Surface Phenomena and Hydrodynamic Effects on the Deposition of *Pseudomonas fluorescens**

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Biofilm adhesion to metals (copper, aluminium and brass) was studied at two different velocities and pH values of 7 and 9. Both bacteria and metals showed negative surface charges at those values of pH, which tends to slow down adhesion. Film densities increased with the fluid velocity and were also affected by the pH and by the growth rate of the bacteria. Long duration tests based on heat transfer measurements were run at five different fluid velocities and at pH = 7, showing in general an asymptotic behaviour and a control of deposition by adhesion and growth phenomena.

On a étudié l'adhésion de films biologiques sur des métaux (cuivre, aluminium et laiton) à différentes vitesses et pour des pH de 7 et 9. Les charges de surface négatives des métaux et des bactéries, obtenues pour ces valeurs de pH, tendent à ralentir l'adhésion. La densité des films augmente avec la vitesse du fluide et est modifiée par le pH ainsi que par la vitesse de croissance des bactéries. Des tests de longue durée utilisant des mesures de transfert de chaleur ont été effectués pour cinq vitesses de fluide différentes à un pH de 7, montrant un comportement asymptotique ainsi qu'un contrôle du dépôt par la croissance et l'adhesion; en outre, la déposition est contrôlée par l'adhésion.

Keywords: biofouling; fouling of metal surfaces; deposition surface effects; biofilm adhesion, density.

Fouling in general is a serious problem in fluid circulating systems leading ultimately to the obstruction of pipes. In heat exchangers this phenomenon creates additional thermal resistance and pressure drop.

Biofouling, defined generally as the accumulation of deposits associated with the growth of living organisms, is a very common phenomenon, especially in cooling water systems. The formation of biological deposits is influenced not only by physico-chemical variables (fluid velocity, solid surface condition, etc.) as in other types of fouling, but also by those intimately related to the growth of living cells (temperature of fluid, pH, oxygen concentration, nutrient concentration). For this reason it is important to examine in detail the design and operating parameters in order to implement methods of combatting the development of this type of deposit.

In this work the influence of fluid velocity, pH and surface characteristics were related to the thickness, weight and thermal resistance of the film. The microorganism utilised was *Pseudomonas fluorescens* since it is present in industrial cooling waters (Bott et al., 1983), and will therefore act as a typical organism.

Materials and methods

Tests for initial film formation

Pseudomonas fluorescens was grown under controlled pH, temperature, agitation and aeration in a fermenter. Nutrient was continuously added and the fluid carrying the microorganisms was circulated in a closed circuit containing test ducts of rectangular and circular cross-section (Miller and Bott, 1981). The test plates (rectangular area = 20 cm^2, circular area = 13.6 cm^2) inserted in these ducts were easily removed, after 48 hours, in order to observe the deposits formed (thickness, weight and microscopic observation) after being dried in the ambient air.

The thickness of the deposit was measured, with an accuracy of ± 5 microns, by means of a micrometer connected to a steel needle and inserted in an electric circuit (Harty and Bott, 1981). By using amplifying instruments the contact point between the surface of the deposit and the needle could be established when the latter encountered its shadow produced by adequate illumination of the system. The inner end of the deposit was detected by lowering the needle through the film until it reached the metal surface indicated by a signal of closure of the electric circuit. A significant number of thickness measurements was carried out on each test plate so that reliable average values could be obtained. The test plates were weighed on an analytical balance, its accuracy being 0.01 mg.

Long duration tests

Tests of 10 days duration were performed and the formation of the deposits on a plate of aluminium was followed, based on heat transfer measurements, utilising different fluid velocities at pH = 7. The fluid contaminated with *Pseudomonas fluorescens*, at a temperature of about 27°C, was circulated along the plate of aluminium located in a perspex duct of semi-circular cross section, 1.8 cm diameter, which was heated by a flow of water at 60°C. The entrance length was about 60 cm, allowing the development of the hydrodynamic and thermal boundary layer. Thermocouples placed in the fluid and in the heat transfer surface (Figure 1) allowed the measurement of temperatures to be made and the heat flux calculated. As the biofilm developed, the variation of the heat transfer resistance with time was determined. Norris' correlation (Kays and Crawford, 1980) was used for the correction of the convective heat transfer coefficient due to the increase in deposit roughness. Cell concentration in the circulating system was sufficiently high to discourage the growth of environmental bacteria. Microscopic observations and growth tests in solid medium were carried out to detect contamination.

