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Influence of Biofilms on Porous Media Hydrodynamics

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5.1 Introduction and Overview

Microbial biofilms form in natural and engineered systems and can significantly affect the hydrodynamics in porous media. Microbial biofilms develop through the attachment and growth of microorganisms, which encase themselves in self-produced extracellular polymeric substances (EPS). Microbial biofilms are, in general, more resistant to environmental stresses, such as mechanical stress, temperature, pH, and water potential fluctuations, than planktonic cells. Biofilm growth in porous media influences porosity, permeability, dispersion, diffusion, and mass transport of reactive and nonreactive solutes. Understanding and controlling biofilm formation in porous media will maximize the potential benefit and will minimize the detrimental effects of porous media biofilms. Subsurface remediation, enhanced oil recovery, and carbon sequestration are only a few examples of beneficial porous media biofilm applications.

5.2 An Introduction to Biofilms

Microbial biofilms have probably been known to exist for as long as we have known about microorganisms. When Anthony van Leeuwenhoek described the "scuff" (plaque) from his teeth in 1683 (see http://www.ucmp.berkeley.edu/history/leeuwenhoek.html [accessed Jan. 09, 2009] or Dixon 2009), the discovery of these "many very little living animalcules, very prettily a-moving," i.e., the mere existence of microorganisms, overshadowed the fact that they were associated with a surface, that is, the teeth. It might be due to the fact that microorganisms associated with surfaces are very difficult to study that the scientific community almost exclusively focused on the study of free-floating ("suspended" or "planktonic") microorganisms well into the twentieth century. The importance of attached microorganisms in nature and engineered systems was really not described until the end of the 1970s when Characklis and Costerton et al. clearly described the abundance of biofilms in many environments (Characklis 1973a,b; Costerton et al. 1978). Bill Characklis' legacy to the biofilm field was recently

acknowledged with the republication of a "vintage article" along with a commentary in biotechnology and bioengineering (Characklis and Bryers 2008).

It took until 1990 for the first book on biofilms to appear. Two of the pioneers in biofilm research, Kevin Marshall and Bill Characklis coedited *Biofilms*, a book still very worthwhile for the beginning biofilm engineer or microbiologist (Characklis and Marshall 1990). Over the past 20 years, numerous books on biofilms have been published, and the interested reader is encouraged to consult those for more detail (Characklis and Marshall 1990; Lappin-Scott and Costerton 1995; Bryers 2000; Evans 2000; Ghannoum and O'Toole 2004; Costerton 2007; Lewandowski and Beyenal 2007). In addition, the Center for Biofilm Engineering at Montana State University is spearheading an effort to develop the "Biofilm Hypertextbook," which can currently be accessed at http://www.biofilmbook.com/.

Biofilms are complex three-dimensional microbial communities attached to a surface (Figure 5.1). There is no one commonly accepted definition for the term "biofilm," although it is generally agreed upon that a biofilm is an aggregate of microorganisms, such as bacteria, algae, fungi, or protozoa, attached to a surface and embedded in a self-produced matrix of EPS. Biofilms can be found on various surfaces and in various industrially, environmentally, and medically relevant systems. They form in completely saturated as well as unsaturated environments such as pipelines, soils, medical implants, blood vessels, biomaterials, tissues, biofilters, cooling towers, ship hulls, river rocks, and a variety of other environments. One of the major differences between suspended cells and biofilms is the commonly large amount of EPS present in biofilms, which, among other potential roles, provides biofilms with structural support. Biofilm communities have been argued to be the predominant form of microbial life in many environments (Costerton et al. 1995; Stoodley et al. 2002b; Costerton 2007), and especially in systems with high surface area to volume ratios, such as porous media, biofilm communities can be expected to dominate (VanLoosdrecht et al. 1990; Bouwer et al. 2000).

The biofilm mode of growth can have significant competitive advantages. Immobilized organisms (e.g., a biofilm on soil particles) in a continuous-flow system (e.g., flowing groundwater containing growth substrates) will have a continuous supply of substrates and nutrients from the flowing fluid or the porous medium itself.

In general, three stages of biofilm development are to be considered: (1) Microbial transport and attachment, (2) biofilm growth, and (3) microbial detachment and propagation. Two animations (Movies 7 and 8) were published as supplementary materials to "The Biofilm Primer" at http://www.springer.com/life+sciences/microbiology?SGWID=0-10037-12-322199-0 (Costerton 2007) and are recommended to be viewed by the novice in this field.

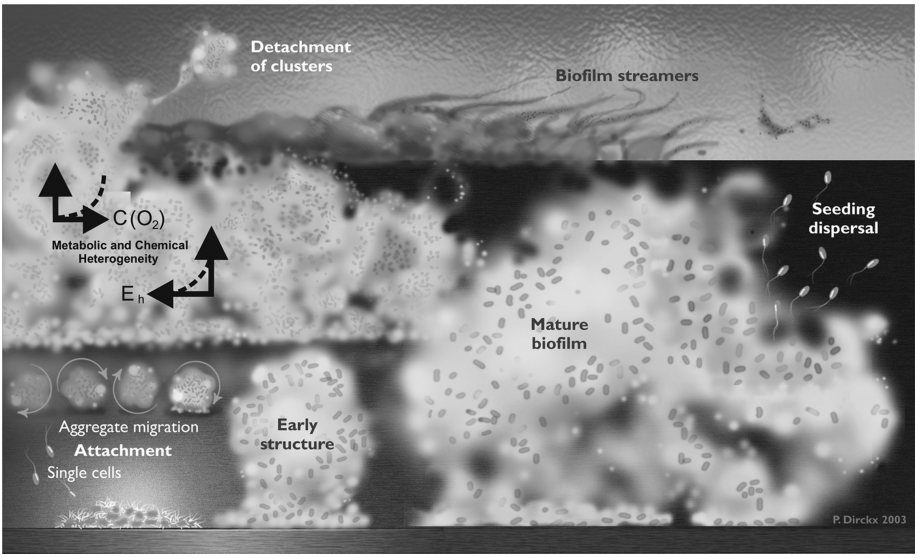

FIGURE 5.1
Schematic of the life cycle of a biofilm. Starting on the bottom left, attachment; growth and development of biofilm structure (bottom center); and fully developed biofilm with detaching cells (bottom right, in this case through seeding dispersal). Top: developed heterogeneous biofilm. Different shades within the biofilm indicate differences in physicochemical conditions (e.g., pH, concentration of nutrient, availability of electron acceptors, etc.). Throughout, mechanically induced stress and strain (e.g., through fluid flow) can cause detachment of clusters, aggregate migration, streamer formation, as well as single cell detachment. Detached cells can colonize surfaces elsewhere to form more biofilm.

5.2.1 Microbial Transport and Attachment

In most situations, if a surface is present, microorganisms tend to attach and make the transition from the planktonic to the attached (sessile) state. The mechanisms and kinetics of attachment in porous media are most frequently described using the colloid filtration theory (Yao et al. 1971). The removal of microorganisms from the flowing fluid has been found to be governed by a large number of parameters, including the properties of the microbial cells, solution chemistry, porous media characteristics, and hydrodynamics. This chapter provides only a brief overview of the parameters and conditions influencing microbial transport in porous media. More detailed reviews are provided, for example, by Bouwer et al. (2000) and Ginn et al. (2002), and the reader is referred to these publications as well as the references therein.

Cell surface properties such as the presence of cell surface molecules (e.g., proteins or carbohydrates), pili, flagella, as well as cell surface hydrophobicity and charge have been shown to play a role in microbial attachment. Motility, chemotaxis, cell buoyant density, size, and shape are cell properties that can influence the transition of cells from the planktonic to the attached state (Bouwer et al. 2000). Many of these parameters can change with the physiological state of the microbes, and recent studies have demonstrated that bacterial starvation can enhance bacterial transport in porous media (Cunningham et al. 2007).

Solute characteristics such as solution ionic strength, pH, temperature, and the presence of organic compounds, such as nutrients or surfactants, can influence the tendency of microbes to remain in suspension or to attach to a surface in porous media (Bouwer et al. 2000). In natural systems, the ability to control these parameters is somewhat limited because of the large amount of (ground) water that would have to be manipulated.

The same is true for porous media characteristics. Pore size and pore size distribution; grain and grain size distribution; mineralogy; roughness; the presence of sorbed, dissolved, or suspended organic matter; and porous media hydrophobicity have been shown to influence microbial attachment and transport in porous media, but these parameters might be difficult to control or manipulate in natural systems (Bouwer et al. 2000).

Many of the models describing microbial transport in porous media are based on the advection dispersion equation and attempt to correlate one or multiple of the parameters listed above to the collision efficiency factor to create a link between the fairly well-understood hydrodynamics and the less well-understood microbe–surface interactions. However, apparent scale dependencies of these parameters, possibly due to the heterogeneity of porous media and microbial populations, have made it difficult to reliably couple our knowledge of the micro- and nano-scale interactions between microbial cells and the porous media with models describing the advective transport of microorganisms (see discussions and references in Ginn et al. [2002] and Thullner and Baveye [2008] for more information).

5.2.2 Biofilm Growth

Once attached to a surface, the development of a biofilm structure will depend on a number of parameters, including availability of growth-limiting nutrients, presence of inhibitors, as well as the prevailing hydrodynamics.

As will be discussed later in detail, direct observations of biofilm formation and growth in porous media is a formidable challenge due to the opaque nature of most porous media. Hence, most of the existing knowledge is based on very simple flow cell experiments with plane (nonporous) surfaces to facilitate microscopic investigations.

The type and extent of biofilm formation depends on the ability of the attached microorganisms to grow and reproduce. Major factors contributing

toward the ability of biofilms to form are as follows: (1) the availability of nutrients and energy (e.g., electron donors and acceptors; macro- and micronutrients), (2) appropriate geochemical conditions (pH, temperature, osmotic pressure, etc.), (3) absence of inhibitors (toxins, antimicrobial agents, waste products), (4) tolerable level of biofilm consuming (e.g., grazing) organisms, and (5) hydrodynamics, which influence mass transport of solutes and can result in mechanical stress acting upon biofilms.

Biofilms are distinctly different from the long-studied planktonic cells (Stoodley et al. 2002b), and research over the past decade has clearly revealed intricate spatial organization in biofilms. A recent review by Stewart and Franklin (2008) nicely summarizes the chemical, physical, and biological (genetic) heterogeneity of microbial biofilms and strategies on how to assess and describe these heterogeneities.

Biofilm communities appear to organize themselves spatially to form continuous films and distinct colonies (also often referred to as mushrooms, towers, streamers, etc.), which can vary in density and spatial organization depending on the culture conditions. Much effort is being expended into understanding organizational structures and processes within biofilms as evidenced in several review papers regarding biofilms as well as recent experimental work investigating differential gene expression in single and multispecies biofilms (Costerton et al. 1995; O'Toole et al. 2000; Tolker-Nielsen and Molin 2000; Hall-Stoodley et al. 2004; Stewart and Franklin 2008; Lenz et al. 2008). Biofilms have been suggested to have tissue-like characteristics (Costerton et al. 1995; Neu et al. 2002), and it has even been postulated that biofilms behave more like a multicellular organism than single cells or even a community of unicellular organisms (Velicer 2003; Crespi and Springer 2003).

Cell–cell communication, the process by which microorganisms can influence each other's behavior via small molecular weight chemical molecules has received significant attention in this context, and the reader is referred to the highly cited works in this area as well as book chapters (Singh et al. 2000; Miller and Bassler 2001; Chen et al. 2002; Sauer et al. 2002; Hentzer et al. 2004; Hall-Stoodley et al. 2004).

