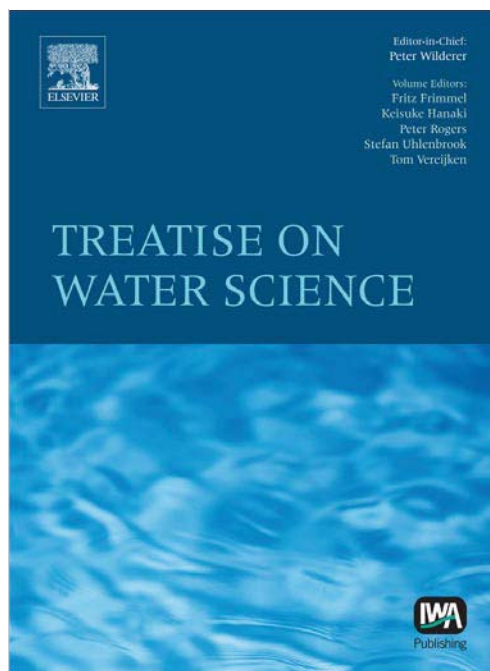


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4.15 Biofilms in Water and Wastewater Treatment

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4.15.1 Introduction

Fundamental principles describing biofilms exist as a result of focused research. The use of reactors for the treatment of municipal wastewater is a common application of biofilms. Applied research exists that provides a basis for the mechanistic understanding of biofilm reactors. The empirical information derived from such applied research has been used to develop design criteria for biofilm reactors and remains the basis for biofilm reactor design despite the emergence of

mathematical models as reliable tools for research and practice. Unfortunately, little information exists to bridge the gap between our current understanding of biofilm fundamentals and reactor-scale empirical information. Therefore, there is a clear dichotomy in literature: micro- (biofilm) and macro- (reactor) scales. This chapter highlights the division. Part I is dedicated to basic research and communicating the state of the art with respect to understanding biofilms. Part II is practice oriented and describes the use of biofilms for the sanitation of municipal wastewater. A basis for addressing this

disconnection is presented by (1) describing the fundamental biofilm principles that can be uniformly applied to biofilms in several disciplines extending from medicine to environmental biotechnology and (2) describing a fundamental-based approach in order to understand and apply biofilms in reactors. The use of mathematical biofilm models is common in both research and practice, but only a cursory presentation of their mathematical description is presented here. Finally, Part III gives examples of undesirable biofilms in water and wastewater industries and describes the attempts to mitigate their effects. Metabolic reactions mediated by microorganisms residing in biofilms promote the biodeterioration of materials, including metals, concrete, and plastics. It is estimated that microbially influenced corrosion (MIC) alone costs the US economy billions of dollars every year.

4.15.2 Part I: Biofilm Fundamentals

4.15.2.1 Biofilm Formation and Propagation

Biofilm formation is a process that consists of a sequence of steps. It begins with the adsorption of macromolecules (e.g., proteins, polysaccharides, nucleic acids, and humic acids) and smaller molecules (e.g., fatty acids, lipids, and pollutants such as polyaromatic hydrocarbons and polychlorinated biphenyls) onto surfaces. These adsorbed molecules form conditioning films which may have multiple effects, such as altering the physicochemical characteristics of the surface, acting as a concentrated nutrient source for microorganisms, suppressing or enhancing the release of toxic metal ions from the surface, detoxifying the bulk solution through the adsorption of inhibitory substances, supplying the nutrients and trace elements required for a biofilm, and triggering biofilm sloughing. Once the surface is prepared, cells begin to attach. The initial stages of biofilm formation are well documented, mostly because acquiring images of microorganisms at this stage of biofilm formation is relatively easy.

The adherence of bacteria to a surface is followed by the production of slimy adhesive substances, extracellular polymeric substances (EPS). These are predominantly made of polysaccharides and proteins. Although the association of EPS with attached bacteria has been well documented in the literature, there is little evidence to suggest that EPS participates in the initial stages of adhesion. However, EPS definitely assists the formation of mature biofilms by forming a slimy substance called the biofilm matrix. **Figure 1** shows the steps in the formation of mature biofilms.

The existence of these three phases of biofilm development, as depicted in **Figure 1**, is generally acknowledged, although the terminology may vary among authors. For example, **Notermans et al. (1991)** called these phases: (1) adsorption, (2) consolidation, and (3) colonization.

Once a mature biofilm has been established on a surface, it actively propagates and eventually covers the entire surface. The mechanisms of propagation in mature biofilms are more complex than those of initial attachment, and several of these mechanisms of biofilm propagation are depicted in **Figure 2**.

Although biofilms can be seen with an unaided eye, imaging their structure, microbial community structure, and

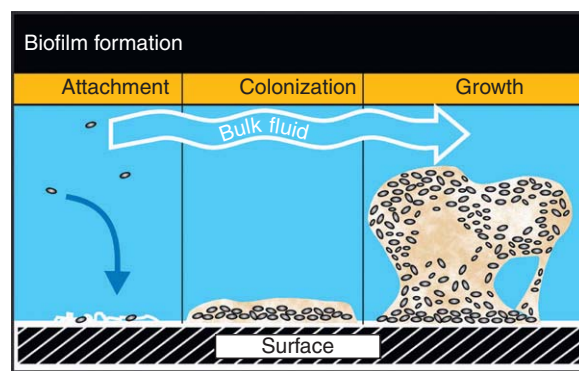


Figure 1 Steps in biofilm formation. © 1995 Center for Biofilm Engineering, MSU-BOZEMAN.

distribution of EPS requires the use of several types of microscopy combined with various probes, such as fluorescent *in situ* hybridization (FISH) probes and fluorescent proteins (FPs) used as reporter genes. The favorite types of microscopy among biofilm researchers are those that allow the examination of living and fully hydrated biofilms. In addition, sophisticated image acquisition devices are often needed that can selectively stimulate and image various probes when more than one type of multicolored probe is used simultaneously. Using these techniques in conjunction with a suitable microscopy, biofilm researchers can detect the presence of the selected physiological groups of microorganisms in the biofilm, their position in the biofilm with respect to other microorganisms and surface, and even their physiological state – dead, injured, or alive. The *in vitro* FISH techniques, popular in medical diagnostics, require that DNA or RNA be isolated from the sample and separated on a gel, and that the fluorescent probes then be added to the sample. The *in situ* variety of the hybridization technique, which is extensively used in biofilm research, does not require isolating DNA or RNA prior to the use of the probes; instead, the probes are hybridized to the respective nucleotide sequences inside the cells (**Biesterfeld et al., 2001; Delong et al., 1999; Ito et al., 2002; Jang et al., 2005; Manz et al., 1999**). *In situ* hybridization uses fluorescence-labeled complementary DNA or RNA probes, often derived from fragments of DNA that have been isolated, purified, and amplified. In microbial ecology, ribosomal RNA in bacterial cells is targeted by fluorescence-labeled oligonucleotide probes. **Figure 3** shows an image of manganese-oxidizing bacteria (MOB) *Leptotrix discophora* stained with a FISH probe (green) and counterstained with propidium iodide (red). Propidium iodide is a general stain which is quite popular with biofilm researchers (**GrayMerod et al., 2005; McNamara et al., 2003; Nanchaiah et al., 2005**).

In mature biofilms, microorganisms are imbedded in the layer of EPS. **Figure 4** shows an image of a mature biofilm acquired using scanning electron microscopy (SEM). It shows microbes embedded in a matrix of EPS attached to a surface, although the EPS in this image were reduced to an entangled network of dry strands because the sample had to be dehydrated before the biofilm was imaged using electron microscopy.

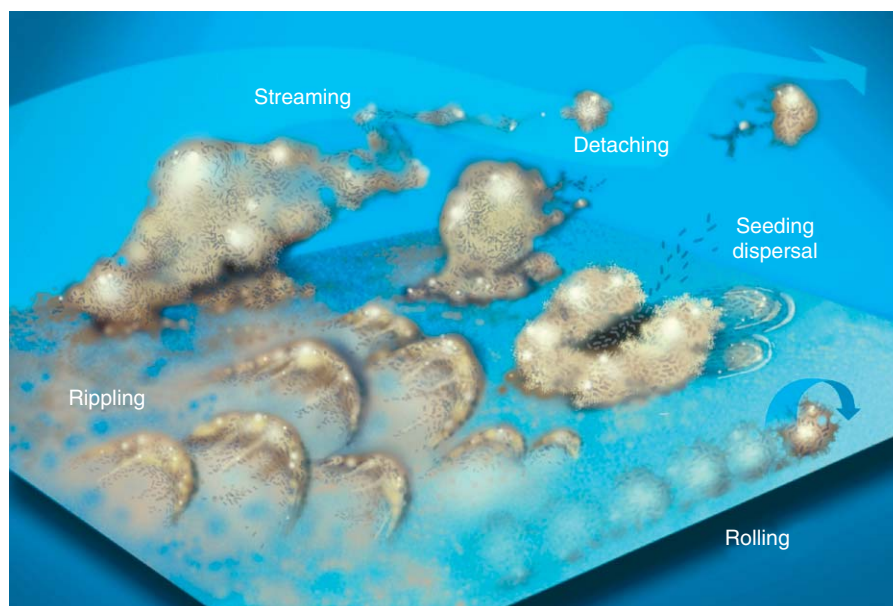


Figure 2 Mechanisms of biofilm propagation (MSU-CBE, P.Dirx).

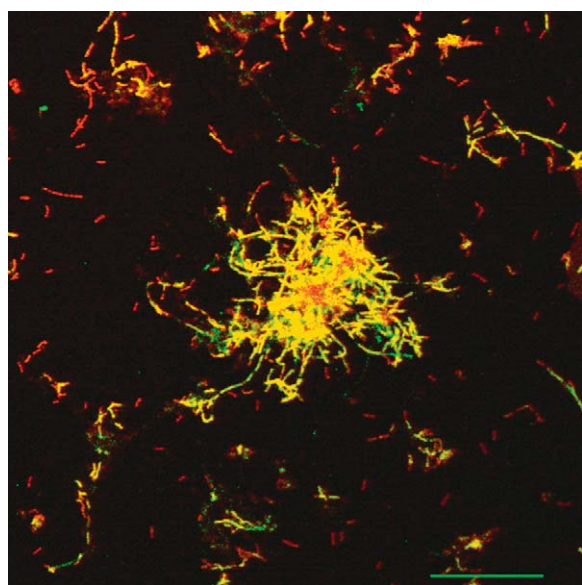


Figure 3 *L. discophora* stained with FISH probes and counterstained with propidium iodide. Red indicates cells that were stained with propidium iodide, and green indicates cells that react positively to the fluorescent FISH probe. Yellow indicates green and red overlay. The scale bar is 20 μm (Campbell, 2003).

4.15.2.2 The Concepts of Biofilms and Biofilm Processes

It is difficult to offer precise definitions of biofilms and biofilm processes that will satisfy everyone who is interested in studying biofilms and biofilm-based technologies. Several currently used definitions have roots in historical approaches to biofilm studies. These approaches initially referred to biofilms as physical objects – microbial deposits on surfaces – but later expanded the concept to consider biofilms as a mode of

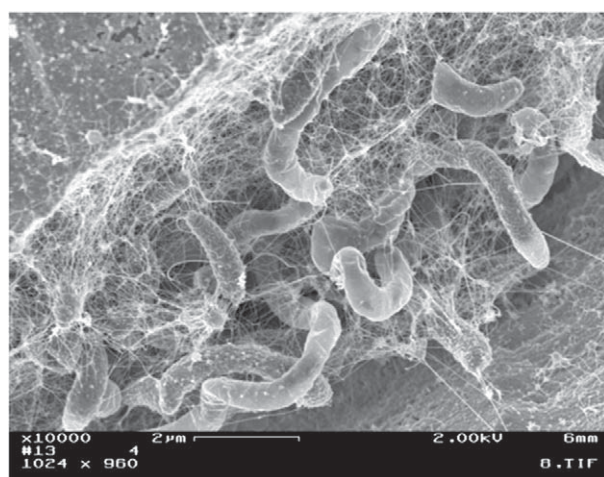


Figure 4 SEM image of a biofilm of *Desulfovibrio desulfuricans* G20 embedded in EPS (Beyenal *et al.*, 2004).

microbial growth, an alternative to microbial growth in suspension. Life scientists often emphasize the definitions that refer to biofilms as a mode of microbial growth. Engineers often find that the definitions that refer to aggregates of microorganisms which are embedded in a matrix composed of microbially excreted EPS and attached to a surface are useful for their applications. Here, we will refer to biofilms as microorganisms and microbial deposits attached to surfaces. We will use the term biofilm processes in reference to all physical, chemical, and biological processes in biofilm systems that affect, or are affected by, the rate of biofilm deposition or the microbial activity in biofilms. Biofilm processes are carried out in biofilm reactors. Colloquially, the terms biofilm reactors and biofilm systems are used interchangeably. However, biofilm systems exist with or without human intervention, while biofilm reactors are produced by our actions.

When we promote or suppress a biofilm process in a biofilm system, or even when we quantify a biofilm process in a biofilm system without affecting its rate, the biofilm system becomes a biofilm reactor. For example, wetlands can be natural or constructed. However, even natural wetlands become biofilm reactors once we start monitoring biofilm processes in them.

We will use the term biofilm system to refer to a group of compartments and their components determining biofilm structure and activity.

Biofilm systems are composed of four compartments:

- the surface to which the microorganisms are attached;
- the biofilm (the microorganisms and the matrix);
- the solution of nutrients; and
- the gas phase (if present).

Each compartment of a biofilm system can have a number of components. The exact number of components in each compartment may vary, depending on the needs of a particular description. For example, for some analyses it may be convenient to identify two components of the biofilm: (1) the EPS (matrix) and (2) the microorganisms. In another study, it may be convenient to identify three components of the biofilm: (1) the EPS, (2) the microorganisms, and (3) the particular matter trapped in the matrix. Similarly, in some studies it may be convenient to single out two components of the surface – (1) the bulk material and (2) the biomineralized deposits – or, if MIC is studied, it may be convenient to describe the surface by identifying three components: (1) the metal substratum, (2) the corrosion products, and (3) the biomineralized deposits on the surface. The needs of the specific study or analysis dictate the number of components identified in each compartment of the biofilm system. Biofilm studies can be characterized as studies of the relations among the compartments, the properties of one or more compartments, or one or more components of a compartment.

Among many factors that are used to quantify biofilm processes, biofilm activity is most often used. Biofilm reactors are often designed and operated to optimize biofilm activity, as are the biofilm reactors used for wastewater treatment discussed later in the text. Typically, biofilm activity is identified with the rate of utilization of the growth-limiting nutrient. In some instances, however, rates other than the rate of substrate utilization or biofilm accumulation are better descriptors of the system dynamics. For example, in studies of MIC, the rate of anodic dissolution of the metal affected by the process may be a more useful descriptor of biofilm activity than the rate at which the growth-limiting substrate is utilized. The choice of the process for evaluating biofilm activity is dictated by the nature of the study, and sometimes by analytical convenience. Monitoring the rate of biofilm accumulation is important in many applications, whether we want to enhance or inhibit the growth of biofilms. The methods employed include optical microscopy (Bakke and Olsson, 1986; Bakke *et al.*, 2001), measuring light intensity reflected from microbially colonized surfaces (Bremer and Geesey, 1991; Cloete and Maluleke, 2005), collecting and analyzing images of biofilm depositions (Milferstedt *et al.*, 2006; Pons *et al.*, 2009), surface sensors based on piezoelectric devices (Nivens *et al.*, 1993; Pereira

et al., 2008), and electrochemical sensors in which stainless steel electrodes change their electrochemical behavior as a result of biofilm deposition (Licina *et al.*, 1992; Borenstein and Licina, 1994).

4.15.2.2.1 Quantifying microbial activity, hydrodynamics, and mass transport in biofilms

Microbial activity (biofilm activity), hydrodynamics, and mass transport in biofilms are difficult to discuss separately as they affect each other in many ways. Biofilm activity at the microscale is quantified as the flux, from the bulk solution to the biofilm surface, of the substance selected for evaluating biofilm activity. Since fluxes at the microscale are quantified locally, rather than averaged over the entire surface area as is done when biofilm activity is evaluated at the macroscale, the concentration profiles of the selected substance must be measured with microsensors to assure adequate spatial resolution. The idealized model of hydrodynamics and mass transfer in biofilms shown in Figure 5 is a good starting point for a discussion of biofilm activity at the microscale.

In this model the overall flow velocity in the main stream is considered to be the average flow velocity, C_b . This decreases toward the surface of the biofilm, as required by hydrodynamics, and reaches concentration C_s at the biofilm surface. The layer of liquid just above the biofilm surface, where the flow velocity decreases as a result of proximity to the surface, is the hydrodynamic boundary layer, and it is denoted by ϕ . As the flow velocity decreases toward the biofilm surface, the mechanism of mass transport changes from being dominated by convection at locations away from the biofilm, where the flow velocity is high, to being dominated by diffusion at locations near the biofilm surface, where the flow velocity is low. As the microorganisms in the biofilm consume nutrients at the rate at which they are delivered and the mass transport becomes less efficient near the biofilm surface, the nutrient concentration decreases near the surface, forming a nutrient concentration profile within the hydrodynamic boundary layer. The layer of liquid above the biofilm surface where the nutrient concentration decreases is the mass transport boundary layer, and it is denoted by L_L and R_L is the mass transfer resistance external to the biofilm.

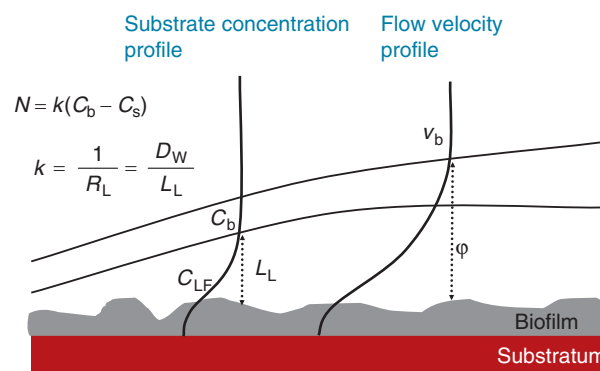


Figure 5 Profiles of flow velocity and growth-limiting nutrient concentration near the surface of an idealized biofilm.

4.15.2.2.2 Biofilm heterogeneity and its effects

The term biofilm heterogeneity refers to the extent of the nonuniform distribution of any selected constituent in any of the compartments of the biofilm system, such as the distribution of the biomass, selected nutrients, selected products of microbial metabolism, or selected groups of microorganisms. Since there are many choices for the constituents selected to evaluate biofilm heterogeneity, the term biofilm heterogeneity is usually combined with an adjective referring to the selected constituent, such as structural heterogeneity, chemical heterogeneity, or physiological heterogeneity. The term biofilm heterogeneity was initially used exclusively to refer to the nonuniform distribution of the biomass in a biofilm. As time has passed, more types of heterogeneity have been described, and the term biofilm heterogeneity is not self-explanatory anymore: the specific feature of the biofilm with respect to which the heterogeneity is quantified needs to be specified. Quantifying biofilm heterogeneity is equivalent to quantifying the extent of nonuniform distributions, such as the distribution of biomass in the biofilm. Several tools from the statistical toolbox are available for evaluating the extent of nonuniform distribution; the most popular is the standard deviation. The procedure for estimating the heterogeneity of a selected constituent of a biofilm is identical with the procedure for evaluating the standard deviation of a set of experimental data with one important difference: the deviations from the average are not due to errors in measurement but reflect a feature of the biofilm – heterogeneity.

One of the most profound effects of biofilm heterogeneity is that microscale measurements in biofilms deliver different results at different locations. This is an obvious concern as most models referring to microbial growth and activity have been developed for well-mixed reactors, in which the result of a measurement does not depend on the location. **Figure 6** shows this effect: three very different profiles of carbon dioxide concentration were measured at three locations in a biofilm.

Because of the biofilm heterogeneity, it is impossible to determine a representative location to make the local measurements of biofilm activity that are used to validate models of biofilm processes. To include the effects of biofilm heterogeneity in mathematical models of biofilm processes, the extent of these effects – the spatial variability of the features measured in biofilms – needs to be evaluated experimentally using tools that can take measurements in biofilms to a high spatial resolution. Such tools are routinely used in biofilm research in the form of microelectrodes and various types of microscopy, often enhanced with fluorescent probes. These types of measurements deliver information about selected locations in the biofilm, and their results are referred to as local properties. The most common such measurements are local biofilm activity, local mass transfer coefficient, local diffusivity, and local flow velocity. The definition of the local mass transport coefficient is derived from the measurement procedure: the coefficient of the mass transport of an electroactive species to the tip of an electrically polarized microelectrode. The local mass transport coefficient is measured using an amperometric microelectrode without a membrane operated at the limiting current condition (mass-transfer-limited). Local diffusivity is computed from these

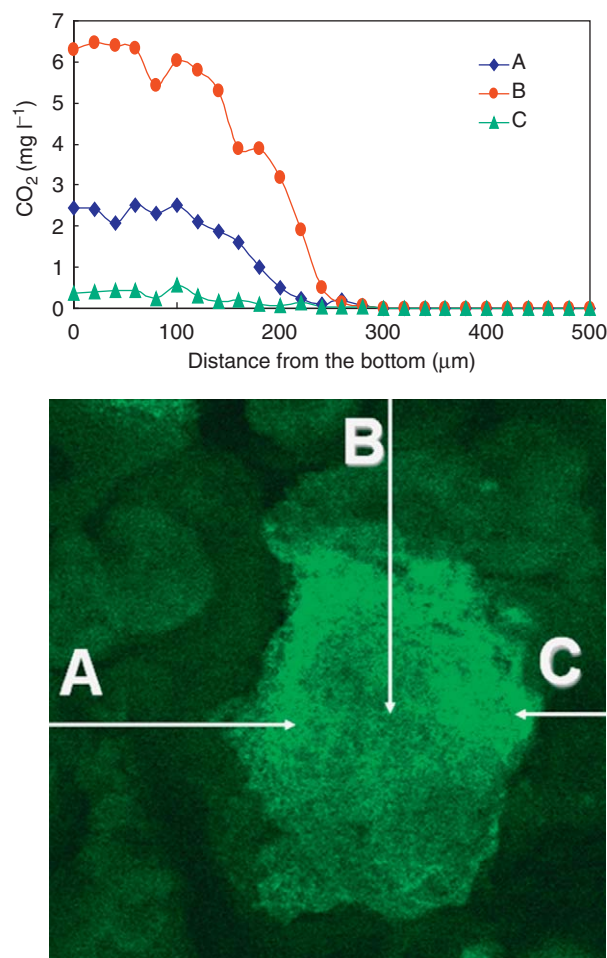


Figure 6 Carbon dioxide concentration profiles measured perpendicularly to the bottom (substratum) at three locations in a biofilm microcolony.

measurements by calibrating local mass transport microelectrodes in gels of known diffusivities (Beyenal *et al.*, 1998).

4.15.2.2.3 Biofilm activity

Biofilm activity in a biofilm reactor can be evaluated from the mass balance on the growth-limiting nutrient in the reactor:

$$\text{Biofilm activity} = \frac{(C_{\text{Influent}} - C_{\text{Effluent}}) \times Q}{A} \quad (1)$$

where C is the concentration of the growth-limiting nutrient (kg m^{-3}), Q the volumetric flow rate in the reactor ($\text{m}^3 \text{s}^{-1}$), and A the surface area covered by the biofilm (m^2). Therefore, biofilm activity at the scale of the reactor is the average flux of nutrients across the biofilm surface, which corresponds to the approach delineated in Equations (12) and (13) used in graphical procedure to evaluate pilot-plant observations.

