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Fructooligosaccharides and β-fructofuranosidase production by *Aspergillus japonicus* immobilized on lignocellulosic materials

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ABSTRACT

This work describes the fructooligosaccharides (FOS) and β-fructofuranosidase (FFase) production from sucrose (200 g/l) by *Aspergillus japonicus* ATCC 20236 immobilized on different lignocellulosic materials including brewer's spent grain, wheat straw, corn cobs, coffee husks, cork oak, and loofa sponge. Transfructosylating (U_t) and hydrolyzing (U_h) activities of FFase were also determined. The FOS production and FFase activity ranged from 128.35 to 138.73 g/l, and 26.83 to 44.81 U/ml, respectively, for cells immobilized in the different carriers. Corn cobs was the best support material since gave the highest results of microorganism immobilization (1.49 g/g carrier), FOS and FFase production, with FOS productivity (6.61 g/l h) and yield (0.66 g/g based on total substrate; 0.73 g/g based on consumed substrate) higher than those obtained by free cells system. Moreover, the ratio U_t/U_h of FFase, parameter of importance for elevated FOS production, was greater for cells immobilized in corn cobs than for free cells. Such results demonstrated that corn cobs can be successfully used as carrier for immobilization of the fungus *A. japonicus*, for the production of FOS and FFase.

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1. Introduction

Fructooligosaccharides (FOS) are fructose oligomers with great potential to improve the quality of many foods because they have functional properties, low caloric value, are not cariogenic, and can be used for diabetic people. In addition, they decrease levels of phospholipids, triglycerides and cholesterol, help gut absorption of calcium and magnesium, and stimulate the bifidobacteria growth in the human colon [1–3]. Therefore, the interest on FOS use in food and pharmaceutical compounds has strongly increased in the last years. As a consequence, is of interest the development of a suitable and economically viable process for industrial production of FOS that permit to obtain high product yield. Actually, FOS is mainly produced on industrial scale from the disaccharide sucrose by microbial enzymes having transfructosylating activity (β-fructofuranosidase—FFase—EC 3.2.1.26). The FOS production yields by this process are normally low (55–60%) since the enzymes involved in the reaction have, besides the transfructosylation activity, hydrolytic activity giving glucose and fructose as reaction by-products [3,4]. In addition, glucose acts as inhibitor of the enzymes, affecting the reaction efficiency [5].

Several studies have been performed aiming to improve the FOS production yield. Most of them were focused on finding new species of microorganisms able to produce enzymes with high FFase activities. It is known nowadays, that most of these enzymes can be found in fungi such as *Aspergillus*, *Aureobasidum*, and *Penicillium* [6–8]. Among them, several *Aspergillus japonicus* strains have been reported as potentially adequate for industrial production of FOS [6]. Another possibility that has also been evaluated to increase the FOS production yield was to determine the best conditions for process variables such as sucrose concentration, nutrient supplementation, fermentation time, temperature and pH of the reaction mixture [9,10]. However, the process yield was not competitively improved when compared to that currently obtained on industrial scale.

A possible alternative that has been few explored for FOS production is the use of immobilized cells. Recently, many studies have been carried out aiming to improve the performance of biotechnological processes by using immobilized-cell systems. Such systems are eco-friendly and have many advantages over suspended cell-systems; one of the most important is that immobilized cells completely maintain their biological functions with increased stability that may often lead to increased cell productivity [11]. As a consequence of the high cell concentration in the fermentation medium, the process efficiency and productivity are also increased [12,13]. In addition, immobilized-cell systems enables easier separation of the cells from the fermentation broth for later use in repeated batch operations, and facilitates isolation and

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requirement of the products [14–16]. However, the correct selection of immobilization carrier is essential to design an effective system to each particular purpose. On the whole, the carrier must be able to maintain its physical integrity, being insoluble and stable under the chemical and thermal conditions of the bioprocess. In addition, it must be resistant to microbial degradation, it cannot react with the substrates and products present in the fermentation medium, and it must be available in large quantity. Furthermore, for industrial purposes, an important criterion is the carrier cost, which, combined with the interest in the reuse of by-products, led to an increased search for cheap and available potential cell carriers [17]. In this sense, the use of lignocellulosic materials as carrier could be an interesting alternative because they are natural, renewable, biodegradable, non-toxic, inexpensive and readily available.

