ELSEVIER

Bioresource Technology 74 (2000) 175–178

BIORESOURCE TECHNOLOGY

Short communication

Solid-state fermentation for the synthesis of citric acid by *Aspergillus niger*

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Received 14 January 1999; received in revised form 23 April 1999; accepted 14 June 1999

Abstract

Solid-state fermentation was carried out to evaluate three different agro-industrial wastes, sugar cane bagasse, coffee husk and cassava bagasse for their efficiency in production of citric acid by a culture of *Aspergillus niger*. Cassava bagasse best supported the mould's growth, giving the highest yield of citric acid among the tested substrates. Results showed the fungal strain had good adaptation to the substrate (cassava bagasse) and increased the protein content (23 g/kg) in the fermented matter. Citric acid production reached a maximum (88-g/kg dry matter) when fermentation was carried out with cassava bagasse having initial moisture of 62% at 26°C for 120 h.

Keywords: Solid-state fermentation; Citric acid; *Aspergillus niger*; Cassava bagasse; Sugarcane bagasse; Coffee husk

1. Introduction

Citric acid is an important commercial product with a global production reaching 736,000 tons per year (Química and Derivados, 1997). Almost the entire quantity of citric acid is produced by fermentation, mainly through submerged fermentation of starch- or sucrose-based media, using the filamentous fungus *Aspergillus niger*. The food industry is the largest consumer of citric acid, using almost 70% of the total production, followed by about 12% by the pharmaceutical industry and 18% for other applications (Shah et al., 1993). There is an annual growth of 3.5–4.0% in demand/consumption rate of citric acid. In Brazil, the entire demand for citric acid is met by imports. This necessitates development of indigenous biotechnological processes with economic feasibility.

Solid-state fermentation (SSF) offers numerous advantages for the production of bulk chemicals and enzymes (Hesseltine, 1977; Pandey, 1992, 1994; Soccol, 1994). In the past decade or so, there has been an increasing number of reports on the use of solid-state fermentation processes for the production of a number of microbial products (Roussos et al., 1994; Nampoothiri and Pandey, 1996; Pandey et al., 1999). This is partly because solid-state processes have lower energy requirements and produce much less wastewater and environmental concerns because disposal is of solid wastes. Traditionally, SSF are characterized by the development of microorganisms in a low water-environment on a non-soluble material that acts both as physical support and source of nutrients; however it is not necessary to combine the role of support and substrate but rather reproduce the conditions of low water activity and high oxygen transference by using a nutritionally-inert material soaked with a nutrient solution (Pandey and Soccol, 1998).

Citric acid production has always been a subject of interest for many workers, e.g., Chaudhary et al. (1978); Eikmeier and Rehm (1984); Hang and Woodmans (1987); Lee et al. (1989); Gutiérrez-Rojas et al. (1996); Lu et al. (1997) and Pintado et al. (1998). Different agro-industrial residues, such as apple pomace, coffee husk, wheat straw, pineapple waste, cassava bagasse, banana, sugar beet cosset, kiwi fruit peel, etc. have been investigated with SSF techniques for their potential to be used as substrates.

The State of Paraná, Brazil, is a large producer of cassava roots. The production of 1997 was estimated to be 3.2 million tonnes (Deral, 1997). Cassava bagasse

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0960-8524/00/$ - see front matter
PII: S0960-8524(99)00107-8

is a solid residue from the starch extraction process, which is generated during the separation stage. It contains fibrous material and about 40% starch (Soccol, 1994). Thousands of tons of cassava bagasse are discarded daily by the cassava processing industries, leading to a big environmental problem. Thus, there is an urgent need to find suitable applications and disposal of this waste. One alternative for its economic utilization is to use it as substrate in fermentation processes for the production of value added products like enzymes and biomass. The aim of this study was to compare citric acid production from cassava bagasse and two other substrates (sugar cane bagasse and coffee husk) in solid-state fermentation using a culture of *A. niger*.

2. Methods

Micro-organisms: Seven strains of *A. niger* were screened for citric acid production in liquid culture which contained (g/l) glucose 120, $(NH_4)_2SO_4$ 3, KH_2PO_4 1, $MgSO_4 \cdot 7H_2O$ 0.5, $MnSO_4 \cdot 7H_2O$ 0.014, $FeCl_3 \cdot 6H_2O$ 0.01 and methanol (2%, v/v) (Kirimura et al., 1987). Based on the results on citric acid yields, one strain, NRRL 2001, was chosen for further studies. The strain was maintained at 4°C on Czapeck Dox Broth slants and renewed once in each month.

Inoculum: *A. niger* spores were produced in Czapeck Dox Broth with agar (50 ml) in a 250 ml Erlenmeyer flask, incubated at 28°C for eight days. A spore suspension was prepared by adding 25 ml distilled water with Tween-80 (0.1%) and was stored at 4°C for a maximum of two weeks. It contained 10^8 spores/ml.

Substrate: Three solid materials, sugar cane bagasse, coffee husk and cassava bagasse were tested. Sugar cane bagasse was obtained from the local market of Curitiba; coffee husk was obtained from an industry of Curitiba, and cassava bagasse was supplied by Companhia Lorenz (Quatro-Pontes, SC, Brazil). These were ground in a mill to a particle size between 0.8–2.0 mm and dried at 55–60°C for 12 h.

