ELSEVIER

0032-9592(95)00004-6

Process Biochemistry, Vol. 30, No. 8, pp. 751–756, 1995

0032-9592/95 $9.50+0.00

Influence of Growth and High Mould Concentration on the Pressure Drop in Solid State Fermentations

Richard Auria,[a,b] Irmene Ortiz, Elba Villegas & Sergio Revah[a]*

[a]Departamento de Ingeniería de Procesos e Hidráulica, Universidad Autónoma Metropolitana- Iztapalapa, Apdo. Postal 55-534, CP 09340 México D.F.
[b]ORSTOM (Institut Français de Recherche Scientifique pour le Développement en Coopération)

(Received 30 August 1994; revised manuscript received 20 December 1994; accepted 8 January 1995)

Aspergillus niger *was grown on Amberlite IRA-900 imbibed with a solution containing high concentrations of sucrose* (S_i = *100, 200, 300 and 400 g/litre) in static aerated fermentors. Growth was followed in dry biomass, biomass protein,* CO_2 *production and pressure drop* (DP). *The* DP *allowed the monitoring of germination, vegetative growth, limitation and the onset of sporulation for the four concentrations of sucrose studied. Concentrations up to 103 mg dry biomass/g dry support were obtained with* S_i = *400 g/litre and these reduced the relative intrinsic permeability to 0·0125. Under this condition the mould occupies 34% of the free space.* DP *increase was related to* CO_2 *production.*

NOTATION

A	Bed cross sectional area (cm^2)
DP	Pressure drop between the inlet and the outlet of the porous bed (mm H_2O)
g	Acceleration due to gravity (cm/s^2)
k	Intrinsic permeability (cm^2)
k_{in}	Initial intrinsic permeability (cm^2)
L	Bed length (cm)
Q	Volumetric flow rate (cm^3/s)
U	Q/A, superficial fluid velocity (cm/s)
V_p	Interparticular volume (cm^3)
V_x	Wet volume biomass (cm^3)
W	Support water content (g/g)
W_s	Biomass dry matter content (g/g)
X	Dry biomass concentration (g/g)

Greek symbols

ε_o	Initial interparticular porosity (cm^3/cm^3)
μ	Fluid dynamic viscosity (g/cm.s)
μ_x	Specific growth rate (h^{-1})
$\rho_s = \rho_x W_s$	Apparent dry biomass density (g dry biomass/cm^3 wet biomass)
ρ_h	Apparent wet support density (g/cm^3)
ρ_x	Wet biomass density (g/cm^3)
ρ_w	Water density (g/cm^3)

INTRODUCTION

Solid substrate fermentation (SSF) has been extensively studied for many microorganisms and their products.[1] SSF can be defined as a four phase system which consists of: (i) a gas which is static or flows through (ii) a solid water insoluble

*To whom correspondence should be addressed.

support which contains (iii) an aqueous static phase where the soluble substrates are dissolved and (iv) the biotic phase formed by the microorganism. An essential requirement for SSF is that the liquid is not in excess. The microorganisms may grow inside the solid support, on its surface or in the interparticular free space. In this multiphase heterogeneous system few relevant parameters can be measured on line to determine the status of the fermentation.[2] This lack of information limits the industrial application of this process.

Some physical parameters that are indirectly related to biomass or growth can be measured directly on line. Among these are the temperature increase and the outlet gas composition from where O_2 consumption or CO_2 production rates can be calculated. These two measurements may be correlated with the metabolic activities of the mould and strongly determine the performance of the fermentation.[3,4] Other parameters include the pH evolution and the pressure drop across the bed. Changes in the composition of the milieu can be followed by infrared spectrophotometry[5] and by the dielectric properties.[6]

Pressure drop (DP) is related to the energy requirements for appropriate aeration. Durand & Chereau[7] reported that the DP can be reduced through agitation. A relationship between growth and pressure drop in static aerated fermentors was first reported by Auria *et al.*[8] with the synthetic resin Amberlite IRA-900 used as a model support. Gumbira Sa'id *et al.*[9] followed the DP during the growth of *Rhizopus oligosporus* on sago beads. In a previous work,[10] it was reported that DP variations with time across an aerated fermentor were correlated with the evolution of the following morphological phases of *Aspergillus niger* growth: (i) germination with no observable DP increase; (ii) vegetative growth with a sharp DP increase; (iii) a limitation phase where no further increase in growth was observed; (iv) sporulation with further increase in DP due to the formation of vesicles bearing conidia; and (v) a final phase where the conidia are liberated and DP decreases. This behaviour was observed with natural supports such as wheat bran and cane bagasse where a clear relationship was established between DP, the evolved CO_2 and the temperature increase between the inlet and the centre of the fermentor. This was further corroborated with another strain of *A. niger* under different CO_2 and O_2 environments in static aerated fermentors used for protease production.[4]

