ABSTRACT: A suite of techniques was utilized to evaluate the correlation between biofilm physiology, fluid-induced shear stress, and detachment in hollow fiber membrane aerated bioreactors. Two monoculture species biofilms were grown on silicone fibers in a hollow fiber membrane aerated bioreactors (HfMBR) to assess detachment under laminar fluid flow conditions. Both physiology (biofilm thickness and roughness) and nutrient mass transport data indicated the presence of a steady state mature biofilm after 3 weeks of development. Surface shear stress proved to be an important parameter for predicting passive detachment for the two biofilms. The average shear stress at the surface of \textit{Nitrosomonas europaea} biofilms (54.5 ± 3.2 mPa) was approximately 20% higher than for \textit{Pseudomonas aeruginosa} biofilms (45.8 ± 7.7 mPa), resulting in higher biomass detachment. No significant difference in shear stress was measured between immature and mature biofilms of the same species. There was a significant difference in detached biomass for immature vs. mature biofilms in both species. However, there was no difference in detachment rate between the two species.

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KEYWORDS: detachment; momentum transport; biofilm; boundary layer; shear stress

Introduction

Biofilm physiology is a temporally and spatially dynamic process that depends on mass transport, hydrodynamics, stage of growth, and chemical/physical stressors such as chemical toxins or abrasion (Cole et al., 2004; Picioreanu et al., 1998; Stoodley et al., 2004, 2005; Wimpenny and Colasanti, 1997). Biofilms actively regulate morphological features (e.g., formation of streamers, microchannels, and mushroom bodies) through differentiation and expression of genes that directly control spatial organization of cells, and biofilm formation is often considered a stochastic process that is dependent on species type as well as growth conditions (Stoodley et al., 2002a, 2004).

Reactors engineered to support biofilm growth (i.e., attached growth reactors) have gained attention in the last few decades due to increased cell density, protection from physical/chemical stressors, and the ability to form stratified community physiology based on mass transfer of nutrients/byproducts. One of the major challenges with biofilm reactors is maximizing convective transport to the biofilm without causing detachment. Uncontrolled biofilm detachment causes a decrease in reactor processing efficiency, and can also lead to human pathogen exposure in downstream processors (Boles et al., 2005). Detailed studies are needed for understanding the detachment of biofilms from different types of engineered biofilm reactors that vary in geometry and operating conditions.

Biofilm detachment depends on temperature, nutrient availability, hydrodynamics, and presence of chemical toxins (Picioreanu et al., 2001; van Loosdrecht et al., 1995). Passive detachment is defined as detachment caused by external forces such as shear, erosion, sloughing, abrasion, and predator grazing; while active detachment...
refers to internally driven mechanisms (Picioreanu et al., 2001; Stoodley et al., 2001). Flow conditions and the geometry of the media are major driving forces for detachment. Biofilms formed under laminar flow are less dense than biofilms formed under turbulent conditions, and have been shown to detach with relative ease (Chen et al., 1998; Ohashi and Harada, 1994). Biofilms grown on flat surfaces form interconnected cell clusters, while biofilms on concave and convex tubular surfaces tend to form continuous biofilms with a relatively uniform cell distribution (Wijeyekoon et al., 2000). While these fundamental observations can be used to predict basic biofilm behavior, to date few detailed studies have been conducted for biofilms grown on concave/convex surfaces. Experiments describing biofilm formation on concave surfaces such as silicone membrane fibers are critical to understanding reactor startup and efficiency of HfMBR, as the biofilm physiology is significantly different than traditional biofilm reactors.

