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SHORT COMMUNICATION

Influence of abrasion on biofilm detachment: evidence for stratification of the biofilm

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Abstract The objective of this paper was to understand the detachment of multispecies biofilm caused by abrasion. By submitting a biofilm to different abrasion strengths (collision of particles), stratification of biofilm cohesion could be highlighted and related to stratification of biofilm bacterial communities using the PCR-SSCP fingerprint method. The biofilm comprised a thick top layer, weakly cohesive and composed of one dominant species, and a thin basal layer, strongly cohesive and composed of a more diverse population. These observations suggest that microbial composition of biofilms may be an important parameter in understanding biofilm detachment.

Keywords Abrasion · Biofilm ecology · Cohesion · Stratification

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Introduction

Biofilm formation and microbial ecology within the biofilm are determined by the balance between growth and detachment processes [1]. Understanding the roles and mechanisms of detachment is important in evaluating biofilm development and persistence in many industrial and natural environments. Detachment occurs when external forces (e.g. shear) exceed biofilm cohesiveness [2, 3]. Different detachment processes can be distinguished: abrasion and erosion are continual detachment of small particles of biofilm, whereas sloughing consists of punctual detachment of large pieces of biofilm. Erosion is caused by shear forces generated by the moving fluid in contact with the biofilm surface, and abrasion is caused by collisions of particles on the biofilm [4].

In the past, biofilms were frequently assumed to be homogeneous structures. Later research demonstrated the heterogeneity of biofilm structure in terms of physical properties (density, diffusivity, etc.) and chemical or microbiological composition, and the concept of stratification appeared [5, 6]. Recently, a stratification of biofilm cohesion could be highlighted by submitting biofilms to different detachment forces [3]. The objective of this paper is to understand biofilm detachment caused by abrasion.

Materials and methods

Biofilm formation

A Couette-Taylor reactor (CTR) (Fig. 1) was used to develop biofilms under a shear stress of 0.27 Pa. The rotating inner cylinder was 0.80 m high, and its radius was 0.097 m. The inner radius of the stationary outer cylinder

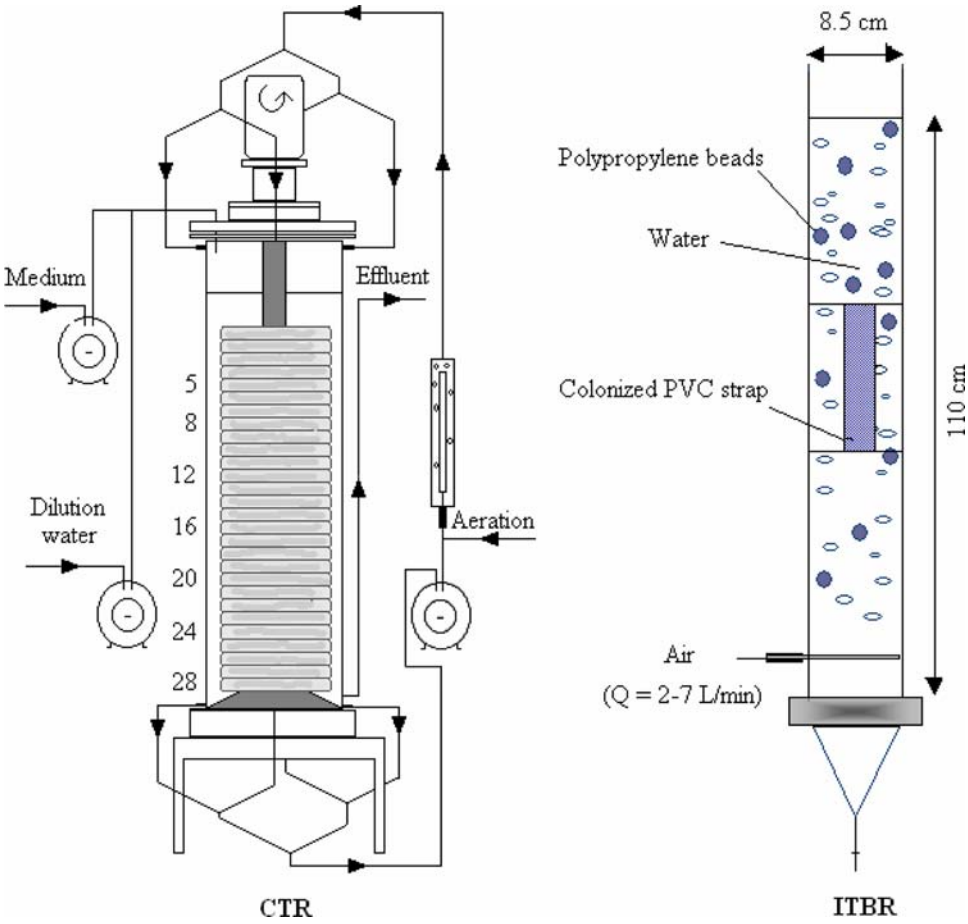

Fig. 1 The Couette-Taylor reactor (CTR) used for biofilm growth and the inverse turbulent bed reactor (ITBR) used for abrasion tests

was 0.12 m. The inner cylinder was surrounded by 28 removable polyvinyl-chloride straps on which biofilms were grown.