Growth studies were also made with the same mould on the synthetic resin Amberlite IRA-900 imbibed with a nutritive solution with an initial sucrose concentration of 65 g/litre. In this case the gas phase permeability of the bed was directly related to the biomass content up to 21·5 mg_{db}/g_{ds} (mg dry biomass/g dry support).[10] In a more recent report,[11] it was shown that permeability data could be used to estimate biomass values up to a concentration of mould biomass of 103 mg_{db}/g_{ds}.

In this report, the relationship between the increase in pressure drop across a packed bed and a number of fermentation parameters were studied when the mould *Aspergillus niger* was grown on a synthetic resin at high initial concentrations of carbon source.

THEORETICAL BACKGROUND

When a gas flows in a laminar regime through a porous bed, the pressure drop (DP) is linked to the superficial fluid velocity (U) by Darcy's equation:[10]

$$U=(k\rho_w g/\mu)(DP/L). \quad (1)$$

During microbial growth in SSF, the intrinsic permeability k varies due to changes in the bed void fraction. The reduction of k can only be due to the increase in biomass which reduces the volume occupied by the gas phase. In this case, the space utilization by mycelia may be characterized by a packing density Ψ, defined as the fraction of the free volume (V_p) occupied by the moulds volume (V_x) and expressed as:

$$\Psi = V_x/V_p, \quad (2)$$

by defining both volumes in terms of densities, Ψ can be expressed as

$$\Psi=(\rho_h(1-W_s)/\varepsilon_o)(X/\rho_s). \quad (3)$$

MATERIALS AND METHODS

Microorganism

Aspergillus niger No. 10, reported previously by Raimbault[12] was used in this study. Conservation

of the strain and spore production have been reported.[8,10]

Support

Amberlite IRA-900 (Rohm and Haas, Philadelphia, PA, USA) was used as a model support. The description and pretreatment of the support have been reported.[8]

Fermentation

For all experiments the inoculum was 10^8 spores per gram dry support. The support was imbibed with the nutritive medium and the spore solution. The initial medium composition was: sucrose, 400 g/litre; $(NH_4)_2SO_4$, 33·03 g/litre; urea, 15·00 g/litre; K_2HPO_4, 12·3 g/litre; $MgSO_4$, 6·15 g/litre; KCl, 6·15 g/litre; $FeSO_4$, 0·123 g/litre. Other initial sucrose solution concentrations (S_i) used were 100 g/litre, 200 g/litre and 300 g/litre while maintaining a C/N ratio of 12 with $(NH_4)_2SO_4$/urea as indicated above. In addition, the following solution of trace elements was used: H_3BO_3 0·0199 g/litre; Cu_2SO_4, 0·0199 g/litre; KI, 0·0039 g/litre; Fe_2Cl_3, 0·0799 g/litre; $MnSO_4$, 0·0159 g/litre; $ZnSO_4$, 0·0159 g/litre; Na_2MoO_3, 0·0079 g/litre which was varied with the sucrose concentration. The pH was adjusted to 2·7. The initial water content and the temperature were 58% and 30°C, respectively.

The experimental setup was presented earlier.[10] One fermentor was provided with two side arms to measure the pressure drop through the bed with a simple U tube manometer that detects pressure variations of 0·5 mm H_2O. Pressure drop (DP) is expressed in mm H_2O. Another fermentor was used to sample gaseous samples of the exit gas to measure the CO_2 gas concentration with a gas chromatograph (Gow-Mac, USA) equipped with thermal conductivity detector and a concentric column (CTR-1, Alltech, USA). Some fermentors[12] were used to evaluate biomass production. These fermentors had 2·0 cm I.D. and 8·0 cm bed height. Aeration was maintained at a constant level with a flow controller (SC440, Veriflo Corp., Richmond, GA, USA). The aeration rates, Q (min^{-1}), used for the biomass, CO_2 gas concentration and pressure drop measurements were respectively 1·3, 2·7 and 4·0 litre air/litre fermentor/min.

