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Liquefaction of sugarcane bagasse for enzyme productionF.M. Cunha¹, A.C. Badino², C.S. Farinas*³, T. Kreke¹, E. Ximenes¹ and M. Ladisch¹

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Bioprocessing of sugarcane bagasse to produce second generation (Gen 2) ethanol is carried out through a sequence of steps: pretreatment, hydrolysis, and fermentation. The localized nature of Brazilian ethanol facilities may benefit from on-site production of cellulase enzymes. This has motivated research on cellulase production using a combination of solid state cultivation and submerged fermentation through Embrapa and UFSCar, at solids loadings of up to 30% w/volume, which may require significant power input in order to achieve adequate mixing during the aerated fermentations. This work addresses enzyme induced liquefaction of sugarcane bagasse in a fed batch reactor based on addition of a cellulolytic strain of *Aspergillus*, discovered in the Brazilian biome, that secretes cellulase, hemicellulases, and β -glucosidase when grown on sugarcane bagasse. In this work *Aspergillus niger* A12 was initially incubated in solid-state cultivation 30% (w/w) for 72h at 32°C. Suspensions of this material were mixed under fed-batch conditions with commercial available endoglucanase (301 IU per gram of dry solids) at 32°C and 50°C, pH 4.8, 290 rpm for 24 h and 48 h. The solids fed-batch intervals were 0, 1, 2, 3, 6, 9 and 12h. The material was liquefied after 48 hours, and the viscosity was slightly lower at 32°C than at 50°C (0.30 and 0.48 Pa.s, respectively, at 100 s⁻¹ shear rate). Effects of liquefaction on enzyme production by the *Aspergillus* sp is discussed, and impacts on enzyme titers compared to solid state fermentations, presented.

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Production of high activity beta-glucosidase strains by screening of strong promoters

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The efficient degradation of natural lignocellulose or microcrystalline cellulose needs balanced cellulase system. Many cellulase systems secreted by filamentous fungi are not optimized for cellulose hydrolysis due to lack of one or more enzyme components. Generally, β -glucosidase is a rate limiting enzyme in cellulolytic enzyme systems secreted by most filamentous fungi. Here, we report a new screening strain *Penicillium documben* peni-1, who can secrete high β -glucosidase (6 U/mL) on high carbon source culture, in addition, more high yield strains (10-15 U/mL) of β -glucosidase are achieved by its overexpression with three strong constitutive promoters (rodA, ubiA PGML) that were screened from *Penicillium oxalicum* 114-2. The results are further partially confirmed on the strain 114-2D15A, a strain that was obtained by deletion of the major amylase amy15A and secreted few extracellular proteins on starch. Our results demonstrated that 114-2D15A is a new homologous expression host for the characterization research of CAZy family proteins of filamentous fungi on starch with those optional strong promoters. Furthermore, it also gives a more convenient way to study the better composition of cellulase enzyme-corresponding different substrates on cellulose.

Keywords: β -glucosidase; Cellulase; Strong promoter; Homologous expression; *Penicillium oxalicum*.

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Breeding of elephantgrass (*Pennisetum purpureum*) for improved biomass yield and biosafety

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Elephantgrass (*Pennisetum purpureum*) is one of the most productive perennial grasses and considered a prime candidate feedstock for lignocellulosic biofuel production in the southern US. However, elephantgrass has some potential for invasiveness due to its production of a vast amount of wind dispersed seeds. New elephantgrass plantings are established from vegetative plant parts. Therefore, unlike most seeded crops, seed production is not necessary for elephantgrass biomass production and its suppression will significantly reduce its potential for invasiveness. Interspecific hybridizations between elephantgrass ($2n=4x=28$) and pearl millet ($2n=2x=14$) results in genotypes that display male and/or female sterility due to their triploid ($2n=3x=21$) nature. Variability in flowering time exists in elephantgrass and selection of late flowering accessions may also suppress the production of seeds since temperature requirements are not met. Genetically distant accessions including high-yielding, late-flowering, non-lodging phenotypes were selected as parents in order to maximize heterosis for biomass yield and enhance biosafety. We will present data describing the biomass yield, yield components, flowering time and seed set of selected interspecific and intraspecific hybrids evaluated in replicated field trials.

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Effect of lignins from different lignocellulosic materials on adsorption of enzyme components

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Non-productive cellulase adsorption onto lignin has been always deemed as having a negative impact during enzymatic hydrolysis of lignocellulosic feedstocks. Therefore, understanding of enzyme-lignin interactions is essential for the development of enzyme mixtures and genetically modified of plant and enzyme. Mill Wood Lignin (WML) from six different lignocellulosic biomass (aspen, pine, corn stover, kenaf and two *Arabidopsis* lines: wild-type and SALK mutant of fah1) were prepared to investigate the effect of the lignin characteristics on adsorption of enzyme components. It was found that lignin sources affected enzyme adsorption by structural features like functional groups and lignin composition. Guaiacyl (G) lignin had higher adsorption capacity on enzyme than syringyl (S) lignin. The low S/G ratio and high uniform lignin fragment size had good correlation with the high adsorption capacity. The higher content of phenolic hydroxyl groups, lower content of carboxylic acid groups resulted in the stronger adsorption affinity for corn stover lignin than kenaf lignin and aspen lignin. The lower aliphatic hydroxyl, by reducing hydrophobic interactions, could explain for the higher adsorption capacity of pine lignin than corn stover lignin. The behavior of mono-component enzyme resulted in adsorption was also studied and found that cellobiohydrolase (CBH) and xylanase were most adsorbed by all lignins, endoglucanase (EG) showed less inhibition, and β -glucosidase (BG) was least affected by lignins, indicating the important role of carbohydrate binding module (CBM) in protein adsorption.