Chemical Engineering Journal 270 (2015) 150–157

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Incorporation of biocides in nanocapsules for protective coatings used in maritime applications

F. Maia [a], A.P. Silva [b], S. Fernandes [b], A. Cunha [b], A. Almeida [b], J. Tedim [a,*], M.L. Zheludkevich [a,c], M.G.S. Ferreira [a]

[a] CICECO, Department of Materials and Ceramic Engineering, University of Aveiro, 3810-193 Aveiro, Portugal
[b] Biology Department and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[c] Institute of Materials Research, Helmholtz-Zentrum Geesthacht, Max-Planck-Str. 1, 21502 Geesthacht, Germany

HIGHLIGHTS

- Successful encapsulation of biocides in silica nanocapsules.
- Expedite and real-time screening method to assess the inactivation of bacteria.
- Antibacterial activity of silica nanocapsules loaded with biocides.
- The developed nanomaterials show high potential to be used in antifouling coatings.

ARTICLE INFO

Article history:
Received 13 October 2014
Received in revised form 16 January 2015
Accepted 19 January 2015
Available online 28 January 2015

Keywords:
Antifouling coatings
Encapsulation of biocides
Silica nanocapsules
Antibacterial activity

ABSTRACT

This work reports the synthesis and characterization of silica nanocapsules with biologically-active compounds 2-mercaptobenzothiazole and 4,5-dichloro-2-octyl-4-isothiazolin-3-one. The resulting particles were characterized by scanning electron microscopy, thermogravimetry and adsorption–desorption isotherms of N_2. The antibacterial activity was assessed for both nanocapsules dispersed in solution as well as incorporated in coating systems, using a recombinant bioluminescent *Escherichia coli* expressing the *luxCDABE* genes from the marine bioluminescent bacterium *Aliivibrio fischeri*. The decrease in light emission of the bacterial model, indicative as decrease of metabolic activity, was directly correlated with the level of biocide detected in solution by UV–Visible spectrophotometry. The results show that the developed nanomaterials show great potential for application in antifouling coatings.

1. Introduction

Marine environments are particularly aggressive for metals and corresponding alloys because of the high salinity, presence of microorganisms, algae, plants and nutrients which lead to the occurrence of biofouling and corrosion issues [1,2]. Despite the settlement of macro and microorganisms on the substrate occurring at the coating/aquatic environment interface, the presence of living organisms can generate a set of specific conditions that weakens the coating. The result is the occurrence and propagation of cracks and pores, thereby contributing to the initiation and progress of corrosion at the metal/coating interface. The economic impact of these processes is considerable and includes costs associated with maintenance operations, replacement of offshore structures, expenditure of extra fuel in maritime transportation and consequent increase in greenhouse gas (GHG) emissions associated with the increase of drag forces [3].

To overcome biofouling, antifouling agents have traditionally been used and added to coating formulations, most notably tributyltin (TBT). However, since 2003, TBT was banned by the International Maritime Organization due to its high toxicity and bioaccumulation [1]. The rapid change enforced by the prohibition of organotin paints, combined with the current low carbon revolution, rising of fuel prices and heightened environmental awareness, have stimulated massive research efforts in the last decade or so in the area of antifouling technology.

In the last couple of years, several reviews reporting current antifouling technologies and highlighting strategies to achieve more environment friendly approaches were published [1,4–8]. Generally, the innovative approaches to prevent the settlement

* Corresponding author at: CICECO, Department of Materials and Ceramic Engineering, University of Aveiro, 3810-193 Aveiro, Portugal. Tel.: +351 234370255x22924.
E-mail address: joao.tedim@ua.pt (J. Tedim).

http://dx.doi.org/10.1016/j.cej.2015.01.076
1385-8947/

of microorganisms and the development of biofilms involve the modification of the physicochemical properties of surfaces or the embedding of antimicrobial compounds in coating materials [9].

Surface functionalization decreases surface energy and prevents adhesion of molecules and microorganisms. This has been achieved by modification of surface topography and biomimetic strategies using natural products, cells and enzymes [6,7], application of sol–gel systems [7,10], by the use of biodegradable polymers [11], self-assembly polymers and nanostructured polymer thin films [12]. On the other hand, the encapsulation of biocides to control the release of bioactive species has been attempted by the entrapment/encapsulation in latex nanocapsules [13] and chitosan/xanthan gum microcontainers with a core–shell structure [14]. An approach based on the combination of nanocontainers loaded with a formulation of corrosion inhibitors and biocides, combining anticorrosive and antifouling properties in the same coating, has been recently proposed [15].

