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Electrophoretic deposition of zinc alginate coatings on stainless steel for marine antifouling applications

L. Abi Nassif ^{1,2}, S. Rioual ¹, W. Farah ², M. Fauchon ³, Y. Toueix ³, C. Hellio ³,
M. Abboud ², B. Lescop ¹

1. Univ Brest, Lab-STICC, UMR 6285, CNRS, F-29200 Brest, France

2. UEGP, Faculté des sciences, Université Saint Joseph, Beyrouth, Liban

3. Univ Brest, BIODIMAR/LEMAR, CNRS UMR 6539, F-29200 Brest, France

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Abstract

The protection of steel against marine biofouling is usually achieved by the application of protective coatings. In this work, an antifouling coating based on alginate biopolymer was developed using the electrophoretic deposition method. Zinc cations have been incorporated into the material to obtain some anti-algae / bacteria properties and calcium cations have been included to contribute to its jellification. The coatings produces were characterized by XRD, SEM, EDX and XPS techniques: the microscopic coatings fully and uniformly covered the steel samples. Results of the biological assays have demonstrated the impact of the coating on marine bacteria and microalgae; the values are comparable to those obtained in bioassays using copper-based alginate coatings. The antifouling effect of the coatings was equivalent to the potency of a high-volume hydrogel effect. These low-cost biocompatible coatings can be attractive in a wide variety of marine applications.

1. Introduction

Marine biofouling can be defined as the undesirable settlement and growth of organisms on biotic and abiotic immersed surfaces in seawater such as ships, oil and gas platforms, pilings, mariculture facilities, buoys and pontoon [1]. Due to the numerous impacted applications, it leads to a significant economic cost [2]. This process is caused by the attachment of organic molecules and organisms such as bacteria, microalgae, macroalgae, or invertebrates (barnacles, mussels, ascidians...) on a wide range of surfaces and can be therefore strongly limited by the use of anti-biofouling coatings. Tributyltin (TBT) based coatings were proven highly toxic **towards** non-target organisms and are now banned since several years [1]. Coatings containing antimicrobial agents such as copper, silver, zinc oxide under different forms (ions, nanoparticles...) have been proposed as substitute to TBT-based paints. However, the toxicity of copper and silver ions is now well-established [3,4] while the one of nanoparticles is still debated [5]. Within this context, the development of biocompatible coatings is a priority. Recently, our group has developed bio-based alginate hydrogels [6,7]. These previous studies have shown that a mixed Zinc / Calcium alginate protects the surfaces by blocking the adhesion of microalgae together with a low toxicity, **a property that is essential for** environmental friendly applications. Steel metal surfaces were protected against biofouling by placing active hydrogel cubes of few cm³ in their surrounding environment [7]. The present work aims at investigating the antifouling property of coatings produced by active alginates.

Coating of surfaces can be performed by numerous techniques such as sol-gel, plasma spray, or electrophoretic deposition (EPD). EPD is a versatile **and inexpensive** technique **with** advantages such as **the need for a** simple apparatus, short processing time and minor restrictions on substrate shape [8,9]. This method consists of applying a potential difference

between an electrode and the conductive substrate. Charged particles or polymer chains in aqueous solution then move towards the substrate under the influence of the electrical field, resulting in coatings between 0.1 to 100 μm thick [10]. Being an anionic polysaccharide, alginate was successfully deposited by EPD [11].

Within the present study, we propose to evaluate the antifouling property of Zinc/Calcium alginate coatings produced by EPD on 316L stainless steel (316L SS). Based on our knowledge, this is the first attempt to report the effect of zinc alginate electrophoretic coating against marine microorganisms (bacteria and microalgae). The coatings were analyzed by Grazing incidence X-ray diffraction, electron microscopy SEM and by X-ray Photoelectron Spectroscopy. Their antifouling properties were tested on microorganisms, which can be considered as pioneer in marine biofilm formation: four bacteria (*Halomonas aquamarina*, *Vibrio aesturianus*, *Vibrio harveyi* and *Pseudoalteromonas elyakovii*) and two microalgae strains (*Halomphora coffeaformis* and *Cylindrothecca closterium*). Copper / Calcium alginate coatings were also produced to highlight the effects of several ions on the growing of marine microorganisms.

