

Synthesis and Characterization of Ferric Sorbate and Aluminum Sorbate as Antifouling Pigments for Marine Paints

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ABSTRACT: Biofouling is a natural process that involves the settlement and growth of organisms on natural or man-made structures and leads to material deterioration, generating severe technical and economic problems. The use of paints has been a practical method to control marine biofouling. However, some biocides currently employed have been banned because of their toxicity. There is a growing need for environmentally safe antifouling systems. The current study describes the synthesis and characterization of two new pigments, ferric sorbate and aluminum sorbate, as potential antifoulants. Laboratory assays with fouling organisms and field trials with antifouling paints were conducted. The antifouling activity of sorbate-pigment-based paints was successful and could be attributed to the combined action of a sorbate anion and low pH values generated by hydrolysis at the paint/seawater interface. After 9 months of exposure in the sea, paints containing synthetic sorbate salts had much lower fouling settlement compared to controls, indicating that these pigments are promising candidates for biofouling control.

INTRODUCTION

Engineered structures emplaced in the marine environment, such as oil-drilling platforms, weather stations, navigation buoys, marine farms, ships and jetties, are constantly threatened by saltwater, temperature fluctuations, and biological colonization.¹ Biofouling is a natural process that involves the settlement and growth of micro- and macroorganisms onto any submerged hard substrate, whether natural or artificial.² The development of marine biofouling on a submerged surface is a fast and sequential process that involves four general steps^{3,4} (Figure 1). The first event includes the accumulation of an

film.⁶ Finally, a great diversity of larvae of marine macrofoulers (hydroids, barnacles, mussels, tube worms, and tunicates) arrives.

Biofouling settlement leads to the deterioration of underwater man-made structures and generates severe technical and economic problems. In particular, these undesirable effects are important in the aquaculture industry and on oil-drilling platforms and ship hulls.⁷ The problems associated with biofouling in aquaculture comprise fouling on infrastructure (immersed structures, such as cages, netting, and pontoons) and stock species (farmed species, particularly shellfish, such as mussels, scallops, and oysters). Besides, marine biofouling modifies the hydrodynamics of ship hulls and consequently increases fuel consumption, promotes corrosion processes, reduces heat-transfer performance on heat exchangers, and causes serious problems on cooling systems for power plants. Another consequence of fouling on ships is the hull transport and/or ballast water and spread of invasive species, which may lead to the exclusion or displacement of native plant and animal species by competition for food, light, and space.⁸

The use of paints on ship hulls has been a practical method to control marine biofouling. However, biocides currently employed in paint formulations, such as lead, arsenic, mercury, tin, and their organic derivatives (TBT and TBTO), have been banned by the International Maritime Organization because they are harmful for human beings and the environment. Even though antifouling paints remain the best option for biofouling control, many formulations still contain toxic compounds, such as cuprous oxide⁹ and other controversial organic compounds.^{4,10,11} As a consequence, there is a growing need for

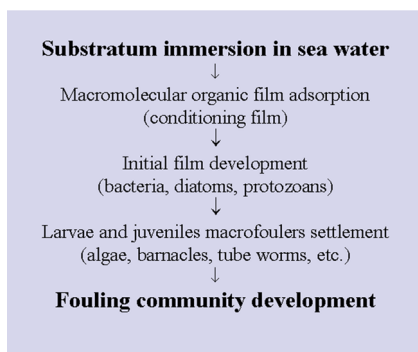


Figure 1. Stages of attachment of marine organisms on surfaces immersed in seawater.

organic conditioning film consisting of protein, proteoglycans, and polysaccharides, making the surface wettable. After approximately 1–2 h, colonization of bacteria occurs involving two distinct phases, a reversible approach phase (“adsorption”) and a nonreversible attachment phase (“adhesion”).⁵ Bacteria secrete extracellular polymeric substances, and with other colonizing microorganisms as diatoms, spores of macroalgae and protozoans constitute the so-called biofilm or primary

Received: November 18, 2013

Revised: January 17, 2014

Accepted: February 16, 2014

Published: February 16, 2014

environmentally safe antifouling systems. Some marine organisms, such as corals, algae, sponges, and ascidians, have been shown to produce antifouling substances, which in nature keeps them free of undesirable encrusting organisms.^{12,13} However, to date, most of these antifouling metabolites have been isolated in low yields from delicate and slow-growing marine organisms, which cannot be harvested on a commercial scale without environmental harm.¹⁴ For this reason, the search for environmentally friendly antifoulants should be directed toward another source of more sustainable nontoxic compounds.

