1
2 **Enhancement glucose oxidase production by solid-state fermentation**
3 **of *Aspergillus niger* on polyurethane foams using mussel processing**
4 **wastewaters.**

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19 **ABSTRACT**

20 In the present work, studies of glucose oxidase (GOD) production in solid-state
21 fermentation on polyurethane foams, using mussel processing wastewaters (MPW) as
22 culture media, were carried out. Initially, the results generated in this experimental
23 modality were compared with those that were obtained in conventional submerged
24 fermentation. Later on, cultures were tested in solid-state with fed-batch performance,
25 defining suitable conditions of operation. These conditions were finally corroborated in
26 experiments with a bioreactor of own design, in which GOD production was improved
27 13 times regarding to the initial cultures.

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29 **Keywords:** Glucose oxidase production; *Aspergillus niger*, solid-state fermentation;
30 solid-state bioreactor; polyurethane foams; mussel processing wastewaters.

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INTRODUCTION

Solid-state fermentation (SSF) is a microbial process that is developed on a solid substrate in absence of free water [1-4]. The origin is linked to the elaboration of numerous fermented products from oriental culinary traditions as, for example, *koji* and *sake*. Thus, the fact that SSF is an especially capable process for the conversion of materials from agricultural products and wastes (flour, vineshoots, straw or wood) together with the simplicity and economy of the bioreactors, and the high quantity of advantages in relation to the submerged fermentation for the microbial production of certain metabolites [5-7], it has motivated in the last years, a new demand in their uses and applications [8-14], as well as an increasing interest in the formal study of the most relevant operational variables for control process [15-20].

One of the biggest problems in SSF scale-up is the absence of commercial designs of solid-state bioreactors due to the difficulty in the standardization of the bioprocess (presence of temperature and humidity gradients of difficult control and correction throughout the fermentation process) and to the limited reproducibility of the experimental results [21,22]. Among the bioreactors used in this kind of cultivation we find out, basically, 3 types: rotating drum, trays and fluidized and packed beds; being classified these ones, according to the aeration and mixture conditions during the fermentation [23,24]. From a historical point of view, tray bioreactor was the most employed due to its simplicity and easy operation [25]. It has the disadvantage derived from the complete heterogeneity of the process, the necessity of large work areas and the only possibility of batch culture operation. Rotating drum bioreactors have been designed with the purpose to maximize the mixture increasing, therefore, the homogeneity of the system and maintaining a perfect aeration of the fermentation [3,25]. Packed bed bioreactor is the most productive due to the homogeneity of the content and the high dissipation of the metabolic heat [26-28]. However, it provokes a

high consumption of sterile water and electricity to compensate the high evaporation and to maintain the fluidity in the process. Moreover, the microbial growth is not homogeneous [2].

On the other hand, glucose oxidase (GOD) is an oxidoreductase enzyme that catalyses the oxidation of β -D-glucose to D-glucono- δ -lactone and hydrogen peroxide, with molecular oxygen as electron acceptor. It is one of the most used bio-catalyst in the alimentary and pharmaceutical fields, as well as the essential component of the biosensors for glucose determination [29-31]. Its production is basically obtained by fermentation, generally by means of *Aspergillus niger* [32-35]. It is also one of the bioproductions with higher economical interest in the depuration and upgrade to the mussel processing wastewaters (MPW). This effluent, generated in the thermal treatment of mussel, is an important factor of pollution and eutrophication in Galician Rias (NW, Spain) due to its large volume of water dumping into the sea with high concentration in organic matter [36]. In previous articles, solid-state fermentation using concentrated MPW on polyurethane foams led to minimal technical, energetic and economical requirements as well as yields increasing on the amylase [37], gibberellic acid [38], citric acid [39] and bacteriocin [40,41] productions.

Based on these considerations, in the present work the feasibility of MPW for GOD production, comparing submerged and solid-state fermentations, is initially studied. Furthermore, a pre-industrial solid-state bioreactor, designed in our laboratory, was used with intermittent fed-batch operation obtaining higher levels of enzymatic activity and more efficiency in the MPW consumption than submerged fermentation.

