Biochemical Engineering Journal 53 (2010) 154–157

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Short communication

Increase in the fructooligosaccharides yield and productivity by solid-state fermentation with *Aspergillus japonicus* using agro-industrial residues as support and nutrient source

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ARTICLE INFO

Article history:
Received 16 July 2010
Received in revised form
10 September 2010
Accepted 17 September 2010

Keywords:
Fructooligosaccharides
Solid-state fermentation
Nutrient
Aspergillus japonicus
β-Fructofuranosidase

ABSTRACT

Corn cobs, coffee silverskin, and cork oak were used as support and nutrient source during the fructooligosaccharides (FOS) production by *Aspergillus japonicus*, under solid-state fermentation (SSF) conditions. The objectives of this study consisted in evaluating the possibility of improving the FOS yield and productivity, besides to finding an alternative to reduce the production costs, and add value to these agro-industrial residues. Fermentation assays were performed by using the materials as solid support, supplemented or not with nutrients. For comparison, assays were also performed using a synthetic material as solid support, under the same operational conditions. All the material residues acted as nutrient source for the microorganism, since FOS production occurred when all of them were used without nutrient supplementation, but not when the synthetic material was used. Among the evaluated materials, coffee silverskin gave the most interesting fermentation results, with a FOS production similar in both supplemented and non-supplemented media. The elevated FOS production (128.7 g/l) and β-fructofuranosidase activity (71.3 U/ml) obtained by using this material suggest SSF of coffee silverskin with *A. japonicus* as an interesting and promising strategy to synthesize both products at the industrial level.

1. Introduction

The development of prebiotic products has gained large attention in the last decades, due to the increased consumer desire to improve health through food. Among the new commercially available prebiotic ingredients, the fructooligosaccharides (FOS), fructose oligomers obtained from sucrose, have attracted special attention due to their important functional properties including low caloric value, non-cariogenicity, effects in decreasing the levels of phospholipids, triglycerides, and cholesterol, help in gut absorption of calcium and magnesium, and usefulness to stimulate bifidobacterial growth in the human colon [1,2].

FOS is produced commercially through the enzymatic synthesis from sucrose by microbial enzymes with transfructosylating activity (β-fructofuranosidases, EC.3.2.1.26, also designed as fructosyltransferases EC.2.4.1.9). Most of these enzymes have been found in fungi such as *Aspergillus*, *Aureobasidum*, and *Penicillium* [3,4], among of which *Aspergillus* species have received particular attention and have been cited as good enzyme producers. In the past few years, several *Aspergillus japonicus* strains have been reported as potentially adequate for industrial production of FOS and β-fructofuranosidase [4,5].

The main drawback of the commercial FOS production by transfructosylation is that the yields are normally low (55–60%) [2,6]. Therefore, and due to the increased demand for using these ingredients in food and pharmaceutical products, there is a great interest in the development of a suitable and economically viable biotechnological process for industrial production of FOS that allow obtaining higher yields and productivities. Most investigations about experimental conditions for FOS and β-fructofuranosidase production are mainly based on submerged fermentation (SmF) experiments. However, solid-state fermentation (SSF) systems appear to be an interesting alternative to obtain higher volumetric productivity and product concentration, with lower capital cost and energy consumption when compared to SmF [7]. SSF requires low water volume and thus has large impact on the economy of the process due to smaller fermenter-size, reduced downstream processing, reduced stirring and lower sterilization costs [8,9]. In addition, the use of low cost agricultural and agro-industrial residues as substrates, contributes also to lower capital and operating costs compared to SmF.

The FOS production by SSF systems have been few explored until nowadays. To the best of our knowledge, only one published study

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doi:10.1016/j.bej.2010.09.012

reported the use of this system for the production of the fructosyltransferase enzyme by *Aspergillus oryzae* [10]. In the present work, three agro-industrial residues were used in SSF systems for the production of FOS and β-fructofuranosidase by *A. japonicus*. The residues included corn cobs, coffee silverskin and cork oak, which were previously found as being materials with ability for immobilization of *A. japonicus* during the FOS production by SmF [11]. Fermentation assays were performed to verify the kinetic behavior of FOS and β-fructofuranosidase production by SSF, and the possibility of using these agro-industrial residues as nutrient source during this process.

2. Materials and methods

2.1. Solid supports

Three agro-industrial residues (corn cobs, coffee silverskin and cork oak) and one synthetic material (synthetic fiber Scotch Brite, 3 M Spain, SA), were used as solid support during the SSF. Corn cobs and cork oak were obtained from local farmers; coffee silverskin was supplied by NovaDelta-Comércio e Indústria de Cafés S.A. (Campo Maior, Portugal), and the synthetic fiber was purchased in a local market. To be used in the experiments, particles with approximately 3 × 3 mm (length × width) were selected and autoclaved at 121 °C for 20 min.