It is clear that the biofilm mode of growth offers a number of competitive advantages to microorganisms, including, but not limited to, protection from chemical and physical environmental stress factors, the trapping of nutrients in systems with low nutrient concentrations, symbiotic or mutualistic community interactions, and enhanced exchange of genetic material (Tolker-Nielsen and Molin 2000; Tolker-Nielsen et al. 2000; Hentzer et al. 2004; Cvitkovitch 2004; Molin et al. 2004).

The presence of EPS, which stabilizes the spatial organization of biofilm communities, appears to be crucial in this context. EPS largely immobilize or at least drastically reduce movement of microbial cells, resulting in a stable, yet not rigid, three-dimensional community, which can provide competitive advantages owing to mutualistic or symbiotic relationships among organisms.

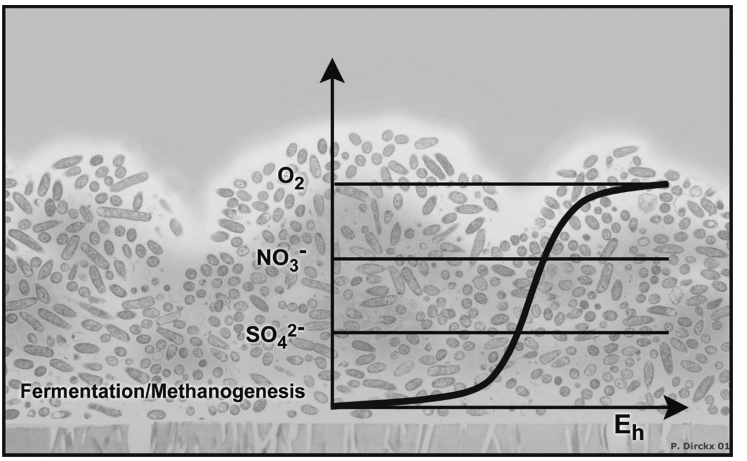

FIGURE 5.2
Schematic of possible spatial organization in a natural biofilm community. Depending on the availability of terminal electron acceptors (e.g., oxygen, nitrate, sulfate), aerobic organisms might establish themselves in the top layer of the biofilm, followed by nitrate-reducing organisms whose activity will increase once oxygen availability decreases, followed by sulfate-reducing organisms, which require a much lower redox potential (indicated by E_h). In the absence of these electron acceptors, anaerobic hydrolysis of complex carbon sources, fermentation, as well as methanogenesis might occur, which can produce sugars, organic acids, alcohols, and other small organic compounds, which can serve as electron donors and carbon sources for the respiratory organisms (aerobes, nitrate- and sulfate-reducing organisms) in the top layers of the biofilm. Carbon cycling not shown in this figure to reduce complexity.

It can be imagined, for instance, that in a natural environment a number of aerobic organisms establish themselves in the top layers of a biofilm consuming the available oxygen and provide anaerobic conditions for nitrate-reducing, sulfate-reducing, and fermentative organisms. The fermentative organisms in turn might produce short-chain organic acids and alcohols, which could become inhibitory in a solely fermentative community but are being consumed by the nitrate-, sulfate-, and oxygen-reducing organisms. Experimental evidence of such stratification has been published (Kuhl and Jorgensen 1992; Ramsing et al. 1993; Okabe et al. 1999) and is outlined in Figure 5.2.

Even without the protection from other community members, the EPS can present a diffusion barrier for solutes allowing organisms deeper inside a biofilm community potentially more time for the initiation of a protective stress response. For reactive solutes, such as oxidative antimicrobials (e.g., chlorine), EPS can also present a reactive barrier. Owing to the high-reaction rate of chlorine with extracellular matrix components, its penetration can

be highly limited (Stewart 2003). Growth-dependent antimicrobials, such as certain antibiotics, might also be less effective against biofilms owing to the presence of slow-growing organisms even in pure culture biofilms (Brown et al. 1988; Gilbert et al. 1990). Overviews of these and other possible mechanisms of biofilm resistance are summarized in a number of articles and book chapters that have been published over the past years (Stewart et al. 2000; Stewart 2003; Fux et al. 2005; Costerton 2007).

The morphology of biofilms in porous media can be highly variable and can range from patchy, colony-like biofilms to continuous films with varying thickness. The significance of such different biofilm morphologies and thicknesses to mass transport of solutes in biofilms and biofilm-affected porous media will be discussed in more detail later (Biofilms in Porous Media and Their Effect on Hydrodynamics).

5.2.3 Microbial Detachment and Propagation

The detachment of microorganisms from microbial biofilms plays an important role in the propagation of biofilms and might ultimately determine the ability of biofilm organisms to survive. Detachment is probably the least-understood process in the life cycle of a biofilm. As shown in nonporous model systems, detachment of biomass can occur in the form of erosion, the loss of single cells, or small clusters of cells from the surface of the biofilm, through hollowing of microcolonies or in the form of small to large sloughing events (e.g., Tolker-Nielsen et al. 2000; Sauer et al. 2002; Stoodley et al. 2002a).

While erosion-like detachment appears to be most highly influenced by hydrodynamics (e.g., changes in shear stress), substrate availability, and the amount of EPS present (Peyton et al. 1995; Paulsen et al. 1997; Kim and Fogler 2000; van Loosdrecht et al. 2002; Ramasamy and Zhang 2005; Ross et al. 2007), massive sloughing events seem to be controlled more by the physiology of the cells. Chemical signaling as well as bacteriophage-induced detachment events have been described in the literature (Stoodley et al. 2001; Wilson et al. 2004; Purevdorj-Gage et al. 2005).

There are reports that detaching cells are metabolically more active than those that remain (Rice et al. 2003) but also reports that bacterial starvation, which should result in decreased activity, increases detachment (Ross et al. 2007).

Detachment of cells is likely to be followed by attachment of cells with subsequent biofilm development as long as the environmental conditions permit. The sequence of attachment, growth and biofilm development, detachment, followed by (re-)attachment is often referred to as the life cycle of a biofilm (outlined in Figure 5.1).

As will be discussed below, the ability to directly observe attachment, biofilm growth, and detachment processes is limited, especially in porous media. However, a thorough understanding of the behavior of biofilms in porous media offers significant opportunities for the development of

environmental and industrial processes as well as the control of detrimental biofilms in medicine and industry. Hence, a number of experimental systems and approaches have been developed to study biofilms in porous media.

5.3 Experimental Systems and Techniques for the Investigation of Biofilms in Porous Media

The direct observation of biofilm processes in porous media is challenging due to the irregular shape and opaque nature of most porous media. However, for the development of effective porous media biofilm technologies it is often necessary to observe the spatial and temporal distribution *and* activity of biofilm cells as well as of the EPS.

A number of reactor designs can be imagined for the investigation of biofilm processes in porous media, and the exact design of laboratory (and industrial) scale reactors will depend on the goal of each study or application. In addition, the mere existence of biofilm cells does not necessarily correlate with metabolic activity since it has been shown that a large portion of biofilm cells can be basically metabolically inactive (Mclean et al. 1999; Hunt et al. 2004; Werner et al. 2004; Sharp et al. 2005; Rani et al. 2007; Kim et al. 2009). The activity of biofilm organisms is governed by a number of mass transfer processes, such as the transport of solvents and solutes into reactors, the possible mass transfer from the gaseous to the liquid phase (e.g., for oxygen as an electron acceptor), the mass transfer of solutes from the liquid phase to the biofilm surface, and simultaneous reaction, diffusion, as well as the sorption of solutes within the biofilm and the supporting porous medium (Mclean et al. 1999).

The remainder of this chapter will mostly focus on approaches for better understanding the influence of biofilms on porous media porosity, permeability, and hydrodynamics. It should be kept in mind that the activity of microorganisms in porous media will ultimately determine the success of advanced biofilm technologies, and, activity measurements should be utilized as much as possible. However, spatially and temporally resolved measurements of biofilm activity are a challenge even in the absence of porous media.

Approaches for measuring biofilm activity include, but are not limited to, traditional microbiological culturing techniques (e.g., plate counts and most probable number techniques), substrate consumption measurements, enzyme assays to assess specific activities, and molecular techniques, such as mRNA (messenger ribonucleic acid) measurements, gene-specific quantitative PCR (polymerase chain reaction), and reporter gene constructs. The interested reader is referred to past (e.g., Fletcher 1979; Poulsen et al. 1993; Lazarova and Manem 1995; Dorn et al. 2004; Teal et al. 2006; Stewart and Franklin 2008; Lenz et al. 2008) and future literature evaluating techniques suitable for activity assessments in porous media environments. A recent review article by

Geesey and Mitchell reemphasizes the need for experimental systems in which hydrodynamics and biological activity can be measured directly (Geesey and Mitchell 2008).

5.3.1 The Challenge of Imaging Biofilms in Porous Media

Destructive (e.g., end-point) and nondestructive measurements have been used to assess the presence, structure, and distribution of biofilms in porous media. Such techniques include visual imaging using high-resolution photography and microscopy, scanning or transmission electron microscopy (SEM or TEM), as well as more recently developed methods such as x-ray tomography, nuclear magnetic resonance (NMR) spectroscopy-, or ultrasound-based imaging techniques.

Noninvasive low-energy techniques such as photography, brightfield or reflective microscopy, NMR imaging, or ultrasound have the advantage of having no or negligible effects on biofilms but often suffer a lack of resolution, depth penetration, or selectivity.

Currently available microscopes and image analysis programs allow determining the thickness of stained or unstained biofilms. However, the applicability of optical techniques to observe biofilms in porous media is limited as most porous media particles are not flat (e.g., sand grains, glass beads). Porous media surfaces are usually rather irregularly shaped, resulting in increased background signal and image blurriness. Confocal scanning laser microscopy (CSLM) combined with fluorescent labeling techniques, and three-dimensional image analysis can reduce the amount of background fluorescence and thus potentially allow for continuous and spatially resolved observation of biofilms in porous media. However, since porous media are opaque and the working distance of high-resolution microscopy objectives is limited, the depth of field of observation is usually very limited.

There have been a few studies in which the refractive index of the fluid and porous media used were chosen in a way to maximize the ability to observe biofilm formation *in situ* over time (e.g., Leis et al. 2005). However, such studies have remained rare and the choice of porous media materials is limited if aqueous solutions are to be used to grow biofilms.

Paulsen et al. (1997) described a model system in which noninvasive microscopic observation combined with measurements of local-flow velocity allowed for estimating the influence of biofilm morphology on convective mass transport. However, such detailed measurements are usually limited to specifically designed experimental systems and one- or pseudo–two-dimensional geometries.

Electron microscopy techniques such as SEM and TEM have been used to estimate the thickness of biofilms on surfaces (Vandevivere and Baveye 1992b; Rinck-Pfeiffer et al. 2000; Hand et al. 2008), however, they cannot be applied directly to porous media systems and can therefore suffer artifacts due to destructive sampling, sample preparation, and the vacuum conditions

necessary for the analysis (Nam et al. 2000). For instance, in one study, the mean biofilm thickness estimates based on SEM images were about 60%–82% less than those obtained through optical microscopy (Jean et al. 2004). Environmental scanning electron microscopy (ESEM) can avoid some of the artifacts possibly introduced during sample preparation and imaging compared to SEM. ESEM does not necessarily require coating of the sample with a conductive material and much less stringent vacuum conditions; however, it can still suffer artifacts associated with the destructive sampling of the porous media.

