Consensus Model Of Biofilm Structure

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Biofilms have been defined in various ways by various researchers. The definition is usually structured to be all inclusive of the many environments that biofilms are found and disciplines that the subject covers. Characklis and Marshall (1990) define a biofilm as consisting of "cells immobilized at a substratum and frequently embedded in an organic polymer matrix of microbial origin". A broader definition is supplied by Costerton et al. (1995) who defined biofilms as "matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces". It might be easiest to define biofilms in terms of what they are not - single cells homogeneously dispersed in fluid, the well mixed batch culture of which much of contemporary microbiology is based. Structural organisation is a characteristic feature of biofilms which distinguishes biofilm cultures from conventional suspended cultures, with or without an association with an interface. Biofilm structure is a recurrent topic of discussion among biofilm researchers generally and has been featured in a number of presentations at the first two British Biofilm Club Gregynog meetings. Much discussion time has been spent in search of a "universal" conceptual biofilm model describing biofilm structure (Handley 1995). The existence of such a model is appealing but given the enormous diversity of biofilms is it possible to characterise all biofilms with a single conceptual model? And if we do agree on a working model how useful will such a model be? Possibly we should not restrict a biofilm model to certain structural constraints but instead look for common features or basic building blocks of biofilms which could be readily incorporated into different structural models in a modular fashion.

RECENT ADVANCES IN BIOFILM IMAGING TECHNIQUES

The recent development of improved imaging techniques such as confocal scanning laser microscopy (CSLM) and direct interference contrast (DIC) has allowed biofilms to be studied in the fully hydrated state (deBeer *et al.*, 1994a, Lawrence *et al.*, 1991, Keevil 1995). CSLM technology coupled with new fluorescent molecular staining techniques has revealed detailed information on the 3-dimensional structure of biofilms and the spatial arrangement of different microbial species within the biofilm (Ramsing *et al.*, 1993, Wagner *et al.*, 1993). The use of flow cells and time lapse image analysis has allowed biofilm development to be studied not only in the hydrated state but under flowing conditions (Lewandowski & Stoodley 1995). The resulting images have allowed better interpretation of high resolution microscopy techniques such as atomic force microscopy (AFM) and scanning electron microscopy (SEM) which require samples to be fixed and dehydrated.

WHY IS A KNOWLEDGE OF BIOFILM STRUCTURE IMPORTANT ?

A knowledge of biofilm structure is important to our understanding and interpretation of biofilm processes and to our ability to predict the influence a biofilm may have on a system. Some of the factors that may be influenced by structure are:

* Ecology and proximity to food chains of the various species within a biofilm. This may be an important factor for multi-step biodegradation processes which are facilitated by different microbial species.
* Mass transfer which determines the rate of supply of nutrients and antimicrobial agents to biofilm cells as well as the removal of metabolites. The application regime of antimicrobial agents in a control strategy may be tailored to particular structural arrangements.
* Biofilm removal may be determined by biofilm structure. In a very heterogenous biofilm large chunks may be more likely to break away while in a relatively smooth homogenous biofilm the erosion of single cells from the surface may be more significant. These processes may have very different consequences for microbial contamination and how a biofilm may be spread through a system.
* Frictional resistance will be strongly influenced by the shape and structure arrangement of a biofilm at high flow, turbulent, systems.

In addition all of these factors will be of major importance to any type of sophisticated mathematical modelling, which will require details concerning mass transfer rates, bacterial growth kinetics and biomass removal.

STRUCTURAL DIVERSITY OF BIOFILMS

Many different biofilm structures have been reported in the literature, these include biofilms composed of mono or mixed cultures from the laboratory, industry or the environment. Most appear to exhibit some degree of heterogeneity. Biofilms growing in aqueous environments are generally found to consist of a patchy arrangement of microbial cells in a slime matrix separated by water filled open spaces. Various descriptive terms have been used in reference to these patches: aggregates, cell clusters, dunes, filaments, fronds, hollow mounds, microcolonies, ridges, streamers and stacks to name a few (Caldwell *et al.*, 1992, Costerton *et al.*, 1994, deBeer *et al.*, 1994a, Gjaltema *et al.*, 1994, Keevil *et al.*, 1993, Lewandowski and Stoodley 1995, Moller *et al.*, 1997 and Siebel and Characklis 1991). There are clearly many terms that are being used to describe biofilm structures and in some cases different terms are being used by different researchers to describe similar structures. It may be useful at this stage of biofilm research to develop some defined taxonomic terms with which to refer to the

more commonly observed biofilm structures. These terms will emerge from discussion groups such as this or evolve themselves from common literature usage.

