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Development of alginate hydrogels active against adhesion of microalgae

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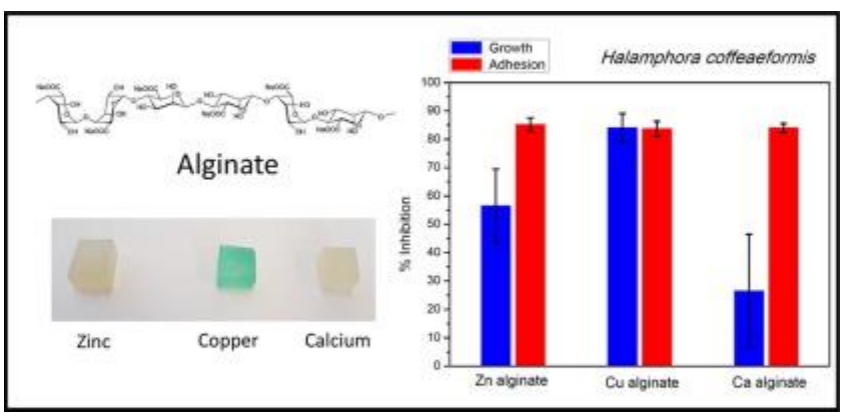
Abstract :

Microorganisms have the ability to settle on nearly all man-made surfaces in contact with seawater and subsequently to form biofilm. Biofilms control and removal is necessary in the sectors of maritime transport, energy... In this work, we present the development of new pure calcium, zinc or copper alginate, but also mixed Ca/Cu and Ca/Zn alginate hydrogels. These materials have been evaluated for their potential inhibition of adhesion of two key biofilm-forming microalgae Halamphora coffeaeformis and Cylindrotheca closterium). All the tested materials have presented high adhesion inhibition about 80%). Copper-base materials present a high toxicity against H. coffeaeformis. Pure zinc alginate is also toxic for this strain. However, the addition of calcium in zinc alginate leads to the toxicity reduction. The toxicity of these materials differs according to the strains. Consequently, mixed zinc/calcium alginate are efficient at inhibiting microalgal adhesion with a low level of cells toxicity. These alginate hydrogels are promising materials because they are efficient, cheap, easy to develop and eco-friendly.

Graphical abstract



Highlights

► Elaboration of alginate hydrogels with different copper/zinc and calcium concentrations. ► High inhibition of marine microalgae adhesion by calcium, copper or zinc alginates. ► Low toxicity of mixed calcium/zinc alginate against microalgae.

Keywords : Biofilm, Adhesion, Alginate, Zinc, Biomaterials, Microalgae

1. Introduction

Marine biofouling starts with the adhesion of organic molecules followed by the attachment and subsequent development of bacteria, diatoms and other microalgae, which form a biofilm [1]. Adhesion and growth of macro-organisms such as macro-algae and invertebrates is then observed. This biofouling community when settled on non-treated surfaces induces a degradation process, which impacts numerous marine and industrial applications. To prevent biofouling formation, the tributyltin (TBT) biocide was extensively used in the past but is now prohibited due to its high toxicity to non-targeted marine organisms [2]. TBT has been replaced gradually by copper, mainly in its oxide or metallic form leading to pollution of seawater, mainly in marinas. Laws reducing the amount of copper in antifouling products are therefore scheduled in many countries. Thus in the United States, Washington became the first state to ban copper-based paints in the beginning of 2020 when owners of recreational boats will be prohibited to buy and apply bottom paints that contain more than 0.5% copper [3]. Within this context, there is an urgent need to develop new eco-friendly materials with antifouling properties.

