0273–2289/86/1303–0181$02.00

Oxygen Limitation on L-Serine Production in a Hollow-Fiber Bioreactor

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Received May 10, 1985; Accepted September 24, 1985

ABSTRACT

Pseudomonas AM1 utilizes glycine and methanol to produce L-serine aerobically (*1*). The consumption of methanol in this bioconversion is stoichiometrically in excess of L-serine production (*2*). Consequently, the oxygen requirement associated with L-serine production is higher than expected for the conversion from glycine.

One method of L-serine production investigated was a technique utilizing a hollow-fiber ultrafiltration cartridge as a bioreactor. Oxygen diffusion limitations appear to impede the consumption of methanol and, consequently, the production of L-serine in such a reactor. Methanol consumption data agree with predictions based on a hollow-fiber diffusion model.

Index Entries: L-serine production; hollow-fiber bioreactor; oxygen; *Pseudomonas* AM1; glycine; methanol; bioconversion.

INTRODUCTION

Hollow-fiber cartridges are used for macromolecular concentration, microbial concentration, cell harvesting, desalting (*3*), continuous fermentation, and cell culture and as bioreactors (*4–6*). The hollow-fiber bioreactor presents a design in which the biocatalyst can be highly concentrated, yet separated from the primary-substrate stream in order to get the highest production with minimal volume. This article concentrates on the use of the hollow-fiber cartridge as a vehicle for bioconversion of glycine and methanol into L-serine, using a temperature sensitive mutant of *Pseudomonas* AM1.

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The question to be addressed is to what extent oxygen diffusion through the membrane wall limits the effectiveness of the hollow-fiber cartridge as a bioreactor in an aerobic system, and, given such a restriction, how can the design be modified to allow high conversion to take place while retaining the most important function of the hollow fiber: separation of biocatalyst and substrate streams in a continuous operation.

MATERIALS AND METHODS

Cells of a temperature-sensitive mutant of the methylotroph, *Pseudomonas* AM1, suspended in 0.05*M* Tris buffer (8.8 g/L dry basis), pH 8.0, bioconversion medium (40 mL), were placed into the shell side of an Amicon H1 X-50-8 hollow-fiber cartridge. This device has a mol wt cut-off of 50,000, an internal fiber diameter of 0.2 mm, a cartridge diameter of 2.3 cm, and a cartridge length of 20.3 cm. It utilizes 1000 vinyl/acrylic copolymer hollow fibers. The fiber length exposed to the cell slurry is 16.5 cm. A 40-mL vol of 0.05*M* Tris buffer, pH 8.0, without cells, was recirculated through the inside of the hollow fibers at a rate of 60 mL/min, with a peristaltic pump, through an agitated substrate reservoir. Methanol and glycine were initially present at 500 m*M* each. Methanol and amino acid levels were measured by GC and HPLC, respectively, during the course of the reaction. Oxygen levels were monitored using a dissolved oxygen probe. A control run, using 11.6 g/L of cells, had 20-mL bioconversion medium in a 500-mL shake flask, and methanol, glycine, L-serine and oxygen were similarly measured.

RESULTS AND DISCUSSION

Bioreactor for L-Serine

The production of L-serine was much greater in the control (65 m*M*) than in hollow-fiber bioreactors with stagnant (2 m*M*) and recirculating cell suspension (3 m*M*) (Fig. 1). Molar yields of L-serine, based on glycine, consumed reflected similar trends: 60% for the control run and 20% for the hollow-fiber system with stagnant cell suspension. Table 1 shows the initial rates of L-serine production and initial uptake rates of methanol and oxygen. The rate of oxygen consumption was calculated to be half of the rate of methanol consumption with the cell suspension.

