The role of biofilms in water reclamation and reuse

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Abstract Biofilms play a major role in many water reclamation and reuse technologies. Normally, wherever there is water, a support surface and nutrients available, a biofilm will form. In some cases, this may result in problems for the water treatment system, due to biofouling or the growth of pathogenic or other unwanted microbes, but more frequently, the biofilm serves a very useful purpose by biodegrading organic contaminants in the water or by converting unwanted inorganic materials into harmless ones. Biofilms are commonly found associated with membrane reactors and filtration systems used in water reclamation and reuse systems, and are often a critical component. They are also found in soils where they may impact water injection or removal systems, or in situ bioremediation. Knowledge of the way biofilms form, how they grow and how to control them is critical for effective design and operation of many water reclamation facilities. This paper explores the modes of formation and growth of biofilms, modern methods for exploring the structure and function of biofilms, and how to control their growth. This paper also presents details on our development of microelectrode sensor arrays for continuous soil pore water quality monitoring.

Keywords Biofilm; biofouling; mass transport; microelectrodes; reclamation; reuse

Introduction
Biofilms play a major role in many water reclamation and reuse technologies. The preferred mode of growth of microorganisms in nature is in the form of biofilms attached to a support surface, rather than as free-swimming organisms, because of the major energy advantages associated with remaining stationary. Normally, wherever there is water, a support surface and nutrients available, a biofilm will form. In some cases, this may result in problems for the water treatment system, due to biofouling or the growth of pathogenic or other unwanted microbes, but frequently, the biofilm serves a very useful purpose by biodegrading organic contaminants in the water or converting unwanted inorganic materials into harmless ones.

Biofilms are commonly found associated with membrane reactors and filtration systems used in water reclamation and reuse systems. They are a critical component of membrane bioreactors and of many biological reactors used for water treatment. They are also found in soils where they may impact water injection or removal systems. They are also the key component in contaminant bioremediation. Knowledge of the way biofilms form, how they grow and how to control them is critical for effective design and operation of many water reclamation facilities. This paper explores the modes of formation and growth of biofilms, modern methods for exploring the structure and function of biofilms, their role in water reclamation systems, and how to control their growth.

The formation of biofilms has long been recognised as an important mechanism of interaction between porous media and biological processes. Biofilm has been defined as an organic material consisting of microorganisms embedded in a polymer matrix of their own making, called extracellular polymeric substances (EPS). In many systems there is a large surface available for microorganisms that can either interact with or attach to these surfaces, colonising them and promoting their survival and growth. Viable cells find
access to sorbed nutrients and increase their ability to grow and survive in changing environmental conditions. Biofilm structures vary with the utilised substrate and the method of growth. The methods of growth and their impact both on contaminant removal mechanisms and on biofouling will be explored.

Biofouling of membranes, filtration systems and other water reclamation systems can become a significant problem if biofilm growth is not controlled. It can lead to clogging of membranes or inactivation of catalytic surfaces, impairing the ability of the system to function properly. The paper contains a discussion of the effects of membrane biofouling.

The need for accurate, robust in situ microscale monitoring of contaminants in biofilm systems is essential for continuous soil pore water quality monitoring. However, necessary in situ monitoring systems do not currently exist. We are developing a suite of self-contained microelectrodes that can be used in the environment or in bioreactors to monitor for such things as pH, ORP, DO, nitrogen, phosphorus and heavy metals. This paper presents details on our development of these microelectrode sensor arrays.

**Biofilm formation**

There are several possible biofilm growth modes. They may grow in flat sheets or in discontinuous patches on the solid surface. Biofilms may also grow in a cluster shape or in a columnar form, allowing for advective transport deep into the biofilm. This can lead to variability in microbial diversity and reaction processes at different locations within the biofilm. The surfaces of biofilms are not usually flat; the rough contours of the surface impact the hydrodynamics of flow past the biofilm, and consequently mass transport from the bulk liquid into the biofilm, as well as promoting detachment of biomass from the biofilm through scour (see Wuertz et al. (2003) for a more thorough description).

