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7ème CONGRES INTERNATIONAL DE LA CORROSION MARINE ET DES SALISSURES

UNIVERSIDAD POLITÉCNICA
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ESPAÑA

SECCION I

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SESSION I Corrosion Marine
Friday 11th November

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Krzysztof Debrowiecki and Kazimierz Boron

MICROENVIRONMENTAL CHANGES AROUND FOULING ORGANISMS AND THEIR CORROSION IMPLICATIONS
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SURFACE MODIFICATION OF CuZn20Al2 ALLOY WITH PERIMONE DERIVATIVES

By

Krzysztof Dąbrowiecki and Kazimierz Boroń

Institute of Inorganic Technology, Technical University, ul. Smoluchowskiego 25, 50-372 Wrocław, Poland

Introduction

The chemical modification of copper alloy surfaces is usually carried out with compounds which are good corrosion inhibitors. The most commonly used inhibitors are benzotriazole and compounds of the structure similar to that of this compound [1].

As follows from a number of reports [2-4], benzotriazole and the compounds similar to it are chemisorbed on the surface of an alloy to form a stable inert layer protecting it against corrosion.

The surface modification of copper alloys improves their stability against outdoor corrosion. It also reduces the rate of corrosion in see waters.

In this work, some other chemicals were tested as surface modifiers for CuZn20Al2 alloy (special brass). The aim was to improve resistance of the brass against corrosion in see waters. The following compounds were selected: 1,10-phenanthroline (F), 1,3-benzodiazole (BD), 2-aminopirimidine (AP), 2-mercapto-4,6-diaminopirimidine (DAP), and 2-mercapto-4,5,6-triaminopirimidine (TAP). Benzotriazole (BTA) was also used, as a reference standard.

In the preliminary experiments, DAP was found to be the most effective corrosion inhibitor. This compound was than studied in detail.

Procedures

The experiments were carried out with CuZn20Al2 brass of the chemical composition presented in Table 1 (according to Polish Standard PN 67/H-87025).

Table 1. Chemical composition of CuZn20Al2 alloy

Components %				Permissible impurities %				
Cu	Al	As	Zn	Fe	Pb	Sb	Bi	P
76.0 -79.0	1.8 -2.3	0.02 -0.06	up to 100	0.07	0.07	0.005	0.002	0.01

The specimens had the form of rings cut out from recrystallized commercial tubes (diameter 19.0/16.0 mm, height 20.0 mm).

The gravimetric corrosion tests were made using an artificial see water at room temperature with contineous aeration.

In the preliminary series of experiments, modifications were carried out by placing the specimens into aqueous solutions of the chemicals specified above and kept at 40°C for 24 hrs. The concentration was 10^{-3} mole/dm³. In the main experiment, the modification conditions were as follows.

- Inhibitor concentration: 10^{-5} to $0.5 \cdot 10^{-2}$ mole/dm³
- Temperature: 20.40.60.80°C
- Time: 3.6.16.24 hrs.

The corrosion resistance was measured by exposing the samples to aerated see water for 5 days, in the preliminary experiments, or for 5.10.20.40, or 60 days in the main experiments.

Results and Discussion

Only the compounds BTA, DAP and BD were found in the preliminary experiments to have the ability of inhibiting brass corrosion.

Table 2. The effectiveness of CuZn20Al2 alloy surface modification after 5 day exposition to see water

Compound	Effectiveness %
BTA	29.3
BD	1.2
DAP	64.5

Detailed experiments were made with DAP, the most effective corrosion inhibitor among the compounds studied. The effects of its concentration and temperature in modification bath on the corrosion rate of brass in an artificial see water are shown in Figs. 1-3. Fig.4 presents the effectiveness of modification vs. time of exposure for the samples modified with 10^{-2} M DAP at 20°C for 24 hrs.

The gravimetric measurements made for CuZn20Al2 alloy in the stationary conditions revealed that the corrosion rate in see water was reduced by a half after treating the brass surface with DAP solution. The effect of concentration of the DAP modifying solution upon the corrosion rate was not very clear. At 20°C, DAP well protected brass when used in the concentration 10^{-3} M. The corrosion rate further decreased as the modification time become longer and longer. The DAP solutions of concentrations $0.5 \cdot 10^{-2}$, 10^{-4} or 10^{-5} used at 20°C either did not affect or increased the corrosion rate of CuZn20Al2 alloy.

At temperature raised to 40°C, the modification with 10^{-3} M DAP substantially reduced the corrosion rate. This rate was more less independent of the time of modification. The modification effectiveness was in this case as high as ca. 88%. An increase in modification temperature had a positive effect on corrosion rate also for DAP solution of concentration $0.5 \cdot 10^{-3}$ M.

For DAP of concentrations 10^{-4} and 10^{-5} mole/dm^3, no sufficient improvement in resistance of the brass against corrosion was observed even at elevated temperatures, 40,60, or 80°C. The rate of corrosion decreased with increasing modification time, and after the 24 hrs treatment it was nearly the same as that for unmodified brass.

For the DPA solution of concentration 10^{-3}, the change in modification temperature in the range 60-80°C did not produce significant differences in corrosion rates. The resulting highly reduced rate of corrosion as compared with unmodified samples remained unchanged independently of modification time.

The lowest rate of corrosion was obtained for the samples

modified with DPA solution of concentration $0.5 \cdot 10^{-2}$ at 60°C. The protection effectiveness of 97% was obtained after a 24 hr. treatment. Further increase of the solution temperature, to 80°C, gave no more improvement.

Conclusions

The results presented above can be summarised in the following conclusions.

1. From among all compounds studied, the best corrosion protection properties had 2-mercapto-4,6-diaminopirimidine (DAP).
2. The protection properties of DAP improved with increasing modification temperature.
3. DAP was found to be better corrosion inhibitor for CuZn20Al2 alloy than benzotriazole.
4. The modification effectiveness did not change with time of exposition of samples to an artificial see water.

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Captions for figures

Fig.1. The rate of corrosion of CuZn20Al2 alloy after 5 days of exposure to artificial see water. Modification conditions: DAP. 10^{-3} mole/dm^3. temp. 20.40.60.80°C.

Fig.2. The rate of corrosion of CuZn20Al2 alloy after 5 days of exposure to artificial see water. Modification conditions: DAP. 10^{-4} mole/dm^3. temp. 20.40.60.80°C.

Fig.3. The rate of corrosion of CuZn20Al2 alloy after 5 days of exposure to artificial see water. Modification conditions: DAP. 10^{-5} mole/dm^3. temp. 20.40,60.80°C.

Fig.4. The modification effectiveness vs. exposure time for CuZn20Al2 alloy in artificial see water. Modification conditions: DAP, 10^{-3} mole/dm^3, temp. 20°C. 24 hrs.

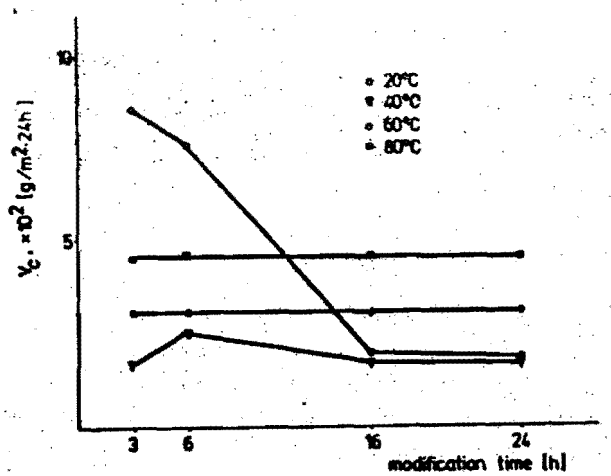
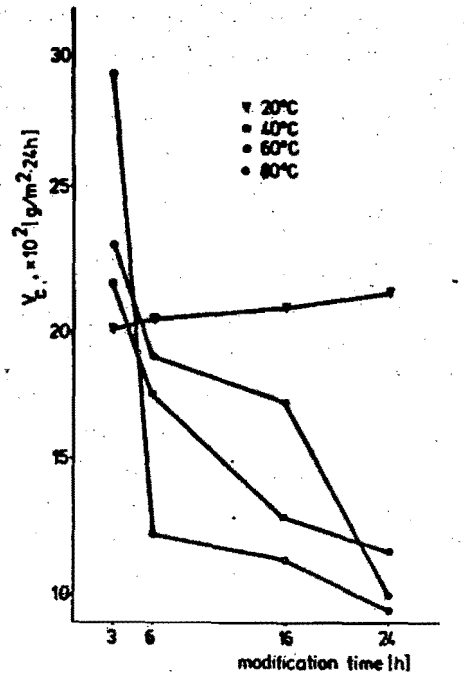


Fig. 1.



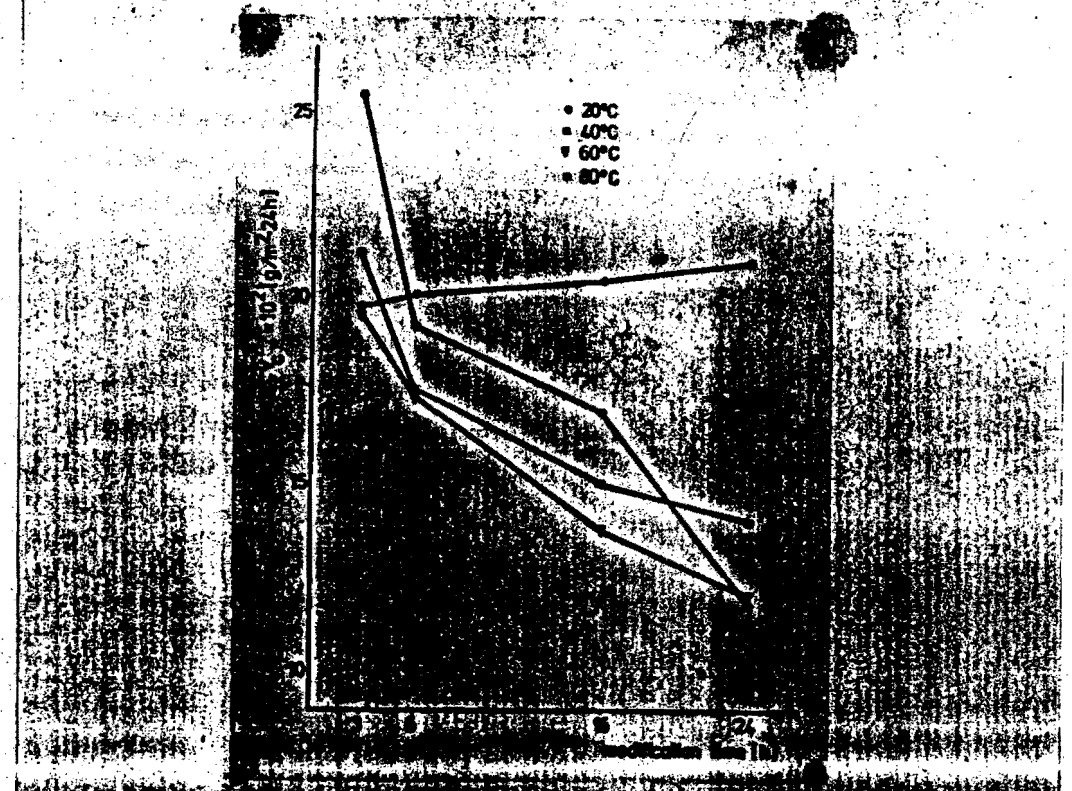


Fig. 4.

Microenvironmental changes around fouling organisms and their corrosion implications

Bellinger, E.G. and Wall, P.*

Department of Environmental Biology,
University of Manchester,
Oxford Road,
Manchester, M13 9PL,
U.K.

* Present address; Dept. Zoology, Cardiff University, Cardiff, Wales.

Abstract
Fouling organisms modify the microenvironment in which they grow. Measurements of such changes can be difficult as the sensors can themselves cause changes. Here microelectrodes have been used to measure pH and pO_2 changes around some live and dead macrofouling organisms growing mainly on steel. Large changes are described within short periods of time. The results presented illustrate the potentially corrosive environments which can develop.

Introduction

Fouling organisms growing at the interface between solid and liquid media are known to modify that boundary layer and hence cause changes in the microclimate at that interface. Such changes in the microclimate, when they occur on man-made structures such as offshore oil rigs, can lead to problems of enhanced erosion (Edyvean & Terry, 1984; Freeman, 1977). Few detailed measurements have been made of the changes, however. Regular or continuous monitoring of microenvironmental changes has been difficult, if not impossible, in the past because the size of the probes used disrupt the environment and may even cause modifications in the environment themselves (Edyvean & Terry, 1983; Woolmington & Davenport, 1983). Great care must be taken in such investigations as the microzones may extend only a few millimetres from the solid substratum so the sensing devices must be on a similar or smaller scale to avoid disruption.

Ion selective electrodes have been used by physiologists for inter and intracellular measurements for several years (Davis & Brink, 1942; Cater & Silver, 1961; Löbbers & Baumgärtle, 1967; Hinke, 1969a & 1969b; Walker, 1971). Their use in ecological measurements has, however, been limited (Revsbech & Ward, 1984; Jorgensen, Revsbech & Cohen 1983). The small size of these electrodes would permit measurements within microenvironments without their gross modification or disruption.

This paper describes the use of microelectrodes to measure pH and pO_2 changes in the microzones around solid surfaces immersed in seawater on which fouling organisms are growing. Some potential implications of these changes to corrosion at the surface are also briefly discussed.

Materials and Methods

(a) Electrodes.

The electrodes used in these experiments have been described in detail elsewhere (Wall & Bellinger, 1982; Wall, 1983) but a summary is given here.

(i) pH electrodes.

The pH electrodes were based on the design of Hinke (1969a&b) where the pH sensitive region of the electrode is exposed on a short point extending from the insulating shank (Fig. 1). The pH and aluminosilicate glass micropipettes used in the electrode construction were drawn using a vertical electrode puller, essentially a vertical version of the equipment originally developed by Alexander and Nastuk (1953). The electrolyte used was 1N HCl and filling was accomplished using a syringe and a very fine drawn out glass capillary.

Once filled, the electrodes were soaked for several days in 1N HCl to allow complete hydrolysis of the pH glass. An internal reference of silver wire coated with silver chloride was

connected to a gold plated pin sealed into the end of the electrode with silicon rubber. Completed electrodes were stored, tip downwards, in a dilute solution of chromic acid. In all experiments an external reference of a standard calomel electrode was used. During experiments each electrode was connected to an electrometer housed in a Faraday cage (see later). The circuit layout for both the pH and pO_2 electrodes is shown in Figure 2.

Calibration of all electrodes used was carried out before and after each experiment. In some cases electrodes were broken during an experiment by an animal's movement or they did not produce a reliable post-experimental calibration. In such cases all recorded data from them was discarded.

(ii) Oxygen electrodes.

The catheter type electrode used was flat ended and of diameter 1 to 1.5 mm. It consisted of a 25 um platinum wire sealed in glass and embedded in epoxy resin with a silver wire acting as a reference (see Fig. 3). When the epoxy had cured, the end of the electrode was carefully ground flat to expose both the platinum and the silver. A membrane was then applied across the flat end by repeatedly dipping it into a solution of collodion in ether/alcohol. A polarising voltage of 700 mV was applied to each electrode and these were not then used for experimental purposes until the response was stable when

immersed in an air saturated buffer solution at a constant temperature. When completed and accepted for use, electrodes were stored in a solution of 0.2 M KCl. Oxygen electrodes were calibrated against solutions of known oxygen content (as measured by means of the Winkler titration method: APHA 1975). Any electrode not exhibiting a linear response was rejected. A zero oxygen solution was obtained by the use of a buffer saturated with pyrogallol scrubbed nitrogen. The 100% saturation reading was obtained by bubbling air through the buffer for 12 hours at a known temperature and pressure.

