Nitrite accumulation from simultaneous free-ammonia and free-nitrous-acid inhibition and oxygen limitation in a continuous-flow biofilm reactor

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ABSTRACT

To achieve nitrite accumulation for shortcut biological nitrogen removal (SBNR) in a biofilm process, we explored the simultaneous effects of oxygen limitation and free ammonia (FA) and free nitrous acid (FNA) inhibition in the nitrifying biofilm. We used the multi-species nitrifying biofilm model (MSNBM) to identify conditions that should or should not lead to nitrite accumulation, and evaluated the effectiveness of those conditions with experiments in continuous flow biofilm reactors (CFBRs). CFBR experiments were organized into four sets with these expected outcomes based on the MSNBM: 1. Control, giving full nitrification; 2. oxygen limitation, giving modest long-term nitrite build up; 3. FA inhibition, giving no long-term nitrite accumulation; and 4. FA inhibition plus oxygen limitation, giving major long-term nitrite accumulation. Consistent with MSNBM predictions, the experimental results showed that nitrite accumulated in sets 2 – 4 in the short term, but long-term nitrite accumulation was maintained only in sets 2 and 4, which involved oxygen limitation. Furthermore, nitrite accumulation was substantially greater in set 4, which also included FA inhibition. However, FA inhibition (and accompanying FNA inhibition) alone in set 3 did not maintained long-term nitrite accumulation. NOB-activity batch tests confirmed that little NOB or only a small fraction of NOB were present in the biofilms for sets 4 and 2, respectively. The experimental data supported the previous modeling results that nitrite accumulation could be achieved with a lower ammonium concentration than had been required for a suspended-growth process. Additional findings were that the biofilm exposed to DO limitation and FA inhibition was substantially denser and probably had a lower detachment rate.

Keywords: ammonium oxidation, biofilm, free ammonia inhibition, oxygen limitation, nitrite accumulation

1. Introduction

In recent years, nitrite accumulation has been spotlighted for its role in shortcut biological nitrogen removal (SBNR) and anaerobic ammonium oxidation (Anammox) (Chung et al. 2007; Strous et al. 1997). By using nitrite as a primary electron accepter, the SBNR process uses 40% less organic electron donor. The Anammox process uses nitrite as an electron acceptor and ammonium as an electron donor to bring about total-N removal without any organic donor. To ensure the practicality of both processes, the key is stable nitrite accumulation in nitrification, which is achieved by securing ammonium-oxidizing bacteria (AOB), but suppressing nitrite-oxidizing bacteria (NOB).

Nitrite accumulation has been associated with inhibition from high or low pH, free ammonia (FA), free nitrous acid (FNA), low dissolved oxygen (DO), and combinations (Jiang et al. 2011, Park et al. 2007; Park and Bae 2009; Park et al. 2010a, b; Hanaki et al. 1990; Bernet et al. 2001). The pH can affect nitrification in two ways: 1) directly by changing the enzyme's reaction mechanism (Van Hulle et al. 2007; Park et al. 2007; Boon and Laudelout 1962; Quinlan 1984), and 2) indirectly by changing the speciation of total ammonium and total nitrite to the inhibitor forms, FA and FNA (Anthonisen e al. 1976; Hellinga et al. 1999; Van Hulle et al. 2007; Carrera et al. 2004; Lee et al. 2004; Park et al. 2010c; Jiang et al. 2011). The FA concentration increases in a basic condition, but the FNA concentration increases in an acidic condition.

Because a biofilm process can be advantageous to secure the accumulation of slow-growing bacteria, such as nitrifiers (Bishop and Zhang 1995; Okabe et al. 1999; Rittmann and Manem 1992), it can provide an advantage or a disadvantage when nitrite accumulation is the goal. The superior retention of slow-growing biomass in a biofilm is good for AOB, but works in the wrong direction for NOB. In addition, a decreased pH through the biofilm caused by acid generation by AOB could help NOB survive by decreasing the FA concentration, which is disadvantageous for nitrite accumulation. On the other hand, depletion of DO inside of the biofilm may limit the activity of NOB, since the NOB are more sensitive to low DO than are the AOB, which is advantageous for nitrite accumulation

(Bernet et al. 2001; Park et al. 2010c). Perez et al. (2009) and Bartrolí et al. (2010) concluded that an oxygen affinity for AOB was the key parameter and stable complete nitrite accumulation was maintained by a constant ratio of DO/TAN in the bulk liquid of the biofilm reactor, respectively. Recently, Park et al. (2010c) suggested that FNA inhibition to accentuate nitrite accumulation can be increased by allowing the pH to decrease in the biofilm.

