Monitoring biofilm detachment under dynamic changes in shear stress using laser-based particle size analysis and mass fractionation

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Abstract Biofilm detachment under a dynamic change in shear stress was monitored using Focused Beam Reflectance Measurements (FBRM, LASENTEC®) and mass fractionation. An annular reactor was used to grow biofilm with glucose as substrate. Changing the rotational speed on the inner cylinder of the annular reactor from 150 RPM to 420 RPM induced a step increase in shear stress. It was observed that the rate of detachment increased rapidly after increasing shear stress and then returned to the previous level. Erosion was the dominant process of detachment under steady state operation, whereas sloughing was dominant following the sudden increase in shear stress. After reaching steady state detachment under high shear conditions, the rotational speed was decreased for a 12-hour period. During this brief period of lower shear, the biofilm adjusted to this new condition. When the shear stress was increased again, another sharp increase in effluent solids concentration was observed. A decrease in density indicates that the biofilm became more vulnerable to shear stress after being subjected to this short period of low shear.

Keywords Biofilm; detachment; laser-based particle size analysis; particle size distribution; shear stress

Introduction

Biofilm processes are widely used in the environmental engineering field for water and wastewater applications. A key aspect of biofilm that differs from suspended culture is the fact that the retention time of the solids (microorganisms) is controlled not by reactor hydraulics but by biofilm detachment (Morgenroth and Wilderer, 2000). While detachment significantly influences biofilm development and performance, we only have a limited understanding of mechanisms governing biofilm detachment. Recently more dimensional mathematical models have been developed that allow for the prediction of biofilm development and mass transport in heterogeneous biofilm structures. Most of these models either neglected detachment (Kreft et al., 1998, Picioreanu et al., 2000) or assumed a constant morphology (Eberl et al., 2000). Picioreanu et al. (2001) developed a two-dimensional biofilm model where local detachment occurred when stress caused by shear forces exceeded the local strength. More experimental data is required to be able to verify models for biofilm detachment, especially for dynamic changes of shear conditions. The purpose of this paper was to study biofilm detachment under dynamically varying shear stress conditions. A novel method of measuring particle size distribution in-line and in real time is introduced as a tool to quantify erosion and sloughing. In many biofilm systems, detachment rates and detachment mechanisms under dynamic shear conditions can determine whether slower growing microorganisms degrading xenobiotic compounds are retained (Stoodley et al., 2001, Peyton and Characklis, 1993). For nitrifying biofilms, Morgenroth and Wilderer (2000) have shown that detachment mechanisms can be a determining factor for competition for substrate and space between heterotrophic and autotrophic biomass. Thus, we expect to obtain quantitative information that will help to improve modeling of detachment in mathematical biofilm models. To understand the detachment behavior of biofilm during dynamic changes in shear stress, this paper studies short-term and long-term
effects of sudden changes in shear on biofilm detachment, based on the hypothesis that biofilms adapt to varying environments quickly and hence the detachment is mostly affected by the recent history of the shear stress acting on the biofilm.

