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Full Length Article

Antibacterial behaviors of Cu_2O particles with controllable morphologies in acrylic coatings

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ABSTRACT

Biofouling, a global issue, exerts an adverse impact on ocean shipping, deep-sea exploration, aquaculture and other industries. The addition of Cu_2O particles to coatings is the most popular and effective method used for biofouling resistance in the ocean circumstance. Herein, Cu_2O particles with three kinds of morphologies, including cube, sphere and cubooctahedron, were fabricated by liquid phase reducing method. Particle size, crystallinity, component, activity in aqueous solution, antibacterial property and copper ion leaching rate were systematically investigated. XRD and XPS jointly reflected that as-prepared Cu_2O particles were highly pure and impurity was absent. Among three kinds of Cu_2O particles, cubooctahedron Cu_2O was the most active, possessed the highest ion leaching rate and exhibited the best antibacterial property. It could be deduced that morphology of Cu_2O particles affected its activity in aqueous solution, then impacted release rate and ultimately influenced antibacterial capacity. Morphology and component of bacteria, before and after being exposed to as-synthesized Cu_2O-acrylic hybrid coatings were also studied and results proved that copper ion was prone to do a damage to the cell membrane of E.coli rather than B. subtilis because of the difference between cell walls.

1. Introduction

No matter which kind of surface, soaked in seawater, is confronted with biofouling, which results from the attachment of microorganism [1]. Due to motion, secretion and reproduction of microorganism [2], biofouling has a detrimental impact on exploitation and utilization of marine resources [3,4] and has become a worldwide problem, which is as severe as corrosion [5,6]. In accordance with statistics, 90% of the total fraction drag is caused by adhesion of hulls [7,8]. As a result, fuel consumption [9] and carbon emission [10] increase. In addition, metabolites produced by bacteria [11], like H_2S and NH_4^+, may accelerate corrosion and affect the security of facilities. Moreover, marine microorganism can migrate to other waters and pose a serious threat to local ecological system by adhering to vessels. Thus, it is of paramount significance to prevent biofouling [12].

Nowadays, there exist a plenty of antifouling technologies such as acoustics method [13], electrolysis method [14], periodic cleaning method and coating coverage method [15,16]. Among them, the coating containing biocides, which works as a protecting barrier to keep the substrate from fouling organisms, plays a crucial role in preventing biofouling [17,18] and accounts for large share of the market [19,20]. In the second half of the twentieth century, organotin compounds [21], especially tributyltin (TBT), were widely utilized in prohibiting attachment of organism [22]. Nevertheless, they are highly toxic to marine organisms, triggering growth defects and aberration [23], and strongly destroy marine ecological balance [24]. International Maritime Organization (IMO) has banned the usage of antifouling coating including organotin compounds [25,26]. At present, there are primarily three kinds of biocides: synthetic biocides, natural biocides and Cu_2O particle. Antifouling property of synthetic biocides is dependent on the sort of substrate, like tannin [27]. Most natural biocides show low yield and are selectivity to fouling microorganism, such as capsaicine [28] and halogenated furanones [29]. Cu_2O particle, highly efficient, broad-spectrum and economical [30], becomes one of the most commercially used antifoulants [31], but to coatings containing Cu_2O particles, the efflux rate of copper ion is unstable which wields an undesirable

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influence on their antifouling performance. Quite a few researchers pay much attention to resins, which encapsulate Cu_2O particles, to cope with the problem [32]. Yonehara et al. [33] found that the kind of alkyl groups and zinc acrylate content exercised a considerable effect on copper ion release rate. Indeed, characteristics of Cu_2O particles also make a potent difference to the antifouling property of coatings.

Via literature research, it could be found that morphology of Cu_2O particles is critically momentous to physical and chemical properties [34-37] which draws researchers' interests and concern. Huan Pang et al. [38] fabricated some Cu_2O particles with different morphologies, like corner-truncated, octahedral, hexa-cone, hexa-pod, cube and so on. By comparing the minimum inhibitory concentration of as-prepared Cu_2O particles, it could be concluded that cube Cu_2O was broad-spectrum, while the antibacterial property of octahedral Cu_2O was dependent on the type of bacteria. Mojtaba Bagherzadeha et al. [39] synthesized octahedra, truncated rhombic dodecahedral, nanocube and sphere Cu_2O particles and investigated their catalytic performance to the same cycloaddition. The result revealed that octahedra Cu_2O was superior to others. However, the size of Cu_2O particles is also essential [40], while most researchers neglect the impact of particle size, and just straightly attribute properties to the morphology. What's more, few people focuses on the way morphology affect Cu_2O particles' performance.

In this research, Cu_2O particles, with similar size and controllable morphologies, were fabricated and a direct relationship between antifouling property and morphology was build up. Moreover, mechanism of antifouling capacity controlled by morphology was studied in details. Since the bacterial adhesion is an impressively vital procedure [41], just following the form of conditional membrane [42] and the first step of irreversible adhesion [20,43], which is conducive to the attachment of algae and barnacle. Thus, antibacterial property was utilized to measure antifouling performance of as-prepared Cu_2O particles. Additionally, via investigating the transformation of morphology and component of bacteria before and after being exposed to as-synthesized Cu_2O-acrylic hybrid coatings, antibacterial mechanism of Cu_2O particle was clarified. The results obtained in this work would be meaningful to design green and effective anti-bacterial hybrid coatings.

