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

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REALIZAÇÃO

Departamento de Engenharia Química – UFSCar

Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA

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FAPESP

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.

CHARACTERIZATION AND PURIFICATION OF β-GLUCOSIDASE FROM *Aspergillus Niger*

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Bioethanol from vegetal biomass is an important route that is hugely studied worldwide. Among sources of cellulosic biomass that can be used to produce energy, especially biofuels, sugar cane bagasse shows a huge potential. The enzymatic hydrolysis of cellulose, although advantageous to environmental, requires intensive research, principally focusing the lowering of the enzyme costs.

Based on this technological demand for the development of agro-industrial processes with strong emphasis on environmental sustainability, the EMBRAPA Agricultural Instrumentation Research Center is focused on a project for the production of bioethanol using sugar cane bagasse and cellulase enzymes in partnership with the Foundation for Research Support of São Paulo (FAPESP) and other foundations in the project BIOEN.

The project goal is to characterize the cellulase enzymes and study its role in vegetal biomass. The plan stated here intends to work in what has already been done in this line of research and its main purpose is the characterization of the cellulase enzymes in a crude and purified state regarding the influence of pH and temperature on the activity and on the thermal stability.

The second part of the project emphasizes the analysis of fermented samples of sugarcane bagasse, by NIR spectrometer from EMBRAPA Agricultural Instrumentation. The spectra will be treated to obtain the positions of spectral bands correspondent to the energy states of cellulose, lignin and other materials involved in the samples. The biomass that will be characterized in this work is sugar cane bagasse from local distilleries.

The influence of the temperature and pH on the activity of crude and purified β-glucosidase from *Aspergillus niger* was evaluated in a range from 22.7 C to 87.2°C and pH values between 2.38 and 6.62. The effect of the temperature and of the pH was undertaken with a full factorial design (22 trials plus 3 central points) involving two independent variables (T and pH). The response variables were enzyme activity, total protein concentration and specific activity. Table 1 shows the factorial design carried out for the β-glucosidase enzyme in a crude state.

The activity of β-glucosidase enzyme was measured accompanying the released glucose in the cellobiose hydrolysis. The glucose concentration was measured by a colorimetric method using a glucose oxidase-peroxidase system. The increase in absorbance was measured at 530 nm.

Figure 1 shows the influence of pH and temperature on crude β-glucosidase activities. It is possible to observe the maximum enzyme activity around the central point of the factorial design. The optimum values for pH and temperature were 4.82 and 52.67 °C, respectively.

Thermal stability of β-glucosidase in absence of substrate at temperatures of 37 and 50°C were evaluated by measuring the residual enzyme activity during 96h in a spans of 24h. Enzyme half-lives were calculated according to Tardioli et al. (2003). The half life of β-glucosidase was 341.5h at 37°C and 148.1h at 50°C.

The purification process will be performed in two steps: (1) ion-exchange adsorption on aminated resin (MANAE-agarose) and (2) bioaffinity adsorption on cellobiose- or glucose-agarose.

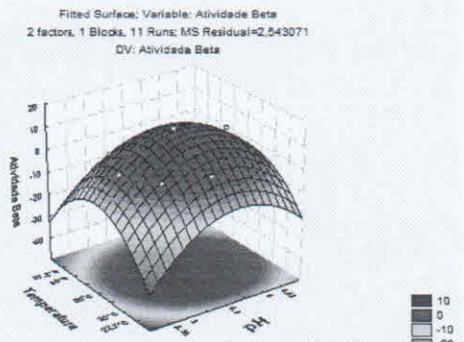
The first step of the purification was performed via adsorption/desorption of the crude enzymatic extract on agarose poorly activated with amino groups (agarose activated with 3 □moles of amino groups per gram of gel). The chromatographic matrix was prepared according Fernandez- Lafuente et al. [1993]. This step aims to eliminate small contaminants proteins from the crude enzymatic extract. Preliminary results showed that 75% of □-glucosidase activity was immobilized on MANAE-agarose while only 44% of protein was immobilized. The results showed that β-glucosidase immobilized on poorly activated amino agarose gel was twice more pure than the crude enzymatic extract.

Bioaffinity chromatography will be performed according to Tardioli et al, 2000, and the purification process will be monitored by protein and activity assays and by chromatographic analysis.

It expected that this work allow evaluating the performance of the crude and purified β-glucosidase on the vegetal biomass (sugar cane bagasse) in order to contribute to the overall process of ethanol production from lignocellulosic residues.

Table 1 - Factorial Design: influence of pH and Temperature on the activity of β-glucosidase

Trial	T (°C)	pH	β-glucosidase (IU/mL)
1	80(1)	6(1)	1.38
2	80(1)	3(-1)	0.23
3	30(-1)	6(1)	4.54
4	30(-1)	3(-1)	0.60
5	87(1,41)	4,5(0)	0.03
6	23(-1,41)	4,5(0)	8.40
7	55(0)	6,6(1,41)	2.71
8	55(0)	2,4(-1,41)	0.37
9	55(0)	4,5(0)	14.00
10	55(0)	4,5(0)	14.03
11	55(0)	4,5(0)	15.95



$$Y = 14.66 - 6.86T + 1.92(pH) - 5.52(pH)^2$$

Figure 1 - Response surface from the influence of pH and temperature on enzyme activity in the raw state

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