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SEASONAL VARIATIONS IN BACTERIAL COLONISATION OF STAINLESS STEEL, ALUMINIUM AND POLYCARBONATE SURFACES IN A SEAWATER FLOW SYSTEM

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Before the construction of an Ocean Thermal Energy Conversion (OTEC) power plant, an experimental station was built near the entrance of the Bay of Brest (France) in order to perform preliminary studies. Bacterial colonisation of three substrata (stainless steel UB6, aluminium AG4 and polycarbonate filters) was observed in a continuous flow seawater system. The study was performed between May 1983 and May 1984 and included seven experiments of 15 days each.

The early events in bacterial colonisation of these materials may be affected by both water quality and type of substratum. If these two parameters influence the adsorption step of the biological process (0 to 1 h), seasonal variation in water quality seems to be more important during the accumulation step (1 h to 6 days), because of its effect on bacterial metabolism.

KEY WORDS: Bacteria, surfaces, adhesion, sea water.

INTRODUCTION

In a natural environment, tubular flow systems invariably become fouled by biofilms. In heat exchangers, biofilms increase energy losses due to increased heat transfer and fluid frictional resistance and can cause material deterioration (Aftring & Taylor, 1979; McCoy *et al.*, 1981).

The development of biofilms in flow systems has been observed in field and laboratory experiments (McCoy & Costerton, 1982; Bott & Miller, 1983; Pederson, 1982). It appears that the progression of biofouling can be divided into three phases (Characklis, 1981). A first phase of transport and adsorption of a wide range of periphytic bacteria to the conditioned surfaces; a second phase of growth and accumulation of adsorbed cells; and a third stationary phase during which detachment of portions of the biofilm leads to a plateau level.

Prior to the construction of an Ocean Thermal Energy Conversion (OTEC) power plant, the French Sea Development and Research Institute, IFREMER (Institut Français pour la Recherche et l'Exploitation de la Mer) constructed an experimental station in the Bay of Brest (France), in order to test different materials and to observe biofilm development in different water quality conditions. Experiments were conducted over one year in 7 periods of 15 days each and colonising bacteria were

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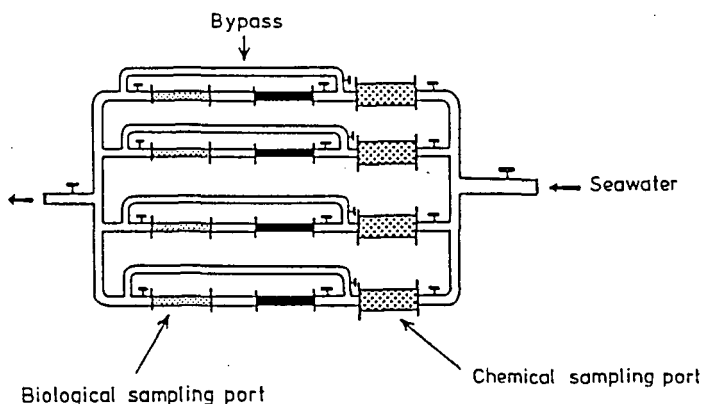


Fig. 1 Schematic representation of the experimental system.

counted on three materials, viz. stainless steel UB6, aluminium AG4 and polycarbonate filters.

The influence of the substrata and seasons on the early stages of biofouling formation was observed and discussed within a statistical context.

MATERIALS AND METHODS

Experimental System

The experimental system consisted of four parallel mounted circular fouling test units (Fig. 1). Seawater flow rate was $0.66 \text{ m} \cdot \text{s}^{-1}$. Individual units could be removed from the reactor without disturbing the others. Each unit was constructed of 0.04 m inside diameter PVC pipe fitted with eight replaceable plugs at sampling ports along the length of the pipe. Two samples of each material studied could be tested simultaneously.

