Appl Microbiol Biotechnol (1987) 26:500–506

Applied and Microbiology Biotechnology

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Intraparticle diffusional effects in immobilized cell particles

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Summary. Immobilized cell technology frequently relies on the entrapment of the biomass in a gel particle, and it is generally observed that mass transfer limitations within the gel particle lead to nonuniform cell distribution. This note addresses the consequence of maintaining a very high cell mass density within a biopolymer particle. We illustrate that conventional effectiveness factor calculations can be used to determine particle sizes which would avoid nonuniform cell growth. The analysis is based on simple Monod kinetics. Special attention is given to near zero order kinetic systems in which the effectiveness factor remains high although the limiting nutrient may be depleted near the center of the particle.

Introduction

The application of immobilized microbial cells has attracted increasing attention in recent years. Among other advantages, the immobilized cell bioreactor avoids cell washout at high dilution rates and can maintain a very high cell mass density. The resulting productivity is higher than other suspension cell processes. The biomass is usually entrapped in a polymer matrix gel bead, for example, calcium alginate or carrageenan, and less frequently polyacrylamide.

If a very high biomass concentration is being entrapped within a particle, the question of diffusional limitations has to be addressed (Black et al. 1984). There is an overwhelming amount of experimental evidence in immobilized *Saccharomyces cerevisiae* work which indicates that cells grow preferentially closer to the surface of gel particles (Wada et al. 1980; Chibata et al. 1983; Beronio et al. 1986). Similar observations if diffusional effects were made in a hollow fiber immobilized yeast bioreactor (Inloes et al. 1983). When yeast cells were maintained in the matrix of an isotropic microporous fiber membrane. A radial distribution of cell packing existed across the membrane, and there was inadequate glucose supply to cells located beyond 0.1 mm from the membrane surface. Observations of diffusional resistance are not limited to immobilized yeast cells. A culture of *Penicillium urticae* resulted in cell growth only within 0.3 mm of a 3 mm carrageenan gel bead (Berk et al. 1984). Similar results were obtained with *Streptomyces aureofaciens* cultured among hollow fibers (Robertson and Kim 1985). Generally, we expect diffusional resistance in a gel particle when the biomass has proliferated to a density much higher than that in a suspension cell culture.

Discussions presented usually allude to the analyses of immobilized enzyme systems or a zero order reaction. However, immobilized cell systems are different from isolated enzymes which function according to the same Michaelis-Menton kinetics irrespective of how low the substrate concentration may become. Thus interpretation of results cannot be based entirely on immobilized enzyme systems as a living cell cannot be maintained for a prolonged period under nutrient starvation conditions. In addition, the cell kinetics may change drastically if the limiting substrate concentration drops below a threshold value. Hence, a strictly zero order kinetic analysis is not necessarily adequate.

The mathematical framework of coupling chemical reaction with intraparticle diffusion is

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well developed with immobilized enzyme systems. Immobilized cell systems can be analyzed appropriately based on similar mathematical tools. To provide appropriate interpretation for immobilized cells, we need to complement the available effectiveness factor analyses with further information on the concentration at the center of the particle. Our objective is to present, making use of available literature data, an assessment on the particle size limitation of immobilized cell particles. The goal is to illustrate that the immobilized cell particle must be sufficiently small to avoid nonuniform cell growth.

Model analysis

If we assume simple Monod growth kinetics, the mathematical description of the immobilized cell particle is very similar to that of immobilized enzyme systems (Moo-Young and Kobayashi 1972; Fink et al. 1973), or is identical to that of microbial flocs (Atkinson and Rahman 1979). Gianetto et al. (1983) used the generalised modulus approach (Moo-Young and Kobayashi 1972) to calculate the effectiveness factor of a bilayer yeast film growth and concluded that there was no diffusional resistance. The following development centers on cell growth in a particle and the consequence of high cell mass densities.

A major assumption in the following analysis is that cell mass density is uniform within the gel particle. We know that the presence of a nutrient gradient will select for a cell mass population on the periphery of the gel particle. Hence the analysis is justified only from the viewpoint that we are looking for conditions under which diffusional resistance is not important and thus a high density cell mass can be maintained uniform within the particle.

