ANAIS

I WORKSHOP DO PROJETO TEMÁTICO FAPESP

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BIOPROCESS SYSTEMS ENGINEERING (BSE) APPLIED TO THE PRODUCTION OF BIOETHANOL FROM SUGARCANE BAGASSE

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Departamento de Engenharia Química

Universidade Federal de São Carlos

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REALIZAÇÃO

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APRESENTAÇÃO

Este "I Workshop do Projeto Temático" tem como principal objetivo a apresentação de propostas e de resultados obtidos durante o primeiro ano de desenvolvimento do Projeto Temático: **"Bioprocess Systems Engineering (BSE) Applied to the Production of Bioethanol from Sugarcane Bagasse"**, financiado pela Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Processo 2008/56246-0), no bojo do programa FAPESP/PRONEX/BIOEN, com vigência de junho de 2009 a julho de 2013. O projeto, proposto conjuntamente pelo Departamento de Engenharia Química da UFSCar e pelo grupo de Bioprocessos da Embrapa Instrumentação Agropecuária, incorpora atualmente colaborações com outros laboratórios e instituições como Instituto de Catálisis y Petroleoquimica (Consejo Superior de Investigaciones Científicas, Espanha), Institute of Resource and Energy Technology (Technishe Universität München, Alemanha), Programa de Engenharia Química da COPPE/UFRJ e do Grupo de Intensificação, Modelagem, Simulação, Controle e Otimização de Processos da UFRGS. O projeto é coordenado pelo Prof. Dr. Roberto de Campos Giordano.

O tema do projeto foi subdividido em **cinco subprojetos interligados**, que buscam promover o conhecimento aprofundado do tema e o desenvolvimento de tecnologia para a produção de bioetanol a partir de bagaço da cana-de-açúcar:

a) Desenvolvimento, implementação e validação de um ambiente computacional integrado amigável, permitindo simulação, otimização, avaliação econômica, análise de CO_2, análise de dados cinéticos e automação de biorreator para processos de produção de etanol lignocelulósico.

b) Cultivos de microrganismos a partir do banco da Embrapa (*Aspergillus sp.*), para a produção de celulases e xilanases usando reatores trifásicos não convencionais, incluindo bagaço pré-tratado no meio.

c) Pré-tratamento físico-químico do bagaço: explosão a vapor, remoção da hemicelulose e deslignificação. Produção de substratos para rotas de produção de bioetanol via fermentação de hexoses.

d) Determinação das condições (sub-)ótimas para a produção de etanol a partir da celulose.

e) Avaliação da produção de etanol a partir da hemicelulose usando enzimas livres e imobilizadas.

CELLULASE PRODUCING MICRORGANISMS: SELECTION OF STRAINS OF *Trichoderma spp*

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The search for microorganism that produces cellulase enzymes is of the great importance in contributing to the viability of biological route for the production of cellulosic ethanol. Among filamentous fungi, the genus *Trichoderma* is characterized as a high enzyme producer. Given the growing demand for the development of processes that reduce the cost of cellulases, which is now one of the technological bottlenecks for the production of cellulosic ethanol, the aim of the present work is to evaluate and select strains of filamentous fungi *Trichoderma spp*, available at Embrapa banks, capable of producing high concentrations of cellulases through the process of solid-state fermentation (SSF).

The methodology developed in the project was divided in four steps until the final selection of the best strains of *Trichoderma spp*. The first step of evaluation consisted in the observation of the growth of 78 pre-selected strains of *Trichoderma* in a medium having a commercial microcrystalline cellulose, Avicel, as the sole carbon source. Only strains able to metabolize the avicel substrate were selected for the next step. The second step of selection, it was used the Congo red test, that consisted in the observation for zone of clearance around the colony and the measurement of enzymatic index (halo colony. hydrolysis halo^{-1}) of each strain. The fermentation test in tubes was the third step on the selection of strains. At this step we evaluated the strains with greater potential to produce cellulases in test tubes, the ones that presented better results passed to the fourth step, solid-state fermentation (SSF) in lignocellulosic substrates. The SSF in column type bioreactor will serve to select the best conditions for fungus growth.

In the first step of selection, 49 strains showed the ability to metabolize the substrate crystalline cellulose, Avicel, producing cellulase enzyme complex (Figure 1). The Congo red test was applied to 49 strains selected in the first step (Figure 2). Only strain *Trichoderma sp* CG 87 showed no halo of hydrolysis. The best enzymatic indices obtained can be seen in Table 1.

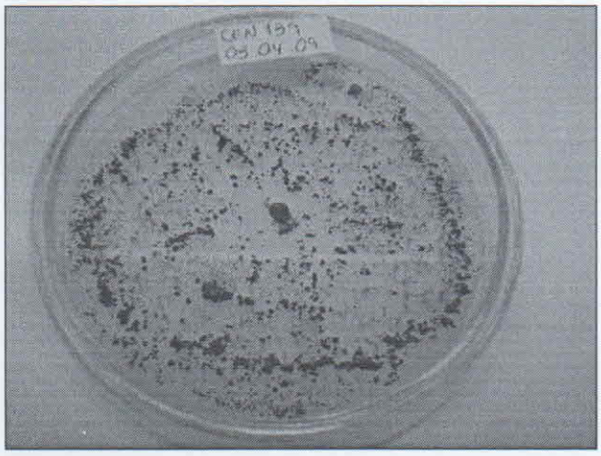

Figure 1. *Trichoderma sp* CEN 139, measure strain capable of metabolizing the cellulosic substrate, avicel.

