Chapter 2

Analytical effectiveness factor calculations concerning product-inhibited fermentations associated with biofilm growth in or around carriers.

C.J. van Ede, H. Dijkman and A.A.C.M. Beenackers[#]; Department of Chemical Engineering, Groningen University, Nijenborgh 4, 9747 AG Groningen, The Netherlands.

Abstract- A reaction engineering model is presented for the bioproduction of chemicals associated with the growth of immobilized biomass in or around carriers.

The model describes multiple-substrate diffusion limitations and first order growth inhibition by one of the products.
Analytical solutions are presented for intra-biofilm substrate and product concentrations, active biofilm thickness, biocatalyst effectiveness factor and degree of catalyst utilization.
Simple criteria for optimal catalyst design are derived.

Where applicable, the presented explicit analytical solutions for the biocatalyst effectiveness factor are much more convenient to incorporate into a macro-reactor model, than the numerical alternatives.

keywords: biocatalyst, immobilization, effectiveness factor, product inhibition.

[#]to whom correspondence should be addressed

Introduction

There is a rapidly growing interest in biomass-growth associated production processes (Krouwel et al.,1983; van der Meer et al.,1986, 1990; Sayles et al.,1990).
The apparently conflicting optimal design specifications of a maximal specific cell growth rate in combination with a high cell concentration, requires sophisticated solutions to control the amount of biomass present in such reactors.

One way of doing this is to immobilize the cells *around* or *in* a carrier (Karel et al.,1985).

In the first case, either a biofilm will repeatedly be built up and subsequently sloughed off (Wagner and Hempel,1988) or a steady state occurs because a continuous shear driven process of sweeping cells from the outer layer of the biofilm is balanced by cell growth.

In the second case, the macro-porous carrier should have pores of diameter at least five times the longest dimension of the bacteria to allow for unhindered biomass growth (Messing et al., 1979,1982). Also in this way a steady state can be achieved in which the synthesis of new cellular material is balanced by the loss of cell material in the effluent of a continuous reactor (van Ede et al.,1990).

The need for good catalyst design criteria and reliable reaction engineering models describing biocatalyst behaviour in immobilized cell processes has attracted many investigators to develop methods for calculating the catalyst effectiveness factor and the degree of utilization (Karel et al.,1985; Radovich, 1985).

However, *analytical* solutions are relatively rare.
Thiele (1939) solved the problem of diffusion accompanied with first order chemical reaction in a porous particle analytically and introduced his well-known reaction modulus.
Moo-Young and Kobayashi (1972) combined analytical solutions for zero and first order substrate consumption in a porous particle to obtain a very accurate approximation for diffusion with reaction following

Monod-kinetics.
Shieh (1981) solved the problem of substrate diffusion with zero or first order reaction in a spherical biofilm around an inert carrier.
The treatment of Moo-Young and Kobayashi (1972) was extended and checked numerically by Vos et al. (1990) to allow a mathematical description of substrate consumption according to Monod-kinetics in biofilms around particles. Here, external diffusion effects also were included.

With immobilized cells inhibition effects will often play an important role (Brink and Tramper,1986; Sayles et al.,1990), because of the usually non-random distribution and high concentration of the biomass in the biocatalyst. Recently, several investigators derived *numerical* solutions for diffusion accompanied with reaction in biofilms, where such inhibition effects are taken into account (Kasche,1983; Brink and Tramper, 1986; Vos et al.,1990).

Although numerical procedures are relatively flexible to adapt, they are time consuming and not very convenient to incorporate in a macro-reactor model. In addition, as far as the authors know, they all are restricted to the description of diffusion limitation by a single substrate independently being present.
Therefore, there is still a need for analytical solutions describing multi-component diffusion with cell growth associated reactions in biofilms, incorporating inhibition effects.

In this paper, it is shown that such analytical solutions can be obtained, if some reasonable and not too rigorous simplifications are made. Kasche (1983) showed that the prediction of an experimental biocatalyst effectiveness factor cannot be better than $\pm$ 20-30 %, because of the experimental error in quantities such as the effective diffusion coefficient and the porosity; the simplifications made in this paper should be viewed in that light.

The approach presented below allows for both an infinite number of substrates and products as long as not more than one product acts as a major growth inhibitor.
For clarity, a substrate relation is written as a model equation for an arbitrary substrate j. If n is the number of substrates, this equation

will represent each of the n similar substrate equations. The only product considered is the inhibitive product because only that species influences the effectiveness calculations.

INTRA-PARTICLE BIOMASS GROWTH AND PRODUCTION.

I. Model description.

For the case where the biomass grows inside bodies containing a stagnant aqueous phase, such as the pores of inert particles, the kinetic model is based on the following assumptions:

- The growth of biomass proceeds according to Monod-kinetics with simultaneous inhibition caused by a product. Instead of using true Monod kinetics, it is assumed that the consumption kinetics for each substrate are of zero order in substrate concentration everywhere in the particle. The range of Monod parameters over which this approach gives accurate results is discussed later.
- Inhibition kinetics often are of the type (Luong, 1985):
  $\mu = \mu_{maxf} [1-\beta C_p]^{\gamma}$.
  With increasing values of C_p, a rapid initial drop in the growth rate followed by a slow decrease to zero occurs when $\gamma < 1$. A slow initial drop in the growth rate followed by a rapid decrease to zero occurs when $\gamma > 1$. Both cases are reported in literature (Luong, 1985).
  Here we assume γ to have the intermediate value of 1, i.e. first order inhibition kinetics. This often is a reasonable first guess in all cases where no actual information on inhibition kinetics is available. With immobilized cells this will be the rule rather than the exception because, unless a product specific micro electrode can be applied, usually no information will be available on actual product concentration gradients over the biofilm.
- Product formation is directly coupled to biomass production. Usually this assumption is valid for biomass growth-associated production processes where a co-factor, such as NADH, is required in the product formation step (van der Meer et al.,1986,1993; van Ginkel et al.,1987).
- Substrate consumption for maintenance is described by the well-known

linear Pirt relationship (Pirt,1975).

- The formation of a homogeneous biofilm of uniform thickness is assumed inside the spherical porous particle.
  The actual film thickness is determined by the growth limiting substrate whose concentration approaches zero at a critical radius r_s. Henceforth this will be called the case of *substrate diffusion limited growth*.
  If all substrates are present in the center of the particle it gets completely filled with biomass, and the growth-kinetics are not hampered by substrate diffusion. This is the case of *non-substrate diffusion limited growth.*
- Under steady state conditions, neither biomass nor product accumulation in the particle is assumed.
- Possible external mass transfer limitations are not included in the model. With external mass transfer films typically in the order of ten microns for aqueous systems this assumption usually will be valid. If not, the model can be easily extended to include an external mass transfer resistance.
- The pH-gradient in the particle is assumed to be negligible. This assumption is not essential, but if H_3O^+ is the principal inhibitory product than the assumption of first order inhibition kinetics probably is too simple (Atkinson,1977).

