# CHAPTER TEN

# BIOFILM REACTORS

LUIS F. MELO[1] AND ROSÁRIO OLIVEIRA[2]

[1] *Department of Chemical Engineering, University of Porto, Rua D. Roberto Frias, 4200-465 Porto, Portugal*

[2] *Department of Biological Engineering, Universidade do Minho, 4710-057 Braga, Portugal*

## ABSTRACT

After presenting the concept of biofilms, reference is made to their importance in industry and health. Although biofilms are also well known for their deleterious effects (biofouling), emphasis is here given to the beneficial use of biofilms in wastewater treatment. The main types of biofilm reactors are briefly described and the rôle of support material in the adhesion and stability of biofilms is explained, taking into account the mechanisms involved in biofilm attachment. Practical procedures for the start-up of biofilm reactors are also mentioned.

Biofilm growth processes are described together with their properties, structure and performance. The advantages and disadvantages of biofilm reactors *versus* suspended biomass systems are discussed.

The main equations of the diffusion-reaction model are developed from engineering science principles. Equations derived from the diffusion-reaction model to calculate the reactor volume are presented, together with experimental values of the kinetic parameters. Practical empirical expressions or rules-of-thumb used in the design of fixed biomass reactors are also given. An overall model to predict the growth rate of biofilms and their final thickness or mass is established. The main problems concerning biofilm reactor modelling are discussed and the "missing links" for an optimised design are identified.

## INTRODUCTION

Micro-organisms, like the vast majority of living creatures, tend to live in communities and form their own specific habitats. It has been estimated that up to 90% of microbial cells in nature grow within agglomerates (Costerton *et al.*, 1987). Microbial films, frequently designated as biofilms, are one of the types of biological agglomerates (together with flocs and granules). They are communities of microorganisms attached to surfaces,

forming a porous matrix which contains the cells, the extracellular polymeric substances (EPS) they produce, and a substantial amount of water (Characklis and Marshall, 1990; Melo *et al.*, 1992). In nature and industry, this biofilm concept is obviously too simple and should be extended to include organic debris and small inorganic particles (clays, metallic oxides, etc.) captured by the polymeric network, as well as adsorbed compounds. Microbial cells in biofilms are often bacteria, but living algae and fungi often appear in significant quantity in such matrices. In some cases, macro-organisms such as mussels and barnacles may attach to the microbial layer. Micro-organisms in biofilms can survive in extreme conditions, with pH ranging from 0 to more than 13, temperatures from –10°C to 120°C or higher, and even in ultra-pure water (Flemming, 1991).

The immobilisation of cells on supports without the formation of an extracellular polymeric matrix will not be considered here as a biofilm process.

Microbial films can be detrimental not only to health (infections on teeth, prosthetic implants, urinary catheters, etc.), but also to engineered systems such as pipes, pumps, valves, reverse osmosis membranes, heat exchangers and ship hulls (Characklis and Marshall, 1990; Flemming and Geesey, 1991; Melo *et al.*, 1992; Bott, 1995; Lapin-Scott and Costerton, 1995). Furthermore, since they grow on almost any surface immersed in aqueous environments offering a minimum availability of nutrients, they can inconveniently develop on the walls of biological reactors as well as on other surfaces existing inside fermenters, such as agitator blades, pH probes, etc. The purpose of the present chapter is, however, to focus on the beneficial aspects of biofilms in reactors, where the microbial layers can be used to degrade unwanted compounds or to obtain desired products. The most common examples in the literature are wastewater treatment facilities containing attached biomass (Harremöes, 1978), but there are also other industrial processes using biofilm technology, such as the recovery of metals through bacterial leaching of ores or the production of vinegar, ethanol (Dempsey, 1990) and citric acid (Briffaud and Engasser, 1979).

## BIOFILM REACTORS – TECHNOLOGICAL FEATURES

### Types of Biofilm Reactors

A biofilm reactor is a biological reactor with fixed biomass. It is usually filled with particles of a carrier material – the "support" – where the microbial film is attached. If the particles are porous, the film forms not only on the surface but also within their pores. A limiting case of a very simple biofilm reactor is a duct where the biomass develops on the walls; such a situation actually occurs in sewers, where the adhered biomass may act as an additional wastewater treatment biological reactor, although in some cases it also contributes to the deterioration of the wall material. Another example is the case of biofilms formed on rocks in contact with mountain streams that help to purify these waters.

In industrial microbial film reactors, the fluid flows in contact with the biofilm particles promoting the exchange of nutrients and metabolic products between the fixed biomass and the surrounding fluid. There is also exchange of mechanical energy between the two



media: on one hand, the liquid exerts hydrodynamic forces on the biofilm enhancing both the detachment of biomass and the compactness of biofilms (these effects are particularly important in turbulent flow systems); on the other hand, the roughness and viscoelasticity of the microbial film increase the pressure drop of the fluid along the reactor.

The tendency for cells to attach to supports in a reactor is determined not only by the physical-chemical properties of the surfaces, but also by the relative values of the specific microbial growth rate and the hydraulic residence time. When the residence time of the fluid in the reactor is small compared to the replication time of the cells, attachment becomes particularly relevant in avoiding the washout of the micro-organisms. The cells will then tend to adhere to the supports if the physical-chemical surface interactions are favourable.

Invariably, suspended biomass may also grow in microbial film reactors, although, if needed, this phenomenon can be minimised in many cases through proper design and operating procedures. Anyhow, since biofilms are dynamic structures, biologically speaking, a part of the biomass that is continuously building up on the supports has to be periodically purged from the system. This can be achieved through proper washing cycles (often, back-washing) in conjunction with external solid-liquid separation or through sedimentation of the detached biomass on the bottom zones of the reactor.

One of the oldest examples of artificial biofilm reactors was promoted by Frederich II of Prussia (Schlegel, 1985) who had lime walls built and put in contact with flowing liquid manure. The presence of ammonium compounds and bacteria in the liquid waste resulted in the development of nitrifying biofilms inside and on the lime stone, which converted ammonium to nitrate and contributed to the formation of calcium nitrate by reaction with the calcium of the lime walls. The purpose was to obtain potassium nitrate for gunpowder production.

In terms of particle-fluid dynamics, microbial film reactors are often classified as fixed bed or expanded bed reactors. The latter include classical fluidised beds (Cooper and Atkinson, 1981; Dempsey, 1990; Trinet *et al.*, 1991; Heijnen *et al.*, 1994; Nguyen and Shieh, 1995; Tavares *et al.*, 1995) where particles move up and down in the bed while the expanded bed as a whole is kept within a well defined zone of the reactor, and the so-called moving beds where the whole expanded bed circulates throughout the equipment together with the fluid, such as in air-lift reactor, moving bed or circulating bed reactors (Heijnen *et al.*, 1990; Tijhuis *et al.*, 1994; Ulonska *et al.*, 1994; Lazarova and Manem, 1997; Rusten *et al.*, 1997; Nogueira *et al.*, 1998). In those reactors, the bed is usually expanded by the liquid, sometimes containing gas bubbles, flowing upwards with a sufficiently high velocity to lift the bed. Recently, biofilm support particles made of low density material, which tend to float in water, have been used in reactors where the bed is expanded by circulating the liquid downwards; this is the so-called inverse fluidised bed (Nikolov *et al.*, 1990; Nikov and Karamanev 1991; Karamanev and Nikolov, 1992, 1996).

Fixed beds can be divided into: i) submerged beds (Hamoda and ABD-El Bary 1987), where the biofilm particles are completely immersed in the liquid (up-flow or down flow circulation); ii) trickling filters (Metcalf and Eddy, Inc. 1987; Briffaud and Engasser, 1979), where the liquid flows downwards split in small isolated streams as it percolates through the biofilm bed, while the gas usually flows upwards, and iii) rotating disk reactors, where the biofilm develops on the surface of vertical disks that rotate within the

liquid. In aerobic processes, the lower part of each rotating disk is periodically submerged in the liquid and the upper zone is in contact with air; in anaerobic or anoxic processes, the disk is (almost) completely submerged at any time, in order to avoid contact of the biofilm with the air. Trickling filter reactors were also adapted to the degradation of volatile organic compounds (VOC) in gaseous effluents (Pederson and Arvin, 1996, 1999; Peixoto, 1998), the biofilm being slightly humidified by water or another liquid.

Membrane biofilm reactors, where the microbial layer is attached to a porous gas permeable membrane, are a promising technology in some situations, including in VOC removal, since they provide a more efficient method of supplying gas to the base of the biofilm (Suzuki *et al.*, 1993; Wilderer, 1995; Freitas dos Santos and Livingston, 1995).

Detailed descriptions and comparative analysis of the advantages and disadvantages of the various types of biofilm reactors, as well as their performances *vis a vis* the conventional activated sludge systems can be found in a number of text books and research papers (e.g., Metcalf and Eddy, Inc. 1987; Eckenfelder *et al.*, 1989; Lazarova and Manem, 1994; Cabral and Tramper, 1994; Willaert *et al.*, 1996).

Table 10.1 summarises the characteristic types of biofilm reactors and some are schematically presented in Figure 10.1.

Most biofilm reactors operate in a continuous mode. An exception is the Sequencing Biofilm Batch Reactor (SBBR), where the tank containing biofilm particles is periodically filled with the feed liquid and discharged (Wilderer, 1995). The latter remains in the reactor during the "reaction period", after which it is drained out. This operation mode is particularly favourable when consecutive processes are involved: for example, in a nitrogen removal process, the biological nitrification (with aeration) and denitrification (without aeration) steps can be carried out in the same unit provided it contains biomass with nitrifying and denitrifying abilities. The operational flexibility of such reactors has been demonstrated to be advantageous in certain processes.

Sometimes, reactors containing microbial granules without support, such as the Up-flow Anaerobic Sludge Blanket reactor (UASB) are also treated as biofilm reactors, mainly as regards the kinetics of substrate consumption (Lettinga and Hulshoff-Pol, 1992; Alphenaar *et al.*, 1993; Brito and Melo, 1997a); aerobic granules have also been developed and tested in nitrification processes (Tijhuis *et al.*, 1995). The granules can be considered to be dense flocs composed by microbes and extracellular polymers, and in fact the problems they pose to the modelling of substrate diffusion and reaction are similar to those

**Table 10.1** Characteristic types of biofilm reactors

| Fixed bed biofilm reactors | Expanded bed biofilm reactors |
|---|---|
| – Trickling filter | – Fluidised bed reactor (& inverse fluidis.) |
| – Submerged filter (downflow or upflow) | – Moving bed reactors: |
| – Rotating disk reactor | – air lift reactor |
| – Membrane biofilm reactor | – circulating bed reactor |
| | – Sequencing batch biofilm reactor (SBBR) |

Reactors containing dense microbial granules not attached to solid supports:
Up-flow anaerobic blanket reactor (UASB)
Expanded granular sludge blanket (EGSB)



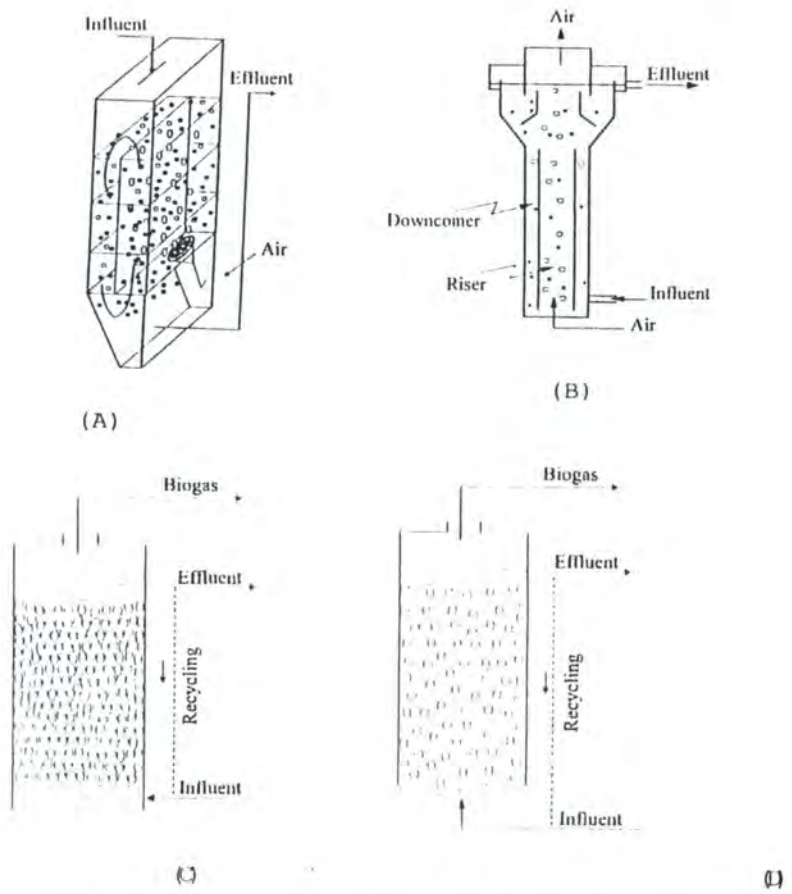


**Figure 10.1** Schematic representation of a circulating bed reactor (A); air-lift reactor (B); anaerobic filter (C) and anaerobic fluidised bed (D).

of biofilms attached to a solid surface. Granules have a lower polymeric content, are usually more compact than microbial films and their formation follows specific distinct steps (Brito and Melo, 1997b).