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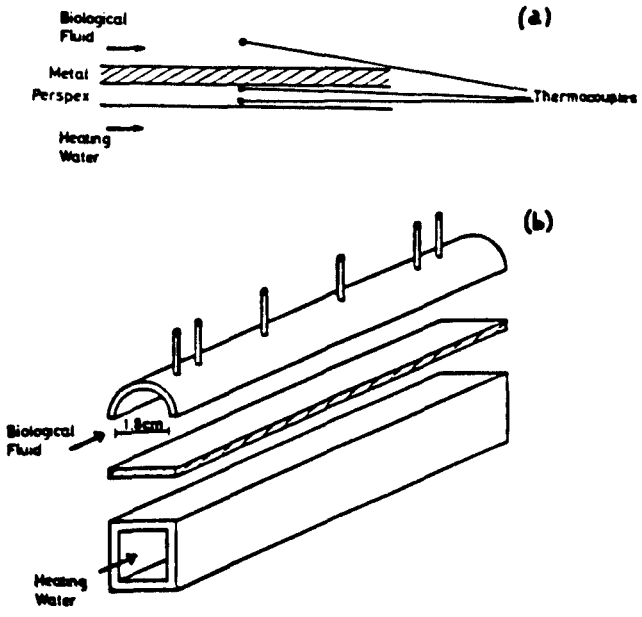

Figure 1 — Test section (a) and thermocouple locations on the cross-section of heat transfer surface (b).

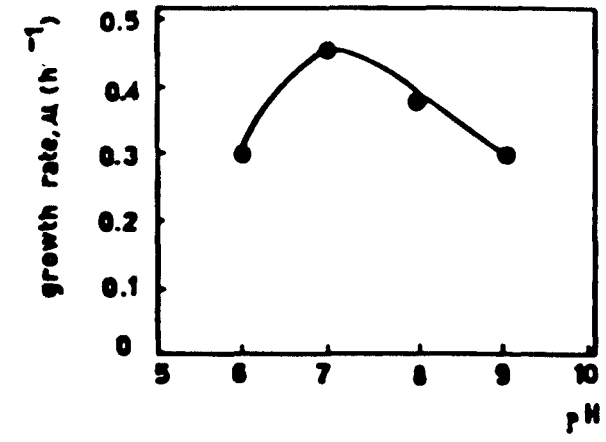

Figure 2 — Growth rate of *Pseudomonas fluorescens* as a function of the pH.

ZETA POTENTIAL MEASUREMENTS

Measurements of zeta potentials were carried out by electrophoresis using a Zetameter apparatus. Under normal electrophoresis experiments the particles of solid conductors, like metals, behave as insulators (Bier, 1967), making this technique an appropriate method for studying the surface properties of such materials. The solids were ground to fine powders and suspensions were prepared at 25 ± 1°C in buffer solutions of ionic strength 10 mol/L, at various pH values. Solutions were left for several hours to equilibrate. Zeta potentials measurements of bacteria were made over the same pH region as those of the solid substances.

Microorganism and medium

Pseudomonas fluorescens was kept in a nutrient agar solid medium prior to growth in liquid culture. This liquid medium was made of glucose (2% w/v), peptone (1% w/v) and yeast extract (0.5% w/v). The microorganism was grown in shake flasks for 10 hours and then introduced into the fermenter system.

TABLE 1
Growth Rate Versus pH

pH	Duplication time, t_D (h)	Growth rate, μ (h^{-1})
6	2.26	0.305
7	1.56	0.457
8	1.81	0.383
9	2.32	0.299

TABLE 2
Isoelectric Points for the Different Substances

Substance	pH (isoelectric point)
Bacteria	3.5
Aluminium	5.3
Copper	4.4
Brass	4.5

Results and discussion

GROWTH RATE OF *PSEUDOMONAS FLUORESCENS*

By following the growth curves of the bacteria, the influence of the pH on the duplication time, t_D (i.e. time for doubling the number of cells) was determined, the results being summarised on Table 1 and illustrated in Figure 2.

In order to study the biofilm formation in the test ducts two different pH values were chosen: pH = 7 as the most favourable for the microorganism and pH = 9 as being less favourable.

SURFACE CHARGES OF BACTERIA AND METALS

The attachment of bacteria during the first period of the fouling process is often dependent on the electrical charges of both the foulant and the surface of deposition (Fletcher and Loeb, 1979). The existence of opposite charges may represent an important factor in the development of greater film thicknesses.