The assumptions formulated and discussed above result in the following reaction engineering model describing intra-particle cell growth and product formation:

$$R_x = \mu\, C_x \tag{1}$$

$$R_p = K_{p/x}\, \mu\, C_x \tag{2}$$

$$(-R_j) = K_{j/x}\, \mu\, C_x + M_{j/x}\, C_x \tag{3}$$

$$\mu = \mu_{maxf}\,(1-\beta C_p) \qquad \text{if all } C_j > 0 \tag{4a}$$

$$\mu = 0 \qquad \text{if any } C_j = 0 \tag{4b}$$

The values of the model constants $K_{p/x}$, $K_{j/x}$ and $M_{j/x}$ depend on the kind of fermentation considered. They can either be measured experimentally or derived theoretically from applying stoichiometrical constraints to the bio-kinetics (Roels,1983; van Ede,1994).

Using the transformation:

$$\hat{C}_p = (1 - \beta C_p) \qquad (5);$$

the intra-film continuity equations for product and substrates are written as:

$$(\mathbb{D}_{eff,p}/r^2)\ d[r^2(d\hat{C}_p/dr)]/dr = KP\ \hat{C}_p \qquad (6)$$

$$(\mathbb{D}_{eff,j}/r^2)\ d[r^2(dC_j/dr)]/dr = KJ1\ \hat{C}_p + KJ2 \qquad (7)$$

Subject to the boundary conditions:

$$C_j = C_j^*, \quad C_p = C_p^* \qquad \text{at } r=r_b$$

$$dC_j/dr = 0, \quad dC_p/dr = 0 \qquad \text{at } r=r_s$$

The constants are defined as:

$$KP = \beta\ K_{p/x}\ \mu_{maxf}\ C_x$$

$$KJ1 = K_{j/x}\ \mu_{maxf}\ C_x \qquad KJ2 = M_{j/x}\ C_x$$

Equations (6) and (7) can be rewritten in a dimensionless form, using

$\zeta_p = [\hat{C}_p/(1-\beta C_p^*)]$, $\zeta_j = (C_j/C_j^*)$, $\lambda = (r/r_b)$,

and the so called Thiele-moduli:

$$\phi_p = r_b\ [KP/\mathbb{D}_{eff,p}]^{0.5} \qquad (8)$$

$$\phi_{j1} = r_b\ [KJ1(1-\beta C_p^*)/(\mathbb{D}_{eff,j} C_j^*)]^{0.5} \qquad (9)$$

$$\phi_{j0} = r_b\ [KJ2/(\mathbb{D}_{eff,j} C_j^*)]^{0.5} \qquad (10)$$

The result is:

$$(1/\lambda^2)\ d[\lambda^2(d\zeta_p/d\lambda)]/d\lambda = \phi_p^2\ \zeta_p \qquad (11)$$

$$(1/\lambda^2)\ d[\lambda^2(d\zeta_j/d\lambda)]/d\lambda = \phi_{j1}^2 \zeta_p + \phi_{j0}^2 \qquad (12)$$

With boundary conditions:

$$\zeta_j = 1, \quad \zeta_p = 1 \qquad \text{at } \lambda=1 \qquad (13)$$

$$d\zeta_j/d\lambda = 0 , \quad d\zeta_p/d\lambda = 0 \qquad \text{at } \lambda=\lambda_s \qquad (14)$$

Equation (11) can be solved analytically, resulting in:

$$\zeta_p = C_1 \left[C_2 \exp[\phi_p(\lambda-\lambda_s)] + C_3 \exp[-\phi_p(\lambda-\lambda_s)] \right]/\lambda \qquad (15)$$

With:

$$C_1 = \left[C_2 \exp[\phi_p(1-\lambda_s)] + C_3 \exp[-\phi_p(1-\lambda_s)] \right]^{-1}$$

$$C_2 = \phi_p\lambda_s + 1$$

$$C_3 = \phi_p\lambda_s - 1$$

This solution of ζ_p is substituted in Equation (12).

The intra-particle substrate profiles can be obtained by integrating Equation (12) once, using the boundary condition given by Equation (14), and then integrating once more and finally using the boundary condition of Equation (13). The result is:

$$\zeta_j = (C_4/\phi_p^2\lambda) \exp[\phi_p(\lambda-\lambda_s)] + (C_5/\phi_p^2\lambda) \exp[-\phi_p(\lambda-\lambda_s)] + C_6\lambda^2/6 - C_7/\lambda + C_8 \qquad (16)$$

With:

$$C_4 = \phi_{j1}^2 C_1 C_2 ; \quad C_5 = \phi_{j1}^2 C_1 C_3 ; \quad C_6 = \phi_{j0}^2 ; \quad C_7 = -C_6\lambda_s^3/3 ;$$

$$C_8 = 1 - (C_4/\phi_p^2)\exp[\phi_p(1-\lambda_s)] - (C_5/\phi_p^2)\exp[-\phi_p(1-\lambda_s)] - C_6/6 + C_7$$

II. Criterion for absence of substrate diffusion limited growth

Biomass growth in the center of the particle is only possible if the depth of penetration of all substrates is sufficient to feed the biomass present there. This will occur if after substituting $\lambda = \lambda_s = 0$ into Equation (16) the resulting ζ_j is positive for all substrates. This can be written as a criterion:

$$\phi_{j1}^2 \left[6/\phi_p^2 - 12/\left(\phi_p[\exp(\phi_p) - \exp(-\phi_p)]\right) \right] + \phi_{j0}^2 < 6 \qquad (17)$$

If all the substrates fit the criterion of Equation (17), the particle is completely filled with biomass, thus $\lambda_s=0$. If not, active biomass is only present in an outer shell, of which the relative thickness $(1-\lambda_s)$

still has to be determined.

It is interesting to evaluate the asymptotic solutions of Equation (17). If product inhibition is so weak that $\phi_p < 1$, this criterion simplifies to:

$$\phi_{j1}^2 + \phi_{j0}^2 < 6 \qquad (17a)$$

For relatively strong product inhibition effects, such that $\phi_p \gg 1$, the criterion reduces to:

$$\phi_{j1}^2/\phi_p^2 + \phi_{j0}^2/6 < 1 \qquad (17b)$$

III. Substrate diffusion limited growth

For substrate diffusion limited growth, the concentration of the limiting substrate becomes zero at $\lambda = \lambda_s$. To detect the rate controlling substrate from the set of substrates that fails the criterion of Equation (17), λ_s is determined for all these substrates from solving Equation (16) after substituting $\zeta_j = 0$ for $\lambda = \lambda_s$:

$$0 = (C_4 + C_5)/(\phi_p^2\lambda_s) + C_6\lambda_s^2/6 - C_7/\lambda_s + C_8 \qquad (18)$$

The substrate yielding the largest value of λ_s will be the actual rate controlling species resulting in a biomass film of thickness:

$\delta = r_b(1-\lambda_{s,j}^{max})$.

Note, that if the substrate consumptions for cell maintenance can be neglected, all ϕ_{j0} are practically zero, so that the diffusion limiting substrate then simply becomes the species with the largest value of the modulus ϕ_{j1}.