A great variety (in shape, dimensions and materials) of supports is used in biofilm reactors, including: rough and rather large pieces of stones and gravel as the ones employed in the first generation of trickling filters (Metcalf and Eddy, Inc. 1987); small particles of sand, basalt or clay in fluidised bed and airlift reactors; particles with complex artificial geometries made of light plastic material used in circulating bed systems and agitated batch reactors; simple flat surfaces such as plastic disks in rotating biological

**Figure 10.2** Illustrative example of plastic supports used in trickling filters.

contactors. The specific area of the supports has been increasing over the last decades (Figure 10.2) in order to improve the efficiency and compactness of the reactors: initially, low specific surface areas of 100 $m^2/m^3$ were available in trickling filters with rock supports and in biological disks; nowadays, fine granular and porous supports with 500 $m^2/m^3$ or more are in use in many submerged, fluidised or moving bed reactors.

## The Importance of the Support

The prerequisite for the formation of a biofilm is the adhesion of the microbial cells to the support surface. Therefore, much effort has been put into investigating adhesion mechanisms (Busscher *et al.*, 1995, Teixeira and Oliveira, 1999, Azeredo *et al.*, 1999). However, in the more recent generation of biofilm-on-carrier reactors other processes might have a greater influence on biofilm formation. The carriers are subjected not only to higher turbulence and liquid shear than most fixed-support biofilms but also to the erosion and abrasion promoted by particle collisions (Gjaltema *et al.*, 1997). This means that the selection of the supports for biomass immobilisation is of great importance to obtain a stable biofilm leading to high reactor efficiency. In this way, the support must favour bacterial adhesion, must have a high mechanical resistance, a low cost and a great availability. To accomplish the first requirement, parameters like surface charge, hydrophobicity, porosity, roughness, particle diameter, density and concentration can be of great importance.

An irreversible initial adhesion is the key factor for the development of a stable biofilm, but the support ability for microbial colonisation is also of crucial importance. This determines the colonisation velocity and ultimately the start-up rate of the reactor. For many years the adhesion mechanism was tentatively interpreted in terms of DLVO theory, with microbial cells considered as colloidal particles (Marshall *et al.*, 1971, van Loosdrecht *et al.*, 1988). Accordingly, the net force of interaction arises from the balance between the Lifshitz-van der Waals forces of attraction and the generally repulsive forces generated during the approach of the electrical double-layers of the interacting species (Oliveira, 1992). This repulsive character is due to the fact that most of the existing solid materials display a net negative charge when immersed in aqueous solutions with pH near



neutrality. Bacterial cells are an example of negatively charged surfaces, especially because in most cases they are only able to survive in mild pH conditions. Therefore, the possibility for generating an electrostatic attraction is to utilise a positively charged support. However, as was said before, in nature only very few materials, like some metallic hydroxides, are able to display such behaviour. Other materials can be engineered in order to be positively charged; this is feasible for laboratory purposes, but it is not economically compatible with large-scale operation.

More recently it was demonstrated that the wettability, or in a reverse sense the hydrophobicity, of solid surfaces strongly influences adhesion either of bacteria, eukariotic cells or proteins (Margel *et al.*, 1993, Prime and Whitsides, 1993, Wiencek and Fletcher, 1997, Taylor *et al.*, 1997, Teixeira and Oliveira, 1999). According to van Oss (1997), hydrophobic interactions are usually the strongest of all long-range non-covalent interactions in biological systems and can be defined as the attraction between apolar or slightly polar entities (molecules, particles or cells) when immersed in water. It must be noted that hydrophobic attraction can prevail between one hydrophobic and one hydrophilic entity immersed in water, as well as between two hydrophobic surfaces (van Oss, 1995). The interaction between two hydrophobic surfaces is favoured in aqueous medium because they can establish a closer contact by squeezing the water in between. In other words, an increasing degree of hydrophobicity enhances adhesion. This has been confirmed by recent studies on the selection of supports for different types of biofilm reactors and using different bacterial strains. One example is the relation found between the higher degree of hydrophobicity of some polymeric materials and the increased biofilm activity of a consortium of autotrophic nitrifying bacteria (Sousa *et al.*, 1997). A more direct relation was established between the number of initially adhered cells of *Alcaligenes denitrificans* and the hydrophobicity of polymeric supports: the number of adhered cells increased linearly with the increase in hydrophobicity (Teixeira and Oliveira, 1999). A linear correlation was also obtained between the amount of attached biomass of an anaerobic consortium and the hydrophobicity of the supports: foam glass, pozzolana, clay and sepiolite (Pereira *et al.*, 1999).

The newest generation of suspended carriers biofilm reactors were designed to have a high biofilm area, which allows for higher loading rates and smaller space requirements. The smaller the diameter of the carrier particles the higher the surface area available for biofilm development. Particles with diameters as small as 0.2 mm have been used in airlift reactors (Heijnen *et al.*, 1992). It is interesting to note that even in fluidised bed reactors it is possible to use dense particles (e.g. basalt) with small diameters without having high pumping power requirements because there is a drastic decrease in density when the particles become covered by the biofilm. Another means to provide more area for biomass attachment is to use rough and/or porous surfaces. Moreover, this is also a way to provide niches to retain micro-colonies, shielding them from the effects of shear forces (Bryers, 1987). For the purpose of colonisation, some authors consider surface roughness as one of the most important parameters (Gjaltema *et al.*, 1997), even more important than internal surface area (Petrozzi *et al.*, 1991). In order to accumulate large quantities of biomass, the porosity must be suitably sized. According to Messing and Opperman (1979 (a) and (b)), the adequacy of the pore size depends upon the cell dimensions and its mode of reproduction: fission, budding or spores (with mycelial growth). For instance, for

microbes reproducing by fission at least 70% of the pores should have diameters up to five times the largest major dimension of the microbial cell. Other authors (Shimp and Pfaender, 1982) also confirmed that surface colonisation is favoured when crevices are microbially sized.

However, micro-organisms retained in pores or niches can be subjected to diffusional resistance to the flux of substrates and products. This is exemplified in a study already mentioned (Pereira *et al.*, 1999), where foam glass, pozzolana, clay and sepiolite were compared as supports for an anaerobic consortium. Sepiolite, although having the highest biomass retention, showed the lowest specific biological activity. This is a consequence of the combined effect of the small pore size of sepiolite and the high amount of attached biomass, promoting a deficient nutrient transport to the cells in the inner zones. Another point to be considered is the accumulation of gaseous metabolites inside porous carriers because this can induce the carriers washout, with a negative effect in the overall performance. This problem is overcome if the carriers have large pores with large internal porous volume because this enables the transport to be mediated also by convective flow.

The carrier concentration determines the available surface area for microbial attachment, but at the same time acts as a controlling factor of biofilm formation. Biofilm formation is balanced by biofilm detachment. In suspended bed reactors, where shear and abrasion is considerable, the detachment is mainly caused by particle particle collisions (Heijnen *et al.*, 1992). The collision frequency depends on the size, the relative velocity and concentration of particles. In an airlift reactor, the rate of biomass detachment was found to be linear with the concentration of particles up to a solids hold-up of 30% (v/v) (Gjaltema, 1996).

### Reactor Start-Up

In biofilm reactors there will always exist a competition between organisms growing in suspension and organisms growing in the biofilm. Significant biofilm formation only occurs under conditions where suspended cells are quickly washed-out (Heijnen *et al.*, 1992). Generally this is accomplished by starting the reactor in batch mode until a significant amount of biomass is reached and then gradually increasing the dilution rate (lowering the hydraulic retention time) until the maximum growth rate of the culture is surpassed. From laboratory experience, in reactors with high shears (namely air-lift, circulating bed and fluidised bed), it is advisable to pre-colonise the supports outside the reactor in a sort of fed-batch mode, especially if the micro-organisms are slow growing. That is to say, the culture medium must be periodically replaced by fresh medium, according to the microbial growth rate (Teixeira and Oliveira, 1998). Otherwise, if directly inserted in the reactor, a great number of carriers will remain bare. In the case of methanogenic bacteria, one method to encourage their growth is to supply a substrate that may be directly metabolised such as methanol (Balaguer *et al.*, 1992). The start up period of anaerobic reactors can also be reduced with the adaptation of the inoculum to the specific substrate properties. It is preferable therefore to use a mix of several sources of active biomass instead of an inoculum from one source only.

When the biofilms are formed by heterotrophs producing large amounts of exopolymers it is common for thick biofilms to develop in the quieter parts of the reactors, as reported by Gjaltema *et al.* (1997). In extreme cases this can lead to the clogging of the reactor. Those situations become worse when working with high C/N ratios, which enhance the production of exopolymers. So, the C/N ratio must be controlled to avoid such undesirable biofilm formation.



## BIOFILM PROPERTIES, FORMATION AND PERFORMANCE

### Composition and Structure

The following characteristics may be considered typical of microbial films:

- they are slimy layers with gel-like consistency and viscoelastic theological behaviour;
- their colours vary from translucid white or yellowish to dark brown or black, depending on the type of prevailing micro-organisms and substrates;
- they can have thicknesses that go from a few microns (films formed in liquids with low substrate concentration) to some millimetres or even centimetres (e.g., in the so-called "white waters" of pulp and paper mills);
- most of the wet biofilm mass is water (frequently, more than 90%);
- the mass of extracellular polymers, typically containing polysaccharides and glicoproteins, may represent 70% or more of the dry weight of the biofilm;
- the fraction of micro-organisms in biofilms depends on the metabolic characteristics of the microbial populations and on the substrate concentration, and it may correspond to 10% to 50% of the dry biomass.

The biofilm matrix is a natural structure built by the micro-organisms in what seems to be a way of protecting themselves from external aggressions – either physical, chemical or biological – and of finding suitable sites where nutrients can be more easily available. The extracellular polymers contribute both to the initial adhesion of the microbial cells to the surface and to the internal cohesion of the matrix due to the links established between the polymeric chains. This network tends to offer some resistance to the diffusion of components, be they toxic compounds or nutrients, and it can favour the adsorption of molecules as well as the capture and accumulation of external particles within the biofilm.

Biofilm structure has been a matter of considerable discussion in recent years (Lappin-Scott and Costerton, 1995; de Beer and Stoodley, 1995; van Loosdrecht *et al.*, 1995). The surface of most biofilms is wavy, sometimes with protruding visco-elastic filaments that penetrate into the water. Microbial biofilms are not homogeneous matrices, showing regions with different concentrations of cells and polymers, together with water containing "voids". There is some controversy over whether the distribution of biomass is based only on randomly localised "clusters" of cells plus biopolymers, separated by water channels (de Beer and Stoodley, 1995), or based on superimposed porous layers of biomass with different densities and compositions (Christensen and Characklis, 1990). Both concepts seem to coexist in practical situations. Each cluster of biomass (cells and biopolymers) may include different layers, containing either distinct microbial populations, or similar populations in different metabolic states, or even just

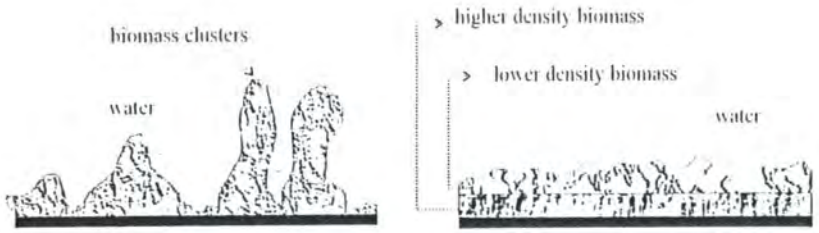


**Figure 10.3** Two conceptual models of biofilm structure.

different concentrations of cells and non-living biomass. It may happen that the cluster structure approaches the layer model as the biofilm grows older and the "void" spaces (water channels) are filled with more biopolymers and cells, as well as with organic and inorganic debris. This statement is supported by data showing the increase of biofilm density with time (Trulear, 1983). The two structural models are schematically presented in Figure 10.3.

### Dynamics of Biofilm Formation

The build up of microbial films is usually preceded by the adsorption of a thin layer of organic macromolecules to the adhesion surface, sometimes comprising also metallic hydroxides or oxides and very fine mineral powders (Chamberlain, 1992). The effect of organic films in promoting the biofouling of surfaces submerged in sea water had also been reported long time ago by Zobell and Allen (1935).

Micro-organisms suspended in the liquid are transported by diffusion, convection or self motility to the initially clean surface. Once at the solid surface, irreversible adhesion is favoured by the formation of "polymer bridges" between the adsorbed layer of macromolecules and the biopolymers excreted by the microbes (Characklis and Marshall, 1990). The "induction or lag period" of the biofilm development includes the adsorption of the organic conditioning film plus the first phase of microbial attachment before exponential growth of the biofilm begins.

Mass transfer mechanisms play an important role in the transport of dissolved nutrients and substrates (oxygen, sugars, organic acids, etc.) to the micro-organisms on the solid surface, providing them with the essential elements for their metabolism. As a result, the biofilm mass increases by means of the growth and multiplication of microbes together with the production of the polymeric matrix. Above a certain thickness, the microbial metabolism inside the biological layer can become limited by the internal resistance to mass transfer of substrates, and also by the fact that the micro-organisms located near the biomass-liquid interface will tend to consume most of the substrate before it reaches the inner zones of the biofilm. At the same time, products from microbial metabolism are carried away from the biofilm. Apart from the initial period of surface inoculation, the transport and adhesion of new micro-organisms to the biofilm seems to have a minor role



in the build up process, as compared to the growth processes that depend on substrate availability (Bott, 1995).

The processes described above contribute to the growth of the biological layer. Simultaneously, detachment processes occur, often promoted by the hydrodynamic forces of the fluid flowing over the biofilm surface, resulting in its erosion or even in the disruption ("sloughing off") of portions of the attached biomass. Even in the absence of fluid shear forces thicker biofilms may also slough off on account of their weaker internal cohesion and lack of nutrients in the inner zones. The competition between the biological growth and the detachment phenomena leads to a final balance where a maximum average thickness (subject to fluctuations due to the periodic detachment) of the biofilm is reached, which is sometimes considered a pseudo-steady state.

