Determination of the Interparticular Effective Diffusion Coefficient for CO_2 and O_2 in Solid State Fermentation

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A simple experimental diffusion controlled fermentor (DCF), coupled with the use of a mathematical model based on mass balance, is proposed to measure the variation of the gas (CO_2 and O_2) diffusion coefficients in solid state fermentation. The DCF was packed with an ion-exchange resin impregnated with a nutritive medium and inoculated with *Aspergillus niger*. The growth conditions in the DCF were very similar to those found in equipment operated with convective oxygen supply. The diffusion coefficient was shown to be very dependent on the biomass concentration within the solid state fermentor, and attained values of less than 5% of the molecular diffusion in air when the biomass in the fermentor reached 27 mg dry/g dry support.

Key words: diffusion coefficient • fermentation • *Aspergillus niger* • solid state fermentor • ion-exchange resin

INTRODUCTION

Solid state fermentation (SSF) is an alternative cultivation system to produce valuable substances from microorganisms. The variety of products that can be obtained and the different characteristics of this process have been extensively reviewed.[6,7] Despite the fact that SSF is widely employed on the industrial level,[6] there is very little information about the engineering aspects. Lonsane et al.[5] reviewed the type of equipment required, the monitoring and control of the process, and its automation and modeling. Additionally, this study includes some recommendations as to the main engineering R&D needs for a more extensive use of SSF.

In SSF, agricultural products are generally used as the support where microbial growth occurs within its mass, on the surface, and in the interparticular free space. Because microbial growth is intimately attached to the support, it is very difficult to measure biomass directly.[6] One of the most widely used methods is to measure growth by O_2 consumption and/or CO_2 production.[11,13] The structure within the fermentor changes with time at different levels; at the intraparticular level, the microorganism invades the available pores and changes the chemical composition of the support; at the interparticular level, growth provokes the coverage of the surface of the support and, in the case of molds, mycelia forms bridges between different particles. The microbial-induced modification of the support brings about changes in the gas mass transfer coefficients.

To study these changes, the use of a support that is not modified (chemically and physically) during the fermentation allows a simplified mathematical representation. In a previous work,[1] it was reported that an ion-exchange spherical resin, impregnated with a nutritive medium and inoculated with a mold (*Aspergillus niger*), could simulate the evolution of a SSF on natural substrates. With this resin, the physical structure of the support is not modified during growth. Also, the size of the internal pores of the resin forces the mold to grow only on the surface and in the interparticular void space. This type of growth reduces the porosity of the fermentor, which brings about changes in the diffusion of the gas. The relation between the porosity and the effective diffusion coefficient has been studied in other systems,[8,12] but not in SSF. For the liquid fermentation, the effective diffusion of the gas in biofilms and bioflocs has been extensively studied and recently reviewed.[3]

This work evaluates the changes in the effective gas (O_2 and CO_2) diffusion coefficients during the evolution of a SSF. To do this, a simple diffusion-controlled fermentor (DCF) was used, which imposes, by growth, a gas (O_2 and CO_2) concentration axial gradient. The coefficients are obtained from data gathered by gas and biomass measurements with time and position, and by applying mass conservation equations.

MATERIALS AND METHODS

Microorganism

All the experiments were made with a strain of *A. niger* (no. 10). This strain is well characterized in terms of the relation between growth, yield, and maintenance coefficient for O_2 and CO_2.[9] Spores were obtained by growing the mold on potato dextrose agar in flasks for 7 days at 35°C and collected with sterile water containing 0.1% Tween-80.

