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EFFECT OF CLAY PARTICLES ON THE BEHAVIOUR OF BIOFILMS FORMED BY *PSEUDOMONAS FLUORESCENS*

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ABSTRACT

This paper presents results obtained with biofilms formed under turbulent flow by a mixed suspension of *Pseudomonas fluorescens* and kaolin particles and compares them with biofilms formed in the absence of clay particles. The results show that the presence of kaolin particles leads to: higher accumulation of biomass in biofilm systems; higher stability of the biofilms when substrate is suppressed; higher values of the respiratory coefficient of the suspended cell cultures; increase in the mass transfer rates throughout the biofilm thickness. The results suggest that the kaolin particles enhance microbial activity in the biofilm and may cause changes in the physical structure of the biofilm, making it a more strong and open matrix.

KEYWORDS

Biofilm; *Pseudomonas fluorescens*; clay particles; Mass transfer;

INTRODUCTION

Microbial films are complex gelatinous structures adherent to surfaces and are composed by microorganisms, their extracellular products and water. However, since most biofilms grow in natural habitats, they may also contain other components, such as small inorganic particles. The presence of these particles may lead to changes in the biofilm structure and activity. Clay particles with equivalent diameters of a few microns are very frequent in rivers and lakes, and are transported in the water streams used in industrial cooling systems, without being removed by filtration operations. The build up of microbials films in heat exchanger tubes may then be affected by these particles.

The interaction of microrganisms and substrates with inorganic inorganic particle in soils, in the absence of fluid flow, was studied by several authors, such as Burns (1979, 1989), Marshall (1989) and Stotzky (1966). In the case of clay particles, it was found that attraction between negatively charged bacteria and the electropositive surfaces of the particles could occur, depending on the pH of the environment. Also, the adsorption of molecules on the surface of clay particles may have an important effect on th activity of the microrganisms : for instance, Filip and Hattori (1984) found that metabolic inhibitors were adsorbed on the particle surfaces, leading to an increase in biomass formation by *Saccharomices cerevisae* in suspension.

Bowen and Dempsey (1992) showed that the addition of solid metal hydroxides and silica to an activated sludge increased its metabolic rate, due to increased surface area available and to possible adsorption of toxic ions such as Cu (II). Stotzky (1966) observed that the microbial respiration was stimulated by the presence of montmorillonite particles, probably due to their ability to maintain the pH of the soil within suitable values, through ion-exchange mechanisms. On the contrary Magdaliniuk *et al.*(1995), concluded that the presence of very small particles of montmorillonite (1-100 nm) reduced the activity of a bacterial suspension, because these particles tend to cover the surface of the cells and hinder mass transfer to and from the environment.

Use of powered clay in an activated sludge plant was found to enhance its nitrification capacity (Chudoba and Pannier, 1994). However, reports on the influence of inorganic particles on biofilms subject to turbulent flow conditions are scarce (Lowe, 1988 ; Pinheiro *et al*, 1988; Bott and Melo, 1992).

The paper presents the results obtained with biofilms formed by a mixed aqueous suspension of *Pseudomonas fluorescens* and kaolin particles, flowing under turbulent conditions, and the results are compared to the ones obtained with biofilms that did not incorporate inorganic particles.

MATERIALS AND METHODS

Bacterial strain - A Gram-negative aerobic bacteria, *Pseudomonas fluorescens* (rod size 1-2 μm x 3-4 μm), was used as a biofilm producer. Present in natural waters, it has a high capacity of adhesion (Vieira *et al.*, 1992). The optimal growth conditions are 27°C, pH 7 and glucose as carbon source. The growth medium used was composed of glucose (0.5%), peptone (0.25%) and yeast extract (0.125%) in distilled water, sterilized at 120°C.

Biofilm studies - The data presented in this work were determined using two different experimental apparatuses described elsewhere (Vieira *et al.*, 1993): the "*Heat transfer test section*", where the accumulation of biofilm was monitored through measurements of the thermal resistance of the biological deposits, as a function of time and velocity, and the "*Mass transfer test section*" used in the evaluation of the biofilm mass transfer coefficient, for different stages of biofilm formation, as a function of velocity and time. Both test sections were connected to a similar "*Biological fluid preparation zone*", where a continuous culture of *Pseudomonas fluorescens* culture was maintained and diluted with tap water. In some runs, kaolin particles (equivalent diameter=18.6μm) were added to the diluted biological suspension.