2. Materials and methods

Material preparation

316L is a well-known austenitic stainless steel containing a high proportion of Cr (about 16 % atomic), Ni (10 %) and Mo (2 %) and a very low proportion of carbon (< 0.04 %). It has excellent corrosion resistance and good mechanical properties. The 316L samples were designed with a circular shape of 13 mm diameter and a thickness of 1 mm. They were treated with silicon carbide paper and cleaned using acetone. Moreover, to improve the adherence of the alginate coating, the roughness of samples was increased by immersion in a

H₃PO₄ solution (C = 1 mol/L) during 10 min. A solution of sodium alginate (Sigma Aldrich 71238, M/G ratio: 0.85) at a concentration of 10 g/L was prepared under by magnetic stirring during 5 hours. The stainless steel and a counter electrode (graphite) were immersed in the sodium alginate solution. The distance between the two electrodes was set at 20 mm and an electrical potential of 5 V was applied. Then, the incorporation of cations such as Ca²⁺, Cu²⁺, Zn²⁺ into the coating was performed via an ion exchange mechanism: the substitution of hydrogen ions in the alginate matrix by cations [12]. This was achieved by immersing the materials in different solutions: CaCl₂ (0.36 mol.L⁻¹), CuSO₄ (0.1 mol.L⁻¹), ZnC₄H₆O₄ (0.1 mol.L⁻¹) for pure coatings and 0.27 mol.L⁻¹ (calcium) and 0.05 mol.L⁻¹ (copper / zinc) for mixed-alginate coatings respectively.

Physicochemical characterization of the coating

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). The chemical composition of the surface was characterized by XPS using an apparatus consisting of an Al Ka X-ray source (Thermo VG) and a cylindrical mirror analyzer (RIBER). The adventitious C(1s) peak was used for the binding energy calibration at 284.8 eV. The crystalline structure of the coated samples was analyzed by grazing incidence angle X-ray diffraction (GIXRD) with an Empyrean PANalytical apparatus using the CuK α radiation (1.5408 Å) with an incidence angle of 0.5°. The main interest of the GIXRD measurements is its high sensitivity to the surface of the material and therefore to the coating.

Biological assays

For the assessment of bioactivity against microfouling organisms, both the inhibition of cell adhesion and growth were evaluated [13]. All the strains were purchased from culture collection: American Type Culture Collection (ATCC) and Algobank (AC) respectively for bacteria and microalgae. The strains used for the experimental work are four bacteria (*Halomonas aquamarina* ATCC 14400, *Vibrio aestuarianus* ATCC 35048, *Vibrio harveyi* ATCC 14126 and *Pseudoalteromonas elyakovii* ATCC 700519) and two microalgae (*Halimnobia coffeaeformis* AC713 and *Cylindrotheca closterium* AC170) model species commonly used in biofouling studies [14].

The algal strains were maintained and grown at 20°C in F/2 medium, under a light exposure regime of 12 hours light (irradiance: 140 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$) / 12 hours darkness. Prior to experiments, microalgal stock solutions at the concentration of 0.1 mg/L chlorophyll *a* were prepared [13]. For the assay of hydrogel coating, experiments were run in 6 replicates. 1 mL of the microalgal stock solution (0.1 mg/L chlorophyll *a*) was transferred to the wells of transparent 24 Iwaki well containing the material to test. The plates were then incubated for 5 days at 20°C under constant light exposure of 12 hours light/darkness (irradiance: 140 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$). Microalgal growth inhibition was measured according to standard protocols [13,14]. Microalgal growth was estimated by chlorophyll *a* quantification using a fluorometric method (excitation at 485 nm and an emission at 645 nm). Results are expressed as percentage of inhibition compared to a control (uncoated steel).

Prior to experiments, bacteria were pre-grown in sterile Marine Bacterial Medium (MBM) (0.5% peptone (Neutralised Bacteriological Peptone, Oxoid Ltd, Basingstoke, UK) in sterile filtered (Whatman 1001-270, pore size 11 μm) natural seawater, at 20 °C [15]. For the assay of hydrogel coating, experiments were run in 6 replicates. 1 mL of the bacterial stock solution (2×10^8 colony forming units/mL) was transferred to the wells of transparent 24 Iwaki well containing the material to test [16]. The plates were then incubated for 72 hours at 20°C.

Bacterial growth inhibition was measured according to standard protocols [15]. Bacterial growth was monitored spectroscopically (Tecan Infinite M200) at 630 nm [17]. Results are expressed as percentage of inhibition compared to a control (uncoated steel).

3. Results and discussion

3.1. Physical characterization of the coatings

Following the procedure presented in the *materials and methods* section, 316L SS samples were coated with alginic acid using the EPD. This process is effective **due to the dissociation of** sodium alginate in anionic Alg^- species: $\text{NaAlg} \rightarrow \text{Na}^+ + \text{Alg}^-$. At the anode (stainless steel electrode), water molecules are decomposed ($2 \text{H}_2\text{O} \rightarrow \text{O}_2 + 4 \text{H}^+ + 4 \text{e}^-$), **which lowers** the pH. The positive potential at the stainless steel electrode attracts the Alg^- species, which react with hydrogen to form the alginic acid gel $\text{Alg}(\text{H})$ on the stainless steel surface. Figure 1 displays the morphology of the surface. As shown in Fig. 1(a), despite of the use of a mixture of ethanol / water (2:3) (v/v), bubbles formation associated with H_2 production during the EPD process is evident. In this case, a constant potential of 5 V was applied during 10 s between the two electrodes. In order to improve the quality of the coating, the deposition process was carried out by two 5 V pulses for 5 s. Fig. 1(b) shows the strong reduction in bubbles quantity by the pulses.

