Biofouling, 1993, Vol. 7, pp. 67–80
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BIOFILM FORMATION: HYDRODYNAMIC EFFECTS ON INTERNAL DIFFUSION AND STRUCTURE

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(*Received 2 March 1992; in final form 4 January 1993*)

Diffusion in microbial films produced by *Pseudomonas fluorescens* under turbulent flow conditions was studied using an inert substance (LiCl). Mass transfer coefficients in the biofilm were measured during formation of the biological deposits and for biofilms developed under different fluid velocities. Mass transfer rates in the biofilm decreased with time, and more quickly in the case of biofilms subjected to high shear stresses. The latter show lower final thicknesses and lower internal diffusivities. The so-called "active layer", if it exists, does not seem to have a fixed thickness (as proposed by some authors), since it will depend on the environmental conditions, particularly on fluid velocities.

KEY WORDS: mass transfer coefficient, internal diffusion, biofilm structure, shear stress effects.

INTRODUCTION

Biofouling, like other types of fouling, constitutes a serious problem in the performance of heat exchange equipment. However, adhesion, growth and maintenance of biofilms are essential to the efficiency of bioreactors used in the purification of polluted waters. In both cases, a deeper knowledge of the fundamental mechanisms of biofilm formation and activity is needed. The build-up of unwanted microbial deposits is believed to proceed by the following steps (Characklis, 1981): (1) formation of an organic conditioning film on the surface; (2) transport of microorganisms from the fluid to the surface; (3) adhesion of microorganisms at the solid-fluid interface; (4) substrate transport and its consumption in the biofilm, resulting in the replication of the attached cells and in their production of exopolymers; (5) removal (detachment) of parts of the biofilm as a consequence of the shear stress exerted by the flowing fluid.

Usually, steps (1) to (4) favour the development of the microbial layer, unless there are problems of inhibition by substrate or by products, while step (5) is an adverse process as far as biofilm growth is concerned. The competition between favourable and unfavourable mechanisms results in a sigmoidal development curve that eventually reaches a plateau (maximum biofilm amount), where the overall rate of steps (1) to (4) is equal to the removal rate (step 5). Removal or detachment may be caused by fluid shear forces and also, in non-laminar flows, by "upsweeps" associated to the phenomena of turbulent bursts (Cleaver & Yates, 1973).

Results published by several authors indicate that, for a given microbial deposit, the detachment rate increases as the biofilm grows, that is, as its mass or thickness increases (Characklis, 1981; Trulear & Characklis, 1982). Peyton and Characklis (1992) studied films produced by *Pseudomonas aeruginosa* and suggest that the detachment rate can be modelled as a first order function of the biofilm thickness. *E. coli* films obtained by Bott and Pinheiro (1977) were clearly thinner at a fluid Reynolds number (Re) of 15 300 than

at Re = 6300. Chang *et al.* (1991) studied biofilm detachment in a liquid-fluidized bed and concluded that increased liquid velocity produced denser and thinner films. The same trend was observed by Pinheiro *et al.* (1988a) for biological deposits formed by *Pseudomonas fluorescens*. Siegrist and Gujer (1985) also found that biofilm density decreased slightly with increasing thickness. On the other hand, Bakke *et al.* (1984) did not detect any effect of shear stress on the thickness or density of the biofilm; these results were obtained in pure laminar flow, with Reynolds numbers between 5 and 30. Also, data presented by Characklis (1981) show that the effect of shear stress on the maximum (plateau) biofilm thickness may not be significant when the substrate loading is low.

Fluid velocities in heat exchangers are, in general, considerably greater than in wastewater bioreactors (typically, 0·3–3 $m \cdot s^{-1}$ *vs* 0·01–20 $m \cdot d^{-1}$) and turbulent regime is the prevailing flow situation. Therefore, hydrodynamic effects tend to be more significant in the first case, affecting the physical and biological structure of the biolayer. In such circumstances, the effects of fluid velocity on biofilm formation may be summarized as follows: (a) higher fluid velocities favour the transport of cells and substrate to the surface, contributing to the growth and replication of cells in the microbial layer and to the production of exopolymers; (b) higher fluid velocities make microbial adhesion more difficult and, at least in turbulent flow conditions, increase the rate of biofilm detachment.

Another effect of fluid shear stress may also be considered. It is thought (Pinheiro *et al.*, 1988b) that, as the biofilm develops, mass transfer rates of nutrients through the microbial layer will depend not only on the film thickness but also on the structure conferred on the deposit by the hydrodynamic conditions. For thick biofilms, the diffusion of nutrients may render inner layers biologically inactive (Capdeville *et al.*, 1988). On the other hand, if the biofilm is thin due to high shear stresses, it can also be more "compact" or more dense and the rate of substrate internal diffusion may still be limiting. There is a need to clarify the relationship between biofilm structure, substrate diffusion and fluid dynamics. This kind of information will be useful not only in controlling biofouling in heat exchangers but also in designing and operating bioreactors, mainly in the optimization of biofilm thickness, in order to improve the overall rate of diffusion and degradation of substrates.