The steady state mass balance of the limiting substrate $\tilde{S}$ in the gel particle is similar to previous analysis with immobilised enzymes (Bailey and Ollis 1986). With the adjustment for Monod kinetics for the substrate consumption, the equation is

$$D_e\left(\frac{d^2\tilde{S}}{d\tilde{r}^2}+\frac{(1+g)}{\tilde{r}}\cdot\frac{d\tilde{S}}{d\tilde{r}}\right)=\frac{1}{Y_{X/S}}\cdot\frac{\mu_{max}\tilde{S}}{(\tilde{K}_m+\tilde{S})}X, \tag{1}$$

where D_e is the effective diffusivity of the substrate in the gel particle. The geometry factor g takes on values of -1, 0, or 1 when equation (1) is applied to slab, cylindrical or spherical geometries, respectively. The simple Monod equation is assumed. The maximum specific growth rate, Monod constant and the cell yield coefficient are denoted by μ_{max}, $\tilde{K}_m$, and $Y_{X/S}$ respectively. The biomass, X, as discussed above is taken as a constant. To follow previous similar analyses in immobilized enzymes (Moo-Young and Kobayashi 1972; Fink et al. 1973), equation (1) is rearranged into a dimensionless form. The substrate concentration $\tilde{S}$ and Monod constant $\tilde{K}_m$ are normalized by the concentration at the surface of the particle $\tilde{S}_S$. The surface concentration is usually relatively large with respect to the Monod constant. However, with a well-mixed continuous flow culture, the surface concentration of the limiting substrate can be rather low. Eq. (1) in dimensionless variables appears as

$$\frac{d^2S}{dr^2}+\frac{(1+g)}{r}\cdot\frac{dS}{dr}=\varphi_0^2\cdot\frac{S}{(K_m+S)}, \tag{2}$$

where $S = \tilde{S}/\tilde{S}_S$ is the dimensionless substrate concentration,
$r = \tilde{r}/R$ is the dimensionless spatial coordinate,
$K_m = \tilde{K}_m/\tilde{S}_S$ is the dimensionless Monod constant,
and $\varphi_0 = R\sqrt{\frac{\mu_{max}X}{Y_{X/S}\tilde{S}_S D_e}}$ is the zero order Thiele modulus.

The boundary conditions to eq. (2), without external mass transfer influence, are

$$r=1,\ S=1 \quad \text{and} \quad r=0,\ \frac{dS}{dr}=0$$

The Thiele modulus (also referred to as the Damköhler number) represents the ratio of the kinetic reaction rate to the substrate diffusion rate. Other authors (Fink et al. 1973; Atkinson and Rahman 1979) have used slightly modified moduli or different dimensionless variables, but we have chosen the present form as it arises naturally from the mass balance and any design calculations can be carried out easily with the dimensionless Monod constant and the zero order modulus. We should point out that a popular approach in analyses of immobilized enzyme systems is to use the pseudo-first order modulus

$$\varphi_1=R\sqrt{\frac{\mu_{max}X}{Y_{X/S}K_m D_e}}.$$

As will be shown in the discussion below, the character of the systems that we are interested in approaches zero order dependence in the substrate concentration. Hence our preference for a zero order modulus. Our goal is to evaluate the concentration at the center of the particle, S_0, and the effectiveness factor, η, as a function of K_m and φ_0. The effectiveness factor represents the ratio of observed reaction rate to the rate obtainable without intraparticle gradients. At steady state, the observed reaction rate is also the mass flux at the particle surface. The values of the effectiveness factor cannot be extracted easily from available literature data, but it is a common concept used in intraparticle diffusion analysis and will be useful in presenting our discussion later. The effectiveness factor in dimensionless variables can be expressed as

$$\eta=\frac{(2+g)\left.\frac{dS}{dr}\right|_{r=1}}{\varphi_0^2\left(\frac{1}{K_m+1}\right)} \tag{3}$$

