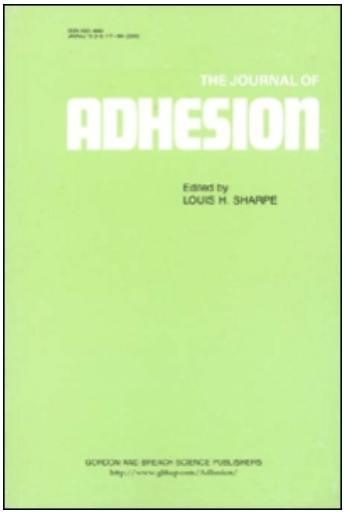

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Factors Influencing the Adhesion of Microorganisms to Surfaces

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Factors Influencing the Adhesion of Microorganisms to Surfaces

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Starvation, growth phase, and carbon source influenced bacterial cell surface hydrophobicity. Both the number and kind of microorganisms that colonized metal surfaces depended on the type of metal and the presence of an imposed electrical potential. No significant differences in attachment and growth of a pure culture were observed when metal surfaces were dipped in an exogenous energy source. The chemical composition of naturally occurring adsorbed organic films on metal surfaces was shown to be independent of surface composition and polarization.

KEY WORDS Adhesion; bioadhesion; cell surface hydrophobicity; metal surfaces; surface composition; surface polarization.

INTRODUCTION

Microbial adhesion to surfaces is a common phenomenon. Many bacteria demonstrate a preference for the surface regions of soils, teeth, plant fibres, roots, etc., where nutrients may be concentrated by the adsorption of dissolved organic material. Because microorganisms act as colloidal particles, their interaction with solid

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surfaces can be anticipated on the basis of colloidal theory. However, microorganisms are more complex than typical colloids as they are capable of independent locomotion, growth in different shapes, and production of extracellular polymeric materials that aid in anchoring the microbe to the surface. This paper will address two factors that have been shown to impact the adhesion of microorganisms to surfaces: the nature of the cell, *i.e.*, hydrophobicity as influenced by starvation, growth phase, and carbon source; and the nature of the substratum, which, in the case of the metals discussed, includes composition, presence and chemistry of a conditioning film, and polarization.

METHODS AND MATERIALS

Experiments were designed to examine (1) the effects of starvation (carbon limitation), growth phase, and carbon source on the cell surface hydrophobicity of the marine bacterium *Deleya marina* (=*Pseudomonas marina*, ATCC 25374)[1] and (2) the effects of metal substratum composition on the attachment of *Vibrio alginolyticus* in the laboratory and of marine bacteria in the natural environment, and (3) the substratum specificity of adsorbed organic materials on different metal surfaces after brief exposures. The pure cultures of these bacteria were maintained on Marine Agar 2216 (Difco, Detroit, Michigan). The media used in the experiments were sterilized by autoclaving at 121°C for 15 minutes. All seawater used in these experiments, both natural and artificial, was filtered through a sterile filter (0.22 μm pore size). All measurements of absorbance to determine bacterial growth and hydrophobicity were made using a Perkin/Elmer Model 552 spectrophotometer

A. Effect of Starvation on Cell Hydrophobicity

D. marina was grown at 26°C to mid-exponential phase (A_{660} approximately 1.1) in 100 ml of Marine Broth 2216 (Difco, Detroit, Michigan) supplemented with [^{3}H]-L-leucine, 0.35 Ci/ml (New England Nuclear, Boston, Massachusetts). Cells were harvested by centrifugation (4000 × g for 15 minutes) and washed twice in 75% Nine Salt Solution (NSS). NSS consists of NaCl, 23.48 g; Na_2SO_4,

1.96 g; $NaHCO_3$, 0.10 g; KCl, 0.33 g; KBr, 0.05 g; $MgCl_2.2H_2O$, 2.49 g; $CaCl_2.2H_2O$, 0.55 g; $SrCl_2.6H_2O$, 0.01 g; H_3BO_3, 0.01 g; deionized distilled water, 1000 ml. After washing, the bacteria were resuspended in 75% NSS, and the bacterial suspension was divided into two aliquots. One part was immediately analyzed for its hydrophobicity (see below), and the other part was further diluted with NSS to give a final concentration of $<2.5 \times 10^7$ cells ml^{-1} and incubated at 26°C for 6 hours. Bacteria were then harvested by centrifugation and resuspended in 75% NSS to a concentration of approximately 10^9 cells ml^{-1}, and their hydrophobicity was determined.

B. Effect of Growth Phase and Carbon Source on Cell Hydrophobicity

D. marina was grown at 26°C to mid-exponential phase (A_{660} approximately 1.1) and stationary phase (A_{660} approximately 1.9) in Marine Broth 2216 and harvested by centrifugation. Bacteria were washed once and resuspended in 75% NSS, and their hydrophobicity was measured.

For studies of the effect of carbon source on hydrophobicity, *D. marina* was grown at 26°C to mid-exponential phase (A_{660} approximately 1.4) and stationary phase (A_{660} approximately 2.5) in a minimal medium consisting of casamino acids, 0.1 g; NH_4Cl, 3.0 g; carbon (glucose or sodium citrate), 5.0 g; $MgCl_2.6H_2O$, 5.3 g; $MgSO_4.7H_2O$, 7.0 g; NaCl, 24.0 g; KCl, 0.7 g; $NaH_2PO_4.H_2O$, 1.08 g; $NaHPO_4.7H_2O$, 3.27 g; deionized distilled water, 1000 ml. Glucose, NaH_2PO_4, and Na_2HPO_4 were prepared as separate solutions, filter sterilized (0.2 μm pore size filter), and added to the other ingredients after autoclaving. Final pH was 7.0. Bacteria were harvested by centrifugation, washed once and resuspended in 75% NSS, and measured for their hydrophobicity.

