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Alginate/albumin in incubation solution mediates the adhesion and biofilm formation of typical marine bacteria and algae

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HIGHLIGHTS

- Porous conditioning layer was characterized by AFM.
- Alginate/albumin in solution enhances adhesion of typical marine microorganisms.
- Alginate/albumin impacts biofilm formation and its surface topography.

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ABSTRACT

Adhesion of microorganisms in the marine environment is one of the initial events responsible for the occurrence of biofouling. A variety of factors play roles in regulating adhesion behaviors and subsequent biofilm formation. Here the study was focused on the influence of the typical marine polysaccharide alginate and the protein albumin on the attachment and colonization of *Bacillus* sp., *Chlorella pyrenoidosa* and *Phaeodactylum tricornutum* to silicon wafers in sterile artificial seawater. The rapid formation of conditioning layers due to the adsorption of the molecules was revealed by atomic force microscopy, and porous layers with the thickness of 3–6 nm further altered the surface roughness and wettability of the substrata. The presence of alginate or albumin in the culture solution tailored the surface properties of *C. pyrenoidosa* and *P. tricornutum*. The thickness, structure heterogeneity, biomass, diffusion distance, and roughness coefficient of the biofilm formed by colonization of the microorganisms were examined and their values showed that alginate/albumin had a significant influence on biofilm formation. The results are relevant to biofouling research on exploring antifouling strategies at the molecular level.

1. Introduction

Biofouling caused by unwanted colonization of sea species usually brings about serious impacts on marine industries, such as increased fuel consumption and frictional resistance [1,2], enhanced corrosion [3], the clogging of membranes and heat exchangers [4] and great economic loss [5]. Many efforts have been devoted to clarifying the mechanism of biofouling in order to prevent it. It was established that the attachment and subsequent biofilm formation of microorganisms on man-made surfaces is the key phenomenon involved in biofouling [4,6]. Generally, as the initial step, the formation of conditioning layers on solid surfaces is attributed predominately to the adsorption of ions, organic molecules (proteins, polysaccharides and lipoproteins) and other matter in the marine environment [7]. Conditioning layers can potentially change the physicochemical properties of substrata significantly, for instance surface tension, roughness, electrostatic charges, and wettability, and in turn significantly inhibit or facilitate biofilm formation [8–10].

In recent years, the influence of conditioning layers on microorganisms' adhesion has been widely investigated. Yet, due to the complex composition of biomacromolecules and difficulties in characterizing the layers, knowledge about the impact of physicochemical features of conditioning layers on biofilm remains elusive. As one of the model macromolecules in biofilm research, albumin is a negatively

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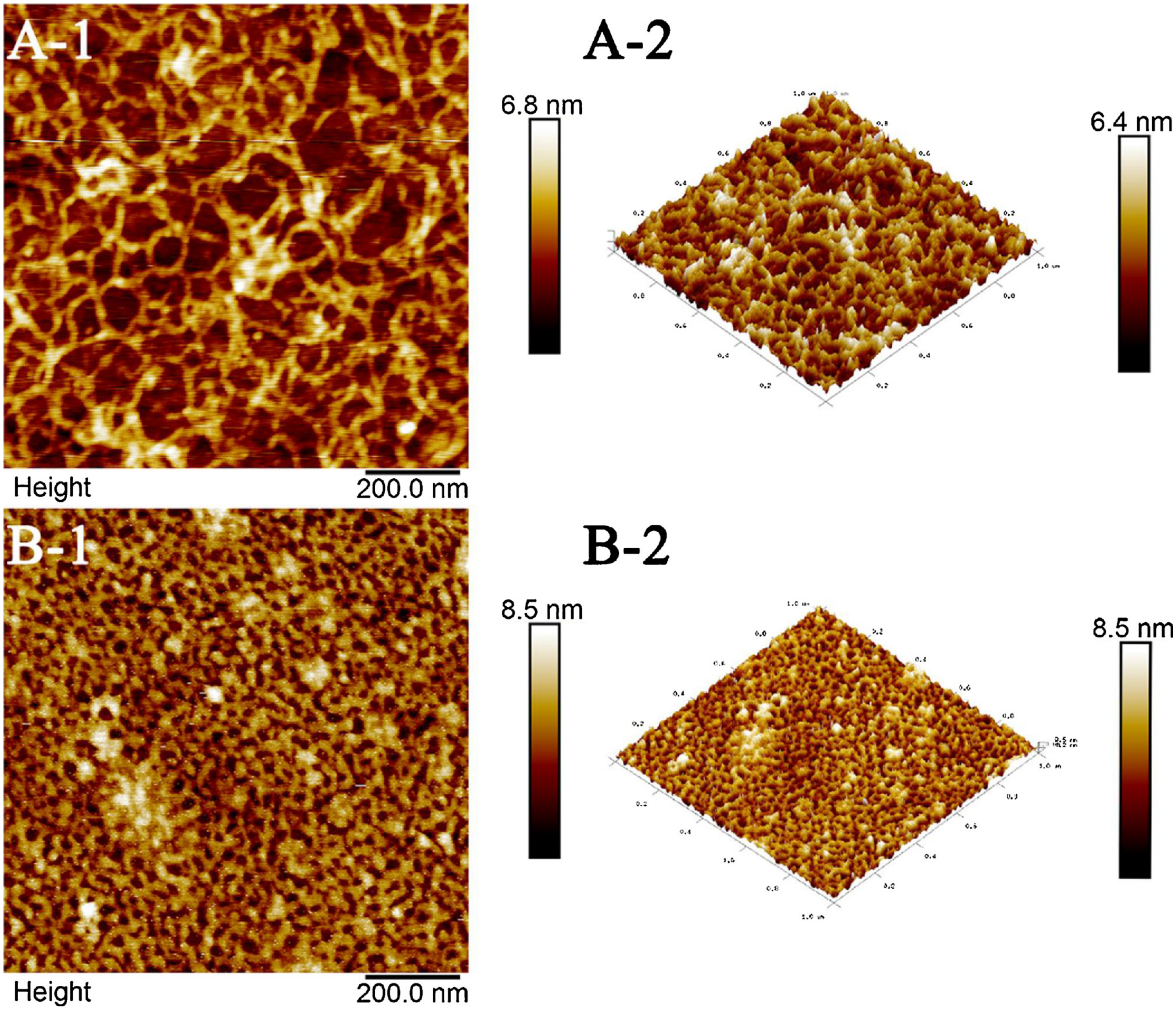