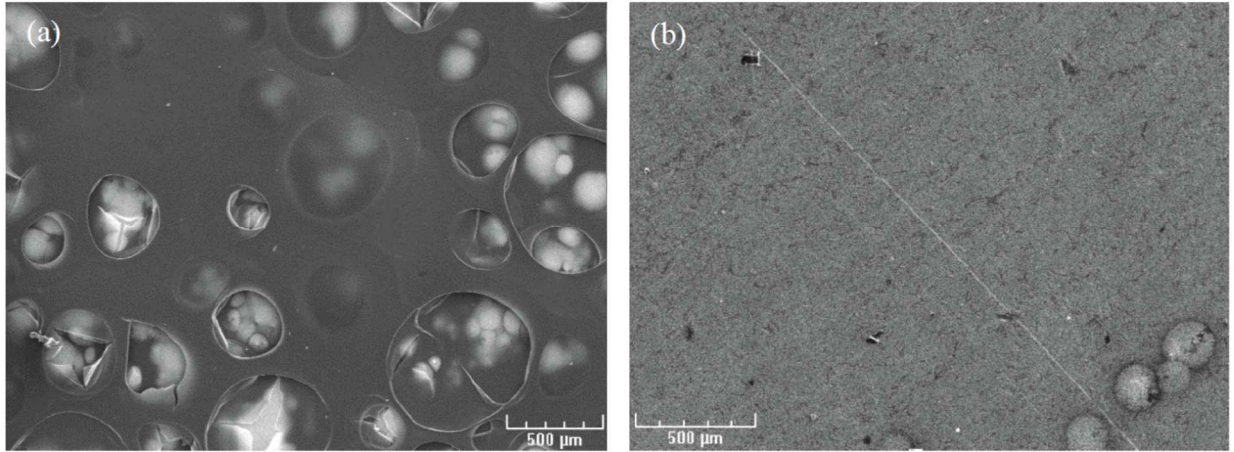


Figure 1. SEM images of the alginate coating layer without (a) and with (b) pulses with a voltage of 5V.

Figure 2(a) presents the GIXRD pattern of the coated sample. The three peaks corresponding to iron in fcc austenite phase without any evidence of martensite phase are observed in agreement with Öztürk *et al* [18]. Additional peaks at 13.3° and 21.0°, attributed to the alginate [19], prove the presence of the alginate coating on stainless steel. The thickness of the coating was estimated by peeling a coating and by obtaining a SEM image. Figure 2(b) shows this SEM image: the coating is a dense layer 2.9 µm thick. As a result, the surface is completely and uniformly covered.

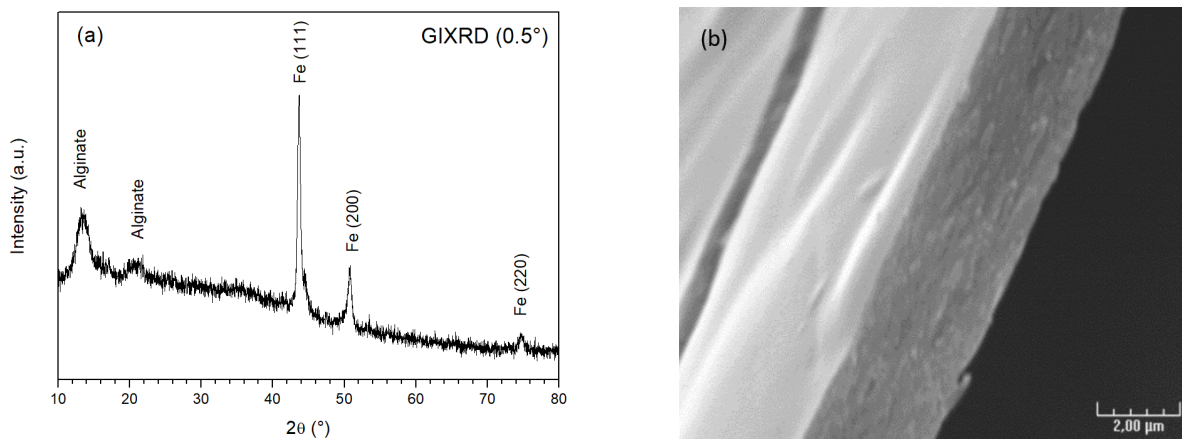


Figure 2. Grazing incidence angle (0.5°) X-ray diffractogram of 316L SS covered by alginate (a) and SEM micrograph of peeling alginate coating layer (b).

