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Benzoates: a new approach to non-toxic marine fouling control

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Keywords

Antifouling paints, Pigments, Marine environment, Biocides, Non-toxic

Abstract

Owing to present and expected future regulations on the use of polluting antifouling compounds, there is a growing need for alternative methods for the prevention of biofouling. Some experiments on the effect of iron benzoate, as a possible biocide agent, on nauplii of *Balanus amphitrite* were carried out. This pigment was used because it is rapidly hydrolysed and consequently it produces a pH decrease. Although anion benzoate has an intense narcotic effect on nauplii, the results clearly demonstrated that the combined action of this compound and a pH decrease (generated by iron benzoate hydrolysis) produce a pronounced antifouling activity, i.e. the synergic effect is greater than separate effects.

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Introduction

Materials submerged in sea water experience a series of discrete physical, chemical and biological events which results in the formation of a complex layer of attached organisms known as biofouling.

The first event that occurs is the accumulation of an organic conditioning film consisting of protein, proteoglycans and polysaccharides, making the surface wettable (Loeb and Neihof, 1975a, b; Dexter, 1978; Baier *et al.*, 1968; Baier, 1984; Lewin, 1984). After approximately one to two hours, the colonization of bacteria involving two distinct phases, a reversible approach phase ("adsorption") and a non-reversible attachment phase ("adhesion") occurs. If a macromolecular pre-adsorbed film does not exist, bacteria do not attach to it. Marshall (1976; 1980a, b) suggested that organic material, accumulated in the solid/water interface, could be a food source for micro-organisms.

The growing bacterial lawn, composed of dead and living cells and the secreted slime, together with the macromolecular film, constitutes the so-called primary film. Bacteria, diatoms, spores of macroalgae and protozoans represent the primary colonizers. Finally, a great diversity of larvae of marine macrofoulers (hydroids, barnacles, mussels, tube-worms, tunicates) arrives.

The sequence of events continues to a level of deposition that eventually reduces ships' speed causing additional fuel consumption, deteriorates protective coatings, starts corrosion processes, increases the frequency of dry-docking periods and maintenance costs. Moreover, biofouling produces a reduction of pipe diameter and water flow in filtering and refrigeration systems.

Undoubtedly, paints are the best way to protect structures from biofouling. However, some of them were forbidden or restricted in their use because they contain heavy metals which may be accumulated in the

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environment. Organotin compounds such as TBT (tri-n-butyltin) and TBTO (tributyltin oxide) have been widely used as antifouling agents in marine paints. In the last decades their toxicity against non-fouling marine organisms was recognized, e.g. shell deformities in oysters, which significantly reduce their commercial value (Simmonds, 1986), mutagenic effect on the development of male sex organs in female gastropod molluscs (i.e. imposex) (Smith, 1981; Bryan *et al.*, 1986; Horiguchi *et al.*, 1994) and the accumulation of this compound in salmon tissues, principally in gills, skin, eyes and intestine (Bruno and Ellis, 1988; Alzieu *et al.*, 1989; Ellis, 1991). Alternatives to toxic coatings are being eagerly pursued in research laboratories around the world. For instance, non-toxic coatings are sought which weaken or eliminate the adhesive bond between marine life and the coating. The organisms would then be dislodged by their own weight or by the motion of the ship through the water (Adkins *et al.*, 1996). Another approach is the use of specific metabolic inhibitors and/or chemical repellents to control both biofilm formation and macrofouling.

Owing to present and expected future restrictive regulations on the use of polluting antifouling compounds, there is a growing need for alternative methods for the prevention of biofouling. Coatings with non-toxic pigments are practical and attractive alternatives to toxic heavy metal based coatings.

In a previous paper (Vetere *et al.*, 1999) the effect of sodium benzoate in laboratory and field studies was considered. This compound showed a narcotic effect on nauplii, cyprids and adults of *Balanus amphitrite*. Narcotic effect of anion benzoate was confirmed in bioassays with solutions of calcium and aluminium benzoate. Larvae exposed in the lab to different concentrations of the sodium benzoate solution could recover when they were transferred to fresh sea water; field trials also demonstrated that sodium benzoate prevents the settlement of this barnacle and retards the growth and development of the green algae *Enteromorpha intestinalis* and the growth of the tubes of the amphipod *Corophium*.

