Biofilm formation dynamics under different growth conditions

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Biofilms are communities of microorganisms which segregate an extracellular polymeric matrix. They are of primary interest due to their relevance in health and food industry but impact also other domains [1]. About half or even more of the microbial infections in humans are caused by bacteria growing as a biofilm. In food industry, biofilms tend to form on production plants and equipment. They also pose sanitation and management problems and extra costs in fish farms, boats, water treatment, etc. Bacteria in biofilms are known to better resist disinfectants, cleaning procedures and even antibiotic attack.

Our current research is aimed at determining the physical and chemical characteristics that contribute to mechanical properties and stability of *Pseudomonas fluorescens* biofilms from experiments in rheology and NMR. To asses the influence of environment, biofilm growth has been carried out in different conditions of agitation and nutrient availability. In this contribution, we present a population dynamics model coupled to nutrient dynamics that can satisfactorily reproduce and explain some of the aspects of bacterial growth in the experiments.

A set of experiments were carried out in vertically disposed coupons half immersed in growth media. Samples at different stages (4, 12, 24, 48, 72, and 96 h) have been extracted to perform cell counting, providing evolution of logarithmic CFU cm⁻² (CFU, colony forming units) data. Different media have been used for different batches, TSB (rich medium), TSB at half its usual concentration, TSB supplemented with glucose and PMS7Ca (minimal medium). Container shaking has a dramatic effect in biofilm growth and morphology. Figure 1 shows (normalized) cell counts in CFU in TSB stirred and static experiments.

The number of bacteria in the biofilm follows a growth, saturation and decay pattern. We interpret these results as follows. In the initial stages growth is exponential as anticipated as expected. Culture medium is not replaced during the experiment. As a result a saturation in population occurs. As nutrients become scarce an competition intensifies, cells either die or leave the biofilm. This can be directly translated into a population dynamics model where carrying capacity is dependent on nutrient concentration and with a rate of feeding proportional to cell number. For suitable parameters the model can reproduce the same behavior (see Fig. 1, blue continuous line). One can expect that static conditions may impact the value of the parameters, and in fact the model can also reproduce the behavior for static conditions by changing some parameter values (see Fig. 1, blue dashed line).

Finally, the system is being analysed also from a different



Fig. 1. Circles are the experimental CFU normalized to value at 24 h, blue stars represent experimental data in static conditions. Blue continuous and dashed lines are the solution of the model for population in stirring and static conditions, respectively. Green continuous and dashed lines are nutrient concentration for stirred and static conditions, also from model results in arbitrary units.

scale and degree of detail by performing mesoscopic dissipative particle dynamics (DPD) simulations of bacterial growth, interaction and extracellular matrix secretion. Bacteria are modeled as rods, where each bacterium is formed by a finite size chain of Brownian particles. They reproduce according to the input reproduction parameter. The extracellular matrix can be modeled either explicitly, as chains of Brownian monomers which generate according to a input production rate of bacteria, or implicitly, as an effective depletion force among bacteria. Both cases have interesting effects in the growth of the modeled biofilm. We expect the population dynamics model to provide valuable information and insight to tune and interpret simulation results. Simulations are a valuable tool to understand rheology of biofilms, which is relevant to asses their stability under external mechanical stress.

[1] M. Ghannoum and G. A. O'Toole (Eds.), *Microbial Biofilms* (American Society of Microbiology Press, 2004).

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