Fermentation: Liquid nutrient medium containing (g/l) $ZnSO_4 \cdot 7H_2O$ 0.2 and $FeCl_3 \cdot H_2O$ 0.014 was sterilized at 121°C for 15 min. After cooling, methanol (4%) was added aseptically to it (Kolicheski, 1995). Sugar cane bagasse and coffee husk were supplemented with 40% of glucose (corresponding to the starch content of cassava bagasse). Solid substrates were inoculated with the spore suspension, mixed with the nutrient medium (10^7 spores/g of dry substrate, inoculum size). Initial moisture contents of the substrates were 90, 65 and 62% for sugar cane bagasse, coffee husk and cassava bagasse, respectively.

Fermentation was carried out in vertical column fermenters of 4-cm diameter × 20-cm length (Raimbault and Alazard, 1980). Each column was charged with a known amount of pre-inoculated moist solid medium (up to 16-cm length) and incubated at 26°C in a water bath. The columns were supplied with humidified air at a flow-rate of 65 ml/min. Fermentation was carried out for a stipulated period and the results reported are the average of two sets of the experiments (standard deviation being less than 3%). The entire column of substrate was taken as initial sample at each sampling time then subdivided.

2.1. Analytical methods

Samples (5 g) were mixed well with 50 ml of distilled water to extract citric acid and sugars. The filtrate so obtained was subjected to high performance liquid chromatograph analysis using a Shimadzu LC-10AD HPLC. A temperature of 60°C and 5 mM H_2SO_4 as the mobile phase at a flow-rate of 0.6 ml/min were used. Citric acid and total sugars were detected in the column eluate by differential refractometer (Shimadzu RID-10A). Protein content was determined by the Stutzer method (Vervack, 1973). pH, moisture, reducing sugars (glucose), total sugars and starch content were determined as described by Soccol (1992).

3. Results and discussion

Initial experiments were carried out with seven strains of fungi in liquid culture and, based on the yields of citric acid, one strain of *A. niger*, NRRL 2001, was selected for further studies (data not shown here).

3.1. Citric acid production with different supports

Initial results of solid-state fermentation, with different solid materials, for citric acid production showed that there was high sugar consumption by *A. niger* in all the three cases (90%, 97% and 87% for sugar cane bagasse, coffee husk and cassava bagasse, respectively) which indicated that the strain had a good affinity for these substrates (data not shown here). Citric acid production was highest for cassava bagasse (88-g/kg dry matter (DM) against 48.7 for sugar cane bagasse and 12.7 g/kg for coffee husk). The substrate-to-product conversion factor was 20.6%, 14.6% and 3.1% for cassava bagasse, sugar cane bagasse and coffee husk, respectively (data not shown). Based on these results on the citric acid production, cassava bagasse was chosen for further studies.

It has been reported that cassava bagasse contains many components such as calcium, phosphorus, vitamin B_2, thiamine and niacin (Hohnholz, 1980). Higher citric acid production by cassava bagasse in comparison to the other two substrates (coffee husk and sugar cane bagasse)

Table 1
Kinetics of citric acid production with cassava bagasse (120 h)

Time (h)	pH	True protein g/kg (DM)	Humidity %	Residual sugars g/kg (DM)	Residual starch g/kg (DM)	Citric acid g/kg (DM)
0	2.0	13.1	62.1	450.5	405.5	0.0
24	1.7	13.7	63.5	369.5	332.9	13.7
48	1.5	14.9	63.6	285.2	256.7	22.5
72	1.4	18.3	64.2	116.2	104.6	56.2
96	1.3	22.2	64.5	56.9	51.2	70.9
120	1.2	23.1	64.8	55.9	50.8	88.1

might be due to the presence of these components in the substrate along with the starch.

3.2. Kinetics of citric acid production with cassava bagasse

Table 1 shows the pattern of fungal growth as monitored by protein content in the fermenting substrate and change in moisture content (humidity) during the 120 h of fermentation. Protein content increased from 13 to 23 g/kg, showing more than 90% increase. There was not much change in the moisture content of the fermenting matter during the course of fermentation. The table also shows data on residual sugars and starch, available in the substrate to *A. niger*. A comparison between residual sugars and starch showed that there was a good proportionate utilization pattern of starch and sugars, which indicated good efficiency of the fungal culture. As is evident from the results, citric acid production started in the initial period of fermentation (at around 12 h) and reached 88-g/kg DM at 120 h, corresponding to a yield of 20% conversion factor (based on consumed sugars/starch). There was a drop in the medium pH, which obviously was due to the formation and accumulation of citric acid.

Generally, it is not possible to increase the protein content of the substrate to a high level in citric acid fermentation. Many authors have reported that a high concentration of citric acid is reached only under limited growth (Grewal and Kalra, 1995). In contrast to this, we have achieved good growth of a fungal culture, as indicated by protein content of the substrate, with citric acid production. High protein content suggests the possibility of utilizing the fermented substrate (after citric acid extraction) as animal feed.

Acknowledgements

One of us (LPSV) thanks CAPES for the award of a fellowship. CRS thanks CNPq, Brazil, for a scholarship under the Scientific Productivity scheme.

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