Influence of Biomass Production and Detachment Forces on Biofilm Structures in a Biofilm Airlift Suspension Reactor

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Abstract: The influence of process conditions (substrate loading rate and detachment force) on the structure of biofilms grown on basalt particles in a Biofilm Airlift Suspension (BAS) reactor was studied. The structure of the biofilms (density, surface shape, and thickness) and microbial characteristics (biomass yield) were investigated at substrate loading rates of 5, 10, 15, and 20 kg COD/ $m^3 \cdot$ day with basalt concentrations of 60 g/L, 150 g/L, and 250 g/L. The basalt concentration determines the number of biofilm particles in steady state, which is the main determining factor for the biofilm detachment in these systems. In total, 12 experimental runs were performed. A high biofilm density (up to 67 g/L) and a high biomass concentration was observed at high detachment forces. The higher biomass content is associated with a lower biomass substrate loading rate and therefore with a lower biomass yield (from 0.4 down to 0.12 $g_{biomass}/g_{acetate}$). Contrary to general beliefs, the observed biomass detachment decreased with increasing detachment force. In addition, smoother (fewer protuberances), denser and thinner compact biofilms were obtained when the biomass surface production rate decreased and/or the detachment force increased. These observations confirmed a hypothesis, postulated earlier by Van Loosdrecht et al. (1995b), that the balance between biofilm substrate surface loading (proportional to biomass surface production rate, when biomass yield is constant) and detachment force determines the biofilm structure. When detachment forces are relatively high only a patchy biofilm will develop, whereas at low detachment forces, the biofilm becomes highly heterogeneous with many pores and protuberances. With the right balance, smooth, dense and stable biofilms can be obtained. © 1998 John Wiley & Sons, Inc. *Biotechnol Bioeng* **58:** 400–407, 1998.

Keywords: abrasion; airlift reactor; biofilm; structure; density; surface shape; thickness; shear; carrier concentration; substrate loading; detachment

INTRODUCTION

Biofilms can be found in a wide range of different systems. Intensive research in the past has revealed a wide variety in biofilm structures. One of the most important aspects, the relation between environmental conditions and biofilm structure, has hardly been studied. Recently, a range of new observation techniques has been used to show the complex nature of biofilm structures (De Beer et al., 1994; Lewandowski et al., 1994). Due to the large influence of substrate surface loading and shear on the biofilm structure (Tijhuis et al., 1995b) and the inhomogeneity of these parameters in many biofilm reactors (Gjaltema et al., 1994; Gjaltema and Griebe, 1995), a clear correlation between these parameters and the resulting biofilm structure is still lacking.

The morphological characteristics of biofilms (biofilm thickness, biofilm density, and biofilm surface shape) are very important for the stability and performance of a biofilm reactor. These factors strongly affect the biomass hold-up and mass transfer in a biofilm reactor (Garrido et al., 1997; Tijhuis, 1995b). The biofilm density has a direct effect on the achievable biomass concentration in the reactor. For aerobic processes, thin biofilms (<150 μm) are favorable (Tijhuis et al., 1994; Van Loosdrecht et al., 1995a). Sloughing phenomena occur more frequently with thicker biofilms (Beeftink, 1987). Therefore, control of biofilm thickness is an important aspect for a stable operation of biofilm processes. Biofilm surface shape is an important parameter for the stability of the reactor too. Especially in particle biofilm processes (e.g., fluid bed reactors), fluffy biofilms and outgrowth lead to instabilities with respect to the separation of biofilm particles from the treated water (Tijhuis et al., 1995b). Suspended particles in the wastewater are easier filtrated from the wastewater by filamentous then smooth biofilms. Also mass transfer characteristics depend on the biofilm structure. Hence, the biofilm structure is important for the overall performance of a biofilm reactor.

Based on a literature review, Van Loosdrecht et al. (1995b) postulated that the balance between biofilm surface loading and detachment forces is the main determining factor for the biofilm structure. Figure 1 shows the schematic representation of the hypothetical influence of substrate surface loading rates and detachment forces on the biofilm

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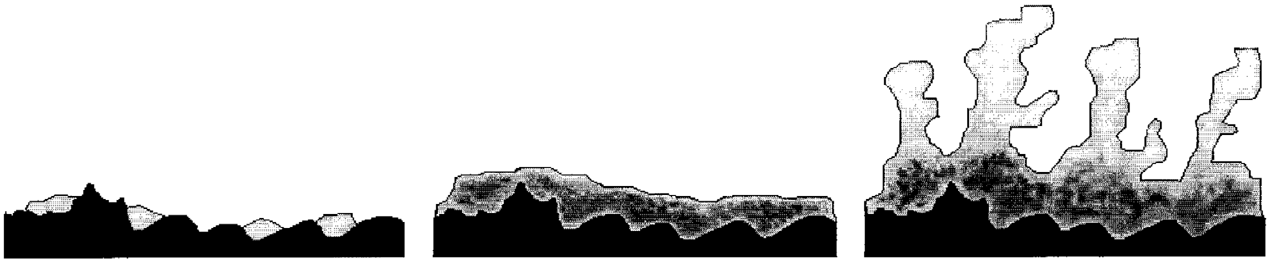

Figure 1. Schematic representation of the postulated influence of substrate surface loading rate (increases from left to right) and detachment force (decreases from left to right) on biofilm structure (adapted from Van Loosdrecht et al., 1995b).

structure. It was suggested that due to the concentration gradient near the biofilm surface, biofilms tend to form a low density heterogeneous structure (Van Loosdrecht et al., 1995b, more details can be found in Picioreanu et al., 1997). In a recent review, Wimpenny and Colasanti (1997) underline the intrinsic heterogeneous growth of biofilms. This trend in biofilm growth can be balanced by detachment forces. When detachment forces are relatively high only a patchy (left panel in Fig. 1) biofilm will develop, whereas under low detachment forces the biofilm becomes highly heterogeneous with many pores and protuberances (right panel in Fig. 1). With the right balance between biomass production (which is directly controlled by the substrate surface loading rate) and detachment force, smooth and stable biofilms will be obtained. According to Van Loosdrecht et al. (1995b) the biomass formed at the biofilm surface intrinsically has a high growth rate and high polymer production rate, leading to a low density structure. When the outgrowth of this outer biofilmlayer is controlled by detachment forces, the substrate can penetrate into the base biofilm. Due to extra biomass growth (in the form of microcolonies) in the base biofilm, the density of the biofilm will increase with increased detachment forces.

