Influence of Mold Growth on the Pressure Drop in Aerated Solid State Fermentors

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The measurement of pressure drop (ΔP) across an aerated fermentation bed is proposed as alternative on-line sensor for the qualitative and, in some cases, quantitative, macroscopic changes in a static solid state fermentor. An increase in the ΔP is correlated with the evolution of the different phases of *Aspergillus niger* growth: germination, vegetative growth, limitation, and sporulation, we observed in the microscope. For the case where the support is not modified during the fermentation and the water content remains constant, i.e., a synthetic resin (Amberlite IRA-900), the gas phase permeability of the bed is directly related to the biomass content. For example, the permeability of the bed is reduced to 5% of the initial value when biomass attains 21 mg dry biomass/g dry support. Biomass was appropriately predicted from the ΔP measurements in an independent test. Experiments with different initial sucrose solution concentrations showed that biomass could not be produced beyond a certain level (21.5 mg dry biomass/g dry support) which suggests steric limitations. For the case of wheat bran and cane bagasse, the increase in ΔP was related qualitatively to the evolution in the growth and the morphology of the mold.

Key words: pressure drop · solid state fermentation · *Aspergillus niger* ·ion exchange resin · permeability · wheat bran · cane bagasse

INTRODUCTION

Valuable substances can be produced from microorganisms using the solid state fermentation (SSF) technique.[15,16] The engineering aspects related to the SSF have not been developed as extensively as the research linked to the probable applications. Lonsane et al.[14] reviewed the equipment, the monitoring and control of the process, the automation and the modeling. In this article are also included some recommendations as to the main engineering R&D areas needed for a more extensive use of SSF. Durand et al.[9] studied some of the constraints related to industrial applications. The most important limitations to SSF are process monitoring and control.

The SSF process can be defined as a four-phase system. The continuous phase is air (or another gas mixture) that usually flows through a solid bed. This solid is composed of a water-insoluble support which contains an aqueous solution of nutrients. This solution is tightly absorbed within the matrix of the insoluble support and no drainage is observed. The fourth phase is the microorganism which grows inside the support and/or on its surface and/or in the interparticulate free space. This multiphase system makes nondestructive on-line monitoring more difficult than in liquid fermentation.

Nevertheless, in SSF, some parameters can be measured directly on-line such as: temperature, gas composition, pH, and gas pressure drop. Biomass or growth have only been estimated indirectly from physical measurements such as temperature, effluent gas composition (O_2 consumption and CO_2 production), composition changes as analyzed by infrared spectrophotometry, and by variation of the dielectric properties.

Temperature variations during the SSF are correlated to the metabolic activities of the mold and strongly determine the performance of the fermentation.[12,17] Control of the temperature has been achieved by evaporative cooling;[3] by changing the inlet gas temperature, relative humidity, and flow[8] and by other methods.[14]

Gas composition may be used to obtain substantial information on the evolution of the fermentation. Water activity (A_w) of the medium is very important in SSF.[17,19] Measurements of the vapor pressure of the gas has been used to evaluate the A_w of the substrate.[10] In SSF the gas phase is near water vapor saturation, however, the equilibrium time between the gas and the liquid phase absorbed within the solid support may be long compared to the biophysical changes brought about by the growth of the microorganism.[10] Several methods have been proposed to control the water content in SSF.[11,22]

Oxygen uptake rate (OUR) and/or carbon dioxide production rate (CDPR) have been used extensively in SSF for growth estimation.[5,18,23,25] These rate measurements offer the advantage of a fast response time and are directly linked to the metabolism of the microorganism. Nevertheless, to estimate microbial biomass from these data the yield coefficients should be evaluated for each microorganism, substrate, and culture conditions.[23] Furthermore, these coefficients may change as a function of growth rate.[21]

Recently, it has been reported[5] that fast biomass determination may be obtained by reflectance infrared spectrophotometry for the growth of *Beauveria bassiana* on

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clay granules. Another recently published technique shows a relation between growth and the change of the dielectric properties of the substrate in Tempeh fermentation. [4]

