Effect of Flow on Formation, Morphology and Wetting Properties of *Pseudomonas fluorescens* Biofilms

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Aristotle University of Thessaloniki · University of Naples, Federico II · IGB-CNR · Marie Curie ITN, Complex Wetting Phenomena

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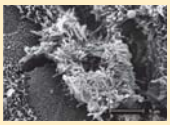
ABSTRACT

Biofilms are sessile, microbial systems, held together by a self-produced matrix of polymeric substance [1]. Biofilms have many implications and issues in different fields, including bioremediation, industrial biofouling, and medical implant contamination. Therefore, the necessity to study their formation and morphology, is essential. Moreover, it is not clear the role of the complexity of biofilm surface morphology and composition on the interaction of the surface with a fluid, that can be simply water or a cleaning solution, especially when they are considering detrimental, like in case of industrial bio-fouling. In this work, *Pseudomonas fluorescens* NCDO 2085 biofilms were grown using different *in vitro*-set ups. In particular, the effect of shear rate on biofilm morphology was investigated, and compared with standard growth conditions, based on static or uncontrolled flow. Biofilm growth kinetics was assessed using turbidimetric and colorimetric techniques. Biofilm morphology was evaluated using CSLM technique. Biofilm structural organization was quantified by image analysis. Preliminary investigation was also done on the interaction of droplets of different fluids with biofilms (*Wetting*).

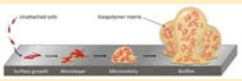
INTRODUCTION

Biofilms are sessile systems, made of communities of microorganisms, embedded by a self-produced matrix of polymeric substances, called EPS [2]. They represent a very common way of living of microorganisms colonies that gives to their members several benefits like mechanical resistance, protection from antibiotics and adaptation to nutrient deficient conditions [3].
Biofilms are ubiquitous systems. They have many implications in many research fields, including bioremediation, waste water treatment, industrial biofouling and medical implant contamination.



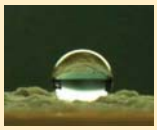
SEM microphotograph of *Pseudomonas aeruginosa* biofilm, scale bar: 5 *μm* [4].

Typically, biofilms formation follows sequential steps: ***initial attachment, cell growth* and *microcolony formation, maturation in 3D structures, and detachment and recolonization***.



Biofilms formation mechanism [5].

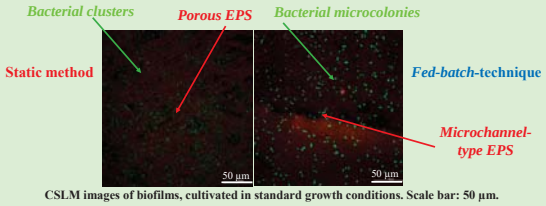
It is also not clear the interactions between these biological structures and fluids, such as water or a cleaning solution. Effective wetting of biofilm surfaces are essential for struggling them with antibacterial agents, in case they are considered detrimental, like in case of industrial biofouling.



Water droplet onto bacterial biofilm colonies [6].

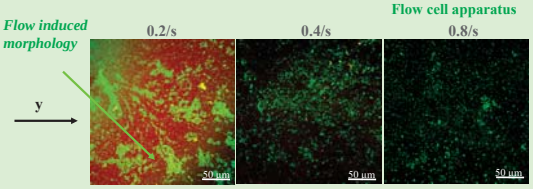
RESULTS

Biofilms, cultivated in standard growth conditions, show different morphologies.



CSLM images of biofilms, cultivated in standard growth conditions. Scale bar: 50 μm.

Biofilm morphology is strictly dependent on the flow conditions. Flow induces the formation of micro-channels at lower shear rates. The tendency to form biofilms attenuates at increasing shear rates. The controlled hydrodynamic conditions induce more uniform coatings, compared to the standard growth methods, above reported. In particular, biofilm appear to be less dense, thinner and less rough, due to the sloughing off phenomena, induced by the flow.

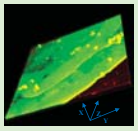


Biofilms under three different shear rate conditions: 0.2/s, 0.4/s and 0.8/s. Scale bar: 50 μm.

In vitro technique	Biodensity, $\frac{\mu m^3}{\mu m^2}$	Bioratio, %	Biofilm Average Thickness, Hav, *μm*	Ra, *μm*
Static method	4.51 ± 1.46	24.74 ± 6.52	4.11 ± 2.53	0.23 ± 0.28
Fed-batch technique	2.70 ± 1.00	22.1 ± 1.6	3.9 ± 0.5	1.4 ± 0.50

Flow cell, $\dot{\gamma}$, 1/s	Biodensity $\frac{\mu m^3}{\mu m^2}$	Bioratio, %	Biofilm Average Thickness, Hav, *μm*	Ra, *μm*
0.2	20.16	70.8	9.55	2.14
0.4	2.24	17.38	7.35	2.04
0.8	2.81	11.99	4.52	0.35

Wettability analysis shows strong biofilm hydrophilicity, due to strong physical-chemical interactions between water and EPS (water adsorption).