IV Biocatalyst effectiveness factor

Analogous to the established approach in conventional chemical heterogeneous catalysis (Westerterp et al.,1987), a biocatalyst effectiveness factor can be defined as:

$$\eta_p = \frac{\text{actual production rate per particle}}{\text{theoretical production rate in absence of internal mass transfer limitations}} =$$

$$-4\pi r_b^2 \varepsilon \mathbb{D}_{eff,p}(dC_p/dr)|_{r=r_b} / \left[\tfrac{4}{3}\pi r_b^3 \varepsilon K_{p/x}\mu_{maxf}(1-\beta C_p^*)C_x\right] =$$

$$\left[3C_1C_2(\phi_p-1)\exp[\phi_p(1-\lambda_s)] + 3C_1C_3(-\phi_p-1)\exp[-\phi_p(1-\lambda_s)]\right]/\phi_p^2 \qquad (19)$$

Further, a degree of catalyst utilization can be defined as $(1-\lambda_s^3)$.
If all substrates satisfy criterion (17) then no substrate diffusion limitation occurs and Equation (19) reduces to the familiar solution for first order kinetics in a porous particle:

$$\eta_p = (3/\phi_p)\ [1/\tanh(\phi_p) \ - \ 1/\phi_p] \qquad (19a1)$$

This indicates that the biocatalyst activity under these circumstances is reduced by product inhibition only. If also that effect is rather weak such that $\phi_p < 1$, Equation (19a1) reduces further to:

$$\eta_p \cong 1 \qquad (\text{for } \phi_p=1;\ \eta_p=0.94) \qquad (19a2)$$

For the case of a negligible product inhibition effect ($\phi_p < 1$), but still substrate diffusion limited growth, another asymptotic solution can be derived. Equation (18), which determines the value of λ_s for the diffusion limiting substrate, is now simplified to a simple cubic equation, of which only one of the three standard roots has a value between 0 and 1. This value is substituted for λ_s in the catalyst effectiveness factor η_p which reduces to the catalyst degree of utilization $(1-\lambda_s^3)$.

The result is:

$$\eta_p = 1 \ - \ \left[\ 0.5 \ + \ \cos[(\psi \ + \ 4\pi)/3]\ \right]^3 \qquad (19b)$$

With:

$$\psi = \cos^{-1}\ [12/(\phi_{j1}^2 \ + \ \phi_{j0}^2) \ - \ 1] \qquad \textit{for} \quad \phi_{j1}^2 \ + \ \phi_{j0}^2 > 6$$

This result is identical to the solution of diffusion accompanied with zero order reaction in a porous sphere (Moo-Young and Kobayashi,1972), with a zero order Thiele-modulus of magnitude $(\phi_{j1}^2 \ + \ \phi_{j0}^2)^{0.5}$.
If $\phi_{j1}^2+\phi_{j0}^2 < 6$ for all substrates (criterion 17a) substrate diffusion is absent so that $\lambda_s=0$; also then, the effectiveness factor reduces to unity, as in Equation (19a2).

In Figures 1 to 6 the dimensionless biofilm thickness $(1-\lambda_s)$ and the biocatalyst effectiveness factor are presented graphically as a function of both ϕ_{j1} and ϕ_{j0} of the λ_s determining substrate for three values of ϕ_p.

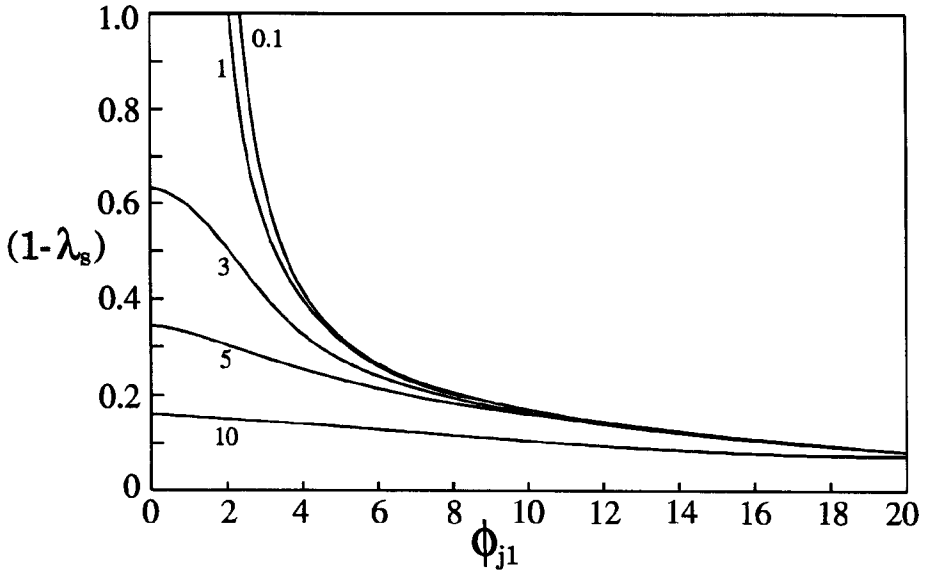

Figure 1. Biofilm thickness vs. substrate Thiele modulus for bacterial growth; $\phi_p < 1$. $\phi_{j0} = 0.1, 1, 3, 5, 10$ as indicated.

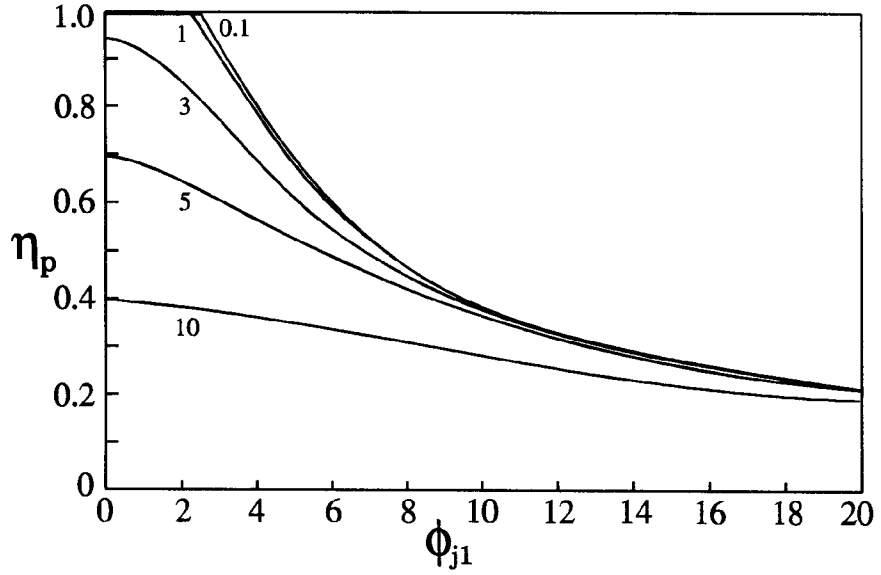

Figure 2. Catalyst effectiveness factor vs. substrate Thiele modulus for bacterial growth; $\phi_p < 1$. $\phi_{j0} = 0.1, 1, 3, 5, 10$ as indicated.

