Where are we with biofilms now? Where are we going?

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Abstract The IWA’s Biofilm VI conference presented a wide range of research on biofilm systems. Particularly popular themes were nitrogen removal, mathematical modelling and microbial ecology. Emerging themes included biofilms with membranes, pathogens in biofilms, biofouling and detachment. Within microbial ecology and mathematical modelling, emphasis was given to N-removal systems, particularly involving nitrifiers and Anammox bacteria. Both themes also recognised the importance of biofilm detachment. Although biofilms on membranes gained attention, little interest was exhibited towards linking biofilms with other advanced materials, such as ceramics, conductors, semi-conductors or nano-materials.

Research presented at Biofilm VI marked major advances in improving water sustainability towards removing BOD and N, but did not address many emerging contaminants, such as oxidised contaminants and endocrine disruptors. Attention to energy sustainability, such as with bio-hydrogen or microbial fuel cells, was minimal. Thus, research reported at Biofilm VI was strong towards “improving the expected” with regard to BOD and N removal, but not yet focused on “exploiting the unexpected” to deal with emerging pollutants and bio-energy.

Keywords Biofilm; microbial ecology; modelling; nitrogen removal; sustainability

Introduction

When conference chairman Mark van Loosdrecht asked me to provide a forward-looking summary of the IWA’s Biofilm VI conference (Amsterdam, 24–27 September 2006), I readily accepted the challenge. My first step was to find a context for a conference summary that also looked ahead to the future of biofilm research and practice. Fortunately, I have been involved in several international projects that have given me a broad perspective on the status and trajectory of environmental biotechnology, including biofilm research. Therefore, I used those experiences as a framework for interpreting the events at Biofilm VI.

First, I synthesised concepts and insights I gained from six international activities in which I have participated in the past few years: Rittmann (2004), Rittmann (2006a, b, c), Rittmann et al. (2006) and Wanner et al. (2006). Integrating ideas from these activities, I developed the following principles that are immediately relevant to biofilm research and practice.

- Environmental biotechnology, the umbrella field that encompasses biofilms, “manages microbial communities to provide services to society”. The most important services are (a) detoxifying (or treating) contaminated water, wastewater, sludge and soils; (b) capturing renewable resources, particularly bio-energy; and (c) directly or indirectly improving human health. Based on current practice and also on future potential, biofilms are at the core of success with environmental biotechnology.

- The key to success in environmental biotechnology is integrating, at all points of research and development, four fundamental tools: understanding the system’s microbial ecology, quantifying the systems performance with modelling, taking advantage of modern materials and going for the biggest benefits. I organise most of this summary around the four keys to success for environmental biotechnology.
Finally, how do we know if our research and development is on the right path? We probably are headed for success if we are improving the proven processes in a major way or exploiting unexpected processes. I look into this aspect at the end of my summary.

Second, I studied the topics of all the papers to be presented orally, and I attended approximately half of them in person. (Most of the conference had two parallel tracks.) Fortunately, conference delegates were provided written proceedings upon registration, and I also had in advance all the abstracts since I was a member of the Programme Committee. I classified the subjects of the approximately 60 papers slated for oral presentation, and I confirmed or updated the classifications for all oral presentations I attended.

Third, I sought out conference-wide themes from the presentations, the discussion after the presentations and information conversations I had or overheard during the conference.

What was hot?
Based on the topics of the oral presentations, three topics were “hot” among biofilm researchers at BiofilmVI. The first was N removal, which was a major topic in 15 oral presentations, or about 25% of the conference papers. Following closely behind in terms of popularity were mathematical modelling (12 presentations) and microbial ecology (11 presentations). Notable to me were four relatively new topics (for IWA biofilm conferences) that were major themes of at least five presentations: membranes (7), fouling (6), detachment (6) and pathogens (5).

Microbial ecology
Since environmental biotechnology manages microbial communities, it is obvious that understanding the system’s microbial ecology is at the core of all research. As a scientific discipline, microbial ecology attempts to understand a community in four ways:

- Structure: what microorganisms are present?
- Potential function: what metabolic capabilities are available within the genomes of the microorganisms in the community? These metabolic capabilities usually include the services that the community provides society.
- Realized function: what metabolic capabilities are actually expressed by the community? These are the services that society obtains in practice.
- Interactions: what are the physical, biochemical and geochemical ways in which different microorganisms interact with each other and their environment?