Previous studies have investigated the *A. japonicus* immobilization by entrapment in supports such as gluten or calcium alginate beads [6,18]. Nevertheless, the immobilization of this strain by attachment in lignocellulosic materials for FOS and FFase production has not been studied yet. Based on the facts pointed above, the present study describes the fermentation performance for FOS and FFase production by *A. japonicus*, using the whole cells immobilized in different lignocellulosic materials, as an alternative to improve the efficiency of this bioconversion process.

2. Experimental

2.1. Carrier's preparation

Six lignocellulosic materials were tested as carriers including brewer's spent grain, wheat straw, corn cobs, coffee husks, cork oak and loofa sponge. All of them with particles sizes of approximately 1 mm, were physical-chemically characterized regarding the water absorption index (WAI—[19]), and critical humidity point (CHP—[20]). WAI was determined by suspending the sample (2.5 g) in 30 ml of distilled water in a tared 60 ml centrifuge tube. The slurry was stirred with a glass rod for 1 min at room temperature (25 °C) and centrifuged at $3000 \times g$, 25 °C for 10 min. The supernatant was discarded, and the WAI was calculated from the weight of the remaining gel and expressed as g gel/g dry weight. The CHP was estimated by adding 1 g of sample in a thermo balance at 120 °C for 60 min. To be used as immobilization carrier, the materials were pre-treated by boiling for 10 min, washed three times with distilled water, and then dried overnight at 60 °C. Prior to use, all of them were autoclaved at 121 °C for 20 min.

2.2. Strain and cell culture

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 25–30 °C for 7–8 days.

2.3. Fermentation conditions and sampling

The used fermentation medium and conditions were based on previous works on FOS production [21,22]. Experiments were carried out in 500 ml Erlenmeyer flasks containing 1 g of carrier and 100 ml of culture medium with the following composition (%, w/v): sucrose 20, yeast extract 2.75, $NaNO_3$ 0.2, K_2HPO_4 0.5, $MgSO_4{\cdot}7H_2O$ 0.05, and KCl 0.05. Steam sterilization of the medium was carried out at 121 °C for 20 min. Flasks were aseptically inoculated with 1.0 ml of a spore suspension containing around 1.8×10^7 spores/ml, which was prepared by scrap down the spores from the PDA plates with a sterilized solution of 0.1% (w/v) Tween 80, and counted in a Neubauer chamber. The inoculated flasks were incubated in a rotary shaker at 28 °C and 160 rpm for 48 h. Cells were immobilized *in situ* in the flasks by natural adsorption through their direct contact with the carrier at the beginning of each fermentation run. Assays under the same fermentation conditions described above but without addition of carrier particles (free cells assays) were also performed for comparison. The pH of all reaction mixtures was not controlled during the experiments.

Samples for analysis were aseptically collected at regular intervals and filtered, using 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), pH and extracellular enzyme activity were measured. The immobilized cells concentration was determined at the fermentation end.

2.4. Analyses

2.4.1. Immobilized cell mass

The amount of biomass attached to the carriers was determined after washing the support material with distilled water for three times, and drying at 105 °C to constant weight. The biomass dry weight was determined from the difference between the mycelium plus carrier and the carrier itself.

2.4.2. FFase activity

Samples of the fermentation media were filtered (through 0.2 μm membranes) to remove the cell mass and the filtrate was utilized as extracellular enzyme source.

The β-fructofuranosidase (FFase) activity was determined by measuring the amount of glucose produced from sucrose [23]. The reaction mixture contained 100 ml of the crude FFase extract, 300 mmol of sucrose and 50 mmol of sodium acetate buffer (pH 5.0) in a total volume of 1 ml. After incubation for 20 min at 30 °C, the reaction was stopped by heating for 5 min at 100 °C. After cooling, the amount of glucose released into the supernatant was measured by high-performance liquid chromatography (see below). One unit (U) of the FFase activity was defined as the amount of enzyme required to release 1 μmol of glucose per min, from sucrose under the above conditions.

2.4.3. Transfructosylating and hydrolyzing activities

The reaction was carried out for 180 min using 0.5 U/ml of FFase in the reaction mixture described above [23]. Transfructosylating (U_t) and hydrolyzing (U_h) activities were determined by measuring the concentrations of 1-kestose and fructose by HPLC, respectively. One unit of transfructosylating activity was defined as the amount of enzyme required to transfer 1 μmol of fructose per min. One unit of hydrolyzing activity was defined as the amount of enzyme required to release 1 μmol of free fructose per min.

