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TESTING OF ANTIBACTERIAL PROPERTIES OF ANTIFOULING PAINT BASED ON QUATERNARY AMMONIUM SALTS

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ABSTRACT: In order to combat the formation of a bacterial film, which is a prerequisite to the fixation of macroorganisms on surfaces immersed in the ocean, hydrolysable paints containing a biocide agent (copper oxide or tributyltin) are generally applied on the under-water surfaces. This action limits the wearability of these paints and discharges toxic pollutants into the marine environment. In the present work, the anti-microbial effectiveness of quaternary ammonium salts, a biocide which is active by contact with membrane cell and needs not be leached out, appears superior in performance, both economically and environmentally to that obtained with conventional antifouling paints containing biocide agents active in the aqueous phase.

RESUME: Pour lutter contre la formation du film microbien, qui conditionne la fixation des macro-organismes sur les surfaces immergées, on utilise généralement des peintures contenant un agent biocide relargable dans l'eau de mer. Ce mode d'action limite la durée de vie de ces peintures et provoque une pollution du milieu marin. Dans le présent travail, l'efficacité anti-microbienne d'un sel d'ammonium quaternaire, agent biocide agissant par contact avec la membrane cellulaire et ne nécessitant pas de lixiviation préalable, apparaît supérieure à celle obtenue avec une peinture classique contenant des agents biocides agissant après passage dans la phase aqueuse.

Introduction

Immersed surfaces exposed to the marine environment are colonized by marine organisms (Paerl, 1985; Maki and Mitchell, 1989). Micro-organisms are soon followed by macroscopic plant and animal organisms such as algae and barnacles. The proliferation of these organisms is often detrimental to many secteurs of work in the oceanic domain (Headerlie, 1984).

Because of these biological accumulations, sonar beacon operations are disrupted, oil rigs are destabilized and subjected to increasing stress, power station cooling circuits undergo considerable losses of charge, shiphulls become increasingly rough (Dawans, 1982) ... each of these disturbances incur additional work costs by increasing routine maintenance and weighing down ships thus increasing their energy consumption (Charalklis, 1984). The result of these accumulations is appropriately termed biofouling.

Various methods are used to slow down and limit biofouling. The most common of these is the application of paints containing biocide agents which inhibit the fixation and proliferation of these organisms. The biocides, of which the most widely used are copper oxide and organostannic derivatives, are slowly released in water. The result is a toxic effect not only on organisms that come in contact with the paint surface but also on the flora and fauna in the marine environment (Alzieu et al., 1980). These environmentally harmful effects have therefore lead

to restrictions in the use of biocide agents containing tin compounds (Anonymous, 1982).

In order to optimize the struggle against biofouling while minimizing the harmful effects on the marine environment, it is clearly a necessity to develop paints in which the biocide agent can be effective without diffusing into the sea water. This requires that the biocide agent not only be fixed in the resin which serves as a binder to the paint be a non-hydrolyzable covalent bond, but also be active on contact with the organisms so that the biocidal effect by maintained after grafting.

It is well known that the formation of a film of microorganisms on an immersed surface precedes the development of macroorganisms, which attach themselves to the film (Cuba and Blake, 1983; Mitchell and Kirchman, 1984). Therefore the most effective method of control against macrofouling is to inhibit the formation of this microbial film.

Quaternary ammonium salts have already been effectively used in the protection of cooling circuits, and could be equally useful in the prevention of biofouling.

In the present work, we have evaluated different formulations containing or not quaternary ammonium salts for their efficiency to stop bacterial colonization which is a precondition to limit macrofouling.

Experimental

Experimental design.

The different antifouling paints consist of one or more active toxic ingredients incorporated in a binder. In addition to this, a volatile organic solvent is mixed in which facilitates the application of paint and various other additives which ensure the cohesion and pigmentation of the coating. Colophane is also added to this mixture. This resin dissolves when in contact with water, forming microcanals in the insoluble vinyl matrix. This network favors the dissolution of biocides contained in the paint.