Materials containing inorganic nanoparticles (NPs) have attracted research interest in recent years. In particular, numerous works focus on ZnO NPs [4-6] due to their low toxicity with respect to other biocide agents such as silver [7]. The release of both ZnO NPs from the matrix and Zn^{2+} ions due to NPs dissolution is the key point for the optimization of antimicrobial properties. However, due to the different nature of these two processes, monitoring this release is highly difficult to realize. Producing material containing only Zn^{2+} ions without any NPs is therefore a promising and environmental friendly solution since it allows a better optimization of the active species release. Elaboration of such material is feasible by inserting Zn^{2+} ions in anionic type materials by sorption. The antibacterial activity of Zn^{2+} liberated from clays and alginates was recently demonstrated in the biomedical domain against bacteria and fungi [8,9]. The aim of the present study concerns the investigation of the antimicrobial property of calcium, zinc and copper alginates on the growth of *Halamphora coffeaeformis* and *Cylindrotheca closterium*, which are pioneer microalga strains involved in surface colonization, biofilm formation and biocorrosion [10]. Limiting the adhesion of microalgae on surfaces consists of a promising alternative approach to achieve reduction of biofilm formation. Moreover, searching for adhesion represents a more environmentally friendly approach because such products have a mode of action different from conventional biocides and present low level of cells toxicity. Thus, the present study reports the anti-adhesive property of the alginates and demonstrates the dual mode of action of the material: inhibition of growth and adhesion of microalgae.

2. Materials and methods

A solution of sodium alginate (Sigma Aldrich 71238) was prepared with a concentration of 50 g/L by magnetic stirring during 5 hours. Solutions of $CaCl_2$, $CuSO_4$ and $Zn(C_4H_6O_4)$ with concentrations from 5.10^{-4} to 0.36 $mol.L^{-1}$ were added to the sodium alginate solution in order to jellify the alginate. The solution was left for 24 hours in order to saturate the alginate with the different ions. The elaborated hydrogels were rinsed with deionized water to remove the excess of ions on the surface. Mixed alginate hydrogels have been elaborated with mixed ion concentrations. The nomenclature for these alginates is Ca_xZn_y / Ca_xCu_y, x and y being the concentrations of the $CaCl_2$ and $Zn(C_4H_6O_4)$ / $CuSO_4$ solutions in $mol.L^{-1}$. The analysis of the morphology of the hydrogels and their chemical composition were performed using field emission SEM (Scanning Electron Microscopy) coupled with EDX (Energy Dispersive X-ray analysis).

For the assessment of bioactivity against microfouling organisms, both the inhibition of cell adhesion and growth were evaluated [11]. The strains used for the experimental work are two model species commonly used in biofouling studies: *Halamphora coffeaeformis* (AC713) and *Cylindrotheca closterium* (AC170) [12]. The procedure is detailed in the electronic supplementary material.

3. Results and discussion

Zinc, copper and calcium alginate hydrogels were produced by immersing sodium alginate in solutions containing Zn^{2+}, Cu^{2+} and Ca^{2+} ions. The composition of these materials was studied by EDX measurements and presented in Table 1. The materials are mainly composed of carbon and oxygen atoms due to the hydroxyl and carboxyl groups of the alginate matrix. The presence of cations in the materials is clearly noted, it can be explained by the ion exchange process between Na^{+} present in alginate and the cations in the solution [13]. Moreover, XPS measurements have proved the presence of Cu^{2+}, Ca^{2+} and Zn^{2+} species. Note that 5 zones in the different parts of the gels were analyzed by EDX and the achieved results display the material composition homogeneity.

	C	O	Cu	Zn	Ca	Na
$Zn_{0.05}$	64.0	28.6		2.5		4.9
$Cu_{0.05}$	58.2	37.4	1.4			3.0
$Ca_{0.36}$	54.5	40.5			4.7	0.3

Table 1. EDX data showing the atomic percentage of each element for the different hydrogels

The influence of alginates on the adhesion and growth of microalgae was evaluated firstly towards *Halamphora coffeaeformis* (see Fig. 1 for results). Zinc alginate leads to a significant reduction of both adhesion (84%) and growth (55%). Similarly, copper alginate induces a similar level adhesion inhibition (84%) but with a significant stronger growth reduction (85%). At the concentration assessed here, both zinc and copper-alginate exhibit a dual mode of action with cells adhesion and growth inhibition. These types of activities lead to efficient antifouling solutions, and undoubtedly increase the length of duration of the surface protection, as the few cells on the surface will then face a very slow growth rate from the biocidal action. However the market requirement nowadays tend to minimize as much as possible the use of biocides in the environment, thus an idea compound will have high activity on inhibition of cells adhesion but without too high biocidal action. In that view, the results obtained for calcium alginate ($Ca_{0.36}$) are of high interest because we recorded significant anti-adhesive property (>85%) with lower effect on growth inhibition (25%).