C. Hydrophobicity Measurements

All measurements of hydrophobicity were determined using the two-phase separation method[2] with hexadecane (Sigma Chemical Co., St. Louis, Missouri) as the water insoluble phase. In the starvation experiment, hexadecane (0.010, 0.025, 0.050, 0.10, and

0.20 ml) was added to duplicate test tubes containing 1.2 ml of the bacterial suspension and vortexed for 2 minutes. The phases were allowed to separate for 15 minutes and 0.5 ml of the water phase was transferred to a scintillation vial containing 15 ml of Aquasol (New England Nuclear, Boston, Massachusetts). Radioactivity was measured by counting for 5 minutes in a Beckman Scintillation Counter. Counting efficiency was determined by the external standard method. The starvation results are presented as the percentage of radioactivity that was left in the aqueous phase (a hydrophilic organism would have a value of 100%). In the growth phase and carbon source experiments, hexadecane (0.17, 0.34, and 0.68 ml) was added to triplicate test tubes containing 4 ml of the bacterial suspension and vortexed for 2 minutes. The phases were allowed to separate as above and the absorbance (A_{400}) of the aqueous phase was measured spectrophotometrically. The results of these experiments are presented as the percent absorbance left in the aqueous phase (a hydrophilic organism would have a value of 100%).

D. Attachment Studies

Coupons (1 cm × 2 cm × 0.16 cm) of Cu Ni 90–10, titanium (Grade 2) and stainless steel (316) were immersed in Marine Broth 2216 for 5 minutes and then were transferred to dishes containing approximately 10^6 cells ml^{-1} of *V. alginolyticus* suspended in natural and in artificial sterile seawater. The cells were grown overnight at 26°C and washed twice in sterile seawater prior to resuspension. Control samples were placed directly into an identical dish of the same bacterial suspension without prior exposure to 2216 broth. Coupons were removed from the bacterial suspensions after 5 minutes, rinsed in seawater to remove unattached bacteria, and incubated for 1 to 4 days at 26°C in sterile seawater to follow increases in cell number. Specimens were removed at various times during the incubation and preserved in 3.7% filtered (0.22 μm pore size) formaldehyde until the bacteria could be counted.

To enumerate bacteria attached to the metals, coupons were stained for 3 minutes with 0.1% acridine orange prepared in 2% formaldehyde, rinsed in deionized distilled water, and allowed to air-dry. Attached cells were then observed and counted under

epifluorescence microscopy. A minimum of 10 random fields was counted for each sample. All stain and rinse solutions were filtered (0.22 μm pore size) immediately prior to use.

Metal coupons were also suspended from styrofoam floats in Eel Pond, Woods Hole, Massachusetts. After exposure to the seawater for varying time periods ranging from 30 minutes to 14 days, the coupons were removed and examined with acridine orange and epifluorescence microscopy as above and scanning electron microscopy (SEM). Coupons were fixed for 24 hours in 2% glutaraldehyde in cacodylate buffered seawater, dehydrated in a series of increasing concentrations of acetone, critical point dried, sputter-coated with gold-palladium before being examined by SEM.

CHARACTERIZATION OF EXPOSED METALS

Surface characteristics of metal foils suspended in marine water (Eel Pond, Woods Hole, Massachusetts) and in estuarine water (Bay St. Louis, Mississippi) were studied. Voltage clamping and chemical characterization of adsorbed dissolved organic materials were limited to the samples exposed in Bay St. Louis.

A. Hydrophobicity/Hydrophilicity and Corrosion Potential

Bubble contact angle determinations were used as a measure of the hydrophobicity/hydrophilicity of the metal surfaces. The contact angles of an air bubble on the test substrata were measured by injecting an air bubble from a syringe (0.25 mm ID) into a chamber containing sterile artificial seawater. The bubble floated 6 to 7 mm from the point of release to rest against a test surface placed horizontally on a stage at the top of the liquid. The average bubble size was 2.0 mm in diameter. Contact angles were measured directly using a Vernier microscope with a goniometer eyepiece. Results represent 12 observations.

Corrosion potential (also called rest potential), the potential of an electrochemical cell consisting of the metal of interest in contact with an electrolyte referenced to a saturated calomel electrode (SCE), was measured for all metal surfaces using an EG&G Princeton Applied Research (Princeton, New Jersey) Model 350

Corrosion Measuring System. The electrolytes in this study were the naturally occurring seawater collected in Eel Pond at the Marine Biological Laboratory in Woods Hole, Massachusetts, and the estuarine water from Bay St. Louis, Mississippi.

B. Voltage Clamping

In the field experiments, imposed electrical potentials were maintained using a high output resistance current source (Figure 1) designed by Dr. Sol M. Gerchakov, University of Miami, Miami, Florida. The principle of operation is that the potential (E) of the power source drops mainly across the resistors, R_1 and R_2, that together have a higher resistance than the electrolyte between the anode and cathode. Thus, any change at the electrode interfaces will result in a relatively small resistance change. The E *versus* saturated calomel electrode (SCE) of either of the electrodes can be selected and maintained.