In a previous study,¹⁵ it was demonstrated that potassium sorbate, a short-chain unsaturated fatty acid, is a promising antifouling compound. Fatty acids are natural metabolites that occur in a wide variety of organisms and have both antimicrobial and antifouling properties.^{16–18}

Potassium sorbate is a salt of (*E,E*)-2,4-hexadienoic acid that is used widely as a food preservative to inhibit the growth of bacteria, mold, yeasts, and fungi (Figure 2). This compound is a

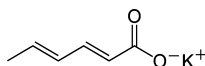


Figure 2. Potassium sorbate structure.

cheap and biodegradable commercial chemical classified as “generally regarded as safe” by the U.S. Food and Drug Administration.^{19,20} Potassium sorbate showed good antisetlement activity at concentrations that were not acutely toxic to barnacle larvae (therapeutic ratio = 3.71), and the effect was reversible, demonstrating that the compound acts via a nontoxic mechanism.¹⁵

Potassium sorbate is highly soluble in water. Moreover, the use of water-soluble compounds could promote the osmotic blistering of the coating when they are submerged in seawater. Further, the soluble antifoulants are quickly leached from the paint film by water penetrating the pores of the coating. As a whole, these facts lead to premature deterioration of the paint films with concomitant loss of antifouling activity. However, it is possible to encapsulate soluble compounds²¹ to prolong the lifetime of paint or to prepare less-soluble sorbates with certain metallic cations to be used as antifouling pigments.

The current study describes the laboratory preparation and characterization of two new pigments, ferric sorbate and aluminum sorbate. The antifouling activity of these pigments was checked by laboratory toxicity tests on two common species of the fouling community at Mar del Plata harbor, *Balanus amphitrite* (Cirripedia, Balanidae) and *Polydora* sp. (Polychaeta, Spionidae). Also, field trials employing Phytigel disks and antifouling paints are presented.

EXPERIMENTAL SECTION

Pigment Preparation and Characterization. Two pigments as potential antifoulants were prepared in the laboratory, ferric sorbate (FeSor) and aluminum sorbate (AlSor). In both cases, a 0.33 M metallic (Fe^{3+} or Al^{3+}) nitrate solution was dropped into a beaker containing a 1 M potassium sorbate solution, under continuous stirring. Once the addition of metallic nitrate was completed, the solution was gently stirred for 1 h. The precipitate was washed three times with a 10^{-3} M potassium sorbate solution to avoid hydrolysis; the supernatant liquid was then decanted, and the solid was filtered off by means of a Büchner funnel. Finally, the precipitate was first

dried at room temperature and then under reduced pressure to completely remove the remaining wetness.

The composition of the sorbate pigments was determined in order to establish their stoichiometric formulas. The iron, aluminum, and sorbate were analyzed in an independent way.

Samples of sorbate pigments were dissolved in hydrochloric acid because in this media cations remain soluble while sorbic acid separates as a solid. Then, sorbic acid was redissolved and diluted in alkaline media and determined directly by high-performance liquid chromatography (HPLC). The chromatographic analyses were carried out in a Shimadzu high-performance liquid chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with two LC-10AS pumps, a CTO-10A column oven, a 7725 Rheodyne manual injector (Cotati, CA), and a model SPD-10A variable-wavelength UV–vis detector using a SCL-10A system controller module and a C-R7A chromatopac integrator. The quantitative HPLC separations were performed at a temperature of 30 °C (controlled by the oven) on a LiChrospher 100 RP-18, 125 × 4 mm, 5 μm particle size, reversed-phase column (Merck). The mobile phase was a 0.5 g/L sodium acetate solution (pH 4.3). The flow rate was 1 mL/min, and the absorbance detector was set at 238 nm.

Both metallic cations were determined by indirect gravimetry, i.e., weighing an aliquot of the pigment into a crucible and then burning it at 1100 °C.

In order to evaluate the contamination grade of the prepared pigments, the nitrate and potassium ions were measured. The potassium content was determined by atomic absorption spectrophotometry, and the nitrate anion was potentiometrically quantified using an ion-selective electrode.