Individual bacterial patches can be composed predominantly of a single species or consist of a mixture of different species (Ramsing *et al.*, 1993, Wagner *et al.*, 1993). The open spaces are generally referred to as channels, pores, or voids. DeBeer *et al.* (1994b) and Stoodley *et al.* (1994) using dye and particle tracers demonstrated that liquid could flow through voids in the biofilm. Further, micro-electrode measurements showed that oxygen concentrations in biofilm channels were significantly higher than in adjacent cell clusters, demonstrating that the channels could facilitate transport through the biofilm (deBeer *et al.*, 1994a, 1996, deBeer and Stoodley 1995). Real time video imaging of biofilms growing in fast flowing water revealed that biofilm streamers oscillated rapidly in the flow (Lewandowski and Stoodley 1995). However, it may take weeks or months for the biofilm to reach maturity. The emerging picture of biofilm structure is one of great diversity with a high degree of spatial and temporal complexity.

WHAT ARE THE FACTORS THAT INFLUENCE BIOFILM STRUCTURE?

There are many parameters that we can hypothesise as playing a role in determining biofilm structure. Some of these connections have been well established while others remain unclear. There are at least four major influences on biofilm structure: 1) surface or interface properties, 2) hydrodynamics, 3) nutrients and 4) biofilm consortia. There are many other influencing variables which can be placed within each of the four main factors:

1) Surfaces
    - Hydrophobicity
    - Roughness
    - Electrochemical properties
2) Hydrodynamics
    - Shear forces
    - Frictional drag
    - Form drag
    - Mass transfer
3) Nutrients / Inhibitors
    - Concentration
    - Mass transfer properties
    - Reactivity
    - Antimicrobial properties
4) Consortia and ecological diversity
    - Food chains and trophic structure
    - Predation
    - Cell signalling
    - Morphotypes present
    - Motility

This list is certainly not meant to be exhaustive but is intended to give an idea of the many factors which may play a significant role in the shaping of a developing biofilm. The dominance of one or more of these factors will depend on the conditions of all of the factors in combination. Only in some extreme cases will the dominant factor be manifestly evident in the biofilm structure. To illustrate this point we will present two examples of biofilm structures, one of which is determined by biology and the other by environment. Myxobacteria are a group of motile gram negative soil bacteria not often associated with biofilm research. However, they meet many of the criteria outlined by various biofilm

definitions. The vegetative rod shaped cells form flat colonies on solid surfaces. The cells spread by gliding and produce a tough slime layer which gives coherence to the colony (Stanier *et al.* 1978). Myxobacteria have a complex life cycle, which includes the formation of fruiting bodies from an aggregation of individual cells (Fig. 1). As such, the myxobacteria are a good example of the elaborate social interaction that is possible among bacteria (Dworkin 1996). Although the mechanisms which cause individual cells to form complex structures are not completely understood, it appears that quorum sensing and cell signalling play major roles in early aggregation and fruiting body morphogenesis. In the case of myxobacteria it is clearly the phenotype of the bacteria which is the determining factor in the development of structure. The majority of bacterial biofilms do not exhibit such a high level of organisation as the myxobacteria. However, it is possible that information concerning these organisms could be used to elucidate mechanisms of inter and intra species communication that may be occurring in more well studied biofilms.

Hydrodynamic forces may also be a determining factor for biofilm structure. When biofilms are grown under turbulent, high shear, conditions the biofilms form drag reducing streamlined bodies as well as transform ripples which migrate downstream (Fig. 2). Often streamers or filaments form which oscillate rapidly in the flow. Such oscillations may reduce drag but increase mass transfer. These types of shapes and forms have parallels with abiotic sedimentary structures, such as ripples or dunes, and larger multicellular organisms, such as marine macroalgae, which are shaped by the external physical forces acting upon them.

The influence of some of the factors may never be easily isolated, especially in mixed cultures. For example an increase in nutrient concentration may result in increased growth rate of biofilm cells, a population shift in a community, or a mixture of both. There may be an infinite number of different structures, which may or may not be reproducible, for a given set of environmental and biological conditions. Given the potentially large number of influencing variables it is possible that biofilms will behave much like chaotic systems which have certain common features but are unpredictable in structural and temporal development.