Average biofilm activity in a reactor is a useful descriptor of reactor performance. However, when the underlying biofilm processes are to be studied, an image of local biofilm activity is often required. This information can be extracted from growth-limiting substrate concentration profiles measured at

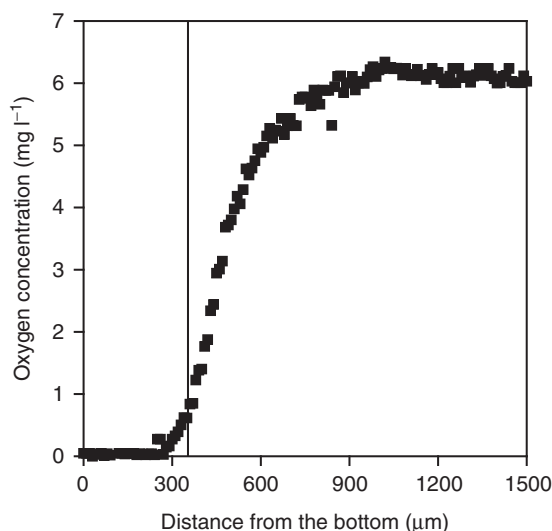


Figure 7 Oxygen concentration profile. The vertical line marks the approximate position of the biofilm surface (Rasmussen and Lewandowski, 1998).

selected locations in the biofilm, as shown in **Figure 7**. The results from the two scales of observation – (1) the local biofilm activity evaluated from the concentration profiles and (2) the average biofilm activity evaluated from the mass balances around the reactor – provide different types of information. The measurements at the microscale deliver information that cannot be extracted from the measurements at the macroscale. For some biofilm processes, it is important to quantify the extreme values of biofilm activity because the locations in the biofilm where these extreme values occur exhibit extreme properties. For example, in studying MIC, which causes highly localized damage to metal surfaces, it is important to evaluate the extreme values of biofilm activity because the extreme, and highly localized, microbial activity in biofilms determines the extent of microbial corrosion. The average biofilm activity estimated from measurements at the macroscale cannot deliver this information.

4.15.2.2.4 Quantifying local biofilm activity and mass transport in biofilms from microscale measurements

The profiles of flow velocity and growth-limiting substrate concentration shown in the conceptual image depicted in **Figure 5** can be measured experimentally. Their interpretation leads to a better understanding of the processes occurring in biofilms. **Figure 7** shows an oxygen concentration profile measured in a biofilm using an oxygen microelectrode.

Nutrient concentration profiles, such as the one shown in **Figure 7**, are composed of two parts, the part above and the part below the biofilm surface. Different factors shape these parts of the profile: the shape of the profile above the surface is dominated by bulk liquid hydrodynamics, whereas the shape of the profile below the surface is dominated by microbial respiration in the biofilm. These two parts are described by different equations but are connected at the biofilm surface by the requirement of oxygen flux continuity. The position of the

surface on the oxygen profile coincides with the inflection point of the nutrient concentration profile. It is not easy to determine the exact position of the surface, though. We use a simplified procedure, explained later in **Figure 16**, to find the approximate position of biofilm surface on concentration profiles measured with microelectrodes. One use of such data is to estimate the local biofilm activity in terms of the flux of the growth-limiting nutrient at the location where the profile was measured. The flux of the nutrient across the biofilm surface, J_{LF} at the location of the measurement is computed as the product of the slope of the concentration profile at the biofilm bulk solution interface by the diffusivity coefficient in water of the substance whose concentration was measured:

$$J_{LF} = D_w \left(\frac{dC}{dx} \right)_{(x-x_s)=0} \quad (2)$$

where D_w is the diffusivity in water of the substance selected for the evaluation of biofilm activity, usually the growth-limiting nutrient ($m^2 s^{-1}$). Diffusivity of this substance in the biofilm is not constant, but instead it varies with distance, as explained below.

Early mathematical descriptions of biofilm activity and the shape of the concentration profile within the biofilm were based on the conceptual model of so-called uniform biofilms, depicting biomass uniformly distributed in the space occupied by the biofilm (Atkinson and Davies, 1974; Williamson and McCarty, 1976). Formally, these early mathematical models of microbial activity in biofilms imitated the models of microbial activity in suspension, with the addition of mass transport resistance. They quantified the equilibrium between the rate of utilization of the growth-limiting nutrient and the rate of mass transport in one dimension, toward the surface:

$$\left(\frac{\partial C}{\partial t} \right)_f = D_f \left(\frac{\partial^2 C}{\partial x^2} \right)_f - \frac{\mu_{\max} X_f}{Y_{x/s}} \left(\frac{C}{K_s + C} \right), \quad 0 \leq x \leq x_s \quad (3)$$

At steady state, this equation delivers

$$D_f \frac{d^2 C}{dx^2} = \frac{\mu_{\max} C X_f}{Y_{x/s} (K_s + C)} \quad (4)$$

Two boundary conditions were generally used to specify the concentrations of oxygen at the bottom and surface of the biofilm:

$$\left(\frac{dC}{dx} \right)_{(x=0)} = 0; \quad C_{(x=x_s)} = C_s, \quad t \geq 0 \quad (5)$$

where D_f is the averaged effective diffusivity of growth-limiting nutrient in the biofilm ($m^2 s^{-1}$); x the distance from the bottom (m); x_s the distance from the biofilm surface in the new system of coordinates (m); X_f the averaged biofilm density ($kg m^{-3}$); $Y_{x/s}$ the yield coefficient (kg microorganisms/ kg nutrient); $\mu_{\max}$ the maximum specific growth rate (s^{-1}); K_s the Monod half-rate constant ($kg m^{-3}$); C the growth-limiting substrate concentration ($kg m^{-3}$); and C_s the growth-limiting substrate concentration at the biofilm surface ($kg m^{-3}$).

These early models were subsequently refined by adding additional factors affecting biofilm processes, such as bacterial

growth and decay in a steady-state biofilm (Rittmann and McCarty, 1980a, 1980b) and then the model was extended to include unsteady states and dual nutrient limitations (Rittmann and Brunner, 1984; Rittmann and Dovantzis, 1983). One of the most popular biofilm models, initially marketed as a software called BIOSIM (Wanner and Gujer, 1986), was later improved to include irregular biofilm structure and renamed AQUASIM (Wanner *et al.*, 1995; Wanner and Reichert, 1996). The growing popularity of the conceptual model of heterogeneous biofilms coincided with the growing popularity of cellular automata (CA) (Wolfram, 1986), and it is not surprising that the heterogeneous biofilm structures were modeled using CA procedures (Wimpenny and Colasanti, 1997a, 1997b). Soon after, Picioreanu *et al.* (1998a, 1998b) improved this model using more realistic assumptions and used differential equations to describe mass transport with the discrete model describing the structure (Picioreanu *et al.*, 1998a, 1998b). Since its early applications, CA remains the most popular model used to generate biofilm structure. Further improvement of the biofilm model came from Kreft *et al.* (2001), who developed a two-dimensional (2-D) multi-nutrient, multi-species model of nitrifying biofilms to predict biofilm structures, that is, surface enlargement, roughness, and diffusion distance. These authors compared the predicted structure of the biofilm with the predictions of the biomass (cells and EPS)-based model developed by Picioreanu *et al.* (1998a, 1998b), and concluded that the two models had similar solutions.

Meanwhile, biofilm researchers urgently needed mathematical description of the biofilm processes that could be used to describe recent progress in understanding biofilm processes. The main problems that needed to be addressed were horizontal and vertical profiles in mass transport and activity in biofilms. These were experimentally verified and the assumption that the effective diffusivity and biofilm density were constant across the biofilm had become difficult to defend. Biofilm diffusivity decreases toward the bottom of the biofilm and biofilm density increases. There have been attempts to include these results in the modeling of biofilm processes but they lead to more complicated mathematical expressions in which diffusivity and biofilm density are functions of distance. To simplify these expressions it is possible to model a biofilm as a stack of layers with constant diffusivity and density, which change from layer to layer rather than continuously. At steady state, this approach delivers the mass transport and activity related to the local properties of the biofilm:

$$\frac{d}{dx} \left(D_{\text{fl}} \frac{dC}{dx} \right) = D_{\text{fx}} \frac{d^2 C}{dx^2} + \frac{dD_{\text{fl}}}{dx} \frac{dC}{dx} = \frac{\mu_{\text{max}} C X_{\text{fl}}}{Y_{\text{x/s}} (K_s + C)} \quad (6)$$

where D_{fl} is the local effective diffusivity of the growth-limiting nutrient ($\text{m}^2 \text{s}^{-1}$) and X_{fl} the local biofilm density (kg m^{-3}).

Accepting that diffusivity and biofilm density are variable introduces two new variables into the equation, and functions describing changes in effective diffusivity and biofilm density need to be quantified before the equations can be solved. Experimental data show that density changes are surprisingly regular in biofilms and can be described as a linear function of biofilm depth. Relative surface-averaged effective diffusivity

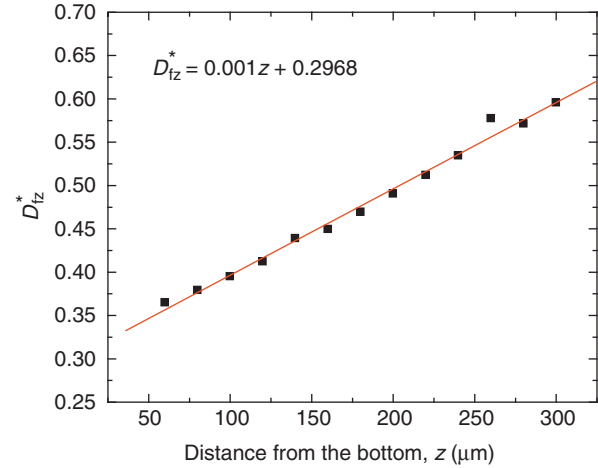


Figure 8 The surface-averaged relative effective diffusivity (D_{tz}^*) is multiplied by the diffusivity of the growth-limiting nutrient in the water to calculate the surface-averaged effective diffusivity (D_{tz}). Since, in the example, the growth-limiting nutrient is oxygen, to calculate the effective diffusivity of oxygen at various distances from the bottom, we must multiply the relative effective diffusivity at various distances from the bottom by the diffusivity of oxygen in water ($2.1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$) (Beyenal and Lewandowski, 2005).

profile, reproduced from Beyenal and Lewandowski (2005), is shown in Figure 8.

Assuming that biofilm density varies with depth in a linear fashion, as shown in Figure 8, the diffusivity gradient (ξ) is constant:

$$\frac{dD_{\text{fx}}}{dx} = \xi \quad (7)$$

At steady state, this simplifies Equation (5) to the form

$$D_{\text{fl}} \frac{d^2 C}{dx^2} + \xi \frac{dC}{dx} = \frac{\mu_{\text{max}} C X_{\text{fl}}}{Y_{\text{x/s}} (K_s + C)} \quad (8)$$

Further, it has been demonstrated that in biological aggregates, including biofilms, density is related to effective diffusivity (Fan *et al.*, 1990):

$$D_{\text{fl}} = 1 - \frac{0.43 X_{\text{fl}}^{0.92}}{11.19 + 0.27 X_{\text{fl}}^{0.99}} \quad (9)$$

Using this equation, we can estimate biofilm density from the variation in local effective diffusivity (Figure 9).

4.15.2.2.5 Horizontal variability in diffusivity and microbial activity in biofilms

Concentration profiles of growth-limiting nutrients, such as the one shown in Figure 7, are taken at a specific location in a biofilm. Based on the results, the biofilm activity at that location can be computed. However, when the next profile is taken at another location, even as close as several micrometers from the first location, the two profiles can be significantly different. This is not surprising, considering that biofilms are heterogeneous. However, it brings into question the practice of

evaluating biofilm activity based on a single measurement at an arbitrarily selected location.

For microscale measurements in stratified biofilms, the selected variable, such as local effective diffusivity or local dissolved oxygen concentration, is measured at locations on a grid (Figure 10). Grids are positioned at various distances from the bottom. The results are then presented as maps of the distributions of the selected parameter at the specified distances from the bottom, as shown in Figure 11.

One of the main advantages of this approach is that it allows us to average the concentrations of oxygen at the selected distances from the bottom and arrive at a representative profile of oxygen that illustrates its distribution across the biofilm and also shows the deviations from the average due to biofilm heterogeneity. The maps of oxygen distributions shown in Figure 11 served to construct the representative profile of oxygen across this biofilm shown in Figure 12.

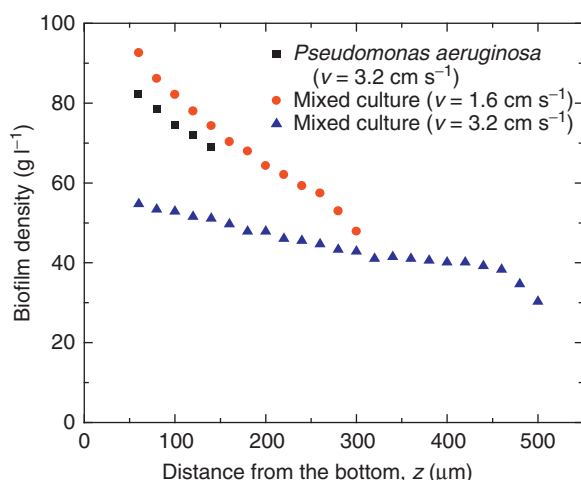


Figure 9 Variation in biofilm density with distance from the bottom (Beyenal *et al.*, 1998).

4.15.2.2.6 Mechanism of mass transfer near biofilm surfaces

When the local nutrient concentrations measured across a biofilm are plotted versus distance, they form a nutrient concentration profile. It would be expected that the shape of the nutrient concentration profile will follow the shape of the local mass transport coefficient profile when they are measured at the same location. It would also be expected that, at locations where the local mass transport coefficient is high, the local nutrient concentration will be high as well, at least higher than at a location where the local mass transport coefficient is low. Figure 13 shows profiles of oxygen concentration and local mass transport coefficient measured at the same location in a biofilm (Rasmussen and Lewandowski, 1998).

As can be seen in Figure 13, the mass transport coefficient profile does not correlate well with the oxygen concentration profile. Approaching the biofilm surface, for example, the oxygen concentration decreases rapidly and reaches quite low levels at the biofilm surface, while the local mass transport coefficient remains quite high at that location. This observation seems difficult to explain: since there is no oxygen consumption in the bulk, the oxygen concentration profile would be expected to follow the shape of the mass transport coefficient profile much closer than it does in Figure 13. However, although these two profiles do not match, each of them is consistent with our knowledge of the system's behavior. We expect to measure a low concentration of oxygen at the biofilm surface: this result fits the concept of a mass transfer boundary layer of high mass transport resistance above the biofilm surface. Measuring a high mass transport coefficient near the biofilm surface is also not surprising because, as we have estimated, convection is the predominant mass transport mechanism in that zone. The two features cannot coexist: high mass transport resistance and convection. To explain this apparent discrepancy, we need to examine the procedure for measuring flow velocity in biofilms. All available flow velocity measurements in biofilms report only one component of the

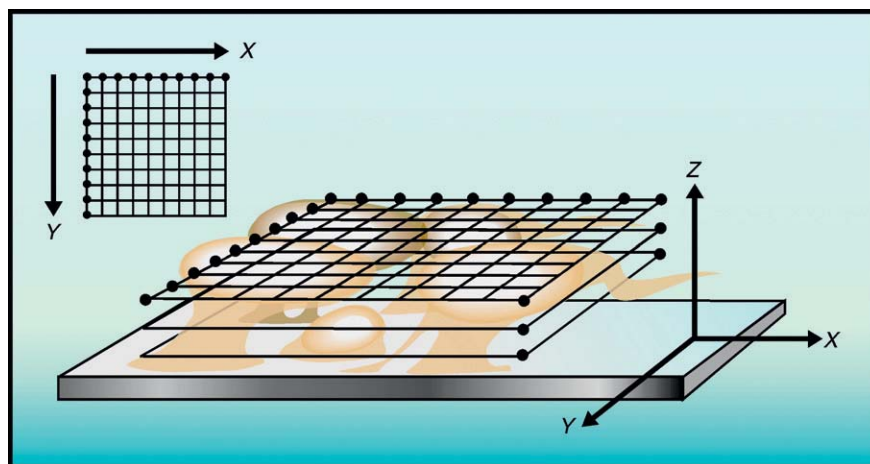


Figure 10 Microscale measurements in stratified biofilms. The selected variable, such as the local effective diffusivity or local dissolved oxygen concentration, is measured at the locations where the gridlines intersect. Such grids are positioned at various distances from the bottom (MSU-CBE, P.Dirx).

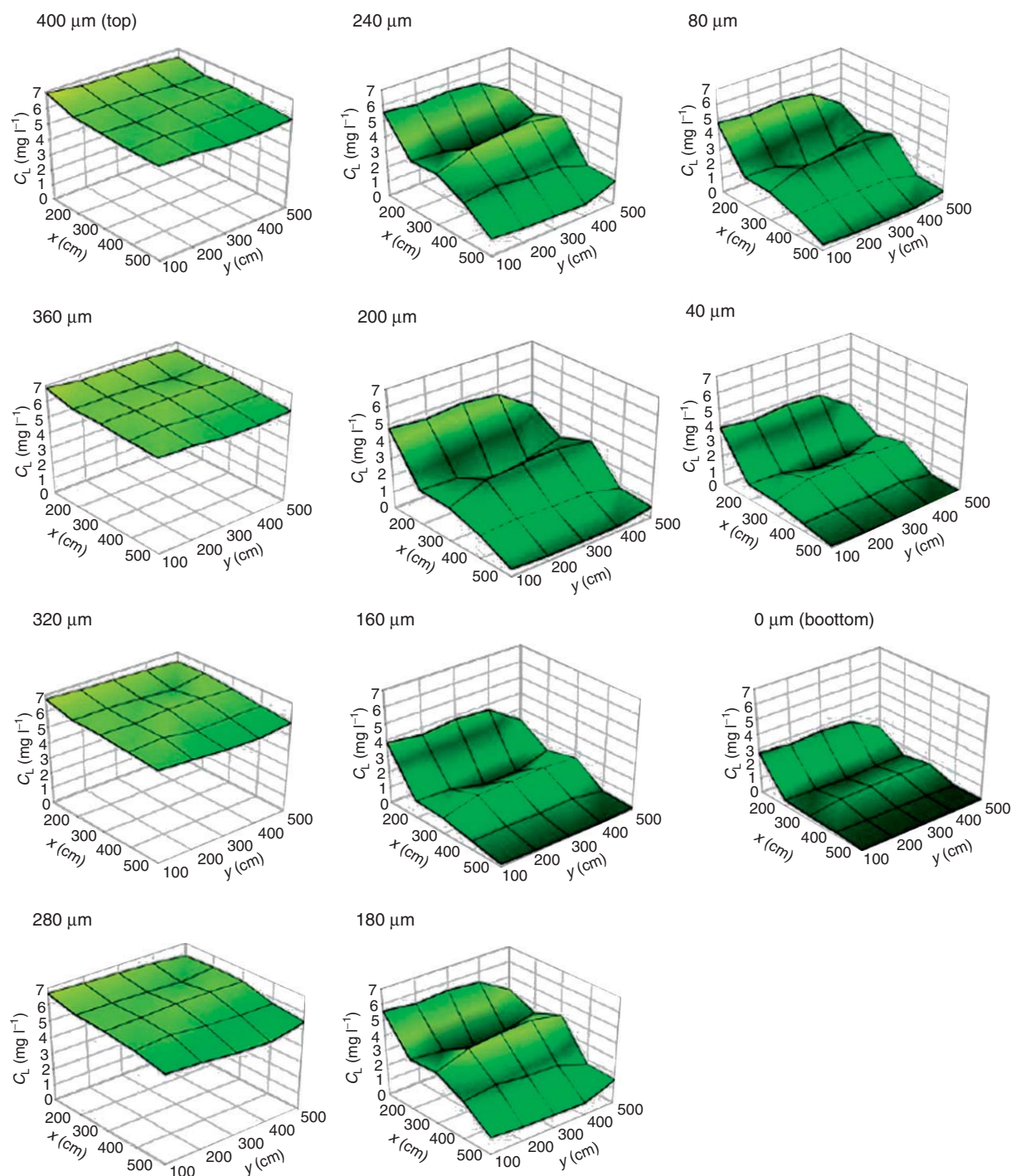


Figure 11 Distribution of oxygen measured in a biofilm at the specified distances from the bottom (Veluchamy, 2006).

flow velocity vector, parallel to the bottom. Based on these results, we estimated that mass transport is controlled by convection near biofilms. However, the convective mass transport rate equals the nutrient concentration times the flow velocity component normal to the reactive surface. The component of the flow velocity parallel to the surface has nothing to do with the convective mass transport toward that surface. Consequently, the estimate of the mass

transport mechanism based on flow velocity holds only in the direction in which the flow velocity was measured. Indeed, when the flow near a surface is laminar, the laminae of liquid slide parallel to the surface, and there is little or no convection across these layers: the mass transport parallel to the surface is convective, while the mass transport perpendicular to the surface remains diffusive. This mechanism is visualized in [Figure 14](#).

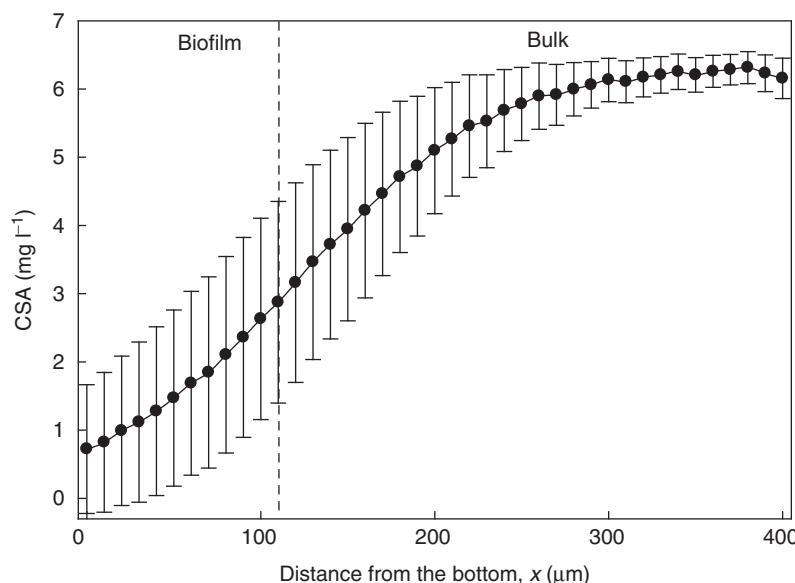


Figure 12 Surface averaged oxygen concentrations (C_{SA}) and standard deviations computed for each data set in Figure 11. The average oxygen concentrations form a representative profile of oxygen concentration, characterizing the area covered with the biofilm, and the envelope of the standard deviation is a measure of the heterogeneity of the measured variable, oxygen concentration in this case (Veluchamy, 2006).

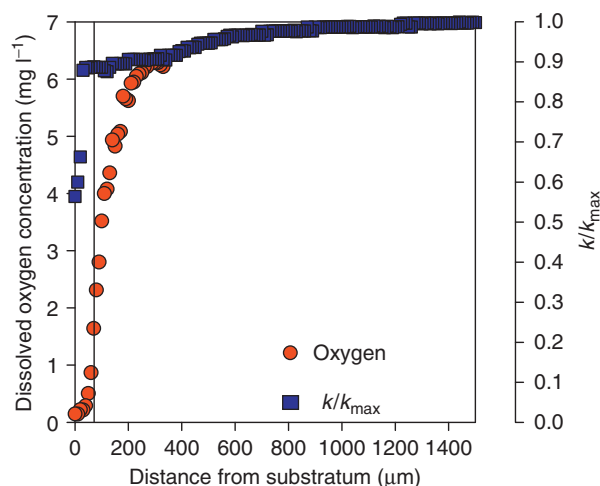


Figure 13 Profiles of oxygen and local mass transfer coefficient through a thin biofilm cluster (■, dissolved oxygen; ●, local mass transfer coefficient). The vertical line marks the observed thickness of the biofilm. At distances of less than 30 μm , the wall effect caused the local mass transport coefficient to decrease. The biofilm thickness was 70 μm in this location. The value of k/k_{max} was only slightly affected by the presence of the biofilm up to a distance of less than 30 μm from the substratum (Rasmussen and Lewandowski, 1998).

4.15.2.2.7 Biofilm processes at the macroscale and at the microscale

Accurate mathematical models are necessary for advances in biofilm research. Biofilm researchers use mathematical models of biofilm processes not only to predict the outcome of these processes, but also to interpret the results of biofilm studies. In the absence of suitable models, the interpretation of biofilm studies is impaired. Biofilm science and technology are relatively young, and mathematical descriptions of biofilm

processes often lag behind the rapidly expanding knowledge of biofilm processes. On the other hand, most of the experience that was accumulated in modeling biofilm processes in water and wastewater treatment was based on the operating reactors with suspended biomass. Biofilm reactors are different, and some effects common in biofilm reactors are much less usual in reactors with suspended biomass.

