The Role of Exopolymers in Biofilm Formation and Composition

Joana Azeredo and Rosário Oliveira*
Centro de Engenharia Biológica-IBQF, Universidade do Minho, 4700 Braga, Portugal

Biofilms consist of microbial cells surrounded by a matrix of large mucous molecules (exopolymers). These polymers stabilise the attachment of microorganisms to surfaces and afford protective and sorptive properties to the cells. The composition of the polymeric matrix determines many important properties of the biofilm such as strength, elasticity, sorption capacity, etc. It was previously assumed that biofilms were structurally homogeneous. However, several studies have recently led to the conclusion that biofilms are characterized by gradients of cell types and physical-chemical parameters and three new conceptual models of biofilm structure emerged. However, these models were based on studies of thin and young biofilms, due to limitations of CSLM that only enables the study of thin biofilms (less than 200 μm). Many of the biofilms used in waste water treatment systems have thickness of 0.5 to 2 mm, an order of magnitude above those that can be observed by CSLM. Therefore the structure of biofilms is still a very controversial subject.

The aim of this work was to study the effect of the amount of the exopolymers produced by bacteria in biofilm formation and composition. For this purpose two mutants of Sphingomonas paucimobilis were used: TR is a high gelane (polysaccharide) producer and CV produces smaller amounts. The bacterial cells were grown in a batch culture after which the cultures were introduced in a reactor continuously fed with aerated S medium with increasing dilution rates. The biofilms were formed around 4 glass cylinders immersed in the reactor. After 12 days the glass cylinders were carefully removed from the reactor and three biofilm layers were detached by submitting the cylinders to increasing velocities of rotation (500, 1000 and 2000 rev./min). The physical properties (wet weight, thickness and volume) of each biofilm portion removed were determined as well as the biochemical composition (DNA, proteins and polysaccharides) of the biofilm matrix. The activity of each biofilm portion was studied by respirometry.

Both biofilms could only be formed onto the glass cylinders when the excreted bacterial polymers were present in the reactor, suggesting that the exopolymer enhanced bacterial adhesion. After 12 days of growth, the biofilm produced by the mutant TR was very thick (about 1mm), while CV produced a much thinner biofilm (0.15 mm).

With 500 rpm 51% of the thickness of the total TR biofilm removed was recovered, the same rotation velocity enabled the detachment of 68% of the thickness of the total removed CV
biofilm. The greatest shear stress used (2000 rpm) detached 12% and 20% of the total biofilm removed, produced by TR and CV respectively. These results suggest that the cohesion forces in TR biofilm (high producer) are stronger than in CV biofilm. The density of both biofilms increases along their depth. However this tendency was more significant in the thinner biofilm produced by CV. This is probably due to the fact that in the outer layer the growth of the biofilm results in an increase of thickness, while in the inner layer the growth of the biofilm is responsible for an increase in density.

Regarding the physical properties, both biofilms revealed a great heterogeneity. The biochemical composition of the matrix per cell was quite homogeneous in the biofilm produced by CV and very heterogeneous in the biofilm produced by TR, where in the inner layer a great accumulation of DNA and proteins was found. This fact was related to cell lysis and to the production of hydrolytic enzymes (according to the results obtained by the API 20NE identification test) due to nutrients limitation.

The possible existence of nutrient limitations in the inner layer of TR biofilm was proved by respirometric assays. The results revealed that the metabolic activity of TR biofilm decreased twice from the outer to the intermediate detached layer and 10 times to the inner layer. CV biofilm revealed a constant metabolic activity along its thickness.

The distribution of cells in both biofilms was in accordance with the biofilm density. The thinner biofilm showed a great accumulation of cells in the inner layer, which is typical of thin biofilms grown in rich media. TR biofilm revealed a more homogeneous cell distribution.