CHEMICAL COMPOSITION AND ACTIVITY OF A BIOFILM DURING THE START-UP OF AN AIRLIFT REACTOR

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ABSTRACT

For the successful operation of a biofilm reactor, it is important to characterise the fixed biomass, its activity and composition. The purpose of this research is to monitor the biofilm characteristics of an airlift reactor, namely its composition in terms of exopolymers (polysaccharides and proteins), attached biomass, thickness, total proteins, as well as its activity in terms of substrate consumption rates. During the early phases of biofilm development, a high exopolymer production was observed, which helped in promoting the initial cell adhesion. The high turbulence with strong abrasion conditions might also trigger the excretion of those polymers. Low levels of extracellular proteins were detected, showing that the total proteins in the biofilm were mainly intracellular. The biofilm accumulation on the carrier was accompanied by an increase in biological activity; however, at the end of the experiment, the substrate consumption rates (surface reaction rate and specific removal rates) decreased, probably due to a reduction in the cellular density within the biofilm.

KEYWORDS

Airlift reactor, biofilm, biofilm composition, biofilm activity, *Pseudomonas fluorescens*

INTRODUCTION

Bacterial growth on surfaces is a phenomenon occurring in a high variety of environments, both in nature and industry. It is an immobilization process that can be exploited to human advantage, such as in bioreactors applied in wastewater treatment. Immobilization favours high biomass concentrations, and therefore high volumetric conversion rates.

In airlift reactors, biofilms are formed on small suspended particles such as basalt or sand grains, which are kept in circulation within the reactors by introducing an air stream. There are some important differences between biofilm formation in an airlift reactor and in conventional biofilm systems, such as, (i) relatively higher specific surface area, (ii) turbulent flow conditions, (iii) spherical biofilm geometry (Heijnen *et al.*, 1992). Due to the large carrier surface area in such a reactor (up to 2000 to 3000 m^2/m^3), high volumetric conversions can be obtained (Tijhuis *et al.*, 1994). Furthermore, high oxygen transfer rates are attainable due to the sparged air and the turbulent conditions in the reactor. This technology is thus potentially capable of achieving high active biomass concentrations in the reactor (Tijhuis *et al.*, 1992).

Biofilm dry mass is mainly composed by two components, namely, microbial cells and EPS (extracellular polymers). This polymeric matrix, often with a large polysaccharide content, is important to biofilm phenomena. The irreversible adhesion between bacterial cells and the support is greatly determined by the

physical properties of the macromolecules at the cell surface, the formation of bridges of extracellular polymers being responsible for anchoring the cells to the substratum.
For a stable and reliable operation of a biofilm airlift suspended reactor, a more detailed knowledge of the biofilm characteristics, such as its thickness, composition and activity is required. Since these properties are affected by the initial conditions of biofilm development, the purpose of this work is to monitor its characteristics during the start-up of an airlift reactor.

MATERIALS AND METHODS

Pseudomonas fluorescens, a gram-negative bacteria, was used as a biofilm producer in a concentric tube airlift reactor (Table 1 and Figure 1) containing basalt particles as support for biofilm attachment. All through the experimental run, a carrier concentration of 50 g/L was maintained in the reactor. The mean equivalent diameter and the density of the particles were 435μm and 3067 kg/m^3, respectively.

Air was sparged from the bottom of the inner tube of the reactor (riser) through a sintered glass filter. The superficial air velocity in the riser was 5.1 cm/s, corresponding to an air flow of about 4.5 L/min whereas the liquid superficial velocity was 0.26 m/s.

Table 1 - Dimensions of the airlift reactor

working volume (L)	total reactor height (m)	Riser internal diameter (mm)	downcomer internal diameter (mm)	riser height (m)
5.9	1.08	43	74	0.89