Tijhuis et al. (1995b) reported that a smooth compact biofilm deteriorated and became heterogeneous with large protuberances when the substrate loading on the biofilm was increased. This showed that the biofilm structure is dependent on the subtle balance between rate of biomass formation and detachment forces. Quantitative results are however, still not available in literature. Hence, in this study, the effect of substrate surface loading and detachment forces on biofilm characteristics was investigated in detail.

Biofilm Airlift Suspension (BAS) reactors have an advantage over many other biofilm reactors (Gjaltema and Griebe, 1995). The biofilm particles are homogeneously distributed in a well-mixed reactor. This means that each particle experience on a time-averaged basis the same substrate loading and detachment force. The detachment force on the biofilm can be easily changed by changing the number of particles in the reactor (Gjaltema et al., 1997a). The amount of carrier added determines directly the number of biofilm particles in the reactor (all carrier particles become covered). Moreover, the biofilm can be easily and representatively sampled without disturbing the remaining films. In this experiment, we have studied the biofilm characteristics (thickness, shape, and density) under steady state conditions as a function of applied substrate loading and detachment force. The results will be discussed in the context of general aspects of biofilm formation and specific aspects for the operation of BAS reactors for wastewater treatment.

MATERIALS AND METHODS

The experimental set-up was similar as reported by Tijhuis et al. (1994). Biofilms were formed in 3-L internal loop airlift reactors. The substrate used was acetic acid and a mineral salts medium, temperature was kept at 30°C and the pH at 7. The hydraulic retention time was 40 min and the superficial air velocity was 2.2 cm/s. Basalt particles were used as carrier for biofilm growth. The basalt had a diameter of 0.2–0.3 mm and a density of 3.0 kg/L. The sampling and analysis procedures are described in Tijhuis et al. (1994). In the experiments, the detachment force was changed by adding different amount of basalt carriers (resulting in different numbers of biofilm particles) and the loading rate was changed by changing the concentration of the substrate in the medium. Biomass density was measured by a dextran blue method according to Garrido et al. (1997).

The biofilm morphology was evaluated by an image analyzer (Galai Cue 2 v.4.70) coupled with stereo microscopy by a CCD camera. The average (ferrets) diameter and the average squared (equivalent) diameter were obtained by analyzing 1000–1200 particles per sample. The average ferrets diameter was used for calculating the biofilm thickness. The biofilm surface or volume is calculated from the equivalent diameter. The average shape factor was determined on the same samples. This factor is equal to the ratio of the perimeter of an equivalent circle and the actual perimeter of the biofilm particle. If this factor is 1 then the particle is exactly spherical.

RESULTS

In the experimental program, three experimental runs (at basalt concentrations of 60, 150, and 250 g/L) with four different loading rates (5, 10, 15, 20 kg $COD/m^3 \cdot d$) were performed. The influence of process conditions on biofilm formation and characteristics were closely monitored during transition and steady-state periods. In this experiment,

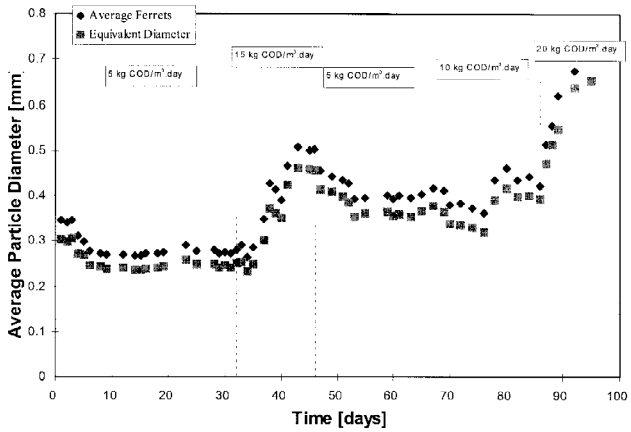

Figure 2. Average biofilm particle diameter vs. time in a BAS reactor with 150 g_{basalt}/L (Experiment E2, see Table I). Average diameter of basalt carrier: 0.2 mm.

''steady state'' was assumed when the biomass concentration, effluent concentration, and biofilm thickness reached stable values. An example of the course of an experiment is given in Figure 2. Average values during steady-state periods are reported in Table I.

At the start of the experiment, as shown in Figure 2, 40 g/L of biofilm particles were used as starting material. This was supplemented with fresh bare basalt particles to obtain the desired basalt concentration (150 g/L), which again determines the final number of biofilm particles. Due to the high amount of bare carriers, the low amount of biofilm covered particles and the low volumetric loading (5 kg/$m^3 \cdot d$), the biofilm particles were eroded and only 10% of the carriers were partially covered after 30 d. The volumetric loading was then increased to 15 kg COD/$m^3 \cdot d$. In two weeks, the particle diameter rose to 0.5 mm with more than 90% of the carriers fully covered. Hereafter, the reactor was changed back to the low loading rate. Now, all the particles remained covered and only a decrease of their diameter occurred. This behavior shows the difference in detachment force between a situation with mainly bare carriers (a high detachment force due to the bare basalt particles) or with full grown biofilms (with low detachment due to already covered basalt particles) as previously reported by Gjaltema et al. (1997a). In order to standardize the results, we have only reported those data related to systems in which more than 90% of the basalt carriers was covered by biofilms.