One effect that is particularly difficult to accommodate in biofilm models is the influence of biofilm heterogeneity on biofilm processes. Biofilm models that describe biofilm processes on the scale of the entire reactor assume that the biofilm is uniformly distributed and its effects do not depend on the location in the reactor. This assumption, which is justified in the case of well-mixed reactors, may or may not be justified in biofilm reactors. With the current sophistication in exploring biofilm processes at the microscale, it is not surprising to observe that the local conditions quantified in biofilms deviate widely from the average conditions described by the biofilm models. One hopes that these deviations from the idealized models cancel each other and that overall, at the macroscale, they do not matter much.

One particularly troubling problem is the definition of and the existence of a steady state in biofilm reactors. Defining a steady state in a biofilm reactor may well be the most important question facing biofilm researchers, both those who focus on experiment and those who focus on modeling. The existence of a steady state is obvious in flow reactors, where microbial growth occurs in suspension. In such reactors, the interplay among the microbial growth rates, biomass concentration, and hydraulic and biomass retention times leads to a steady state in which process variables do not change for a long time. In contrast, the reasons for the existence of a steady state in a biofilm reactor are much less clear because an important condition for a steady state is not satisfied in a biofilm reactor: the concentration of biomass in a

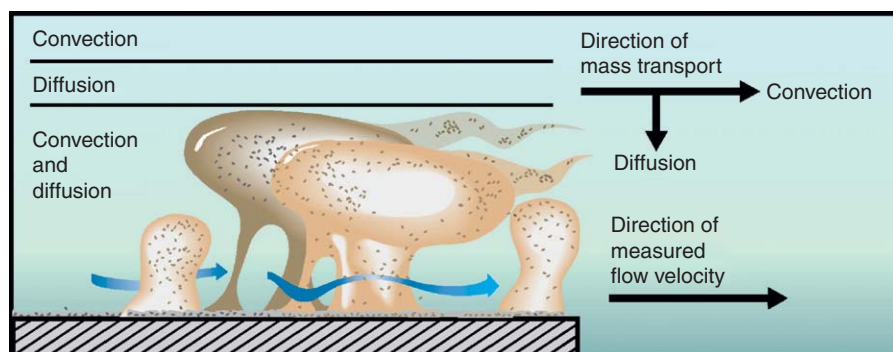


Figure 14 Alternating zones of convective and diffusive mass transport in heterogeneous biofilms. This hypothetical model of mass transport is consistent with the results in **Figure 13**. Mass transport in the space occupied by the biofilm is convective, but the amount of nutrient delivered to this space is limited by the diffusive mass transport just above the biofilm surface (MSU-CBE, P.Dirx).

biofilm reactor is not a simple function of retention time and growth rate. Some biofilm technologies actually take advantage of this fact and grow biofilm microorganisms using retention times at which the microorganisms would be washed out from reactors operated with suspended microorganisms. Practically, this problem corresponds to the fact that we are uncertain what function describes detachment in biofilms, and what mechanisms are involved in biofilm detachment, except perhaps for shear stress. The mechanism of biofilm sloughing remains unknown. A steady state for the biomass concentration assumes that the same amount of biomass is generated as is removed by various processes, particularly biofilm detachment. One can argue that if the biofilm reactor is large enough, the microscale biofilm processes will average out on the scale of the reactor, and that this average may be stable even if the components of the average vary over time. This argument, even if it is true, however, does not settle the issue. A question follows: how large does the reactor have to be to ensure that the variations in the microscale biofilm processes average out and the reactor reaches a steady state at the macroscale?

There are also difficulties at the microscale. Experimentally measured concentration profiles and flow velocity profiles corroborate the conceptual model shown in **Figure 5**. However, when it comes to interpreting experimental data, the idealized image of biofilms in **Figure 5** is not adequate for many reasons. One reason is shown in **Figure 15**: the difficulty with locating the position of the biofilm surface. The position of the biofilm surface is important: one of the boundary conditions in the equation describing biofilm activity and mass transport specifies the conditions at the biofilm surface. As can be seen in **Figure 15**, however, locating it is not trivial.

This problem has been addressed experimentally by judiciously locating the surface on a nutrient concentration profile at the location where the profile ends its curvature near the bottom. The rule of locating the biofilm surface at that location has been developed based on the results of studies in which an oxygen electrode and an optical sensor were used to measure the oxygen concentration profile and detect the biofilm surface, where optical density changed (**Figure 16**). The position of the biofilm surface coincides with the location where the oxygen profile becomes linear. The biofilm surface in **Figure 7** was positioned using this principle.



Figure 15 Surface of a biofilm grown at a flow velocity of 0.81 m s^{-1} (Groenenboom, 2000).

4.15.2.2.8 Biofilms in conduits

Among the many possible effects that biofilms may have in water conduits, we will discuss two effects in more detail: (1) the effect on flow characteristics – pressure drop in conduits and (2) the effect on material performance – MIC.

Flow velocity near the biofilm surface.

It is well known that flow velocity affects biofilm processes. **Figure 5** shows an example of the effect of flow velocity on mass transport dynamics near the biofilm surface. However, biofilm also affects flow velocity: flow velocity near a wall covered with biofilm is different from that near a wall with no biofilm. **Figure 17** shows this effect.

The effect of biofilm on flow velocity distribution most certainly influences the dynamics of mass transfer. However, this is not the only effect that biofilm has on hydrodynamics. For example, it is well known that biofilms increase the pressure drop in conduits, but it is not clear what the mechanism of this process is or how to quantify it. To predict pressure drop in pipes the Moody diagram is used, which correlates the Reynolds number and the relative roughness to provide the friction factor, f . This friction factor is then

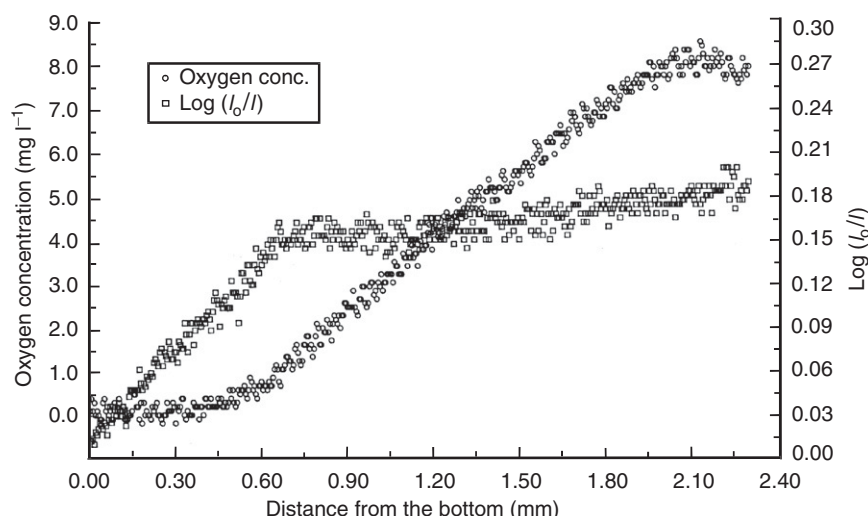


Figure 16 Profiles of oxygen concentration and optical density in a biofilm. A combined microsensor – an oxygen microelectrode and an optical density microprobe – permitted locating the biofilm surface at 0.60 mm from the bottom. This distance, when marked on the oxygen concentration profile, indicates that the biofilm surface is at the beginning of the linear part of the oxygen profile within the mass transfer boundary layer; I is the local light intensity, and I_0 is the maximum light density (Lewandowski *et al.*, 1991).

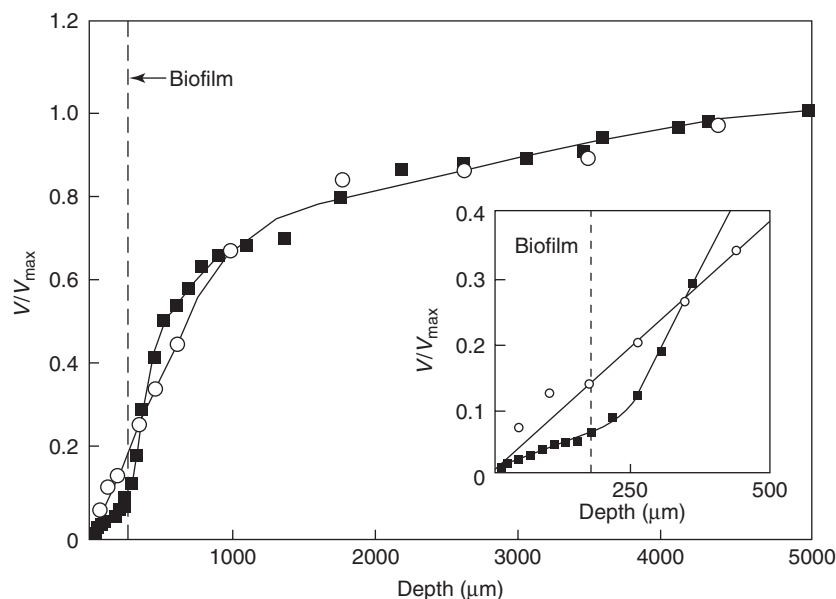


Figure 17 The flow velocity profile near a wall covered with a biofilm is different from the flow velocity profile near the same wall without the biofilm (DeBeer *et al.*, 1994).

plugged into the Darcy–Weisbach equation to calculate the pressure drop:

$$H_L = f \frac{l}{D} \frac{V^2}{2g} \quad (10)$$

where H_L is the head loss due to friction, l the pipe length, V the average fluid velocity, g the gravitation constant, D the pipe diameter, and f the friction factor provided by the Moody diagram. When the flow velocity increases, the thickness of the boundary layer decreases, and the roughness elements protrude through the boundary layer, further affecting the drag and the pressure drop.

Unfortunately, the Moody diagram is of little help in predicting the pressure drop in conduits covered with biofilms. The pressure drop in such conduits is caused by different factors than the pressure drop in conduits without biofilms because different mechanisms are responsible for the shape of the pressure drop in each of these conduits. These differences sometimes demonstrate themselves in the form of puzzling experimental results, such as decreasing pressure drop resulting from increasing flow velocity, which is a consequence of the elastic and viscoelastic properties of biofilms. Microcolonies are made of bacterial cells embedded in gelatinous EPS that can change shape under stress. At high flow velocities the hydrodynamic boundary layer separates from the

microcolonies, causing pressure drag downstream of the microcolony and pulling the material in this direction. The microcolonies slowly flow under the strain, forming elongated shapes that we call streamers. Streamers are often seen when biofilms grow at high flow velocities. The streamers contribute to pressure drop by moving rapidly and dissipating the kinetic energy of the flowing water. Another important consequence of a streamer's oscillations is that they are transmitted to the underlying microcolonies, which also oscillate rhythmically. This system reacts with turbulent boundary layers much differently than the rigid surface roughness elements of clean pipes do. One way to gain experimental access to the interactions between flowing water and biofilm is to monitor flow velocity profiles.

Imaging flow velocity profiles makes it possible to evaluate the effect of biofilm formation on the flow in conduits by quantifying its effect on the entry length in the conduit. The hydrodynamic entry length is defined as the distance needed to develop a steady flow, after the water has passed through the entrance to the reactor. If the presence of biofilm makes the entry length longer, then the biofilm contributes to flow instability, and vice versa. There is a simple relation between the Reynolds number and the entry length: the higher the Reynolds number, the longer the entry length. This effect was used as a base for quantifying the effects of biofilm on the flow in conduits. Flow velocity distribution was measured in a rectangular reactor when the flow velocity was increasing from one measurement to another. As the flow velocity and the Reynolds number increased, the flow stability was monitored in a rectangular conduit using nuclear magnetic resonance (NMR) imaging. The results, shown in Figure 18, demonstrate that the presence of biofilm actually made the flow more stable. The entry length was shorter and the flow reached stability closer to the entrance in the presence of biofilm than in its absence.

It is difficult to interpret this result immediately because it is well known that the presence of biofilm increases pressure drop in conduits: traditionally, pressure drop in pipes is related to friction. As pressure drop is larger in biofilm-covered pipes, a natural conclusion was that biofilms must increase friction and therefore the presence of the biofilm should introduce flow instability rather than reduce it.

The relation between flowing water and biofilms is determined by two facts: (1) biofilms are made of viscoelastic polymers which actively interact with the oscillations generated by the flow of water and (2) the flow of water affects the biofilm structure. Based on what we now understand, at low flow velocities biofilms can effectively smooth surfaces and stabilize the flow because the oscillating layer of elastic polymeric matrix can effectively damp the vibrations coming from the flowing water. This effect delays the onset of turbulence in conduits covered with biofilm and explains the results shown in Figure 18. However, as the flow velocity increases further, the elastic polymeric matrix must oscillate faster and faster and, eventually, the frequency of its oscillation cannot follow the frequency of the incoming eddies. At that point the biofilm oscillation is out of phase and the biofilm not only fails to damp the flow instabilities but also actively introduces instability by randomly oscillating at a different frequency than the incoming eddies. The pressure drop in the conduit

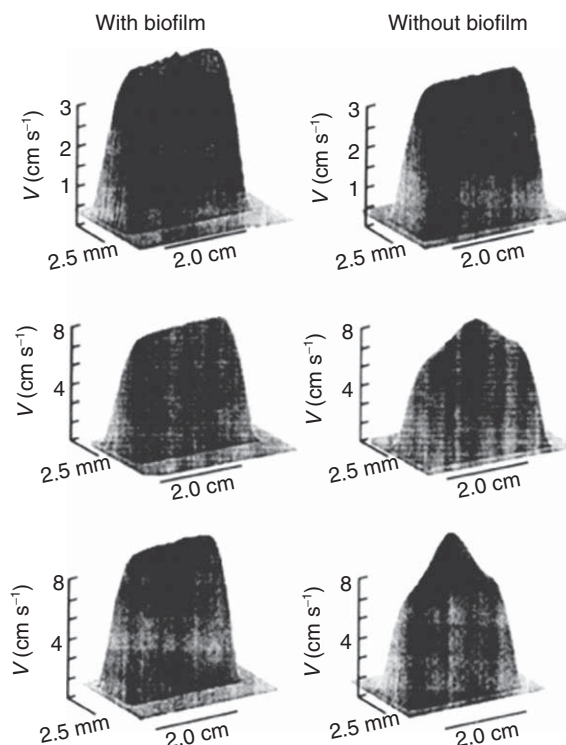


Figure 18 Flow velocity profiles in a rectangular conduit whose walls were colonized with a biofilm. The increasing flow velocity did not affect the character of the velocity profiles in the reactor with biofilm. On the other hand, the same increase in velocity had a pronounced effect on the reactor without biofilm.

increases rapidly. This effect was, in early biofilm works, mistaken for a similar effect caused by rough surface elements. For example, Picologlou *et al.* (1980) observed a considerable increase in frictional resistance after the film thickness reached a value approximately equal to the calculated thickness of the hydrodynamic boundary layer for a clean surface. In clean pipes covered with surface roughness elements, when flow velocity increases the boundary layer becomes thinner and at some flow velocity the boundary layer thickness is smaller than the height of the roughness elements. When this happens, the roughness elements protrude through the boundary layer and cause an additional drag, which exhibits itself in a sudden increase of the pressure drop for flow velocities exceeding this critical flow velocity. This model was commonly accepted and was used to explain the pressure drop in conduits covered with biofilms, although even at that time some authors warned that this might not be the true mechanism of the process (Characklis, 1981). Currently, there are no models that can account accurately for pressure drop in conduits covered with biofilm.

4.15.3 Part II: Biofilm Reactors

Biological systems treating municipal wastewater require (1) the accumulation of active microorganisms in a bioreactor and (2) the separation of the microorganisms from treated effluent. In suspended growth reactors, such as the activated

sludge process, microorganisms grow and biofloculate; the resultant flocs are suspended freely in the bulk phase. Floculated bacteria are then separated from the bulk liquid by sedimentation or membranes. Clarifier-coupled suspended growth reactors rely on return activated sludge, or underflow, from the coupled clarifier to provide the desired active biomass concentration in the bioreactor. Consequently, clarification unit processes may be limited by the hydraulic loading rate (HLR) or solids loading rate (SLR). Biofilm reactors retain bacterial cells in a biofilm that is attached to the fixed or free moving carriers. The biofilm matrix consists of water and a variety of soluble (C) and particulate (X) components that include soluble microbial products, inert material, and EPS. Without suspended biomass, the bioreactor is decoupled from the liquid–solids separation unit. Active biomass concentrations inside the biofilm are large at 10–60 g of volatile suspended solids (VSS) l⁻¹ of biofilm. This biomass range can be compared with the range of concentrations expected for suspended growth reactors, which is typically 3–8 g VSS l⁻¹ of reactor volume. The lower value in this range is associated with clarifier-coupled activated sludge processes, and the upper range with membrane bioreactors. In biofilm reactors, bacteria attached to carriers periodically detach from the biofilm matrix and exit the system in the effluent stream. **Figure 19** provides a conceptual illustration of different biofilm reactor types.

Biofilm reactors can be classified based on the number of phases involved – gas, liquid, solid – according to the biofilm being attached to a fixed or moving carrier within the reactor. They are also classified based on how electron donors or acceptors are applied to seven basic types as listed below (adapted from Harremoes and Wilderer (1993)):

1. Three-phase system – fixed biofilm-laden carrier, bulk water, and air. Water trickles over the biofilm surface and

air moves upward or downward in the third phase (e.g., trickling filter (TF)) (**Figure 19(a)**).

2. Three-phase system – fixed (or semifixed) biofilm-laden carrier, bulk water, and air. Water flows through the biofilm reactor with gas bubbles (e.g., aerobic biologically active filter (BAF)). Gravel is a fixed media and polystyrene beads are semifixed (**Figures 19(b)** and **19(c)**).
3. Three-phase system – moving biofilm-laden carrier, bulk water, and air. Water flows through the biofilm reactor. Air is introduced with gas bubbles (e.g., aerobic moving bed biofilm reactor (MBBR)) (**Figure 19(g)**).
4. Two-phase system – moving biofilm-laden carrier and bulk water. Water flows through the biofilm reactor with the electron donor and electron acceptor (e.g., denitrification fluidized bed biofilm reactor (FBBR)) (**Figure 19(g)**).
5. Two-phase system – fixed biofilm-laden carrier material and bulk water. Water flows through the biofilm reactor with the electron donor and electron acceptor (e.g., denitrification filter) (**Figures 19(b)** and **19(c)**).
6. Three-phase membrane system – a microporous hollow-fiber membrane with biofilm and water on one side and gas on the other, diffusing through the membrane to the biofilm (e.g., membrane biofilm reactor (MBfR)) (**Figure 19(h)**).
7. Two-phase membrane system – a proton exchange membrane separating a compartmentalized biofilm-laden anode from a compartmentalized cathode with water on both sides, but with the electron donor on one side and electron acceptor on the other (e.g., biofilm-based microbial fuel cell (MFC)).

Biofilms are ubiquitous in nature and in engineered systems and can be used beneficially in municipal water and wastewater treatment. Biofilm and suspended growth reactors can meet similar treatment objectives for carbon oxidation,

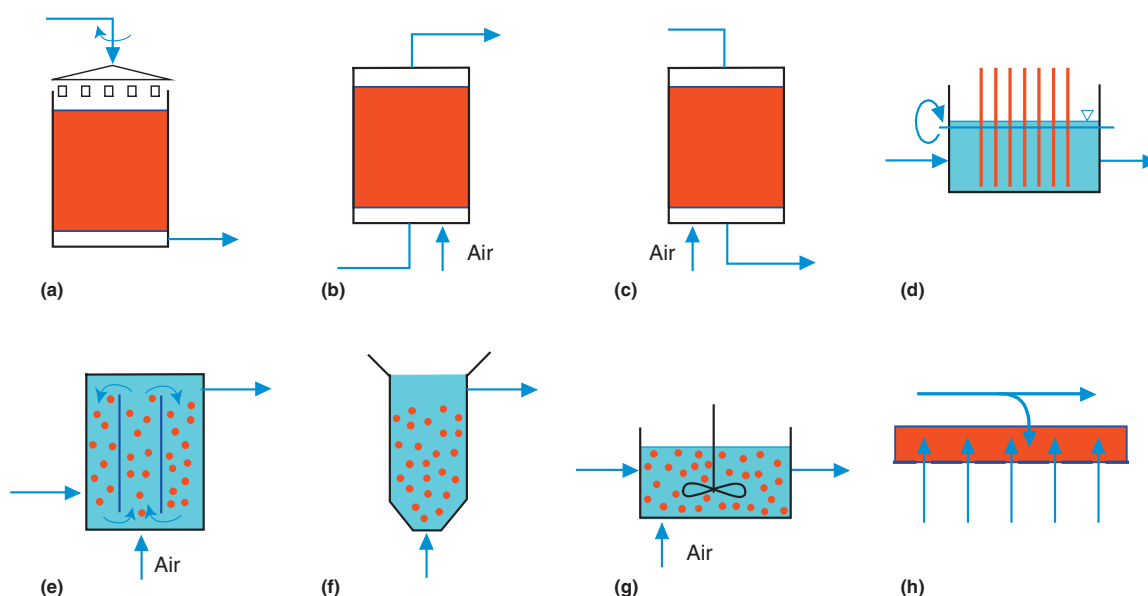


Figure 19 Types of biofilm reactors: (a) trickling filter; (b) submerged fixed bed biofilm reactor operated as up flow or (c) down flow mode; (d) rotating biological contactor; (e) suspended biofilm reactor including airlift reactor; (f) fluidized bed reactor; (g) moving bed biofilm reactor; and (h) membrane attached biofilm reactors. From Morgenroth (2008) *Modelling biofilm systems*. In: Henze M, van Loosdrecht MCM, Ekama G, and Brdjanovic D (eds.) *Biological Wastewater Treatment – Principles, Modelling, and Design*, pp. 457–492. London: IWA Publishing.

nitrification, denitrification, and desulfurization. Biofilm reactors have also been used for the treatment of a variety of oxidized contaminants including perchlorate and bromate. The same microorganisms are responsible for biochemical reactions in both activated sludge and biofilm systems, and respond in the same way to local environmental conditions (i.e., pH, temperature, electron donor, electron acceptor, and macronutrient availability) (Morgenroth, 2008). A key component to be considered by anyone who is evaluating a biofilm reactor is the effect of multiple substrates and biomass fractions and the manner in which the reactor is affected by mass-transport limitations. Substrates typically considered are:

1. soluble compounds, including electron donors (e.g., readily biodegradable chemical oxygen demand (rbCOD), NH_4^+ , NO_2^- , and H_2), electron acceptors (e.g., O_2 , NO_3^- , NO_2^- , and SO_4^{2-}), and nutrients and buffers (e.g., PO_4^{3-} , NH_4^+ , and HCO_3^-) and
2. particulate compounds, including electron donors (e.g., slowly biodegradable COD (sbCOD)), active biomass fractions (e.g., heterotrophic and autotrophic bacteria), inert biomass, and EPS.

4.15.3.1 Application of Biofilm Reactors

This section exists to provide the reader with a general overview of biofilm reactor applications. While general biofilm reactor applicability is described here, several treatment scenarios exist that are not conveniently generalized yet warrant the use of biofilm reactor technology. Water-quality regulations exist to protect human health and the water environment. Organic matter and the nutrients such as nitrogen and phosphorus are major contributors to water-quality impairment. In municipal wastewater-treatment scenarios, biofilm reactors are generally applied for the removal of carbon-based organic matter and/or nitrogenous compounds. Specifically, these biofilm reactors may achieve carbon oxidation, combined carbon oxidation and nitrification, tertiary nitrification, or tertiary denitrification. Biofilm reactors are not commonly used for biological phosphorus removal.

Biofilm reactors treating industrial wastewaters have been applied to meet treatment objectives similar to those in municipal wastewater treatment and industrial pretreatment. The objective of pretreatment is to process industrial waste streams until their characteristics are similar to raw sewage (see Metcalf and Eddy (2003) for a description). As a result the industry can then discharge their treated wastewater into municipal sewers where further processing is accomplished at a municipal wastewater-treatment plant. Biofilm reactors are common for industrial applications because the processes are reliable, robust, easy to operate, and resilient to toxic or shock loading.

4.15.3.1.1 Techniques for evaluating biofilm reactors

Several approaches exist to evaluate biofilm reactors. The primary objective of a biofilm or biofilm reactor model is to predict soluble substrate flux (J) through the biofilm surface. This flux ($\text{ML}^{-2}\text{T}^{-1}$) can be used to obtain an estimate of the (1) overall biofilm reactor performance, (2) required biofilm surface area, (3) electron acceptor (e.g., dissolved oxygen), (4)

external electron donor (e.g., methanol or hydrogen), and (5) biosolids management requirements. This section discusses the relative benefits and limitations to some general methods of evaluating biofilm reactors. The use of mathematical biofilm models is common in both research and practice, but only a cursory presentation of their mathematical description is presented. Excellent resources exist describing aspects of mathematical modeling of biofilms and biofilm reactors (for additional information, see Wanner *et al.* (2006) and Morgenroth (2008)).