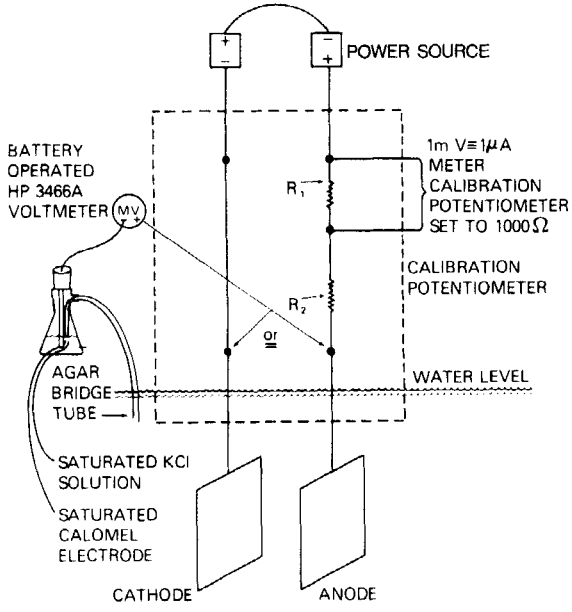

FIGURE 1 A high impedance current source used to maintain imposed electrical potentials in field studies.

The electrodes used for the voltage clamping experiments consisted of a strip of titanium foil (12 cm × 1 cm × 0.16 cm) sandwiched between two glass microscope slides filled with room-temperature vulcanizing rubber (RTV 112), which insulated the strip from the electrolyte. One end of the strip was attached to the leads from the high output resistance current source, the other end was exposed for the purpose of collecting absorbed dissolved organic material (ADOM). All electrodes were cleaned with trichloroethylene and were secured to a styrofoam block (Figure 2). The entire block, with attached electrodes, was placed into a glass enclosure. At the time of exposure the glass case and its contents were forced beneath the air/water interface, and the block was removed and allowed to float to the surface. Leads were then attached and the timed exposures started. Samples were collected in a similar fashion. These precautions were taken to avoid passing the electrodes through the organic-rich neuston layer and to ensure that the organics deposited on the foil surface could be attributed to adsorption processes. The number of bacteria attached to the titanium was counted using epifluorescence microscopy as described as above.

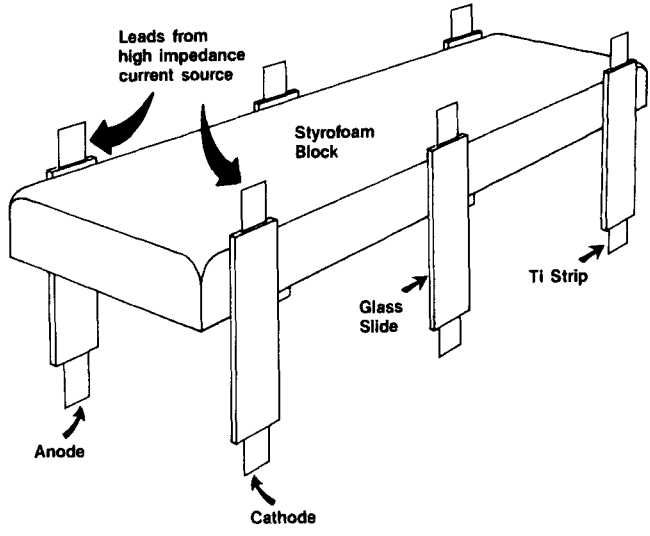

FIGURE 2 Electrodes for voltage clamping experiments.

C. Pyrolysis

Water samples for pyrolysis were placed in quartz boats, 50 µl at a time, and dried at 60°C in a laminar flow hood. This process was repeated until each boat contained the nonvolatile residue from a total 200 µl sample. All sample manipulations were carried out in a laminar flow hood.

Metal foil strips were placed in individual quartz boats (200 mm × 6 mm ID) in the coil of a Data System Pyroprobe (Series 100). The coil and the boat were inserted into an interface that was attached to the capillary injection port of a Finnigan 9600 gas chromatograph. A continuous stream of 99.99% methane gas flowed through the interface after passing through an oxygen and water scrubber. The flow rate was unknown, but the pressure was adjusted so that the ionizer of the mass spectrometer was maintained at a pressure of 0.25 Torr (33.3 Pa). The temperature of the interface was 200°C. Each sample was pyrolyzed with a 20°C/msec ramp for 20 sec at nominal temperatures of 600°C and 900°C. The temperature is nominal for the samples in the quartz boat, although fully attained by the coil.

Mass Spectrometry

The pyrolyzates were swept into a Finnigan 4000 mass spectrometer through an empty stainless steel, glass-lined capillary column (30 cm × 0.3 mm ID) at a temperature of 190°C. Methane, ionized by electrons with an energy of 130 ev, was used to ionize the pyrolyzates. The resulting pyrolyzate ions were quadrupole filtered and counted with an electron multiplier at a potential of −1500 V. The scan range was 66 to 323 amu over 1 sec. The resulting data were processed by the Finnigan INCOS data system. Nominal masses were assigned using perfluorotributylamine (FC 43) reference spectra acquired the same day as the analyses. Details related to operating parameters have been described elsewhere.[3,4] The similarity between mass spectra, termed purity, was determined by an algorithm in the INCOS system. Briefly, the intensities of up to 50 of the most significant ions in the mass spectrum were weighted with the square root of their masses. The result was calculated as a vector in a multidimensional vector space. Purity was then determined using the following equation:

$$\text{purity}(A, B) = 1000 \cos^2 \theta,$$

where θ is the angle between the vectors produced from spectra A and B. A purity value of 1000 would indicate that two spectra were identical. Purity values reflect similarities and differences in the individual ions and in the intensities of those ions. Reconstructed ion chromatograms were drawn by the computer by tracing the total ion count for each scan. The reconstructed ion counts (RIC) from these pyrograms were adjusted for the area of the foil or volume of liquid and were used for quantification of the pyrolyzates.

