

## Influence of different substrates on the formation of biofilms in a biofilm airlift suspension reactor

J.C. Villaseñor<sup>\*,\*\*</sup>, M.C.M. van Loosdrecht<sup>\*</sup>, C. Picioreanu<sup>\*</sup> and J.J. Heijnen<sup>\*</sup>

<sup>\*</sup>Kluyverlaboratory for Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands.

<sup>\*\*</sup>Chemical Engineering Department, University of Castilla-La Mancha, Campus Universitario, 13071 Ciudad Real, Spain.

**Abstract** The effect of different C<sub>1</sub> carbon substrates (formate, formaldehyde, methanol) on biofilm formation in biofilm airlift suspension reactors was studied. The biofilm structure depended on the type of substrate. There seems to be a trend that more reduced substrates or substrates allowing a lower biomass growth rate lead to more dense biofilms. Variations in detachment forces (biomass hold up and carrier concentration) during the experiments affected biofilm characteristics and detachment. Detachment of biofilm biomass under non-growing conditions was studied. The detachment experiments clearly showed that detachment of a growing biofilm is larger than for a non-growing biofilm subjected to the same detachment forces. It is hypothesized that the growth zone of a biofilm forms a more weakly attached biofilm. A trend between biofilm density and the weakly attached fraction of biomass in the biofilm was found.

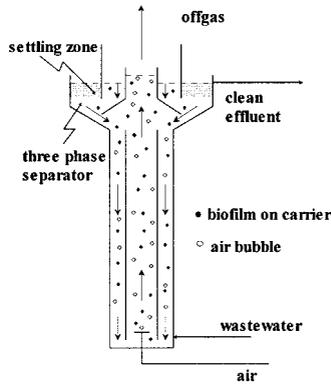
**Keywords** Airlift reactor; biofilm density; detachment; formate; formaldehyde; methanol

### Introduction

The activated sludge process has some inherent disadvantages, e.g., high surplus biomass production, low sludge age, and a great area requirement for reactors and settlers. Immobilisation of the sludge as biofilms offers solutions for these problems. An important aspect for aerobic biofilm processes is the diffusion of substrate into the biofilm. For a good efficiency, a relatively small thickness and a high amount of biofilm area per unit volume is needed. This can be achieved with small suspended particles as carrier material for the biofilms. An example of such a reactor is the Biofilm Airlift Suspension reactor (BAS reactor, Figure 1), in which biofilms are developed on small suspended particles, (Heijnen *et al.*, 1993). Due to the large carrier surface area in this reactor (up to 4000 m<sup>2</sup> m<sup>-3</sup>), high volumetric substrate conversions are possible. The reactor is well mixed and highly turbulent (liquid velocity is 60 m min<sup>-1</sup> approximately) meaning shear or detachment forces have a large influence on biofilm development.

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 (Tijhuis *et al.*, 1996). The biofilm density has a direct effect on the achievable biomass concentration in the reactor. Dense biofilms allow large amounts of biomass in the reactor and thus high volumetric removal rates. Moreover, the settling in the three phase separator strongly depends on the density of the biofilm particles. Especially in fluidised biofilm reactors, fluffy biofilms and outgrowth lead to instabilities with respect to the separation of biofilm particles from the treated water (van Loosdrecht *et al.*, 1995). Hence, control of the biofilm structure is an important aspect for stable operation of biofilm processes, however there are no good engineering correlations to predict this structure.

Van Loosdrecht *et al.* (1995) postulated that the balance between biofilm surface growth and detachment processes is the main determining factor for the biofilm morphology. Different factors, like substrate loading rate, dilution rate and shear forces, were experimentally verified with acetate as substrate (Tijhuis *et al.*, 1996; Kwok *et al.*, 1998). It was



**Figure 1** BAS reactor

observed that biofilm density and biofilm shape factor decreased (less dense and more fluffy and rougher biofilms) as biomass surface loading rate increased or the detachment force decreased.

The detachment process is also important because of the wash-out of biomass produced. This determines the sludge removal and eventual need for post-treatment. Detachment is generally assumed to be caused by shear forces (Characklis and Marshall, 1990). The effect of different factors like shape, size and concentration of particles were previously studied (Gjaltema *et al.*, 1997a; Gjaltema *et al.*, 1997b) under non-growth conditions. However, it has also been observed that biomass growth has an important contribution to the overall detachment rate under growing conditions (Speitel and DiGiano, 1987; Tijhuis *et al.*, 1995).