MATERIALS AND METHODS

Bacterial Strain

The microorganism used to produce biofilms was *Pseudomonas fluorescens*, a Gram-negative aerobic bacterium present in natural waters. Previous studies revealed this bacterium to have a high capacity for adhesion to metallic surfaces under operating conditions similar to those used in the present work (Vieira *et al.*, 1992).

Experimental System

Biofilm formation was monitored through heat transfer and mass transfer measurements carried out in vertical test sections inserted in a continuous flow system (Fig. 1).

A pure culture of *P. fluorescens* was grown in a 4 l fermenter (fermenter 1), continuously fed with a sterile concentrated nutrient solution consisting of glucose (0·5% (w/v)), peptone (0·25% (w/v)) and yeast extract (0·125% (w/v)). The culture was

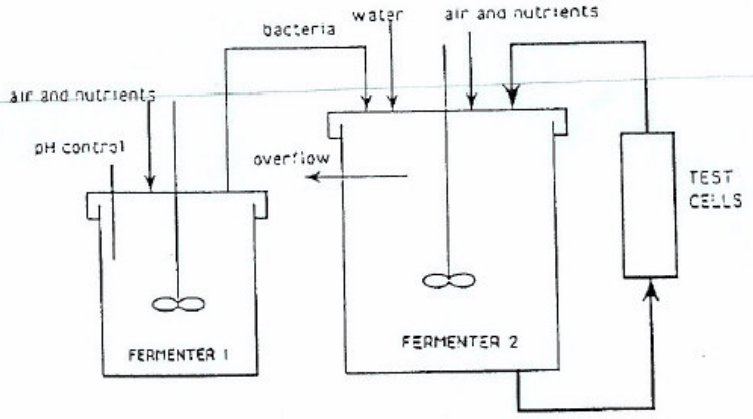


Fig. 1 Continuous flow experimental system.

aerated, kept at 27°C, and at pH 7, the latter being controlled by the addition of 2M NaOH.

In the heat transfer tests, a 25 l mixing vessel (fermenter 2) was fed with water at a flow rate of 20 $l \cdot h^{-1}$. The mixing vessel (fermenter 2) used in the mass transfer tests had a volume of 12 l and was fed with water at 10 $l \cdot h^{-1}$. In both cases, bacteria coming from fermenter 1 were inoculated in the mixing vessel in order to obtain a suspension with 6×10^7 cells $\cdot$ ml^{-1}. The glucose concentration in both mixing vessels was 20 ppm, maintained by means of constant addition of the sterile concentrated nutrient, at a flow rate of 200 $ml \cdot h^{-1}$ (heat transfer tests) or 100 $ml \cdot h^{-1}$ (mass transfer tests). An overflow stream coming out from this vessel kept the liquid level constant. The temperature was 27°C and the pH was 6·5–7·0. The residence time in each of the mixing vessels was 72 min.

The biological suspension was pumped up, passing through the test sections (heat or mass transfer cells) and back to the mixing vessel. The total volume of the system comprising fermenter 2, tubes and test sections was 33 l and 16 l for the heat transfer and the mass transfer measurements, respectively.

The Heat Transfer Test Section

Heat transfer experiments were carried out in order to measure the thermal resistance of the biological deposits (R_f) as a function of time and fluid velocity. The heat transfer test sections were mounted vertically and made of 50 cm long semi-circular perspex ducts with aluminium deposition surfaces. Two similar ducts were used with hydraulic diameters of 2·2 cm and 2·06 cm (Figure 2). One face of the aluminium plate was in contact with the biological fluid, while the other was in contact with a perspex wall, which is a part of a rectangular cross section duct through which water circulated at 60°C. A thermal grease of high conductivity was spread between the aluminium and perspex surfaces to reduce thermal contact resistances. The heat transfer area of the deposition plate was $0{\cdot}99 \times 10^{-2}$ m^2.

To measure the heat flux between the warm water and the biological fluid, two thermocouples (T_1 and T_2) were imbedded in the perspex wall, while a third (T_3) was

