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HORIZON PAPER

Biofilm engineering: linking biofilm development at different length and time scales

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Abstract Biofilms are heterogeneous and dynamic systems. Evaluation of biofilm structure and function at the microscale has been greatly advanced through the application of multidimensional imaging, in-situ identification of the microbial community composition, function, and genetic regulation. Biofilm reactors are being applied for advanced biological treatment processes and their overall (macroscale) operation is well understood and controlled. What is missing is the link between micro and macroscale. In this horizon paper we suggest how understanding the overall biofilm ecosystem will require an integrated evaluation of the different length and time scales.

Keywords Biofilm · Length scale · Time scale · Modeling · CLSM

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1 Introduction

Life was very organized in the early days of biofilm research: In academia, microbiologists focused on identifying microbial distributions, interactions, and mechanisms of structure formation at the cellular and microcolony level. Engineering science developed kinetic expressions to predict overall biofilm reactor performance assuming a one dimensional homogeneous biofilm. And most practitioners neglected biofilm heterogeneity and mass transport limitations altogether and resorted to empirical design equations for full scale processes. Academicians (microbiologists as well as engineers) and practitioners were all interested in biofilms but focused on different questions and largely went their separate ways. There was no need to talk.

Nowadays biofilm research is not so compartmentalized anymore. Engineers have realized that optimizing overall reactor performance can be closely linked to microscale interactions within the biofilm. Mass transport limitations are not only reducing the overall efficiency of bacteria in the biofilm, but can also create ecological niches within the biofilm that are beneficial for the overall reactor performance. One example is a novel treatment process for nitrogen removal where ammonia is oxidized to nitrite in aerobic regions of the biofilm and nitrite is reduced in anaerobic zones using ammonia as the electron donor (Jetten et al. 2003). On the other hand, microbiologists have started to appreciate full-scale