The structure and biological activity of microbial films depend on the history of their formation, including not only the specificities of the microbial populations involved in this process, but also the effects of environmental parameters such as the liquid velocity, temperature and pH, the nature and concentration of the substrate(s) and the surface properties (Bott, 1995; Characklis and Marshall, 1990; Messing and Oppermann, 1979a; Mott and Bott 1991; Vieira *et al.*, 1993). Biofilm development is favoured when the temperature and pH approach the optimum values for microbial growth, although it should be stressed that the conditions inside the biofilm are different from those in the surrounding liquid. The pH is particularly affected by the metabolic products excreted by the micro-organisms in the attached layer. A well documented example is the nitrogen biological removal process, which includes the nitrification and denitrification steps. While the pH inside nitrifying biofilms tends to decrease due to the production of $H^+$ when $NH_4^+$ is oxidised to $NO_3^-$, the opposite occurs in biofilms containing denitrifying micro-organisms (Harremöes, 1978).

The composition of the liquid, particularly the nature and concentration of nutrients and substrates, has a direct influence on biofilm development. A higher carbon/nitrogen ratio seems to favour bacterial attachment and the production of biopolymers (Veiga *et al.*, 1992), leading to an increase in biomass concentration in the reactor. Experiments were carried out where the substrate was suppressed from the liquid (Bott, 1995; Vieira and Melo, 1995) and, as a result, part of the microbial film detached from the surface within a few hours or, at the most, one day. This period of time was remarkably increased when small clay particles (around 10 $\mu$m) were incorporated in the biofilm during its development (Vieira and Melo, 1995).

The liquid velocity in contact with the biofilm is a major parameter that affects the dynamics of its development and its structure. In turbulent flow, higher velocities tend to originate thinner biofilms; although nutrient mass transfer to the biofilm surface increases with velocity at an almost linear rate, hydrodynamic detachment forces have a stronger impact, since they are proportional to the square of the velocity. However, some experiments carried out with water at velocities below 1 m.s$^{-1}$ showed an increase of the biofilm thickness with the velocity, meaning that in those cases substrate mass transfer was the process controlling the biofilm growth rate (Bott, 1995). Such situations are usually favoured by low bulk substrate concentrations.

Additionally, the liquid velocity has also a significant effect on the structure of the microbial layer: Christensen and Characklis (1990) reported a linear increase in biofilm

density (dry mass per unit wet volume) with shear stress; Vieira *et al.* (1993) measured densities of 14 kg.m$^{-3}$ and 21 kg.m$^{-3}$ for water velocities of 0.34 m.s$^{-1}$ and 0.54 m.s$^{-1}$, respectively.

Biofilms formed under low velocities, particularly in non-turbulent conditions such as those occurring in many wastewater treatment bioreactors, can be very thick – sometimes preventing substrates from reaching the inner zones – and/or have a very "loose" and "fluffy" consistence. In such cases, there is a high probability of occurring the detachment of biomass lumps ("sloughing off") resulting in an unstable operation of the bio-reactor.

Hermanowicz (1999), using two-dimensional modelling, predicted that higher shear stresses and substrate concentrations lead to more compact layers and that a decrease in the liquid velocity results in a more open biofilm structure with protuberances extending from the biomass into the flowing liquid.

## Biological Activity

Here, biological activity is considered as the rate at which biofilms metabolise substrates and nutrients. Basically, it depends on the nature and concentration of the microbial species present in the biofilm, on the chemical composition and mass transfer properties of the surrounding fluid and on the physical structure of the attached biomass. The latter is also affected by the environmental conditions, including the hydrodynamics and the surface properties and morphology, as discussed before.

The distribution and metabolic state of the micro-organisms within a biofilm is a most sensitive aspect in terms of its performance. If the consumption of substrate in the upper part of the biofilm and/or the mass transfer resistance offered by the polymeric network lead to substrate depletion in the inner zones, the latter will remain fairly inactive as regards that substrate; this means that the bacteria in those zones will either be able to survive with residual nutrients or change their metabolism and start consuming other compounds existing in the liquid (which may correspond to the development of new species or strains). It has been shown by different authors (e.g., Trulear, 1980; Capdeville *et al.*, 1992) that in thicker biofilms only a small portion of its mass is in fact active in metabolising a given substrate: for example, Trulear (1980) found that a mono-species biofilm fed with 2 mg/m$^2$.min of glucose had the same substrate consumption rate when its thickness was 25 $\mu$m as when its thickness was 100 $\mu$m, a few days later. Capdedville *et al.* (1992) showed that the active biomass in aerobic biofilms formed under different substrate concentrations was the same (around 0.1 mg/cm$^2$), in spite of the total mass of the several biofilms being quite different. Hamdi (1995) defined a critical diameter for flocs and biofilms, above which there will be inactive zones within the biomass.

There are cases where the activity of biofilms is dependent on the relative concentrations of different substances in the liquid. For instance, in an ammonia oxidation process carried out in rotating disk systems, it was found that if the ratio of bulk oxygen to ammonia is below 2.5 $gO_2/gN\text{-}NH_4^+$ the ammonia consumption rate will be limited by the oxygen concentration (Gönenç and Harremöes, 1985). Other authors (Nogueira *et al.*, 1999), working with thinner nitrifying biofilms in a circulating bed



reactor, under turbulent flow, obtained a lower critical value of 1.5 $gO_2/gN\text{-}NH_4^+$, which could be explained by the reduced resistance to oxygen transport within the thinner biofilms.

The following is an interesting example (Mendez *et al.*, 1989) of the effect that the history of a biofilm has on its performance: two anaerobic biofilm reactors using clay particles as supports for the fixed biomass were fed with the same carbon source, but with different carbon/nitrogen ratios (250/7.5 and 250/1.5). The reactor with less nitrogen content presented a higher concentration of the attached biomass than the other, although the suspended biomass concentration was the same in both. The conversion rate and the methane production rate obtained in the two reactors were also similar. However, significant differences appeared when pulses of volatile fatty acids were introduced: the nitrogen deficient reactor showed a lower conversion rate of these fatty acids, meaning that its biofilm had less active bacteria (and probably much more polymers) than the other. In fact, the specific activity of the nitrogen deficient microbial layer was one third of the biofilm fed with a greater amount of nitrogen compounds. It can then be said that thicker biofilms do not necessarily correspond to more active ones, mainly if their mass is essentially composed of extracellular polymers. Much depends on the amount of active bacteria they contain.

## Biofilms *versus* Suspended Biomass

The basic advantage of biofilm reactors over suspended biomass systems (either with dispersed cells or with flocs) is that the former are able to retain much more biomass – 5 to 10 times more, per unit volume of the reactor – substantially reducing its wash out and allowing for a more stable operation with a higher biomass concentration. As a consequence, the investment in downstream liquid-solid separation equipment is much smaller, the reactors are more compact and offer a greater flexibility in terms of the hydrodynamic operating conditions (different flow rates or hydraulic residence time can be chosen without the risk of washing out the biomass). The structure of the biofilm matrix favours the resistance of its microbial cells not only to hydraulic shocks but also to toxic substances that can unexpectedly get into the reactor with the liquid stream.

A further advantage of biofilms is that they offer enhanced possibilities of transferring metabolites from one species to another, due to their spatial proximity. A study on an anaerobic fixed bed reactor carried out by Miyhara and Noike (1995) demonstrated that the degradation of long chain fatty acids was more easily accomplished in the biofilm than in the suspended biomass, because the lipolytic bacteria that produce hydrogen are surrounded by hydrogen consuming bacteria (methanogenic). This spatial arrangement is possible in an aggregated biomass and not in dispersed biomass, and it favours the interspecies exchange of hydrogen, which is determinant for the success of the anaerobic process.

However, fixed biomass does not necessarily have higher biological activity per unit of organic dry mass than the suspended cells or flocs, in terms of substrate consumption rate, partly on account of the internal diffusional limitations caused by the polymeric matrix. For example, in nitrification experiments (Wiesmann, 1994), values around 0.2 g of $N\text{-}NH_4^+$

per gram of dry biomass and per day were obtained for both activated sludges and biofilms. Since the total biomass concentration was significantly higher in the fixed biomass systems, it seems that either there were less active nitrifying bacteria in the biofilm than in the activated sludge, or each cell in the biofilm had, in average, a lower biological activity than one cell in the suspended biomass.

This raises a very important question about the metabolic state of the cells in biofilms: are their yield coefficients, maximum specific growth rates, saturation constants, etc., the same as when they are in suspension? Biofilm modelling has been developed by considering that the main differences in biological activity result from the fact that the substrate diffusional limitations are more severe in a biofilm than in a suspended biomass system. However, it does not seem correct to assume that the cells in the biofilm act in every other aspect as if they were freely dispersed, because the microenvironment around them can be totally different; not only the substrate concentrations can be lower than in suspended cultures, but also the distances between cells are much smaller. Additionally, there are increasing evidences of phenotypic changes in cells when they go from a planktonic growth mode to biofilms (Costerton and Lappin-Scott, 1995).

## BIOFILM REACTOR MODELLING

Biofilm reactors are usually calculated on the basis of lumped empirical parameters, the values of which are assumed to be known from previous experience. An example of such parameters is the so-called "eliminated load" (mass of substrate consumed per unit time and unit volume of the reactor), frequently referred to in the design of wastewater treatment plants (Harremöes and Henze, 1995). Hence, there are no generalised relationships between the lump parameters and operational or design variables like substrate concentration, liquid velocity, hydraulic residence time, biofilm thickness, support characteristics, etc.

As yet, not even the existing unstructured mathematical models based on a phenomenological approach of mass transfer and biological reaction rates (e.g., AQUASIM, see: Reichert 1994, 1995; Wanner and Reichert, 1996) are commonly used by the practical designers, on account of the lack of sound estimates of the kinetic and diffusion coefficients. In this section, a simple diffusion-reaction model, applied to a biofilm system, will be presented with the aim of estimating the bio-reactor volume and of offering the reader a basis for a quantitative analysis of the underlying mechanisms that affect the reactor performance. This can also be helpful in terms of the understanding of further developments of more sophisticated mathematical tools available for the design of bio-reactors.

### Diffusion-Reaction Model: Calculation of Substrate Consumption Rate for a Single Substrate and a Single Microbial Species in Steady-state Biofilms

The model will be described for the case of single limiting substrate and single microbial species in the biofilm, and the latter will be supposed to be in "steady state conditions".



By this, it is meant that the amount of attached biomass, its thickness and the rate of substrate consumption in the biofilm are constant with respect to time. Moreover, the equations will be applied to describe the case of a flat biofilm, that is, a microbial layer attached to one side of a flat particle or thin biofilms attached to particles of other shapes; here, the meaning of "thin" depends on the relative dimensions of the biolayer and the support particle: a biofilm may be considered "thin" if its thickness is smaller than roughly 30–50% of the radius of the support particle (supposing the latter is spherical). The model is derived from the well-known heterogeneous catalysis approach in chemical engineering (Froment and Bischoff, 1979), and its application to enzyme reactors and wastewater treatment biofilm reactors has been fully reported by several authors in the last decades (Harremöes, 1978; Cabral and Tramper, 1994; Harremöes and Henze, 1995).

Let $\mathbf{r_f}$ be the reaction rate inside the biofilm, that is, the substrate consumption rate per unit volume of wet biofilm ($kg.m^{-3}.s^{-1}$), $\mathbf{y}$ the distance inside the biofilm, measured from the liquid-biofilm interface, and $\mathbf{J}$ the substrate flux through the biofilm, referred to the unit area of a microbial layer attached to a supposedly flat surface ($kg.m^{-2}.s^{-1}$). A mass balance to the substrate across an element of thickness $\mathbf{dy}$ inside the biofilm results in:

$$\frac{dJ}{dy} = -r_f \tag{1}$$

Assuming unidimensional mass transfer in the biofilm, Fick's law will be written as:

$$J = -D_f \frac{dS_f}{dy} \tag{2}$$

where $\mathbf{S_f}$ is the substrate concentration inside the biofilm at a distance $\mathbf{y}$ from the biofilm-liquid interface, and $\mathbf{D_f}$ is the effective diffusion coefficient (also called "effective diffusivity") of the substrate in the microbial layer. This coefficient may not be equal to the molecular diffusivity of the same compound in the liquid phase, on account of the tortuosity and porosity of the biofilm and of the fact that convective flow (and not only molecular diffusion) may also take part in the transport of substrates inside the microbial matrix (Stoodley *et al.*, 1994).

The steady-state diffusion-reaction differential equation is obtained from Equations (1) and (2):

$$D_f \frac{d^2S_f}{dy^2} = r_f \tag{3}$$

Once the expression for the biological reaction rate ($\mathbf{r_f}$) is known, the next problem is the integration of Equation (3). In the present case, an equivalent of the traditional Monod formula will be used to describe the kinetics of biomass production and substrate consumption in the biofilm. It should be stressed that the Monod model was developed only for describing the specific growth rate of suspended cells in simple situations (e.g., without substrate or product inhibition, in dilute solutions). Therefore, the application of such a model to a system where cells are entrapped within a polymeric matrix built by themselves, should be carried out with extreme caution, since the environment around the cells can be totally different from the one encountered by dispersed cells in solution.