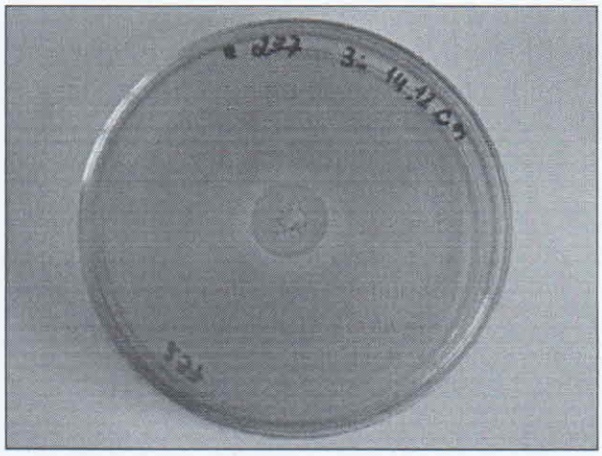

Figure 2. Observation for zone of clearance around the colony using the dye Congo red. Strain *Trichoderma harzianum* CEN 237.

Preliminary experiments were performed in order to evaluate the methodology of fermentation in test tubes. The results for CMCase activity are shown in Figure 3. The best results on the third selection step was reached in three weeks of fermentation, with CMCase activity up to 25.47 $U.g^{-1}$ for fungus *Trichoderma harzianum* CEN 155. Studies by Castro (2006) showed best results with the fungus *Aspergillus niger* ATCC 16404, gave values equal to 14 $U.g^{-1}$ for the CMCase enzyme. The maximum value attained by a strain of the genus *Trichoderma* by this same author was 0.6 $U.g^{-1}$ by the fungus *T. harzianum* IOC 4042.

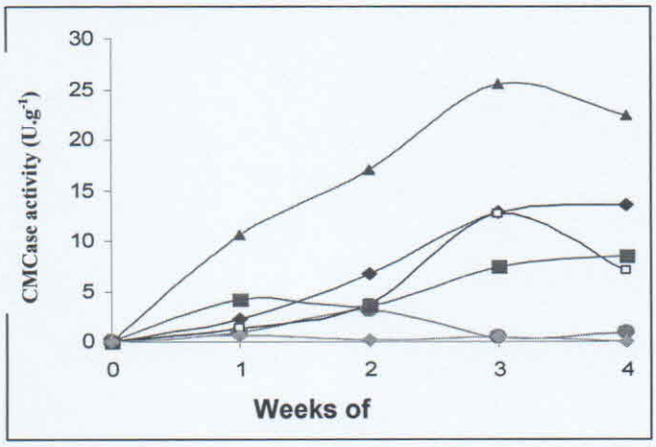

Figure 3. Production the CMCase in a test tube. (-▲-) *Trichoderma harzianum* CEN 139; (-♦-) *T. koningii* CEN 142; (-□-) *T. harzianum* CEN 155; (-■-) *T. harzianum* CEN 202; (-✹-) *T. atroviride* CEN 223; (-✦-) *T. sp* LCB 46.

Table 1. Strain with better index enzimatic and its Standard deviation.

Fungi	Enzimatic Index	Standard deviation
139	1,84	0,215
142	1,84	0,233
145	1,45	0,055
151	1,46	0,103
155	1,61	0,118
156	1,64	0,078
159	1,56	0,200
201	1,51	0,183
238	1,57	0,137
241	1,72	0,131
248	1,51	0,071
104NH	1,52	0,197

In order to evaluate cellulase production under SSF, a kinetic experiment was carried out aimed to obtain the best time of the fungi growth, so it can achieve the highest enzyme productivity. As a result, the higher CMCase activity level of 5.74 Ug^{-1} was achieved on the eighth day. Based on these results, we used the statistical methodology of factorial experimental design to select the significant variables in the process of SSF. The variables studied were: volume of inoculum, substrate moisture content and wheat bran to sugarcane bagasse ratio. After statistical analysis, the only variable significant at 90% confidence level was the wheat bran to sugarcane bagasse ratio. This variable and the moisture content were then selected for a central composite design to optimize the fermentation parameters: The CMCAse activity reached 11.16 Ug^{-1}, but is believed to still be able to reach even higher values for the production of cellulases. More experiments will be conducted for the optimization of moisture variable in SSF, and its evaluation in a column bioreactor in order to improve even more the potential of the best cellulases producers strains.

CASTRO, A.M. **Produção e Propriedades de Celulases de Fungos Filamentosos, Obtidas a Partir de Celulignina de Bagaço de Cana-de-Açúcar (*Saccharum* spp.).** 2006. 212f. Tese de Mestrado. Programa de Pós-Graduação em Tecnologia de Processos Químicos e Bioquímicos, Universidade do Rio de Janeiro, Rio de Janeiro, 2006.

MEKALA, N.K. et al. Cellulase Production Under Solid-State Fermentation by Trichoderma reesei RUT C30: Statistical Optimization of Process Parameters. **Applied Biochemistry Biotechnology**. v.151, p.122-131, 2008.

SÁNCHEZ, C. Lignocellulosic residues: Biodegradation and bioconversion by fungi. **Biotechnology Advances**, v. 27, p. 185-194, 2009.

TEN, L.N., IMA, W-T., KANGA, M-K.M.S., LEEA, S-T. Development of a plate technique for screening polysaccharide-degrading microorganisms by using of insoluble chromogenic substrates. **Journal Microbiol. Methods**. v.56, p.375-382, 2004.