Journal of the Taiwan Institute of Chemical Engineers 000 (2015) 1–7

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

Mg–Al mixed metal oxide film derived from layered double hydroxide precursor film: Fabrication and antibacterial properties

Yi Wang [a], Dun Zhang [a,*], Dandan Lv [a,b], Yan Sun [a]

[a] Key Laboratory of Marine Environmental Corrosion and Bio-fouling, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[b] University of Chinese Academy of Sciences, 19 (Jia) Yuquan Road, Beijing 100039, China

ARTICLE INFO

Article history:
Received 22 December 2014
Revised 25 April 2015
Accepted 9 May 2015
Available online xxx

Keyword:
Layered double hydroxide
Mixed metal oxide
Magnesium oxide
In situ growth
Antibacterial film

ABSTRACT

Mg–Al layered double hydroxide (LDH) precursor films are fabricated *in situ* by urea hydrolysis using pure Al as both the substrate and Al source. X-ray diffraction patterns and scanning electron microscopy images show that Mg–Al LDH films are fabricated *in situ*. After calcination at 500 °C for 4 h, Mg–Al mixed metal oxide (MMO) films that inherited the microstructure of LDH precursor films are obtained. The surface concentration of nano-MgO dispersed in the MMO matrix and the microstructure of the films may be tailored by varying the crystallization time of LDH precursor films. The superhydrophilic property of MMO films arising from their microstructure can reduce the adsorption and adhesion of bacteria and retard biofilm formation. Moreover, nano-MgO dispersed in the MMO matrix has high bactericidal activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Vibrio fischeri*. Mg–Al MMO films obtained in the present work have excellent antibacterial activities that are due to synergistic effects and thus have potentially applications in antibacterial coatings.

1. Introduction

Bacteria are able to attach to solid surfaces, colonize, proliferate, and eventually form biofilms. This is a widespread problem in the food industry [1], hospitals [2], and marine [3] and aerospace construction applications [4]. Bacterial biofilms serve as reservoirs for pathogen development, which could lead to infectious diseases [5]. In marine environments, marine bacterial biofilm can provide an easily accessible platform to which other marine species, such as diatoms and algae, can attach and proliferate, thereby inducing significant economic losses [6]. Therefore, considerable effort has been directed toward developing antibacterial coatings that can resist bacterial adhesion.

A variety of techniques have recently been developed to fabricate antibacterial coatings, including antibacterial polymer immobilization [7], photo-activated self-cleaning coating [8], bactericidal nanomaterial incorporation [9–12], and structural surface modification [13]. Among these techniques, bactericidal nanomaterial incorporation and structural surface modification have received considerable attention because of their long-term durability and environmentally safety.

Nanostructured MgO (nano-MgO) is reported among the most attractive inorganic nanomaterials because of its wide applications in various areas [14]. Nano-MgO exhibits a unique antibacterial mechanism [15,16]. Similar to other metal oxide nanomaterials, the toxicity of nano-MgO is commonly attributed to the production of reactive oxygen species (ROS), which can cause the cell membrane damage by lipid peroxidation [15]. In aqueous environments, the formation of superoxide anions (O_2^-) on its surface is considered one of the key factors influencing the antibacterial activity of nano-MgO [17]. Leung et al. [18] recently found that nano-MgO clearly demonstrated robust toxicity toward *Escherichia coli* (*E. coli*) in the absence of ROS production and this excellent antibacterial activity could be related to the cell membrane damage because of the attachment of nano-MgO on the cell membrane. Moreover, as a low-cost and environmental friendly nanomaterial, nano-MgO is expected to be used as an inorganic antibacterial agent in antibacterial coatings. Immobilization of nano-MgO to fabricate antibacterial coatings, however, remains a challenge to most researchers because of its powder form. As a result, we aim to find an alternative method to immobilize nano-MgO in antibacterial coatings.