**Background**

Detachment can be classified into four categories (Bryers, 1988): 1) erosion: small particles from the surface of biofilm detaching into bulk fluid; 2) sloughing: large piece of biofilm detaching from inner part or base of the biofilm; 3) abrasion: detachment by collision of carrier medium in fluidized bed systems; 4) grazing: removal of biomass due to the consumption by higher organisms such as protozoa. This study is focused on the first two processes, erosion and sloughing, which are assumed to be significantly affected by shear stress. Erosion is caused by shear forces of the moving fluid in contact with the biofilm surface and is assumed to be effective over the entire surface of the biofilm. Sloughing, in contrast, refers to the detachment of relatively large portions of the biofilm whose characteristic size is comparable to or greater than the thickness of the biofilm itself. During sloughing a fraction of the biofilm can be removed down to the substratum but detachment is not effective for the entire surface of the biofilm. In the present study, the biofilm was up to 800 µm thick and sloughing was operationally defined as detachment of particles larger than 200 µm. Differentiation between erosion and sloughing is important for many environmental systems. In the mixed culture biofilm where slow-growing nitrifiers dominate near the base and fast-growing heterotrophs dominate near the surface, erosion will mostly remove heterotrophs living near the surface of the biofilm, whereas the sloughing will remove the nitrifiers as well (Okabe et al., 1996). Disinfection of biofilms in drinking water systems has been shown to be limited by the penetration of the disinfectant into thick biofilms (Xu et al., 1996). Thus, it can be expected that the disinfection of detached biofilm particles will also be influenced by the size of the detached particle. There have been a number of studies developing expressions for mechanisms of detachment and its application to the engineered and natural systems. Rittmann and McCarty (1980) proposed to model detachment as a continuous process. Consequently, a steady state biofilm thickness can be calculated from the balance of biofilm growth and detachment. Trulear and Characklis (1982) used an annular reactor to study the growth and detachment of biofilm. They found that detachment increases with fluid velocity and mass of biofilm. Bakke (1986) used steep changes in shear stress levels and found that the detachment sharply increased immediately after the increase of shear stress but rapidly decreased back to the previous level. A decrease in shear stress did not influence observed detachment rates. Peyton and Characklis (1993) studied erosion under different shear stress levels using an annular reactor. They used different but constant shear stresses and concluded that detachment is not affected by the shear stress. Stoodley et al. (2001) reported particle size distributions for a biofilm grown under constant shear conditions in a flow cell. Size distributions were measured by microscopic examination of filtered effluent. Although single cells and aggregates of 10 cells or less accounted for over 90% of the detached particles (by number), they contained only approximately 50% of the total effluent biomass concentration. Conversely, a disproportionately high percentage of the total cells occurred in the larger aggregates.

**Materials and methods**

Biofilms were grown in an annular reactor (Biosurfaces Inc., Bozeman, MT) with 1 litre of water volume. The total internal surface area available for biofilm growth was 1,600 cm². The influent contained glucose (25 mg COD/l) as a sole electron donor. Nutrients and trace element composition was assessed as described by Larsen and Harremoës (1994). In Figure 1, an overview of the experimental set up is shown. The influent was separated into a con-
centrated feed solution that was diluted with tap water before it entered the annular reactor. A granular activated carbon bed of 4 litres was used to dechlorinate the tap water. Oxygen concentrations in the dilution water of 35 mg/l were achieved by aeration with pure oxygen. The combined influent flow of dilution water and concentrated substrate was 52 ml/min, resulting in a hydraulic retention time of 19.2 min. Bulk water from the reactor was recirculated at 500 ml/min. To prevent unwanted accumulation of biomass on the top and bottom surfaces of inner cylinder, where the shear stress is not uniform, surfaces were cleaned daily except for the sidewalls of the inner and outer cylinder.

**Focused beam reflectance measurement (FBRM™)**
To quantify the detachment of different particle sizes, particle concentration and size distribution were measured. Since there was no particulate component in the influent, all of the particles measured were assumed to originate from the biofilm as a result of detachment events. Hence, by analyzing the particles in the bulk phase, we could differentiate between the two detachment mechanisms of erosion and sloughing. The detached particles were analyzed by laser-based focused beam reflectance measurement (FBRM, LASENTEC, WA). The FBRM probe (Figure 2) has a diameter of 1.9 cm and was inserted directly in a flow cell in the recirculation loop (Figure 1). In Figure 2, a schematic of the FBRM principle is shown. A rotating laser beam is emitted that is focused just outside the tip of the probe. When the laser beam hits a particle, it is reflected and the duration of this reflection is proportional to the chord length of the particle.