"Biological fluid preparation zone"- A pure culture of *Pseudomonas fluorescens* was grown continuously in a 4 liter fermenter, fed with the medium described before. The pH was controlled at 7 by addition of 2 M NaOH, and the culture was agitated at 120 rpm and aerated. The pure culture was pumped to a 12 liter tank and continuously fed with 10 l/h of filtered tap water at 27°C and with 100 ml/h of the growth medium described before. In some runs, a kaolin suspension was also added in order to obtain a clay concentration of 150 ppm. Convenient aeration and agitation was performed. The biological fluid obtained (cell concentration = 6×10^7 cells/ml, glucose concentration = 20 ppm, with or without kaolin particles and temperature= 27 °C) was then pumped to the test sections.

"The heat transfer test section" - The heat transfer measurements were carried out in two parallel semi-circular test sections made of perspex of hydraulic diameter 2.02 cm, mounted vertically, containing each one a 50 cm long test plate (Figure 1a).
The deposition surface was an aluminium plate, and its upper face, on which the biofilm was formed, contacted the biological fluid. The other face was in contact with a perspex wall of a duct that transported water at 60°C. Two thermocouples were imbedded in the perspex wall, and a third one was immersed in the biological fluid (Figure 1b). The heat flux was determined in three different positions of the test cell.

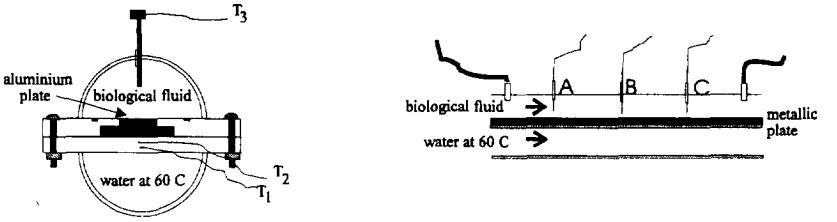


Figure 1 - Schematic diagram of the heat transfer test cell
a) cross section view
b) longitudinal view

" *The mass transfer test section*" - This test section, geometrically similar to the heat transfer section, was made of perspex, was semi-circular, was positioned vertically and included a test cell of 15 cm long and hydraulic diameter 2.06 cm, where the mass transfer measurements were carried out. It had two compartments, Compartment I and II, separated by a 10 cm long membrane, of 0.21×10^{-2} m^2 mass transfer area, with 0.22 µm pore diameter, impermeable to the bacteria but not to solutes. The biological suspension circulated in Compartment I, and the biofilm grew on the membrane. Water similar to that used in the biological suspension, circulated in Compartment II.

Determination of the mass transfer coefficients: Mass transfer through the biofilm was studied using lithium chloride, an inert substance not consumed by bacteria (Vieira *et al.*, 1993). Once a day, during one hour, a constant concentration of lithium chloride (100 ppm) was maintained in Compartment I. Li+ was transported across the biofilm and the membrane, accumulating in Compartment II. Samples were taken every five minutes from Compartment II, and the ion concentration determined in a flame photometer.

Respiratory coefficients - The respiratory coefficients of bacterial cultures in the presence of different kaolin concentrations were determined by cutting off the aeration and agitation in the batch cultures, and following the oxygen concentration in the medium (Dynamic method described in Bailey and Ollis, 1987).

CALCULATION METHODS

Evaluation of the thermal resistance of biofilms - The overall heat transfer coefficient at any instant t of biofilm formation was calculated by:

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

and the biofilm thermal resistance by

$$R_f = \frac{1}{U} - \frac{1}{U_0} - \frac{1}{h_0}\left[\left(\frac{f_0}{f}\right)^p - 1\right] \qquad (2)$$

where K_W is the thermal conductivity of the wall where the thermocouples T_1 and T_2 are imbedded, and y_W is the distance between them. U_O is the overall heat transfer coefficient and h_O is the overall convective heat transfer coefficient. f and f_O are respectively the friction factor of the surface with and without biofilm. p is a

factor calculated using the Norris correlation $p = 0.68\,Pr^{0.215}$, where Pr is the Prandlt number of the biological fluid. The values of h_0 were determined for each test cell, as a function of velocity, without biofilm and then the effect of roughness was taken into account in equation (2). Six measurements of temperature were made every day. Each value of the overall heat transfer coefficient, U, was calculated using three values of those determinations, thus obtaining 2 values of R_f per day. The so-called "biofouling curves" are plots of R_f values as a function of time.