Biofilms in HfMBR are immobilized on the exterior (convex) surface of hollow polydimethylsiloxane (PDMS) membranes. For aerobic HfMBR, oxygen diffuses across the semi-permeable PDMS fiber wall to the biofilm from the attachment site, and also from the bulk liquid phase into the biofilm (known as counter-diffusion) (Wang et al., 2009). HfMBR have demonstrated improved gas transfer, high areal conversion rates (see Rector et al., 2006 for description of areal conversion rates), low energy demand, low maintenance, and reduced volatile stripping (Casey et al., 1999, 2000; Cote et al., 1989) relative to traditional co-diffusion biofilm reactors. HfMBRs are widely used for a number of applications, including nitrification (Suzuki et al., 2000), acetonitrile degradation (Li et al., 2009), simultaneous organic carbon removal and nitrification (Yamagiwa et al., 1998), single stage nitrification and denitrification (Hibiya et al., 2003; Terada et al., 2003), treatment of raw sewage (Pankhania et al., 1994), urine treatment (Tansel et al., 2005, 2006), and sulfide removal (Sahinkaya et al., 2011).

Previous studies have evaluated the physiology of mature Pseudomonas aeruginosa and Nitrosomonas europaea biofilms on PDMS fibers (Jaroch et al., 2011; McLamore et al., 2009, 2010a). These monoculture HfMBR studies provided fundamental data describing metabolism and stress response under various operating conditions. However, to date no studies have investigated the detachment of these monoculture biofilms from PDMS fibers under conditions commonly used in wastewater processing.

This paper employs a suite of techniques to measure physiology, shear stress, and detachment rate for a chemolithoautotrophic (N. europaea) and a chemooorganoheterotrophic (P. aeruginosa) biofilm grown on hollow fiber membranes. Basic physiology (metabolic flux, roughness, thickness) was measured during biofilm development. Once biofilms achieve steady state conditions, detailed passive detachment studies were conducted using a range of fluid velocities in a constructed flow cell.

Materials and Methods

Biofilm Culture

P. aeruginosa (Schroeter) Migula (ATCC 27853) and N. europaea Winogradsky (ATCC 19718) were purchased from the American Type Culture Collection (Manassas, VA). All reactors were inoculated with 1 mg/L of suspended monoculture bacteria. Biofilms were grown in autoclaved upflow hollow fiber membrane-aerated bioreactors (HfMBR) (McLamore et al., 2007). Non-porous PDMS (Silastic®) silicone membranes (Dow Corning Co., Midland, MI) were attached to reactor caps using threaded ferrules (Direct Industry, Sarasota, FL), which allowed individual fibers to be easily removed when needed. Filtered air was pumped through the membrane lumen at a transmembrane pressure of 10 kPa. P. aeruginosa was grown on glucose-enriched trypticase soy broth; 10 mM glucose and 10 mM Tris buffer (pH 7.4; Sigma–Aldrich, St. Louis, MO) according to McLamore et al. (2010a). N. europaea was grown in ATCC 2265 media (McLamore et al., 2009, 2010a).

HfMBRs effluent water quality was analyzed weekly, and steady state performance was based on the criteria outlined in McLamore et al. (2009). All reactors were located in a sterilized environmental growth chamber within a class 10 clean room. Reactor sampling procedures are described in McLamore et al. (2009, 2010a,b). Silicone membrane fibers (5 mm long each) were removed from reactors and attached to the constructed flow cell using the threaded ferrules to limit biofilm detachment during transfer.

Biofilm Thickness and Roughness

Biofilm thickness was measured using a high resolution Pulnix progressive scan camera (JAI, Inc., San Jose, CA) with an Optem 70XL zoomscope (Fairport, NY) and a National Instruments frame grabber (Austin, TX) with custom optical profiling software described in McLamore et al. (2010a,b). Briefly, the biofilm was focused at 7× magnification using a stepped motor platform (McLamore et al., 2009) attached to a vibration isolation table (Technical Manufacturing Corporation, Peabody, MA). The location of the biofilm surface relative to the fiber wall was recorded using a frame locator at ten locations along each biofilm. All measurements were collected at a standard position controlled by the stepped motor to ensure accuracy relative to the fiber wall. After measurement of the 5 mm long biofilm sample, the fiber was rotated in 30° increments using the threaded swivel ferrules in the flow cell. Average thickness was calculated using all values measured during rotation. Biofilm thickness was calculated by subtracting the average vertical position of the bare membrane from the position of the biofilm surface, and values averaged for three replicate biofilm samples.