Despite some ambiguity of what mechanisms are at work, researchers have reported evidence that biofilm processes can accomplish nitrite accumulation (Fux et al. 2004; Chung et al. 2007; Yamato et al. 2008; Perez et al. 2009; Park et al., 2010c; Brockmann and Morgenroth, 2010). In particular, Park et al. (2010c) developed the multi-species nitrifying biofilm model (MSNBM), which has three biomass types -- AOB, NOB, and inert biomass -- and can track the effects of DO, FA, and FNA inhibition on the growth of the two groups of nitrifiers in the biofilm. MSNBM simulation results explain that a biofilm can be advantageous for accumulating nitrite while simultaneously maintaining a low ammonium concentration, because FA inhibition can occur at the surface of the biofilm, while FNA inhibition and oxygen limitation occur inside the biofilm. These factors can be simultaneously regulated by aeration intensity, influent ammonium concentration, and buffer concentration (Flora et al. 1999; Perez et al. 2009; Park et al. 2010c).

We explore the simultaneous effects of oxygen limitation and FA and FNA inhibition in a nitrifying biofilm by operating a continuous-flow biofilm reactor (CFBR) with different oxygen and influent ammonium concentrations. The experimental results are compared with the simulated results of the MSNBM to ascertain whether the proposed benefits of carrying out SBNR can be achieved in practice or not.

2. Material and Methods

2.1. Seeding microorganisms and mineral medium

The biomass used in all CFBRs originated from a 50-L sequencing batch reactor (SBR) operated as a typical nitrification process: 2 cycles per day, with aeration time = 10 hr and decant and filling times = 1 hr during one cycle. In the 50-L reactor, the DO was maintained over 4 mg/L, the temperature was $27^{\circ}C \pm 2^{\circ}C$, and the pH was held near 8 (± 0.2) by injecting sodium bicarbonate to balance the alkalinity consumed by ammonium oxidation.

After the mixture of seeding sludge and feeding solution was introduced into a CFBR, it was recirculated for 5 days to allow the microorganism to attach onto the biofilm surface of the reactor. After 5 days, CFBR operation began, and the original suspended sludge from the SBR gradually washed out. The feed solution to the SBR and CFBR contained 100 mgN/L of $(NH_4)_2SO_4$ in a mineral medium that contained (in mg/L): K_2HPO_4 390, $MgSO_4 \cdot 7H_2O$ 100, $FeSO_4 \cdot 7H_2O$ 4, $CaCl_2$ 8, $MnSO_4 \cdot H_2O$ 10, $NaHCO_3$ 1020, and KCl 14. This medium composition also was used for the activity batch tests of NOB.

2.2 Reactor configuration and operation

Each 1-L (working volume, 10cm×10cm×10cm) CFBR was made of polyacrylic plastic (Fig. 1) and contained an air diffuser, a mixing paddle, and a detachable substratum divided into four sections (each 5cm×5cm×1.5cm deep). The detachable substrata were used to obtain a biofilm sample easily. The total biofilm surface area of the reactor, including all geometric structures onto which the biofilm could accumulate, was 750 cm^2. The CFBR was submerged in a water bath for temperature control. The reactor's liquid contents were completely mixed, and Fig. S1 in the Supporting Information (SI) shows results of tracer tests that demonstrate complete mixing.

During a 60-day initiation period, four CFBRs were operated with identical conditions that minimized inhibition. After each CFBR was seeded with a one-liter mixture of sludge from the SBR and feed medium containing 60 mgN/L of total ammonium nitrogen (TAN), the CFBR was operated at a flow rate of 2 L/day and hydraulic retention time (HRT) of 12hr with 6.5 mgDO/L, 30°C, and pH 7±0.5 in the bulk liquid. The initial biomass concentration was approximately 4,000 mgVSS/L.