RESULTS

A. Hydrophobicity Experiments

The effects of starvation (carbon limitation) on the hydrophobicity of *D. marina* cells grown to mid-exponential phase are presented in Figure 3. The data indicate that starved bacteria became more hydrophobic than bacteria that were not starved. When *D. marina* was grown to both mid-exponential and stationary phases in Marine

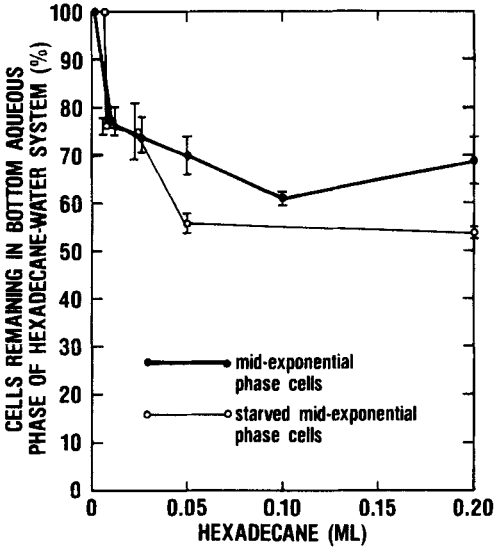

FIGURE 3 Affinity of starved and non-starved mid-exponential phase *Deleya marina* cells to hexadecane as a function of hexadecane volume. Results are expressed as percentage of the initial radioactivity left in the aqueous phase as a function of hexadecane volume.

Broth 2216, the stationary phase bacteria were more hydrophobic (Figure 4). The carbon source on which the bacteria were grown also appeared to influence their hydrophobicity (Figure 5). *D. marina* grown on glucose to mid-exponential and stationary phases were more hydrophobic than their counterparts grown on citrate. As was observed with Marine Broth 2216, stationary phase bacteria were also more hydrophobic than the mid-exponential bacteria grown on the same carbon source.

B. Metal Characterization

All of the metal surfaces used in this experiment were hydrophilic with bubble contact angles of less than 15°. Corrosion potential data indicate differing electrochemical behaviours of the test metals. Potentials for stainless steel and titanium became more positive immediately after immersion while those for copper-nickel became more negative before reaching a stable potential. The stable

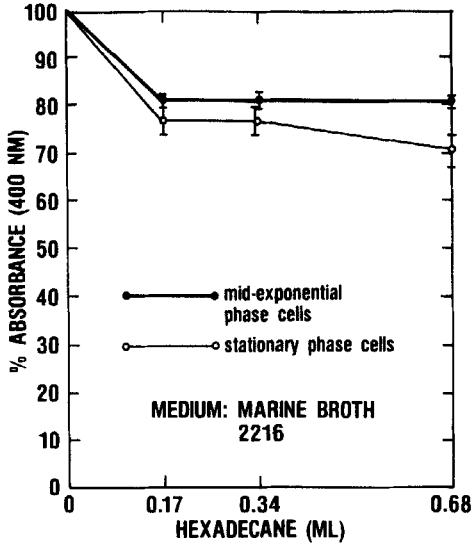

FIGURE 4 Affinity of mid-exponential and stationary phase *Deleya marina* cells to hexadecane as a function of hexadecane volume. Results are expressed as percentage of the initial absorbance (A_{400}) remaining in the aqueous phase as a function of hexadecane volume.

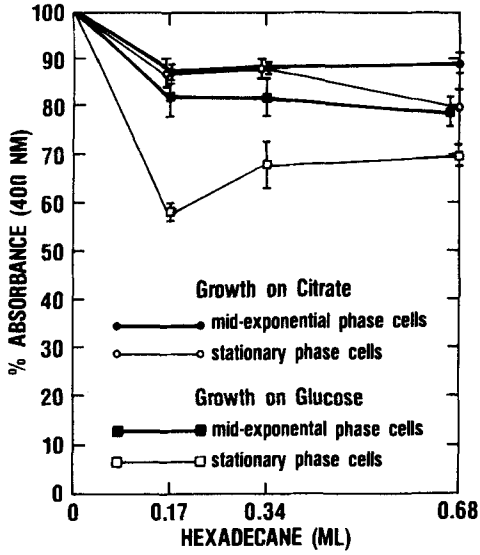

FIGURE 5 Affinity of mid-exponential and stationary phase *Deleya marina* cells grown on glucose or citrate to hexadecane as a function of hexadecane volume. Results are expressed as percentage of the initial absorbance (A_{400}) remaining in the aqueous phase as a function of hexadecane volume.

corrosion potentials *versus* SCE in the estuarine and marine waters, respectively, were: titanium, +0.22 and +0.02; stainless steel, +0.10 and +0 04; and copper-nickel, −0.22 and −0.31.

C. Attachment Studies

The numbers of bacteria that attached and grew on the metal surfaces were substratum dependent. Differences in bacterial attachment were also observed between experiments run in artificial seawater and in filter-sterilized natural seawater.

Under all experimental conditions, copper-nickel supported a smaller population of *V. alginolyticus* than either titanium or stainless steel. Figure 6 shows the results of experiments conducted in natural seawater without prior exposure to 2216 broth. With time, the numbers of attached cells on copper-nickel appeared to decrease. This behaviour was not observed in experiments using

