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BIOCOLLOIDS AND BIOSURFACES

ADHESION OF *Pseudomonas fluorescens* TO METALLIC SURFACES

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ABSTRACT

Deposition of *Pseudomonas fluorescens* on aluminium, brass and copper plates was studied in a flow system. The number of bacteria deposited on aluminium was greater than on the other two types of metals. The results are discussed in terms of the mechanisms (transport and/or adhesion) that may control the rate of the deposition process. Adhesion data are compared with the predictions given by thermodynamic models.

INTRODUCTION

Biofouling is caused by the deposition and growth of living organisms on surfaces. It occurs quite often on the metallic surfaces of heat exchange equipment, such as condensers, and can lead to severe economical losses.

In a flowing system the overall biofouling process is the result of a sequence of events wich include: a) adsorption of macromolecules (proteins, carbohydrates, soluble substrates) by the solid surface, forming the so-called "conditioning film"; b) transport of bacteria and substrates from the bulk fluid to the surface; c) adhesion of microorganisms to the surface; d) cell growth and reproduction and, in some cases, production of extracellular material by the microorganisms; e) detachment and removal of parts of the biofilm, due to the shear forces of the flowing fluid. It is usually accepted [1,2] that the effect of shear forces increases with the thickness of the deposited layer.

As regards the process of cell transport and adhesion, it should be stressed that these events are sequential steps and as such, the slowest one will determine the rate of deposition (considering deposition as the result of transport and adhesion).

The rate of particle or bacterial transport increases with the fluid velocity, whereas adhesion becomes more difficult as the velocity increases, at least in turbulent flow [2]. Therefore, it is quite important to identify in each case, the controlling mechanism, in order to interpret the data from deposition studies.

As regards adhesion, a thermodynamic model has been widely used by several authors, as a means of interpretating and predicting this phenomenon. According to it, and neglecting electrical charges and specific biochemical interactions [3], adhesion is energetically favourable if the change in the free energy of adhesion is negative ($\Delta F_{adh} < 0$). The latter can be calculated if the surface free energies of the interacting bodies are known.

There are different approaches for the determination of the surface free energies, such as the ones proposed by van Oss *et al.*[4] and Busscher *et al.*[5]. Both take into account the dispersion and polar components of the surface free energy of the solid and liquids involved. This approach has proven to be valid not only for low energy surfaces, but also for high energy surfaces on which spreading pressures may become significant [5,7].It can be used to predict correctly a number of widely varying biological adhesion phenomena as well [6,11,12].

Recently, van Oss *et al.* [4,8], in order to obtain more molecularly relevant data, considered the surface free energy dispersion component to be due to all three types of Lifshitz-van der Waals forces (γ^{LW}), while the polar component arises from interactions that are predominantly acid/base in nature (γ^{AB}), comprising an hydrogen-donating (γ^{+}) and an hydrogen-accepting (γ^{-}) parameter.

In the present study, different types of metallic surfaces were used with the purpose of determining their ability to be fouled by a given microorganism. Simultaneously, methodologies for the prediction of adhesion can be assessed.

MATERIALS AND METHODS

Organism and Growth Conditions. *Pseudomonas fluorescens,* a bacterium present in natural waters, was selected for this study due to its ability to form biofilms. A pure culture of the bacterium was

mantained at 27 °C, pH=7, using glucose (5%), peptone (2.5%) and yeast extract (1.25%) as culture medium.

Flow System. This culture was continuously inoculated in a vessel containing water at 27 °C and pH 6.5, in order to maintain a concentration of bacteria of $6x10^7$ cells/ml.

Bacterial deposition was studied on metal plates (aluminium, copper and brass) of 10 cm x 2 cm fitted in the plane wall of semi-circular test sections, where the aqueous suspensions of bacteria circulated at velocities of 0.265 m/s and 0.46 m/s, parallel to the plates. There was no addition of nutrients, in order to obtain only deposition without significant biological growth. The plates were removed after 2, 4 and 6 hours of exposure, according to each experiment, rinsed and the bacteria were heat fixed at 160 °C for 2 minutes [9]. The plates were observed by scanning electron microscopy (magnification 1200 x), and several photos were taken, representative of the surface of the whole plate, obtaining the average number of bacteria deposited per cm^2.

Solid Substrata. Commercial aluminium, copper and brass were used as substrata for adhesion. The metal plates were polished twice and detergent cleaned before every experiment.

Contact Angle Measurements and Estimation of Surface Free Energies. Contact angle measurements and estimation of surface free energies were made on bacterial lawns and on metal surfaces.

Bacteria were deposited on membrane filters to produce a lawn of 50-100 stacked cells, suitable for contact angle measurements [6,10]. After a standard drying time of 30 min., plateau contact angles [6,13] were determined at 25 °C using sessile drops of water, formamide, diiodomethane, α-bromonaphthalene, and a series of water/n-propanol mixtures.