Table 1 lists the operating conditions for each reactor. Reactor 1 was maintained with the same operating condition throughout the full 200-day experimental period, and it served as a control with minimal inhibition. Reactor 2 was operated with a condition of potential DO limitation: The DO concentration was reduced to 3.8 mg/L at day 61 and then further reduced to 2.5 mg/L at day 161. In Reactor 3, the influent TAN concentration was increased from 50 mg/L to 150 mg/L to cause potential FA inhibition at a sufficient DO concentration of 7.6 mg/L. Reactor 4 combined the conditions of reactors 2 and 3 to impose both types of limitation/inhibition together. Table S1 of SI explains why these conditions could have achieved the desired types of inhibition.

2.3 Analytical measurements

For all liquid samples, the concentrations of TAN, TNiN, and total nitrate nitrogen (TNaN) were measured according to *Standard Methods* (APHA, AWWA, WEF, 1998). Temperature, pH (Orion, 720A), and DO (Orion, 850) were detected using selective electrodes. In case of mixed liquor volatile suspended solid (MLVSS) leaving the CFBR in the early phase of seeding, it also was analyzed by the volatile suspended solid (VSS) method in *Standard Methods* (APHA, AWWA, WEF, 1998).

2.4 Thickness of the biofilm

The thickness of the biofilm was measured by using a stereomicroscope linked to a closed-circuit television (CCTV) (Olympus, SZ-CTV). Near the end of the experiments (~ day 200), a detachable substratum from the bottom of each CFBR was carefully moved

to the microscopic stage to capture images perpendicular to the top of the detachable substratum, as depicted in Fig. 1. The images of the biofilm captured by CCTV were transferred to OriginPro 7.5 (OriginLab), which has a digital ruler. The average thickness of the biofilm was taken from 100 points having an arithmetic interval located along the edge of substratum. As soon as an image was taken, the substratum piece was replaced into the CFBR for further experiments.

2.5 NOB activity in the biofilm

NOB-activity assays were executed through batch tests for the specific nitrite-utilization rate. When all CFBR operations were finished, day 200, the biofilm was removed by a soft brush and suspended by using a stirrer. A 15-mL volume of the suspended-biofilm sample was taken for MLVSS measurement, and the rest of biomass was used for the batch test. For the batch test to obtain the specific nitrite utilization rate, $NaNO_2$ was introduced to give a 30-mgTNiN/L initial concentration in a 1-L volumetric flask that contained mineral medium, with pH 7 and temperature of 30°C. The decline of the nitrite concentration over time gave the specific nitrite-utilization rate.

2.6 Simulations with MSNBM

We used the MSNBM by Park et al. (2010c) to simulate the conditions of the experiments. The MSNBM model is developed to simulate a shortcut nitrogen removal (SBNR) in biofilm with different operational parameters: buffer, pH, loading, DO, etc. The feature of model is including the pH model. One modification was that we included suspended reactions by the seeding bacteria between days 0 and 5. Table 2 shows the kinetic parameter values used. The maximum substrate utilization rate ($\hat{q}$) and the half-maximum-rate concentration (K_S) were adjusted for temperature, as needed, by using 25°C kinetic values from Rittmann and McCarty (2001) and temperature correction according to Novak (1974). Most kinetic parameters for AOB and NOB are from Rittmann and McCarty (2001). Diffusion coefficients for nitrogen species and oxygen were taken from

Picioreanu et al. (1997). The values related to direct pH, FA, and FNA inhibitions were from Park et al. (2007) and Park and Bae (2009). Operating parameters, i.e., flow rate (Q), volume of reactor (V), and biofilm surface area (A), are from the experiment of this study. The biofilm density was an estimated value that is presented in Results and Discussion.

The simulation conditions to evaluate the experiments followed the same conditions in Table 1. In the case of Reactor 1, the influent TAN concentration, DO, and pH were maintained with 60 mg/L, 8 mg/L and 7, respectively. When the operational parameters were changed to those of Reactors 2 and 4 at day 61, the input values to begin the simulation were the simulated results at day 61, i.e. biomass distribution and biofilm depth. However, simulation results for Reactor 3 after day 61 used the original initial values, since severe biofilm sloughing occurred and required a new seeding.