MATERIALS AND METHODS

Microbiological methods, culture media and experimental units

The GOD-producing strain used was *Aspergillus niger* (CBS 554-65). The composition of the MPW and their treatment for the preparation of the culture media were previously described in detail [42,43]. The media used in this bioproduction were:

G-01: Used in the preliminary cultivations and following the modification carried out by Munk et al. [44], consisted in a 6M MPW, that is, 60 g l⁻¹ of total sugars, chemical oxygen demand (COD): 15 gO₂ l⁻¹ and 2.10 g l⁻¹ of proteins (0.34 g l⁻¹ of N and 0.03 g l⁻¹ of P). This MPW substrate was supplemented with 13.88 g l⁻¹ of Ca(NO₃)₂·4H₂O (1.65 g l⁻¹ of N), 2.10 g l⁻¹ of KH₂PO₄ (0.47 g l⁻¹ of P), 0.10 g l⁻¹ of MgSO₄·7H₂O and 60 mM of CaCO₃.

G-02: It was defined with 6M medium, as basic formulation, supplemented with 11.90 g l⁻¹ of CaCO₃ (119 mM), 0.36 g l⁻¹ of KH₂PO₄ (0.10 g l⁻¹ of P), and 0.10 g l⁻¹ of MgSO₄·7H₂O. In this case, the only concentration of N (0.34 g l⁻¹) was contributed by the protein source of MPW medium.

G-03: This medium was formulated for GOD production in the solid-state bioreactor taking into account the optimal concentrations of N and P obtained in Mirón et al. [35]. Thus, 12M solution (120 g l⁻¹ of total sugars; COD: 30 gO₂ l⁻¹) with 2.20 g l⁻¹ of proteins (0.35 g l⁻¹ of N, 0.03 g l⁻¹ of P) was supplemented with 6.3 g l⁻¹ of Ca(NO₃)₂ (0.75 g l⁻¹ of N), 0.76 g l⁻¹ of KH₂PO₄ (0.17 g l⁻¹ of P) and 250 mM of CaCO₃.

Initial pH was adjusted to 7.0 and sterilized at 121°C for 15 min. The inocula consisted of a spore suspension at the concentration required to obtain an initial population of 6.75 × 10⁶ spores ml⁻¹. Experimental conditions were:

Submerged fermentation (SF): In 250 ml Erlenmeyer flasks with 50 ml of G-01 broth. Incubations were carried out at 30°C with orbital shaking at 200 rpm.

Batch solid-state fermentation (bSSF): In 50 ml Erlenmeyer flasks loaded with 0.26 g of polyurethane foams ($\varnothing = 5$ mm, $\rho = 40$ g dm⁻³) and 2.96 ml of liquid phase using G-01 medium (11.4 ml g⁻¹, equivalent to 50% of saturation) with the corresponding concentration of spores early indicated. Incubations were carried out at 30°C in a closed water-bath, to avoid evaporation of liquid phase, and shaking the experimental units manually each 12 h to avoid possible compaction due to micellar growth.

Solid-state fermentation with fed-batch (fbSSF): In 250 ml Erlenmeyer flasks loaded with 0.3 g of polyurethane foams ($\varnothing = 5$ mm, $\rho = 20$ g dm⁻³). Using the same proportion of liquid phase than previous case (11.4 ml g⁻¹; 3.42 ml of G-02 medium each experimental unit), 25% of saturation was obtained. The experimental estimate of evaporation in this system was of 0.175 ml h⁻¹. Thus, and ignoring the possible variations due to the microbial growth, it is necessary to consider that the addition of 2.1 ml of liquid phase every 12 h it would maintain a 17% of average saturation with a variation between 9.6 and 25%. These feedings entail, by experimental unit, an intake of 126 mg of glucose each 12 h and a capacity for neutralizing the production of 98.8 mg of gluconic acid (73% of potential production) in the same period of time.