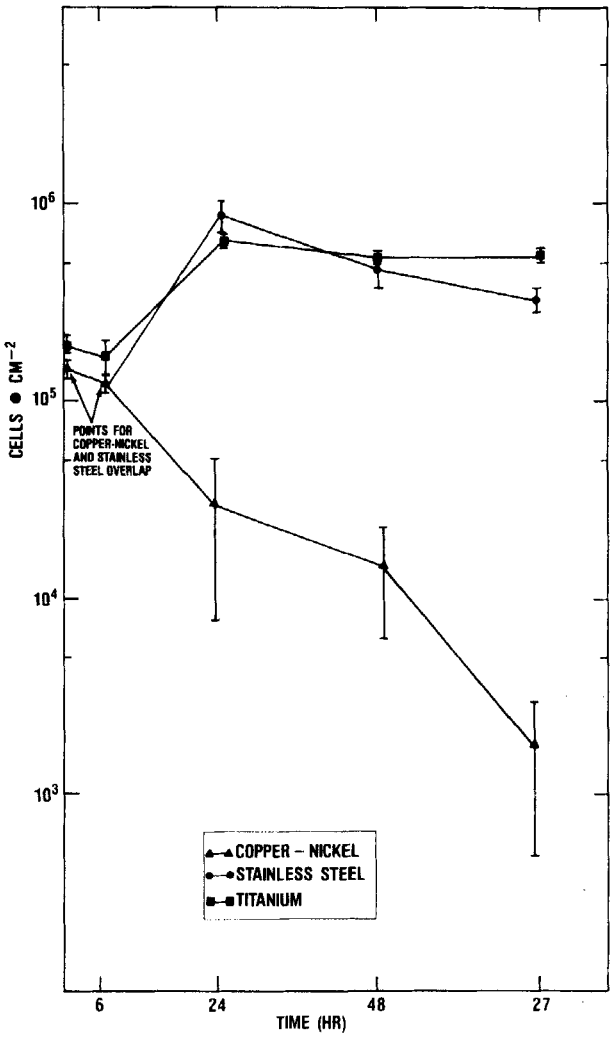

FIGURE 6 Growth of *Vibrio alginolyticus* on copper–nickel (90–10), titanium (grade 2), and stainless steel (316) in natural seawater.

either titanium or stainless steel. No significant differences in attachment and growth of *V. alginolyticus* were seen between any of the metals dipped in 2216 broth and the controls. Data are presented in Figures 7 and 8 for the titanium and stainless steel, respectively. However, there were differences in the numbers of

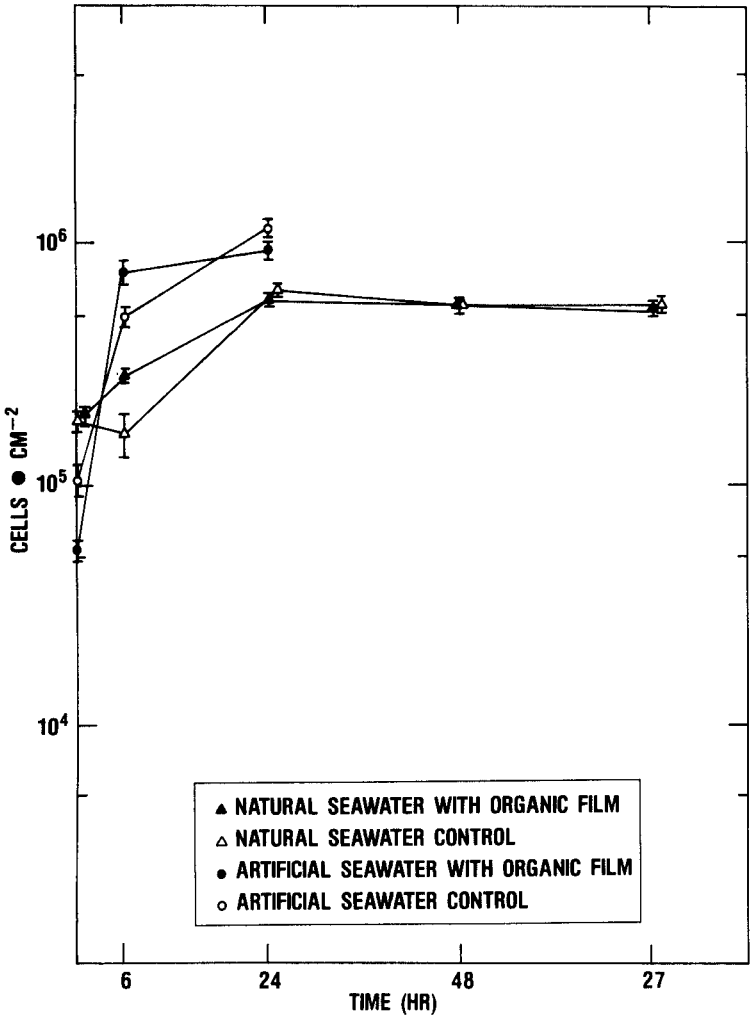

FIGURE 7 Growth of *Vibrio alginolyticus* on titanium (grade 2).