Layered double hydroxides (LDHs) are a family of synthetic anionic clays composed of 2D brucite $Mg(OH)_2$-like layered inorganic materials [19]. They are expressed by the general formula $[M_{1-x}^{2+}M_x^{3+}(OH)_2]A_{x/n}^{n-}\cdot mH_2O$, where M^{2+} and M^{3+} are divalent and trivalent metal ions, respectively, coordinated octahedrally to hydroxyl groups to form infinite 2D layers by edge sharing. Here,

* Corresponding author. Tel.: +86 532 82898960; fax: +86 532 82898960.
E-mail address: zhangdun@ms.qdio.ac.cn, zhangdun@qdio.ac.cn (D. Zhang).

http://dx.doi.org/10.1016/j.jtice.2015.05.017
1876-1070/

A^{n-} is the interlayer anion compensating for the positive charge of the brucite-like layers [19]. Given their flexible compositions, various functional LDHs with a wide range of properties have been prepared [20]. A Mg–Al LDH compound can be readily transformed into the corresponding Mg–Al mixed metal oxide (MMO) by heating to a certain temperature. This process involves dehydration (100–250 °C), dehydroxylation (350–450 °C), and decarbonation (420–470 °C) in series [21]. The nano-MgO phase formed can be well dispersed over the Mg–Al MMO matrix [22]. Therefore, calcination of Mg–Al LDH films would be an effective approach to immobilize nano-MgO to form antibacterial films. Spin coating [23], electrophoretic deposition [24], solvent evaporation [25], the layer-by-layer technique [26], and *in situ* growth [27] processes have recently been developed to fabricate LDH films. Compared with deposition, adhesion between the LDH film and the substrate is much stronger when *in situ* growth is employed because of chemical bonding between two phases [28]. *In situ* growth is not limited by the shape of the substrate and may thus be applied in a wide range of uses. Mg–Al LDH films can be fabricated by *in situ* growth on pure Al [29], porous anodic alumina/Al [30], and Mg alloy [31] substrates. They are ideal precursors for fabricating Mg–Al MMO films on these substrates. However, the potential of Mg–Al MMO films as antibacterial coatings has not been explored. In this work, we demonstrate for the first time that Mg–Al MMO films exhibit intrinsic antibacterial activity.

This work describes the successful fabrication of Mg–Al MMO antibacterial films on pure Al substrates by calcination of Mg–Al LDH precursor films synthesized using *in situ* growth. The structure and morphology of the synthesized films were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), and atomic force microscopy (AFM). The resultant Mg–Al MMO films showed satisfactory bactericidal performance toward four species of bacteria, including *E. coli*, *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Vibrio fischeri (V. fischeri)*, and resisted their attachment on the film surface. The excellent antifouling activities obtained in this work can be attributed to the synergistic effects of nano-MgO dispersed in the Mg–Al MMO matrix and the unique superhydrophilic microstructure of the resultant MMO films. The simple but versatile approach proposed in this work can facilitate the preparation of highly efficient antibacterial coatings based on inorganic nano-MgO.

2. Materials and methods

2.1. Materials

The pure Al substrate (purity >99.99%, thickness 0.2 mm) was purchased from Beijing Cuibolin Non-Ferrous Technology Developing Co., Ltd. Other analytical reagent-grade chemicals were supplied by Sinopharm Chemical Reagent Co., Ltd. Milli-Q water was used in the experiments (Millipore, USA).

2.2. Bacterial culture

All bacteria were provided by the Key Laboratory of Experimental Marine Biology, Chinese Academy of Sciences. Bacteria were seeded and cultured in the following media: *E. coli*, *S. aureus*, and *P. aeruginosa* in Luria-Bertani (LB) medium; *V. fischeri* in 2216E Zobell marine agar. A single bacterial colony was inoculated overnight in the appropriate bacterial medium at 180 rpm at 30 °C. One milliliter of the bacterial suspension was centrifuged at 12,000 rpm for 5 min and washed twice with sterile phosphate buffer solution (PBS) (pH 7.4). Finally, 1 mL of water was added to the suspension, which was then mixed vigorously. The optical density of the final bacterial suspension was measured using a Hitachi U2900 UV–vis spectrophotometer (Japan), and the cell concentration was calculated using a standard curve of four species. The final cell concentrations of *E. coli*, *S. aureus*, *P. aeruginosa*, and *V. fischeri* were 6.90×10^7, 6.65×10^7, 6.89×10^7, and 9.67×10^7 CFU/mL, respectively.