When we consider a slab geometry, an analytical solution can be obtained for the effectiveness factor (Moo-Young and Kobayashi 1972):

$$\eta=\frac{2}{\varphi_0}\cdot(1+K_m)\cdot\left\{(1-S_0)+K_m\cdot\ln\left(\frac{K_m+S_0}{K_m+1}\right)\right\}^{1/2}, \tag{4}$$

where S_0 is the center concentration. The center concentration is expressed implicitly as

$$\int_{S_0}^{1}\left\{(S'-S_0)+K_m\cdot\ln\left(\frac{K_m+S_0}{K_m+S'}\right)\right\}^{1/2}dS'=\sqrt{2}\cdot\varphi_0, \tag{5}$$

where S' is the integration variable.

The important point to note is that the system is defined

clearly by the two parameters: zero order modulus and the dimensionless Monod constant, viz.,

$$\eta = \eta(\varphi_0, K_m) \quad \text{and} \quad S_0 = S_0(\varphi_0, Km) \tag{6}$$

Numerical solutions are required for cylindrical and spherical geometries. The following results for spherical geometries are obtained from the steady state solution of the transient form of eq. (2), and an implicit finite difference scheme with Newton itertions (Davis 1984).

Results and discussion

According to conventional chemical engineering wisdom, the effectiveness factor is adequate to determine the significance of diffusional resistance within a catalytic or enzymatic particle. In the following discussion concerning living immobilized microbial cells, we shall emphasize the need to also assess the value of the substrate concentration at the center of the particle, and the consequences for a system whose behavior approaches that of zero order.

The results of eq. (3) are usually presented as plots of effectiveness factor versus a modulus. The modulus may be that of a pseudo-first order modulus, zero order modulus or other modified forms, and the choice is usually made on the basis of the preference of the form of the plots (Moo-Young and Kobayashi 1972; Fink et al. 1973; Atkinson and Rahman 1979). For the purpose of our discussion, we only need a common feature from these analyses. That is, the effectiveness factor approaches unity when the modulus is adequately small, usually a number less than unity.

The fundamental difference between enzymatic and living cell systems should be emphasized. With immobilized enzyme systems, the presence of a concentration gradient within a particle will only diminish the effectiveness of the enzyme utilization. In the absence of deactivating agents, the catalytic activity of the enzyme theoretically will remain intact. The same situation does not hold with immobilized cells. A living cell system cannot be maintained in a starvation medium for prolonged periods. The reaction kinetics with respect to a substrate commonly exhibits near zero order behavior when the substrate is in abundance. However, certain nutrient components may need to be maintained above a threshold value for operation of metabolic pathways. For example, the level of dissolved oxygen is very often critical in antibiotic fermentations. The concentration at the center of the particle provides the most severe criterion to determine whether a particular nutrient is maintained above a certain specified value.