Solid-state fermentation in bioreactor (SSF): The experimental unit was a bioreactor of 20 l with 50 g of inert support consisting of cylindrical particles (8 × 8 mm) of polyurethane foams ($\rho = 20$ g dm⁻³), with a 75% of occupation coefficient of the bioreactor volume. Incubations were carried out at 30°C. The remainder of experimental

conditions are summarized in Table 1. The solid-state bioreactor used for GOD production presented the following features:

1: A structure constituted by a cylindrical tube of glass ($\varnothing=16$ cm and length=100 cm) with hermetic catch by means of lids of rigid nylon. This cylinder is externally covered by an electric resistance in band, regulated by a thermostat for control and maintenance of fermentation temperature. A series of entrances are situated along its top longitudinal line. These holes are employed as follows: a) entry of fresh medium dosed by means of a multiple peristaltic pump; b) aeration entrances connected to a compressor with exit pressure controlled by a manometer and flow measured using flow meter; c) exits for aeration and metabolic gases, with filter of glass wool for the retention of spores; d) entrances for temperature and humidity sensors. A little number of exits is also disposed along its bottom longitudinal line.

2: The lateral lids sustain a longitudinal central axis connected to an engine with rhythm operation and adjustable speed. The axis has internally a single lead worm divided in two halves with steps of opposite directions and separated by a dead central space. Culture homogenization is achieved by means of two scrapers with neoprene border, situated each one of them on a metallic bar fixed to two external disks that rotate due to the engine action. The scrapers also allow longitudinal displacement of two internal disks, capable of reciprocal displacement due to be coupled to the thread of the axis. Furthermore, they work as pistons in the extrusion process of polyurethane foams for the products recovery. The extreme situation of the external disks can be fixed in different positions in order to vary the effective volume of the bioreactor.

3: Two kinds of bolts that work as clutches coupling or uncoupling the turn transmission of the axis to the two types of disks. Thus, two operation stages are produced:

164
165 a) Incubation stage: the turn of the axis is transmitted to the external disks and their
166 corresponding turn to the scrapers.

167
168 b) Extrusion-expansion stage: the turn of the axis is not transmitted to the external disks
169 but it generates the mutually approach of the internal disks. When extrusion is finished,
170 the turn of the engine is reversed until the return of the disks to the incubation position.

171

172 ***Extraction of the samples***

173 At pre-established times, the samples were taken using the following protocols:

174
175 *Submerged fermentation* (SF): The total content of each experimental unit was filtered
176 through glass fibre filters, dividing the sample in two aliquots: mycelium and medium.
177 The mycelium was rinsed in distilled water and dried to constant weight at 108°C. This
178 weight was used as an estimation of the biomass. The filtered medium was filtered
179 again through membranes of cellulose acetate of 0.8 µm and used for the
180 corresponding analytical determinations.

181
182 *Batch solid-state fermentation* (bSSF): As in the previous case, the whole of the
183 experimental unit was repeatedly washed with distilled water by means of extrusion in a
184 syringe until a total volume of 5 times the initial. Subsequently, it was centrifuged at
185 4,000 g for 20 min. The supernatants were employed for analytical measures.

186
187 *Solid-state fermentation with fed-batch* (fbSSF): A similar protocol of extraction than
188 bSSF was carried out, but in this case, sterile conditions were maintained in the
189 extrusion process with the subsequently recharge of sterile fresh medium.
190 Postincubated samples were similarly handled than bSSF.

Solid-state fermentation in bioreactor (SSF): In sterile conditions, the load of the bioreactor was evacuated by compression of the internal disks and extrusion of the solid support (polyurethane foams), thus obtaining ~95% of the incubated medium. The system was subsequently recharged with sterile fresh medium. Postincubated media was centrifuged under the same conditions than bSSF and the corresponding supernatants analyzed.