2.4.4. Sugars and FOS concentrations

FOS (1-kestose, 1-nystose, and 1-β-fructofuranosyl nystose) and other residual sugars (sucrose, glucose, and fructose), were directly analyzed by high-performance liquid chromatography (HPLC), as previously described [24]. A system composed by an equipment LC-10 A (Jasco, Japan) with a Prevail Carbohydrate ES column (5 μm, 250 mm × 4.6 mm, Alltech) at room temperature and refractive index detector, was used. The response of the refractive index detector was recorded and integrated using the Star Chromatography Workstation software (Varian). A mixture of acetonitrile and 0.04% ammonium hydroxide in water (70/30, v/v) was used as mobile phase at a flow rate of 1.0 ml/min. Before injection, the samples were filtered through 0.2 μm filters and diluted with Milli-Q water when needed. The sugars and FOS concentrations were determined from standard curves made with known concentrations of each compound. Sucrose and oligomers were perfectly separated and eluted on the order of their degree of polymerization (sucrose

being the first compound eluted, and the pentasaccharide (1-β-fructofuranosyl nystose) being the last one).

The total yield of FOS (Y_{FOS} in g/g) was calculated as the proportion of the sum of 1-kestose (Y_{GF2}), 1-nystose (Y_{GF3}), and 1-β-fructofuranosyl nystose (Y_{GF4}), to initial sucrose concentration. The FOS productivity (Q_P) was calculated as the total FOS production (g/l) by fermentation time (h), while the FOS yield was calculated as the total FOS production (g/l) by total substrate (g/l) or only by consumed substrate (g/l).

2.5. Statistical analysis

All the fermentation experiments were conducted at least in duplicate and the average values are reported. The results were analyzed by analysis of variance (ANOVA) and the difference among samples was verified by using the Tukey's range test. A p value of less then 0.05 was regarded as significantly different. Statgraphics Plus for Windows, version 2.1, was the software used for data analysis.

3. Results and discussion

3.1. Carrier's physical–chemical properties

Initially, the stability of the carriers during the total fermentation time (48 h) was evaluated by performing assays with carrier particles added to the fermentation medium without cells. All the carriers were quite stable and did not show any tendency to dissolve during experiments. In the later step, all the carriers were thus assayed for determination of the WAI and CHP values, physical–chemical properties of great importance when the potential of different materials for use as cell immobilization carrier or as substrate for solid-state fermentation is evaluated. WAI indicates the sample ability to absorb water, and depends on the availability of hydrophilic groups to be bonded with water molecules and on the gel forming capacity of macromolecules [25]. Materials with high WAI values are better for microorganism cultivation because they facilitate the species growth and development. Several material carriers used in the present work for immobilization of *A. japonicus* presented high WAI, the highest value being observed for wheat straw (Table 1).

Nevertheless, the WAI is not the only property to be considered when choosing a material for cells immobilization, the CHP is also very important. Materials must have low CHP to facilitate the microorganism cultivation because high values mean that a low proportion of water is bounded to the material, and consequently, the species development will be affected. In this case, when comparing the different lignocellulosic residues, the lowest CHP value was found for corn cobs (Table 1). Since the same material did not present the highest WAI and the lowest CHP values, it cannot be concluded through these two properties what will be the best material for immobilization of *A. japonicus*. Nevertheless, it will be possible to estimate which of them that can be of major influence for cells adhesion and growth.

Table 1
Water absorption index (WAI) and critical humidity point (CHP) for the different lignocellulosic materials used as cells immobilization carrier.

Immobilization carrier	WAI (g/g dry matter)	CHP (%)
Cork oak	3.35	58
Corn cobs	3.77	50
Brewer's spent grain	9.03	60
Wheat straw	9.95	57
Loofa sponge	7.76	56
Coffee husks	8.30	55