The measurement of pH is generally made after diluting and homogenizing a sample of the SSF in distilled water. Variations in the pH may result from the consumption of substrates and/or the production of primary or secondary metabolites and, thus, could be used as an indicator of metabolic activity. On-line measurements have been reported,[4,8] but no relation to biomass or growth was established. Control has been achieved generally by initial medium formulation (increased buffer capacity, urea/ammonium sulphate relation, etc.)[14] or by addition of neutralizing agents during fermentation in agitated vessels.[8,13]

The pressure drop (ΔP) of air as it passes through the fermentor is a relevant engineering parameter. It defines the energy requirements for appropriate aeration, Durand and Chereau[8] reported that the (ΔP) can be reduced through agitation. In a previous work, it was reported[1] that an increase in the (ΔP) could be observed when *Aspergillus niger* was grown on an ion-exchange resin (Amberlite IRA-900, Rohm & Haas, Philadelphia, PA) in a static fermentation. The variation of the (ΔP) was linked to an increase in the biomass, because growth of the mold causes a reduction in the bed void fraction.

In this article, the possibility of using (ΔP) as an on-line measurement to estimate biomass is explored. This will be done, first, by establishing qualitative relations between the (ΔP) and the morpholgical changes that occur during the fermentation on a model substrate. Then, with the model substrate, quantitative relations will be proposed which link the biomass and the (ΔP) through the gas-relative permeability obtained using Darcy's Law. Finally, the results are extrapolated to natural substrates (cane bagasse and wheat bran).

THEORETICAL BACKGROUND

In fluid flow through a porous bed there are viscous and kinetic phenomena. The pressure drop, (ΔP), through the bed is linked to the superficial fluid velocity by the Ergun equation:

$$(\Delta P/L) = \alpha U + \beta U^2 \qquad (1)$$

where: ΔP, pressure drop between the inlet and the outlet of the porous bed (cm); L, bed length (cm); Q, volumetric flow rate (cm^3/s); A, bed cross sectional area (cm^2); $U = Q/A$, superficial fluid velocity (cm/s); and α, β, proportionality constants.

The first term in Eq (1) represents the drop in pressure due to the viscous effects, while the second term is linked to the inertia of the fluid.

The fluid flow pattern through the substrate can be described by the particle Reynolds number[20]:

$$N'_{Re} = \rho U D_p/\mu \qquad (2)$$

where: N'_{Re}, particle Reynolds number; ρ, fluid density (g/cm^3); D_p, mean particle diameter (cm); and μ, fluid dynamic viscosity (g/cm·s).

In most SSF applications, gas flows in a laminar regime ($N'_{Re} < 1$) which reduces Eq. (1) to Darcy's equation:

$$(\Delta P/L) = \alpha U = (1/K)U \qquad (3)$$

where K is the hydraulic conductivity.

This equation is valid for liquids with constant density. When the fluid is a gas, the equation can be used by taking into consideration a mean gas density between the inlet and the outlet. The intrinsic permeability, k, may be defined as:

$$k = K\mu/\rho_w g \qquad (4)$$

where: ρ_w, water density (g/cm^3); and g; acceleration due to the gravity (cm/s^2).

Darcy's equation has been justified theoretically in simple porous models[7] where the intrinsic permeability is defined as:

$$k = \varepsilon^3 D_p^2/72t^2(1 - \varepsilon)^2 \qquad (5)$$

where: t, tortuosity factor (1.58 for spheres); ε, bed void fraction, ($\varepsilon = (1 - V_s)/V_t$), V_t, the total reactor volume; and V_s the volume occupied by the solid. V_s includes the volumes occupied by water, the insoluble solid, and the microorganism.

During microbial growth in SSF, k varies due to the changes in the bed void fraction; the V_s changes due to water adsorption or desorption, water production by reaction, substrate (soluble and insoluble) consumption, and biomass production. V_t may also vary due to packing or swelling. These changes also affect the particle diameter and the tortuosity of the bed.

For the case of a SSF system where the solid phase, total bed volume, and water content remain constant, such as in the model substrate reported previously,[1] the reduction in the reactor bed porosity can only be due to the increase in biomass which reduces the volume occupied by the gas phase.