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Support

An anionic ion-exchange resin with macroreticular structure (Amberlite IRA-900, Rohm and Hass) was used througout the study. The description and pretreatment of the support have been reported.[1]

Fermentation

The culture medium 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. The culture medium was sterilized for 15 minutes at 115°C. The pH was then adjusted to 2.7. The spore suspension was then added to give a final concentration of 10^8/g dry support. The resin was then added to obtain a final humidity of 58%, and the fermentors were packed with 5.0 g of dry support each. The initial porosity of the bed, (ε_o = 40%) was calculated from the packed weight using a density of the wet Amberlite of 1.07 g/cm^3. The experimental setup for the diffusion-controlled fermentor (DCF) was a modification of the one proposed by Rathbun and Shuler[10] (Fig. 1). Growth was made in fermentors of 7.4 cm height (h) and 2.0 cm dia.; 5 fermentors were used for each experiment, and placed in an incubator at 35°C. The bottom base (Z = h) was closed. Through the upper end (Z = 0) vapor-saturated air (10 L/h at 35°C) was fed perpendicular to the axial gas transfer, the gas flow used was in the range used by Rathbun and Shuler.[10] One fermentor had 5 gas sample ports in the axial direction, the other 4 were used for analysis. Temperature was followed inside the fermentors throughout the experiment with needle type K thermocouples.

To compare the biological behavior of the mold in the DCF, a convective fermentor (CF), where aeration is supplied through the bed, was used.[1]

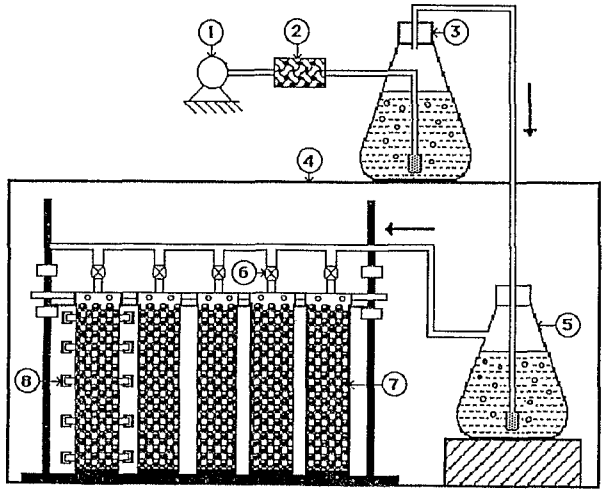

Figure 1. Experimental setup of the diffusion controlled fermentor. 1. Air supply, 2. air filter, 3. prehumidifier, 4. incubator, 5. humidifier, 6. air valves, 7. fermentors, 8. gas sample ports.

Analysis

The extraction of the biomass protein from the support, and its measurement by the Lowry method and the pH evaluation, has been reported.[1] Dry biomass was obtained from the protein measurement using a factor of 0.21 g protein/g dry biomass measured by the Lowry method. To measure the CO_2 and O_2 concentrations, a gas sample (100 μL), was taken from the ports periodically with a gas tight syringe, and were simultaneously evaluated with a gas chromatograph (Gow-Mac) equipped with thermal conductivity detector and a concentric column (CTR-1, Alltech).

MATHEMATICAL MODEL

The model is based on 3 hypotheses:

- the diffusion of O_2 and CO_2 is unidirectional;
- the variation of the effective diffusion coefficient depends only from the biomass produced by growth which occupies the interparticular void space; and
- the gas yield and maintenance coefficients [Eq. (3)] are constant throughout the experiment.

The conservation equation [Eq. (1)] for O_2 in the gas phase is:

$$\rho_g \frac{\partial \varepsilon C_\alpha}{\partial t} = \rho_g \frac{\partial J_\alpha}{\partial z} - \hat{R}_\alpha \tag{1}$$

The flux (J_α) is defined by the Fick law [Eq. (2)]:

$$J_\alpha = -\mathbf{D}_\alpha \frac{\partial \varepsilon C_\alpha}{\partial z} \tag{2}$$

The rate $\hat{R}_\alpha$ can be related to the dry biomass Xd through the yield Y_α and the maintenance coefficient m_α [Eq. (3)] as suggested by Sato et al.[11]

$$\hat{R}_\alpha = \frac{1}{V_r}\left[\frac{1}{Y_\alpha}\frac{\partial Xd}{\partial t} + m_\alpha Xd\right] \tag{3}$$