3. Results and discussion

3.1. Biofilm-development period of all reactors: days 0 to 60

Fig. 2 presents the experimental and simulated results from all reactors during the biofilm-development period, days 0 to 60. The four CFBRs gave similar trends of nitrogen concentrations. Nitrification due only to the suspended seed occurred during the initial 5 days, after which the biofilm started to take over nitrification as the VSS washed out to below 10 mg/L. Experimental results between days 10 and 20 fluctuated because of intermittent plugging of the feeding tube in this period, but the reactors were stable after day 20, when nitrification to NO_3^- was fully stabilized.

The lines in Fig. 2 show the results of simulation by the MSNBM. Because the tubing problem was not simulated, the results have the poor correlation between days 10 and 20, but match well with the experimental results thereafter. The biofilm thickness (not shown in the figure) predicted by the MSNBM at day 5 was 17 μm, and it increased to 54 μm at day 10. The predicted FA inhibition value can be calculated with the form $1/(1+FA/K_{FA,AOB\ or\ NOB})$. Rapid biofilm growth was possible, because FA inhibition in the reactor (calculated by $1/(1+FA/K_{FA})$) was less than 20% of the maximum potential given in Table S1 of supported information, which was insignificant for preventing biofilm growth. As TAN decreased out to 10 days, FA inhibition upon of NOB further weakened, and full nitrification was achieved.

3.2 Minimal limitation and inhibition in Reactor 1

The results for Reactor 1 after day 60 are shown in Fig. 3a. An unexpected problem of DO control caused nitrite accumulation during days 60 to 100. However, reactor 1 returned to stable full nitrification after day 100, and the model simulations are nearly identical with the experimental results. We operated the system with a high surface loading (1.6 kgN/1000 m^2-d at 60 mgN/L), compared to a standard trickling filter (0.5-0.8 kgN/1000 m^2-d), rotating biological contactor (0.2-0.6 kgN/1000 m^2-d), and circulating bed

biofilm reactor (< 1 kgN/1000 m^2-d) (Rittmann and McCarty 2001). Despite the high loading, the system was able to maintain full nitrification; one reason was that it did not have any organic donor in the influent, which kept it relatively free from DO and space competition with heterotrophs.

3.3 DO limitation in Reactor 2

Fig. 3b shows the experimental results of Reactor 2, which had DO limitation. As the DO concentration was decreased (from 3.8 mg/L from day 61 and then to 2.5 mg/L from day 161), the effluent concentrations of ammonium and nitrite increased, while nitrate decreased. The simulated results showed that DO limitation should have caused prompt decreases of TNaN and increases of TNiN and TAN, but the experimental results responded more slowly and with considerable fluctuations. Under more severe DO limitation (2.5 mg/L), the full nitrification efficiency fell significantly to ~60%, since both TAN and TNiN increased to approximately 10 mgN/L.

3.4. FA inhibition in Reactor 3

Reactor 3 was reseeded at day 61, since severe biofilm sloughing occurred at that time. It was restarted with an influent TAN concentration of 150 mgN/L. Shown in Fig. 4a, the experimental results in Reactor 3 demonstrate nitrite accumulation for only about 20 days. Eventually, full nitrification set in and was stable. The early responses in TAN and TNiN concentrations resemble those in Fig. 2, but the peaks were higher due to increased TAN loading.

The simulated curves in Fig. 4a capture the trend of experimental data well, although ammonium and nitrate sometimes fluctuated in the experiments. The higher TAN concentration (150 mgN/L) and pH (= 8) in the influent might have caused temporary NOB suppression at first. In this case, the maximum potential inhibition values (that is, $1/(1+FA/K_{FA,AOB\ or\ NOB})$) are 1/(1+15/10) = 0.4 for AOB and 1/(1+15/0.75) = 0.05 for NOB. (See Table S1 in SI for details.) Thus, NOB were more severely inhibited at the early

phase of elevated TAN concentration in the reactor (as high as ~80mg/L), and this was responsible for nitrite accumulation at first. However, inhibition of NOB was insufficient after day 20, because the TAN concentration was decreased by AOB, which caused a decrease of FA inhibition at the surface of the biofilm; furthermore, a pH decline inside the biofilm minimized the impact of FA inhibition, while FNA inhibition always was insufficient to prohibit NOB growth. The maximum FNA inhibition value to NOB that was shown around day 70 was calculated as 0.92 (that is, 92% of the non-inhibitory reaction was secured), which was insufficient to prohibit net NOB growth. These factors eventually led to full nitrification in the experiments and the model output. Bartrolí et al. (2010) demonstrated the feasibility of maintaining stable complete nitrite accumulation by maintaining the DO/TAN ratio in the bulk liquid of the biofilm reactor below 0.25. The complete nitrification in the reactor 3 indirectly supported their suggestion as the DO/TAN ratio in the bulk liquid was over 10.