General Observation

One to four days after the COD loading rate was increased, a fluffy and less dense biofilm was always observed on the outer layers of the particles. These fluffy biofilms slowly eroded (except at a loading of 20 kg COD/$m^3 \cdot d$) as the effective substrate surface loading decreased (due to an increase in biofilm specific surface area associated with the increased particle radius). The reverse of this phenomenon was also observed when the substrate loading was decreased. Thin, smoother, and denser outer layers were observed. These changes of COD loading with time sometimes resulted in multi-layered, heterogeneous biofilms.

During initial biofilm formation, most of the initial observed covered carrier particles were smaller particles. This was then followed by biofilm growth on the larger particles. These observations are in line with other experiments showing that larger particles have a higher collision energy than smaller particles (Gjaltema et al., 1997b). Obviously a high shear or detachment force does not allow easy colonization.

The results are summarised in Table I. Visual observations indicated that biofilms were more dense, rigid, and smooth when the reactor was run at higher basalt concentration. Dense biofilms lead to higher biomass accumulation in the reactor with a subsequent decrease in biomass loading rate. This in turn leads to a lower observed biomass yield.

Table I. Overview of the steady-state experimental results.

Experiment no.	Volumetric loading rate (kg COD/$m^3 \cdot d$)	Basalt concentration (g/L)	Substrate surface loading rate (g COD/$m^2 \cdot d$)	Biomass surface production rate (g VSS/$m^2 \cdot d$)	Biomass concentration (g/L)	Sludge production rate (g/L · d)	Biomass yield (g biomass/g acetate)	Biofilm thickness (μm)	Biofilm shape factor (−)	Biofilm density (g/L)	Biomass detachment rate (g/g · d)
1	5	60	3.54	1.06	1.80	1.41	0.32	95	0.49	25	0.75
2	5	150	1.57	0.46	2.35	1.44	0.31	75	0.65	37	0.62
3	5	250	0.94	0.19	3.45	0.94	0.22	50	0.51	38	0.27
4	10	60	6.39	2.04	2.37	3.01	0.34	120	0.47	28	1.33
5	10	150	2.68	0.73	3.63	2.74	0.29	90	0.61	34	0.90
6	10	250	1.38	0.18	10.16	1.03	0.14	70	0.63	53	0.10
7	15	60	7.25	2.79	2.74	4.82	0.41	145	0.41	23	1.76
8	15	150	2.98	0.59	6.51	2.70	0.21	125	0.51	30	0.42
9	15	250	2.06	0.29	12.94	1.79	0.15	80	0.72	67	0.15
10[a]	20	60	7.70	2.82	3.94	7.82	0.39	230	0.33	17	2.11
11[a]	20	150	3.54	0.43	10.19	2.34	0.13	170	0.51	31	0.28
12[a]	20	250	2.46	0.28	16.33	2.34	0.12	140	0.23	43	0.15

[a]Steady states were not obtained. Results were taken 2–4 d before washouts.

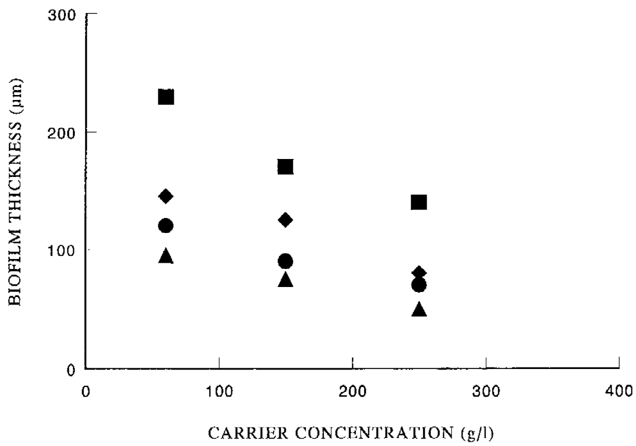

Figure 3. Relation between biofilm thickness and carrier concentration at different substrate loading rates. Loading rates: (▲) 5, (●) 10, (♦) 15, (■) 20 kg COD/$m^3 \cdot$ d.

At volumetric substrate loading rates of 20 kg COD/$m^3 \cdot$ d, steady states were not observed for any of the three experiments. The systems seemed to be unstable at these conditions. Filamentous micro-organisms were regularly observed under these conditions. The highest volumetric solids hold-ups that led to instability due to bioparticle washout were 26%, 38%, and 40% for Experiments 10, 11, and 12 respectively. Clearly, the maximum solids hold-up in these BAS reactors is around 40%, which is related to the loading of the three-phase separator. The lower maximum hold-up in Experiment 10 was mainly due to an inefficient separator. In Experiments 11 and 12 an improved separator was used.

DISCUSSION

Effect of Detachment Force (Particle Concentration) on Biofilm Thickness, Density, and Surface Shape

The biofilm thickness markedly decreases with increasing carrier (biofilm particle) concentration in the BAS reactor (see Fig. 3). The higher detachment force on the biofilms due to particle-particle interaction clearly leads (as expected) to thinner biofilms. From Figure 3 it can also be seen that the effect of detachment can be counterbalanced by the substrate loading rate. The effect of particle shear on biofilm thickness is stronger on biofilms grown at higher substrate loading rates. Because these are less dense (see below), this could indicate that low density biofilms are more susceptible to detachment forces. It seems there is a linear relation between applied carrier concentration and biofilm thickness. This indicates that at a carrier concentration of around 450 g/L (volumetric basalt hold-up 15%), there will be no biofilm formation at all, this being independent of the substrate loading rate.

