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

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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.

PRODUCTION OF CELULLASES IN SOLID CONTAINING MEDIUM: INDIRECT METHOD FOR QUANTIFICATION OF *Aspergillus niger* CELLULAR BIOMASS

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The production of ethanol from lignocellulosic biomass, known as second generation ethanol, has been calling a lot of attention recently. The enzymatic route for the conversion of biomass into biofuels is an alternative of lower environmental impact, but still requires the development of technologies to reduce the cost of the enzymes, one of the major bottlenecks on the production of cellulosic ethanol. The existing technologies for enzyme production use fermentation process that can be conducted both in liquid medium, called submerged fermentation (SmF), or in a solid medium, the solid-state fermentation (SSF). Each process has well known advantages and disadvantages. Among the bioreactors available for SmF, there are the mechanical agitated and the pneumatic reactors, including the bubble-column and the air-lift bioreactors, the last one with growing applications in cultivations of filamentous fungi. In this context, the work in which this study is included aims the production of celullases in a combined fermentation process, that aggregates the advantages of SSF and SmF in one equipment, a pneumatic bioreactor. However, for starting up a fungae cultivation as the proposed combined process, it is necessary to standardize the inoculum prepraration step, in order to allow the transition from SSF to SmF. The fungi cultivation begins as SSF and continues as SmF with the addition of liquid medium. Due to the difficulties in measuring cellular biomass in the presence of solids, is necessary to develop a methodology for indirect quantification of this biomass. Thereby, the present work has as objective to evaluate the indirect quantification of cellular biomass during cultivation of *Aspergillus niger* aiming the production of the inoculum for pneumatic bioreactors cultures.

In order to estimate cellular biomass we used a methodology that consists on measuring the consumption of substrate, in this case glucose. The cultivation was initiated as SSF in sugar cane bagasse. This phase was visually monitored by images in order to evaluate the right moment to add the glucose containing liquid medium. The parameters evaluated were the volume of submersion, the agitation rate and the incubation time as SSF. To follow the cell growth during SSF, samples were collected at 24 h intervals during 72 h and the glucose consumed was quantified by an enzymatic method (LaborLab,Brazil) The results showed that by using a sample of 5g of solid substrate incubated for 24 h as SSF at 32 °C, and then continuing the cultivation as SmF during 48 h in the presence of 200 mL of medium (Mandels, 1976) under 200 rpm and at the same temperature, it was possible to obtain an appropriated suspension to be used as inoculum. In addition, the incubation as SSF for 24 h allowed fungi growth predominantly as mycelial morphology.

Mathematical models that describe the fungi growth on the combined fermentation were further identified and validated. Experiments in liquid medium in the absence of bagasse were conducted for the determination of the growth kinetic parameters by measuring both variations on glucose and biomass concentrations during the cultivation. Mandel medium supplemented with 30 g/L of glucose was used in these experiments. The cell and glucose concentrations were measured by dry weight method and enzymatic method, respectively. The values of the biomass yield on glucose, $Y_{x/s}$, as well as the maximum growth rate, $\mu_{máx}$, were calculated by linear regression of data belonging to the exponential growth phase. The software Análise de Bioreatores Anabio 1.2 (Silva et al., 2003) was used to simulate a simple unstructured model, consisting on mass balance equations, with growth kinetics described by Contois model. The simulations showed that the growth follows the Contois model, with $\mu_{máx}$ of 0,034h^{-1}, $Y_{x/s}$ of 0,297 g/g and death constant of 0,005h^{-1}. The fit of the simulated results to the experimental data with the parameters determined by Anabio is showed on Figure 1.

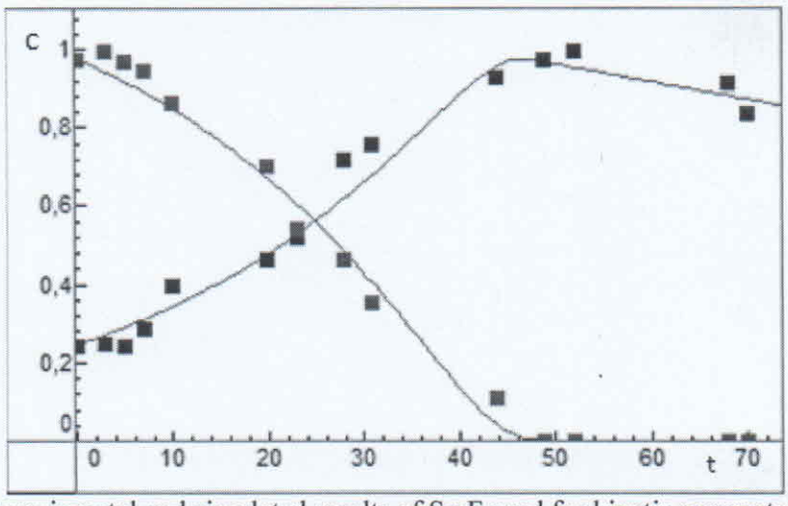

Figure 1– Experimental and simulated results of SmF used for kinetic parameter estimation.

After estimation of the model parameters, they were used to simulate the profile of glucose consumption and generate the simulated profile of cellular growth in the medium containing solids. Glucose concentration data were introduced to Anabio software and the glucose simulated curves were visually adjusted to the experimental data by attributing values to the initial cellular concentration (C_{xo}). Figure 2 shows the simulated cell concentration profile for the experiment carried out with mycelia from 24 h of SSF incubation. The developed methodology for indirect quantification of biomass showed to be reliable, because the proposed model fitted very well to experimental data and just one parameter (C_{X0}) was adjusted.

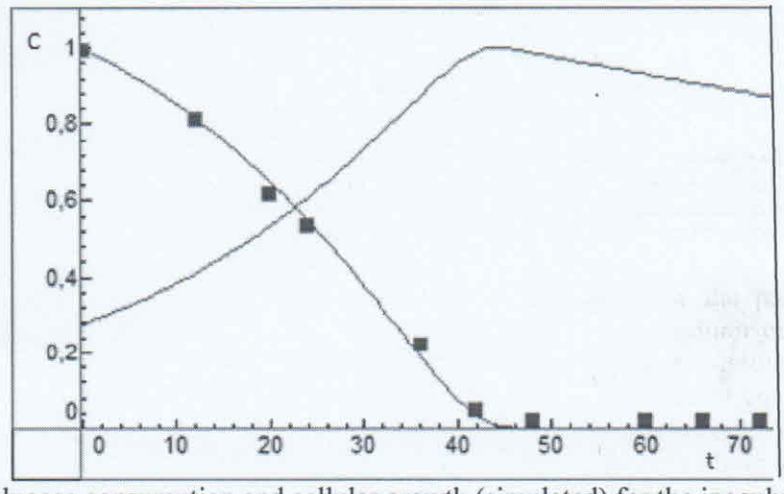

Figure 2 – Glucose consumption and cellular growth (simulated) for the inoculum preparation with 5g of bagasse and 200 mL of Mandels medium added after 24 h of SSF incubation. C_{xo}=3,5 g/L

Concerning the standardization of the inoculum preparation for starting up pneumatic bioreactor SmF and SSF mixed cultures, the results showed that the best procedure to obtain higher cell concentrations in a shorter time is by incubating as SSF during 24 h, followed by the addition of 200 ml of Mandels medium and stirring at 200 rpm for 48 h. This condition of inoculum production also showed additional features, such as the predominant presence of mycelial morphology and the fluidity of the resulting suspension.

References:

MANDELS, M.; STERNBERG, D. Recent advances in cellulases technology. J. **Ferment Technol.**, v. 54, p. 267-286, 1976.

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