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EXPERIMENTAL EVALUATION OF POLLUTION POTENTIAL OF ANIONIC SURFACTANTS IN THE MARINE ENVIRONMENT

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Abstract—A study of the influence of temperature and salinity on the biodegradation of a commercial anionic surfactant (LAS) in waters and sediments of the Bay of Cádiz (SW Spain) is reported. The assays were carried out using batch reactors, and the surfactant concentrations used were similar to those found in the urban effluents which discharge into the bay. Temperature was found to have a decisive effect on the degradation rate. At temperatures of 20–25°C, degradation exceeded 90% within less than 10 days of assay whereas at temperatures under 10°C, degradation scarcely took place (the percentage of degradation did not exceed 5% after 21 days of assay). Variation in salinity, particularly marked during wet seasons, disturbs the activity of bacterial flora and slows down biodegradation of surfactant. All this means that the pollution potential of urban waste containing detergents undergoes significant seasonal variation. The rate of surfactant degradation was remarkably accelerated in the presence of sediments, except in those tests where anoxic conditions were established. This occurs where there is high benthic oxygen demand from sediments containing high percentage of easily-biodegradable organic matter.

Key words—biodegradation, surfactant, microorganisms, pollution, sea, water, sediments, near-shore ecosystem

INTRODUCTION

Bays, estuaries and other littoral ecosystems are highly productive, and are frequently the site of the larval development of large numbers of fishes, molluscs and crustaceans, which is of great importance to fisheries and marine aquaculture. Renovation of sea-water emplaced in the Bay of Cádiz, owing to flushing, is generally small, which makes the environmental conditions (essentially temperature and salinity) of these ecosystems particulary sensitive to climatic and metereological variation.

Located on the coastline, these zones are the first to receive the large quantities of waste products from human activities which are discharged in the sea. Particularly important among the pollutants which reach the marine environment, are anionic surfactants, habitually used in the manufacture of commercial detergents. Their importance stems from their hazardous effects on marine organisms (Sprague, 1969; Marchetti, 1968; Swedmark and Braaten, 1971), especially on larvae and alevins (Walne, 1968; Hidu, 1965; Granmo, 1972), and from the increasing use being made of them. Despite the importance of the subject, very few studies have been made on the biodegradation of these surfactants in the marine environment (Flores *et al.*, 1980a, b; Sales *et al.*, 1981). This is due basically to the great diluting power traditionally attributed to the sea, and the fact that the effects of detergent dumping in the sea are less spectacular than those of dumping in inland aquatic ecosystems (foam, mass deaths of fish, and so on).

When surfactants are dumped in the sea, the high ionic strength of the medium causes a fall in their critical micelle concentration (Corrin and Harkins, 1947; Shinoda, 1967), and consequently their solubilities are greatly reduced. As a result, these materials accumulate in the sediments close to waste outlets. This presents an obvious danger to benthic communities, which are of great importance in the marine food-chains.

This paper reports on a study of the influence of temperature and salinity on the rate of degradation in sea-water of one of the surfactants most widely used in the manufacture of commercial detergents (sodium dodecylbenzene sulfonate, LAS). Also studied is how the presence of sediments affects the process of biodegradation. Sediments normally have a large specific surface, which means, among other things, that they have a high bacterial content. The tests were conducted with sea-water and sediments from the Bay of Cádiz (SW Spain). This is one of the areas of the European coast with the greatest potential for the development of marine aquaculture; in previous papers (Sales *et al.*, 1983; Establier *et al.*, 1985) it has been shown that the pollution levels being produced there by urban and industrial waste are becoming really serious.