The physicochemical characteristics of pigments, such as the pH and solubility, in artificial seawater were also determined at room temperature. For this, saturated solutions were obtained as follows: 10 g of sorbate pigments were incorporated into a glass beaker containing 1000 mL of artificial seawater. Then, the suspensions were stirred for 24 h to achieve the equilibrium solubility. Finally, saturated solutions were obtained from the suspensions by filtration employing a Büchner funnel. The sorbate concentration of the saturated solution was measured by HPLC as described above, and the pH was determined with a benchtop pH meter.

Laboratory Bioassays. Bioassays were performed using larvae of *B. amphitrite* and *Polydora* sp., two common species of the fouling community at Mar del Plata harbor, Argentina (38° 08' 17"S and 57° 31' 18"W). *B. amphitrite* adults were collected from Club de Motonáutica piers, and *Polydora* sp. larvae were obtained with a 25 μm zooplankton net at the site. In the laboratory, all organisms were conditioned in artificial seawater (ASTM D1141/75, pH 8.2) at 20 ± 1 °C with suitable aeration and natural light. Adult barnacles were fed a daily diet of *Artemia salina* nauplii and *Polydora* sp. larvae with cultures of the diatom *Skeletonema costatum*. Newly released *B. amphitrite* larvae (nauplii I) were transferred to a beaker containing filtered seawater; they molted and became nauplii II approximately 1 h after release. Some nauplii II actively swimming toward a light source were selected for bioassays.

For the experiments, $1/2$, $1/4$, $1/8$, $1/16$, $1/32$, and $1/64$ dilutions from saturated solutions of FeSor and AlSor were prepared. Before the bioassays were performed, some dilutions were buffered in order to fit their pH value close to the artificial seawater value, i.e., 8.1–8.2. A total of 30 nauplii II of *B. amphitrite* and 30 larvae of *Polydora* sp. (15–16 setigerous stage, i.e., close to the tube-forming stage) were used for the

toxicity assays. Larvae were added using a Pasteur pipet to small crystallizing dishes containing 50 mL of testing solutions. A 50 inactivity time (It_{50}) was estimated, i.e., the time required for 50% of the test organisms to become inactive for each concentration.²² The inability of larvae to stay in the water column and the loss of a phototactic reaction were scored as toxic responses.²³

All bioassays were carried out with three replicates of each treatment and repeated twice with separate batches of larvae. In all cases, the experiments were compared with the controls (filtered artificial seawater).

In the laboratory, toxic compounds are not differentiated from narcotic compounds, and for this reason, larvae were placed in seawater to determine if they recovered. In order to detect if the effect of compounds on larvae was temporary (narcotic) or permanent (toxic), refreshing experiments were also carried out. For the experiments, larvae exposed to solutions were removed and placed in vessels with clean artificial seawater. Then, the percentage of larval recovery was estimated.

Field Trials. Phytigel Assays. Phytigel disks were exposed in the sea for 28 days during summer months (December to March) when the highest peak of recruitment of fouling species occurs.^{4,24}

The procedure for immobilization of pigments into the gel was adapted from that described in the literature.²⁵ Gels were made by dissolution of 7.2 g of Phytigel (Sigma Chemical) in 180 mL of distilled water in a microwave oven. After cooling at 50 °C, 0.1 g of each sorbate pigment suspended in 10 mL of distilled water was incorporated into the gel matrix with vigorous stirring. The gel/pigment mixtures were poured into polystyrene Petri dishes (9 cm diameter) containing a nylon mesh to reinforce the structural integrity and to prevent detachment of gel. Likewise, gels without any compound added were used as controls. Petri dishes were placed on rectangular aluminum structures and hung from the marina at 50 cm below the water line for 28 days. Results from gels were used as a screening test in order to establish whether sorbate pigments might be adequate to be included in antifouling paint formulations.

Tests were carried out in triplicate. The settlement of fouling organisms was estimated as the percentage cover on each gel using a dot-grid estimate method.²⁶

Antifouling Paints. Soluble matrix paints were formulated with colophony as the film-forming material and oleic acid as the plasticizer. Colophony is a brittle solid (glass transition temperature of about 30 °C) obtained from exudation of pine and fir trees. Water white rosin is the common variety used to formulate antifouling paints, which contain abietic acid as the main component²⁷ (Figure 3).

In all cases, sorbates were incorporated as antifouling pigments and calcium carbonate was added to complete the pigment formula. The pigment volume concentration was 37%.

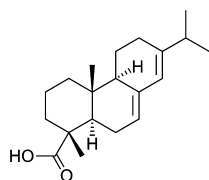


Figure 3. Abietic acid structure.