Biofilm surface roughness was calculated using custom optical profiling software based on McLamore et al. (2010b).
_root mean square (RMS) values were calculated using Veeco software (RMS is the standard deviation of the Z values measured in contact mode) (Auerbach et al., 2000).

**Flow Field Measurements**

Flow visualization was conducted using a micro particle image velocimetry (μ-PIV) apparatus (Santiago et al., 1998). Briefly, 3 μm fluorescent particles (1% solid volume fraction, excitation: 542 nm, emission: 612 nm; Duke Scientific, Palo Alto, CA) were seeded in deionized water at a concentration of 6.7 × 108 particles/mL for the target fluid. A double-pulse Nd:YAG laser (Gemini15 120 mJ/pulse, New Wave Research, Portland OR) was used to produce high illumination for the flow plane. The illuminated plane in the targeted flow with particles was evaluated with a Sony high resolution camera (1,300 × 1,030 pixels, pixel size = 6.45 μm, 12 bit; Lavision, Ann Arbor, MI). A series of paired images between two distinct time intervals (Δt) were recorded from the illuminated plane. The images were then processed with Education Particle Image Velocimetry software (http://www.edpiv.com/) to determine the distance (Δx) particles travel during the time interval. The particle velocity was calculated using the spatially averaged cross-correlation algorithm in combination with median filtration, 9-point Gaussian peak detection, vector validation, and a smoothing algorithm.

The hydrodynamic boundary layer (HBL) thickness near biofilms was measured using μ-PIV. A syringe pump was used to control the flow rate (5, 10, and 15 mL/min) in the constructed flowcell for each experiment. All biofilm samples were taken from a single membrane fiber, and a new sample from the HiMBR was used for each experiment. Before each measurement, fluid was pumped for at least three hydraulic retention times to allow for flow stabilization. Clean, sterile membranes were used as a control for all measurements. The thickness of the HBL was defined as the location in the direction perpendicular to flow (y) where the measured velocity (u) was 99% of the free stream velocity (u∞).

**Calculation of Shear Stress and Dimensionless Parameters**

The surface shear stress (τₛ; see Supplementary Fig. S1) was calculated using the linear portion of the velocity profile according to Equation (1), where μ is the viscosity of the solution and y is vertical distance from biofilm surface:

\[
\tau_s = \mu \frac{\partial u}{\partial y}
\]  

For calculating the axial Reynolds number (Re), the flowcell was treated as an annular tube (Fig. S1; Equation 2); where D is the hydraulic diameter (D = Do − Di), Do is the diameter of the flow cell, and Di is the outer diameter of the silicone membrane (see Supplementary Fig. S1):

\[
Re = \frac{\rho u \Delta x}{\mu}
\]

All measurements were performed in the geometric center of the flowcell where L/D (length to diameter ratio) was greater than 3 (L was the distance from the inlet to the measurement location). An L/D ratio greater than 3 ensures that fully developed flow exists, and minimizes entrance, stagnation, and exit effects (Higuchi et al., 2005).

**Physiological Flux**

Metabolic flux was measured using the self-referencing (SR) microsensor technique described in the literature for oxygen (McLamore et al., 2010a), ammonium (McLamore et al., 2009), and glucose (Jaroch et al., 2011). SR is a technique that directly monitors concentration gradients (ΔC) during oscillation of a microsensor by a constant distance (Δx) at the surface of a biofilm. Flux values (J) were calculated using Fick’s first law of diffusion (J = −ΔCΔx⁻¹). SR discretely corrects for signals produced by ambient drift and noise using phase sensitive detection (reviewed in detail by McLamore and Porterfield, 2011).

Physiological oxygen flux was measured using a SR optical oxygen sensor, which was constructed by immobilizing an oxygen-quenched fluorescent dye (platinum tetrakis pentafluorophenyl porphyrin) on the tip of a tapered optical fiber (5 μm tip diameter) (McLamore et al., 2010a). For _P. aeruginosa_ biofilms, substrate (glucose) flux was measured using a nanomaterial-mediated glucose biosensor (McLamore et al., 2011) that was fabricated by entrapping glucose oxidase within a Nafion/carbon nanotube layer on the tip of a Pt/Ir wire. For _N. europaea_ biofilms, substrate (NH₄⁺) flux was measured using a microelectrode fabricated by immersing a Ag/AgCl wire in a tapered glass capillary containing electrolyte and a liquid membrane selective for NH₄⁺ (McLamore et al., 2009). The diffusion coefficients used to calculate ammonium, glucose, and oxygen flux may be found in the literature (McLamore et al., 2009, 2010a, 2011).