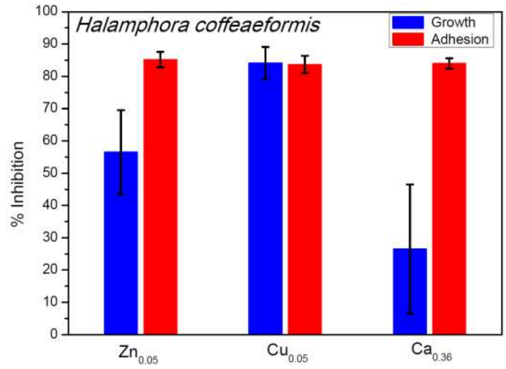


Fig. 1. Growth and adhesion inhibition to *H. coffeaeformis* by zinc, copper and calcium alginate gels.

Following the Biocidal Products Regulation (Regulation (EU) 528/2012), antifouling candidate should work within the called "therapeutic window" with EC_{50} values (efficient concentration leading to 50% inhibition) being inferior to LC_{50} values (concentration leading to 50% mortality). From our results, it appears that the antimicrobial property of alginates can then be adjusted to reach this limit by considering alginates containing different amounts of the three considered ions. Thus, Fig. 2 presents the growth inhibition and anti-adhesive property of various mixed CaZn (or CaCu) alginate hydrogels assayed towards *Halamphora coffeaeformis* and *Cylindrotheca closterium*. The Zn (or Cu) concentration was between 5.10^{-2} to 5.10^{-4} mol/L. For *H.coffeaeformis* (Fig. 2a,b), the influence of the alginates on the cells adhesion is very similar to that previously found with $Zn_{0.05}$, $Cu_{0.05}$ and $Ca_{0.36}$. A reduction of the growth inhibition is observed for $Zn_{0.05}Ca_{0.27}$ and $Cu_{0.05}Ca_{0.27}$, with respect to $Zn_{0.05}$ and $Cu_{0.05}$. The inhibition growth for the $Zn_{0.05}Ca_{0.27}$ alginate becomes lower than the 50% limit. EDX measurements have shown that the main difference between pure and mixed alginates concerns the presence of calcium ions which replace sodium ones. The reduction of the cells mortality (related to inhibition of growth) is therefore not associated with a variation of the number of Zn^{2+}/Cu^{2+} ions in the matrix. This reduction should therefore be ascribed to a different release of Zn/Cu ions between sodium and calcium alginates. This can be attributed to the solubility of the two matrices. As shown in Fig. 2(a,b), the subsequent decrease of Cu/Zn concentration does not change drastically the results.