X-ray tomography techniques for imaging porous media are available, but microscale imaging of biofilms in porous media has not yet been established completely. Some promising work has been published in the recent years, which shows the principal feasibility of performing microtomography on porous media to image the transport and deposition of fluids and colloids in porous media (e.g., Wildenschild et al. 2005; Li et al. 2006; Gaillard et al. 2007). However, the lack of x-ray–detectable absorption properties that are specific to the presence of biofilms or suitable biofilm-labeling techniques have slowed the progress in this field. Furthermore, the applicability of high-energy techniques to biological systems is limited because of the potential damage that can occur during exposure.

Nonoptical techniques such as NMR (e.g., Hoskins et al. 1999; Paterson-Beedle et al. 2001; Seymour et al. 2004a,b, 2007; Metzger et al. 2006; McLean et al. 2007; Hornemann et al. 2009), ultrasound (e.g., Shemesh et al. 2007), or complex conductivity (Davis et al. 2006) imaging of biofilms have immense potential for imaging biofilms in opaque media. However, their application is currently restricted because of their limited availability, resolution, and because of the interference that natural substrates, such as natural soils, sand, or stone cores, have on their signal.

5.3.2 Porous Media Biofilm Reactors

A large variety of bench- and pilot-scale porous media biofilm reactors have been used to evaluate the effect of biofilm accumulation on porous media hydrodynamics and mass transport.

Columns, ranging from a few millimeters to several meters in length and millimeter to approximately 1 m diameter, are probably the most commonly used reactor types. Unfortunately, natural porous media themselves as well as most column materials are opaque and do not lend themselves to direct optical interrogation. Hence, high-optical quality microscopic flowcells and flat-plate reactors, which can represent natural porous media or fractures more or less closely, have been used frequently to investigate fundamental processes of biofilm development in porous media (e.g., Cunningham et al. 1991, 1995; Sharp et al. 1999a, 2005; Yarwood et al. 2002, 2006; Nambi et al. 2003; Knutson et al. 2005; Ross et al. 2007; Willingham et al. 2008). These reactors can consist of materials that contain certain patterns or can be filled

with thin layers of porous media (sand, glass beads, or similar). Depending on their size and optical quality, these reactors allow for direct optical or microscopic observations of biofilm formation and mass transport (see Figure 5.3 for examples). Capillary flowcells also allow for direct microscopic interrogations and are sometimes used to represent single pores.

While there are no review articles available, which summarize use and operation of reactors for the investigation of biofilm formation in porous media, there are reviews and special issues available related to the possibilities of visualizing colloid transport in porous media, such as a recent special section in *Water Resources Research* (2006, Vol. 42, Issue 12) and, in specific, an article by Ochiai et al. (2006).

The challenges in both fields—colloid transport and biofilm formation in porous media—are similar: (1) Aqueous phase measurements are simply not sufficient to completely understand the fundamental microscale processes affecting colloid transport or biofilm development and (2) The direct observation of microscale processes is complicated because of the opaque nature of porous media.

Nevertheless, a fairly large body of literature on the influence of biofilm formation on porous media hydrodynamics is available and will be described in the next section. Such studies generally rely on measurements of differences in hydraulic head, flow rate, and (particulate or dissolved, reactive or nonreactive) tracer through characteristics, sometimes combined with direct, real-time observation of biofilm distribution in columns, capillaries, network models, or larger lysimeter-like (two-dimensional/quasi three-dimensional) reactors (Paulsen et al. 1997; Sharp et al. 1999a; Rinck-Pfeiffer et al. 2000; Yarwood et al. 2002; Thullner et al. 2002a; VanGulck and Rowe 2004; Sharp et al. 2005; Arnon et al. 2005a; Yarwood et al. 2006; Castegnier et al. 2006; Seki et al. 2006; Ross et al. 2007; Rees et al. 2007).

More recently, with the broader availability of computer-controlled microscale fabrication opportunities, microscale reactors have been developed and employed in our and other laboratories, which allow for significantly improved possibilities for the noninvasive observation of reactive transport processes in porous media model reactors (Wan et al. 1994; Dupin and McCarty 1999; Kim and Fogler 2000; Nambi et al. 2003; Knutson et al. 2005; Willingham et al. 2008; and Figure 5.4).

Larger scale laboratory studies have also been conducted, mostly as precursors to field-scale demonstrations (Figure 5.5). In general, it has been observed that the increased complexity of two- or pseudo–three-dimensional systems can result in lesser permeability reductions, presumably due to the possibility of flow around biofilm- or mineral-clogged areas (Cunningham et al. 1997, 2003; Kildsgaard and Engesgaard 2001; Thullner et al. 2002a; Seki et al. 2006). Direct observations and simulations clearly show diverted flow around biofilm-clogged areas (Thullner et al. 2002a; Seki et al. 2006).

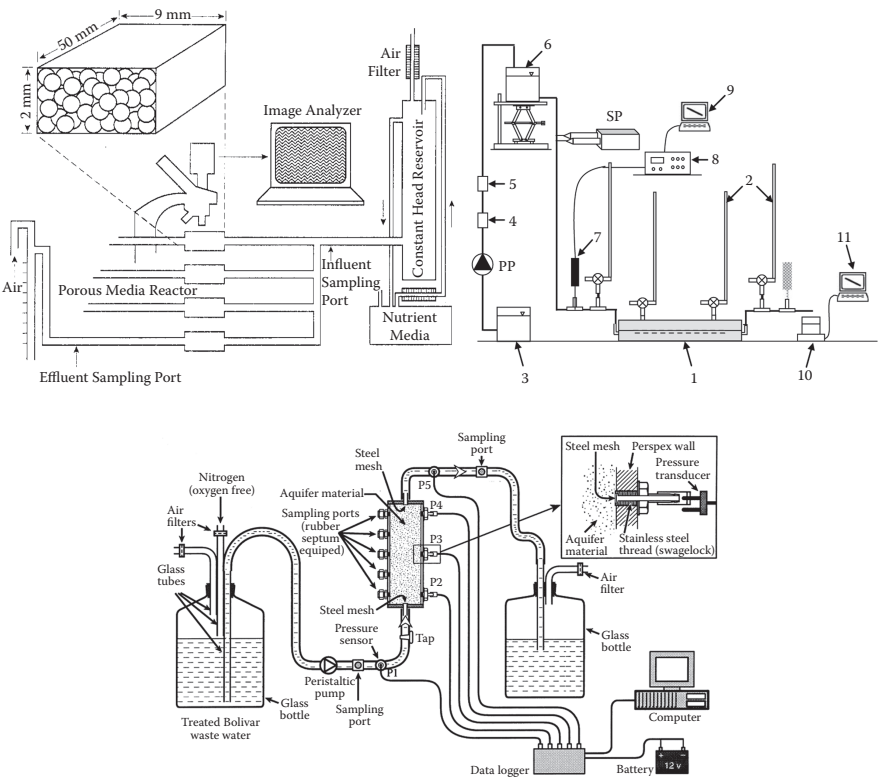

FIGURE 5.3
Schematics of experimental setups to study biofilm formation in porous media. Top left: Capillary flowcells used by Cunningham et al. (1991); Top right: artificial fracture setup used by Castegnier et al. (2006); Bottom: column setup utilized by Rinck-Pfeiffer et al. (2000). The two flowcells systems (top) both allow for direct optical interrogation of the system. The column setup (bottom) allows for more realistic porous media conditions but does not permit direct optical interrogation. The fracture and column flow systems allow for limited spatially resolved assessments of hydraulic conductivity due to multiple piezometers or pressure transducers. See cited sources for more detailed explanations of the experimental setups. (Top Left: Reprinted with permission from Cunningham, A. B., Characklis, W. G., Abedeen, F., and Crawford, D., *Env. Sci. Tech.,* 25, 1991. ©1991, American Chemical Society. Top Right: Reprinted from, Castegnier, F., Ross, N., Chapuis, R.P., Deschenes, L., and Samson, R., *Water Res.,* 40, 2006. ©2006—with permission from Elsevier. Bottom: Reprinted from, Rinck-Pfeiffer, S., Ragusa, S., Sztajnbok, P., and Vandevelde, T., *Water Res.*, 34, 2000. ©2006—with permission from Elsevier.)

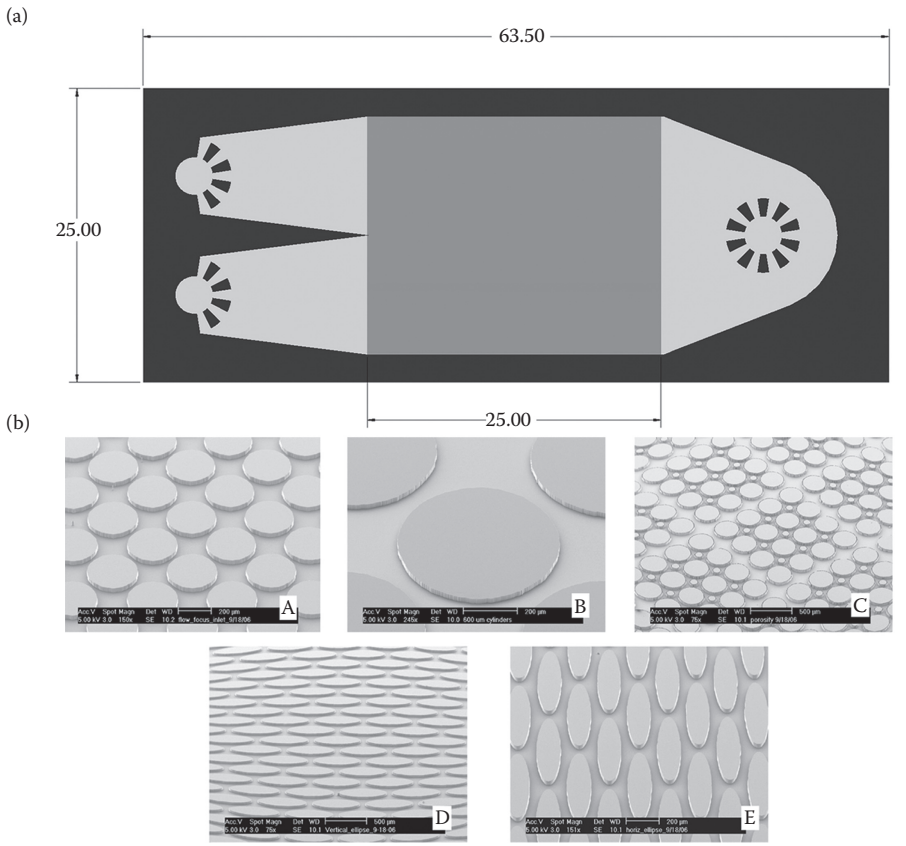

FIGURE 5.4
Technical drawing and pictures of selected microscale reactors for the study of biofilm formation in porous media. (a) Microscale porous media reactor developed at Montana State University; (b) SEM images of porous media structures employed by Willingham et al. (2008). (Reprinted with permission, Willingham, T.W., Werth, C.J., and Valocchi, A.J., *Env. Sci. Tech.*, 42, 2008. ©2008, American Chemical Society.)