Preparation of Substrata

The stainless steel UB6 and aluminium AG4 samples were finely polished ($1 \mu\text{m}$ diamond polisher) to obtain a mirror-type surface. Each sample was cleaned with acetone and then sterilised in a Pasteur oven ($1 \text{ h } 30 \text{ min}$ at 150°C). The polycarbonate filters were dyed with Irgalan black (Hobbie *et al.*, 1977), fitted on PVC plugs with rubber rings, sterilised with UV radiation (24 h) and introduced into the fouling test unit before the beginning of an experiment.

Experimental Procedures

Experiments were conducted in 7 periods of 15 days from May 1983 to May 1984. After the beginning of an experiment (start of the seawater flow through the test unit), samples were removed after 1, 6, 12 h, 1, 2, 4, 6, 8, 10 and 15 days. Samples were removed from the fouling test unit after stopping the flow. Metallic samples were fixed in seawater solutions containing 2.5% glutaraldehyde and filters were fixed in 2% formaldehyde. Between experimental runs, the test units were removed, brushed, chlorinated (7% chlorine) rinsed with fresh water and provided with clean test surfaces before the flow was restarted.

Samples for determination of seawater quality parameters (temperature, salinity, dissolved oxygen and pH) were taken weekly at high tide during the one year experimental period.

Simultaneously, samples were taken for the determination of bacterial concentrations in seawater at 0.3 m below the water surface and in the units.

From temperature and dissolved oxygen data, the percentage of dissolved oxygen was determined using the modified version of a formula by Dopp (1984) taking both salinity and elevation above sea level into account.

$$DO = \frac{P - 0.925.T + 0.282}{1.499.T + 51.92}$$

where DO = dissolved oxygen concentration ($\text{g}\cdot\text{m}^{-3}$)

P = barometric pressure at sea level (mmHg)

T = temperature

From temperature and flow rate data the shear stress was obtained (Davies, 1972):

$$\tau = (0.2.v.Re^{-0.125})^2$$

where τ = shear stress ($\text{kg}\cdot\text{m}^{-1}\cdot\text{s}^{-2}$)

$v = 18.988 + 0.1212.T)^{-4.5}$ is kinetic viscosity, ($\text{m}^2\cdot\text{s}^{-1}$), related to temperature (Huisman, 1977)

Re = Reynolds number (—)

Cell Counts

Substratum samples were used for direct observation of biofilm development. Metallic surfaces were prepared for scanning electron microscopy (SEM) according to Anderson (1951). Samples were observed with a scanning electron microscope (JEOL JSM) at 35 kV accelerating voltage and a magnification of 2000 or 4000 times. On each sample, 10 or 20 fields were counted. Polycarbonate filters were prepared for fluorescence microscopy according to Hobbie *et al.* (1977), and observed with an epifluorescence microscope (Bausch & Lomb "balpan"). Cells were counted on 10 fields at a magnification of 1000 times. Cell concentrations in seawater samples were determined with epifluorescence microscopy (Hobbie *et al.*, 1977).

Cell Volume

During the January and May 1984 experiments, the cell volume of bacteria attached to the polycarbonate surfaces was measured according to Fry and Davies (1985) with an image analyser (OPTOMAX PS2). Photographic slides of epifluorescent microscopic fields were taken with a Canon AE1 camera using automatic exposure and Kodak Ektachrome film (400 ASA). The slides were then projected and the contours of clearly detectable cells were drawn. These drawings were viewed with the camera of the image analyser, fitted with a macro 50 mm lens, and dimensions of 50–200 cells per sample were determined. From these data, cell volume was calculated.