(b) Experimental procedures.

All experiments were carried out in a temperature controlled tank placed on a teflon runner mounted sliding steel platform inside a one metre cube Faraday cage (Fig. 4). The cage was provided with a large hinged door at the front for access and was grounded to earth to screen out radio frequency interference. The seawater flowing through the experimental tank was pumped at a controlled rate from a temperature controlled reservoir. For all experiments involving algae, illumination was provided using daylight fluorescent tubes.

Mild steel and marine plywood plates, approximately 20 m x 15 m, were exposed in the marine environment for several months to allow a heavy growth of macrofouling organisms to develop. The steel plates had been placed subtidally and were exclusively colonized by the barnacle Balanus tintinnus, some of which were alive and healthy and others of which were dead with only the shells remaining. The dead shells, however, accumulated debris and microorganisms providing a sheltered environment of their own. The wooden plates were exposed in the intertidal region and were covered with mats of the alga. Enteromorpha sp. with occasional bryozoans. There were also large quantities of sediments retained amongst the algal filaments. These plates were also cleansed and stabilised as before. Illumination was provided on a 12 hourly cycle using daylight fluorescent tubes. Additional steel plates were exposed in salt water dock areas at Liverpool (UK) where they were colonised by mixtures of mussels (Mytilus edulis) and the ascidian Ciona intestinalis. Dead organisms were removed and the plates were placed in clean seawater for 5 days at constant temperature to stabilise.

pH and pO_2 concentrations from the external environment through the fouling to the substrate were recorded. The electrodes were held in a rigid perspex carrier attached to a universally jointed arm on the micromanipulator. To record the profiles the position of the recording tip of the electrode was incremented through the fouling towards the substrate. Increments of 1 mm could be accurately controlled by using the fine worm drive of the manipulator. After each increment the DVM reading was allowed to stabilise for one minute prior to recording the voltage.

when the profiles were made into the barnacles shells, control recordings of the pH and pO_2 at the same depth outside the shell were made.

Results

(a) pH and pO_2 profiles.

The results for pH and pO_2 profile through both algal and animal fouling are given in Figs. 5 to 8.

Typical profiles down through a mat of Enteromorpha are shown in Fig. 5. Whilst typical diurnal fluctuations occurred during photosynthetic and non-photosynthetic periods (Fig. 5a), marked decreases in pH were measured away from the surface of the mat down towards the substrate on which it was enclosed (Fig. 5b). These falls in pH amounted to as much as 2 units over 9 mm depth.

Figure 6a shows the changes in pH and pO_2 between live barnacles with depth in a static environment. A steady decrease in pO_2 occurred as the plate surfeces were approached. pH initially fell on one profile before rising but rose steadily with depth on the second profile. Similar changes are shown in Fig. 6b with depth into the shells of dead barnacles. Both oxygen and pH levels were relatively steady and similar to the ambient environment until the actual dead shell was entered, when a marked drop in both pO_2 and pH occurred.

The results of microenvironmental changes around mussels are given in Figs. 7 and 8. Measurements were made around individual mussels with time (Fig. 7d&b). In the first experiment electrodes were placed, as indicated (Fig. 7a), around individual mussels in gently flowing seawater. The results show a gentle increase in pO_2 over a six hour period. The pH amount of the exhalent siphon remained constant at around 8.1 (the ambient pH being 8.0) but rose steadily near the inhalent siphon to 8.6. In order to determine the effects of flow on local environmental conditions, the experiments were repeated but the flow of seawater was turned off for a 2.5 hour period in the middle of the experiment. The results are given in Fig. 7b. pO_2 levels rose steadily in flowing conditions but fell markedly, after a short lag, when flows ceased showing a 20% fall in 90 min. Levels were rapidly restored once the flow was switched on again. No such marked variations were observed in pH around the exhalent siphon where a steady increase was observed, especially close to the animal. Conditions around indivdual organisms, whilst of interest, are less representative of field conditions from around groups or clumps. The experimehts were thus repeated within dense clumps of mussels fouling a plate. These results are given in Figs. 8a (around mussels) and 8b (within byssal threads). In static conditions around the mussels themselves pO_2 fell rapidly (45%) whereas pH rose slightly and then fell after 2 h. When flowing conditions were resumed after 3 h, the pO_2 rose to the original levels. The pH rose markedly at the electrode near to the exhalent

siphon (3) but remained steady and ultimately fell slightly (2) in the general fouling mass.

Within the bysaal threads of mussel clumps, where more detritus tends to accumulate, greater changes were observed (Fig. 8b). With no flow oxygen concentrations dropped from 110 mmHg to 35 mmHg but rose rapidly to 150 mmHg when flow was restored. pH within the thread clumps dropped from 7.8 to 7.4 (3) and 8.2 to 7.85 (2) in static conditions, whilst at the edge of the threads it remained fairly constant at around 7.75. When flow was restored pH initially rose (by 0.4 units at site 3) but it then fell only to start rising again at the end of the experiment.

Discussion

From the results obtained it can be clearly seen that fouling species have the ability to greatly modify their local environments.

Light and dark period fluctuations in algal mats were observed and were in close agreement with those reported by Terry and Edyvean (1981) with increases in both pH and pO_2 during light periods. Whilst this happened at the surface of the mat where filaments were freely projecting into the water, deep into the mat nearer to the solid substratum where shading from light and accumulation of decomposing algal material occurred, pH values fell markedly. Concurrent with this drop in pH was a fall in

oxygen. Such temporal and spatial variations in pO_2 and pH would certainly give rise to corrosion cells at the substrate surface, probably resulting in corrosion pitting.

The normal metabolic activities of fouling macroinvertebrates, together with their propensity to accumulate detritus around themselves, will also lead to microenvironmental modifications. Respiratory activities will tend to remove oxygen from the water and input components such as ammonia. Many aquatic invertebrates are ammonotelic, i.e. the bulk of their nitrogenous wastes are excreted as ammonia. The experiments recording pO_2 and pH changes around individual animals show such changes and clearly indicate the impact of water movement on the development of environmental changes. In experiments where no water movements occurred there was a rapid decrease in pO_2. There could also be a marked decrease in pH (Fig. 6b). When flowing conditions are resumed there may or may not be a subsequent pH increase depending upon the amount of shelter produced by the organisms in the clump. Changes will be influenced by the proximity of inhalent or exhalent siphons. The density and the thickness of the clump will also have an influence in regulating water movement. Hence in thick clumps silt and detritus accumulation is more common towards their base and flow is restricted. Breakdown of organic detrital materials consumes oxygen resulting in reducing conditions. Measurements have shown that sulphids quickly build up in such deposits and sulphate reducing bacteria may be common. SRB concentration in

the deposits would tend to be low at the start of experiments as conditions were not favourable (Postgate, 1979). The continuous production of rich feacal/pseudofeacal material or sulphonium salts by Enteromorpha (Baas-Becking and Wood, 1955), however, would rapidly alter the environment and the numbers of SRB were noted to increase from < 1 SRB ml^{-1} to at least 1 x 10^7 SRB ml^{-1} after a month.

Microbial corrosion can affect metals in a number of ways (Iverson, 1972; Miller and King, 1975; Miller, 1981). pH can affect the activity of the main organisms involved, the SRB, who have an optimum pH range between 7.0 and 7.2 (Graff, 1981) although this can be influenced by other factors. In addition, they require a carbon and nitrogen source as well as various salts and an electron acceptor (Herbert et al., 1985). All of these factors are available in the debris accumulating between marine fouling and the substratum so providing a potentially corrosive environment.

Even where such accumulation does not take place variable changes in oxygen and pH concentrations across the surface of steel such as can be caused by fouling organisms can give rise to corrosion cells leading to pitting corrosion.

There is an initial tendency for the pH to rise as a result of the excretory activities of fouling animals and this is unlikely to give rise to corrosion problems. Eventually, however, feacal

materials and detritus will build up around organisms where both low pH and pO_2 develops providing a potentially corrosive environment. Flow does modify this build up but, in the experiments carried out, did not prevent it. Rather it regulated the depth of deposit, reducing its thickness in fast currents. It should also be recognised that in any fouling population some individuals will be dying or dead. Their decomposing remains will provide localised corrosive areas where anaerobic conditions and SRB's will be found. Fouling generally provides a habitat for SRB and, as it is seldom uniform, will create localised environmental differences which increase the likelihood of pitting.

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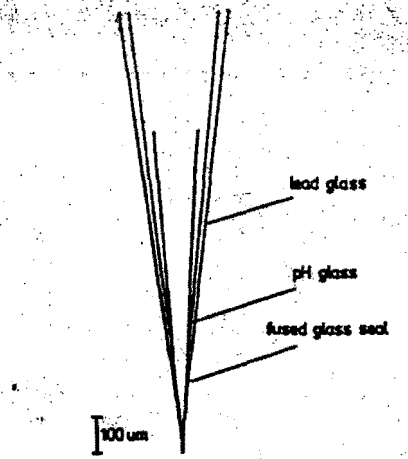


Fig.1. Hinke type pH electrode

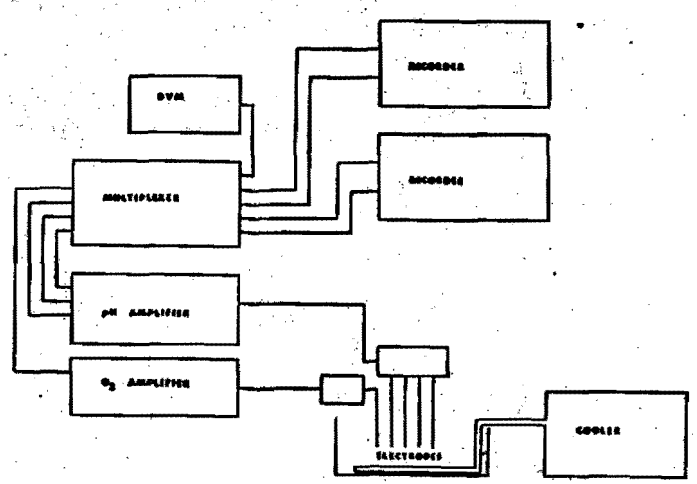


Fig.2. Circuit layout for both pH and pO_2 electrodes

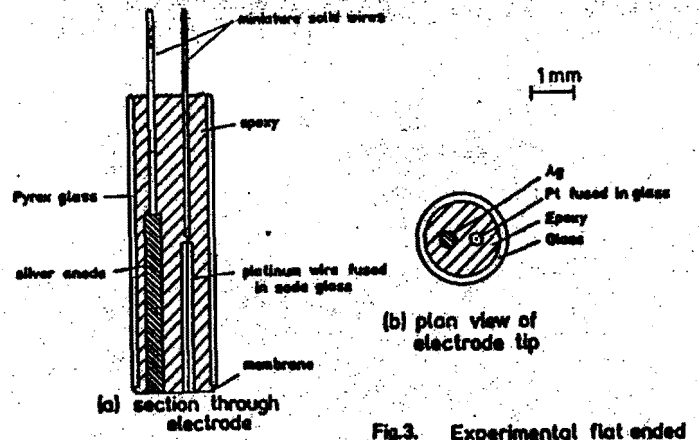


Fig.3. Experimental flat ended oxygen electrode

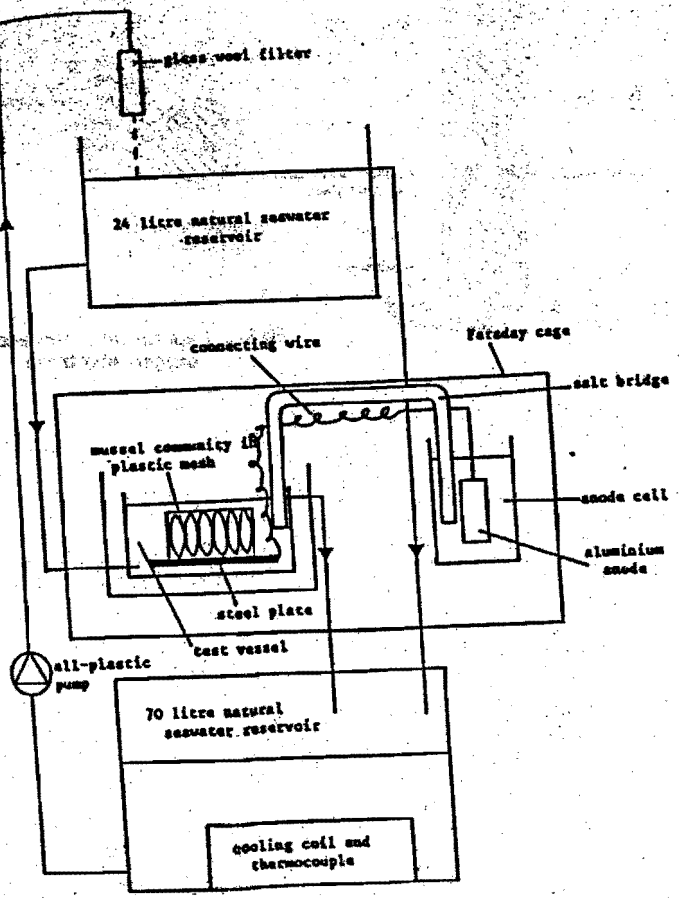
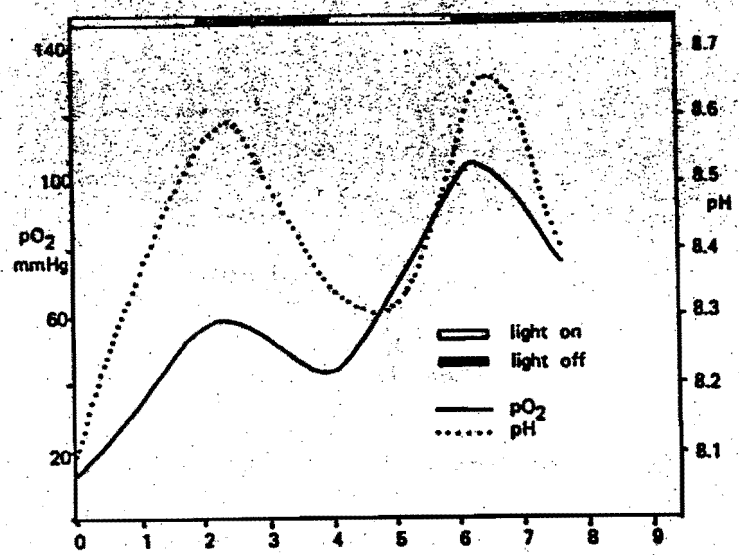


Fig. 4. Experimental lay-out showing arrangement of Faraday cage and flow through chamber. Also indicated is the arrangement for cathodic protection of the steel plate for longer term experiments if required.

Fig. 5a. Changes in pO_2 and pH within the surface layers of a dense mat of Enteromorpha sp. fouling a metal plate.



