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Poster 15

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

V. Sóti[1], S. Lenaerts[2], Iris Cornet[1]
Universiteit Antwerpen, [1]Faculty of Applied Engineering and [2]Faculty of Bioscience Engineering, Antwerp, Belgium
iris.cornet@uantwerpen.be

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.

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

Luís Alves[1], S.A. Ladeira[2], B.F. Arez[1], S.M. Paixão[1]
[1]LNEG - Laboratório Nacional de Energia e Geologia, U. Bioenergia - LEN, Lisboa, Portugal; [2]UENF - Universidade Estadual do Norte Fluminense Darcy Ribeiro, LTA-CCTA, Campos dos Goytacazes, RJ, Brazil
luis.alves@lneg.pt; susana.alves@lneg.pt

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 X_2 and X_3, in the hydrolizates produced.

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

Susana M. Paixão[1], S.A. Ladeira[2], B.F. Arez[1], M.L.L. Martins[2], J.C. Roseiro[1], Luís Alves[1]
[1]LNEG - Laboratório Nacional de Energia e Geologia, U. Bioenergia - LEN, Lisboa, Portugal; [2]UENF - Universidade Estadual do Norte Fluminense Darcy Ribeiro, LTA-CCTA, Campos dos Goytacazes, RJ, Brazil
susana.alves@lneg.pt; luis.alves@lneg.pt

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

enzymatic hydrolysis to obtain sugar-rich syrups that will be more readily fermented to bioethanol. The delignification was carried out by autoclaving the biomass with KOH and the influence of KOH concentration (1-10%) and the autoclave time (10-60 min) were evaluated through a statistical design. Experimental distribution for two factors according to the Doehlert uniform design was used to produce response surfaces. The responses studied in this design were the percentage of hemicellulose, lignin and total polysaccharides. The results showed that from the two factors evaluated, the KOH concentration was the one that most influenced the response observed and that the treatments of SCB with KOH 5-10% for 35 minutes of autoclave at 121ºC and 1 atm led to the highest rates of lignin extraction. Using KOH treatment, a significant reduction of lignin content in SCB was observed, namely from 19% to 5%. Scanning electron micrographs of SCB pre-treated with 10% KOH for 35 minutes demonstrated a change in the structure of the material, with the appearance of broken structures, which can be attributed to the alkaline treatment. To validate the experiments, the SCB pretreated in the optimal conditions (95% of total polysaccharides) was further hydrolyzed with commercial enzymes and the enzymatic hydrolysis performance was evaluated.

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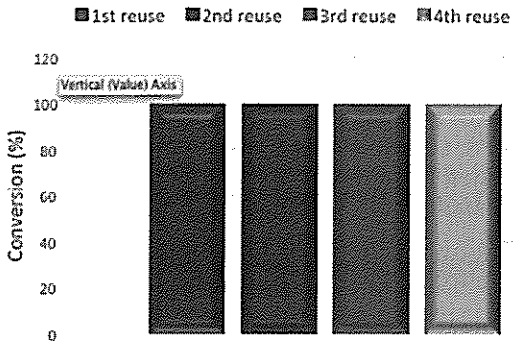
MESOPOROUS Zr-SBA-16 CATALYSTS FOR GLYCEROL VALORIZATION PROCESSES: TOWARDS BIORENEWABLE FORMULATIONS

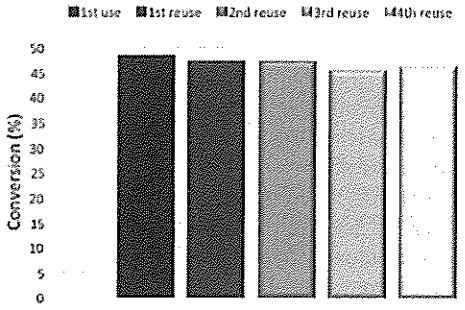
Camino González-Arellano, L. Parra, R. Luque
University of Alcalá, Departamento de Química Orgánica and Química Inorgánica, Alcalá de Henares, Spain
camino.gonzalez@uah.es

Glycerol has the potential to be a versatile building block in biorefineries, it is a nontoxic, biodegradable compound, it will provide important environmental benefits to new platform products. In the present work, Zr-containing SBA-16 materials with varying Si/Zr ratios were utilized in glycerol valorization for the production of esters, via reaction with levulinic acid, which is a biomass-derived platform molecule, like glycerol, and glycerol formal via acetalisation with paraformaldehyde. Materials were found to be highly active and selective for the production of valuable compounds from glycerol using benign by design solventless protocols which employ mild reaction conditions. Quantitative conversion was achieved in the esterification of glycerol with levulinic acid with moderate selectivites to diacetylglycerides, being ZrSBA-16(25) most active in the reaction as expected by its large concentration of acid sites. Comparably, ZrSBA-16(100) exhibited optimum activities under optimized conditions in the acetalisation of glycerol with paraformaldehyde as formaldehyde source. Investigated catalysts were generally highly stable and reusable under the investigated conditions, with a particularly outstanding recyclability of Zr-SBA-16(25) in glycerol esterification, the catalyst could be successfully reused up to four times under identical reaction conditions, without any decrease in activity and selectivity. Gratifyingly, Zr-SBA-16(50) could also be successfully used in the acetalisation with paraformaldehyde without loss of activity and selectivity.

A



B



Expertiments of catalyst recycling in **A:** esterification esterification of glycerol with levulinic acid using ZrSBA-16(25) as catalyst, 50 mg catalyst, 1 mmol glycerol, 5 mmol levulinic acid, 140 ºC, 15 h reaction. **B:** acetalization of glycerol with paraformaldehyde using Zr-SBA-16 (50) as catalyst, 1 mmol glycerol, 1 mmol of paraformaldehyde at 100ºC, 100 mg of catalyst, without solvent for 8h.

Reference:

1. C. Gonzalez-Arellano, L. Parra and R. Luque, *Catal. Scien. Tech.*, **2014**, DOI: 10.1039/c4cy00230j

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CANDIDA LIGNOHABITANS FOR ORGANIC ACID PRODUCTION FROM LIGNOCELLULOSIC MATERIAL

Martina Bellasio[1], H. Marx[1], D. Mattanovich[1,2], M. Sauer[1,2]
[1]Dept. of Biotechnology, BOKU – VIBT University of Natural Resources and Life Sciences, Vienna; [2]Austrian Centre of Industrial Biotechnology (ACIB GmbH), Vienna, Austria
martina.bellasio@boku.ac.at

Introduction: Microbial conversion of lignocellulosic biomass as basis for chemical production depends on microbial cell factories, which efficiently convert hexoses as well as pentoses into the desired products.
Concept and Methodology: We suggest the unconventional yeast species, *Candida lignohabitans*, for the efficient fermentation of biomass derived feedstocks. Glucose, galactose, mannose, arabinose and xylose are efficiently metabolized by *Candida lignohabitans*, in pure form or as mixtures.
To test the performance of this microorganism on real substrates a variety of lignocellulosic materials including saw dust, wood chips, christmas tree and *Miscanthus* have been pretreated by steam explosion and subsequently enzymatically hydrolysed. Liquefaction of the biomass was successful and led to hydrolysates with sugar concentrations of 50-80 g/L.