The original Monod equation is:

$$\mu = \mu_{max} \frac{S}{K_s + S} \quad (4)$$

where $K_s$ is the affinity (or "saturation") constant of the suspended cell culture; it can be interpreted as the substrate concentration for which the specific growth rate ($\mu$) will be equal to half the maximum specific growth rate ($\mu_{max}$) and the higher is its value, the lower is the affinity of the micro-organism with the substrate. The specific growth rate is defined as the mass of new cells produced per unit mass of existing cells and per unit time ($kg.kg^{-1}.s^{-1}$). **S** is the bulk substrate concentration in the solution.

The adaptation of the Monod concept to biofilms implies the introduction of a few modifications. Since the active cells in a biofilm produce not only new cells but also a substantial amount of exopolymers, a new variable, called "specific biofilm production rate" ($\mu_p$), should be defined: it represents the mass of cells and exopolymers (dry biofilm) produced per unit time and per unit mass of the biofilm. Then, the Monod equation will be transformed into:

$$\mu_p = (\mu_p)_{max} \frac{S_f}{K_s + S_f} \quad (5)$$

$(\mu_p)_{max}$ being the maximum value of the specific biofilm production rate and $S_f$ the substrate concentration inside the biofilm. Note that the value of $K_s$ in a biofilm is not necessarily the same as in a suspended culture.

If $(X_f)_a$ is the active cell density in the microbial film (that is, the mass of active cells per unit volume of wet biofilm, $kg.m^{-3}$), and $Y_{f/S}$ is the mass of dry biofilm (cells plus exopolymers) produced per unit mass of substrate consumed in the biofilm (kg of dry biofilm/kg substrate), the following relationship applies:

$$r_f = (\mu_p)_a \frac{(X_f)_a}{Y_{f/S}} \quad (6)$$

where $(\mu_p)_a$ is the mass of dry biofilm produced per unit time and per unit mass of active cells in the biofilm. If the whole biofilm is biologically active, then $(\mu_p)_a = \mu_p$.

The biofilm reaction rate equation will then be given by:

$$r_f = (r_f)_{max} \frac{S_f}{K_s + S_f} \quad (7)$$

In this equation, $(r_f)_{max}$ is the maximum substrate consumption rate per unit volume of wet biofilm. Substitution of Equation (7) into Equation (1) results in the following equation, which has to be integrated to obtain $r_f$:

$$D_f \frac{d^2S_f}{dy^2} = (r_f)_{max} \frac{S_f}{K_s + S_f} \quad (8)$$

Solutions of this equation are generally obtained by numerical methods. The analytical integration of the equation is however possible in particular situations, such as in the case of intrinsic first order (low substrate concentrations, $S_f$ substantially lower than $K_s$) and



zero order reactions (high substrate concentrations, $S_f$ substantially higher than $K_s$). In those cases, as indicated in Figure 10.4, Equation (8) will be reduced to:

$$D_f \frac{d^2S_f}{dy^2} = (r_f)_{max} S_f \quad \text{(first order reaction)} \quad (9)$$

or to:

$$D_f \frac{d^2S_f}{dy^2} = (r_f)_{max} \quad \text{(zero order reaction)} \quad (10)$$

As shown in Figure 10.4, the reaction rate constants for the two limiting cases are:

$$\text{First order biofilm reaction } (s^{-1}): \quad k_{1f} = \frac{(r_f)_{max}}{K_s} \quad (11)$$

$$\text{Zero order biofilm reaction } (kg.m^{-3}.s^{-1}): \quad k_{0f} = (r_f)_{max} \quad (12)$$

So far, we have only described the reaction and diffusion phenomena inside the microbial layer. In the more general case, where the external mass transfer (in the liquid medium) may also control the consumption rate, the mass transfer resistance in the liquid film next to the biofilm surface should be incorporated in the model. In steady state conditions, the rate of external mass transfer will be equal to the overall rate of diffusion and reaction in the biofilm. Therefore, if $J_e$ is the external mass transfer rate of substrate in the liquid:

$$J_e = k_m(S - S_f) \quad (13)$$

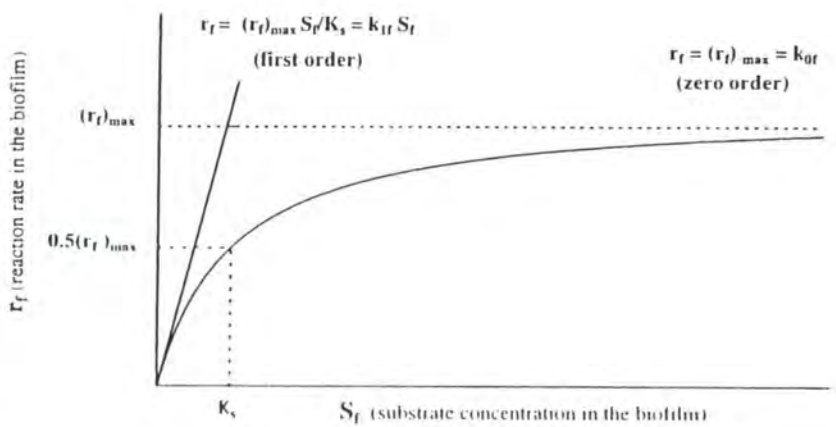


**Figure 10.4** Graphic representation of the Monod type reaction rate model and its two limiting cases (first order and zero order).

where $k_m$ is the external mass transfer coefficient, and S and $S_i$ are the substrate concentrations in the bulk liquid and at the biofilm-liquid interface, respectively, then:

$$J_s = r_f \tag{14}$$

In biofilm reactors, it is more common to calculate the "surface reaction rate", $r_A$ ($kg_{substrate}.m^{-2}.s^{-1}$), that is, the substrate consumption rate per unit area of biofilm. This variable is related to $r_f$ through:

$$r_A = r_f L_f \eta \tag{15}$$

where $L_f$ is the thickness of the microbial layer and $\eta$ is the overall efficiency of the biofilm (see below).

The following integrated expressions may be obtained to estimate the substrate consumption rate per unit surface area of biofilm:

a) First order intrinsic reaction:

$$r_A = \frac{S}{\frac{1}{k_m} + \frac{1}{\eta_i k_{1f} L_f}} \tag{16}$$

The "biofilm internal efficiency" ($\eta_i$) represents the ratio between the actual substrate consumption rate and the substrate consumption rate that the biofilm would display if there were no internal diffusional limitations. This parameter is given by:

$$\eta_i = \frac{\tanh \phi}{\phi} \tag{17}$$

and $\phi$ is the Thiele modulus for a first order reaction for a flat plate:

$$\phi = \sqrt{\frac{k_{1f} L_f^2}{D_f}} \tag{18}$$

The Thiele modulus represents the ratio between the reaction rate and the internal diffusion rate. When the Thiele modulus has a small value, the biological reaction will be the rate limiting step. For high values of $\phi$, internal diffusion will control the rate of substrate consumption. The more general expression of the Thiele modulus, which can be applied to reactions of any order "n" is:

$$\phi = \phi \sqrt{\frac{(n+1) k_{nf} L_f^2 S_i^{n-1}}{2 D_f}} \tag{19}$$

$k_{nf}$ is the rate constant for a reaction of order "n".

The concentration profile in the biofilm (Figure 10.5), in the case of first order kinetics, is:

$$\frac{S_f}{S_i} = \frac{\cosh\left[\phi\left(1 - \frac{y}{L_f}\right)\right]}{\cosh(\phi)} \tag{20}$$

where y is the distance inside the biofilm (measured from the liquid biofilm interface) corresponding to the concentration $S_f$.



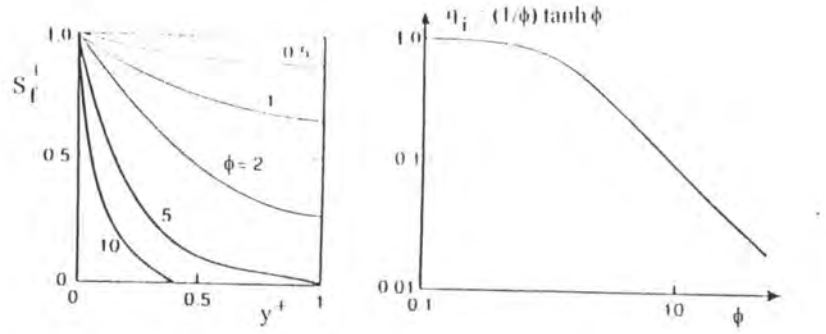


**Figure 10.5** Dimensionless concentration ($S_f^+ = S_f/S_i$) as a function of the dimensionless distance inside the biofilm ($y^+ = y/L_f$) for a flat biofilm with first order kinetics at several values of the Thiele modulus ($\phi$) and efficiency factor ($\eta_i$).

Sometimes, the observed reaction rate per unit area of biofilm ($r_A$) is written as:

$$r_A = \eta k_{1f} L_f S \tag{21}$$

where $\eta$ is the overall biofilm efficiency for a flat plate:

$$\frac{1}{\eta} = \frac{1}{\eta_i} + \frac{k_{1f} L_f}{k_m} = \frac{1}{\eta_i} + \frac{\phi^2}{Bi} \tag{22}$$

*Bi* is the mass transfer Biot number which represents the ratio between the maximum external mass transfer rate and the maximum internal mass transfer rate:

$$Bi = \frac{k_m L_f}{D_f} \tag{23}$$

Equations (16) and (21) are simply two different forms of the same expression.

b) Zero order intrinsic reaction:

$$r_A = \frac{k_m S}{2\lambda^2}\left[\sqrt{1 + 4\lambda^2} - 1\right] \tag{24}$$

where:

$$\lambda = \frac{k_m \sqrt{S}}{\sqrt{2 k_{nf} D_f}} \tag{25}$$

$\lambda$ is a dimensionless variable that represents the ratio between the external mass transfer rate and the internal coupled diffusion-reaction rate.

If the external mass transfer is the limiting step of the overall process, then:

$$r_A = k_m S \tag{26}$$

If the internal phenomena of diffusion and reaction are much slower than the external mass transfer (consumption rate limited by internal processes), then:

$$r_A = \sqrt{2k_{ol}D_lS} \tag{27}$$

When applying the equations for zero order kinetics, two extreme situations can be easily identified: either the biofilm is fully penetrated by the substrate or not. A new parameter $\beta$, which represents the degree of substrate penetration in the biofilm, is related to the reciprocal of the Thiele modulus for zero order intrinsic reactions:

$$\beta = \frac{\sqrt{2}}{\phi} = \sqrt{\frac{2D_lS_l}{k_{ol}L_f^2}} \tag{28}$$

Here, $\beta$ corresponds to the biofilm internal efficiency ($\eta_f$) for a flat plate geometry and zero order reaction.

bi) If the substrate fully penetrates through the whole microbial film ($\beta > 1$), the surface reaction rate will be given by:

$$\text{no external mass transfer limitations}: \quad r_A = k_{ol}L_f \tag{29}$$

or by:

$$\text{control by external mass transfer}: \quad r_A = k_mS \tag{26}$$

and the concentration profile will be:

$$\frac{S_l}{S_l} = \frac{\left(\frac{y}{L_f}\right)^2}{(\beta)^2} - 2\frac{\left(\frac{y}{L_f}\right)}{(\beta)^2} + 1 \tag{30}$$

In a fully penetrated biofilm, the reaction is of zero order throughout the whole biological layer.

bii) When the substrate cannot fully penetrate the microbial film ($\beta < 1$), then the surface reaction rate is given by Equation (27), above (no external mass transfer control):

$$r_A = \sqrt{2k_{ol}D_lS} = k_{ol}L_f\beta \tag{27}$$

In this case, the overall process (also named as the "apparent" or "observable" reaction) has a half-order dependency on the bulk substrate concentration. The concentration profile (Figure 10.6) inside the biofilm in the partially penetrated film is then:

$$\frac{S_l}{S_l} = \frac{\left(\frac{y}{L_f}\right)^2}{(\beta)^2} - 2\frac{\left(\frac{y}{L_f}\right)}{\beta} + 1 \tag{31}$$

The reaction rate constants for the first and zero orders are related by:

$$k_{ol} = k_{1l}K_s \tag{32}$$

where $K_s$ is the affinity constant of the Monod model.

It can be shown (Harremöes and Henze 1995) that, by defining parameter $\alpha$ as:

$$\alpha = \sqrt{\frac{k_{ol}L_f^2}{K_sD_l}} \tag{33}$$



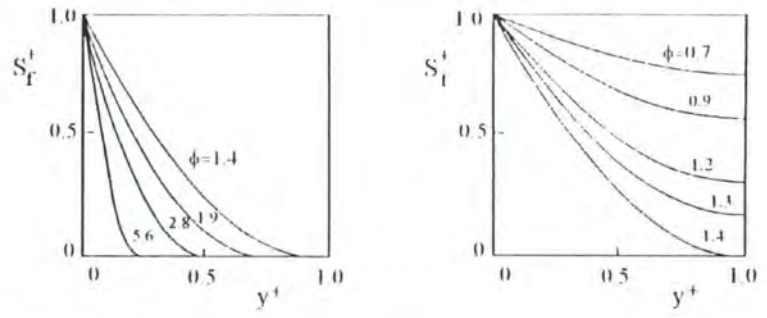


**Figure 10.6** Dimensionless concentration ($S_f^+ = S_f/S_l$) as a function of the dimensionless distance inside the biofilm ($y^+ = y/L_f$) for a flat biofilm with zero order kinetics at several values of the Thiele modulus ($\phi$): (A) biofilm partially penetrated by the substrate; (B) biofilm fully penetrated by the substrate.

only first order or zero order reactions (and no half-order apparent reaction) can exist when $\alpha < 2$. If the concentration is low and the biofilm is thin (1st order process) or if the concentration is very high and the biofilm is totally penetrated (zero order process), diffusional limitations will not affect the order of the overall process; that is, an intrinsic zero order reaction in the biofilm will yield an apparent overall zero order reaction (process), and an intrinsic first order reaction will yield an apparent first order reaction.