5.4 Biofilms in Porous Media and Their Effect on Hydrodynamics

5.4.1 The Relationship of Porous Media Hydrodynamics and Biofilm Structure

This section will discuss the influence of biofilm growth on porous media hydrodynamics including porosity, permeability, and dispersivity. During this

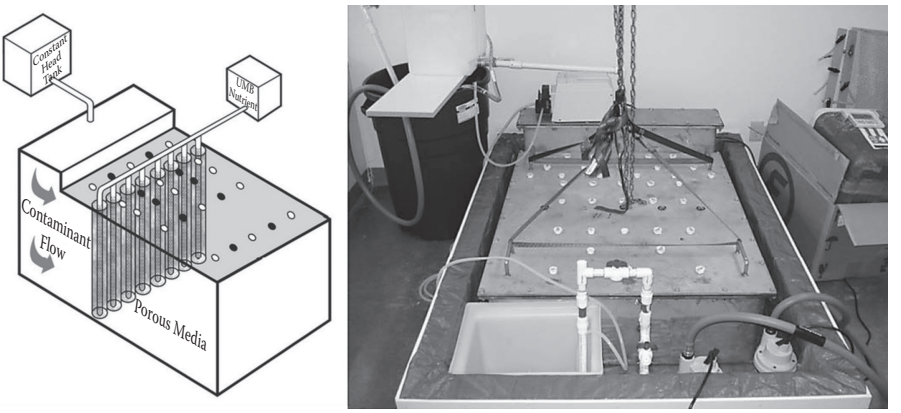

FIGURE 5.5
Schematic and picture of 3 × 4 × 1 ft^3 rectangular lysimeter utilized by Cunningham et al. (1997).

discussion, the reader should keep in mind that not only biofilm growth influences the hydrodynamics of porous media but that the prevailing hydrodynamic conditions also influence biofilm growth characteristics; that is, it is likely that, over time, biofilm structure responds to changes in mass transport and adapts by adjusting its structure to ultimately optimize the mass transport of substrates and products as well as the stability of the biofilm. This interplay of hydrodynamics and biofilm growth will be discussed in more detail later.

It is commonly accepted that EPS and the microbial cells themselves, can affect the hydrodynamics in porous media. Moreover, there is significant experimental evidence that biofilms do not always accumulate homogeneously in porous media but that there can be significant spatial and temporal variability.

It has been observed repeatedly that discrete clusters are formed at least during the initial phase of biofilm accumulation and that the distribution of biofilms in porous media is influenced by the availability of nutrients, the effective porous media particle size, and local hydrodynamics (Taylor and Jaffe 1990b; Baveye et al. 1992; Vandevivere and Baveye 1992b; Rittmann 1993; Vandevivere 1995; Vandevivere et al. 1995; Sharp et al. 1999a; Rowe et al. 2000; Nam et al. 2000; Hill and Sleep 2002; Thullner et al. 2002a; Thullner et al. 2002b; Sharp et al. 2005; Seifert and Engesgaard 2007; Thullner and Baveye 2008).

Continuous biofilms occasionally form in porous media, but it is often observed that flow channels and low-permeability zones dominate (Sharp et al. 1999a, 2005; Stewart and Fogler 2001; Hill and Sleep 2002; Seymour et al. 2004a, 2007; Viamajala et al. 2008).

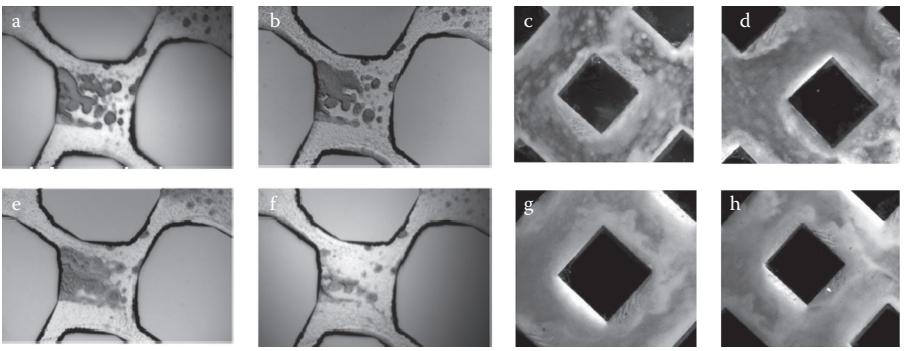

FIGURE 5.6
Microscopy images of biofilm forming in continuous-flow porous media environments. Flow from left to right in all images. Images (a), (b), (e), (f) are from Nambi et al. (2003), biofilms appear to be forming preferentially on the downstream edge of the porous media elements, which are approximately 300 μm in diameter; pore throats are approximately 35 μm in size. (a) 25 days, (b) 32 days, (e) 39 days, and (f) 44 days after inoculation. Images (c), (d), (g), (h) are depicting thick biofilm development in a porous media reactor over time. Porous media elements (black) are 1 mm^3; cross-section of flow channels is 1 mm^2. The reactor design is explained in more detail in Section 5.4.5. Images taken at effluent, (c and d) and influent region (g and h) of reactor. (Reprinted with permission Nambi, I.M., Werth, C.J., Sanford, R.A., and Valocchi, A.J. *Env. Sci. Tech.*, 37, 2003. ©2003, American Chemical Society.)

On the pore scale, it appears that biofilms accumulate initially in regions that are somewhat protected (Figure 5.6), that is, areas low in shear stress, and biofilm growth subsequently expands on the leeward side of porous media particles (Nambi et al. 2003; Stoodley et al. 2005; Knutson et al. 2007). The relatively high-shear forces in the pore throats of porous media are generally not favorable for initial attachment and thick biofilm development. High-shear environments generally result in relatively thin and smooth biofilms while lower shear environments usually produce thicker and rougher biofilms (Nam et al. 2000).

Oscillations in pressure drop across reactors have been related to changes in biomass distribution, the development of flow channels within the porous media filled with biomass, and creation of plugged regions where advective flow had occurred previously (Stewart and Fogler 2001). This behavior is probably more pronounced under constant flow conditions, which are employed more commonly in the laboratory than under constant head conditions, which are more commonly observed in the subsurface.

Under both types of flow conditions (constant head and constant flow), the most significant effects are usually observed in the influent regions of

biofilm-affected porous media, where electron acceptor and donor supply is the greatest (see case study below as well as Hosokawa et al. 1992; Chen et al. 1994; Jennings et al. 1995; Ince et al. 2000). Decreasing availability of electron donors, acceptors, or other important nutrients (e.g., phosphorous or nitrogen) can be as responsible for a decrease in biofilm formation as the presence of inhibitory compounds. There are definite reports that the availability of carbon can influence the formation of biofilms in porous media (Hand et al. 2008). However, there are also reports that indicate increased biofilm growth in the effluent regions as well as where carbon might be limited (Kim et al. 2006; Wheeler 2009). This might be partially explained by the increased availability of another limiting growth factor, for example, the availability of oxygen. In column systems, which are repeatedly opened or which are connected to gas-permeable tubing, such as silicon tubing, the entry of oxygen into the systems can result in increased microbial growth in those regions.

Changes in hydrodynamics in biofilm-affected porous media are not always solely due to biofilm growth itself but also often due to the biofilm induced formation of minerals, such as di- or tri-valent (e.g., Fe, Ca, Mg) carbonate, sulfate, sulfide, and phosphate minerals (Mclean et al. 1997; Benner et al. 1999; Rinck-Pfeiffer et al. 2000; VanGulck and Rowe 2004). In at least one case of investigating the effect of biofilm growth in porous media, the majority of the "clog material" was identified as calcium carbonate (Rowe et al. 2000).

5.4.2 Porosity

When describing the porosity of biofilm-affected porous media one has to consider at least three types of porosity: (1) the overall porosity of the porous medium, (2) the effective porosity of the porous medium, and (3) the internal porosity of the biofilm itself.

The internal porosity of biofilms was initially believed to be negligible. Biofilms were treated as hydrogels with little relevant internal structure until the mid-1990s when it was demonstrated that advective flow can occur within biofilms through channels formed during biofilm growth (Stoodley et al. 1994; Okabe et al. 1998). Such channels can be tens of micrometers in size, allowing for significant advective mass transport.

However, in most cases the influence of biofilm growth on porosity has been investigated on scales at which the internal porosity of biofilms becomes insignificant. Hence, in most experimental and modeling work, the overall (or bulk) porosity and effective (i.e., available for advective fluid flow) porosity have been assessed. However, one recent study suggests the importance of including the internal porosity (and thus permeability) of biofilms to more accurately describe permeability changes in biofilm-affected porous media (Thullner and Baveye 2008).

Differences in effective and overall porosity can be drastic, and their effect on localized and bulk permeability depends on the location of the formed

biofilm plugs. As biofilms grow, they initially decrease the free pore space; however, the overall porosity might not be significantly affected. If biofilms form on the inside of large pores or fractures, even a large change in porosity due to the presence of relatively thick biofilms can have a negligible effect on overall permeability. In contrast, if biofilms form in regions where their potential to affect fluid flow is great, such as pore throats, fracture entrances, and so on, a small change in overall porosity can have a significant effect on localized or overall permeability. In addition, as discussed by Sharp et al. (2005), Paulsen et al. (1997) and in Section 5.4.5, areas basically excluded from flow through thick biofilm growth can become accessible to flowing fluid owing to sudden detachment events, such as sloughing of large biofilm clusters that previously had blocked certain pores. Hence, methods, which allow spatially resolved measurements of porosity and differentiation between bulk and effective porosity are necessary to assess spatial and temporal changes.

Changes in porosity have mostly been assessed through direct microscopic observation combined with image analysis or are based on tracer breakthrough curves. Changes in media porosity can vary widely depending on the pore size distribution of the media as well as the method of measurement. Seifert and Engesgaard (2007) discuss problems with tracer breakthrough curve-based porosity estimates of biofilm-affected porous media in detail and discuss the validity of dual-porosity models for the description of hydrodynamics in biofilm-affected porous media (Seifert and Engesgaard 2007). Along with many other authors, Seifert and Engesgaard and Bielefeldt et al. point out that traditional porosity, permeability relationships, such as the Kozeny-Carman equation, generally underpredict the change in hydraulic conductivity (Bielefeldt et al. 2002b; Seifert and Engesgaard 2007).

5.4.3 Permeability

By far the most frequently used parameter to describe the influence of biofilm formation on porous media properties is permeability (k), expressed in dimensions of L^2 (length squared, e.g., m^2 or meter squared). In many published research papers permeability is expressed in the form of hydraulic conductivity (K, L/T, e.g., m/d or meters per day). The conversion is simple and uses the fluid density (ρ, M/L^3, e.g., kg/m^3), gravitational constant (g, L/T^2, e.g., m/sec^2), and viscosity (μ, (M/L)/T, e.g., (kg/m)/sec) according to the following equation:

$$k = K\frac{\mu}{\rho \bullet g}$$

For the purpose of comparing different studies (Table 5.1), 15°C was utilized as a reference with water as the fluid of interest so that $\rho = 999.099$ kg/m^3; $\mu = 1.14 * 10^{-3}$ (kg/m)/sec; $g = 9.807$ m/sec^2. Table 5.1 summarizes

TABLE 5.1
Initial and Final (or Lowest) Permeability Reported for Experiments in which Biofilm Growth in Porous Media or Fractures Was Promoted

Initial Permeability (cm^2)	Final or Lowest Permeability (cm^2)	Log (Reduction)	Porous Medium	Inoculum	Source
1.75E–04	1.53E–04	0.1	Sand	*Klebsiella oxytoca* and *Burkholderia cepacia*	Komlos et al. 2004
6.42E–08	5.71E–08	0.1	Sintered glass bead model cores	*Klebsiella pneumoniae* (starved)	MacLeod et al. 1988
3.49E–06	2.33E–06	0.2	Berea sandstone	*Pseudomonas aeruginosa* (killed)	Kalish et al. 1964
1.75E–04	1.01E–04	0.2	Sand	*Klebsiella oxytoca* and *Burkholderia cepacia*	Komlos et al. 2004
6.82E–08	4.17E–08	0.2	Sintered glass bead model cores	*Pseudomonas* sp. (killed)	Shaw et al. 1985
3.49E–07	1.75E–07	0.3	Berea sandstone	*Pseudomonas aeruginosa* (killed)	Kalish et al. 1964
3.49E–06	1.16E–06	0.5	Berea sandstone	*Micrococcus roseus* (killed)	Kalish et al. 1964
3.49E–06	1.05E–06	0.5	Berea sandstone	*Proteus vulgaris* (killed)	Kalish et al. 1964
3.49E–06	1.05E–06	0.5	Sandstone cores	*Klebsiella pneumonia*	Lappin-Scott et al. 1988b
6.42E–08	1.86E–08	0.5	Sintered glass bead model cores	*Klebsiella pneumoniae* (starved)	MacLeod et al. 1988