MATERIALS AND METHODS

Microorganism *Aspergillus niger* (No. 10), reported previously by Raimbault,[21] and A. *niger* (ANH-15), an acid protease producer strain (kindly supplied by Dr. Chow, Université de Technologie de Compiègne, France) were used throughout the study. They were kept on potato dextrose agar and transferred monthly. Spores were obtained by growing the mold on this media in flasks for 7 days at 35°C and collected in sterile water containing 0.1% Tween-80.

Substrates Amberlite IRA-900 (Rohm &. Haas) was used as a model support. Wheat bran and cane bagasse were brought from commercial outlets.

Support Pretreatment The Amberlite IRA-900 was pretreated as reported earlier.[1] Wheat bran was milled and sieved to obtain the 10- to 20-mesh fraction (D_p between 2.0 and 0.85 mm) and sterilized for 15 minutes at 115°C. Cane bagasse was sieved to obtain the 40- to 50-mesh fraction (D_p between 0.42 and 0.29 mm), which was sterilized and air dried.

Fermentation For all experiments, the inoculum was 10^8 spores per gram dry support. In the fermentations carried out with Amberlite and cane bagasse, they were imbibed with the nutritive medium and the spore solution. The medium composition was: sucrose, 65 g/L; $(NH_4)SO_4$, 5.37 g/L; urea, 2.44 g/L; K_2HPO_4, 2 g/L; $MgSO_4$, 1 g/L; KCl, 1 g/L; $FeSO_4$, 0.02 g/L. The C/N ratio was 12. For some experiments, the initial sucrose solution concentrations (S_i) used were 90 g/L, 130 g/L, or 200 g/L, keeping the same $(NH_4)SO_4$/urea ratio and a C/N of 12. The initial water content was 58% for the resin and 70% for the cane bagasse. Wheat bran was imbibed with a water solution of $ZnSO_4$ and $FeSO_4$ to obtain a final concentration of 0.025 and 0.4 mg/g dry support, respectively, and with the spore solution to obtain an initial humidity of 50%. The temperatures used were 35°C for *A. niger* No. 10 and 30°C for *A. niger* ANH15.

The experimental setup is presented in Figure 1. The fermentor has two side arms to measure the pressure drop through the bed with a differential pressure transducer (Cole Parmer Model 7352-16, USA) that detects pressure variations of 0.3 mm H_2O with a precision of ± 1%. The side arms were positioned just below and above the bed and 10 mm were allowed between the top of the bed and the upper plug of the fermentor. Pressure drop ($\Delta P/L$) is expressed in millimeters H_2O/mm fermentor height. The gas evolved was dried and used to measure the CO_2 produced with an IR detector (Beckman Instruments, Fullerton, CA). The fermentor had a 2-cm i.d. and 6.5-cm bed height; for some experiments a 4-cm i.d. and 15-cm bed height fermentor was used. Aeration was controlled with a Matheson mass flow controller (Matheson Dynablender 8259, USA). The aeration rates, Q (min^{-1}), used were from 1.7 to 17 l air/L fermentor · min. The maximum observed pressure drops were 40 cm H_2O. To prevent water condensation, which might provoke undesirable variations in the $\Delta P/L$ measurement, the fermentors and the outcoming air were maintained at the same temperature of the bath.

In some experiments, simultaneous fermentations were performed to evaluate biomass production and for microscopy observation. Sixteen fermentors, as those described by Raimbault,[21] were used with the same substrate and under the identical conditions of the pressure drop experiments. These fermentors were used for analysis. Aeration was kept constant with a flow controller (SC440, Veriflo Corp., Richmond GA).

Analysis The biomass protein and residual sugar extraction from the Amberlite and the protein measurement by the Lowry method have been reported previously.[1] Biomass is evaluated from the protein assuming that the dry biomass contains 21% protein as measured by the Lowry method. Biomass, X, is reported as mg dry biomass/g dry support. Residual sugar was measured by the phenol sulfuric method as described by Dubois et al. [6]

RESULTS AND DISCUSSION

Figure 2 shows the evolution of the pressure drop with time for the growth of *A. niger* No. 10 on Amberlite IRA-900 for two different aeration rates and initial sucrose solution concentrations. In these experiments the water content (58%), the total bed volume (V_t). and the resin diameter distribution were measured and found to remain constant throughout the fermentation. Due to the fact that the resin is not assimilated and that growth occurs only

