Pergamon

Wat. Sci. Tech. Vol. 39, No. 7, pp. 273–278, 1999

0273–1223/99 $20.00 + 0.00

PII: S0273-1223(99)00178-X

BIOFILM MODELING: PRESENT STATUS AND FUTURE DIRECTIONS

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ABSTRACT

Biofilm models are commonly used as simulation tools in engineering applications and as research tools to identify and fill gaps in our knowledge of biofilm processes. While models used in engineering applications rely on simplifying assumptions to make them practical, recent experimental evidence of biofilm heterogeneity questions the validity of these assumptions. On the other hand, research models are becoming more complex and use advanced computational tools to mathematically investigate which factors determine the structural heterogeneity and the population dynamics of biofilms. One of the goals of advanced models is to evaluate the relevance of three-dimensional heterogeneities to the predictive capability of traditional biofilm models. In addition, biofilm models are used to evaluate experimental observations when studying a diversity of biofilm-related phenomena. Given the variety of applications of biofilm models and the different approaches that modelers have taken in recent years, a specialist group was convened to evaluate the present status and determine future directions of biofilm modeling research. The education of scientists and engineers on the fundamentals of biofilm models, the development of mathematical models for real-time control of biofilm processes, and the ability to "engineer" the biofilm structure and function (or performance) were identified as the most important objectives for the practical application of biofilm models. As mathematical research tools, biofilm models are directed towards gaining a better understanding of biofilm structure and population dynamics. Specific topics identified as priorities on biofilm research include the behavior of specialist microorganisms, the elucidation of attachment and detachment mechanisms, the determination of mechanical properties of exopolymeric substances, and the study of ecological interactions among different microorganisms. The need to evaluate parameter sensitivity in the different models was identified as an essential component of modeling research. A group decision from this meeting was to initiate a collaborative effort to identify similarities and differences among current modeling approaches. Such comparative analysis will enhance our understanding of biofilm processes and mathematical approaches, and will facilitate the future use of biofilm models by scientists and engineers involved in biofilm research.

KEYWORDS

Biofilm, modeling, education, 3D heterogeneity, detachment, EPS.

INTRODUCTION

Mathematical models have been used for the last three decades as tools to simulate the behavior of microbial biofilms. The initial models described biofilms as uniform steady-state films containing a single type of organism (Figure 1a), governed exclusively by one-dimensional (1D) mass transport and biochemical transformations (Atkinson and Davies, 1974; Rittmann and McCarty, 1980). Later, stratified dynamic models (Figure 1b) able to represent multisubstrate-multispecies biofilms (Wanner and Gujer, 1986) were developed. Although these 1D models were advanced descriptions of multispecies interactions within the biofilm, they were not able to represent the characteristic structural heterogeneity that has been recently elucidated through experimental observations. The new biofilm models provide sophisticated two- and three-dimensional (3D) descriptions of the microbial biofilm (Figure 1c), and incorporate not only mass transport and transformations (Picioreanu *et al.*, 1998), but also hydrodynamics (Picioreanu *et al.*, 1999) and population dynamics (Noguera *et al.*, 1999). This evolution in model complexity has paralleled the advances in computational tools. While hand calculators were the tools used in the seventies, the biofilm models of today reflect the availability of fast personal computers and advanced parallel processing.

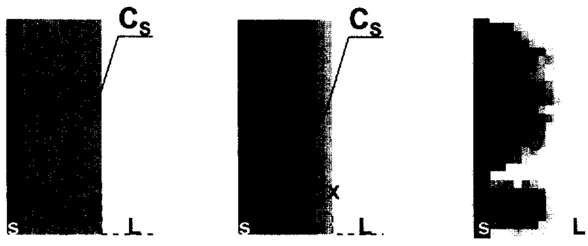

Figure 1. Evolution of biofilm models from (a) uniform biomass distribution and one-dimensional substrate gradient in the 70s, to (b) one-dimensional stratified biomass and multisubstrate-multispecies biofilms in the 80s, to (c) multidimensional distribution of biomass and substrates at the end of the 90s. L is the bulk liquid, b is the biofilm, and s is the substratum. C_S represents the concentration of substrate, and C_X is the concentration of cells in the biofilm, both being one-dimensional representations of laterally averaged values from slices parallel to the substratum surface.