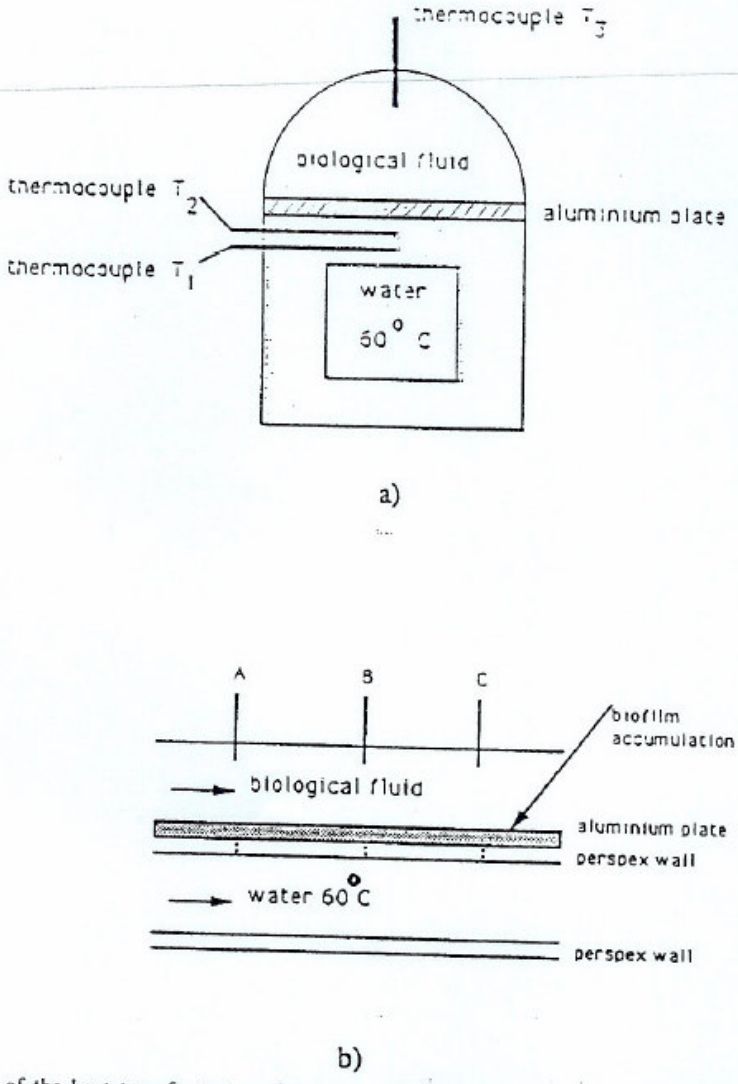


Fig. 2 Detail of the heat transfer test sections. (a) = transversal cross section; (b) = longitudinal cross section.

immersed in the biological suspension that circulated in the semicircular duct. The heat flux was measured in three different axial positions (A, B and C) along the plate (Figure 2b).

Biofilms were formed under different Reynolds numbers, ranging from 7800 to 15 500 (fluid velocities = 0·34 m · s^{-1} to 0·61 m · s^{-1}). The heat flux between the two fluids was determined six times a day from temperature measurements. The pressure drop along the plate was determined using a differential manometer. The residence time of the biological fluid in the test sections was less than 1·5 s, in all cases.

Evaluation of Biofilm Thermal Resistances

The overall heat transfer coefficient at any instant of time (U) was calculated by:

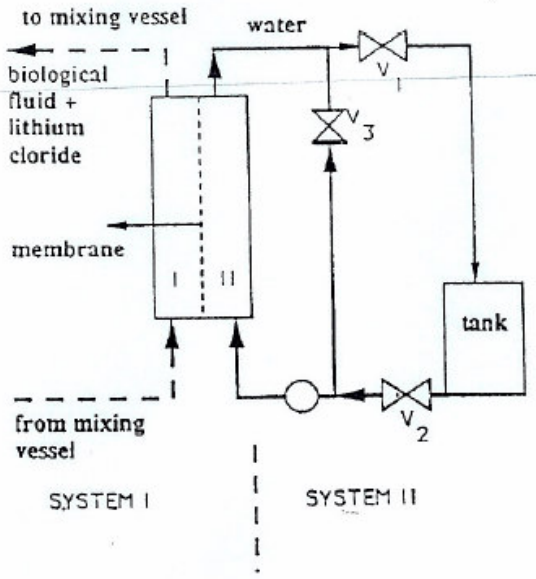


Fig. 3 Mass transfer system, including test section (membrane cell).

$$U = \frac{k_w}{y_w} \frac{(T_1 - T_2)}{(T_1 - T_3)} \qquad \text{(Eqn 1)}$$

and the thermal resistance of the biofilm by (Pinheiro *et al.*, 1988a):

$$R_f = \frac{1}{U} - \frac{1}{U_o} - \frac{1}{h_o}[(f_o/f)^p - 1] \qquad \text{(Eqn 2)}$$

k_w is the thermal conductivity of the wall containing thermocouples T_1 and T_2 and y_w is the distance between these two thermocouples. U_o and h_o are, respectively, the overall and the convective heat transfer coefficient with the clean surface (no biofilm). f and f_o are the fraction factors measured with the fouled and clean surfaces, respectively. Parameter p was estimated using the correlation proposed by Norris (Kays & Crawford, 1980), *viz.* $p = 0{\cdot}68.Pr^{0{\cdot}2\overline{15}}$, where Pr is the Prandtl number of the test fluid. Values of h_o for different Reynolds numbers had been previously obtained by conventional heat transfer methods based on the "Wilson plot" (Knudsen, 1981). The effect of biofilm roughness could thus be taken into account in Equation 2. An average value of U was calculated from each of three measurements, therefore obtaining two values of R_f per day in each of the positions A, B and C. These values were plotted as a function of time, yielding the so-called "biofouling curves" (see Results).