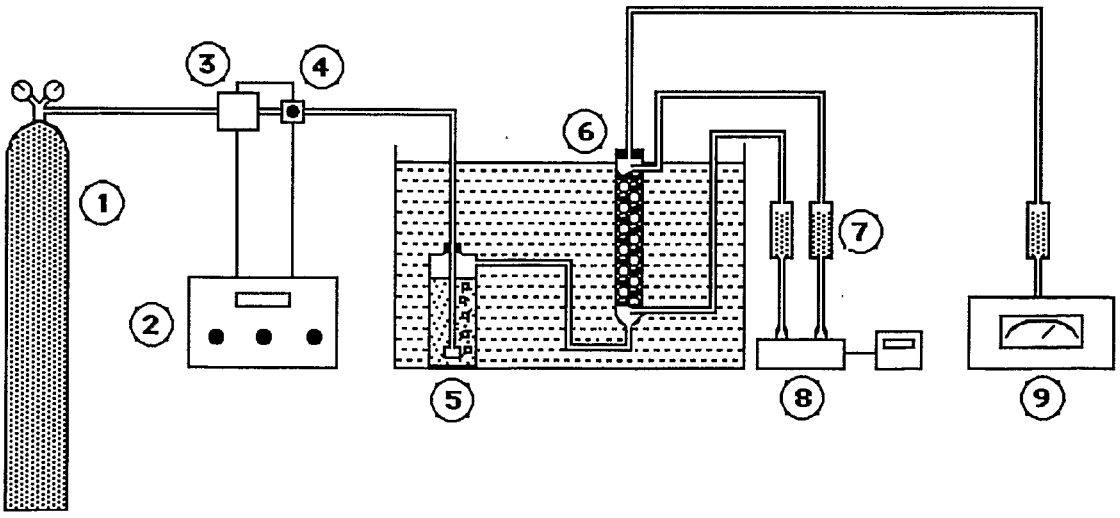

Figure 1. Experimental apparatus: 1, air supply; 2, flow controller; 3, flow meter; 4, control valve; 5, humidifier; 6, fermentor; 7, gas dehumidifier; 8, pressure transducer; 9, infrared CO_2 analyzer.

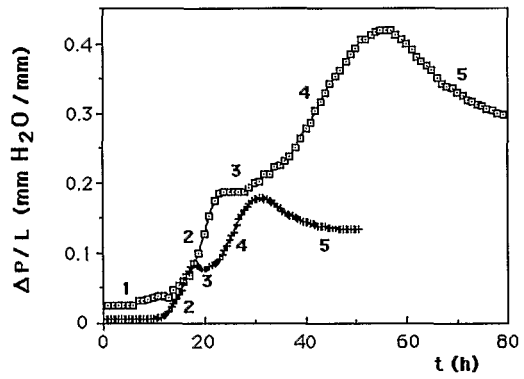

Figure 2. Variation of pressure drop ($\Delta P/L$) with fermentation time for the growth of *A. niger* No. 10 on Amberlite IRA-900 at: (− + −) $Q = 2.1$ min^{-1}; initial sucrose solution concentration, $S_i = 65$ g/L; (−⊡−) $Q = 4$ min^{-1}, $S_i = 200$ g/L.

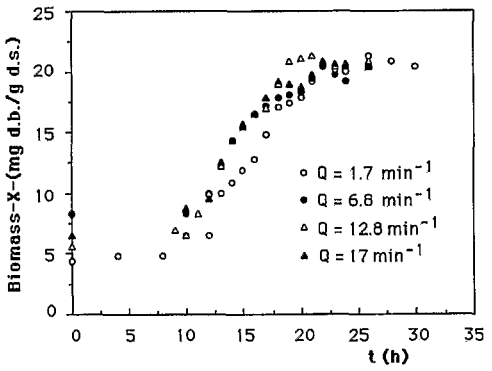

Figure 3. Dry biomass concentration X variation with time for different air-flow rates for the growth of *A. niger* No. 10 on Amberlite IRA-900.

on the surface of the resin,[1] it may be concluded that the increase in the $\Delta P/L$ can only be due to the occupation of the free interparticular space by the mold. Schematically, the evolution of the fermentation can be divided in five regions which correspond to changes in the $\Delta P/L$ trends and to the macroscopic morphology of the mold as observed under the microscope.

