

POLYMERIC BIOCIDES AS INHIBITORS OF BIOFOULING IN MARINE APPLICATIONS

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ABSTRACT

The deposition of microorganisms occurs continuously and dynamically to all surfaces submerged in seawater. This phenomenon is called bioaccumulation (biofouling). Marine biofouling represents one of the greatest problems in marine technology. To circumvent this problem, antifouling paints or coatings, i.e. matrices containing biocidal species, are used to protect the submerged surfaces and objects. With the tightening of legislation on the use of biocidal species, research actually focuses on designing environmentally friendly or environmentally acceptable biocidal materials.

Polymeric quaternary ammonium compounds are promising biocidal materials, due to their low toxicity and broad antimicrobial spectrum. In the present work, the effect of the method of attachment of such biocidal species onto the polymeric backbone is investigated. In fact, quaternary ammonium ions are either counterions (PSSAmC16) of a negatively charge polymer or they are covalently attached (P(AA-co-VBCHAM88)) on the polymer chain. Moreover, a copolymer combining both methods of attachment (P(SSAmC16-co-VBCHAM30)) is also studied.

An adequate experimental set-up has been constructed to study the evolution of biofouling using lagoon water under accelerated biofouling conditions. It is found that the homopolymer PSSAmC16 as well as the copolymer P(SSAmC16-co-VBCHAM30) effectively prevent biofouling, indicating that these polymers are promising for potential marine applications (aquaculture nets, antifouling paints, etc).

Keywords: polymeric biocides, quaternary ammonium, electrostatic binding, covalent attachment, marine biofouling

1. Introduction

Marine biofouling is a natural phenomenon representing one of the greatest problems in marine applications (navigation, aquaculture nets, etc) (Rao *et al.*, 2009, Ross *et al.*, 2004), as a consequence of the continuous and dynamic deposition of microorganisms such as barnacle, tube worms and algae on the submerged surfaces (Almeida *et al.*, 2007, Gunn *et al.*, 1987). To circumvent these problems, several methodologies have been evolved within the last decades, most of which are based on biocidal agents.

Among the several recent approaches in antifouling materials, the use of polymeric biocidal agents is of significant interest, since the advantages of the polymeric nature of the material are combined with biocidal action (Beyth *et al.*, 2006). A class of polymers that are widely used are the cationic polymers with incorporated biocidal quaternary ammonium groups (Campoccia *et al.*, 2013, Jaeger *et al.*, 2010, Munoz-Bonilla and Fernandez-Garcia, 2012). In these polymers, biocidal groups can be temporarily attached through electrostatic interaction or permanently

attached to the polymeric chains through covalent bonding (Banerjee *et al.*, 2011, Guo, *et al.*, 2013, Jellali *et al.*, 2013, Koromilas, *et al.*, 2014, (a) and (b), Tiller *et al.*, 2001).

In the present work, the potential biocidal action of polymers containing quaternary ammonium groups is investigated. In fact, the biocidal action, in terms of biofouling prevention, of polymers containing quaternary ammonium groups, electrostatically bound or covalently attached, is compared to that of a polymer combining both types of biocidal incorporation. The chemical structure of the polymers is shown in Figure 1. The biocidal groups are electrostatically attached in the case of poly(cetyl trimethylammonium styrene sulfonate), PSSAmC16, while they are covalently attached in the case of poly(acrylic acid-co-vinylbenzylhexadecyltrimethylammonium chloride), P(AA-co-VBCHAM88), containing 88mol% VBCHAM units. Finally, the combined action is investigated in the case of the random copolymer P(SSAm16-co-VBCHAM30), containing 30%VBCHAM units.

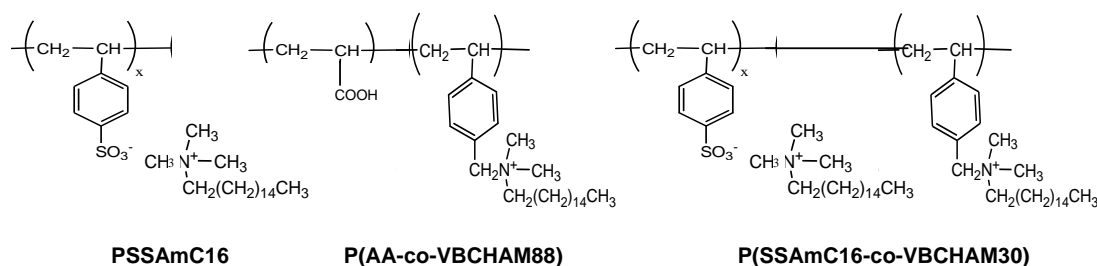


Figure 1: Chemical structures of the polymers used in the present study as potential polymeric biocides.