An experimental design (Ochsenbein, 1981) was carried out to overview the most significant factors in the formulation of a paint and to avoid laborious, empirical evaluation.

In order to obtain a coating presenting the best bactericide activity, it was necessary to determine firstly the relative importance of three principal parameters, namely the rates of quaternary ammonium salts (trioctylmethyl ammonium chloride), colophane and copper oxide. These three parameters were represented by three variables, respectively X_1, X_2, X_3, taking the values -1 and $+1$ in an experimental framework with pre-defined minimum and maximum limits. In the case of copper oxide, the rate of 40% corresponds to regular concentrations found in commercial paints.

The causal relations of these parameters were calculated as a function of the researched property, Y, the antimicrobial effect (see definition below):

$$Y = b + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{123}X_1X_2X_3 \quad (1)$$

Preparation of the samples.

Eight different formulations were prepared and added to a vinylic paint base as shown in Table 1, and then spread on PVC 4 × 2 cm plaquettes. A blank sample consisted of a non-coated PVC plaquette.

Evaluation of antibacterial effect.

The plaquettes were immersed in a $10m^3$ seawater basin and collected after 4 hours, 1, 2, 4, 7, 10 and 21 days. Free-living bacteria were eliminated by a quick washing of the collected plaquettes into 0.2 micronfiltered seawater.

The surface-linked bacteria colonized were scraped with a sterile cotton swab which was then submitted to sonication for 5 min (Bransonic water bath 55 MH, 100 W) in 9 ml of sterile physiological serum (Vianna Doria and Bianchi, 1982). The resulting bacterial suspension and successive dilutions were platted on 2216 E culture medium (Oppenheimer and ZoBell, 1952). After 15 days of in-

Table 1. Rates of methyl trioctylammonium chloride, colophane and copper oxide in eight formulations

Formulation	Methyl trioctyl ammonium chloride %	Colophane %	Copper oxide %
A	0	1	0
B	10	1	0
C	0	5	0
D	10	5	0
E	0	1	40
F	10	1	40
G	0	5	40
H	10	5	40

cubation at 20°C, the viable heterotrophic bacteria, which constitute the dominant microbial type in marine waters (ZoBell, 1947), were counted. This method of enumeration based on culture medium was preferable to that of direct count of microbial cells by microscopic epifluorescence, which does not permit a distinction between living and dead cells. In this particular case where the bacteria are exposed to biocide agents, the direct microscopic count could result in a large margin of error.

The number of bacteria, N_i, was plotted for each sample i as a function of time and the area, S_i, under the curve $\log_{10}$ (Ni) = f(t) was calculated. The anti-microbial effect, Y_i, is defined as $Y = (S_0 - S_i)/S_0$ where S_0 refers to the blank sample. Thus Y_i is an index varying between 0 if the sample has no anti-microbial activity and approaching 1 if its activity is very high ($S_i \approx 0$).

Results

During the course of the three week experimental period, a progressive colonization was observed of all surfaces. The evolution of the colonization of bacteria on the different plaquette preparations in function of immersion time, is presented in figure 1.

After 4 hours of immersion, there was evidence of microbial colonization on plaquettes. In the absence of an inhibitor (see sample O without paint and paint A and C with colophane but without biocide), colonization was already on the order of 1000 viable cells cm^{-2} of exposed surface, and the highest count observed was on the non-painted polyvinylchloride.

The surfaces covered with the paint containing copper oxide as inhibiting agent were colonized during the first 4 hours of immersion, the viable cell count being about 100 cells cm^{-2}, whatever the colophane concentration (see figure 1, formulations E and G).