2. Experiment

2.1. Materials

Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), sodium hydroxide (NaOH), glucose ($C_6H_{12}O_6$), copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$), ethanol and glutaraldehyde 25% aqueous solution were purchased from Sinopharm Chemical Regent Co., Ltd. Polyvinylpyrrolidone (PVP) with a molecular weight of 8000, ascorbic acid ($C_6H_8O_6$), tryptone and yeast extract were obtained from Aladdin Industrial Corporation. Self-polishing zinc acrylate resin was supplied from Wuxi Honor Shine Chemical Co., Ltd. 2216E agar and 2216E liquid medium were gained from Qingdao Hope Bio-Technology Co., Ltd. 316 stainless steels were provided by Shanghai Baosteel Group Corporation. All chemicals, excluding resin, were analytic grade regent and used without further purification. Deionized water was used in all experiments.

Gram-positive bacterium (B. subtilis, CMCC63501) and Gram-negative bacterium (E. coli, CMCC44102) were provided by Shanghai Luwei Technology Co., Ltd.

2.2. Preparation of Cu_2O particles with different morphologies

2.2.1. Preparation of cube Cu_2O

In the first stage, 100 mL 0.2 M $CuSO_4$ solution was put in a round-bottomed flask and blended by a magnetic stirrer. At the same time, 100 mL 0.4 M $C_6H_{12}O_6$ solution was heated to 50 °C. Then, 100 mL 0.6 M NaOH solution was dropwise added into the flask and the feeding time was 30 min. After that, temperature of the mixed solution was increased to 50 °C and $C_6H_{12}O_6$ solution was poured into it. The above system was continuously stirred for another 30 min at 50 °C. The crystals were collected by suction filtration and washed with distilled water and ethanol several times to remove impurities. Afterwards, the obtained products were placed in a vacuum oven at 80 °C for 6 h.

2.2.2. Preparation of sphere Cu_2O

The preparation method of sphere Cu_2O was similar to cube Cu_2O. The distinctions were that the concentration of NaOH solution was 1 M and the feeding time was 60 min.

2.2.3. Preparation of cubooctahedron Cu_2O

0.34 g $CuCl_2 \cdot 2H_2O$ and 6.66 g PVP were firstly dissolved into 200 mL distilled water at 55 °C in a round-bottomed flask. Then, 20 mL 3 M NaOH solution was added into the flask drop by drop with magnetic stirring. After being stirred for 30 min, 20 mL 0.6 M $C_6H_8O_6$ solution was introduced dropwise into the mixed solution, and the system was stirred sequentially for 1 h. The products were achieved by centrifugation. Eventually, the crystals were dried at a vacuum oven at 80 °C for 6 h.

2.3. Preparation of Cu_2O/self-polishing zinc acrylate resin hybrid coating

Cu_2O powders and self-polishing zinc acrylate resin were blended by ultrasonic vibration with the mass ratio of Cu_2O powders 5%. Then, 0.3 g mixture, to uniform the thickness of coatings, was covered to a piece of 316 stainless steel by glass rod and the smoothness of coating was attributed to strong liquidity of self-polishing zinc acrylate resin. Hybrid coatings were named after the morphology of Cu_2O particles.

2.4. Characterization of Cu_2O particles

Physical and chemical properties of prepared Cu_2O powders were determined by scanning electron microscopy (SEM, Hitachi S-4800, Japan), X-ray diffraction (XRD, Bruker D8 ADVANCE, Germany), Confocal Micro-Raman spectroscopy (Raman, Renishaw, UK), Fourier transform infrared spectroscopy (FTIR, Thermo Nicolet 6700, USA), X-ray photoelectron spectroscopy (XPS, Kratos AXIS ULTRA, UK) and Dynamic Light Scattering (DLS, Malvern Zetasizer Nano ZS, UK). Surface morphology of Cu_2O powders was observed using SEM with a secondary electron detector. SEM images were attained with working distance of 9.2 mm, operated at 8 kV. All samples were sprayed with platinum to improve electrical conductivity. Chemical constituents and crystal information were measured by X-ray diffraction with a Cu-Ka radiation (λ = 0.15406 nm), utilizing a scanning rate of $7^\circ\ min^{-1}$. 2 θ ranges from 10° to 90°. Structural information of as-fabricated Cu_2O was obtained by Raman and FTIR. Surface composition and chemical valence were investigated via XPS which was more accurate than XRD and could be utilized to quantitative analysis. Surface charge and stability were studied using Dynamic Light Scattering, with deionized water as solvent and ultrasonic shaking 5 min.

2.5. Antibacterial assays

B. subtilis and E. coli cells were stored in agar plates. Then, they were transferred into 2216E medium and LB medium respectively via inoculation. To activate these bacteria, medium was placed in constant temperature incubator shake for 24 h at 120 rpm, with the temperature of 37 °C. Later, the bacteria was obtained by centrifugation at 3000 rpm for 5 min. B. subtilis and E. coli cells were cleaned three times with saline solution and synthetic seawater, respectively, to wash residual medium. Afterwards, a microplate reader (MD SpectraMax 190, USA) was employed to determine the concentration of cells with wave length of 600 nm and a moderate concentration, 10^5–10^6 CFU/mL, of bacteria liquid was achieved via dilution. Subsequently, cube-Cu_2O, sphere-