For $\alpha > 2$, the apparent (or observable) "half-order" process will predominate for intermediate values of the substrate concentration, although the intrinsic order is zero. The half-order case emphasises the existence of strong diffusional limitations inside the biofilm.

Figure 10.7 shows the internal efficiency factor as a function of a "dimensionless observable modulus" ($\psi$) for two different geometries, spheres and slabs. The new modulus $\psi$ is defined as:

$$\psi = \frac{r_AL_f}{D_lS_l} \tag{34}$$

### *Limiting substrate*

Many biological degradation processes involve at least two nutrients or, more precisely, a nutrient/substrate and an electron acceptor; they are of the oxidation-reduction (redox) type. In order to use the diffusion-reaction model described above, it is essential to identify the limiting substrate (substance), that is, the first one to reach zero concentration (that is, to be completely consumed) within the biofilm. This depends on the mass transfer and the reaction rates of the two components in the microbial layer. For the case of intrinsic zero order kinetics, and taking into account the penetration depths ($\beta$), it can be easily shown (Harremöes and Henze 1995) that:

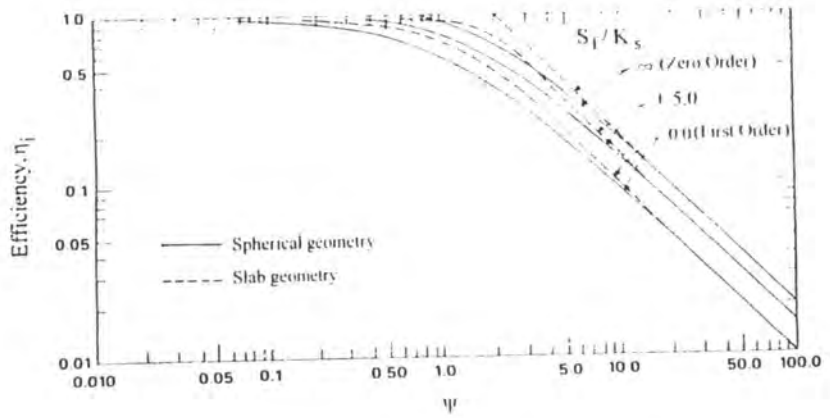


**Figure 10.7** Biofilm internal efficiency as a function of the "dimensionless observable modulus" $\psi$ (equation 34).

i) the oxidant will be the limiting factor if:

$$\frac{S_{ox}}{S_{red}} < \frac{(D_f)_{red}}{(D_f)_{ox} N_{ox,red}} \tag{35a}$$

where the subscripts "**ox**" and "**red**" refer to the oxidant and reductant substance, respectively, and $N_{ox,red}$ is the stoichiometric ratio between the two reactants;

ii) the reductant will be the limiting factor if:

$$\frac{S_{ox}}{S_{red}} > \frac{(D_f)_{red}}{(D_f)_{ox} N_{ox,red}} \tag{35b}$$

The previous equations apply to a flat biofilm. They can also be used when the biofilm is formed around a particle, provided that the microbial layer is thin. In the case of thick biofilms around small spherical supports or of microbial granules without any support surface, a spherical geometry should be considered in the derivation of the model, and the variable $L_f$ in the Thiele modulus should be replaced by $r_p/3$ ($r_p$ is the radius of the spherical biofilm-particle). The reader should look in specialised textbooks on heterogeneous catalysis for further modifications to the model equations when using spherical or cylindrical particles.



## Calculation of Reactor Volume

Let **Q** be the volumetric flow rate of the liquid through the system, $S_1$ the inlet substrate concentration and $S_2$ the substrate concentration at the reactor outlet. A simple mass balance to the substrate, from the inlet to the outlet of the reactor, results in:

$$Q(S_1 - S_2) = r_A A_f \tag{36a}$$

In the case where the substrate concentration varies continuously along the reactor, it is advisable to write a differential balance:

$$QdS = -r_A\, dA_f \tag{36b}$$

$A_f$ is the surface area of the biofilm (the external mass transfer area), which will be here supposed to be smooth, that is, without filaments or protuberances. The case of spherical support (or carrier) particles will be considered below, although the biofilm can be modelled as a flat geometry.

### *Ideal continuous stirred tank reactor*

Let $r_p$ be the radius of the bare carrier particles (i.e., without biofilm) and $L_f$ the thickness of the microbial layer. The surface area of the biofilm can then be related to the reactor volume ($V_R$) through:

$$A_f = \frac{3V_R(1-\varepsilon)}{L_f + r_p} \tag{37}$$

where $\varepsilon$ is the porosity of the reactor (liquid volume over reactor volume, the latter containing liquid and particles with biofilm). Therefore, the reactor volume will be given by:

$$V_R = \frac{Q(S_1 - S_2)(L_f + r_p)}{3r_A(1-\varepsilon)} \tag{38}$$

In an ideal stirred tank reactor, the substrate concentration in the liquid will be the same in every point and equal to the outlet concentration. Therefore, the observed reaction rate $r_A$ (per unit surface area of biofilm) will be given by equations (16) to (18) in the case of intrinsic first order reaction, with $S = S_2$, and by Equations (24) and (25) in the case of intrinsic zero order reaction, also with $S = S_2$.

If the biofilm is thin, $(L_f + r_p)$ can be replaced by $r_p$.

### *Ideal plug flow reactor*

In this case, the following simplified expressions can be used (Harremoes, 1978; Harremöes and Henze, 1995), assuming pseudo-homogeneity in the reactor (i.e., uniform substrate concentration along the cross section area of the reactor):

i) First order reaction:

$$V_R = \frac{Q(L_f + r_p)}{3(1-\varepsilon)k_1 L_f \eta} \cdot \ln\left[\frac{S_1}{S_2}\right] \tag{39}$$

ii) Zero order reaction with full substrate penetration ($\beta > 1$) and no external mass transfer limitations:

$$V_R = \frac{Q(L_f + t_p)}{3(1-\varepsilon)k_{ol}L_f}(S_1 - S_2) \tag{40}$$

iii) Zero order reaction with partial substrate penetration ($\beta < 1$) and no external mass transfer limitations:

$$V_R = \frac{\sqrt{2S_1}(L_f + t_p)Q}{3(1-\varepsilon)\sqrt{k_{ol}D_f}}\left[1 - \sqrt{\frac{S_2}{S_1}}\right] \tag{41}$$

### *Transition between different reaction orders*

Biofilms may be subject to different operating conditions within the same reactor, particularly in plug flow systems. Near the reactor outlet, a substantial amount of substrate will have been consumed and its concentration will be low, giving rise to a first order reaction in the biofilm. Near the reactor inlet, if the concentration is large enough, the biofilm may be completely penetrated (zero order overall process) or partially penetrated (half order overall process). Therefore, when calculating bioreactors, it is useful to have a way of detecting which reaction order will prevail in the biofilm system at each zone of the reactor. The following simple criteria can be applied:

i) Transition from zero order to half order overall process:

The critical substrate concentration will be defined, by considering $\beta = 1$, as:

$$S_{crit} = \frac{L_f^2 k_{ol}}{2D_f} \tag{42}$$

Therefore, if $S > S_{crit}$, the overall process (apparent reaction) will be of zero order. On the other hand, if $S < S_{crit}$, the overall process will be of half-order.

ii) Transition from half order to first order overall process:

In this case, the transition point will be defined in relation to the Monod saturation constant. When $S > 2\ K_s$, the apparent reaction will be of half-order. In the case of $S < 2\ K_s$, the process will be of first order.

### *Values of the diffusion-reaction parameters: illustrative examples*

In spite of the limitations of the diffusion-reaction model, mainly those related to the supposed homogeneous distribution of the active biomass in the microbial layer, many reports have been published on its application to different substrates and different biofilm reactors. The purpose of such studies was often to obtain values of the biofilm kinetic parameters by fitting the model to experimental data on substrate consumption rates in laboratory or larger scale reactors. Most of the time, the external mass transfer resistances were not taken into account, and only the *apparent* kinetic constants were estimated. Since it is considered that in wastewater treatment processes using biofilms the apparent kinetic



order is in most cases 1/2 (Harremoës, 1978). Table 10.2 indicates typical values of the apparent half order constant ($k_{1/2ap}$), which, from Equation (27), is:

$$k_{1/2ap} = \sqrt{2k_{ol}D_f} \tag{43}$$

Although the data in Table 10.2 display some scattering, the order of magnitude of the $k_{1/2ap}$ values is the same and there are no large inconsistencies in aerobic heterotrophic and autotrophic systems. This is somehow remarkable in view of the considerable differences between the cases studied (different reactors, different substrates, etc.), often without a real accurate control of the measured variables. Anyhow, design

**Table 10.2** Apparent half order kinetic constants in wastewater treatment ($k_{1/2ap}$)

| Biological process and limiting metabolic factor | Reactor type | $K_{1/2ap} \cdot 10^5$ ($kg^{1/2} \cdot m^{-1/2} \cdot s^{-1}$) | Reference |
|---|---|---|---|
| Aerobic, heterotrophic biomass Oxygen | Fixed bed | 0.12 | Grasmick *et al.* (1982) |
| Aerobic, heterotrophic biomass Oxygen | Rotating drum | 0.12–0.15 | Harremoës *et al.* (1980) |
| Aerobic, heterotrophic biomass Glucose | Rotating drum | 0.12 | Harremoës (1978)<br>Harremoës *et al.* (1980) |
| Aerobic, heterotrophic biomass Toluene | Waste gas trickling filter | 0.07–0.11 | Pedersen and Arvin (1996) |
| Nitrification, autotrophic biomass Oxygen | Rotating disk | 0.075–0.11 | Gönenç and Harremoës (1985) |
| Nitrification, autotrophic biomass Oxygen | Submerged filter | 0.05–0.10 | Çeçen and Gönenç (1994) |
| Nitrification, autotrophic biomass Oxygen | Circulating bed reactor | 0.15 | Nogueira *et al.* (1998) |
| Nitrification, autotrophic biomass Ammonium | Rotating disk | 0.06 | Gönenç and Harremoës (1985) |
| Anaerobic, methanogenic biomass Acetate | Downflow filter | 0.12 | Hamoda and Kennedy (1987) |
| Anaerobic, methanogenic biomass Acetate | UASB | 0.05 | Brito and Melo (1997) |
| Anaerobic, methanogenic biomass Molasses | Upflow filter | 0.01–0.18 | Gönenç *et al.* (1991) |
| Anoxic, denitrifying biomass Methanol | Rotating drum | 0.10–0.20 | Jansen and Kristensen (1980)<br>Jansen (1982) |
| Anoxic, denitrifying biomass Nitrate | Rotating drum | 0.02–0.11 | Jansen and Kristensen (1980)<br>Jansen (1982) |
| Anoxic, denitrifying biomass Nitrate | Rotating disk | 0.11 | Watanabe (1978) |

engineers do not seem yet to rely on such values for the efficient design of biofilm reactors.

So far, there are no widely acceptable values for other model parameters, such as the effective diffusion coefficient (Harremoës 1978; Henze and Harremoës 1995) and the Monod saturation constant, which may be quite different from the values obtained in suspended cell cultures, both of them having strong effects on the results yielded by the model.

Some authors advocate that the diffusion coefficient in biofilms should be taken approximately as 80% of the value in water, but there is much disagreement on the experimental values published in different sources (for example, some values are even larger than the coefficient in water). Table 10.3 presents only a few illustrative values of the diffusion coefficients in different biofilm systems.

Such large differences in diffusivities may be due to a variety of factors (Fan *et al.*, 1990, de Beer *et al.*, 1997) related to the nature of diffusing substance, the microbial species present and the physical structure of the biofilms. The latter is in turn very much dependent on the conditions under which the microbial layer was developed, among others the nature and concentration of the substrate, the hydrodynamic parameters (fluid velocity, turbulence), the presence of toxic substances, etc. Information about the precise chemical, microbiological and hydrodynamic parameters that affected the history of formation of the biofilm are missing in many of the publications reporting diffusivity values. Some authors have tried, with a certain degree of success, to correlate the diffusivity with biofilm properties such as the cell or biomass density in the attached layer. Fan *et al.* (1990) presented the following correlation based on the experimental data of a significant number of authors:

$$\frac{D_f}{D_w} = 1 - \frac{0.43 X_v^{0.92}}{11.19 + 0.27 X_v^{0.99}} \qquad (44)$$

which shows that the diffusion coefficient decreases with the increase in the cell density ($X_v$, kg.m$^{-3}$) within the biofilm. $D_w$ is the diffusion coefficient in water (variable $X_v$ seems to represent biomass dry density and not only cell density, in spite of the authors having used this last name). Although this type of correlation is a step forward in the estimation of diffusivities, relationships between the biomass density in biofilms and the

**Table 10.3** Measured or estimated effective diffusion coefficients in biofilms

| Diffusing component | Diffusion coefficient $\times 10^9$ (m$^2$/s) | $D_f$ (biofilm)/ $D_f$ (water) (%) | References |
|---|---|---|---|
| Oxygen | 2.2 (20 °C) | 105% | Kissel *et al.* (1984) |
| Oxygen | 2.55 (20 °C) | 120% | Williamson and McCarty (1976) |
| Oxygen | 1.75 (29 °C) | 66% | Noguera *et al.* (1998) |
| Lithium chloride | 0.33 (35 °C) | 33% | Nilsson and Karlsson (1989) |
| Glucose | 0.08–0.63 (20 °C) | 15%–117% | Ohmura and Ohmura (1982) |



reactor operating conditions are needed to predict diffusion coefficients for proper reactor design.