The same liquids were also used for the contact angle measurements on the metallic surfaces.

Surface free energies were estimated according to two approaches.

First, the water/n-propanol and α- bromonaphthalene contact angles were least square fitted to [5,7]:

$$(\cos\theta + 1).\gamma_l = 2\ (\gamma_s^d.\gamma_l^d)^{1/2} + 2\ (\gamma_s^p.\gamma_l^p)^{1/2} - \pi_e \qquad [1]$$

in wich "d" and "p" denote the dispersion and polar surface free energy components of the surface and the liquids employed, respectively. π_e is the equilibrium spreading pressure [5]. All liquid parameters are known through calibration experiments [7].

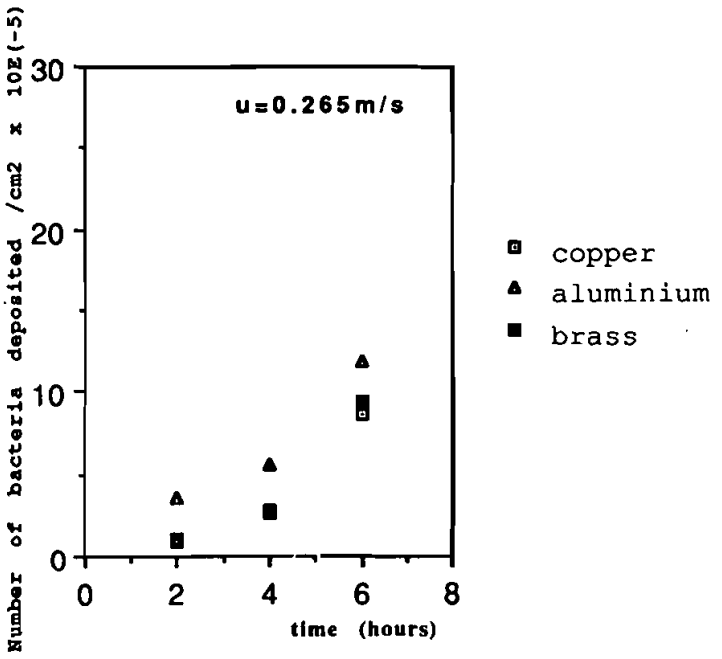

Figure 1 - Number of bacteria deposited per cm^2 on metal plates vs time, u=0.265 m/s

In the approach of van Oss [4,8], where the spreading pressures are neglected, the pure liquid contact angles are inserted in the following equation:

$$(\cos\theta + 1).\gamma_l = 2(\gamma_{sv}^{LW}.\gamma_l^{LW})^{1/2} + 2(\gamma_{sv}^{+}.\gamma_l^{-})^{1/2} + 2(\gamma_{sv}^{-}.\gamma_l^{+})^{1/2} \quad [2]$$

to yield a Lifshitz-van der Waals γ_{sv}^{LW} surface free energy component of the bacteria and a hydrogen-donating γ_{sv}^{+} and hydrogen-accepting γ_{sv}^{-} surface free energy parameter, which together determine the Acid-Base surface free energy component of the bacteria according to:

$$\gamma_{sv}^{AB} = 2\ (\gamma_{sv}^{+}.\gamma_{sv}^{-})^{1/2} \quad [3]$$

Surface free energies and their components, of all the pure liquids used are referred to in literature [11].

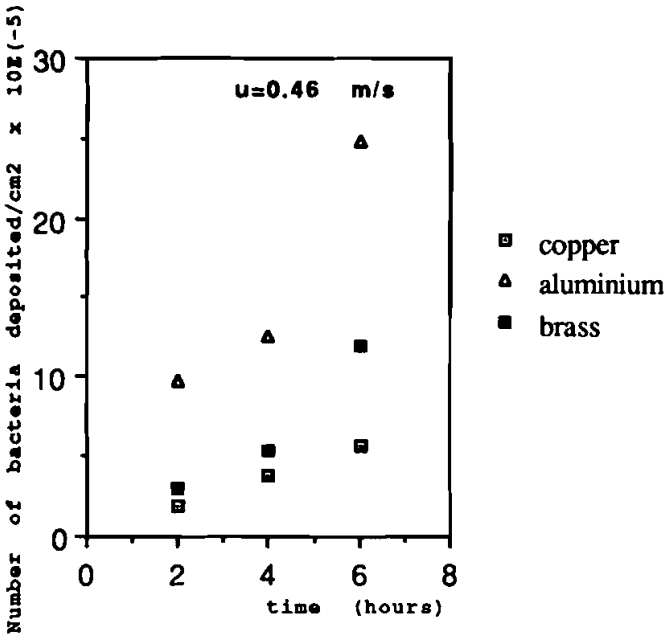

Figure 2 - Number of bacteria deposited per cm^2 on metal plates vs time, u=0.46 m/s

RESULTS AND DISCUSSION

Initial adhesion results of *Pseudomonas fluorescens* on aluminium, copper and brass after 2, 4 and 6 hours of exposure to the flowing biofluid, are given on Figure 1 (fluid velocity(u) = 0.265 m/s) and on Figure 2 (fluid velocity(u) =0.46 m/s).