The biofilm density clearly increases with increasing detachment force due to particle-particle collisions (Fig. 4). Similar observations have been made in fluidized bed reactors (Chang et al., 1991) and in BAS reactors with nitrifying biofilms (van Benthum et al., 1996). Research on nitrifying and heterotrophic bacteria show that under similar reactor conditions (temperature, shear) the slow growing nitrifyers form a much denser biofilm than fast growing heterotrophs (Tijhuis et al., 1994, 1995b), similar observations are reported for methanogenic and acidifying bacteria (Alphenaar, 1995). These observations all indicate that with increasing detachment forces, relative to the surface growth, the biofilm density increases. Differences in biofilm density are often explained by assuming the presence of different types of organisms, however, there is clear evidence from pure culture studies that hydrodynamic conditions have a direct influence on the biofilm density. Vieira et al. (1993) have shown that the density of a pure culture biofilm increases with increasing shear stress on the biofilm. This could indicate that a physical rather than a biological phenomenon is underlying the relation between detachment force and biofilm density.

The relation between detachment force and biofilm density (Fig. 4a) can be explained as follows: The increase in

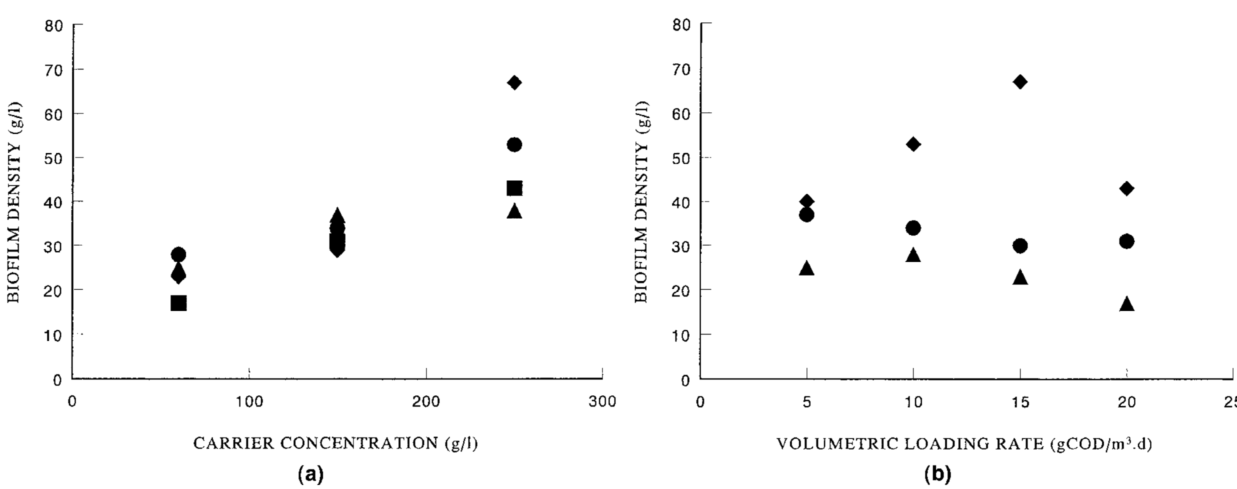

Figure 4. Influence of carrier concentration **(A)** and volumetric substrate loading rate **(B)** on biofilm density expressed as gram of biomass per L of biofilm volume (excluding pores). Symbols: **A:** (▲) 5, (●) 10, (♦) 15, (■) 20 kgCOD/$m^3 \cdot$ d; **B:** (▲) 60, (●) 150, (♦) 250 g-carrier/L.

collision frequency due to increased particle numbers, and hence higher detachment force (Gjaltema et al., 1997b), removes fluffy and less dense, newly grown biomass on the outer layers of the particles. The removal of these protuberances or less dense layers enables substrate to diffuse into the older inner biofilm layers. The growth of cells in the inner layer results in microcolony formation and thereby, to an increase in the average biofilm density. It was also expected that at constant particle (basalt) concentration, biofilm density should decrease as volumetric substrate loading increases (Van Loosdrecht et al., 1995b). There is however no specific trend between volumetric loading and applied carrier concentration observed (Fig. 4b). This can be explained by the fact that when the substrate loading increased, biofilm thickness increased too. An increase in biofilm thickness is associated with an increase in biofilm surface area (spherical geometry), which reduces the surface loading but increases the detachment forces (Gjaltema et al., 1997b). Hence, depending on which factors dominate, biofilm density can either increase or decrease as the volumetric loading increases at different carrier concentrations.

An increased biofilm density (i.e., amount of biomass per volume of biofilm) allows a higher biomass hold-up in the reactor. The latter is mainly depending on the solids hold-up. Figure 5 shows that there is a linear relation between biomass concentration and amount of carrier added. Of course, the volumetric loading rate also has a clear influence on the biomass accumulation. Relatively high biomass concentrations (up to 16 gVSS/L) for heterotrophic biofilms were obtained.

The biofilm shape factor (a measure for the biofilm irregularity, a smooth circular structure has a shape factor of 1) is plotted as a function of the basalt concentration for all volumetric substrate loadings (Fig. 6). As basalt concentration, and therefore the detachment force increases, the biofilms obtain in general a more circular and smooth shape. There is however, no unambiguous trend between surface shape and volumetric substrate loading rate at each basalt concentration. This is due to the same reason as discussed above for the relation between loading rate and biofilm density.

