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Production of bio-ethanol from soybean molasses by *Saccharomyces cerevisiae* at laboratory, pilot and industrial scales

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ABSTRACT

The aim of this work was to develop an economical bioprocess to produce the bio-ethanol from soybean molasses at laboratory, pilot and industrial scales. A strain of *Saccharomyces cerevisiae* (LPB-SC) was selected and fermentation conditions were defined at the laboratory scale, which included the medium with soluble solids concentration of 30% (w/v), without pH adjustment or supplementation with the mineral sources. The kinetic parameters – ethanol productivity of 8.08 g/L h, $Y_{P/S}$ 45.4%, $Y_{X/S}$ 0.815%, m 0.27 h^{-1} and μ_X 0.0189 h^{-1} – were determined in a bench scale bioreactor. Ethanol production yields after the scale-up were satisfactory, with small decreases from 169.8 L at the laboratory scale to 163.6 and 162.7 L of absolute ethanol per ton of dry molasses, obtained at pilot and industrial scales, respectively.
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1. Introduction

Natural energy resources such as petroleum and coal have been consumed at high rates over the last decades. The heavy reliance of the modern economy on these fuels is bound to end, due to their environmental impact (and the corresponding pressure of society) and to the fact that they might eventually run out. Therefore, alternative resources as ethanol are becoming more important. Bio-ethanol is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilization of the fossil fuels (Cardona and Sánchez, 2007). Some biological processes have rendered possible routes for producing ethanol in large volumes using the cheap substrates (Gunasekaran and Raj, 1999).

The worldwide production of bio-ethanol (all grades) reached around 51 billion liters in 2006, of which 17 billion were produced in Brazil from sugarcane. The United States produced around 18 billion liters from maize. Studies on bio-ethanol production from the cellulosic materials are being financed by the US Department of Agriculture (RFA, 2008).

Ethanol can be used directly as a fuel, but most often it is blended with gasoline to yield gasohol (Staniszewski et al., 2007). The Brazilian National Bio-Fuel Program, initiated in 1975, stimulated the substitution of gasoline for sugarcane alcohol for automobile use, and intensified the use of a mixture of ethanol and gasoline as fuel for common cars (Soccol et al., 2005). Anhydrous ethanol is added to gasoline at a 20–26% proportion in volume (Cortez et al., 2003). Today, about 3 million automobiles run on 100% alcohol, and about 60% of all new motor vehicles produced in Brazil are "flex", i.e. they can run on any mixture of alcohol/gasoline, as well as on 100% alcohol (Grad, 2006).

A worldwide interest in the utilization of bio-ethanol as energy source has stimulated studies on the cost and efficiency of industrial processes for ethanol production. Intense research has been carried out for obtaining efficient fermentative organisms, low-cost fermentation substrates, and optimal environmental conditions for fermentation to occur (Cysewski and Wilke, 1978). Even though the fermentative process for ethanol production is well known, the production costs are still the key impediment for the wide use of ethanol as fuel. Therefore, the development of fermentation processes using economical carbon sources is important for the ethanol production in a commercial scale (Cazetta et al., 2007).

The Brazilian production of soybean is estimated in 56.6 million tons (2006/2007), which represent around 30% of the global production (IBGE, 2007). Soybean molasses is a co-product generated in the production of protein-concentrate soybean meal. The protein-concentrate is a soy bran with around 70% protein (in dry basis), obtained by the extraction of sugars from de-oiled soybean meal using a mixture of water/ethanol as solvent (Fig. 1), its major

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Nomenclature

°Brix	percentage (w/v) of soluble solids	r_S	sugar consumption rate, dS/dt (g/L h)
S_0	initial sugar concentration (g/L)	r_X	biomass formation rate, dX/dt (g/L h)
S_f	final sugar concentration (g/L)	r_P	product formation rate, dP/dt (g/L h)
X_0	initial biomass concentration (g/L)	μ_S	specific sugar consumption rate, $r_S \cdot X^{-1}$ (h^{-1})
X_f	final biomass concentration (g/L)	μ_X	specific biomass production rate, $r_X \cdot X^{-1}$ (h^{-1})
P_0	initial ethanol concentration (g/L)	μ_P	specific ethanol production rate, $r_P \cdot X^{-1}$ (h^{-1})
P_f	final ethanol concentration (g/L)	$Y_{X/S}$	biomass yield from sugar, r_X/r_S (g/g)
m	maintenance coefficient (h^{-1})	$Y_{P/S}$	ethanol yield from sugar, r_P/r_S (g/g)

application field being the animal feed industry. An important Brazilian soybean-processing company produces 600 tons per day of protein-concentrate, generating 220 tons per day of molasses (Siqueira, 2006).