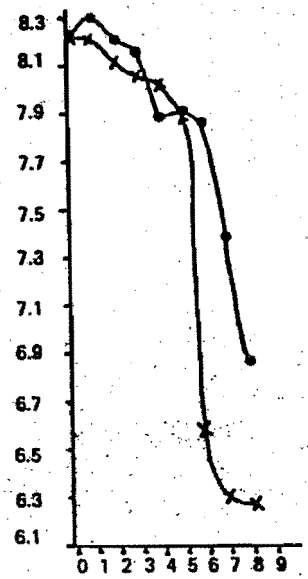
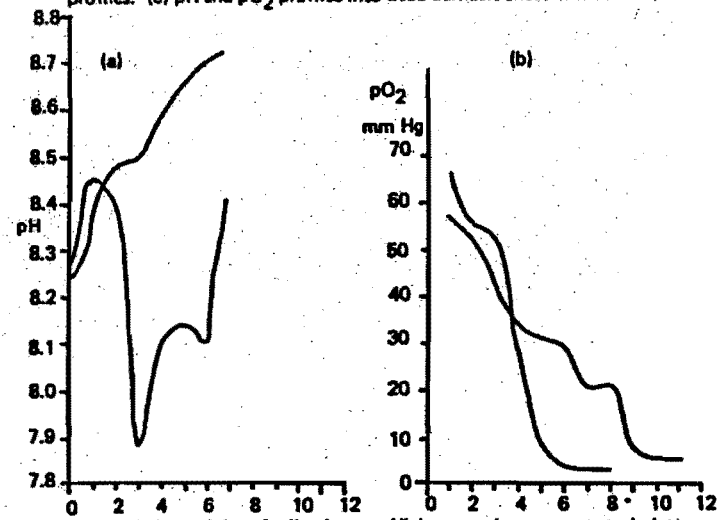
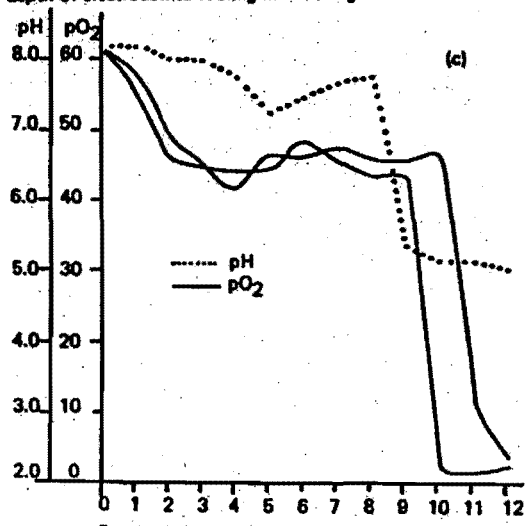


Fig. 5b. pH profiles into a mat of Enteromorpha

Fig.6. pH and pO_2 profiles into macrofouling communities dominated by Balanus crenatus on a steel plate. (a) two pH profiles. (b) two pO_2 profiles. (c) pH and pO_2 profiles into dead barnacle shells withdetritus.





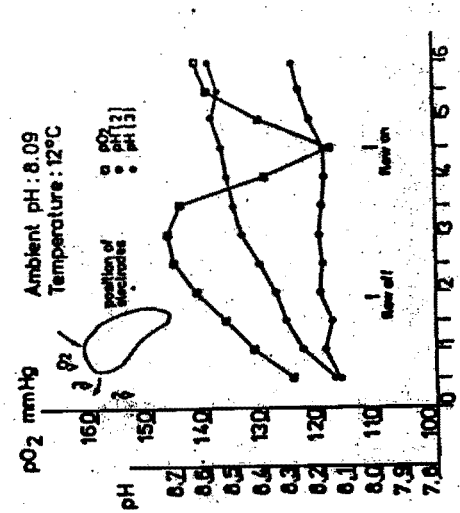


Fig. 7b. Mean pH and pO_2 around an isolated mussel in static and flowing conditions.

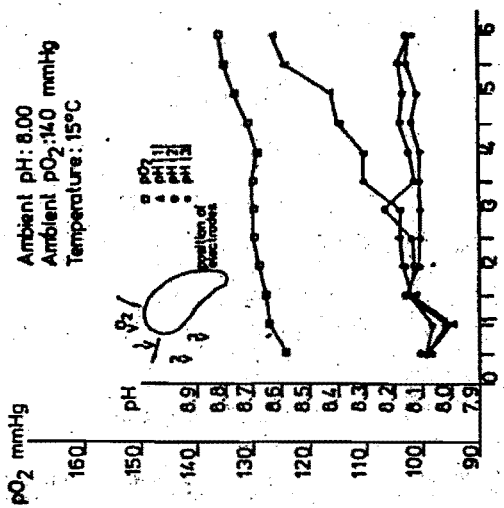


Fig. 7a. Mean pH and pO_2 in the microenvironment around an isolated mussel (Mytilus edulis) in constantly flowing conditions. Arrows indicate inhalent and exhalent siphons.

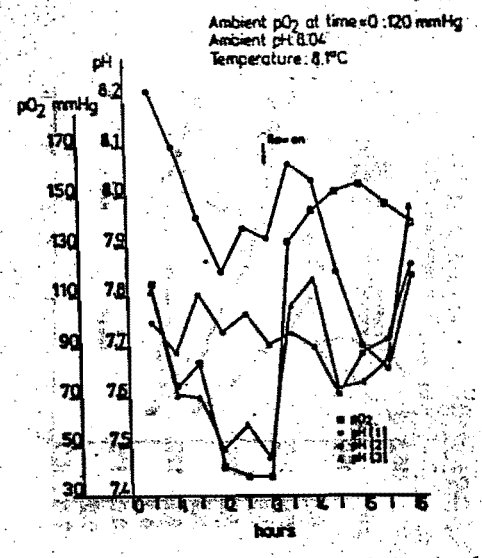


Fig. 8b. Mean pO_2 and pH within byssal threads of a clump of Mytilus edulis in static and flowing conditions

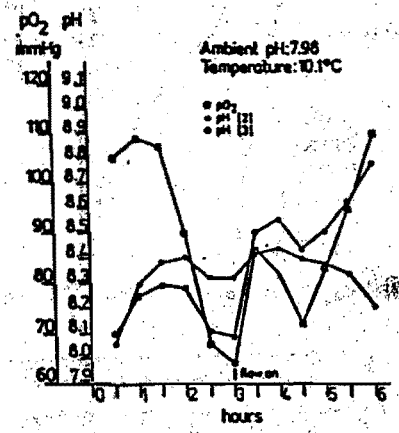


Fig. 8a. Mean pO_2 and pH within a mussel dominated clump of fouling organisms in static and flowing conditions

The effects of cathodic protection on the microflora and performance of antifouling paints.

Bellinger,E.G. & Mitchell,A.J.
Dept. of Environmental Biology
Manchester University
Oxford Road
Manchester, M13 9PL.
England

Abstract

Experimental steel billets coated with Copper/Tin antifouling paint were exposed in seawater in the field and laboratory.Some were subjected to cathodic protection by impressed current (-966mv). SEM and EDAX measurements indicated enhanced leaching rates of copper on the protected paint after 14 days.The protected paint also showed reduced bacterial activity but enhanced diatom and protozoan colonization. Tin was leached by contact not by diffusion. Other mechanisms involved in toxin release are also noted.

Introduction

Applications of protective coatings and cathodic protection (C.P.) are recognised corrosion control methods for marine steel structures. Cathodic protection prevents corrosion by introducing electrical currents from external sources to counteract the normal electrochemical corrosion reactions. Coatings such as antifouling paints form a barrier to prevent the flow of corrosion current between the naturally occurring anodes and cathodes or within galvanic couples (Rogers, 1968; Munger, 1980). Antifouling paints also incorporate biocides such as Tributyltin Fluoride (TBTF) and Cuprous Oxide (Cu_2O) (Phillips, 1973; Evans, 1981). Leaching of these biocides discourages the settlement and growth of biofouling organisms which can cause deterioration of paint films and enhance corrosion (Forteath et al., 1984).

As corrosion protection has become more critical applications of antifoulings in conjunction with C.P. have become more common, resulting in research investigating the compatibility of various paint formulations to different levels of C.P. (Simpson et al., 1980; Munger, 1980). Relatively few investigations have observed the effects of C.P. on the antifouling performance of paints.

In the present investigation an antifouling formulation used commercially in conjunction with C.P. was protected by an impressed current (-966 mv) and exposed at the Menai Strait, Anglesey. Observations were made on the short term effects the impressed current had on a) the various components of the paint film, and b) biofilm development.

MATERIALS AND METHODS

(1) Preparation of Painted Steel Billets

Thirty-six steel billets (8-10 mm^2 and 3 mm depth) were cut from standard steel plates. The dimensions of the billets were chosen to (a) facilitate a less destructive method of preparation prior to scanning electron microscopy, and (b) allow direct observations of the whole billet surface.

Single non-standard (insulated) nickel wires were spot welded along one side of each billet and encapsulated in a flexible polyurethane resin. Each billet was then sand blasted and primed with a 50 μm layer of aluminium primer. A commercial PVC antifouling paint containing Tributyltin Fluoride (TBTF) and Cuprous Oxide (Cu_2O) as biocides was then applied to give a dry film thickness of 100-150 μm.

(2) Impressed Current System

A rigid plastic disc was drilled with thirty-six 1 mm holes approx. 0.5-1.0 cm apart. Each painted billet was fixed into position by threading the nickel wire through the disc into a waterproof junction box. Eighteen of the billets (receiving impressed current) were wired into a block connector receiving d.c. from a potentiostat. The remaining control billets were fixed firmly within the junction box without any d.c. supply. A non-consumable platinum anode and a calomel reference electrode were fixed to the perimeter of the plastic disc and connected to the potentiostat. The design of the potentiostat allowed the protection current to be maintained at a pre-set level of -966 mv.

(3) Field Exposures

Field exposures were carried out at the Menai Strait, Anglesey, Grid ref. OS Sheet 114 SH 563724, during May and June. Certain organic molecules and bacteria in general are known to accumulate in the surface microlayer (neuston) of seawater (Norkrans, 1980). To avoid contamination of the painted surfaces by these agents, the painted billets were immersed in a 4 l container of sterile (0.22 um) filtered seawater which was then submerged in seawater where the billets could be removed underwater. Eighteen billets from each treatment were removed over a 14 day (336 hr) exposure period; three replicates from each being removed after exposures of 1, 5, 11, 24, 168 and 336 hours.

After field exposures the billets were placed into (0.22 um) filtered seawater to remove loose debris, fixed for 10 minutes in 2% w/v glutaraldehyde in seawater, and desalted in distilled water for 10 minutes. Samples for scanning electron microscopy were then air dried, mounted onto 1 cm diameter stubs and carbon coated. The surface of each billet was examined using a Cambridge scanning electron microscope (S360) with energy dispersive X-rey microanalysis (EDAX). After EDAX analysis the samples were removed and coated with gold to allow more detailed SEM of the surface.

(4) Laboratory Exposures

Painted steel billets were prepared as described (1-2) and immersed in a marine recirculating flow system containing seawater collected from the Menai Strait, Anglesey. The recirculating system at 14°C ± 1°C maintained mixed populations of bacteria, protozoa, green algae (Enteromorpha) and diatoms (Nitzschia, Navicula and Amphora). Painted

billets were removed after 12 days exposure and prepared as in (3).

Results

Samples of cathodically protected painted steel billets exposed for various time intervals were examined by electron microscopy. Carbon coated specimens were subjected to EDAX analysis. They were then gold coated for direct visual examinations of the surface features and biota.

Examples of the spectra obtained are given in Fig. 1-2. The main peaks represent the major components of the paint, i.e. the matrix, which was PVC based and was represented by the chlorine peak and the pigment/ toxins represented by the iron, copper and tin peaks. The relative proportions of each elemental peak varied with time and between the 0 mv and -966 mv experiments. These changes are summarised in Table I. The amounts represent the changes in relative proportions of each element within the surface layers of the paint film. The amounts of both tin and copper fell over the 336 h period, as can be seen from Fig. 3, although the rate of change differs between them. Iron appeared to increase initially until after day 168 when it fell markedly. There were significant differences between unprotected and protected (at -966 mv) paints for iron and copper but not for tin.

Observations of the pain surface showed several changes with time (Plate I to IV). The pristine (unexposed) paint (Plate Ia and b) clearly showed rectangular slots on the surface which characterised areas of high initial tin concentration. Many slots were partially covered by the matrix material. There were also many densely packed granules, both large (mainly copper) and small (mainly iron) being visible and often protruding from the surface. After exposure in seawater for 11 h more slots appeared and they had less matrix cover (Plate Ic to If). the granules did not protrude from the surface in such a pronounced manner. Both 0 mv and -960 mv protected paints were similar at this stage (Plates Ic-d and Ie-f respectively). With increasing exposure time differences between the protected and unprotected paints became apparent. After 24 h more slots were present with sharper edges on the unprotected paint (Plate IIa). There was also less obvious granulation and fewer larger copper based particles. On the -966 mv paint however, two types of areas with different appearances had developed (Plate III a to c). One had more extensive film with less obvious slots, the other had clearer slots though still less than at 11 h. Granulation was dense but less clearly defined in these latter areas. After 7 days a film had started to develop more extensively over the unprotected (0 mv) sample, making the slots and granulation less clear (Plate IIb). Film also continued to develop on the -966 mv samples (Plate III d to f) but was uneven in its thickness. Although some slots were obvious some were becoming less clear, as were the granulations.

Finally, after 14 days exposure the film development on all samples was more extensive with the 0 mv showing both slots and pores becoming overgrown (Plate II c to f). Granulation was impossible to see because of the thickness of the film. On the -966 mv samples (Plate IV) the film was more extensive than at 7 days but many slots were still obvious. Many pores were still present but they often appeared deeper than before. Pores appeared to be formed from bumps in the surface (Plate IVf, label 1) which broke open (2) and developed into deeper

holes (3). The rectangular slots also showed a tendency to deepen with exposure as the paint matrix cavity bottom eroded away revealing more deep-seated pores, also probably produced by dissolution.

Observations of the painted surfaces indicated that slime formation and bacterial colonisation occurred on both the 0 mv and -966 mv samples. Obvious bacterial colonisation was, however, more frequent on the unprotected paints (Plate II d,e,f). Bacteria were seen to have invaded cavities (Plate IVc) which were originally sites of high leaching. A range of bacterial types were present ranging from coccoid to rods to filaments. Occasional diatoms were also present and although not common on the field exposed samples, were more frequent on the -966 mv protected paints (Plate IIIe). Also found on these samples were small peritrich protozoans (Plate IIIa and c). These had disappeared by 14 days and any remains had been overgrown by the film.

In the laboratory experiment where higher temperatures (15°C) and a richer diatom flora was present, diatom colonisation on the -966 mv protected paints was much more obvious. A number of species were represented on the -966 mv painted surface including Amphora and Achnanthes. Colonisation by bacteria in the laboratory based experiments followed a similar pattern to the field exposures.

Discussion

Different paint formulations have different mechanisms of action. It has been reported in the literature that in Cu_2O based paints the toxins are released by contact leaching (Phillips, 1973; Evans, 1981). In contrast they reported that organometallics, such as tributyl tin compounds, are molecularly dispersed through the system maintaining a uniform concentration through the film so that biocide release at the paint surface results in more diffusing upwards to replace it and resulting in a steady slower leaching rate. Whilst this may be true of some tin compounds it was not true of the TBTF formulation used here. Observations under SEM clearly showed the tin to be present predominantly as needle-like particles, not just at the surface (I to IV) but also throughout the depth of the film. Consequently leaching occurred by contact as with the Cu_2O component of the paint.

If one considers the concentration changes in main metallic components from the paint film, i.e. the two biocide Cu_2O, $SnBut_3F$ and the piquant iron oxide, it can be seen (Fig. 3 and Table I) that there are considerable differences. Copper and tin decrease markedly over the period whereas iron shows an apparent slight increase. The decreases in copper concentration occurred rapidly at the start of the exposure period, evened out then fell rapidly again over the final phase. This would be expected if contact leaching was occurring as exposed particles of copper would leach rapidly on immersion in seawater. There would then be a lag as slightly less available material became exposed. This would then leach. This pulsing effect would be expected to smooth out with time as particles become available for leaching on a more random basis. This effect of particle leaching can be seen on the surface as, once it has occurred, a pore is formed and these can be seen in Plates I to IV, more being seen at later exposures than earlier ones. Plate IVF indicates a possible sequence of pore formation. Although tin showed a more steady loss rate there was still evidence of a slight pulsed release effect as would be expected during the earlier exposures for contact leaching processes.