RESULTS

The progression of areal cell density was analysed by plotting logarithm of areal cell density versus time. The characteristics of cell adsorption and cell accumulation were

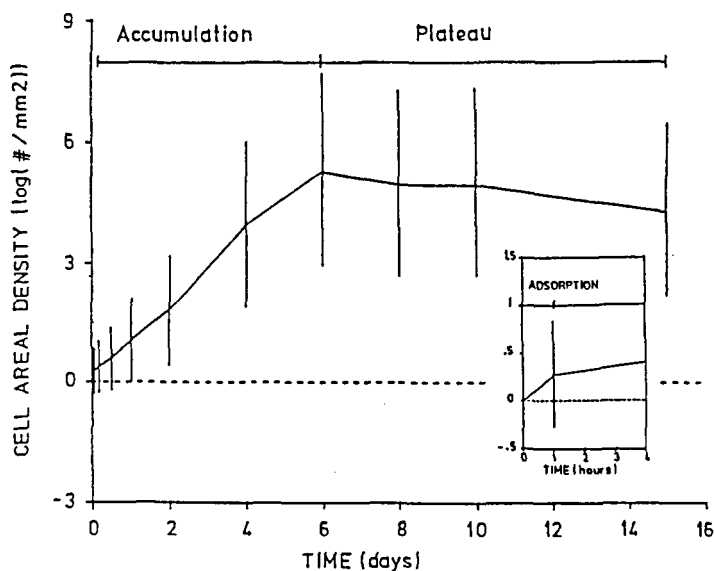


Fig. 2. Means and standard deviations of areal cell density on aluminium for September 1983 based on 40 data points for 1, 6 and 12 h and 1 and 2 days and 20 data points for 4, 6, 8, 10 and 15 days.

determined (a) for the adsorption phase: between time = 0 and the time at which an initial plateau is reached (1 h), and (b) for the accumulation phase: the time at which areal cell density starts to deviate from the initial plateau and maximum areal cell density (steady state) is reached.

Data points for each phase were fitted to a linear equation using linear regression. A characteristic time progression of areal cell density is shown by the means and the standard deviations of the data points (Fig. 2). The phases are between 0 and 1 h (adsorption phase), between 1 h and 6 days (the accumulation phase) and over 6 days (the plateau phase).

The initial bacterial densities observed after 1 h of immersion on the three materials ranged from 20 to 800 cells mm^{-2} . After 24 h immersion, these densities were slightly higher. The maximum densities occurred in spring and summer, up to 10^5 cells mm^{-2} . In winter, the values were around 10^4 cells mm^{-2} .

Values for the rate of cell adsorption are calculated from the initial bacterial density for the period between 0 to 1 h. Mean values and standard deviation of adsorption rate for different substrata were calculated from 20 or 40 data points (Fig. 3). Similarly, mean values and standard deviation of accumulation rate for different substrata were calculated from 20 or 40 data points (Fig. 4). The starting and ending points for the accumulation phase vary. Summary statistics on rates of adsorption and accumulation are given in Table 1. In both Figs 3 and 4, differences between rates appear to be restricted to the first half of the investigated period, i.e. from May to November 1983. Thereafter, differences are roughly within the standard deviation. Also, both rate of adsorption and rate of accumulation on filter material appear different from rates of adsorption and accumulation on both metal surfaces in the first half of the investigated period. Analysis of variance related to the effect of substratum and/or season on rate of adsorption, indicated that at the 95% level of significance, both substratum

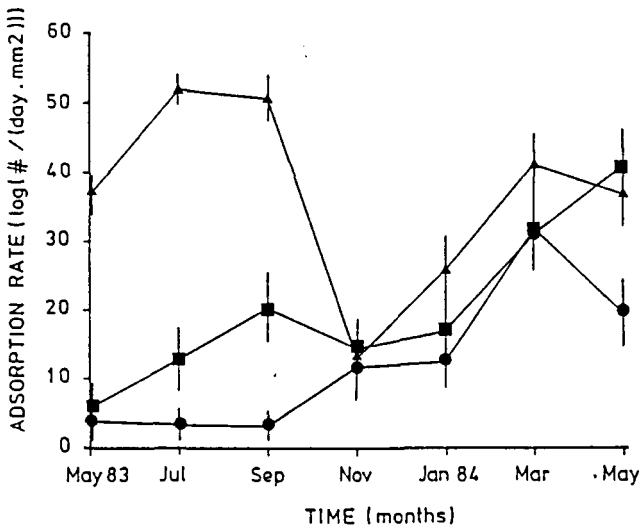


Fig. 3. Rate of cell adsorption. ▲ = filter; ■ = stainless steel; ● = aluminium. Data points are means of 20 or 40 measurements; error bars indicate standard deviation.