Table 1 presents the chemical composition of 316L SS covered and not covered by Alg(H) obtained from EDX experiments. The uncovered surface is composed of mainly carbon, iron, chromium and nickel [20] in accordance with the composition of steel. For surfaces coated with alginic acid (Alg(H)), carbon and oxygen atoms concentrations increase due to the presence of hydroxyl and carboxyl groups in the alginate coating matrix. The presence of sodium can be noted due to the use of sodium alginate in the initial solution. Immersion of the Alg(H) coating in solutions containing copper, zinc or calcium ions then leads to a change in its composition. Indeed, as shown in Table 1, the presence of these ions in the coating is clearly demonstrated. This result can be explained by the ion exchange process that occurs between H^+ and Na^+ of Alg(H) and the cations present in the solution. As this Table shows, the presence of chloride in Alg(Ca) is also observed due to the use of $CaCl_2$ in the solution [7].

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

| | C | O | Fe | Cr | Ni | Mo | Si | Al | Na | Ca | Cu | Zn | Cl |
|-----------------|------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 316L SS | 39.2 | 4.6 | 37.6 | 9.8 | 4.6 | 1.9 | 1.0 | 1.3 | - | - | - | - | - |
| Alg (H) | 73.3 | 14.1 | 7.9 | 2.1 | 0.9 | 0.4 | 0.2 | - | 1.1 | - | - | - | - |
| Alg (Ca) | 67.4 | 24.3 | 3.7 | 1.2 | 0.3 | 0.4 | 0.2 | - | - | 1.9 | - | - | 0.6 |
| Alg (Cu) | 66.0 | 28.4 | 2.0 | 0.7 | 0.2 | 0.4 | 0.1 | - | - | - | 2.2 | - | - |
| Alg (Zn) | 71.3 | 19.5 | 4.5 | 1.3 | 0.4 | 0.5 | 0.2 | - | - | - | - | 2.3 | - |

The ionic states of the various elements on the surface were determined by XPS. Figure 3 shows the survey spectra of uncovered and alginate-covered 316L SS up to a binding energy of 1150 eV. The uncovered steel spectrum is dominated by carbon, oxygen, iron and

chromium species in accordance with the previous XPS data [20] and with our EDX data. The Fe(2p) and Cr(2p) spectra (not shown here) are dominated by structures at 710.0 (Fe 2p_{3/2}), 724.1 (Fe 2p_{1/2}), 575.6 (Cr 2p_{3/2}) and 585.6 eV (Cr 2p_{1/2}). These positions prove the existence of oxidized species at the surface [21] to form a passive layer. It should be noted that small contributions of the metallic species Fe⁰, Cr⁰ are also reported indicating the **thinness** of the oxide layer. As reported in Figure 3, the XPS spectra measured on alginate coatings differ greatly from those obtained on steel. The total disappearance of the iron and chromium peaks confirms the complete covering of the surface. **The baseline observed in the uncovered surface is classical for iron material [22], but the baseline of the covered surface spectra is different. This observation is consistent with the total masking of the 316L SS surface.** Structures associated to the Na(1s), Cu(2p) or Zn(2p) atoms at high binding energy demonstrate the presence of these species in the coatings according to our EDX data.