The natural buffering system of sea water becomes highly inadequate for marine life when large amounts of foreign substances are added. Most of the organisms grow and

complete their life cycles in a narrow pH range. Consequently, it is useful to know such pH tolerance limits, because it must be possible to develop an environmentally safe antifouling system by using compounds that cause changes in pH values (Mor, 1968).

The current study describes some experiments on the effect of iron benzoate as a possible biocide agent on nauplii of *Balanus amphitrite*. This pigment was used since it is rapidly hydrolysed and consequently it produces a pH decrease. In this sense, the effects on nauplii of the pH and the iron benzoate dissolved in sea water and leached from an antifouling paint were studied.

Materials and methods

Plankton samples were taken at the Club de Motonáutica (38° 02' S - 57° 32' W, Mar del Plata, Argentina) using a plankton net (25 µm). Samples were collected to obtain different barnacle larval stages. Nauplii of *Balanus amphitrite* were isolated under stereomicroscope and maintained in artificial sea water (ASTM D 1141/75), pH 8.2, in a culture chamber at 20°C ± 1°C, 14h of light and 10h of dark.

In order to establish the tolerance of larvae to different pH, buffer solutions ranging between pH 3 and 9 using acetic acid, phosphoric acid, magnesium oxide and iron benzoate in artificial sea water were prepared. The solutions were prepared immediately before each experiment and adjusted to a given pH.

Laboratory tests to evaluate the efficiency of the antifouling paints with iron benzoate as a pigment were carried out. A series of 8 × 16cm acrylic tiles was painted with soluble matrix antifouling paints containing, by volume, iron benzoate 28.5%, ww rosin 29.0%, phenolic varnish 5.8% and solvent 36.7%. After three coats of paint, final dry film thickness was 150 µm. A series of plates were put in 1-litre crystallizing vessels for one to seven days, in order to obtain solutions of pH 7.5, 7, 6.5 and 6 by the leaching from the paints. On the other hand, the effect of rosin and paint without iron benzoate was studied.

Thirty nauplii of *Balanus amphitrite* were used for the bioassays. Larvae were injected (using a Pasteur pipette) in small crystallizing vessels containing a volume of 50ml of each solution.

To study the "refreshing effect", larvae were removed from the test solutions, placed

in vessels with artificial sea water and the recovery or non-recovery was estimated.

All experiments were compared with controls without adding testing compounds. In all cases, bioassays were achieved by using artificial sea water, pH 7.9-8.2, salinity of 35-37%, light and room temperature (18-20°C) and suitable aeration.

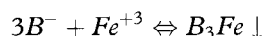
Field tests were carried out at the Club de Motonáutica. Painted plates were hung from the marinas at 50cm below the water line. Unpainted acrylic panels and panels with paint without iron benzoate were used as controls. The tests were performed in three replicates.

Results

Laboratory tests

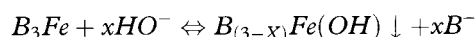
Iron benzoate hydrolysis

Iron benzoate formation could be described as follows:

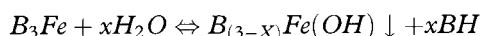


where B^- is the anion benzoate ($C_6H_5COO^-$).

As a general rule, ferric salts show a marked tendency to hydrolysis. The hydrolysis reaction may be expressed by means of the following general equations depending on the pH of the medium:

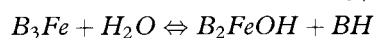


or



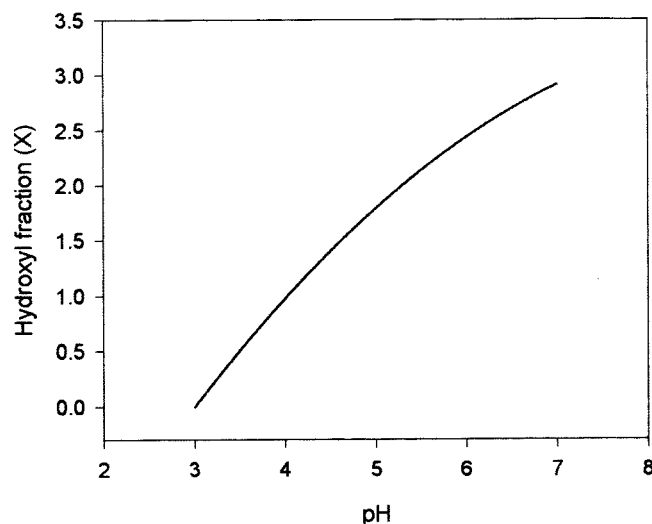
The relationship between x and the pH of the medium was obtained from experimental acid-base titration curves (Figure 1). It could be observed that, as pH increased, the x value also increased; that is to say the hydrolysis degree increased.