Analysis

Biomass was estimated as protein using the Lowry method[8] and a factor of 0·21 g protein/g dry biomass. Biomass was also extracted directly from the resin as reported elsewhere.[11] Biomass, X, is expressed as mg_{db}/g_{ds} (mg dry biomass/g dry support). Water activity was measured with an Aqualab CX-2 water activity system (Decagon Devices, Inc., Pullman, WA, USA); a precision of $\pm 0{\cdot}003$ units is reported for this equipment.

RESULTS AND DISCUSSION

Several fermentations were performed with *A. niger* on Amberlite IRA-900 imbibed with culture medium containing initial sucrose concentrations in the liquid of 100, 200, 300 and 400 g/litre. Figures 1 and 2 show the evolution of the pressure drop (DP), the evolved CO_2 and the dry biomass concentration (X), obtained from protein measurements, with time for $S_i = 100$ and 300 g/litre respectively. For each of both cases a clear relationship between the increase of these three variables was observed. The DP and biomass behaviour followed that found previously[10] for an $S_i = 65$ g/litre with an initial DP of 0·5 mm H_2O. The pattern of fermentation therefore included the germination phase, where the DP was due only to the initial packing, followed by a second phase with a duration of about 10 h corresponding to vegetative growth where the free inter particular space was occupied by the mycelia, and the DP increased as more biomass was produced. A third phase, lasting about 5 h, corresponded to the limitation phase, the substrate being depleted and, while no DP increase was observed, the protein decreased thereafter probably due to changes in the mould composition. In the fourth phase, beyond 25 h and 29 h for an $S_i = 100$ g/litre and $S_i = 300$ g/litre, respectively, sporulation began. The DP increased due to the reduction of the free space by the formation of vesicle bearing conidia which can attain up to 100 μm. A fifth phase, which was not followed, occurred at longer incubation times and signalled the end of sporulation and the liberation of the conidia. Here the DP stabilizes and then decreases.

Maximum values of X, as obtained from dry weight, were 17 mg_{db}/g_{ds} and 76 mg_{db}/g_{ds} which correspond to the DP of 7 mm H_2O and 17 mm H_2O of the limitation phase. In comparison, the biomass values obtained from protein were 26 mg_{db}/g_{ds} and 83 mg_{db}/g_{ds}. This difference for the biomass for the value at 100 g/litre was the largest found between the two methods among all the

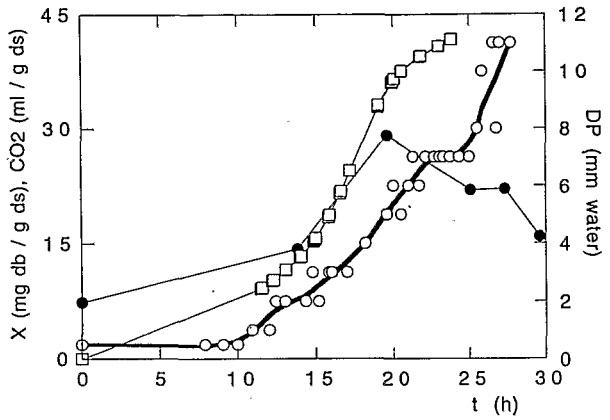

Fig. 1. Evolved CO_2, pressure drop (DP) and dry biomass concentration (X) variations with time for the growth of *A. niger* No. 10 on Amberlite IRA-900 for initial sucrose solution concentration of 100 g/litre. CO_2 (□), pressure drop (○), dry biomass concentration (●).

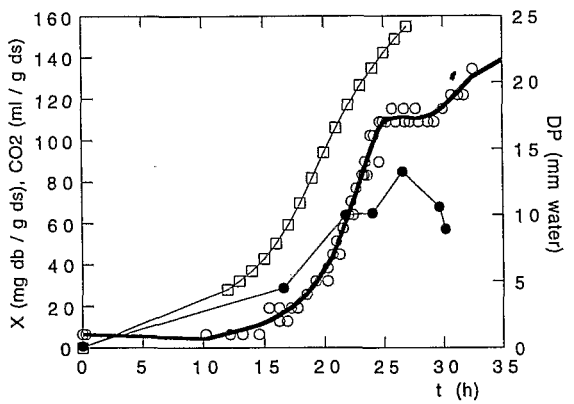

Fig. 2. Evolved CO_2, pressure drop (DP) and dry biomass concentration (X) variations with time for the growth of *A. niger* No. 10 on Amberlite IRA-900 for initial sucrose solution concentration of 300 g/litre. CO_2 (□), pressure drop (○), dry biomass concentration (●).

experiments. By comparison with previous results[10] with $S_i = 65$ g/litre, the same trend was observed but higher final values of biomass were obtained (21·5 mg_{db}/g_{ds} with $S_i = 65$ g/litre). On the onset of the limitation phase 25% and 28% of the initial carbon in the substrate was transformed into CO_2 for the low and the high initial sucrose.