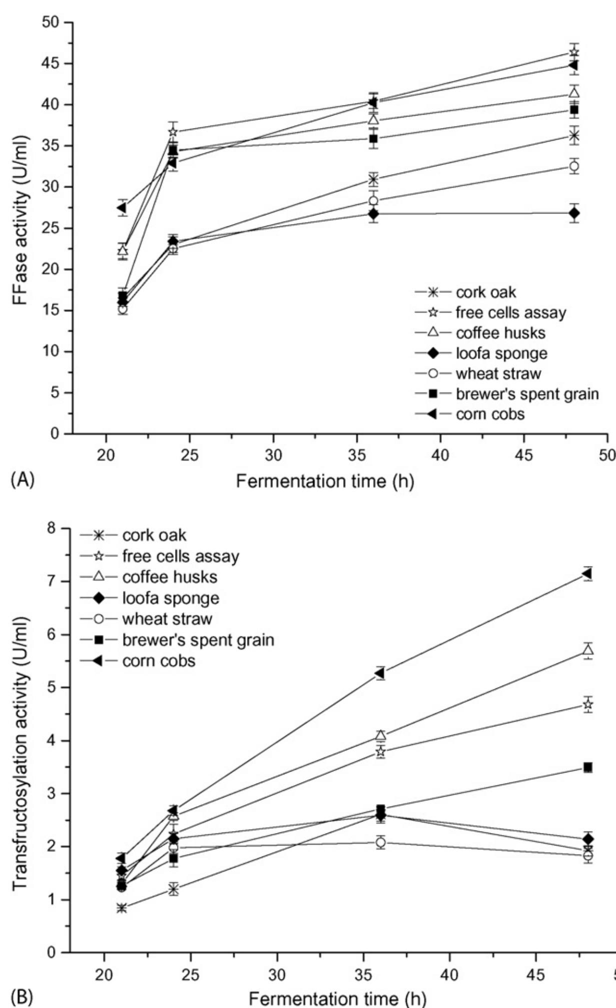


Fig. 1. FFase activity (A) and transfructosylating (U_t) activity (B) during the sucrose fermentation by *A. japonicus* immobilized or not in different lignocellulosic materials.

3.2. FFase activity

The FFase activity results for the different assays are presented in Fig. 1A. Note that cells immobilized in corn cobs presented good performance, giving the highest value (44.81 U/ml) among the immobilized systems. This activity was 8.6% higher than that obtained when the fungus was grown in coffee husks; and about 67% higher than that measured in cells immobilized in loofa sponge, which gave the lowest FFase activity values. Maybe some nutritional component of corn cobs (not identified in the present work) favored the FFase production by the microorganism.

Another important aspect regards the fermentation pH. The pH is a variable that contributes mostly to changes in the fructosyltransferase and hydrolytic rates [10]. The optimal initial pH values for FFase production depend on different strains, composition of the fermentation medium and growth conditions [26]. For *A. japonicus* JN-19, the FFase production and growth were maxima at pH 5.5, being restrained at acidic conditions or pH greater than 6.5 [26]. In the present work, the pH of the media was set at 7.0 before inoculation and was not controlled during the experiment, being gradually decreased during the cultivations (Fig. 2). The final pH values of the fermented media using cells immobilized in corn cobs and coffee husks was near to 6.0, value close to the ones reported as optima for the FFase activities by other fungus strains [10,27,28]. This fact

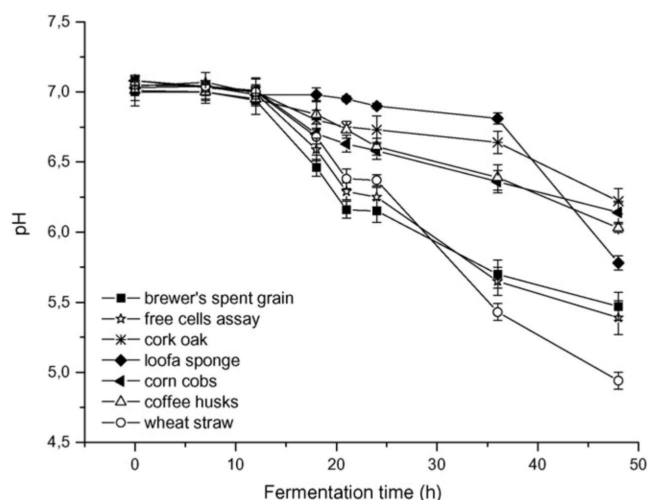


Fig. 2. Kinetic behavior of pH during the sucrose fermentation by *A. japonicus* immobilized or not in different lignocellulosic materials.