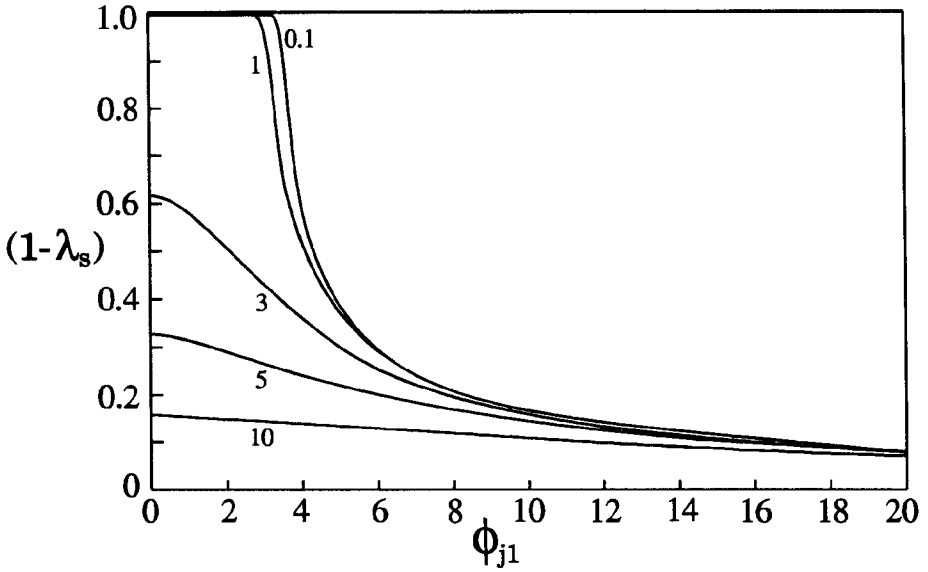

Figure 3. Biofilm thickness vs. substrate Thiele modulus for bacterial growth; $\phi_p = 3$. $\phi_{j0} = 0.1, 1, 3, 5, 10$ as indicated.

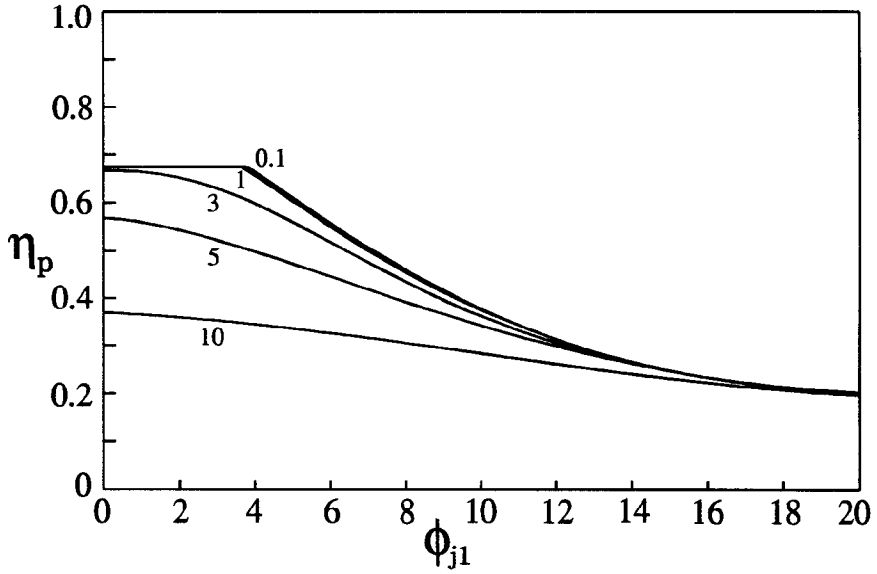

Figure 4. Catalyst effectiveness factor vs. substrate Thiele modulus for bacterial growth; $\phi_p = 3$. $\phi_{j0} = 0.1, 1, 3, 5, 10$ as indicated.

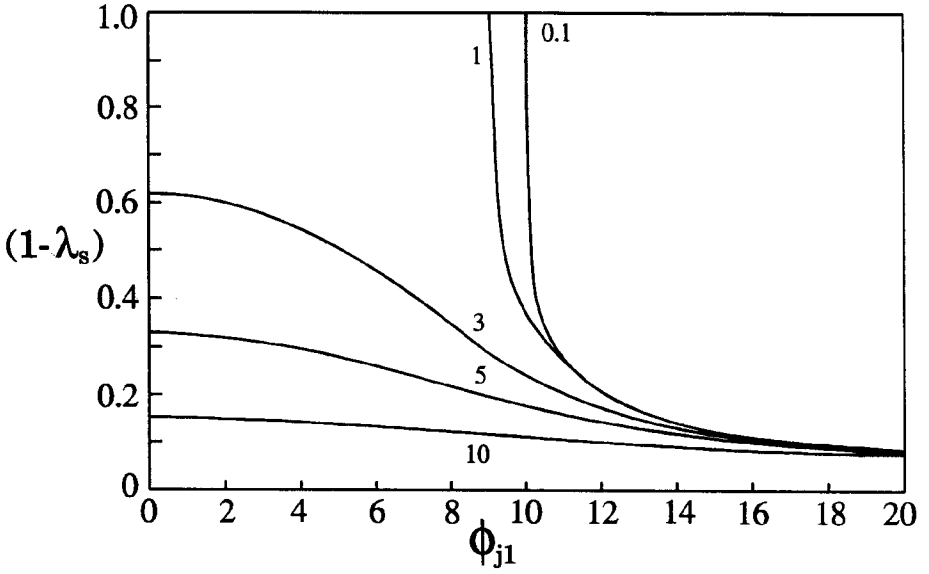

Figure 5. Biofilm thickness vs. substrate Thiele modulus for bacterial growth; $\phi_p = 10$. $\phi_{j0} = 0.1, 1, 3, 5, 10$ as indicated.

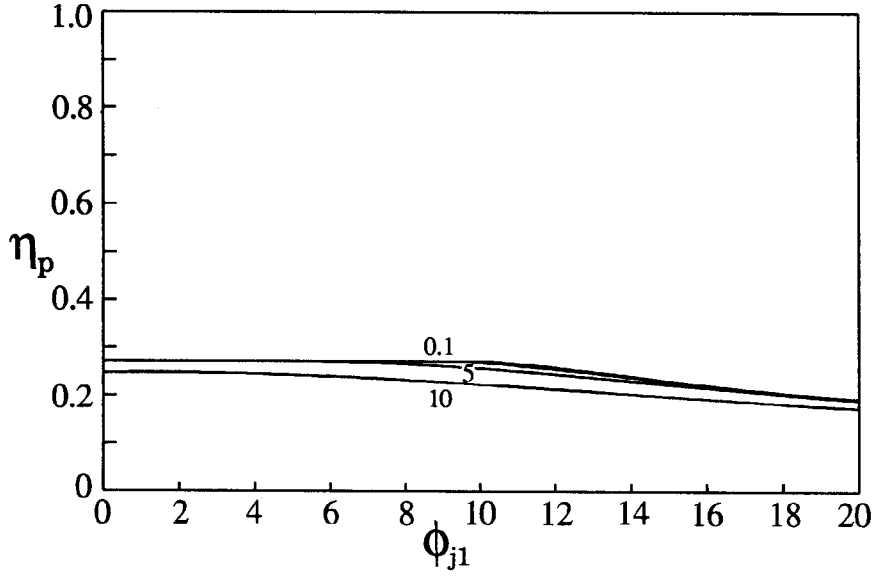

Figure 6. Catalyst effectiveness factor vs. substrate Thiele modulus for bacterial growth; $\phi_p = 10$. $\phi_{j0} = 0.1, 1, 3, 5, 10$ as indicated.

Depending on the biochemical system applied, large variations are possible in biofilm density, kinetic rate constants, substrate and product diffusion coefficients and in solubilities. Nevertheless, the range of numerical values selected for the moduli is such that most known biofilm systems are believed to be covered by it (Atkinson and Mavituna, 1983).

For $\phi_p < 1$ the product gradient in the particle is negligible, so the results are hardly influenced by this modulus. If the particles are completely filled with biomass $[(1-\lambda_s)=1]$ the maximal effectiveness factor is obtained in conformity with Equation (19a1). The effect of the product inhibition modulus ϕ_p is shown in some more detail in Figures (7) and (8).