The plaquettes prepared with the quaternary ammonium salts underwent very little colonization, the population sampling being on the order of about 10 cells cm^{-2}. Nevertheless the preparation containing both the ammonium and colophane at the strongest concentration (see formulation D), allowed a colonization 10 times

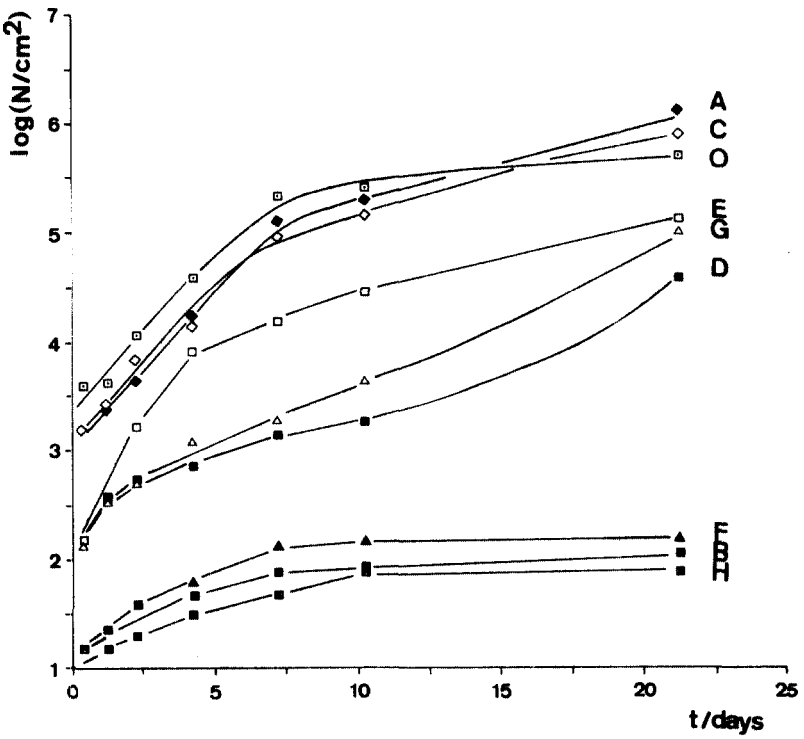

Fig. 1. Time-plot of the decimal logarithm of the number of viable bacteria per cm^2 for the eight formulations described in Table 1.

greater.

At the end of the three-week period, all surfaces underwent a certain amount of colonization. In the absence of a biocide, colonization is intense, on the order of 10^5 to 10^6 viable bacteria per cm^2. In the presence of copper oxide, the figures are very close, from 9.10^4 to 1.10^5 with the concentrations of colophane ranging from 1 to 5%. In the presence of quaternary ammonium salts, the bacterial development is strongly inhibited, viable cell counts being on the order of some 30 to 100 cells. cm^{-2}. However in the presence of the strongest concentration of colophane (5%), the bacterial count reached $3.4\ 10^4$ cm^{-2}

Discussion

At first sight, the eight samples present different behaviour according to the biocide agents they contain. As expected, the worse behaviour is shown by the samples containing no biocide (A, C and O : 10^6 bacteria. cm^{-2} after 3 weeks). The best behaviour is that of the samples containing a quaternary ammonium salt (F, B and H: 10^2 bacteria. cm^{-2} after 3 weeks). In between lie the samples containing Cu_2O alone (E and G: 10^5 bacteria. cm^{-2} after 3 weeks). The sample D (containing a quaternary ammonium salt) shows a loss of efficiency after 10 days. The same trend id observed for sample G containing Cu_2O.

The coefficientsain equation (1) are :

$b = +0.450$ $\quad a_1 = +0.265$

$a_2 = -0.016$ $\quad a_3 = +0.104$

$a_{12} = -0.057$ $\quad a_{13} = -0.013$

$a_{23} = +0.068$ $\quad a_{123} = +0.041$

This shows that quaternary ammonium salts are much more active than Cu_2O ($a_1 > a_3$) and that the effect of colophane alone is very low. On the other hand, the interaction coefficients are relatively low, but the three variables cannot be considered as independant. The positive coefficient a_{23} indicates that colophane has a beneficial effect on the activity of Cu_2O (probably by accelerating the dissolution of the low soluble oxide) whereas the effect of colophane is detrimental on the activity of the well soluble ammonium salt (negative coefficient a_{12}). This also suggests an activity of the quaternary ammonium salt included in the paint surface by direct contact with the cell membrane of bacteria, thus making unnecessary the leaching out of the biocide.