Conventional concepts concerning the internal structure of biofilms have been varied. It was previously assumed that biofilms could be modelled in a one-dimensional (perpendicular to the substratum) fashion. It is now known that this is not normally the case. Biofilms are highly heterogeneous in structure. This has a significant impact on how the biofilm grows and on mass transport of materials into and out of the biofilm. It also is one of the factors governing detachment of biomass from the biofilm, a process that keeps the remaining biofilm in a more active state, but which also results in suspended solids in the effluent.

Many have questioned whether these microscale variabilities have any impact when the scale is extrapolated several orders of magnitude to a full-scale biofilm system. There is still no definitive answer to this question because useful scale-up models have not been fully developed yet. However, knowledge of the inner workings of a biofilm can provide useful insight into critical processes that govern biofilm behaviour under various environmental and operational conditions (Bishop, 2003).

**Soil biofilms**

Bioremediation treatment technologies for contaminated soils can be used for both in situ and ex situ stimulation, involving the enhancement of the biodegradation rate of the organic contaminants within affected soil or in compost heaps or slurry bioreactors (Baker and Herson, 1994). Usually, ex situ methodologies provide better-controlled conditions (Schwartz and Scow, 2001), but they also require excavation and transportation of the soil, which results in high costs of treatment. On the other hand, in situ bioremediation has acquired more importance in the last few years as an attractive alternative for biodegradation; in this case, it requires the stimulation of the degradative activities of endogenous microbial populations by providing nutrients (called biostimulation) and/or
external electron acceptors (Johnson and Scow, 1999), or repeated inoculation (called bioaugmentation) to increase the bacterial population (Schwartz and Scow, 2001).

Several studies have been conducted on soil pore water, generally using black-box approaches, which often provide a poor description of the actual rate of disappearance of the contaminants. However, little research has been reported on the biofilm surrounding the soil particles, which is the primary site where biodegradation occurs. The role of biofilms in bioremediation may be crucial to understanding microbial community development, mechanisms of contaminant fate and transport in soils, and possible rate-enhancing processes for biodegradation. The structural forms and chemical composition of biofilms in porous media for biodegradation are currently not well understood because of their complexity and the numerous biotic and abiotic processes. Gaining an understanding of the phenomena of biomass growth on soil surfaces is the first step to understanding how to optimise bioremediation.

The formation of biofilms has long been recognised as an important mechanism of interaction between porous media and biological processes. Biofilm has been defined as an organic material consisting of microorganisms embedded in a polymer matrix of their own making, called extracellular polymeric substances (EPS) (Bishop, 1997). In soil systems there is a large surface available for microorganisms that can either interact or attach to these surfaces, colonising them and promoting their survival and growth. Viable cells find access to sorbed nutrients and increase their ability to grow and survive in changing environmental conditions.

Biofilm structures vary with the utilised substrate; however, there are investigations that support the concept of continuous biofilm formation on the outer surfaces of the soil grain (Taylor and Jaffe, 1990), which leads to the growth of layers of maximum thickness; this was later described as the “open pore” model for permeability reduction. Other studies (Vandevivere and Baveye, 1992) suggest that bacterial aggregates develop in the pore spaces between coarse soil grains, giving sparse and heterogeneous coverage and limited exopolymer content. Mixed culture biofilm was developed in a simulated sandstone aquifer (Paulsen, 1997) showing biofilm colonisation at the beginning, followed by formation of larger cell clusters, and finally development of a “bioweb” network of fibrillar strands, varying in size and shape depending on liquid flow velocity.

Recent studies on the bioremediation of PAHs in soils (Ebihara and Bishop, 2002; Rodriguez, 2005), using confocal scanning laser microscopy (CSLM) to examine the physical structure of the soil biofilm, indicated the presence of continuous surface biofilms on sand grains that were approximately 15–30 μm in thickness. A variety of biological aggregate structures ranging from 20 to 50 μm in diameter was also observed: large cluster-and-protrusion-type structures, cell aggregate bridging and a thick bioweb-type growth containing viable cells developing in the pore spaces between soil grains.