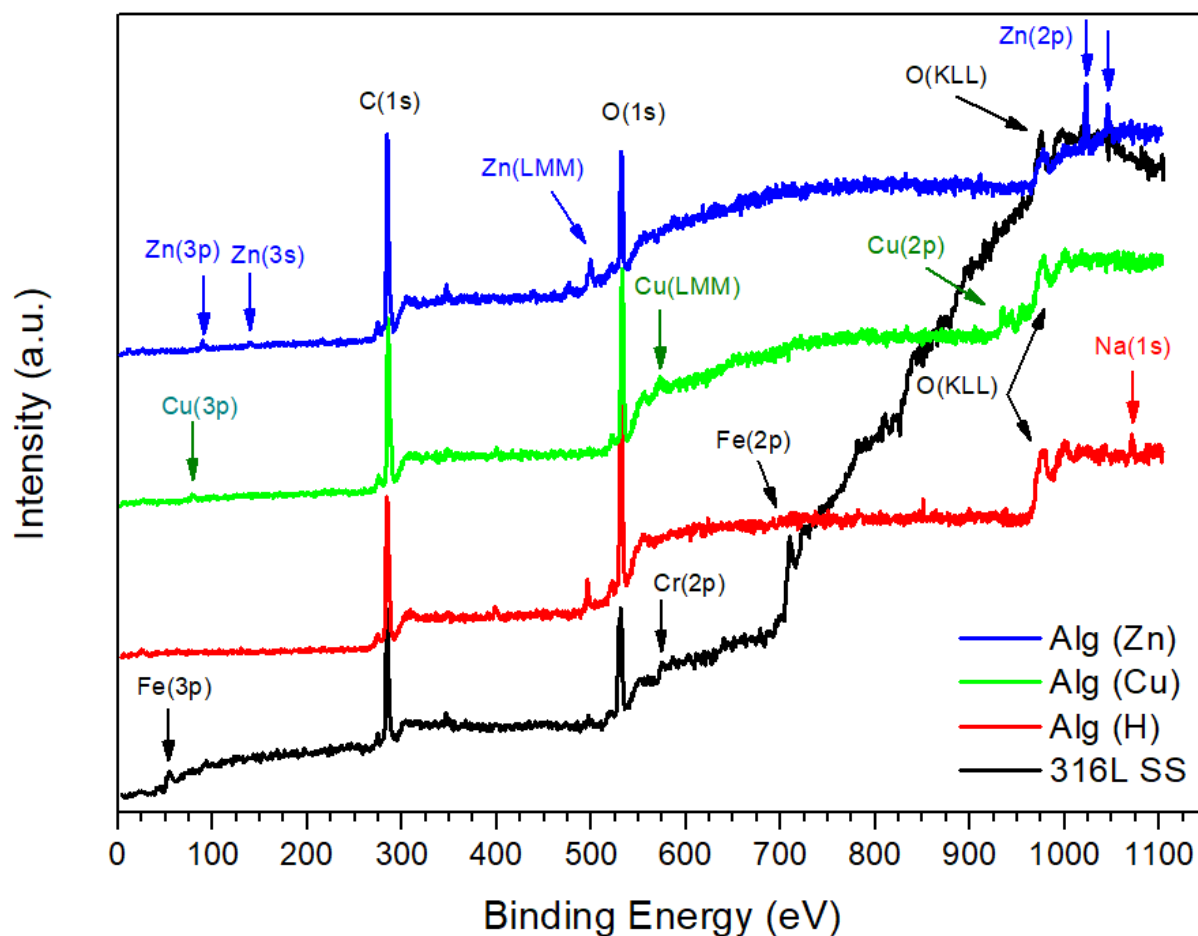


Figure 3. Full XPS spectra of uncovered and alginate-covered 316L SS.

Figure 4(a) shows the O(1s) spectra of the **various** alginate coatings and steel. For steel, it presents two broad structures at 529 and 532 eV. The low energy part is attributed to iron and chromium oxides [23], while the high energy part is related to hydroxide and carbonates species present at the surface [23]. **For** alginate-covered steels, **no oxide-associated peaks are observed and only one** peak at about 532 eV is observed. This peak can be attributed to the chemical groups of alginate such as the C – OH groups of alcohol, C – O – C groups of acetal and hemiacetal and carboxylate groups. The peak shift with the cation with the alginate is explained by the interaction of the cations with the functional groups of the alginate [12,24].

