KINETICS OF BIOFILM SYSTEMS

Dr.ir. M.C.M. van Loosdrecht
Dept. Biochemical Engineering-Delft university of Technology
MAY 1993

Contents

1. INTRODUCTION 1
1.2 Biofilm formation
1.3 Kinetics of biofilm systems

2. SUBSTRATE CONVERSION BY BIOFILM SYSTEMS 4
2.1 Substrate transport from bulk liquid to biofilm (external mass transfer)
2.2 Diffusion and conversion in a biofilm
(internal mass transport)
Diffusion equation
Active layer thickness or penetration depth
Substrate surface flux
First order reaction kinetics
Overall substrate flux in a reactor with flat biofilms
2.4 Mixed population biofilms
2.5 Effect of inhibitors on biofilm kinetics

3. GROWTH OF BIOFILMS 13
3.1 Competition with suspended cells
3.2 Biofilm growth
3.3 Overall biofilm model

4. EFFECT OF NON IDEALITY OF BIOFILMS 14
Effect of a rough biofilm surface
Effect of sloughing
Layered biofilm structures

5. CONCLUSIONS 15

References 16

APPENDIX A: Sherwood number relations for different reactor types [7,8] ... 17

APPENDIX B: Overall effectiveness factors for biofilm systems 19

1. INTRODUCTION

In the previous chapter "Biomass retention using flocs and biofilms" it has been discussed that use of biofilms offer good potentials for the optimization of the volumetric conversion capacity of treatment systems.

When a surface is in contact with a liquid which contains nutrients and (undefined) microorganisms, these organisms will attach to the surface. The attached cells will start growing and produce a polymeric matrix by which they are "immobilized" on the surface. In principle there is always competition between suspended and biofilm cells for nutrients. Attached bacteria do in general not have a different physiology from suspended cells [14]. Due to diffusion limitation attached cells will experience lower substrate concentrations then suspended cells. Biofilms will therefore in general only occur under "wash-out" conditions for suspended cells (i.e. plug flow conditions or when $D > \mu_{max}$).

Besides for environmental biotechnological applications there is a strong interest in biofilm processes because of their role in fouling processes (e.g. in pipelines, heat exchangers, ship hulls, or teeth). This has led to an extended amount of literature and many different biofilm models. Here we only want to give an introduction to biofilm processes fit for engineering purpose and discuss several of the used biofilm processes.

1.2 Biofilm formation

The net formation of biofilms is determined by the following biological, physical and chemical processes (fig. 1)[2,13]:

* Adsorption of (in)organic molecules to the surface, resulting in the "conditioning" of the substratum. Most natural and process waters contain organic macromolecules and nutrients. These molecules can adsorb to the solid surface and thereby change the physico-chemical properties of the surface. As adsorption of molecules is a much faster process then adhesion of cells, initial adhesion is rather governed by the properties of the conditioning layer than by the surface itself.
* Transport of microorganisms to the surface. Microorganisms can reach a surface by three different modes:
  - Diffusive transport. Bacteria exhibit a non-negligible Brownian motion (average displacement 40 μm/h). This motion can only contribute significantly to bacterial transport under quiescent conditions or in a viscous sublayer surrounding the surface.
  - Convective transport. This is due to fluid dynamic forces. Convective transport will be the dominant factor under flow conditions.
  - Active transport. Once a bacterium is in the vicinity of a surface, it may chemotactically response to any concentration gradient.
* Initial adhesion. Initial adhesion is mainly a physicochemical process and can be divided in two separate stages, namely reversible and irreversible adhesion. The initial adhesion of cells is determined by the physicochemical properties of the cell and solid surface. Usually cells initially adhere in a reversible manner, which means that they can be easily removed by liquid shear [13].