The dry biomass, Xd, is related to the porosity between particles ε [Eq. (4)]:

$$Xd = \rho_x W_s V_r[\varepsilon_o - \varepsilon] \tag{4}$$

By combining Eqs. (1) through (4), with the relation $\rho_s = \rho_x W_s$:

$$\rho_g \frac{\partial \varepsilon C_\alpha}{\partial t} = -\rho_g \frac{\partial}{\partial z}\left[\mathbf{D}_\alpha \frac{\partial \varepsilon C_\alpha}{\partial z}\right] + \frac{\rho_s}{Y_\alpha}\frac{\partial \varepsilon}{\partial t} - m_\alpha \rho_s[\varepsilon_0 - \varepsilon] \tag{5}$$

By integration of eq. (5) on the z axis for each time t:

$$\mathbf{D}_\alpha(z_1,t)\frac{\partial[\varepsilon(z_1,t)C_\alpha(z_1,t)]}{\partial z} = \frac{\rho_s}{\rho_g}\frac{1}{Y_\alpha}\int_0^{z_1}\frac{\partial \varepsilon}{\partial t}(z,t)\mathbf{d}z - m_\alpha\frac{\rho_s}{\rho_g}\varepsilon_0 z_1 + m_\alpha\frac{\rho_s}{\rho_g}\int_0^{z_1}\varepsilon(z,t)\,\mathbf{d}z - \int_0^{z_1}\frac{\partial[\varepsilon(z,t)C_\alpha(z,t)]}{\partial t}\mathbf{d}z \tag{6}$$

with the following boundary condition:

$$J_\alpha(Z_h, t) = 0.$$

where Z_h is the height of the fermentor.

From the experimental data, 4 polynomial approximations were obtained that expressed the gas and the biomass *Xd* concentrations as a function of Z and t, respectively. These fitted polynomials were derived and integrated analytically and used in Eq. (6) to obtain the effective diffusion coefficient. A similar treatment can be made for the production of CO_2 by changing the sign in eq. (1) for $\hat{R}_\alpha$.

RESULTS AND DISCUSSION

The experimental setup allowed isothermal conditions, and the resin kept a constant water content (58%) and constant diameter distribution in the fermentors throughout each experimental run. These findings were similar to those reported in the CF.[1] Also, no axial pH gradients were found, and the pH variation with time was similar to that observed in CF (Fig. 2).

Figure 3 represents the time evolution of the mold biomass at different heights. Biomass concentration in the upper (high O_2) part of the reactor attains similar values to those reported previously[1] for the CF. The biomass production is improved in the lower part of the reactor probably due to the increased CO_2 concentration as biomass accumulates.[2] By the effect of growth, the biomass occupies the empty interparticular space thus reducing the porosity of the bed [Eq. (4)]. The reduction in the porosity is very dependent on the apparent density of the dry biomass which is composed by two factors: (1) the wet biomass density, ρ_x: and (2) the dry matter content of the biomass, W_s. The values of ρ_x and W_s are in the range of 1.05 to 1.35 g/cm^3 and 0.1 to 0.3 g/g, respectively.[4] Using the extreme values and eq. (4) for the maximum dry biomass attained (135 mg/reactor), the final porosity of the bed lays between

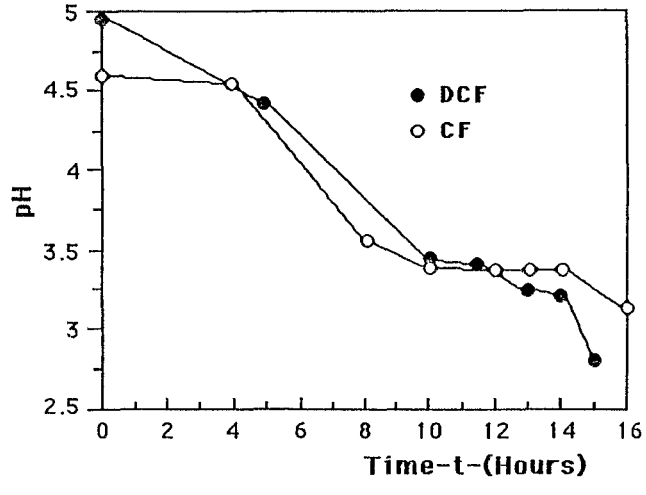

Figure 2. pH variation with time in the diffusion controlled fermentor (●) and convective fermentor (○).