Since the surface electrical charges of particles and microorganisms vary with the pH, the evaluation of the electrokinetic potentials for the *Pseudomonas fluorescens* and for the surface materials was undertaken. The experimental results are plotted in Figure 3, where zeta potentials are presented as a function of pH.

From these curves the isoelectric points (points of zero charge) of each substance were determined, as shown on Table 2.

This type of data indicates that, for instance, at pH = 4 bacteria would have negative charges and all the metals would be positive. In these circumstances, one could expect a high level of adhesion during the period of colonization. However, since *Pseudomonas fluorescens* is not able to grow at a pH as low as 5, no experimental runs were performed at such conditions.

For pH = 7 or pH = 9 all the substances present in the system are electro-negatively charged and the differences between their zeta potentials are small; indeed, the measured differences could be attributed to experimental errors. From

TABLE 3
Mass and thickness of the deposits

		Copper				Aluminium				Brass			
		Cylind.		Rect.		Cylind.		Rect .		Cylind.		Rect.	
v m/s	pH	M mg	y μm	M mg	y μm	M mg	y μm	M mg	y μm	M mg	y μm	M mg	y μm
0.43	pH = 7	4.1	52	4.7	54	3.9	47	3.4	44	2.8	31	1.6	31
	pH = 9	2.3	42	3.0	47	2.5	44	2.1	46	1.9	39	2.4	38
0.13	pH = 7	1.8	60	1.9	55	1.6	45	1.3	50	0.9	35	1.0	33
	pH = 9	1.7	58	2.4	62	2.0	50	1.8	48	0.7	35	1.0	38

TABLE 4
Biofilm Densities (kg/m^3)

	Cylindrical duct				Rectangular duct			
	pH 7		pH 9		pH 7		pH 9	
v (m/s)	0.43	0.13	0.43	0.13	0.43	0.13	0.43	0.13
Copper	57.9	22.0	40.3	21.6	43.5	17.3	31.9	19.3
Aluminium	61.0	26.1	41.8	29.4	38.6	13.0	22.8	18.0
Brass	66.4	18.9	35.8	15.1	25.8	15.2	31.6	13.2

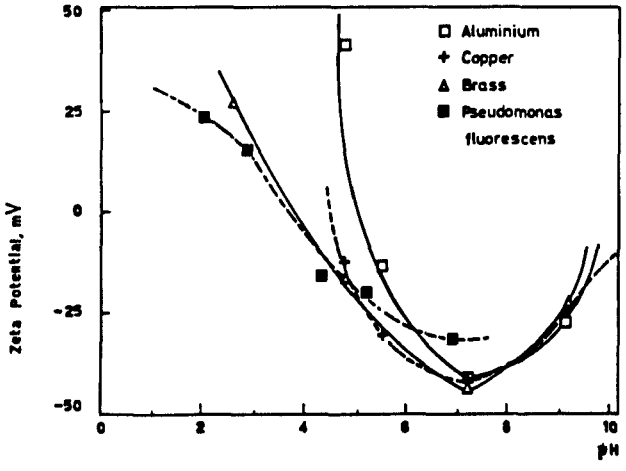

Figure 3 — Zeta potentials as a function of the pH.

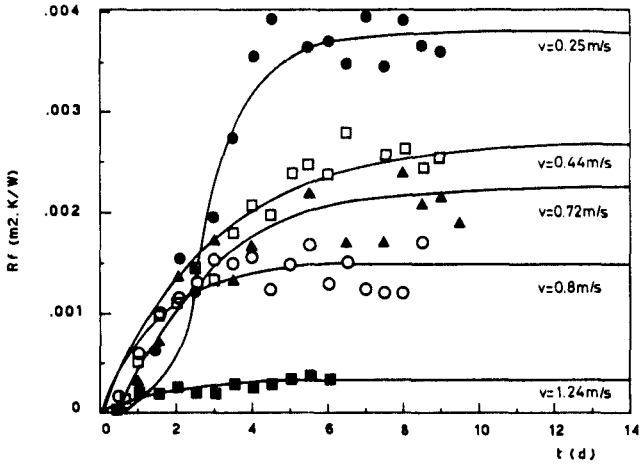

Figure 4 — Fouling curves of *Pseudomonas fluorescens* on aluminium surfaces.

these observations it can be concluded that the surface charges do not seem to be responsible for significant differences in the bioadhesiveness during the first period of fouling formation with *Pseudomonas fluorescens* grown at pH = 7 or pH = 9 on metal surfaces such as brass, aluminium and copper.