The approaches discussed here include a graphical procedure, empirical models, semiempirical models, and mechanistic mathematical models.

4.15.3.1.2 Graphical procedure

A graphical procedure can be used to determine the total hydraulic load (THL) required to decrease a substrate concentration, and by definition the biofilm surface area required to provide a desired substrate concentration remaining in the effluent stream. These items can be determined directly. The graphical procedure can be used to determine effluent substrate concentration from any series of continuous flow stirred tank reactors (CFSTRs). A stepwise procedure must be used when a series of CFSTRs will be used. Antoine (1976) and Grady *et al.* (1999) developed the graphical procedure described here and the approach is valid for any biofilm-based CFSTR. If multiple stages are expected to have different characteristics, then the graphical method requires different flux curves to describe system response in each of the CFSTRs.

The procedure requires a graphical representation of substrate flux (J) as a function of bulk-liquid substrate concentration (C_B). This relationship between flux and bulk-liquid substrate concentration can be obtained from numerical simulations, full-scale or pilot-plant observations. In practice, this graphical procedure is typically used to extend pilot-plant observations to full-scale biofilm reactor design criteria. The process designer should recognize that the relationship between flux and bulk-liquid substrate concentration is based on the system and location. Therefore, the flux curve required to implement the graphical procedure may not be obtained from or correlate well with values reported in the literature or from different systems. As a result, the process designer should consider carefully the conditions under which the flux curve was developed before applying results. A flux curve representing mass transfer and environmental conditions characteristic of a specific system and operating mode may not be the representative of different biofilm reactor types designed to meet the same treatment objectives. A flux curve generated for the same biofilm reactor type under similar operating conditions, however, may offer some direction in the absence of system-specific numerical simulation or pilot/full-scale observations.

When using the graphical procedure to evaluate pilot-plant observations, fluxes should be compared to rates in full-scale systems. Any flux that deviates significantly from those reported for biofilm reactors in published studies should be used only after careful consideration. Pilot or experimental systems may promote a greater flux than expected. The basis for the graphical procedure is a material balance on a

biofilm-based CFSTR:

$$0 = \underbrace{Q \cdot C_{in,i}}_{\text{mass per time input}} - \underbrace{Q \cdot C_{B,i}}_{\text{mass per time output}} - \underbrace{J_{LF,i} \cdot A}_{\text{biofilm transformation rate}} - \underbrace{r_{B,i} \cdot V_B}_{\text{suspended growth transformation rate}} \quad (11)$$

where Q is the flow rate through the system ($\text{m}^3 \text{d}^{-1}$); $C_{in,i}$ the influent concentration of soluble substrate i (g m^{-3}); $C_{B,i}$ the effluent, or bulk-liquid, concentration of soluble substrate i (g m^{-3}); $J_{LF,i}$ the flux of soluble substrate i across the biofilm surface equal to the average biofilm activity in the reactor, as shown in Equation (1) ($\text{g m}^{-2} \text{d}^{-1}$); A the biofilm surface area (m^2); $r_{B,i}$ the rate of substrate i conversion because of suspended biomass ($\text{g m}^{-2} \text{d}^{-1}$); and V_B the bulk-liquid volume (m^3).

Assuming that transformation occurring in the bulk liquid is negligible, the suspended growth transformation rate (Equation (11)) can be neglected. Rearranging Equation (11) provides the rationale for the graphical procedure:

$$J_{LF,i} = \underbrace{\frac{Q}{A} \cdot C_{in,i}}_{\text{const.}} - \underbrace{\frac{Q}{A} \cdot C_{B,i}}_{\text{slope}} \quad (12)$$

The slope, or $(-Q/A)$, is referred to as the operating line and represents the total hydraulic load on each stage. Figure 20 illustrates the graphical method.

The flux curves have been created based on observations in the first and second stage of a post-denitrification biofilm reactor. The ordinate represents nitrate–nitrogen flux and the abscissa nitrate–nitrogen concentration remaining in the effluent stream. The graphical solution indicates that the

first-stage denitrification biofilm reactor effluent nitrate–nitrogen concentration is approximately 3.9 mg l^{-1} . The second-stage effluent nitrate–nitrogen concentration is approximately 1.1 mg l^{-1} with fluxes of approximately 1.6 and $1.1 \text{ g m}^{-2} \text{d}^{-1}$ in the first and second stage, respectively. The graphical procedure depends on the substrate flux curve(s). The method requires development of multiple flux curves if the performance characteristics of respective stages vary significantly. When using pilot-plant data to generate a flux curve, appropriate scale considerations must be given when designing the pilot unit and experiments.

4.15.3.2 Empirical and Semi-Empirical Models

Empirical models can be implemented easily by hand or using a spreadsheet, but they have limited applicability because of their black-box consideration of system parameters. Because environmental conditions and bioreactor configuration affect biofilm reactor performance, a system can respond differently from the description provided by an empirical model. The limited descriptive capacity of empirical models typically results from parameter values and model features based on data that were obtained from few system installations or operating conditions. Therefore, the engineer or scientist should be aware of conditions under which system-specific model parameters have been defined. Significant sources of variability in values include differences in biofilm carrier type and configuration, the extent of concentration gradients external to the biofilm surface, and biofilm composition. Despite their ease of implementation, empirical models can produce results that vary 50–100% of actual system performance.

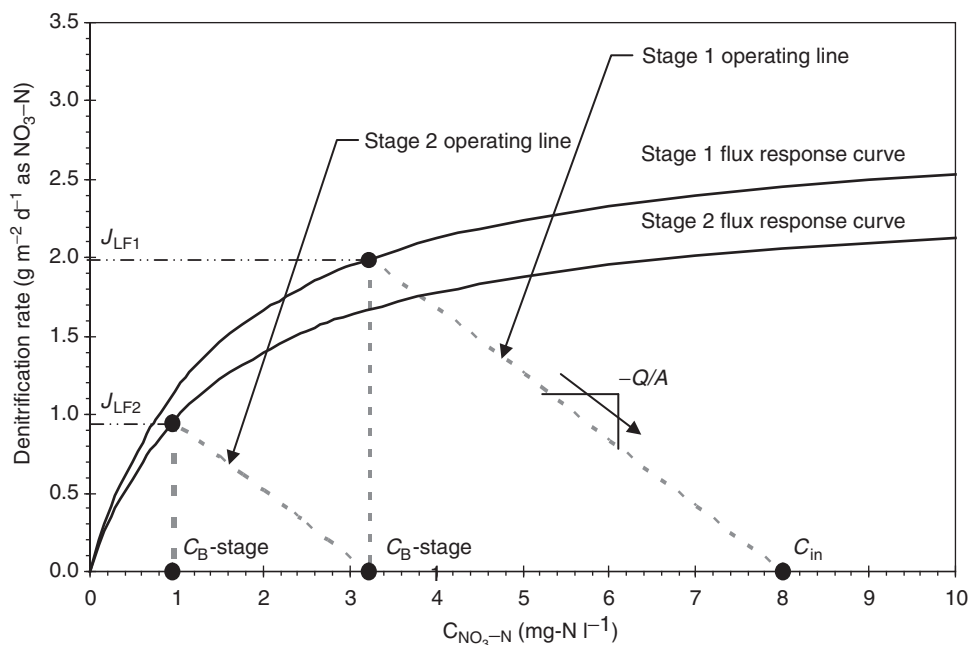


Figure 20 Graphical procedure for describing the response of a denitrification moving bed biofilm reactor to defined conditions, including (1) first- and second-stage operating lines and (2) flux curves based on observations at a pilot-scale denitrification moving bed biofilm reactor (Boltz *et al.*, 2010b).

Coefficient values, and sometimes the empirical models, are typically created to describe system response for the removal of a specific material. The models can be used as an indicator of system viability to meet treatment objectives with respect to the specific process governing transformation. Empirical models are, however, inadequate for describing complex processes such as the explicit evaluation of two-step ammonium oxidation first to nitrite by ammonia-oxidizing bacteria and then to nitrate by nitrite-oxidizing bacteria. Therefore, empirical models have limited application in defining the conditions that either promote or deter complex processes in biological systems.

Historically, biofilm reactors have been designed using empirical criteria and models, but this trend is changing. One should recognize that the coefficients in empirical models describing biofilm reactors include system, and many times, location-specific mass-transfer resistances (Grady *et al.*, 1999). For this reason, the values typically differ from apparent or intrinsic values reported in the literature. Once a flux has been determined, Equation (11) can be rearranged, neglecting bulk-phase conversion processes, to calculate the material concentration remaining in the effluent:

$$C_{B,i} = C_{in} - \frac{J_{LF,i} \cdot A}{Q} \quad (13)$$

If sufficient data exist to allow for the development of parameter values and mathematical relationships capable of describing a complete range of conditions expected when treating municipal wastewater, then empirical models can be used. The addition of model components to account for specific phenomenon encroaches on the premise of mechanistic mathematical model development. For this reason, a distinction is made between empirical and semi-empirical models. Gujer and Boller (1986) and Sen and Randall (2008) provided an example of the latter describing nitrifying TFs, and MBBRs and IFAS systems, respectively.

4.15.3.3 Mathematical Biofilm Models for Practice and Research

Mathematical modeling can be used to describe certain features of a biofilm or biofilm system (such as a bioreactor) by selecting and solving mathematical expressions. Biofilm reactor research and design commonly involve the use of mathematical biofilm models. These mathematical models are tools that allow the user to efficiently evaluate a variety of complex scenarios. Empirical models fail to provide information that is a concern for biofilm researchers and environmental protection such as biofilm composition and competition among bacteria for multiple substrates and space inside the biofilm, and the influence of individual processes on the interaction between several bacterial types. Mathematical biofilm models have been used as a research tool, but only recently modern biofilm reactors have encouraged the use of biofilm models in engineering practice. Submerged and completely mixed biofilm reactors allow for the application of modern biofilm knowledge, and are conducive to simulation with existing biofilm models (Boltz and Daigger, 2010). As a result, a majority of existing wastewater-treatment plant simulators have been improved to include a biofilm reactor module(s) that is based on the mathematical description of a 1-D biofilm. A user should understand the mathematical biofilm model basis, underlying assumptions, and limitations before applying the model to research or design.

A biofilm schematic is shown in Figure 21. The schematic illustrates diffusion and reaction occurring inside a 1-D biofilm. In addition, concentration gradients external to the biofilm surface are illustrated in the manner that they are modeled, namely an external mass transfer resistance represented by a mass transfer boundary layer.

The partial differential equation describing molecular diffusion, substrate utilization inside a biofilm, and dynamic accumulation has been presented as Equation (3). It should be emphasized that the basis for a mathematical description of the 1-D biofilm, as described by Equation (3), is simultaneously

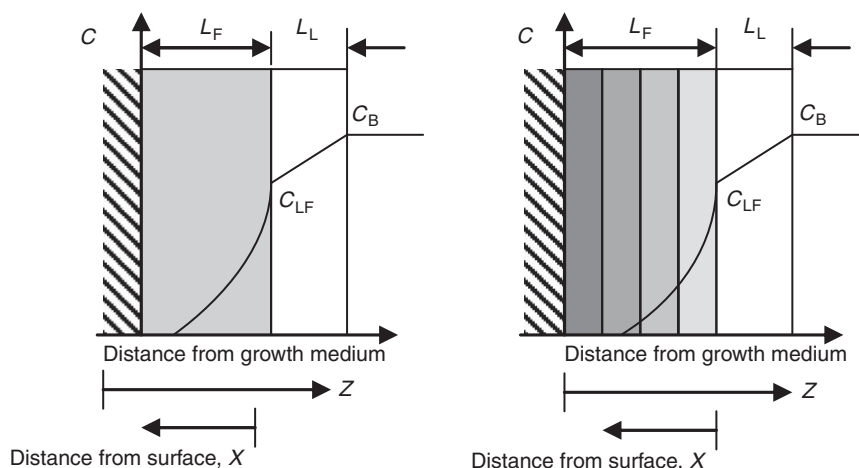


Figure 21 Schematic of a 1-D biofilm of thickness L_F having an assumed homogeneous (a) and heterogeneous, or layered, (b) biomass distribution. Soluble substrate concentration profile is illustrated with a bulk-liquid concentration (C_B) decreasing through a mass transfer boundary layer of thickness L_L until reaching the liquid–biofilm interfacial concentration (C_{LF}), and then decreasing through the biofilm.

occurring molecular diffusion and biochemical reaction. Molecular diffusion is based on Fick's law. Monod-type kinetics is typically applied to describe the biochemical transformation rate. Analytical solutions to Equation (3) are available only for first- and zero-order rate expressions and assuming steady state. Zero-order kinetics are valid if the bulk-liquid substrate concentration is well above the half-saturation concentration (i.e., $C_{B,i} > K_i$), and first-order kinetics is applicable for low substrate concentrations (i.e., $C_{B,i} < K_i$). Solving the second-order differential equations requires constants that can be derived from two boundary conditions described by Equation (5). From the concentration profile ($C_{f,i}(x)$) the flux through the biofilm surface (J_{LF}) is calculated as Equation (2). This substrate flux, J_{LF} is used in biofilm reactor material balances (see Equation (11)). The concentration gradient external to the biofilm surface is not explicitly modeled. Rather, it is modeled as a mass transfer resistance:

$$J_{MTBL} = \frac{1}{R_L}(C_{B,i} - C_{LF,i}) \quad (14)$$

Here, J_{MTBL} is the substrate flux in the stagnant liquid layer and R_L the mass transfer resistance external to the biofilm. It is helpful to visualize R_L by introducing the concept of a mass transfer boundary layer. Defining the thickness of this mass transfer boundary layer provides a more intuitive understanding compared to the mass transfer resistance. Resistance to mass transfer and the mass transfer boundary layer thickness are related according to Equation (15):

$$R_L = \frac{L_L}{D_w} \quad (15)$$

Here, L_L is the mass transfer boundary layer thickness and D_w the solute diffusion coefficient in the water phase. The substrate flux through the mass transfer boundary layer (Equation (15)) is linked to the substrate flux across the biofilm surface (Equation (2)). This provides an additional Equation (16) (boundary condition) that is required to calculate the additional unknown value of the substrate concentration at the liquid–biofilm interface (J_{LF}):

$$J_{MTBL} = J_{LF} \quad (16)$$

One of the most difficult aspects of choosing an approach to model biofilms and biofilm reactors is to choose the appropriate level of complexity. An overview of the different model approaches is provided below (after Takács *et al.*, 2010):

- *0-D biofilm.* One aspect of modeling biofilms is that bacteria are retained in the system and are not washed out with effluent water. The simplest approach for biofilm modeling would be to assume that all biomass in the reactor is exposed to bulk phase concentrations neglecting the effect of mass transport limitations (i.e., 0-D). In wastewater treatment biofilms are relatively thick and are usually mass-transfer-limited. Thus, the 0-D modeling approach that neglects mass transfer limitations is not useful except in special cases.
- *1-D homogeneous biofilm (single limiting substrate).* This approach takes into account mass transfer limitations into

the biofilm and the corresponding effects on concentration profiles and substrate flux into the biofilm. It is assumed that active bacteria are homogeneously distributed over the thickness of the biofilm. The approach is valid only if calculations are performed for the limiting substrate which has to be determined *a priori* by the user as described in Morgenroth (2008). The flux of the nonlimiting substrates can be calculated based on reaction stoichiometry.

- *1-D homogeneous biofilm (multiple substrates and multiple biomass components).* One key aspect of modeling biofilms is to evaluate the competition and coexistence of different groups of bacteria and local environmental conditions. Local process conditions can be accurately determined by calculating penetration depths for different soluble substrates. Based on the fluxes the growth of individual groups of bacteria can be determined. To simplify calculations it can be assumed that all bacterial groups are homogeneously distributed over the thickness of the biofilm (Rauch *et al.*, 1999; Boltz *et al.*, 2009a).
- *1-D heterogeneous biofilm.* Different groups of bacteria are competing in a biofilm not only for substrate but also for space where bacteria toward the surface are less influenced by mass transport limitations. Bacteria growing toward the base of the biofilm are often rate limited by substrate availability resulting from mass transfer limitations. On the other hand, these bacteria are better protected from detachment. These 1-D heterogeneous biofilm models must keep track of local growth and decay of the different bacterial groups and of detachment to calculate biomass distributions over the biofilm thickness.
- *2-D and 3-D biofilm models.* Practically, biofilms are not as smooth and flat as is assumed in 1-D biofilm models. Mathematical models have been developed that predict the development of biofilms in two or three dimensions, the influence of the heterogeneous structure on fluid flow, and ultimately the combination of fluid flow and biofilm structure on substrate availability and removal inside the biofilm. For most questions related to practical biofilm reactor studies, such multi-dimensional models are not necessary. However, it is important for model users to recognize that biofilm structure influences local fluid dynamics and external mass transport, which are simultaneously affected by biofilm reactor appurtenances and mode of operation. Such interactions are not accounted for in existing 1-D biofilm models due to a rigid segregation of the bulk phase, mass transfer boundary layer, and biofilm (which is assumed to have a uniform thickness and smooth surface). Multi-dimensional biofilm models have been used to quantify the influence of biofilm structure on local fluid dynamics and external mass transport (Eberl *et al.*, 2000).

Different scales of heterogeneity are relevant for biofilm reactors. The length scale of the biofilm thickness, which is on the order of 100–1000 μm , is taken into account in 1-D and multi-dimensional biofilm models. Substrate fluxes from these simulations can then be integrated into models describing overall reactor performance where the length scale is on the order of 1 m. However, heterogeneities can also be observed in biofilm reactors in between these scales where, in some cases, patchy biofilms are observed and where certain

parts of the biofilm support medium is bare while at other areas dense biofilms develop ($\sim 1\text{--}10\text{ cm}$). These heterogeneities in between the small and the large scale are typically not considered in biofilm models and it is not clear to what extent they are relevant (Takács *et al.*, 2010).

No simple and general recommendations can be given on what approach is the most appropriate for describing biofilm reactors. Wanner *et al.* (2006) provided a detailed description of different modeling approaches and a discussion on how the modeling approaches compare for different modeling scenarios. Many commercially available wastewater-treatment plant simulators used for biofilm reactor design and evaluation takes into account multiple substrates and biomass fractions in either a heterogeneous or a homogeneous 1-D biofilm. Examples of software, and references to the biofilm model that constitutes the biofilm reactor module, that is applied to design, optimize, and evaluate, typically pilot- or full-scale biofilm reactors are summarized in Table 1.

4.15.3.4 Biofilm Model Features

Excellent guides exist that describe the mathematical modeling of biofilms (see Wanner *et al.*, 2006; Morgenroth, 2008). However, the state of biofilm modeling is subject to several uncertainties. In the context of this chapter, Boltz *et al.* (2010a) summarized the following items which cause uncertainty when using 1-D biofilm models to describe biofilm reactors: (1) the fate of particulate substrates, (2) biofilm distribution in the reactor and the effect biofilms have on reactor components, (3) dynamics and fate of biofilm detachment, (4) quantifying concentration gradients external to the biofilm surface, and (5) a lack of generally accepted biofilm reactor

model calibration protocol. Parameter estimation and model calibration are serious concerns for process engineers who apply biofilm models in engineering practice. Therefore, parameters that are critical components of biofilm reactor models (that use a 1-D mathematical biofilm model) are introduced, including: attachment (k_{at}) and detachment (k_{det}) coefficients, the mass transfer boundary layer, rate-limiting substrate diffusivity coefficient inside the biofilm ($D_{i,rate\text{--}limiting}$), and the biokinetic parameters maximum growth rate (μ) and the rate-limiting substrate half-saturation coefficient ($K_{i,rate\text{--}limiting}$) (Boltz *et al.*, 2010b).

4.15.3.4.1 Attachment and detachment process kinetics and rate coefficients

An accurate mathematical description of particle attachment and detachment processes is a critical component of biofilm (reactor) models. Unfortunately, attachment/detachment process mechanics are poorly understood. Conceptually, particles suspended in the bulk liquid are hydrodynamically transported to the vicinity of the biofilm. From the bulk phase, particles are subjected to concentration gradients external to the biofilm surface. Particles enter the biofilm matrix through channels, crevasses, and other structural irregularities where they attach to the biofilm surface (see Reichert and Wanner (1997) for a description of particle transport within the biofilm matrix). Once entrapped, the particles can be hydrolyzed by extracellular polymeric enzymes resulting in soluble substrate that diffuses into the biofilm. Then, the soluble substrate is subject to well-known biochemical transformation processes that yield biomass. Alternatively, particles that have attached to the biofilm surface from the bulk phase remain unaltered and exit the system after detaching from the biofilm

Table 1 Biofilm models used in practice (Boltz *et al.* 2010b)

Software	Company	Biofilm model type and biomass distribution	Reference
AQUASIM TM	EAWAG, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland (www.eawag.ch/index_EN)	1-D, DY, N; heterogeneous	Wanner and Reichert (1996) (modified)
AQUIFAS TM	Aquaregen, Mountain View, California (www.aquifas.com)	1-D, DY, SE and N, heterogeneous	Sen and Randall (2008)
BioWin TM	EnviroSim Associates Ltd., Flamborough, Canada (www.envirosim.com)	1-D, DY, N, heterogeneous	Wanner and Reichert (1996) (modified), Takács <i>et al.</i> (2007)
GPS-X TM	Hydromantis Inc., Hamilton, Canada (www.hydromantis.com)	1-D, DY, N, heterogeneous	Hydromantis (2006)
Pro2D TM	CH2M HILL Inc., Englewood, Colorado (www.ch2m.com/corporate)	1-D, SS, N(A), homogeneous (constant L_f)	Boltz <i>et al.</i> (2009a; 2009b)
Simba TM	ifak GmbH, Magdeburg, Germany (www.ifak-system.com)	1-D, DY, N, heterogeneous	Wanner and Reichert (1996) (modified)
STOAT TM	WRc, Wiltshire, England (www.wateronline.com/storefronts/wrcgroup.html)	1-D, DY, N, heterogeneous	Wanner and Reichert (1996) (modified)
WEST TM	MOSTforWATER, Kortrijk, Belgium (www.mostforwater.com)	1-D, DY, N(A) ^a , N ^b , homogeneous ^a , heterogeneous ^b	Rauch <i>et al.</i> (1999) ^a , Wanner and Reichert (1996) (modified) ^b

^aRauch *et al.* (1999) is linked with the definition 'N(A)' and 'homogeneous'.

^bWanner and Reichert (1996) (modified) is linked with the definition 'N' and 'heterogeneous'.

1-D, one dimensional; DY, dynamic; N, numerical; N(A), numerical solution using analytical flux expressions; SE, semi-empirical; SS, steady-state. Hydromantis, Inc. (2006) Attached growth models. In: *GPS-X Technical Reference*, pp. 157–185 (unpublished).

matrix. Most of the heterogeneous 1-D biofilm models listed in **Table 1** describe the rate of particle attachment (r_{at}) as a first-order process ($r_{at} = k_{at} \cdot X_{TSS,bulk}$) depending on an attachment rate coefficient (k_{at}) and the bulk-liquid particle concentration. Boltz and La Motta (2007) presented a model describing variability in this parameter with influent particle concentrations. The researchers postulated that increasing particle concentrations ultimately reduced the biofilm surface area available for particle attachment; thereby, the particle attachment coefficient decreases until reaching a plateau. The plateau was considered commensurate with a condition in which a minimum biofilm area was consistently available as a result of continuously detaching biofilm fragments (during steady operating conditions – variable hydrodynamics can influence biofilm structure). Given the current state of the science, experimental data are required to develop/validate or evaluate existing approaches for simulating the fate of particles in biofilm reactors.