Others variables, pH, oxygen tension and bacterial concentration, have been held constant. In the case of pH, the variations during degradation are very little

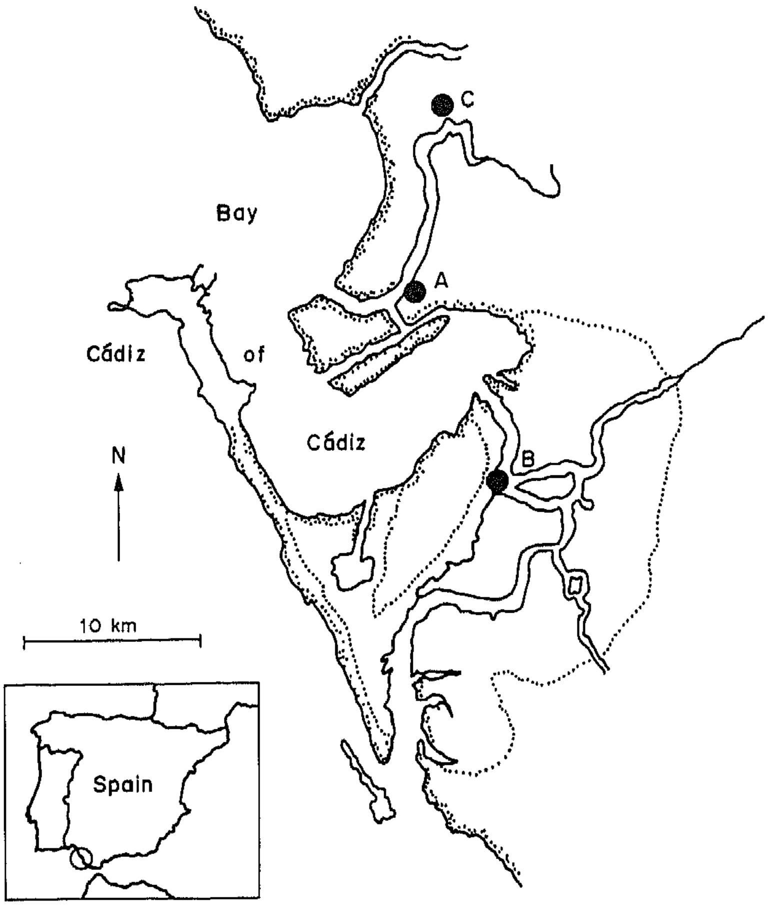

Fig. 1. Location of water and sediment sampling points.

and they do not affect the final results (Dobarganes and Ruiz-Cruz, 1977). For oxygen content, trying to reproduce faithfully the natural conditions requires a different value in each assay (the solubility of oxygen in sea-water depends on salinity, etc.).

EXPERIMENTAL

Figure 1 shows the locations from which the sediments and water for the biodegradation test were taken. Water samples to study the influence of temperature on the rate of degradation were taken at point A. Working temperatures were 5, 10, 15, 20 and 25°C. The influence of sediments on the degradation process was tested with samples from points A and B. For the test at different salinities, sea-water was used from a salt-pond located at point C which had undergone severe evaporation (S = 65‰). Other salinities at which surfactant degradation was studied (50, 32 and 16‰) were obtained by diluting with sterile distilled water. These last tests were conducted in the presence of sediments taken at location A. The main physical-chemical characteristics of the water and sediments used, along with the micro-organism content, are shown in Tables 1 and 2.

The degradation experiments were conducted using an adaptation of the "river die-away test" (Swisher, 1970). For the tests done with sea-water only, 500 ml of water were put in a 1000 ml flask for each of the working temperatures. During the experiments, the flasks were stoppered with hydrophobic cotton. The aliquots for determining residual surfactant concentration were taken by means of a pipette inserted to half the depth of the solution.

In the test in the presence of sediments, a separate solution was used for each determination. In these cases, 250 ml flasks were used, with 100 ml of water and 25 g of sediment wet weight. This ratio of water volume and sediment weight is similar to that used by other authors to study processes of transference of heavy metals and nutrients through the water–sediment interface (van Kessel, 1978; Lu and Chen, 1977).