The aim of this work is to study the effect of different C-sources on the biofilm formation and detachment processes. Hereto different C1 carbon sources (formate, formaldehyde, methanol) were used.

### Materials and methods

For this research, concentric-tube airlift reactors combined with a three phase separator were used (Figure 1). All operating conditions were the same as reported by Tijhuis *et al.* (1994), but the initial carrier concentration was  $80 \text{ g L}^{-1}$  (3% on volume). Three different carbon sources (methanol, formate and formaldehyde) were used in the experiments, with the same volumetric substrate loading rate ( $5 \text{ kg COD m}^{-3} \text{ d}^{-1}$ ). The other medium components were the same as reported by Tijhuis *et al.* (1994).

The reactors were inoculated with 25 ml of biofilm particles obtained from a full-scale BAS reactor. During a period of more than two months, influent and effluent were monitored for soluble and particulate (biomass) COD. The biofilm development was followed by biomass dry weight measurements. Biofilm morphology (particle diameter and shape factor) was evaluated by image analysis (the average shape factor – ratio of the perimeter of an equivalent circle and the actual perimeter of the particle – is 1 for spherical particles). Biofilm density was calculated using the particle volume measured by image analysis, the VSS content and the solids hold-up in the reactor.

Detachment of biofilm biomass under non-growing conditions was studied in dynamic experiments in which the substrate loading rate was changed to zero by removing the organic substrate in the wastewater, but keeping the rest of experimental (hydraulic) conditions constant. During these experiments the effluent suspended biomass concentration was measured until the detachment rate remained nearly constant.

The microbial maximum specific growth rates, determined in batch tests with biofilm bacterial suspensions, and the maximal yield of biomass on substrate were calculated as reported by Tijhuis *et al.*, (1994).

**Table 1** Summary of experimental results. Numbers with arrows indicate a gradual change during the period

Substrate:	Formate	Formaldehyde	Methanol (I)	Methanol (II)
Operation period (d)	20–40	30–60	40–75	90–100
Bare basalt concentration (g L <sup>-1</sup> )	7	75	8	0
Biomass concentration in the reactor (gVSS L <sup>-1</sup> )	5 →12	1.3 →5	8 →22	25
Biofilm covered basalt particles (%)	90	2	92	100
Solids hold-up	0.15 →0.4	0.05 →0.2	0.1 →0.2	0.48
Detachment of newly formed biomass (%)	50	85	56	56
Particle diameter (mm)	0.45 →0.7	2.1	0.5 →0.55	0.6 →0.8
Shape factor	0.7	0.7	0.8	0.65
Biofilm density (gVSS L <sup>-1</sup> )	20–30	25–35	100–120	40–60
Specific biofilm surface area (m <sup>2</sup> m <sup>-3</sup> )	1700 →3100	100 →400	1800 →2400	4000 →4300
Surface specific detachment rate (gVSS m <sup>-2</sup> d <sup>-1</sup> )	0.2 →0.1	3.5 →1.5	0.3 →0.1	0.1
Maximum Yield (gVSS gCOD <sup>-1</sup> )	0.15	0.17	0.17	0.17
μ <sub>max</sub> (d <sup>-1</sup> )	8	6	3.5	3.5