Analytical methods

Glucose was estimated by means of 4-aminoantipirina reaction (Lee et al., 1970). Citric acid was measured by the spectrophotometric method of Marrier and Boulet [45]. For GOD determination was used the method of Lloyd and Wheland [46] taking into account the modification carried out by Fiedurek et al. [47]. Gluconic acid was estimated by dehydrogenase phosphogluconate method [48]. The determination of the total nitrogen was carried out with the method of Havilah et al. [49] applied to digests obtained by the classic procedure of Kjeldahl. Finally, protein-nitrogen was determined by the product $6.25 \times \text{nitrogen}$, being estimated the level of soluble proteins following Lowry method [50]. All analyses were carried out in duplicate.

Numerical methods

Fitting procedures and parametric estimations calculated from the experimental results were carried out by minimisation of the sum of quadratic differences between observed and model-predicted values, using the non linear least-squares (quasi-Newton) method provided by the macro Solver of the Microsoft Excel XP spreadsheet.

RESULTS AND DISCUSSION

Preliminary tests. Submerged fermentation (SF) versus solid-state fermentation (bSSF)

The aim of these preliminary experiments was to compare GOD and gluconic acid productions in two cultivation modalities: submerged and solid state (with polyurethane foams as solid support) fermentation, both in batch culture.

In the case of bSSF, the difficulties to obtain accuracy determinations of biomass (in particular using experimental units of low volume), it gave rise to dispense with these values. The results showed in Figure 2 revealed that:

1: In the experimental conditions specified, bSSF was faster than SF in both senses, consumption of nutrients and production of metabolites.

2: In both modalities, protein-nitrogen consumptions were similar, reaching quickly an asymptotic value equivalent to 75-85% of the initial level. This behaviour was in agreement with previous results [51-53] in which the proteins of MPW were not completely consumed, showing that a fraction of these not was susceptible of use as nitrogen source. For all tested fungi, the fraction of “nitrogen useful” represented around 75% of the initial concentration in the effluent.

In these circumstances, it seems clear that the difference of uptakes among the two cultures comes given by the differences in the intake of inorganic nitrogen, much quicker in the case of bSSF, and without tendency to the stabilization in the final phase of the incubation as it can be showed in SF.

3: In both cases citric acid production is obtained after to achieve the maximum gluconic acid production. The well-known associations between increase of citric

production drop of gluconic production and acid pH values [54-56] are clearly showed in SF but less clear in bSSF. The biomass profile in SF indicates that an important conversion of gluconic acid to biomass is established from ~40 h. Though biomass quantification in bSSF was not employed, gluconic profile would allow to suppose that similar conversion could also happen in this case. However, pH kinetic shows significant differences in both fermentations with an asymptotic phase at 4.5 from 20 hours in bSSF. A possible explanation of this behaviour comes suggested by the higher rate of inorganic nitrogen uptake in bSSF that could lead to a faster process of reduction from N-NO₃ to N-NH₂ with the corresponding consumption of protons.

4: Though GOD maximum productions were not significantly different in both cultures, the conversion of glucose in gluconic was much more efficient in bSSF. Probably, this higher efficiency can not be exclusively attributed to pH profile and it could be related with physical factors in bSSF as, for instance, its lower restrictions to gases transfer.

5: Taking into account the stoichiometric relationships in the catalytic conversion glucose → gluconic acid → calcium gluconate, 1 g of Ca⁺² can neutralize 9.79 g of gluconic acid. Thus, the concentration of Ca⁺² in the culture medium (60 mM; 2.41 g l⁻¹) could neutralize a maximum concentration of 120 mM (23.54) of gluconic acid. However, the highest production was obtained at 16.5 g l⁻¹ therefore it is difficult to suppose that Ca⁺² effects could represent a restrictive factor under the experimental conditions used.

6: Finally, regarding to the specificity of bSSF it can be pointed out that Ramesh and Lonsane [57] observed a significant reduction of the catabolic repression of α-amylase production by *Bacillus licheniformis* in SSF. The authors attribute the responsibility from this phenomenon to the lower rate of nutrients and metabolites diffusion under the

characteristic conditions of low water activity defined by SSF. Although in this work a bacterium is used, our results suggest, on the contrary, that the specificity of SSF seems to be associated with the higher metabolic rate, possibly depending on the factors that facilitate the oxygen transfer.