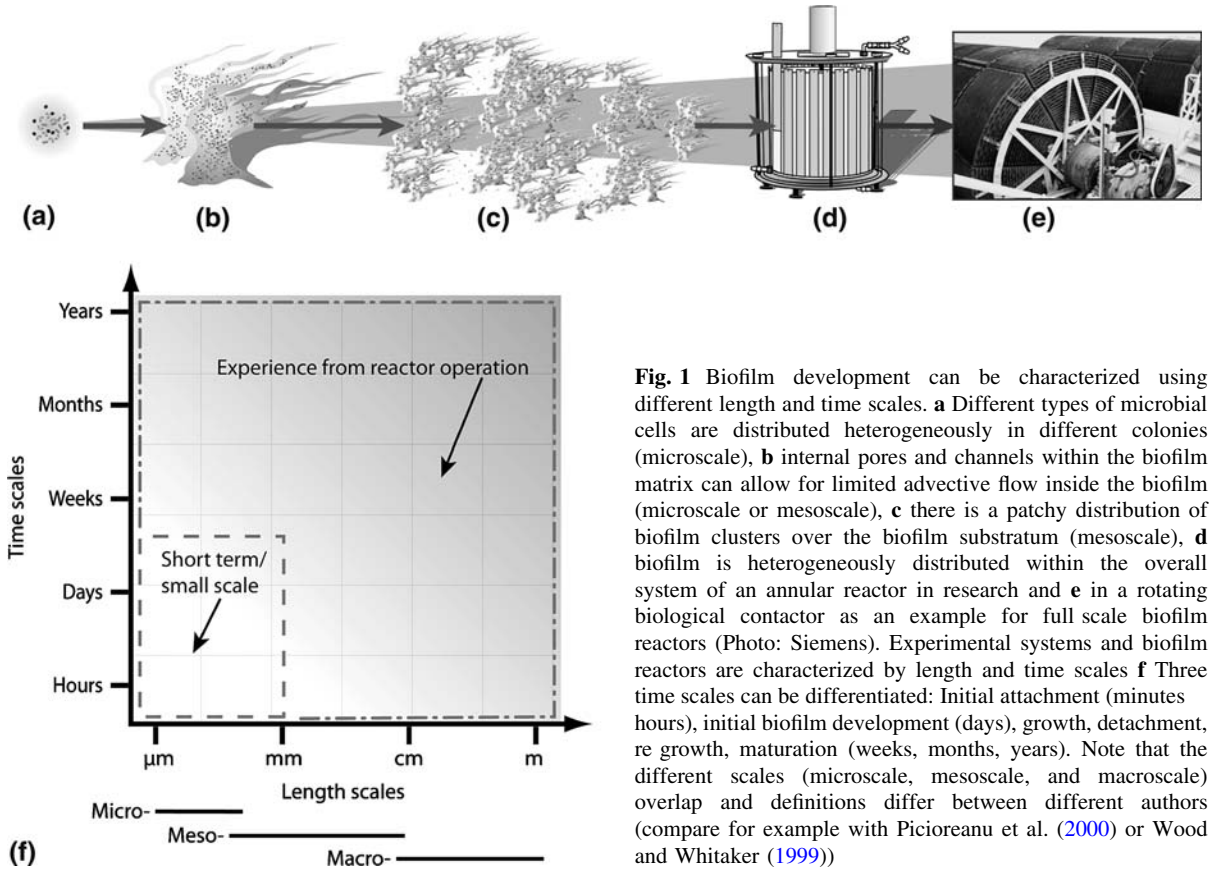

Fig. 1 Biofilm development can be characterized using different length and time scales. **a** Different types of microbial cells are distributed heterogeneously in different colonies (microscale), **b** internal pores and channels within the biofilm matrix can allow for limited advective flow inside the biofilm (microscale or mesoscale), **c** there is a patchy distribution of biofilm clusters over the biofilm substratum (mesoscale), **d** biofilm is heterogeneously distributed within the overall system of an annular reactor in research and **e** in a rotating biological contactor as an example for full scale biofilm reactors (Photo: Siemens). Experimental systems and biofilm reactors are characterized by length and time scales **f** Three time scales can be differentiated: Initial attachment (minutes hours), initial biofilm development (days), growth, detachment, re growth, maturation (weeks, months, years). Note that the different scales (microscale, mesoscale, and macroscale) overlap and definitions differ between different authors (compare for example with Picioreanu et al. (2000) or Wood and Whitaker (1999))

wastewater treatment reactors as complex ecosystems where ecosystem development can be influenced by modifying reactor operation (Daims et al. 2006). While engineers and microbiologists have become aware of and more interested in each other's contributions, linking approaches and findings from the different fields of biofilm research goes further and remains a challenge. Integration of the different areas of biofilm research is complicated by the fact that biofilm heterogeneity and function strongly depend on the spatial and temporal scale of observation (Fig. 1). The purpose of this paper is to identify some key questions of interest and opportunities that arise when linking the different scales of biofilm systems.

2 Relevant questions for engineering applications

Some aspects of biofilms are well understood: The degradation of soluble contaminants in biofilm reactors, for example, is well studied and mathematical models reliably predict flux of soluble substrate and overall reactor performance (Wanner et al. 2006). Other aspects related to biofilm structure and biomass distribution within the overall reactor are not so well understood and cannot be reliably predicted using today's mechanistic mathematical models:

- What are the main factors influencing biofilm growth and detachment? How will growth and detachment influence biofilm structure and the formation of heterogeneous biofilms at different scales (Fig. 1)? Examples of how heterogeneity can influence overall system performance are patchy biofilm distribution influencing contaminant removal for biofilms grown on sorptive media (Herzberg et al. 2003) and the scale and extent of patchiness determining microbially influenced corrosion. Detached biomass in addition degrades water quality (e.g., through the release of single

cells or large cell aggregates in a drinking water distribution network). What factors govern re-attachment downstream in the system?
- Growth and detachment are system dependent. How can we influence biofilm development through reactor design, operating conditions, or by choosing surfaces with specific morphology, roughness, or surface chemistry?
- How do suspendered and attached bacteria interact in a biofilm reactor (e.g., biofilms seeding the suspended biomass and vice versa) and what are their relative contributions to the overall reactor performance?
- What is the fate of different size fractions of particulate organic matter entering the biofilm reactor? What determines attachment, hydrolysis, and degradation of particulate organic matter in a biofilm?