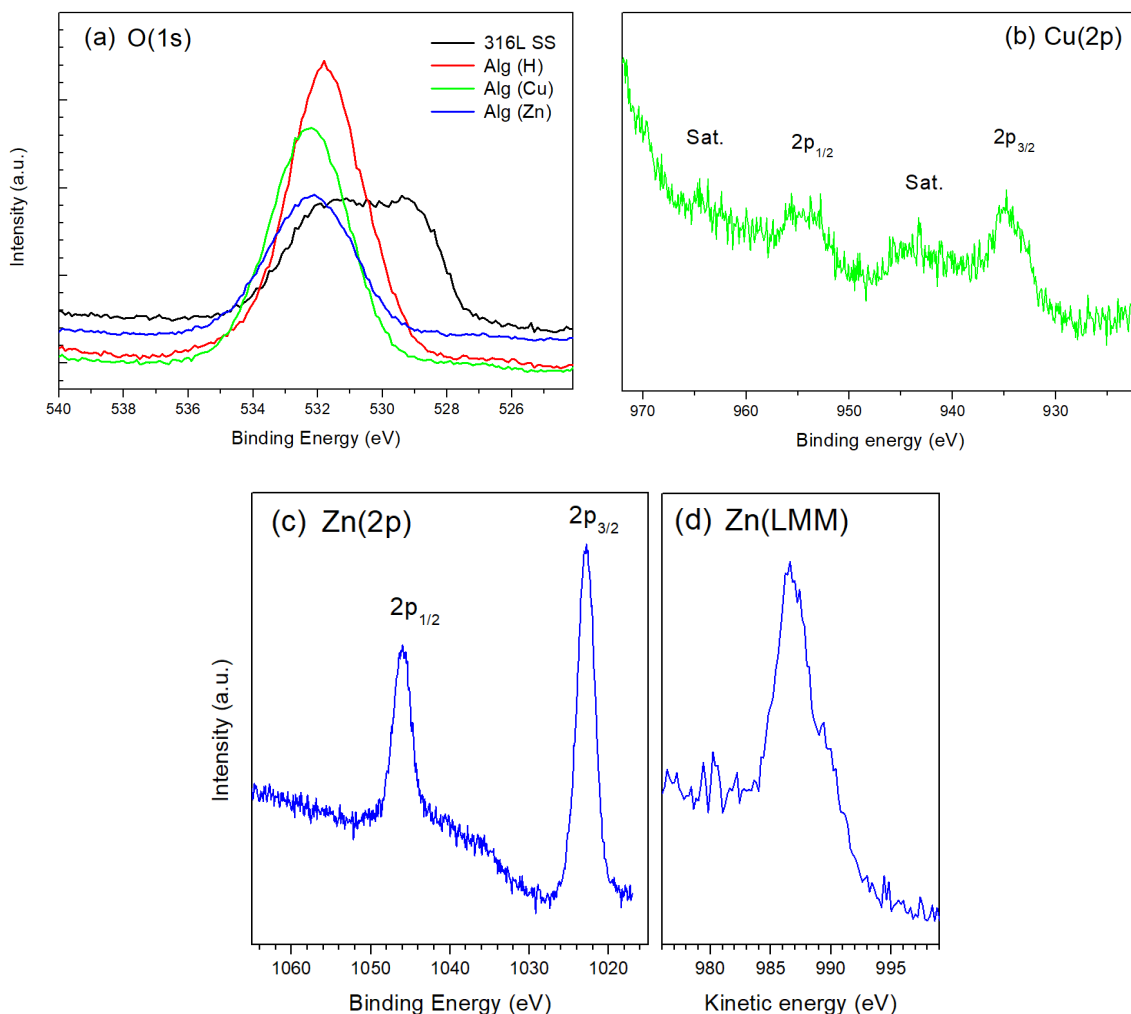


Figure 4. O(1s), Cu(2p), Zn(2p) XPS and Zn(LMM) Auger spectra of uncovered and alginate-covered 316L SS.

Fig. 4(b,c) presents Cu and Zn spectra of the alginate coatings. In Fig. 4(b), the Cu(2p) spectrum shows peaks corresponding to the 2p_{3/2} (934.5 eV), 2p_{1/2} (953.6 eV) and associated satellites (943.6 / 962.7 eV). The existence of the well-known shake-up satellites proves that copper ions are in the Cu²⁺ form [25], as usually observed in biomaterials [26]. These positions can be related to the interaction between Cu²⁺ ions and hydroxyls [25]. The high FWHM value (full width at half maximum) of the 2p peaks can be explained by the complexity of the chemical environment such as oxygen or carboxylate species [27]. The Zn(2p_{3/2}) spectrum of the zinc alginate coating, shown in Fig. 4(c), has a symmetrical peak at

1022.6 eV. As the position of this peak was not sufficient to determine the chemical environment, the Zn(LMM) Auger spectrum was measured. It is shown in Fig. 4(d). The position of the Zn Auger peak is at 986.8 eV. Note that a shoulder at 990.1 eV can be attributed to the Na(KLL) Auger peak. The estimated α parameter value of 2009.4 eV is consistent with the Zn^{2+} bonding with hydroxyl and/or carboxylate species [28]. Thus, the XPS data prove the existence of Cu^{2+} and Zn^{2+} ions at the coating surface.