The pH of an aqueous suspension of iron benzoate measured with a glass electrode was found to be equal to 4.2. From these experimental data it was deduced that a hydrolysis degree with $x \cong 1$ was attained. Consequently, hydrolytic decomposition of B_3Fe can be expressed as follows:



The stoichiometric of the basic ferric benzoate formed by the hydrolysis reaction implies the release of one mole of benzoic acid (BH) and, as a consequence, the pH of the medium become stabilised in the vicinity of 4.

Figure 1 Hydrolysis degree of iron benzoate as a function of pH



In order to understand this last statement, it must be taken into account that benzoic acid is a weak one and generates a buffer system, which pH is determined by the Henderson-Hasselbalch's equation:

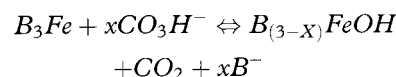
$$pH = -\log Ka + \log \frac{[B^-]}{[BH]}$$

where $Ka = 6.3 \times 10^{-5}$ is the acid constant of benzoic acid:

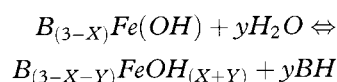
$$pH = 4.2 + \log \frac{[B^-]}{[BH]}$$

This last equation points out that the pH of the medium is 4.2 when the concentration of the acid and the basic forms are equal.

When iron benzoate is suspended in sea water, the alkaline hydrolysis induced by bicarbonate anion takes place:



then the basic iron benzoate, formed according to the preceding reactions, further reacts with water to yield the formation of additional benzoic acid:



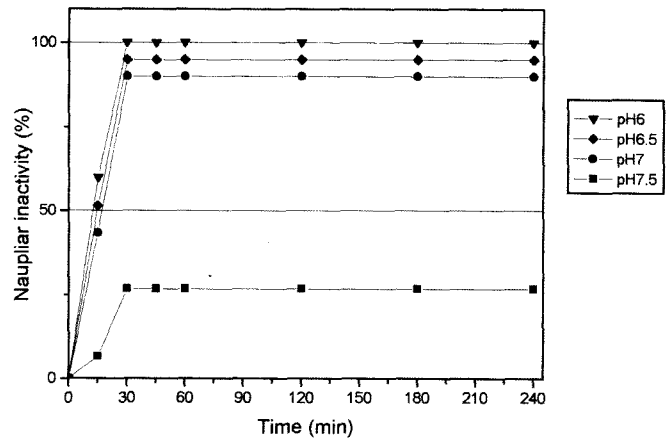
As was shown previously, the final pH will become stabilized in the vicinity of 4. When enough iron benzoate is available in the suspension, the hydrolysis may continue and the pH of the medium may be lower than 4 as a result, especially at the paint interface.

Bioassays

The larval activity was dependent on the pH of the solution, i.e. it was observed that there was a marked interval close to normal pH in which larvae showed a normal activity and below and above these values the activity was rapidly interrupted. In solutions with pH close to normal sea water (8.2-8.3) the behaviour of the organisms was similar to the control experiments and the low percentages of mortality or inactivity observed were those expected for laboratory experiments. In pH solutions below 7.5 and above 8.5 they became immediately quiescent, immobilized their appendages and stopped their swimming movements. When larvae lost their activity they fell to the bottom of the vessels with the result that the number could readily be counted at suitable time intervals. Figure 2 shows naupliar inactivity percentage in function of time for solutions of different pH obtained from acetic and phosphoric acids. In a similar way, Figure 3 shows larval response in pH values obtained from iron benzoate paints leaching. These percentages values plotted against time gave curves from which the time required for 50 per cent of the test nauplii to be quiescent was calculated. However, when larvae were put in fresh artificial sea water they could recover and followed their development. Therefore, we use It_{50} to indicate the 50 inactivity time (Vetere *et al.*, 1999).

Although anion benzoate has an intense narcotic effect on nauplii, the results clearly demonstrated that the combined action of this compound and a pH decrease (generated by iron benzoate hydrolysis) produce a

Figure 3 Naupliar inactivity percentage versus immersion time in leachate from paints



pronounced antifouling activity, i.e. the synergic effect is greater than separate effects (Figure 4).

