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Production of ethanol from soybean hull hydrolysate by osmotolerant *Candida guilliermondii* NRRL Y-2075

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Abstract

In this research, we studied the use of soybean hull hydrolysate (SHH) as a substrate for ethanol and xylitol production using an osmotolerant strain of *Candida guilliermondii*. The best acid hydrolysis of soybean hull achieved a recovery of 85 and 62% of xylose and mannose, respectively. Among detoxification treatments, activated charcoal 10% (w/v) showed the best results. Kinetic parameters obtained from the cultivation on four-fold concentrated SHH have shown that the osmotic pressure of this medium is higher than that supported by most osmophilic yeasts, revealing the osmotolerant characteristic of *C. guilliermondii* NRRL Y-2075. When cultivations were carried out on two times concentrated SHH, we obtained high yields of ethanol production, showing the prospect of SHH as a candidate for this biofuel production. Although xylose was present in high concentrations, no xylitol was produced, probably due to the presence of furfural acting as external electron acceptor or some varying cofactor preference of xylose reductase in this yeast strain.

Keywords: *Candida guilliermondii*; Soybean hull; Hydrolysate; Ethanol production; Xylitol

1. Introduction

Lignocellulosic biomass is the most abundant and renewable organic compound in the biosphere; it comprises three major groups of polymers: cellulose, hemicellulose, and lignin (Lee, 1997). Biomass in the form of agro-industrial wastes is produced in large quantities generating environmental problems and loss of potentially valuable resources (Kuhad and Singh, 1993).

Soybean (*Glycine max*) is the most cultivated plant worldwide, with its production reaching 96.2 millions of hectares, resulting in 216.3 millions tons of grains per year (USDA, 2006). Soy hulls represent the major by-product of soybean processing industry and constitute about 8% of the whole seed (Gnanasambandan and Proctor, 1999). According to this data, approximately 17.30 millions tons of soybean hull are produced every year. The insoluble carbohydrate fraction of soy hull cell walls consists of 30% pectin, 50% hemicellulose and 20% cellulose (Snyder and Kwon, 1987).

Hemicellulose is the second most common polysaccharide in nature (Saha, 2003). Hemicelluloses are heterogeneous polymers composed of hexoses (glucose, mannose, and galactose), pentoses (xylose and arabinose) and uronic acids (Jeffries and Jin, 2000; Kuhad and Singh, 1993). The utilization of hemicellulose for bioconversion to value-added fuels and chemicals has attracted much attention, particularly, due to xylose-fermenting yeasts ability to produce polyols and ethanol from xylose (Jeffries, 2006). However, in order to use these materials in bioprocesses, either chemical or enzymatic hydrolysis of them must be performed, in order to render the sugars soluble and available to microorganisms. Diluted sulphuric acid has been the most used so far for converting hemicellulose into ferment-

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able sugars with high reaction rates, especially because it is a low cost technology (Jeffries and Jin, 2000). The liquid phase from the hydrolysis reaction, containing xylose and other hemicelullosic monosaccharides, besides other compounds derived from lignin, can then be utilized for bioconversion.

Xylitol is a five carbon sugar alcohol, which has attracted much attention due to its high sweetening power, anticarcinogenic properties, and possible uses in diabetics diets and products. These properties, together with its high negative heat of solution, make xylitol an attractive sucrose substitute in the food industry (Emodi, 1978; Pepper and Olinger, 1988). The chemical production of xylitol needs expensive refining treatments, granting, therefore, biotechnological research for its production, being yeasts the most studied producers of this polyol (Nigam and Singh, 1995).

Ethanol is a renewable energy source produced by cultivation of sugars and it is an attractive alternative for the partial replacement of fossil fuel (Jeffries, 2006; Sun and Cheng, 2002). Traditionally, fermented sucrose from sugarcane, in Brazil, and maize starch, in the USA, are presently the major sources of ethanol. However, the use of agro-industrial by-products, such as soybean hull, for the commercial production of ethanol is a promising technology, which depends upon a list of factors, among them the use of xylose-assimilating yeast strains; hydrolysate composition (monosaccharides and inhibitors); and the control of oxygen supply during cultivation (Kuhad and Singh, 1993; Sun and Cheng, 2002).