The surfactant studied was a commercial linear-chain sodium dodecybenzene sulfonate, with 90.3% active matter. Immediately before each test, the surfactant was dissolved in sterilized, distilled water, then added to the flask. Total quantities of surfactant to be degraded at the beginning of each experiment are shown in Table 1. The quantities selected are similar to those found in the urban waste discharging in the Bay of Cádiz (Flores *et al.*, 1980a).

Table 1. Physical-chemical characteristics and micro-organism counts of sea-water samples used in degradation tests

Assay	Temperature (°C)	Salinity (‰)	pH	DO (mg/l)	Anionic surfactant (μg DSNa/l)	Aerobic micro-organisms (col./ml)	Surfactant initial amount (mg)
1–5	5–25	33.3	8.03	Saturation	62.7	1.71×10^4	0.48–0.53*
6–7	20	33.8	8.10	7.61	90.0	8.0×10^3	2.00–2.50
8	25	16.0	8.12	9.78	30.1	4.2×10^3	1.74
9	25	32.2	8.11	6.50	70.2	8.5×10^3	1.71
10	25	49.9	8.13	2.71	97.9	1.28×10^4	1.66
11	25	65.1	8.14	1.50	115.3	1.69×10^4	1.80

*Range.

Table 2. Physical-chemical characteristics and micro-organism counts of sediment samples used in degradation tests

	Sediment		
	A	B	C
Loss of weight 105°C (%)	23.20	36.32	23.10
Loss of weight 450°C (%)	9.76	13.48	4.90
Organic C (%) w/w dry	0.47	1.92	0.52
Organic N (%) w/w dry	0.045	0.247	0.030
Clay (%) w/w dry	26.6	56.0	68.7
Silt (%) w/w dry	28.4	30.2	10.3
Sand (%) w/w dry	45.0	13.8	21.0
Anionic surfactant (μg DSNa/g sed. dry)	4.0	28.0	3.3
Fe (%) w/w sed. dry	3.60	3.69	3.20
Mn (ppm) w/w sed. dry	518	342	375
Zn (ppm) w/w sed. dry	130	184	330
Cu (ppm) w/w sed. dry	20	47	13.7
Pb (ppm) w/w sed. dry	72	82	21.5
Cd (ppm) w/w sed. dry	1.36	2.47	2.17
Aerobic micro-organisms [(col./g) × 10^4 (sed. wet)]	10	20	24
Anaerobic micro-organisms [(col./g) × 10^4 (sed. wet)]	6	7.2	2.3

All experiments were duplicated and in all cases two samples were taken and analysed separately. The different values obtained from analysis, show a difference of $<5\%$. The flasks were kept in the thermostats with a temperature control accuracy of ± 0.1°C. All material used in conducting the experiments and taking samples was previously sterilised.

Anionic surfactant matter was determined using the methylene blue method proposed by Abbot (1962). In order to use this method with sediment sample, the surfactant had to be solubilised beforehand. To do this, three consecutive extractions were made with 5 ml of CH_3OH–NH_4OH (2N) 2:1 (v:v) per gram of sediment. The extraction were carried out over 20 min at ambient temperature, while shaking continuously. This procedure is a modification of the technique described by Neufahrt *et al.* (1983) and permits the extraction of over 95% of the surfactant in the sediment.

Dissolved oxygen (DO), pH and salinity of the sea-water used in the tests were determined by the methods described by Strickland and Parsons (1968). Micro-organism counts were done according to Harrigan and McCance (1976). The characteristics determined in the sediment included organic C (Gaudette *et al.*, 1974), organic N (Anonymous, 1973), weight-loss at 110 and 450°C and content in various heavy metals (Fe, Mn, Zn, Cu, Pb and Cd). These were determined by atomic absorption spectroscopy after extraction from the sediment sample with HCl–HNO_3 (10:1) (Gómez-Parra *et al.*, 1984). Granulometric analysis was carried out by the chain hydrometer method (De Leenheer *et al.*, 1965).