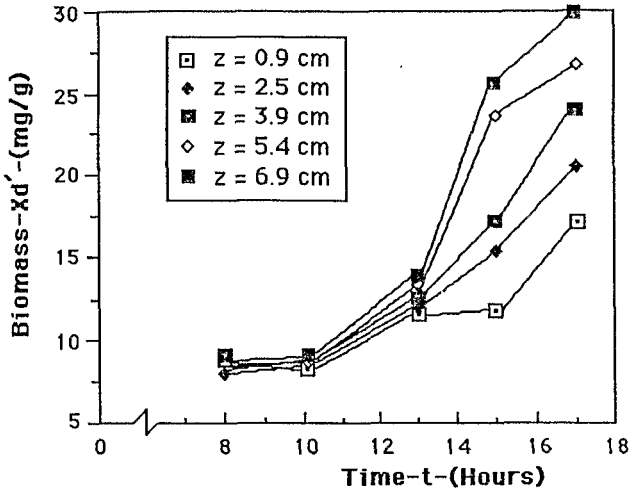

Figure 3. Dry biomass concentration variation with time at different bed height in DCF. $Z = 0$ at uppermost end.

0.345 for the low density ($\rho_x = 1.05$ g/cm^3 and $W_s = 0.1$ g/g) to 0.386 for the high density ($\rho_x = 1.35$ g/cm^3 and $W_s = 0.3$ g/g) with a value of 0.376 for the mean ($\rho_x = 1.2$ g/cm^3 and $W_s = 0.2$ g/g). The porosity for the low-density biomass is similar to that reported as maximum by Laukevics et al.[4] taking in account steric hindrance for several molds.

In Figures 4 and 5 the variation of the local O_2 and CO_2 concentration is shown. For O_2, it is represented as the initial (atmospheric O_2) minus the measured value. In both gases, for each height, the gradient increases with time up to 16 hours, then it tends to decrease. The extreme O_2 and CO_2 concentrations are 14% and 11%, respectively; these values represent an aerobic environment with a high CO_2 concentration for the mold. It is assumed that the constants in eq. (3) remain constant under these conditions. Also, the time at which the gradient was maximum (16 hours) corresponds to that when the maximum O_2 consumption and CO_2 variation was observed in CF.[1] From Figures 2 through 5, it can be considered that the growth behavior of the mold is similar in the DCF and in the CF, for the height of the DCF used.

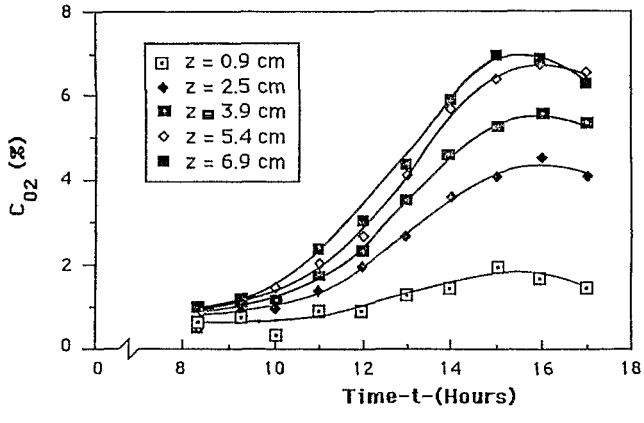

Figure 4. Variation of consumed O_2 with time at different bed height in DCF. $Z = 0$ at uppermost end.

