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OPTIMIZATION OF PACKED BED REACTOR FOR DETOXIFICATION OF LIGNOCELLULOSIC XYLOSE RICH FRACTION (XRF)

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During pretreatment of lignocellulosic biomass, fermentation inhibitors are formed that lead to reduced ethanol productivities (Almeida et al. 2007). Therefore, in many cases, detoxification of the hydrolysate is needed. Previous experiments show that, among the formed inhibitors, light molecular-weight phenolic compounds have the highest inhibiting effect (Jönsson et al. 1998). Detoxification of phenolic compounds can be performed in several ways. In this work the focus is on enzymatic bioconversion.

Laccase enzyme has several possible applications because of its capacity to transform phenolic substrates, one of them is lignocellulose hydrolysate detoxification. Often authors suggest immobilization of the enzyme to increase the enzyme's lifetime and tolerance, and, at the same time, enable the recovery of the catalyst (Champagne and Ramsay 2007). For engineering purposes it is important to determine the immobilized enzyme activity. However, activity measurements with initial rate experiments can be difficult because of adsorption of substrate to the carrier material occurring simultaneously with the reaction (Champagne and Ramsay 2007).

Lignocellulosic xylose rich fraction (XRF) detoxification was investigated with immobilized laccase enzyme from *Trametes versicolor* on 3-chloropropyltrimethoxysilane (CPTS) activated silica gel support. The role of adsorption was investigated, and the flow rate was optimized for maximal efficiency.

A packed/fluidized bed reactor was built for the experiments. The flow rate profile was controlled during the whole process. Samples were analyzed spectrophotometrically at 280 nm, either manually or automatically with a flow through detector.

The role of adsorption on the overall detoxification was investigated by comparing the active catalyst with inactivated catalyst. It can be seen that in the early stage of the column operation (<1h) the adsorption effect is more significant than the real enzymatic removal. Therefore adsorption has to be taken into account in immobilized laccase experiments and initial rate activity measurements have to be treated with care.

The effect of flow rate on immobilized enzyme performance was determined after saturating the surface with substrate in order to minimize the error of adsorption. Reactor efficiency was calculated as phenolics removal over time, which showed a clear maximum at the stage of fluidization.

PROPERTIES OF *Anoxybacillus* sp. 3M XYLANASES AND FURTHER APPLICATION TOWARDS SUGAR RICH HYDROLYSATES

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This study aimed to optimize the production of xylanases by *Anoxybacillus* sp. strain 3M, a thermophilic bacterium isolated from terrestrial hot springs (temperature of 90°C) samples collected on S. Miguel, Azores, Portugal, in batch fermentation testing several agroindustrial byproducts as inducer substrates (BSG - Brewer's spent grain, wheat straw, sugarcane bagasse, and corn cobs). In addition, the xylanases produced by this bacterium with the best inducer substrate were characterized for their optimal pH, temperature and stability. The results for xylanase production showed that the higher levels of xylanases were obtained in growth medium containing 1% (w / v) BSG (1.35 U/mL), but the xylanolytic activity was also observed when wheat bran (1.32 U/ml), sugarcane bagasse (0.80 U/mL), corn cobs (0.30 U/mL) and commercial xylan (0.21 U.ml⁻¹) were used as substrates. The extracellular crude enzymatic extract from *Anoxybacillus* sp. 3M was then characterized for its optimal temperature and pH and stability. The best enzyme activity was observed at a temperature of 60 °C and pH 5.3, and the enzyme retained 100% of its original activity after 96 h at 60 °C and pH 7.0. Zymogram of native gel analysis of the different culture supernatants revealed the presence of an enzyme complex with a molecular weight of 420 kDa. This xylanase may be considered as a biocatalyst thermotolerant and it is interesting for biotechnological applications. Further application of *Anoxybacillus* 3M crude enzymatic extract to BSG and commercial xylan revealed the presence of xylose and xylooligosaccharides, mainly X₂ and X₃, in the hydrolyzates produced.

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KOH FOR ENHANCED SUGARCANE BAGASSE DELIGNIFICATION AND FURTHER PRODUCTION OF SUGAR-RICH HYDROLYZATES BY ENZYMES APPLICATION

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Lignocellulosic biomass is envisaged as an important raw material for bioethanol production due to its low cost and high availability. Sugarcane bagasse (SCB), a fibrous residue of cane stalks left over after crushing and extraction of the juice from sugarcane; it is one of the largest cellulosic agro-industrial by-products. Tons of SCB are produced in Brazil as a waste of sugar and ethanol industries. This lignocellulosic by-product is a potential renewable source for 2G-bioethanol production. Usually, SCB is pretreated using alkaline and/or acid treatments viewing higher ethanol yields. The main goal of this study was to optimize the delignification of SCB towards the higher availability of glucans and xylans for further