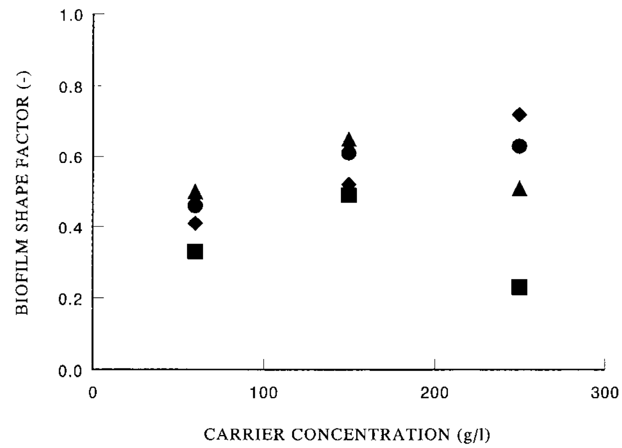

Figure 6. Observed effect of the carrier concentration and volumetric loading rate on the shape factor of the biofilm particles (Shape factor 1: fully circular biofilm). Loading rate: (▲) 5, (●) 10, (♦) 15, (■) 20 kg COD/$m^3 \cdot$ d.

Effect of Growth (Biomass Surface Production Rate/Substrate Surface Loading Rate) on Biofilm Thickness, Density, and Surface Shape

The relation between observed biofilm thickness and substrate surface loading rate is shown in Figure 7. The biofilm thickness increases with the substrate surface loading rate. Clearly a higher substrate loading rate leading to a higher biomass production rate more easily balances the detachment forces resulting in thicker biofilms. Probably the results plotted in Figure 7 can best be interpreted as follows. With increasing surface loading rate the biofilm thickness gradually increases, almost independent of the detachment force. However, above a critical substrate surface loading

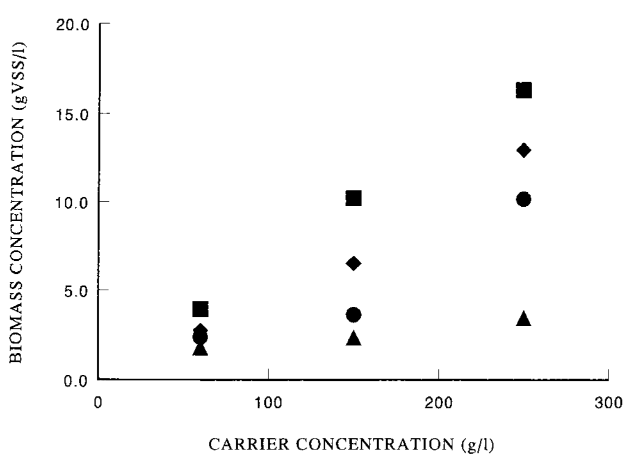

Figure 5. Biomass concentration as a function of basalt concentration and loading rate; loading rate: (▲) 5, (●) 10, (♦) 15, (■) 20 kgCOD/$m^3 \cdot$ d.

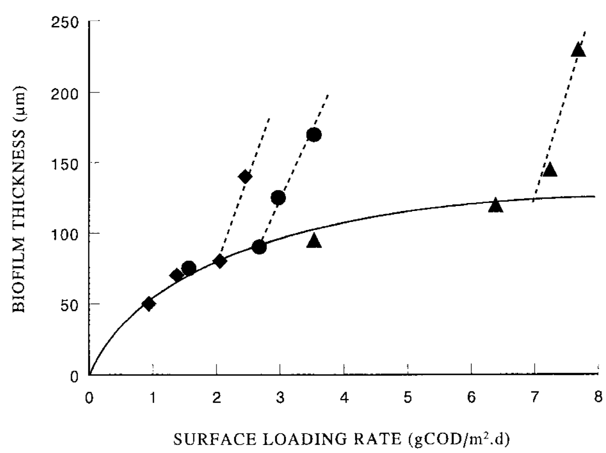

Figure 7. Effect of substrate surface loading rate and carrier concentration on biofilm thickness. Carrier concentration: (▲) 60, (●) 150, (♦) 250, kg/m^3.

rate (which depends on the detachment force) the detachment does not control the outgrowth of the biofilm anymore and the thickness increases rapidly with increasing loading rate. Eventually this leads to wash-out of biofilm particles.

The steady-state biofilm density is plotted as a function of the biomass surface production rate (g VSS/$m^2_{\text{biofilm surface area}}$ · d) in Figure 8. The biofilm density clearly decreases as the biomass surface production rate increases. This is in agreement with expectations. As the biomass surface production rate increases, more biomass will be formed by the outer biofilm layer. These newly formed biofilm layers have in general, lower densities (cells grow more dispersed in the newly formed gel matrix, whereas in an existing gell matrix the cells form microcolonies). When the majority of the substrate is consumed by the cells in the outer layer, less substrate remains for the base biofilm and no increase in density can occur. This results in an overall lower average biofilm density.

The effect of the biomass surface production rate on the biofilm shape factor is shown in Figure 9. As with biofilm density, the biofilm shape factor decreases when the biomass surface production rate increases. This is in accordance with the recognition that biofilms intrinsically tend to form more heterogeneous structures, which is balanced by the applied detachment force. In Experiment 12 (250 g-basalt/L, 20 kg COD/m^3 · d), a very low biofilm shape factor was obtained (0.23). The observed low shape factor was due to the excessive growth of filamentous micro-organisms, probably formed due to the high COD loading and a decrease in the detachment force. At this high solids hold-up, the flow in the reactor strongly changed from highly turbulent to more laminar (visual observation), hence the detachment force decreased and could not compensate the biomass production rate.