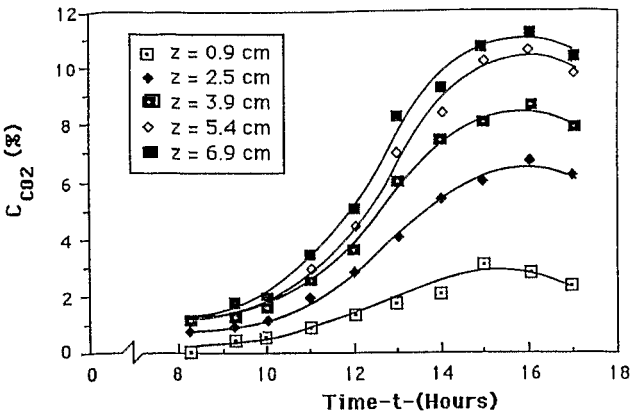

Figure 5. Variation of CO_2 with time at different bed height in DCF. $Z = 0$ at uppermost end.

Figures 6 and 7 show the change of the effective diffusion coefficient of O_2 and CO_2 with the dry biomass (Xd'). These values were obtained with eq. (6), using $\rho_x = 1.2$ g/cm^3 and $W_s = 0.2$ g/g. As expected, there was a decrease in the effective diffusion coefficients as the biomass increases. The behavior of the D_{O_2} and the D_{CO_2} was not evaluated at biomass concentrations below 7 mg/g, because the method used could not measure spores or very small biomass concentrations. At the highest biomass concentrations, the effective diffusion coefficient for both the O_2 and the CO_2 was more than 20 times lower than the molecular diffusion coefficient $D_{m\alpha}$ ($D_{mO_2} = 0.21$ cm^2/s, $D_{mCO_2} = 0.16$ cm^2/s). The behavior of the D_{O_2} and the D_{CO_2} as a function of the biomass follows the same general pattern as reported by other authors.[3] The influence of ρ_x and W_s on the effective diffusion coefficient showed deviations of +10% for the high-density and −10% for the low-density extreme.

Neale and Nader[8] studied the effective diffusion within porous media composed by impermeable spheres.

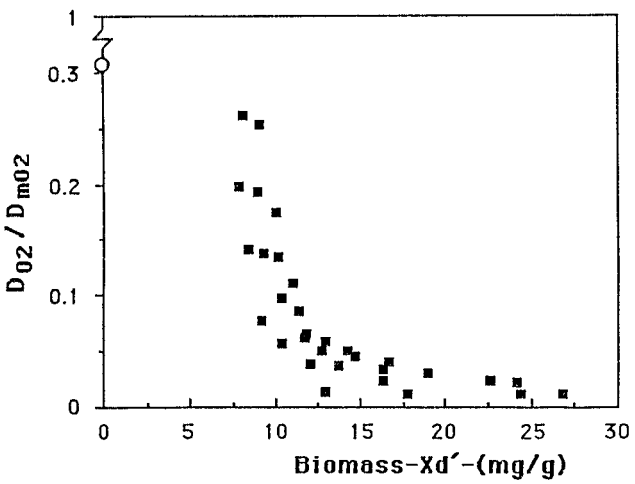

Figure 6. Effect of the dry biomass concentration on the diffusivity of oxygen in DCF. The mark (○) on the y-axis correspond to the calculated value according to the model reported by Neale and Nader.[8]