Field trials

After two months' exposure in the sea, paints containing iron benzoate were effective in inhibiting organism settlement. Only a low density of macroalgae (*Enteromorpha* and *Ectocarpus*) was observed on the illuminated side of the panel. Algae had a very weak bond to the substrate and consequently they were easily detached and sloughed off when they were taken off from sea water. Paint films were maintained in good condition, and were not altered by algal detachment. The

Figure 4 Fifty inactivity time (It_{50}) versus pH

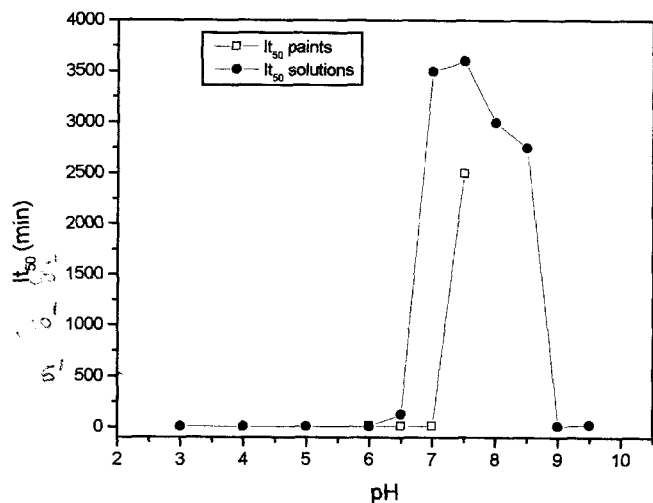
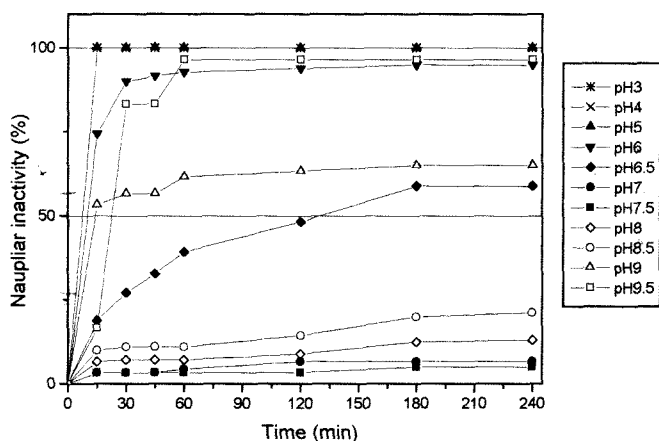


Figure 2 Naupliar inactivity percentage versus immersion time in different solutions



barnacles *Balanus amphitrite*, which are 1mm in diameter, *Polydora ligni*, *Enteromorpha*, *Ectocarpus*, with strong adhesive bond were registered on paints without iron benzoate and on acrylic panels (as controls).

In a solvent-borne paint, which we used in this study, there is a migration of organic solvent to the surface of the film. This concentration of solvent in the vicinity of the surface film may cause some of the fouling inhibition and this is sometimes referred to as the "burst effect" (Weisman *et al.*, 1992). In this sense we proved that both resin and solvent had no effect on larvae activity.

The paints significantly limited the strength of the joint between fouling and panel, making the bond so weak that it can be broken by the weight of the fouling or by the motion of the water. These results were similar to those expected for another type of paint like "fouling-release coatings" (Brady, 2000; Hare, 2000).

Short-term experiments are useful in screening different inhibitors for biofouling control but it is necessary to confirm the results with long-term assays. It is our goal to extend the study to other fouling organisms in the lab and to optimise formulation variables as with conventional paints earlier.

Conclusions

The hydrolysis of iron benzoate produces not only a decrease of pH but also an increase in soluble benzoate (and/or benzoic acid) concentration. In the paint/sea water interface the pH value is about 4. It could be concluded that the sum of the two factors (pH + anion benzoate) is greater than their individual effects. This behaviour could be mainly demonstrated in pH 6.5 and 7 due to time required to immobilize 50 per cent of the larval population with a marked decrease in the leaching obtained from iron benzoate paints.