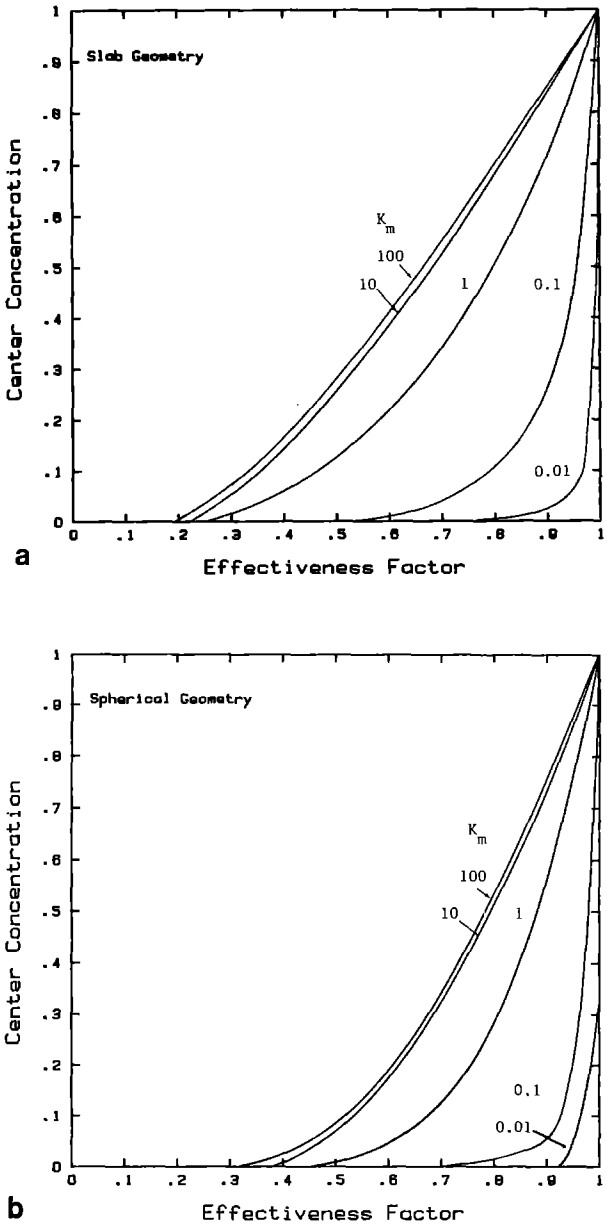

Fig. 1. The variation of dimensionless center concentration of substrate with effectiveness factor at different dimensionless Monod constants. **a** Slab geometry, **b** spherical geometry. In both figures, the case with a first order reaction virtually merges with the $K_m = 100$ curve

Figure 1 illustrates this idea. With a first order reaction, the center concentration generally decreases as the effectiveness factor falls below one. With a zero order reaction, the effectiveness factor remains unity for as long as the center concentration has a finite non-zero value. With a slab geometry, we can describe this phenomenon

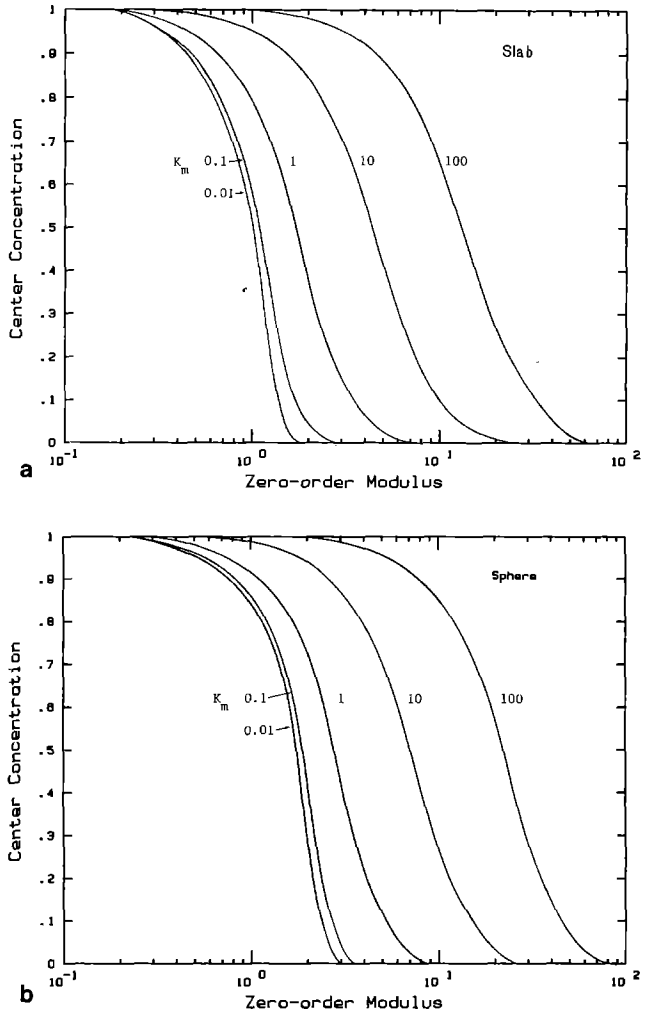

Fig. 2. The dimensionless center concentration of substrate as a function of the zero order modulus at different dimensionless Monod constants. **a** Slab geometry, **b** spherical geometry