(*Continued*)

TABLE 5.1
(Continued)

Initial Permeability (cm^2)	Final or Lowest Permeability (cm^2)	Log (Reduction)	Porous Medium	Inoculum	Source
2.33E–07	5.82E–08	0.6	Berea sandstone	*Bacillus cereus* (killed)	Kalish et al. 1964
1.75E–04	3.68E–05	0.7	Sand	*Klebsiella oxytoca* and *Burkholderia cepacia*	Komlos et al. 2004
3.49E–06	4.65E–07	0.9	Berea sandstone	*Bacillus* sp.	Raiders et al. 1986
2.64E–01	2.88E–02	1.0	Artificial fracture	Groundwater community	Castegnier et al. 2006
2.33E–07	2.33E–08	1.0	Berea sandstone	*Micrococcus flavus* (killed)	Kalish et al. 1964
1.14E–05	1.16E–06	1.0	Sand	Mixed community, previously exposed to nitrate	Kildsgaard and Engesgaard 2001
4.65E–05	3.49E–06	1.1	Sand	*Pseudomonas* sp.	Cusack et al. 1992
2.33E–09	1.75E–10	1.1	Sand plus kaolinite	*Beijerinckia indica*	Dennis and Turner 1998
1.05E–05	8.35E–07	1.1	Sandy limestone aquifer material	Indigenous community	Rinck-Pfeiffer et al. 2000
6.98E–06	4.65E–07	1.2	Hanford loam	Indigenous	Allison 1947
4.89E–04	3.42E–05	1.2	Screened soil	*Pseudomonas fluorescens*	Cunningham et al. 2003
5.82E–06	3.49E–07	1.2	Berea sandstone, aerobic	Indigenous	Raiders et al. 1986
4.65E–05	2.33E–06	1.3	Sandy loam	Indigenous	Allison 1947

9.70E–07	5.00E–08	1.3	0.12 mm sand	*Pseudomonas aeruginosa*	Cunningham et al. 1991
4.65E–05	2.33E–06	1.3	Sand	*Pseudomonas* sp.	Cusack et al. 1992
3.49E–06	1.75E–07	1.3	Berea sandstone	*Micrococcus flavus* (killed)	Kalish et al. 1964
5.70E–05	2.09E–06	1.4	#70 quartz sand	Airport soil community	Bielefeldt et al. 2002b
1.51E–06	5.82E–08	1.4	Berea sandstone	*Bacillus subtilis* (killed)	Hart et al. 1960
1.97E–09	7.90E–11	1.4	Sandstone cores	*Klebsiella pneumonia*	Lappin-Scott et al. 1988b
3.95E–09	1.54E–10	1.4	Sandstone cores	*Klebsiella pneumonia*	Lappin-Scott et al. 1988b
2.10E–05	7.35E–07	1.5	1 mm glass beads	*Pseudomonas aeruginosa*	Cunningham et al. 1991
3.49E–04	1.16E–05	1.5	Ottawa 50–70 sand	Indigenous	Gupta and Swartzendruber 1962
2.09E–01	6.98E–03	1.5	artificial fracture	Top soil community	Hill and Sleep 2002
6.98E–06	2.33E–07	1.5	Berea sandstone, anaerobic	Indigenous	Raiders et al. 1986
3.49E–05	8.14E–07	1.6	Exeter sandy loam	Indigenous	Allison 1947
2.17E–06	5.00E–08	1.6	0.54 mm sand	*Pseudomonas aeruginosa*	Cunningham et al. 1991
4.77E–04	1.12E–05	1.6	Field scale	*Pseudomonas* sp.	Cunningham et al. 2003
3.95E–09	8.69E–11	1.7	Sandstone cores	*Klebsiella pneumonia*	Lappin-Scott et al. 1988b

(*Continued*)

TABLE 5.1
(Continued)

Initial Permeability (cm^2)	Final or Lowest Permeability (cm^2)	Log (Reduction)	Porous Medium	Inoculum	Source
3.19E–06	5.00E–08	1.8	0.7 mm sand	*Pseudomonas aeruginosa*	Cunningham et al. 1991
1.86E–06	2.68E–08	1.8	Sandy soil	*Azotobacter chroococcum*	Kim et al. 2006
3.49E–06	4.65E–08	1.9	Sandstone cores	*Klebsiella pneumonia*	Lappin-Scott et al. 1988b
9.87E–08	1.18E–09	1.9	300 um glass beads	*Leuconostoc mesenteroides*	Stewart and Fogler 2001
5.82E–04	6.40E–06	2.0	#30 quartz sand	Airport soil community	Bielefeldt et al. 2002b
1.97E–09	1.97E–11	2.0	Sandstone cores	Produced water	Lappin-Scott et al. 1988b
3.95E–09	3.95E–11	2.0	Sandstone cores	*Klebsiella pneumonia*	Lappin-Scott et al. 1988b
6.42E–08	6.42E–10	2.0	Sintered glass bead model cores	*Klebsiella pneumonia*	MacLeod et al. 1988
1.05E–05	7.37E–07	2.0	Quartz sand	Subsurface strains	Vandevivere and Baveye 1992a
1.28E–06	1.06E–08	2.1	Sandy soil	*Azotobacter chroococcum*	Kim et al. 2006
1.16E–06	9.08E–09	2.1	Sandy soil	*Azotobacter chroococcum*	Kim et al. 2006
6.59E–02	5.27E–04	2.1	Limestone fracture	groundwater community	Ross et al. 2001
6.43E–08	4.50E–10	2.2	Sintered glass bead model cores	*Pseudomonas* sp.	Shaw et al. 1985
6.46E–08	3.88E–10	2.2	Sintered glass bead model cores	*Pseudomonas* sp.	Shaw et al. 1985

1.16E–08	5.82E–11	2.3	Sand plus kaolinite	*Beijerinckia indica*	Dennis and Turner 1998
3.95E–09	1.97E–11	2.3	Sandstone cores	*Klebsiella pneumonia*	Lappin-Scott et al. 1988b
4.30E–04	1.75E–06	2.4	Quartz sand	Airport soil community	Bielefeldt et al. 2002a
4.07E–04	1.28E–06	2.5	#50 quartz sand	Airport soil community	Bielefeldt et al. 2002b
4.02E–01	1.21E–03	2.5	Artificial fracture	Groundwater community	Castegnier et al. 2006
5.84E–08	1.75E–10	2.5	Sintered glass bead model cores	*Pseudomonas* sp.	Shaw et al. 1985
5.82E–08	1.40E–10	2.6	Sand plus kaolinite	*Beijerinckia indica*	Dennis and Turner 1998
3.95E–09	7.90E–12	2.7	Sandstone cores	*Klebsiella pneumonia*	Lappin-Scott et al. 1988b
6.42E–08	1.28E–10	2.7	Sintered glass bead model cores	*Pseudomonas* sp. (killed)	Shaw et al. 1985
1.05E–07	1.86E–10	2.8	Sand plus kaolinite	*Beijerinckia indica*	Dennis and Turner 1998
6.98E–08	1.05E–10	2.8	Sand plus kaolinite	*Beijerinckia indica*	Dennis and Turner 1998
1.16E–07	1.75E–10	2.8	Sand plus kaolinite	*Beijerinckia indica*	Dennis and Turner 1998
4.32E–01	4.32E–04	3.0	Artificial fracture	Groundwater community	Castegnier et al. 2006
1.16E–07	1.16E–10	3.0	Compacted, silty sand	*Beijerinckia indica*	Dennis and Turner 1998

(*Continued*)

TABLE 5.1
(Continued)

Initial Permeability (cm^2)	Final or Lowest Permeability (cm^2)	Log (Reduction)	Porous Medium	Inoculum	Source
1.16E–07	1.16E–10	3.0	Sand plus kaolinite	*Beijerinckia indica*	Dennis and Turner 1998
3.95E–09	3.95E–12	3.0	Sandstone cores	produced water	Lappin-Scott et al. 1988b
3.95E–09	3.95E–12	3.0	Sandstone cores	*Klebsiella pneumonia*	Lappin-Scott et al. 1988b
8.38E–05	8.38E–08	3.0	Sand	*Arthrobacter* sp.	Vandevivere and Baveye 1992b
2.33E–07	1.63E–10	3.2	Sand plus kaolinite	*Beijerinckia indica*	Dennis and Turner 1998
2.95E–03	1.47E–06	3.3	Porous medium	wastewater treatment plant	Taylor and Jaffe 1990b
3.07E–04	6.46E–08	3.7	Loamy soil	Indigenous	Frankenberger et al. 1979
8.14E–04	8.14E–08	4.0	Two-dimensional lysimeter	*Klebsiella oxytoca*	Cunningham et al. 1997
1.05E–05	7.37E–09	4.0	Quartz sand	Subsurface strains	Vandevivere and Baveye 1992a
3.03E–03	1.16E–07	4.4	Sand	*Klebsiella pneumonia*	Cunningham et al. 1997
3.46E–02	1.16E–07	5.5	Sand	*Klebsiella pneumonia*	Cunningham et al. 1997
3.87E–01	1.16E–06	5.5	Glass beads	Landfill leachate, evidence of $CaCO_3$	VanGulck and Rowe 2004

the changes in permeability observed during studies in which biological growth reduced the permeability of porous media or fractures.

The reduction of porous media permeability is generally believed to occur through the accumulation of biomass and polysaccharides. Permeability reductions of three orders of magnitude or less are generally reported, but greater reductions have been reported. The largest reductions reported have been clearly associated with the coprecipitation of carbonate minerals (VanGulck and Rowe 2004; Castegnier et al. 2006).

Like biofilm accumulation, permeability reduction is usually not homogeneous but rather spatially and temporally varied with higher production of biomass, and thus greater permeability reduction, at the influent (Rinck-Pfeiffer et al. 2000; VanGulck and Rowe 2004; Castegnier et al. 2006). Permeability reduction also appears to be more pronounced in fine-textured materials than in coarse-textured ones (Vandevivere 1995; Vandevivere et al. 1995).

Once achieved, decreased porous media permeability can often be maintained even during periods of environmental stress for the biofilm organisms, such as starvation, toxic upset or similar, as demonstrated in one- and two-dimensional flow fields in the laboratory (Cunningham et al. 1997; Kim and Fogler 2000; Kim et al. 2006) as well as in a field-scale demonstration (Cunningham et al. 2003). This persistence is usually attributed to the presence of large amounts of EPS that do not degrade readily. These observations are supported by Kim and Fogler who observed no EPS degradation in batch experiments for a period of about 2 years (Kim and Fogler 1999).