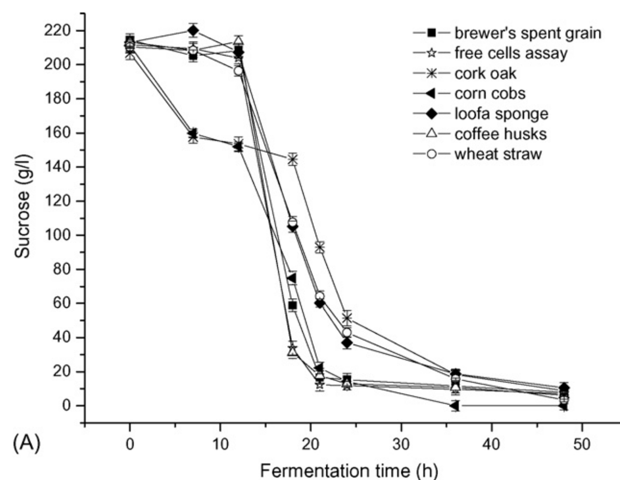
could explain the high FFase activity obtained at the fermentations' end.

High FFase activity is very important for the industrial production of FOS [29]. Therefore, corn cobs appear to be a material of great potential to be used as immobilization carrier aiming to maximize the FOS production. However, it is known that FFases commonly posses both hydrolytic (U_h) and transfructosylating (U_t) activities, the first one being responsible for the sucrose hydrolysis to monomer sugars, whereas the second one acts on the sucrose conversion to FOS. The U_t/U_h ratio indicates the relative strength of the transfructosylating activity of the produced strain, and for an efficient production of FOS, high U_t/U_h ratio is preferable [29]. In the present work, maximum U_t was obtained from cells immobilized in corn cobs (Fig. 1B). Cells immobilized in coffee husks also gave elevated U_t values, while the cells immobilized in the other carriers gave lowest results.

It is worth mentioning that although free cells presented FFase activity higher than those observed for immobilized cells (Fig. 1A), their U_t value was lower than those observed for cells immobilized in corn cobs and coffee husks. By comparing the U_t/U_h ratio for the different assays, the highest value (4.15) was found after 36 h cultivation for cells immobilized in corn cobs, which was 30% higher than that observed for the free cells system.

3.3. FOS production

The strain *A. japonicus* adhered in all the evaluated carriers, and produced FOS in all media, but both concentrations of immobilized cells and FOS varied for each case. The time course of sucrose consumption and FOS production is shown in Fig. 3. All media presented similar kinetic behaviors with an initial lag phase among 0 and 12 h approximately, followed by an almost complete sucrose consumption between 12 and 24 h. In this interval of time, sucrose was rapidly converted into glucose and 1-kestose (GF_2), and after completed 24 h fermentation the FOS production attained the most elevated level in almost all media. Maximum production of FOS was attained in 21 h fermentation with cells immobilized in corn cobs. In this case, a final product containing GF_2 (46.83%), GF_3 (16.31%), GF_4 (2.75%), residual sucrose (10.53%), glucose (20.93%) and fructose (2.65%) was obtained. Fig. 4 shows the chromatogram profile obtained for the assay of FOS production by *A. japonicus* immobilized in corn cobs, after 21 h fermentation. Note that sucrose and oligomers were perfectly separated and eluted on the order of their degree of polymerization; sucrose was eluted at 8.60 min, trisaccharide at 12.08 min, tetrasaccharide at 16.18 min and pentasaccharide at 22.45 min.



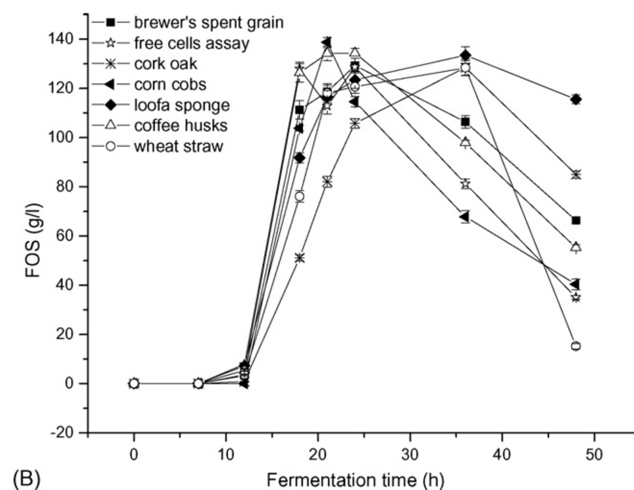


Fig. 3. Sucrose uptake (A) and FOS production (B) using *A. japonicus* immobilized or not in different lignocellulosic materials.