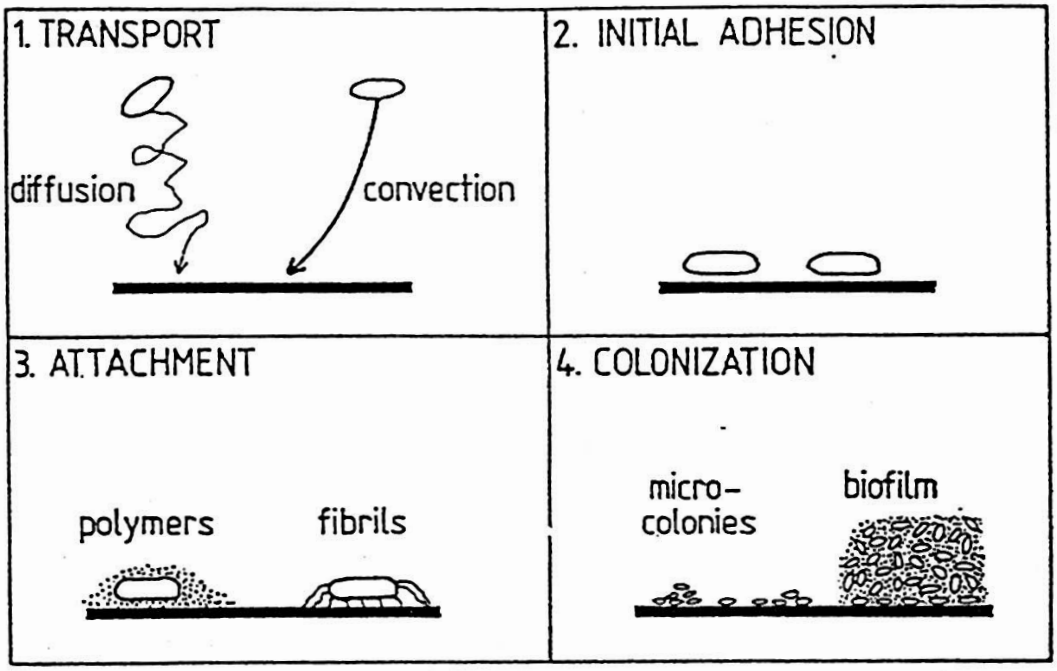

Figure 1

* Attachment After cells have been deposited on the solid surface, special cell surface structures (e.g. fibrils or polymers) may form a strong connection between the cell and solid surface. Polysaccharides have been shown to be essential for the development of surface films, but not for the initial adhesion of bacteria [1].
* Biofilm formation When sufficient nutrient components are present in the liquid, attached cells start growing and producing more polymers. Hereby a biofilm is formed. Cells or inorganic particles from the liquid might be incorporated into the biofilm matrix.
* Detachment Cells and other biofilm components can be transferred into the bulk liquid by several processes:
  - Erosion. Erosion is the continuous loss of small portions of the biofilm. It is highly dependent on fluid dynamic conditions. The rate of erosion increases with fluid shear stress.
  - Sloughing. Sloughing refers to a rapid massive loss of biofilm. It generally occurs with thick biofilms and as a result to sudden changes in e.g. the substrate loading to the biofilm. Sloughing is a stochastic process opposed to the continuous process of erosion.
  - Abrasion. Abrasion is the loss of biofilm due to collision between particles and the biofilm. Especially biofilms in fluidized beds will be strongly subject to abrasion.

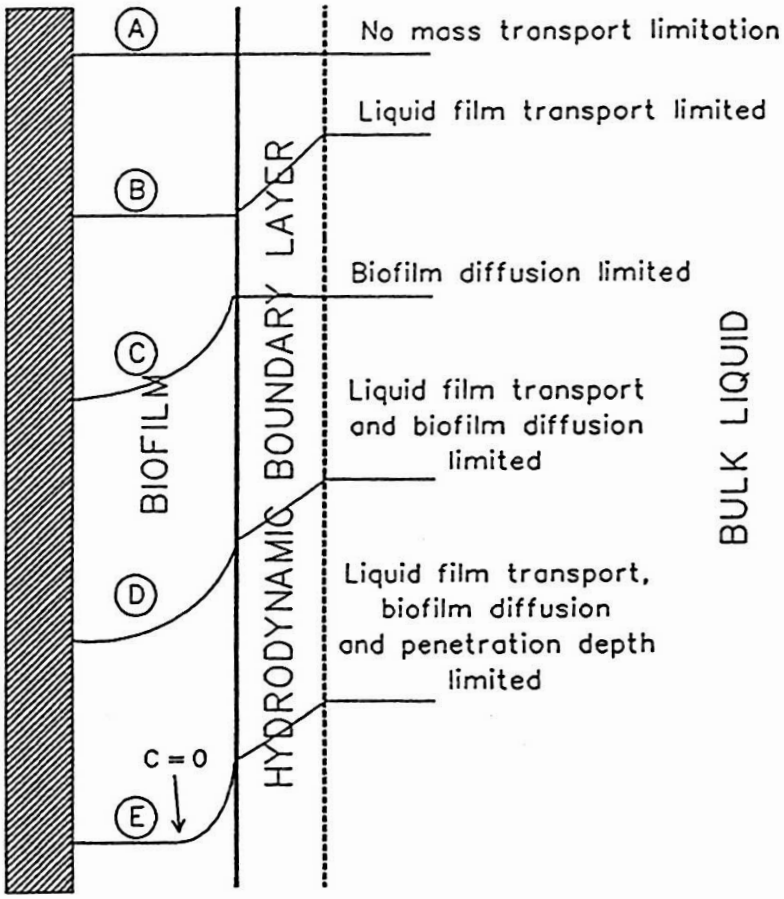

Figure 2

1.3 Kinetics of biofilm systems

In the description of biofilm processes mass transfer plays a dominant role (Fig. 2). This means that in contrast to suspended growth the kinetics of a biofilm are not directly related to the substrate concentration in the liquid. Various mass transport situations can be distinghuised (see figure 2):

A Virtually no limitation of transport in the liquid boundary layer or the biofilm. The kinetics of the system can be described with conventional substrate kinetics for suspended cell systems.

B Strong mass transfer limitation in the boundary layer, no limitation in the fully penetrated biofilm.

C No limitation in the boundary layer, limitation in the fully penetrated biofilm.

D Substrate transport limitation in both the boundary layer and the biofilm.

E As D but also conversion limitation because the biofilm is not fully penetrated by the substrate.

The role of mass transfer processes makes it necessary to define the biofilm morphology. Since biofilm morphology is often relatively complex, generally a strong simplification is made when models for biofilm processes are made (Fig. 3). Here we will initially use this simplification, later the effects of this simplification on biofilm processes will be discussed.