The same sequence of cavity formation (in the case of the test were "slot" shaped) followed by secondary holes forming in the cavity bottoms releasing further deeper toxins (Plate IV d and g). As cavities were produced they became invaded by seawater, hence one would expect increases in sodium and chlorine levels at the same time at which the toxins are decreasing. This was found to be so (Fig. 3) supporting the view that seawater penetration of the paint gradually increases with time.

Differences were found in toxin level reductions between the 0 mv and -966 mv samples. For both tin and copper lower levels of metal were present after 14 days in the -966 mv paints. The difference was most marked with the copper. Electrochemical reactions involving copper tube placed at a potential higher than -370 mv compared with tin (-960 mv) in the context of this experiment. It is thus more likely that copper based reactions would proceed more rapidly at the negative potential applied (-966) as it is further from it, compared with tin which is almost at equilibrium with it. If this is so copper leaching from paints could be enhanced by an impressed current cathodic protection system possibly shortening the life of the paint's antifouling ability.

Bacterial colonisation was reduced on the -966 mv paint. This could have been due to unfavourable electrostatic charges on the surface. This effect can play an important role in the attachment of bacteria to surfaces (Marshall, 1980). As bacteria appear to show greater toxicity to copper (Dempsey, 1981) it is more likely that enhanced copper leaching on the -966 mv samples caused the reduced activity. The lower toxicity of tin to bacteria (as reported by Dempsey, 1981) or its lower leaching rate was supported by the relatively rapid recolonisation of the 'slots' by microorganisms after the initial surge of leaching. The impressed current seemed to have the opposite effect, however, on diatom colonisation where in the laboratory experiments larger numbers were found on protected rather than unprotected paints.

Conclusions

1. Evidence is presented indicating that some tin based toxins undergo contact leaching not diffusion leaching.

2. The use of C.P. impress current (at -966 mv) on this antifouling paint lead to enhanced leaching rates of toxins.

3. Less rapid bacterial colonisation occurred on protected paints.

4. More larger microfouling (Diatoms and Protozoans) organisms occurred on the protected paints.

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FIG.1. Spectrum From Pristine (TBT/Cu_2O) Paint Using EDAX.

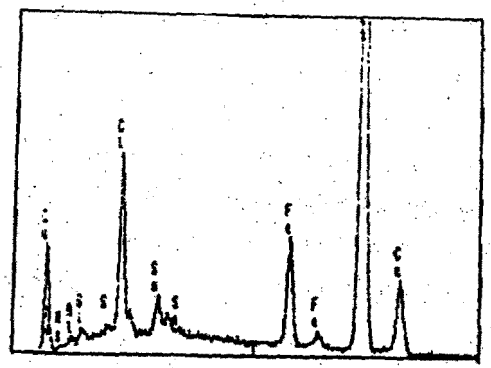


FIG.2. Spectrum From TBT/Cu_2O After 14 Days Field Exposure At ~950mv.

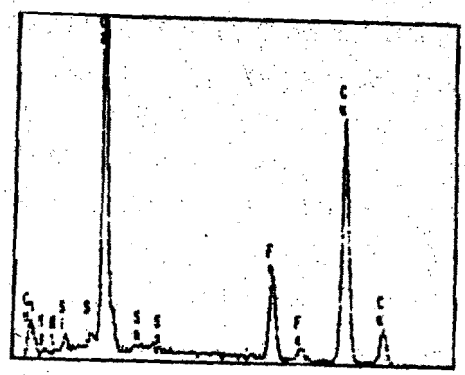
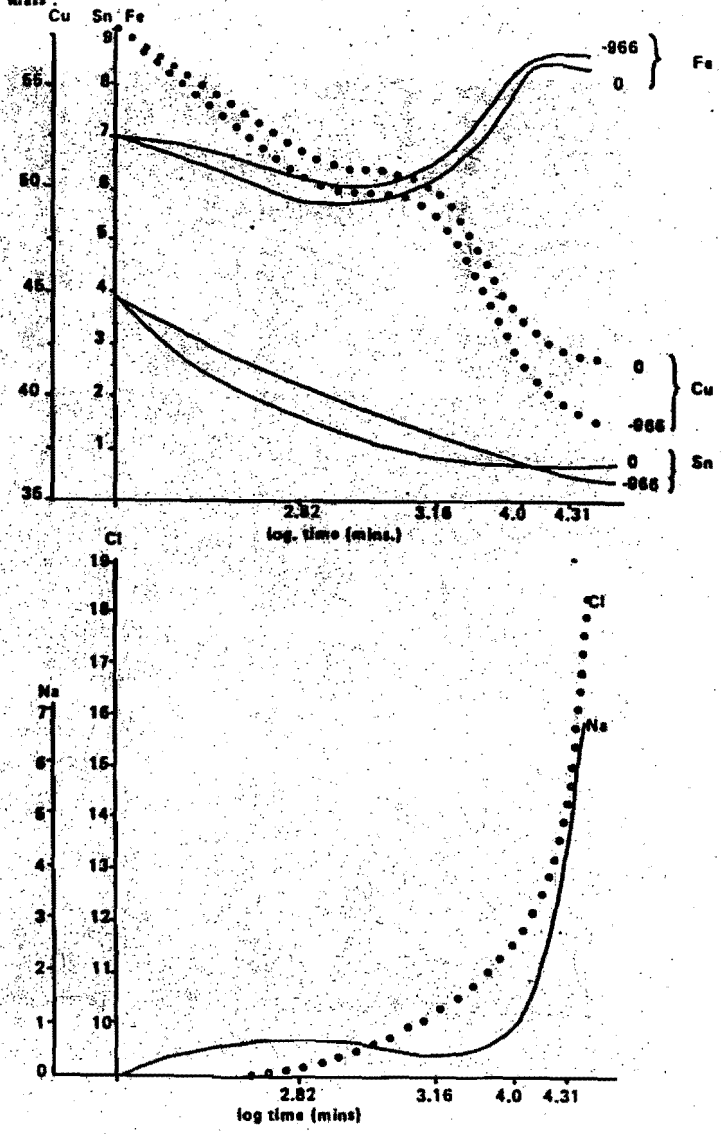


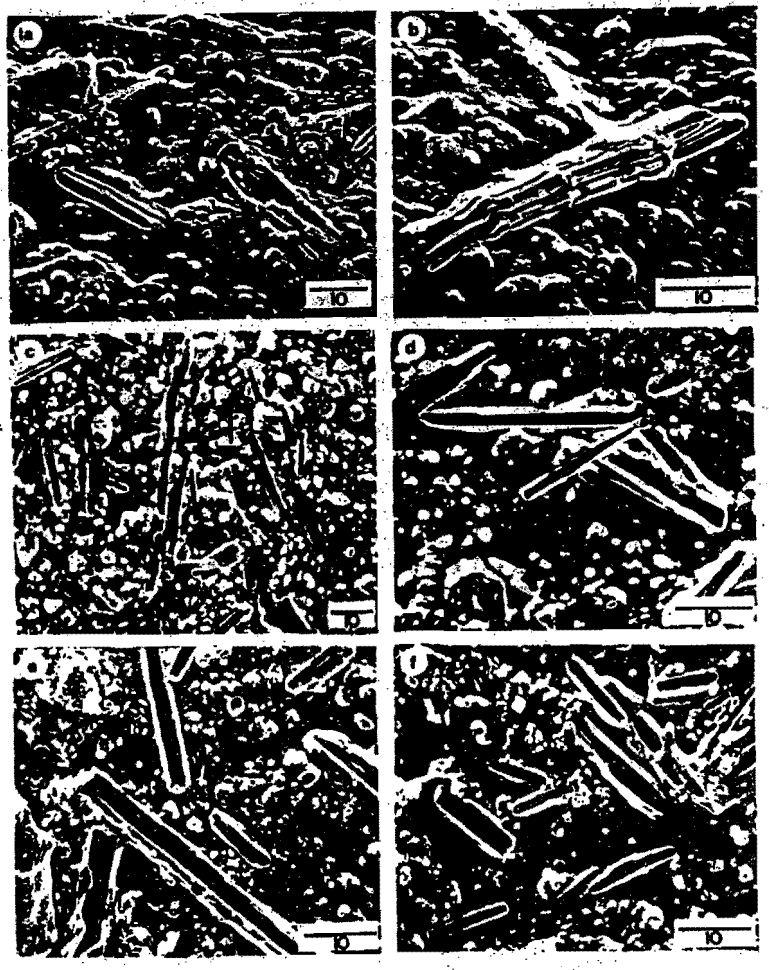
Figure 3. The changes in relative proportions of various components of the paint film with time. The vertical axis units are the percentage of that element per unit mass.



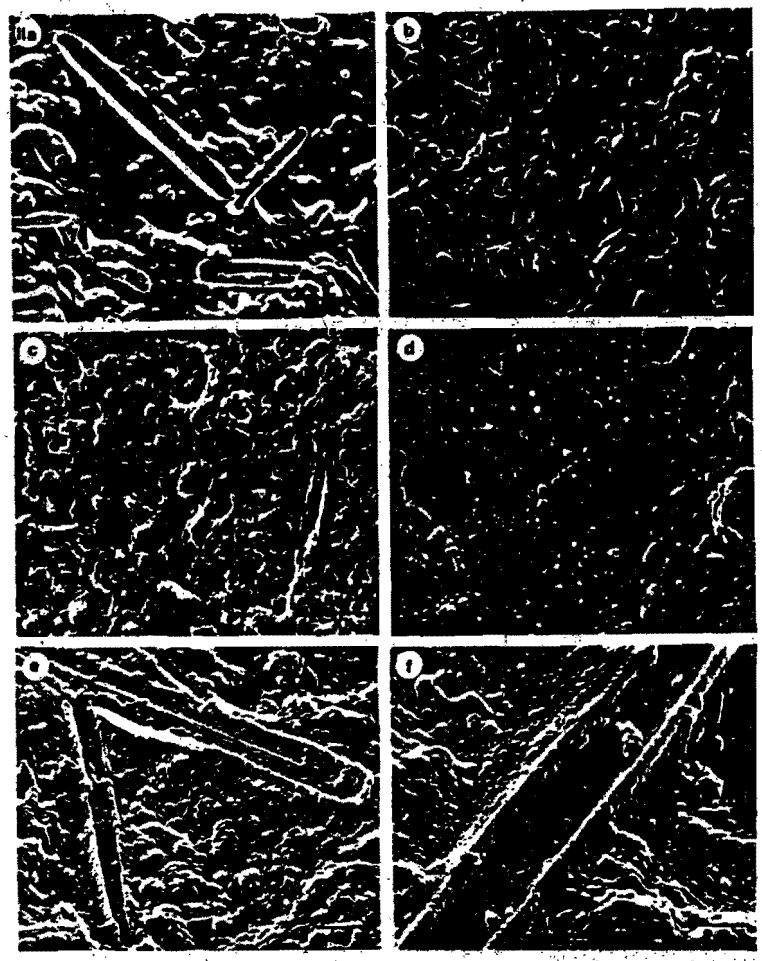
		Cu	Fe	Sn	Cl	Na
Pristine		57.36	7.04	3.74	9.08	-
11 hr	0	50.49	5.76	1.62	9.09	0.70
	966	49.83	6.19	2.11	8.88	0.50
24 hr	0	50.83	6.48	1.14	10.14	0.56
	966	51.74	6.69	1.57	11.96	0.16
7 d	0	44.61	8.78	1.21	10.71	0.27
	966	42.21	7.99	1.10	12.02	8.59
14 d	0	40.84	8.04	0.98	18.86	6.65
	966	37.26	8.64	0.76	19.17	6.64

Table 1. Relative proportions of various elements in protected (-966 mv) and unprotected (0 mv) paint after different periods of exposure.

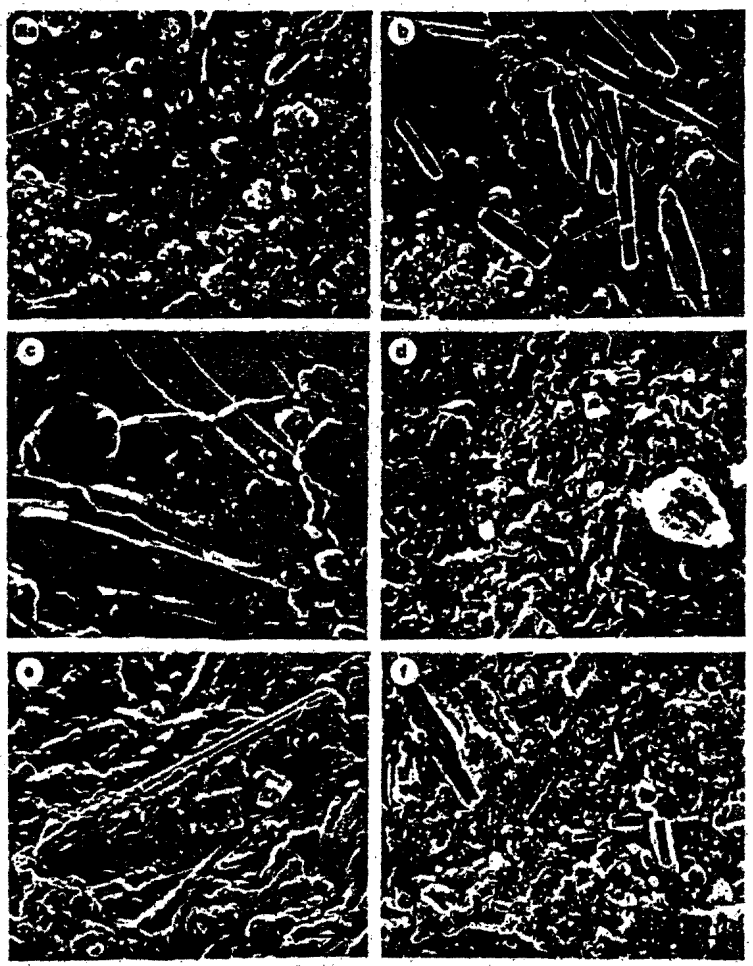
Plates 10a-f. (a-b) TBT/Cu$_2$O, Pristine. X 1.86K. Rectangular slots (high tin concentration). Partially covered by matrix, numerous granules evident. (c-d) TBT, 0 wy; 11 hr. X 1.14K (c), X 1.86K (d) and (e-f) TBT, -986 wy; 11 hr X 186K. Numerous distinctive slots indicative of TBT leaching, granulation less protrusive.