## Results

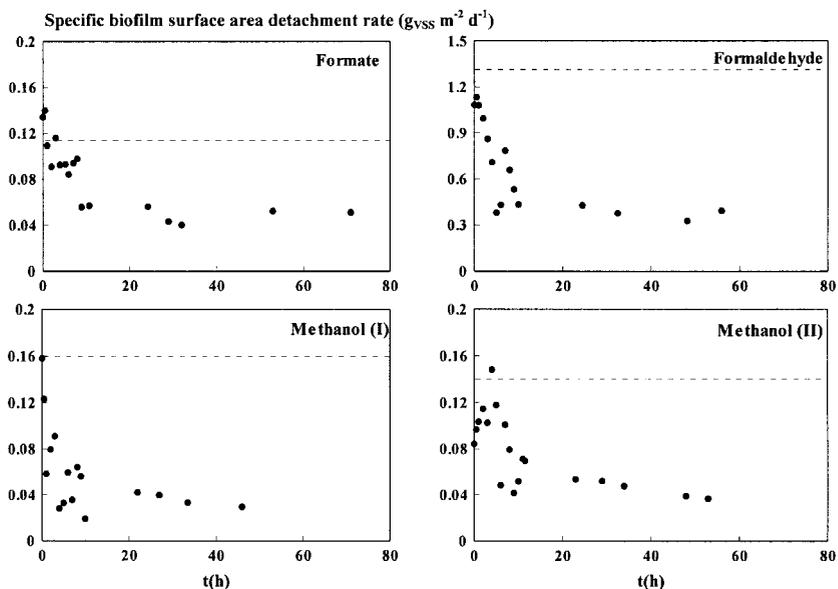
### Dynamics of biofilm formation

Biofilms were cultivated on three different C1 compounds with different degree of reduction: formic acid (0.35gCOD/g), formaldehyde (1.07 gCOD/g) and methanol (1.5 gCOD/g). In all experiments the basalt was suspended homogeneously in the reactor. After some days, the organic substrate was completely converted and biomass appeared in the effluent. Biofilms became visible after a few days. A real stationary state was never reached in the experiments. The results shown for each experiment (table 1) correspond to a dynamic period in which the biofilm density remained constant. Accumulation of biomass was always observed at a constant rate in each experiment, while the amount of suspended biomass in the effluent remained approximately constant (slightly decreased) during the entire experiment.

Formic acid was completely removed from the effluent after 14 days of operation. From this day, biofilms quickly developed in the reactor and the fraction of biofilm covered particles reached approximately 90% one week later and then remained constant. Biofilm diameter, surface area and solids hold-up increased in time, but shape factor remained constant. Not only biofilm covered basalt particles but also granules (biofilm particles without basalt inside) were observed. Good settling of biofilm particles and granules was observed in the three-phase separator.

In the second experiment, complete formaldehyde conversion was reached within two weeks. Foam appeared at the top of the reactor, which remained for more than five weeks. Many biomass granules appeared at the same time that biofilms developed (2–3 weeks after the starting day). The biofilms obtained were large (approximate diameter 2 mm) with various basalt particles inside. Biofilm size remained constant and also did biofilm shape. A quick change from bare basalt particles to fully biofilm covered particles was not detected in this experiment. The fraction of covered particles increased very slowly and the new biomass in the reactor was accumulated on bare basalt particles, so the diameter of biofilms remained constant but the biofilm surface area and solids hold-up increased in time.

Methanol was completely removed from the effluent in 12 days. Biofilm development became visible at day 20 and the basalt particles were nearly completely covered (90–95%



**Figure 2** Detachment rates after feeding was stopped with constant hydrodynamic conditions. Dashed lines indicate stationary detachment rate under growth conditions

fully covered particles) at day 45. Biofilm diameter, biofilm surface area and solids hold-up increased in time, and shape factor remained constant. This experiment continued for more than 100 days. After approximately day 80 the biofilm diameter and the solids hold-up reached very high values. The excessive biomass accumulation in the reactor, caused a clear decrease in the liquid flow velocity. The three-phase separator was nearly fully filled with biofilms and high amounts of were washed-out. During days 90–100 the biofilm density strongly decreased and a second, apparently less dense, outer biofilm layer appeared.

#### Detachment under non growing conditions

Figure 2 shows the results of the detachment experiments under non-growing conditions. The specific biofilm surface area detachment rate is plotted as a function of time. From the results it is clear that the overall detachment rate in the BAS reactor decreased drastically to a constant value, different from zero, after the substrate loading was put to zero. Two phases could be distinguished in the wash out of biomass. Initially, some biomass washed out, with large fluctuations in the amount. After some hours the wash out stabilised at a lower level remaining roughly constant until the end of the experiment. The experiments were finished when a constant or slowly decreasing detachment rate was measured. At that moment, less than 9% of the total biomass at the start of the detachment experiment was washed out in all cases.

#### Discussion

Experimental results shown in table 1 indicate that the use of C1 organic compounds with different level of reduction as the sole carbon source in synthetic wastewater influenced the biofilm structure. The influence of some factors, like solids hold-up, bare basalt concentration and the type of micro-organism developed on the biofilm structure, can be analysed in a qualitative way according to the results obtained.

