A special conference of the
Society for Industrial Microbiology

33rd SBFC

Symposium on Biotechnology for Fuels and Chemicals

May 2 - 5, 2011
Sheraton Seattle
Seattle, WA
www.simhq.org/meetings/sbfc2011/index.asp

large multi-modular family 9 enzyme, CbhA, including the X1, CBM4 and the immunoglobulin-like modules were studied using molecular dynamics and small angle x-ray scattering.

Y. J. Bomble, G. T. Beckham, J. F. Matthews, M. R. Nimlos, M. E. Himmel, and M. F. Crowley (2010) *J. Biol. Chem. (DOI:* 10.1074/jbc)

Poster 5-31

Two-step hydrolysis with thermostable liquefying and secondary saccharifying enzymes

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Conventional biotechnical conversion of lignocellulose biomass to fermentable sugars is usually based on mesophilic enzymes at about 45°C in separate hydrolysis and 35°C in simultaneous saccharification and fermentation. In order to reach the technological minimum of about 4% ethanol concentration in the broth required for an economically feasible distillation increased initial biomass content is needed. In conventional processes this could, however, lead to poor enzymatic conversion due to *e.g.* diffusion limitations. An approach to overcome this problem is to liquefy the feedstock first - preferably at increased temperatures where viscosities are inherently lower - to achieve better flowability of the high-solids substrate and thus guarantee improved mass transfer conditions for the enzymatic hydrolysis. After a high-temperature liquefaction using thermostable enyzmes the hydrolysis can be continued at conventional temperatures using mesophilic enzymes. This two-step process concept has been in the focus of the present work using novel thermostable enzymes in the pre-hydrolysis (55°C) and a commercial cellulase mixture in the secondary hydrolysis (35°C, 45°C) of hydrothermally pretreated wheat straw. A thermostable enzyme mixture, tailored to efficiently reduce the viscosity of the high-solids substrate, was used for the liquefaction. Various pre-hydrolysis times, temperature profiles, and enzyme loadings in the two-step process were investigated in detail. The obtained conversions were evaluated in comparison with commercial preparations. The use of thermostable enzymes for liquefaction combined with traditional saccharification or SSF offers several advantages and allows more flexible process design.

Poster 5-32

Indirect method for quantification of cellular biomass in a solid containing medium used for cellulase production

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The cost of cellulase enzymes is one of the major bottlenecks on the economics of cellulosic ethanol. Enzyme production can be carried out using submerged (SmF) or solid-state fermentation (SSF). Each process has different advantages and disadvantages. In this study, we are evaluating celullase production in a combined fermentation process, which aggregates the advantages of SSF and SmF in one equipment, a pneumatic bioreactor. Due to the difficulties in measuring cellular biomass in the presence of solids, we developed a methodology for indirect quantification of this biomass. The cultivation of *Aspergillus niger* was initiated as SSF using sugar cane bagasse as solid substrate and a liquid medium containing glucose was added after 24h of growth. Samples were collected during 72h and the glucose consumed was quantified. Experiments in liquid medium in the absence of bagasse were conducted in parallel for the determination of the growth kinetic parameters by measuring both variations on glucose and biomass during the cultivation. The software Anabio 1.2 was used to simulate a simple unstructured model. The simulations showed that the growth followed the Contois model, with μmax of 0,034h^{-1}, Yx/s of 0,297g/g and death constant of 0,005h^{-1}. The model parameters were used to simulate the profile of glucose consumption and generate the simulated profile of cellular growth in the medium containing solids. The developed methodology for indirect quantification of biomass showed to be reliable, since the proposed model fitted very well to experimental data and only one parameter needed to be adjusted.

Poster 5-33

Using Amazon forest fungi and agricultural residues as a strategy to improve cellulase production efficiency

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The successful strategy to reduce cellulase enzymes costs includes both microorganism selection and improved fermentation process conditions. The Amazon biome is the world's largest reserve of biological diversity, presenting special soil and climate characteristics ideal for microorganism growth. Enzymes production using low cost and easily available agricultural residues as substrates can contribute to cost reduction. This work addresses both cellulase cost reduction approaches (microorganism selection and improvement of fermentation process) by describing the isolation, screening and selection of biomass-degrading fungi species from the Amazon forest and analyzing the enzymatic complex produced by a selected strain of *Aspergillus fumigatus* cultivated using different agro-industrial residues (wheat bran, sugar cane bagasse, soybean bran, and orange peel) as substrate in solid state fermentation (SSF). The highest levels of endoglucanase (CMCase) corresponded to 160 IU/g and it was obtained using soybean bran as the carbon source, after 96h of cultivation at 35°C. This enzymatic extract was used to run a zymogram analysis that showed 3 bands of endoglucanase activity. The CMCase activity was higher at 65°C and pH 3-3.5, indicating that this microorganism produces a thermophilic and acid endoglucanase. The fungal isolates from the Amazon forest were highly efficient producers of cellulases and xylanases as well.

Poster 5-34

Effect of cellulolytic lignins on enzyme adsorption and hydrolysis in filter paper test systems

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In an environmentally benign process biomass can be used as source of fuel ethanol. This is produced by enzymatic hydrolysis of the cellulose and hemicelluloses to monomeric sugars, which are then fermented to ethanol. The biomass is usually pretreated under high temperature and pressure in aqueous solution to improve enzyme accessibility and enhance the hydrolysis. The residual hydrolysis resistance is believed to be driven by the adsorption of enzymes to lignin and/or the formation of a physical barrier by lignin in respect to accessibility to cellulose.

In the present work the influence of the adsorption of enzymes to lignin is examined in model systems containing filter paper. Lignin from pretreated materials was isolated by extensive enzymatic hydrolysis, which removes practically all carbohydrates producing a cellolytic lignin (CL). Four CL samples were produced from wheat straw and corn stover, where two different pretreatment processes were applied. CL samples do adsorb enzymes but the impact of this on initial hydrolysis yield of filter paper test systems is small. Presently little attention is given to the state and chemical composition of CL which is discussed in relation to enzyme adsorption and overall hydrolysis yield.