Figure 3 shows the evolution of the DP, in mm H_2O, for the four different initial conditions ($S_i = 100$ g/litre, 200 g/litre, 300 g/litre and 400 g/litre). The four curves describe the fermentation behaviour described above. The germination phase, detected when the DP started to increase was delayed as the S_i was increased. The DP at the onset of the limitation phase increased as S_i was increased.

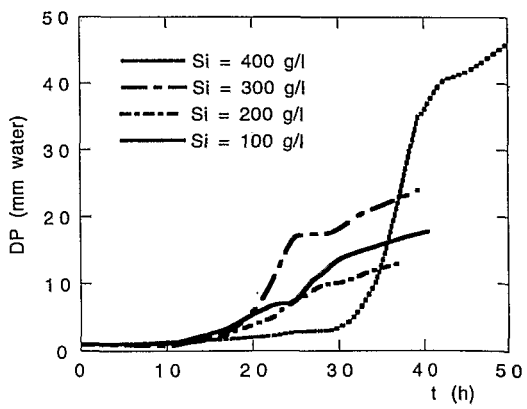

Fig. 3. Variation of pressure drop (DP) with time for the growth of *A. niger* No. 10 on Amberlite IRA-900 for different initial sucrose solution concentrations, $S_i = 100$ g/litre; 200 g/litre; 300 g/litre; 400 g/litre.

Figure 4 shows the relationship between the biomass and the relative permeability (k/k_{in}). The (k/k_{in}) was obtained from eqn (1) using an initial DP of 0·5 mm H_2O.[10] The data utilized for this graph was obtained from the fermentations with S_i of 100 g/litre, 200 g/litre, 300 g/litre and 400 g/litre and that previously reported for 65 g/litre.[10] In the previous publication the maximum biomass obtained was 21 mg_{db}/g_{ds} and a straight line was used to establish the relation between (k/k_{in}) and biomass. As seen in Fig. 4 the experimental data beyond 21 mg_{db}/g_{ds} showed an asymptotic trend.

Even at the highest biomass concentrations the continuity of the gas phase was not broken, i.e. the air passage was not plugged and this behaviour corresponds well with the mesh-like growth of the mycelia. There was a clear relationship between the two parameters, nevertheless some of the experimental points found with $S_i = 65$ g/litre lay below the fitted line. This behaviour may be due to problems in the measurement of the k_{in}. Small variations in this value, which is obtained from the initial DP, offset the graph. This fact is not relevant in the course of one experiment but may present some problems between different experiments, especially when doubts arise on the reproducibility of the k_{in}. Variations in the initial DP, and hence in k_{in}, may also occur when there are changes in the substrate (i.e. diameter of the particles and packing heterogeneities).

The relationship between the dry biomass concentration and the biomass packing density is described by eqns (2) and (3). The value of Ψ depends on the apparent dry biomass density (ρ_s)

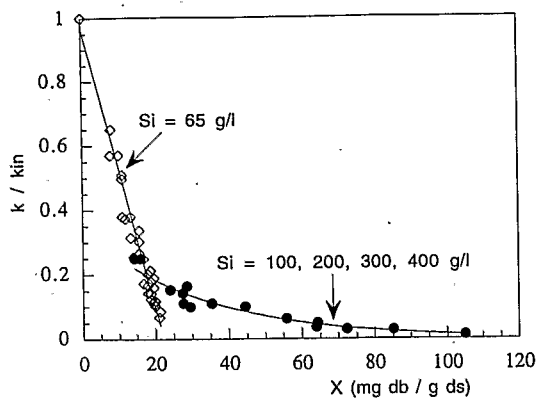

Fig. 4. Variation of the relative permeability, k/k_{in} (●) with dry biomass concentration (X) for different initial sucrose solution concentration, S_i=100 g/litre; 200 g/litre; 300 g/litre; 400 g/litre. (◇) Relation of k/k_{in} and with biomass for S_i=65 g/litre.[10]