These questions are not only relevant for practical engineering applications but also for fundamental biofilm research. Answering these questions will not be possible based on microscale investigations or the assessment of macroscale system performance alone. It will require a new approach to biofilm research leading to an improved understanding of how microscale, mesoscale, and macroscale are linked with each other.

3 Current approaches to characterize biofilms

Molecular microbiological tools, advanced imaging techniques, and microsensors have significantly improved our understanding of the in-situ development of biofilm structure and interactions within a biofilm (Stoodley et al. 2002). For these studies, biofilms are frequently grown in 96-well plates or in flow channels coupled with light or confocal laser scanning microscopy (CLSM). Compared to the range of time and spatial scales of biofilms in engineered and natural systems (Fig. 1f), experiments in well plates or flow channels are short and small. In flow channels, temporal development is usually limited to the period where the channels can be stably operated, typically not more than 2 weeks. The spatial scale is confined by the image size of cameras used for CLSM (on the order of 100 200 μm).

But as practitioners always suspected, a biofilm with a total area of 1 m^2 is not simply the sum of biofilm grown in 1,000 flow channels, even though the total areas roughly correspond. The practical problem of scale-up of biofilm systems harbors a plethora of relevant and timely research questions. Mechanisms and processes at the microscale depend on and influence interactions on larger spatial scales and the evaluation of process at one scale needs to be linked to all other scales (Battin et al. 2007; Raes and Bork 2008). Battin et al. (2007) compare detachment and grazing activity of protozoa in biofilms with a forested landscape where wind-fall of trees results in gaps in the canopy. Macroscopic gaps in the forest canopy influence dispersal of microscopic seeds, much like previously detached areas in biofilms provide opportunities for new biofilm to develop.

The forest-biofilm comparison by Battin et al. (2007) can be taken one step further by taking into account dynamic changes of the landscape. A wind-fall gap in the forest canopy is not static. Shrubs and bushes will take over the wind-fall area, followed by various succession stages until, after years, old-growth forest will be re-established. Likewise, biofilms are dynamic systems where biofilm re-develops locally on detached areas. The resulting heterogeneous biofilm resembles the mosaic-cycle concept originally developed for beech forests (Remmert 1991; Wissel 1991): A typical beech forest is composed of patches of vegetation in various successional stages, with old growth forest as the climax. An important factor in the development of a patchy vegetation distribution is dieback of trees, synchronized by solar damage to the suddenly exposed bark of mature beech trees after windfall of a neighboring beech tree. Exposed mature trees are more susceptible to dieback than non-exposed individuals and thus re-set the development of an area to an early state. Now, a similar developmental state with no mature trees is present at the windfall site and in the neighboring areas. The local interactions result in beech forests developing a mosaic of distinct patches of 100 to 150 m (Wissel 1992) this can be regarded as the mesoscale of beech forests. The basis for mosaic cycles, the combination of a developmental cycle and a synchronizing factor that links neighboring areas, is also given in biofilms. Cyclic development in biofilms with an attachment stage, growth, maturation, and detachment/dispersal have been suggested (Sauer et al. 2002). A synchronizing factor could be increased detachment shear forces for biofilm next to a larger open patch (Stoodley et al. 1999). Following the mosaic cycle concept, a system may be homogeneous

both at the microscale and at the macroscale but patchy and heterogeneous at a mesoscale (Milferstedt et al. 2009). In our previous work we have demonstrated some initial indication of cyclic behavior of biofilms when observed at the mesoscale (Milferstedt et al. 2008). What would be the characteristic size of mosaic patches for different biofilm systems? Data and systematic evaluations of biofilm dynamics linking the different scales are scarce.