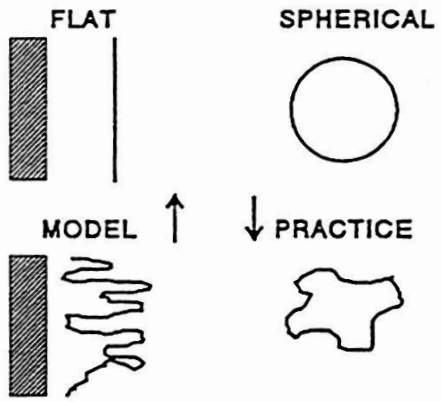

Figure 3

In general the intrinsic metabolism of microorganisms in biofilms will not differ from suspended growth. However several points have to be taken into account:

- Mass transfer from the liquid to the biofilm. Not only the mass transfer of solutes but also the mass transfer of suspended solids has a, not well understood, role in biofilm processes.
- Mass transfer in the biofilm. The diffusion of solutes can be reasonably well described by Fick's law for diffusion. The transport of solids and cells inside the biofilm is still a point of much research.
- Competition in a biofilm is not only for the substrate but also for space.

Although it is often neglected, it is worthwhile to realize that activated sludge flocs are also immobilized cell systems for which diffusion processes can be rate limiting. Below a general treatment of diffusion and conversion by immobilized cell systems will be given. Most attention will be given to simple biofilm models. For more detailed models concerning biofilm growth reference is made to the literature [16].

2. SUBSTRATE CONVERSION BY BIOFILM SYSTEMS

2.1 Substrate transport from bulk liquid to biofilm (external mass transfer)

The conversion by a biofilm is in general equal to the substrate transport velocity from the liquid to the biofilm. In general only one substrate is rate limiting. It is needed in each process to determine which of the substrates (e.g. organic carbon or oxygen) is rate limiting.

The transport of solutes from the liquid phase to the biofilm is analogous to the transport of e.g. oxygen from the gas phase to the liquid phase (see chapter "gas-liquid interfaphase transport). The substrate surface flux is generally described as the product of a mass transfer coefficient and a concentration difference (driving force):

$$J_S = k_l \cdot (C_{Sl} - C_{Si}) \qquad \text{eq. 1}$$

with: J_S: Surface substrate flux ($g/m^2.s$)
k_l: Mass transfer coefficient (m/s)
C : Substrate conc. at interphase (i) or in the liquid (l) (g/m^3)

The mass transfer resistance is assumed to be due to a "stagnant liquid boundary layer" in which all the diffusion takes place. In this case the substrate gradient can be

described by Fick's law (fig. 4). From this it follows that:

$$k_l = D/\delta_s \qquad \text{eq. 2}$$

with: D: Diffusion coefficient (m^2/s)
δ_s: Thickness of the liquid boundary layer (m)

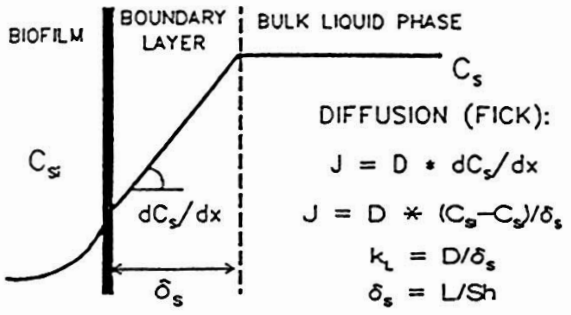

Figure 4

The thickness of the boundary layer depends on the reactor and biofilm geometry and on the hydrodynamic conditions. In general the thickness is derived from a (semi-)empirical estimation of the dimensionless Sherwood number (Sh, which represents the ratio of external mass transport by convection and diffusion):

$$\delta_s = L/Sh \qquad \textbf{eq. 3}$$

With: L: Characteristic length of the system (e.g. particle diameter, d)

The Sherwood number for a system can be calculated from empirical relations. As an example a relation for the Sherwood number of spherical particles in liquid flow is (for $0<Re<3.10^5$) [7]:

$$Sh = 2 + \frac{0.66}{[1+(0.84Sc^{1/6})^3]^{1/3}} \cdot \frac{(Re.Sc)^{1.7}}{1 + (Re.Sc)^{1.2}} \qquad \textbf{eq. 4}$$

With: Re: Reynolds number ($v_s.d_p/\nu$)
Sc: Schmidt number (ν/D)
ν: Kinematic viscosity (m^2/s)
d_p: Particle diameter (m)
v_s: Liquid velocity (m/s)

A more detailed overview on the liquid/solid mass transfer process can be found in [8]. Several Sherwood relations are included in appendix 1.

To determine the mass transfer coefficient (k_l) the following parameters have to be known:
- Diffusion coefficient
- Viscosity of liquid
- Density of the liquid
- Liquid velocity
- Characteristic length

The first three parameters are known and fixed constants for a certain substrate and waste water, whereas the latter two are dependent on the type of reactor used. Table 1 summarizes the external mass transfer coefficients for different treatment systems. Also the maximal flux of oxygen to the biofilm is calculated. Hereto it is assumed that C_{Si} = 8 g/m^3 (air saturation) and C_{Si} = 0 g/m^3.