Conclusion

Anti-bacterial activity of the tested formulations appears to be, on the whole, superior to the previous and most widely used antifouling paints. In the conditions of the study, the colonization observed in the presence of quaternary ammonium salts is 1000 times less than that formed in the copper oxide preparation during the three-week immersion. This treatment seems all the more effective when observed that the samples containing an ammonium salt showed a stabilized cell count at the end of the first week of immersion, while the level of colonization for the copper oxide based paintings still showed no sign of stabilization after three weeks.

According to their larger efficiency, quaternary ammonium salts could advantageously replace the diffusible metal salts such as copper oxide in antifouling marine paints since the prevention of bacterial colonization is a prerequisite for macrofouling control. Nevertheless the solubility of these compounds is not compatible with a long-term activity. This prompted us to graft quaternary ammonium salts onto vinylic binder by a non-hydrolyzable covalent bond and to determine their biocidal activity under the same experimental conditions as used in this work (Mellouki et al., to be published). It was anticipated that such resins could lead to long-term antifouling coatings without leaching out of toxic compounds harmful to the marine environment.

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References

Anonymous, 1982. French Decree N° 82-782, 1982, september, 14. Official Journal from 09/16/1982, pp. 2792.

Alzieu C., Sanjuan J., Deltreil J.P. and Borel M., 1986. Tin Contamination in Arcachon Bay: Effects on Oyster Shell Anomalies. Marine Pollution Bulletin, 17, n°11, pp. 494-498.

Characklis W.G., Zelver N., Turakhia M., 1984. Microbial films and energy losses. In : Ed. by .J.D. Costlow and R.C. Tipper. Marine biodeterioration : an interdisciplinary study. Naval Institute Press, Annapolis,

Md, pp. 75-81.

Cuba T.R., Blake N.J., 1983. The initial development of a marine fouling assemblage on a natural substrate in a tropical estuary. Bot. marina, 26, pp. 259-264.

Dawans F., 1982. Les peintures marines antisalissures à base de polymères organostanniques. Revue de l'Institut Français du Pétrole, 37, n°6, pp. 767-807.

Haderlie E.C., 1984. A brief overview of the effects of macrofouling. In : Ed. by J.D. Costlow and R.C. Tipper. Marine boideterioration : an interdisciplinary study. Naval Institute Press, Annapolis, Md, pp. 163-166.

Maki J.S., Mitchell R., 1989. L'adhésion microbienne aux surfaces et ses conséquences. In : Ed. by M. Bianchi, D. Marty, J.C. Bertrand, P. Caumette, M. Gauthier. Micro-organismes dans les écosystèmes océaniques. Masson, Paris, pp. 389-411.

Mellouki A., Bianchi A., Périchaud A. and Sauvet G. (to be published). Bacterial colonization evaluation on several antifouling polymers.

Mellouki A., Dhainaut A., Périchaud A. and Sauvet G. (to be published). Vinyl copolymers with permanent antibacterial activity.

Mitchell R., Kirchmann D., 1981. A biochemical mechanism for marine biofouling. Oceans, September 1981, pp. 537-541.

Ochsenbein M., 1981. Informations Chimie, n° 216/217, pp. 181-187.

Paerl H.W., 1985. Influence of attachment on microbial metabolism and growth in aquatic ecosystems. In : Ed. by D.C. Savage and M. Fletcher. Bacterial adhesion. Mechanisms and physiological significance. Plenum Press, New York: 363-400.

Vianna-Doria E., Bianchi A., 1982. Comparaision de deux méthodes d'extraction des sédiments. C.R. Acad. Sci., Paris, 294 (III), pp. 467-470.

ZoBell C.E., 1946. Marine microbiology. Chronica Botanica, Waltham, Mass. 240 pp.

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