The composition of this paint, expressed as volume percentages, was as follows: 37.6% colophony, 6.8% oleic acid, 27.3% xylene/white spirit, 4.1% calcium carbonate, and 24.2% sorbate pigments.

The procedure for preparation of the paint involves three stages. First of all, rosin and oleic acid are dissolved in a mixture of solvents to obtain the “vehicle”. Then, a ball mill is loaded with vehicle + pigments and dispersed for 24 h. After that, paints are filtered through a Lycra grid in order to eliminate environmental dust and/or bigger particles.

Paints were applied to sandblasted acrylic panels (128 cm²) with a paint brush to reach a dry-film thickness of 100 ± 5 μm. A series of unpainted panels were used as controls to establish fouling community development. All panels were placed on rectangular aluminum structures and submerged in a marina in Mar del Plata harbor at 50 cm below the water line. Painted and unpainted panels were evaluated after 3, 6, and 9 months. All tests were performed in triplicate.

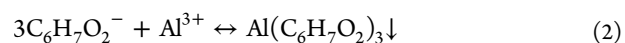
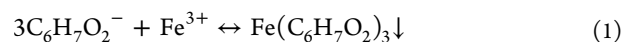
After exposure in the sea, abundance percentages for each species of fouling settled on panels were estimated using a dot-grid method.²⁶

Statistical Analysis. All statistical analyses were performed with *Statistica 8.0*. The normality assumption was verified with the Shapiro–Wilk test.²⁸ The differences between treatments and controls were determined by one-way analysis of variance, followed by the Tukey posthoc test. The differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Pigment Characterization. The composition of FeSor was 14.3% iron, 84.9% sorbate, 0.02% potassium, and 0.01% nitrate. The AlSor composition was 7.5% aluminum, 92.3% sorbate, 0, 03% potassium, and 0.01% nitrate. Analysis of these results revealed that there was 3 mol of sorbate (C₆H₇O₂) per each 1 mol of metallic cation; then, it was concluded that the stoichiometric relationship for metallic laboratory-prepared pigments was Fe(C₆H₇O₂)₃ and Al(C₆H₇O₂)₃, respectively.

Pigment precipitation could be described as follows:



It is important to remark that the methodology employed to prepare these pigments at laboratory scale is quite simple and, perhaps, it could be industrially scaled. Moreover, the pigment composition is highly reproducible when prepared and run under the same conditions. Additionally, raw materials employed in this process are cheap industrial products that are commercially available in the market.

The experimental solubilities of pigments in seawater were 64 mg/100 mL and 151 mg/100 mL for FeSor and AlSor, respectively. Otherwise, the solubility of potassium sorbate was close to 60g/100 mL, i.e., 3 orders of magnitude larger than those of FeSor and AlSor pigments, and consequently this compound result is inappropriate for antifouling formulations. Conversely, the solubility values obtained for FeSor and AlSor pigments showed that they were suitable for inclusion in antifouling coatings.^{29–32}

The pH values of the pigment suspensions were around 3.7–3.8, suggesting that when they were suspended in seawater, hydrolysis reactions took place.

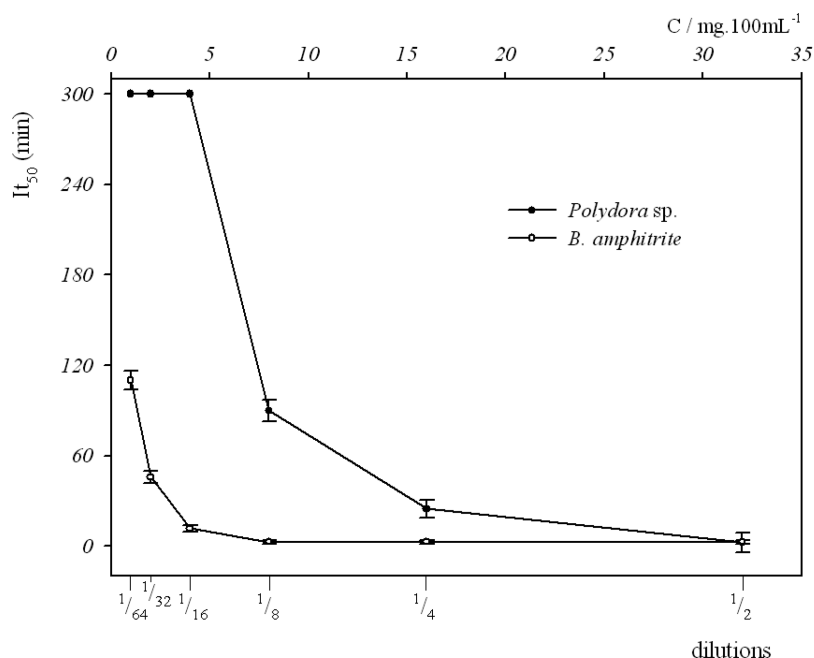


Figure 4. Larval It_{50} versus FeSor solutions.