The Monod saturation constant in biofilms is often considered similar to the one in cell suspensions, but this may be quite erroneous on account of the microenvironment surrounding the micro-organisms being quite different in a biological layer from that in a dispersed cell culture, leading to distinct metabolic states (Fletcher 1992a, 1992b).

## Mathematical Models for Multisubstrate and Multispecies Biofilms

The diffusion-reaction model presented above applies to simple situations where only one microbial species and one substrate are present. Sometimes, for the sake of simplicity, while incurring possible errors, the model has been applied to mixed cultures where one or more substrates are involved in the biochemical reactions. Empirical parameters can be obtained to describe the consumption of substrates in that specific situation, but it will not be advisable to try to apply those values to any other case. More complex models were published and tested for the cases where multisubstrate and/or multispecies are present, as well as when inhibiting factors or particulate material affect the biological process. A limited number of references is given below, for the reader interested in more specialised models or in further developments of the above model: Wanner and Reichert, 1996; Reichert, 1994, 1995; Coelhoso and Rodrigues, 1995; Bryers, 1993; van Ede *et al.*, 1993; San *et al.*, 1993; Droste and Kennedy, 1986; Wanner and Gujer, 1986.

Comparison of the behaviour of plug flow and continuous stirred reactors may be found in several published sources (Moser 1988; Rodrigues *et al.*, 1983).

The recent investigations on the composition and spatial distribution of biomass and void spaces within biofilms, using microelectrodes, confocal laser microscopy and molecular probes, led to the development of new multidimensional modelling strategies and techniques which may contribute to a better understanding and quantification of the activity, population dynamics, stability, morphology of microbial films, as well as of the dynamics of their transient growth processes (Wanner 1995; Ritmann *et al.*, 1999; Picioreanu *et al.*, 1999; Noguera *et al.*, 1999; Hermanowics, 1999).

## Practical Design Procedures for Reactor Calculation

For practical design purposes, empirical equations are still the most common method to calculate biofilm reactors in wastewater treatment processes. Trickling filters, the classical technology, have received more attention from the designers. One of the well known mathematical formulae for this case is (Metcalf and Eddy, Inc., 1987):

$$\frac{C_2}{C_1} = \exp\left[-K_1 Z \frac{A_s}{Q}\right] \qquad (45)$$

$C_2$ and $C_1$ are the substrate concentrations at the outlet and the inlet of the reactor, respectively, expressed as mg.L$^{-1}$ of soluble $BOD_5$ (5-day biological oxygen demand,

that is the dissolved oxygen used by the micro-organisms in the biochemical oxidation of soluble organic matter during an incubation period of 5 days). **Z** is the depth of the filter (m), $\mathbf{A_v}$ is the specific area of support per unit volume of reactor ($m^2.m^{-3}$), $\mathbf{A_s}$ is the cross sectional area of the filter ($m^2$) and **Q** the volumetric flow rate of the wastewater to be treated ($m^3.s^{-1}$). $\mathbf{K_T}$ is the observed removal rate constant ($m.s^{-1}$) at temperature T, which has been correlated with temperature through:

$$K_T = K_{20\,C} \cdot 1.08^{(T\,-\,20)} \tag{46}$$

For municipal wastewater, an approximate value of $K_{20\,C} = 0.10$ m.day$^{-1}$ was suggested. For industrial wastewaters, values of $\mathbf{K_T}$ should be determined experimentally in pilot-plant studies using the same wastewater and support particles as the ones in the real case.

Practical rules recommended by different sources for the design of biological disk reactors show large differences (Harremöes and Henze 1995; McGhee 1991); values from 5 to 60 kg BOD.$m^{-2}$.day$^{-1}$ have been reported for domestic wastewater. Rotating speed at the tip of the disks should be around 20 m.min$^{-1}$.

## Overall Model for Biofilm Transient Development

The diffusion-reaction model was established for biofilms in steady state and does not allow calculations of the transient development of the microbial layer. The value of the steady-state biofilm thickness should be known in order to apply the model to reactor design. To predict the final biofilm thickness and mass or the time needed to reach steady state, biofilm growth models are needed. A simple overall model (Melo and Vieira, 1999) is presented below giving biofilm mass as a function of time.

Let $\mathbf{m_f}$ be the mass of attached biofilm per unit surface area, at a given time **t**. The change in $m_f$ with time is the result of two competitive parallel phenomena; the production of biomass by the micro-organisms in the biofilm and the removal of attached biomass (biofilm detachment) caused by the hydrodynamic forces:

$$\frac{dm_f}{dt} = M_p - M_d \tag{47}$$

$\mathbf{M_p}$ – "biofilm production flux" (increase in biofilm mass per unit time and unit surface area, associated to the production of biomass – cells plus extracellular polymers – as the result of the microbial activity within the biofilm), kg m$^{-2}$ s$^{-1}$.

$\mathbf{M_d}$ – "biofilm detachment flux" (decrease in biofilm mass per unit time and per unit surface area, associated to the detachment of parts of the biological deposit caused by the fluid forces), kg m$^{-2}$ s$^{-1}$.

The "biofilm detachment flux" is assumed to be proportional to the amount of biomass attached to the surface, since the probability of existing "weak zones" in a thick biofilm is higher than in a thinner one. Therefore:

$$M_d = b m_f \tag{48}$$



where **b** is proportional to the hydrodynamic forces acting upon the biofilm surface and varies inversely with the cohesiveness of the biofilm (i.e., **1/b** represents the "mechanical strength" or the "resistance to detachment" of the biofilm).

As regards the "production flux" ($M_p$), the colonisation of the clean surface by bacteria coming from the fluid is an essential feature only in the first hours of biofilm formation. Experimental results have shown that the subsequent growth of the biolayer is mainly due to the activity of the micro-organisms located in the attached film and not to the transport of new bacteria from the liquid to the biofilm surface (Bott and Miller, 1983; Melo and Vieira, 1999). Due to this biological activity and to diffusional limitations, the substrate concentration may in some cases decrease down to zero within the biofilm before reaching the surface of the support. Thus, modelling of the "biofilm production flux" ($M_p$) must take into account two different situations (named below as *i* and *ii*) during the build up of the biofilm layer. A mono-species biofilm will be considered here.

### *i) Thick biofilm, partially penetrated by the substrate*

In this case, there is an "active layer" located in the outer part of the biofilm, and a "non-active" layer that occupies the inner part of the biofilm, close to the support. The latter contains microbial species with residual activity as regards the main substrate, plus polymeric substances and, possibly, other microbial species that do not use that substrate. If the biofilm is partially penetrated, then the active layer will have a constant thickness along the time, equal to the maximum depth of substrate penetration. On the contrary, the thickness of the inner layer can increase with time due, for instance, to the production of polymeric material by the micro-organisms in the active layer, which will result in an overall increase of the total amount of attached biomass.

As indicated before, let $\mu_p$ be the "biofilm specific production rate", that is, the mass of biofilm produced by the active layer per unit time and per unit mass of biofilm. Thus, at a given time t:

$$\mu_p = (\mu_p)_a \frac{(m_f)_a}{m_f} \tag{49}$$

where $(\mu_p)_a$ is the (constant) biomass specific production rate of the active zone, i.e., the mass of biofilm produced per unit time and per unit mass of active layer, and $(m_f)_a$ is the mass of active layer per unit surface area (constant with time). Therefore:

$$M_p = \mu_p m_f = (\mu_p)_a (m_f)_a = \text{constant} \tag{50}$$

### *ii) Thin biofilm, completely penetrated by the substrate*

In this case, the biofilm is biologically active (as regards the main substrate) throughout its entire depth, i.e.:

$$\mu_p = (\mu_p)_a \tag{51}$$

The mass of this active layer increases with time as the biofilm builds up, until its thickness reaches the maximum penetration depth. From this point on, case *i)* applies. It

should be stressed, however, that the number of micro-organisms in the biofilm does not increase proportionally to the biofilm mass, because the result of their activity is not only the production of new microbial cells but also of extra cellular substances (biopolymers). The latter, although not biologically active, can be a major fraction of the biofilm mass. As a consequence, the rate of biomass produced per unit mass of biofilm will decrease with time, meaning that the *specific* activity (i.e., per unit mass) of the active layer will get lower as its mass builds up. Therefore, in a completely penetrated biofilm, it does not seem unreasonable to assume that $\mu_p$ is inversely proportional to the mass of active layer at each instant of time:

$$\mu_p = (\mu_p)_a \propto \frac{1}{(m_f)_a} \tag{52}$$

where the symbol $\propto$ indicates proportionality. Since all the biofilm is active ($m_f = m_{fa}$), the following equation may be applied to case ii):

$$M_p = (\mu_p)_a (m_f)_a = \text{constant} \tag{53}$$

### *Biofilm growth equation*

In both cases, *i)* and *ii)*, $M_p$ is constant. Replacing $M_x$ in Equation (47) by Eq. (48):

$$\frac{dm_f}{dt} = M_p - bm_f \tag{54}$$

which, upon integration, results in the final equation of the overall model:

$$m_f = m_f^*[1 - \exp(-bt)] \tag{55}$$

where $m_f^* = (M_p/b)$ is the maximum mass of biofilm, at steady state. Graphically, Equation (55) represents a curve that tends to an asymptotic value of $m_f$ for t = infinite.

$M_p$ can be modelled in more detail by using the concepts and equations of heterogeneous catalysis summarised in the preceding sections (Vieira and Melo, 1999). Assuming that the biomass yield in the biofilm (that is, the mass of cells plus polymers produced per unit mass of substrate consumed by the active cells in the biofilm) is known, the substrate consumption rate, i.e., the overall apparent reaction rate ($r_A$), can be calculated according to:

$$r_A = \frac{M_p}{Y_{f/s}} \tag{56}$$

By experimentally monitoring the growth curves of biofilms produced by a given microbial culture under different operating conditions, values of the parameters $m_f^*$ and b can be correlated with variables such as substrate concentration, liquid velocity, temperature, pH, etc. This will enable the prediction of the steady-state biofilm mass for any set of operating conditions within the range of applicability of those parameters.



## CONCLUDING REMARKS

Microbial film reactors are still calculated by means of practical design criteria that are not based upon sound phenomenological equations such as the diffusion-reaction models. Those procedures have been used for many years in designing wastewater biofilm reactors but, although many processes are operating in quite acceptable conditions, some design errors and, most probably, over- design are the natural consequence of the lack of more reliable and precise calculation methods (Harremöes and Henze, 1995). Over-designed reactors imply excessive capital costs, and can also result in operational problems such as the instability of the bio-reactor due to the formation of thick biofilms that tend to detach or slough off, causing periodic poor performances. Efficient control of the microbial layer thickness has been discussed by several authors in the last decade (Capdeville *et al.*, 1992; Lazarova and Manem, 1994, 1997; Tijhuis *et al.*, 1994) as a way of achieving an enhanced reactor stability.

The major problems in biofilm modelling are not the unavailability of more or less sophisticated mathematical tools. They result from the lack of correlations able to produce values of the diffusion coefficients, the biological kinetic parameters and the biofilm thickness as a function of the operating conditions and reactor characteristics; from the lack of capacity to relate the composition and structure of the microbial film, particularly the density and spatial distribution of active cells, to the conditions under which the biofilm was formed; and from the lack of accurate information on the biomass yield in the biofilm. Obviously, all this implies a deeper knowledge of the microbial metabolism inside the biological matrix, including a better understanding of the physiological state of the micro-organisms and their kinetics in the specific micro-environment that surrounds them in a biofilm. The concurrent efforts of both engineering science (to develop semi-empirical models that relate intrinsic parameters to external operating and design variables) and biological science (to shed light on the behaviour of micro-organisms in attached biomass systems) are clearly needed.