3.5 FA inhibition and DO limitation in Reactor 4

Reactor 4 was operated under DO limitation and potential FA inhibition after day 60. Fig. 4b shows that the TAN concentration in the reactor rapidly increased after elevation of the influent concentration (from 60 to 150 mg/L), pH (from 7 to 8), and DO limitation (from 6.5 to 3.8 mg/L). Though it slowly decreased with time, full nitrification did not recover throughout the period of 3.8 mgDO/L, where the DO/TAN ratio was below 0.2 in most cases. When the DO concentration was decreased further to 2.5 mg/L (day 160), the TAN concentration further increased accordingly, resulting in almost complete suppression of NOB activity. The AOB activity was also affected as shown with the decreased TNiN accumulation in Fig. 4b. Comparing to the results in Fig. 4a (in which the reaction conditions were identical except for the DO concentration that was two times higher), it was clear that the DO concentration was one of the critical parameters to control nitritation as indicated in the literature (Perez et al. 2009; Bartrolí et al., 2010). Important to note was that FA inhibition had been another critical parameter for nitritation in Fig.4b since Fig.

3b did not accumulate nitrite much in which the DO concentrations were identical but the average FA concentration in the reactor was much lower.

The model curves are similar to the trends in the experimental data when DO was 3.8 mg/L (Fig. 4b). However, the profile of the TNaN was not an exact match, because the DO concentration was not well controlled, having a standard deviation of ±1.2 mg/L. Similarly, Park et al. (2010b) showed unstable TNiN accumulation when the actual DO concentration was close to a minimum oxygen concentration (Park et al., 2010a) for AOB survival or over a minimum oxygen concentration for NOB survival. When the DO concentration was 2.5 mg/L (day 161-200), again the model simulation correctly captured the overall trends in TAN and TNiN concentrations, although the data and model gave a significant discrepancy. One possibility for the discrepancy might be the complexity delivered by the growth of heterotrophic bacteria on the soluble microbial products (SMP) produced by the nitrifiers (Furumai and Rittmann, 1992; de Silva et al., 2000a,b; Merkey et al. 2009). Another possibility is an increase in the biofilm density and thickness that results in diffusion resistance of TAN into biofilm; we discuss biofilm density and thickness in next section.

Park et al. (2010c) reported that a biofilm system might be able to maintain a stable nitrification at a lower TAN concentration than a suspended system, since FNA inhibition and DO limitation can deepen NOB suppression inside the biofilm. A suspended system needed approximately 80 mgTAN/L to suppress nitrite oxidation (Park et al., 2010b). while we observed nitrite accumulation at 50±30 mgTAN/L from day 61 to day 160 under similar DO and pH.

3.6. Measurement of biofilm depth and estimation of biofilm density

Fig. 5 shows MSNBM-simulated profiles of biofilm composition, as well as the measured and simulated biofilm depths at the end of operation of each reactor. The predicted results show that higher DO and substrate concentrations led to thicker biofilms, and the simulated and experimental values for Reactors 1, 2, and 3 are very close to each other. However, the experimental value is much larger for Reactor 4. The experimental result for Reactor 4 appears to be inconsistent with the experimental result for the other reactors. For example, the biomass accumulation for Reactor 4 ought to be considerably less than for Reactor 3, since Reactor 3 had a substantially higher rate of nitrification. The apparent inconsistency can be explained if the biofilm detachment rate for Reactor 4 were considerably smaller than for the other reactors, which we discuss below.

