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Ethanol production from soybean molasses by *Zymomonas mobilis*

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ABSTRACT

This work deals with the utilization of soybean molasses (a low cost byproduct) to produce ethanol, an important biofuel, using the microorganism Zymomonas mobilis NRRL 806, a gram negative bacterium. At the first part of the work, laboratorial scale tests, using 125 mL flasks were performed to evaluate the effect of three variables on ethanol production: soybean molasses concentration (the sole carbon and nitrogen source), pH and period of previous aerobial phase. The optimal soybean concentration was around 200 g L^{-1} of soluble solids, pH between 6.0 and 7.0, and the period of previous aerobial phase did not provide significant effect. At the second part, kinetic tests were performed to compare the fermentation yields of Zymomonas mobilis NRRL 806 in flasks and in a bench scale batch reactor (it was obtained respectively 78.3% and 96.0% of the maximum theoretical yields, with productions of 24.2 and 29.3 g L^{-1} of ethanol). The process with a reactor fermentation using Saccharomyces cerevisiae LPB1 was also tested (it was reached 89.3% of the theoretical maximum value). A detailed kinetic behavior of the molasses sugars metabolism for Z. mobilis was also shown, either in reactor or in flasks. This work is a valuable tool for further works in the subject of ethanol production from agro-industrial by-products.

1. Introduction

The imminent ending of the non-renewable natural energetic resources brings the necessity of researches to obtain new renewable natural energetic resources, as well as the maximization of productivity and lowering the costs of already known processes [1]. Biomass-based energy sources constitute an important alternative to help solving this problem [2].

When organic substrates are fermented, such as agro-industrial residues like sugarcane molasses [3], wheat stillage [4], fish residues [5], pineapple waste [6], among others, carbon sources can be converted into ethanol, an important and well known biofuel [7]. This strongly helps solving an environmental problem related to residues treatment and disposal and also helps to aggregate value to an agro-industrial residue as long as to decrease the costs with the substrates for the fermentation.

Usually, yeasts like Saccharomyces cerevisiae carry out alcoholic fermentations [8]. However, some works have shown that the bacterium Zymomonas mobilis provides many advantages over the yeast, such as: higher theoretical yields, higher ethanol tolerance and ability to grow under anaerobic conditions [3,4–9]. It is able to produce ethanol via an anaerobic version of the Entner-Doudoroff pathway [10,11].

In the present work, soybean molasses, a by-product of soybean processing that results from the extraction of sugars from de-oiled soybean meal, was used as the sole carbon and nitrogen source in order to carry out the alcoholic

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fermentation. The molasses was supplied by a soybean processing company (IMCOPA) located in the Southern Region of Brazil, in the concentrated form (600 g L^{-1} of soluble solids), and was stored at room temperature, during no more than 3 months. This material is rich in fermentable sugars (fructose, glucose and mainly sucrose, as far as stachyose and raffinose) as shown in Table 1. Besides, it has considerable amounts of proteins, fibers, ash and inorganic salts.

Soybean molasses is produced during soy protein concentrate production, as shown in Fig. 1:

The objective of this work was to evaluate the best levels of some important variables for fermentation (concentration of soybean molasses, pH and time of aerobial phase). Another goal is to show the profile of consumption of sugars during the fermentation as well as the profiles of ethanol production in a bench scale batch reactor. Finally, to make a comparison between the performance of Zymomonas mobilis and Saccharomyces cerevisiae in respect of ethanol production, productivity and yields.

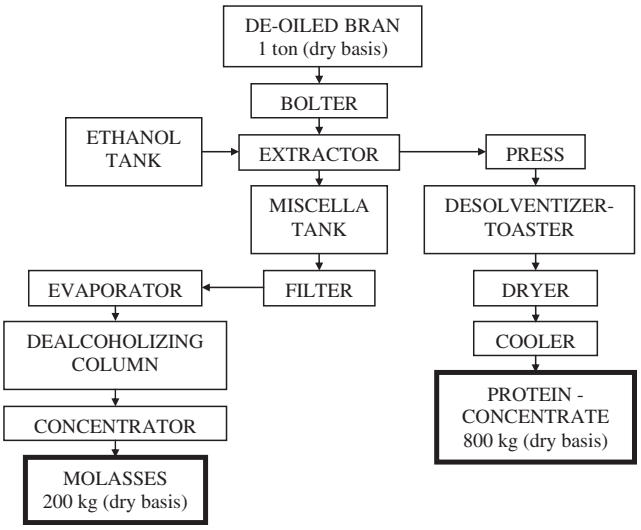

Fig. 1 – Soybean classical processing and molasses generation – adapted from [12].

2. Material and methods

2.1. Strains

The microorganism used at this study was Zymomonas mobilis NRRL 806. The cells were inoculated in ZM medium: sucrose (20 g L^{-1}), yeast extract, (10 g L^{-1}) and peptone, (10 g L^{-1}); the growth was performed at shaker (12.6 rad s^{-1}) for 21 h at 30 °C, and stored for no more than 3 weeks in refrigerator (4 °C).

The yeast used at the reactor tests was a strain of Saccharomyces cerevisiae from Biotechnological Processes Laboratory – UFPR, named S. cerevisiae LPB1.

2.2. Adaptation of the strain

Progressive adaptation of Zymomonas mobilis in soybean molasses was done through successive transferences from the seed culture (10% inoculation rate). Initially, it was used soybean molasses at 50 g L^{-1} of soluble solids (first transference), and thereafter (second and third transferences) soybean molasses at 100 g L^{-1} of soluble solids. The stock culture was stored for no longer than 3 weeks at 4 °C. These cultures were grown at the same conditions described previously.

Table 1 – Composition of soybean molasses. Average of three different samples – adapted from [12].

Component	% mass fraction of dry material
Total Carbohydrates	57.3
Glucose	0.243
Fructose	0.127
Galactose	0.254
Sucrose	21.3
Lactose	–
Other disaccharides	7.10
Raffinose	9.68
Stachyose	18.6
Proteins	9.44
Lipids	21.2
Fibers	5.7
Ash	6.36

2.3. Inoculum

The inoculum was prepared from the stock culture, using the same culture medium. The growth was carried out at 30 °C for 21 h in shaker (12.6 rad s^{-1}).

2.4. Preparation of the culture media

The concentrated soybean molasses received from local industries with 550–650 g L^{-1} of soluble solids was diluted with distilled water to produce the culture media with the desired sugar concentration, without any supplementation of other nitrogen or carbon sources, or salts. The total soluble solids concentration was determined with the aid of a saccharimeter. The pH was adjusted to the desired value with 1000 mol.m-3 HCl or 3000 mol.m-3 NaOH, depending on the case. The material was sterilized at 121 °C during 15 min.

2.5. Experimental design

Three variables were evaluated in this plan, each of them in two different levels: concentration of soybean molasses (the sole carbon and nitrogen source), pH of the media and time of previous aerobial phase. Some central points were also considered, as described in Table 2. The chosen levels were based in preliminary studies (not shown). An analysis of variance (ANOVA) with a randomized complete block design was performed.