The Mass Transfer Test Section

The mass transfer experiments were carried out in a cell with a semi-circular geometry identical to those used in the heat transfer experiments (hydraulic diameter = 2·06 cm) (Fig. 3). This test section is also vertical and has two compartments (I and II) separated

by a 10 cm long membrane of 0·22 μm pore diameter that does not allow bacteria to pass through but is permeable to solutes. The mass transfer area of the membrane was $0{\cdot}21 \times 10^{-2}$ m^2. The biological suspension flowed across compartment I and the biofilm built up on the membrane. Water similar to that used in the microbial suspension circulated in compartment II. Pressure drop along the test section was measured by means of a differential manometer.

Diffusion through the biofilm was studied using an "inert" compound (lithium chloride) which is not consumed by the bacteria. A fixed amount of LiCl was introduced in the mixing vessel and the lithium concentration was kept constant (50–100 ppm) by a continuous input of LiCl solution in the flow system connected to compartment I of the mass transfer cell. Tests were carried out with the biological suspension circulating at different Reynolds numbers in the turbulent flow regime (Re = 8250 to 14 700), corresponding to fluid velocities between 0·34 and 0·61 m · s^{-1}.

Compartment II is part of a circuit (system II) that includes as 20 l tank of water (Fig. 3). The velocity of the water flowing across compartment II was such that a pressure balance was maintained across the membrane. When the LiCl concentration reached a steady-state value in compartment I, valve V_3 was opened and valves V_1 and V_2 were closed. The volume in system II was then reduced to 790 ml, but the velocity in compartment II was maintained. Lithium started to diffuse through the biofilm and the membrane, and to accumulate in system II. Samples of 5 ml were taken at known time intervals and analysed in a flame photometer. After 1 h, the experiment was finished by stopping the input of LiCl into the mixing vessel of system I. Valves V_1 and V_2 were opened and value V_3 closed. The water containing LiCl was collected and water started to circulate in the original system II.

This 1 h experiment was carried out without biofilm, before starting-up the biofouling test, and with biofilm at different stages of formation (different days). The whole procedure was repeated for other tests at different fluid velocities.

Separate batch tests were made in order to determine possible effects of LiCl on the biological activity of *P. fluorescens*. Specific growth rates were found to be the same in the absence of LiCl and in the presence of 1000 ppm of this substance.

Evaluation of Mass Transfer Coefficients

For each biofouling test (each fluid velocity), the overall mass transfer coefficient can be calculated at different stages of biofilm development by measuring the change in lithium concentration with time in the two compartments of the cell. If C_I and C_{II} are the lithium concentrations in compartments I and II, respectively, V is the volume of system II and A is the mass transfer area, a mass balance can be made, resulting in:

$$V \frac{dC_{II}}{dt'} = - k_T A (C_I - C_{II}) \qquad \text{(Eqn 3)}$$

where k_T is the overall mass transfer coefficient that includes external and internal resistances (fluid, membrane and biofilm resistances), and t′ is the time during which lithium accumulates in system II. Upon integration, Equation 3 yields:

$$C_I - C_{II} = [C_I - C_{II}(t' = 0)] \exp(- k_T A\, t'/V) \qquad \text{(Eqn 4)}$$

Experimental data ($C_I - C_{II}$ versus t′) were correlated according to Equation 4 using 7 or more values of C_I, C_{II} and t′ obtained over 1 h. A value of k_T was then calculated, representing the overall average mass transfer coefficient during measurement time

(1 h). For each of these 1 h measurements, the mass transfer coefficient in the biofilm (k_b) could be calculated from:

$$\frac{1}{k_b} = \frac{1}{k_T} - \frac{1}{k_T(t=0)} \qquad \text{(Eqn 5)}$$

where $k_T(t=0)$ is the initial overall mass transfer coefficient, when the biofilm was not yet formed on the membrane.

During each biofouling test, the above procedure was repeated, usually once a day, and values of k_T (overall coefficient) and k_b (biofilm coefficient) could be obtained at different phases of biofilm development, that is, for different thicknesses of microbial layer. Therefore, a series of "k_b *vs* time" curves could be obtained under different hydrodynamic conditions. It should be noted that such values represent average mass transfer coefficients for the whole deposit, since there may be changes in the diffusivity throughout the biofilm.

Measurement of Biofilm Thickness and Density

In some of the experiments, the final thickness and density of the microbial films were determined. Thickness measurements were carried out (for dry and wet biofilms) with a micrometer-based device similar to the ones described by Harty and Bott (1981), Melo and Pinheiro (1984) and Pinheiro *et al.* (1988a). Since the area of the deposition plates was known, the volume of the biofilm could be evaluated.