Steady-state biofilm models have assumed a constant biofilm thickness in which case biofilm growth is balanced by internal loss (e.g., decay and hydrolysis, or endogenous respiration) and/or detachment. This approach has been successfully applied to simulate biofilm reactors at steady state, but their dynamic simulation requires that a detachment model is included despite rather limited mechanistic understanding. The rate (Morgenroth and Wilderer, 2000; Boltz *et al.*, 2010a) and category (i.e., abrasion, erosion, sloughing, and predator grazing) of detachment can have a significant influence on biofilm reactor simulation and performance (Morgenroth, 2003). Kissel *et al.* (1984) stated that problems inherent to biofilm detachment modeling include a poor understanding of fundamental (biofilm detachment) process mechanics and the inability to predict exactly at what location inside the biofilm that detachment will occur. Detachment location is important when taking into account a heterogeneous biofilm distribution throughout the reactor either by combining multiple 1-D simulations or by 2- or 3-D modeling (Morgenroth *et al.*, 2000). Unlike attachment, Boltz *et al.* (2010a) described eight different biofilm detachment rate expressions (r_{det}) for the heterogeneous 1-D biofilm models listed in **Table 1**. Detachment rate equations can be categorized based on the aspect controlling detachment: biofilm thickness (L_f), shear, or growth/activity. Mixed-culture biofilms, such as those growing in a combined carbon oxidation and nitrification MBBR, are subject to competition for substrate between fast-growing heterotrophic and slow-growing autotrophic organisms (primarily for dissolved oxygen). Morgenroth and Wilderer (2000) performed a modeling study that demonstrated ammonium flux was significantly influenced by the mode of simulated detachment. Essentially, biofilm (thickness) dynamics influenced competition for substrate between heterotrophic and autotrophic organisms; high variations in biofilm thickness dynamics favored the faster growing heterotrophic organisms.

4.15.3.4.2 Concentration gradients external to the biofilm surface and the mass transfer boundary layer

Biofilms growing virtually in all full-scale biofilm reactors are subject to some degree of substrate concentration gradients

external to the biofilm surface. Concentration gradients external to the biofilm surface are not explicitly simulated in 1-D biofilm models. Rather, the reduction in concentration of any substrate is modeled as a mass-transfer resistance, R_L ($= L_L/D_w$). Based on the observation that the external mass-transfer resistance, R_L , is more dependent on biofilm reactor bulk-liquid hydrodynamics than biofilm thickness or surface heterogeneity, the impact of R_L can be accounted for by empirical correlations (Boltz *et al.*, 2010a). However, a realistic description of hydrodynamic effects ultimately depends on an accurate estimate of the mass-transfer boundary layer thickness L_L . Therefore, the mass-transfer boundary layer thickness is an important facet of biofilm-reactor models that use a 1-D biofilm model. Despite the potential significant impact the mass-transfer boundary layer thickness may have on biofilm-reactor model results and process design, factors influencing the interface between the biofilm model and reactor scale is one important feature of biofilm-reactor models that is not well understood.

4.15.3.4.3 Diffusivity coefficient for the rate-limiting substrate inside the biofilm

Soluble substrates are primarily transported into biofilms by a combination of advection and molecular diffusion. Generally, the most important mechanism is molecular diffusion (Zhang and Bishop, 1994). The largest component of biofilm is water, but the diffusivity of a solute inside the biofilm is generally less than that in water because of the tortuosity of the pores and minimal biofilm permeability. Consequently, an effective diffusivity must be applied. Many biofilm reactor models treat this value as 80% of the diffusivity in water (i.e., $D_w = D_f/0.80$) (Stewart, 2003). However, it has been demonstrated that the effective diffusion coefficient ($D_{f,i}$) for any soluble substrate i can vary with depth inside the biofilm (Beyenal and Lewandowski, 2000). The effective diffusivity decreases with depth because of increasing density and decreasing porosity and permeability of the biofilm with depth. Flow velocity past the biofilm is a major influencing factor determining biofilm density. Varying liquid velocity in the vicinity of the biofilm surface can influence a soluble substrate effective diffusivity inside a biofilm. Consequently, the varying flow rate can affect the rate of internal mass transfer and transformation rates (Bishop, 2003). Turbulent, high-shear stress environments result in planar and denser biofilms while quiescent, low-shear stress environments will result in rough and less dense biofilms (van Loosdrecht *et al.*, 1995). Picioreanu (1999) defined a growth number ($G = L_f^2 \cdot \mu_{max} \cdot X_f / (D_f \cdot C_B)$) that can be related to biofilm roughness. According to Picioreanu (1999), the biofilm may have a dense solid matrix and a flat surface when $G < 5$. However, if $G > 10$ the biofilm may develop complex structures such as mushroom clusters and streamers.

4.15.3.4.4 Parameters: estimation and variable coefficients

A parameter is an arbitrary constant whose value characterizes a system member. Biokinetic parameter estimation is a serious concern for those who seek to use biofilm models for biofilm reactor process design and research because most parameter values cannot be measured directly in full-scale municipal

wastewater-treatment plants (Brockmann *et al.*, 2008). Parameters exist for every aspect of biofilm models, including stoichiometry, kinetics, mass transfer, and the biofilm itself. A majority of parameter values in modern process models (e.g., those described by Henze *et al.* (2000)) have a substantial database that serves to define a relatively narrow range of values that are applicable to a majority of municipal wastewater-treatment systems. Existing biofilm models are relatively insensitive to changes in a majority of the biokinetic parameter values, most of which are described by Henze *et al.* (2000), within a range of values reported in the literature except for, as an example, the autotrophic nitrifier maximum growth rate (μ). However, the mathematical description of some processes includes variable, or lumped, parameters. These parameter values are often system specific and subject to significant uncertainty. The lumped parameters account for an incomplete mechanistic description of the simulated process. Lumped parameters in a majority of biofilm models, including those described in this chapter, are the oxygen affinity constant for autotrophic nitrifiers ($K_{O_2,A}$), endogenous respiration rate constants (b_{res}), attachment rate coefficient (k_{at}), detachment rate coefficient (k_{det}), mass-transfer boundary layer thickness (L_L), ratio of diffusion in biofilm to diffusion in water (D_f/D_w). Identifying parameter subsets that require definition for biofilm model calibration has been the subject of several investigations by Smets *et al.* (1999), Van Hulle *et al.* (2004), and Brockmann *et al.* (2008).

4.15.3.4.5 Calibration protocol

Application of a dynamic biofilm model to describe full-scale municipal wastewater-treatment processes requires a calibration of the selected model. *Ad hoc* expert-based trial and error and standardized systematic approaches have been used to calibrate process models. Sin *et al.* (2005) presented a critical comparison of systematic calibration protocols for activated sludge models. These protocols have many similarities that are applicable to biofilm reactor models including goal definition, data collection/testing/reconciliation, and validation. The major differences between protocols reported by Sin *et al.* (2005) are related to the measurement campaign, experimental methods for influent wastewater characterization, and parameter subset selection and calibration. The major differences speak to areas of systematic calibration protocols for activated sludge models that will almost certainly be exasperated when creating systematic protocol for calibrating a biofilm reactor model. Certainly, additional tests will be required to characterize the physical attributes of both suspended growth and biofilm compartments, and mathematical biofilm models have more parameters than activated sludge models. Furthermore, the biofilm compartment parameters must be estimated from bulk-phase measurements in order to have a timely and cost-effective approach to calibrating biofilm reactor models.

Sin *et al.* (2008) reported the calibration of a dynamic biologically active (continuously backwashing) filter model using traditional expert-based manual trial and error. The researchers manipulated system-specific parameters related to attachment, detachment, and biofilm thickness. After calibration, Sin *et al.* (2008) successfully tested the calibrated

model for another period. Similarly, Bilyk *et al.* (2008) reported the calibration of a denitrification filter model by adjusting assumed biofilm thickness and incorporating the assimilative denitrification reaction. Both of these biofilm reactor model calibration efforts were based on bulk-phase measurements, but only Sin *et al.* (2008) utilized measured characteristics of the biofilm. Such adjustments to system-specific biofilm and biokinetic parameters in order to match observed data may not produce a properly calibrated model that is capable of describing a variety of design conditions for a wastewater-treatment plant. As previously discussed, the attachment coefficient, for example, has been experimentally demonstrated (and described mathematically) to change as a function of particle (total suspended solids) load (Boltz and La Motta, 2007). Then, it may be argued that adjusting the attachment coefficient (during calibration) to match an observed dataset would naturally render the calibrated model incapable of describing another scenario with a different particle load. Suffice it to say that a reliable and transparent description of recommended approaches for the application and calibration of biofilm models are required for the models to gain general acceptance, understanding, and become effectively used in engineering practice. Protocol defining methodology for sampling, testing, evaluating and applying data to mathematical biofilm reactor models is required. It is likely that existing biofilm reactor models will require improvement for reliable dynamic simulation in practice.

4.15.3.5 Biofilm Reactors in Wastewater Treatment

Biofilm reactors play an important role in environmental biotechnology, but many aspects of their design and operation remain poorly understood. Biofilm reactors can be traced to origination of modern water sanitation. Corbett (1903) reported the use of continuously distributed sewage flow over a fixed bed, and Stoddart (1911) reported the use of a coarse biofilm-covered medium dosed with a continuous trickling flow. These accounts are acknowledged as the creation of the TF process. Approximately 100 years following these reports significant advances in the design, academic understanding, and mathematical modeling of biofilms have led to the development of new and emerging biofilm reactors conducive to fundamentally based design approaches and the application of fundamentally based design and operation procedures for traditional biofilm reactors. Two processes – mass transfer and biochemical conversion – are characteristics of all biofilm reactors and influence biofilm structure and function. Compartments that are common to every biofilm reactor exist to optimize mass-transfer and biochemical conversion.

4.15.3.5.1 Biofilm reactor compartments

Biofilm reactors have five primary compartments: (1) influent wastewater (distribution) system; (2) containment structure; (3) biofilm carrier; (4) effluent water collection system; and (5) an aeration system (for aerobic processes and scour) or mixing system (for anoxic processes that require bulk-liquid agitation and biofilm carrier distribution). Five components influence local conditions inside the biofilm: (1) biofilm carrier surface (i.e., substratum); (2) biofilm (including both particulate and liquid fractions); (3) mass-transfer boundary

layer; (4) bulk liquid; and (5) gas phase (when significant). The components typical of biofilm reactors are described in context of some commercially available biofilm reactors. The five biofilm reactors described include the MBBR, BAF, FBBR, rotating biological contactor (RBC), and TF.

4.15.3.5.2 Moving bed biofilm reactors

The MBBR is a two- (anoxic) or three- (aerobic) phase system with a buoyant free-moving plastic biofilm carrier that requires mechanical mixing or aeration to distribute carriers throughout the tank. The process includes a submerged, completely mixed biofilm reactor and liquid–solids separation unit (Ødegaard, 2006). A range of pollutant loading and bulk-phase external carbon sources in denitrification MBBRs and dissolved oxygen concentrations in carbon-oxidation and/or nitrification MBBRs have been applied, and system response evaluated (Lazarova and Manem, 1994). It has been demonstrated that MBBRs are capable of processing wastewater to meet a variety of effluent water-quality standards ranging, for example, from the US Environmental Protection Agency definition of secondary treatment (30 mg TSS l⁻¹ and 30 mg BOD₅/l monthly average) to more stringent enhanced nitrogen removal limits (e.g., total nitrogen less than 3–5 mg l⁻¹) under a variety of loading conditions. The MBBR process is capable of meeting similar treatment objectives as the activated sludge process for carbon oxidation, nitrification, and denitrification, but the MBBR makes use of a smaller tank volume than a clarifier-coupled activated sludge system. Biomass retention is clarifier independent; therefore, solids loading in liquid–solids separation unit are significantly reduced when compared with the activated sludge process. Because it is a continuously flowing process, the MBBR does not require a special operational cycle for biofilm thickness control (e.g., backwashing in a BAF or flushing in a TF). Hydraulic head loss and operational complexity is minimal. The MBBR offers much of the same flexibility to manipulate the process flow sheet (to meet specific treatment objectives) as the activated sludge process. Multiple reactors can be configured in series without the need for intermediate pumping or return activated sludge pumping (to accumulate mixed liquor). Liquid–solids separation may be achieved with a variety of processes including sedimentation basins, dissolved air flotation, cloth-disk and membrane filters. The MBBR is well suited for retrofit installation into existing municipal wastewater-treatment plants.

An MBBR may be a single reactor or several reactors in a series. Typically, each MBBR has a length-to-width ratio ($L:W$) in the range of 0.5:1–1.5:1. Plans with an $L:W$ greater than 1.5:1 can result in nonuniform distribution of the biofilm carriers. MBBRs contain a plastic biofilm carrier volume up to 67% of the liquid volume. Screens are typically installed with one MBBR wall and allow treated effluent to flow to the next treatment step while retaining the free-moving plastic biofilm carriers. Aerobic MBBRs use a diffused aeration system to evenly distribute the plastic biofilm carriers and meet process oxygen requirements. On the other hand, anoxic MBBRs use mechanical mixers to evenly distribute the plastic biofilm carriers because there is no process oxygen requirements. Each process mechanical component is submerged. Figure 22

depicts the Williams-Monaco WWTP, Commerce City, Colorado, a two-train bioreactor that consists of four MBBRs in series.

The biofilm carriers are extruded or molded from either virgin or recycled high-density polyethylene (HDPE). Table 2 summarizes characteristics of several commercially available plastic biofilm carriers. The carriers are slightly buoyant and have a specific gravity between 0.94 and 0.96 g cm⁻³. Both native and biofilm-covered plastic biofilm carriers have a propensity to float in quiescent water. Biofilms primarily develop on the protected surface inside the plastic biofilm carrier. For this reason, the specific surface areas of plastic biofilm carriers listed in the table exclude areas not inside the plastic carrier. The listed bulk-specific surface area, which is based on 100% carrier fill, is characteristic of a plastic biofilm carrier. The net specific surface area is characteristic of plastic biofilm carrier and fill percentage. For example, if a plastic biofilm carrier has a 500 m² m⁻³ bulk-specific surface area, then the net specific surface area at 50% carrier fill is 250 m² m⁻³. Similarly, the net liquid volume displacement at 50% carrier fill is 0.0725 for a plastic biofilm carrier having a characteristic 0.15-bulk-liquid volume displacement (at 100% carrier fill).

Plastic biofilm carriers are retained in an MBBR by horizontally configured cylindrical screens or vertically configured flat screens as shown in Figure 23. Aerobic zones typically contain cylindrical screens; anoxic zones contain the flat wall screens. Cylindrical screens are desired. They extend horizontally into the upward-flowing air bubbles imparted by the diffuser grid which aids in scouring any accumulated debris. Energy imparted by the mechanical mixers is insufficient to dislodge debris accumulated on the flat wall screen. Therefore, scouring of flat screens is accomplished with a sparging air header in a denitrification MBBR. Removing the debris retained on a screen aids in maintaining hydraulic throughput. Hydraulically, an MBBR is commonly designed to process a maximum approach velocity (based on the tank cross-sectional area perpendicular to forward flow) in the range 30–35 m h⁻¹. Screen area is defined by the maximum allowable head loss through the screens, which is typically in the range of 5–10 cm. The screen superficial hydraulic load is typically in the range of 50–55 m h⁻¹ for average design conditions. The screens and their supporting structural assemblies, if required, are typically constructed from stainless steel and may be from wedge-wire mesh or perforated plates.

Low-pressure diffused air is applied to aerobic MBBRs. The airflow enters the reactor through a network of air piping and diffusers that are attached to the tank bottom. Airflow has the dual purpose of meeting process oxygen requirements and uniformly distributing plastic biofilm carriers. To promote uniform distribution of the plastic biofilm carriers, the diffuser grid layout and drop pipe arrangement provide a rolling water circulation pattern. Coarse-bubble diffusers are typically used in moving bed reactors (Figure 25). Coarse-bubble diffusers typically used in MBBRs are stainless steel pipes with circular orifices along the underside. These coarse-bubble diffusers are less affected by scaling and fouling because of the large dimension and turbulent airflow through the discharge orifice (Stenstrom and Rosso, 2008). As a result, coarse-bubble diffusers require less maintenance than fine-bubble diffusers. The coarse-bubble diffusers are designed with a structural end

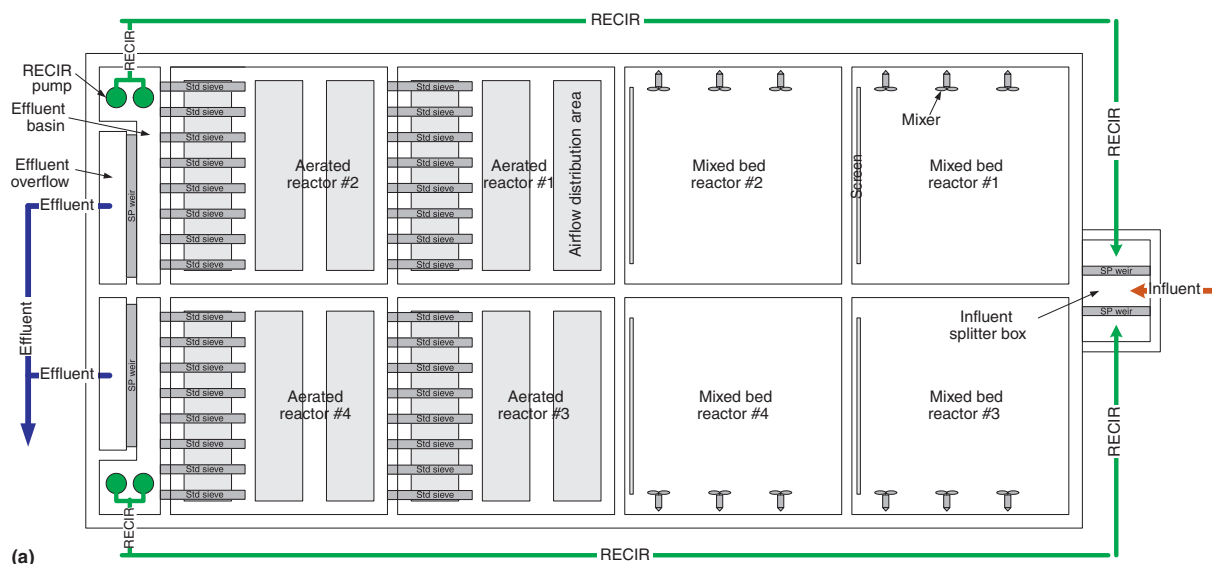


Figure 22 (a) Moving bed biofilm reactor at the Williams-Monaco Wastewater Treatment Plant, Colorado, USA. (b) Schematic representation of the photographed system which illustrates the system consisting of two parallel trains each with four reactors in series.

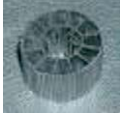
support that enables them to withstand the weight of plastic biofilm carriers when the MBBR is out of service and drained.

Denitrification MBBRs use mechanical mixers to agitate the bulk of the liquid and to distribute plastic biofilm carriers uniformly throughout the tank. The mechanical mixers are typically rail-mounted submersible (wet motor) units. State-of-the-art submersible mechanical mixers typically have a maximum 120-rpm impeller speed and a minimum of three blades per impeller. The mixer uses a stainless steel backward-curve propeller with a round bar welded along its leading edge to avoid damage to the plastic biofilm carriers and impeller wear. The mixer has a large diameter impeller with a fairly low rotational speed (90 rpm at 50 Hz and 105 rpm at 60 Hz). The plastic biofilm carriers float in quiescent water. As a result, the mixers need to be located near the water surface but not so close as to create an air-entraining vortex. A slight negative

inclination of mixer orientation helps maintain the rolling-water circulation pattern and uniformly distribute plastic biofilm carriers (see Figure 24). Rail-mounted units facilitate access to the mixer when maintenance is required. The mixers are typically sized to input 25 W m^{-3} of reactor volume.

Carbon-oxidizing MBBRs are classified as low-rate, normal-rate, or high-rate bioreactors. Low-rate carbon-oxidizing MBBRs promote conditions for nitrification in downstream reactors. High- and normal-rate MBBRs are strictly carbon-oxidizing bioreactors. In the absence of site-specific pilot-scale observations or a calibrated mathematical model, high-rate MBBRs are typically designed to receive a filtered BOD_5 load in the range of $15\text{--}20 \text{ g m}^{-2} \text{ d}^{-1}$ at 15°C . This corresponds to total BOD_5 loads as high as $45\text{--}60 \text{ g m}^{-2} \text{ d}^{-1}$ at 15°C (Ødegaard, 2006). To reach secondary treatment effluent standards, a hydraulic residence time less than 30 min is not

Table 2 Moving bed biofilm reactor plastic biofilm carrier characteristics^a

Manufacturer	Name	Bulk specific surface area, weight, gravity	Nominal carrier dimensions (depth; diameter)	Carrier photo
Veolia Inc.	AnoxKaldnes™ K1	500 m ² m ⁻³ 145 kg m ⁻³ 0.96–0.98	7.2 mm; 9.1 mm	
	AnoxKaldnes™ K3	500 m ² m ⁻³ 95 kg m ⁻³ 0.96–0.98	10 mm; 25 mm	
	AnoxKaldnes™ Biofilm Chip (M)	1,200 m ² m ⁻³ 234 kg m ⁻³ 0.96–1.02	2.2 mm; 45 mm	
	AnoxKaldnes™ Biofilm Chip (P)	900 m ² m ⁻³ 173 kg m ⁻³ 0.96–1.02	3 mm; 45 mm	
Infilco Degremont Inc.	ActiveCell™ 450	450 m ² m ⁻³ 134 kg m ⁻³ 0.96	15 mm; 22 mm	
	ActiveCell™ 515	515 m ² m ⁻³ 144 kg m ⁻³ 0.96	15 mm; 22 mm	
Aquise	ABC4™	600 m ² m ⁻³ 150 kg m ⁻³ 0.94–0.96	14 mm; 14 mm	
	ABC5™	660 m ² m ⁻³ 150 kg m ⁻³ 0.94–0.96	12 mm; 12 mm	
Entex Technologies Inc.	BioPortz™	589 m ² m ⁻³	14 mm, 18 mm	

^aAs reported by manufacturer.

Modified from Boltz JP, Morgenroth E, deBarbadillo C, *et al.* (2010b) Biofilm reactor technology and design. In: *Design of Municipal Wastewater Treatment Plants*, WEF Manual of Practice No. 8, ASCE Manuals and Reports on Engineering Practice No. 76, 5th edn, vol. 2, ch. 13, (ISBN P/N 978-0-07-166360-1 of set 978-0-07-166358-8; MHID P/N 0-07-166360-6 of set 0-07-166358-4). New York: McGraw-Hill.

recommended. Medium-rate MBBRs designed for meeting basic secondary treatment standards are typically designed for a loading of 5–10 g BOD₅ m⁻² d⁻¹ at 10 °C, depending on the choice of liquid–solids separation process. Values in the higher range are used when coagulation occurs before the separation unit; values in the lower range are used without coagulation. Studying a pilot-scale combined carbon oxidation and nitrification MBBR receiving primary effluent and a (tertiary) nitrification MBBR receiving secondary effluent while maintaining a 4–6 g m⁻³ bulk-liquid dissolved-oxygen concentration in both units, Hem *et al.* (1994) observed that a total BOD₅ load of 1–2 g m⁻² d⁻¹ resulted in nitrification rates

from 0.7 to 1.2 g m⁻² d⁻¹, a total BOD₅ load of 2–3 g m⁻² d⁻¹ resulted in nitrification rates from 0.3 to 0.8 g m⁻² d⁻¹, and a total BOD₅ load greater than 5 g m⁻² d⁻¹ resulted in virtually no nitrification.