Solid-state fermentation with fed-batch (fbSSF)

In general terms, the results obtained in this modality showed a very scarce improvement with regard to the previous batch fermentation. Even so, these results suggest, at the same time, that fed-batch culture can be performed with a higher efficiency if glucose necessities are supplemented. In addition, the possibility of inhibition by product can be suspected in this bioconversion.

Experimental data (Figure 3) show that enzyme production rate drops from 36 h whereas apparent GOD values remain constant until the end of the culture. Similar tendency was reached by gluconic acid production. These experimental data were fitted to asymptotic equations of hyperbolic and exponential negative type:

$$X = \frac{K \cdot t}{b + t} \quad (1)$$

t : time
 X : response
 K : maximum response
 b : time of semi maximum response

$$X = K + a \cdot \exp(-\mu \cdot t) \quad (2)$$

t : time
 X : response
 X_0 : initial value of response
 K : maximum response
 $a = X_0 - K$
 μ : specific rate

In both cases, the highest correlations between observed and predicted values ($r^2_{\text{GOD}}=0.952$; $r^2_{\text{GnH}}=0.982$) were obtained with the exponential model. As its numerical

derivatives are more easy-to-handle, the theoretical values (continuous line in Figure 3) were used for the calculation of the corresponding production rates.

An interpretation of the dynamics observed in the enzyme production could rely on the fast drop of glucose concentration (from 62.7 g l⁻¹ to stabilized value of ~3.5 g l⁻¹ at 36 h). Under these conditions, it would be necessary to suppose that GOD production rate (a primary metabolite) is a direct consequence of the moderate increase in the biomass production rate that it also promotes a moderate concentration of substrate.

Keeping in mind that an important amount of the glucose added in the fed-batch culture was transformed to gluconic, an elementary balance indicates that substrate consumed for biomass maintenance followed a constant slope with the time-course of the fermentation. The latter revealed that biomass remained active throughout incubation period (gluconic increase could simply be due to the initial enzyme activity in the first hours of culture). If, in spite of such an activity, the net increase of GOD was null from ~36 h, it could be accepted that the microorganism used the excess of glucose from the fermentative process in the compensation of osmotic problems associated to the progressive increase of salts and gluconic concentration in the medium. This dynamic reduces step by step the fraction of substrate that could be employed for growth.

The falling tendency in the GOD production rate could admit a complementary interpretation that also resorted to the low levels of GOD achieved. However, this low production of enzyme should be enough to promote a significantly higher catalysis. A plausible justification of this discrepancy could be sustained by the high concentration of the metabolic products, with the corresponding drop of water activity, that especially at the end of the culture they would suppose strong restrictions to the solutes diffusion.

Another explanation, not necessarily excluding to the first one, it would be the possibility that gluconic acid accumulation induces inhibition by product.

A tentative evaluation of this hypothesis can be obtained by fitting numerical data to the Michaelis-Menten equation with competitive mechanism of inhibition by gluconic acid:

$$V = \frac{V_m \cdot S}{K_m \cdot (1 + K_i \cdot I) + S} \cdot E \quad (3)$$

where: V : gluconic acid production rate
 S : substrate concentration (glucose)
 I : inhibitor concentration
 E : enzyme concentration
 V_m : maximum rate
 K_m : Michaelis-Menten constant
 K_i : inhibition constant

and comparing real rate of gluconic production with the expected rate taking into account the values of E , S and I .

Using quasi-Newton method in the minimization of the sum of quadratic differences between observed and predicted values according equation (3), the obtained results are shown in Figure 4 (right). Although at initial times the fit seems reasonable, the divergence increase with incubation time. This lack of fit is higher when other kinetic suppositions are tested (for example, non-competitive and uncompetitive inhibition). Apart from this, the numerical value of the V_m , K_m and K_i parameters was not incoherent but not very realistic, at least if they are compared with the kinetic features of GOD in solution [58]. It is clear, therefore, that the supposed inhibition by product does not explain totally the fall in the gluconic production rate and its existence should be further studied with a more specific experimental design.