**Analysis**
Dissolved Oxygen (DO) was measured continuously using an Oxi 197 DO meter (WTW Weilheim, Germany) in the recirculation. Chemical Oxygen Demand (COD) was measured using the closed reflux method with HACH DR3000U spectrophotometer. Effluent
samples were collected and mass distributions were measured by quantifying particles between 1 and 20 µm, between 20 and 500 µm, and above 500 µm. Poretics® polycarbonate membrane filters (Osmonics, Minnetonka, MN) with pore sizes of 1 µm and 20 µm were used. These membranes are track-etch type filters that have a smooth surface and cylindrical and very uniform pores. A stainless steel sieve was used to remove particles larger than 500 µm. When measuring the particle concentration, the sample was carefully poured onto the membrane filter only up to the point where clogging and build-up of a filter cake would occur. Biofilm thickness was measured by adopting the method of Arvin (1991) where the biofilm volume is calculated from the decrease in bulk water volume within the reactor. The average biofilm thickness was calculated by dividing the biofilm volume by the surface area available for biofilm growth. Biofilm density was measured using the removable coupons (1.2 cm × 15 cm) on the inner cylinder. For each measurement a coupon was removed from the annular reactor, dried and biofilm density was calculated from dry mass and biofilm thickness measurements.

Results and discussion

Shear stress and detachment characteristics

The development of the biofilm during the initial growth phase and the two subsequent detachment experiments (Experiments “A” and “B”) is shown in Figure 3. Biofilm was grown for 2 weeks at a constant rotational speed of the inner cylinder of 150 RPM, which corresponds to a shear stress of approximately 1.1 N/m² at the surface of the biofilm. During start-up, both biofilm thickness and effluent suspended particle concentrations increased. After 10 days of operation, effluent solids concentration reached a steady level of about 10 mg/l. After 14 days of operation, shear stress was increased by increasing the rotation of the inner cylinder to 420 RPM corresponding to a shear stress of 3.1 N/m² (Experiment A). Growth under high shear conditions was maintained for 16 days followed by a 12-hour period of low shear (150 RPM). After the 12-hour period shear was increased again to the previous level (420 RPM) (Experiment B).

Increasing the shear stress was always associated with a significant increase in effluent suspended solids concentration and resulted in two detachment peaks. In Figure 3, the first detachment peak is shown where effluent suspended solids concentrations increased to 600 mg/l followed by a decrease of effluent suspended solids over the next two hours,
bringing the concentration level back to the steady state value. These observations can be explained by an instantaneous detachment event after increasing shear stress followed by a washout of detached particles from the completely mixed bulk phase of the annular reactor. Bakke (1986) has reported similar instantaneous detachment events for a tubular reactor. However, detachment after the first and the second increase in shear stress showed different characteristics. The total mass of detached particles was 0.78 and 0.28 g for the first and the second peak, respectively. The amount of detached biofilm in the second event corresponds to about twice the expected biofilm growth under the previous 12-hour low shear conditions. Thus, the second detachment event can be partially explained with new biofilm grown under the previous low shear conditions. In addition, the 12-hour period of low shear has caused the biofilm to be more susceptible to shear stress and induced a significant detachment of the biofilm that had previously been grown under high shear conditions. It appears that shear forces not only influence biofilm strength during biofilm growth but even short term exposures to a lower shear conditions can reduce biofilm strength. Other differences between the two detachment events were that after the second detachment event the effluent particle concentrations did not decrease as would be expected from washout of the completely mixed bulk phase. After the second detachment event, settling of effluent particles was worse compared to the first event and the supernatant after settling was very turbid.

Biofilm densities were measured after reaching steady state detachment (10 days of operation), at the end of the high shear period (day 30), and at the end of the subsequent low shear period (day 30.5). For these three samples, biofilm densities were 7,222, 12,284, and 8,556 g dry mass/m³, respectively. Thus, during the 12-hour period of low shear conditions, the density of the biofilm decreased by 30%. This decreased overall biofilm density can only partially be explained with growth of less dense biofilm during the low shear conditions. Wasche et al. (2000) had reported an increasing biofilm density for biofilms grown under constant high shear conditions. However, it is likely that the density of the biofilm that had previously been grown under high shear conditions also decreased during the short-term exposure to low shear conditions. This short term influence of shear on biofilm density and biofilm structure needs to be further investigated. Effluent COD concentrations did not appear to be affected by shear conditions. Higher shear can decrease the thickness of the concentration boundary layer but may also alter biofilm morphology, which influences mass transport inside the biofilm. Overall, these two effects may cancel each other out (Liu and Tay, 2002).