quantitatively as follows:

$$\begin{aligned} &\varphi_0 \leq \sqrt{2}, \quad \eta = 1 \quad \text{and} \quad S_0 = 1 - \varphi_0^2/2 \\ &\varphi_0 > \sqrt{2}, \quad \eta = \sqrt{2}/\varphi_0 \quad \text{and} \quad r^* = 1 - \sqrt{2}/\varphi_0, \end{aligned} \tag{7}$$

where r^* is the position where the concentration of the substrate S drops to zero. Similar results can be obtained for a spherical particle.

$$\begin{aligned} \text{When } \varphi_0 \leq \sqrt{6}, \quad &\eta = 1 \quad \text{and} \quad S_0 = 1 - \varphi_0^2/6 \\ \varphi_0 > \sqrt{6}, \quad &\eta = 1 - r^{*3} \end{aligned} \tag{8}$$

and r^* is given implicitly as

$$\frac{\varphi_0^2}{6}(1 - r^*)[2r^{*3} - r^* - 1] + 1 = 0$$

which is similar to a derivation by Krouwel and Kossen (1980).

Thus the zero order curve follows the right ordinate (where effectiveness factor is one) in Fig. 1 until the center concentration becomes zero. Then the curve follows the bottom abscissa until the effectiveness factor is zero, theoretically for an infinitely fast reaction. The important message is that with zero order reaction, we can have a very low center concentration even though the effectiveness factor remains rather high. Similar observations can be made with systems which behave close to a zero order system. The pertinent instance is cases with small dimensionless Monod constants such that the system is close to zero order with respect to the substrate uptake rate. This has important implications to immobilized cell systems as we shall illustrate with examples which bear characteristics of this nature. A comparison of the slab and sphere cases in Fig. 1 indicates that for any given effectiveness factor, the center concentration of a spherical particle is lower.

Recall Eq. (6), the center concentration is defined by the zero order modulus and the dimensionless Monod constant. The numerical solution is shown in Fig. 2. For a given zero order modulus, the concentration becomes increasingly lower for smaller K_m which is in agreement with earlier discussion. We can also consider that large K_m inhibits the substrate uptake, and thus a less severe concentration gradient within the particle. For a given modulus and K_m, the slab geometry provides a lower center concentration. Hence calculations based on the slab geometry give a very conservative design for spherical particles. This observation is not in contradiction to Fig. 1 since the effectiveness factor of a spherical particle is higher than that of a slab geometry for the same φ_0 and K_m (Fink et al. 1973).

With immobilized yeast ethanol production, carbon dioxide gas bubbles can be formed within the gel particle. The evaluation of the center substrate concentration is potentially useful in assessment of gas bubble nucleation within the particle. Krouwel and Kossen (1980) provided the analysis for a strictly zero order system. However, such analyses are not fruitful until one can also assess the critical saturation pressure. Generally, observations indicate that carbon dioxide bubbles are formed only in gel particles above 3—4 mm diameter (Dalili 1984).

To analyse circumstances in which a substrate critical threshold value exists, it is more convenient to design the particle system based on Fig.