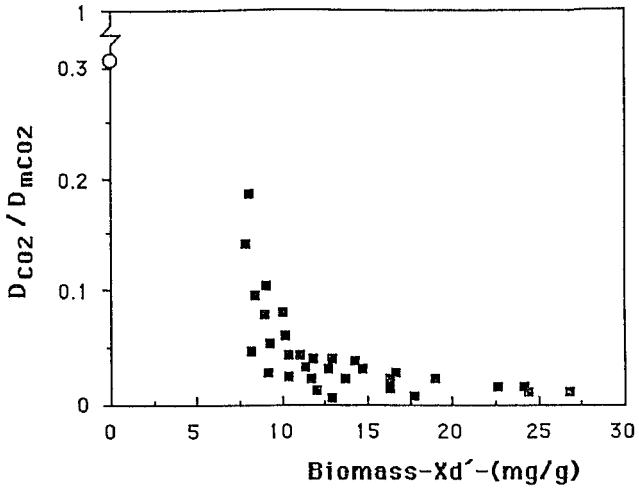

Figure 7. Effect of the dry biomass concentration on the diffusivity of carbon dioxide in DCF. The mark (○) on the y-axis correspond to the calculated value according to the model reported by Neale and Nader.[8]

The effective diffusion is related to the molecular diffusion through the porosity by the relation: $D_\alpha/D_{m\alpha} = 2\varepsilon(3 - \varepsilon)$, which is independent of the size distribution of the particles. For the experimental system reported in this work, the initial condition, which corresponds to a value of $\varepsilon_0 = 0.4$ with no mold, sets an initial $D_\alpha/D_{m\alpha}$ value of 0.31 according to the Neale and Nader model. When growth occurs, less variation in the $D_\alpha/D_{m\alpha}$ value would be predicted with the Neale and Nader model as a function of the porosity than the results obtained through the use of eq. (6). For the case of SSF, the occupation pattern of the void space differs from the conditions set by the Neale and Nader model. The reduction in the porosity in SSF is made by superficial growth on the sphere particles and by the formation of bridges between the adjoining particles.

CONCLUSIONS

The DCF packed with homogeneous and inert support proved to be a feasible way to calculate the variations in the effective diffusion coefficients for CO_2 and O_2. Despite the difficulty in measuring the density and water content of the mold, this methodology can give suitable values for the diffusion coefficient (±10%). The variation obtained in the effective diffusion coefficient as a function of the biomass shows a much steeper decrease as that which could be predicted by the porosity using models such as that reported by Neale and Nader.[8] This may be related to the complexity of the mold growth.

In the case of supports that are usually used in SSF (rice, bran, wheat, etc.), there are other macroscopic changes that were not observed with the ion-exchange resin used in this study. First, there may appear intraparticular diffusion limitations due to the internal growth. Second, the packing of the substrate may vary;

initially, it may depend on the size, form, distribution of size, and the pressure exerted on packing the fermentor. As the fermentation proceeds there may be variations in the packing due to substrate utilization. The method reported in this study might be applied for natural supports depending upon the importance of the macroscopic changes during the fermentation.

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NOMENCLATURE

C	gas concentration (cm^3/cm^3)
D	effective diffusion coefficient (cm^2/s)
D_m	molecular diffusion coefficient (cm^2/s)
J	flux of gas (cm/s)
m_{O_2}	maintenance coefficient of O_2 (6.08×10^{-8} mol O_2/g *Xd*/s)
m_{CO_2}	maintenance coefficient of CO_2 (3.47×10^{-8} mol CO_2/g *Xd*/s)
$\hat{R}$	rate of gas consumption (for O_2) or production (for CO_2) (mol/cm^3/s)
t	time (s)
Vr	reactor volume (23.24 cm^3)
Ws	biomass dry matter content (g/g)
Xd	dry biomass (g)
Xd'	dry biomass per gram dry support (g/g)
Y_{O_2}	yield coefficient of O_2 (496.0 g *Xd*/mol O_2)
Y_{CO_2}	yield coefficient of CO_2 (492.8 g *Xd*/mol CO_2)
z	height (cm)

Greek Letters

ε	interparticular porosity (cm^3/cm^3)
ρ_g	gas density (mol/cm^3)
ρ_x	wet biomass density (g/cm^3)
$\rho_s = \rho_x W_s$	apparent dry biomass density (g/cm^3)

Subscripts

α	gas constituents (O_2 or CO_2)
O	initial condition

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