With increasing values of ϕ_p, be it because of a higher production rate, a lower product diffusivity or a larger particle diameter, lower effectiveness factors are obtained. In contrast, the corresponding film thicknesses increase with increasing ϕ_p because of the decreasing substrate consumption rates caused by the increasing product inhibition effect resulting from the larger product concentration levels inside the catalyst particle. This is shown in both Figures 7 and 8 and in Figure 9, where $\zeta_j = C_j/C_j^*$, $(1-\zeta_p) = 1 - (1-\beta C_p)/(1-\beta C_p^*)$, and the specific growth rate relative to the growth rate in the bulk solution, all are presented as a function of radial position for two values of ϕ_p. Note that the value of $(1-\zeta_p)$ represents the normalized effect of product inhibition relative to the inhibitive effect found in the bulk of the solution.

As expected from criterion (17) and (17b) increasing values of ϕ_p above $\phi_p=1$ allow for larger values of ϕ_{j1} and/or ϕ_{j0} before the biomass growth becomes diffusion controlled.

The higher the substrate consumption rate per unit pore volume the larger the value of ϕ_{j1} and/or ϕ_{j0} will be and the smaller both the biofilm thickness and the catalyst effectiveness factor become.

The ratio ϕ_{j1}/ϕ_{j0} is a measure of the ratio of the product inhibited substrate consumption for bacterial growth and the substrate consumption for bacterial maintenance. Its numerical value decides whether growth or maintenance controls the degree of catalyst utilization.

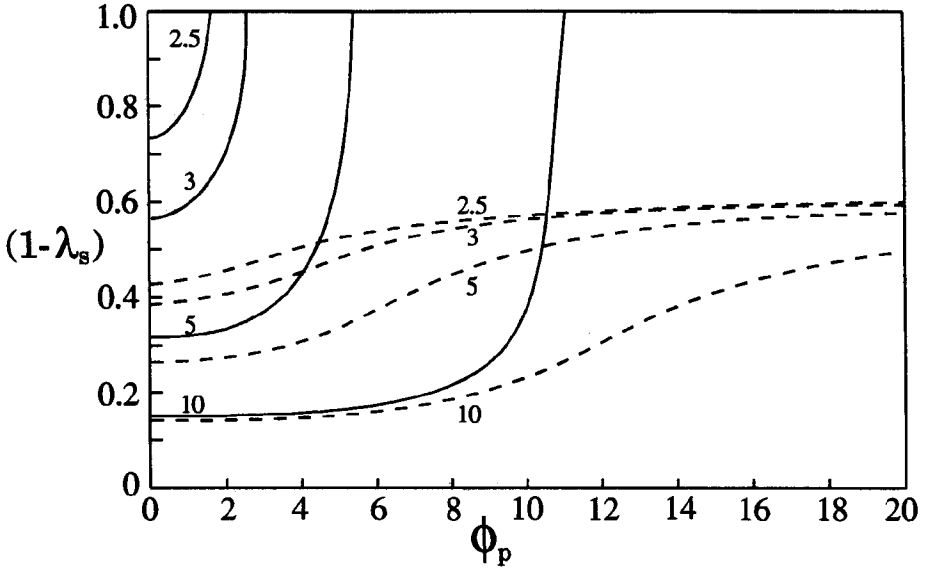

Figure 7. Biofilm thickness versus product Thiele modulus.
—— $\phi_{j0} = 1$ - - - $\phi_{j0} = 3$ ϕ_{j1} = 2.5, 3, 5, 10 as indicated.

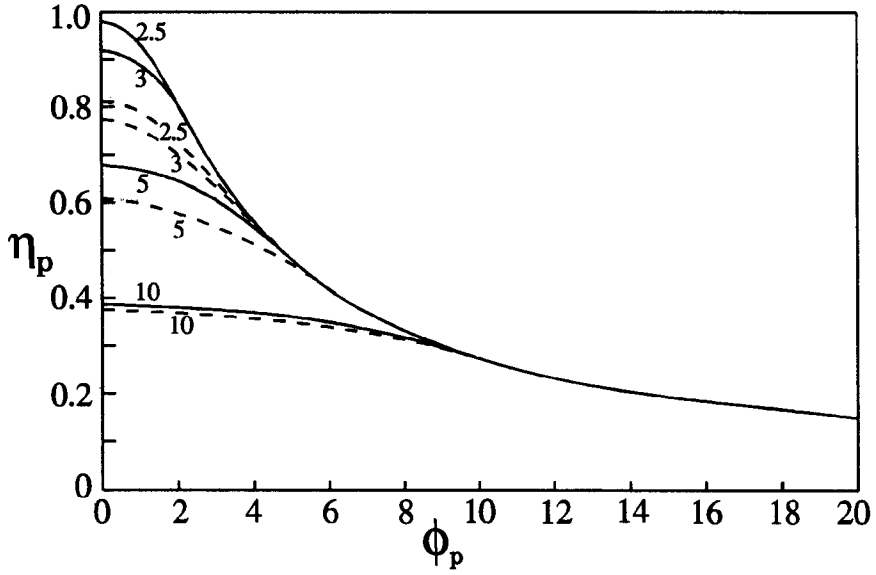

Figure 8. Catalyst effectiveness factor versus product Thiele modulus.
—— $\phi_{j0} = 1$ - - - $\phi_{j0} = 3$ ϕ_{j1} = 2.5, 3, 5, 10 as indicated.

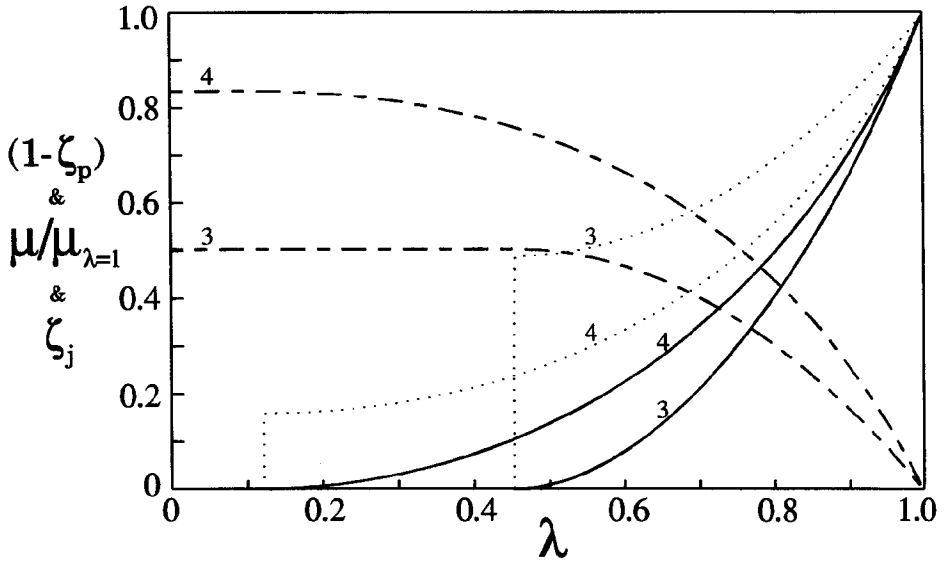

Figure 9. Dimensionless substrate concentration (ζ_j), normalized level of product inhibition ($1 - \zeta_p$), and growth rate relative to the growth rate in the bulk of the solution, versus the dimensionless carrier radius.
$\phi_{j1} = 4$; $\phi_{j0} = 1$; $\phi_p = 3$ or 4 as indicated.