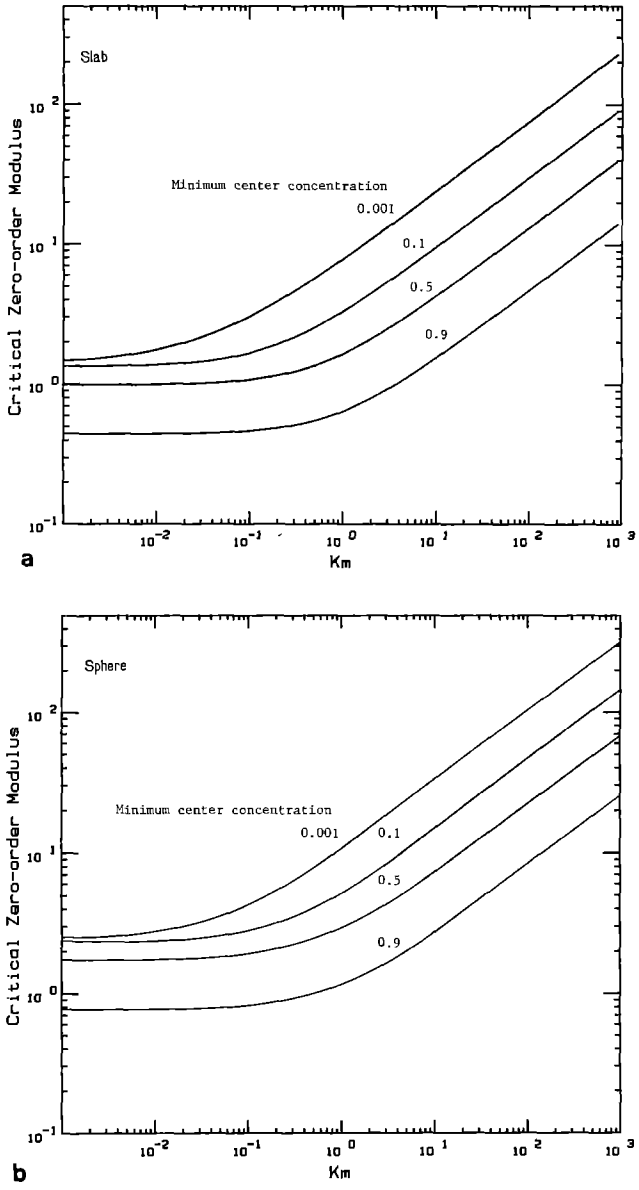

Fig. 3. The critical zero order modulus as a function of the dimensionless Monod constant at different specified minimum dimensionless center concentrations. **a** Slab geometry, **b** spherical geometry

3. For a system with a required minimum center concentration and a known Monod constant, the critical zero order modulus can be calculated from which the maximum particle size can be evaluated. The design becomes more stringent, that is smaller critical modulus, as the minimum center concentration is higher. The criterion also is more stringent with small K_m or systems which approach that of a zero order. The critical modulus becomes larger when the system approaches pseudo-first order, or large K_m. It should be emphasized that all results are based on dimensionless center concentrations and K_m. Thus a large K_m also means deprivation of substrate on the surface of the particle, and we should be aware of the actual surface concentration.

With Figs. 2 and 3, we can determine with certainty the gel bead diameter which would provide for not only a high effectiveness factor, but also the necessary nutrient concentration at the center of the particle. For microbial systems which have a small Monod constant, the cell growth approaches zero order with respect to the limiting substrate concentration. Hence, if we can maintain the substrate center concentration above a certain threshold level, we can maintain a uniform cell mass density within the particle and also satisfy the assumption we made on eq. (1).

Finally, we illustrate with two examples. The results of an immobilized cell system are presented in Table 1. A cell mass density of 11.6 g/liter is typical of immobilised yeast reactor start up, while 250 g/liter is close to the highest immobilized cell density possible (Nagashima et al. 1984). The two glucose surface concentrations are typical of aerobic cell growth and anaerobic fer-

Table 1. Particle size and cell mass loading effects on immobilised yeast cell particles. Basis of calculations (Tanaka et al. 1984; Tyagi and Ghose 1982): Glucose is limiting substrate. Maximum specific growth rate = 0.25 h^{-1}, cell yield coefficient = 0.08 g/g, Monod constant = 0.476 g/l, and effective diffusivity = 6.8×10^{-6} cm^2/s