After 24 h fermentation, the total FOS concentration declined gradually, as result of the sucrose exhaustion and formation of FOS with longer chain (GF_3 and GF_4) from 1-kestose (GF_2). However the accentuated reduction in the FOS yield, observed in several media (Fig. 3B), suggests also that the presence of a high glucose concentration may have inactivated the transfructosylation activity of FFase and increased the FOS hydrolysis, leading to an increase in the

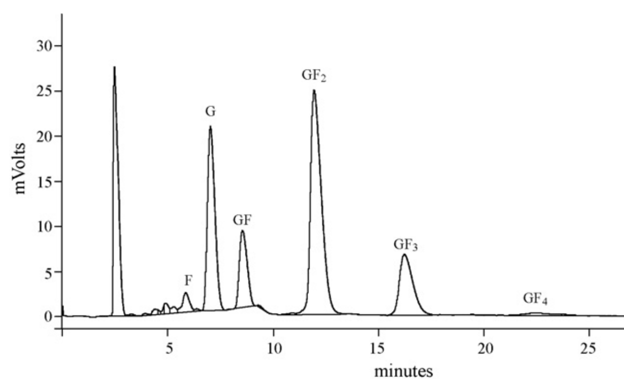


Fig. 4. HPLC chromatogram of FOS production by *A. japonicus* immobilized in corn cobs, after 21 h fermentation. F: fructose, G: glucose, GF: sucrose, GF2: 1-kestose, GF3: 1-nystose, GF4: 1-β-fructofuranosyl nystose.

Table 2
Fermentative parameters of FOS production by *Aspergillus japonicus* immobilized in different lignocellulosic materials, and using free whole cells.

	FOS (g/l)	Fermentation time (h)	$Y_{P/S}$ total substrate (g/g)	$Y_{P/S}$ consumed substrate (g/g)	Q_P (g/l h)	Cells immobilized after 48 h (g/g carrier)
Cork oak	128.47 ± 3.33	36	0.62 ± 0.02	0.68 ± 0.02	3.57 ± 0.09	0.65 ± 0.13
Corn cobs	138.73 ± 2.03	21	0.66 ± 0.01	0.73 ± 0.01	6.61 ± 0.09	1.49 ± 0.15
Brewer's spent grain	129.28 ± 2.98	24	0.60 ± 0.01	0.65 ± 0.01	5.39 ± 0.12	1.06 ± 0.18
Wheat straw	128.35 ± 3.01	36	0.61 ± 0.01	0.66 ± 0.02	3.57 ± 0.08	1.24 ± 0.11
Loofa sponge	133.57 ± 3.33	36	0.63 ± 0.02	0.69 ± 0.02	3.71 ± 0.09	0.86 ± 0.02
Coffee husks	134.29 ± 2.04	24	0.65 ± 0.01	0.69 ± 0.01	5.60 ± 0.08	1.46 ± 0.12
Free cells assay	128.68 ± 2.01	24	0.61 ± 0.01	0.64 ± 0.01	5.36 ± 0.08	–

free fructose and glucose concentrations. In fact, glucose and fructose concentrations increased in all the media during the running time, attaining elevated levels (between 50–80 g/l and 12–27 g/l, for glucose and fructose, respectively) after 48 h fermentation. Similar decrease on FOS yield was observed by Sánchez et al. [30] during the sucrose fermentation by *Aspergillus* sp. N74 in a mechanically agitated airlift reactor.

Among the evaluated carriers, cork oak provided the lowest immobilized cells concentration although the FOS production was similar to those achieved by free cells system (Table 2). This means that even with the lowest immobilized cells concentration, *A. japonicus* was able to produce FOS with capacity similar to that of free cells. All the other evaluated carriers gave highest immobilized cells concentration than cork oak, and the FOS production was at least similar to that achieved in the free cells assay. Corn cobs and coffee husks can be detached among the other carriers since they immobilized the major amounts of cells and yielded the highest FOS concentrations. Nevertheless, cells immobilized in corn cobs attained the maximum FOS production in a shorter time 21 h), giving as a consequence the highest value of FOS productivity (6.61 g/l h). Such result was 23.3% higher than that achieved in the free cells assay. In fact, it has been demonstrated for different fermentation systems the productivity increase by using immobilized cells [12,13]. For FOS production, particularly, Chien et al. [6] also observed that the larger the amount of cell-immobilized preparation, the faster the reaction rate and, consequently, a shorter time was necessary to reach the maximum FOS concentration in the reaction mixture. In the present work, the FOS production was also faster in the medium containing a larger quantity of immobilized cells (Table 2).