Physiological flux was continuously monitored at five positions along the surface of 5 cm biofilm segments for 20 min unless otherwise indicated. All flux values represent averages of the arithmetic mean (at least 10 min of continuous recording) at five different positions (n = 3 replicates), and error bars for all physiological flux data represent the standard error of the arithmetic mean.

**Measuring Biomass**

Detached biomass was measured using either standard solids testing (EPA, 2010) or using a Beckman Multisizer 4
Biofilm Development and Detachment Studies

For time course studies of biofilm development, PDMS fibers were placed in the constructed flowcell and inoculated with 1 mg/L of monoculture cell suspensions. The transmembrane pressure was maintained at 10 kPa throughout all experiments. Biofilms were exposed to media as previously described, and liquid effluent was recirculated to maintain a steady state behavior of biofilm thickness, roughness, and HBL thickness is similar to correlations observed for biofilms grown on inert media (Bishop et al., 1997) and flat sheets (Shanahan and Semmens, 2007). Detailed experiments investigating the effect of fluid shear on detachment in this study were measured between 2.9 and 46.8 mm s$^{-1}$, which represents a recycle ratio of 20–350 at the loading rates used in this study based on the transport model developed by Rector et al. (2006).

Statistical Analysis

Where indicated, statistical tests were used to test for significance; either analysis of variance (ANOVA) or student’s $t$-test. For all tests, the significance level ($\alpha$) was 0.05.

Results and Discussion

Biofilm Development

One week old N. europaea biofilms grown at $Re = 20$ ($u_{\infty} \approx 23$ mm s$^{-1}$) had a thickness of 13 ± 11 μm and a roughness of 60 ± 3 μm. One-week-old P. aeruginosa had a thickness of 10 ± 6 μm and a roughness of 18 ± 2 μm (Fig. 1, panels A and B). Biofilm thickness and roughness were not significantly different during the first week of growth ($P = 2.2 \times 10^{-3}$, $\alpha = 0.05$). After 3 weeks of growth at $Re = 20$, N. europaea and P. aeruginosa biofilms had an average thickness of 199 ± 12, and 25 ± 12 μm, respectively; and an average roughness of 82 ± 7, and 18 ± 8 μm, respectively. After 2 weeks, N. europaea and the P. aeruginosa thickness and roughness exhibited a stable value after 3 weeks that did not significantly change ($P = 1.1 \times 10^{-2}$, $\alpha = 0.05$), indicating steady state growth. However, the thickness and roughness of mature N. europaea was significantly larger than the mature P. aeruginosa biofilms. Following the lag in biofilm thickness during week 1, the change in thickness (during week 1–2) was calculated to estimate exponential growth rate. Based on this calculation, N. europaea growth rate (1.11 μm h$^{-1}$) was significantly higher than the P. aeruginosa growth rate (0.07 μm h$^{-1}$). These values are different from those reported by other researchers, as specific growth rates reported for planktonic P. aeruginosa (0.29 h$^{-1}$; Beyenal et al., 2003) are much higher than N. europaea (0.017–0.088 h$^{-1}$; Kindaichi et al., 2006; Prosser, 1989). However, the physiology of the growth conditions are significantly different (Stoodley et al., 2002a, 2004). To further investigate this observed pattern of growth between N. europaea and P. aeruginosa biofilms, substrate and oxygen flux were measured during development.