Thus, experimental results suggest that fbSSF process could improve its efficiency maintaining the criterion of compensating the evaporation with the addition of culture broth but inserting the following modifications:

1: Feeding increase with glucose until the concentration of half-consumption detected in the first ~36 h.

2: Reduction of the addition intervals to ~6-12 h in order to diminish the width of the oscillation in the saturation percentage.

3: Extrusion and wash of the support to intervals of ~24-48 h with reinitiate without fresh inoculum, maintaining the operation as long as a decreasing in the production is not detected.

Solid-state fermentation in the bioreactor (SSF)

In Table 1 experimental conditions of SSF are summarized. The position of the external disks was fixed with the purpose that the support load was approximately equivalent to 75% of the effective volume in order to avoid possible difficulties in the movement of the scrapers.

Starting with a liquid phase equivalent to 40% of saturation, the operatory stage was principally directed to maintain that level, compensating by evaporation a feeding of 7 ml g⁻¹day⁻¹. Using a 12M medium, this feeding implies an entrance of 0.85 g g⁻¹day⁻¹ of glucose. This flow is sensibly lower than the half-consumption of the fermentation (conversion + consumption ~2.5 g g⁻¹day⁻¹), therefore, it makes foresee a slower process of those previously described but, however, more suitable to identify possible deviations of the process. After to verify the linearity between aeration flow and

evaporation rate in a wide interval of values (data not shown), the initial level of aeration flow was established in 20 l min⁻¹. This value was enough to determine the evaporation of liquid phase equivalent to which is inserted with the feeding.

The process was carried out with intermittent extrusion each 12 h, followed of washes every 24 h which were performed by means of the addition of sterile distilled water in the moment of maximum compression of the support. Subsequently, the expansion was completed and another compression was carried out in order to collect the postincubated medium that flows towards the bottom exists before to reload the system with a volume of fresh medium equivalent to the extracted one. The assembly of this experimental system on a laboratory scale allowed to obtain, in all moment, exact approaches of the liquid present phase.

Figures 5 and 6 depict the experimental results of these fermentations. Firstly, it should be pointed out that though the first samples showed the insufficient evaporation of the liquid phase, we did not decided to alter the experimental design and to continue with the system until its derive. The causes of this deviation could be due to the fact that the calibration of the evaporation carried out with support and water in absence of nutrients and biomass, does not exactly reproduce the real condition in the bioreactor.

The gluconic acid formation and the corresponding accumulation of calcium gluconate was very intensive with what its elimination would require more energetic washes that the strictly necessities for enzyme extraction. This accumulation was also the cause of pH drop that it was more pronounced than previous cultivation, reaching a value of stabilization not very appropriate for GOD production with the subsequent fall in the yield.

Finally, the moderate feeding with glucose not only led to a slower dynamic, but rather it was clearly insufficient (see Figure 6) with low levels at 30 h that continued falling in a progressive way until almost exhaustion at 90 h.

Even so, and in spite of this whole of unfavourable conditions, it should be pointed out that GOD production for support unit was of $8 \text{ EU g}^{-1} \text{ h}^{-1}$ for the first 48 h of incubation. This value means more than double of the maximum production (at 20 h) and productive period as well as the multiplication of results obtained in fbSSF by a factor of 13. On the other hand, it proved that the scale-up, bioreactor multiplied by a factor of ~160 the flasks volume, does not change process dynamics if the relationships among basic variables are maintained constants. Moreover, this system was robust and its productive yield could be improved replacing, as much as possible, carbonate addition by NaOH in the feeding medium in order to help the buffer capacity of its protein concentration. Further experiments should be made taking into account these considerations.