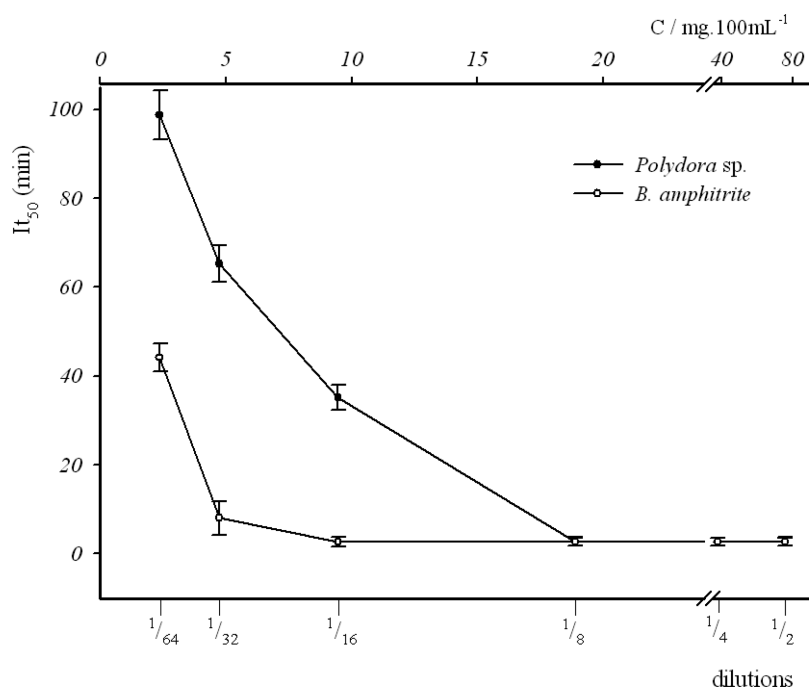
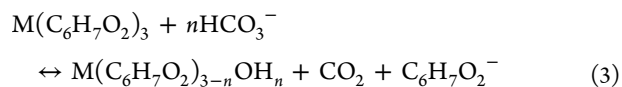
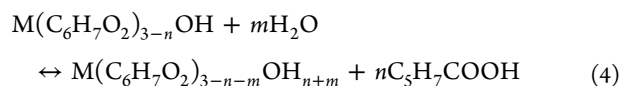


Figure 5. Larval It_{50} versus AlSor solutions.



where $M = Fe^{3+}$ or Al^{3+} . Then, the basic sorbate reacts with water to give sorbic acid:



When enough sorbate pigment is available in the suspension, hydrolysis may proceed and eventually metallic hydroxides and sorbic acid could be formed. In fact, reaction (4) is responsible

of the pH measured in the aqueous extract and especially at the paint/seawater interface.³³ The natural buffering system of seawater becomes highly inadequate for marine life when a large amount of acids or alkaline substances are added. Most of the organisms grow and complete their life cycles in a narrow range of pH. For this reason, laboratory bioassays were carried out, neutralizing the pH of the test solutions close to the artificial seawater value. In contrast, the hydrolytic acidity afforded by the pigments at the paint/seawater interface could be a powerful tool to develop an environmentally safe antifouling system.³²