## NOMENCLATURE

| | |
|---|---|
| $A_f$ | surface area of biofilm ($m^2$) |
| $A_s$ | cross sectional area of the filter ($m^2$) |
| $A_v$ | specific area of support per volume of reactor ($m^2.m^{-3}$) |
| b | reciprocal of the resistance to detachment |
| Bi | Biot number |
| $C_1$ | substrate concentration at reactor inlet, as soluble $BOD_5$ ($mg.L^{-1}$) |
| $C_2$ | substrate concentration at reactor outlet, as soluble $BOD_5$ ($mg.L^{-1}$) |
| $D_f$ | effective diffusion coefficient or effective diffusivity ($m^2.s^{-1}$) |
| $(D_f)_{ox}$ | effective diffusivity of the oxidant ($m^2.s^{-1}$) |
| $(D_f)_{red}$ | effective diffusivity of the reductant ($m^2.s^{-1}$) |
| $D_w$ | diffusion coefficient in water ($m^2.s^{-1}$) |
| dy | element of thickness (m) |
| J | substrate flux through the biofilm ($kg.m^{-2}.s^{-1}$) |

| | |
|---|---|
| $J_e$ | external substrate flux (kg.m$^{-2}$.s$^{-1}$) |
| $k_m$ | external mass transfer coefficient (m.s$^{-1}$) |
| $K_s$ | affinity (or saturation) constant of a suspended cell culture (kg.m$^{-3}$) |
| $k_n$ | rate constant for a reaction of order n |
| $K_T$ | observed removal rate constant at temperature T (m.s$^{-1}$) |
| $k_{0f}$ | zero order biofilm reaction rate (kg.m$^{-3}$.s$^{-1}$) |
| $k_{1f}$ | first order biofilm reaction rate (s$^{-1}$) |
| $k_{1/2ap}$ | apparent half order constant (kg$^{1/2}$.m$^{-1/2}$.s$^{-1}$) |
| $L_f$ | thickness of the microbial layer (m) |
| $M_d$ | biofilm detachment flux (kg.m$^{-2}$.s$^{-1}$) |
| $m_f$ | mass of attached biofilm per surface area (kg.m$^{-2}$.s$^{-1}$) |
| $m_f^*$ | maximum mass of biofilm at steady state |
| $M_p$ | biofilm production flux (kg.m$^{-2}$.s$^{-1}$) |
| n | order of reaction |
| $N_{ox\ red}$ | stoichiometric ratio between the oxidant and reductant |
| Q | volumetric flow rate (m$^3$.s$^{-1}$) |
| $r_A$ | reaction rate per unit area of biofilm or surface reaction rate (kg.m$^{-2}$.s$^{-1}$) |
| $r_f$ | reaction rate inside the biofilm (kg.m$^{-3}$.s$^{-1}$) |
| $(r_f)_{max}$ | maximum reaction rate per unit volume of wet biofilm (kg.m$^{-3}$.s$^{-1}$) |
| $r_p$ | radius of the bare carrier particles (m) |
| $r_{pf}$ | radius of the biofilm particles (m) |
| S | bulk substrate concentration in the solution (kg.m$^{-3}$) |
| $S_{crit}$ | critical substrate concentration (kg.m$^{-3}$) |
| $S_f$ | substrate concentration inside the biofilm (kg.m$^{-3}$) |
| $S_f'$ | dimensionless concentration inside the biofilm ($S_f/S_L$) |
| $S_L$ | substrate concentration at the biofilm-liquid interface |
| $S_{ox}$ | concentration of oxidant substance (kg.m$^{-3}$) |
| $S_{red}$ | concentration of reductant substance (kg.m$^{-3}$) |
| $S_1$ | substrate concentration at reactor inlet (kg.m$^{-3}$) |
| $S_2$ | substrate concentration at reactor outlet (kg.m$^{-3}$) |
| t | time (s) |
| T | temperature (°C) |
| $V_R$ | reactor volume |
| $(X_f)_a$ | active cell density in the biofilm (kg.m$^{-3}$) |
| $X_s$ | cell density within the biofilm (kg.m$^{-3}$) |
| y | distance inside the biofilm measured from the liquid-biofilm interface (m) |
| y' | dimensionless distance in the biofilm ($y/L_f$) |
| $Y_{f/s}$ | mass of dry biofilm (kg) |
| Z | depth of the trickling filter (m) |

Greek Symbols

| | |
|---|---|
| $\alpha$ | parameter defined by equation (33) |
| $\beta$ | degree of substrate penetration in the biofilm |
| $\varepsilon$ | reactor porosity |
| $\phi$ | Thiele modulus |



| | |
|---|---|
| $\eta_i$ | biofilm internal efficiency |
| $\eta$ | biofilm efficiency based on external substrate concentration |
| $\lambda$ | external mass transfer rate /internal coupled diffusion-reaction rate |
| $\mu$ | specific growth rate (s$^{-1}$) |
| $\mu_{max}$ | maximum specific growth rate (s$^{-1}$) |
| $\mu_p$ | specific dry biofilm production rate per unit mass of biomass (s$^{-1}$) |
| $(\mu_p)_a$ | specific dry biofilm production rate per unit mass of active biomass (s$^{-1}$) |
| $(\mu_p)_{max}$ | maximum specific biofilm production rate (s$^{-1}$) |
| $\psi$ | dimensionless observable modulus, defined in equation (34) |

## REFERENCES


Alphenaar, P.A., Perez, C. and Lettinga, G. (1993) The influence of substrate transport on porosity and methanogenic activity of anaerobic sludge granules. *Appl. Microb. Biotechnol.*, **39**, 276–280.

Azeredo, L., Visser, J. and Oliveira, R. (1999) Exopolymers in bacterial adhesion: interpretation in terms of DLVO and XDLVO theories. *Colloids and Surfaces B: Biointerfaces*, **14**, 141–148.

Balaguer, M.D., Vincent, M.T. and Paris, J.M. (1992) Anaerobic fluidized bed reactor with sepiolite as support for anaerobic treatment of vinasse. *Biotechnology Techniques*, **14**, 433–438.

Bott, T.R. (1995) Biological growth on heat exchanger surfaces. In Bott, T.R. *Fouling of Heat Exchangers*, chapt. 12, pp. 223–267, Elsevier, Amsterdam.

Brilland, J. and Engasser, J.M. (1979) Citric acid production from glucose. II Growth and excretion kinetics in a trickle flow fermenter. *Biotechnol. Bioeng.*, **XXI**, 2093–2111.

Brito, A.G. and Melo, L.F. (1997a) A simplified analysis of reaction and mass transfer in UASB and EGSB reactors. *Environmental Technology*, **18**, 35–41.

Brito, A.G. and Melo, L.F. (1997b) Granulation during the start up of a UASB reactor used in the treatment of low strength wastewaters. *Biotechnol. Letters*, **19** (4), 363–367.

Bryers, J.D. (1987) Biologically active surfaces: processes governing the formation and persistence of biofilms. *Biotechnology Progress*, **3**, 57–68.

Bryers, J. (1993) Evaluation of the effectiveness factor for a multiple species biofilm. *Biofouling*, **6**, 363–380.

Busscher, H.J., Bos, R. and van der Mei, H.C. (1995) Initial microbial adhesion is determinant for the strength of biofilm adhesion. Hypothesis. *FEMS Microbiology Letters*, **128**, 229–234.

Cabral, J.M.S. and Tramper, J. (1994) Bioreactor design. In *Applied Biocatalysis*, edited by Cabral, J.M.S., Best, D., Boross, L. and Tramper, J., pp. 333–370, Harwood Academic Publishers, Switzerland.

Çeçen, F. and Gönenç, I.E. (1994) Nitrogen removal characteristics of nitrification and denitrification filters. *Water Sci. Technol.*, **29**, 409–416.

Capdeville, B., Nguyen, K.M. and Rols, J.L. (1992) Biofilm modelling: structural, reactional and diffusional aspects. In *Biofilms Science and Technology*, edited by Melo, L.F., Bott, T.R., Fletcher, M. and Capdeville, B., pp. 251–276, Kluwer Academic Publishers, Dordrecht.

Chamberlain, A.H.L. (1992) The role of adsorbed layers in bacterial adhesion. In *Biofilms Science and Technology*, edited by Melo, L.F., Bott, T.R., Fletcher, M. and Capdeville, B., pp. 59–67, Kluwer Academic Publishers, Dordrecht.

Characklis, W.G. and Marshall, K.C. (1990) *Biofilms*, John Wiley and Sons, New York.

Christensen, B.E. and Characklis, W.G. (1990) Physical and chemical properties of biofilms. In *Biofilms*, edited by Characklis, W.G. and Marshall, K.C., pp. 93–130, John Wiley and Sons, New York.

Costerton, J.W., Cheng, K.J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M. and Marrie, T. (1987) Bacterial biofilms in nature and disease. *Ann. Rev. Microbiol.*, **41**, 435–464.

de Beer, D. and Stoodley, P. (1995) Relation between the structure of an aerobic biofilm and transport phenomena. *Water Sci. Technol.*, **32**, 11–18.

de Beer, Stoodley, P. and Lewandowski, Z. (1997) Measurement of local diffusion coefficients in biofilms by microinjection and confocal microscopy. *Biotechnol. Bioeng.*, **53**, 151–158.

Dempsey, M. (1990) Ethanol production by *Zymomonas mobilis* in a fluidised bed fermenter. In *Physiology of Immobilized Cells*, edited by de Bont, J.A.M., Visser, J., Mafiasson, B. and Tramper, J., pp. 137–148. Elsevier Science Publishers B.V., Amsterdam.

Droste, R.L. and Kennedy, K.J. (1986) Sequential substrate utilization and effectiveness factor in fixed biofilms. *Biotechnol. Bioeng.*, **XXVIII**, 1713–1720.

Ecknenfelder, W.W., Argaman, Y. and Miller, E. (1989) Process selection criteria for the biological treatment of industrial wastewaters. *Environmental Progress*, **8** (1), 40–45.

Fan, L.-S., Leyva-Ramos, R., Wisecarver, K.D. and Zehner, B.J. (1990) Diffusion of phenol through a biofilm grown on activated carbon particles in a draft tube three-phase fluidized-bed bioreactor. *Biotechnol. Bioeng.*, **35**, 279–286.

Fletcher, M. (1992a) Bacterial metabolism in biofilms. In *Biofilms-Science and Technology*, edited by Melo, L.F., Bott, T.R., Fletcher, M. and Capdeville, B., pp. 113–124. Kluwer Academic Publishers, Dordrecht.

Fletcher, M. (1992b) Metabolism and adhesion: report on workshop discussion. In *Biofilms Science and Technology*, edited by Melo, L.F., Bott, T.R., Fletcher, M. and Capdeville, B., pp. 673–675. Kluwer Academic Publishers, Dordrecht.

Flemming, H.-C. (1991) Introduction: biofilms as a particular form of microbial life. In *Biofouling and Biocorrosion in Industrial Water Systems*, edited by Flemming, H.-C. and Geesey, G.G., pp. 1–6. Springer-Verlag: Berlin, Heidelberg, New York.

Freitas dos Santos, L.M. and Livingston, A.G. (1995) Membrane attached biofilms for VOC wastewater treatment. I: novel in situ technique. *Biotechnol. Bioeng.*, **47**, 82–89.

Froment, G.F and Bischoff, K.B. (1979) *Chemical Reactor Analysis and Design*. New York: John Wiley & Sons.

Gjaltema, A. (1996) Biofilm development: growth versus detachment. PhD Thesis, Biochemical Engineering Department, Technical University of Delft, Delft, The Netherlands.

Gjaltema, A., van der Marel, N., van Loosdrecht, M.C.M. and Heijnen, J.J. (1997) Adhesion and biofilm development on suspended carriers in air-lift reactors: hydrodynamic conditions versus surface characteristics. *Biotechnology, and Bioengineering*, **55**, 880–889.

Gönenç, I.E. and Harremöes, P. (1985) Nitrification in rotating disk systems- I, Criteria for transition from oxygen to ammonia rate limitation. *Water Research*, **19**, 1119–1127.

Gönenç, I.E., Orhon, D and Baikal, B.B. (1991) Application of biofilm kinetics to anaerobic fixed bed reactors. *Water Sci. Technol.*, **23**, 1319–1326.

Grasmick, A., Elmaleh, S. and Ben Aim, R. (1980) *Water Research*, **14**, 613.

Hamdi, M. (1995) Biofilm thickness effect on the diffusion limitation in the bioprocess reaction: biofloc critical diameter significance. *Bioprocess Engineering*, **12**, 193–197.

Hamoda, M.F. and ABD-El Bary, M.F. (1987) Operating characteristics of the aerated submerged fixed-film (ASFF) bioreactor. *Water Research*, **21** (8), 939–947.

Hamoda, M.F. and Kennedy, K.J. (1987) Biomass retention and performance in anaerobic fixed film reactors treating acetic acid wastewater. *Biotechnol. Bioeng.*, **30**, 272–281.

Harremöes, P. (1978) Biofilm kinetics. In *Water Pollution Microbiology*, Vol. 2, edited by Mitchell, R., pp. 82–109, John Wiley and Sons, New York.

Harremöes, P. and Henze, M. (1995) Biofilters. In *Wastewater Treatment: Biological and Chemical Processes*, edited by Henze, M., Harremöes, P., Jansen, J. la Cour and Arvin, E., pp. 143–193, Springer-Verlag, Berlin.

Harremöes, P., Jansen, J. la Cour and Kristensen, G.H. (1980) Practical problems related to nitrogen bubble formation in fixed film reactors. *Progr. Water Technol.*, **12**(6), 253–269.



Heijnen, J.J., Mulder, A., Weltvrede, R., Hols, J., van Leeuwen, H.L.J.M. (1990) Large scale anaerobic/aerobic treatment of complex industrial wastewater using immobilized biomass in fluidized and air lift suspension reactors. *Chem. Eng. Tech.*, **13**, 145–220.

Heijnen, J.J., van Loosdrecht, M.C.M., Mulder, A. and Tijhuis L. (1992) Formation of biofilms in a biofilm air lift suspension reactor. *Water Sci. Technol.*, **26**, 647–654.

Hermanowics, S.W. (1999) Two-dimensional simulations of biofilm development: effects of external environmental conditions. *Water Sci. Technol.*, **39**, 107–114.

Jansen, J. la Cour (1982) Fixed film kinetics- kinetics of soluble substrates. *PhD Thesis*, Department of Environmental Engineering, Technical University of Denmark, Lyngby.

Jansen, J. la Cour and Kristensen, G.H. (1980) Fixed film kinetics- denitrification in fixed films. *Report 80–59*, Department of Environmental Engineering, Technical University of Denmark, Lyngby.

Karamanev, D.G. and Nikolov, L.N. (1992) Bed expansion of liquid-solid inverse fluidization. *AIChE Journal*, **38** (12), 1916–1922.

Karamanev, D.G. and Nikolov, L.N. (1996) Application of inverse fluidization in wastewater treatment: from laboratory to full-scale reactors. *Environmental Progress*, **15** (3), 194–196.

Kissel, J.C., McCarty, P.L. and Street, R.L. (1984) Numerical solution of mixed-culture biofilm. *J. Environ. Eng. Div. ASCE.*, **110**, 393–411.

Lappin Scott, H.M. and Costerton, J.W. (1995) *Microbial Films*. Cambridge University Press, Cambridge.