Densities were determined by weighing the plates with and without biofilm in an analytical balance.

RESULTS

Effects of Fluid Velocity on Biofilm Thermal Resistance, Thickness and Density

The "biofouling curves" in Figure 4 represent the average values of R_f determined in positions A, B and C of the heat transfer test section. The curves are representative of the effects of time and Reynolds number on biofilm formation. As expected, biofilm thermal resistance increases with time and the final amount of biofilm (R^{∞}_f, the maximum value of R_f) decreases as the fluid velocity increases.

Values of the asymptotic thermal resistance are given in Table 1 for several Reynold numbers. Other values, obtained also with *P. fluorescens* biofilms in test sections with the same geometry but different diameters, have been published elsewhere (Pinheiro *et al.*, 1988a). The conclusions are the same in all cases. The final thickness of the microbial films was also found to decrease with increasing velocity, as shown in Table 2. Conversely, higher densities were obtained for films formed under higher velocities. The data shown in Tables 1 and 2 confirm that the thermal resistance of the biolayer decreases when its thickness decreases.

The biofilms were observed to have a "wavy" surface with thicknesses that differ about 250 μm between the "valleys" and the "peaks" of the deposit.

An additional experiment was carried out to study the effect of fluid velocity on the relative cohesive strength (sometimes called mechanical resistance) of the microbial deposits. Two different fluid velocities (0·36 $m \cdot s^{-1}$ and 0·61 $m \cdot s^{-1}$), corresponding to Reynolds numbers of 9200 and 15 500, were tested in the heat transfer sections. The two

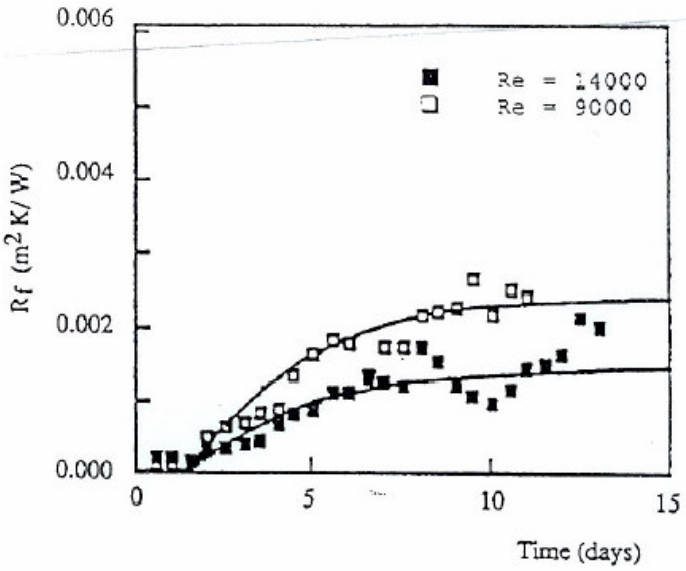


Fig. 4 Typical biofouling curves.

Table 1 Asymptotic thermal resistances *vs* Reynolds numbers

Reynolds number	7800	9000	9200	13 100	14 000	15 500
Hydraulic diameter of the test section (cm)	2·06	2·2	2·2	2·06	2·2	2·2
Fluid velocity ($m \cdot s^{-1}$)	0·34	0·35	0·36	0·54	0·55	0·61
$R^{\infty}_f \times 10^4$ ($m^2 K \cdot W^{-1}$)	25	23	24	16	14	12

Table 2 Effect of fluid velocity on biofilm thickness and density

Reynolds number	7800	13 100
Fluid velocity ($m \cdot s^{-1}$)	0·34	0·54
Average thickness of wet biofilm (μm)	710	500
Average thickness of dry biofilm (μm)	170	120
Density		
kg of dry biofilm · m^{-3} of dry biofilm	50	76
kg of dry biofilm · m^{-3} of wet biofilm	14	21

biofilms built up until they reached the "plateau", that is, the asymptotic thermal resistance. Afterwards, the velocity in the two test sections was steadily increased to a common value ($1 m \cdot s^{-1}$), corresponding to a shear stress of $10 N \cdot m^{-2}$. This means that two "stable" films, formed under different hydrodynamic conditions, were subjected to higher shear stress than they had experienced during their development.

The results can be seen in Figure 5. The thermal resistance of the biofilm formed at lower velocities was substantially reduced, while the other biofilm resisted the increase in shear stress. Significant amounts of microbial film were observed to detach in the first case. Therefore, it can be said that the cohesive strength is considerably higher in the case of the biofilm formed at higher shear stress.

Mass Transfer Coefficients in the Biofilm; Effects of Time and Fluid Velocity

Table 3 presents values for the overall mass transfer coefficient of an "inert" substance (LiCl) diffusing through the membrane of the mass transfer cell, at time $t=0$, for tests performed at three different Reynolds numbers. This coefficient includes the clean membrane and the fluid resistances to diffusion.