5.4.4 Dispersion and Diffusion

Just like permeability and porosity, dispersivity (which relates the dispersion coefficient to velocity) is influenced by biofilm growth in porous media. Most of the experiments reveal a two- to eight-fold increase in dispersivity (Sharp et al. 1999b, 2005; Hill and Sleep 2002; Bielefeldt et al. 2002b; Arnon et al. 2005b), although order of magnitude changes in dispersivity have also been observed (Taylor and Jaffe 1990a; Bielefeldt et al. 2002a; Seifert and Engesgaard 2007). In general, dispersivity increases over time in biofilm-affected porous media but often reaches semistable values once the biofilm has reached a pseudosteady state (Sharp et al. 1999a, 2005; Bielefeldt et al. 2002b). Hill and Sleep stated that the dispersion coefficient increased logarithmically with hydraulic conductivity reduction in biofilm-affected fractures (Hill and Sleep 2002).

Increases in dispersivity have mostly been attributed to increases in tortuosity of the porous medium, the development of no flow zones, or increased influence of diffusive transport into and out of the developed biofilms (see for example, Sharp et al. 1999a, 2005; Seymour et al. 2004a,b). Seymour et al. demonstrated that diffusion out of no flow zones can clearly influence the macroscale dispersion in biofilm-affected porous media (Seymour et al. 2004b).

In most cases, the dispersivity in biofilm-affected porous media has been estimated on the basis of tracer tests and subsequent parameter fitting in mathematical models (e.g., Taylor and Jaffe 1990a; Sharp et al. 1999b; Kildsgaard and Engesgaard 2001; Hill and Sleep 2002; Bielefeldt et al. 2002a,b; Sharp et al. 2005; Arnon et al. 2005b). However, recently more advanced techniques have been developed.

Magnetic resonance microscopy (MRM) techniques are ideally suited to provide nondestructive, spatially, and temporally resolved measurements of hydrodynamic dispersion and velocity in porous media while allowing limited biofilm imaging (10–100 sec of μm resolution). The recent work by Seymour et al. clearly demonstrates the strength of this technique and already indicates the development of anomalous hydrodynamic dispersion in biofilm-affected porous media (Seymour et al. 2004b; Seymour et al. 2007).

In the future, this technique will allow for the correlation of local and bulk dispersivity with the distribution of biofilms in porous media environments. This capability will ultimately allow for the development of improved models for the description of reactive transport in biofilm-affected porous media. MRM techniques as well as other techniques capable of spatially resolving dispersion and diffusion phenomena will also allow for the evaluation of the importance of diffusive transport processes in biofilms.

The importance of diffusion on biofilm processes in general has been reported widely (Williamson and McCarty 1976a,b; Debeer et al. 1994; Stewart 1996, 1998; Xu et al. 1998, 2003; Stewart and Costerton 2001) and its influence on reactive transport in porous media affected by biofilms should be considered to explain micro- and macroscale processes (Seymour et al. 2004a,b, 2007).

5.4.5 Constant Head versus Constant Flow

Most experimental systems for the investigation of the influence of biofilm on porous media hydrodynamics have been operated under constant flow conditions by using pumps. Although there are situations in industry and the environment where constant flow conditions are encountered, constant head conditions are more commonly encountered in subsurface environments. For instance, most aquifers are subject to constant head conditions. This section will give an example that clearly demonstrates differences in biofilm development and its influence on porous media hydrodynamics under constant flow and constant head conditions.

Significant biofilm growth in porous media will reduce the pore space available for advective flow. Figures 5.7 and 5.8 provide a comparison of tracer studies and images, which compare solute transport through two-dimensional mesoscale (3.8 cm × 8.5 cm) porous media reactors. Similar studies demonstrating the utility of these reactors for monitoring reactive and nonreactive transport in porous media were conducted with *Vibrio fischeri* (Sharp et al. 2005). It became apparent in the studies by Sharp et al. (2005) that electron

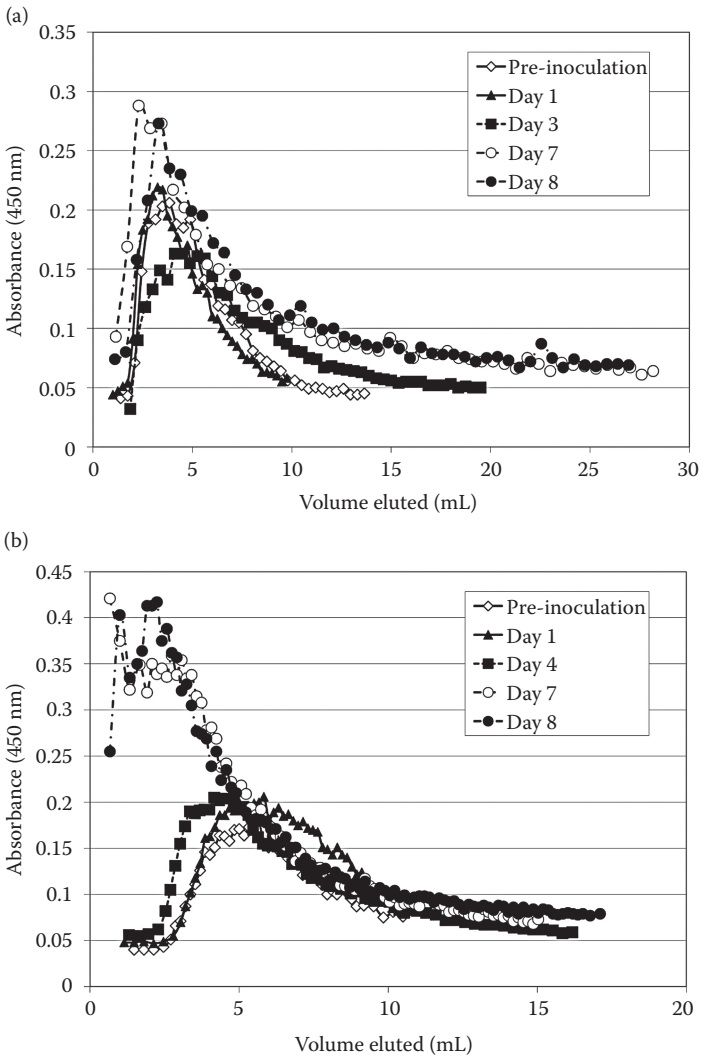

FIGURE 5.7
Tracer breakthrough curves acquired over time for two different flat-plate porous media reactor systems: a constant flow system (a) and a constant head system (b). The constant flow system clearly shows an accelerated breakthrough after 7 days, while the constant head system shows increased retention and dispersion of the dye tracer beginning on day 3. Figure 5.8 shows pictures for the 7 day tracer study for each system (as well as for a clean ["pre-inoculation"] reactor).

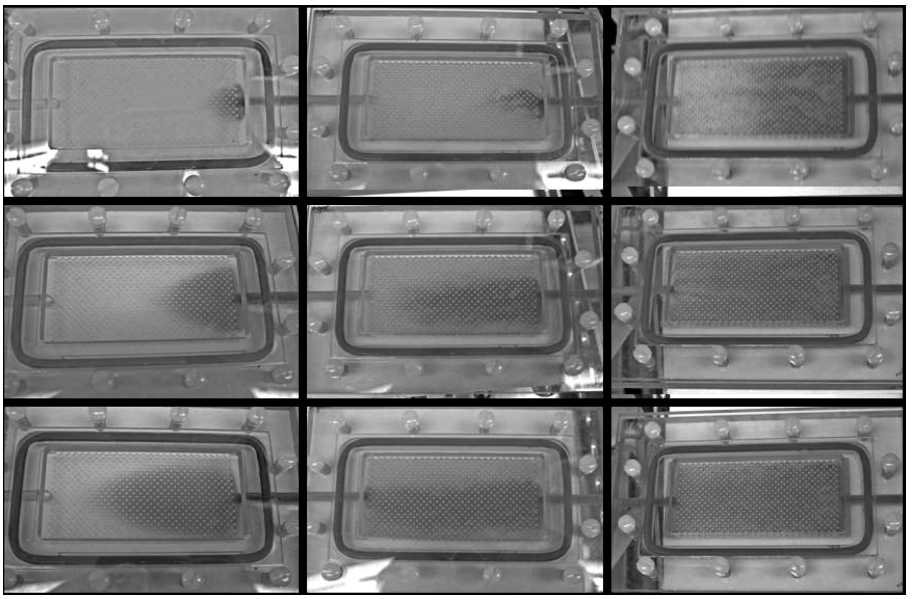

FIGURE 5.8
Images of flat-plate porous media reactor systems during representative times of tracer studies. Left column: clean reactor before inoculation; center column: constant flow system after 7 days; right column: constant head system. Duration from time of tracer injection increases from top to bottom of each column. The pictures correspond to the day 7 tracer curves in Figure 5.7.

acceptor (i.e., oxygen) availability limited biofilm growth and activity. To circumvent electron acceptor availability-related limitation of biofilm growth, a fermentative microorganism, *Cellulomonas* sp. strain ES6 (Sani et al. 2002; Smith et al. 2002; Viamajala et al. 2008) was utilized in the studies summarized in Figures 5.7 and 5.8.

A comparison of biofilm development and its influence on hydrodynamics between constant head and constant flow conditions becomes immediately obvious. The tracer breakthrough characteristics in the clean (not biofilm-affected) reactors are similar for the constant head and constant flow conditions (Figure 5.7). However, differences in the characteristics of tracer breakthrough become obvious very quickly (compare images in Figure 5.8, taken after 7 days in both reactors). Under constant flow conditions significant changes in tracer breakthrough characteristics become obvious after 7 days, when the initial breakthrough of tracer occurs very quickly, indicating a significant reduction in effective porosity. In addition, some of the dye injected tends to remain in the biofilm-affected constant flow reactor for an extended period

of time as indicated by elevated absorbance readings at large eluted volumes after 7 and 9 days. This prolonged retention of dye is likely due to no flow regions of the biofilm-affected reactor and diffusion-limited transport of dye back into the main flow path after the initial front of dye has passed through the reactor. Prolonged retention of dye is even more evident in the biofilm reactor operated under constant head. While the initial breakthrough of dye seems to be relatively unaffected under constant head conditions throughout the experiment, dye retention due to slow (likely diffusion-limited) transport of dye out of the biofilm toward the end of each tracer study becomes significant after 3 days already.

It is clear from the research in our laboratories that there are significant differences between biofilm growth patterns under the different flow regimes and that there is a need for a more thorough understanding of the reasons for these differences. Under constant flow conditions a decrease in effective porosity will increase the fluid velocity and thus in most cases the influent pressure as well as shear stress within the porous medium (data not shown). Biofilms grown under continuous-flow conditions in a two-dimensional flat-plate reactor appear to reach a pseudosteady state in which the average hydraulic residence time changes only slightly although the location of the primary flow path seems to be changing with time (Sharp et al. 1999a, 2005; Arnon et al. 2005b). The time until a pseudosteady state is reached and the extent of porosity reduction depends on the microorganism(s) present, growth conditions (e.g., temperature, electron acceptor availability, pH, etc.), flowrate, and similar parameters. Equivalent observations for constant flow conditions were obtained in our laboratories using MRM in pseudo one-dimensional porous media columns (Seymour et al. 2004b, 2007).

The existence of a "critical shear stress," that is, a shear stress above which significant detachment occurs, has been proposed (Kim and Fogler 2000) and makes intuitive sense under constant flow conditions. Once biofilm growth begins to restrict pore spaces the localized flowrate and associated shear stress increase with decreasing effective porosity and can result in increased biofilm detachment.

In constant head systems, such as many shallow aquifers, a decrease in pore space results in a decrease in overall porosity and, likely, permeability. Since the influent head remains constant, such a decrease in overall permeability will result in a decrease in flowrate according to Darcy's Law.

$$Q = -KA\frac{dh}{dl}$$

where Q is the flow rate (L^3/T, e.g., m^3/sec), K is the hydraulic conductivity (L/T, e.g., m/d), A is the cross-sectional area of flow (L^2, e.g., m^2), dh is the difference in hydraulic head across the reactor (L, e.g., m), and dl the length of the reactor (L, e.g., m).