RESULTS AND DISCUSSION

Influence of temperature on degradation

The main physical-chemical characteristics of the water along with the micro-organism counts, are shown in Table 1 (see assays 1–5). Table 3 shows percentages of degradation reached on different days of testing, for the five temperatures studied. Generally speaking, it can be seen that there was hardly any degradation at low temperatures (5–10°C), while at 25°C over 90% of surfactant had disappeared 15 days after the start of the experiment. At intermediate temperatures, only a small amount of degradation took place.

In the test run at 5, 10 and 15°C, the rate of degradation turned out to be independent of residual surfactant concentration (zero order kinetics). For the results obtained at 20 and 25°C, various polynomials were tried, between grade 0 and grade 6, in order to find the best correlation between degradation rate and residual surfactant concentration. Data were processed on a DIGITAL PDP-11 computer using a programme supplied by the makers. At both temperatures, the best results were obtained for a second-degree polynomial whose equations are:

$$(20°\mathrm{C}) \qquad v = \frac{\mathrm{d}C}{\mathrm{d}t} = -4.72 \times 10^{-4} C^2 + 0.28\,C - 17.06$$

$$(25°\mathrm{C}) \qquad v = -\frac{\mathrm{d}C}{\mathrm{d}t} = -4.78 \times 10^{-4}\,C^2 + 0.28\,C - 0.28$$

where t is time (in days) and C is the residual amount of LAS (μg) at time t.

The dependence on temperature of the rate at which the degradation process occurs, is related to the metabolism of the micro-organisms responsible for degradation. From this, it can be stated that the pollution potential of urban waste containing surfactants undergoes marked seasonal variation. Thus, in coastal areas where water temperature ranges from 5

Table 3. Percentage degradation reached on different days of testing in experiments conducted at different temperatures

	Time (days)													
Temp (°C)	1	2	3	4	5	6	7	8	9	10	11	13	15	21
5	0.9	1.1	1.3	1.5	1.7	1.8	2.0	2.0	2.4	2.6	3.1	3.4	3.6	4.2
10	0.3	1.7	2.1	4.2	7.5	9.0	11.5	13.3	14.0	15.0	20.0	28.6	33.1	36.0
15	2.3	4.2	5.8	9.4	13.4	17.5	22.2	25.0	27.5	30.2	37.1	42.6	44.8	47.9
20	1.1	2.5	5.2	6.3	8.7	11.3	15.5	17.3	20.6	24.2	29.2	40.5	48.3	69.7
25	1.7	3.7	6.5	9.9	14.3	20.0	26.4	38.5	41.0	48.8	62.7	87.0	92.7	97.0

to 10°C (frequent winter temperatures), the adverse effects of the discharging of anionic surfactant products will be considerably enhanced owing to their longer presence in the medium. This is particularly important in coastal areas with little water renewal if any kind of aquaculture is carried on, since it is in winter that a large number of marine species spawn, and their larvae and alevins are especially sensitive to surfactants.

At temperatures below 10°C there is very little bacterial activity (Brisou, 1980) and the only degradation agents are psychrophilic micro-organisms (Harrigan and McCance, 1976) which are relatively scarce. This is supported by the fact that in the experiments conducted at 5 and 10°C, there was no appreciable growth in the number of aerobic micro-organisms. These went from 11,000 col./ml to 31,000 and 144,000 col./ml, respectively. At these temperatures it is a fair assumption that the slightness of surfactant loss is due, to a greater or lesser extent, strictly to chemical processes.

In those tests where the rate of degradation can be expressed in terms of concentration as a second-degree polynomial (tests at 20 and 25°C), the independent term has a negative value in both cases. Qualitatively speaking, this term may be related with some inhibition of bacterial development (Bayley and Ollis, 1980). This is concordant with the fact that its absolute value is smaller when temperature is higher.

