

Cellulose crystallinity index

The results of the cellulose crystallinity index (Cr_I) are shown in Figure 2. In untreated biomass, Cr_I was around 76%.

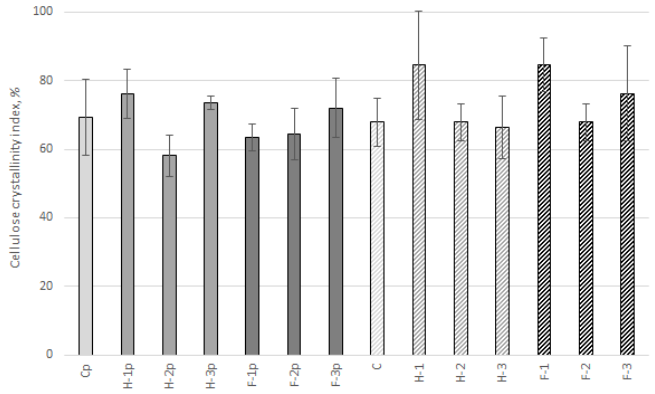

Fig. 2. The cellulose crystallinity index of the control sample (C), samples containing hydrogen peroxide (H 1-3) and samples containing Fenton reagent (F 1-3), both plasma treated (p) and non-treated

Analysis of variance showed no significant difference between samples whatsoever. The reason for this could lay in the fact that the applied treatments probably affect hemicellulose more than cellulose. Therefore, the structure of cellulose remains almost intact. Shortly after mixing the samples containing hydrogen peroxide with ferrous salt, hydroxyl radicals are produced. These radicals can cause damage and rapid degradation of cellulose and hemicellulose depending on the conditions applied (*Yang et al., 2019*). The high crystallinity index of corn stalk used in this study (76%) makes it harder for hydroxyl radicals to attack the solid cellulose structure, leaving the hemicellulose more susceptible to applied treatments. To elucidate the mechanism of carbohydrate degradation during these treatments, it is necessary to apply techniques for specific component analysis, such as HPLC, FTIR, or SEM analysis. A small increase in the amount of glucose was found in filtrates remaining after the treatment (no data shown). The presence of small glucose content further confirms that, when subjected to the treatments applied in this study, the carbohydrate fraction of biomass is slightly affected. To enhance the level of degradation, the parameters of the experiment, such as biomass: Fenton reagent ratio, duration of both plasma treatment and Fenton reaction, should be adjusted.

CONCLUSION

According to the results obtained so far, there is a possibility to improve the degradation of lignocellulose by combining different AOPs. The combined treatment with non-thermal plasma/Fenton reagent showed slightly better results than the Fenton process or the non-thermal plasma treatment alone. To improve these processes and achieve better biomass decomposition, the optimal ratio of biomass: hydrogen peroxide: Fe^{2+}-salt and the optimal duration of plasma treatment should be determined. Furthermore, the effect of different Fe^{2+}- or Fe^{3+}-salts on the Fenton reaction should be examined. To improve delignification, the use of enzymes that can degrade lignin, such as lignin peroxidase, versatile peroxidase, manganese peroxidase, or laccase could also be considered.

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