### Biofilm density

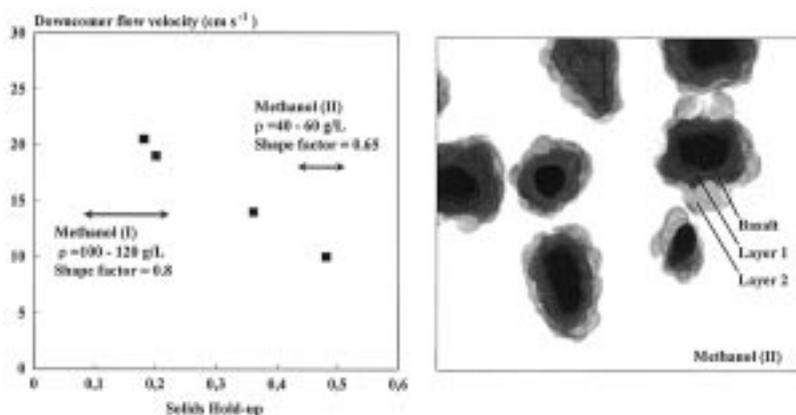
Biofilm density clearly depends on the kind of substrate used. An important difference was observed between the biofilms developed with formate ( $\rho=20\text{--}30\text{ gVSS L}^{-1}$ ) or formaldehyde ( $\rho=25\text{--}35\text{ gVSS L}^{-1}$ ), and the biofilms developed with methanol ( $\rho=100\text{--}120\text{ gVSS L}^{-1}$ ). This difference indicates that the biofilm density could be influenced by the degree of reduction of the substrate or the growth rate of the bacteria. The methanol degraders had a lower maximal growth rate and methanol is more reduced substrate than formate or formaldehyde. It means a higher requirement of diffusion of  $\text{O}_2$  per mol of substrate. This trend was also previously observed in the development of biofilms in BAS reactors using acetate or  $\text{NH}_4^+$  as substrates and using the same operating conditions (Tijhuis *et al.*, 1994; van Benthum *et al.*, 1996). The results show a possible trend, however more experimental work is needed in order to understand better the possible mechanistic relations.

### Effect of biomass hold-up

Biofilm biomass accumulation was detected in all experiments, which caused continuous solids hold-up increase. In Figure 3(a) the liquid velocity in the downcomer is plotted as function of the solids hold-up in the reactor when methanol was used as substrate, and a clear inverse relationship can be observed. In Figure 3(a) also the two periods mentioned in Table 1 are indicated. After the first period (days 40 to 75), the solids hold-up increased causing a lower liquid flow and less turbulence in the reactor. This led to a decrease in biofilm density and shape factor. An apparently less dense outer biofilm layer appeared (Figure 3(b)). It is supposed that this change was caused by a decrease in the shear forces, which is a consequence of the excessive solids hold-up and consequently lower liquid flow velocity in the reactor. A similar behaviour was reported by Tijhuis *et al.* (1995) for acetate grown biofilms. Because of the lower density, the solids hold-up increased more quickly leading to a further reduction in reactor turbulence. This eventually results in wash-out of large amounts of biofilm particles. So, from a practical point of view, the solids hold-up should be controlled, even when the operating conditions and the wastewater characteristics remain constant, in order to control the biofilm structure in a desired range.

### Stationary biomass detachment

The fraction of generated biomass that was detached during the biofilm formation was clearly higher with formaldehyde (85%) than with formate (50%) and methanol (56%). The



**Figure 3** Experiment with methanol. (a) Downcomer flow velocity as function of the solids hold-up in the reactor. (b) Two-layers biofilms developed as a consequence of lower shear forces

same difference can be observed in the specific biofilm surface detachment rate (Table 1). This difference could be due to the higher fraction of bare basalt present in the reactor in this experiment. This trend agrees with previous research (Gjaltema *et al.*, 1997c) which found a strong positive relationship between the biofilm detachment rate and the concentration of bare carrier particles. A higher concentration of bare basalt causes higher detachment rates and thereby a higher amount of biomass in the effluent. It is unclear why the biofilms in the case of formaldehyde did not fully cover all basalt. Until now no other substrate with such behaviour was found. The higher fraction of bare basalt will have had an impact on the biofilm density (Gjaltema *et al.*, 1997a; Kwok *et al.*, 1998). It is likely that when all basalt would get covered the biofilm density decrease to 20–25 g L<sup>-1</sup>.