Table 1 Estimation of liquid/solid mass transfer coefficients

	Liquid velocity (m/h)	Particle diameter (cm)	Liquid volume fraction (-)	Sh (-)	δ_s (μm)	k_l (m/d)	Maximal O_2-Flux ($g/m^2.d$)
FILTER SYSTEMS							
- Aquifers	0.004	0.06	0.4	2.3	200	0.38	3.04
- Slow sand filter	0.04	0.06	0.4	5	100	0.83	6.64
- Rapid sand filter	5	0.07	0.4	25	20	3.6	28.8
- Anaerobic filter	0.08	4	0.9	11	3600	0.028	---
MOVING BED							
- Fluid bed	33	0.1	0.7	34	20	3.4	27.2
- UASB reactor	1	0.3	0.5	14	200	0.35	---
TRICKLING FILTER		Spec. Area (m^2/m^3)					
- Low rate	0.08	0.58	0.4	11	1500	0.06	0.48
- High rate	1.67	0.9	0.9	39	20	0.35	2.8
MIXED REACTORS	Settling velocity (m/h)	Diameter particle (μm)	Power input (W/m^3)				
- Activated sludge	0.06	10	40	2.1	5	39	312
- Activated sludge	1.45	50	40	2.8	17.8	16	128
- Activated sludge	13	150	40	6.2	24.1	7.5	60
- Air lift suspension	96	500	40	23	21.7	6.5	52
- Lagoon	1.45	50	5	2.8	17.8	11	88
- Single cells		1		2	0.5	172	1376

It is now possible (using eq.1) to determine the mass flux of substrate to the biofilm provided the concentration at the interphase (C_{Si}) is known. This concentration must be obtained from a combination of the external and internal mass transfer processes. Therefore the internal mass transport must be known.

2.2 Diffusion and conversion in a biofilm (internal mass transport)

Diffusion equation

The substrate concentration in a biofilm is determined by the substrate conversion process and diffusion. The latter process can be described by Fick's law with an effective diffusion coefficient (D_{eff}). The diffusion in gel like materials is retarded due to the presence of the polymer. In general the effective diffusion coefficient is 80 to 90 % of the

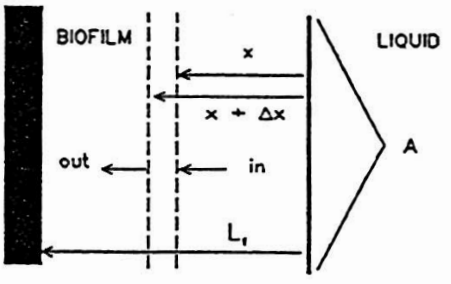

Figure 5

diffusion coefficient in liquid. Also the presence of biomass in the biofilm retards the diffusion. In general one can estimate the effective diffusion coefficient by multiplying the diffusion coefficient in water with the water fraction inside the biofilm.

The concentration of solutes in each point in a biofilm can be obtained from a mass balance over the biofilm. For the substrate a steady state mass balance around a small layer inside a biofilm reads (fig. 5):

$$A \cdot D_{eff} \cdot \left(\frac{\delta C_s}{\delta x}\right)_x - A \cdot D_{eff} \cdot \left(\frac{\delta C_s}{\delta x}\right)_{x+\Delta x} - A \cdot \Delta x \cdot r_s = \frac{dC_S}{dt} = 0 \qquad \text{eq. 5}$$

with: x: depth inside biofilm (m)
r_S: Substrate conversion rate per volume of biofilm ($g/m^3.h$)

The steady state mass balance for a substrate in a flat biofilm is now obtained by taking the limit Δx . 0 of eq. 1:

$$D_{eff} \cdot \frac{d^2C_S}{dx^2} - r_S = 0 \qquad \text{eq. 6}$$

A similar approach for a spherical biofilm yields:

$$D_{eff} \cdot \left(\frac{d^2C_S}{dr^2} + \frac{2}{r} \cdot \frac{dC_s}{dr}\right) - r_S = 0 \qquad \text{eq. 7}$$

with: r: distance from the center of particle (m)

The boundary conditions to solve these differential equations are as follows.

- At the interface (x = 0) the concentration is equal to the interface concentration as derived from the external mass transfer relation:

$$x = 0: \quad C_S = C_{Sl} \qquad \text{eq. 8}$$

- When the biofilm is not penetrated fully with the rate limiting substrate, then at the penetration depth (.) $C_s = 0$.
  At point ., at the solid/biofilm interface, or in the centre of a spherical biofilm particle (granule, aggregate, floc) there is no net transport of substrate:

$$\left(\frac{dC_S}{dx}\right) = 0 \qquad \text{eq. 9}$$

Above equations can be solved analytically if zero or first order kinetics apply, for Michaelis Menten kinetics numerical methods have to be applied.

Active layer thickness or.penetration depth

Often it is possible to assume zero order kinetics for the conversion process. In these cases it is possible to calculate an active layer thickness for the biofilm. This thickness can be calculated from the diffusion equations. For a flat biofilm one obtains for the penetration depth (δ_{fl}):

$$\delta_{fl} = \sqrt{\left(\frac{2 \cdot D_e \cdot C_{Si}}{q_s^{max} \cdot C_{Xf}}\right)} \qquad \text{eq. 10}$$

For a spherical biofilm one obtains:

$$3 \cdot \delta_{sph}^2 - \frac{2 \cdot \delta_{sph}^3}{r_p} = \frac{6 \cdot D_e \cdot C_{Si}}{q_s^{max} \cdot C_{Xf}} \qquad \text{eq. 11}$$

Combining eq. 10 and 11 leads to eq. 12:

$$\delta_{fl} = \delta_{sp} \cdot \sqrt{1 - \frac{2 \cdot \delta_{sp}}{3 \cdot r_p}} \qquad \text{eq. 12}$$

Clearly the penetration depth in a flat biofim is always smaller than in a spherical biofilm. The effect of the ratio δ_{sph}/r_p on the "correction term" in eq. 12 is shown in figure 6. It is clear that for small (< 0.1) δ_{sph}/r_p a flat biofilm geometry can be assumed. Since for aerobic growth the penetration depth is less then 100 μm already for particles with a radius of 1 mm a flat biofilm geometry can be used for calculating the conversion capacity.