Yeasts are the most commonly used microorganisms for ethanol fermentation. Anaerobic cultivation of *Saccharomyces cerevisiae* generates, besides ethanol, carbon dioxide, glycerol and cell biomass as the most significant byproducts. Carbon dioxide is an inevitable fermentation product, but the off-gas can be sold as a high-quality raw material and is, therefore, more of a logistic problem. Glycerol can be produced as a compatible solute during osmotic stress (Brandberg et al., 2007).

The main objective of this work was to develop an economical bioprocess to produce bio-ethanol from soybean molasses by a selected strain of the yeast *S. cerevisiae*, through experiments at laboratory, pilot and industrial scales. Fermentative assays evaluated the effects of initial soluble solids concentration, supplementation with mineral sources, pH, operational mode and addition of antifoam and dispersant agents.

2. Methods

2.1. Characterization of the soybean molasses

The soybean molasses was received from a soybean-processing company in the concentrated form (75–80% soluble solids); this material is stable, and was stored at room temperature, being diluted with distilled water to concentrations of 15–30% soluble solids prior to fermentation tests. The percentage (w/v) of soluble solids (°Brix) was determined with a portable refractometer for sugar – Instrutherm, model RT-30 ATC (Singh et al., 1996).

The carbohydrate composition of soybean molasses was determined by HPLC (High Performance Liquid Chromatography; see Section 2.2). Protein concentration was determined by the Kjeldahl method. Lipids concentration was determined by gravimetric analysis after solvent extraction with hexane (Soxhlet method). Ashes were quantified by gravimetric analysis after burning samples at 550 °C for 5 h. Fibers concentration was calculated by difference. Moisture content was determined by gravimetric analysis after drying at 105 °C to constant weight.

2.2. Determination of biomass, sugars and ethanol concentrations

Biomass concentration in cells/mL was quantified with a Neubauer counting chamber; biomass concentration in g/L was determined by gravimetric analysis after drying to constant weight. The viability of yeast cells was determined by methylene-blue staining (Alfenore et al., 2002).

Individual sugars and ethanol were quantified by HPLC (Varian Liquid Chromatography: solvent delivery module 240; column valve module 500; RI Detector 350; Workstation software 5.0) using a Shodex KS-801 column, that separates sugars by molecular size, at

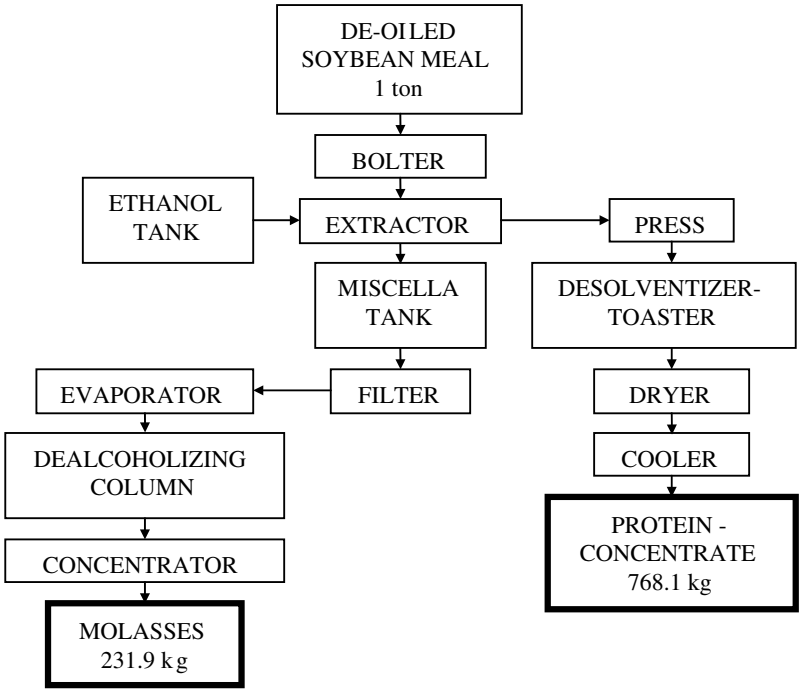

Fig. 1. Production process of the soybean protein-concentrate.

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