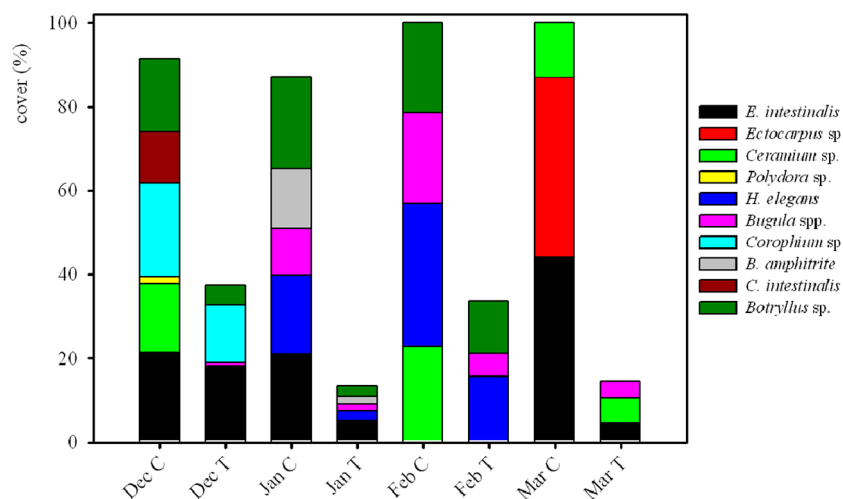


Figure 6. Fouling cover percentage on gel disks [C, control; T, treatments (FeSor)].

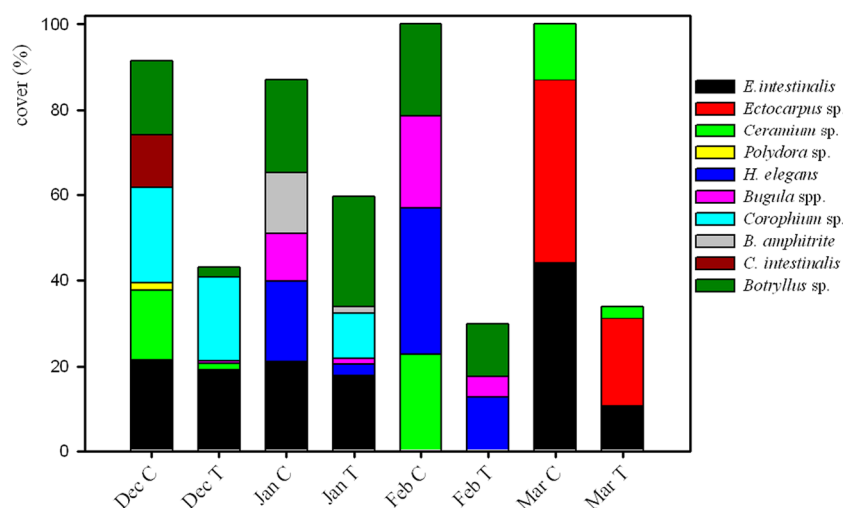


Figure 7. Fouling cover percentage on gel disks [C, control; T, treatments (AlSor)].

Laboratory Assays. Larval Toxicity Test. Laboratory tests were conducted using larvae exposed to increasing concentrations of FeSor and AlSor solutions in seawater (Figures 4 and 5).

The toxicity test showed that nauplii II of *B. amphitrite* and 15–16 setigerous larvae of *Polydora* sp. were affected in a similar way by solutions of sorbate pigments; i.e., larvae responded with a loss of phototactic response, and they became immediately quiescent, immobilized their appendages, and stopped their swimming movements. However, it is important to note that barnacle nauplii were more sensitive than *Polydora* sp. to both sorbate solutions, even from the lowest concentrations (i.e., $1/64$ dilution). A comparison of both sorbate solution curves demonstrated that AlSor affected the larval activity more rapidly than FeSor. In all cases, the inhibitory response was in a concentration-dependent manner.

It_{50} experimentally determined for *B. amphitrite* nauplii exposed to sorbate solutions was similar to that obtained in SCNCu solutions.³⁴ From an environmental point of view, these results represent an advantage because a satisfactory antifouling effect was reached with copper-free pigments.

After 24 h of exposition, nauplii and *Polydora* sp. larvae were transferred to fresh artificial seawater and the percentage recovery was estimated. In all concentrations, all larvae

recovered swimming movements and were able to complete their development; that is to say, sorbate compounds have a temporary effect. Reversible antifouling activity, meaning that larvae can settle normally after leaving the environment containing the antifouling substance, is a direct measure and evidence for nontoxicity of compounds. However, it has only been experimentally proven for a few compounds.^{15,35}

Field Trials. Phytigel Assays. The development of effective antifouling coatings requires field trials. Field antifouling assays are more ecologically relevant and more sensitive to detection of the activity spectrum of antifouling substances.³⁶

From the beginning of the summer, both sorbate pigments were capable of inhibiting the settlement of a broad range of fouling organisms in field experiments.