Complex mixtures of organic compounds and other contaminants are usually present in contaminated sites. This may influence biodegradation patterns and changes in biofilm growth and development. Biodegradation studies of naphthalene, phenanthrene and pyrene were conducted in sole-substrate systems and in binary and tertiary mixtures to examine substrate interactions on biofilms in porous media systems (Rodriguez and Bishop, 2005). It was shown that phenanthrene and pyrene could not be degraded as sole carbon sources in the system, but binary systems of the three- and four-ring PAHs with acetate and naphthalene supplements stimulated their degradation, with up to 87.9 and 70.1% removal efficiencies, respectively. However, in the tertiary systems the presence of phenanthrene inhibited pyrene degradation. Adsorption of PAHs to sand media was determined to be negligible. This indicates the complexity of trying to predict renovation patterns.
Membrane bioreactors

Membrane bioreactors (MBR) can be defined as the combination of two basic processes – biological degradation and membrane separation – into a single process where suspended solids and microorganisms responsible for biodegradation are separated from the treated water by a membrane filtration unit (Manem and Sanderson, 1997). MBRs are already an essential component of water reclamation and reuse for sustainability purposes, and their application will grow in the future. They can be used for drinking water treatment, wastewater treatment and water desalination.

As alternative sources of water supply are sought to meet the increasing domestic and industrial needs, and increasingly stringent water and wastewater quality requirements are established, public water utilities and industrial dischargers are being forced to adopt more sophisticated water treatment technologies to meet compliance requirements. Biological membrane-based technologies may be successful in turning alternative sources into potable water, as well as generating water of high purity standards. A recent study by Frost and Sullivan (Anon, 2004) indicated that revenue from membrane-based water purification, desalination and waste treatment totalled over $750 million in 2003, and is projected to reach $1.3 billion in 2010. For Europe, the market was estimated to be $43 million in 2002 (Anon, 2003).

MBRs for wastewater renovation combine the conventional activated sludge process with membrane filtration, thus eliminating the need for traditional gravity clarifiers for the effluent. They retain particulates and slow-growing microorganisms, thereby treating more recalcitrant organics; and retain most pathogens, thereby reducing chemical disinfection requirements. The effluent has fewer suspended solids and a lower turbidity. All biological solids are retained in the system, with excess growth removed as waste-activated sludge. In addition, inorganic nutrients (nitrogen and phosphorus) are greatly reduced (DiGiano et al., 2004).

There are two modes of MBR operation – with an external membrane module for solids recovery and with the membrane module located inside of the bioreactor. There has been a strong recent trend towards submerged membranes. In both cases, the role of the membrane is to provide a barrier against suspended solids and to serve as a support for biofilm development. However, it is possible for the biofilm to develop to the point where fouling occurs and passage of water through the membrane is impeded.

Biofouling

Fouling may arise from particle deposits on the membrane surface, macromolecules adsorbing onto the surface or into the membrane material, or pore blocking (Thomas et al., 2000). It also can result from excess biofilm and EPS material. This results in a decrease in the permeate flux and an increase in head loss. Factors that affect the rate and extent of biofouling include: (1) the membrane material type, pore size and pore distribution; (2) operating conditions such as pressure, cross-flow velocity and turbulence; and (3) solution characteristics, including solids concentration and nature of the bulk fluid.

Solids retention time (SRT) in the system can affect membrane biofouling. It has been shown that fouling increases with increased SRT since particles are more severely deposited on the membrane surface (Han et al., 2005). Much of the total fouling resistance is due to soluble compounds, especially biopolymers (Huang et al., 2000) and extracellular polymeric substances (Cho and Fane, 2002; Rosenberger and Kraume, 2002). Microscopic analysis of the fouled membrane in one study showed that biopolymer residues modified the membrane surface, making it friendlier to bacterial attachment, leading to easier biomass accumulation. Bacteria were bridged by biopolymers to form the sludge cake attached to the membrane (Chu and Li, 2005). Fane and Chang (2002) reported
that submerged hollow fibre membranes performed better with the smallest diameter fibres, and when the fibres were loose rather than tight, because the movement minimised solids deposition.