- — $1 - \zeta_p$ —— ζ_j ····· $\mu/\mu_{\lambda=1}$

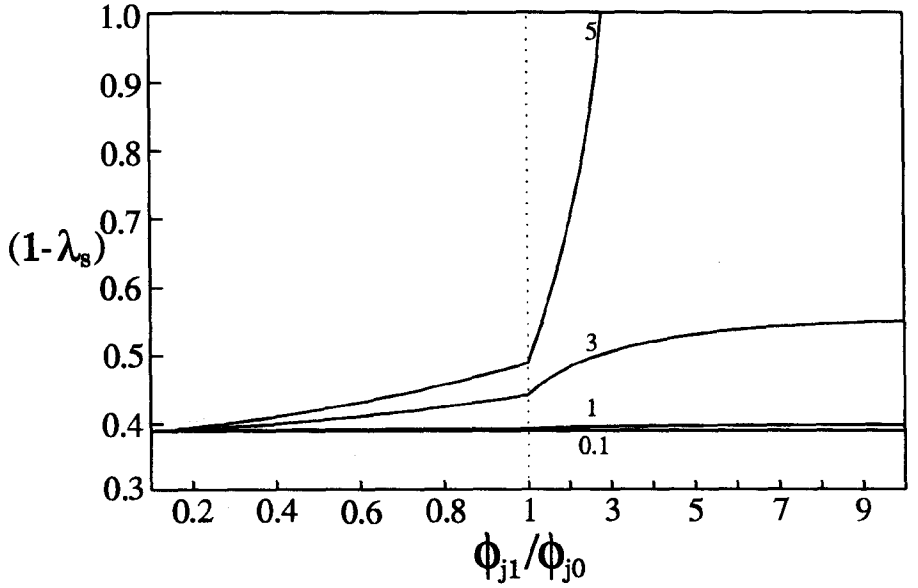

Figure 10a. Biofilm thickness vs. the ratio of the substrate modulus for growth and maintenance: $\phi_{j1}^2 + \phi_{j0}^2 = 18$. ϕ_p = 0.1, 1, 3, 5 as indicated.

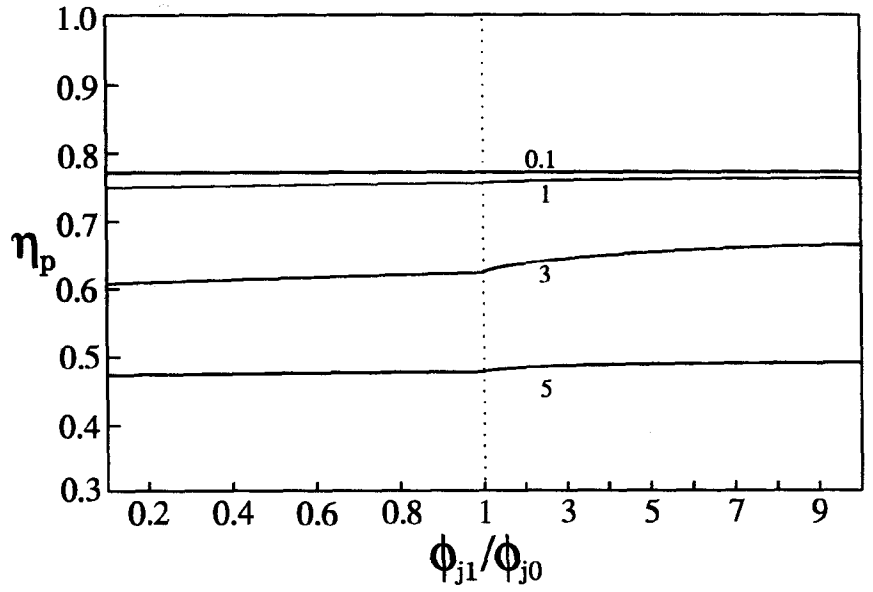

Figure 10b. Effectiveness factor vs. the ratio of the substrate modulus for growth and maintenance: $\phi_{j1}^2 + \phi_{j0}^2 = 18$. ϕ_p = 0.1, 1, 3, 5 as indicated.

Its influence is demonstrated in Figures 10a and 10b. As can be concluded from Figure 10b, the catalyst effectiveness factor is hardly influenced by the ratio ϕ_{j1}/ϕ_{j0}. It should be realized that the numerical value of ϕ_{j1}/ϕ_{j0} is dictated by the system except for the value of C_p^*.

V. Model deviations from true Monod kinetics

In the model discussed above the value of μ_{maxf} is considered to be independent of all n substrate concentrations in the biofilm:

$$\mu_{maxf} = \mu_{max} \left[1/(1+\phi_{m,1}^2)\right] \times \left[1/(1+\phi_{m,2}^2)\right] \times \ldots \times \left[1/(1+\phi_{m,n}^2)\right] \quad (20)$$

In this equation $\phi_{m,j}$ represents the Monod modulus for substrate j:

$$\phi_{m,j} = \left[K_{m,j}/C_j^*\right]^{0.5} \quad (21)$$

However, for true Monod kinetics, we actually have:

$$\mu_{maxf} = \mu_{max} \left[\zeta_1/(\zeta_1+\phi_{m,1}^2)\right] \times \left[\zeta_2/(\zeta_2+\phi_{m,2}^2)\right] \times \ldots \times \left[\zeta_n/(\zeta_n+\phi_{m,n}^2)\right]$$

The analytical solutions therefore are approximations, deviating more or less markedly from the exact solutions depending on the values of the various ϕ_m's. Therefore it is of interest to find the critical values of the Monod moduli for which the analytical solution is still an accurate approximation.

The model regards the *n-1* substrates with $\zeta_j > 0$ at λ_s as non diffusion limiting. This is correct only if the value of the Monod-term inside and outside the biofilm is about the same. Therefore, for all non diffusion limiting substrates, it should be checked whether:

$$\text{Monod-term}|_{\lambda=\lambda_s} \;/\; \text{Monod-term}|_{\lambda=1} =$$

$$\left[\zeta_j|_{\lambda=\lambda_s}(1 + \phi_{m,j}^2)\right] \;/\; \left[\zeta_j|_{\lambda=\lambda_s} + \phi_{m,j}^2\right] > 0.9 \quad (22)$$

With:

$$\zeta_j|_{\lambda=\lambda_s} = \left[2\phi_{j1}^2/\phi_p\right]/\left[(\phi_p\lambda_s+1)\exp[\phi_p-\phi_p\lambda_s] + (\phi_p\lambda_s-1)\exp[\phi_p\lambda_s-\phi_p] \right]$$

$$- \phi_{j1}^2/\phi_p^2 - \phi_{j0}^2[1+\lambda_s^2+2\lambda_s^3]/6 + 1$$

Although the diffusion limiting substrate will certainly fail this criterion, because $\zeta_j=0$ at $\lambda=\lambda_s$, the analytical solution is still a good approximation, if the fraction of the biofilm where the Monod-term of this substrate changes is very small.

The deviations from the exact solution both with respect to biofilm thickness and catalyst effectiveness factor are shown in Figure 11 as a function of the Monod modulus for the diffusion limiting substrate.