Overall, the prediction and control of biofilm formation in porous media remains difficult since the ability to observe biofilm development spatially and temporally is severely limited even in laboratory systems. Mathematical modeling can aid in the development of biofilm-based technologies, but the development of conceptual and mathematical models is not only limited by a lack of highly resolved experimental data but also by computational challenges.

5.5 A Few Notes on Modeling

The experimental observations summarized above clearly show that biomass is often distributed heterogeneously in porous media. To properly model biofilm processes in porous media, models should take into account the influence of microscale heterogeneities and distributions. Unfortunately, it can become computationally burdensome to model a large (meter to hundreds of meter) scale system on the microscale. Hence, compromises have to be made with respect to the desired accuracy at the microscale and the computational feasibility for larger-scale systems.

5.5.1 Macroscopic versus Microscopic Models

Bulk-scale models using analytical or fast numerical solutions are computationally efficient and work well for cases where large volume averaging is appropriate, that is, where microscale processes are negligible in relation to the overall behavior of the system (Clement et al. 1996). Such models are capable of modeling bulk changes for parameters, such as porosity, specific surface area, permeability, and dispersivity, in dependence of biofilm formation in porous media but in general they do not assume any specific pattern for microbial growth but instead use macroscopic estimates of the average biomass concentration.

Microscale models, which, in contrast, treat biofilm-affected porous media as multidimensional on the pore scale, can potentially predict localized clogging, which can have a significant effect on the overall hydrodynamics of a system, but become computationally demanding if they are to predict the effect of localized (i.e., pore scale) biofilm growth on the bulk properties of the porous medium.

Over the past years, a number of papers have been published that address the issues associated with bulk-scale modeling, while attempting to obtain computationally efficient, appropriate descriptions of bioclogging processes in porous media. The pore-network model approach allows for the simulation of porosity and hydraulic conductivity changes without having to describe the process of biofilm development on the microscale for every single pore (Dupin

and McCarty 2000; Thullner et al. 2002b, 2004). In this context, it is rightfully pointed out by Thullner et al. that there are no published, theoretically-derived hydraulic conductivity versus porosity relationships that account for interpore connections in more than one dimension and heterogeneous biofilm distributions (Thullner et al. 2002b).

In addition, it has been proposed to consider the importance of advective flow within biofilms themselves. There is experimental evidence that advective flow occurs within biofilms (Stoodley et al. 1994; Debeer et al. 1994) but until relatively recently these findings had not been included into mathematical modeling approaches. It is now being suggested to model the biofilm phase itself as a porous medium (Zhang and Bishop 1994; Nguyen et al. 2005; Zacarias et al. 2005; Kapellos et al. 2007a,b). The inclusion of fluid flow through the EPS matrix and biofilm microchannels has been demonstrated to result in improved description of biofilm processes (Seifert and Engesgaard 2007; Thullner and Baveye 2008).

5.5.2 Mixed Domain (Hybrid) Models

More recently, the use of multiscale models has been described, which solve the Navier–Stokes and Brinkman equation numerically and combine the approach with a cellular automaton approach or Lagrangian-type simulations of detached fragment trajectories (Kapellos et al. 2007a,b).

A hybrid Lagrangian particle dynamics model capable of describing biofilm formation in porous media is described in detail in Chapter 7 of this book. Such a model might be better suited to incorporate the heterogeneity of porous media and microbial populations, and to ultimately improve our ability to describe hydrodynamics and mass transport in biofilm-affected porous media.

5.6 Porous Media Biofilms in Nature and Technology

Biofilms are recognized to be present in many industrial, environmental, and medical systems. Initial work mostly focused on the eradication of biofilms by treatment with antimicrobials, but more recently many of the "typical" biofilm properties have been recognized to be potentially advantageous for engineered applications (Petrozzi et al. 1993; Bouwer et al. 2000; Sauer et al. 2002; Stoodley et al. 2002b; Cvitkovitch 2004; Molin et al. 2004; Massoudieh et al. 2007; Costerton 2007; Stewart and Franklin 2008).

Despite the fact that biofilm growth and organization are not yet completely understood (as discussed earlier), it is clear that the establishment of organized biofilm communities can be utilized for benefit. The close proximity of organisms to each other, allowing for the possibility of cell–cell communication, exchange of genetic elements (DNA, RNA), colocation of physiologically

different organisms that can facilitate the exchange of metabolites, and the increased resistance to environmental stresses, make biofilms very attractive for technology development.

Owing to their increased tolerance to environmental stress and toxic compounds, biofilm reactors are frequently proposed to be used for the treatment of recalcitrant compounds. The range of such compounds spans from surfactants to herbicides and organic solvents to dyes (Mol et al. 1993; Petrozzi et al. 1993; Jerabkova et al. 1999; Mondragon-Parada et al. 2008). In addition, biofilms can influence colloid transport (Kim and Corapcioglu 1997; Stevik et al. 2004; Muris et al. 2005; Morales et al. 2007). The transport of pathogens (biological colloids) and colloid-mediated contaminant transport through porous media are both areas of importance for public health.

Biofilm technologies are available, and there are ongoing research and development efforts in the areas of subsurface biofilm barriers, hazardous waste treatment, biofilters, enhanced oil recovery, acid mine drainage treatment, filters, and infiltration systems in water and wastewater treatment, biofilms as biosorbents, air pollution control, and municipal solid waste leachate control.

Changes in porosity, permeability, dispersion, and diffusion can be desirable or detrimental for a given application. For instance, in the case of subsurface biofilm barriers for the control of groundwater flow, maximum porosity and permeability reduction is desired. In contrast, in biotrickling filters for wastewater treatment an optimal biofilm thickness is desired, which results in maximum removal of solutes while maintaining fairly high permeability.

To limit the discussion of biofilm technologies in porous media in this chapter, it should be pointed out that moving bed reactors, such as suspended (fluidized) bed reactors, will be excluded from consideration. The extent of mixing and abrasion of biofilm by the carrier material is significantly different from, for instance, filters, soils, and packed bed reactors, and the topic would become too complex to be discussed in sufficient detail here. Membrane systems such as reverse osmosis filtration cartridges are also excluded from consideration, although these networks of flow channels could be considered a special form of porous medium, and biofilm growth (biofouling) in these systems can significantly affect flow as indicated by increasing backpressure in (bio-) fouled membrane systems.

A major factor in the performance of biofilm reactors is the limited mass transport of solutes into the biofilm where the active biomass is located. Transport limitations of electron acceptors and donors can significantly affect the performance of porous media biofilm reactors, especially if the reaction depends on oxygen, which has a very limited solubility in water (Kirchner et al. 1992; Joannis-Cassan et al. 2007). Hence, it is extremely important to understand flow dynamics and solute reactive transport in biofilm-affected porous media (Iliuta and Larachi 2004).

It should also be kept in mind that, in the environment and industrial systems, biofilms are likely to accumulate solutes and possibly significant amounts of minerals. Iron oxides, calcium carbonate, and sulfur-containing

minerals are the most frequently described inorganic constituents of biofilms in porous media (Mclean et al. 1997; Cooke et al. 2005). The precipitation of minerals can result in semipermanent to permanent encrustation of biofilm cells or clogging of certain areas in a porous medium. These effects might or might not be desirable depending on the goal of a given application.

The following sections will summarize a few biofilm technologies in porous media in detail and discuss research and development needs associated with the further development of these technologies.

5.6.1 Subsurface Biofilm Barriers for the Control and Remediation of Contaminated Groundwater

Subsurface biofilm barriers are engineered structures developed through the growth and activity of microorganisms in soils. The establishment of permeable, impermeable, and semipermeable biofilm barriers has been proposed for the control and remediation of contaminated soil and groundwater (Cunningham et al. 1997; Waybrant et al. 1998; Benner et al. 1999; Hiebert et al. 2001; Nyman et al. 2002; Ludwig et al. 2002; Cunningham et al. 2003; Komlos et al. 2004).

The establishment of low-permeability biofilm barriers (Figure 5.9) has been the focus of a number of research and development efforts. These barriers are designed to provide maximum reduction of permeability by promoting thick biofilm growth to either reduce the flow of groundwater through certain areas of the subsurface or direct groundwater into a certain direction (e.g., an area where treatment occurs). Maximum permeability reduction is usually obtained by promoting the production of copious amounts of EPS by indigenous microorganisms or through bioaugmentation with organisms known to produce copious amounts of EPS.

These barriers have been shown to reduce porous media permeability by several orders of magnitude (Cunningham et al. 1997, 2003; Ross et al. 1998, 2007; Hiebert et al. 2001; Komlos et al. 2004). More recently, the biofilm-promoted precipitation of carbonate minerals has been proposed to be advantageous for long-term biofilm barrier stability (Gerlach et al. 2009).

In addition, these subsurface biofilm barriers have been shown to effectively remove solutes. The consumption of soluble electron acceptors such as oxygen as well as the removal of contaminants of concern such as nitrate (Hiebert et al. 2001; Cunningham et al. 2003) has been shown. Cunningham et al. (2003) described the establishment of a nitrate-remediating biofilm barrier in a 130 ft wide, 180 ft long, 21 ft deep test cell. Starved cells of *Pseudomonas fluorescens* strain CPC211a were injected and biofilm growth was stimulated through the injection of a growth nutrient mixture composed of molasses, nitrate, and other additives. The biofilm barrier reduced the soil hydraulic conductivity by 99% from an initial value of 0.042 cm/sec and reliably reduced nitrate concentrations by 93% or more from an initial value

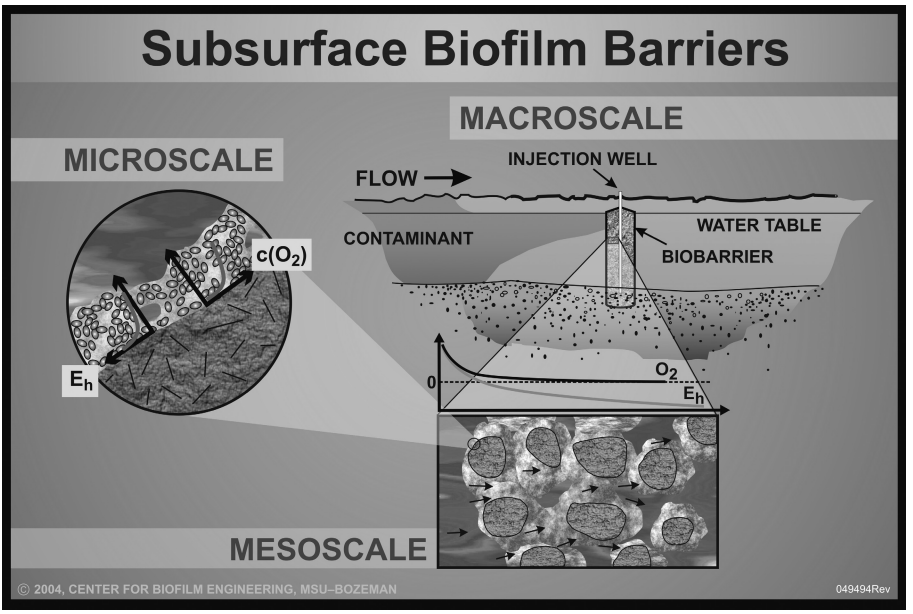

FIGURE 5.9
Schematic of a low-permeability subsurface biofilm barrier. Such barriers have been established at the field scale through the injection of bacteria and/or nutrients. Thick biofilm growth is promoted in the pore space, which reduces soil permeability. Nutrient and electron acceptor consumption create gradients (as indicated by decreasing oxygen concentrations and redox potential [E_h]) along the direction of groundwater flow (see mesoscale schematic) and transversally into the biofilm (see microscale schematic).

of approximately 100 mg/L (Cunningham et al. 2003). This biofilm barrier was designed for maximum reduction of permeability; however, the observation of nearly complete nitrate removal has led to additional research efforts designed to optimize permeability and contaminant transformation rates in reactive subsurface biofilm barriers.