The beginnings of microbial ecology can be traced back to the 1940s and 1950s (Rittmann, 2006a), but microbial ecologists seldom could answer the fundamental questions, because classical tools, such as microscopy and selective culturing, were insufficient. The stricture began to be relieved in the late 1980s, when the first molecular-biology tools began to come into play (Rittmann and McCarty, 2001; Rittmann, 2002). The first tools were directed towards detecting ribosomal RNA (rRNA), which is a chronometer of phylogenetic evolution and a meter for phylogenetic identity, or community structure (Stahl, 1986). Today, many molecular-biology tools are available to elucidate community structure, and the number and type are increasing steadily (Rittmann, 2006a; Rittmann et al., 2006; Stahl and Wagner, 2006). Furthermore, new genomics and proteomics tools are coming along to investigate community function at the levels of transcription (genomics) and translation (proteomics) (Rittmann, 2006a; Stahl and Wagner, 2006). The molecular-biology tools yield the fine details of community structure and function, and they are ideal complements to mass-balance tools that define the overall community function, which is the ultimate goal of an environmental biotechnology (Rittmann, 2002).
Participants in BiofilmVI embraced microbial ecology and the modern tools. Eleven presentations had microbial ecology as a major theme, and microbial ecology was a sub-theme of many other presentations. The strongest focus was on microbial ecosystems involved in N removal, and special attention was given to nitrifying bacteria, Anammox bacteria, and situations in which the two groups coexisted inside a biofilm or granule. Figure 1 (left) shows a good picture of ammonia oxidisers and Anammox bacteria together in a high-rate biofilm reactor.

A second important focus within microbial ecology at the conference was on pathogens. This included notorious protozoa (Cryptosporidium and Giardia), Helicobacter pylori and Legionella. Molecular biology tools were used to detect these pathogens within biofilms (Figure 1 (right)) and to understand how biofilm can be essential for the survival of pathogens, especially in drinking-water distribution systems.

Third, biofilm researchers have begun to realise that detachment is a very important factor that controls the biofilm’s community structure, along with its physical structure. Six oral presentations dealt explicitly with the causes of detachment, how detachment affects the community, or both.

**Mathematical modelling**

Without question, mathematical modelling has become a well-accepted tool for biofilm research. BiofilmVI had 12 oral presentations in which modelling was the featured aspect. Much of the attention was directed toward N-removal systems, whose community structures are inherently complex. Significant attention was also given to detachment, motility and other factors that control the physical structure of the biofilm. Figure 2 gives some examples of model outputs that relate to these feature topics.

Modelling is gaining importance because it is the ultimate tool for quantitatively and systematically linking all the components and processes in a complex microbial ecosystem, such as a biofilm (Wanner et al., 2006). The components include the various microbial types, extracellular polymeric substances (EPS), inert or dead biomass, the metabolic substrates for all the microbial species, metabolic products from the reactions they carry out, alkalinity, hydrogen ion (for pH), and more. Processes include the metabolic reactions, transport of solutes and biomass, acid/base reaction, precipitation/dissolution, and physical deformations due to forces acting on the biofilm. Many of the processes, particularly the metabolic ones, are the services that the community provides human society.

**Figure 1** Examples of using molecular biology tools to detect microorganisms of particular interest at BiofilmVI. (Left) FISH photomicrograph of ammonia oxidizers (green) and Anammox bacteria (red) coexisting in a biofilm of a high-rate, Anammox-based biofilm reactor (Tsushima et al., 2007). (Right) FISH image of Helicobacter pylori in biofilm on a coupon exposed to drinking water (Bragança et al., 2007). Subscribers to the online version of Water Science and Technology can access the colour version of this figure from http://www.iwaponline.com/wst
Advanced materials

The third key to success is using advanced materials, which can include membranes, ceramics, conductors, semi-conductors and nano-materials. This strategy is especially valuable with biofilm systems, because the biofilm can accumulate directly on the surface of the advanced material. In addition, most of the advanced materials are reactive surfaces, not inert surfaces. Surface reactions open up many opportunities to make the biofilm particularly effective in achieving one of society’s goals.

Using advanced materials has a good history in biofilm technology (Rittmann and McCarty, 2001). Biological towers and rotating biological contactors came to prominence in the 1970s after the advent of strong, lightweight plastics. A little later, various biological aerated filters (BAFs) were developed based on small, lightweight, pellet-like carriers that allow high specific surface area and good bed aeration. Granular activated carbon also became an excellent biofilm carrier due to its outstanding biomass retention and ability to adsorb poorly biodegradable organics.

Presentations at BiofilmVI addressed advanced materials, but not in an overwhelming way. Seven presentations focused on the roles of biofilm with membranes. Four were on fouling of ultra- or micro-filtration membrane, or a bad interaction of biofilm with the material. Three presentations exploited good biofilm accumulation on a membrane; these were about aerobic membrane biofilm reactors, which are useful for total-N removal. Interestingly, no oral papers addressed the hydrogen-based membrane biofilm reactor (e.g. Nerenberg et al., 2002; Rittmann, 2006b), which is uniquely useful for reducing a large range of oxidised contaminants, e.g. nitrate, perchlorate, bromate, selenate, chromate and chlorinated solvents. The hydrogen-based MBfR is a perfect example of a

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**Figure 2** Example of model outputs on themes of high interest at BiofilmVI. (Top left) Explanation of why nitrifiers (blue and green) form dense clusters at the base of a biofilm (Matsumoto et al., 2007). (Top right) Illustration of what conditions (the dark valley) favour total-N removal in a totally autotrophic membrane biofilm reactor (Terada et al., 2006). (Lower Left) Illustration of how motility causes the biofilm to spread out, not for clusters (Picireanu et al., 2007). (Lower Right) Mechanical behaviour of a biofilm cluster in response to hydrodynamic stress that can lead to detachment (Alkquist and Klapper, 2007). Subscribers to the online version of *Water Science and Technology* can access the colour version of this figure from [http://www.iwaponline.com/wst](http://www.iwaponline.com/wst).
reactive surface since it is the means of delivering the cells’ electron donor, H₂, virtually directly to the microorganisms.