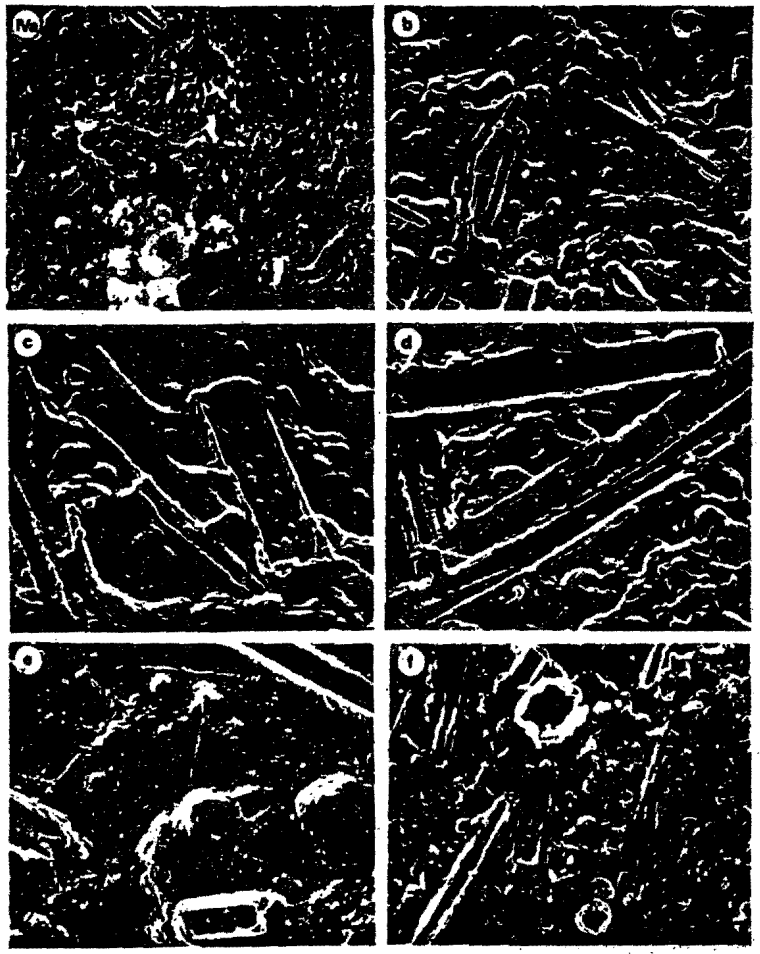
Plates 11a-f. (a) MBT/Cu$_2$O, 0 wy; 24 hr. X 1.86K. Numerous slots incorporated in smooth matrix. (b) TBT, 0 wy, 7 d. X 1.86 K. Extensive film development obscuring paint surface. (c-f) TBT, 0 wy; 14 d. (c) X 1.86K Film development surrounding pores. (d-f) Colonization by rod bacteria; settlement evident around (d) and within slots (e-f). X 5.13 (d); X 3.08K (e), X 6.68K (f).



Plates IIIa-f. (a-c) TBT/Cu_2O, -966 mv; 24 hr. (a) X 1.49K. Biofilm development showing Peritrich attachment (b) X 1.86K. Numerous slots, some appear shallow (c) X 8.05K Peritrich overlying paint film. (d-f) TBT, -966 mv; 7 d. (d) X 1.86K Extensive film development obscuring paint, (e) X 2.56K Diatom being incorporated into biofilm, (f) X 1.86K Pores and slots evident through thinner biofilm.



Plates IVa-f. (a-f) TBT/Cu_2O, -966 mv, 14 d. (a) X 803 Biofilm development quite extensive, slots and pores visible, (b) X 1.86K Pores and slots penetrating deep into paint film. (c-e) Bacterial colonization around and within slots, many slots form distinctive troughs, some overlying each other. (c-d) (c) X 4.41K, (d) X 4.23K, (e) X 4.39K, (f) X 1.86K Numerous pores at various stages of development.



7.º CONGRESO INTERNACIONAL DE CORROSIÓN MARINA E INCRUSTACIONES

7th INTERNATIONAL CONGRESS ON MARINE CORROSION AND FOULING

7ème CONGRES INTERNATIONAL DE LA CORROSION MARINE ET DES SALISSURES

UNIVERSIDAD POLITÉCNICA
Valencia, 7-11 Noviembre, 1988
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SECCIÓN II

Biología marina
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SESSION II Marine biology
Friday 11th November

SURFACE PROPERTIES ON NON-TOXIC ANTIFOULING PAINT FILM
K Nanishi, M Murase, Y Yonehara, M Kishihara and T Hirama

MICROFOULING ON ALUMINIUM PANELS PLACED IN THE TROPICAL WATERS
N B Bhosle, K Venkat and A B Wagh

EFFECT OF TRIBUTYLTIN OXIDE (TBTO) ON THE OVARIAN MATURATION OF THE PRAWN, *CARIDINA WEBERI*
R Nagabhushanam, B Indira, R Sarojini and Mary Frances Thompson

EFFECT OF BIOACTIVE MATERIALS FROM SOFT CORALS ON BACTERIA ASSOCIATED WITH COMMON FOULER
Vitalina Mary, Avelin Mary, R Sarojini and R Nagabhushanam

SURFACE PROPERTIES OF NON-TOXIC ANTIFOULING PAINT FILM

K.Nanishi, M.Murase, Y.Yonehara, M.Nishihara and T.Hirama
Kansai Paint Co.Ltd.
Hiratsuka, Japan

ABSTRACT

As many researchers have pointed out, silicon compound is one of the most promising materials for non-toxic antifouling paints. But its mechanism of preventing marine foulings is not clear. On the other hand thrombosis formation has been attracted attention as a similar phenomenon to marine foulings. In order to make the relationship between these two phenomena clear, proteins' adsorption was examined on various silicons. Several interesting coincidents between the two phenomena were observed.

1. Introduction

It has been indicated that lov energy surface and also surface which is in specific range of energy have good antifouling property. 1)2) This is sometimes explained with the surface energy. But it is not adequate to explain. For example, teflon resin has lower surface energy than silicon resin, but teflon resin shows poorer antifouling performance than silicon resin.

Marine fouling phenomenon is the result of adhesion and coagulation of viscous liquid.3)4)5) In this sense, it closely resembles the formation of thrombosis. Recently in the medical field, many artificial anti-thrombosis polymer materials have been developed. One of the most intersting materials possesses a unique surface structure which is composed of separated hydrophobic and hydrophilic portions. Such a surface can be obtained by changing portions of HEMA (hydroxy ethyl methacrylate...hydrophilic component) and styrene(hydrophobic component) when the domain size and the figure of the surface also change. These changes are predominating factors for obtaining effevtive polymer surface. Based on this findings, authors investigated the relation between adsorption of proteins and amount of marine foulings on candidate polymers by the use of various phase-separated surface.

2. Experiment

(1) Materials

As hydrophobic component (A), methyl-phenyl-siloxane resin (R/Si=1.5) and dimethyl siloxane rubber (R/Si=2) were selected. As hydrophilic component (B), silicon resin modified with polyethylene glycol was selected. Some structual models by using component (A) and (B) are shown in Figure 1.

The methyl-phenyl siloxane resin in 75% toluon solution, the dimethyl siloxane rubber in 50% fast volatile solution and the silicon resin modified with polyethylene glycol in 100% N.V. liquid.

Silicon resin model formula, which were composed of the methyl-phenyl siloxane and the silicon resin modified with polyethylene glycol, and the silicon rubber model formula, which were composed of the dimethyl siloxane rubber, paraffin vax and the silicon resin modified with polyethylene glycol, were prepared for following tests.

(2) Sea immersion test

On 1.0 x 300 x 100 mm steel plates, epoxy A/C was applied in two coats (100 microns x2) and then each sample was applied to 30-50 microns thickness, and left for drying for 1 week at room temperature.

Silicon resin model formula and silicon rubber model formula were immersed at Shimizu Orido Bay from August 1986 to March 1987 and at Yokosuka Bay from March 1987 to January 1988, respectively. The amount of fouling organisms was valued at fouling percent every month.

(3) Surface energy

The surface energy was induced from the contact angle by using Fowkes' and Owens' formula. The surface was cleaned off dirts and slime, which adhered during sea immersion, and presented to the measurement.

(4) Surface structure

The surface dried for a week after application was dyed with Osmium acid 6) and observed with a S.E.M.(Scanning Electro Microscope).

(5) Adsorption of proteins

Adsorption amount was obtained by the Folin's method.7) The outline is as follows:

1mm diameter glass beads, which have about 2,000 ㎠ areas, were immersed in 2.4% Non Volatile solution, and dried while shaking. After that they were washed in buffer solution and then the air on beads surface were completly evacuated. Adsorbed proteins (Albumine, γ-

globrine and Fibrinogen were measured by the spectro scopic difference using 750 nm light source.

3. Result

(1) Silicon resin model formula

The polarity of the films, adsorption of proteins and amount of adhered organisms are tabulated in Table 1.

It was found that there is an optimum combination ratio (hydrophobic/hydrophilic = component (A) / component (B)) for minimizing the fouling amount, which was between 85/15 ~ 80/20 by wt. in series. Surface energy dose not coincide with this change. Polar component of the surface energy increases steadily up to 80/20 and then rises dramaticaly. The reason seems to be the effct of the hydrophilic component.

In accompany with the increase of the hydrophilic component the surface structure changes as shown in Figure 2. Phase separation becomes most clear at the ratio around 85/15, showing the smallest domain size. The domain sizes are 0.8 μm and 1.2 ~ 1.5 μm, at the ratio of 85/15 and 70/30 ,respectively.

It was found that there is an optimum ratio for each protein where protein adsorption can be kept at a minimal level. These optimum combination ratios between component (A) and component (B) are on 90/10 for Albumin, 80/20 for Fibrinogen and 85/15 for γ-Globulin, respectively. These optimum combination ratios; i.e. from 90/10 to 80/20, are found to well coincide with the minimal fouling amount. See Figure 3.

(2) Silicon rubber model formula

The same experiments as those for silcon resins were carried out with silicon rubbers and paraffin vax which are widely used as non-toxic antifouling paints.

Amount of marine fouling and surface energy are shown in Table 2. In this table any notable relationship was not found, either.

The phase contrast microscope showed clear phase separation, it is seen in Figure 4. Although the S.E.M. showed vague phase separation, it is not assumably appropriate to take the same dyeing method for silicon resin model formula's experiment. Table 3 shows, except for the formulation containing praffin vax, low adsorption of the proteins correlated to good anti-fouling performance. Though the formulation containing paraffin wax shows rather high adsorption value, it's anti-fouling performance belongs to the best group. The result of immersion is shown in Table 3.

4. Discussion

Although it is not well known about anti-thrombosis mechanism by the phase separation with hydrophobic and hydrophilic portions, following explanation is sometimes adopted. As there is an observation that blood platelet does not change their figure on phase separated block polymer with hydrophobic and hydrophilic portions, it is considered that phase separation achieves something effective to restrain coagulation of blood.

The restriction of coagulation has a relation with the adsorption of blood platelet which may be affected directly by the domain of surface structure. Albumin ,γ-Globline and Fibrinogen are the representative blood plasma proteins which have been investigated of their adsorption phenomena on various materials. 8) It is very interesting that if Albumine is said to be selectively adsorbed on the hydrophilic site and γ-Globline selectively adsorbed on the hydrophobic site to accumulate themselves making domain, which may restrain the function and coagulation of blood.

It is well known that adhesion of marine fouling is due to viscous polymer liquid. 9) As for mytilus, the adhesive of foot (edulis) is mainly composed of tyrosine and takes quinon type cross-linking mechanism. In marine foulings, as the first of all organic molecular irreversible adhesions proteins adsorption takes place and proteins forms "conditionig film".10)11)12) As marine organisms adhere onto such a film, it is similar to the thrombosis in the sense that the proteins of blood plasma adhere on polymeric films beforehand.

In our experiment, except for paraffin wax , the polymer with hydrophilic and hydrophobic phase separation showed a good anti-fouling performance and showed a low adsorption of proteins. It suggests that not only surface energy but also morphorogy are important factors to prevent marine foulings. The fact that there are different minimum values respectively for Albumine ,γ-Globrine and fibrinogen means that the difference may only come from their characteristics. This might be

able to be proved by competitive adsorptions which are commonly used as a useful method with medical polymers.

Although paraffin wax worked effectively for preventing marine foulings. the proteins' adsorption did not decrease unlike with the other materials. Thus paraffin wax's anti-fouling performance doesn't rely on the proteins' adsorption.

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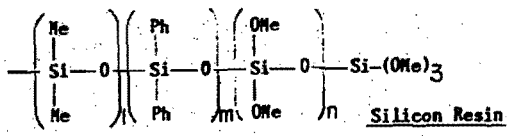
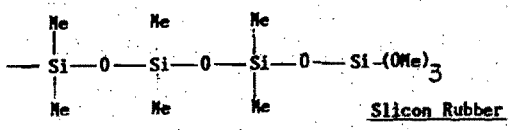
Hydrophobic component (A)



Silicon Resin



Silicon Rubber

Hydrophilic component (B)

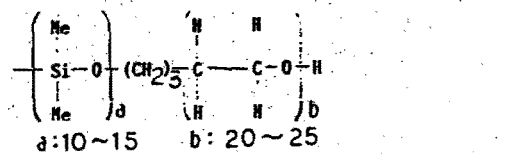


Figure 1. Model polymers used in the study

Table 1 Interrelationships of polarity of the films, adsorption of proteins and amount of adhered organisms

	(A)/(B) (wt)	*1 Polarity (γ^P/γ)	Adsorbed protein *2 ($\mu g/cm^2$) A	F	G	Adhered area percentage of algae and barnacles
1	100 / 0	0.9/25.7	2.22±0.01	1.58±0.01	0.79±0.04	60%
2	95 / 5	1.1/23.6	1.20	0.95	0.36	20
3	90 / 10	1.3/24.0	1.08	0.87	0.04	2
4	85 / 15	2.9/23.8	1.30	0.79	0.01	0
5	80 / 20	2.7/23.2	1.42	0.84	0.04	0
6	75 / 25	5.1/20.1	1.35	0.88	0.09	1
7	70 / 30	4.8/19.7	1.44	0.85	0.14	5

*1 (A):Hydrophobic component; (B):Hydrophilic component
*2 A: Albumin. F:Fibrinogen. G:r-Globulin

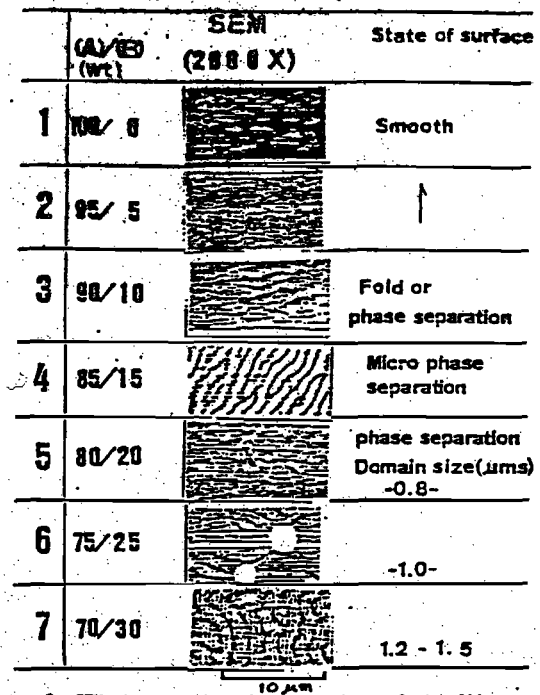


Figure 2. SEM photographs of the surface of the film systems

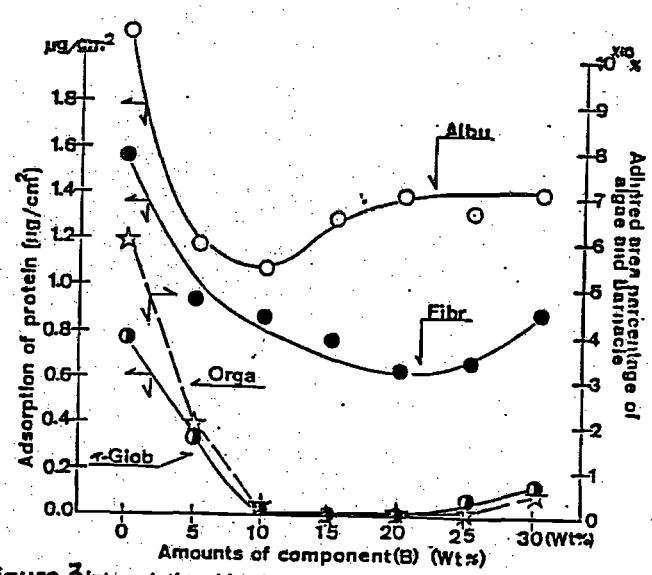


Figure 3 Interrelationships between ratio of component(B), adsorption of protein and adhesion of marine organisms
○; Albumin, ●; Fibrinogen, ⊙; π-Globulin
☆; Adhesion of marine organisms

Microfouling on aluminium panels placed in the tropical waters

N.B. Bhosle, K Venkat and A.B. Wagh
National Institute of Oceanography, Dona Paula
Goa - 403 004 (India)

ABSTRACT : Microfouling on aluminium panels placed in shelf, slope and deep oceanic waters of the Arabian Sea was studied. Microfouling biomass (as dry weight and/or organic carbon) was higher in the shelf waters (< 200 m) as compared to slope (> 200 m) and deep oceanic waters. Amongst the organisms studied, bacteria were first to appear on the aluminium surfaces and showed irregular growth pattern during initial stages of fouling (192 h). The number of fouling diatoms decreased as the depth increased. Nitzschia, Licmophora and Navicula were the most dominant diatoms. Carbohydrate and lipids were the most abundant constituents in the fouling material as compared with protein.