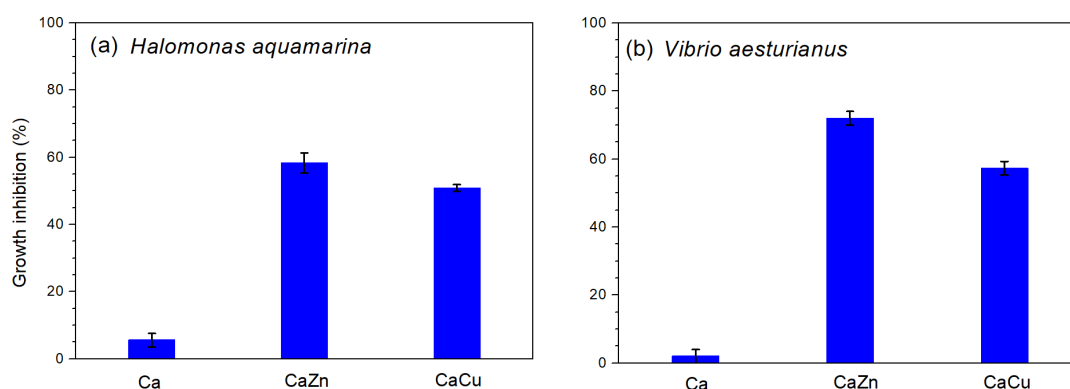
3.2. Biological assays of the materials

When microorganisms attach to surfaces, they form a biofilm surrounded by a complex polymer matrix. This fixed and communal form of life offers many advantages, including protection against toxic agents. Thus most microbial consortia in the natural environment are found in the form of biofilms. Communication between cells plays an important role in synchronizing processes within the biofilm and is done through the quorum sensing (QS) system. QS can modulate the regulation of gene expression ([29]Lami 2019). It is important to note as well that it was proven that marine bacteria and microalgae can interact via quorum sensing molecules ([30] Joint et al., 2002). In order to develop new antifouling solutions, specific experiments can be carried out to determine the mode of action of molecules on the quorum sensing. However, this is not possible when testing new surfaces. For this reason, we have chosen to evaluate the effect of surfaces on cell adhesion and growth. These biological phases occur before the formation of biofilms, and it is therefore relevant to develop innovative surfaces able to control or inhibit them.

The biofilm formation is initially governed by the adhesion of microorganisms on surfaces, with a dominance of bacteria and microalgae. Consequently, the influence of alginate coatings on the growth of pioneer marine microalgae and bacteria was evaluated. To decrease the toxicity of the materials against the organisms, copper/zinc quantity was reduced

with respect to alginates studied in the previous part. Calcium ions were also integrated in the alginates to enhance the insolubility property of the coating and to eventually enhance anti-fouling property [6]. The calcium and copper/zinc concentrations were set at 0.27 and 0.05 mol.L⁻¹ respectively. These coatings are named as CaZn and CaCu. The influence of these coatings compared to a pure calcium alginate (Ca) is investigated to highlight the role of different ions in reducing the growth of bacteria/microalgae.

Figure 5 presents the growth inhibition of four marine bacteria (Fig. 5(a-d)) and two microalgae (Fig. 5(e,f)) when considering CaZn, CaCu and Ca coatings. Uncoated 316L SS samples were used as control. As observed in Fig. 5(a-d), the calcium alginate coatings leads only to a weak effect against bacteria with less than 15 % of growth inhibition. Fig. 5(a,b,d) show that the inclusion of zinc and copper in alginate leads to a bacteria growth inhibition between 50 and 70 %. No major difference are observed between CaZn and CaCu alginates. Copper and zinc coatings are therefore effective against marine bacteria, as demonstrated previously on microalgae [6]. In contrast to these results, for *Vibrio harveyi* in Fig. 5(c), the growth inhibition with CaZn alginate is negligible and close to the control and calcium coating data. In this case, the CaCu coating is definitely more efficient. This effect can be explained by the fact that many *Vibrio harveyi* harboured antimicrobial resistant genes and thus can be found to be resistant to specific antifouling coatings [31].