We have recently investigated the release of biocidal cetyl trimethylammonium cations (AmC16) from PSSAmC16 embedded in poly(methyl methacrylate matrices) in aqueous NaCl solutions (Bekiaris *et al.*, 2015). It was evidenced from this study that the AmC16 release is maintained to lower levels when the NaCl concentration is less than 0.5 M, as a consequence of the weaker ion exchange process under such low salinity conditions. For this reason, for the present study we have chosen to use lagoon water. In addition, in order to simulate more realistic conditions, fish nets samples were used, which were adequately covered with the potential biocidal polymer, eventually using a water-insoluble polymer matrix. To test possible biocidal action a 5L set-up was constructed filled with lagoon water. Moreover, the system was illuminated in order to accelerate biofouling.

2. Materials and methods

The synthesis of the polymeric biocides has been performed according to protocols described elsewhere (Koromilas, *et al.*, 2014, (a) and (b)). Briefly, PSSAmC16 was prepared through precipitation of the material upon mixing the anionic polyelectrolyte poly(sodium styrene sulfonate), PSSNa, with the oppositely charged surfactant cetyl trimethylammonium bromide, CTAB, in aqueous solution. For the preparation of the copolymer P(AA-co-VBCHAM88), the monomer VBCHAM was first synthesized through quaternization of N,N-dimethylhexadecylamine, HAM, with the monomer vinyl benzyl chloride, VBC. Then, the copolymer was obtained through free radical copolymerization of VBCHAM with acrylic acid, AA, in a common organic solvent. Finally, the copolymer P(SSAm16-co-VBCHAM30) was synthesized through free radical copolymerization of VBCHAM with the monomer cetyl trimethylammonium styrene sulfonate, SSAmC16. The monomer SSAMC16 was prepared, following a similar to the previously described protocol for the preparation of the respective homopolymer PSSAmC16.

To perform the study, fish nets were immersed in ethanolic polymer solutions (~5% (w/v)) and let dried in air for some hours, as well as at 60°C overnight. The polymer uptake of the nets was ~ 10% by weight. Then the nets were hung in the constructed set-up, filled with lagoon water

(salinity 3.2‰). The nature of the (micro)organisms evolved was also examined through optical microscopy.

3. Results and discussion

The experimental set-up for the present study is shown in Figure 2. The tanks are filled with 5 L lagoon water, being in continuous agitation, in order to permit facile oxygenation. Moreover, water is illuminated throughout the investigation to accelerate biofouling. One of the tanks is used as control, containing exactly the same water and being under the same conditions. For the control experiment, the net used was not covered with any polymer. At least two net samples were immersed in each tank.

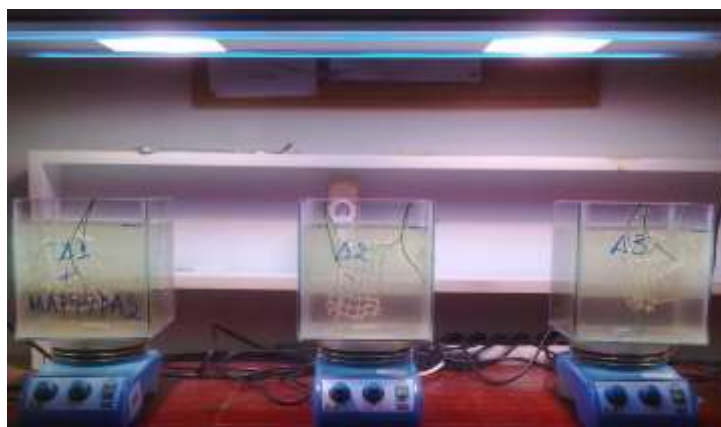


Figure 2: The experimental set-up for the accelerated biofouling studies.

The appearance of the tanks at the beginning of the study and after 20 days under accelerated biofouling conditions is shown in Figure 3. Both the control and the coated nets are white and clear at the beginning of the experiment. After 20 days, biofouling is clearly observable in the tank and on the surface of the control net. Similar is the case of the net covered with P(AA-co-VBCHAM88), indicating that the biocidal efficacy of this polymer is not sufficient to prevent biofouling under these conditions. In contrast, the tank and the net covered with PSSAmC16 remain completely clear after 20 days, demonstrating the high efficiency of this polymer to act as a biofouling inhibitor. Finally, the copolymer P(SSAmC16-co-VBCHAM30) seems also to prevent efficiently biofouling, since the appearance of the net covered with this polymer is quite similar to that covered with PSSAmC16 after 20 days of investigation.