Effect of Particle Concentration and Biomass Surface Production Rate on Biomass Yield

The biomass yield decreased as the particle concentration increased in the reactor (Fig. 10a). Due to the increased

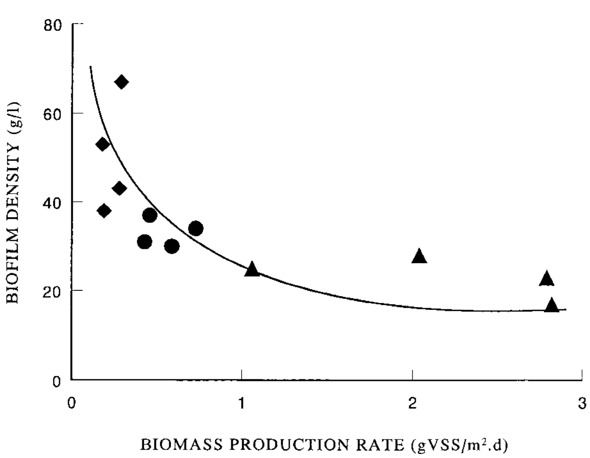

Figure 8. Relation between the biomass surface production rate and observed biomass density of the biofilm. Carrier concentration: (▲) 60, (●) 150, (♦) 250, kg/m^3.

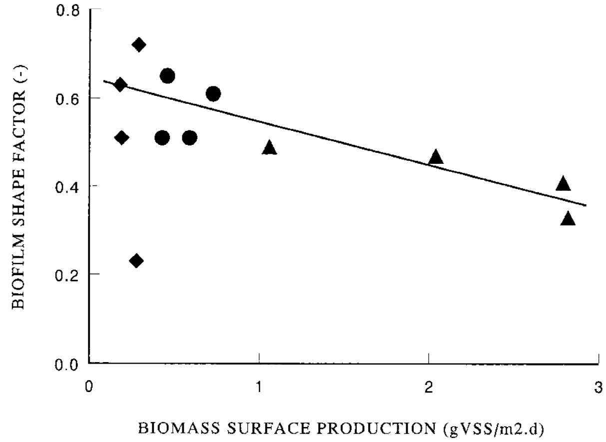

Figure 9. Relation between the biomass surface production rate and biofilm shape factor. Carrier concentration: (▲) 60, (●) 150, (♦) 250, kg/m^3.

biomass hold-up in the reactor at higher particle concentration, a lower sludge loading is obtained, which again results in a decreased sludge production. If the biomass yield is plotted against the average growth rate (volumetric biomass production rate divided by the biomass concentration) the influence of maintenance requirements on the observed yield becomes clear (Fig. 10b). From these data a maximal yield on acetate of 0.23 C-mol biomass per mol HAc and a maintenance coefficient of 0.37 mol HAc/C-mol · d are obtained. These values are similar to previously obtained coefficients (Tijhuis et al., 1994). Clearly with respect to a desirable low sludge production, a high carrier concentration is beneficial.

Effect of Particle Concentration on Detachment Forces for a Growing and Non-Growing System

One is tempted to assume that the detachment of biomass from a biofilm is predominantly governed by the detachment forces acting on the biofilm. However, it has been shown that under identical detachment conditions, detachment is much higher for growing than for non-growing biofilm systems (Peyton and Characklis, 1993; Speitel and DiGiano, 1987; Tijhuis et al., 1995a). Gjaltema et al. (1997a) investigated the effect of particle concentration on the detachment force under non-growth conditions. The detachment force increased with increasing particle concentration. This is easily understood, as higher concentration of particles will lead to higher collision frequency and hence, higher detachment force. These kind of experiments under non-growth conditions easily lead to a general perception that a high detachment force (here—particle concentration) is not desirable for good biofilm formation. This perception can be quite misleading and deceiving when we look into growing biofilms. In a growing system, growth and detachment processes are much more complex and we should look at more factors. Due to growth processes especially the biofilm structure (density, shape factor) and strength can

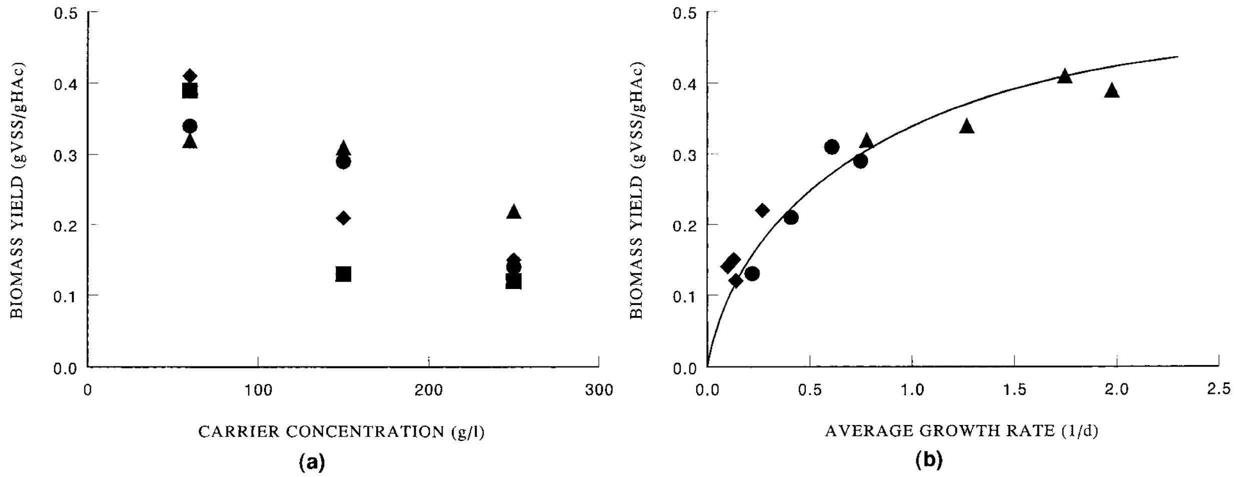

Figure 10. Observed biomass yield as a function of carrier concentration **(A)** and average specific growth rate **(B). (A)** Loading rate: (▲) 5, (●) 10, (◆) 15, (■) 20 kg COD/m^3 · d; **(B)** Carrier concentration: (▲) 60, (●) 150, (◆) 250, kg/m^3.