After 28 days of exposure in December, marked inhibition on the settlement of the red algae *Ceramium* sp., the sand tube building polychaete *Polydora* sp., and the solitary ascidian *Ciona intestinalis* was recorded for both FeSor and AlSor ($p < 0.05$). However, attachment of the common green algae *Enteromorpha intestinalis* was not affected by these compounds.

The most relevant results obtained from January and February on sorbate gels were the strong inhibition of calcareous species, particularly *B. amphitrite* and *Hydroides elegans*. Also, significant differences on the settlement of the

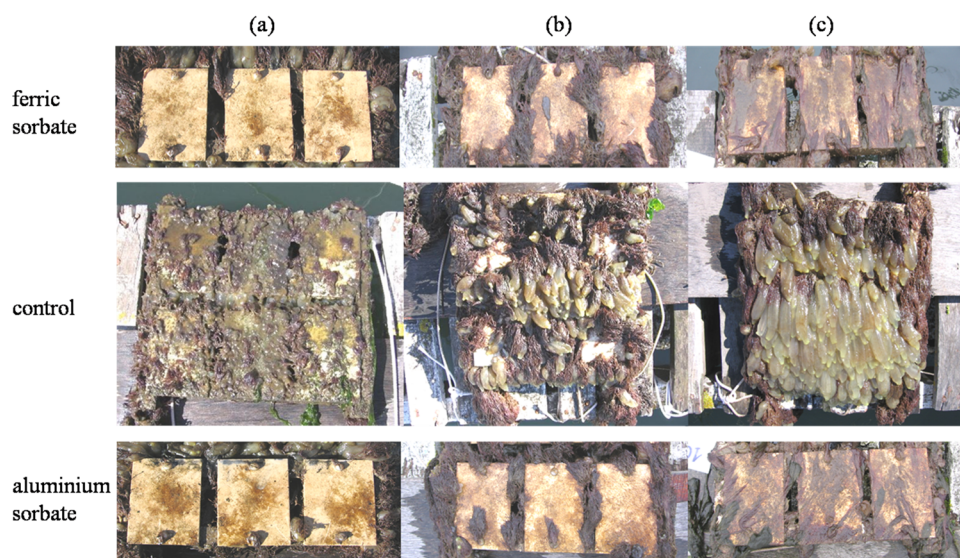


Figure 8. Panels immersed at Mar del Plata harbor: (a) 3 months; (b) 6 months; (c) 9 months.

arborescent bryozoan *Bugula neritina* were observed. A particular case was registered for the colonial ascidian *Botryllus* sp. because it was virtually absent in all replicas except on January gel disks when settled at a similar percentage as the control. Results from February samples indicated that the percentage of larval settlement of *H. elegans* and *B. neritina* on treated gel disks was significantly lower than that of a control disk; also, the complete absence of algal species, *Polydora* sp. and *B. amphitrite* was detected ($p < 0.05$).

In late summer (March gel disks), the recruitment of species drastically diminished and the fouling community was dominated by seaweeds. Both FeSor and AlSor maintained their antifouling activity and affected algal settlement (Figures 6 and 7).

Unlike the majority of recently reported antifouling compounds, FeSor and AlSor are effective against multiple species in Phytigel assays. This characteristic gives sorbates the potential to achieve a better performance on the broad spectrum of fouling organisms and encourages their incorporation in marine coatings.

Antifouling Paints. Antifouling coatings are film-forming products that act as controlled release vehicles of antifoulants. A reasonable number of active antifouling compounds have already been identified from different sources, but only a few included in a paint formulation have been studied. Laboratory preparation and evaluation of antifouling paints in the sea represent a stage in fouling control research that is not usually carried out.

After 9 months of exposure in the sea, paints containing FeSor or AlSor had much lower fouling settlement compared to that of controls, indicating that these compounds were strongly resistant to biofouling of Mar del Plata harbor. This performance was observed from the first and second samplings after 3 and 6 months of immersion, respectively.