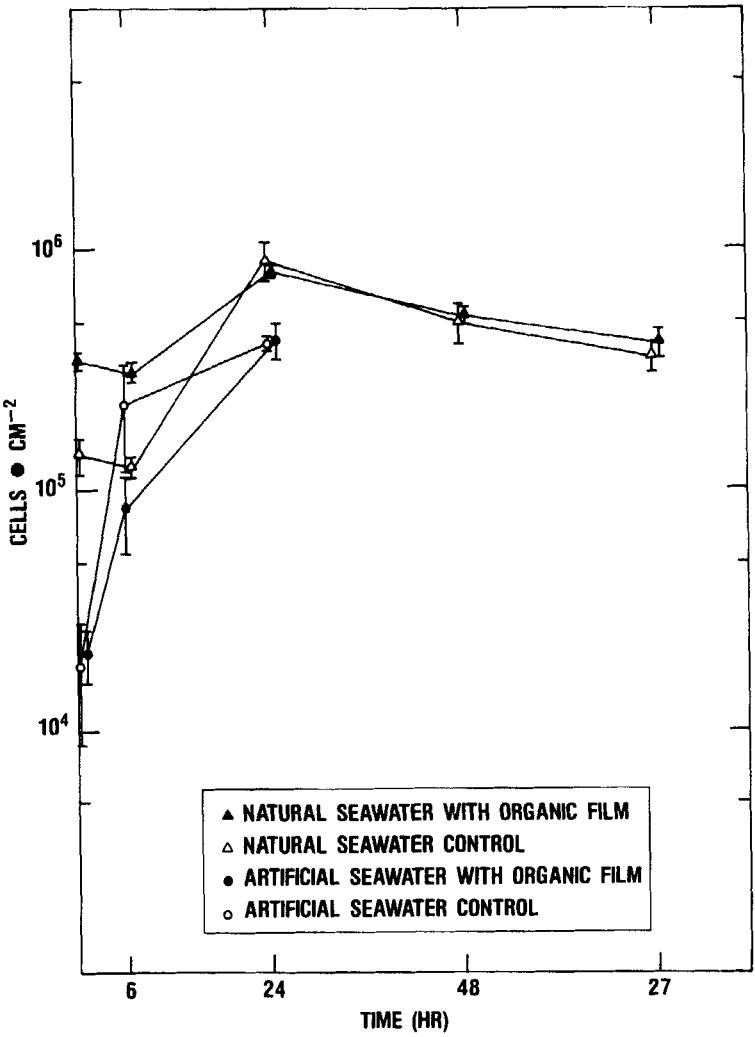

FIGURE 8 Growth of *Vibro alginolyticus* on stainless steel (316).

attached cells when exposures in artificial seawater were compared to exposures in natural seawater.

SEM observations of metals exposed to natural seawater *in situ* supported the results of the pure culture experiments. Large, diverse communities of bacteria, protozoa, and algae were seen on both titanium and stainless steel surfaces, while 90–10 copper–nickel supported few protozoa or algae and only sparse populations of bacteria (Figure 9a and b). Some morphological differences in the bacterial communities could also be seen. Bacteria on the copper alloys were often filamentous in form and less often developed than the large microcolonies seen on steel and titanium.

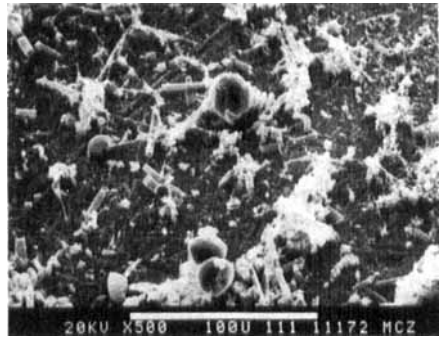

FIGURE 9a SEM of 7-day marine biofilm on stainless steel (316); scale bar = 100 μm.

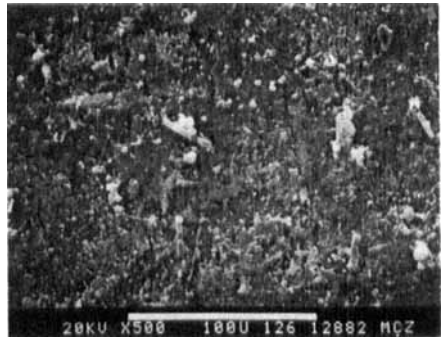

FIGURE 9b SEM of 14-day biofilm on copper–nickel (90–10); scale bar = 100 μm.

CHARACTERIZATION OF ADSORBED DISSOLVED ORGANIC MATERIAL (ADOM)

A. The Results of the Pyrolysis-Chemical Ionization Spectrometry

Pyrolysis-chemical ionization mass spectrometry used to characterize adsorbed dissolved organic material (ADOM) from the estuarine waters of Bay St. Louis, Mississippi, on three metal substrata as a function of time are presented in Table I. These data have been published elsewhere.[5] The top row of numbers compares the organics present in Bay St. Louis water with those sorbed on the surfaces as a function of time. In all cases the highest purity values for this comparison were found at the 15-minute exposure. The adsorption process appears to be selective, *i.e.*, the sorbed organics do not closely resemble those found in the water column, and this selectivity becomes more apparent as exposure time increased. Numbers in the lowermost continuous diagonal line are the purity values for the replicate samples, which were 600 or greater with an average standard deviation of ±37. In between these two lines are all other purity comparisons. Purity values for all comparisons decreased during the first hour of exposure, indicating that the chemical composition of the adsorbed material varied among the substrata. However, after 4 hours the purity values for the adsorbed material on all surfaces were 690 ± 20 or greater, which indicates a

TABLE I
Purity values for comparisons of pyrolyzates from metal surfaces.

	CN(¼)	CN(½)	CN(1)	Cn(4)	SS(¼)	SS(½)	SS(1)	SS(4)	Ti(¼)	Ti(½)	Ti(1)	Ti(4)
BSL	451	330	176	261	372	298	375	319	229	170	237	150
CN(¼)	730				358				346			
CN(½)		699				254				154		
CN(1)			698				389				195	
CN(4)				720				736				778
SS(½)					696				260			
SS(¼)						725				227		
SS(1)							701				192	
SS(4)								748				731
Ti(¼)									657			
Ti(½)										638		
Ti(1)											643	
Ti(4)												792

Numbers in ( ) indicate exposure time in hours.

great deal of similarity. Indeed, the purity values among the various substrata were indicative of replicate films.

The impact of initial surface charge on the quantity and the chemical composition of adsorbed material was investigated by using a high output resistance current source. Titanium was voltage clamped at the empirically determined potential of zero charge (pzc), −0.7 V, reported by Dhar *et al.*[6], and at potentials above (+1.3 V) and below (−2.7 V) the pzc. In such a circumstance, the sign of the charge can be deduced. Potentials positive to the pzc result in a positive charge, and those negative to the pzc result in a negative surface charge. However, the magnitude of the charge cannot be evaluated. The quantity and the chemical composition of the absorbed films were then examined using pyrolysis/mass spectrometry.