INITIAL ADHESION TESTS

The values for the total mass and thickness of the deposits obtained at different values of pH, fluid velocities and surface materials are indicated in Table 3, corresponding to the two geometrically different test ducts. The two fluid velocities (0.13 m/s and 0.43 m/s) correspond to the following Reynolds numbers: 6.5×10^3 and 2.0×10^3 for the cylindrical duct; $1.1. \times 10^4$ and 3.3×10^3 for the rectangular duct. These results are summarised in Table 4 in terms of biofilm densities.

It can be stressed that the film density increases with the fluid velocity for the same pH (see Table 4), which is in accordance with the idea previously expressed by Bott and Pinheiro (1977) of a higher compactness of the films formed under high Reynolds numbers. In fact, as shown by Table 3 the thickness of the deposits does not seem to change significantly with the fluid velocity (the accuracy of the thickness measurements is $+5$ μm), whereas the mass (accuracy 0.01 mg) of the deposits is always higher for higher Reynolds numbers; note that this effect is greater for pH = 7.

An increase in the velocity tends to make the cell adhesion more difficult, but on the other hand it favours the growth (reproduction) of the microorganisms already deposited, since it results in a higher rate of diffusion of nutrients and oxygen to the bacteria. This conclusion is confirmed by the data presented on Table 4 by comparing the densities of biofilms grown at different pH levels and higher velocity conditions. In fact, there is in general a significant

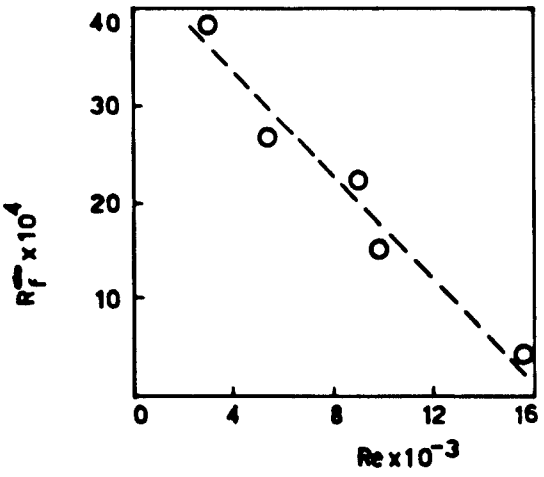

Figure 5 — Asymptotic thermal resistance versus Reynolds number.

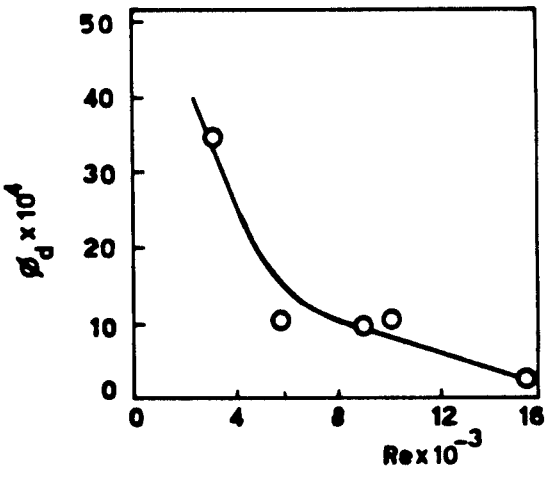

Figure 6 — Deposition flux versus Reynolds number.

decrease in the film density from pH = 7 to pH = 9 for $v = 0.43$ m/s. This suggests that the most important factor is the growth of bacteria rather than the phenomena of bacteria transport or adhesion. The growth rate is about 53% greater when using pH = 7 for *Pseudomonas fluorescens* grown in shake flasks (Table 1). Thus, the greater film densities for pH = 7 can be explained by the fact that the reproduction of the microorganisms is favoured by higher velocities. The apparent increase in biofilm density for the brass plate in the rectangular duct is believed to reflect experimental errors in the measurement of thickness.

For lower velocities ($v = 0.13$ m/s), the differences in film densities for the two pH levels employed are not significant. It appears that the growth process is not influencing the formation of the biofilm as in the case of higher velocities, i.e., the growth may be limited by nutrient availability.

It is interesting to note that deposits on the brass surface appear to be thinner than on the other metals. This may be due to the presence of zinc ionic species which were reported to inhibit adhesion of microorganisms (Duddridge et al., 1981).

Long duration tests

Curves like those represented on Figure 4 were obtained for five different fluid velocities, all the other conditions being kept constant.

