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Short Communication

A more unifying hypothesis for biofilm structures

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Abstract

The 'unifying' hypothesis for biofilm structures as recently proposed by Wimpenny and Colasanti deals with only one dimension of a two-dimensional force field action upon the biofilm. A 'more unifying' hypothesis is therefore proposed which states that the interaction between the substrate gradient (rather than concentration) at the biofilm interface and detachment forces influence the biofilm structure.

Keywords: Biofilm; Structure; Cellular automaton; Model

In a recent paper Wimpenny and Colasanti [1] excellently review the observations on conceptual biofilm models. They conclude, based on literature observations and by the use of cellular automaton models, that "the biofilm structure is largely determined by substrate concentration". The title of their review (A unifying hypothesis for the structure of microbial biofilms) however, strongly provokes reactions since the hypothesis deals with only one force acting on the formation of biofilms. Previously [2] we proposed a general working hypothesis for the development of biofilm structures, stating that the biofilm is influenced by substrate availability as well as detachment forces. The large amount of biofilm literature (specific growth rate 0.17 year^{-1} [1]) and the wide range of journals publishing papers on biofilms (>30) makes it virtually impossible for anyone to read everything and similar concepts seem to be developed in microbiological as well technological research fields. In this discussion we want to introduce briefly the essentials of our hypothesis and compare this to the hypothesis of Wimpenny and Colasanti.

Wimpenny and Colasanti recognise that there exist non-porous as well as porous biofilms. This balanced view is highly appreciated in a research field where the porous biofilm dogma seems to have replaced the smooth biofilm dogma. They, however, use a peculiar comparison of biofilm ecosystems at different substrate concentrations. From this comparison it is concluded that biofilms in oligotrophic environments are most heterogeneous, whilst those from eutrophic systems are most homogeneous. This comparison ignores the general ecological feature that oligotrophic ecosystems have usually a more diverse (i.e. heterogeneous) population. It is therefore questionable whether this comparison is warranted.

It is more logical to reason from the similarity between particle growth by crystallisation or flocculation and biofilm growth. From the crystallisation literature it is well known that if a diffusion gradient exists (i.e. low concentration) more porous crystals

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will be formed, whereas if the crystallisation is not hindered by diffusion (i.e. at high concentrations) dense crystals are formed [3]. This is explained by the fact that at the outgrowing tips a higher concentration exists, leading to faster growth of these tips compared to the base crystal. This has been verified by, for example, the diffusion limited aggregation cellular automaton models [3]. Similarly, for biofilms growing in a diffusion gradient, it can be expected that a porous heterogeneous structure will be formed. At lower substrate concentrations a steeper diffusion gradient will be formed more easily than at higher concentrations. This reasoning is supported by the studies of Wolfram [4] and Matsushita [5]. The formation of a substrate gradient depends not only on the concentration in solution, but also on the external mass transfer resistance (i.e. mixing of the system) and the rate at which the substrate is consumed by the biofilm. Therefore, in our view, the main conclusion of Wimpenny's hypothesis should be phrased as "biofilm structure is largely determined by the substrate concentration gradient at the biofilm-liquid interface".

The substrate availability is, however, only one dimension of a two-dimensional hypothesis needed to explain the observations on biofilm structures. In our experimental systems, biofilm formation on suspended carriers in a three phase reactor, we have shown that under identical substrate loading conditions the biofilm can either be smooth, dense and non-porous or highly porous [2,6]. In these experiments the difference in biofilm structure was caused by the shear applied. A higher shear (detachment force) leads to a less porous biofilm. Based on these and other literature observations we hypothesised that the biofilm structure is the result of two counteracting forces: an intrinsic tendency of biofilms to grow as filamentous porous structures which is counteracted by detachment. In the end it is a balance between substrate gradient (or biomass production rate) and the shear rate at the biofilm surface which determines the biofilm structure. This can be stated in simple words by: fast growing tips lead intrinsically to porous biofilm structures, at higher shear rates these tips are 'shaved off' resulting in a dense biofilm.

Based on the above discussion we would like to propose the following hypothesis for biofilm structure formation: "the biofilm structure is largely determined by the substrate concentration gradient at the biofilm-liquid interface and the detachment forces working on the biofilm".

On top of this simple two-dimensional model several complicating factors exist such as biomass yield and extracellular polysaccharide (EPS) production. Organisms with a higher yield on the rate determining substrate will form a more porous biofilm than those with a lower yield. Organisms with a lower yield produce relatively less new biofilm material and the influence of detachment forces is then also larger. On the other hand production of more EPS might result in a faster formation of new biofilm material and consequently less influence of shear, resulting in a more heterogeneous biofilm.

Wimpenny and Colasanti reject the 'morphogenetic' view towards porous biofilm structures, as regularly expressed by other authors, we strongly support this opinion. When we presented our mechanistic hypothesis on biofilm structures at a biofilm structure conference in 1995 we were accused of overlooking the poetry of biofilms. In our view one should however try to recognise the basic physical processes of the biofilms. On top of this the microbial ecology will play its role and add the poetry biologists prefer to observe.

Finally we also want to make a short comment on the 'biofilm cellular automaton model'. The model presented is a first step towards a possible biofilm model. At this stage it is, however, not significantly different to the diffusion limited aggregation (DLA) models for crystal growth. The major difference between crystal growth and biofilm growth is that a crystal only grows at the surface, whereas growth of the biofilm occurs in the bulk of the biofilm. The model presented only describes the growth in the outermost cell layer, just like in a crystal. Probably the conclusions reached will not be strongly different, but we feel that a model can only be called a biofilm model if the growth occurs in the bulk of the biofilm as a function of the substrate gradients occurring in the biofilm.

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