The exact solution, which accounts for true Monod/Pirt kinetics with linear product inhibition, was obtained by solving numerically Equations (6) and (7) with $\mu_{maxf}= \mu_{max}\ [\zeta_j/(\zeta_j+\phi^2_{m,j})]$ using Runge-Kutta integration (RK-Package, Reactor Research Foundation, University Delft, The Netherlands) and a shooting method with variable stepsize.

As can be seen from Figure 11, the analytical solution for the effectiveness factor is accurate within 5% for a wide range of ϕ_{j0}, ϕ_{j1} and ϕ_p values if:

$$\phi_{m,j} < 0.06 \qquad (23a)$$

Figure (11) further shows that for increasing ϕ_p and decreasing ϕ_j values higher critical $\phi_{m,j}$ values are allowed.

The analytical calculated biofilm thickness is accurate within 5% , provided that:

$$\phi_{m,j} < 0.01 \qquad (23b)$$

Theoretically larger deviations in film thickness may occur for $\phi_{j0}/\phi_{j1} < 1/20$ since the particle will become completely filled with biomass for $\phi_{j0}=0$. However, in practice this situation will be rarely met because substrate consumption for maintenance generally is more then 0.25 % of the maximal substrate consumption (Atkinson and Mavituna, 1983).

The analytical solutions derived in this paper are expected to be applicable to all types of oil/water bioreactors and waste water purification processes where a low soluble organic substrate controls the biomass growth.

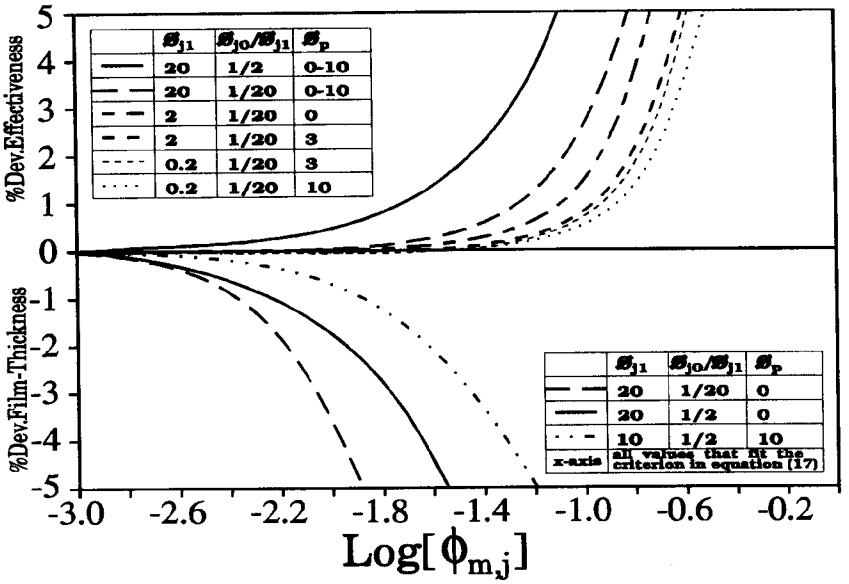

Figure 11. Deviations from exact solution in % versus Monod modulus. Parameters: ϕ_{j1} , ϕ_{j0}/ϕ_{j1} and ϕ_p .

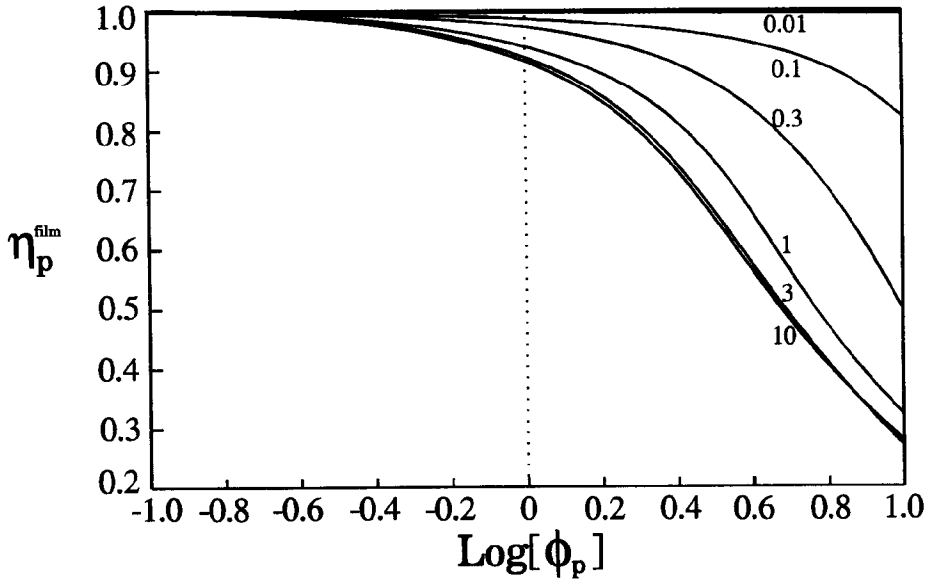

Figure 12. Biofilm effectiveness factor vs. product Thiele modulus for the shear controlled regime. δ/r_s = 0.01, 0.1, 0.3, 1, 3, 10 as indicated.

VI. Biocatalyst design

The ideal catalyst will have an effectiveness factor η_p of approximately one. This will be obtained for $\phi_p < 1$ and when Equation (17a) is simultaneously satisfied for all substrates: $\phi_{j1}^2 + \phi_{j0}^2 < 6$

This requires a critical maximal particle diameter, depending not only on kinetic and diffusion parameters but also on the external boundary conditions C_j^* and C_p^*. The latter values greatly depend on reactor operation parameters. The value of C_p^* is subject to an economical optimization between high specific production rates favoured by a low value of βC_p^* and minimum downstream separation costs, usually promoted by increased product concentrations. It is here that the present reaction engineering model for the biocatalyst must be integrated with a relevant macro-reactor model.

BIOMASS GROWTH AND PRODUCTION IN FILMS AROUND PARTICLES.

I. Model description.

Also for biomass growing in films around carriers (e.g. sand or lava particles), the biofilm thickness can be controlled by the substrate of which the intra-biofilm supply rate by diffusion is the rate limiting step. If so, this case is perfectly similar to the case of intra-particle biomass growth, discussed above. However, it is also possible that a shear driven process of attrition controls the thickness of the biofilms around the particles.

If the shear stress in the reactor is sufficiently low, be it because of a low particle loading or a low degree of turbulence in the reactor, substrate diffusion limitation may become the dominating factor (Wagner and Hempel,1988). Then the biofilm grows continually until the inner cells die because of lack of substrate. Subsequently the biofilm comes off the carrier after which a new biofilm is built up. In such a cyclic process, no steady state biofilm thickness is obtained. If this mechanism prevails, an non-stationary-mass-transfer-with-reaction model of the type of Higbie's penetration theory has to be applied. We have not found analytical solutions yet according to this model with the type of kinetics under consideration.

However, in most applications (e.g. Tang and Fan,1987) the biofilm is controlled by the shear stress in the reactor and, as shown by recent investigations of Mulder and Heijnen (1988), also by the roughness of the carrier. Then a steady state can be achieved. If so, the biocatalyst effectiveness factor is influenced by product inhibition effects and shear controlled film thickness only.
Analogous to the derivation of a criterion for non-substrate diffusion limited intra-particle cell growth, a similar criterion for non-substrate diffusion limited extra-particle cell growth under shear controlled biomass growth can be derived.