Oxygen and substrate flux increased over the first 15 days of continuous growth; NH$_4^+$ flux was measured for N. europaea biofilms; glucose flux was measured for P. aeruginosa biofilms (Fig. 1, panels C and D), and did not significantly change after approximately 3 weeks of growth at $Re = 20$ ($P = 7.3 \times 10^{-6}$, $\alpha = 0.05$). Based on the steady state parameters defined by McLamore et al. (2009, 2010a), oxygen and substrate flux were at steady state after 3 weeks. No significant difference between oxygen and...
substrate flux was observed for *N. europaea* and *P. aeruginosa* biofilms. The steady state flux values measured for mature *N. europaea* and *P. aeruginosa* were not significantly different than previous values reported in the literature for HfMBR biofilms operated at $Re = 35$ (McLamore et al., 2009, 2010a; Jaroch et al., 2011). The observed steady state metabolism, surface roughness and thickness after approximately 3 weeks at $Re = 20$ indicate that *N. europaea* and *P. aeruginosa* biofilms reached steady state growth. For thin biofilms on gas-permeable membranes (such as *P. aeruginosa* in these experiments), oxygen flux from the membrane lumen can diffuse into the bulk liquid, and measurement of microprofiles using traditional microelectrodes may not account for this experimental bias. The SR fiber optic sensors used in these studies can monitor oxygen flux from the bulk liquid into the biofilm. This is a major technical advantage that allows direct measurement of the metabolic oxygen uptake along the surface of the biofilm (McLamore et al., 2010a,b).

Poplawski et al. (2008) proposed that biofilm morphology depends on the non-dimensional ratio ($R$) of the maximum biomass growth rate to the maximum substrate transport rate. In their studies, biofilm growth was either growth rate limited (high $R$ values) or nutrient transport limited (low $R$ values). When the growth rate is limiting, substrate is easily transported and will penetrate most of the biofilm. These biofilms tend to be flat, compact and fast-growing. When transport limits growth, the substrate penetrates only the top thin layers of the biofilm. In this growth state, Poplawski et al. (2008) concluded that cells tend to protrude out of the biofilm, and biofilm roughness is significantly higher than growth limited biofilms; the higher surface area increases mass transport of nutrients. Other researchers have noted that transport limited biofilms with a low specific growth rate may have an increased production of EPS, although this is often species specific (Boe-Hansen et al., 2002; Kindaichi et al., 2004, 2006; Okabe et al., 2005; Rittmann et al., 1994). Based on the growth rates estimated using biofilm thickness (Fig. 1A) and the maximum substrate flux (Fig. 1D), the modified $R$ ratio defined by Poplawski et al. (2008) for *N. europaea* ($8.5 \pm 1.2$) was significantly higher than *P. aeruginosa* ($0.5 \pm 0.3$). Based on these calculated ratios, *N. europaea* biofilms should have a higher roughness than *P. aeruginosa*. This trend was confirmed based on the data in Figure 1B.

To further investigate the rate limiting growth state of the developing biofilms, effluent biomass was measured from the flow cells during development (Fig. 2A). A short lag
The observed exponential response in Figure 2A is similar to trends observed in the literature and stochastic detachment models (Bohn et al., 2007; Picioreanu et al., 2000; Stoodley et al., 2001, 2002b; Xavier et al., 2005). The lag phase for *P. aeruginosa* (2–3 days) was shorter than *N. europaea* (5–6 days); lag time was defined as less than a 5% change in effluent biomass. During the first 10–12 days, *P. aeruginosa* growth rate was higher than *N. europaea* biofilms (i.e., lower effluent biomass; Fig. 2B). Interestingly, after 12–14 days, the biomass measured in the *P. aeruginosa* flowcell effluent \((17.8 \pm 1.4 \text{ mg-biomass } \text{L}^{-1})\) was significantly lower than *N. europaea* effluent biomass \((49.7 \pm 2.7 \text{ mg-biomass } \text{L}^{-1})\). This difference in biomass was likely due to the increased thickness of *N. europaea* biofilms relative to *P. aeruginosa* (Fig. 1A), and may also be related to relatively high roughness of *N. europaea* biofilms after 2 weeks of growth.

To investigate possible correlations between detachment, thickness, roughness, and species type, detailed shear-induced detachment studies were conducted using mature biofilms.