Since the cell concentration in the hollow-fiber shell is comparatively low (8.8 g/L), the lack of L-serine production cannot be attributed to cell "overcrowding," which has been observed at high cell concentrations by others (*2,7*). Rather, since the catalyst is effectively isolated from direct contact with the atmosphere, this reduction in productivity can be attributed to the limitation of oxygen diffusion. When the cell suspension was recirculated at 60 mL/min to disrupt any significant concentration gradi-

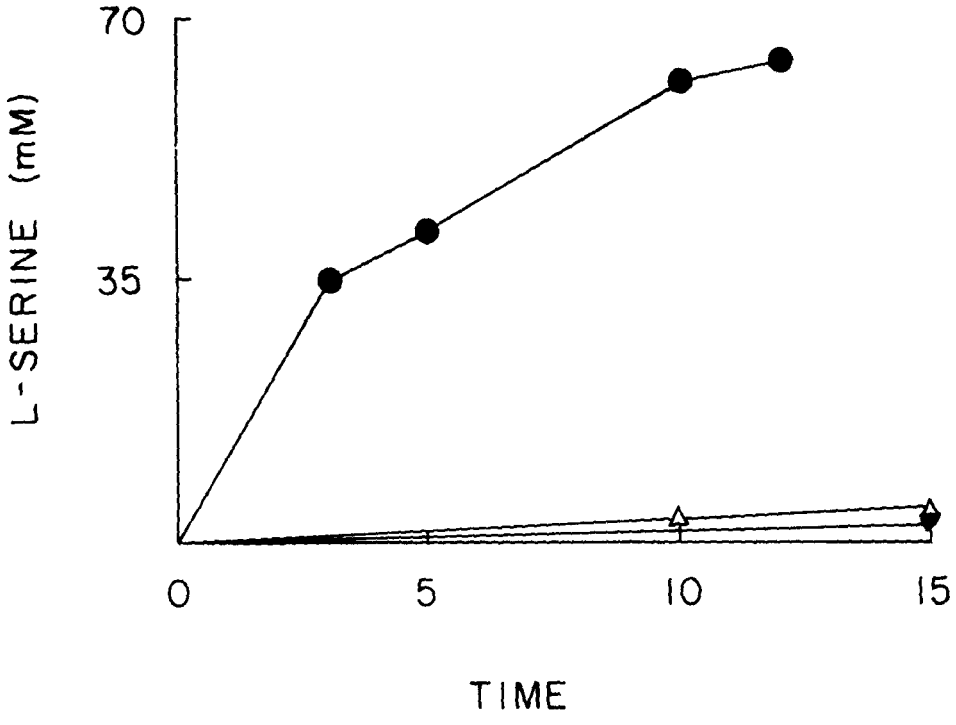

Fig. 1. L-serine production. (●), control-shake flask; (△), hollow-fiber bioreactor with recirculating cell suspension; and (▼), hollow-fiber bioreactor with stagnant cell suspension.

ents in the annular portion of the hollow fiber, the results (Fig. 1 and Table 1) also showed very low L-serine production along with low methanol consumption rate; the values are far below the control. These results are consistent with the fact that even with recirculation, sufficient resistance to oxygen transfer exists in the hollow fiber, medium, and boundary layer to severely limit the rate of reaction. The reason such low conversions are obtained is that all the cells and a large portion of the volume of

TABLE 1
Bioconversion of Glucose and Methanol into L-Serine, Using a Hollow-Fiber Bioreactor

Reactor	L-Ser, mM	O_2, % sat	mmol/g cell/h		
			g_{ser}	g_{MeOH}	g_{O_2}
Control	65	100	0.94	16.1	8.05
Hollow fiber w/stagnant cell suspension	2	67	0.34	1.05	0.53
Hollow fiber w/recirculatng cell suspension	3	67	0.36	1.36	0.68

substrate (50%) are enclosed in an oxygen limited vessel—the hollow-fiber cartridge. Dissolved oxygen levels were measured at 67% of saturation in the reservoir.

Oxygen-Limited Model

To test the validity of the oxygen-diffusion limited hypothesis, a model for diffusion in cylindrical coordinates into the catalytic region was used (*8–10*). The individual hollow fibers are modeled as parallel tubes with a corresponding extratubular annulus, the volume of which is equal to the total cell-suspension divided by the number of fibers. The individual hollow-fiber can be modeled as a tube with a cell-free solution at uniform oxygen concentration, surrounded by a hollow-fiber wall that allows all materials, but not the whole cells, to pass through it, which is, in turn, surrounded by an annulus or shell of suspended cells (Fig. 2).