Lazarova, V. and Manem, J. (1994) Advances in biofilm aerobic reactors ensuring effective biofilm activity control. *Water Sci. Technol.*, **29**, 319–327.

Lazarova, V. and Manem, J. (1997) An innovative process for waste water treatment: the circulating floating bed reactor. *Water Sci. Technol.*, **34**, 89–99.

Lettinga, G. and Hulshoff-Pol, L.W. (1992) UASB-process design for various types os wastewaters. In *Design of anaerobic processes for the treatment of industrial and municipal wastes*, edited by Malina, J. and Pohland, F., pp. 119–145. Technomic Pub., Lancaster.

Margel, S., Vogel, E.A., Firment, L., Watt, T., Haynie, S. and Sogah, D.Y. (1993) Peptide, protein and cellular interactions with self-assembled monolayer model surfaces. *J Biomed. Mater. Res.*, **27**, 1463–1476.

Marshall, K.C., Stout, R. and Mitchell, R. (1971) Mechanisms of the initial events in the sorption of marine bacteria to surfaces. *J. Gen. Microbiol.*, **68**, 337–348.

McGhee, T.J. (1991) *Water Supply and Sewerage*, 6th Edition, McGraw-Hill, Inc., Singapore.

Melo, L.F., Bott, T.R., Fletcher, M. and Capdeville, B. (1992) *Biofilms- Science and Technology*, Kluwer Academic Publishers, Dordrecht.

Melo, L.F. and Vieira, M.J. (1999) Physical stability and biological activity of biofilms under turbulent flow and low substrate concentration. *Bioprocessing Engineering*, **20**, 363–368.

Mendez, R., Pan, M. and Lema, J.M. (1989) Effect of C/N/P ratio on the performance of a DSFFR working at low organic loading rates. *Water Sci. Technol.*, **21**, 1673–1676.

Messing, R.A. and Opperman, R.A. (1979a) Pore dimensions for accumulating biomass. I. Microbes that reproduce by fissing or by budding. *Biotechnology and Bioengineering*, **XXI**, 49–58.

Messing, R.A. and Opperman, R.A. (1979b) Pore dimensions for accumulating biomass. II. Microbes that form spores and exhibit mycelial growth. *Biotechnology and Bioengineering*, **XXI**, 59–67.

Metcalf & Eddy, Inc. (1987) *Wastewater Engineering: Treatment and Disposal*, 2nd ed., Tata McGraw-Hill Publishing Company, New Dehli.

Miyahara, T. and Noike, T. (1995) Behavior of suspended solids and anaerobic bacteria in an anaerobic fixed bed reactor. *Water Sci. Technol.*, **30**, 75–86.

Mott, H.E.C. and Bott, T.R. (1991) The Adhesion of Biofilms to Selected Materials of Construction for Heat Exchangers. *9th Intern. Heat Transf. Conf.*, Jerusalem, **5**, 21–26.

Nguyen, V.T. and Shieh, W.K. (1995) Evaluation of intrinsic and inhibition kinetics in biological fluidized bed reactors. *Water Research*, **29** (11), 2520–2524.

Nikolov, L.N. and Karamanev, D.G. (1990) The inverse fluidized bed reactor; a new laboratory scale apparatus for biofilm research. *J. Ferment. Bioeng.*, **69** (4), 265–267.

Nikov, I. and Karamanev, D. (1991) Liquid-solid mass transfer in inverse fluidized bed. *AIChE Journal*, **37** (5), 781–784.

Nilsson, B.K. and Karlsson, H.T. (1989) Diffusion rates in a dense matrix of methane-producing microorganisms. *J. Chem. Technol. Biotechnol.*, **44**, 255–260.

Noguera, D.R., Pizarro, G., Stahl, D.A. and Ritmann, B.E. (1999) Simulation of multispecies biofilm development in three dimensions. *Water Sci. Techn.*, **39**, 123–130.

Nogueira, R., Lazarova, V., Manem, J. and Melo, L.F. (1998) Influence of dissolved oxygen on the nitrification kinetics in a circulating bed biofilm reactor. *Bioprocess Engineering*, **19**, 441–449.

Oliveira, R. (1992) Physico-chemical aspects of adhesion. In *Biofilms– Science and Technology* edited by Melo L.F., Bott T.R., Fletcher M. and Capdeville B., pp. 45–58. Dordrecht, Boston, London: Kluwer Academic Publishers.

Onuma, M. and Omura, T. (1982) Mass transfer characteristics within microbial systems. *Water Sci. Technol.*, **14**, 553–568.

Pederson, A.R. and Arvin, E. (1996) Toluene removal in a biofilm reactor from waste gas treatment. *3rd International IAWQ Special Conference on Biofilm Systems*, Copenhagen.

Pederson, A.R. and Arvin, E. (1999) The function of a toluene-degrading bacterial community in a waste gas trickling filter. *Water Sci. Technol.*, **39**, 131–137.

Peixoto, J. and Mota, M. (1998) Biodegradation of Toluene in a Trickling Filter. *Bioprocess Engineering*, **19**(5), 393–397.

Pereira, M.A., Alves, M.M., Azeredo, J., Mota, M. and Oliveira, R. (1999) Physico-chemical properties of porous microcarriers in relation with the adhesion of an anaerobic consortium. *Journal of Industrial Microbiology*, in press.

Petrozzi, S., Kut, O.M. and Dunn, I.J. (1993) Protection of biofilms against toxic shocks by the adsorption and desorption capacity of carriers in anaerobic fluidized bed reactors. *Bioprocess Engineering*, **9**, 47–59.

Picioreanu, C., van Loosdrecht, M.C.M. and Heijnen, J.J. (1999) Discrete- differential modelling of biofilm structure. *Water Sci. Techn.*, **39**, 115–122.

Prime, K.L. and Whitside, G.M. (1993) Adsorption of proteins onto surfaces containing end-attached oligotethylene oxide: a model system using self- assembled monolayers. *Journal of American Chemical Society*, **115**, 10714–10721.

Reichert, P. AQUASIM-A tool for simulation and data analysis of aquatic systems. *Water Sci. Technol.*, **30**, 21–30.

Reichert, P. Design techniques of a computer program for the identification of processes and the simulation of water quality in aquatic systems. *Environmental Software*, **10** (3), 199–210.

Ritmann, B.E., Pettis, M., Reeves, H.W. and Stahl, D.A. (1999) How biofilm clusters affect substrate flux and ecological selection. *Water Sci. Techn.*, **39**, 99–105.

Rusten, B., Kolkinn, Odegaard, H. (1997) Moving bed biofilm reactors and chemical precipitation for high efficiency treatment of wastewater from small communities. *Water Sci. Technol.*, **35**, 71–79.

San, H.A., Tanik, A. and Orhon, D. (1993) Micro-scale modelling of substrate removal kinetics in multicomponent fixed-film systems. *J. Chem. Tech. Biotechnol.*, **58**, 39–48.

Schlegel, H.G. (1985) *Allgemeine Mikrobiologie*. Thieme, Stuttgart.

Shimp, R.J. and Pfaender, F.K. (1982) Effect of surface area and flow rate on marine bacterial growth on activated carbon columns. *Applied and Environmental Microbiology*, **44**, 2, 471–476.

Sousa, M., Azeredo, J., Feijó, J. and Oliveira, R. (1997) Polymeric supports for the adhesion of a consortium of autotrophic nitrifying bacteria. *Biotechnology Techniques*, **11**, 751–754.



Stoodley, P. de Beer, D., Lewandowski, Z. (1994) Liquid flow in biofilm systems. *Appl. Environm. Microbiol.* 2711–2716.

Suzuki, Y., Miyahara, S. and Takeishi, K. (1993) Oxygen supply method using gas permeable film for wastewater treatment. *Water Sci. Technol.*, **28**, 243–250.

Tavares, C.R.G., Sant'Anna, J.R., G.L. and Capdeville, B. (1995) The effect of air superficial velocity on biofilm accumulation in a three phase fluidized- bed reactor. *Water Research*, **29** (10), 2293–2298.

Taylor, G.T., Zheng, D., Lee, M., Troy, P.J., Gyanamalt, G. and Sharma, S.K. (1997) Influence of surface properties on accumulation of conditioning films and marine bacteria on substrata exposed to oligotrophic waters. *Biofouling*, **11**, 31–57.

Teixeira, P. and Oliveira, R. (1998) The importance of surface properties in the selection of supports for nitrification in airlift bioreactors. *Bioprocess Engineering*, **19**, 143–147.

Teixeira, P. and Oliveira, R. (1999) Influence of surface characteristics on the adhesion of Alcaligenes denitrificans to polymeric substrates. *J. of Adhesion Science and Technology*, **13**, 1287–1294.

Tijhuis, L., Huisman, J.L., Hekkelman, H.D., van Loosdrecht, M.C.M. and Heijnen, J.J. (1995) Formation of nitrifying biofilms on small suspended particles in airlift reactors. *Biotechnol. Bioeng.*, **47**, 585–595.

Tijhuis, L., van Loosdrecht, M.C.M. and Heijnen, J.J. (1994) Formation and growth of heterotrophic aerobic biofilms on small suspended particles in airlift reactors. *Biotechnol. Bioeng.*, **44**, 595–608.

Trulear, M.G. (1980) Dynamics of biofilm processes in an annular reactor. *MSc Thesis*, Rice University, Texas, USA.

Trulear, M.G. (1983) Cellular reproduction and extracellular polymer formation in the development of biofilms. *PhD Thesis*, Montana State University, Bozeman, USA.

Ulonska, A., Deckwer, W.D. and Hecht, V. (1994) Degradation of quinoline by immobilized *Comamonas acidovorans* in a three-phase airlift reactor. *Biotechnol. Bioeng.*, **46**, 80–87.

van Ede, C.J., Bollen, A.M. and Beenackers, A.A.C.M. (1993) Analytical effectiveness factor calculations concerning product-inhibited fermentations associated with biofilm growth and maintenance. *Can. J. Chem. Eng.*, **71**, 901–910.

van Loosdrecht, M.C.M., Eikelboom, D., Gjaltema, A., Mulder, A., Tijhuis, L. and Heijnen, J.J. (1995) Biofilm structures. *Water Sci. Technol.*, **32**, 35–43.

van Loosdrecht, M.C.M., Lyklema, J., Norde, W. and Zhender, A.B (1988) Bacterial adhesion: a physico chemical approach. *J. Gen. Microbiol.*, **68**, 337–348.

van Oss, C.J. (1995) Hydrophobicity of biosurfaces – origin, quantitative determination and interaction energies. *Colloids and Surfaces B. Biointerfaces*, **5**, 91–110.

van Oss, C. J. (1997) Hydrophobicity and hydrophilicity of biosurfaces. *Current Opinion in Colloid Interface Science*, **2**, 503–512.

Veiga, M.C., Mendez, R. and Lema, J.M. (1992) Development and stability of biofilms in bioreactors. In *Biofilms-Science and Technology*, edited by Melo, L.F., Bott, T.R., Fletcher, M. and Capdeville, B., pp. 421–434. Kluwer Academic Publishers, Dordrecht.

Vieira, M.J. and Melo, L.F. (1995) Effect of Clay particles on the behaviour of biofilms formed by Pseudomonas fluorescens. *Wat. Sci. Tech.*, **32**(8), 45–52.

Vieira, M.J. and Melo, L.F. (1999) Intrinsic kinetics of biofilms formed under turbulent flow and low substrate concentration. *Bioprocessing Engineering*, **20**, 369–375.

Vieira, M.J., Melo, L.F. and Pinheiro, M.M. (1993) Biofilm Formation: Hydrodynamic Effects on Internal Diffusion and Structure. *Biofouling*, **7**, 67–80.

Vieira, M.J., Oliveira, R., Melo, L.F., Pinheiro, M.M. and Martins, V. (1993) Effect of Metallic Ions on the Adhesion of Biofilms Formed by *Pseudomonas fluorescens*. *Colloids and Surfaces B: Biointerfaces*, **1**, pp. 119–124.

Wanner, O. (1995) New experimental findings and biofilm modelling concepts. *Water Sci. Technol.*, **32**, 133–140.

Wanner, O. and Gujer, W. (1986) A multispecies biofilm model. *Biotechnol. Bioeng.*, **XXVIII**, 314–328.
Wanner, O. and Reichert, P. (1996) Mathematical modeling of mixed-culture biofilms. *Biotechnol. Bioeng.*, **49**, 172–184.
Watanabe, Y. and Ishiguro, M. (1978) Denitrification kinetics in a submerged rotating biological disk unit. *Progr. Water Technol.*, **5**, 187–195.
Wiesmann, U. (1994) Biological nitrogen removal from wastewater. *Advances in Biochemical Engineering/Biotechnology*, **51**, 113–154.
Wilderer, P. (1995) Technology of membrane biofilm reactors operated under periodically changing process conditions. *Water Sci. Technol.*, **31**, 173–183.
Willaert, R.G., Baron, G.V. and de Backer, L. (1996) *Immobilised Living Cell Systems: Modelling and Experimental Methods*, Chapter 3, John Wiley and Sons, Chichester, England.
Williamson, K. nd McCarty, P.L. (1976) A model of substrate utilization by bacterial biofilms. *J. Water Poll. Contr. Fed.*, **48**, 9–24.
Wiencek, K.M. and Fletcher, M. (1997) Effects of substratum wettability and molecular topography on initial adhesion of bacteria to chemically defined substrata. *Biofouling*, **11**, 293–311.
Zobell, C.E. and Allen, E.C. (1935) The significance of marine bacteria in the fouling of submerged surfaces. *J. Bacteriol.*, **20**, 239–251.