Biodegradation in the presence of sediments (see assays 6–7, Table 1)

Figure 2 shows the results of the test conducted with sediments. For reference purposes, it includes the variation of the percentage of degradation with time, in a test using water without sediment. All these experiments were run at 20°C. It can be seen that in both cases, because of the presence of sediment, the degradation rate is very high with only a few days of assay. This means that the period of acclimatisation of the bacterial flora was substantially shortened.

However, the two types of sediment behaved very differently. With type A, 90% degradation was reached before day 10 of the test. With type B, although there was rapid degradation at the outset, from day 6 to 7 on, it scarcely occurred. The discrepancies must lie in the differences between the two types of sediments used, both in terms of their physical nature and of the degree of pollution of their place of origin.

The granulometry of the A-type sediments shows an important sand component and consequently a low organic matter content. The B-types, on the other hand, show a predominance of clay, with a substantial amount of organic matter. As regards their degree of contamination, type A comes from a channel running into the Bay of Cádiz, with a low contamination level, whereas type B was taken about 500 m from the main waste outlet for the town of San Fernando (*c.* 100,000 inhabitants). There, the concentration of heavy metals is very high and the water layers close to the sediment are in reduced conditions.

The oxidising conditions at the start of the tests (7.61 mg O_2/l) encouraged bacterial action on a large number of organic compounds easily biodegradable in such conditions and present in appreciable concentrations in the sediments from area B (compounds with high organic N content). This degradation occurs at the same time as that of the added surfactant. The high benthic demand in the oxidation of the organic substances in the sediment exhausted the available oxygen in a few days. Figure 3 shows the evolution of the number of aerobic col./ml during the assay and corroborate the previous hypothesis. In this figure, the number of aerobic micro-organisms increase to day 9. Immediately, the diminution of

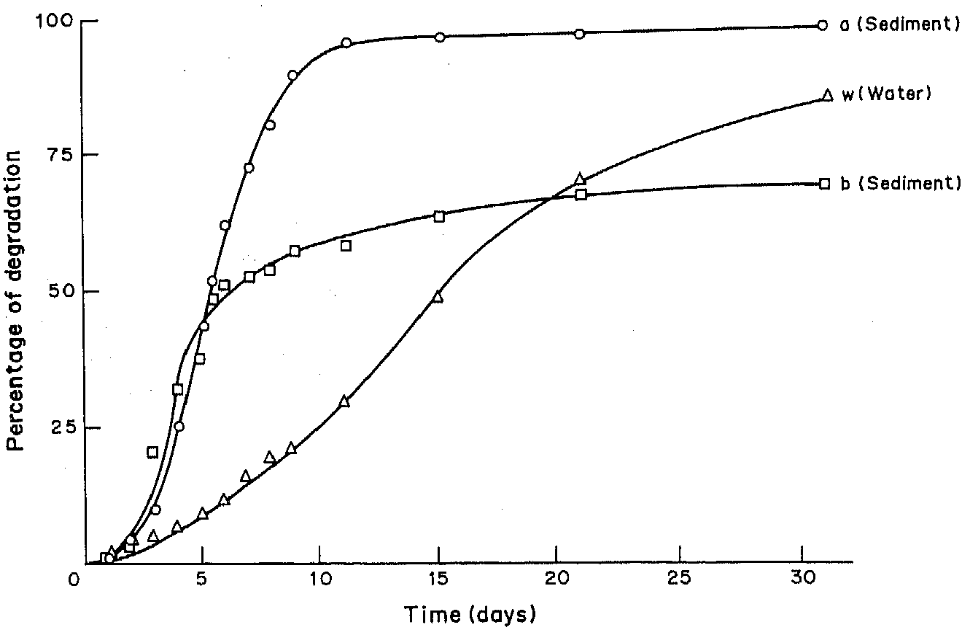

Fig. 2. Variation of percentage degradation with time at 20°C (a and b, assay with sediments A and B; w, only water).