**Mass fractionation of effluent particles by membrane filters**

Different size fractions of effluent suspended solids were obtained from sequential filtration (Figure 5). Total suspended solids concentration increased sharply then decreased
The decrease of effluent suspended solids concentrations is similar to washout of a spike tracer added to a completely mixed reactor. Thus, it can be concluded that detachment increased very rapidly following the increase in shear stress. Immediately after increasing the shear stress, the major fraction of effluent solids was in the large size fraction (>20 µm) (Experiment “A”). These detached particles are significantly larger compared to normal operating conditions where detached particles are dominated by particles smaller than 20 µm. Thus, a change in shear stress not only increased the total amount of detached particles but also resulted in a change of detachment mechanism from erosion to sloughing. Increasing the shear stress after a 12-hour period of low shear conditions resulted in the second detachment event (Figure 6).

For experiment “B”, the fractionation using membrane filters could not produce meaningful data and only the total amount is presented. For peak “B”, the concentration of effluent particles was not as high as peak “A” and the resulting measurement error is so great that obtaining reasonably accurate mass fractionation was impossible. Low precision of size fractionation using membrane filters motivated the pursuit of a novel method and the use of a laser-based in-line particle size monitor FBRM.

Particle size distribution analysis by FBRM

On-line monitoring of particle size distributions within the reactor with FBRM allows for a direct observation of changing detachment mechanisms. The laser-based particle monitor was capable of generating the particle size distribution in a time interval as short as 10 seconds. With this novel method, it is possible to track the variation in particle size distribution in real time.

Figure 7 (a–d) shows the variation of number-based particle size and volume average size distribution from an experiment conducted to reproduce the conditions at experiment “B”. In Figures 7a and c, size distributions just before the change of RPM are shown using number or volume-based distributions, respectively. In Figure 7b and d, corresponding size distributions representing conditions after increasing the shear are shown. The influence of increased shear resulted in a decrease of the average number based particle size (Figure 7a, b). At the same time increase shear resulted in an increase of the average volume based particle size (Figure 7c, d). Combining these two observations, it can be concluded that an increase of shear resulted in a sloughing event where detached biomass was dominated by larger particles. At the same time, sloughing was accompanied by an increased number of small particles.

In Figure 8, these variations of average particle size are shown for number and volume-based distributions over time. The average number-based particle size decreased from 20 µm to 6 µm following the increase in shear but the average volume-based size increased from 70 µm to 250 µm. Both averages return to normal level after detached particles have been washed out of the reactor. The changes of mean particle sizes over time fit well with
the expected washout of detached particles from the completely mixed annular reactor suggesting that observed changes in particle size were caused by washout rather than breakup of particles in the annular reactor. Overall, detached particles were not simply the higher concentration of the same types and size particles as the steady state detachment, but particles with a completely different size distribution.

**Conclusions**

1. Laser-based particle size analysis can be used as an innovative tool for in-situ and on-line monitoring of biofilm detachment.
2. Detachment from biofilms exposed to constant shear stress was dominated by erosion. A sudden increase in shear stress caused significant increase of both the concentration and also the volume-based average of the particle size.
3. Erosion of small particles dominates the number-based size distribution while sloughing dominates the mass of detachment biofilm. An increase in the shear stress resulted in both an increase in the amount of detached particles and also a shift of the particle size distributions.

4. Short-term (i.e., 12-hour) exposure to lower shear stress decreased the overall biofilm density and biofilm susceptibility to shear stress. It seems that the biofilm strength is governed to a significant degree by the very recent history of shear conditions. The mechanisms responsible for changing the structure of the biofilm previously grown under high shear stress are currently not well understood.

References