Low permeability and reactive as well as permeable and reactive subsurface biofilm barriers have been proposed (Figure 5.10). Komlos et al. (2004, 2006) evaluated the possibility of establishing biofilm barriers capable of simultaneously providing a reduction in soil permeability and trichloroethylene (TCE) degradation. These studies led to the development of readily deployable strategies for controlling the relative abundance of different species in defined mixed-culture biofilms in porous media (Komlos et al. 2004, 2005, 2006).

Permeable reactive subsurface barriers for the treatment of chlorinated solvents, heavy metals, and radionuclides have been proposed and employed in

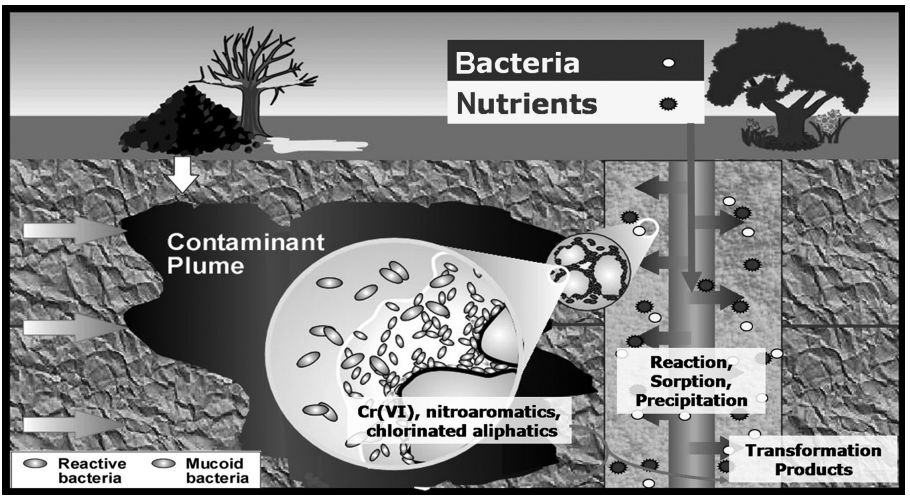

FIGURE 5.10
Schematic of a reactive biofilm barrier. Depending on the goal of the barrier (reduction in permeability [groundwater flow] *and* degradation of contaminants or only degradation of contaminants but no hydraulic manipulation) a different degree of permeability reduction, and thus biofilm growth, is desired. The barrier can be established through the injection of nutrients (carbon source, electron donor, electron acceptor, etc.) and bacteria (if necessary). Contaminant transformation, sorption, and precipitation will all play a role in the ultimate fate of the contaminants.

field situations. Zero-valent iron-based barriers are probably the most common type of permeable subsurface barrier employed (Gould 1982; Cantrell et al. 1995; O'Hannesin and Gillham 1998; Fiedor et al. 1998; Scherer et al. 2000; Wilkin et al. 2003), and the influence of attached microorganisms on their performance has been noted (Gerlach et al. 2000; Gu et al. 2002; Shin et al. 2007). Furthermore, biologically active permeable reactive barriers for the control and treatment of acidic mine wastewater have been proposed and demonstrated in the field (Waybrant et al. 1998; Benner et al. 1999; Ludwig et al. 2002; Golab et al. 2006; Davis et al. 2007). Chemical *in situ* redox manipulation has also been demonstrated in situations where trench and fill methods are impractical owing to depth limitations (Istok et al. 1999; Seaman et al. 1999).

On the basis of the success and relative ease of implementation of these technologies, combined with the possibility of establishing biologically active zones in the deep subsurface through the careful control of distribution and activity of microorganisms, the development of permeable subsurface biofilm barriers has immense economical potential. Especially the possibility of employing such treatment strategies in the deep subsurface through the

use of injection wells has resulted in a flurry of research and development efforts that contribute toward the continued development of such permeable reactive subsurface biofilm barriers.

5.6.2 Deep Subsurface Biofilms for Enhanced Oil Recovery and Carbon Sequestration

The initial recovery of oil from deep subsurface reservoirs is typically estimated to reach between 10% and 35% of a reservoir's oil. Secondary recovery, which most often involves waterflooding, can increase recovery by 20% or more. Tertiary recovery, often also called secondary enhanced oil recovery, further increases the recovery of oil from these reservoirs. Thermal recovery, chemical flooding, miscible displacement (such as gas injection), and microbially enhanced oil recovery (MEOR) have been explored as tertiary techniques.

Microbially enhanced oil recovery is proposed to be applicable in a number of different ways, such as production of biosurfactants or gases to enhance oil mobility, selective plugging of high-permeability channels in reservoir rock to increase sweep efficiency, and *in situ* biocracking, during which microbes break down long alkane chains to produce higher solubility shorter alkane chains.

A flurry of research and development efforts in the 1980s and 1990s investigated the use of microorganisms to enhance oil recovery from deep subsurface oil-bearing formations, and detailed information can be found in a number of books and reviews (Zajic and Donaldson 1985; Kosaric et al. 1987; Donaldson et al. 1989; Yen 1990).

Biofilms are thought to be effective in selectively plugging water-filled high-permeability areas, also called thief zones. Selectively reducing the permeability of such high-permeability areas would allow fluids used for enhanced secondary or tertiary oil recovery, such as water or supercritical CO_2 ($scCO_2$), to more effectively mobilize residual oil in low-permeability areas of the formation. Significant research and development efforts have been conducted to develop this technology (e.g., Shaw et al. 1985; MacLeod et al. 1988; Lappin-Scott et al. 1988a,b; Cusack et al. 1992; Lappin-Scott and Costerton 1992) yet widespread reports of successful implementation of this technology are lacking. This may be due to difficulties in evaluating the economics of using biofilm-mediated plugging of high-permeability areas in the deep subsurface. Determining the location of biofilms in the deep subsurface is even more challenging than the imaging of biofilms in laboratory-scale reactors as described earlier. Hence, MEOR is continuing to be proposed for application in the oilfield but has yet to gain widespread acceptance, and thus application.

The potential of using biofilms to enhance the deep subsurface sequestration of carbon dioxide has been proposed as well (Davis et al. 2006; Mitchell et al. 2008a; Mitchell et al. 2009). Recent work by Mitchell et al. (2008, 2009)

demonstrated the utility of biofilms in sandstone cores to reduce the permeability by more than 95% at elevated pressures (8.9 MPa) and moderate temperatures (32°C). Biofilm organisms under these conditions were also demonstrated to survive exposure to $scCO_2$ and maintain the decreased permeability of the core even under starvation conditions.

5.6.3 Porous Media Biofilm Reactors in Industry and Waste Treatment

Other industrial and environmental systems and applications in which porous media biofilms play a significant role are mostly related to water and waste treatment.

Attached microorganisms in water filtration, which can form within, at the influent, or effluent of filtration devices, can have detrimental or beneficial effects on water treatment processes. Obviously, excessive microbial growth can reduce the hydraulic conductivity of filtration devices and result in increased maintenance requirements, reduced run times, increased frequency of backflushing cycles, or increased need to exchange filter materials. However, porous media biofilms have also been observed to facilitate the sorption or degradation of organic particulates and solutes as well as the oxidative precipitation of problem metals, such as reduced iron and manganese, during drinking water treatment.

The use of trickling filters and infiltration systems in wastewater treatment has been practiced for a long time and microorganisms, more or less immobilized in porous media, have been used for the removal of wastewater constituents. Both, large-scale applications of these technologies as well as fundamental research assessing the role of biofilms in these technologies are ongoing (Ileri and Muslu 1996; Iliuta and Larachi 2004; Wanko et al. 2005, 2006; Mauclaire et al. 2006).

Some of the characteristics discussed earlier, such as enhanced resistance to adverse conditions and enhanced-metabolic capabilities of biofilm communities, have also led to an increased use of immobilized microbial communities in hazardous waste treatment. The treatment of synthetic dyes or tannery wastewater in fixed-bed biofilm reactors are two examples of such processes (Song et al. 2003; Tse and Yu 2003).

Gas-phase treatment using biofilters has also been practiced for an extended period of time, especially for the control of odors, such as hydrogen sulfide, and volatile organic compounds, such as methanol or acetone. While such technologies are already being applied on the industrial scale (Shareefdeen and Baltzis 1994; Kennes and Thalasso 1998; Shareefdeen et al. 2002; Elmrini et al. 2004), fundamental research targeted at understanding the physico- and biochemical processes controlling the efficiency of such vapor-filtration devices is ongoing (Hwang et al. 1997; Tang et al. 1997; Wani et al. 1997; Ramirez et al. 2008). Overall, biofilm processes in unsaturated porous media have been studied much less extensively than in saturated porous media

and much work has yet to be done to better understand and consequently capitalize on the existing potential.

5.7 Conclusions and Outlook

Biofilms can have a significant influence on hydrodynamics and thus on mass transport in porous media. Biofilm growth quickly induces heterogeneity into even highly uniform porous media and the dynamics of biofilm development are—at this point—somewhat unpredictable owing to the influence biofilm growth and hydrodynamics have on each other. Hydrodynamics, nutrient availability, physicochemical conditions (e.g., temperature, pH), presence (or production) of inhibitory or stimulating compounds, community composition and structure are only some of the factors that have been shown to influence the development of biofilms in porous media. The interplay of hydrodynamics and biofilm growth leads to an oscillatory behavior, which appears to reach a pseudosteady state at certain scales in time and space. The ability to predict the size of these relevant scales remains a challenge.

In general, porous media permeability and porosity decrease during biofilm formation while dispersivity generally increases. Although rarely observed, opposite effects might also occur.

The immense beneficial (and detrimental) potential of porous media biofilms has led to the development of large-scale technologies, such as subsurface biofilm barriers and biofilm reactors, as well as strategies on how to control biofilms in porous media environments (e.g., around injection wells where excessive biofilm growth is undesirable).

Owing to the challenges of studying porous media biofilms in detail, a number of these developments have been based more or less on science-based understanding. The (mostly microscale) research summarized in this chapter has been designed to develop the fundamental knowledge necessary to develop science-based engineering strategies for porous media biofilm technologies.

Even without a complete understanding of biofilm processes in porous media, the potential of promoting subsurface biofilm growth for benefit has been widely acknowledged and technology development in the areas of oil production, contaminant remediation, carbon sequestration, soil stabilization, and waste treatment proves its economic potential.

The widely accepted inherent resistance of biofilm organisms to environmental stresses has made biofilm barriers an attractive strategy. Even exposure for extended periods of time to toxins; absence of nutrientsl; low water potential; highly saline, acidic, or basic conditions; and supercritical carbon dioxide have been shown to affect biofilm barriers in porous media only slightly (Sturman et al. 1995; Warwood et al. 1995; Cunningham et al. 1997; Dennis and Turner 1998; Bouwer et al. 2000; Hiebert et al. 2001; Cunningham et al. 2003; Komlos et al. 2004; Mitchell et al. 2008a,b, 2009).

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