change, resulting in more resistance against applied detachment forces.

Figure 11 clearly shows that in growing systems, unlike non-growing systems, the detachment force decreased with increasing detachment force. At high particle concentration, more biomass is accumulated in the system because the interaction forces between the particles lead to a compact, stable, and dense biofilm. Hence, contrary to general belief high detachment forces can lead to a lower detachment in these reactors.

A General Hypothesis for the Structure of Microbial Biofilms

The results of the experiments presented in this article clearly underline our previous assumption that the biofilm structure is a resultant of the interaction between biomass production, which is for biofilm systems supposed to be intrinsically heterogeneous, and biomass detachment, which depends on the applied detachment force and the biofilm structure itself. Biofilm systems will adapt to detachment by a mechanism that makes them less susceptible for detachment forces.

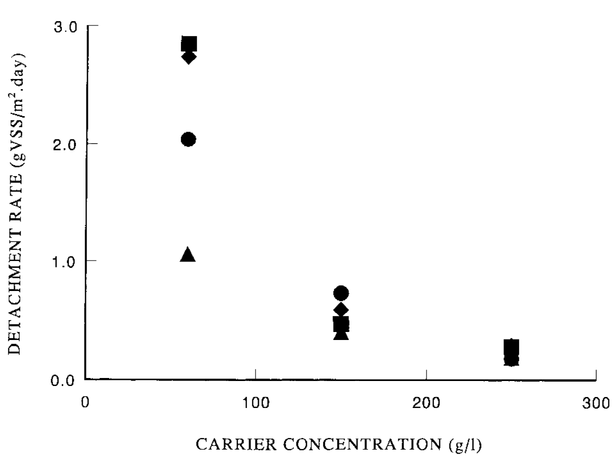

Figure 11. Biomass detachment force at different carrier concentration. Loading rate: (▲) 5, (●) 10, (◆) 15, (■) 20 kg COD/m^3 · d.

This research has experimentally underlined the interaction between biofilm growth (depending on substrate availability and biomass yield) and detachment forces, however it does not show the exact mechanisms underlying the observed behavior. That biofilms tends to form an intrinsic heterogeneous structure is nowadays an accepted fact. Wimpenny and Colasanti (1997) relate this to the substrate concentration in the medium. In our view, it is however better to relate this to the substrate gradient at the biofilm surface (Picioreanu et al., 1997; Van Loosdrecht et al., 1997). Just as it has been shown for crystal growth, diffusion limited growth intrinsically leads to a heterogeneous outgrowth of the (crystal or biofilm) surface. This has been elegantly shown by the works of Cooper et al. (1968), Wolfram (1984), and Matsushita and Fujikawa (1990). Their experiments are however, performed in systems without detachment and cannot explain the effect of detachment process on the biofilm density, for example. Clearly detachment leads to a more homogeneous and smooth biofilm structure. Our hypothesis that due to control of surface growth by a high detachment force more substrate diffuses into the biofilm and leads therefore, to a higher biofilm density is not yet mechanistically explained. It is also not fully clear why a dense biofilm is more resistant to detachment forces. Possibly this is only the result of the fact that a dense biofilm is generally more smooth and therefore less susceptible for detachment forces.

A higher detachment from growing biofilms as compared to non-growing biofilms (Tijhuis et al., 1995a) can be explained by the assumption that biofilm growth occurs preferentially in protuberances. These are continuously formed and detached, in non-growing biofilms this process does not occur, resulting in lower detachment at the same detachment force. In order to fully elucidate the mechanisms governing the architecture of biofilms, future experimental work should be concentrated on the exact detachment mechanism and the mechanism that determines the density of biofilms.

CONCLUSION

The influence of reactor conditions (substrate loading rate and applied detachment forces) on the performance of the BAS reactor and on the structures of biofilms growing on biofilm particles was studied. In most cases, steady state was achieved between 10 to 30 d after changing to a new COD loading.

Biofilm thickness increased as a function of substrate surface loading rate or biomass surface production rate. For a given volumetric substrate loading rate, the biofilm thickness decreased as carrier concentration increased. This was due to two reasons: increased detachment forces and decrease in biomass surface production rate (higher carrier concentration leads to a higher biofilm surface area).

It was observed that biofilm density and biofilm shape factor decreased (less dense and more fluffy, more protuberances, or rougher biofilms) as biomass surface production rate increased. Biofilm density and shape factor also decreased as the detachment force in the reactor decreased.

Contrary to a non-growing system, detachment of biomass is lower with higher carrier concentration. This contradicts the general belief that high detachment force is not desirable for a stable biofilm formation. Clearly a higher carrier concentration, which results in higher biofilm density and higher biomass concentration is desirable, as this will reduce biomass yield considerably thereby reducing the amount of excess sludge produced.

