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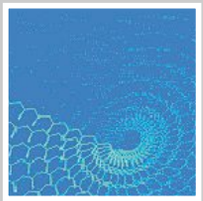

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Interfacial Rheology of Bacterial Biofilms at Air/Water and Oil/Water Interfaces

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Biofilms are highly complex biological assemblies composed of bacterial cells embedded in an extracellular polymeric matrix. Biofilms are ubiquitous and can cause severe environmental and health problems. Therefore it is intriguing to understand the influence factors on formation and destruction of bacteria biofilms.[1,2] In this contribution, we study the kinetics of bacterial attachment as well as the biofilm formation of model bacteria at both the air/water and oil/water interface through interfacial rheology and tensiometry.[1,3,4]

In the first step of biofilm formation, bacteria attach to hydrophobic interfaces. This was measured with interfacial rheology (Fig. 1) and pendant drop tensiometry. Bacteria with very little hydrophobicity (*e.g. Escherichia coli*) formed very weak adsorption layers whereas bacteria with a high hydrophobicity (*e.g. Pseudomonas putida KT2442)* formed strong adsorption layers against oil.

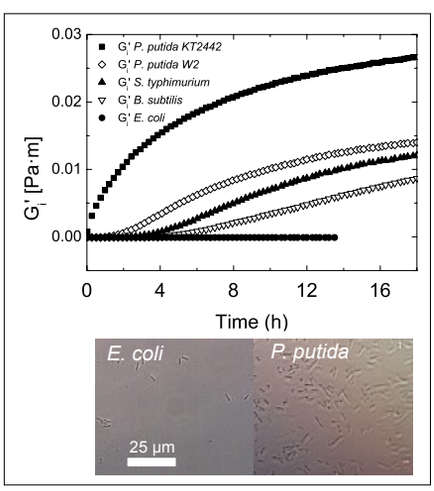

Fig. 1. Interfacial elasticity measurements of bacteria at the mineral oil/water interface. Microscopic images of the water/oil interface of *E. coli* and *P. putida*.

With growing bacteria, the several stages of the biofilm lifecycle of different bacteria were observed (Fig. 2 A and B).

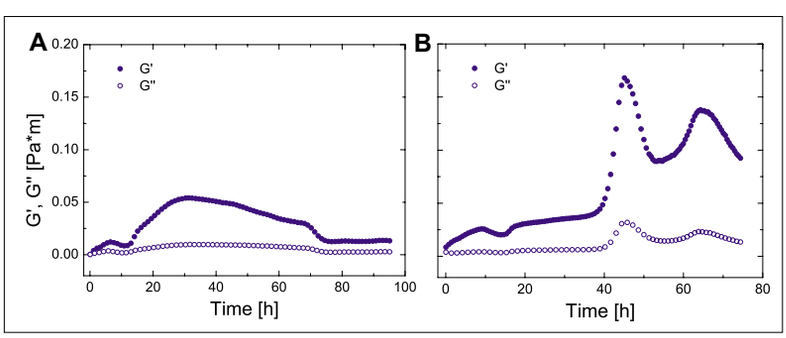

Fig. 2. The elastic and viscous moduli are plotted as a function of time for (**A**) *P. fluorescens* and (**B**) *E. coli*.

Electrophoretic mobility measurements and bacterial adhesion to hydrocarbons (BATH) tests were performed to characterize selected bacteria. To validate interfacial rheology and tensiometry measurements, we monitored biofilm formation utilizing both confocal laser scanning microscopy and light microscopy. In Fig. 3, the biofilm formation of *P. putida KT2442,* a bacterium used in bioremediation, is shown at the water/oil interface after 48 h- and after 2–3 weeks.

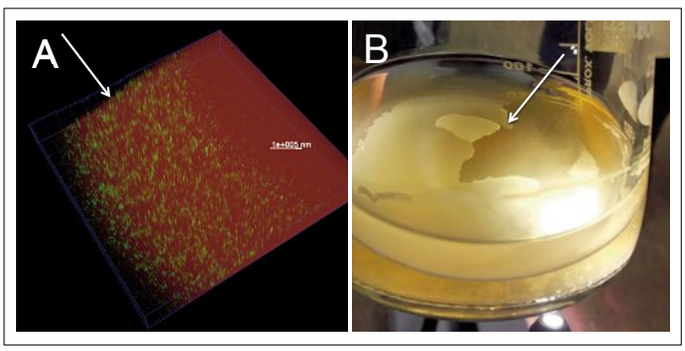

Fig. 3. (**A**) Confocal laser scanning microscopy image of *P. putida* KT2442 at the water/oil (red) interface. (**B**) The biofilm formed after 2–3 weeks is shown. The arrows indicate the position of the interface.

Using this combination of techniques, we were able to observe the elasticity and tension development over time, from the first bacterial attachment up to biofilm formation. Interfacial rheology proved to be a valuable tool for studying biofilms as the influence of temperature, media type, bacterial strain, pH and surfactant concentration could be observed successfully during biofilm formation.[1,4]

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