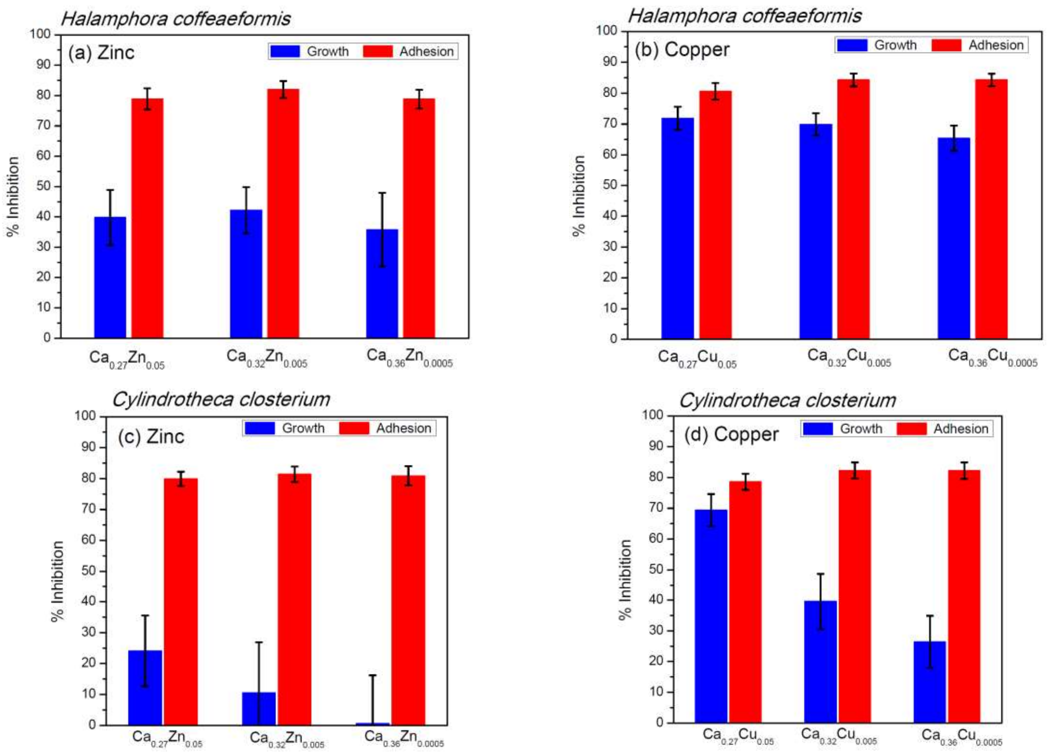


Fig. 2. Growth and adhesion inhibition of *H.coffeaeformis* (a,b) and *Cylindrotheca* (c,d) by zinc (a,c) and copper (b,d) alginate hydrogels with different concentrations.

Fig. 2(c,d) presents the assays performed on *C. closterium*. The anti-adhesive property is similar to that already observed earlier. However, in contrast to the previous strain, a clear relationship is observed between the concentration of Zn^{2+} (Cu^{2+}) ion in mixed alginates and the inhibition of growth. This behavior is explained by different sensitivities of microalgae to the cations and enables the development of antimicrobial materials specific to some strains.

4. Conclusion

Calcium, zinc and copper alginate hydrogels have been elaborated with different ion concentrations. The obtained materials have been tested on two microalgae strains: *H.coffeaformis* and *C.closterium*. All the tested materials have presented high adhesion inhibition. Mixed zinc/calcium alginate protects the surfaces by blocking the microalgae adhesion together with a low toxicity: this property is essential for an antifouling material. Consequently, these alginate hydrogels are promising materials because they are cheap, easy to develop, eco-friendly and in the therapeutic window.

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Figure
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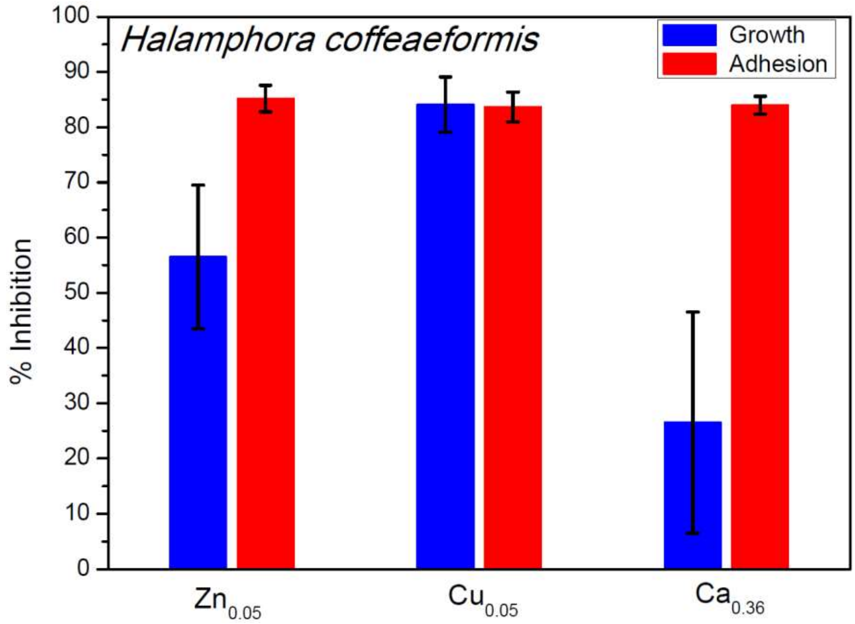
Figures and captions