These curves show the asymptotic behaviour dictated by the equation:

$$R_f = R_f^{\infty} [1 - \exp(-\beta t)]$$

By fitting this equation to the experimental points, values of R_f^{∞}, and also ϕ_d ($\phi_d = \beta \cdot R_f^{\infty}$) were obtained. The deposition flux (ϕ_d) includes the processes of mass transfer, adhesion and biological growth, i.e., all the processes that contribute to the increase of the amount of deposits, as opposed to those which affect the removal flux.

The variation of R_f^{∞} and ϕ_d with the Reynolds number is shown in Figures 5 and 6. As can be seen from Figure 5, the asymptotic value of the heat transfer fouling resistance (R_f^{∞}) decreases with the increase of the Reynolds number, which means that the increase in the rate of removal is much higher than any possible increase in the deposition flux.

The plots of the deposition flux versus the Reynolds number also show a decrease in ϕ_d with an increase in Re, but the shape of the curves is somehow different from the reported cases of inorganic fouling where adhesion controls deposition (Watkinson and Epstein, 1970; Melo and Pinheiro, 1986). In fact, as shown in Figure 6, for values of Re higher than about 5000, the change of ϕ_d with Re is much less steep than for Re < 5000. The following qualitative explanation is suggested:

There are three processes competing for the control of the deposition rate: (a) mass transfer of bacteria and of nutrients (including oxygen); (b) adhesion of bacteria to the solid–fluid interface, and (c) growth (reproduction) of the microorganisms in the deposit. They occur in series or in parallel, such as indicated below:

Mass transfer of bacteria → Bacteria adhesion

Mass transfer of nutrients → Bacteria reproduction

If the processes are consecutive, the slower step will control the deposition rate. If they are parallel (simultaneous), the faster process will be the dominant one.

For the whole range of Reynolds numbers studied, the transport of bacteria seems to be faster than the adhesion phenomenon (Pinheiro, 1981). This suggests that the latter will tend to control the deposition rate, otherwise ϕ_d would increase with the Reynolds number rather than decrease.

For Re < 5000 the biological growth rate may still be rather small compared to the adhesion rate, on account of the relatively low level of nutrients (oxygen, glucose, etc.) diffusing to the deposit. An increase in the fluid velocity tends to reduce the possibilities of adhesion; as this is the dominant step, the deposition rate will decrease significantly.

However, on increasing Re above 5000, the acceleration of the transport of nutrients to the biofilm will begin to have a marked effect on the reproduction rate of bacteria, while adhesion will continue to decrease with higher velocities. That is, the main competition will be between two parallel processes: adhesion versus reproduction of bacteria, their rates being additive. The decrease in the adhesion rate seems to be compensated by an increase in the biogrowth rate, leading to a much more flattened curve of ϕ_d versus Re.

Conclusions

It may be concluded from this work that the build-up of biological deposits is very much dependent upon the

reproduction of bacteria, once the surface is contaminated, provided sufficient nutrient is available at the biofilm.

In fact, different types of tests using a biological fluid at pH = 7 and 9 containing *Pseudomonas fluorescens* showed that:

—The reproduction rate of these bacteria is higher at pH = 7.

—The density of the initial layers of the biological deposit is larger at pH = 7 than at pH = 9 only in the case of higher fluid velocities, that is, when there are greater mass transfer rates of nutrients to the biofilm.

—At pH = 7, increasing the Reynolds number decreases the deposition rate (which includes transport, adhesion and reproduction), but that decrease becomes less and less steep as the fluid velocity grows higher. Again, there seems to be a prevalence of the reproduction processes at larger Reynolds numbers, due to a greater availability of nutrients.

The nature (composition) of the deposition surface did not appear to have a strong effect on the initial biofilm formation, which agrees with the similarity of the zeta potentials measured for the different metalic surfaces (copper, aluminium and brass). However, somewhat thinner deposits were found on the brass surfaces, probably on account of Zn toxicity effects.

Nomenclature

M = total mass of the deposit, mg
R_f = thermal resistance of the deposit, $m^2 \cdot K/W$
R_f^{∞} = asymptotic value of thermal resistance of the deposit, $m^2 \cdot K/W$
Re = Reynolds number
t = time, s
t_D = duplication time, s
v = fluid velocity, m/s
y = thickness of the deposit, μm
β = constant, s^{-1}
ϕ_d = deposition rate, $m^2 \cdot K/J$

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