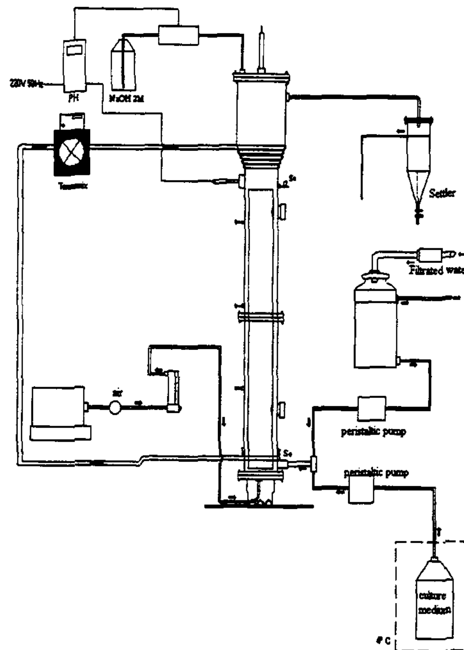

Figure 1 - Schematic representation of the airlift reactor

The temperature and pH in the reactor were maintained at 22°C and 7, respectively. The reactor was inoculated with a batch culture, the culture medium consisting of 5 g glucose/L, 8.75 g KH_2PO_4/L, 3.75 g K_2HPO_4/L, 1.25 $(NH_4)_2SO_4$/L and 0.125 g $MgSO_4.7H_2O$/L. At the beginning, the reactor was set in batch operation for two days, in order to promote the initial adhesion of microorganisms to the particles. Then, *continuous operation was started with an inlet glucose concentration of 53 mg/L and an initial dilution rate of* 0.1 h^{-1}, which is lower than the maximum growth rate of *Pseudomonas fluorescens* ($\mu_{max} = 0.30\ h^{-1}$, obtained in batch conditions). After this adjustment period, the dilution rate was stepwise increased to 1 h^{-1}.

In the course of the test, glucose in the influent and effluent streams was quantified using the GOD-POD method (Sera-Pack GOD/POD Method - Ames) in order to determine the surface glucose reaction rate (r_{fa}, mg of glucose consumed per unit time and unit of area of carrier) and the specific glucose removal rate (q_S, mg of glucose consumed per unit time and unit mass of volatile biofilm).

Freely suspended biomass in the effluent stream was determined as Total Suspended Solids (TSS). To determine the immobilized biomass, biofilm particles were removed from the reactor, dried at 103°C and afterwards, placed in a furnace at 550°C for 2.30 hours. Biofilm mass (volatile mass) was estimated as the difference between the dried and the burned mass. The mass of the support particles was determined by washing the burned sample several times with distilled water to remove ashes and then placing it again in an *oven to dry. Attached volatile biomass was expressed as* $g_{volatile\ biofilm}/g_{Basalt}$.

The exopolymers were extracted with glutaraldehyde, according to a method adapted from the one reported by Azeredo *et al.* (1998). Biofilm samples were placed in an Erlenmeyer flask with 20 mL of glutaraldehyde 3% and kept in an orbital shaken incubator (80 rpm) at room temperature for at least 2 days. Afterwards, the supernatant was filtered through a 0.20 μm filter and dialysed against distilled water during 3 days. On the other hand, the treated sample was then submitted to another extraction with glutaraldehyde. Thereafter, polysaccharides were determined by the method of Dubois *et al.* (1956) and extracellular proteins were estimated according to Lowry *et al.* (1951) method developed by Sigma (procedure 5656).

When determining total proteins, a sonication (Vibracell-model CV 600) process (power 30 W and 12 min in 20s cycles) was employed before applying the appropriate analytical method. The extracellular and total proteins and polysaccharides were expressed as $mg/g_{volatile\ biofilm}$.

When steady state was reached in the reactor, particle projected areas (A_2) and perimeters (P_2) of the biofilm covered particles were measured using an image analysis system. After being washed to remove nonadherent biomass, biofilm samples were observed by means of a lens and a camera connected to the computer system. The projected area and the perimeter of about 600 biofilm pellets were determined. Afterwards, these particles were placed in a furnace in order to remove the biomass. Then they were submitted to the same procedure to determine the corresponding projected area (A_1) and perimeter (P_1) without biofilm.

The following expression was used to estimate biofilm thickness (L): $L = \frac{A_2 - A_1}{\left(\frac{P_1 + P_2}{2}\right)}$ (1)

Removal of carrier material from the reactor due to sampling was compensated by adding new basalt particles.

RESULTS AND DISCUSSION

By applying a dilution rate of 1 h^{-1} (Figure 2), the growth of suspended biomass was prevented. Data from this figure clearly show an increase in biofilm volatile mass with time, finally attaining a roughly steady-state value. In this situation, the detachment rate should balance the growth rate of the biomass on the carrier and the amount of attached biomass remains fairly constant. This figure also indicates a decrease in suspended biomass concentration in the effluent with time, as expected. At high dilution rates ($D \geq 0.5\ h^{-1}$), the suspended biomass can be regarded as recently detached biofilm mass. Biofilm development in such a system is strongly

influenced by liquid shear and abrasion, the latter being caused by particle-particle and particle-reactor wall interactions. When a higher number of bare carrier particles become covered with biofilm, their surfaces become smoother (no sharp edges) and the impact of the abrasion mechanisms will be lower, reducing biofilm detachment.