Zhang et al. (2006) recently reported a study of the microorganisms responsible for surface colonisation of membranes in membrane bioreactors that lead to biofilm formation and ultimately biofouling. In this study, the composition of the planktonic and sessile microbial communities inhabiting four laboratory-scale membrane bioreactor (MBR) systems were compared using amplified ribosomal DNA restriction analysis (ARDRA) and 16S ribosomal DNA gene sequencing. The ARDRA results suggest that the microbial communities on membrane surfaces could be very different from the ones in the suspended biomass. Phylogenetic analysis based on the 16S rDNA sequences provided a list of bacteria that might be the pioneers of surface colonisation on microfiltration membranes. The results further suggested that research on the mechanisms of cell attachment in such an engineering environment could be critical for future development of appropriate biofouling control strategies.

Biofilm monitoring with microelectrodes

Microscale in situ measurement of various constituents in aqueous, biofilm and soil environments is essential for proper monitoring of environmental conditions at a specific location and to determine impacts of environmental stressors. Other environmental applications include the monitoring of stream or lake sediments, water and wastewater treatment reactors, and water distribution systems.

Many of these measurements are currently made using chemical electrodes of various types, including those for measuring pH, dissolved oxygen, oxidation-reduction potential and various cations and anions. Unfortunately, most of these electrodes are relatively large in size, on the order of 1–3 cm in diameter. They can be used to monitor bulk liquid concentrations when there is sufficient volume to wet the electrode contacts, but they are often inappropriate for measurements in small volumes of liquids or in soils. Further, their size makes it impossible to make spatial measurements over small distances, as needed for biofilm monitoring.

Over the past 15 years, microelectrodes have been developed with tip diameters of 1–10 μm. Much of the earliest work on microelectrodes for use in physiology was performed at the University of Cincinnati by Gesteland et al. (1959). During the last decade, microelectrodes have been widely applied in the field of microbial ecology, giving valuable information on the microscale distribution of oxygen consumption (Zhang and Bishop, 1994), photosynthesis (Glad et al., 1996), sulphate reduction (Yu and Bishop, 2001) and nitrification and denitrification (Li and Bishop, 2004). More microelectrodes relevant to microbial ecology, such as CH4, NO2, K+ and CO2, have been developed recently, indicating the dynamics of this field. Figure 1 shows a typical set of profiles that can be measured in a biofilm.

Recently, we have developed a new generation integrated microelectrode system that produces a very stable response, even when the measurement is carried out outside of a Faraday cage where signals from most conventional microelectrodes are usually inhibited by external electrical noise. These new microelectrodes are easier to fabricate and are more robust than conventional microelectrodes. The tip size of the integrated microelectrodes is approximately 200 nm². The integrated microelectrode exhibits better signal stability and substantially shorter response times (usually only a few milliseconds) than commercial electrodes. We expect that the newly developed integrated microelectrodes may have a wide range of applications in biofilms, reactors, contaminated soils and sediments (Figure 2).
Conclusions

It is estimated that by 2025, one-quarter of the world’s population will experience severe water scarcity (Seckler et al., 1999). New technologies for reusing and renovating water will be needed to meet this increased demand. Soil biofilm systems are already widely used for water renovation. It is likely that membrane bioreactors will play a significant role in developing alternative sources of potable water. They produce high quality water, can remove a wide variety of contaminants, reduce the size of the treatment facility required, and can be used for both water and wastewater treatment as well as for desalination. However, one of the biggest challenges still facing the membrane bioreactor industry is preventing biofouling. As new membranes are developed that can minimise fouling, and as proper operating conditions are established, the market for membrane bioreactors should flourish.
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