Preface

This book is for biofilm researchers. It should be of benefit to both those beginning biofilm research and those with active biofilm research programs who wish to expand them by the addition of new tools, procedures, and research protocols.

The essential components of any research program are tools and reliable experimental procedures. Our approach to biofilm research emphasizes the use of conceptual, computational, physical, and virtual tools. Although modern science is driven not by tools but by hypotheses, we do not make excuses for celebrating tools in our research. Facility with tools determines competence in empirical sciences. The history of the natural sciences demonstrates that major advances in the understanding of natural processes follow the development of relevant tools. It is hardly a coincidence that rapid advances in microbiology followed the invention of the microscope. It is also interesting to speculate that biofilms were overlooked by life scientists for so long because the tools for their exploration were not ready.

One way of building a new research program, or expanding an existing program, is to look up what others have done and emulate their work. It is common, and expected, that researchers spend time developing research tools and mastering research procedures, and that they describe these tools and procedures in research papers. Such descriptions are rarely exhaustive, however, and anyone who attempts to reproduce another's work described in a research paper knows that this pursuit is not trivial in any branch of the empirical sciences. There are several reasons for the difficulty in reproducing results of biofilm research. Some are trivial and easily overcome, such as describing experimental conditions and experimental procedures carefully; others are not so trivial, such as reproducing physiological states of microorganisms from one study to another. The latter difficulties are research-specific, and one way to deal with them is to standardize research protocols, as standardizing tools and experimental protocols can improve the reproducibility of results. However, consistent standardization of protocols can be fully accomplished only for routine procedures, and in exploratory research these protocols may be modified between experiments to accommodate new information. Although we do not aspire to standardizing biofilm research protocols, our book offers a system of compatible tools and measurements that can be used to conduct biofilm studies and interpret their results in a consistent manner. This system can be implemented in both new and existing biofilm research programs.

We define biofilm research tools broadly as devices and procedures used to study biofilms, and the system includes the following:

- *conceptual tools*, such as the conceptual model of stratified biofilms and the concept of a local mass transport coefficient
- *physical tools*, such as biofilm reactors and microsensors
- *computational tools*, such as computer programs for computing biokinetic parameters from microelectrode measurements
- *virtual tools*, such as software packages for the 3-D reconstruction of biofilms from images taken with a laser confocal microscope

Each of these tools can be used separately, or in combination with other tools, and we strive for consistency within the system. For example, the results obtained using the physical tools of the system, such as the microsensors, can be interpreted using the computational tools of the system, such as programs for computing biokinetic parameters from the growth-limiting nutrient concentration profiles measured by the microsensors. Generations of our students and visitors to our laboratory have tested and refined these tools. We have also presented them at various conferences and tested them thoroughly during the summer workshops that we offer to international biofilm

researchers. This book describes our approach to studying biofilm processes, an approach that has been refined during twenty years of biofilm research.

In addition to the tools for studying biofilm processes, we also present definitions of concepts common in biofilm research. We find it useful to define these concepts for our purposes, although our definitions may vary from those used by other researchers. It is well known that biofilms are studied by disparate groups of researchers, such as corrosion engineers and biomedical researchers. Not surprisingly, these groups of researchers find it difficult to communicate their advances in understanding biofilm processes. Certainly some of these difficulties are caused by using different terminologies. However, most of the difficulties that we see in communicating the advances in understanding biofilm processes are caused by using concepts that are vaguely defined and tools that have not been standardized. As an example we cite the common measurement of biofilm thickness: if biofilm thickness is not defined precisely and the procedure for measuring biofilm thickness is not standardized, reporting biofilm thickness is entirely meaningless, particularly the thickness of a heterogeneous biofilm which varies from one location to another. Although the selection of tools to study biofilms is dictated by the field of exploration and it is unrealistic to expect that corrosion engineers and biomedical researchers will ever use the same physical tools to study biofilms, they can and should be using the same conceptual tools, computational tools, and virtual tools. Using the example of biofilm thickness again, that term should be standardized to both groups of researchers. Leaving definitions to intuition leads to difficulties in communicating results, and we try to explicitly define all terms we use. This book proposes a system of tools that can be used to explore biofilm processes, from the principles of mass transport and microbial activity in biofilms, through experimental exploration of various biofilm processes and data interpretation, to mathematical modeling of these processes. We know that such a system is needed.

Conducting biofilm studies using consistent and reproducible procedures is the first step toward establishing a reputable biofilm research program. The next step is using consistent and reproducible procedures to interpret the results of such studies. Results of biofilm studies, like empirical results acquired by other branches of sciences, are interpreted qualitatively using relevant conceptual models and quantitatively using relevant mathematical models. These models evolve over time, and as a branch of research matures, the conceptual models are adequately represented by the mathematical models and the entire process of making measurements and interpreting the results becomes well established. In the branches of research in which the mechanisms of the observed processes are still being discussed, as is the case in biofilm research, the conceptual models are changing much faster than the mathematical models, and this creates problems in interpreting the results of empirical studies: some recently discovered, or hypothesized, important variables affecting biofilm processes are not represented in the mathematical models of these processes. For obvious reasons, mathematical models have difficulty quantifying the effects of variables represented in the conceptual models if they are not represented as variables in the mathematical models. In an extreme case, the assumptions of the mathematical model may deny the significance of the variables represented in the conceptual models. In such instances, the existing mathematical model cannot be used to interpret the empirical data. For example, a mathematical model of biofilm processes that assumes that biofilms have constant effective diffusivity cannot be used to interpret the measured variations of effective diffusivity in biofilms. This is obviously an inconvenience, because mathematical models of biofilm processes are used to interpret the results of empirical studies. To avoid such difficulties, the system of tools and procedures we use to study biofilm processes uses a procedure that can quantify the relations among the measured variables and verify the predictions of the existing mathematical models of biofilm processes: we use the conceptual model of stratified biofilms.