INTRODUCTION

Solid surfaces exposed to seawater adsorb organic matter, such conditioned surfaces play an important role in the subsequent attachment of microfouling organisms including bacteria, diatoms, protozoa etc. (Loeb and Neihof, 1977; Gerchakov and Udey, 1984). Adsorption of organic matter and subsequent growth of microorganisms on the surfaces is known as microfouling. The development of microfouling layer may play an important role in attracting the larvae of many animals (Crisp and Rayland, 1960; Barnes,

1970) leading to macrofouling. Such fouled surfaces may reduce heat transfer properties of heat exchanger (Petkovich et al., 1978; White and Benson, 1984), impede flow by increasing frictional resistance (Characklis, 1973), and may enhance metal corrosion (Gerchakov and Udey, 1984).

Very little information is available on the development of microfouling on metal surfaces exposed to tropical marine environments. Therefore, in this paper, preliminary data on the initial stages of microfouling on aluminium metal surfaces have been presented and discussed.

MATERIALS AND METHODS

Microfouling studies on aluminium panels were carried out in the shelf (< 200 m), slope (> 200 m) and oceanic waters of the Arabian Sea during oceanographic cruises of the research vessels R.V. Gaveshani and O.R.V. Sagar Kanya. The stations occupied during these cruises are shown in Fig. 1 and Table 2

Preparation of panels

Aluminium panels (15 x 10 cm) were cleaned with concentrated hydrochloric acid and repeatedly washed with tap water followed by distilled water. Finally, dried in an

oven and kept covered until used. For enumeration of bacteria, small panels (2 x 6 cm) were cleaned as above and were sterilized before use.

Deployment of panels

At eleven stations (Fig. 1) aluminium panels were kept suspended in the surface seawater (~ 1 m) from the ship. The panels were retrieved after 3 to 6 h and evaluated for microfouling biomass as organic carbon. At these stations surface water samples were also collected and filtered through GF/C glass fibre filters to know particulate organic carbon (POC).

A single station (15° 08' N, 73° 16' E) in the shelf water (Fig. 1) was occupied to study the initial biological and biochemical stages of microfouling on aluminium panels with respect to time and depth. Time series experiment was carried out as described above except that the panels were removed at 6 h interval during first 24 h and thereafter every 24 h upto 192 h.

Depth related changes in the microfouling was studied by suspending aluminium panels at 10, 25, 40, 60 and 80 m using 'I' mooring system at the above station. Mooring was deployed on March 17 and retrieved on March 27, 1986.

Three oceanic stations (Table 1) were also occupied. At these stations microfouling was studied by deploying aluminium panels at 1000 and 2400 m using deep sea mooring.

Biological and biochemical analysis of microfouling material

Microfouling material formed on the above panels at various stations and with time and depth was scrapped using brush and filtered seawater. Samples were then diluted to a known volume and subsamples were taken for different biochemical and biological analyses.

Subsamples for diatoms were preserved with Kefers solution. In laboratory they are allowed to settle for 24 h and then counted and identified using inverted microscope.

For the analysis of dry weight (oceanic station only), organic carbon, carbohydrate, protein and lipid, a known volume of subsamples was filtered through preignited (450° C, 3 h) and preweighed GF/C glass filters. Organic carbon and lipid were estimated as suggested by Parsons et al. (1984). Carbohydrate and protein were analysed using phenol-sulphuric acid (Dubois et al., 1956) and Folin-phenol reagents (Lowry et al., 1951, respectively.

Aseptic techniques were used to scrape and prepare dilutions for bacterial analysis. Two or three dilutions were prepared in sterile seawater. Duplicate aliquots were filtered through Millipore filters (0.2 um pore size) and filters were placed on ZoBell's marine nutrient agar (No. 2216) plates. The plates were incubated at 20 +/- 2 ^{o}C to estimate of bacterial numbers.

RESULTS

Microfouling biomass (as organic carbon) and the POC of the surface water at eleven station are shown in Table 1. Microfouling biomass as well as POC varied from station to station. In general stations occupied in shelf waters (< 200 m) showed higher microfouling than those of slope waters (> 200 m) and deep oceanic waters (Table 2). High microfouling was often but not always associated with higher POC of the surface waters of the shelf and slope stations. Microfouling biomass did not show any appreciable diffrecnce with space and depth in deep water of the oceanic stations (Table 2).

Amongst the microorganisms studied, bacteria were the first to colonize the aluminium surface and did not show any definite growth pattern during 192 h of deployment (Table

4). More or less similar pattern was recorded for diat[illegible] and their numbers were very low (2 to 0 cells/[illegible] sq.) and rarely changed during the exposure period of 192 h.Diatoms Triceratium, Navicula, Biddulphia, Rhizosolinia were observed in the microfouling material of the time series.

Ten different diatoms were observed in the microfouling material collected from differnt depths (Table 3). Nitzschia was the most abundant group at all the depths. Total number of fouling diatom decreased as depth increased. Of the ten different diatoms present, four belonged to centrales and six pennales. At 10, 25 40 and 80 m depth three to four types of diatoms were recorded, whereas at 60 m seven types of diatoms were present. Bacterial analyses in the microfouling material collected from different depth could not be carried out due to practicle difficulties on board the ship.

Microfouling biomass and its biochemical composition varied with time and depth (Table 4 and 5). As observed for bacteria and diatoms no definite pattern was noticed during initial stages (192 h) of microfouling. Lipid and carbohydrates were most abundant in the microfouling material during time series experiment and also at various

depths.

DISCUSSION

Several parameters such as carbon nitrogen, ATP and carbonate content (Aftring and Taylor, 1979; Mayack et al., 1984), heat transfer resistance (Characklis, 1973) and total dry weight of fouling material (Burton and Margrey, 1979) have been used to quantify the extent of biofouling. We used organic carbon and/or dry weight content to assess the extent of microfouling on aluminium panels with space (Stations), time and depth. Microfouling was relatively low in slope (> 200 m) and oceanic waters as compared with shelf waters (< 200 m). This may be due to the differences in the phisico-chemical and biological factors at these three environments. It is suggested that sea area, exposure time and depth played an important role in the development of microfouling (Yanshun et al., 1984). This is evident from the data presented here (Table 1 to 5). We have deployed aluminium panels from the ship to estimate microfouling biomass. It is,therefore, possible that microfouling was perhaps overestimated due to contamination from the ship. Presumably, this effect was small because we observed considerable differences in the microfouling biomass in shelf and slope waters. Microfouling was maximum at 25 m (Table 5). This is in agreement with our earlier

observations carried out in the nearby area of Bombay High region wherein maximum microfouling was obtained at 30 m over a period of three years (Wagh, unpubl. results).

Various workers have described the developing stages of the primary slime film and ecological succession of complex microfouling communities in the temperate and sub-tropical environments (Mitchell and Krichman 1984; White and Benson, 1984; Yanshun et al., 1984). These studies suggest that bacteria were the first organisms to appear on the various surfaces placed in seawater. Our observation on the microfouling material formed on aluminium panels is in agreement with the above findings. Irregular growth patterns of bacteria and diatoms in earlier stages of microfouling observed on the aluminium panels seems to be a regular phenomenon on many surfaces (Corpe, 1972; Yanshun et al., 1984). This irregular growth pattern may be the result of reversible attachment of bacteria to surfaces which are removed due to small stress and/or due to grazing by protozoa (Marshall et al., 1971; Marshall, 1976).

After bacterial adhesion diatoms were the second group of colonists on the aluminium panels. Although microscopic investigations of the microfouling have revealed the presence of diatoms in large numbers, few studied have been

found on these organisms (Caron and Seiburth, 1981; Cooksey et al., 1984). Diatoms which foul manmade surfaces belong to a restricted group of organisms and are usually of pennate type (Characklis and Cooksey, 1983; Cooksey et al., 1984). In the present study in addition to pennates, centrales were also recorded. Of the various diatoms observed Triceratium was the first to appear after 48 hrs on aluminium panels followed by other diatoms. The predominance of Navilula and Licmophora on the subsurface panels (Table 3) indicated the common appearence of these organisms on non-toxic surfaces exposed to various types of environments.

Biochemical analysis of the microfilm showed large amount of lipid, carbohydrate and protein which may serve as a food source for the settlement of larvae of many macrofouling organisms.

ACKNOWLEDGEMENTS

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Table 1 Microfouling biomass on aluminium panels placed in the surface waters at various stations and particulate organic carbon (POC) of surface waters at these stations

Station	Position Lat.	Position Long.	Station depth (m)	Sampling depth (m)	A	B
3717	15°00.0'N	69°45.0'E	3700	1	55.92	415.00
3719	17°00.0'N	68°30.0'E	3500	1	31.48	375.69
3721	19°00.0'N	67°15.0'E	3400	1	66.29	416.81
3723	21°00.0'N	66°00.0'E	3600	1	68.64	676.72
3725	20°00.0'N	71°00.0'Ë	68	1	346.02	1002.33
3734	18°00.0'N	72°15.0'E	68	1	234.65	693.93
3735	18°00.0'N	72°00.0'Ë	90	1	321.36	700.93
3736	18°00.0'N	71°45.0'E	80	1	237.64	806.06
3744	17°30.0'N	73°00.0'E	45	1	109.97	647.65
3746	17°00.0'N	72°30.0'E	85	1	158.34	643.45
3748	17°00.0'N	72°00.0'E	93	1	114.55	695.31

A= Microfouling biomass (ugC/h/100 cm^2)

B = POC (ug/l^{-1})

[illegible]

[illegible]
May 1987.

Station	Position		Depth (m)			
	Lat.	Long.	100%		50%	
	N	E	A	B	A	B
Eastern Arabian Sea	15° 27'	69° 44'	81	42	10	509
Central Arabian Sea	14° 04'	74° 46'	104	521	14	587
Western Arabian Sea	16° 18'	60° 28'	[illegible]	[illegible]	90	500

A = no. of [illegible]/100 cm²/day; B = no. of [illegible]/100 cm²/day.

Table 3 Type and number of microfouling diatoms on aluminium panels placed at various depths at a station (15°08'N, 73°16'E)

Sr.No.	Name of the genus	Depth (m)				
		10	25	40	60	80
1	Hyalodiscus	—	—	—	6	—
2	Coscinodiscus	—	—	—	2	—
3	Planktoniella	—	—	—	—	3
4	Biddulphia	—	—	—	4	—
5	Asterionella	—	—	—	2	—
6	Thalassiothrix	8	—	10	15	—
7	Grammatophora	—	15	—	—	—
8	Licmophora	93	24	19	4	—
9	Navicula	2	2	10	6	3
10	Nitzschia	718	591	789	44	10
	Total	821	632	828	83	16

Table 4 Variation in microfouling and its composition with time at a station (15° 08' N, 73° 16' E)

Duration (hrs)	Organic Carbon @	Carbohydrate *	Protein +	Lipid +	Bacteria +
6	284.90	26.06	11.55	42.50	0.91×10^4
12	152.90	11.73	8.92	30.65	0.32×10^4
18	189.64	16.38	18.16	40.28	1.23×10^4
24	163.13	11.77	4.10	18.96	4.20×10^4
48	141.68	7.04	3.15	17.85	2.63×10^4
72	118.16	11.67	3.46	12.21	0.68×10^4
96	174.57	14.78	7.90	10.09	0.42×10^4
120	194.15	6.78	3.27	16.25	
144	239.39	13.11	4.90	45.85	
168	214.33	11.[illegible]	3.74	50.61	
192	219.43	12.11	4.12	38.90	0.45×10^4

@ = ugC/100 cm^2 ; * = ug/100 cm^2 ;

+ = Bacteria/24 cm^2

Table 5 Microfouling biomass and its biochemical composition at different depths at a station (15° 08'N, 73° 16'E)

Depth (m)	Organic @ carbon	Carbohydrate *	Protein *	Lipid *
10	804.40	166.24	98.14	329.11
25	1326.34	126.29	115.78	336.30
40	1105.18	277.28	109.50	303.24
60	1052.33	100.88	88.97	314.70
80	1103.87	151.20	82.14	336.32

@ = ugC/100 cm^2

* = ug/100 cm^2

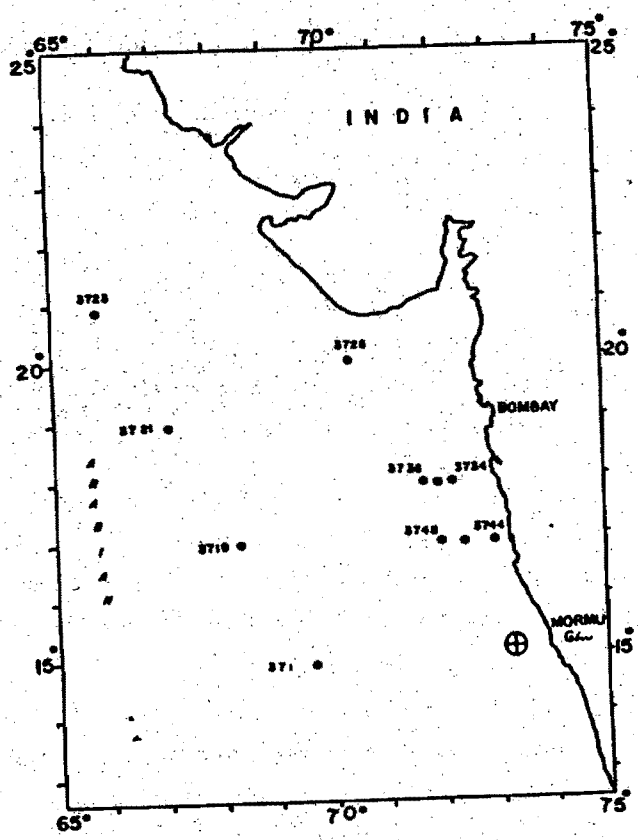
65°
70°
75°
25°
20°
15°
INDIA
BOMBAY
3728
3726
3731
3736
3734
3748
3744
3710
371
MORMU
Gulf

Fig: 1

EFFECT OF TRIBUTYLTIN OXIDE (TBTO) ON THE OVARIAN MATURATION OF THE PRAWN, CARIDINA WEBERI

R. NAGABHUSHANAM[1,2], B. INDIRA[1], R. SAROJINI[1], and MARY FRANCES THOMPSON[2].

1. Department of Zoology, Marathwada University, Aurangabad 431 004, India.

2. American Institute of Biological Sciences, Washington, D.C. 20001, U.S.A.

Abstract

Tributyltin oxide (TBTO) at lethal and sublethal concentrations inhibited the ovarian maturation in the prawn, Caridina weberi. This was evident by the decrease in the ovarian index, regression of vitellogenin from oocytes, reduction in the number of oocytes per unit area of the ovary and general decrease in the oocyte diameter.