Substrate surface flux

A zero order reaction rate can be assumed if the substrate concentration at the biofilm interface is much larger then the K_S value. In this case the majority of the substrate is consumed at q_S^{max}. For a fully penetrated biofilm (i.e. no diffusion limitation) the substrate flux can be expressed as:

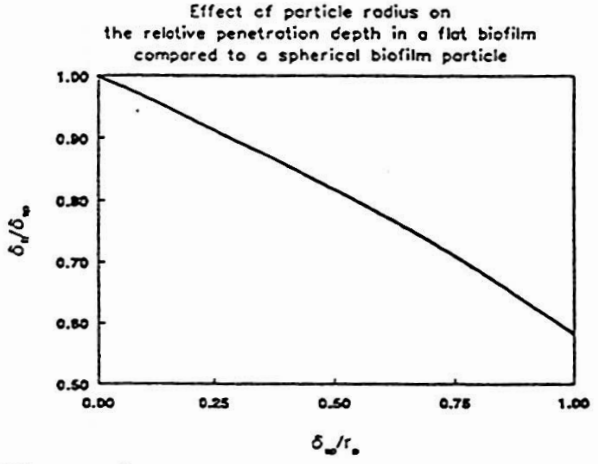

Figure 6

For a flat biofilm:

$$J_S = q_S^{max} \cdot C_{Xf} \cdot L_f \qquad \text{eq. 13}$$

with: C_{Xf}: Biomass concentration inside the biofilm (g/m^3)
L_f: Biofilm thickness (m)

For a spherical biofilm:

$$J_S = \frac{q_S^{max} \cdot C_{Xf} \cdot r_p}{3} \qquad \text{eq. 14}$$

For a partly penetrated flat biofilm the above derived relation for the penetration depth can be substituted in eq. 14 and 15 to obtain the equations for the surface flux. For a flat biofilm a "half order" surface flux is obtained:

$$J_S = \sqrt{2 \cdot q_S^{max} \cdot C_{Xf} \cdot D_e} \cdot \sqrt{C_{Sl}} = k_{0.5} \cdot \sqrt{C_{Sl}} \qquad \text{eq. 15}$$

with: $K_{0.5}$: Bulk half order rate constant

For a partly penetrated spherical biofilm and zero order kinetics one obtains:

$$J_S = \frac{q_S^{max} \cdot C_{Xf} \cdot [r_p^3 - (r_p - \delta_{sp})^3]}{3 \cdot r_p^2} \qquad \text{eq. 16}$$

where δ_{sp} from eq. 11 must be substituted.
In many practical cases the above half order rate expression (eq. 15) can very well describe the substrate conversion in biofilm systems [5]. This is mainly due to the fact that K_S values for most substrates (e.g. 0.05 mgN-NH_3/l or 0.01 mgO_2/l) are very low compared to the bulk concentration, while the biofilm is relatively thick. It should however be noted that $k_{0.5}$ is not an intrinsic microbial constant but depends on the system, and extrapolation of obtained values to different biofilm reactors should only be done with great care. Moreover often the half order rate constant is fitted on the overall conversion data. This means that when external diffusion limitations occur this is also incorporated in the obtained rate constant.

In figure 7 a comparison is made between a half order rate expression and a Michaelis Menten type of equation. Both relations can describe the experimental data. If one however wants to use the data for extrapolation erroneous results can be obtained.

Occurence of diffusion limitation becomes visible when one experimentally determines the K_S value. Large values compared to data for pure cultures indicate serious diffusion limitation. (e.g. K_S for nitrification in pure cultures: 0.05 mg/l and in activated sludge flocs: 1 mg/l)

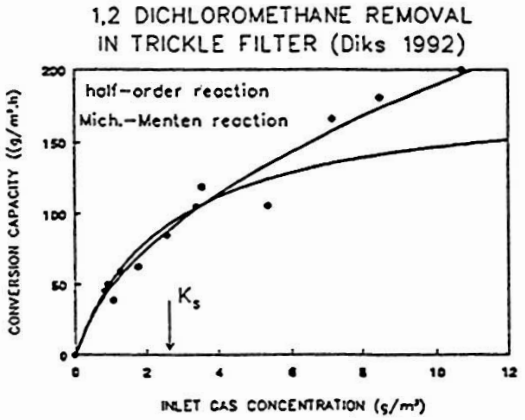

Figure 7
Comparison M.M. and half order reaction. Both curves are drawn through the first nine data points

First order reaction kinetics

When the substrate concentration at the interface is lower than the K_s value first order kinetics can be applied. The substrate flux for a flat biofilm gives [11]:

$$J_s = \frac{q_s^{max} \cdot C_{xf} \cdot L_f \cdot C_{sl}}{K_s} \cdot \epsilon \; ; \quad \epsilon = \frac{\tanh\alpha}{\alpha} \; ; \quad \alpha = \sqrt{\frac{q_s^{max} \cdot C_{xf} \cdot L_f}{K_s \cdot D_{eff}}} \qquad \text{eq. 17}$$

ε is the "first order efficiency" of the biofilm. ε is equal to one (i.e. no influence of diffusion on the conversion kinetics) when . << 1.