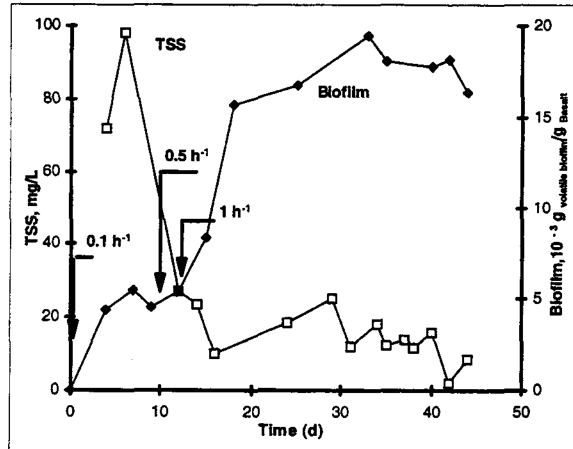

Figure 2 - Biofilm accumulation on the carrier and suspended biomass concentration (TSS) in the effluent

Image analysis measurements (A_1=0.2243 mm^2; A_2=0.2912 mm^2; P_1=1.7629 mm; P_2=1.9912 mm) showed an average steady-state biofilm thickness of about 36 μm. This value indicates that, in spite of the glucose being easily degradable by the cells, the strong hydrodynamic forces in the reactor seem to be the prevailing mechanism in the biofilm formation, resulting in a very thin biofilm.

Figure 3 indicates an increase in biofilm extracellular proteins and polysaccharides at the beginning of the experiment, decreasing with time and finally attaining a lower and more stable value, around day 20. The high extracellular polymer production in the initial period of biofilm formation corresponds to the initial cell adhesion, in particular when the carrier surface is only partially covered by isolated microcolonies. Furthermore, at the beginning, with higher shear stress and abrasion levels, those polymers can play an important role in biofilm cohesion. Since the abrasion effect of particle collisions will diminish during the run, the exopolymers production per unit mass of volatile biofilm will tend to decrease.

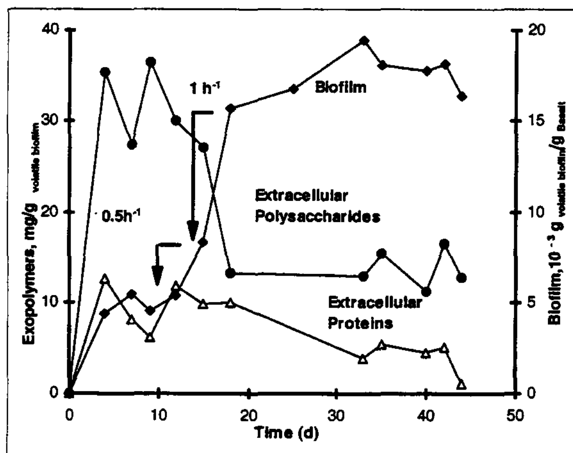

Figure 3 - Extracellular polysaccharides and proteins as function of time of operation

The total protein content (Figure 4) is much higher than the extracellular proteins, which means that biofilm proteins are mainly intracellular, in the present case.

It should be noted that until day 10 (i.e., before the dilution rate was increased up to 0.5 h^{-1}), the substrate consumption in the reactor was due not only to the biofilm, but also to the significant amount of suspended biomass (Figure 5 and 2).