CONCLUSIONS

The main conclusions generated in the present work can be summarised in the following points:

1: It was proven that mussel processing wastewaters (MPW), partially saccharified by enzymatic methods, constitutes a suitable medium for *Aspergillus niger* fermentation in both modalities, submerged and solid state, in order to produce glucose oxidase (GOD) and gluconic acid.

2: Solid state fermentation of *Aspergillus niger* on polyurethane foams soaked with MPW constitutes a better, simpler and faster modality of culture in comparison to

submerged one. This system improved the productivity of the fermentative process and facilitated the fed-batch as well as the recovery of the final products by extrusion.

3: Finally, these results were applied to the design and construction of a cylindrical bioreactor of 20 l with continuous aeration, with discontinuous and coupled feeding and mixture as well as unloading by extrusion and intermittent wash without fresh inoculum. In these conditions, experimental scale was multiplied by a factor of ~160 maintaining similar metabolic behaviour and increasing preliminary productions by a factor of ~13.

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FIGURE CAPTIONS

Figure 1: Schematic diagram of solid state bioreactor. S: scrapers of homogenization; ED: external disks, base for the subjection of the scrapers and together with them constitute the skeleton of the system; ID: internal disks with reciprocal movement along the axis of the bioreactor for the extrusion of the support; A: entrances and exists of aeration; B: entrances for fresh medium; C: holes for the recovery of the product.

Figure 2: Comparison between the results obtained in SF (white symbols) and in bSSF (black symbols) by *Aspergillus niger*. B ($\square$): biomass in SF; G: glucose; GnH: gluconic acid; CIT: citric acid; GOD: glucose oxidase; Nc: nitrogen uptake (∇ $\blacktriangledown$: nitrogen-protein; $\bigcirc$ $\bullet$: total nitrogen). Error bars are the confidence intervals ($\alpha = 0.05$, $n = 3$).

Figure 3: Global balances of fbSSF (extensive values, per experimental unit –eu–). Left: GOD production with fit to the equation (1) in continuous line. Right: point line: total added glucose; $\square$: total glucose uptake; $\bigcirc$: gluconic production (fit to the equation (2) in continuous line); ∇ : glucose uptake per biomass production. Error bars are the confidence intervals ($\alpha = 0.05$, $n = 3$).

Figure 4: Gluconic acid production rates. The continuous line to the right shows the profile of the predicted values to the GOD, glucose and gluconic acid concentrations supposing Michaelis-Menten kinetic with inhibition by product, according to the equation (3).

Figure 5: SSF results. L: liquid phase per gram of support; G and GOD: glucose and glucose oxidase production per unit of volume of liquid phase.

Figure 6: SSF results. ΣG : accumulate value of added glucose ($\square$) and consumed (∇) per g of support. ΣGOD : accumulate value of GOD production per g of support. GOD: GOD production rate per intervals ($\bullet$) and per period average ($\bigcirc$). GOD/Gc: yield of GOD production on consumed glucose per intervals ($\bullet$) and per period average ($\bigcirc$).

TABLES

Table 1: Experimental conditions in the biorreactor with 50 g of polyurethane foams as support.

CONDITIONS		
	Bioreactor	Rotation: periods of 5 minutes (3 rpm) per hour
	Support	50 g ($\rho=20 \text{ g dm}^{-3}$; $\varnothing= 8 \text{ mm}$) ~ 75% of the total capacity
	Aeration flow	20 l min^{-1}
	Saturation	Expected: 40 %.
G-03 MEDIUM		
		12M
	N	1.1 g l^{-1} of N as $(\text{CaNO}_3)_2 \cdot 4\text{H}_2\text{O}$
	P	0.2 g l^{-1} of P as KH_2PO_4
	Ca	250 mM CaCO_3
	Relationships C/N and C/P	43 and 240
FEEDING		
	Average flow	$7 \text{ ml g}^{-1} \text{ day}^{-1}$
	Glucose	$0.85 \text{ g g}^{-1} \text{ day}^{-1}$
WASH		
		200 ml of sterile distilled water

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6