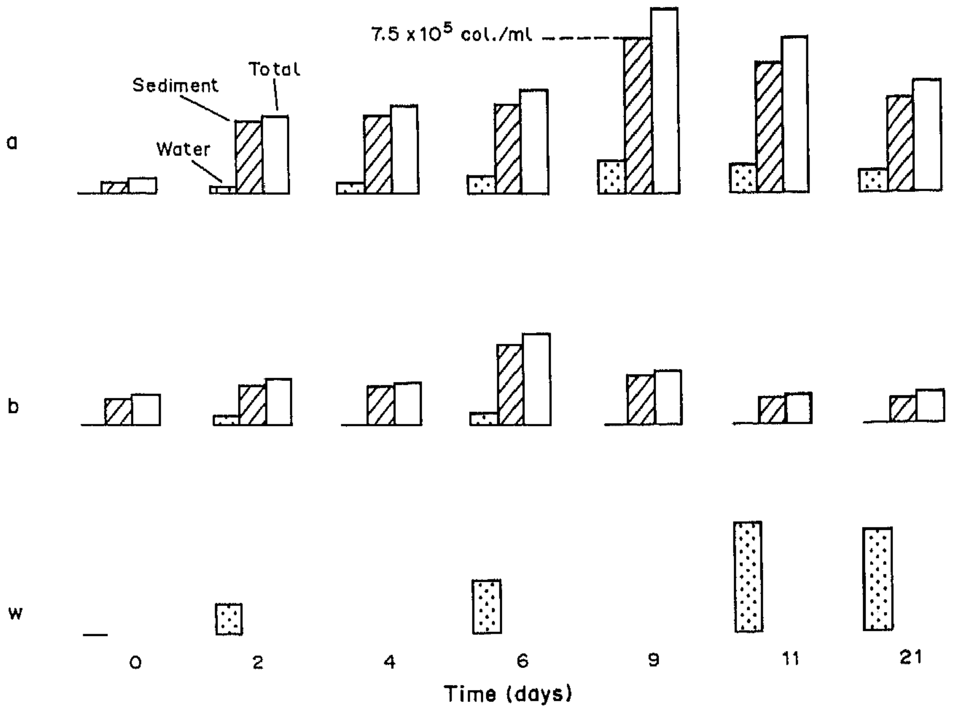

Fig. 3. Variation with time of the number of aerobic micro-organisms at 20°C (a and b, assay with sediments A and B; w, only water).

oxygen content in the medium had to take place since degradation is little from that moment. These results are in agreement with those found by other authors (Flores *et al.*, 1980a, b) in previous papers, the evolution of oxygen content during the degradation process has been shown.

From that moment on, surfactant degradation had to take place through anaerobic pathways which must be carried out by the microorganisms present. These are generally less efficient than aerobic processes, as is proven by the fact that there was only appreciable degradation of surfactant with B-type sediment while there was enough dissolved oxygen in the supernatant water to sustain aerobic bacterial activity.

Besides this, in the experiments with these sediments there could have been some inhibition of bacterial growth owing to the toxicity of some of the heavy metals which are present at very high concentration (Pb, Cu). There are two decisive factors affecting the toxicity of heavy metals: the direction in which the transfer of metals takes place between the sediment and the interstitial water; and their speciation when in solution (Förstner and Wittman, 1979), both conditioned by the redox potential value. Hence, if the physical-chemical conditions in the medium became modified in the course of the biodegradation experiments, this could induce the appearance of bacteriostatic substances.

Influence of variation in the medium's salinity (assays 8–11)

This study includes the simulation of another phenomenon affecting the way in which the surfactant degradation occurs in the natural environment, viz. the substantial decrease in salinity undergone by the water column during the winter months in certain coastal ecosystems, as a result of rainfall. The experiments exaggerated the range of variation in salinity normally to be found in nature. Thus, starting with sea-water taken from a salt-pond which had undergone heavy evaporation (S = 65.1‰), this was diluted by 0, 65.2, 101.1 and 203.4%. The salinity of the interstitial water in the sediment used (Table 2) was 38.6‰.