Table II compares the purity values of the mass pyrograms produced from the titanium foils exposed in Bay St. Louis. These comparisons indicate that surface charge had no impact on the composition of the adsorbed film. The films collected on the unpolarized strips are identical to those collected with imposed voltages. Furthermore, after 4 hours the average RIC among all the samples was 28,000/cm^2, with a standard deviation of ±820. This result indicates that virtually the same amount of material sorbed to all the exposed titanium surfaces, regardless of imposed potential and surface charge.

The average number of attached bacteria for polarized and unpolarized titanium after 24, 48 and 72 hours is presented in Figure 10. More bacteria attached to the negatively charged titanium than to the unpolarized, polarized to the pzc or the positively charged surface.

TABLE II

Purity values for comparisons of pyrolyzates from polarized and unpolarized titanium surfaces.

		Imposed Potential in Volts		
	Unpolarized	-2.7	-0.7	+1.3
Unpolarized	732	686	603	594
Imposed Potential in Volts -2.7		790	712	532
Imposed Potential in Volts -0.7			712	568
Imposed Potential in Volts +1.3				796

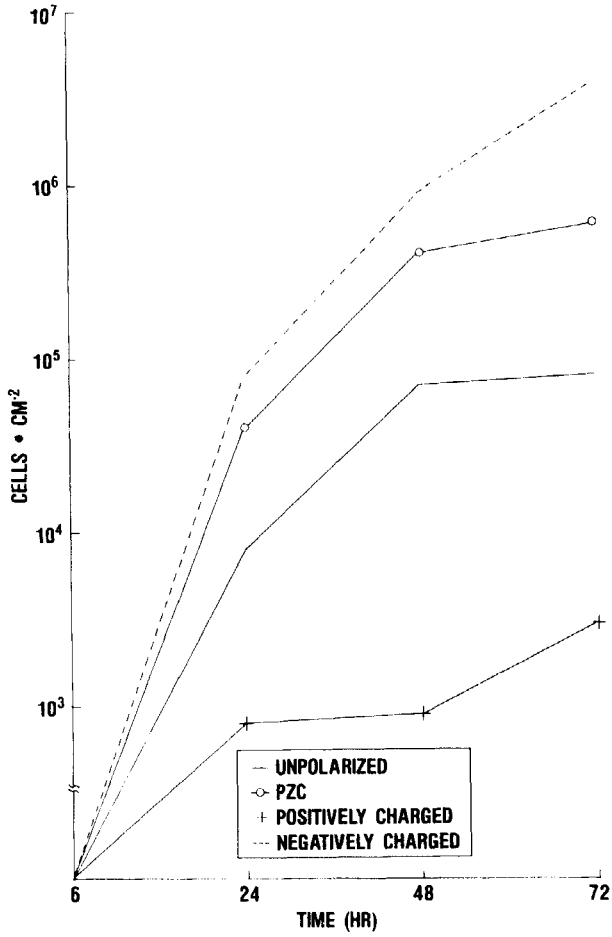

FIGURE 10 Numbers of bacteria attached to polarized and unpolarized titanium surfaces.

DISCUSSION

Bacterial attachment to surfaces includes a reversible and an irreversible phase.[7] The former is an instantaneous attraction which holds a bacterium near a surface, while the latter is time dependent and may involve extracellular material.[8] A bacterium that is only

reversibly adsorbed to a surface may still be able to scavenge nutrients from that surface.[9] Hydrophobicity is one of several short-range attractive forces responsible for the reversible adsorption of bacteria to solid surfaces.[8]

The methods used to measure cell surface hydrophobicity are limited in that they may only detect a single type of hydrophobic interaction, while a bacterium may have hydrophobic regions that are very complex in nature.[9,10] The two-phase separation method for measuring cell surface hydrophobicity works under the assumption that the more hydrophobic bacteria will disappear from the aqueous phase as they become adsorbed to the hydrocarbon (in this case hexadecane) phase.[2] Due to the high interfacial tension existing between the aqueous and hydrocarbon phases it has been suggested that this method determines differences between cells that already exhibit a high degree of surface hydrophobicity.[11] The method has been used by a number of investigators, and their results have been summarized by Rosenberg.[12]

Our results indicated that starvation (carbon limitation) of *D. marina* resulted in cells that were more hydrophobic than cells that were not starved. After starving various strains of marine bacteria in a similar manner, Kjelleberg and Hermansson[11] also observed an increase in cell surface hydrophobicity accompanying the general starvation responses of division without growth and continuous size reduction of cells. Starving bacteria may have their survival enhanced by adhering to a surface[13] where they can scavenge adsorbed nutrients.[14,15] Although hydrophobicity may be important in the initial phases leading to irreversible binding to a surface, Kjelleberg and Hermansson[11] did not always observe parallel increases in cell surface hydrophobicity and irreversible binding of starved bacterial cells.

An increase in the cell surface hydrophobicity of *D. marina* was observed when cells were harvested at stationary phase from that of cells harvested at mid-exponential phase. Similar results were reported for *Acinetobacter caloaceticus* RAG-1,[16] *Serratia marcescens*,[2,17] and *Streptococcus pyogenes*.[10] It was suggested that capsular polysaccharides of the mid-exponential phase cells interfered with adherence to the hexadecane in two of the cases.[10,16] The cause of the increase in hydrophobicity of *D. marina* with age of the culture is unknown at present. Finally, we observed an increase in

surface hydrophobicity when cells were grown on glucose over cells grown on citrate. At present we do not know why *D. marina* grown on glucose is more hydrophobic.