Diameter (cm)	$\bar{S}_S = 10$ g/l, $K_m = 4.76 \times 10^{-2}$					
	$X = 11.6$ g/l gel			$X = 250$ g/l gel		
	φ_0	η	S_0	φ_0	η	S_0
0.5	3.04	0.901	5.4×10^{-3}	14.1	0.271	0.
0.3	1.83	0.988	0.485	8.47	0.430	0.
0.2	1.22	0.996	0.767	5.65	0.601	4.8×10^{-8}
0.1	0.608	1.0	0.941	2.82	0.929	0.0197
0.05	0.304	1.0	0.985	1.41	0.994	0.687
0.03	0.183	1.0	0.995	0.847	0.999	0.886
Diameter (cm)	$\bar{S}_S = 100$ g/l, $K_m = 4.76 \times 10^{-3}$					
	$X = 11.6$ g/l gel			$X = 250$ g/l gel		
	φ_0	η	S_0	φ_0	η	S_0
0.5	0.962	1.0	0.847	4.47	0.681	0.
0.3	0.577	1.0	0.945	2.68	0.977	3.5×10^{-5}
0.2	0.385	1.0	0.976	1.79	0.999	0.473
0.1	0.192	1.0	0.994	0.893	1.0	0.868
0.05	0.096	1.0	0.999	0.447	1.0	0.967
0.03	0.058	1.0	0.999	0.268	1.0	0.988

mentation conditions. It should be mentioned that at 100 g·glucose/liter, the simple Monod equation is not adequate as ethanol inhibition effects will become important. Thus the calculations here only serve to illustrate the change in results as the dimensionless Monod constant becomes smaller. There are several obvious observations. At a low cell mass loading, diffusional effects are not important. This is essentially the basis of Gianetto and co-workers' calculations (1983). When the cell mass density is kept at a high value which is desired of immobilized cell systems, intraparticle diffusional effects cannot be ignored. Particles at 1 mm diameter or bigger have an extremely low center concentration even though the effectiveness factor may be very close to unity. To maintain a very high and uniform cell mass, the particle has to be less than 1 mm diameter.

Similar inferences can be drawn from the example in Table 2, which represents an immobilizec *Streptomyces parvulus* system for the production of actinomycin D. In this case, dissolved oxygen is the limiting substrate, and the dissolved oxygen has to be maintained above 5 ppm for the *Streptomyces parvulus* to function properly (Dalili 1984). Because of difficulties in extracting information on the cell mass, two values of oxygen uptake rate are used to simulate low and high cell density situations in Table 2. Oxygen has a slightly higher diffusivity than glucose. Nevertheless, a gel bead diameter under roughly 1 mm is necessary to keep the center concentration above 5 ppm at a high cell mass loading. These results are consistent with the observation with immobilized *Penicillium urticae* that mycelia only propagate within 0.3 mm of a gel bead (Berk et al. 1984).

Hence, both examples show that the gel particles for cell entrapment must be sufficiently small to avoid nonuniform cell growth, and the scale of the theoretical prediction agrees well with experimental evidence provided in the literature. Most immobilized cell work uses gel beads around 3—5 mm diameter. These particles are prepared out of convenience with a syringe needle. Particles smaller than 1 mm diameter are not difficult to prepare. Concentric air stream atomizers have been used with success to prepare very small and monodispersed gel bead particles (Rehg et al. 1986).

Table 2. Particle size and cell mass loading effects on immobilised *Streptomyces parvulus* particles. Basis of calculations (Tanaka et al. 1984; Dalili 1984): Limiting substrate is dissolved oxygen, maximum specific growth rate = 0.14 h^{-1}, Monod constant = 4.85 ppm, surface concentration of dissolved oxygen = 9 ppm, effective diffusivity = 2×10^{-5} cm^2/s. The amount of cell mass is represented by the overall oxygen uptake rate, OUR. $K_m = 0.54$

Diameter (cm)	OUR = 66 mg/l h			OUR = 660 mg/l h		
	φ_0	η	S_0	φ_0	η	S_0
0.5	2.53	0.896	0.446	7.98	0.460	8.6×10^{-4}
0.3	1.52	0.965	0.768	4.79	0.672	4.2×10^{-2}
0.2	1.01	0.985	0.893	3.19	0.833	0.254
0.1	0.51	0.997	0.973	1.60	0.961	0.744
0.05	0.25	1.0	0.993	0.80	0.991	0.932
0.03	0.15	1.0	0.998	0.48	0.998	0.975

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Received October 9, 1986/Revised April 2, 1987