#### **Biomass detachment without feeding**

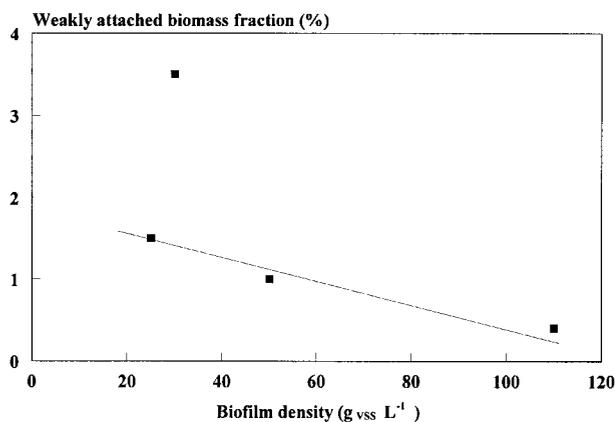
Figure 2 shows the specific biofilm surface detachment rates measured after the feeding of the reactors stopped, leaving all hydrodynamic conditions (i.e. shear rate) identical. From the results is clear that the overall detachment rate in the BAS reactor decreased drastically to a constant value, different from zero, after the substrate loading was put to zero. The mechanism actually involved in the detachment process observed during the experiments is not clear. It is evident that a major difference exists between the detachment rate of substrate fed or non-fed biofilms under the same hydrodynamic conditions. Tjihuis *et al.* (1995) proposed two different hypothesis to interpret the same measured phenomena: a gradient in strength in the biofilms or the initial removal of biofilm protrusions.

Once the substrate loading rate was put to zero, the detachment rate decreased but detachment did not disappear. Table 2 shows the ratio between the overall detachment rates under fed and non-fed conditions. It can be seen that the constant non-fed overall detachment rate is 45% (formate) to 20% (methanol) of the detachment rate under growth conditions. Clear differences between these ratios in the different experiments were not detected, and a clear possible relationship between these ratios and the different biofilm properties were not found. It is important to notice that in the case of formaldehyde, the relative decrease is the same despite higher shear forces due to a high bare basalt content. This implies that the difference in detachment rates under growth and non-growth conditions is more likely a characteristic of the biofilm than of the prevailing detachment forces.

Table 2 shows the amount of biomass that was quickly detached (12 h approximately) before the overall non-fed detachment rate was reached. According to the hypothesis proposed by Tjihuis *et al.* (1995), this amount of biomass could mean an external portion of the biofilm that is weakly attached. Therefore, the outer part of the biofilm could be much more susceptible to shearing and abrading forces than the inner parts of the biofilm. This is a very small fraction of the total amount of biomass, and it could be the part of the biofilm where growth mainly occurs. During the experiments, the weak outer shell of the biofilms could be sheared off during the first 10–15 hours. Thereafter stronger parts of the biofilms came under the influence of the same shearing and abrading forces resulting in a decreasing detachment rate. From the results shown in Table 2 a possible relationship between biofilm density and the weakly attached fraction of biomass in the biofilm was found. This relationship is shown in Figure 4 (line means trend only). One of the experimental data was not included in the general trend (experiment with formaldehyde) because of the strongly different shearing conditions. It seems that a more dense biofilm has a lower fraction of external biomass weakly attached. Biofilm volume and surface decreased due to the removal of this fraction. This decrease was higher in the case of less dense biofilms. Anyway, the maximum biofilm volume reduction measured was approximately 8% (experiment with formaldehyde) or 63  $\mu\text{m}$  (3%) in particle diameter reduction, which is not an important change in the solids hold-up. The thickness of the “rapid detachable layer” is in accordance

**Table 2** Results obtained from the detachment experiments

Substrate:	Formate	Formaldehyde	Methanol (I)	Methanol (II)
Overall detachment rate under non-growth conditions ( $\text{gVSS d}^{-1}$ )	0.50	0.58	0.22	0.29
Ratio between overall detachment rate ( $\text{gVSS d}^{-1}$ ) under non-growing and growing conditions	0.45	0.32	0.17	0.22
Amount of biofilm biomass quickly detached during the first 12 h in the detachment experiments (%)	1.5	3.5	0.4	1.0
Removal time of biofilm biomass under non-growing conditions ( $\text{d gbiofilm}^{-1}$ )	2	1.8	4.5	3.5

**Figure 4** Relationship between density and the weakly attached biofilm fraction

with the general depth as which substrate penetrates the biofilm. This would underline the assumption that the fast growing part of the biofilm is more susceptible to detachment than the slower growing part.

