Improvement of enzymatic saccharification of corn cob by microwave-assisted peroxide treatment

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Lignocellulosic biomass is composed of polysaccharides, cellulose and hemicellulose, and aromatic polymer lignin. These three structural components are interlinked together making lignocellulosic biomass greatly resistant to biodegradation. Lignin limits the accessibility of hydrolyzing enzymes to the cellulosic and hemicellulosic fractions and leads to unproductive enzyme adsorption. A common lignocellulose biorefinery process includes the pretreatment of biomass for the disruption of the complex polymer structure, subsequent enzymatic hydrolysis of pretreated biomass to break down polysaccharides, and fermentation of released simple sugars to final products such as biofuels or biochemicals. So far, different pretreatment strategies have been studied to disrupt a complex polymer structure of biomass and remove lignin. Advanced oxidation processes have been usually applied in the treatment of wastewater, soil remediation, and detoxification of hazardous chemicals. Recently they also attacked considerable attention in the treatment of lignocellulosic biomass (Xia *et al*, 2022, Ho *et al*, 2019, M'arimi *et al*, 2020). These pretreatments are based on substrate oxidation with in situ generated active radicals as a driving force. The efficiency of using H_2O_2 in these processes relies on its decomposition under certain conditions and the formation of intermediate products which selectively react with lignin leading to biomass delignification (Ho et al, 2019). Recently, the coupling of microwave irradiation and advanced oxidation processes has been shown to increase the reaction rate and exert a stronger effect in wastewater treatment (Xia *et al*, 2022).

This work aimed to investigate how peroxide treatment coupled with microwave irradiation affects the characteristics of the cell wall of corn biomass and whether changes in the composition and structure of treated biomass lead to more efficient enzymatic hydrolysis. This study was performed using corn cob (obtained from a local farm in Vojvodina province, Serbia). It was grounded and sieved and a fraction with a particle size of 400 μm-1 mm was subjected to microwave irradiation with H_2O_2 as an oxidizing agent under alkali conditions. Pretreated biomass was further subjected to the enzymatic hydrolysis with Cellic® CTec2. The saccharification efficiency was assessed by determining the concentration of reducing sugars, total hexose, and pentose sugars. In addition, the accessibility of cellulose was determined by the Congo dye staining as described in the literature (Wiman *et al*, 2012), while lignin content was studied by the acetyl bromide method. Finally, enzyme adsorption experiments were performed with the same cellulase preparation as in the enzymatic hydrolysis, and cellulase adsorption was characterized by the Langmuir adsorption isotherm.

The obtained results showed that H_2O_2 under alkaline conditions primarily reacts with lignin resulting in almost 70% delignification (Table 1). Enzymatic accessibility of corn cob determined by the Congo red dye assay showed a significant increase of cellulose surface area when biomass was treated with H_2O_2 coupled with the microwave. As lignin interlinked with hemicellulose acts as a physical barrier, removal of these components during pretreatment increases the surface area of cellulose available for binding of cellulolytic enzymes during hydrolysis. Consequently, hydrolysis of treated biomass resulted in a significantly higher concentration of reducing sugars, total hexose, and pentose sugars compared to untreated biomass (Table 1).

Table 1. Comparison of characteristics of treated and untreated corn cob.

	Untreated	Microwave-assisted alkali H_2O_2
Lignin (%)	23.18	6.92
Cellulose surface area (m^2/g)	57.36	131.78
Saccharification efficiency		
Reducing sugars (g/l)	4.39	33.04
Hexose sugars (g/l)	5.22	17.33
Pentose sugars (g/l)	2.35	19.16

Fitting the data obtained in the enzyme adsorption experiment to the Langmuir isotherm corresponding parameters for untreated and treated biomass were calculated and summarized in Table 2. Biomass treated with H_2O_2 coupled with microwave had a higher maximum enzyme adsorption capacity. Also, treated biomass had a lower Langmuir constant indicating the lower adsorption affinity of the treated sample. These results are in accordance with other studies which revealed that some lignocellulosic substrates adsorbing more cellulase and having smaller adsorption affinity could have a higher enzymatic hydrolysis efficiency compared to other substrates (Lan *et al*, 2020), which could be explained by lignin content and enzyme unproductive binding.

Table 2. Langmuir adsorption parameters for treated and untreated corn cob.

	Untreated	Microwave-assisted alkali H_2O_2
*q_{max} (mg/g)	0.77	1.04
**K_L (ml/mg)	11.85	7.39
***R^2	0.89	0.95

*q_{max} is the maximum adsorption capacity of cellulase preparation to the substrate
**K is the Langmuir adsorption constant, representing the affinity between enzyme and substrate
***R^2 is the R-squared value

This work contributes to a better understanding of the effects of the peroxide treatment coupled with microwave irradiation on lignocellulosic biomass and enables the development of a more efficient biomass fractionation process. This way combination of microwave and oxidation processes could help develop time- and cost-saving treatment for lignocellulose processing into biofuels and/or biochemicals.

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