II. Criterion for shear controlled biofilm growth

In a typical shear controlled situation, the actual film thickness will be much smaller than the maximal film thickness. The value of the maximal thickness is determined by the concentration of a diffusion limiting substrate, approaching zero at the carrier surface, r_s. This maximal film thickness can be calculated analogous to the method presented above for intra-particle biofilm formation.
The maximal biofilm thickness is found from Equation (16) as follows. After substituting $\lambda=\lambda_s$ and $\zeta_j=0$ a set of values $\lambda_{s,j}$ is found. The highest value of $\lambda_{s,j}$ thus found is the relevant one. The corresponding j represents the diffusion controlling substrate. The maximal biofilm thickness now follows from:

$$\delta_{maximal} = [r_{b,max} - r_s] = r_s [1/\lambda_{s,max} - 1] \qquad (24)$$

The film thickness in the reactor is shear controlled if the actual film thickness is significantly smaller than the maximal film thickness as defined in Equation (24). Thus:

$$\delta_{actual} < \alpha \cdot \delta_{maximal} \qquad for\ \alpha < 1 \qquad (25)$$

The value of the constant α depends on the accuracy of both the experimentally observed film thickness and the theoretically calculated maximal film thickness. However, in practice it is easier to check if the biofilm thickness is in the shear controlled regime by defining a critical dimensionless carrier radius:

$$\lambda_{s,crit} = \alpha r_s/(r_s + \delta_{actual}) \qquad (26)$$

Next, $\lambda_s = \lambda_{s,crit}$ is substituted in Equation (16).
The film thickness is shear controlled if $\zeta_j|_{\lambda_{s,crit}} > 0$ for all substrates.

III. Biocatalyst effectiveness factor for shear controlled biofilm growth

Equation (19) gives an expression for the biocatalyst effectiveness factor. For shear controlled external biofilm growth, λ_s is determined by the shear stress in the reactor, contrary to the values of the Thiele moduli controlling λ_s for intra-particle biofilm formation.
In calculating the product formation rate in the absence of internal mass transfer limitations, the complete sphere is considered to be potentially productive, according to the definition of η_p in Equation (19). So defined the catalyst effectiveness factor will always be smaller then one, because the inert carrier volume can't contribute to the production. This inconvenience can be avoided by defining the biofilm effectiveness factor for external biofilm growth as:

$$\eta_p^{film} = \eta_p/[1-\lambda_s^3] \qquad (27)$$

Figure 12 shows the influence of the product inhibition modulus on the biofilm effectiveness factor for several values of the ratio of biofilm thickness and carrier radius, δ/r_s. For values of $\phi_p < 1$, the product inhibition effect is again negligible, resulting in values for $\eta_p^{film} \cong 1$.
With increasing values of δ/r_s the intra-film diffusion resistance is raised and lower effectiveness factors are obtained for $\phi_p > 1$.
For $\delta/r_s \gg 1$ the biofilm effectiveness factor becomes a function of ϕ_p only. Equation (27) then reduces to Equation (19a1).
For $\delta/r_s \ll 1$ the solution for η_p^{film} is hardly influenced by the value of r_s. Then the biofilm effectiveness factor can be approximated by:

$$\eta_p^{film} = \tanh(\phi_p^{\delta})/\phi_p^{\delta} \qquad (28)$$

With: $\phi_p^{\delta} = \phi_p \delta/r_b = \delta \left[KP/\mathbb{D}_{eff,p}\right]^{0.5}$

IV. Biocatalyst design

In principle, and in analogy with the design of a biocatalyst for intra-particle cell growth, the ideal biofilm effectiveness factor η_p^{film} value of one is obtained if the product inhibition effect is negligible.

This will be the case if $\phi_p < 1$, in other words the biofilm should be thinner than a critical thickness.

Note however, that the overall biocatalyst effectiveness factor η_p decreases with decreasing film thickness because of the relative increase of the catalyst volume fraction consisting of inert carrier material. The average biofilm thickness in the reactor will be maximal at the point where substrate limitation threatens to occur. Provided that the level of product inhibition is still negligible here, the biocatalyst effectiveness factor reaches its optimal value in this situation.

Therefore, the shear stress effect in the reactor should be controlled to create thick biofilms, of which the thickness is only marginally lower than the thickness where substrate diffusion limitation effects come into play or where the specific growth rate is substantially lowered by product inhibition.

The shear stress in the reactor can be controlled by the choice of reactor type, adjusting the power input for mixing, the carrier diameter and solids loading, and the roughness of the particles.

Conclusions

A reaction engineering model is presented for the bioproduction of chemicals associated with growth of immobilized biomass in or around carriers, which describes multiple-substrate diffusion limitations and first order growth inhibition by one product.

Analytical solutions are presented for intra-biofilm substrate and product concentrations, active biofilm thickness, biocatalyst effectiveness factor and degree of catalyst utilization. Simple criteria for optimal catalyst design are derived. An infinite number of substrates and products can be taken into account as long as only one product inhibits biomass growth.

Where applicable, the presented explicit analytical equations for the biocatalyst effectiveness factor are much more convenient to incorporate in a macro-reactor model than the existing numerical alternatives. Even if the resulting macro-reactor model has to be solved numerically, in

which case the biocatalyst effectiveness has to be recalculated in every integration step, still solutions will be obtained much easier and faster than from using a nested numerical procedure for the calculation of the biocatalyst effectiveness factor.

Nomenclature

C Concentration in reaction phase, kg/m^3

$\hat{C}$ Transformed concentration in reaction phase, $[1-\beta C]$, dimensionless

$\mathbb{D}_{eff}$ Effective diffusion coefficient in biofilm, m^2/s

$K_{j/x}$ Bacterial specific substrate constant, kg $_{substrate}$ /kg $_{biomass}$

$K_{p/x}$ Bacterial specific product constant, kg $_{product}$ /kg $_{biomass}$

K_m Monod constant, kg/m^3

$M_{j/x}$ Bacterial specific substrate maintenance rate constant, kg $_{substrate}$ /kg $_{biomass\ s}$

r Radius, m

R_i Reaction rate of component i, kg/m^3s

Greek letters:

β Product inhibition constant, m^3/kg

δ Biofilm thickness, m

ε Porosity catalyst

ϕ_{j0} Thiele modulus for diffusion limited substrate consumed for bacterial maintenance, Equation (10)

ϕ_{j1} Thiele modulus for diffusion limited substrate consumed for bacterial growth, Equation (9)

ϕ_m Modulus for Monod kinetics, Equation (21)

ϕ_p Thiele modulus for inhibitive product, Equation (8)

η Catalyst effectiveness factor defined in Equation (19)

η^{film} Biofilm effectiveness factor defined in Equation (28)

λ Dimensionless radius $[r/r_b]$

ψ Dimensionless parameter used in Equation (19b)

μ Specific growth rate constant, s^{-1}

ζ Dimensionless concentrations $[\zeta_p = \hat{C}_p/(1-\beta C_p^*);\ \zeta_j = C_j/C_j^*]$

Subscripts:

b	Outer boundary biofilm
j	Substrate "j"
max	Maximal
maxf	Maximal in biofilm
p	Product
s	Inner boundary biofilm
x	Biomass
*	At outer boundary biofilm

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