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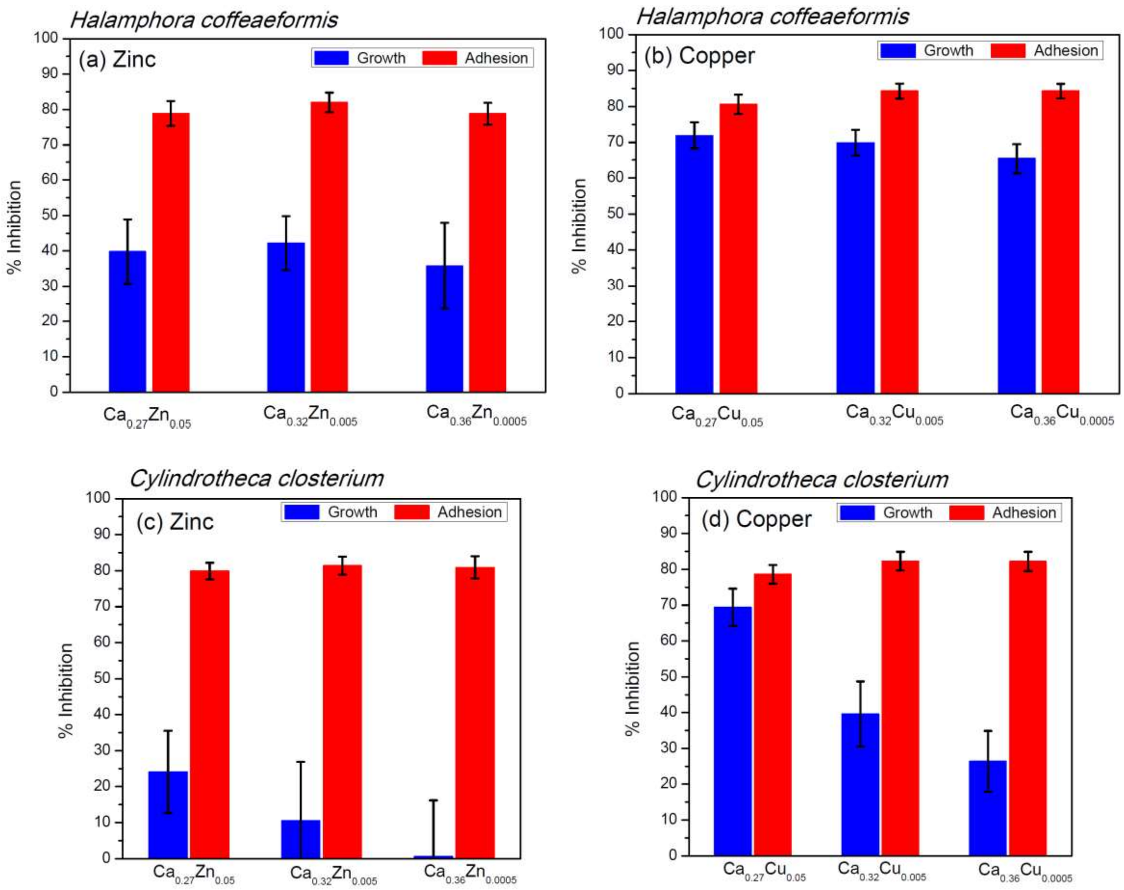

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