Fig. 1. PeakForce QNM AFM height images (-1) and the 3D images (-2) of surface after adsorption of alginate (A) and albumin (B).

charged protein with extraordinary ligand binding capacity [11]. Pre-adsorbed albumin showed a surprising inhibitory effect on bacterial adhesion to polymer, ceramic, and metal surfaces [12,13]. Studies performed by Tang et al. showed that increased concentration of -NH_2 group and more hydrophilic albumin inhibited adhesion and colonization of *Staphylococcus Epidermidis* [14]. Meanwhile, albumin suppresses initial bacterial adhesion due to the lack of specific interactions between *Staphylococcus epidermidis* and albumin [15]. It was claimed that, at higher ionic strength, the protein would result in more compressed, giving rise to a denser core with fewer interaction sites for adhesion [16]. However, it was reported that bovine serum albumin conditioning layer significantly promoted the adhesion of *Pseudomonas aeruginosa* PAO1 at a low ionic strength but hindered its adhesion at a higher ionic strength [17]. As an extensively used polysaccharide [18], alginate has been noticed because it is the major component of extracellular polymeric substances (EPS) and possesses similar macroscopic physicochemical properties of conditioning layers [8,19], which triggers severe fouling of membranes and coatings [20]. The presence of alginate conditioning layer enhanced the initial adhesion of *Pseudomonas aeruginosa* PAO1 on microscope slides through polymeric interactions between alginate and bacterial surfaces [17]. Moreover, the adhesion of *Burkholderia cepacia* to alginate-coated slides was enhanced with increasing ionic strength [8]. However, the over-expression of alginate can reduce deposition and the adhesion of *Pseudomonas aeruginosa* due to steric shielding of cell surface features [10]. Hwang et al. extracted EPS from *Burkholderia cepacia* and *Pseudomonas aeruginosa* and then placed bare slides into the EPS solution to form a conditioning layer, and they surprisingly found that the conditioning layer formed by EPS hindered the adhesion of *Burkholderia cepacia* and *Pseudomonas aeruginosa* [21]. It is clear that the influence of biomacromolecule conditioning layers on bacterial adhesion varies depending on the species and conformations of the macromolecules, the surface properties of bacteria, and the ionic strength of culture solutions.

It is well known that the adhesion of microorganisms and subsequent biofilm formation is influenced by the physicochemical features of both the conditioning layers and the microorganism cells [21]. The response of microorganisms to conditioning layers pre-formed by several typical biomacromolecules at the initial stage has been studied [8,11,17,21]. Yet knowledge about the impact of biomacromolecules in suspension on the adherence of microorganisms and subsequent development of biofilm remains elusive. A fundamental understanding of the interactions between macromolecules in suspension and marine microorganisms is essential for understanding biofouling processes. In the present study, alginate and albumin were chosen as the typical biomacromolecules for building simplified models of conditioning layers. The influence of alginate/albumin on adhesion and biofilm