2.2. Microorganism and cultivation conditions

The strain *A. japonicus* ATCC 20236 was used in the experiments. The strain was maintained on potato dextrose agar (PDA – Difco) plates at 4 °C, and the spores were maintained mixed with glycerol solution in ultra-freezer at −80 °C. For the production of spores the strain was grown on PDA medium, at 28–30 °C for 7–8 days.

2.3. Solid-state fermentation (SSF)

The SSF cultivations were performed in Petri dishes containing approximately 3.0 g of the previously sterilized materials. For the experiments, the materials were moistened with a 200 g/l sucrose solution to attain 70% moisture content, inoculated with a concentrated spore suspension to give 2×10^6 spores/g material, and statically incubated at 28 °C during 48 h. Cultivations were performed with the sucrose solution supplemented or not with the following nutrients (g/l): yeast extract (27.5), $NaNO_3$ (2.0), K_2HPO_4 (5.0), $MgSO_4 \times 7H_2O$ (0.5), and KCl (0.5). Steam sterilization of the media was carried out at 112 °C for 15 min. The spores' suspension was prepared by scrap down the spores from the PDA plates with a sterilized solution of 1 g/l Tween 80, and counted in a Neubauer chamber.

Samples for analysis were collected at regular intervals. The total content of each Petri dish was collected as a sample and 2 ml of sterilized distilled water was added to facilitate the fermented broth extraction. After the water incorporation to the fermented solid material, the mixture was submitted to a vacuum filtration process to extract the fermented broth, which was subsequently filtered in 0.2 μm filters. In the filtered broth, FOS (1-kestose, 1-nystose, and 1-β-fructofuranosyl nystose), residual concentration of other sugars (sucrose, fructose, and glucose), and extracellular enzyme activity were measured. All the results obtained in these analyses were calculated considering only the moisture content present in the material, being thus corrected by the dilution caused during the water addition to extract the fermented broth. All the experiments were conducted in duplicate.

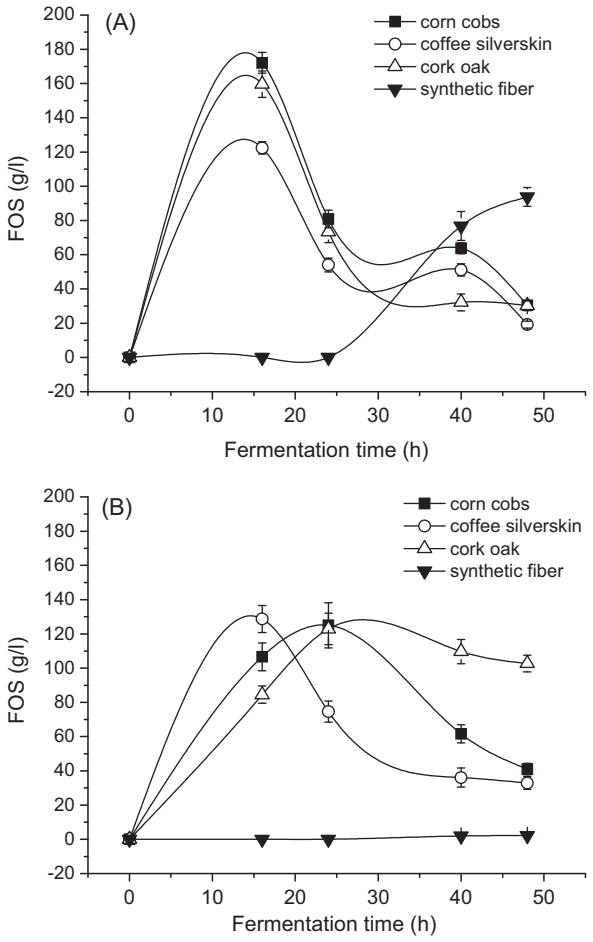

Fig. 1. FOS production by *Aspergillus japonicus* under solid-state fermentation conditions using different materials as solid support. Assays supplemented (A) or not (B) with nutrients.

2.4. Analytical methods

The concentration of sucrose, glucose, fructose, 1-kestose, 1-nystose, and 1-β-fructofuranosyl nystose, and the β-fructofuranosidase (FFase) activity were determined as previously described [11].

2.5. Fermentative parameters calculation

The total FOS concentration was calculated by the sum of the concentrations of 1-kestose, 1-nystose, and 1-β-fructofuranosyl nystose. The FOS yield ($Y_{P/S}$, g/g) was determined by the ratio between total FOS (g/l) and consumed sucrose (g/l). FOS productivity (Q_P) was calculated as the total FOS (g/l) by fermentation time (h).

3. Results and discussion

Fig. 1 shows the FOS production by *A. japonicus* under SSF conditions using the different materials as solid support. Fig. 1A shows the results obtained for the assays supplemented with nutrients; while Fig. 1B shows the results obtained for the assays where no nutrients were added to the media (the materials were moistened with sucrose solution only). As can be noted, all the material residues used in the experiments acted as nutrient source for the microorganism since FOS production occurred from all of them but not when using the synthetic fiber (Fig. 1B). Among the three tested