Evaluation of mass transfer coefficients - The overall mass transfer coefficient was evaluated, for each biofilm formed, at different stages of biofilm development. The change in lithium concentration in Compartment I (C_I) and in Compartment II (C_{II}) was determined. The volume of the water where lithium accumulated, V, is 790 ml and A is the mass transfer area of the membrane. A mass balance in this system gives rise to the equation:

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

where k_T is the overall mass transfer coefficient, including the biofilm, the membrane and the external mass transfer resistances, and t' is the time during which lithium ion accumulate in the Compartment II. Upon integration the previous equation gives:

$$\left(C_I - C_{II}\right) = \left(C_I - C_*\right)_{t'=0}\exp\left(-\frac{A}{V}k_T t'\right) \qquad (4)$$

Experimental data of (C_I-C_{II}) as a function of t' were correlated according to this equation, thus obtaining the value of K_T for that situation.
The biofilm mass transfer coefficient, k_b was calculated using the following equation:

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

where k_T (t=0) is the overall mass transfer coefficient without biofilm.

This procedure was carried out once a day for each biofilm, obtaining a curve of "k_b *versus* time"

RESULTS

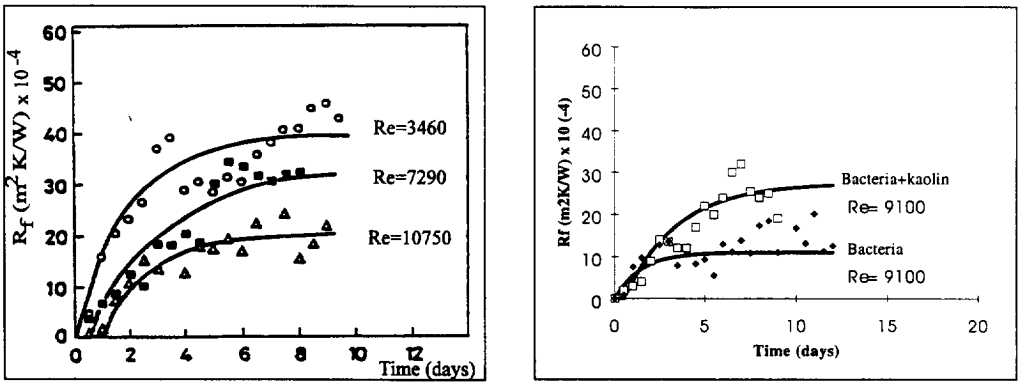
The heat transfer test section was used to :

- Evaluate the effect of the fluid velocity on the accumulation of biofilms
- Observe the effects of supressing the substrate from the water stream after the biofilm reached its "steady-state" (maximum thickness).

The mass transfer test section was used to determine the changes of the mass transfer coefficient within the biofilm, as a function of time and of the water velocity.

As can be seen in Figure 2, the assymptotic thermal resistance of the microbial film (which is considered to be proportional to the biofilm thickness, Vieira *et al.*,1993) is highly dependent on the hydrodynamic

conditions, decreasing as the fluid velocity increases. The amount of the microbial layer accumulated is higher when mixed suspensions of bacteria plus kaolin particles are used.

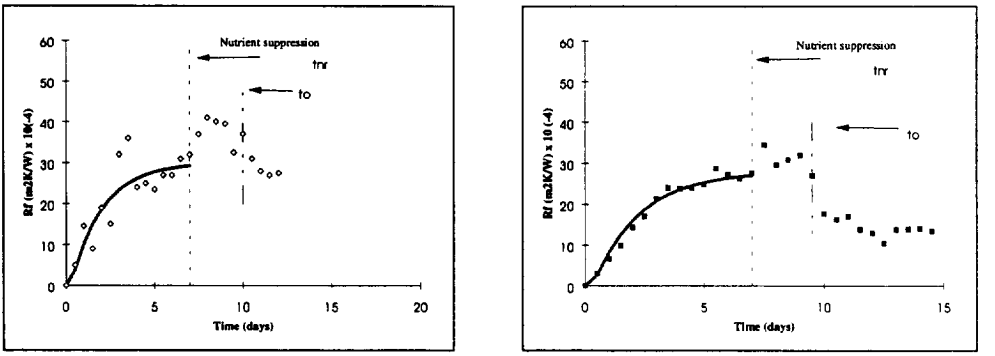


a) b)