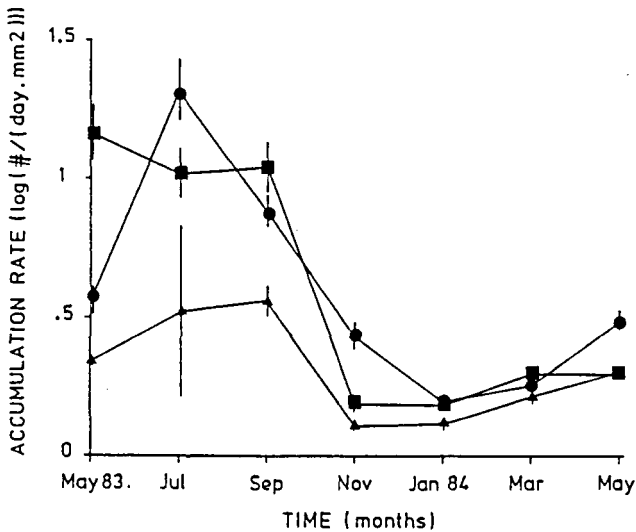


Fig. 4. Rate of cell accumulation. ▲ = filter; ■ = stainless steel; ● = aluminium. Data points are means of 20 or 40 measurements; error bars indicate standard deviation.

Table 1 Summary statistics on rates of adsorption and accumulation

Stainless steel										
Date Mo/Ye	Log Adsorption Phase					Log Accumulation Phase				
	Slope (*)	SDev (*)	CoVar (%)	R2 (—)	Period (days)	Slope (*)	SDev (*)	CoVar (%)	R2 (—)	Period (days)
May 1983	5.92	3.33	56.25	0.075	0-0.047	1.17	0.10	8.57	0.497	0.5-4
Jul 1983	12.90	4.51	34.96	0.174	0-0.047	1.02	0.09	9.01	0.471	0.5-4
Sep 1983	20.38	5.01	24.58	0.298	0-0.047	1.04	0.09	8.68	0.474	0.5-4
Nov 1983	14.40	4.30	29.86	0.224	0-0.047	0.19	0.03	14.46	0.257	2-10
Jan 1984	17.09	4.44	25.98	0.275	0-0.047	0.19	0.02	13.28	0.264	2-10
Mar 1984	31.08	5.56	17.89	0.445	0-0.047	0.30	0.02	8.35	0.549	4-10
May 1984	40.89	5.40	13.21	0.595	0-0.047	0.29	0.02	6.18	0.655	2-10
Aluminium										
Date Mo/Ye	Log Adsorption Phase					Log Accumulation Phase				
	Slope (*)	SDev (*)	CoVar (%)	R2 (—)	Period (days)	Slope (*)	SDev (*)	CoVar (%)	R2 (—)	Period (days)
May 1983	3.83	2.67	69.71	0.050	0-0.047	0.55	0.04	7.07	0.592	2-10
Jul 1983	3.29	2.30	69.91	0.050	0-0.047	1.31	0.11	8.33	0.595	1-4
Sep 1983	3.11	2.17	69.77	0.050	0-0.047	0.88	0.06	6.49	0.668	1-6
Nov 1983	11.77	4.49	38.15	0.150	0-0.047	0.43	0.04	10.35	0.357	1-8
Jan 1984	12.43	3.98	32.02	0.200	0-0.047	0.20	0.02	8.81	0.372	1-15
Mar 1984	31.90	5.40	16.93	0.472	0-0.047	0.25	0.02	7.36	0.539	4-15
May 1984	19.65	4.93	25.09	0.298	0-0.047	0.49	0.03	6.39	0.608	1-8
Filter										
Date Mo/Ye	Log Adsorption Phase					Log Accumulation Phase				
	Slope (*)	SDev (*)	CoVar (%)	R2 (—)	Period (days)	Slope (*)	SDev (*)	CoVar (%)	R2 (—)	Period (days)
May 1983	36.71	2.90	7.90	0.804	0-0.047	0.34	0.02	4.51	0.820	1-10
Jul 1983	51.98	2.06	3.96	0.971	0-0.047	0.52	0.31	59.63	0.708	1-6
Sep 1983	50.68	3.21	6.33	0.929	0-0.047	0.56	0.05	9.37	0.663	1-4
Nov 1983	12.97	4.62	35.62	0.293	0-0.047	0.11	0.02	14.88	0.315	2-10
Jan 1984	25.84	4.96	19.20	0.588	0-0.047	0.12	0.02	17.87	0.315	2-10
Mar 1984	41.05	4.28	10.43	0.829	0-0.047	0.22	0.02	10.65	0.531	4-10
May 1984	36.85	4.42	11.99	0.785	0-0.047	0.31	0.02	4.99	0.821	2-10

