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Sustainable succinic acid production from rice husks

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ABSTRACT

In this study, an investigation was carried out into the production of succinic acid by *Actinobacillus succinogenes* using the residual biomass rice husks as a sustainable carbon source. The rice husks were submitted to acid hydrolysis in autoclave and in a pressurized polytetrafluorethylene vessel. The hydrolysis conditions were optimized with the aid of a factorial design. The best results were obtained with a pressurized reactor using HCl 2.2% (v v^{-1}), at a temperature of 174 °C (59 bar), 46 min reaction time, and producing 19.0 g L^{-1} glucose and 3.01 g L^{-1} xylose. The hydrolysate was detoxified through a combination of pH regulation and adsorption on active carbon; it was subsequently, fermented in anaerobic medium at 37 °C; the nutrient concentration and the agitation speed were also optimized by factorial design. After 54 h static fermentation of the rice husks hydrolysate, supplemented with 8.40 g L^{-1} yeast extract and 1.40 g L^{-1} $NaHCO_3$, an amount of 12.5 g succinic acid L^{-1} was produced, which corresponds to a yield of 59.9%. This confirms that, rice husks can definitely be used as substrate to produce succinic acid and other priority chemicals

1. Introduction

Lignocellulosic residues are important sustainable sources of energy and chemicals. A potential source of residual biomass can be derived from rice processing, during which huge quantities of rice husks are discharged, and this disposal of refuse poses hazards to the environment. According to the Brazilian National Supply Company (CONAB, 2012/2013), the country produced 12.1 million tons of grain in the 2013/2014 harvest, which corresponds to around 2.4 million tons of rice husks (RH).

RH are relatively rich in carbohydrates: 37.10% cellulose, 19.50% hemicellulose and 17.60% lignin (Armesto et al., 2002; Ndazi et al., 2007; Saha and Cotta, 2008) and can be fractionated and subsequently converted into high added value chemicals (Bevilaqua et al., 2013; Martins et al., 2007; Rambo et al., 2013, 2011). One of these, Succinic acid (SA), is a versatile chemical intermediary, which is widely employed in the food and pharmaceutical industry, and classified by the US Department of Energy as one of the most promising biologically-based chemicals (Werpy and Petersen, 2015).

The *building block* SA is commonly produced from fossil raw materials, and this leads to the exhaustion of scarce resources, environmental pollution and high final prices (Bechthold et al., 2008; Zeikus et al., 1999). Recent studies suggest that the SA production by means of microbial fermentation has the potential to solve many problems caused by synthetic modes of production, to ensure the sustainability of the process.

The acidic hydrolysis of RH allows the separation of the lignin fractions and converts cellulose and hemicellulose into hexoses and pentoses, which can be metabolized by different kinds of microorganisms for the production of SA, such as the *Bacteroides fragilis* (Isar et al., 2006), *Actinobacillus succinogenes* (Borges and Pereira, 2011; Guettler et al., 1996; Liu et al., 2008; Xi et al., 2011), *Anaerobiospirillum succiniciproducens* (Datta and Glassner, 1992), *Mannheimia succiniciproducens* (Oh et al., 2009) and genetically modified *Escherichia coli* (Okuda et al., 2007; Vemuri et al., 2002).

In accordance with the results obtained by using bacteria isolated from bovine rumen, *A. succinogenes* is a most promising microorganism for the production of SA since it makes use of the vast majority of sustainable carbon sources, such as arabinose, cellobiose, fructose, galactose, glucose, lactose, mannose, xylose and saccharose, under anaerobic conditions. Sugar cane and agave bagasse, and wheat, rice and corn straws, are also described as a source of fermentable sugars for the production of AS with *A. succinogenes,* by obtaining final concentrations of up to 55 g SA L^{-1}, depending on the applied medium concentration and supplementation (Borges and Pereira, 2011; Corona-Gonzalez et al., 2008; Liu et al., 2012; Zheng et al., 2009).

For this reason, RH, as a source of cellulose and hemicellulose, represents a sustainable, cheaper raw feedstock for the SA making,

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here, by fermentation of the hydrolysate by *A. succinogenes*. Left–over RH is an undesirable material, and a cause of environmental damage, but which can be converted into a sustainable solution.

Experiments were planned for the optimization of both, hydrolytic and fermentative processes, with the aim of maximizing, first the production of fermentable sugars and, secondly, the formation of SA.

2. Material and methods

2.1. Chemicals

The analytical reagents used were succinic acid (≥ 99%), α-D-glucose anhydrous (96%), D-(+)-xylose (≥ 99%), D-(-)-arabinose (≥ 98%), and 5-hydroxymethylfurfural (from Sigma-Aldrich, Steinheim, Germany); formic, hydrochloric and sulfuric acids (Merck, Darmstadt, Germany); NaOH, $Ca(OH)_2$, $NaHCO_3$ and active carbon (Synth, São Paulo, Brazil), yeast extract (Acumedia, Lansing, EUA), and quaternary ammonium tannate (Acqua Química, São Paulo, Brazil). The aqueous solutions were prepared in ultrapure water (Milli-Q System, 18.2 MΩ cm).

2.2. Pretreatment of rice husks

RH was ground (Romer Labs Micro Mill, São Paulo, Brazil) to increase the surface area and reduce the crystallinity of the cellulosic fraction. Particles between 0.18–0.30 mm in size were collected (Bertel, São Paulo, Brazil), then washed with distilled water and dried at 50 °C for 24 h.