2.3. Fabrication of the Mg–Al MMO films

Mg–Al LDH precursor films were fabricated by *in situ* growth on pure Al substrates. Pure Al substrates were cleaned with acetone, ethanol, 0.5 wt.% NaOH solution, and water in sequence before use. In a typical procedure, 4.000 mmol $Mg(NO_3)_2 \cdot 6H_2O$ and 2.425 mmol urea were dissolved in water to form a clear solution with a total volume of 75 mL. Pure Al substrates with a lateral size of 10.5 cm × 5 cm were rolled into tubular shape and immersed vertically in the solution and then heated at 70 °C for 12, 24, 36, and 48 h, respectively. Afterward, substrates were withdrawn, rinsed with water, and dried at room temperature. The as-prepared Mg–Al LDH precursor films were heated at 500 °C for 4 h with a heating rate of 2 °C/min, and then slowly cooled to room temperature to finally form Mg–Al MMO films. Mg–Al LDH precursor films fabricated over different crystallization times were denoted as LDH-12, LDH-24, LDH-36, and LDH-48, respectively. The corresponding Mg–Al MMO films were denoted as MMO-12, MMO-24, MMO-36, and MMO-48, respectively.

2.4. Characterization

XRD measurement was performed on a Rigaku D/max-Ultima III diffractometer (Japan) under the following conditions: 40 kV, 30 mA, graphite-filtered Cu K_α radiation ($\lambda = 0.1542$ nm). FT-IR spectra were collected on a Nicolet iS10 infrared spectrophotometer (USA) using KBr disks with a sample/KBr mass ratio of 1:100. SEM images were taken from a Hitachi S-3400 scanning electron microscopy (Japan). AFM images were taken with Agilent5400 (USA) in tapping mode with a speed of 0.2 L/ns. Elemental analyses (Mg and Al) were performed by inductively coupled plasma (ICP) emission spectroscopy with a Shimadzu IPC-7500 instrument (Japan) using samples diluted in HNO_3. Static water contact angle was measured with a contact angle meter (JC2000C1, China) at room temperature.

2.5. Antibacterial tests

The antibacterial and antifouling activities of the final Mg–Al MMO films obtained on the pure Al substrate were characterized using *E. coli*, *S. aureus*, *P. aeruginosa*, and *V. fischeri*. MMO films were cut into rectangular bars approximately 2 cm long and 1 cm wide with a razor blade. The pure Al substrate with a same size was used as control.

For the bactericidal test, 100 μL of the bacterial suspensions was uniformly spread on Mg–Al MMO films and the pure Al substrate, which were then incubated at 30 °C for 24 h without shaking. After incubation, all film samples were washed with 1 mL of sterile PBS (pH 7.4). The bacterial suspensions obtained were diluted to 100 times for plate counts according to the 10-fold dilution method. All measurements were done in triplicate and average value is reported. The bactericidal efficacy (%) was calculated using the following equation [17]:

$$\text{bactericidal efficacy}\ (\%) = (N_R - N_E)/N_R \times 100\% \quad (1)$$

where N_R is the alive number of bacteria in control group and N_E is the alive number in experiment group.

For the antifouling test, 200 μL of the bacterial suspensions was added to 20 mL of LB medium (pH 7.0) in 50 mL sterile tubes. The MMO-24 sample and the pure Al substrate were then immersed into the tubes. The bacteria were subsequently cultured at 180 rpm at 30 °C for 24 h. After culturing, the film samples were removed from the solution, washed with sterile PBS (pH 7.4) to remove non-adherent bacteria, and fixed in glutaraldehyde for SEM observation.

Please cite this article as: Y. Wang et al., Mg–Al mixed metal oxide film derived from layered double hydroxide precursor film: Fabrication and antibacterial properties, Journal of the Taiwan Institute of Chemical Engineers (2015), http://dx.doi.org/10.1016/j.jtice.2015.05.017