

# Evaluation of pre-treatment methods for improving the enzymatic hydrolysis of lignocellulosic waste

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Lignocellulosic biomass is a sustainable and low-cost carbohydrate source that could be converted into high-value products, such as biofuels, organic acids and enzymes, providing huge benefits to the environment and society. Despite being the most abundant renewable raw material with promising application in different biorefinery processes, in developing countries majority of lignocellulosic biomass remains largely unused and is commonly discarded or combusted. The main obstacle for exploitation of lignocellulosic materials is complex physical and chemical structure that makes their bioprocessing rather costly and inefficient (Chen et al., 2017). To be effectively utilized, fractionation of lignocellulose into its main components (cellulose, hemicellulose, and lignin) through an adequate pre-treatment is highly required. Pre-treatment of lignocellulosic biomass has a crucial role in depolymerisation of cellulose, increasing the porosity and surface area of the material, thus facilitating the subsequent enzymatic hydrolysis and enabling release of fermentable sugars into the bulk liquid. Up to now, several pre-treatment strategies have been investigated, and they can be broadly categorised into physical, chemical, and biological methods (Ravindran and Jaiswal, 2016). In addition, combination of two or more available processes has been frequently applied as a possible solution for increasing the pre-treatment efficiency. The choice of the pre-treatment should be made considering physical and chemical nature of particular lignocellulosic material, but also taking into account an economic viability and potential environmental impact.

The aim of this work was to evaluate and compare several pre-treatment methods in order to improve subsequent enzymatic hydrolysis, thus enabling efficient bioprocessing of low-cost and unused lignocellulosic biomass into high-valuable products. The study was performed using corn husk (obtained from PKB Corporation Belgrade, Padinska Skela, Serbia). Corn husk was grounded and sieved and afterwards, a fraction with particle size of 400-800  $\mu\text{m}$  was subjected to the pre-treatment methods applying dilute sulfuric acid, sodium-hydroxide solution, microwave assisted alkali treatment, and plasma assisted enzymatic treatment. Pre-treated biomass was subjected to the enzymatic hydrolysis using cellulase complex Cellic<sup>®</sup> CTec2 (from Novozymes A/S, Bagsvaerd, Denmark). The efficiency of each pre-treatment was assessed by determining the concentration of hydrolyzed products using 3,5-dinitrosalicylic acid method (Miller, 1959) and HPLC analysis. The saccharification efficiency of pre-treated biomass was compared with untreated samples.

According to the obtained results, corn husk showed comparable susceptibility to the chemical pre-treatment performed by alkaline and dilute acid solutions. Reducing sugar concentrations obtained after enzymatic hydrolysis of alkali and acid pre-treated corn husk were 25.65  $\text{g L}^{-1}$  and 23.94  $\text{g L}^{-1}$ , respectively. Further, microwave assisted alkali pre-treatment and plasma assisted enzymatic treatment significantly facilitated subsequent enzymatic hydrolysis, recording maximal sugar concentration of 32.52  $\text{g L}^{-1}$ . In addition, microwave and plasma assisted processes have been less intensive in energy consumption and shorter in processing time, showing lower negative environmental impact compared to the conventional chemical pre-treatments conducted under high temperature or high pressure conditions. Also, performing at atmospheric pressure and room temperature, pre-treatment using plasma prevents formation of inhibitory compounds. Generated inhibitory and toxic compounds may exhibit negative effect on the growth of microorganisms, which are used for fermentative biorefinery processes on treated and hydrolyzed lignocellulosic substrates. Thus, efficient novel treatments can significantly increase process efficiency and decrease overall cost of biomass processing. In this way treated corn husk could be used as carbohydrate source for the production of lactic acid or/and other valuable products.

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