

Understanding the Development and Formation of Biofilms

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A biofilm is a complex aggregation of micro-organisms growing on a solid substrate. Biofilms occur in a range of everyday situations, from pipe and ship fouling to dental caries. Formed from bacterial colonies, the formation and maturation of biofilms is not a well understood process, nor is the mechanism regulating bacterial colony size and species. Biofilms generally form on surfaces immersed in fluid and are generally resistant to antibiotics, disinfectants and cleaning fluids.

Biofilms grow in a three stage process as shown in Figure 1(a). The initial stage includes the attachment of bacteria to the substratum. Bacterial growth and division then leads to the colonisation of the surrounding area and the formation of the biofilm. Recent work in our laboratory has shown that bacteria (*Rhodobacter sphaeroides*) do not act individually to form biofilms, but congregate into long chains to help initiate the early stages of biofilm formation as shown in Figure 2.

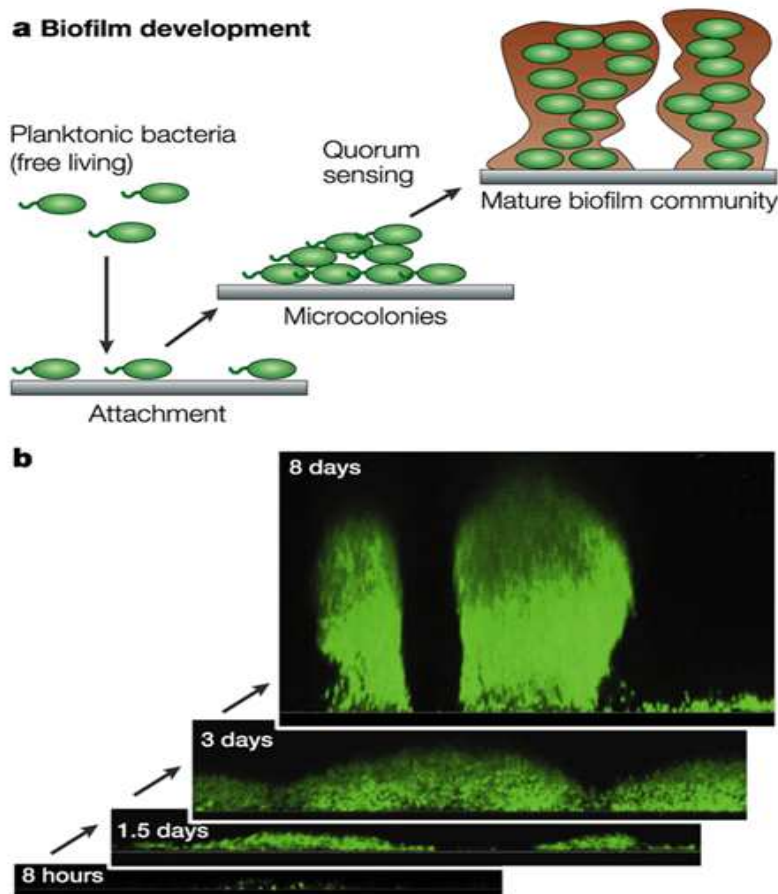


Figure 1: The stages of biofilm formation.