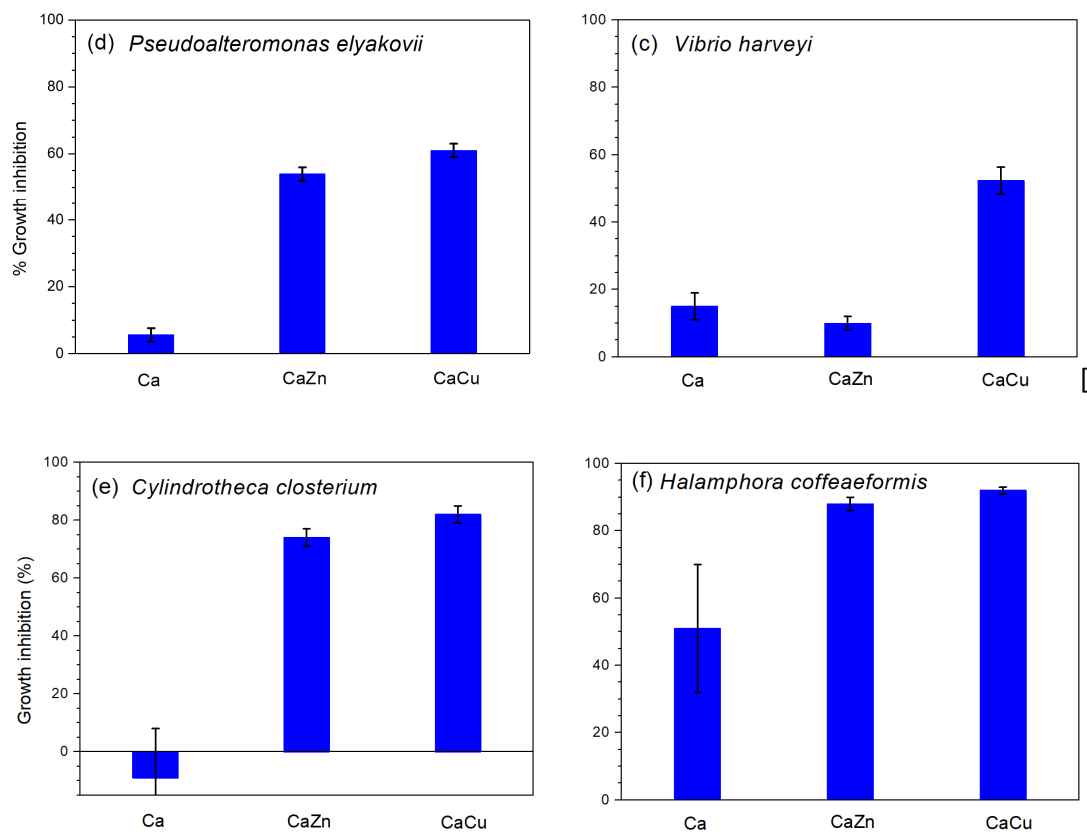


Figure 5. Evaluation of growth inhibition of four bacteria: *Halomonas aquamarina* (a), *Vibrio aesturianus* (b), *Vibrio harveyi* (c), *Pseudoalteromonas elyakovii* (d) and two microalgae strains: *Cylindrotheca closterium* (e) and *Halomphora coffeaeformis* (f).

After successively assessing the influence of coatings against bacteria development, two microalgae strains (*Cylindrotheca closterium* and *Halomphora coffeaeformis*) were studied. Firstly, Fig. 5(e) presents the assays performed on *Cylindrotheca closterium*. The influence of calcium alginate on the inhibition is negligible or maybe slightly negative: the coating could enhance alga growth [32,33]. The behaviors of CaCu and CaZn coatings are similar to those observed with bacteria (*Halomonas aquamarina*, *Vibrio aesturianus*, *Pseudoalteromonas elyakovii*) with a high growth reduction (about 75 %) and no major difference between copper and zinc action. Similar results were previously obtained using hydrogels [6]. Indeed, the reduction of growth was observed to depend only on the availability of copper and zinc ions in the alginate material, independently of calcium ions.

Indeed, it was observed that the reduction in growth was only dependent on the availability of copper and zinc ions in the alginate material, and not on the availability of calcium ions.

Figure 5(f) presents the results using the microalga *Halomphora coffeaformis*. Both CaZn and CaCu coatings lead to a strong reduction of algal growth (more than 85 %), a value definitely higher than for *Cylindrothecca closterium*. In this case, the calcium-alginate coating presents a non-negligible inhibition effect (Fig. 5f). This result is in agreement with our previous study performed with bulk hydrogels [6]. The high efficiency of CaZn and CaCu alginate may therefore be explained by the synergical effect of both Cu(Zn) and Ca ions in the inhibition process.

The calcium-zinc alginate coatings present a clear growth inhibition effect on all tested bacteria and microalgae. The role of the copper and zinc ionic species seems to be nearly identical in the reduction of growing process. Only results achieved on *Vibrio harveyi* show a different sensitivity to zinc and copper ions. Concerning the role of calcium ions, almost no influence of calcium ions on the inhibition is observed, excepted for the microalga *Cylindrothecca closterium*. The anti-bacterial and microalgal efficiency of the coating is ascribed to the release of ions in its surrounding environment. The similar sensitivity to the bacteria / microalgae to the zinc or copper alginate coatings can be explained by the equivalent quantity of metal ions (Cu^{2+} , Zn^{2+}) in the material [34] as shown by our EDX data. Stanic *et al* [34] have explained the antimicrobial activity of the polymer including metal ions (Cu^{2+} , Zn^{2+} ...) by the following mechanism: ions form strong bonds with thiol, imidazole, amino and carboxyl groups of microorganism membrane proteins, causing structural changes of the membrane. The membranes exhibit a significant increase in permeability, leaving the microorganism cells incapable of properly regulating transport through the plasma membrane. This leads to the death of the cells. The difference observed on *Vibrio harveyi* between CaCu

and CaZn can be related to the efficiency of the copper and zinc ions to bond with the different groups or to resistance effects.

Therefore, our results showed the effectiveness of zinc alginate coating against marine fouling. One of the interests of the coating is that it is based on a natural, abundant and cheap product: alginate. This biosourced material is then not toxic against marine microorganisms. In addition, the EPD technique requires only a low-voltage power supply and does not require any thermal annealing. As a result, the price of the coating is greatly reduced to few cents.

4. Conclusion

This study highlights the feasibility of producing a uniform alginate coating containing zinc and calcium ions by electrophoretic deposition on stainless steel. Its characterization demonstrates the presence of ions in their 2+ state into the coating initiated by exchange process between the initial alginic acid coating and cations present in solutions. Biological assays have shown an effective growth inhibition of bacteria and microalgae. This result is consistent with previous studies performed with bulk alginate and is explained by the release of ions from the coating. Therefore, the proposed method should be considered as a promising antifouling technique due to its low price, easy to develop and environmentally friendly. For further development, corrosion protection and the service lifetime of coatings must be addressed.

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