During microcolony formation planktonic cells are undergoing reversible attachment, cohesion, or coaggregation as planktonic cells begin to undergo physiological changes and produce a dense exopolymeric matrix. Thus, one would expect the passive (i.e., externally driven) detachment rate to be relatively high during this growth phase. In natural systems (e.g., rivers) where the number of cells entering a control volume is dynamic, the detachment rate for a mature biofilm is approximately equal to the attachment rate under steady state conditions (Kwok et al., 1998; Xavier et al., 2005). However, in a closed microbial system such as a bioreactor under constant loading, no cells are continuously entering the system, and the detachment rate is equal to the growth rate under steady state conditions.

**Surface Shear Stress**

To estimate the effect of fluid shear on biofilm detachment, microscale velocity profiles were measured near the surface of mature biofilms for three different flow rates \((5, 10, \text{ and } 15 \text{ mL/min})\) using a µ-PIV (all biofilms were developed and transferred to flowcells as previously described). The measured velocity profiles followed predicted behavior based on boundary layer momentum transport theory. As expected, the measured velocity near all biofilm surfaces was approximately zero (due to the no-slip condition at the biofilm–liquid interface) and velocity exponentially increased with distance from the biofilm surface (Fig. 3A). Surface shear stress (defined as the force at the biofilm surface caused by hydrodynamic drag) was calculated using Equation (1) and the measured linear portion of the velocity–distance plots \(\left(\frac{du}{dy}\right)\) in Figure 3A. A representative plot of calculated \(\frac{du}{dy}\) values for a mature *N. europaea* biofilm is shown in Figure 3B. Velocity profiles above all biofilms and RMS values may be found in the Supplementary Section (Fig. S2). Linear regression for all \(\frac{du}{dy}\) plots had an \(R^2\) value >0.98.

Hydrodynamic boundary layer (HBL) thickness was calculated at various fluid velocities using µ-PIV velocity profiles. Biofilm roughness for mature biofilms ranged from 350 to 595 \(\mu\text{m}\) (recall Fig. 1B). HBL thickness decreased with increasing free stream velocity, which is consistent with expected trends for momentum transport describing compressed HBL under increasing fluid shear (Picioreanu et al., 2000) (Fig. 4A). Relatively thin biofilms (e.g., 1-week-old *P. aeruginosa* biofilms) had an average HBL thickness which was not statistically different than a clean (sterile) membrane \((P = 4.3 \times 10^{-4}, \alpha = 0.05)\). All other biofilms (i.e., mature *P. aeruginosa* and all *N. europaea* biofilms) had an HBL thickness that was significantly greater than sterile PDMS fibers \((P = 1.5 \times 10^{-5}, \alpha = 0.05)\). HBL thickness for
*N. europaea* biofilms was significantly larger than *P. aeruginosa* biofilms. The largest HBL values were observed for mature *N. europaea*, which had the highest surface roughness (82 ± 8 μm), followed by microcolony *N. europaea* (60 ± 3 μm).

Using the measured *du/dy* values and Equation (1), surface shear was calculated for *Re* values between 1 and 40 for *N. europaea* and *P. aeruginosa* biofilms (both 1-week-old and mature biofilms ≥4 weeks old were measured). As expected, increased mean stream velocity resulted in an increase in surface shear stress for all biofilms. For both species of biofilm, there was no significant difference between the measured shear stress for 3-week-old and 1-week-old biofilms (*P* = 5.0 × 10⁻³, α = 0.05). However, the measured average shear stress at the surface of *N. europaea* biofilms (54.5 ± 3.2 mPa) was approximately 20% larger than the shear for *P. aeruginosa* biofilms (45.8 ± 7.7 mPa).

### Passive Detachment

In preliminary experiments using mature biofilms, biomass was measured following transfer of samples from HfMBR to ensure sampling did not cause significant detachment. After transfer to the flowcell, the samples were allowed to equilibrate to new conditions for 1 h, liquid was removed, and biomass was measured. For six replicate biofilms, detachment due to sample transfer was near the lower limit of detection for the coulter counter (*N. europaea* was 0.22 mg-biomass L⁻¹; *P. aeruginosa* was 0.13 mg-biomass L⁻¹).