Many studies have provided visual records of the attachment of various microorganisms to metallic and nonmetallic surfaces in the sea.[18,19] It is not clear which properties of metal surfaces and/or their adsorbed organic films have the greatest influence on attachment and how these properties differ among the various alloys that we studied. Data are scarce concerning the quantitative behavior of specific film-forming bacteria in response to metals of known composition.[20] Some studies have shown that different metals may harbour quite different types of microbial flora.[21,22] Our SEM observations support the results of these workers.

Though all metals are susceptible to fouling by microorganisms, different types of metals appear to be susceptible in different degrees, at least upon initial immersion in the water. Pure culture experiments carried out using 90–10 copper–nickel showed a decrease in cell number with time. The data suggest either a toxic effect from copper ions or perhaps a loss of cells due to sloughing-off of loosely bound corrosion products.[23]

Surfaces immersed in seawater immediately adsorb a conditioning film of complex macromolecules that reportedly favors the subsequent growth of microorganisms.[24] We expected that bacteria attached to metals dipped in a protein-rich broth would multiply more quickly than those on metals that were not dipped. The results of our experiments do not support this idea, as no significant differences in cell numbers were seen between the two treatments. It is possible that many of the bacteria associated with the filmed surfaces are reversibly adhered and thus washed away during the experimental procedure. These loosely attached bacteria comprise an important and active component of the biofilm community,[25] but the reversible nature of their adhesion makes accurate counts difficult.[11]

Studies on the growth and attachment behavior of marine bacteria often use artificial seawater (ASW) as the test medium. ASW has the advantage of controlled composition and lack of interference from varying organic content, etc. Evidence strongly suggests, however, that results of experiments on marine microfouling differ when the analyses are conducted in natural seawater (NSW). The presence of dissolved organics in the NSW used in our

experiments may at least partially explain the differences in attachment and growth seen in the two media. An exogenous energy source at low concentration has been shown[7] to enhance attachment, perhaps accounting for the higher numbers of initially attached cells in experiments using NSW. On the other hand, nutrient limited cells adhere very efficiently to surfaces and, in response to starvation, fragment (resulting in more cells), shrink in size, and exhibit a drastic decrease in endogenous metabolism.[13,15] It is possible that a starvation response in either the ASW or the NSW treatments influenced our results.

The possibility that the observed qualitative and quantitative differences in the microbial communities developed as a response to substratum specific adsorption of dissolved organic constituents from the water column was also investigated. Pyrolysis-chemical ionization mass spectrometry was used to characterize adsorbed dissolved organic material from the estuarine waters on metal substrata as a function of time. The chemical composition of the adsorbed film varied among the substrata after the first hour of exposure. Surface properties such as corrosion potential and charge may influence the bulk chemical composition during this time period. After 4 hours all adsorbed films became uniform, and the quantity and composition of the adsorbed material was substratum independent for the metals that were tested. Microorganisms did not adhere to the substrata during the 4-hour exposure.

Surface charge did not impact the bulk chemistry of the adsorbed films over a wide potential range. Morrissey *et al.*[26] made a similar observation in the case of fibrinogen adsorbed on platinum at pH 7.4; applying potentials beginning at 0.2 V/SCE resulted in no change in the steady-state rest potential value of adsorbance until +0.4 V/SCE was reached. At this potential and above, a rapid increase in adsorbance occurred, the rate of which was roughly proportional to the applied potential. Data obtained for serum albumin and γ-globulin indicated a similar behavior, with potentials for enhanced adsorption of +0.6 V and +0.8 V/SCE, respectively. At physiological pH, these proteins possess a net negative charge. The observed behavior was consistent with electrostatic attraction at high anodic potentials, resulting in enhanced adsorption. The potentials of enhanced adsorption did not correlate with the protein isoelectric points.[26]

The possible relationship between potential and charge on the

phenomenon of thrombosis on synthetic implants has been extensively explored.[27] The implantation of metal electrodes, which cover a wide range of the electromotive series, indicated that metals registering negative potentials/NHE were generally free of thrombus deposits, while those that were positive/NHE were covered with deposits.[28] However, attempts to produce a nonthrombogenic metal by imposing an external potential have not succeeded.[29] The dependence of the adsorption of individual blood proteins upon potential has been investigated using capacitance techniques,[30] cyclic voltammetry,[31] potentiostatic and qualitive ellipsometric techniques,[32] and by internal reflection spectroscopy.[33]

The charge imposed on a metal surface and the resulting interfacial pH depend on the extent and direction of polarization, and on the nature of the metal.[34] In contrast, the surface charge of bacterial cells depends on the interfacial pH, which determines the degree of protonation of iogenic groups associated with the cell. Therefore, the electrophoretic mobility of bacteria toward polarized surfaces is directly affected by charge. It has been demonstrated by others that surface charge can influence the adhesion of glutaraldehyde-fixed red blood cells.[35,36] The effects of polarization and induced charge on bacterial attachment have been attributed to the enhancement of anodic and cathodic reactions induced by polarization.[35]

CONCLUSIONS

The adhesion of bacteria from solution to surfaces is strongly influenced by the conditions at the solid–solid interface. It is apparent from the present study, as well as the previous summarized work,[12] that a number of factors, including starvation, growth phase, and carbon source, can influence the surface hydrophobicity of a cell and that changes in hydrophobicity of a cell can also influence the ability of that cell to adsorb reversibly or irreversibly to a substratum.

The number and kind of microorganisms found attached to metal substrata were found to be substratum specific. We have also demonstrated that bacterial adhesion to metal surfaces in estuarine environments can be influenced by surface charge induced by

electrochemical polarization. In addition, the development of differing microbial communities must be due to surface properties that are expressed in the presence of the ubiquitous film of dissolved organic matter or to unique properties of the adsorbed film other than bulk chemical composition.

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