Overall substrate flux in a reactor with flat biofilms

We have now derived relations for the mass flux over the biofilm-liquid interface based on the internal and external diffusion processes. At the interface both mass fluxes should equal each other. This means that we can now, by equalizing the appropriate relations, derive the substrate conversion as a function of the bulk liquid substrate concentration. As an example the surface flux for a partly penetrated flat biofilm is obtained by combining eq. 1 and 15:

$$J_s = k_l \cdot \left(C_s - \frac{J_s^2}{k_{0.5}^2} \right) = \sqrt{\frac{0.25 \cdot k_{0.5}^2}{k_l^2} + k_{0.5}^2 \cdot C_S} - 0.5 \cdot \frac{k_{0.5}^2}{k_l} \qquad \text{eq. 18}$$

The overall reactor volumetric substrate conversion rate is obtained by multiplying the surface substrate flux by the specific surface area. Above relation has as its limits eq. 1 for small k_l values (strong external transport limitation), or eq. 15 for high k_l values (no external transport limitation). The influence of external transport limitations can be found as the quotient of eq. 18 and 15. This leads to:

$$\frac{J}{J_{k_l=\infty}} = -0.5 \cdot \alpha + \sqrt{1 + 0.25 \cdot \alpha^2} \qquad \text{eq. 19}$$

In this equation . is a dimensionless number (the Hatta number) expressing the ratio between the maximum conversion rate and the maximal external transport velocity:

$$\alpha = \frac{\text{max. } \textit{conversion rate}}{\text{max. } \textit{ext. transport rate}} = \frac{k_{0.5} \cdot \sqrt{C_S}}{k_l \cdot C_S} \qquad \text{eq. 20}$$

Figure 8 shows the effect of . on the quotient of the actual flux and the flux without ext. transport limitation. When the transport rate is much larger then the conversion rate . becomes 0 and the ratio between the fluxes is equal to 1. When the maximal conversion rate is equal to the maximal transport rate . is 1 and the ratio between the fluxes is 0.61. Based on rough estimations for the different constants (q_s^{max}=0.5 g/g.h, C_{xf}=30 g/l, k_l=0.016-0.16 m/h (table 1), C_S=1mg/l) leads to a value for the Hatta number of 18 - 1.8. This shows that external diffusion limitations can have serious effects on the overall substrate conversion rate in a biofilm proces. Finally one should

realize that often the external transport proces is not explicitly taken into account, however it will be incorporated in the obtained rate coefficient.

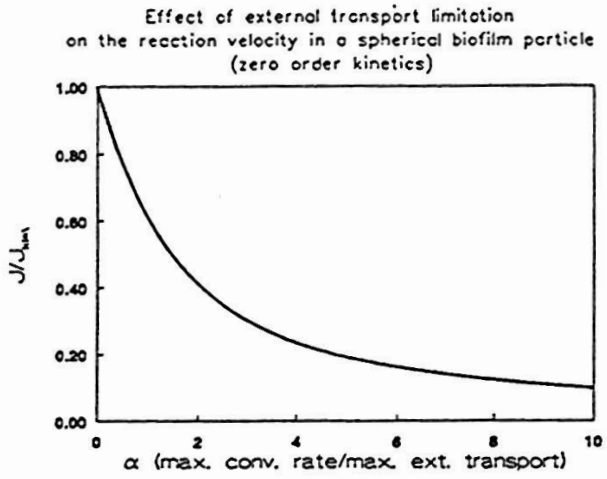

Figure 8

In table 2 the penetration depth and flux of the rate limiting substrate to a flat biofilm has been calculated for a completely mixed reactor and for the entrance of a plug flow reactor.

Table 2 *Substrate flux and penetration depth for different substrates and reactor types. For a completely mixed reactor effluent substrate concentrations are used. For the plug flow reactor calculations are made for the reactor entrance, i.e. influent substrate concentrations are used. The values for the plug flow reactor are underlined.*

Rate limiting substrate	Substrate concentration (mg/l)		Penetration depth (μm)		Substrate flux (g/m².d)	
Oxygen	3	*3*	100	*100*	10	*10*
Acetate (aerobic)	15	*150*	120	*400*	20	*60*
Acetate (anaerobic)	15	*800*	120	*1100*	20	*160*
Ammonium	5	*70*	60	*200*	15	*50*
Dichloromethane	0.01	*1*	5	*150*	0.6	*20*
	Completely mixed reactor/*Plug flow reactor*					

From above table 2 it is clear that for aerobic processes in general the diffusion of oxygen will be the rate limiting process. Since the oxygen concentration is always low there is no advantage in this respect for the use of a plug flow over a completely

mixed reactor. For anaerobic processes the use of a plug flow type of reactors can be beneficial because of the high substrate concentrations in the first part of the reactor. Due to a greater penetration depth, higher surface specific conversion rates are possible.

It is also clear from table 2 that the penetration depth of substrates is (especially for aerobic processes) very low. Therefore thick biofilms are in general not beneficial. The capacity of a biofilm process is then mainly determined by the specific surface area of the reactor.

2.4 Mixed population biofilms

Until now it has been assumed that the transformation process inside the biofilm is performed by one type of organism. In many environmental processes mixed populations are present. Above description of the diffusion processes equally applies in this case. Also the competition phenomena discussed in another lecture still apply. Besides competion for substrates, however, also competition for space occurs.