Oxygen diffusion occurs through the hollow-fiber wall into the annular cell-containing region, where it is utilized by the cells in methanol consumption (Fig. 2). The material conservation equation for radial diffusion in the outer annular region can be written as:

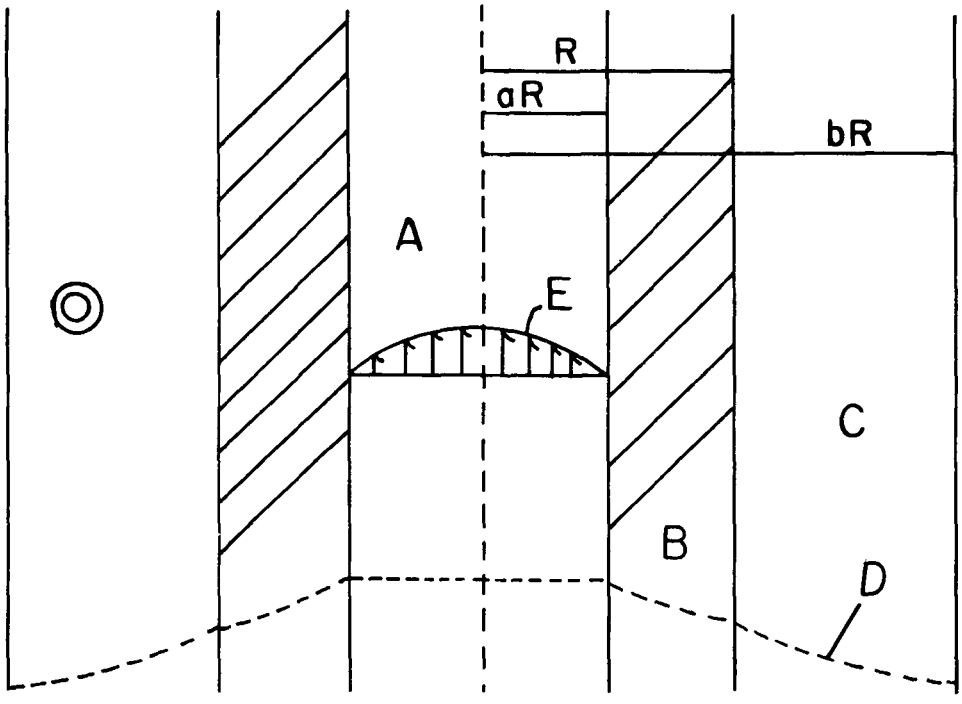

Fig. 2. Cross-section of hollow fiber. (A) is hollow fiber interior; (B) is hollow fiber wall; (C) is the annulus; (D) is the concentration profile; and (E) is the velocity gradient within the fiber. *aR* is the distance from the center of the fiber to the inner wall surface, *R* is the distance from the center to the outer wall surface and *bR* is the distance from the center to the half-way point to the next hollow fiber.

$$\frac{D}{r}\frac{d}{dr}r\frac{dC(r)}{dr} - R = 0 \tag{1}$$

where D = diffusivity (cm^2/s); r = distance from the hollow-fiber center (cm); R = rate of reaction; and $C(r)$ = concentration as a function of r (mM).

If the rate of reaction is assumed to be first-order in oxygen [$R = k(O_2)$], the equation becomes:

$$\frac{dC^2(r)}{dr^2} + \frac{1}{r}\frac{dC(r)}{dr} - \frac{k}{D}C(r) = 0 \tag{2}$$

where k = the first-order rate constant.

This expression is a form of Bessel's equation, and a general solution can be obtained:

$$C(r) = AI_0(Pr) + BK_0(Pr) \tag{3}$$

where $P = (R/D)^{0.5}$; I_0 and K_0 are hyperbolic bessel functions of 0th order of the first and second kind, respectively, and A and B are constants.