(*) = $\log(\text{cell number} \cdot \text{day}^{-1} \cdot \text{mm}^{-2})$

SDev = Standard Deviation

CoVar = Coefficient of Variation

R2 = R-squared, measure of goodness of fit

($p = 0.0009$) and season ($p = 0.0056$) have an effect on adsorption levels. In addition, the interactive effect between the two variables substratum and season on the rate of adsorption was significant at the 95% level ($p = 0.0051$).

The temperature, salinity, dissolved oxygen saturation percentage and the derived parameter, shear stress, are shown in Fig. 5. Temperature shows the characteristic effects of seasonal changes, resulting in a 10°C change between the extremes. The effect

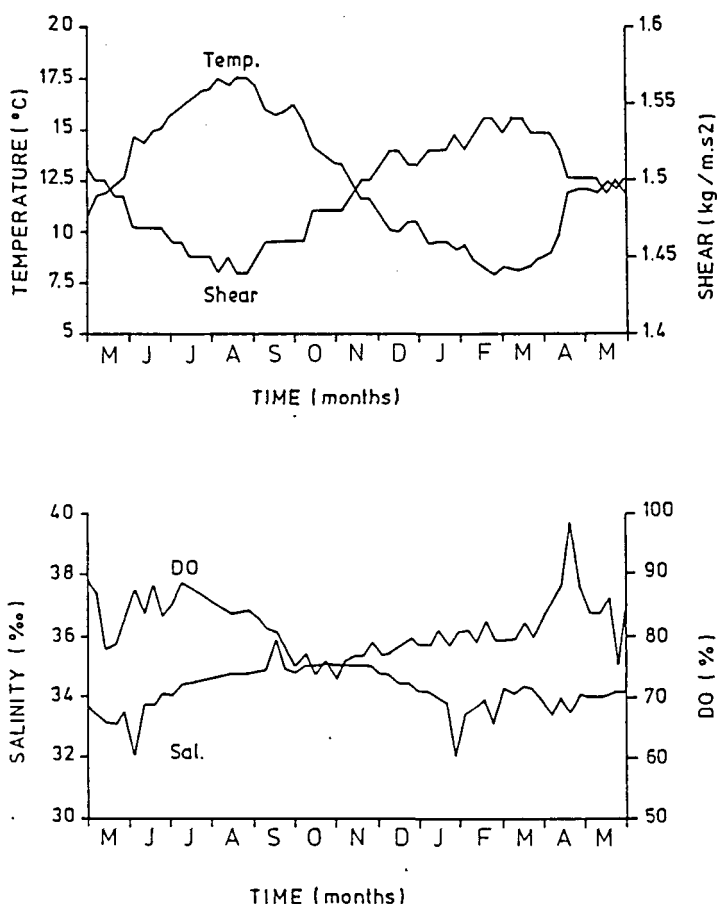


Fig. 5. Temperature (Temp), salinity (Sal), dissolved oxygen saturation percentage (DO) and shear stress (Shear) over the experimental period.

of temperature variations is mirrored by variations (although relatively small) in shear stress through its effect on viscosity. Seasonal variations in salinity were also small, although twice a sudden drop was observed (from 34 to 32%). The DO saturation percentage was always 75% or more. In the first 4 months of the experimental period, 85% was recorded while a sudden peak almost reaching saturation occurred in early Spring 1984. DO saturation concentration does not seem to be clearly related to variations in temperature.