The results of the various test are shown in Table 4. It will be seen that percentages of degradation vary widely in a single day and bear no clear relationship with the salinity of the medium. Figure 4 shows the rate of degradation as a function of the dilution of the sea-water used, for the following days of testing: day 1 (adaptation phase), day 4 (exponential growth phase of number of micro-organisms), day 7 (diminishing phase) and day 12 (static phase). The rate of

Table 4. Percentage degradation reached on different days of testing in experiments conducted at different salinities

	Time (days)												
Salinity (‰)	1	2	3	4	5	6	7	8	9	10	12	15	21
16.0	3.0	8.6	13.4	32.8	59.3	85.5	86.8	89.0	91.0	92.2	93.8	94.8	95.3
32.2	3.5	10.7	17.9	42.1	75.0	89.9	92.0	93.0	94.0	95.0	95.2	95.6	96.2
49.9	1.6	3.5	6.3	15.7	34.3	85.6	89.3	90.7	92.1	92.9	93.8	95.1	96.1
65.1	4.4	11.4	21.0	33.6	47.1	57.1	74.3	84.2	91.4	93.7	94.6	95.9	96.9

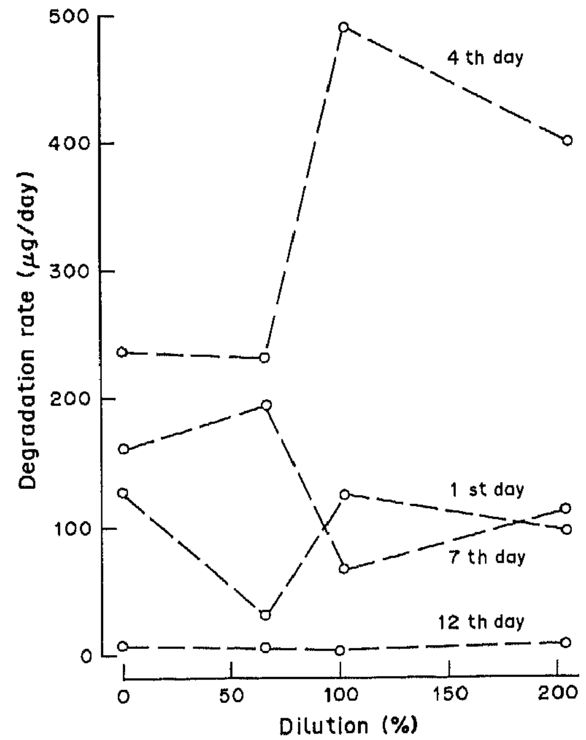

Fig. 4. Degradation rate on different assay days in experiments conducted at different dilutions of original sea-water ($S = 65.1‰$).

degradation was calculated as $[C(t_i) - C(t_{i-1})]/[t_i - t_{i-1}]$.

During the first day of testing, the highest rate of degradation occurred with 32.2‰ salinity (101% dilution), which is the closest to the initial value of the water sediments. The lowest degradation rate at the start of the experiment was found at 49.9‰ salinity (65% dilution), where there is a coincidence of the adverse effects of dilution of supernatant water and increased salinity of interstitial water. During this period, the relatively high degradation rate at 16.5‰ (203.4% dilution) may be attributed to the more efficient surfactant degradation affected by less halophilous micro-organisms (Buchanan and Gibson, 1974). The results also suggest that the adaptation period of the these bacteria to the less saline medium is short.

As the test proceeded and the amount of surfactant in the medium diminished, the degradation rates tended to become equal.

CONCLUSIONS

Temperature was found to have a decisive effect on the degradation rate. At temperatures of 20–25°C, the percentage of degradation increases and the adaptation phase of micro-organisms becomes considerably shorter.

The degradation improves when it occurs in sea-water that is in contact with sludge, it can be attributed to the great concentration of bacteria as well as the physical-chemical characteristics.

The salinity of the environment does not influence the percentage of degration that is measured at the end of the assay, though it does during the time in which the degradation is operating.

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