CSLM 3 D reconstruction of the water droplet onto biofilm.

Type of substrate	CA center, °	CA periphery, °
Uncoated	68.50 ± 1.0	66.19 ± 1.4
Biofilm Coated (24 h)	37.04 ± 0.83	36.81 ± 0.6

MATERIALS AND METHODS

Biofilms were cultivated at **30°C, pH 7, in aerobic conditions**. For the purpose, the following components were chosen:

- *Pseudomonas fluorescens* NCDO 2085, AR 11 strain, extracted from diary industry production;
- A minimal salts medium as a culture medium;
- Glass coupons as substrates.

Three different *in vitro* set-ups were selected for biofilm cultivation:

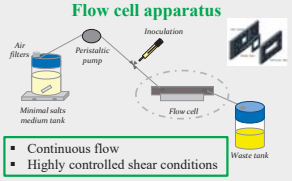
Static method

- No flow
- Diffusion mechanism

***Fed-batch*-technique**

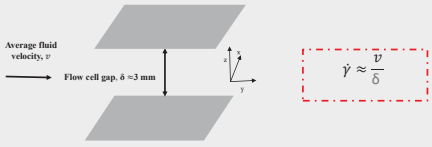
- Dynamic conditions
- Culture medium replacements

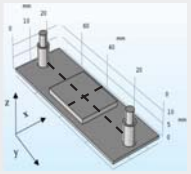
Flow cell apparatus



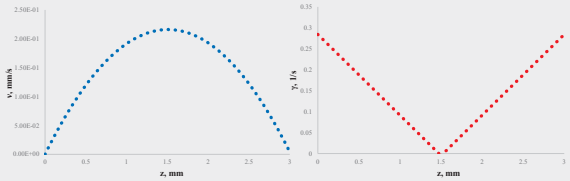
- Continuous flow
- Highly controlled shear conditions

In controlled flow conditions, the role of wall shear rate,$\dot{\gamma}$, on biofilm formation was assessed and evaluated as the following:





Numerical simulation of the flow conditions

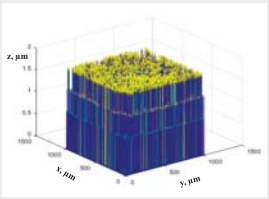


Velocity (left) and shear rate (right) profiles, evaluated at the flow cell center point.

Biofilm images were acquired via **CSLM.** Bacterial cells were stained using the green fluorescent dye, **SYTO®9,** and α-mannose residues bonds within polymeric matrix stained by the red fluorescent **TRITC conjugate Concanavalin A**.
Biofilm structural organization was quantified by **image analysis,** in terms of the following geometrical parameters:

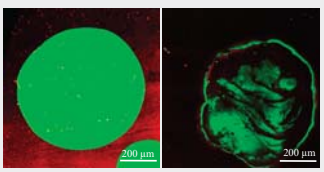
- **Bio-density,** $\frac{\mu m^3}{\mu m^2}$: volume occupied by biomass pixels (**voxels**) for unit of surface.
- **Bioratio, %**: biovolume/totalvolume.
- **Biofilm Average Thickness, Hav, μm**.
- **Arithmetic Average Roughness Profile, Ra, μm,** $R_A = \frac{1}{n}\sum_1^n |y_i|$

Where y_i is the deviated profile roughness determined from the center lines, n the number of space points [7].

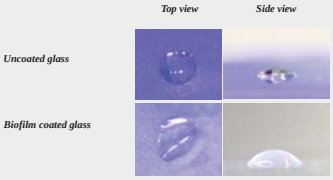


Biofilm Thickness Profile.

Preliminary wetting properties of biofilms, formed at solid-liquid interfaces, were assessed by determining **thermodynamic contact angles** of 20 μL water droplets. CSLM was also used to evaluate qualitatively local droplet contours. For the aim, water droplets were stained with the green fluorescent **FITC Isomer I**. Biofilms were stained with **TRITC coniugate Concanavalin A.**



CSLM images of FITC stained water droplet onto negative control (left) and onto a biofilm coated substrate (right). Scale bar: 200 μm.



Comparison between water droplet shape onto uncoated and biofilm coated coupons.

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