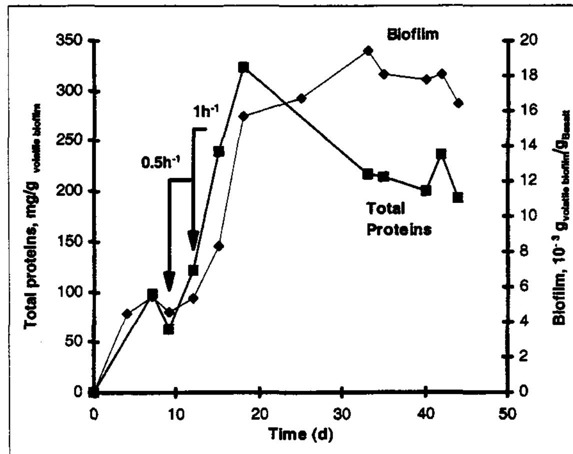

Figure 4 - Total proteins as function of time of operation

Between days 10 and 16-18, the substrate consumption rates continued to rise (Figure 5), meaning that the number of active cells on the support also increased. This is in agreement with the data in figure 4, showing an increase in total proteins (related to the mass of cells). As reported by other authors (Tijhuis *et al.*, 1994), three stages of the biofilm formation were visually observed in this run: first, only the bare carrier, then some microcolonies or "patchy" biofilm on the carrier surface and, finally (after about 20 days), a continuous biofilm surrounding the support. Therefore, the increase in the consumption rates was probably the result of the spreading of new colonies on the carrier surface.

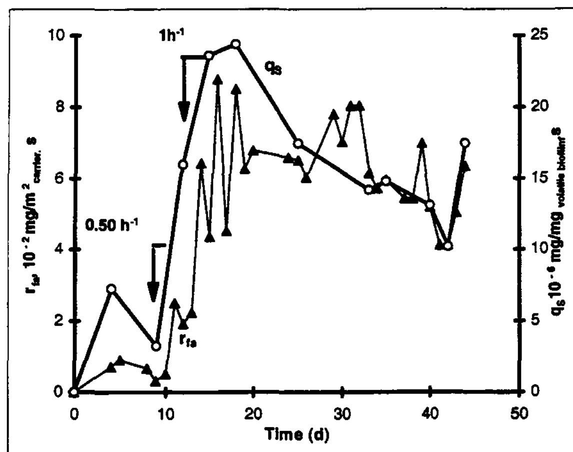

Figure 5 - Surface glucose reaction rate (r_{fa}) and specific glucose removal rate (q_S) as function of time of operation

Figure 5 presents a decrease in the consumption rates after days 16-18. Since in such a thin biofilm (36 μm) the existence of inactive zones is not expectable, that decrease can be explained by a reduction in the cellular density within the biofilm. In fact, at the beginning, the biofilm is basically constituted by cells and some polymers, which means that almost all the attached biomass may produce new cells and exopolymers. After this initial phase, only a fraction of the biofilm mass (the cells) can produce new cells and exopolymers (these are not biologically active), leading to a decrease in the cellular density with time. In fact, using total proteins (approximately intracellular proteins, in the present case) to assess the amount of cells, a decrease in cell content in the biofilm after an initial period of development can be detected (Figure 4) which confirms the proposed reduction in cellular density. This cellular density decrease results in lower consumption rates (specific substrate uptake rate and surface glucose reaction rate) (Figure 5). Moreover, the application of a diffusion-reaction model to this biological film (Lopes, 1997) confirms the existence of a completely penetrated biofilm (Figure 6), as expected, and demonstrates that the reaction rate inside the film is of zero order.

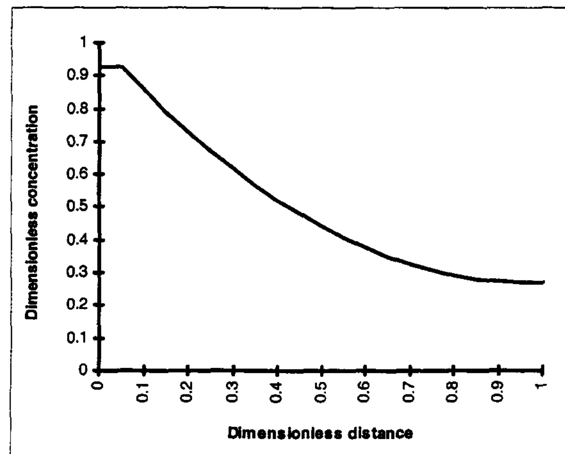

Figure 6 - glucose concentration profile predicted by a diffusion-reaction model

CONCLUSIONS

The following main conclusions are:

1 - The high exopolymer production detected in the early phase of biofilm formation is appropriate for promoting cell adhesion and biofilm cohesion in high shear stress and abrasion conditions.

2 - From the results, it could be concluded that biofilm proteins were mainly intracellular.

3 - Biofilm development results in an increase of its activity, namely, surface glucose reaction rate (r_{fa}) and specific glucose removal rate (q_S), followed by a decrease, at the end of the experiment. This decrease was explained by a reduction in the cellular density inside the biofilm instead of a loss of biological activity, as confirmed by the substrate concentration profile determined by a diffusion-reaction model applied to the data.

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