The table that is part of Fig. 5 provides the amounts of each type of biomass computed from the simulated results of day 200. One key finding is that the mass of AOB and NOB in Reactor 1 and 3 are almost the same. This shows that active biomass in the biofilm is similar when the degree of nitrification is the same: full nitrification for both reactors. In the model, we assume that the daily detached biofilm length is 6% of total biofilm length (i.e., 0.06/day specific detachment rate) and the detachment phenomenon occurs at the surface of biofilm. Since 6% of biofilm length in Reactor 3 is 31.1 μm and that in Reactor 1 is 14.4 μm, the biomass detached from Reactor 3 is more active. Since Reactors 1 and 3 have active-biomass fractions of 0.72 and 0.83, respectively, at the surface of the biofilm, the ratio of active biomass detached from the surface is 1 : 2.5 (i.e., 0.72*14.4 μm : 0.83*31.1 μm). This ratio is the same as the influent-loading ratio (1.6 kg/1000 m^2-d : 4 kg/1000 m^2-d = 1 : 2.5). The correspondence of ratios indicates that the higher biomass synthesis with higher loading was balanced by higher detachment of active biomass, even though the total active biomass was similar in the two reactors. However, the higher loading gave a substantially larger accumulation of inert biomass, which was located away from the outer surface and comparatively free from detachment loss.

The AOB mass with oxygen limitation was similar in Reactor 2 and 4, but the NOB mass was different. Similar to the comparison of Reactors 1 and 3, loss from the surface

of the biofilm was the reason why the AOB mass was similar in Reactor 2 and 4. However, Reactor 4 had complete suppression of NOB, while Reactor 2 had only partial suppression of NOB; thus, Reactor 4 had no NOB.

The biofilm density was calculated by dividing the total biomass in the whole reactor with the biofilm volume (volume = average depth × total surface area). Table 3 shows that the measured biofilm densities of Reactor 1, 2, and 3 were similar, giving an average value of 20±2.5 mg/cm^3, which is close to the value used in the simulations (18 mg/cm^3, Table 1). However, the density of Reactor 4 was much higher (38 mg/cm^3). While these variations are in the range of biofilm density reported in the literature (e.g., Rittmann and McCarty, 2001), the high density for Reactor 4 strongly affects the biofilm thickness. Biofilm density can be affected by microbial species and physical forces (e.g., Christensen and Characklis, 1990; Vieira et al. 1993; Trinet et al., 2001; Sharma et al., 2005; Garny et al. 2008), and Laspidou and Rittmann (2004) presented a consolidation model to describe the increase of density over time. The results in Table 3 suggest that low DO concentration and high TAN made the biofilm denser in Reactor 4, although the cause-and-effect relationship cannot be determined from these results alone. Additional simulations with the higher density (38 mg/cm^3) produced a similar biofilm thickness (587 μm), but the detachment rate had to be much smaller, 0.015/day.

3.7. Biofilm activity of each reactor

NOB activity in the biofilm was observed through batch tests, as shown in Fig. 6. Since the slope of each line represents the specific nitrite utilization rate (q), the slope should be smaller as the NOB of the biomass decreased. The slopes from the batch tests (inset in Fig. 6) match well with the simulated results in Fig. 5 (the ratio of NOB/total biomass). The concentration of NOB in the biofilm increased at higher DO concentration, thus, giving a steeper slope. With DO limitation (Reactor 2 and 4), Reactor 4 had a nearly zero slope, which indicated that Reactor 4 had little NOB due to DO limitation and FA inhibition.

4. Conclusions

We explored the simultaneous effects of oxygen limitation and FA and FNA inhibition in the nitrifying biofilm, giving special focus to testing the conditions that the MSNBM identifies for nitrite accumulation in biofilms. CFBR experiments were organized into four sets with these expected outcomes based on the MSNBM: 1. Control, giving full nitrification; 2. oxygen limitation, giving modest long-term nitrite build up; 3. FA inhibition, giving no long-term nitrite accumulation; and 4. FA inhibition plus oxygen limitation, giving major long-term nitrite accumulation. Consistent with MSNBM predictions, the experimental results showed that nitrite accumulated in sets 2 – 4 in the short term, but long-term nitrite accumulation was maintained only in sets 2 and 4, which involved oxygen limitation. Furthermore, nitrite accumulation was substantially greater in set 4, which also included FA inhibition. However, FA inhibition (and accompanying FNA inhibition) alone in set 3 did not maintained long-term nitrite accumulation. NOB-activity batch tests confirmed that little NOB or only a small fraction of NOB were present in the biofilms for sets 4 and 2, respectively. The experimental data supported the previous modeling results that nitrite accumulation could be achieved with a lower ammonium concentration than had been required for a suspended-growth process. Additional findings were that the biofilm exposed to DO limitation and FA inhibition was substantially denser and probably had a lower detachment rate.

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