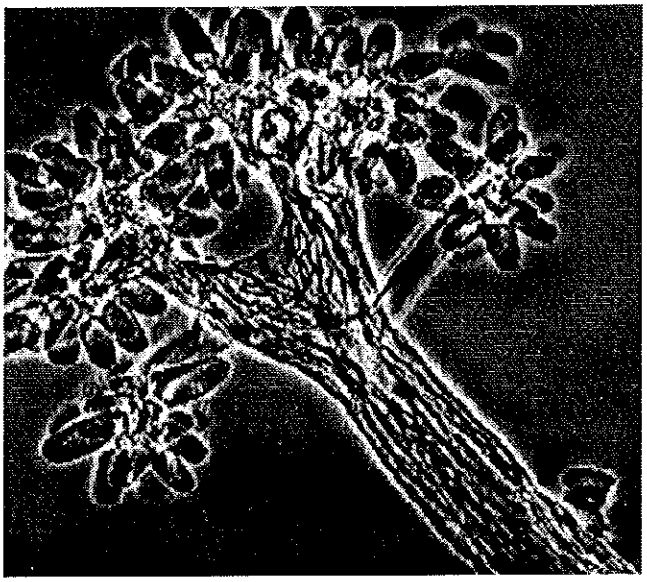

Fig. 1. Fruiting bodies of myxobacteria illustrate the complex structures that can be formed from social interaction between individual bacteria. The fruiting bodies are about 150 microns tall. The image was taken from the ASM website www.asmusa.org/pcsrc/mlp/mlp.htm and used with permission from Hans Reichenbach, GBF, Braunsweig, Germany.

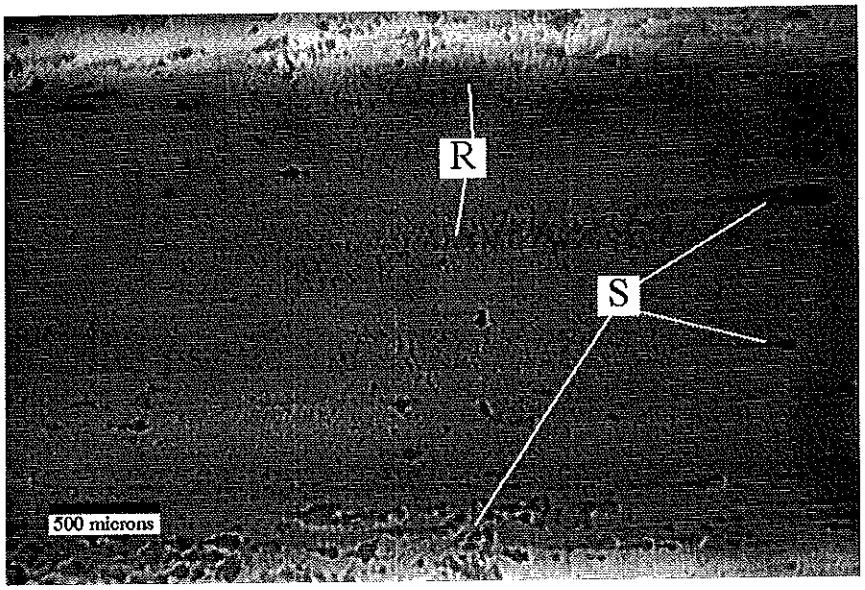

Fig. 2. Biofilm grown under turbulent flow in a square (3 x 3 x 200 mm) glass flow cell. The biofilm was composed of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, and *Xanthomonas maltophilia*. The structure of the biofilm suggests a strong influence from the hydrodynamic forces. Ripple beds (examples indicated by "R") formed in the centre channel and streamlined cell clusters were predominant near the corners (examples indicated by "S"). Flow was from right to left. Scale bar = 500 microns.

IS IT IMPORTANT TO QUANTIFY BIOFILM STRUCTURE ?

A major problem when comparing different biofilm structures is that such comparisons are generally descriptive. To be able to truly assess the impact of a variable or set of variables on the structure of a biofilm the structure will need to be quantified in some manner. There are a number of different measurements that are easily made, for example average thickness, surface area coverage and volume. However, none of these parameters gives any meaningful information on structure in either 2 or 3 dimensions. Some researchers are currently using fractal analysis in an attempt to quantify structure (Hermanowicz *et al.*, 1996, Zahid and Ganczarcyzk 1994). Although this approach adds a greater degree of sophistication to structure quantification one fractal dimension alone is not enough to uniquely describe a structure, since different structures may have the same fractal dimension. Another problem is that fractal analysis is extremely sensitive to image quality. Uneven illumination, out of focus haze, and thresholding levels will all influence the fractal dimension. Currently fractal analysis is performed on 2-dimensional images. The increasing amount of 3-dimensional information from CSLM and other techniques will almost certainly cause greater problems for structural quantification.

IS A CONSENSUS MODEL OF BIOFILM STRUCTURE DESIRABLE OR NECESSARY ?

This is a question that we should continue to ask as we search for a universal or consensus biofilm structure. There are advantages and disadvantages for the development of such a conceptual model. An advantage in having a consensus is that it provides a common language and understanding with which to discuss biofilms. It is also useful to have a benchmark by which to compare different biofilms. A consensus model may be of greatest use to the mathematical modellers who require some idea of spatial organisation to describe mass transfer processes, growth kinetics and mechanisms of biomass removal.