The model of stratified biofilms divides the space occupied by the biofilm into a finite number of uniform layers, parallel to the surface, and the properties of these layers are characterized empirically. This approach has proven to be quite productive. In the system of tools and procedures

described here, the conceptual model of stratified biofilms constitutes a framework for interpreting the results of biofilm studies conducted with the use of various physical tools of the system, such as microsensors and confocal microscopy. The results of the studies, interpreted in accordance with the concept of stratified biofilms, can be further analyzed using the computational and virtual tools of the system. Representing a biofilm as an assemblage of individual layers, each constituting a unit of biofilm structure, gives the researcher the flexibility needed to verify the importance of various factors affecting biofilm activity in mathematical modeling. It also makes it possible to combine the results of various measurements conducted within the space occupied by the biofilm. For example, sets of growth-limiting nutrient concentration profiles are used to develop maps of the growth-limiting nutrient concentration at various levels in the biofilm, at exactly the levels at which confocal images were acquired. Mathematical models of biofilm processes can be calibrated using results of relevant measurements, and model predictions can be verified experimentally, using the same type of measurements. Therefore, interpreting the results of the measurements using the concept of stratified biofilms makes it possible to verify existing mathematical models of biofilm processes, such as the models predicting the distribution of the rate of metabolic activity, and even to amend them with factors representing missing variables to improve the accuracy of the predictions. The model of stratified biofilms generates representative profiles of variables characterizing biofilm structure and activity.

Most topics covered in this book can be described as microscale biofilm research, although we make frequent references to macroscale biofilm processes. The terms microscale and macroscale are not self-explanatory, and associating them with any linear dimensions is arbitrary. To communicate the scale of our observations, we associate the term *microscale* in biofilms with the linear dimensions that are used to refer conveniently to the smallest dimension of the biofilm, biofilm thickness, and to typical objects within the space occupied by the biofilm, such as individual microorganisms. The term *macroscale* is associated with the linear dimensions that are used to refer conveniently to the dimensions of biofilm reactors. One goal of biofilm engineering is to predict the performance of macroscale biofilm processes based on measurements conducted at the microscale. Using our assertions, this goal can be expressed as defining the relations between the biofilm processes occurring in the space occupied by the biofilm with the processes occurring in the reactor. This book makes an effort to accomplish this goal.

Although the title of this book promises that the content covers the fundamentals of biofilm research, it cannot possibly cover the entire spectrum of biofilm research, and necessarily some topics have had to be considered more important than others. Since we, the authors of this book, are engineers—environmental and chemical—we emphasize the aspects of biofilm research that are related to process analysis, engineering systems, applications of biofilms and mathematical modeling. In our research, we often use tools designed by life scientists, by molecular biologists in particular, such as fluorescent in situ hybridization (FISH) probes, reporter genes and mutated microorganisms, but we do not make these probes in our laboratory nor do we perform the genetic manipulations necessary to modify the microorganisms. We collaborate with research groups that make these probes and genetically modified microorganisms; the probes and microbes are provided to us ready to use. In this text, we describe how to use molecular probes and genetically modified microorganisms. For relevant procedures for making the probes and manipulating the microorganisms, the reader must refer to more specialized sources.

This book may serve as any of the following:

- Compendium of knowledge about biofilms and biofilm processes
- Set of instructions for designing and conducting biofilm studies
- Set of instructions for making and using various tools useful in biofilm research
- Set of computational procedures useful in interpreting results of biofilm research
- Set of instructions for using the model of stratified biofilms for data interpretation, analysis, and making predictions by extrapolating trends

A CD is attached to the end of the book that contains several software packages that can be used in microscale biofilm research. The procedures imbedded in these packages are compatible with the experimental procedures described in this text and they include:

- Computer programs for extracting numerical parameters from images of biofilms
- Computer programs for computing the growth-limiting nutrient concentration profiles in stratified biofilms
- Computer programs for calculating biokinetic parameters from growth-limiting nutrient concentration profiles measured by microelectrodes
- Computer programs for controlling micropositioners and a data acquisition system for measuring chemical concentrations in biofilms using microelectrodes

We refer to using MATLAB® very often in this book, both for calculations and to control instruments used in our research. The source codes of the MATLAB programs are given on the CD and the use of the programs is described in the corresponding chapters. Before you use the CD, copy the entire CD to your hard drive. If you use a different directory structure or folder names different from those given on the CD, you must revise the path definitions in the corresponding MATLAB programs or you will receive error messages. The programs on the CD have been tested by several graduate students and by us.

Finally, we wrote this book to help biofilm researchers, and we would like to know whether we have succeeded. Therefore, to monitor the opinions of our readers and to address emergent issues, we have set up a homepage, http://www.fundamentalsofbiofilmresearch.com/, and an e-mail address, FundamentalsofBiofilmResearch@erc.montana.edu. Please feel free to send us your opinions about the book and to make comments and suggestions. We will use this information to improve future editions of this text. We will be periodically updating the homepage with information about the availability of new or updated software packages and new or modified experimental procedures.