Introduction

The use of antibiofouling materials on shrimps is necessitated by the damage some organisms may cause to wooden ships. The most widely used paints contain copper which is active only when it leaches from the paint as ion. This limits the useful life of the paints effectiveness ends. These paints are also viewed as an environmental problem because the leached copper is an environmental contaminant.

Tributyltin (TBT) compounds are presently used as the active component in marine antifouling paints as a replacement for, or in addition to copper compounds. Tributyltin compounds display high toxicity to aquatic organisms. TBT has been shown to accumulate in cyprinidont fish, exposed under experimental

conditions (Ward et al., 1981) and has been found in bivalve molluscs. Ritchie et al. (1974) in a long term experiment examined the acute and sublethal effects of TBTO on the snail, Biomphalaria glabrata and found that when newly hatched snails were chronically exposed to 1-10 ng/litre, growth, maturation rate and subsequent egg production significantly decreased. The results of laboratory experiments and field studies on the mollusc, Nucella lapillus (Gibbs and Bryan, 1986) indicate that with exposure to tin leached from antifouling paint at a concentration of 0.02 µg/l, imposex (development of male characters) advances at a faster rate and the female becomes sterile before reaching maturity.

The prawn which forms a big contributor as food in aquaculture may face the same fate as that of the molluscs (Ritchie et al., Gibbs and Bryan, 1986), unless something is known about the effect of TBT on its gonadal growth and maturation and suitable preventive measures are taken. In the present study the commercially important prawn, Caridina weberi is exposed to lethal and sublethal concentrations of TBT and the effect of it on the ovarian maturation was studied.

Material and Methods

The prawns, C. weberi were collected from Paithan, near Aurangabad, Maharashtra, India and kept in the laboratory under constant environmental conditions (Temperature, 25 ± 1°C ; Photoperiod, 12L : 12D) for acclimatization for one week. Then

they were exposed to 0.060 ppm (LC_{50} for 24 hours), 0.042 ppm (LC_{50} for 48 hours), 0.040 ppm (LC_{50} for 72 hours) and 0.028 ppm (LC_{50} for 96 hours) for 24, 48, 72 and 96 hours respectively. For sublethal effects, the prawns were exposed to 1/10 of 48 hours LC_{50} value i.e. 0.0042 for 30 days. After the lethal and sublethal exposures, the animals were sacrificed, then ovaries were dissected out for studying the effect of TBT. The criteria used for assessing the damage done to the ovaries after TBT exposure are, ovarian index cell and nuclear diameters, nature of coplasm and oocyte stages.

Results

The ovarian index (OI) showed a significant decrease in lethal exposure. The OI of control prawns was 5.047 ± 0.054, which decreased to 4.235 ±0.023 by 96 hrs. At sublethal levels the OI decreased from 3.608 ± 0.379 (7 days) to 0.506 ± 0.115 (30 days) (Table 1). The cytoplasm of the oocytes was acidophilic, indicating the presence of yolk, in the control prawns, but changed gradually to basophilic nature (absence of yolk material) during lethal and sublethal exposures (Table 1). The oocyte diameter and nuclear diameters increased gradually upto 48 hrs. but later at lethal and sublethal exposure they showed a gradual decrease (Table 1).

The ovaries of control prawns had a large number of vitellogenic oocytes (Fig.1) indicating that the prawns are in a mature condition. Within 24 hours of exposure to TBTO, only previtellogenic oocytes could be seen in the ovaries (Fig. 2). This condition

prevailed at 48, 72, 96 hrs and also at sublethal exposure (Fig. 3 to 8). There were more vitellogenic oocytes in the ovaries of control prawns (Fig. 1). This number decreased gradually by the end of 96 hours (Fig. 5). In the early part of the sublethal exposure (7 and 15 days) only previtellogenic oocytes could be seen (Fig. 6, 7). At the end of the sublethal treatment (30 days) the ovaries showed many oogonial cells (Fig.8). These results indicate that TBTO not only completely inhibited the growth of the oogonia in the ovaries but also that there was regression in the ovarian maturation.

Discussion

Tributyltin oxide was shown to be slow acting toxin at very low concentrations in the experiments reported here. Exposure to levels as low as 0.3 µg/litre for 8 weeks significantly reduced survival of Gammarus oceanicus larvae exposed to single tributyltin compounds (Laughtin et al., 1984). Long term exposure (30 days) to TBTO brought about a decrease in the production of vitellogenic oocytes in the prawn, C. Weberi . Ritchie et al. (1974) in a long-term experiment like ours, examined the acute and sublethal effects of TBTO on the snail, Biomphalaria glabrata. They found that when newly hatched snails were chronically exposed to 1-10 µg/litre of TBT, growth, maturation rate and subsequent egg production significantly decreased. Salmon et al. (1981) exposed rainbow trout (Salmo gairdneri) yolk sac fry to tributyltin chloride. Even in concentrations as low as 0.2 µg/litre, they observed decrease in growth, liver hyperplasea and decreased

thymus cell counts. In our present study, we found a decrease in the number of vitellogenic oocytes in the ovaries of C. yeberi after lethal and sublethal exposure to TBTO. However, there seems to be little comparable information for other crustacean species. Smith (1981 a,b) found strong evidence that imposex, a phenomenon involving the superimposition of male sex characters onto the female, was induced in the American mud snail, Nassarius obsoletus by exposure to organotin compounds leaching from anti-fouling paints. Gibbs and Bryan (1986) noticed reproductive failure in female populations and a general decline in the number of females in such populations which are close to the sources of tributyltin contamination. In the present study, we found the mature ovaries of C. yeberi having vitellogenic oocytes after TBTO contamination lost their yolky material and became previtellogenic even as early as 24 hours. After 30 days, the ovaries had only oogonial cells. This itself is an indication, that TBTO is not only inhibiting the ovarian maturation, vitello-genesis and consequent egg release, but also bringing back the ovary to an immature condition. It was reported by Laughlin et al. (1986), that the tissues having high lipid content will accumulate more TBT. In their studies on Mytilus edulis, they found that the gonads with maturing gametes contained a very lipid-rich material, and these tissues invariably had the highest bioaccumulation of TBT. Probably in C. yeberi also the TBTO is directly accumulated in the mature ovaries, where there are vitellogenic oocytes. This accumulated TBTO by some chemical

reactions which are not known at present, either lypolysing the vitellogenin or transferring it to other tissues. This may be the reason for the appearance of previtellogenic oocytes in the ovaries after lethal and sublethal treatment with TBTO.

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Table - 1

Effect of TBTO on the ovary of the prawn M. weberi

	Control	24 hrs	48 hrs	72 hrs	96 hrs	7 days	15 days	30 days
Ovarian index	5.047 ± 0.054	4.368 ± 1.79	-	-	4.235± 2.31	3.068± 3.79	1.916± 0.247	0.506± 0.115
Oocyte diameter (u)	98.0 ± 2.45	92.5 ± 2.75	109.5± 3.45	97.0± 1.06	93.0± 0.69	91.5± 1.29	92.5± 0.88	-
Nuclear diameter (u)	42.0± 1.03	29.0± 0.60	44.4± 1.53	34.0± 0.54	40.5± 0.57	31.5± 0.54	32.0± 0.83	-
Nature of cooplasm	Acido-philic	Baso-phile	Baso-phile	Baso-phile	Baso-phile	Baso-phile	Baso-phile	-
State of oocytes	Vitello-genic	Pre-vitello-genic	Pre-vitello-genic	Pre-vitello-genic	Pre-vitello-genic	Pre-vitello-genic	Pre-Vitello-genic	Oogonia

Fig. 1 T.S. ovary of control C. weberi showing vitellogenic oocytes X 250.

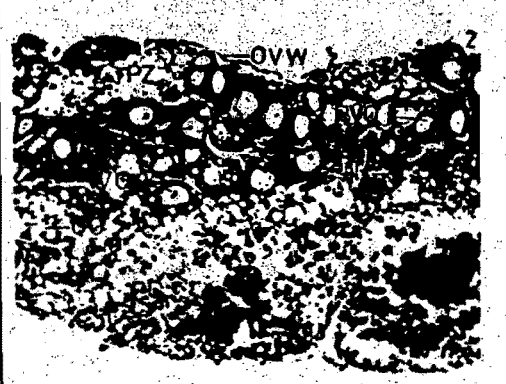


Fig. 2 T.S. ovary of C. weberi 24 hours after exposure to TBTO x 250

Fig. 3 T.S. ovary of C. weberi 48 hours after exposure to TBTO x 250

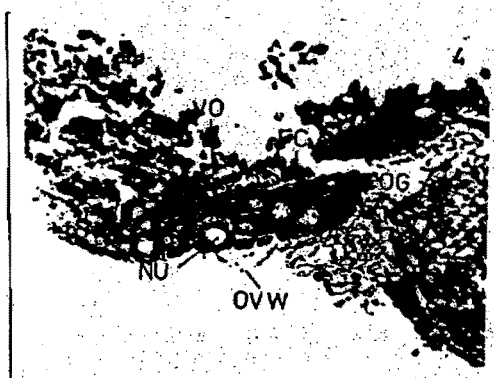


Fig. 4 T.S. ovary ofC. weberi 72 hours after exposure to TBTO x 250

Fig. 5 T.S. ovary of C. weberi 96 hours after exposure to TBTO x 250

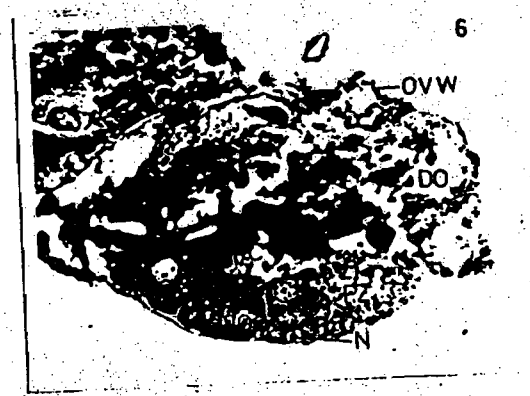


Fig. 6 T.S. ovary of C. weberi 7 days after exposure to TBTO x 250

Fig. 7 T.S. ovary of C. weberi 15 days after exposure to TBTO x 250

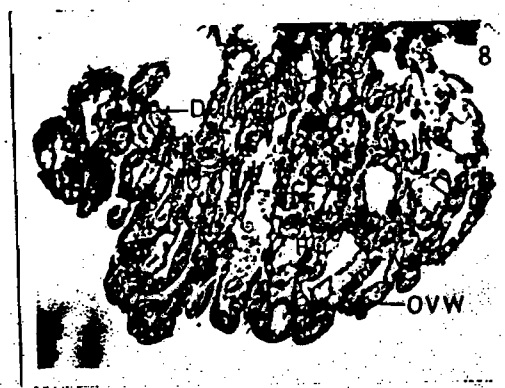


Fig. 8 T.S. ovary of C. weberi 30 days after exposure to TBTO x 250

DO - Degenerating oocytes
EPL - Epithelial layer
FC - Follicle cell ; NU - Nucleolus; N- Nucleus
OVW - Ovarian wall ; OG - Oogonial cell,
PC - Phagocyte
PVO - Previtellogenic oocyte
PZ - Proliferating zone
V - Vacuoles
VO - Vitellogenic oocyte

EFFECT OF BIOACTIVE MATERIALS FROM SOFT CORALS ON BACTERIA ASSOCIATED WITH COMMON FOULER

Sr. VITALINA MARY, O.S.M., Sr. AVELIN MARY O.S.M., R. SAROJINI* and R. NAGABHUSHANAM*

St. Mary's College, Tuticorin, 628 001, India

* Department of Zoology, Marathwada University, Aurangabad - 431 004, M.S., INDIA

Abstract

Bacteria associated with Balanus amphitrite were isolated and identified based on various morphological, physiological and biochemical characters. They are Vibrio, Alcaligenes, and Aeromonas and all the eight bacterial isolates were gram - negative. Methylene chloride extract from three soft corals i.e., Solenocaulon tortuosum, Echinogorgia complexa and Juncella juncea were found to be inactive. Methylene chloride extract from Suberogorgia suberosa prevent the motile bacteria from growing on the treated surface.

Introduction

Among first organisms to appear on a submerged surface, are bacteria, which may become securely attached after as little as one hour. Larvae of macroorganisms, if different have similar attachment kinetics. The adhesion of a primary population of microorganisms to marine surfaces has been studied extensively (Cundell and Mitchell, 1977, Marshall, 1978, Corpe, 1978). Thebacterial film then attracts a diverse film with protozoa, algae and fungi that eventually is covered with multicellular macrofouling (Corpe, 1970 a; Mars Zalek, et al., 1979). The slime layer consists of loose slime, entrapped organic detritus, and firmly attached bacteria with holdfast mechanisms.

A strong preference for slime surfaces has been observed in harnacle, and sedentary polychaetes such as Spirorbis borealis (Knight Jones, 1951). Barnacles, bryozoans and oysters are among the most serious fouling problems, on ship bottoms and pilings. The presence of extracts, fattyacid - derived butenolides, prostaglandins, and nitrogen heterocycles (Baker and Murphy, 1976; Tursch et al., 1978) with antibacterial, antialgal, antiprotozoal, ichthyotoxic and several other types of biological activity strongly suggests that chemical defence is operative in gorgonians, and more specifically, that primary film formation is inhibited in many of these animals (Perkins and Chemizko 1973; Tursch, 1976; Weinheimer et al., 1977). The present study was designed to isolate and identify the bacteria associated with the common fouler barnacle and the biological activity of soft coral extracts on bacterial isolates.

Material and Methods

Isolation of Bacteria

Balanus amphitrite were collected from the bottom of the ship aseptically in sterile petridish for bacterial isolation.

Approximately 10 grams of Balanus amphitrite sample was aseptically transferred to a sterile glass mortar, slightly crushed with a sterile pestle and mixed well in 99 ml of sterile aged sea water. After giving a full shake with vortex, serial dilutions were prepared by adopting standard procedures given by Rodina (1972). 1.0 ml of the inoculum was transferred

to sterile glass petridishes and pour plated. The plates were incubated at room temperature for 24 hours. The colonies developed in the petridishes were counted after 24 hours and represented on dry weight basis. For the enumeration of plate - viable bacteria, culture medium, Marine 2216 Agar (Difco Laboratories, Detroit, MI) was used.

Morphological observations : Cultural characters, including the appearance of cultures, the size of the colonies, their outline, elevation, translucency and whether they are colourless, white, or otherwise pigmented were noted.

Microscopical Observations : Gram - stain is a differential stain requiring a primary stain and counter stain. The primary stain is crystal violet which is followed by an iodine solution.

Subcultures in peptone broth and on agar plates: Since it was impossible to examine in detail all the colonies that grew on the count plate, a limited number of colonies (8) were isolated from the primary plates and were subcultured both in peptone broth. Further purification was done by repeated streaking on the sea water agar medium of the same composition.