In order to see the probability that the bacteria will interact with the surface, surface free energies of adhesion were calculated.

The values obtained for the contact angle with pure liquids on *Pseudomonas fluorescens* and metallic surfaces are presented on tables 1 and 2.

Table 3 indicates the change in the interfacial free energy of adhesion, ΔF_{adh}, of the three phase system consisting of bacteria, solid substratum - aluminium, copper and brass - and water determined by the methods proposed by Busscher *et al.* and by van Oss *et al.*.

Table 1 - Pure liquids contact angles on *Pseudomonas fluorescens*

Liquids	Contact angle,θ
water	38
formamide	38
α-bromonaphthalene	32
methyleneiodide	50

Table 2 - Pure liquids contact angles on metallic surfaces.

	Contact angles θ		
	aluminium	copper	brass
Liquids			
water	89	80	86
formamide	79	75	53
α-bromonaphthalene	15	29	21
methyleneiodide	54	47	51

Table 3 - Surface free energies of adhesion calculated by the approaches of Busscher *et al.*[5] and van Oss *et al.* [4]

Metallic surfaces	ΔF_{adh} ($mJ.m^{-2}$) (Busscher et al) [5]	ΔF_{adh} ($mJ.m^{-2}$) (van Oss et al.) [4]
aluminium	+ 7.67	- 3.84
copper	+ 6.94	- 5.32
brass	+ 6.61	-13.90

Figures 1 and 2 show that deposition is greater on aluminium, than on brass and copper, for both velocities. Increasing the velocity, a pronounced increase in the number of deposited bacteria on aluminium is observed. Therefore, for the aluminium surface the controlling step of the process seems to be the transport of microorganisms to the surface, adhesion occurring rather easily.

There is no clear indication that transport is the controlling step in the case of the brass and copper plates, since no significant increase of deposition with the fluid velocity can be detected. When the copper surface was used, there was even a decrease in the number of bacteria deposited during the 6 hours experiment for the higher velocity. In both cases (brass and copper), adhesion must surely play an important role in determining the rate of deposition.

Calculation of ΔF_{adh} by the approach of van Oss *et al.* (Table 1) denotes that adhesion of bacteria is always a favourable process. Furthermore, adhesion is expected to be more pronounced on brass, than on copper, in accordance with the experimental results.

The values of the free energy of adhesion calculated by the methodology of Busscher and co-workers, are positive (Table 1). However, these authors [12] found that microorganisms adhere to solid substrata even when the energy of adhesion is positive. They assumed that $\Delta F_{adh} > 0$ denotes reversible adhesion and $\Delta F_{adh} < 0$ irreversible adhesion.

Both thermodynamic approaches neglect the effect of electric charges and microbial factors. The zeta potentials of the metals and of the bacteria are negative in the case under study [14]. This confirms that there must be other types of interaction acting in the process of bacterial adhesion In fact, the surface composition of the bacteria, the existence of pili, flagella and capsules, and the modification of the substratum characteristics by macromolecules present in the aqueous phase, must be taken into account.

These macromolecules (glycoproteins and other substances excreted by bacteria), adsorbed by the substratum after a few minutes of exposure, affect bacterial adhesion since steric interactions or bridging can occur between the macromolecules and polymers of the bacterial surface [15], and can modify the substratum surface free energy to levels where adhesion is favourable [16,17].

In some circumstances the solid surface may be toxic to the bacteria.For instance, bacterial metabolism can be affected by the existence of copper ions [18]. This happens not only with the copper plates but also with the brass plates.The latter may also inhibit

bacterial adhesion due to the presence of zinc ions [9]. Presumably, these effects explain why adhesion to aluminium is greater than to the other plates, contrary to the results shown in Table 1.

In the above discussion, the removal effects were discarded since the thickness of the biofilm was extremely small (there was not even a complete monolayer of bacteria on the plates).

CONCLUSIONS

Initial deposition of *Pseudomonas fluorescens* suspended in a stream of water was more pronounced on aluminium plates than on brass and copper. The number of bacteria deposited increased with fluid velocity when aluminium was used.

The interpretation of the results in a flowing system should take in consideration not only the adhesion process but also the effect of mass transfer mechanims, since one or the other may act as the step controlling the rate of bacterial deposition.

The thermodynamic approaches are useful tools to predict adhesion, although they need to be improved mainly as regards the influence of specific biochemical factors associated to the morphology and metabolism of microorganisms. The approach of van Oss and co-workers showed, in the present study, a better agreement with the experimental results.

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