The amount of experimental evidence describing some biofilms as heterogeneous entities in structure and composition contradicts the simplifying assumptions of the original 1D models and has challenged engineers to create a more accurate mathematical description of biofilms. This challenge has resulted in an increasing model complexity, derived from the inclusion of an ever increasing number of parameters to explain the overall biofilm structure. Current models predict the formation of microcolonies, the development of heterogeneous colonization patterns, the sloughing of large biofilm sections, etc., and could be further expanded to simulate experimentally observed phenomena such as formation of streamers and advective flux through microchannels. Nevertheless, the real challenge to the modeler is not to create models that include as many parameters as possible, but rather, to determine the level of significance of these parameters and their importance in the description of the different biofilm processes. Moreover, the mathematical evaluation of parameter significance is essential to define the required level of accuracy of experimental measurements.

In order to foster an organized framework for biofilm modeling for the coming years, a specialist group was convened to discuss the current status and future directions of biofilm modeling. The meeting took place during the IAWQ International Specialty Conference on Microbial Biofilms (Lake Bluff, IL, U.S.A). The participants and their home institutions are listed in Table 1.

CURRENT STATUS OF BIOFILM MODELING

Biofilm models have been primarily used for research purposes. Their application in the design of full-scale operations is far from reaching the acceptance level that other models already have (e.g., the IAWQ activated sludge models). This limited use in design can be explained by a combination of factors.

- Biofilm models are perceived as *complicated mathematical* entities.
- Simplifications and assumptions used in 1D models are often *not supported by experimental observations*.

- There are many *phenomena not considered* in the models, such as the fate of particulate substrates, the activity of higher organisms, and the role of exopolymeric substance (EPS) production.
- There is a general *lack of trust* in the capability of the models to make accurate and reliable predictions.
- The *usefulness* of biofilm models for the design of full-scale systems *is not fully appreciated.* Many engineers prefer to use simple empirical correlations for design, while models are mostly used as troubleshooting tools when operational problems arise.
- Biofilm models have *not been adequately distributed* or commercialized.
- *Parameters* used in biofilm models are sometimes *difficult to estimate.*

On the other hand, biofilm models are more frequently used by practicing engineers as a simulation tool to analyze the performance of biofilm processes. The models provide engineers with the means to evaluate the significance of several parameters, allowing them to search for explanations of performance problems. Thus, it is possible to formulate hypothetical modifications in operation and to simulate process behavior in response to operational changes before full-scale implementation.

The use of biofilm models continues to be prominent in the research arena. Their development is driven by the scientific interest to understand the basic principles determining biofilm formation, composition, structure, and function. Significant modeling efforts are currently concentrated on investigating 3D heterogeneity and population dynamics. For example, mathematical models are now being used to investigate how water velocity regulates the erosion and sloughing of biofilm sections (Picioreanu *et al.*, 1999), how the thickness or shape of the boundary layer determines biofilm structure and activity (Picioreanu *et al.*, 1998; Hermanowicz, 1999; Picioreanu *et al.*, 1999; Rittmann *et al.*, 1999), or how microbial heterogeneity and microcolony formation affect the 3D structure of a biofilm (Noguera *et al.*, 1999).

There is also an increasing interest in utilizing mathematical simulations to elucidate other potentially important aspects of biofilm research, such as the significance of biofilm structure in cometabolic degradations, the survival of specialist microorganisms within the biofilm (Pedersen and Arvin, 1999), the role of EPS formation, and the detachment mechanisms.