For anaerobic processes several microorganisms are involved in the conversion of e.g. COD to methane (acetogens, methanogens, hydrogen producers and consumers). For this process a more detailed biofilm model has been developed [9,15].

The competition for space can be illustrated for a combined COD and NH_4 oxydizing biofilm. It has been shown that the slow growing nitrifyers grow in deeper biofilm layers, whereas the fast growing COD-oxydizers are found at the outside of a biofilm [16]. Moreover with increasing COD surface loading rates, the ammonium conversion rate is decreased. This is because nitrifyers are forced deeper into the biofilm where they experience greater mass transport limitations [12].

For aerobic processes not only the aerobic part of the biofilm can contribute to the conversion process. Due to oxygen diffusion limitation anaerobic zones can develop inside thick (>100 μm) biofilms. This might for instance lead to denitrification. Although this seems profitable in the process design it can be questioned whether it is an optimal form of denitrification. In a COD/N-removing treatment system nitrifyers and COD-oxidizers will compete with each other. Due to the higher growth rate and oxygen affinity of COD oxidizers, nitrifyers will be found mainly inside the biofilm [3,16]. Due to the fact that nitrifyers are generally in deeper biofilm layers, nitrate formation will only occur when the soluble COD is almost consumed. This means that denitrification in deeper anoxic layers of the biofilm can only occur on COD resulting from decaying biomass, which is not a very rapid process.

2.5 Effect of inhibitors on biofilm kinetics

In general biofilm bacteria will observe lower substrate concentrations than suspended bacteria. This leads to less effect of inhibitors on biofilm conversion processes. In the case of substrate inhibition biofilm conversion rates can be even higher than suspended cell conversion rates (i.e. $\eta_{ov} > 1$) [17]. Especially in the conversion of toxic xenobiotic compounds biofilm processes might therefore be

advantageous.

In the case of product inhibition the opposite might occur. The product concentration in the biofilm will be higher then in the bulk. The conversion inside the biofilm will be therefore extra inhibited.

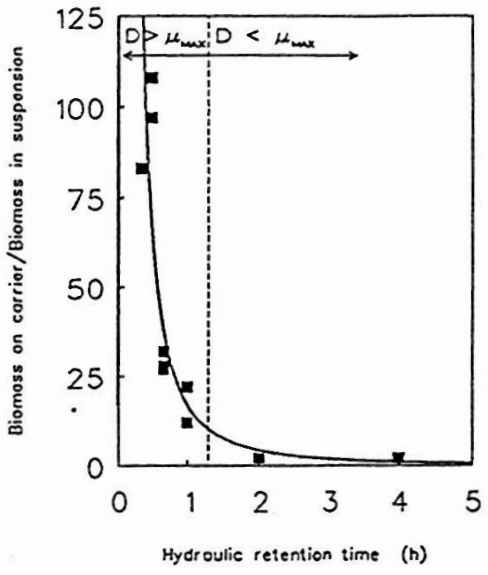

Figure 9

3. GROWTH OF BIOFILMS

3.1 Competition with suspended cells

In order to obtain biofilms in a bioreactor the competition between biofilm (or floc or aggregate) bacteria and suspended bacteria must be to the advantage of the biofilm bacteria. Due to diffusion limitation the growth rate of biofilm cells will be always smaller then the growth rate of suspended cells. Moreover the amount of cells in suspension will result from growth of suspended cells and the detachment of biofilm cells. Therefore biofilms will only occur when the dilution rate is somewhat larger than the maximal growth rate of the bacteria. The effect of the dilution rate on the biofilm accumulation in an airlift reactor is shown in figure 8 [6].

3.2 Biofilm growth

A major difference between biofilm and suspended growth processes is that in a (completely mixed) suspended culture the biomass residence time is determined by the dilution rate of the process.

The growth rate in a biofilm is determined by two processes:

(i) advective transport due to formation of biomass in deeper cell layers.

(ii) detachment of biomass at the biofilm liquid interface.

The equilibrium thickness of a biofilm is determined by the surface loading rate (i.e. biomass production) and detachment rate.

The advective transport rate is a function of the depth of the biofilm, since in deeper cell layer less biomass will be produced. Therefore the biomass residence time in the biofilm depends on the position inside a biofilm where the biomass is formed. The structure of a biofilm will be strongly determined by the maximal growth rate of the organisms. Organisms with a low maximal growth rate will be found predominantly in deeper layers of the biofilm. This has been found for COD and N oxidizing biofilms where the slow growing nitrifyers are found in deeper layers than the heterotrophic biomass [16].

3.3 Overall biofilm model

Several mathematical models for biofilm processes have been developed. The model of Wanner [15] is probably the most general one and is based on the IAWPRC model for activated sludge processes. This model allows to predict the change in spatial distribution of different microbial species in a biofilm in time. Although this model is the best available model several limitations make it difficult to use it for accurate process calculations. (i) The model describes only a one-dimensional substrate flux, perpendicular to the biofilm surface. The biomass density over the biofilm is considered constant. These features ignore the inhomogeneities observed in actual biofilm systems. Moreover segregation of biomass in biofilms has a profound influence on the reactor performance [4]. (ii) The attachment /detachment processes at the biofilm surface can be taken into account, however there are no good theoretical descriptions of these processes. Moreover even experimental data are scarce. (iii) The biofilm interface is considered to be smooth (i.e. ideal, fig. 2). Due to these limitations biofilm models have the potential to qualitatively but not quantitatively describe biofilm reactor performance.

