Conference Proceedings
DCE23-Doctoral Congress in Engineering
June 2023, Porto Portugal

Received: 31 March, 2023
Revised: 12 May, 2023
Published: 16 June, 2023

Development of antifouling surfaces coated with chitosan from *Loligo opalescens* for marine applications

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Abstract
Marine biofouling is often associated with biofilm formation on submerged surfaces, which causes economic and environmental problems. Although several biocides and cleaners have been used to control biofouling, new eco-friendly antifouling approaches are required. Chitosan (CS) is a polysaccharide widely used due to its outstanding properties, namely its antimicrobial activity. This work aimed to produce and characterise poly(lactic acid) (PLA)-CS surfaces with CS of different molecular weight (Mw) at different concentrations for application in marine paints. The antimicrobial performance of these surfaces was assessed against *Cobetia marina* for 7 weeks under controlled hydrodynamic conditions. All CS surfaces exerted a significant antimicrobial action by reducing the number of culturable cells up to 69% compared to control, being this activity dependent on CS Mw. Optical Coherence Tomography analysis corroborated these results, showing reductions in biofilm thickness of up to 36%. Overall, CS coatings represent a promising approach to reducing biofouling in marine environments.

Author Keywords. Chitosan, marine biofouling, marine waste, biofilm formation, antifouling coatings

Type: Research Article
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1. Introduction

In marine environments, natural and artificial submerged structures are quickly colonised by marine organisms in a process known as biofouling (Faria et al. 2020). This natural and complex process is responsible for several economic, industrial, environmental, and health problems (Tian et al. 2020). The attachment of fouling organisms to marine vessels increases drag resistance, which leads to higher fuel consumption and environmental pollution, changes the physicochemical properties of the surfaces, and contributes to species invasion (Tian et al. 2020; Lacoursière-Roussel et al. 2016). Since biofilm formation is one of the first steps of this process, a potential strategy to delay macrofouling is to prevent adhesion and biofilm formation by marine bacteria. Although several biocides, cleaners, and antifouling agents have been used to mitigate biofilm formation, their negative impact on marine ecosystems and human health (Amara et al. 2018) stresses the need to develop novel sustainable and eco-friendly approaches to prevent marine biofouling in ship hulls such as bio-based coatings. Chitosan (CS) is a polysaccharide obtained by deacetylation of chitin, which can be extracted from marine sources and microorganisms. Besides the use of CS enables the valorization of fish processing industry waste, CS has been widely used due to its interesting biological properties, namely the antimicrobial activity, which depends on a set of properties such as molecular weight (Mw), degree of deacetylation, concentration, and source (Kong et al. 2010; Chandrasekaran, Kim, and Chun 2020). The present study aimed to (i) produce and characterise poly(lactic acid) (PLA) surfaces coated with CS of different Mw and concentrations obtained from the *Loligo opalescens* pen, and (ii) evaluate the antifouling activity of these surfaces against *Cobetia marina* under nutritional conditions, temperature, and hydrodynamics that mimics the conditions typically found in some marine environments.

2. Materials and Methods

A combination of enzymatic and alkaline treatments was used to extract CS from *Loligo opalescens* pens, which was depolymerised into derivatives of different molecular weight (Mw). After dip coating the PLA films with 0.5% and 1% (w/v) of CS and its derivatives, the physical and chemical properties of the produced surfaces were assessed by Atomic Force Microscopy, contact angle measurements, and Scanning Electron Microscopy. The antifouling assays were performed with *Cobetia marina* for 7 weeks at 25 ˚C under controlled hydrodynamic conditions that mimic a ship in a harbor. Våatanen Nine Salt Solution (VNSS) medium was prepared as previously described (Holmström et al. 1998) and used to simulate the nutritional conditions found in marine environments. The biofilms formed were analysed by colony-forming unit counts to determine the number of culturable cells and by Optical Coherence Tomography (OCT) to analyse the biofilm thickness and architecture.

3. Discussion

The analysis of biofilm composition revealed that PLA films coated with 0.5% and 1% (w/v) CS exerted a significant antimicrobial effect by reducing the number of culturable cells by up to 58% and 69%, respectively, compared to the control. Regardless of the CS concentration, the results suggest that CS coatings with lower Mw CS had higher antimicrobial activity since the most active surfaces were those coated with CS1, CS2, and CS3 with 186, 129, and 61 kDa, respectively (Lima et al. 2022).

OCT imaging was used to assess the thickness and architecture of *C. marina* biofilms. Regarding biofilm thickness, significant reductions were observed for all functionalized surfaces compared with PLA. This antifouling effect was higher on biofilms developed on PLA-CS1, PLA-CS2 and PLA-CS3 surfaces, achieving thickness reductions of up to 36%. In general, no significant differences between the concentrations of 0.5% and 1% (w/v) CS were observed in biofilm culturability and thickness. Concerning *C. marina* biofilm architecture, regardless of the CS concentration, biofilms developed on PLA-CS surfaces presented visible differences in their structures compared with those grown on PLA films. Biofilms formed on the functionalized surfaces were more homogeneous and compact, while those formed on the control surface presented more prominent and irregular structures. These results were corroborated by the biofilm cell counts and thickness, revealing that both native CS and its derivatives prevented *C. marina* biofilm development (Lima et al. 2022).

4. Conclusions

CS coatings showed long-term and Mw-dependent antifouling performance against *C. marina* biofilms under the marine environmental conditions mimicked in this work. Overall, the incorporation of CS in marine paints may be a promising eco-friendly antifouling approach to reduce biofouling on ship hulls, simultaneously contributing to the valorization of marine waste.

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Acknowledgments

This work was financially supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE) were funded by national funds through the FCT/MCTES (PIDDAC); project HealthyWaters (NORTE-01-0145-FEDER-000069) was supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); CVMAR + I—Industrial Innovation and Marine Biotechnology Valorization" project was funded by INTERREG V Espanha Portugal (POCTEP) (0302_CVMAR_I_1_P). The research was also supported by the SurfSAFE project funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 952471. A.I.B. thanks the Portuguese Foundation for Science and Technology (FCT) for the financial support of her work contract through the Scientific Employment Stimulus— Individual Call (ref. 2020.03447.CEECIND). R.T.-S. acknowledges the receipt of a junior researcher fellowship from the Project PTDC/CTM-COM/4844/2020 (NanoCAT) supported by national funds through the FCT/MCTES (PIDDAC). M.L. and M.J.R. also acknowledge FCT for their PhD grants (2022.11196.BD and SFRH/BD/140080/2018, respectively). J.A.V. and J.V. also thank Xunta de Galicia by Xunta de Galicia (Grupos de Potencial Crecimiento, IN607B 2018/2019) for the financial support. Support from the EURO-MIC COST Action (CA20130) is acknowledged.