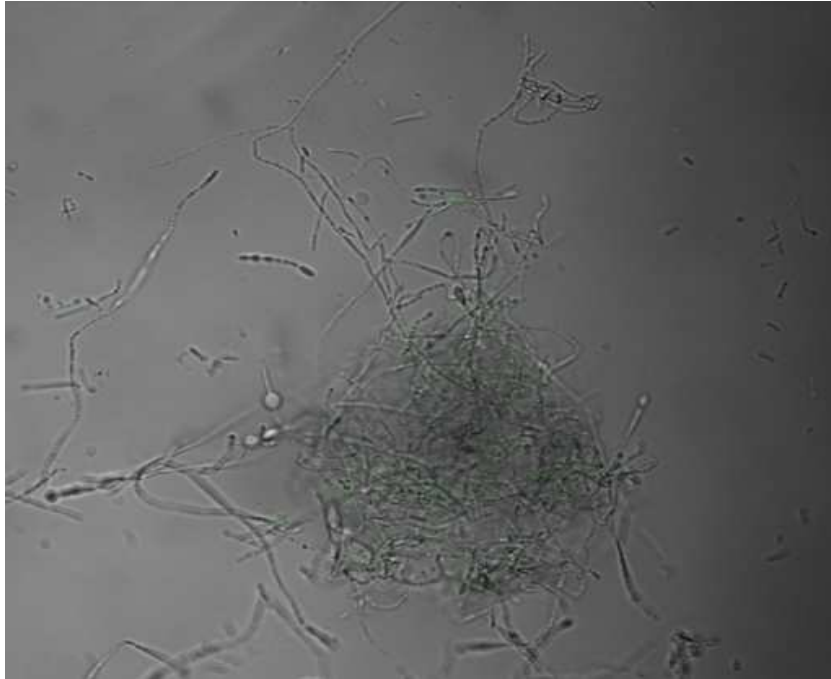


Figure 2: Individual bacteria which have formed chain like structures during early biofilm formation.

Mature biofilms, as shown in Figure 1(a), are a complex heterogeneous structure of dormant and actively growing bacteria colonies along with further enzymes, excretory products and small channels forming part of the overall structure. In certain cases the biofilm will form pillar like structures as seen in Figure 1(b).

Bacteria move chemotactically and use the process of quorum sensing to communicate. Different species use different molecules for communication; some sense only their own molecules, others sense their own and other species' molecules. There is also evidence that colonies near the biofilm surface behave differently from those in the bulk. It has been shown that mutant colonies which lack quorum sensing or the ability to move chemotactically form less well defined, looser structures.

The outer regions of the biofilm are generally well oxygenated with the inner regions being quite anaerobic. Bacteria dividing on the edge of the biofilm can often be sloughed off to form biofilms elsewhere. It has been shown that the metabolic activity of the bacteria is higher in the outer more nutrient rich regions of the biofilm (near or in contact with the fluid flow) as demonstrated in Figure 3, but the number of bacteria in these regions is actually quite small. In contrast, the majority of bacteria within a biofilm are found towards the inner regions, usually in a more dormant state, as demonstrated in Figure 1(b).

The Study Group participants are asked to look at providing answers to the following questions.

- 1) What role does bacterial chain formation play in the formation and stabilization of new biofilm colonies?
- 2) Is it possible to produce a model of biofilm development which includes the effects of quorum sensing and the chemotactic pathway?
- 3) Why are biofilms so effective in combating the effects of antibiotics and other such reagents and what role does their heterogeneous structure play in this? If nutrients can diffuse into the inner regions, why are agents harmful to the bacteria only able to penetrate the outer regions?

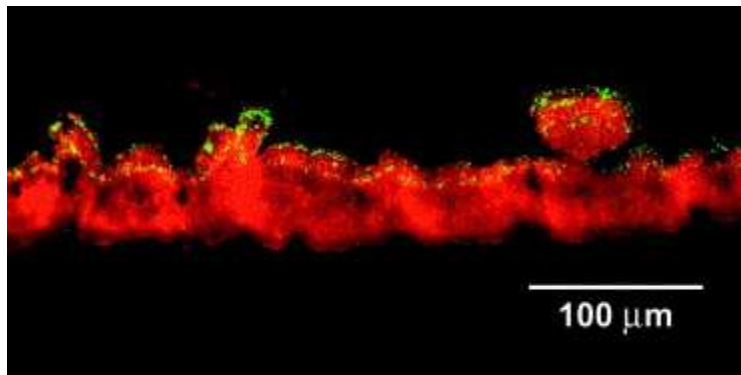


Figure 3: Metabolic activity within a biofilm. The green dots indicate GFP labelling, showing metabolic activity on the outer regions of the biofilm near the fluid flow.

References

1. A good introduction to biofilms can be found at <http://www.erc.montana.edu/CBEssentials-SW/bf-basics-99/default.htm>
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