4. EFFECT OF NON IDEALITY OF BIOFILMS

Previous discussion of the kinetics of biofilm has been done with a strongly idealized biofilm. In practice two processes make that this idealized approach can only be used with great care.

Effect of a rough biofilm surface

In general biofilms do not have a smooth interface with the liquid phase (figure 2). In reactors with high shear rates on the biofilm (e.g. fluidized bed systems) more or less smooth biofilms are formed. In reactors with less shear a biofilm with filaments will be formed. The length and number of these filaments seem to be correlated with the shear rate on the biofilm. In these cases the estimation of the mass transfer coefficient based on the liquid film theory is a conservative estimation. A rough biofilm surface has a higher surface area. Moreover due to the movement of the protrusions from the biofilm the hydrodynamic boundary layer is mixed up with the bulk liquid. Resulting in a substrate concentration at the biofilm surface more close to the bulk concentration. These two effects result in a greater mass transfer of substrate to the biofilm (i.e. a higher interfacial substrate concentration) than calculated on the basis of an idealized biofilm. Neglecting this non-ideality of biofilms can result in for instance overestimation of diffusion coefficients.

The external mass transfer of non smooth biofilms is greatly influenced by the mixing intensity of the bulk liquid. It has recently been shown that for these type of biofilm systems the conversion is related to the turbulent intensity of the liquid and filament density of the biofilm [10].

Effect of sloughing

The detachment of biomass from a biofilm is not only determined by a continuous erosion of cells from the biofilm surface, but also by discrete sloughing phenomena. In this case large parts of a biofilm are removed. This phenomenon makes that the structure of a biofilm will be less ordered then deduced from many biofilm models.

The deviation from ideality can not easy be predicted. Mainly because there are almost no quantitative experimental data available. A general trend which can be deduced from the literature shows that more effect of sloughing processes and surface roughness has to be taken into account for biofilms grown in reactors with low shear rates on the biofilm surface.

Layered biofilm structures

In relatively thick aerobic biofilms the inner part can become anaerobic. This means that extra conversion can occur inside these biofilms. This is not the place to extensively discuss this topic, therefore only some general remarks are made. The occurence of different layers inside biofilms can easily be accounted for in structured biofilm models.

It is often suggested that anaerobic layers inside biofilms are beneficial for denitrification. In several publications [e.g. 12,15,16] it is however shown that nitrifying organisms grow mainly in deeper biofilm, i.e. on places where the COD concentration has diminished due to conversion. This means that denitrification in the anaerobic layers of the biofilm can only use endogeneous material (or decayed biomass) as substrate. This is of course not as efficient as denitrification on soluble COD. Separation of denitrification and nitrification in two biofilm reactors (or in two compartments in the same reactor) will lead therefore to a more efficient nitrogen removal process.

5. CONCLUSIONS

Biofilm processes are governed by diffusion processes. Both liquid film diffusion as well as internal biofilm diffusion may limit the conversion rate. As a first approximation the socalled "half order" reaction rate can generally be applied. But one has to be aware that it is difficult to translate the thereby obtained rate coefficients to other reactor systems.

The main deviations from theoretical calculations are due to non ideality of the biofilms. Some attempts are made in literature to take this into account, but no satisfying results have been presented.

References

1. Allison, D.G., and I.W. Sutherland (1987) The role of exopolysaccharides in adhesion of fresh water bacteria. J. Gen. Microbiol. 133:1319-1327.
2. Characklis W.G., K.C. Marshall (1990) Biofilms, John wiley and sons Inc.
3. Gujer, W. verhaal nitrificeerders
4. Gujer, W., (1987) The significance of segregation of biomass in biofilms. Wat. Sci. Tech. 19:495-503.
5. Harremoes, P., (1978) Biofilm kinetics. Wat. Poll. Microbiol. 2:82-109.
6. Heijnen, J.J., M.C.M. van Loosdrecht, A. Mulder, L. Tijhuis (1992) Formation of biofilms in a biofilm airlift suspension reactor. Wat. Sci. Tech. 26:647-654.
7. Hooijmans, C.M., S.G.M. Geraats, J.J.M. Potters and K.Ch.A.M. Luyben (1990) Experimental determination of mass transfer boundary layer around a spherical biocatalyst particle. Biochem.Eng.J. 44:B41-B48.
8. Kissel, J.C., (1986) Modeling mass transfer in biological waste water treatment processes. Wat.Sci.Tech. 18:35-45.
9. Kissel, J.C., T Grotenhuis
10. Kugaprasatham, S., H. Nagaoka, and S. Ohgaki (1992). Effect of turbulence on nitrifying biofilms at non-limiting substrate conditions. Wat. res. 26:1629-1638.
11. Levenspiel, O., (1980) Chemical reaction engineering. Wiley, New York.
12. Rittman, B.E., and J.A. Manem, (1992) Development and experimental evaluation of a steady state, multispecies biofilm model. Biotechn. Bioeng. 39:914-922.
13. Van Loosdrecht M.C.M. et al. (1988) Bacterial adhesion: a physico chemical approach. Microb.Ecol. 17:1-15.
14. Van Loosdrecht M.C.M. et al. (1990) Influence of interfaces on microbial activity. Microb.Rev. 54:75-87.
15. Wanner O. and W./ Gujer (1986) A multispecies biofilm model. Biotech.Bioeng. 27:314-328
16. Watanabe
17. Inhibitie constantes biofilm