The boundary conditions on Eq. [2] are:

$$C(r) = C(R) \text{ at } r = R \tag{4}$$

and

$$(d/dr)C(r) = 0 \text{ at } r = bR \tag{5}$$

The following equation for the concentration in the annulus as a function of radius is obtained:

$$C(r) = C(R)G(r) \tag{6}$$

where:

$$G(r) = \left[\frac{K_1(PbR)I_0(Pr) + K_0(Pr)I_1(PbR)}{K_0(PR)I_1(PbR) + K_1(PbR)I_0(PR)}\right] \tag{7}$$

If the concentration within the fiber wall is the same as within the fiber tube, then $C(R) = C_0$, and the concentration profile for the annular region can be developed.

If, however, there exists a concentration profile within the wall region, the following equation applies:

$$\frac{D}{r}\frac{d}{dr}r\frac{d}{dr}C(r) = 0 \tag{8}$$

with the solution:

$$C(r) = A' \ln (r) + B' \tag{9}$$

where A′ and B′ are constants, and the following boundary conditions are applicable:

$$C(R) = A' \ln (R) + B' \text{ at } r = R \tag{10}$$

$$C(aR) = A' \ln (aR) + B' \text{ at } r = aR \tag{11}$$

and

$$\frac{d(Cr)}{dr} \text{ wall} = \frac{dC(r)}{dr} \text{ annulus at the interface} \tag{12}$$

Using the appropriate expressions for $C(r)$ for the wall and the annulus regions, the following equation for the oxygen concentration as a function of r, $C(r)$, can be obtained:

$$C(r) = C_0\left(\frac{1}{RG(R)P \ln(a) + 1}\right)G(r) \tag{15}$$

where $G(R)$ is $G(r)$ at $r = R$.

The flux of substrate, N/A (mol/cm^2/sec) can also be computed into the annular region by the following expression:

$$N/A = D\frac{d}{dr} C(r) \text{ evaluated at } r = R \tag{16}$$

The volumetric oxygen consumption rate, N/V, can be elevated by multiplying the flux by the ratio of the area (A) of the hollow fiber at $r = R$ to the corresponding annular volume (V):

$$N/V = C_0\left(\frac{P}{RG(R)P \ln(a) + 1}\right)\frac{A}{V}DG(R) \tag{17}$$

The specific oxygen consumption rate q_{O_2}, can be obtained by dividing the volumetric rate by the cell concentration within the hollow fiber.

Substituting measured values for this cartridge [$R = 0.25$ cm; $aR = 0.1$ cm; $bR = 0.36$ cm; $P = (R/D)^{0.5} = 100$] from $D = 1 \times 10^{-5}$cm^2/s for a well dispersed cell suspension (*7*), $k = 0.1$/s (from experimental measurement, and $C_0 = 0.000134M$ within the fiber) gives a consumption rate of 0.88 mmol/g cell/h. This model predicts a decrease in oxygen consumption as measured by methanol consumption, which is quite similar to measured experimental values (Table 1).

However, certain approximations within this model serve to compromise its accuracy. The kinetic rate is not first-order, but needs to be approximated for the sake of the model. The diffusivity through the annular region of the fiber will not be that for oxygen in a cell-free medium, and the diffusivity through the fiber wall may not be equal to that through the annulus, although, given the highly porous nature of the fiber wall, it may be very close.

Furthermore, the concentration profile within the fiber will not be uniform (as assumed) and some resistance to diffusion will be offered by the boundary layers along the substrate stream/fiber wall interface. Also, the thickness of the fiber wall is variable, and an average estimate is used in this model. Finally, the annular region is not, in fact, an annulus with a uniform concentration of catalyst. The interfiber region is modeled as

an annulus for the sake of mathematical workability, and the catalyst is taken as being uniform in concentration, although some adhesion of the cells to the hollow fibers were observed.

Despite these shortcomings, the model presents an accurate description of the concentration profile around the hollow fibers. It serves to explain the reduction of methanol consumption rate in the hollow fiber. Also, it provides a quantitative guideline for methanol consumption in this system, and, as such, can be used as a predictive tool for evaluation of further reactor designs along these lines.

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