An industrial water consortium, i.e. 2 L of paper machine process water, was used as inoculum. The reactor was initially run as a batch culture for 20 h to allow attachment before being switched to a continuous culture mode with a retention time of 20 h. The growth medium consisted of glucose (2 g L^{-1}) and minerals as described in [7]. The temperature was 45°C, the pH remained between 7 and 7.5 and the dissolved oxygen concentration was higher than 4 mg L^{-1} during the whole experiment.

Abrasion tests

The abrasion tests were carried out in an inverse turbulent bed reactor (ITBR) in which polypropylene beads (diameter = 3.15–4.00 mm, density = 970 g L^{-1}) were fluidised by a gas flow (Fig. 1). The colonised straps were placed along the wall of the ITBR, which was filled with a NaCl solution having the same ionic strength as the growth medium. Abrasion characteristics (particle collision frequency and pressure) increase with gas velocity U_g and with solid concentration $\varepsilon_S/\varepsilon_{S0}$ [8], and strongly affect biofilm detachment rate [9, 10]. For abrasion tests, operating conditions inducing low hydrodynamic strengths were selected (U_g = 10.7 mm s^{-1}, $\varepsilon_S/\varepsilon_{S0} = 0.016$).

Sampling

For microbial analyses, biofilms that remained attached after abrasion tests were removed from the straps with sterile razor blades and placed in Petri dishes before being transferred to 2 mL microtubes with 200 µL 4 M guanidine thiocyanate–0.1 M Tris (pH 7.5) and 60 µL 10% N-lauryl sarcosine and frozen at −20°C for storage. The biofilm parts that detached from the straps during the abrasion tests were collected by sampling the liquid phase in the ITBR. Total biofilm biomass was quantified by determining the mass of attached volatile solids (AVS) per support surface. AVS was obtained by measuring the sample weight loss between drying at 105°C for 24 h and burning at 550°C for 2 h.

Total DNA extraction and PCR-SSCP

Total biofilm DNA was extracted according to [7]. Bacterial communities were analysed by the PCR-single strand

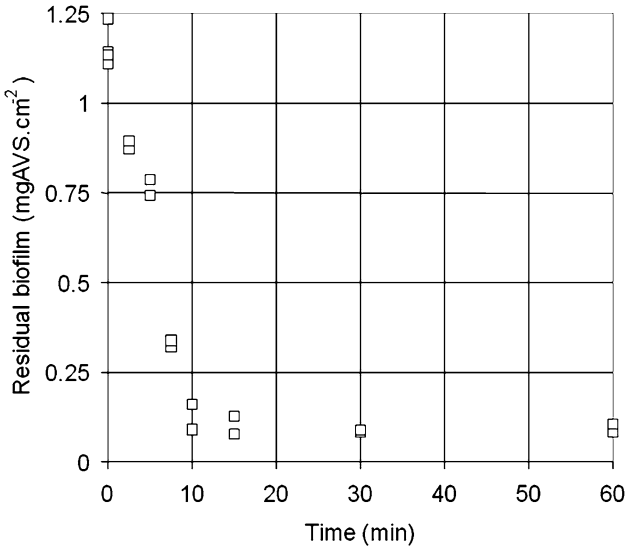

Fig. 2 Example of detachment kinetics of biofilm in the ITBR, $U_g = 10.7$ mm s^{-1}, $\varepsilon_S/\varepsilon_{S0} = 0.016$. The total biofilm biomass was expressed as attached volatile solids (AVS) per support surface

conformation polymorphism (SSCP) technique [11]. Briefly, highly variable regions of the 16SrRNA gene were amplified by PCR. PCR-SSCP products were denatured and then separated by capillary electrophoresis using an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA).

Results and discussion

Biofilms developed in the CTR for 1 week were submitted to abrasion tests in the ITBR for periods of 2.5, 5, 7.5, 10, 15, 30 or 60 min. The detachment kinetics of biofilms exposed to particle abrasion were determined [3]. A massive fraction of the initial biofilm amount (>80%) detached during the first 10 min, whereas a residual amount of biofilm remained attached on the supports until the end of the test (Fig. 2). This revealed that the biofilm was composed of a thick top layer that detached easily, and a thin basal layer that resisted hydrodynamic forces and thus was characterised by high cohesion strengths. Abrasion tests also showed that biofilm cohesion increased with depth as previously described [12, 13].