Cell concentrations in seawater samples were determined weekly (Fig. 6). In both the flowing seawater and in the surface seawater near the experimental site, bacterial numbers can be considered constant during the experimental period.

Cell volume for the rod shaped bacteria varies during the immersion time and appears to be affected by the season (Fig. 7). In January 1984, most adsorbed cells were relatively small (approx. $0.1 \mu\text{m}^3$). The cell volume progressively increased (to approx. $0.4 \mu\text{m}^3$). In May 1984, initial volumes were also small ($0.2 \mu\text{m}^3$), increased dramatically in the following 12 h ($0.8 \mu\text{m}^3$) and then gradually decreased to approximately $0.2 \mu\text{m}^3$ towards the end of the experiment.

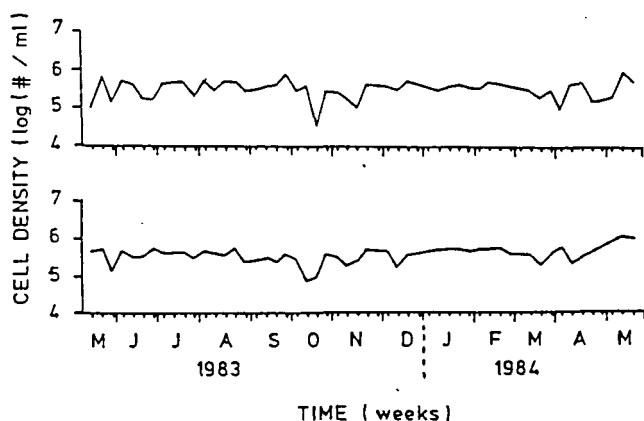


Fig. 6. Cell concentration in seawater.

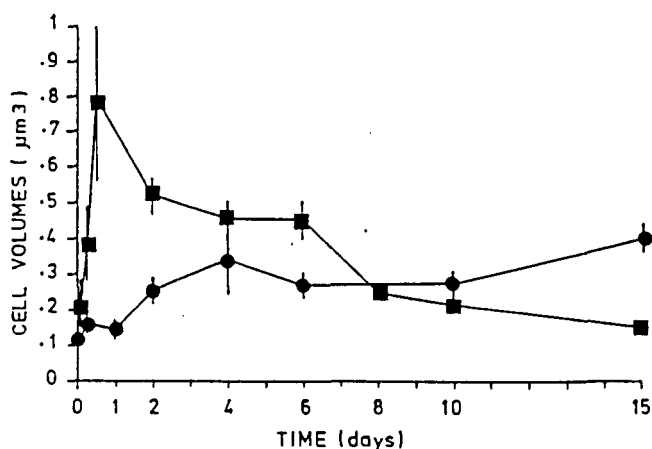


Fig. 7 Fixed rod-shaped bacterial volumes in summer (■) and in winter (●).

DISCUSSION

The process of biofilm formation appears to be affected by both type of substratum and season, but the effect varied according to the phase of colonisation.