All evaluated media gave FOS yield ($Y_{P/S}$) at least similar to that of free cells system (Table 2), independently of the carrier used for cells immobilization. Such yields were also similar or higher than the maximum theoretical normally found for FOS production by microorganisms (55–60%, w/w) [3,4]. Once more, the highest values were attained from cells immobilized in corn cobs and coffee husks. When considering only the consumed substrate, the $Y_{P/S}$ value attained for cells immobilized in corn cobs was 14% higher than that obtained by free cells. Such difference was statistically significant, as shown in the analysis of variance (ANOVA) given in Table 3.

The ANOVA decomposes the variance of the data into two components: a between-group component and a within-group component. The *F*-ratio, which in this case equals 22.59, is a ratio of the between-group estimate to the within-group estimate. Since the *p*-value of the *F*-test is less than 0.05, there is a statistically significant difference between the means of $Y_{P/S}$ for the 7 fermentations, at the 95.0% confidence level. To determine which means are significantly different from which others, a multiple comparison procedure was performed (Table 4), and the Fisher's least significant difference (LSD) procedure was the method used to discriminate among the means. At the top of Table 4, note that 5 homogenous groups were identified using columns denoted with letters. Within each column, the levels containing similar letters form a group of means within which there are no statistically significant differences. The bottom half of the output in Table 4 shows the estimated difference between each pair of means. The asterisk placed next to the pairs indicates that these pairs show statistically significant differences at the 95.0% confidence level. It can be thus easily observed in this table that the FOS production yield was statistically similar among various carrier groups. Nevertheless, the value

Table 3
Analysis of variance (ANOVA) for the FOS yield during the sucrose fermentation by *A. japonicus* immobilized in different lignocellulosic materials and using free whole cells[a].

Source	Sum of squares	DF	Mean square	*F*-ratio	*p*-value
Between groups	0.0126	6	0.0021	22.59	0.0003
Within groups	0.0007	7	0.0001		
Total (Corr.)	0.0132	13			

[a] FOS yield regarding the consumed substrate.

Table 4
Multiple comparison procedure to discriminate among the FOS yield means obtained during the sucrose fermentation by *A. japonicus* immobilized in different lignocellulosic materials and using free whole cells[a].

Immobilization carrier	Mean	Homogeneous group
Free whole cells (FHC)	0.635	a
Brewer's spent grain (BSG)	0.650	ab
Wheat straw (WS)	0.655	abc
Coffee husks (CH)	0.670	bcd
Cork oak (CO)	0.675	cd
Loofa sponge (LS)	0.685	d
Corn cobs (CC)	0.735	e

Contrast	Difference	+/− Limits
BSG–LS	−0.035*	0.023
BSG–CH	−0.020	0.023
BSG–CO	−0.025*	0.023
BSG–FHC	0.015	0.023
BSG–CC	−0.085*	0.023
BSG–WS	−0.005	0.023
LS–CH	0.015	0.023
LS–CO	0.010	0.023
LS–FHC	0.050*	0.023
LS–CC	−0.050*	0.023
LS–WS	0.030*	0.023
CH–CO	−0.005	0.023
CH–FHC	0.035*	0.023
CH–CC	−0.065*	0.023
CH–WS	0.015	0.023
CO–FHC	0.040*	0.023
CO–CC	−0.060*	0.023
CO–WS	0.020	0.023
FHC–CC	−0.100*	0.023
FHC–WS	−0.020	0.023
CC–WS	0.080*	0.023

[a] FOS yield regarding the consumed substrate.
* Denotes a statistically significant difference.

achieved with cells immobilized in corn cobs was totally different of the values obtained in the other fermentations.

To summarize, based on the criteria of cells adhesion, FOS and FFase production, and U_t/U_h ratio, corn cobs was the best support material for the immobilization of *A. japonicus*. This fact would suggest that materials with low CHP can be better for cells immobilization than materials with high WAI, although both properties are required to have good cells adhesion to the carrier, and to facilitate the species growth and development.

4. Conclusions

According to the obtained results, it can be concluded that the use of *A. japonicus* ATCC 20236 immobilized in corn cobs may have potential for industrial application in FOS production, because high amounts of cells adhered to this material and produced FOS and FFase enzyme with high productivity and U_t/U_h ratio. It is evident that optimization of the nutritional and process parameters is useful for establishing a process with a long operational life. Nevertheless, the results achieved in the present work, without any optimization study, are very promissory and give us a positive expectative to maximize FOS production at industrial level.