Other biofilm–material connections covered by presentations at BiofilmVI included two papers on bio-corrosion and three on various biological aerated filters. On the other hand, no oral presentations mentioned exploiting ceramics, semi-conductors, conductors or nano-materials as biofilm carriers. This suggests to me that biofilm research and technology are ripe for dramatic advances as these advanced materials are linked to biofilms.

**Big benefits**

From my viewpoint, the biggest benefits to society will accrue as environmental biotechnology fully addresses water and energy sustainability. What could be more essential to the survival of modern human society than water and energy sustainability? Environmental biotechnology is perfectly suited to enhance both types of sustainability, because microbial communities are uniquely able to purify water and convert complex forms of energy into convenient renewable forms (Rittmann, 2006a).

In terms of water sustainability, the biofilm community – as reflected by BiofilmVI – is doing a “bang up job” with research and development towards reclaiming water that is contaminated with oxygen demand and nitrogen. The largest single theme was N removal, and BOD removal was involved throughout the conference. We have new and old technologies, and we are putting out best minds towards understanding the ecology, doing the modelling, and generally improving performance for these rather traditional water pollutants.

The same cannot be said for a range of emerging water contaminants: oxidised inorganics, such as selenate, perchlorate and chromate; oxidised organics, such as chlorinated aliphatics, chlorinated aromatics, nitro-aromatics and fluorinated aliphatics; metals, such as cadmium, zinc and nickel; and endocrine disruptors, such as pharmaceuticals and plasticisers. Microbial technologies to detoxify most of these emerging contaminants exist, but have not yet reached the “radar screen” of most biofilm researchers and practitioners. Again, I see opportunity for new research and technology advances, and I think that this area will be linked closely with advances in the utilisation of advanced materials.

Energy sustainability was hardly touched in BiofilmVI. I found only one oral paper on bio-energy: methane generation with an upflow anaerobic sludge blanket reactor, a well-established (albeit still very interesting) technology. I found nothing on other methanogenesis approaches, biologically produced hydrogen, or biologically produced electricity with a microbial fuel cell (MFC). In reality, research on bio-hydrogen and MFCs is widespread and growing worldwide. Thus, it is only a matter of time until they begin to take a prominent place at biofilm conferences. Biofilm processes will be essential for all the energy-conversion systems, because all rely on slow-growing microorganisms whose stable retention is enhanced by being in a biofilm.

The MFC is a great case-in-point. In an MFC, a biofilm grows on the anode of a fuel cell. The biofilm bacteria oxidise an organic fuel (i.e. oxygen demand) and transfer most of the electrons to the anode, a solid conductor. The electrons flow through an external circuit and reduce O₂ to H₂O at the cathode. In effect, the MFC carries out typical aerobic respiration, but the electrons must flow through a circuit before reaching the terminal electron acceptor. The electron flow provides us directly with electrical energy.

Why is the MFC such an exciting new development? The reason is that using a biofilm as the catalyst at the anode opens up, for the first time, the possibility to use renewable, organic fuel for fuel-cell technology. Furthermore, it does so without combustion, which eliminates all combustion-related air pollution and also offers the potential to roughly double the energy-capture efficiency from any combustion process (Rittmann, 2006a).
The key to an MFC is the biofilm on the anode, which is reactive as an electron collector, the intermediary leading to the terminal electron acceptor.

**Improve the expected, exploit the unexpected**

Figure 3 is a variation of the famous “S curve” for the development of a new process. The left part of the S curve is for very new technologies. They are “starting from scratch” and need creative, persevering inventors and the help of brave, forward-looking “early adopters”. This is where we benefit most by “exploiting the unexpected”, such as having biofilm grow and work on the surface of a membrane or an anode. The right part of the S curve is for mature technologies that have proven their value in the marketplace; competition often is for price or for subtle improvements in performance. This is where we need to “improve the expected” by better engineering or taking advantage of better materials.

The figure also identifies the locations (in my view) of some key environmental biotechnologies. BOD removal, such as by activated sludge, is very mature, and far to the right. Its performance is relatively expected, but the process’s performance and/or cost-effectiveness can be improved. Contemporary examples include using membrane separator instead of a settler (Daigger et al., 2005) or creating a hybrid biofilm-suspended-growth reactor (Downing and Nerenberg, 2007). At the other extreme are processes to reduce a range of oxidised contaminants or to produce bio-hydrogen or bio-electricity. Success in these areas probably will use approaches far afield from what is in current use with mature technologies: i.e. exploit the unexpected.

**References**