1. Germination phase: no growth is observed, only swelling of the spores which remain attached to the surface of the resin, the pressure drop remains constant. Initial pressure drop is only due to the support packing of the reactor. A delayed germination was observed at initial substrate concentration of 200 g/L. This behavior has been reported previously.[19]
2. Vegetative growth: hyphal elongation occupies the free interparticular space. The pressure drop increases as more biomass is produced.
3. A limitation phase: no further increase in biomass is observed; this phase corresponds in both experiments to a sucrose consumption of about 95%. There is no increase in $\Delta P/L$ and, in some cases, a small decrease is observed.
4. After the limitation phase, the sporulation process is initiated. Vesicle bearing conidia are progressively formed with diameters which attain 100 μm. These bodies reduce the interparticular space, causing an increase in the $\Delta P/L$.
5. In this phase, a liberation of the spores occurs. The spores were observed on the hyphae, on the surface of the support, and on the filter that was at the exit of the fermentor beyond the pressure measurement arm. Also, the hyphae showed a smaller diameter. A decrease in $\Delta P/L$ was observed.

Figure 3 shows the influence of the aeration rate on the mold growth for the first three phases, for an initial sucrose solution concentration of 65 g/L. It may be observed that the measured biomass production rate is independent of the aeration rate in the 1.7 to 17 min^{-1} range. For the lowest aeration rate (1.7 min^{-1}) the hyphal growth was initiated later but similar growth rates were observed. The biomass concentration in the limitation phase was independent of the aeration rate as reported by Raimbault,[21] and attained values of about 21.5 mg of dry biomass/g dry support. This value is lower than the biomass obtained in conditions where the gas composition is different from air (e.g., reduced O_2 and high CO_2 partial pressures), such as the diffusion-controlled fermentor (DCF) described in a previous study,[2] where higher values of biomass (up to 30 mg dry biomass/g dry support) were obtained.

Figure 4 shows the evolution of the pressure drop and the dry biomass as a function of time at different gas flow rates for the experiments depicted in Figure 3. A clear relation between the increase of both $\Delta P/L$, and X with time is observed for the different flow rates. The variation in the absolute values of $\Delta P/L$ (maximum values of 0.08, 0.42, and 0.85 for air flows of 1.7, 6.8, and 14.9 min^{-1}, respectively) can be explained by the Darcy equation [Eq. (3)].

To relate the $\Delta P/L$ data to the conductivity associated with the gas phase, an initial bed, K_{in}, of 16.9 cm/s was obtained with Eq. (3) and by measuring the $\Delta P/L$ at different flow rates. By comparison, a slightly lower value of K_{in} (13.4 cm/s) was obtained with Eqs. (4) and (5) using an average D_p of 0.630 mm, [1] a tortuosity factor of 1.58, and an initial porosity (ε) of 0.36. Figure 5 shows the relation of the relative permeability (k/k_{in}) as a function of the measured biomass for the same time at different flow rates. Data was taken only from the vegetative growth and limitation phases from the experiments shown in Figure 4. The ratio (k/k_{in}) is equivalent to the ratio k/k_{in}, considering that the experiments were performed at the same temperature (i.e., same μ). K was obtained from Eq. (3) and, for K_{in}, a value of 16.9 cm/s was used. As may be observed in Figure 5, there is a strong reduction in the relative permeability (i.e., 5% of the initial permeability is found for an X concentration of 21 mg dry biomass/g dry support). This variation in the relative permeability has strong effects on other transport parameters as has been shown for the effective gas (O_2 and CO_2) diffusion coefficients.[2] Reliable experimental measurements for biomass below 7 mg dry biomass/g dry support could not be made due to the