Figure 2 - Effect of the fluid velocity (or Reynolds number) on the accumulation of biofilm
a) with kaolin particles; b) with and without kaolin particles

Data on the respiratory coeffcient revealed a stimulation of oxygen consumption by the suspended bacteria when kaolin particles were present (0.019 kg/m^{3}s in a suspension containing 0.1 g/litre of kaolin particles *versus* 0.004 kg/m^{3}s when there were no kaolin particles present).

Figure 3 illustrates how the suppression of nutrients affects the biofilms. Some time after cutting off the supply of nutrients, the amount of the microbial layer starts to decrease,this decrease being much less pronounced at lower fluid velocities. Table 1 shows that the percentage of biomass removed from biofilms formed by bacteria alone and by bacteria plus kaolin increases with fluid velocity.



a) b)

Figure 3 - Effect of nutrient suppression on biofilms formed at different velocities by suspensions of bacteria and kaolin particles- a) Re=4200 ; b) Re=9100

Table 1 - Loss of fixed biomass after suppressing the nutrient supply

	Bacteria suspension			Bacteria+kaolin suspension			
Reynolds Number	4200	6830	8955	4200	7505	9100	11763
Fluid velocity (m/s)	0.34	0.54	0.72	0.34	0.60	0.73	0.95
Biomass removed	21 %	79 %	91 %	15 %	63 %	83 %	82 %

The behaviour of the two types of biofilms is similar in this respect. However, the period of time between the suppression of substrate and the "collapse" of the biofilm is higher when kaolin particles are present (Table 2).

Table 2 - Period of time between the suppression of nutrients from the flowing suspension and the collapse of the biofilm

Type of biofilm	Fluid velocity (m/s)	Reynolds number	t_{nr} (days)	t_o (days)	$\Delta t\ (t_o - t_{nr})$ (days)
bacteria	0.34	4200	7.5	9.5	2.0
bacteria	0.52	6830	7.5	8.5	1.0
bacteria	0.71	8955	7.0	8.0	1.0
bacteria+kaolin	0.34	4300	7.0	9.5	2.5
bacteria+kaolin	0.60	7500	7.5	10.0	2.5
bacteria+kaolin	0.73	9100	7.5	9.5	2.0
bacteria+kaolin	0.95	11176	7.5	10.0	2.5

Possible changes in the structure of the microbial layer caused by the incorporation of clay particles were indirectly verified by measuring the mass transfer coefficient of an inert substance (LiCl) through the biofilm. The results (Figures 4 and 5) show that the mass transport is enhanced in biofilms containing kaolin particles.

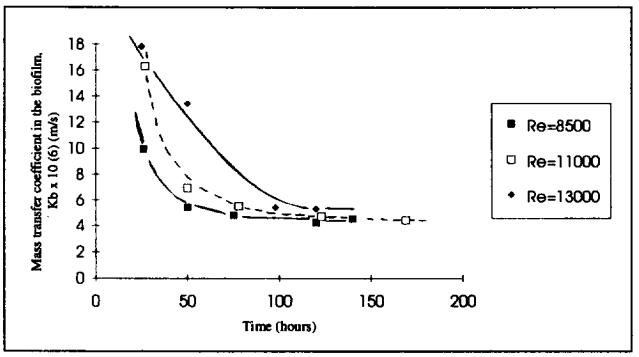


Figure 4 - Evolution with time of the mass transfer coefficient in biofilms formed by bacteria/kaolin particles for different hydrodynamic conditions.