For all biofilms measured (conditions between *Re* = 10–60), detachment from *N. europaea* biofilms was significantly higher than detachment from *P. aeruginosa* biofilms (approximately 37 ± 18% higher for all samples tested; Fig. 5). Detachment of mature biofilms (≥4 weeks) was significantly larger than detachment of 1-week-old biofilms.
The highest measured detached biomass concentration ($170 \pm 13$ mg-biomass L$^{-1}$) was measured for mature ($\geq 4$ weeks old) *N. europaea* biofilms at $Re = 61$ (69 mm s$^{-1}$). Detachment of mature *P. aeruginosa* was slightly lower ($161 \pm 13$ mg-biomass L$^{-1}$), but significantly different. In preliminary experiments, velocities above 85 mm s$^{-1}$ ($Re \approx 75$) caused total detachment of microcolony biofilms and detachment from hollow fibers (data not shown). Interestingly, passive detachment of mature *N. europaea* biofilms was significantly larger than mature *P. aeruginosa* biofilms, especially when $Re$ was smaller than 60. This phenomenon was consistent with the fact that the measured average shear stress at the surface of *N. europaea* biofilms was approximately 20% larger than the shear for *P. aeruginosa* biofilms. Another reason may be that *N. europaea* biofilm has a much thicker biofilm and therefore more cells/polymers were able to detach from the surface. Other studies have reported relatively high extracellular polymer (EPS) content in *N. europaea* biofilms (Boe-Hansen et al., 2002; Kindaichi et al., 2004, 2006; Okabe et al., 2005; Rittmann et al., 1994). While the production of EPS is a metabolic burden, it is also a survival mechanism that provides protection from, stressors, predators, and possibly passive detachment (Lim et al., 2011; Purevdorj-Gage et al., 2005). While no significant difference in metabolism (substrate or oxygen flux) was observed, the relatively high roughness of *N. europaea* biofilms increased the overall surface area under the influence of fluid shear. Detailed studies to confirm the link between EPS production and passive detachment are outside the scope of this work, but these studies are needed to understand the correlation between physiology and detachment rate.

Passive biofilm detachment has been studied in a number of engineered bioreactors, including airlift suspension reactors (Kwok et al., 1998), granular sludge reactors (Liu and Tay, 2002), as well as catheters (Fux et al., 2004), glass cover slides and flow cells (Klapper et al., 2002; Stoodley et al., 2002b). Although empirical studies and the development of mathematical models have increased our understanding of the relationship between biofilm formation and mass/momentum boundary layer transport (Eberl et al., 2000; Matsumoto et al., 2007; Xavier et al., 2005), there are minimal published in situ data quantifying transport phenomena in membrane bioreactors. In addition to the unique physiology of HFMBR (i.e., counter diffusion biofilms), fundamental studies such as these improve our basic understanding of biofilm formation on hollow fibers. Wijeyekoon et al. (2000) highlights how subtle differences in geometry can result in relatively large differences in phenotype formation.

**Conclusions**

A unique set of experimental methods was used to quantify the correlation between biofilm physiology, fluid-induced shear stress, and detachment in hollow fiber membrane aerated bioreactors using two monoculture biofilms. Surface shear stress was demonstrated to be an important parameter in the prediction of passive detachment events. No significant difference was noted when comparing shear stress for immature and mature biofilms of the same species. Surface shear stress was higher for *N. europaea* biofilms (54.5 ± 3.2 mPa) when compared with *P. aeruginosa* biofilms (45.8 ± 7.7 mPa), resulting in higher biofilm detachment. There was a significant difference in detached biomass for immature vs. mature biofilms in both species. However, there was no difference in detachment rate between the two species. Results from this research indicate that the operation of nitrifying HFMBR with mean stream velocities $\approx 23$ mm s$^{-1}$ may optimize reactor performance. This information would be particularly applicable during initial biofilm development when detachment rates of nitrifying communities may be considerably higher and may have implications for rapid reactor startup, shock loading, and cell-templated layering of communities in bioreactors.

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References


two references that are not complete.