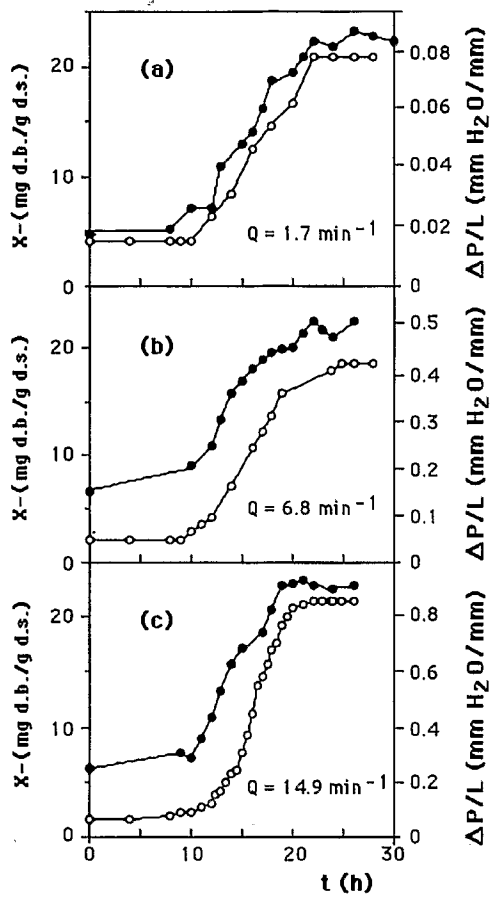

Figure 4. Dry biomass concentration X (●) and pressure drop ($\Delta P/L$) (○), variation with time for the growth of *A. niger* No. 10 on Amberlite IRA-900 for different air-flow rates, Q, for initial sucrose solution concentration of 65 g/L. (a) $Q = 1.7$ min^{-1}; (b) $Q = 6.8$ min^{-1}; (c) $Q = 14.9$ min^{-1}.

sensibility of the Lowry analysis. The relation of (k/k_{in}) with biomass may be expressed as:

$$(k/k_{in}) = 0.976 - 0.044X, \quad \text{with} \quad r^2 = 0.981. \quad (6)$$

To corroborate this relation, an independent experiment was performed under different conditions (reactor: 4-cm diameter, 15-cm long, initial sucrose solution concentration = 90 g/L and aeration = 1.8 min^{-1}). Biomass was both measured and estimated with Eq. (6) using the permeability obtained through the measured $\Delta P/L$ and Eq. (3). The results are shown in Figure 6. In this case the equation was used to estimate the biomass below the detectable protein concentration. The biomass, estimated from the on-line measurement of the $\Delta P/L$, is related quantitatively to the growth, evaluated from protein content. Figure 7 compares the estimated biomass with two other on-line measurements: the temperature increase, and the CO_2 production rate (CDPR). Both variables are related to the metabolic activities of the mold and represent rate measurements while the biomass estimated from the $\Delta P/L$ is a purely physical integrated variable. The $\Delta P/L$ measurement, besides giving a good estimate of the biomass produced, gives a clear indication of the onset of the vegetative growth and when the limitation phases is attained.

In an attempt to increase the biomass at the end of the vegetative phase to values previously obtained in the DCF,[2] the initial sucrose solution concentration was increased. Growth was monitored by $\Delta P/L$ and the use of Eq. (6). For these experiments, it was considered that the biomass density, which is implicitly considered in Eq. (6), could be extrapolated to higher initial sucrose concentrations. For initial glucose concentrations of 90, 130, and 200 g/L, a value of 21.5 mg dry biomass/g dry support, (20.0 ± 1.5 mg dry biomass/g dry support) was obtained. This biomass corresponds to a minimum (k/k_{in}) value of 0.05. It is interesting to note that, despite the threefold variation in the initial substrate concentration, the maximum biomass reaches a plateau. This may suggest the presence of steric limitations as suggested by Laukevics et al.[13] For the maximum biomass obtained (21.5 mg dry biomass/g dry support) and assuming[13] average values of wet biomass density (1.2 g/cm^3) and dry matter content (0.2 g dry biomass/g wet biomass), a packing density of 0.07 cm^3 wet biomass/cm^3 space available for growth is found. This value agrees with those reported by Laukevics et al.[13] who

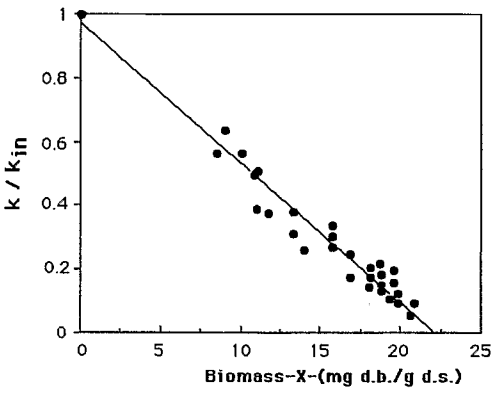

Figure 5. Variation of the relative permeability, k/k_{in}, with dry biomass concentration, X.