The overall mass transfer coefficient decreased with time during biofilm formation, as shown in Figure 6. When the biofilms reach, or approach, their maximum amount ($t\rightarrow\infty$), the overall mass transfer coefficients are not substantially different for the several fluid velocities.

Using the data shown in Figure 6 and Table 3, values of the mass transfer coefficients in the biofilm (k_b) were calculated through Equation 5. These values, plotted in Figure 7, represent the reciprocal of the average mass transfer resistance in the microbial layer and show a trend similar to the one observed in the case of the overall mass transfer coefficients.

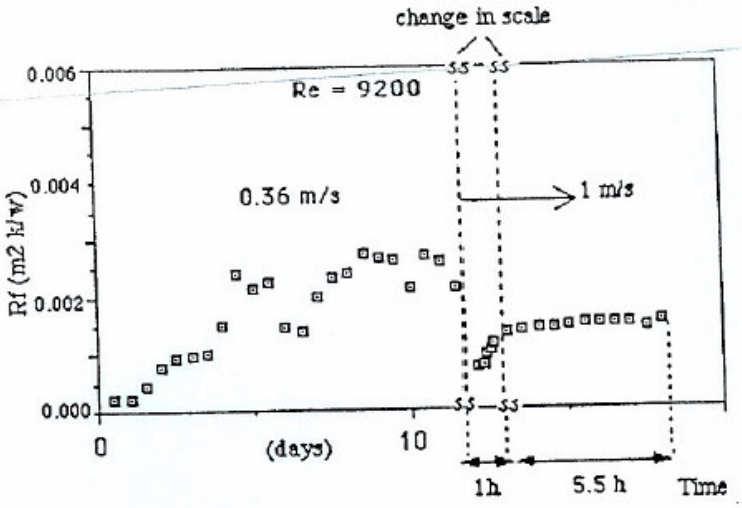
As in the heat transfer tests, the effect of roughness on the mass transfer coefficients was evaluated, but was found to be negligible. This is due to the relatively high values of the external mass transfer coefficients of the turbulent fluid (3 to 5 times higher than the values of $k_T(t=0)$ in Table 3) that mask the effect of increased roughness.

DISCUSSION

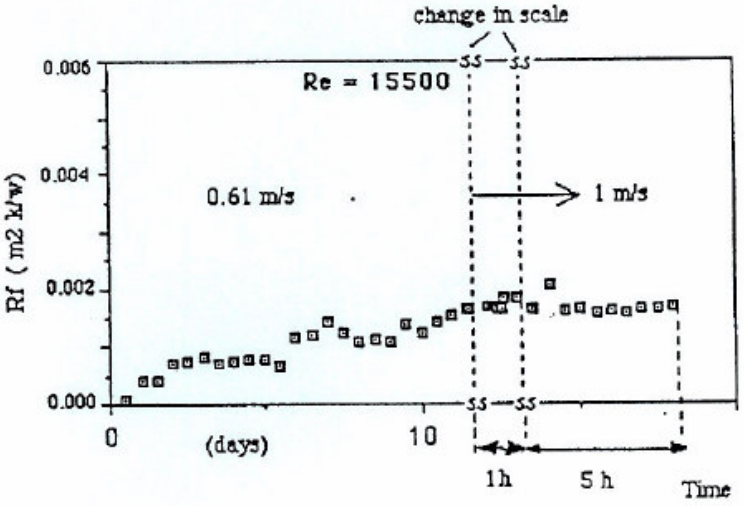
From the results presented in Figure 4 and Tables 1 and 2 it can be concluded that both the final thickness and the final thermal resistance are lower when biofilms are formed under higher shear stresses (in turbulent flow). Figure 7 shows that the internal mass transfer coefficients at steady state ($t\rightarrow\infty$) are not significantly different for biofilms subjected to different hydrodynamic conditions, within the range of fluid velocities studied.

The biofilm mass transfer coefficient can be interpreted as the quotient between internal diffusivity and thickness. Therefore, in the case of microbial films grown under higher shear stresses (thinner biofilms), the average diffusivity in the biolayer will be lower (at $t\rightarrow\infty$) than in the case of biofilms grown under lower shear stresses. The structure of the biofilm appears to be more compact (higher density) when the biofilm is subjected to stronger fluid shear forces, and, as a consequence, internal diffusion becomes more difficult. This may be interpreted as a natural response of biological mechanisms to an unfavourable situation; the bacteria reinforce the extracellular polymeric matrix thus protecting themselves against the more aggressive hydrodynamic conditions of the surrounding fluid. The increase in biofilm density (Table 2) and cohesive strength (Fig. 5) with fluid velocity supports this hypothesis.