A disadvantage of a consensus model is that it may be conceptually restrictive and non-inclusive of many biofilms. This is apparent in the literature where definitions of biofilms are

individually tailored to fit a particular system of interest and often end up being clumsy and awkward to use. If every system requires its own specific definition then we may have to rethink the usefulness of a "consensus" model.

Instead of trying to define a single structural model it may be more useful to agree on a terminology that can be used to refer to the structures that are the building blocks of biofilms. Two basic units may be 1) the variously shaped aggregates of microbial cells in a slime matrix and, 2) the thin "base film" which is often reported as ranging from a sparse monolayer of cells up to being a few cell layers thick. It may also be useful for descriptive and modelling purposes to think of the interstitial "voids" and "channels" that separate the aggregates as being an integral part of the biofilm system. These may be included as the third basic unit. Many different types of biofilms could be described using such basic units. This approach may allow greater flexibility of application and yet still provide the framework for a common descriptive language.

STRUCTURAL MODELS AND MATHEMATICAL MODELS

Because biofilms play an important role in many industrial processes biofilm research has always had a strong engineering component. For predictive purposes it is useful to be able to model biofilm processes mathematically. Biofilms have long been utilized for waste water treatment and the modeling effort is well developed in this field (Harremoes 1978). Most biofilm models are one dimensional and assume that the biofilm is composed of homogenous flat layers and all fluxes are in the Z-plane. Generally it is assumed that convective mass transfer only occurs outside the biofilm (in the bulk liquid) and only diffusive mass transfer occurs within the biofilm layer. Although somewhat simplified in their assumptions such models have been suitable for use in waste water treatment systems for many years (Gujer and Wanner 1990). Recently 2 dimensional cellular automata models have been applied to biofilms in an effort to incorporate the observed structural heterogeneity (Wimpenny and Colasanti 1997, Hamilton 1997). Wimpenny and Colasanti (1997) have succesfully used such a model to demonstrate the possible influence of nutrient concentration on biofilm structure in a diffusion limited static system.

The description of macroscale processes will almost certainly require some degree of spatial averaging. It is yet unclear if detailed 2 or 3 dimensional information at the microscopic scale is necessary to predict overall performance at the macroscale. There is a danger that as more complexity is incorporated into mathematical models the models become unwieldy and possibly more restricted. Further, as the models become more sophisticated, other layers of complexity will continually be revealed (Dibdin 1995) which will be very difficult if not impossible to describe mathematically. For example it is probably impossible to adequately model a filamentous biofilm waving in a turbulent flow (a complex 4 dimensional system). Yet experimental data describing mass transfer processes and biofilm growth kinetics in such a system may be spatially averaged and readily incorporated into a one dimensional model.

Another approach is to use expert systems for predicting biofilm processes. Expert systems are "smart databases" that use empirical observations to predict a certain outcome given a set of conditions. However, as with mathematical models, expert systems should be kept as simple as necessary and their predictive value validated against observed situations. It is possible that an expert system constructed from the observations of many different researchers will become the framework for a consensus view on biofilm structure and processes.

CONCLUDING REMARKS

In this paper we have discussed some of the issues that are pertinent to our understanding of the factors that shape biofilm structure and the relevance that such structure may have on biofilm processes. We have highlighted some of the considerations that should be taken into account as we search for a "consensus" structural biofilm model. We have not attempted to make any recommendations on whether or not a consensus model of biofilm structure should be adopted, or indeed nominated potentially suitable candidates. However, hopefully this paper will stimulate further discussion among the group to determine if a true consensus actually exists.

Structural organisation is a property that distinguishes biofilm microorganisms from the so called completely mixed suspended broth cultures. There are numerous types of structures which often consist of common building blocks, adapted to the biological and environmental forces which shape a particular biofilm. Structural adaptability is yet another powerful weapon in the biofilm arsenal which allows microorganisms to live in community structures in a multitude of different environmental conditions. The ability to quickly respond and adapt not only phenotypically but also structurally allows microorganisms to survive under rapidly changing conditions. Living in close proximity to each other biofilm cells can take advantage of proximity to food chains, easily swap genetic material, and benefit from the protective properties of an interconnecting slime matrix. Although the morphologies of the basic building blocks of biofilms, the microbial cells, are, for the most part, genetically controlled we consider that the overall biofilm structure is probably not. This allows the possibility for biofilms composed of the same microbial consortia to form different structures in response to a given set of biological and environmental conditions. Now we need to define a common language to describe these structures.

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