4.15.3.5.3 Biologically active filters

BAFs have natural mineral, structured or random plastic media that supports biofilm growth and serves as a filtration medium. Solids accumulated from filtration and biochemical transformation are removed by backwashing. Media density influences BAF configuration and backwash regimes. BAF

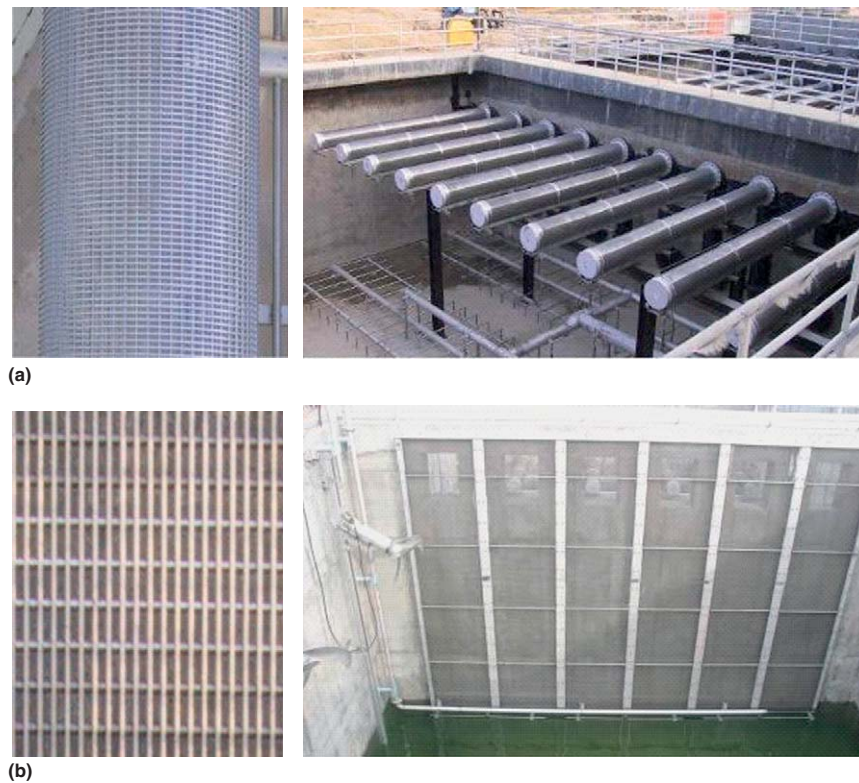


Figure 23 (a) Horizontal cylindrical screens constructed of wedge wire. Stainless steel coarse-bubble diffusers typically used in aerobic MBBRs are also pictured on the tank floor. (b) Flat wall screen constructed of wedge wire. A single air-header is pictured. Air is periodically introduced to scour debris accumulated on the screen.

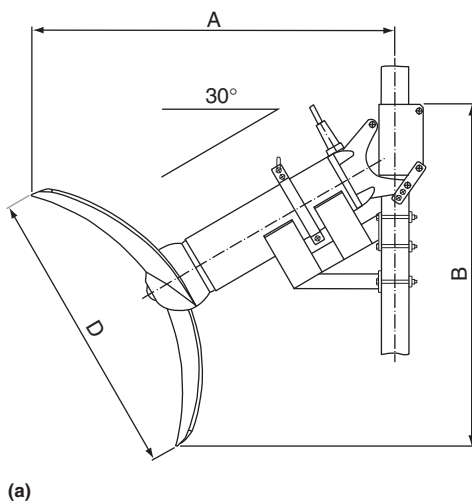


Figure 24 (a) Schematic and (b) picture of mechanical mixers that are specially designed for anoxic moving bed biofilm reactors.

influent requires preliminary and primary treatment. Historically, the acronym BAF has meant biological aerated filters which have been used to aerated biofilters used for secondary treatment. However, Boltz *et al.* (2010b) revised the acronym BAF to cover all BAFs, including those that operate under anoxic conditions for denitrification. BAFs are characterized by their media configurations and flow regime.

Downflow BAFs with media heavier than water include the Biocarbone[®] process, which was marketed during the 1980s for secondary and tertiary treatment, and packed-bed tertiary denitrification filters such as the Tetra Denite[®] process. These BAFs are backwashed using an intermittent counter-current flow. Upflow BAFs with media heavier than water such as the Inflico Degremont Biofor[®] process have been used for

secondary and tertiary treatment. The systems make use of expanded clay or another mineral media. These BAFs are backwashed using an intermittent concurrent flow. BAFs with floating media such as the Veolia Biostyr® process have also been used for secondary and tertiary treatment, and uses polystyrene, polypropylene, or polyethylene media. These BAFs operate with an intermittent backwash counter-current flow. Continuous backwashing filters operate in an upflow mode and contains media that is heavier than water. The media continuously moves counter-current to the wastewater flow (i.e., downward), and is continuously channeled to a center air lift where it is scoured, rinsed, and returned to the top of the media bed. Nonbackwashing submerged filters consist of a submerged static media bed, and have been called submerged aerated filters (SAFs). Solids are not retained in these filters. Therefore, nonbackwashing submerged filters require a dedicated liquid–solids separation process.

A downflow BAF with media heavier than water, such as the Tetra Denite® filter, is illustrated in Figure 25. The

Denite® process has been used since the late 1970s for meeting stringent total nitrogen limits while providing a filtered effluent. Methanol or another external carbon source is added to the influent wastewater stream to promote biological denitrification. A typical installation includes 1.8 m of 2–3 mm diameter sand media over 457 mm of graded support gravel. In a downflow denitrification BAF, the backwash cycle typically consists of a brief air scour followed by an air–water backwash and water rinse cycle. Backwash water and air scour flow rates are typically 15 and 90 m³ m⁻² h⁻¹, respectively. Backwash water usage is typically 2–3% of the average flow being treated. Nitrogen gas accumulates in the media. A releasing mechanism is pumping backwash water up through the media bed for a short duration. The denitrification capacity between nitrogen release cycles typically ranges from 0.25 to 0.5 kg NO_x-N m⁻².

An upflow BAF with media heavier than water, such as the Infilco Degremont Biofor®, is illustrated in Figure 26. The Degremont Biofor® operates such that solids are trapped

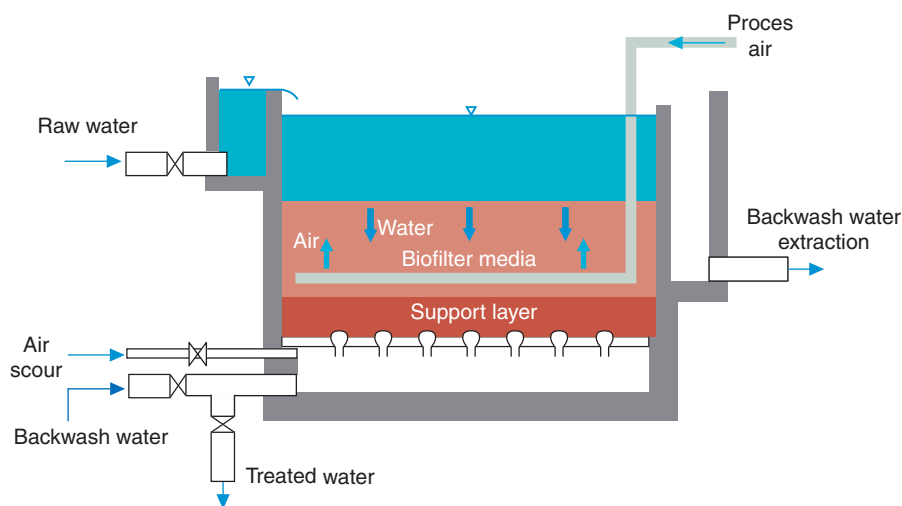


Figure 25 Downflow BAF with media heavier than water (e.g., Biocarbone® and Tetra Denite®). From ATV (1997) *Biologische und weitergehende Abwasserreinigung* (German), 4th edn. Berlin: Ernst and Sohn as presented by Tschui (1994).

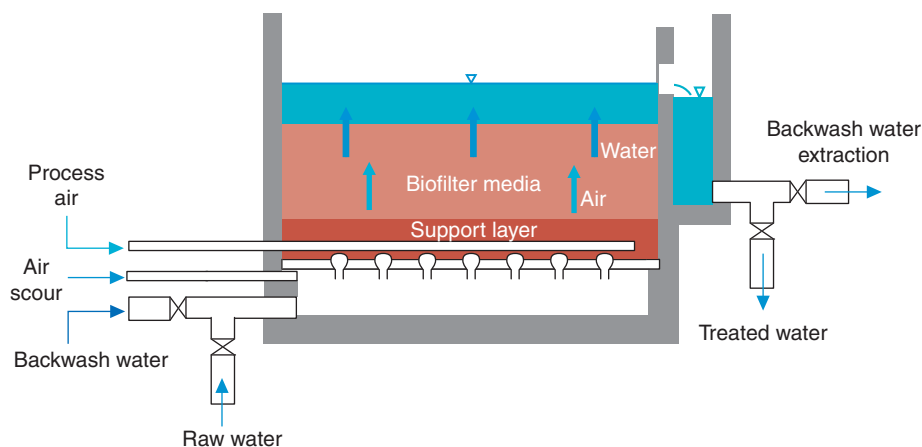


Figure 26 Upflow BAF with media heavier than water (e.g., Infilco Degremont Biofor®). From ATV (1997) *Biologische und weitergehende Abwasserreinigung* (German), 4th edn. Berlin: Ernst and Sohn as presented by Tschui (1994).

mostly in the lower part of the filter medium during normal operation and are removed through backwashing and applying scour air. As the backwash consists of concurrent scour air and backwash water, accumulated solids travel up through the media bed before being released at the top. Three types of media can be used in the Biofor[®] depending on the application; the media types include expanded clay, expanded shale (both in the form of spherical grains with an effective size of 3.5 or 4.5 mm), and angular grains (with an effective size of 2.7 mm). The media form a submerged, fixed bed in the bottom of the reactor. The media bed typically has a height of 3–4 m with approximately 1-m freeboard. The grains-specific surface area is approximately $1640 \text{ m}^2 \text{ m}^{-3}$. Influent water to the bed flows through a plenum and nozzle air/water distribution system. The nozzles are installed in a false floor located approximately 1 m above the filter floor. Nozzles in the false floor are subject to clogging. Therefore, backwash water and scour air flow through the same plenum/nozzle system. Process air is introduced through separate air diffusers located in the media bed above the inlet nozzles. A key issue with the backwash of sunken media systems is the potential for boils during backwashing. The flow will short-circuit through the line of least resistance. This will result in a boil, or violent eruption of the flow through the point of least resistance. Similar short circuits and boils can also occur if the nozzles are blocked. These boils can result in excessive media loss during backwashing. Therefore, to achieve even backwashing the water must be well distributed across the BAF plan area. Therefore, the headloss across the distribution system must be greater than the headloss through the bed.

An upflow BAF with floating media, such as the Veolia Biostyr[®], is illustrated in Figure 27. These processes use a floating bed of media to provide area for biofilm development and filtration. Coarse-bubble aeration diffusers exist at the bottom of the media to enhance the contact of air, water, and biomass (Rogalla and Bourbigot, 1990). The Biostyr[®] process uses light weight expanded polystyrene (specific gravity of 0.05). Alternatively, the Biobead[®] process uses

recycled polypropylene with a specific gravity slightly lower than 1. The Biostyr[®] reactor is partially filled with (2–6 mm) polystyrene beads. Process objectives determine selection of the bead size; larger beads can be more heavily loaded. The beads, which are lighter than water, form a floating bed in the upper portion of the reactor, typically a height of 3–4 m with approximately 1.5 m of freeboard. The top of the bed is restrained by a slab fitted with filtration nozzles to evenly collect treated wastewater. The clean specific surface area of spherical beads is $1000\text{--}1400 \text{ m}^2 \text{ m}^{-3}$. In the bottom of the reactor, influent is distributed by troughs formed in the base of the cells. Process air is distributed through diffusers located along the bottom of the reactor or within an aeration grid in the media bed. The latter is used if an anoxic zone is required for denitrification. Backwashing consists of counter-current air scour and backwash water flow. The Biobead[®] BAF process is similar to Biostyr[®], except that the media is larger and heavier, using polypropylene or polyethylene with a density of approximately 0.95. To prevent media attrition, a metal grid is fixed near the top of the reactor. Upflow floating media BAFs may also require a certain number of mini-backwashes (typically 4–8 and, in extreme cases, more than 10) to bump the filter, remove some solids, and lower headloss to achieve a complete filtration cycle of 24 or 48 h (which is the time between normal backwashes). The requirement for mini-backwashes plus normal backwashes can generate a significant backwash water volume. During demonstration testing in San Diego, California, USA, a single-stage carbon-oxidation BAF with floating media generated a backwash water volume in the range of 10.3–13.9% of influent flow, compared to a sunken media BAF which produced a backwash water volume in the range of 7.4–7.9% (Newman *et al.*, 2005).

An upflow continuous backwash BAF, such as the Parkson Dynsand[®], is illustrated in Figure 28. Moving bed, continuous backwash filters operate in an upflow mode and consist of media heavier than water. The media continuously moves downward, counter-current to the wastewater flow. These filters are used widely for tertiary suspended solids and turbidity

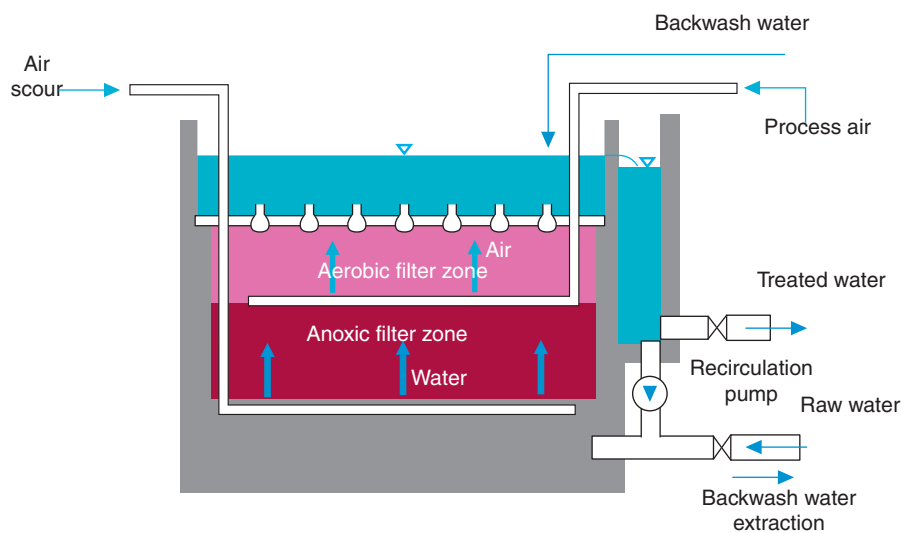


Figure 27 Upflow BAF with floating media (e.g., Veolia Biostyr[®]). Adapted from ATV (1997) *Biologische und weitergehende Abwasserreinigung* (German), 4th edn. Berlin: Ernst and Sohn as presented by Tschui (1994).

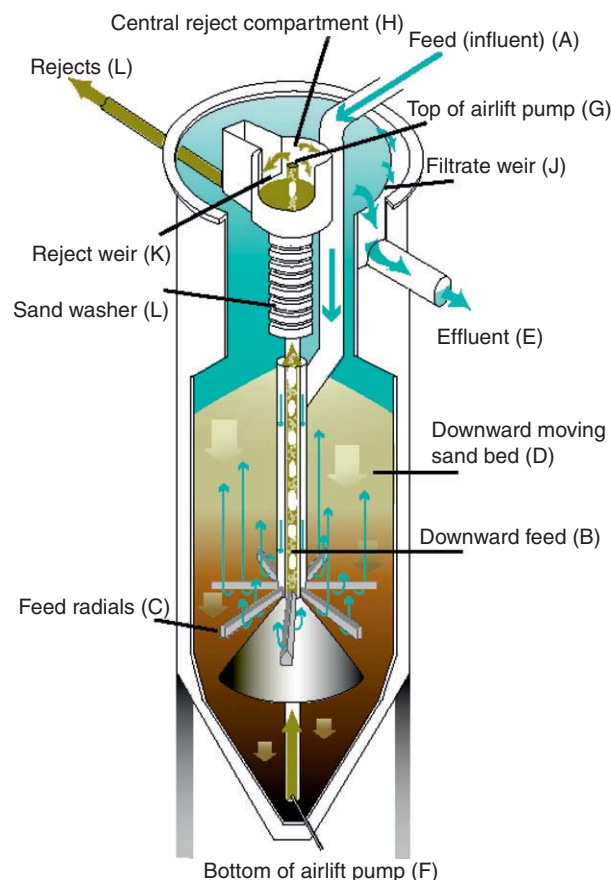


Figure 28 Parkson Dynasand® process schematic, continuous backwash BAF.

removal but have also been applied to separate stage nitrification and denitrification. Two commercially available systems using this technology are the Parkson DynaSand® and Paques Astrasand® filters. The filter cells are supplied as 4.65-m² modules with center airlift assembly. The effective media depth is typically 2 m, and sand media size typically ranges from approximately 1 to 1.6 mm. Influent wastewater enters the filter bed through radials located at the bottom of the filter. The flow moves up through the downward-moving sand bed and effluent flows over a weir at the top of the filter. The media, with the accumulated solids, is drawn downward to the bottom cone of the filter. Compressed air is introduced through an airlift tube extending to the conical bottom of the filter and rises upward with a velocity exceeding 3 m s⁻¹ creating an air pump that lifts the sand at the bottom of the filter through the center column. The turbulent upward flow in the airlift provides scrubbing action that effectively separates solids from the media before discharge to a wash box. There is a constant upward flow of liquid into the wash box (backwash water) controlled by the wash box discharge weir. Moving bed filter manufacturers typically set the reject weir to provide a wash water flow rate equivalent to approximately 10% of the forward flow at an average filter loading rate of 4.9 m h⁻¹. The backwash frequency is quantified by the bed turnover rate. To maintain sufficient biomass for denitrification, the bed turnover rate must be reduced to approximately 100–250 m h⁻¹.

Several media types are available for use in BAFs. Media selection is integral to meeting treatment objectives, flow and backwashing regimes. Typically, media can be categorized as mineral media and plastic media. In most cases, mineral media is denser than water and plastic media is buoyant. The media needs to resist breakdown from abrasion during backwashing and chemical degradation by constituents in municipal wastewater. Commercially available BAF systems and their media are listed in [Table 3](#).

Backwashing BAFs maximizes solids capture and filter run time. Proper backwashing requires filter bed expansion and rigorous scouring followed by efficient rinsing. Accumulation of solids and media (mud balling) results in wastewater short-circuiting and can result in excessive media loss. Feed characteristics and type of treatment provided by the BAF affect solids production and frequency requirements for backwashing. Biomass yield in tertiary BAF systems is typically low, so backwashing is relatively infrequent (i.e., one backwash per 36–48 h). Reactor characteristics and media type influence backwash frequency. More openly structured media capture fewer solids which reduces backwash frequency. During backwashing the media bed is typically expanded or fluidized (depending on the system) to allow for grain separation and free movement in order to remove as much accumulated solids as possible. [Table 4](#) compares typical BAF backwashing requirements.

BAFs designed for carbon oxidation and suspended solids removal in secondary treatment typically have volumetric BOD loading rates in the range of 1.5–6 kg m⁻³ d⁻¹. Average and peak HLRs for secondary and tertiary treatment systems are typically in the range of 4–8 and 10–20 m h⁻¹, respectively. As BAFs for secondary treatment are typically placed immediately downstream of primary clarifiers, the applied volumetric mass loading rate is almost always the limiting design parameter. Combined carbon oxidation and nitrification will proceed when the organic loading at lower temperatures is limited to 2.5 kg BOD m⁻³ d⁻¹ ([Rogalla et al., 1990](#)). Under these conditions a total Kjeldahl nitrogen removal rate of 0.4 kg N m⁻³ d⁻¹ may be achieved. Inversely, [Rogalla et al. \(1990\)](#) found that nitrification decreases when soluble COD loadings approach 4 kg m⁻³ d⁻¹. Ammonium removal of 80–90% can be achieved for ammonium loads in the range of 2.5–2.9 kg m⁻³ d⁻¹ ([Peladan et al., 1996](#)).

4.15.3.5.4 Expanded and fluidized bed biofilm reactors

Expanded bed biofilm reactors (EBBRs) and FBFRs use small media particles that are suspended in vertically flowing wastewater, so that the media becomes fluidized and the bed expands. Individual particles become suspended once the drag force of the relatively fast flowing wastewater (30–50 m h⁻¹) overcomes gravity and they are separated. In municipal applications, fluidized beds are typically used for tertiary denitrification. Design criteria for denitrifying FBFRs are listed in [Table 5](#). When treating groundwater or industrial wastewater, FBFRs are used for the removal of oxidized contaminants such as nitrate and perchlorate.

Suspension of the media maximizes the contact surface between microorganisms and wastewater. It also increases treatment efficiency by improving mass transfer because there

Table 3 Biologically active filter systems and commercially available media

Process	Supplier	Flow regime	Media	Specific gravity	Size (mm)	Specific surface area (m ² m ⁻³)
Astrasand [®]	Paques/Siemens	Upflow ^a	Sand	> 2.5	1–1.6	
Biobead [®]	Brightwater F.L.I.	Upflow	Polyethylene	0.95		
Biocarbhone [®]	OTV/Veolia	Downflow	Expanded shale	1.6	2–6	
Biofor [®]	Degremont	Upflow	Expanded clay	1.5–1.6	2.7, 3.5, and 4.5	1400–1600
Biolest	Stereau	Upflow	Pumice/pouzzolane	1.2		
Biopur	Sulzer/Aker Kvaerner	Downflow	Polyethylene		Structured	
Biostyr [®]	Kruger/Veolia	Upflow	Polystyrene	0.04–0.05	3.3–5	1000
Colox [™]	Severn Trent	Upflow	Sand	2.6	2–3	656
Denite [®]	Severn Trent	Downflow	Sand	2.6	2–3	656
Dynasand [®]	Parkson	Upflow ^a	Sand	2.6	1–1.6	
Eliminite [®]	FB Leopold	Downflow	Sand	2.6	2	
Submerged activated filter	Severn Trent	Up/down	Slag	2–2.5	28–40	240
			Washed gravel	2.6	19–38	

^aMoving bed.

From Boltz JP, Morgenroth E, deBarbadillo C, *et al.* (2010b) Biofilm reactor technology and design. In: *Design of Municipal Wastewater Treatment Plants*, WEF Manual of Practice No. 8, ASCE Manuals and Reports on Engineering Practice No. 76, 5th edn, vol. 2, ch. 13 (ISBN P/N 978-0-07-166360-1 of set 978-0-07-166358-8; MHID P/N 0-07-166360-6 of set 0-07-166358-4). New York: McGraw-Hill.

Table 4 Summary of biologically active filter (BAF) backwashing (BW) requirements

	Backwash water rate, m h ⁻¹	Air scour rate, m h ⁻¹	Total duration min ^c	Total backwash water volume per cell ^c	Total backwash water volume per cell ^d
Upflow, sunken media					
Normal BW	20 (8.2)	97 (5.3)	50	9.2 m ³ m ⁻²	12 m ³ m ⁻²
Energetic BW ^a	30 (12.3)	97 (5.3)	25	9.2 m ³ m ⁻²	10 m ³ m ⁻²
Upflow, floating media					
Normal BW	55 (22.5)	12 (0.65)	16	2.5 m ³ m ⁻³ media ^e	2.5 m ³ m ⁻³ media ^e
Mini-BW ^b	55 (22.5)	12 (0.65)	5	1.5 m ³ m ⁻³ media ^e	1.5 m ³ m ⁻³ media ^e
Downflow, sunken media	15 (6)	90 (5)	20–25	3.75–5 m ³ m ⁻²	3.75–5 m ³ m ⁻²
Upflow, moving bed ^f	0.5–0.6	Continuous through air lift	Continuous	55–67 m ³ d ⁻¹	55–67 m ³ d ⁻¹
	(0.2–0.24)				

^aEnergetic backwash once every 1–2 months depending on trend in “clean bed” headloss following normal backwash.^bMini-backwash applied as interim measure when pollutant load exceeds design load.^cBackwash duration reflects total duration of the typical backwash cycle, which includes valve cycle time and pumping and nonpumping steps. The duration of each step is adjustable via programmable logic controller and supervisory control and data acquisition control systems.^dThe total backwash water volume includes drain and filter to waste steps, where applicable.^eBackwash volume requirements for upflow floating media BAF typically are based on media volume rather than cell area because depths vary.^fContinuous backwash filter BW is based on a standard 4.65 m² cell and a typical weir setting for reject flow of approximately 2.3–2.8 m³ h⁻¹ cell⁻¹.