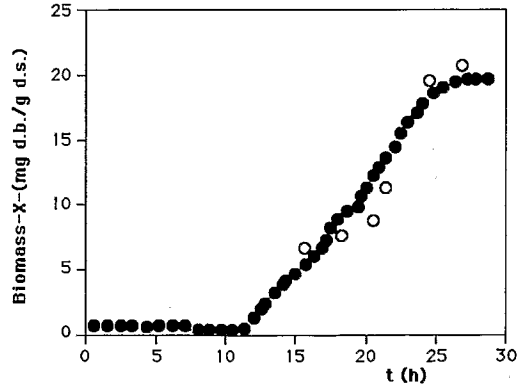

Figure 6. Dry biomass concentration, X, variation with time for the growth of *A. niger* No. 10 on Amberlite IRA-900 with initial sucrose solution concentration of 90 g/L and $Q = 1.8$ min^{-1}. (•) Estimated values from pressure drop ($\Delta P/L$); (O) experimental values. Fermentor size 4 cm i.d. and bed height 15 cm.

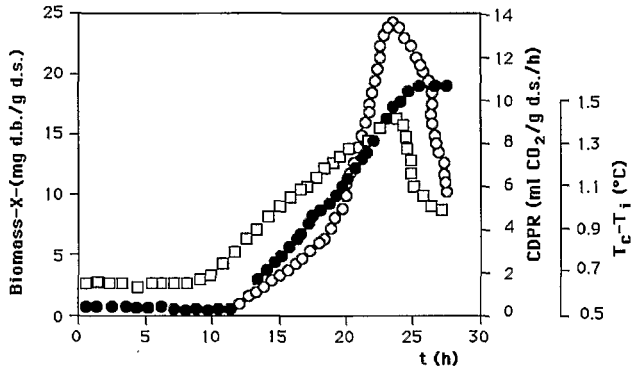

Figure 7. Variation of dry biomass concentration, X (•); carbon dioxide production rate, CDPR (O); and temperature difference between fermentor center and the inlet temperature, $T_c - T_i$ (□), with time for the growth of *A. niger* No. 10 on Amberlite IRA-900. $S_i = 90$ g/L and $Q = 1.8$ min^{-1}. Fermentor size 4 cm i.d. and bed height 15 cm.

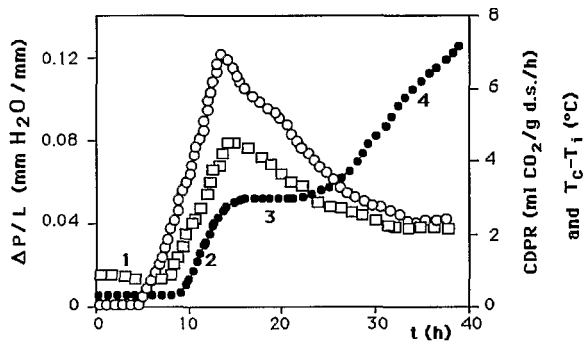

Figure 8. Variation of pressure drop, $\Delta P/L$ (•); carbon dioxide production rate, CDPR (o); and temperature difference between fermentor center and the inlet temperature, $T_c - T_i$ (□), with time in the fermentation of *A. niger* ANH-15 on wheat bran. $Q = 2.7$ min^{-1}, initial water content = 50%. Fermentor size 4 cm i.d. and bed height 15 cm.

give a range of 0.05 to 0.08 as common limiting values where growth stops due to steric limitations.