Although the biocidal activity of encapsulated materials has been experimentally demonstrated, the potential toxic effects introduced by capsules and their performance in coatings with different properties in terms of propensity to biocide release have not been sufficiently addressed. In this work we investigated two compounds with known biocidal properties, in their free and encapsulated form: 2-mercaptobenzothiazole (MBT) which has a minimum inhibitory concentration (MIC) for *Escherichia coli* of 50 ppm [16] and the antifouling agent, 4,5-dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) with a MIC of 16 ppm for *E. coli* [17]. These compounds were encapsulated in silica nanocapsules [18] and the resulting materials were fully characterized by scanning electron microscopy (SEM), thermogravimetric analysis (TG/DTA) and adsorption/desorption isotherms. The antibacterial activity was assessed using a recombinant bioluminescent *E. coli* expressing the *luxCDABE* genes from the marine bioluminescent bacterium *Alivibrio fischeri* [19]. This expedite method allow the real-time monitoring of the inactivation of the bacterium as result of the biocide release from silica nanocapsules. The activity observed for the encapsulated biocides, either in solution or incorporated into coatings applied on carbon steel substrates was correlated with the release profiles of biocides obtained by UV–Visible spectrophotometry as a function of time.

2. Experimental

2.1. Materials

The active compounds used for encapsulation were 2-mercaptobenzothiazole (MBT) from Sigma–Aldrich and the marine antifouling agent SEA-NINE™ 211 N, which is 30% of 4,5-dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) in xylene, obtained from Rohm and Haas Company. For the preparation of capsules, cetyltrimethylammonium bromide (CTAB) (99%) and tetraethoxysilane (TEOS) (99.9%) were purchased from Sigma–Aldrich. Ammonia solution (NH_4OH) (25–28%), sodium hydroxide (NaOH), sodium chloride (NaCl), ethyl ether (99.5%) and buffer solutions were obtained from Riedel-de-Haën. Phosphate buffered saline (PBS) pH 7.4, containing 0.138 M NaCl; 0.0027 M KCl, was prepared with Sigma–Aldrich reagents. The recombinant bioluminescent strain of *E. coli* was provided by Alves et al. [19]. All the chemicals were analytic grade and were used without further purification.

2.2. Encapsulation of MBT and DCOIT in Silica Nanocapsules, SiNC

The encapsulation of MBT was performed as reported in our previous paper [18]. The encapsulation of DCOIT (SEA-NINE™ 211 N) was performed in a similar way. Briefly, an aqueous solution containing 0.1 g of CTAB dissolved in 35 mL of water was prepared and placed under stirring. Then, 0.25 mL of ammonia solution was added. In parallel, 5 mL of DCOIT solution (SEA-NINE™ 211 N) was diluted in 20 mL of ethyl ether, and added to the aqueous solution under constant stirring, forming an oil-in-water emulsion. After 30 min of emulsion stabilization 2 mL of TEOS was added to the emulsion. The reaction was left during 24 h at room temperature and under constant stirring. The resulting nanocapsules were filtered under vacuum and washed several times with distilled water and dried at room temperature.

2.3. SiNC characterization

The morphology of obtained nanocapsules was characterized by scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDS), (Hitachi S-4100 system with electron beam energy of 25 keV). Particle size distribution was determined using a free software named "ImageJ" for image processing and analysis with Java [20]. Thermogravimetric analysis (TG/DTA) was carried out in a Sataram–Labsys system under air atmosphere, with a heating rate of 10 °C min^{-1} from room temperature up to 800 °C. The textural properties were evaluated based on the adsorption–desorption isotherms of N_2 at −196 °C, performed on Gemini V2.00 equipment (Micromeritics Instrument Corp.) – samples were previously degassed at 180 °C for 6 h. The specific area (S_{BET}) was calculated by the BET method (Brunauer, Emmett and Teller) [21,22].

2.4. Preparation of substrates and coating

Carbon steel AISI 1008 samples were blasted with glass microspheres until reaching a roughness of ≈30–40 μm, followed by degreasing with an alkaline solution (10% Extran (Merck)), rinsed with water and ethanol, and dried with hot air.

Two types of formulation were used to coat carbon steel substrates: (i) water-based and (ii) solvent-based. The first one was a water-reducible, hydroxyfunctional polyacrylic dispersion (Bayhydrol® A 145), used in combination with aliphatic polyisocyanates (Bayhydur® 304) with fast drying at room temperature, from Bayer (Germany).

The second one was a bi-component Sumadur120 without pigments composed by an epoxy based resin (component A) cured with a polyamide (component B) from Sherwin Williams Sumare (Brazil). In general, a specific amount of nanocontainers loaded with biocides was added to the component A, mixed, and followed by addition of component B, in a 1:1 proportion between both components. Carbon steel samples were coated with the prepared formulations using a brush. In Table 1 the composition of all the coatings prepared is shown.

2.5. Microbiological studies of MBT@SiNC and DCOIT@SiNC in suspension and immobilized in coatings

The antimicrobial effect of biocides MBT and DCOIT encapsulated in silica nanocapsules was evaluated using a recombinant bioluminescent strain of *E. coli* [19], in liquid suspensions and immobilized in a paint for carbon steel. Bacterial cells suspensions (10^7 cell mL^{-1}) in PBS were exposed to the nanocontainers, during 6-h time course experiments. The concentration of nanocontainers was that corresponding to 20 and 200 mg L^{-1} MBT or 8 and 80 mg L^{-1} DCOIT. The inactivation was assessed, in real time, as the decrease in bacterial light emission, read in a luminometer (TD-20/20, Turner Designs). A negative control corresponding to cell suspensions in PBS without any amendment and controls corresponding to an equivalent concentration of SiNC without