Droste *et al.*, (1990), when investigating the initial build-up of anaerobic biofilms at low fluid velocities (0·05 to 1·5 $cm \cdot s^{-1}$), observed the formation of "attached" and "loose" biofilms, the latter resulting from weakly bound layers at the film surface. These authors suggest that the loose microbial aggregates are an "intermediate stage



a)



b)

Fig. 5 Application of the same shear stress (10 N · m⁻²) to two biofilms formed under different fluid velocities. (a) = 0·36 m · s⁻¹; (b) = 0·61 m · s⁻¹.

Table 3 Overall mass transfer coefficient without biofilm, $K_T(t=0)$, for different Reynolds numbers (Re)

Re	*Fluid velocity* ($m \cdot s^{-1}$)	$k_T(t=0) \times 10^6$ ($m \cdot s^{-1}$)
8250	0·34	5·2
9805	0·40	6·6
14 700	0·61	7·8

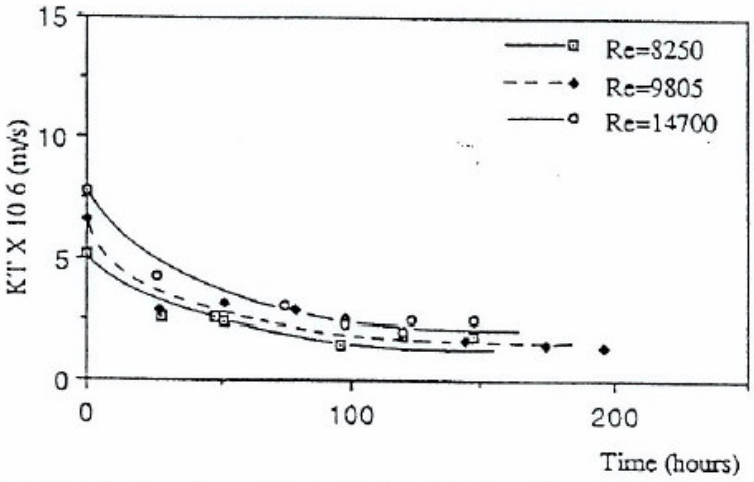


Fig. 6 Overall mass transfer coefficient of LiCl as a function of time and Reynolds number.

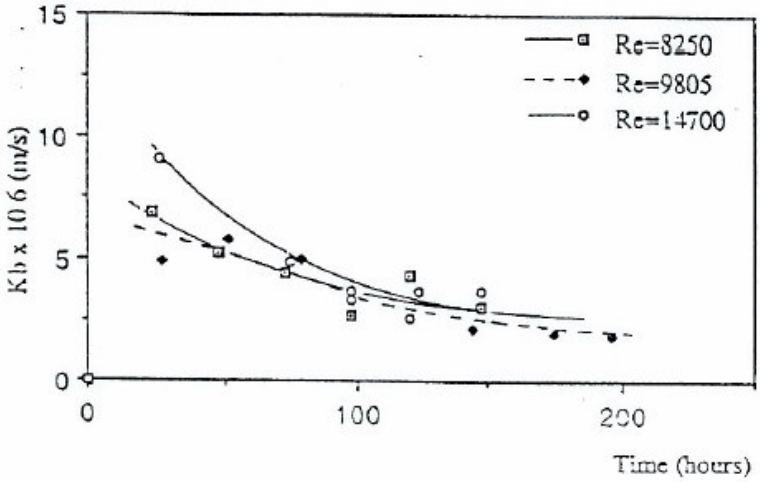


Fig. 7 Mass transfer coefficients of LiCl in the biofilm as a function of time and Reynolds number.

between suspended biomass and attached biomass" in low velocity biofilm systems. "Loose" biofilm was collected from the liquid adjacent to the "attached" biofilm, and the amount was found to be inversely related to the fluid velocity.

Siegrist and Gujer (1985) also observed a more compact layer near the deposition surface and a light structure with filaments at the top of biofilms formed in a stirred reactor. It was assumed that, although molecular diffusion predominates in the compact zones, eddy diffusion may become important in the more "open" zones. Therefore, in cases where the latter occupy a major part of the biofilm, the average apparent diffusivity will be relatively high. Siegrist and Gujer did not report values of shear stresses or Reynolds numbers in their paper although, as confirmed by the present results, the hydrodynamic environment appears to play a decisive rôle in determining biofilm properties. In their case, eddy diffusion effects can be of importance in the upper layers of the biofilm, whereas in the case of Droste *et al.*, (1990) eddy diffusion was not present since the flow was laminar.

It was not possible in the present work to observe or to measure significant amounts of loose biofilm in the liquid near the attached layers or to have pictures of layers of different compactness within the biofilm. However, in similar hydrodynamic conditions, the existence of inner "hard" layers and "loose" outer layers has been identified in inorganic deposits (Taborek *et al.*, 1972; Melo & Pinheiro, 1986) and the outer layers found to be easily removed by the liquid flow. Melo and Pinheiro (1986) determined that the fraction of the loose layer in the inorganic deposit decreased with an increase in the fluid velocity, which is in accordance with the results of Droste *et al.*, (1990) for anaerobic biofilms. If it is assumed that a similar kind of structure exists in the biofilms produced by *P. fluorescens*, then it can be said that, under high shear stresses, the compact layers will predominate over the loose layers, leading to lower molecular diffusivities.