These experiments confirmed the hypothesis postulated by Van Loosdrecht et al. (1995b), that the ratio between biofilm surface loading (proportional to biomass surface production rate, when biomass yield is constant) and detachment force determines the biofilm structure. When detachment forces are relatively high only a patchy biofilm will develop, whereas at low detachment forces the biofilm to become highly heterogeneous with many pores and protuberances. With the right balance, smooth and stable biofilms can be obtained.

References

Alphenaar, A. 1995. Anaerobic granular sludge. Ph.D. thesis, Wageningen Agricultural University, The Netherlands.

Beeftink, H. H. 1987. Anaerobic bacterial aggregates: Variety and variation. Dissertation, University of Amsterdam, The Netherlands.

Chang, H. T., Rittmann, B. E., Amar, D., Heim, R., Ehrlinger, O., Lesty, Y. 1991. Biofilm detachment mechanisms in a liquid fluidized bed. Biotechnol. Bioeng. **38**: 499–506.

Cooper, A. L., Dean, A. C. R., Hinselwood, C. 1968. Factors affecting the growth of bacterial colonies on agar plates. Proc. R. Soc. London Ser. B **171**: 175–199.

De Beer, D., Stoodley, P., Roe, F., Lewandowski, Z. 1994. Effects of biofilm structures on oxygen distribution and mass transport. Biotechnol. Bioeng. **43**: 1131–1138.

Garrido, J. M., van Benthum, W. A. J., van Loosdrecht, M. C. M., Heijnen, J. J. 1997. Influence of dissolved oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. Biotechnol. Bioeng. **53**: 168–178.

Gjaltema, A., Arts, P. A. M., Van Loosdrecht, M. C. M., Kuenen, J. G., Heijnen, J. J. 1994. Heterogeneity of biofilms in rotating annular reactors: Occurrence, structure, and consequences. Biotechnol. Bioeng. **44**: 194–204.

Gjaltema, A., Griebe, T. 1995. Laboratory biofilm reactors and on-line monitoring. Wat. Sci. Technol. **32**: 257–261.

Gjaltema, A., Vinke, J. L., van Loosdrecht, M. C. M., Heijnen, J. J. 1997a. Abrasion of suspension biofilm pellets in airlift reactors: Importance of shape, structure and particle concentration. Biotechnol. Bioeng. **53**: 88–89.

Gjaltema, A., van Loosdrecht, M. C. M., Heijnen, J. J. 1997b. Abrasion of suspension biofilm pellets in airlift reactors: Effect of particle size. Biotecnol. Bioeng. **55**: 206–215.

Lewandowski, Z., Stoodley, P., Altobelli, S., Fukushima, E. 1994 Hydrodynamics and kinetics of biofilm systems—recent advances and new problems. Wat. Sci. Techn. **29**: 223–229.

Matsushita, M., Fujikawa, H. 1990. Diffusion limited growth in bacterial colony formation. Physica A **168**: 498–506.

Peyton, B. M., Characklis, W. G. 1993. A statisticasl analysis of the effect of substrate utilization and shear stress on the kinetics of biofilm detachment. Biotechnol. Bioeng. **41**: 728–735.

Picioreanu, C., Van Loosdrecht, M. C. M., Heijnen, J. J. 1998. A new combined differential discrete cellular automaton approach for biofilm modelling—Application for growth in gel beads. Biotechnol. Bioeng. **58:** 101–116.

Speitel, G. E., DiGiano, F. A. 1987. Biofilm shearing under dynamic conditions. Environ. Eng. **113**: 464–475.

Tijhuis, L., van Loosdrecht, M. C. M., Heijnen, J. J. 1994. Formation and growth of heterotrophic aerobic biofilms on small suspended particles in airlift reactors. Biotechnol. Bioeng. **44**: 595–608.

Tijhuis, L., van Loosdrecht, M. C. M., Heijnen, J. J. 1995a. Dynamics of biofilm detachment in biofilm airlift suspension reactors. Biotechnol. Bioeng. **45**: 481–487.

Tijhuis, L., Hijman, B., Van Loosdrecht, M. C. M., Heijnen, J. J. 1995b. Influence of detachment, substrate loading and reactor scale on the formation of biofilms in airlift reactors. Appl. Microbiol. Biotechnol. **45**: 7–17.

Van Benthum, W. A. J., Garrido, J. M., Tijhuis, L., Van Loosdrecht, M. C. M., Heijnen, J. J. 1996. Formation and detachment of biofilms and granules in a nitrifying biofilm airlift suspension reactor. Biotechnol. Prog. **12**: 764–772.

Van Loosdrecht, M. C. M., Tijhuis, L., Wihdieks, A. M. S., Heijnen, J. J. 1995a. Population distribution in aerobic biofilms on small suspended particles. Wat. Sci. Technol. **31**: 163–171.

Van Loosdrecht, M. C. M., Eikelboom, D., Gjaltema, A., Mulder, A., Tijhuis, L., Heijnen, J. J. 1995b. Biofilm Structures. Wat. Sci. Technol. **32**: 35–43.

Van Loosdrecht, M. C. M., Picioreanu, C., Heijnen, J. J. 1997. A more unifying hypothesis for biofilm structures. FEMS Microbiol Ecol. **24:** 181–183.

Vieira, M. J., Melo, L. F., Pinheiro, M. M. 1993. Biofilm formations: Hydrodynamic effects on internal diffusion and structure. Biofouling **7**: 67–80.

Wimpenny, J. W. T., Colasanti, R. 1997. A unifying hypothesis for the structure of microbial films based on cellular automation models. FEMS Microbiol. Ecol. **22**: 1–16.

Wolfram, S. 1984. Cellular automata as models of complexity. Nature **311**: 419–424.