Table 2 also shows the theoretical time (expressed as time to remove 1 g of biomass in the reactor) needed for removal of attached biomass due to detachment under non growing conditions. In any case a large period of time is always needed. This is an important practical aspect in operation with BAS reactor. As an example, taking into account the total amount of biomass in the reactor, more than 300 days would be needed for the removal of the methanol-grown biofilms. The reactor can remain a large period of time without feeding, without a direct detrimental effect on the biofilms. Of course, at prolonged periods of non-feeding (weeks), biomass decay will also contribute to the “wash out” of the biofilms (Gjaltema *et al.*, 1997a).

## Conclusions

The biofilm structure clearly depends on the type of substrate. There seems to be a trend that more reduced substrates or substrates allowing a lower growth rate lead to more dense biofilms. However the trend in these experiments is too weak to generalise this at this moment. The observed effects of increased solids hold-up underline previous conclusions that decreasing detachment forces lead to less dense, more heterogeneous biofilms. The detachment experiments clearly show that detachment of a growing biofilm is significantly larger than for a non-growing biofilm under the same detachment forces. This fact should get more attention when detachment studies are performed.

## Acknowledgements

We are grateful to the University of Castilla-La Mancha that funded Mr. Villaseñor's visit to the Kluyver Laboratory.

## References

- Characklis, W.G. and Marshall, K.C. (1990). *Biofilms*. John Wiley and Sons, New York.
- Gjaltema, A., Vinke, J.L., van Loosdrecht, M.C.M. and Heijnen, J.J. (1997a). Abrasion of suspended biofilm pellets in airlift reactors: importance of shape, structure and particle concentrations. *Biotech. Bioeng.*, **53**(1), 88-99.
- Gjaltema, A., van Loosdrecht, M.C.M. and Heijnen, J.J. (1997b). Abrasion of suspended biofilm pellets in airlift reactors: effect of particle size. *Biotech. Bioeng.*, **55**(1), 206-215.
- Gjaltema, A., van der Marel, N., van Loosdrecht, M.C.M. and Heijnen, J.J. (1997c). Adhesion and biofilm development on suspended carriers in airlift reactors: Hydrodynamic conditions versus surface characteristics. *Biotech. Bioeng.*, **55**(6), 880-889.
- Heijnen, J.J., van Loosdrecht, M.C.M., Mulder, R., Weltevrede, R and Mulder, A. (1993). Development and scale-up of an aerobic biofilm air-lift suspension reactor. *Wat. Sci. Tech.*, **27**(5-6), 253-261.
- Kwok, W.K., Picioreanu, C., Ong, S.L., van Loosdrecht, M.C.M., Ng, W.J. and Heijnen, J.J. (1998). Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor. *Biotech. Bioeng.*, **58**(4), 400-407.
- Speitel, G.E. and DiGiano, F.A. (1987). Biofilm shearing under dynamic conditions. *J. Env. Eng.*, **113**(3), 464-474.
- Tijhuis, L., van Loosdrecht, M.C.M. and 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. and Heijnen, J.J. (1995). Dynamics of biofilm detachment in biofilm airlift suspension reactors. *Biotechnol. Bioeng.*, **45**(6), 481-487.
- Tijhuis, L., Hijman, B., van Loosdrecht, M.C.M. and Heijnen, J.J. (1996). 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-Fernández, J.M., Tijhuis, L., van Loosdrecht, M.C.M. and Heijnen, J.J. (1996). Formation and detachment of biofilms and granules in a nitrifying biofilm airlift suspension reactor. *Biotech. Progress*, **12**, 764-772.
- Van Loosdrecht, M.C.M., Eikelboom, D., Gjaltema, A., Mulder, A., Tijhuis, L. and Heijnen, J.J. (1995). Biofilm Structures. *Wat. Sci. Tech.*, **32**(8), 35-43.