The structure of biological deposits and their relationship to the internal diffusion of substrates is also dependent on other factors, such as the nature of the fluid and of the microbial species. This was confirmed, for different fluids, by Siegrist and Gujer (1985) who observed biofilms where the compact zone extended more or less over the entire film (low average diffusivities) and by Applegate and Bryers (1991) who found that biofilms subjected to either carbon or oxygen limitations had different resistances to shear stress.

It is sometimes considered that the penetration of the substrate in the biofilm is limited to a certain distance (the "penetration depth") which determines the thickness of the so-called "active layer", the part of the biofilm that is actively consuming the nutrients and contributing to the growth of the biological deposit. Some authors (*e.g.* Kornegay & Andrews, 1968) suggest that the active layer has a thickness of 70 μm with the microbial species in the remaining part of the biofilm ("inactive" or "deactivated" layer) being limited to residual activities, such as enzymatic reactions without cell growth and reproduction or exopolymer production (Capdeville *et al.*, 1988). The present results indicate that, if such an active layer (or active cells) exists, its thickness will probably vary with the ambient conditions, particularly with the shear stress under which the biofilm is formed. The use of microelectrodes could be very helpful in confirming this statement, since they allow the measurement of concentrations in different positions within the biofilm.

Two further points should be stressed. First, the data shown in this paper are based on mass transfer of an "inert" substance. When a real substrate is moving through the biofilm, it is being simultaneously consumed, which modifies its concentration profile in the microbial layer when compared to the "inert" case. However, it should be noted

that during the present experiments the biofilm was continuously being fed by a glucose substrate. If the results were based on measurements of the substrate concentration instead of lithium concentration, this would not alter the observed effects of fluid velocity on the mass transfer resistance offered by the biofilm, from a qualitative viewpoint (the changes in biofilm structure with fluid velocity would be identical). However, it would be problematic to measure substrate concentration on compartment II of the mass transfer cell, since much of the substrate would be consumed by the biofilm. Second, the "wavy" aspect of biofilm surfaces shows that the mass transfer area of biofilms and of clean surface can have different values. It was not possible to quantify such differences and to include them in the evaluation of mass transfer coefficients; an increase in the mass transfer area during biofilm formation would result in lower values of k_b. It is conceivable, however, that those effects could be similar for the several biofilms and that the overall conclusions would not be affected.

CONCLUSIONS

Internal diffusion in biofilms is strongly dependent on the hydrodynamic conditions imposed by the fluid flowing over the microbial layer, at least under turbulent conditions. High fluid velocities, corresponding to high shear stresses, result in thinner, more dense and more cohesive biofilms. The internal diffusivity of a "non-degradable" substance (LiCl) was lower in the thinner, more compact biofilms. The results indicate that there is not a unique value for the diffusivity of a given substrate within biofilms. Data on mass transfer coefficients in the biofilm also suggest that the so-called "biologically active layer" may have different thicknesses, depending on operating conditions such as fluid velocity. More detailed studies on biofilm structure are needed to establish the existance of zones of different compactness within the film and to relate them to possible changes in diffusivity.

Acknowledgements

The authors are grateful to JNICT, Portugal, for the financial support of the research work, through Project No 87509.

NOMENCLATURE

A = mass transfer area, m^2
C_I, C_{II} = lithium concentrations in compartments I and II, $kg \cdot m^{-3}$
f = friction factor
f_o = friction factor for clean surfaces
h_o = convective heat transfer coefficient with non-fouled surface, $W \cdot m^{-2} K$
k_b = mass transfer coefficient in the biofilm, $m \cdot s^{-1}$
k_T = overall mass transfer coefficient $m \cdot s^{-1}$
$k_T(t=0)$ = overall mass transfer coefficient with the clean membrane, $m \cdot s^{-1}$
k_w = thermal conductivity of the material between thermocouples T1 and T2, $W \cdot mK^{-1}$
Pr = Prandtl number ($c_p \mu / k$), where c_p, μ and k are the heat capacity, the viscosity and the thermal conductivity of the fluid

R_f = heat transfer resistance of the biofilm, $m^2K \cdot W^{-1}$
R'_f = asymptotic value of R_f, corresponding to the steady state maximum biofilm amount, $m^2K \cdot W^{-1}$
U = overall heat transfer coefficient, $W \cdot m^{-2} K$
U_o = overall heat transfer coefficient with the clean surface, $W \cdot m^{-2} K$
t = time, s
t' = time for lithium accumulation in system II, s
T_1, T_2, T_3 = temperatures of thermocouple 1, 2 and 3, respectively, K
V = volume of system II, m^3
y_w = distance between thermocouples 1 and 2, m

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