The transport and adsorption step is assumed to take place in the first hour of exposure because after about 1 h, cell counts remain relatively stable for some time before increasing again. Thus, the number of adsorbed cells after 1 h are presumed to be a good indicator of adsorption rate. The cell densities observed after 1 h of exposure ranged between 20 to 800 cells·cm⁻² (Fera & Prieur, 1986). Similar results were generally observed on different non-toxic surfaces, in the natural environment (Dexter *et al.*, 1975; Marszalek *et al.*, 1979), or under controlled laboratory conditions (Nelson *et al.*,

1985). In this study, both substratum and season affected the cell adsorption rate. Fluid shear stress and concentration of suspended cells can influence this process (Fletcher, 1977; Bott & Miller, 1983; McCoy *et al.*, 1981), but these parameters were almost constant in the experiments. Thus, any variation in the rates is probably due to differences in substratum characteristics or to seasonal change in water quality (e.g. temperature).

With regard to differences between the three materials, the sticking efficiency is undoubtedly a function of substratum properties (e.g. hydrophobic vs. hydrophilic, rough vs. smooth) (Dexter *et al.*, 1975; Fletcher & Loeb, 1979; Absolom *et al.*, 1983). The initial surface tension of the polished metallic alloys used in these experiments were 35.5 ± 1.5 dyne·cm⁻¹ for the stainless steel and 36.5 ± 1.5 dyne·cm⁻¹ for the aluminium. Thus, these surfaces appeared to be hydrophilic and very similar. Because of surface roughness, it was impossible to measure the surface tension of the filter material. Lee (1968) gave a value of 42 dyne·cm⁻¹ for polycarbonate, but the staining of filters certainly reduced this value (Baier, 1970). Adsorption rate appeared to be substantially higher for the filter material than for both metallic surfaces in the first 6 months of the experimental work. Thereafter, differences, if any, were less apparent. This agrees with the literature concerning *in situ* and *in vitro* experiments, when the surface tension of the materials is up to 25 dyne·cm⁻¹ (Dexter *et al.*, 1975). It is also possible that the roughness of the filters enhanced this phenomenon.

For all the materials, an increase in cell adsorption rate was observed during the winter months. In this case, the hydrophobicity of the bacterial cell surfaces may be very important. Thus, for the same substratum, hydrophobic cells adhere to a greater extent than hydrophilic cells (Van Loosdrecht *et al.*, 1987a). This fact is very important, although cell hydrophobicity can change as a function of growth condition (Malmqvist, 1983; McEldowney & Fletcher, 1986; Van Loosdrecht *et al.*, 1987b). Kjelleberg and Hermansson (1984) reported an increase in hydrophobicity during the starvation process. Because the nutrient concentration of sea water generally decreased during the winter period, the bacterial cell surfaces could show a proportionally greater tendency to attach. On the other hand, Kjelleberg *et al.* (1985) observed that the "oligotrophic bacteria" (capable of growth on low nutrient media) were more adhesive than copiotrophic bacteria and were predominant on surfaces during the initial attachment phase.

The second step of biofilm formation is the accumulation phase. Its rate was maximum during the summer months and decreased in winter. The increase in bacterial numbers on a surface is due to both the adhesion of new cells and the accumulation of daughter cells from previously attached bacteria. Since cell numbers in the sea water were virtually constant during the experiments, we estimate that the rate of the first process remains relatively stable. However, because of the attachment of primary bacterial colonisers, the adhesion properties of the surface vary during this process (Baier, 1973; Daniels, 1980; Characklis *et al.*, 1982).

Thus, any differences in rates is presumed to be due to water quality and its action on the metabolism of the attached bacteria. During the summer months, a very rapid increase in cell volume (4 times in 12 h) was observed, which permits bacteria to enter a growth phase (Kjelleberg *et al.*, 1982). This rapid change in cell size suggested that the copiotrophic bacteria were probably the main source of biomass increase at the interface (Kjelleberg *et al.*, 1982). On the other hand, in winter, the increase in cell volumes was very slow, parallel to the slow and regular accumulation rate of the biofilm.

In conclusion, both substratum and season have an apparent effect on the rate of adsorption while the rate of accumulation appeared to be affected mainly by season.

The season may influence cell surface characteristics of early bacterial colonisers and, in later stages of colonisation, favour the growth of different nutritional groups.

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