References

- Adkins, J.D., Mera, A., Roe-Short, M., Pawlikowski, G. and Brady, R. Jr (1996), "Novel non-toxic coatings designed to resist marine fouling", *Prog. Org. Coat.*, Vol. 29 No. 1-4, pp. 1-5.
- Alzieu, C.L., Sanjuan, J., Michel, P., Borel, M. and Dreno, J.P. (1989), "Monitoring and assessment of butyltins

- in Atlantic coastal waters", *Mar. Pollut. Bull.*, Vol. 20, p. 22.
- Baier, R.E. (1984), "Initial events in microbial film formation", in Costlow, J.D. and Tipper, R.C. (Eds), *Marine Biodeterioration: An Interdisciplinary Study*, E&FN Spon Ltd, London, p. 57.
- Baier, R.E., Shafrin, E.G. and Zisman, W.A. (1968), in *Science*, Vol. 162, p. 1360.
- Brady, R.F. Jr (2000), "No more tin. What now for fouling control?", *J.P.C.L.*, pp. 42-8.
- Bruno, D.W. and Ellis, A.E. (1988), "Histopathological effects in Atlantic salmon, *Salmo salar* L., attributed to the use of tributyltin antifoulant", *Aquaculture*, Vol. 72, p. 15-20.
- Bryan, G.W., Gibbs, P.E., Hummerstone, L.G. and Burt, G.R. (1986), "The decline of the gastropod *Nucella lapillus* around south-west England: evidence for the effect of tributyltin from antifouling paints", *J. Mar. Biol. Assoc. UK*, Vol. 66, p. 611.
- Dexter, S.C. (1978), "Influence of substratum critical surface tension on bacterial adhesion - *in situ* studies", *J. Coll. Interf. Sci.*, Vol. 70, p. 346.
- Ellis, D.V. (1991), "New dangerous chemicals in the environment: lessons from TBT", *Mar. Pollut. Bull.*, Vol. 22, pp. 8-10.
- Hare, C.H. (2000), "Marine fouling and coatings for its control", *J. P. C. L.*, pp. 50-65.
- Horiguchi, T., Shiraishi, H., Shimizu, M. and Morita, M. (1994), "Imposex and organotin compounds in *Thais clavigera* and *T. bronni* in Japan", *J. Mar. Biol. Assoc. UK*, Vol. 74 No. 3, pp. 651-69.
- Lewin, R. (1984), "Microbial adhesion is a sticky problem", *Science*, Vol. 224, p. 375.
- Loeb, G.J. and Neihof, R.A. (1975a), "Marine conditioning films", *Adv. Chem. Ser.*, Vol. 145, p. 319.
- Loeb, G.J. and Neihof, R.A. (1975b), "Applied chemistry at protein interfaces", in Baier, R.E. (Ed.), *Adv. in Chem. Series 145*, Amer. Chem. Soc., Washington, DC, pp. 1-25.
- Marshall, K.C. (1976), *Interfaces in Microbial Ecology*, Harvard University Press, Cambridge, MA.
- Marshall, K.C. (1980a), in Berkley, R.C., Lynch, J.M., Melling, J., Rutter, P.R. and Vincent, B. (Eds), *Microbial Adhesion to Surfaces*, Ellis Horwood, Chichester, UK, p. 187.
- Marshall, K.C. (1980b), in *Bioscience*, Vol. 30, p. 246.
- Mor, E. (1968), "Influence du pH du substratum sur la fixation des organismes du fouling", *Proceedings of the 2nd International Congress on Marine Corrosion and Fouling*, Athens, Greece, pp. 445-52.
- Simmonds, M. (1986), "The case against tributyltin", *Oryx*, Vol. 20 No. 4, pp. 217-20.
- Smith, B.S. (1981), "Male characteristics on female mud snails caused by antifouling paints", *J. Appl. Toxicol.*, Vol. 1 No. 1, p. 22.
- Vetere, V., Pérez, M., García, M., Deyá, M., Stupak, M. and del Amo, B. (1999), "A non-toxic antifouling compound for marine paints", *Surface Coatings International*, Vol. 82 No. 12, pp. 586-9.
- Weisman, G., Sundberg, D., Cimini, R., Brown, M., Beno, B. and Eighmy, T. (1992), "Controlled release antifouling coatings. I. Approaches for controlled release of 2,4-dinitrophenolate and benzoate into seawater", *Biofouling*, Vol. 6, pp. 123-46.