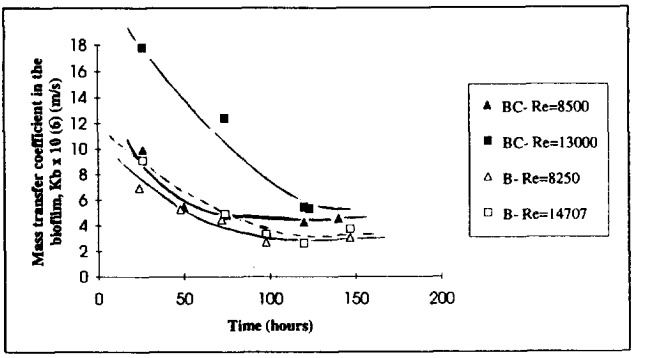


Figure 5 - Mass transfer coefficients in the biofilms :BC- with kaolin particles
B - without kaolin particles

DISCUSSION

The data show that the presence of kaolin particles results in :

(a) a higher accumulation of biomass (Figure 2 b)
(b) a higher stability of the biofilms when substrate is suppressed (Table 2)
(c) higher values of the respiratory coefficient of the suspended cell cultures
(d) an increase in the mass transfer rates throughout the biofilm thickness. (Figure 5)

In respect to (a), it could be argued that the increase in the thermal resistance of the microbial layer is due mainly to the low thermal conductivity of the clay particles. Lowe et al. (1988) measured the mass of *Pseudomonas fluorescens* biofilms formed with and without kaolin particles and observed also a much higher mass in the first case, in spite of the mass fraction of particles in the biofilm being rather low (around 5-10%). Furthermore, the organic content of the biofilm almost do doubled when clay particles were present. The higher values of the respiratory coefficient also point out to an increased biological activity of the bacteria in the presence of clay particles, in acordance with Stostzky (1965) and Bower and Dempsey (1992). One reason for the enhancement of biofilm activity could be the fact that the clay particles adsorb organic molecules (e.g., glucose) and, as such, they can act as substrate reservoirs where the microrganisms will preferentially adhere, wether they are in the aqueous suspension or within the biofilm itself (it should be noted that, contrary to the experiments of Magdaliniuk *et al.*(1995), the clay particles used in this work are much larger than the microbial cells). Since clay particles are known to promote ion-exchange (keeping pH at a suitable value for growth), they could also help in maintaining suitable environmental conditions for the bacterial metabolism.

Another aspect that should be taken into account is the apparent modification of the physical structure of the biological matrix when kaolin particles are incorporated. The increased stability of the biofilm, which resisted for a longer period of time to a sudden reduction of substrate concentration, and the higher values of the mass transfer coefficient within the bacteria-kaolin biofilms suggest that the inorganic particles may contribute to a more expanded and mechanically stronger structure.

A final remark should be made on the effect of the fluid velocity on both types of biofilms. Although the mass transfer coefficients through the biolayer are quite similar for the different hydrodynamic conditions, the biofilms formed under higher velocities are thinner and more compact (Vieira *et al*, 1993). If, in Table 1, the values of the biomass removed after suppressing the substrate are interpreted as representing the fraction of the biofilm that is more dependent on substrate concentration (that is, the so-called "active layer"), then this fraction is higher in biofilms formed under higher fluid velocities. All these observations lead to the

conclusion that, when biofilms are used in wastewater bioreactors, higher velocities make them more stable and therefore more suitable for effluent treatment purposes.

NOMENCLATURE

A - mass transfer area, m^2
C_I, C_{II} - lithium concentration in Compartment I and II, kg/m^3
f - friction factor of the surface with biofilm
f_o - friction factor of the clean surface
h_o - convective heat transfer coefficient with non fouled membrane, W/m^2K
k_b - mass transfer coefficient in the biofilm(m/s)
k_T - overall mass transfer coefficient (m/s)
$k_{T(t=0)}$ - overall mass transfer coefficient with the clean surface (m/s)
K_w - thermal conductivity of the wall, W/mK
Pr - Prandlt 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 - biofilm thermal resistance, m^2K/W
t' - time of lithium accumulation in Compartment II, s
T_1, $T_{2,,}$ T_3 - temperatures of thermocouples 1,2 and 3, respectively, K
U - overall heat transfer coefficient, W/m^2K
U_o - overall heat transfer coefficient with the clean surface, W/m^2K
V - volume of water where lithium accumulated, m^3
y_w- distance between thermocouples 1 and 2, m

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