After the end of the biofouling study, the nets were inspected through optical microscopy. Characteristic photos of the nets modified with the polymers P(AA-co-VBCHAM88) and P(SSAmC16-co-VBCHAM30) are shown, as typical examples, in Figure 4. It is clear that an intense biofouling occurs in the first case, while in the second case bioaccumulation is hardly observable.

Finally, the nets were dried and their masses were compared to their initial ones. The variation of the % weight gain is seen in Figure 5. As a consequence of bioaccumulation a large weight gain (~ 15%) is observed in the case of the control. A similar weight gain is also found in the case of the net covered with P(AA-co-VBCHAM88), in agreement with the previous observations indicating that this polymer can not effectively prevent biofouling. In contrast, the % weight gain is practically zero in the cases of the nets covered with the other two polymers, PSSAmC16 and P(SSAmC16-co-VBCHAM30), as a consequence of their high antifouling efficiency. The slightly negative values observed in the case of PSSAmC16, though within experimental error, could be attributed to the slight solubility of this polymer in water under these salinity conditions.

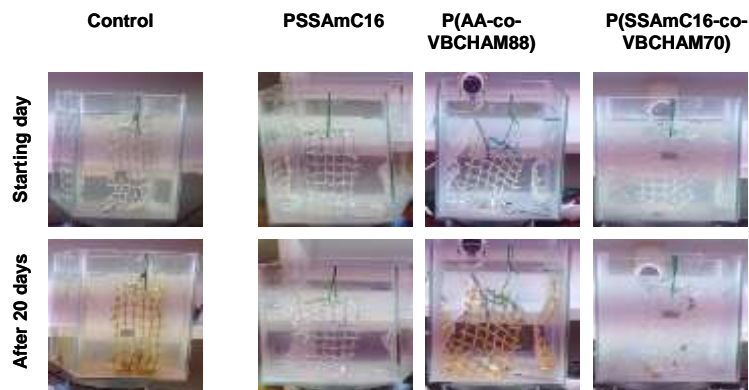


Figure 3: Appearance of the tanks at the beginning of the study and after 20 days under accelerated biofouling conditions.

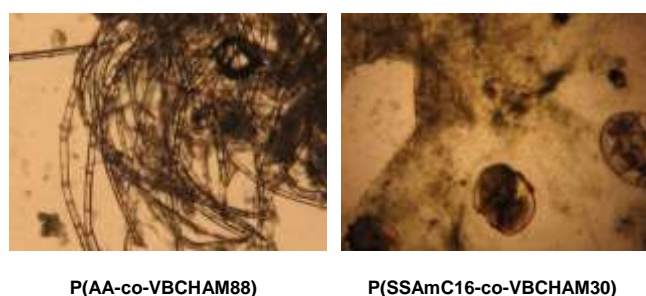


Figure 4: Representative optical microscopy photos of the nets after 20 days under accelerated biofouling conditions.

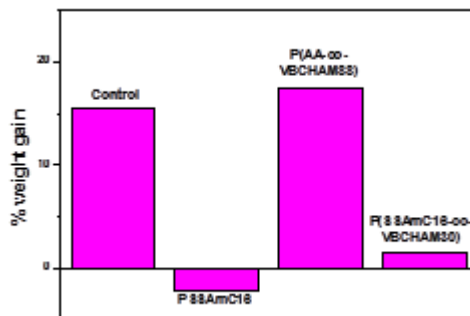


Figure 5: Variation of the % weight gain of the nets after 20 days under accelerated biofouling conditions.

4. Conclusions

The potential biocidal action, in terms of biofouling prevention, of polymers containing quaternary ammonium groups was investigated in the present work. The quaternary ammonium groups were electrostatically bound (PSSAmC16) or covalently attached (P(AA-co-VBCHAM88)) onto the polymeric backbone. In addition, the copolymer P(SSAmC16-co-VBCHAM30), combining both types of biocidal incorporation, was elucidated.

Fish nets, covered with these polymers, were investigated under accelerated biofouling conditions, using natural lagoon water. It is found that the homopolymer PSSAmC16 and the copolymer P(SSAmC16-co-VBCHAM30) effectively prevent biofouling. These results indicate that these polymers are promising for potential marine applications, for instance aquaculture nets, antifouling paints, etc.

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