From Boltz JP, Morgenroth E, deBarbadillo C, *et al.* (2010b) Biofilm reactor technology and design. In: *Design of Municipal Wastewater Treatment Plants*, WEF Manual of Practice No. 8, ASCE Manuals and Reports on Engineering Practice No. 76, 5th edn, vol. 2, ch. 13 (ISBN P/N 978-0-07-166360-1 of set 978-0-07-166358-8; MHID P/N 0-07-166360-6 of set 0-07-166358-4). New York: McGraw-Hill.

is significant relative motion between the biofilm and flowing wastewater. Because of the balance of forces involved in particle fluidization and bed expansion, the smallest particles are found at the top and the largest at the bottom of the fluid bed. Therefore, the media particles should be graded to a relatively tight size range. The degree of bed expansion determines whether a bed is deemed expanded or fluidized. The transition lies between 50% and 100% expansion over the static bed height. This discussion assumes the upper limit: beds less than double static bed height

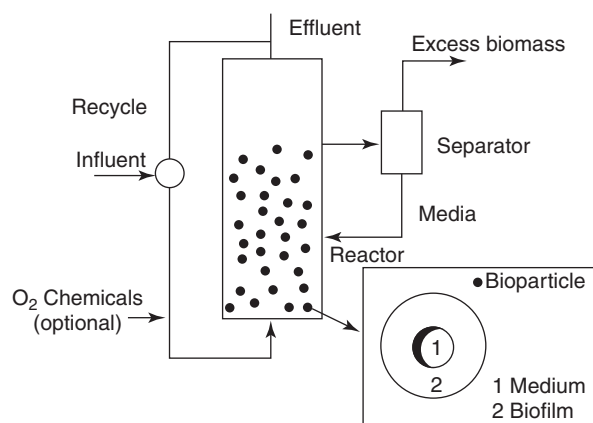
(<100% expanded) are considered expanded; those more than double the static bed height (>100% expanded) are fluidized. A lower degree of bed expansion is advantageous, because it requires a lower flow velocity, less energy, and increases effective biomass concentration, which reduces the reactor footprint. In aerobic processes, however, it increases volumetric oxygen demand because of increased biomass concentration.

The FBFR/EBFR is illustrated in **Figure 29**. The system consists of a column in which the particles are fluidized and a

Table 5 Design criteria for denitrifying fluidized bed biofilm reactors

<i>Parameter</i>		<i>Value</i>	
	<i>Unit</i>	<i>Range</i>	<i>Typical</i>
Packing			
Type		Sand	Sand
Effective size	mm	0.3–0.5	0.4
Sphericity	Unitless	0.8–0.9	0.8–0.85
Uniformity coefficient	Unitless	1.25–1.50	≤ 1.4
Specific gravity	Unitless	2.4–2.6	2.6
Initial depth	m	1.5–2.0	2.0
Bed expansion	%	75–150	100
Empty-bed upflow velocity	m h ^{−1}	36–42	36
Hydraulic loading rate	m ³ _{effluent} m ^{−2} _{Bioreactor area} d ^{−1}	400–600	500
Recirculation ratio	Unitless	2:1–5:1	3:1
NO ₃ [−] – N loading:			
13 °C	kg m ^{−3} d ^{−1}	2.0–4.0	3.0
20 °C	kg m ^{−3} d ^{−1}	3.0–6.0	5.0
Empty-bed contact time	min	10–20	15
C:N (methanol)	Unitless	3.0–3.5	3.2
Specific surface area ^a	m ² m ^{−3}	1000–3000	2000
Biomass concentration ^a	mg l ^{−1}	15 000–40 000	30 000

^aSpecific surface area range based on sand particles; alternate media used in fluidized bed reactors such as carbon or glassy coke may have a different specific surface area range. From Boltz JP, Morgenroth E, deBarbadillo C, *et al.* (2010b) Biofilm reactor technology and design. In: *Design of Municipal Wastewater Treatment Plants*, WEF Manual of Practice No. 8, ASCE Manuals and Reports on Engineering Practice No. 76, 5th edn, vol. 2, ch. 13 (ISBN P/N 978-0-07-166360-1 of set 978-0-07-166358-8; MHID P/N 0-07-166360-6 of set 0-07-166358-4). New York: McGraw-Hill.

**Figure 29** Fluidized bed biofilm reactor process flow diagram (Shieh and Keenan, 1986).

recycle line that is used to maintain a fixed, vertical hydraulic flow.

In this way, bed expansion is kept constant and biofilm covered particles are retained independent of influent flow. Aeration typically is achieved during recycle, during which influent wastewater mixes with effluent recycled from the top of the bed. If aeration is conducted within the fluidized bed, then a significant volume of gas disturbs the fluidized state by causing turbulence and increased force of interparticle collisions. This can cleave biofilm from the substratum. Nevertheless, this approach has been used. The advantage of adding air to the recycle stream is that biomass is not stripped from the media by turbulence of rising gas bubbles; therefore, the treated effluent typically has a lower suspended concentration (Jeris *et al.*, 1981).

Process flow enters at the bottom of the reactor and flows through a distribution system to ensure even dispersion and uniform fluidization. Silica sand (0.3–0.7 mm diameter) and granular activated carbon (GAC; 0.6–1.4 mm) are typically used. Other materials, however, have been used at pilot scale, such as 0.7–1.0 mm glassy coke (McQuarrie *et al.*, 2007). Small carrier particles (1 mm) provide a large specific surface area for biofilm growth (up to 2400 m² m⁻³ when expanded 50%), which is one of the key advantages of this process technology.

In a study of tertiary nitrification of activated sludge-settled effluent using a pilot-scale EBBR, Dempsey *et al.* (2006) found that the process also removed up to 56% CBOD and 62% TSS from the influent stream. Removal of these materials was attributed to the activities of protozoa (free-living and stalked) and metazoa (rotifers, nematodes, and oligochaetes) as shown in Figure 30.

4.15.3.5.5 Rotating biological contactors

The RBC process has been applied where average effluent water-quality standards are less than or equal to 30 mg l⁻¹ BOD₅ and TSS. The RBC employs a cylindrical, synthetic media bundle that is mounted on a horizontal shaft. Figure 31 illustrates the shaft-mounted media.

The bundled media is partially submerged (typically 40%) and slowly (1–1.6 rpm) rotates to expose the biofilm to substrate in the bulk of the liquid (when submerged), and to air (when not submerged). Detached biofilm fragments suspended in the RBC effluent stream are removed by liquid–solids separation units. The RBC process is typically configured with several stages operating in series. Each reactor-in-series may have one or more shafts. Parallel trains are

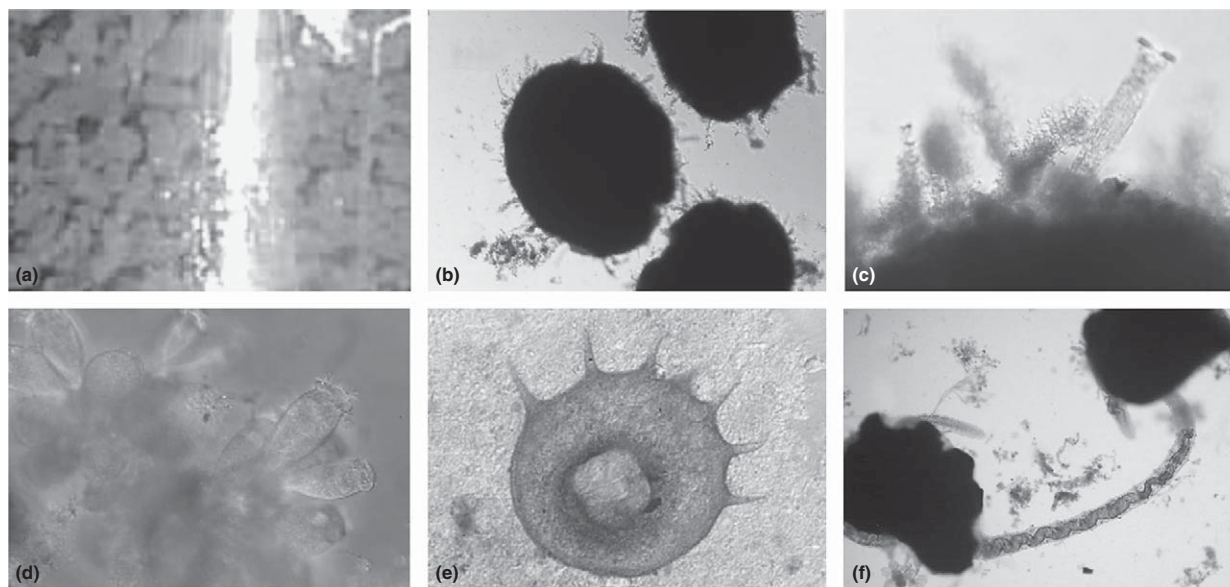


Figure 30 Particulate biofilms with associated protozoa and metazoan from expanded bed: (a) bioparticles in expanded bed; (b) bioparticles with surface attached; (c) closeup of rotifer attached to bioparticle; (d) stalked protozoa on surface of particulate biofilms; (e) testate amoeba grazing on biofilm; and (f) oligochaete worm grazing on bioparticles (Dempsey *et al.*, 2006).

implemented to provide additional surface area for biofilm development.

Media-supporting shafts typically are rotated by mechanical drives. Diffused air-drive systems and an array of air-entraining cups that are fixed to the periphery of the media (to capture diffused air) have been used to rotate the shafts. RBCs have failed as a result of shaft, media, or media support system structural failure; poor treatment performance; accumulation of nuisance macrofauna; poor biofilm thickness control; and inadequate performance of air-drive systems for shaft rotation. Typically, the RBC tank is sized at $4.9 \times 10^{-3} \text{ m}^3 \text{ m}^{-2}$ of media for low-density units. Disks typically have a 3.5-m diameter and are situated on a 7.5-m-long rotating shaft. The RBCs may contain low- or high-density media. Low-density media has a $118 \text{ m}^2 \text{ m}^{-3}$ biofilm active specific surface; high-density units have $180 \text{ m}^2 \text{ m}^{-3}$. Low-density media typically are used in the first stages of RBC systems which are designed for BOD⁵ removal to reduce potential media clogging and weight problems resulting from substantial biofilm accumulation. High-density media typically is used for nitrification.

Mechanical shaft drives consist of an electric motor, speed reducer, and belt or chain drive. Typically, 3.7-kW mechanical drives have been provided for full-scale RBCs. Air-driven shafts require a remote blower for air delivery. Air headers are equipped with coarse-bubble diffusers. The air flow rate is typically in the range of $4.2\text{--}11.3 \text{ m}^3 \text{ min}^{-1}$ per shaft. Air quantity required by systems using air-driven shaft rotation, however, must be evaluated on a site-specific basis. Mechanical drive units have been designed for operation from 1.2 to 1.6 rpm. Air-drive units have been designed for 1.0–1.4 rpm. Ideally, shaft rotational speed is consistent. The development of an evenly distributed biofilm is desirable to avoid an uneven weight distribution, which may cause cyclical loadings in mechanical-drive systems and loping (uneven rotation) in air-driven shaft rotating systems. A loping condition often

accelerates rotational speed and, if not corrected, may lead to inadequate treatment and the inability to maintain shaft rotation. Air-drive systems should provide ample reserve air supply to maintain rotational speeds, restart stalled shafts, and provide short-term increased speeds (2–4 times normal operation) to control excessive or unbalanced biofilm thicknesses. Available data indicate that in excess of an $11.3 \text{ m}^3 \text{ min}^{-1}$ airflow rate per shaft may be required to maintain a 1.2-rpm shaft rotational speed during peak organic loading conditions (Brenner *et al.*, 1984). Large-capacity air cups (150 mm diameter) typically are provided in the first stage of the process to exert a greater torque on the shaft and reduce loping.

The RBC process is typically covered to avoid ultraviolet (UV) light-induced media deterioration and algae growth, to prevent excessive cooling, and to provide odor control. RBCs have been installed in buildings or under prefabricated fiberglass-reinforced plastic (FRP) covers (as pictured in Figure 31).

4.15.3.5.6 Trickling filters

The TF is a three-phase biofilm reactor with fixed carriers. Wastewater enters the bioreactor through a distribution system, trickles downward over the biofilm surface, and air moves upward or downward in the third phase where it diffuses through the flowing liquid and into the biofilm. TF components generally include an influent water distribution system, containment structure, rock or plastic media, and underdrain and ventilation system. Wastewater treatment using the TF results in a net production of total suspended solids. Therefore, liquid–solids separation is required, and is typically achieved with circular or rectangular secondary clarifiers. The TF process generally includes an influent/recirculation pump station, the TF(s), and liquid–solids separation unit(s).

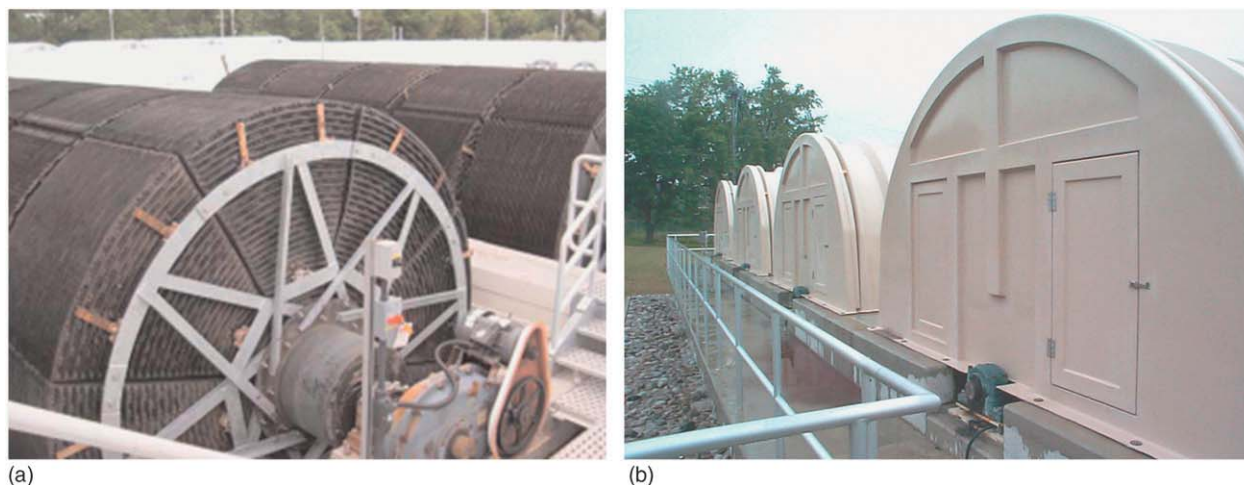


Figure 31 Photograph of the Envirex[®] rotating biological contactor cylindrical synthetic media bundle mounted on a horizontal shaft (a) and rotating biological contactor covers (b). Photographs courtesy Siemens Water Technologies.



Figure 32 (a) Hydraulically driven rotary distributors use variable frequency drive controlled gates that either open or close distributor orifices which adjust with varying pumped flow rates to maintain a constant preset rotational speed. (b) Electrically driven rotary distributor. Photographs courtesy WesTech, Inc.

Primary effluent or screened and degritted wastewater is either pumped or flows by gravity to the TF distribution system. Essentially, there are two types of TF distribution systems: fixed-nozzle and rotary distributors. Because their efficiency is poor, distribution with fixed nozzles should not be used (Harrison and Timpany, 1988). Rotary distributors may be hydraulically or electrically driven. A properly designed rotary distribution system allows for effective media wetting and the intermittent application of wastewater to biofilm carriers. The intermittent application of influent wastewater allows the biofilm to have periods of resting which primarily serves as a process aeration mechanism. Poor media wetting may lead to dry pockets, ineffective treatment zones, and odor. An electrically or modern hydraulically driven rotary distributor

(Figure 32) controls rotational speed independent of the influent wastewater flow rate, and may be used to maintain the desired hydraulic dosing rate.

Ideal TF media provides a high specific surface area, low cost, high durability, and high enough porosity to avoid clogging and promote ventilation (Metcalf and Eddy, 2003). TF media types include rock (RO), random (RA) (synthetic), vertical flow (synthetic) (VF), and cross-flow (synthetic) (XF). Both VF and XF media are constructed with smooth and/or corrugated plastic sheets. Another commercially available synthetic media, although not commonly used, is vertically hanging plastic strips. Horizontal redwood or treated wooden slats have also been used, but are generally no longer considered viable because of high cost or limited supply. Modules

of plastic sheets (i.e., self-supporting VF or XF modules) are used almost exclusively for new and improved TFs, but several TFs with rock media exist, and have proven capable of meeting treatment objectives when properly designed and operated. **Table 6** compares the characteristics of some TF media. The higher specific surface area and void space in modular synthetic media allow for higher hydraulic loading, enhanced oxygen transfer, and biofilm thickness control in comparison to rock media. Rock media has, ideally, a 50-mm diameter, but may range in size. Due to structural requirements associated with the large unit weight of rock, rock-media TFs are shallow in comparison to synthetic-media TFs. Their large surface area makes them more susceptible to excessive cooling. Generally, rock media is considered to have a low specific surface area, void space, and high unit weight. Although recirculation is common, the low void ratio in rock-media TFs limits hydraulic application rates. Excessive hydraulic application can result in ponding, limited oxygen transfer, and poor bioreactor performance.

Performance of existing rock-media TFs may sometimes be improved by providing mechanical ventilation, solids contact channels, and/or deepened secondary clarifiers that include energy dissipating inlets and flocculator-type feed wells. [Grady](#)

et al. (1999) suggested that under low organic loading (i.e., $<1 \text{ kg BOD}_5 \text{ d}^{-1} \text{ m}^{-3}$) rock- and synthetic-media TFs are capable of equivalent performance. However, as organic loading increases, synthetic-media TFs are less susceptible to operational problems and have reduced potential for plugging.

Synthetic TF media has a higher specific surface area and void space, and lower unit weight than rock media. Modular synthetic media is generally manufactured with the following specific surface areas: $223 \text{ m}^2 \text{ m}^{-3}$ as high density, $138 \text{ m}^2 \text{ m}^{-3}$ as medium density, and $100 \text{ m}^2 \text{ m}^{-3}$ as low density. Both VF and XF media are reported to remove BOD_5 and $\text{NH}_3\text{-N}$ ([Harrison and Daigger, 1987](#)), but sufficient scientific evidence exists to surmise that there is a difference in the treatment efficiency of TFs constructed with XF and VF media even when manufactured with virtually identical specific surface areas.

Plastic modules with a specific surface area in the range of $89\text{--}102 \text{ m}^2 \text{ m}^{-3}$ are well suited for carbon oxidation and combined carbon oxidation and nitrification. [Parker *et al.* \(1989\)](#) recommended medium-density XF media against the use of high-density XF media in nitrifying TFs. This is supported by observations from a pilot-scale nitrifying TF application data and conclusions of [Gujer and Boller \(1983, 1984\)](#)

Table 6 Properties of some trickling filter media

Media type	Material	Nominal size (m)	Bulk density (kg m ⁻³)	Specific surface area (m ² m ⁻³)	Void space (%)
Rock					
River		0.024–0.076	1442	62	50
Slag		0.076–0.128	1600	46	60
Plastic ^a					
Cross flow		0.61 × 0.61 × 1.22	24–45	100, 138, and 223	95
Vertical flow		0.61 × 0.61 × 1.22	24–45	102 and 131	95
Random ^b		0.185 ø × 0.051 H	27	98	95

^aManufacturers of modular plastic media: (formerly) BF Goodrich, American Surf-Pac, NSW, Munters, (currently) Brentwood Industries, Jaeger Environmental, and SPX Cooling.

^bManufacturers of random plastic media: (formerly) NSW Corp. and (currently) Jaeger Environmental.

which show lower nitrification (flux) rates for lower-density modular synthetic media. The researchers claim that lower rates occur with high-density media due to the development of dry spots below the flow interruption points (i.e., higher-density media has more flow interruptions and, therefore, less effective wetting). Using medium-density media also reduces plugging potential. Vertically oriented modular synthetic (VF) media is generally accepted as being ideally suited for high-strength wastewater (perhaps industrial) and high organic loadings such as with a roughing TF. In some cases, XF media has been placed in the top layer to enhance wastewater distribution and VF media comprises the remainder of the TF media. Typically, the top layer of a TF's modular plastic media is covered with FRP or HDPE grating. The grating protects modular plastic media from deterioration by UV light and potential structural damage that may result from water-induced load exerted during periods of high-intensity dosing. **Figure 33** illustrates a typical TF column and a picture of the grating.

Rock and random synthetic media are not self-supporting and require structural support to contain the media within the bioreactor. These containment structures are typically precast or panel-type concrete tanks. When self-supporting media such as plastic modules are used, other materials such as wood, fiberglass, and coated steel have been used as containment structures. The containment structure serves to avoid wastewater splashing, and to provide media support, wind protection, and flood containment. In some cases TF containment structures have been designed to allow flooding of the media, which increases operator flexibility in controlling macrofauna accumulation.

The TF underdrain system is designed to meet two objectives: collect treated wastewater for conveyance to downstream unit processes and create a plenum that allows for the transfer of air throughout the TF media (Grady *et al.*, 1999). Clay or concrete underdrain blocks are commonly used for rock-media TFs because of the required structural support. A variety of support systems including concrete piers and reinforced fiberglass plastic grating are used for other media types. The volume created between concrete and media bottom creates the underdrain.

TFs require oxygen to sustain aerobic biochemical transformation processes. The VF of air through the media can be induced mechanically or by natural draft. Natural air ventilation results from a difference in ambient air temperature outside and inside the TF. The temperature causes air to expand when warmed or contract when cooled. The net result is an air-density gradient throughout the TF, and an air front either rises or sinks depending on the differential condition. This rising or sinking action results in a continuous air flow through the bioreactor. Natural ventilation may become unreliable or inadequate in meeting process air requirements when neutral temperature gradients do not produce air movement. Currently, the provision of adequate underdrain and effluent channel sizing to permit free air flow is standard. Passive devices for ventilation include vent stacks on the TF periphery, extensions of underdrains through TFs side walls, ventilating manholes, louvers on the sidewall of the tower near the underdrain, and discharge of TF effluent to the subsequent settling basin in an open channel or partially filled pipes. Drains, channels, and pipes should be sufficiently sized to prevent submergence greater than 50% of their cross-sectional area under design hydraulic loading. Ventilating access ports with open grating covers should be installed at both ends of the central collection channel. Large diameter TFs typically have branch channels (to collect the treated wastewater). These branches should also include ventilating manholes or vent stacks installed at the TF periphery. The open area of the slots in the top of the underdrain blocks should not be less than 15% of the TF area. One square meter gross area of open grating in ventilating manholes and vent stacks should be provided for each 23 m² of TF area. Typically, 0.1 m² of ventilating area is provided for every 3–4.6 m of TF periphery, and 1–2 m² of ventilation area in the underdrain area per 1000 m³ of TF media. Another criterion for rock-media TFs is the provision of a vent area at least equal to 15% of the TF cross-sectional area.

Mechanical ventilation enhances and controls air flow with low-pressure fans that continuously circulate air throughout the TF. Therefore, a majority of new and improved TFs use low-pressure fans to mechanically promote air flow. The air flow resulting from natural draft will distribute itself. This will

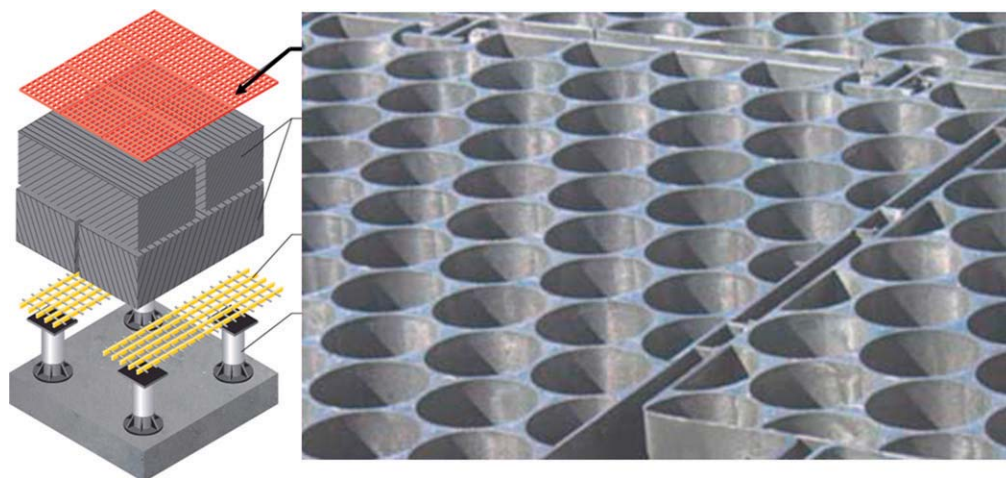


Figure 33 Skid-resistant (polyethylene or fiberglass-reinforced plastic) grating placed on top of a typical modular plastic media trickling filter column.

not occur with mechanical ventilation. Pressure loss through synthetic TF media is typically low, often less than 1-mm H₂O/m of TF depth (Grady *et al.*, 1999). The low pressure drop typically results in low fan power requirements ($\sim 3\text{--}5\text{ kW}$). The head on the fan is typically less than 1500-mm H₂O. Unfortunately, the low pressure drop allows air to rise upward through the TF media without distributing itself across the bioreactor section. Therefore, fans are typically connected to distribution pipes. The air flow distribution piping has openings sized such that air flow through each is equal and air flow distribution is uniform. The pipes typically have a velocity in the range of 1100–2200 m h⁻¹ in order to further promote uniform air flow distribution. Air flow requirements are calculated based on process oxygen requirements and characteristic oxygen-transfer efficiency which is typically in the range of 2–10%. The mechanical air stream may flow upward to downward. Down-flow systems can be designed without covers. However, covers are required for systems that do not have air distribution through a network of pipes under the media. Covering TFs offers a wintertime benefit of limiting cold airflow and minimizing wastewater cooling. Mechanical ventilation and covered TFs may be used to destroy odorous compounds.