Table 1. Participants on the IAWQ specialist meeting on biofilm modeling, Lake Bluff, IL, USA (October 11, 1998)

Participant	Institution
Erik Arvin	Technical University of Denmark, Denmark
Paul L. Bishop	University of Cincinnati, USA
Poul Harremoës	Technical University of Denmark, Denmark
Slawomir W. Hermanowicz	University of California at Berkeley, USA
Mark van Loosdrecht	Delft University of Technology, The Netherlands
Daniel R. Noguera	University of Wisconsin - Madison, USA
Akiyoshi Ohashi	Nagaoka University of Technology, Japan
Satoshi Okabe	Hokkaido University, Japan
Cristian Picioreanu	Delft University of Technology, The Netherlands, and University Politehnica of Bucharest, Romania
Bruce E. Rittmann	Northwestern University, USA
Makram T. Suidan	University of Cincinnati, USA
Oskar Wanner	EAWAG, Switzerland

OBJECTIVES OF MODELING

The IAWQ International Specialty Conference on Microbial Ecology of Biofilms (Lake Bluff, IL, U.S.A.) provided updated information on current issues in biofilm research. In general, biofilm models can be broadly classified into two categories.

- Biofilm models for *practical engineering applications*, such as design, troubleshooting, real-time operation, and education.
- Advanced models used as *research tools* to investigate specific processes occurring within microbial biofilms. The application of these models is primarily intended to fill gaps in our knowledge of biofilm dynamics.

Regardless of the type of application, biofilm models should be *realistic*. That is, a model should not attempt to include all possible phenomena occurring within a biofilm, but should be able to accurately represent the specific phenomena that it is intended to simulate. The sensitivity of model outputs to changes in input parameters will determine which are the critical processes that regulate the biofilm behavior under investigation. However, the relative insensitivity of model output to changes in a parameter does not necessarily mean that the parameter (or process) should be eliminated from the model.

The combination of mathematical models and adequate sensitivity analyses provide useful insights into the degree of accuracy needed in the experimental evaluation of biofilm parameters. For example, the kinetic behavior of microorganisms in biofilms might be significantly different from that of planktonic cells. Thus, determining accurate intrinsic parameters might be of significance when analyzing substrate utilization rates or microcolony formation in a multispecies biofilm. However, the accuracy of kinetic parameters might be relatively unimportant when analyzing other phenomena such as attachment and detachment mechanisms.

Experimental biofilm research can also benefit from simulations aimed at revealing which new parameters need to be measured. For instance, recent attempts to mathematically elucidate mechanisms of biofilm erosion and sloughing have highlighted the need to experimentally measure mechanical properties of EPS, such as elasticity and stress resistance (Picioreanu *et al.*, 1999). With experimental methodologies for their measurement starting to be developed (Ohashi *et al.*, 1999), the sensitivity of model outputs to variations in EPS properties can be helpful to ascertain the appropriateness of experimental designs.

In relation to *practical engineering applications*, the current objectives of biofilm modeling include biofilm engineering, real-time control, and applications in education. These objectives are briefly described below.

- *Biofilm Engineering*. An essential objective of biofilm modeling is to gain an insight into the interactions between the processes involved in biofilm formation so that it would be possible to "engineer" the biofilm structure and its function. For example, we envision the manipulation of the environmental conditions to generate dense biofilm structures that will be easily separated from a liquid phase (e.g., granules in UASB reactors and in fluidized bed or airlift reactors), multi-layered biofilms that would block corrosion of metal surfaces, or rough biofilm structures with high capacity for removal of particulate material.
- *Real-Time Control*. The ability to control biofilm systems on-line requires the generation of mathematical models that incorporate the activity of the biofilms and the stochastic behavior of system inputs and biofilm activity. The generation of such biofilm models is an essential development towards the goal of real-time control of biological treatment processes.
- *Education*. Biofilm models are also learning tools. If mathematical models of biofilms are to be used as design and simulation tools, it is essential to teach the fundamentals of these models to future generations of scientists and engineers. Moreover, a better understanding of basic physical and computational principles, as well as of the benefits and limitations of existing models, would contribute to an increased appreciation of the mathematical model as a basic tool for research and practical applications.

The current use of biofilm models as *research tools* has broader objectives, most of them related to gaining a better understanding of biofilm structure, population dynamics, and structural heterogeneities.