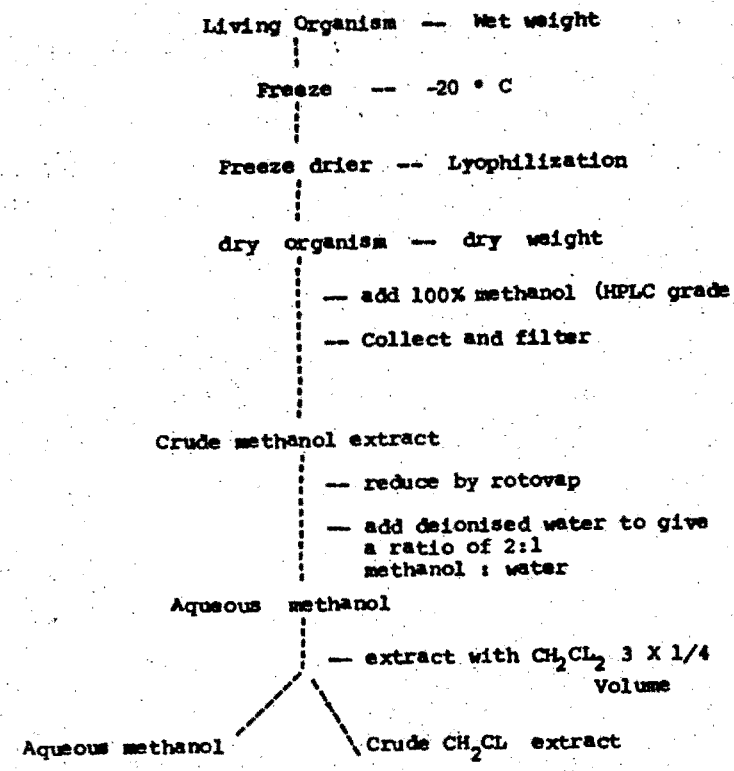
Soft coral extract : Soft Corals were collected at Tuticorin, South east coast of India. The four soft corals were Solenocaulon tortuosum, Subergorgia suberosa, Echinogorgia Complexa and Juncella juncea. Methylene chloride extract was

prepared (Scheme 1)

SCHEME 1

FLOW DIAGRAM OF EXTRACTION PROTOCOL

METHYLENE CHLORIDE EXTRACTION



Known amount of extract was taken and methylene chloride was removed by vaccum. Solids were dissolved in sea water and concentration series were prepared.

Pour plate method

One ml of the strain was transferred into a petridish. About 15 - 20 ml of the nutrient agar medium was poured into the petridishes at an ear bearing temperature aspetically. The dishes were rotated in clockwise and anticlockwise direction for thorough mixing. When the agar solidified, four small discs were placed at various points on the agar surface and each disc was loaded with 5 ul of each concentration.

Results

Enumeration of viable bacteria associated with Balanus amphitrite

The total bacterial population in the pour plates, which are incubated at room temperature for 24 hours ranged from 48 X 10^3 / g to 88 X 10^3/g on dry weight basis. A total of eight strains were isolated and identified upto genera level with the help of the scheme given by Usio and Asise... (1962) and the results were compared with Bergey's manual of Determinative Bacteriology (1975).

Identification of bacteria

Pour plates, which are incubated at room temperature for 24 hours are observed for colony characteristics. Figures 1 to 3 show different morphological charactersof the colony. Some are dull white or orange in colour while others are colourless.

Some colonies are translucent, round and regular. Rhizoid colonies are also observed.

Gram - staining

The main groups of bacteria are distinguished by microscopical observations of their morphology and staining reactions, initially in the gram - stained preparations. In the present observations, all the eight bacterial isolates are gram - negative. Table 1 shows the identification of bacteria on the basis of morphological, microscopical observations and biochemical analysis.

Antibiotic Sensitivity Test

The antibiotic, pencillin, diffuses through the agar occupying a circular zone around the original spot. The growth of the bacteria was seen in all places except the circular zone where the antibiotic was present. Strains 1 to 5 showed negative results. Figure 4 shows the nutrient agar plates with strains 1 (A) and strain 2 (B) Vibrio sp. indicating antibiotic pencillin resistance. Strains 6 to 8 showed positive results. Nutrient agar plate with Alcalegenes sp strains 6 (A) and 7 (B) showing antibiotic sensitivity is shown in Figure 5.

Effect of methylene chloride extract of soft corals on bacterial isolates :

The methylene chloride extract loaded on the disc, diffused outwards from each disc into the surrounding agar and produced a diminishing gradient of concentration. On incubation

the bacteria grow on areas of the plate except those around the material to which they are sensitive, and the width of each growth - free 'Zone of Inhibition' is a measure of their degree of sensitivity to the material.

No 'Zone of Inhibition' was observed in all the eight strains when four different concentrations of methylene chloride extract from Solenocaulon tortuosum 70 mg. Echinogorgia complexa 105 mg, and Juncella juncea 205 mg, based on the wet weight of the original animal. Other three concentrations were 10 mg. 100 µg. and 1 µg based on the wet weight of the original animal (Figures 6 - 8).

It was interesting to note the 'Zone of Inhibition' in all the strains when Suberogorgia suberosa was loaded on the disc with four different concentrations i.e., 195 mg, 10 mg, 100 µg and 1 µg based on the wet weight of the original animal (Figure 9).

Discussion

Gram - negative bacteria predominate in the sea. Most of the bacteria are small rods, being either straight or helicoidal and motile (Zo Bell and Upham 1944). In our observations of bacterial isolates associated with common fouler Balanus amphitrite the bacterial population was predominated by gram negative forms. Three groups of heterotrophic bacteria were identified based on various morphological, physiological and biochemical characters. They are Vibro, Alcaligenes and Aeromonas. 'Hanging drop' preparations show that common

slimimg strains of Vibro are motile. Our nutrient agar pour plates show peak density of morphologically different strains.

Organisms that cannot be distinguished by morphology, exhibit distinct differences in their biochemical reactions. Bacteria differ widely in their ability to metabolise carbohydrate to use them as a source of carbon and energy. The present analysis shows that except the strain Aeromonas, all the other seven strains produce no detectable gas. Zobell and Upham (1944) stated that although most marine bacteria can assimilate the simple carbohydrate, little acid and no detectable gas is produced as a rule. The corrosion enhancing effect of sulfate reducers of the genus Desulfovibrio on corrosion processes has been attributed to their ability to remove hydrogen by hydrogenase activity from cathodic sites of corrosion cells (Gerchakow et al., 1984). Iverson (1966) presented direct evidence for cathodic depolarization. Similar observations were made in the case of nitrate - reducing bacteria which use NO_3^- as a terminal electron donor. In our eight bacterial isolates seven show nitrate reduction.

P. terogorgia citrina contains compounds structurally related to the fatty acid derived butenolides, which exhibit antibiotic activity from P. anceps and P. guadalupensis (Schmitz and Lorance, 1971). On site bioassays indicated that alcohol extracts of several gorgonians possessed considerable cytotoxic, ichthyotoxic and antibacterial activity (Jacobs et al., 1981). The diterpenes jeunicin and eunicin have been isolated as the

major cembranolides in the gorgonian Eunicea mammosa from Jamaica and Bimini, respectively. Both were found to be cytotoxi against the National Cancers Institute's KB cell line. The crude extract of the Bimini gorgonian showed confirmed anti-ncoplastic activity in National Cancers Institute in vivo bioassay against p - 388 lymphocytic (Alfred et al., 1980).

In the present study bioactive materials from four soft corals are neither highly toxic, nor exactly like antibiotics. The crude methylene chloride extract from Suberogorgia suberosa prevent the motile bacteria from growing on the treated surface. Other three methylene chloride soluble crude extracts from Solenocaulon tortuosum, Echinogorgia complexa and Juncella juncea are non - toxic. These crude extracts should be purified and it is assumed that the purified material could be used as a means of preventing microbial biofilm formation on the marine surface. This technique may provide promising result in controlling slime rather than the toxic heavy metals.

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TABLE 1 IDENTIFICATION OF BACTERIA ON THE BASIS OF MORPHOLOGICAL, MICROSCOPICAL OBSERVATIONS

Samples	Gram Stain	Morphology	Colony appearance	Size of the Colony	Motility	Hugh and Leifson's Test: Formative Acid only	Acid and Gas	Cytochrome Oxidase Test	Catalase Test	Reduction of Nitrate	Formation of H_2S
Strain 1	-	Slender, Straight rods	Translucent	0.5 mm	+	+	+	+	+	+	-
Strain 2	-	Curved, Straight rods	Translucent, Yellow	0.2 mm	+	+	-	+	+	+	-
Strain 3	-	Stout rods	Translucent, White	0.4 mm	+	+	-	+	+	+	-
Strain 4	-	Short rods	White	0.4 mm	+	+	-	+	+	+	-
Strain 5	-	Straight rods	Orange	0.4 mm	+	+	-	+	+	+	-
Strain 6	-	Slender, Straight rods	Translucent, Colourless	0.3 mm	+	+	+	-	+	+	-
Strain 7	-	Short, stout rods	Dull White	1.0 cm	-	-	-	+	+	+	-
Strain 8	-	Long Straight rods	White	0.3 mm	+	+	-	+	+	+	-
	-	Long Straight rods	Translucent	0.2 mm	-	+	+	-	+	-	-

Fig.1 Pour plates with nutrient agar in which bacterial colonies are developed at 24 h incubation period. Note several morphological different colonies.

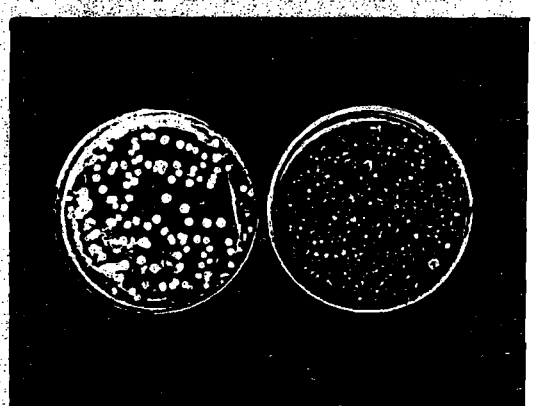

Fig.2 Pour plates with nutrient agar in which bacterial colonies are developed at 24 h incubation period. Note several morphological different colonies.

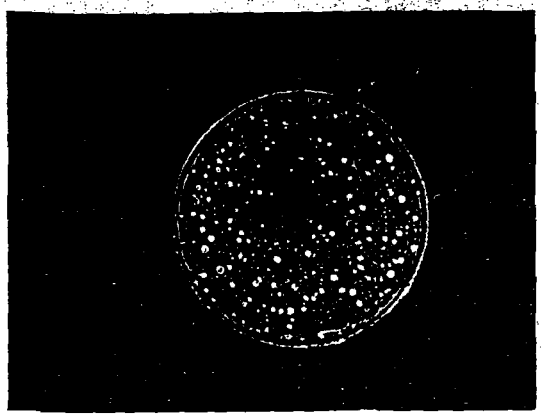

Fig.3 Pour plate with nutrient agar in which bacterial colonies are developed at 24 h incubation period. Note several morphological different colonies.

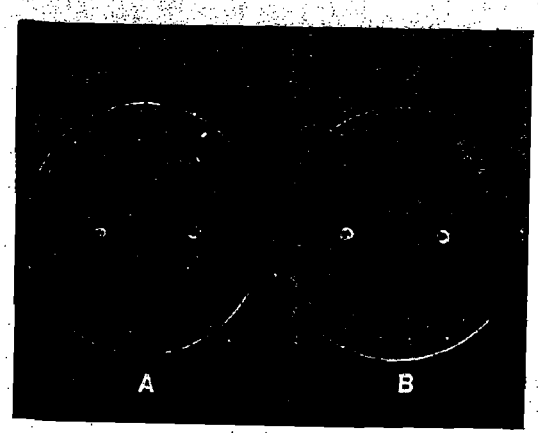

Fig.4 Nutrient agar plates with strains 1 (A) and (2) (B) Vibrio sp. showing antibiotic pencillin resistance.

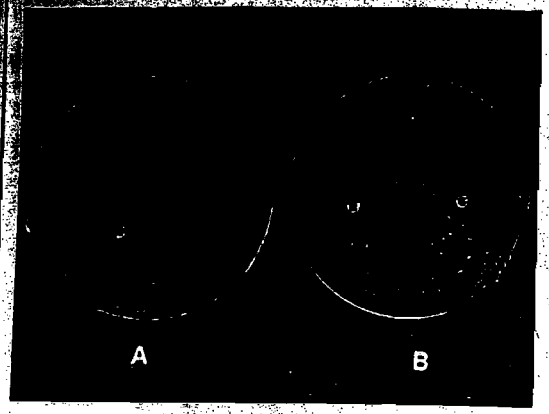

Fig.5 Nutrient agar plate with Alcaligenes sp. strains 6(A) and 7(B) showing antibiotic sensitivity. Note the 'Zone of inhibition'.

Fig.6 Vibrio sp. Nutrient agar plate showing no sensitivity when methylene chloride extract of Solenocaulon tortuosum was loaded.

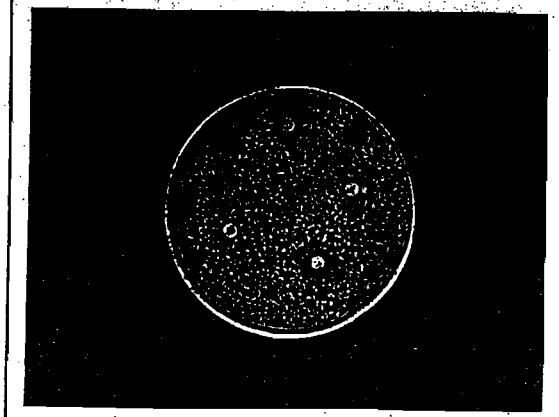

Fig.7 Alcaligene sp. Nutrient agar plate showing slight sensitivity when methylene chloride extract of Echinogorgia complex was loaded.

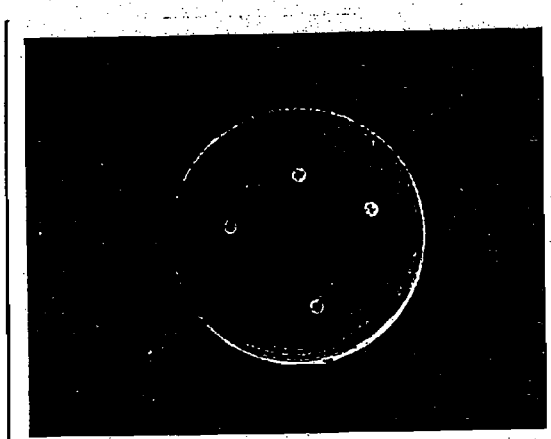

Fig.8 Alcaligene sp. Nutrient agar plate showing no sensitivity when methylene chloride extract of Juncella juncea was loaded.

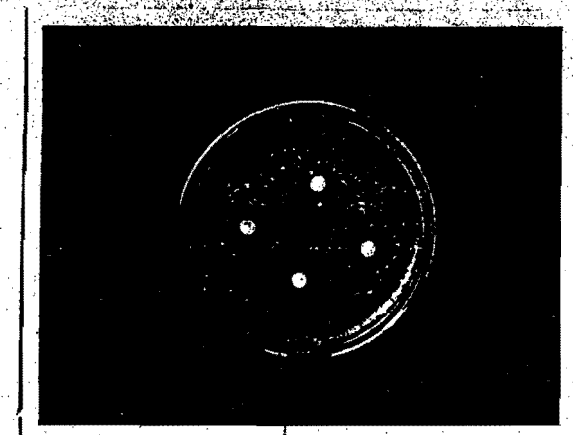

Fig.9 *Vibrio* sp. Nutrient agar plate showing 'Zone of Inhibition' when methylene chloride extract of *Suberosa* was loaded.