Strong reductions in the (k/k_{in}) as a function of the volume occupied by the continuous gas phase is observed in other systems such as in water infiltration in soil.[24] In this case, as the soil bed is wetted by water infiltration, a continuous reduction in the k/k_{in} of the gas phase is observed. The effect is due to the increased water volume, which reduces the volume occupied by the gas phase. Increase water infiltration eventually causes the k/k_{in} to become zero as the continuity of the gas phase is broken. In SSF, there are no such physical barriers that may from discontinuous zones because of its mycelial, mesh-like structure. The fact that biomass was not able to grow beyond 21.5 mg of dry biomass/g dry support, under the conditions studied, agrees well with the value of 22.2 mg of dry biomass/g dry support predicted by Eq. (6) when k/k_{in} is zero. Nevertheless, the fact that higher biomass concentrations have been obtained with the same support and microorganism, but under different environments, may suggest that this steric limitation is not absolute for a given microorganism but it is strongly dependent on the particular conditions where growth is taking place.

Figures 8 and 9 show the evolution of the $\Delta P/L$ for the growth of the two strains of *A. niger* on wheat bran and cane bagasse. The final water content was 55% for wheat bran and 72% for cane bagasse. The biomass cannot be estimated from the described equations for these supports because they cannot be considered, as with the Amberlite, to have constant properties throughout the experiment. This is due to the fact that the bran and the bagasse are modified by microbial growth. The changes that these supports undergo are consumption due to the hydrolysis of starchy and protein material, intraparticular mold growth, water absorption, and packing changes, among others. Nevertheless, the mold growth imposes a behavior on the $\Delta P/L$ that overcomes the substrate variations and the four phases can be clearly distinguished. In Figure 8, a similar tendency to that shown in Figure 7 with the resin may be observed for the $\Delta P/L$, the temperature, and the CDPR. In Figure 9, a similar $\Delta P/L$ evolution may be observed.

CONCLUSIONS

The $\Delta P/L$ may be used as an alternative on-line sensor to obtain qualitative and, under certain conditions, quantitative data of biomass production in SSF. The $\Delta P/L$ is related by simple equations to the permeability of the bed which reflects the occupation of the fermentor gas phase by the microorganism. Although this measurement may be sensitive to variations in the biomass density, especially when different initial substrate concentrations are used, it has the advantage of reflecting a totally physical phenomena, and may be used as a complement to other sensors which gather information more closely related to the microbial metabolism, such as temperature, pH, OUR, and CDPR. This complementary effect has been observed in the case of the OUR and CDPR, where increasing the gas flow rate reduces, by dilution, the inlet and outlet difference in the gas composition, whereas the $\Delta P/L$ is increased. The $\Delta P/L$, in comparison, may indicate of some other macroscopic changes in the fermentor that are not easily detected by

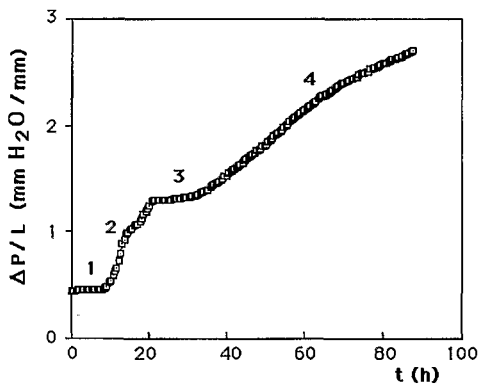

Figure 9. Variation of pressure drop ($\Delta P/L$) with time in the fermentation of *A. niger* No. 10 on cane bagasse. $S_i = 130$ g/L, $Q = 3.5$ min^{-1}, initial water content = 70%.

temperature, pH, OUR, or CDPR, such as the onset of sporulation. From the engineering point of view, the $\Delta P/L$ measurement may indicate certain operating problems such as abnormal changes in the packing and water condensation and drainage. For the general SSF, where agricultural products are used both as substrate and support, $\Delta P/L$ data cannot be directly used to obtain an estimate of the produced biomass. For this case, the changes in the permeability may not only be due to the biomass occupation of the gas phase, but also to macroscopic changes in the support such as substrate consumption, water absorption and evaporation, packing changes, and water condensation. Nevertheless, the method proved to be highly reproducible for agricultural products, and may be used for monitoring and control if correlated with other measurements such as product formation, pH, outlet O_2 and CO_2 concentrations, etc. Furthermore, very precise and sensitive equipment can be easily obtained that may be connected to a computer for measurement or/and control. On the other hand, a simple U-tube manometer can be used, the only limitation being that the gas flow rate must be regulated.

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