2.3. Acid hydrolysis of rice husks

Pretreated biomass was submitted to acidic hydrolysis in two different reactors, a regular laboratory autoclave (Phoenix, São Paulo, Brazil) and a pressurized polytetrafluorethylene (PTFE) vessel (Berghof, Eningen, Germany).

By means of pressurized acidic hydrolysis (PAH), the parameters evaluated were acid concentration (HCl or H_2SO_4; 1–7% v v^{-1}); reaction time (30–110 min) and temperature (135–185 °C); the pressure varied in-between 46–62 bar, by a biomass: acid solution ratio of 1 g : 10 mL.

The effect of the acid concentration (HCl or H_2SO_4; 1–7% v v^{-1}) was evaluated through hydrolysis involving autoclaving as well as the reaction time (30–110 min) and the biomass: acid solution ratio of 1–7 g : 10 mL. A Central Composite Rotatable Design (CCRD) was planned for the 3 independent variables with 8 possible combinations for the 2 levels studied (2^3); plus 4 axial essays and 3 repetitions of the central point, making a total of 17 experiments for each reactor and each kind of acid; after this, the results were evaluated by the *Statistica* 8.0 software (StatSoft, Tulsa, OK, USA).

2.4. Detoxification of the hydrolysate

The efficiency of the different detoxification methods of RH acid hydrolysates: adsorption in active carbon; combination of active carbon and change of pH; and flocculation, was evaluated by measuring the effect of reducing the concentrations of acetic and formic acids, and phenols – and taking constant account of the loss of fermentable sugars.

As a result of the detoxification with active carbon, 2.5% (mv^{-1}) of this adsorbent was added to the RH hydrolysate and stirred at 400 rpm and 50 °C for 30 min. After this, the solution was vaccum filtred through a cellulose nitrate membrane (47 mm × 0.45 μm) (Marton et al., 2006).

The combined detoxification process enabled the hydrolysate pH to be regulated with a 1 mol L^{-1} $Ca(OH)_2$ solution up to pH 7.0, and thereafter down to pH 2.5, with a 1 mol L^{-1} H_3PO_4 solution, following the adsorption in active carbon 2.5% (mv^{-1}).

The pH regulation of the acid hydrolysate was also tested with 1 mol L^{-1} CaO up to pH 8.0, followed by flocculation with polymeric quaternary ammonium tannate 5% v v^{-1}, under stirring at 200 rpm for 45 min. The hydrolysate was finally centrifuged at 4000 rpm, for 10 min and then filtered through a cellulose nitrate membrane (47 mm × 0.45 μm).

After the detoxification, the hydrolysate was adjusted to pH 7.0 with a 6 mol L^{-1} NaOH solution and stored at 4–8 °C until autoclaving (15 min, 120 °C).

2.5. Microorganism and inoculum preparation

The microorganism used for the fermentation of the RH hydrolysate was *A. succinogenes* DSM 22257 (DSMZ, Braunschweig, Germany). The liquid medium for inoculum preparation contained (in g L^{-1}) the following: hydrolyzed enzymatic casein (17), hydrolyzed enzymatic soya bran (3), sodium chloride (5), dipotassium phosphate (2.5), and dextrose (2.5).

The cultivation media was kept under orbital agitation at 150 rpm, 37 °C, for 24 h.

2.6. Fermentation process of the rice husks hydrolysate

The RH hydrolysate (200 mL) was fermented with 10% inoculum (v v^{-1}) within the Erlenmeyer flask in the hybrid oven/orbital shaker (Marconi, São Paulo, Brazil). The best temperature and pH for the *A. succinogenes* fermentation were 37.5 °C and pH 7.0, in anaerobic medium (at a CO_2 flow of 37 mL min^{-1}) (Zheng et al., 2009; Liu et al., 2008).

The additional fermentation conditions, such as nutrient concentration (0–2 g L^{-1} $NaHCO_3$, 2–10 g L^{-1} yeast extract) and agitation speed (0–300 rpm) were optimized by means of a factorial design.

The time of fermentation was 96 h, with aliquots being taken at intervals of 6 h in order to accompany the consumption of the sugars and the production of SA.

2.7. Determination of sugars and by-products by HPLC-RID

The SA concentration, subproducts and sugars (glucose, xylose and arabinose), as well as the sugar consumption during the fermentation stage, were determined by high-performance liquid chromatography, coupled to a refractive index detector (HPLC-RID). The equipment comprises a Shimadzu Chromatograph equipped with a LC-20AT pump, DGU-20A5 degaser, SIL-20A autosampler, CBM-20A communicator module, LC Solution Software, and RID-10A detector (Shimadzu, São Paulo, Brazil).

The best separation was obtained with a chromatographic column - Aminex HPX-87H (300 mm x 7.8 mm), using mobile phase 5 mM H_2SO_4, isocratic mode, with a flow of 0.4 mL min^{-1}, at 40 °C. The injection volume was 20 μL; for this experiment, the samples were diluted in ultrapure water (1:10 v v^{-1}) and, after this, passed through a syringe filter (PTFE 13 mm × 0.22 μm).

2.8. Extraction of the succinic acid

The fermentative medium was subjected to solid phase extraction (SPE) of the SA, and different cartridges were tested: polymeric Strata-X (styrene-divinylbenzene, 3 mL, 200 mg) (Phenomenex, Torrance, EUA), reverse phase Strata-X (C18, 3 mL, 200 mg) (Phenomenex, Torrance, EUA) and ion exchange Chromabond® SB (quaternary ammonium, 3 mL, 500 mg) (Macherey-Nagel, Dueren, Germany).

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