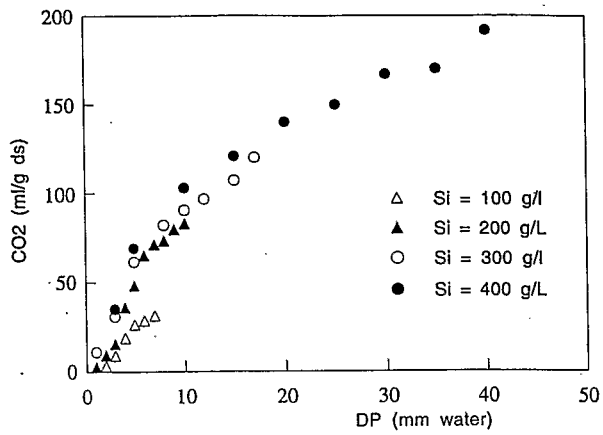

Fig. 5. Variation of evolved CO_2 with pressure drop (DP) for different initial sucrose solution concentration, S_i=100 g/litre; 200 g/litre; 300 g/litre; 400 g/litre.

which is obtained from the density (ρ_x) and the biomass dry matter content (W_s). While a wide range of values has been established for these parameters,[11] it is possible that they change little during growth and these experiments proved to be highly reproducible. Using average values of ρ_x and W_s, Ψ corresponds to 34% of the free inter-particular space when the biomass reached 103 mg_{db}/g_{ds} for S_i of 400 g/litre assuming average values[10] of ρ_x of 1·2 g/cm^3 and W_s of 0·2 g dry biomass/g wet biomass and ρ_s=0·24 g dry biomass/cm^3.

The measurement of DP allows the possibility of coupling a purely physical phenomenon with a biological response. For example, in Fig. 5 there is a clear relationship between the evolved CO_2 and the DP, for the four initial substrate concentrations studied and only in the vegetative growth phase. There is not a linear relationship between these variables for the complete duration of the growth phase, due to the non-linearity between the DP and the biomass (X) which is responsible for the CO_2 evolution. Estimation of the exponential specific growth rate μ_x from either CO_2 production or DP give similar results as a linear relationship can be approximated at the onset of the growth phase. The μ_x is inversely related to the initial substrate concentration as depicted in Fig. 6. Similar results have been previously reported.[13]

Another interesting biological parameter that can be easily detected with the DP measurement is duration of the germination as shown in Fig. 7 from data collected from Fig. 3. It has been

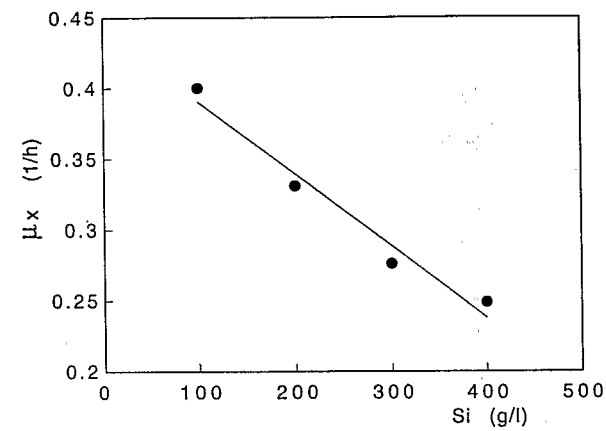

Fig. 6. Variation of the specific growth rate μ_x with initial sucrose solution concentration, S_i.

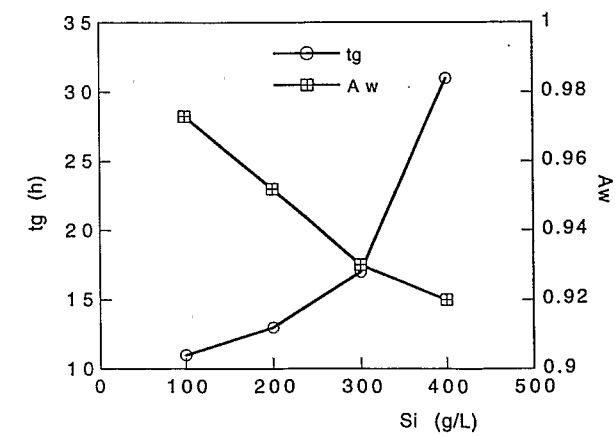

Fig. 7. Variation of the germination time, tg, and initial water activity, Aw, with initial sucrose solution concentration, S_i.

shown,[13] that the germination time correlates with the water activity (Aw) of the medium. The germination phase starts with a step of water absorption by the spore that allows the internal biochemical reactions to proceed. The rate of this water absorption is dependent on the osmotic pressure of the liquid phase. The time for spore germination increased to 31 h when $S_i = 400$ g/litre.