A critical unit in the TF process is the pump station that lifts primary effluent (or screened raw sewage), and recirculates unsettled trickling effluent (here, referred to as underflow) to the influent stream. In general, TF underflow is recirculated to the distribution system to achieve the hydraulic load (influent + recirculation) required for proper media wetting and biofilm thickness control, and decouple hydraulic and organic loading. TF influent is generally pumped to allow TF underflow to flow by gravity to the suspended growth reactor (or solids contact basin), secondary clarifier, or other downstream of the TF. When fit with weirs, a single pump station can be used to convey both influent and recirculation streams.

TFs can be classified as roughing, carbon oxidation, carbon oxidation and nitrification, and nitrification. Table 7 summarizes characteristics of each TF. The performance ranges are associated with average design conditions. Single day or average week observations may significantly be greater.

4.15.4 Part III. Undesirable Biofilms: Examples of Biofilm-Related Problems in the Water and Wastewater Industries

Biofilms are unavoidably associated with water environments, so biofilm control, a component of many industrial processes, is especially important in water and wastewater treatment. Depending on the particular setting, biofilms may cause process performance problems, material performance problems, health problems, and esthetic problems. The specific problems that biofilms cause in industrial settings are as diverse as the technological processes affected by the biofilms. In this section, we discuss four biofilm-related problems that have been reported in the water and wastewater industries:

1. biofilms on metal surfaces and MIC;
2. biofilms on concrete surfaces and crown corrosion of sewers;
3. biofilms on filtration membranes in drinking water treatment; and
4. biofilms on filtration membranes in wastewater treatment.

4.15.4.1 Biofilms on Metal Surfaces and MIC

In the manufacturing of metals and metal alloys, raw materials – the ores – are chemically reduced and their internal chemical energy increases. These materials are used by microorganisms as sources of energy in a sequence of processes in which the chemical energy of the affected material decreases, bringing the energy levels of the products closer to

Table 7 Trickling filter classification

Design parameter	Roughing ^a	Carbon oxidizing (cBOD ₅ removal) ^a	Carbon oxidation and nitrification ^a	Nitrification ^a
Media typically used	VF	RO, XF, or VF	RO, XF, or VF	XF
Wastewater source	Primary effluent	Primary effluent	Primary effluent	Secondary effluent
Hydraulic loading m ³ d ⁻¹ m ⁻²	52.8–178.2	14.7–88.0	14.7–88.0	35.2–88.0
BOD ₅ and NH ₃ – N Load kg m ⁻³ d ⁻¹ g m ⁻² d ⁻¹	1.6–3.52 NA	0.32–0.96 NA	0.08–0.24 0.2–1.0	NA 0.5–2.4
Conversion (%) or effluent concentration (mg l ⁻¹)	50–75% filtered cBOD ₅ conversion	20–30 mg l ⁻¹ cBOD ₅ and TSS ^b	<10 mg l ⁻¹ as cBOD ₅ ; <3 mg l ⁻¹ as NH ₃ – N ^b	0.5–3 mg l ⁻¹ as NH ₃ – N ^b
Macro fauna	No appreciable growth	Beneficial	Detrimental (nitrifying biofilm)	Detrimental
Depth, m (ft)	0.91–6.10	≤12.2	≤12.2	≤12.2

^aApplicable to shallow trickling filters. gpm ft⁻², gallons per minute per square foot of trickling filter plan area.

^bConcentration remaining in the clarifier effluent stream.

From Boltz JP, Morgenroth E, deBarbadillo C, *et al.* (2010b) Biofilm reactor technology and design. In: *Design of Municipal Wastewater Treatment Plants*, WEF Manual of Practice No. 8, ASCE Manuals and Reports on Engineering Practice No. 76, 5th edn, vol. 2, ch. 13, p. 238 (ISBN P/N 978-0-07-166360-1 of set 978-0-07-166358-8; MHID P/N 0-07-166360-6 of set 0-07-166358-4). New York: McGraw-Hill.

the energy levels of the materials from which they were made. MIC can affect a variety of materials, both metallic and nonmetallic. If nonmetallic materials are affected, the term biodeterioration of materials is more often used than MIC, although this terminology is not very consistent and, for example, the term crown corrosion of sewers, which in fact refers to the biodeterioration of concrete, is quite popular among water professionals.

Accelerated corrosion of metals in the presence of microorganisms stems from microbial modifications to the chemical environment near metal surfaces (Beech *et al.*, 2005; Geiser *et al.*, 2002; Lee and Newman, 2003; Lewandowski *et al.*, 1997). Such modifications depend, of course, on the properties of the corroding metal and on the microbial community structure of the biofilm deposited on the metal surface (Beech and Sunner, 2004; Dickinson *et al.*, 1996; Flemming, 1995; Olesen *et al.*, 2000, 2001). Beech *et al.* (2005) described MIC as a consequence of coupled biological and abiotic electron transfer reactions, that is, redox reactions of metals enabled by microbial ecology (Beech *et al.*, 2005). Hamilton (2003) attempted to generate a unified concept of MIC but found common features in only some of the possible mechanisms (Hamilton, 2003). It is unlikely that a unified concept of MIC can be generated at all. Rather, there are many mechanisms by which microorganisms may affect metal surfaces, and we demonstrate some of them here. These do not exhaust the possibilities, of course, but are rather used to exemplify the possible mechanisms.

As we have restricted the discussion of MIC to metal surfaces only, it is convenient to define corrosion as anodic dissolution of a metal. In this way we can easily separate the corrosion reaction, the anodic dissolution of the metal, from many other anodic reactions that can occur at a metal surface covered with a biofilm. These other anodic reactions deliver electrons originating from substances metabolized near the metal surface, but only the reaction in which the metal itself is oxidized is defined as corrosion. The presence of the other anodic reactions causes confusion in MIC studies, as the current between the anode and the cathode is made up of electrons originating from many anodic reactions occurring at the surface, not only from the corrosion reaction. Microorganisms generate chemical environments that are conducive to corrosion reactions even if they do not take part in the process themselves. As in most industrial processes microorganisms are always present on metal surfaces, it is not immediately obvious whether the microorganisms attached to the surface accelerate the corrosion process or are just innocent bystanders. The only way to resolve this is by demonstrating that a specific mechanism of MIC is present because a product of microbial metabolism consistent with this mechanism can be detected.

Many mechanisms of MIC have been proposed. Accelerated corrosion may result from the action of acid-producing bacteria, such as *Thiobacillus thiooxidans* and *Clostridium acetivum*; iron-oxidizing bacteria, such as *Gallionella*, *Sphaerotilus*, and *Leptothrix*; MOB, such as *L. discophora*; or hydrogen-producing bacteria. These mechanisms have been studied and the results described in numerous publications. We describe here representative examples of such mechanisms: the effects of differential aeration cells, sulfate-reducing bacteria (SRB corrosion), and MOB corrosion.

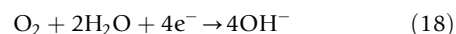
4.15.4.1.1 Differential aeration cells on iron surfaces

MIC caused by differential aeration cells is an example of a nonspecific mechanism of MIC, because it depends on the presence of biofilm, and not on the type of microorganisms that reside in the biofilm. If the oxygen concentrations at two adjacent locations on an iron surface are different, then the half-cell potentials at these locations are different as well. The location where the oxygen concentration is higher will have a higher potential (more cathodic) than the location where the oxygen concentration is lower (more anodic). The difference in potential will give rise to a current flow from the anodic locations to the cathodic locations and to the establishment of a corrosion cell. This is the mechanism of differential aeration cells, and the prerequisite to this mechanism is that the concentration of oxygen varies among locations (Acuna *et al.*, 2006; Dickinson and Lewandowski, 1996; Hossain and Das, 2005). Indeed, many measurements using oxygen micro-sensors have demonstrated that oxygen concentrations in biofilms can vary from one location to another (Lewandowski and Beyenal, 2007).

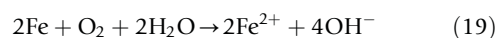
If the anodic reaction is the oxidation of iron,



and the cathodic reaction is the reduction of oxygen,



then the overall reaction describing the process is



The Nernst equation quantifying the potential for this reaction is

$$E = E^\circ - \frac{0.059}{4} \log \frac{[\text{Fe}^{2+}]^2 [\text{OH}^-]^4}{p(\text{O}_2)} \quad (20)$$

Figure 34 visualizes this mechanism.

4.15.4.1.2 SRB corrosion

SRB causes corrosion of cast iron, carbon, and low alloy steels and stainless steels. SRB corrosion of potable water mains is a common (US EPA, 1984) and well-recognized problem (Seth and Edyvean, 2006; Tuovinen *et al.*, 1980). MIC caused by SRB is an example of a mechanism that depends on the activity of a specific group of microorganisms in a biofilm. The corrosion of mild steel caused by SRB is the most notorious case of MIC, and it provides a direct and easy-to-understand link between microbial reactions and electrochemistry (Javaherdashti, 1999). According to the mechanism that was originally proposed by Von Wohlzogen Kuhr in 1934, SRB oxidizes cathodically generated hydrogen to reduce sulfate ions to H_2S , thereby removing the product of the cathodic reaction and stimulating the progress of the reaction (Al Darbi *et al.*, 2005). This mechanism was later found to be inadequate to explain the field observations. More involved mechanisms were implicated in this type of microbial corrosion, including the puzzling effect of oxygen, which can stimulate what is apparently an anaerobic process. It is now certain that the

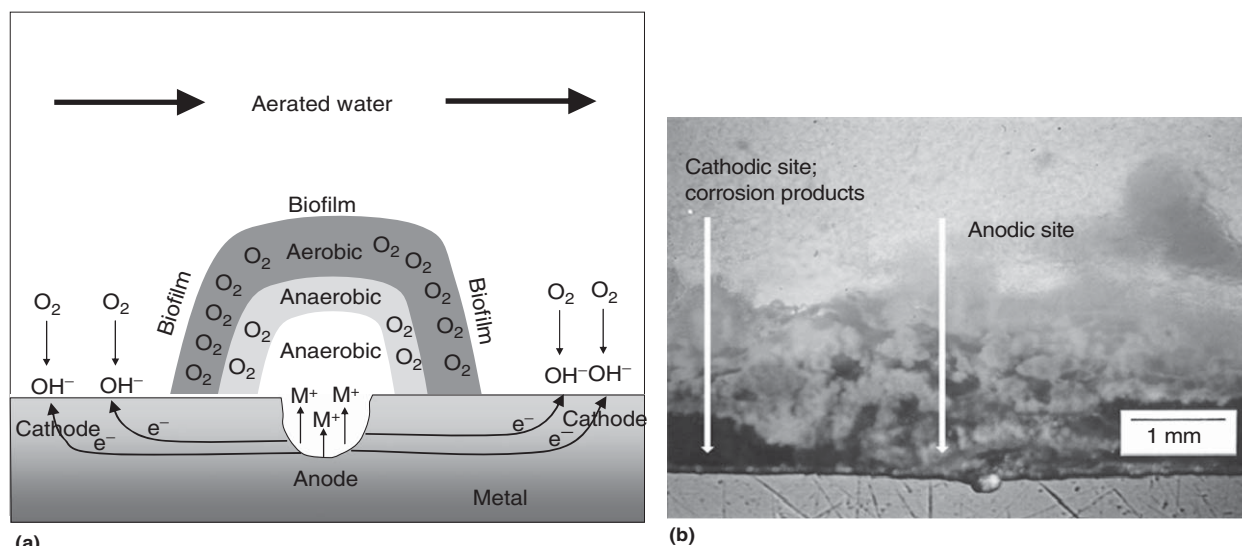
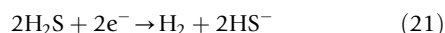


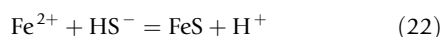
Figure 34 Biofilm heterogeneity results in differential aeration cells. (a) This schematic shows pit initiation due to oxygen depletion under a biofilm (Borenstein, 1994). (b) An anodic site and pit under the biofilm and corrosion products deposited on mild steel.

possible pathways for cathodic reactions include sulfides and bisulfides as cathodic reactants (Videla, 2001; Videla and Herrera, 2005). The currently accepted mechanism of SRB corrosion is composed of a network of reactions that reflects the complexity of the environment near corroding metal surfaces covered with biofilms; the following paragraphs illustrate some of this complexity.

The process starts with the microbial metabolism of SRB producing hydrogen sulfide by reducing sulfate ions. Hydrogen sulfide can serve as a cathodic reactant, thus affecting the rate of corrosion (Antony *et al.*, 2007; Costello, 1974):

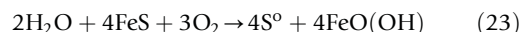


Ferrous iron generated from anodic corrosion sites precipitates with the metabolic product of microbial metabolism, hydrogen sulfide, forming iron sulfides, FeS_x :



This reaction may provide protons for the cathodic reaction (Crolet, 1992). The precipitated iron sulfides form a galvanic couple with the base metal. For corrosion to occur, the iron sulfides must have electrical contact with the bare steel surface. Once contact is established, the mild steel behaves as an anode and electrons are conducted from the metal through the iron sulfide to the interface between the sulfide deposits and water, where they are used in a cathodic reaction. Surprisingly, the most notorious cases of SRB corrosion often occur in the presence of oxygen. As SRB is anaerobic microorganisms, this fact has been difficult to explain. This effect of oxygen can be explained based on a mechanism in which iron sulfides (resulting from the reaction between iron ions and sulfide and bisulfide ions) are oxidized by oxygen to elemental sulfur, which is known to be a strong corrosion agent (Lee *et al.*, 1995). Biofilm heterogeneity plays an important role in this process, because the central parts of microcolonies are anaerobic while the outside edges remain aerobic

(Lewandowski and Beyenal, 2007). This arrangement makes this mechanism of microbial corrosion possible, because the oxidation of iron sulfides produces highly corrosive elemental sulfur, as illustrated by the following reaction:



Hydrogen sulfide can also react with the oxidized iron to form ferrous sulfide and elemental sulfur (Schmitt, 1991), thereby aggravating the situation by producing even more elemental sulfur, and closing the loop through production of the reactant used in the first reaction, FeS :



The product of these reactions – elemental sulfur – increases the corrosion rate. Schmitt (1991) has shown that the corrosion rate caused by elemental sulfur can reach several hundred mpy (Schmitt, 1991). We have demonstrated experimentally that elemental sulfur is deposited in the biofilm during SRB corrosion (Nielsen *et al.*, 1993), thereby detecting the component vital for this mechanism to occur. It is also well known that the sulfur disproportionation reaction that produces sulfuric acid and hydrogen sulfide is carried out by sulfur-disproportionating microorganisms (Finster *et al.*, 1998). Also, several microbial species, such as *T. thiooxidans*, can oxidize elemental sulfur and sulfur compounds and produce sulfuric acid:



In summary, the SRB corrosion of mild steel in the presence of oxygen is an acid corrosion:

Anodic reaction:



Cathodic reaction:



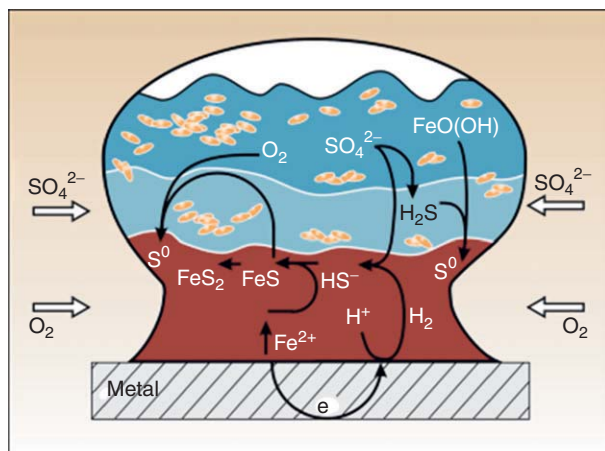


Figure 35 The SRB corrosion of mild steel in the presence of oxygen is an acid corrosion (Lewandowski *et al.*, 1997).

The mechanism of SRB corrosion involves several loops, cycles in which reactants are consumed in one reaction and recycled in other reaction; the process is spontaneous at the expense of the energy released by the oxidation of the metal. This mechanism also demonstrates how the reactants and products of corrosion processes are included in the metabolic reactions of the microorganisms. For example, hydrogen, the product of the cathodic reaction above, is oxidized by some species of SRB to reduce sulfate and generate hydrogen sulfide, H_2S (Cord-Ruwisch and Widdel, 1986), which is the reactant in the first reaction we referred to in this section. Hydrogen sulfide then dissociates to bisulfides:



which are then used in the reactions described above.

Figure 35 shows the network of reactions described above.

4.15.4.2 Biofilms on Concrete Surfaces: Crown Corrosion of Sewers

The mechanism of crown corrosion of sewers is very similar to the mechanism of MIC corrosion of metals caused by SRB. In sewers, SRB reduces sulfate ions to sulfides, which are oxidized by oxygen to elemental sulfur. Then the elemental sulfur is further oxidized, mainly by *T. thiooxidans*, but also by other *Thiobacillus* species, such as *T. novellus/intermedius* and *T. neapolitanus*, in a complex ecosystem on the sewer pipe (Vincke *et al.*, 2001). As a result, sulfuric acid is produced, which dissolves the concrete and damages the sewers (Padival *et al.*, 1995; Islander *et al.*, 1991; Sand and Bock, 1984). The following reactions illustrate this action:



Crown corrosion of sewers depends on the presence of biofilm on the concrete surface and on the generation of sulfuric acid in immediate proximity to the concrete surface.

4.15.4.3 Biofilms on Filtration Membranes in Drinking Water Treatment

The common use of membranes in various technologies of water and wastewater treatment is probably the most visible mark of the changes that occurred in these applications in the last decade, and it is expected that filtration membranes will be even more popular in the future than they are now (Shannon *et al.*, 2008). The traditional use of membranes in water treatment has been in the desalination of sea and brackish waters using the reverse osmosis (RO) process, and there is a large body of knowledge accumulated on this application. RO membrane filtration is becoming even more popular as the cost of desalination decreases because of various improvements in the technology that reduce the energy consumption and because of the use of new materials that produce less expensive and more robust membranes (Veerapaneni *et al.*, 2007). Membrane processes have been introduced into other types of water treatment, besides desalination, such as water softening (Conlon *et al.*, 1990). The main advantages of using membrane filtration in water treatment are that the process does not require using chemicals and that the membrane modules have a smaller footprint than the conventional treatment facilities. Membrane filtration can be used instead of other traditional processes in water treatment, such as coagulation, sand and activated carbon filtration, or ion exchange, without the necessity of adding chemicals to the water, which helps prevent the formation of disinfection byproducts, for example. Membrane filtration can be used alone in water treatment or in combination with other processes, in hybrid arrangements. For example, it can be used in combination with powdered activated carbon (PAC) to remove disinfection byproducts that exist in the raw water (Khan *et al.*, 2009). Excessive biofouling of membranes is a problem in all membrane applications, but RO and nanofiltration (NF) processes are the most sensitive to biofouling (Vrouwenveldera *et al.*, 2009). Much research has been done toward understanding the process of biofilm formation on these membranes and developing methods for cleaning the membranes. The removal of biofilm from RO membranes can be accomplished by mechanical or by chemical methods, or by a combination of mechanical and chemical methods. Mechanical methods include flushing with water or with water and air. Mechanical cleaning can be used alone or it can be followed by chemical cleaning. The simplest method of mechanical cleaning is the forward flush, in which the water flow rate above the membrane is increased to increase the shearing force and remove the deposits from the membrane. To increase the shearing force even further, air can be introduced into the conduit delivering the cleaning water. The air bubbles introduce additional instability into the flow field and increase the shearing force exerted on the surface. The backward flush is based on reversing the direction of filtration: cleaning water is filtered in the opposite direction and the particles trapped in membrane pores are removed. Depending on the contaminants deposited on the membranes, the surface can be cleaned chemically using various type of chemicals. If the deposits are predominantly inorganic scale, then the chemical cleaning can include agents that act mostly on scale, such as hydrochloric acid (HCl) or nitric acid (HNO₃). If the

biofilm is the main problem, then the cleaning substance may include antimicrobial agents to remove the biofilms. Two types of antimicrobial agents are in common use for this purpose: oxidizing and nonoxidizing biocides. The oxidizing biocides popular in membrane cleaning processes include chlorine, bromine, chloramine, chlorine dioxide, hydrogen peroxide, peroxyacetic acid, and ozone. Nonoxidizing biocides include formaldehyde, glutaraldehyde, and quaternary ammonium compounds. One recent study targeted cell–cell communications in biofilms to develop a novel approach in controlling membrane fouling (Yeon *et al.*, 2009).

Much effort has been directed toward the development of membranes with new or modified materials that can resist biofouling and toward modifying the surfaces of ultrafiltration (UF) and NF membranes by the graft polymerization of hydrophilic monomers that resist biofouling or allow more aggressive chemical treatment of the membranes (Hester *et al.*, 2002; Wang *et al.*, 2005; Asatekin *et al.*, 2006, 2007). According to recent studies, in spiral-wound membrane modules, biofilm accumulation has a major impact on the spacer channel but the actual fouling of the membrane contributes to the overall pressure drop to a much smaller extent than previously assumed (Vrouwenveld *et al.*, 2009).

4.15.4.4 Biofilms on Filtration Membranes in Wastewater Treatment

Membrane filtration is used in two types of wastewater technologies: (1) membrane bioreactors (MBRs) and (2) membrane biofilm reactors (MBfRs). This terminology is somewhat confusing: the names sound similar, and the fact that the obvious acronyms for the two technologies are the same does not help. It is therefore customary to call the MBRs and the MBfRs. From the biofouling point of view, microbial growth on membranes is undesirable (Le-Clech *et al.*, 2006) while in MBfRs biofilm growth on the membrane is necessary for process performance. MBfRs are used to deliver dissolved gases, such as oxygen, hydrogen, and methane, to the microorganisms attached to the membrane (Brindle and Stephenson, 1996; Brindle *et al.*, 1998; Suzuki *et al.*, 2000; Lee and Rittmann, 2000; Pankhania *et al.*, 1999; Modin *et al.*, 2008).

MBRs are used to replace gravity settling in the secondary sedimentation tanks used in traditional biological wastewater treatment; for example, the activated sludge process where membrane processes can be used to separate the biomass of suspended microorganisms from the effluent. The membranes used in MBRs are typically UF membranes. MBR technology is well established in wastewater treatment: it has been implemented on large scales (Melin *et al.*, 2006), and textbooks have been published describing its application (Stephenson *et al.*, 2000; Judd, 2006). Using membrane filtration to replace gravity settling has many advantages, and one of them is avoidance of the notorious problems with sludge bulking that plague many activated sludge treatment plants. Membranes in MBRs suffer from biofouling, which decreases the permeate flow (Howell *et al.*, 2003; Young *et al.*, 2006; Kimura *et al.*, 2005). Large-scale operations suffer from this problem, particularly the irreversible fouling that cleaning does not remove (Wang *et al.*, 2005). The most common solution to the excessive accumulation of biomass is bubbling air near the

membrane's surface, which creates high shear and removes the biomass (Hong *et al.*, 2002).

Basic studies on biofilm formation (Davies *et al.*, 1998) indicate that bacteria regulate their group behaviors, such as biofilm formation, in response to population density using small signal molecules called autoinducers, or quorum-sensing molecules. It is expected that interference with microbial communication systems in biofilms may lead to novel approaches to preventing biofouling in many areas. Three strategies for interfering with autoinducer molecules have been proposed: blockage of autoinducer production, interference with signal receptors, and inactivation of autoinducer molecules (Rasmussen and Givskov, 2006). In a recent study, Yeon *et al.* (2009) demonstrated that inactivating the autoinducer molecules in a batch-type MBR reactor decreased the amount of EPS deposited on the membrane and that interfering with cell–cell communication in biofilms can alleviate the fouling of filtration membranes.

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