The aims of our work were to develop an acid hydrolysate of soybean hull to a maximum yield of xylose and minimum formation of inhibitory compounds and to treat this hydrolysate in order to reduce such inhibitory compounds with sodium hydroxide and activated charcoal. We also studied the cultivation capabilities of *Candida guilliermondii* NRRL Y-2075 in two and four fold concentrated SHH, detoxified or non-detoxified, in terms of substrate consumption, biomass and alcohols production.

2. Methods

2.1. Microorganism, maintenance and inoculum

Candida guilliermondii NRRL Y-2075 used in this study was maintained on YM agar slants containing (g L^{-1}): yeast extract, 3; malt extract, 3; peptone, 5; glucose, 10; agar, 20. The medium used for inoculum of synthetic media contained (g L^{-1}): yeast extract, 3; malt extract, 3; peptone, 5; xylose, 30; glucose, 10; whereas the medium used for inoculum of SHH contained (g L^{-1}): yeast extract, 3; malt extract, 3; peptone, 5; glucose, 30. Xylose and glucose were autoclaved separately at 120 °C, 15 min. Inocula were prepared by cultivation of the yeast in 1000 mL Erlenmeyer flasks containing 200 mL of the medium. Cells were cultivated at 140 rpm, 30 °C for 24 h and were subsequently recovered by centrifugation (1000*g*, 10 min). The cell pellet was washed and suspended directly into the cultivation broth.

2.2. Soybean hull hydrolysate preparation

Soybean hulls were supplied by The Solae Company (Esteio, Brazil). The hulls were milled to a particle size less than 1 mm in diameter. The tested conditions for the hydrolysis were temperature (100, 120, 125 °C); sulphuric acid solutions (0.2; 0.8; 1.4% (v/v)). The solid/liquid ratio was kept at 1/10 and the reaction time was 1 h. After the hydrolysis, the remaining solid material was separated by filtration and the filtrate was stored at 4 °C. The optimal hydrolysis condition, which was defined as yields of liberated xylose, was used for cultivation experiments. The liquid phase from the optimal hydrolysis was concentrated two-fold (SHH-2C) and four-fold (SHH-4C) under vacuum in an evaporator at 70 °C.

2.3. Detoxification of soybean hull hydrolysate

Detoxification assays were carried out over the SHH-4C to evaluate the effects of activated charcoal that minimizes the concentration of the main cultivation inhibitors. Charcoal was mixed with SHH-4C, previously adjusted to pH 2.5, in different concentrations (1; 2; 5; and 10% (w/v)) and was stirred in an orbital shaker at 140 rpm, 30 °C, for 1 h. The pH of treated SHH-4C was then adjusted to 5.5 with NaOH pellets.

2.4. Media and cultivation conditions

Experiments in synthetic medium were used to compare the cultivation kinetics with those over hydrolysates. The synthetic medium consisted of (g L^{-1}): yeast extract, 3; malt extract, 3; peptone, 5; xylose, 30. The pH of non-detoxified hydrolysates was adjusted to 5.5 with NaOH pellets. No supplementation was added to the hydrolysates media SHH-2C, whereas for some experiments with SHH-4C we supplemented this medium with yeast extract (3 g L^{-1}) or yeast extract (3 g L^{-1}) plus peptone (5 g L^{-1}). The cultures were inoculated at an initial cell concentration of 1–1.5 g L^{-1}. Cultivations were carried out in 125 mL Erlenmeyer flasks with a total medium volume of 50 mL in an orbital shaker at 120 rpm, 30 °C, for 168 h for synthetic media and 120 h for hydrolysates media. Samples were taken at 24 h time intervals to determine the concentrations of cell mass, residual sugars, xylitol and ethanol in the broth. All experiments were carried out in duplicates. The oxygen volumetric transfer rate (k_1a), for both synthetic and SHH media, were measured in 2 L bioreactor (BBraun, Germany) fitted with a polarographic oxygen probe, using the dynamic gassing-out method.

2.5. Analytical methods

Monosaccharides (glucose, mannose, xylose, and arabinose), xylitol and glycerol concentrations were determined by HPLC (Perkin–Elmer Series 200, USA) with a refractive index (RI) detector and a Phenomenex RHM-monosaccharide