PCR-SSCP analyses of biofilm bacterial communities before and after abrasion tests are presented in Fig. 3. The SSCP profiles of the portion of biofilms that detached during the abrasion tests were the same as those of the initial biofilm, regardless of the duration of the test. The community profiles of the residual biofilms that remained on the substratum after abrasion periods of 3.5 and 5 min contained the same dominant species as the initial biofilm (peak D). After 13 min, the residual biofilm profile displayed two peaks (peaks A, E) in addition to peak D. After 30 and 60 min, the residual biofilms contained five dominant species (peaks A–E).

Microbiological analyses showed that the composition and diversity of the biofilm bacterial community changed with biofilm depth. The biofilm that detached during the shortest abrasion tests (<13 min) corresponds to the initial top layer; it was always composed of one dominant species. The biofilm that remained attached after the longest abrasion tests (>30 min) corresponded to the basal layer; it was composed of five major species. It should be noted that analyses of the residual biofilm revealed the presence of species that were not abundant enough to be detected in the whole initial biofilm. In the top layers, the most competitive species dominated as described in young biofilms, whereas in the basal layers diversity was higher, probably due to biofilm maturation [14, 15].

Combining analyses of microbiological composition to measurements of biofilm detachment confirmed the concept of stratification of biofilm structure. Our observations suggest that the biofilm was structured in two layers: a thick top layer, weakly cohesive and composed of one dominant species, and a thin basal layer, strongly cohesive and composed by a more diverse population with five main species.

The relationship between biofilm properties and cohesiveness remains poorly understood. Experimental studies showed that dense biofilms are cohesively stronger than porous biofilms [16]. Models describing the heterogeneous mode of growth of biofilm explain why the top layer is more susceptible to shear forces. The new biomass that forms preferentially at the biofilm surface with a high growth rate and a low density is relatively loose and fluffy, whereas the oldest biomass, located deeper in the biofilm, is dense and tightly packed [1, 17]. Cohesiveness of the biofilm has also been related to the extracellular polymer substances (EPS) matrix properties [13, 18]. Differences in biofilm cohesion were associated with differences in biofilm microbial composition, suggesting that community structure may be an important parameter in understanding biofilm detachment.

The results presented in this work may have practical implications in biofilm disinfection. It is known that disinfectants fail to penetrate the full depth of the biofilm. Top layers are sensitive to biocides whereas basal layers are less sensitive due to differences in the physiological state associated with lower growth rate and diffusion resistance in denser parts of the biofilm matrix [19]. The higher diversity observed in the basal layer provides a species reservoir that includes the dominant species of the top layer and thus could enhance the rapid re-growth of this top layer. This could explain biofilm resistance to disinfection treatments and could be taken in consideration when choosing effective biocides.

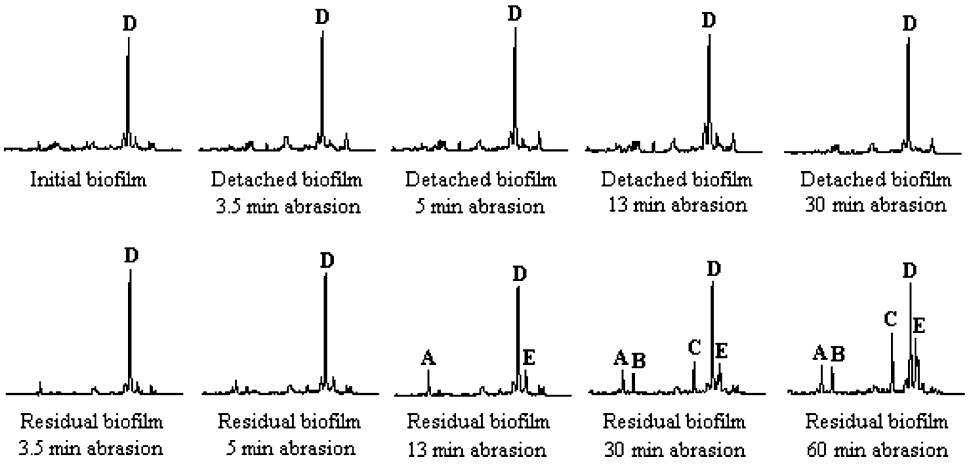

Fig. 3 PCR-single strand conformation polymorphism (SSCP) profiles of the initial biofilm, biofilms detached during abrasion tests, and biofilms that remained attached after abrasion tests (residual biofilms)

Conclusion

This study of biofilm detachment confirmed the stratified structure of biofilms. The multispecies biofilm developed in the CTR presented a thicker top layer, weakly cohesive and composed of one dominant species, and a thin basal layer, strongly cohesive and composed of a more diverse population. These observations suggest that community structure may be a factor influencing biofilm cohesion.

This work also showed that it would be interesting to study the spatial organisation of biofilm microbial communities further to better understand the processes driving biofilm community structure and to better control biofilm formation and detachment.

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