- *Relevance of 3D heterogeneity*. With the abundant experimental evidence showing that biofilm structures are heterogeneous, the simplifying assumptions of 1D models are in question. Paramount to the development of useful models for biofilm engineering is the critical evaluation of these original assumptions. Furthermore, the development of experimental and mathematical tools to study 3D heterogeneity poses an additional challenge to the researcher seeking answers from the vast amount of 3D information generated. Thus, it is fundamental to propose and develop unifying parameters to describe biofilm structure and to investigate trends within the biofilms (Lewandowski *et al.*, 1999). It is equally significant to evaluate the importance of biofilm heterogeneity on overall biofilm reactor performance.

- *Behavior of specialist microorganisms.* The survival of a specific organism within a biofilm is often the most interesting aspect of a biofilm process. For example, the ability of pathogens to colonize biofilms in water distribution systems dictates the level at which these biofilms need to be controlled. Furthermore, the success of a bioremediation approach might depend on the survival and persistence of an individual strain within a multispecies biofilm. Modeling efforts need to be directed towards elucidating the important mechanisms involved in competition for space and resources within biofilms.
- *Microbial Ecology.* Novel experimental methods are continuously producing more evidence of the heterogeneous nature of multispecies biofilms. Even though it is possible to develop hypotheses on the ecological interactions among different microorganisms based on the experimental observations, mathematical modeling is a key tool to evaluate the adequacy of the hypotheses. Furthermore, model simulations and predictions are critical in the development of additional experimental tests to help prove or disprove a specific hypothesis.
- *Microorganisms as producers.* An important concept to be recognized in multispecies biofilm models is the production of substances by microorganisms and the ecological implications of this activity. Microbial products of interest include chemical transformation products, soluble organic compounds from autotrophic bacteria, quorum sensing factors, and EPS substances.
- *Analysis of potential detachment mechanisms.* Advanced mathematical modeling of biofilms is being used to understand the effect of hydrodynamic flow and shear forces on the erosion and sloughing mechanisms in biofilms. These mathematical efforts need to be complemented with experimental information on mechanical properties of biofilms, such as elasticity and tension resistance as a function of EPS and cell content.
- *Elucidation of processes determining particle behavior (including microorganisms).* Increased attention should be placed on elucidating the mechanisms involved in the consumption of particulate organic matter by microbial biofilms. Similarly, the importance of transport of microorganisms to and from the biofilm (attachment and detachment) has not yet been mathematically evaluated, even though this phenomenon can be extremely important in defining the microbial ecology of the biofilm.

FUTURE WORK

Biofilm research is driven by the scientific and practical interest to understand, control, and engineer biofilms in a variety of scenarios, including pollution control, prevention of corrosion and biofouling, minimization of bacterial regrowth in water distribution systems, biomedical applications, etc. Mathematical modeling plays a central role in understanding biofilm dynamics, and is a key tool to link microscale phenomena occurring within the biofilm with macroscale indicators of full-scale process performance. With the increasing power of computational tools, the mathematical efforts have been diversified, and different approaches are being used to model similar concepts. A significant task for the coming years is to determine what types of modeling approaches provide better insight into the different processes occurring within biofilms.

Thus, it is essential to conduct comparative analyses among the different modeling approaches to determine relevant similarities and differences and to elucidate which type of model applies to a given situation. This could be accomplished by selecting a "model biofilm system" to compare output simulations from multidimensional models such as those presented by Noguera *et al.* (1999) and Picioreanu *et al.* (1998; 1999) with those from existing 1D models (Rittmann and Manem, 1992; Wanner and Reichert, 1996). This comparative evaluation needs to be integrated with sensitivity analysis of the different parameters to further elucidate differences in model outputs. The outcome of this comparison would be a reduction of model complexity, a clear identification of the parameters that need to be experimentally determined, and an assessment of the relative importance of accuracy of parameter estimation.

Finally, the interaction between mathematical modeling and experimental research cannot be overemphasized. Models provide the means to mathematically evaluate hypotheses and determine guidelines for adequate parameter estimation. Experimental observations reveal the appropriateness of model assumptions and provide realistic values to be used in model simulations. Only through the combination of modeling and experimental research will it be possible to acquire the knowledge and ability to "engineer" the biofilm structure and its function.

ACKNOWLEDGEMENTS

The meeting of the specialist group on biofilm modeling was sponsored by IAWQ. We would like to thank all the participants for their insightful comments during the preparation of this report.

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