FeSor and AlSor pigments released from the paint produced an inhibitive microlayer at the paint surface/seawater interface that deters the settlement of microscopic larvae or spores of fouling organisms. Only a thin slime composed of some diatoms (*Melosira*, *Achnanthes*, and *Grammatophora*) and protozoans (*Vorticella*) was observed on the paint surface. In contrast, control panels were heavily invaded by microfouling

species, seaweeds (*E. intestinalis* and *Ceramium* sp.), and other macrofouling organisms (mainly *Polydora* sp., *Bugula*, *C. intestinalis*, and *Botryllus* sp.; Figure 8; $p < 0.05$). This is consistent with previous studies that established strong inhibition of the sorbate anion included in a varnish on both microfouling (particularly diatoms) and macrofouling species (mainly *E. intestinalis*, *Ectocarpus* sp., *B. amphitrite*, *Bugula* spp., *C. intestinalis*, and *Botryllus* spp.).¹⁵

Several controlled-release films with soluble sorbates (e.g., sorbic acid and potassium sorbate) have been studied in order to develop antimicrobial materials for the food-packaging industry. The film-forming materials most frequently used for active packages include natural^{37,38} (e.g., shrimp chitosan, cornstarch) and synthetic organic polymers^{39–41} [poly(ethylene-co-methacrylic acid), cellulose acetate, epoxy-poly(vinylpyrrolidone)] as well as hybrid polymers⁴² [poly(vinyl alcohol) + bacterial cellulose]. Additionally, antimicrobial inorganic silica films obtained via a sol-gel technique were also reported.⁴³ However, this is the first report on the use of FeSor or AlSor for coatings, particularly in antifouling paints.

The performance of sorbate-pigment-based paints, with regard to their antifouling activity, could be attributed to the combined action of at least two factors: the sorbate anion and pH.

It was well established that the pH of the environment could affect the growth and development of a wide variety of organisms. The pH of the world's oceans remains relatively constant at pH 8.2, but that of estuaries and freshwater bodies can vary extensively from one location or season to another.^{44,45} In the case of antifouling paints, the pH of the boundary layer in which antifoulants function will be moderated by the type of paint matrix and by compounds leaching from the paint. In this sense, sorbate pigments hydrolyze, decreasing the pH value at the paint/seawater interface in a way similar to that of other antifouling pigments, such as ferric benzoate.³³

The activity of many organic biocides is affected by changes in the pH, especially those that ionize. Weak acids tend to become more toxic at low pH values, while the toxicity of basic biocides is increased with increasing pH.⁴⁶ These mechanisms directly affect the development and growth of organisms and

are responsible of antifouling action. Altering the environmental pH can cause changes in the cell physiology, such as charge distribution on bacterial cell walls, and consequently affect the uptake of charged biocides.⁴⁷

Depending on the pH of the environment, sorbate in solution exists in equilibrium between the dissociated state (sorbate anion) and the undissociated state (sorbic acid). As shown in eqs 3 and 4, the formation of sorbic acid is favored during the progress of hydrolysis of the sorbate pigments. Sorbic acid is a neutral compound that is lipid-permeable and is able to diffuse into the cell, reaching equilibrium when the inside and outside concentrations of sorbic acid are equal. Inside, a new equilibrium is formed between sorbic acid and sorbate, releasing protons into the cytoplasm and causing inhibition of many metabolic functions.⁴⁸ Furthermore, the lipophilic tail of the sorbate molecule has been shown to disrupt the membrane and interfere with membrane proteins.⁴⁹

The role of fatty acids on the larval settlement is controversial. Some authors theorize that fatty acids such as palmitoleic, linoleic (18:2), arachidonic (20:4), and eicosapentaenoic acids induce larval settlement though low percentages of metamorphosis.⁵⁰ However, experiments on the polychaete *Capitella* sp. demonstrated that linoleic acid and eicosatrienoic acid induced larval settlement and metamorphosis in this worm.⁵¹ In contrast, studies confirm that a branched-chain fatty acid produced by a marine bacterium isolated from deep-sea sediment effectively inhibited the larval settlement of biofouling polychaete *H. elegans*.¹⁶

The present study provides evidence that synthetic sorbate salts, such as FeSor and AlSor, can effectively prevent fouling either in the laboratory or in more ecologically realistic field assays. An ideal antifouling compound should be repellent at the paint surface but harmless in the surrounding environment. Overall, this investigation demonstrates that sorbates can act as antifouling agents in a nontoxic manner. These compounds also have the advantage that they can be easily manufactured and thus have lower cost for commercial use. For these reasons, FeSor and AlSor are promising candidates for antifouling technology.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CICPBA), Universidad Nacional de La Plata (Argentina), and Universidad de Buenos Aires for their economical support. The authors thank Claudio Cerruti for HPLC measurements. They also thank the Club de Motonáutica de Mar del Plata for permission to use their marine testing site.

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