4 How to bridge the gap between the different scales?

The common ultimate goal for researchers and practitioners is to develop an understanding of how external factors influence biofilm development to such an extent that it provides the basis for purposefully influencing or "engineering" biofilm structure and function. What new experimental and modeling approaches are needed to study mechanisms of biofilm development over the entire range of relevant length scales microscale, mesoscale, and macroscale (Fig. 1) and to better understand interactions between these different scales?

4.1 Experimental approaches

At the microscale, CLSM in combination with fluorescent in situ hybridization (Manz et al. 1999) and specific fluorescent dyes (Staudt et al. 2004) allows to image the three dimensional distribution of different types of microorganisms or extracellular polymeric substances within the biofilm structure, respectively. CLSM can further be combined with microelectrodes to evaluate local substrate utilization. In the past the application of CLSM has mostly been limited to short-term experiments in flow channels under well controlled but also very simplified conditions. New experimental approaches are needed that allow to monitor longer term and larger scale biofilm development. These experimental approaches can be based on in-situ observations in novel types of flow channels or in-situ or ex-situ monitoring of biofilms in their natural environment.

Imaging at the mesoscale and macroscale is possible, but comes at the expense of lower spatial resolution and the ability to directly observe the distribution of different types of organisms. Local biofilm accumulation and mesoscale or macroscale distribution can be quantified by optical measurements of the biofilm thickness at different locations (Bakke and Olsson 1986), measuring the optical density (Bakke et al. 2001), by scanning the optical density over larger areas (Milferstedt et al. 2006), or optical coherence tomography (Haisch and Niessner 2007). Determining biofilm distribution and structure in porous media (such as soil or biologically activated filters) is difficult. Magnetic resonance imaging can be use to determine biomass distribution if the biofilm is grown in a suitable reactor (Hoskins et al. 1999). For most systems, however, biofilm distribution is determined based on grab samples providing only the overall amount of biofilm but not their spatial distribution. Mesoscale or macroscale quantification of biomass distribution will depend on and needs to be linked to the microbial community distribution and also microscale structure.

4.2 Modeling approaches

New mathematical approaches need to be developed to help integrate information obtained at the different scales of observing biofilm heterogeneity. Because of the interactions between the scales, upscaling from a smaller (or downscaling from a larger) scale will likely be difficult.

Engineers are successful in predicting macroscale biofilm reactor performance using 1-D mathematical models that average biofilm composition and substrate concentrations in planes parallel to the substratum. These 1-D models take substrate gradients into the biofilm into account but often grossly simplify external mass transfer resistance and mixing conditions along the length of the reactor. With the advent of experimental methods to quantify microscale heterogeneity using CLSM, multidimensional mathematical models were developed that allow to both predict and to evaluate the relevance of the formation of microscale heterogeneous structures. The resolution of these multidimensional models is usually the bacterial cell they are also referred to as agent based models. While it is in principle possible to apply these agent based models to mesoscale or macroscale questions, there are practical limitations in such an approach.

Models evaluating mesoscale or macroscale heterogeneity could build on approaches from other areas of ecology. In forest ecology, mesoscale and macroscale dynamics are modeled using pattern oriented models

(Grimm et al. 2005; Rademacher et al. 2004; Schlicht and Iwasa 2007). These models predict the development of overall patterns (also referred to as pattern oriented models) but do not resolve a system down to the individual agent. The challenge for both experimental approaches and for mathematical modeling will be to link information from agent based (microscale), pattern oriented (mesoscale), and overall reactor models (macroscale) with each other (Fig. 1a e).

5 Perspective

Better understanding and control of biofilms will require both engineers and microbiologists to evaluate biofilms at broader range of length and time scales (Fig. 1f). Linking different scales is difficult but also holds significant promise in developing a comprehensive understanding of ecological mechanisms in general and biofilm development in specific (Battin et al. 2007; Grimm et al. 2005; Raes and Bork 2008). An improved understanding of biofilm development over different spatial and temporal scales in biofilm systems (e.g., biofilm reactors) holds promise to ultimately deliver approaches to purposefully influence biofilm development the basis for true biofilm engineering.

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