CONCLUSIONS

DP, evolved CO_2 and dry biomass concentrations, were used to monitor the growth of *Aspergillus niger* on Amberlite IRA-900 with initial substrate solution concentrations ranging from 100 g/litre to 400 g/litre. *DP* measurements allowed the detection of the different phases of growth. The response of *DP* followed the same behaviour found earlier for lower substrate concentrations. The relative intrinsic permeability decreased to a value of 0·0125. It is estimated that under these conditions the biomass occupies 34% of the free inter particular space with a biomass concentration of 103 mg_{db}/g_{ds}. There was a good relationship between *DP* and CO_2 evolution for the range of initial substrate concentrations studied.

The use of *DP* as an on-line measurement is subject to the same restrictions of other sensors. The sensitivity can be improved by increasing the air flow (eqn (1)) or by using a sensor with a higher sensitivity. For an augmented airflow the *DP* response is increased and this may be useful considering that CO_2 production would be more difficult to measure as CO_2 is more diluted in an increased airflow. On the other hand a more sensitive pressure sensor could be useful if air flow can be finely controlled to reduce background noise. This work supports early results and confirms that *DP* can be a valuable parameter in the study of growth of moulds at high concentrations.

ACKNOWLEDGEMENTS

This work was performed under research agreements between the UAM (Mexico) and the ORSTOM (France). The authors acknowledge the Mexican Research Council (CONACYT) for their contribution to the financial support of this study.

REFERENCES

1. Doelle, H. W., Mitchell, D. A. & Rolz, C. E., *Solid Substrate Cultivation*. Elsevier Applied Science, London, 1992.
2. Mitchell, D. A., Biomass determination in solid state cultivation. In *Solid Substrate Cultivation*, ed. H. W. Doelle, D. A. Mitchell & C. E. Rolz. Elsevier Applied Science, London, 1992, pp. 53–64.
3. Sargantanis, J., Karim, M., Murphy, V., Ryoo, D. & Tengerdy, R., Effect of operating conditions on solid substrate fermentation. *Biotechnol. Bioeng.*, **42** (1993) 149–58.
4. Villegas, E., Aubague, S., Alcantara, L., Auria, R. & Revah, S., Solid state fermentation: acid protease production in controlled CO_2 and O_2 environments. *Biotech. Adv.*, **11** (1993) 387–97.
5. Desgranges, C., Georges, M., Vergoignan, C. & Durand, A., Biomass estimation in solid state fermentation. *Appl. Microbiol. Biotechnol.*, **35** (1991) 206–9.
6. Davey, C. L., Peñaloza, W., Kell, D. B. & Hedger, J. N., Real-time monitoring of the accretion of *Rhizopus oligosporus* biomass during the solid-substrate tempeh fermentation. *World J. Microbiol. Biotechnol.*, **7** (1991) 248–59.
7. Durand, A. & Chereau, D., A new pilot reactor for solid state fermentation: application to the protein enrichment of sugar beet pulp. *Biotechnol. Bioeng.*, **31** (1988) 476–86.
8. Auria, R., Hernandez, S., Raimbault, M. & Revah, S., Ion exchange resin: a model support for solid state growth fermentation of *Aspergillus niger*. *Biotechnol. Techn.*, **4** (1990) 391–6.
9. Gumbira, Sa'id E., Greenfield, P. F., Mitchell, D. A. & Doelle, H. W., Operational parameters for packed beds in solid-state cultivation. *Biotech. Adv.*, **11** (1993) 599–610.
10. Auria, R., Morales, M., Villegas, E. & Revah, S., Influence of mold growth on the pressure drop in aerated solid state fermentors. *Biotechnol. Bioeng.*, **41** (1993) 1007–13.
11. Auria, R. & Revah, S., Pressure drop as a method to evaluate mold growth in solid state fermentors. In *Advances in Bioprocess Engineering*, ed. E. Galindo & O. T. Ramirez. Kluwer Academic Press, The Netherlands, pp. 289–94.
12. Raimbault, M., Fermentation en milieu solide: Croissance de champignons filamenteux sur substrat amylacé, Thèse de Doctorat, U.P.S. Toulouse, France, 1980.
13. Oriol, E., Schettino, B., Viniegra-Gonzales, G. & Raimbault, M., Solid-state culture of *Aspergillus niger* on support. *J. Ferment. Technol.*, **66** (1988) 57–62.