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References

[1] S.I. Mussatto, I.M. Mancilha, Carbohyd. Polym. 68 (2007) 587–597.
[2] P.T. Sangeetha, M.N. Ramesh, S.G. Prapulla, Trends Food Sci Tech. 16 (2005) 442–457.
[3] J.W. Yun, Enzyme Microb. Technol. 19 (1996) 107–117.
[4] K. Nishizawa, M. Nakajima, H. Nabetani, Food Sci. Technol. Res. 7 (2001) 39–44.
[5] K.J. Duan, J.S. Chen, D.C. Sheu, Enzyme Microb. Technol. 16 (1994) 334–339.
[6] C.-S. Chien, W.-C. Lee, T.-J. Lin, Enzyme Microb. Technol. 29 (2001) 252–257.
[7] A.K. Balasubramaniem, K.V. Nagarajan, G. Paramasamy, Process Biochem. 36 (2001) 1241–1247.
[8] Ghazi, A. Gómez de Segura, L. Fernández-Arrojo, M. Alcalde, M. Yates, M.L. Rojas-Cervantes, F.J. Plou, A. Ballesteros, J. Mol. Catal. B: Enzym. 35 (2005) 19–27.
[9] P.T. Sangeetha, M.N. Ramesh, S.G. Prapulla, J. Food Eng. 68 (2005) 57–64.
[10] R.C. Fernandez, C.A. Ottoni, E.S. Silva, R.M.S. Matsubara, J.M. Carter, L.R. Magossi, M.A.A. Wada, M.F.A. Rodrigues, B.G. Maresma, A.E. Maiorano, Appl. Microbiol. Biotechnol. 75 (2007) 87–93.
[11] M. Fenice, R.D. Giambattista, E. Raetz, J.-L. Leuba, F. Federici, J. Biotechnol. 62 (1998) 119–131.
[12] Y. Cohen, Bioresour. Technol. 77 (2001) 257–274.
[13] J.C. Santos, S.S. Silva, S.I. Mussatto, W. Carvalho, M.A.A. Cunha, World J. Microbiol. Biotechnol. 21 (2005) 531–535.
[14] S.V. Ramakrishna, R.S. Prakasham, Curr. Sci. 77 (1999) 87–100.
[15] J.C. Santos, S.I. Mussatto, G. Dragone, A. Converti, S.S. Silva, Biochem. Eng. J. 23 (2005) 1–9.
[16] M. Skowronek, J. Fiedureck, Enzyme Microb. Technol. 38 (2006) 162–167.
[17] S.S. Silva, S.I. Mussatto, J.C. Santos, D.T. Santos, J. Polizel, Appl. Biochem. Biotechnol. 141 (2007) 215–227.
[18] R. Cruz, V.D. Cruz, M.Z. Belini, J.G. Belote, C.R. Vieira, Bioresour. Technol. 65 (1998) 139–143.
[19] R.A. Anderson, H.F. Conway, V.F. Pfeifer, E. Griffin, Cereal Sci. Today 14 (1969) 11–12.
[20] W. Horwitz (Ed.), AOAC—Association of Official Analytical Chemists, Official Methods of Analysis of the Association of Official Agriculture Chemistry, Washington, 1980.
[21] C. Dorta, R. Cruz, P. Oliva-Neto, D.J.C. Moura, J. Ind. Microbiol. Biotechnol. 33 (2006) 1003–1009.
[22] W.-C. Chen, Process Biochem. 33 (1998) 267–271.
[23] J. Yoshikawa, S. Amachi, H. Shinoyama, T. Fujii, FEMS Microbiol. Lett. 265 (2006) 159–163.
[24] L.G. Dias, A.C.A. Veloso, D.M. Correia, O. Rocha, D. Torres, I. Rocha, L.R. Rodrigues, A.M. Peres, Food Chem. 113 (2009) 246–252.
[25] M.H. Gómez, J.M. Aguilera, J. Food Sci. 48 (1983) 378–382.
[26] L.-M. Wang, H.-M. Zhou, J. Food Biochem. 30 (2006) 641–658.
[27] M. Hirayama, N. Sumi, H. Hidaka, Agr. Biol. Chem. 53 (1989) 667–673.
[28] L. L'Hocine, Z. Wang, B. Jiang, S. Xu, J. Biotechnol. 81 (2000) 73–84.
[29] W.-C. Chen, C.-H. Liu, Enzyme Microb. Technol. 18 (1996) 153–160.
[30] O. Sánchez, F. Guio, D. Garcia, E. Silva, L. Caicedo, Food Bioprod. Process 86 (2008) 109–115.