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Microbial attachment and adsorption–desorption kinetic of tightly bound extracellular polymeric substances on model organic surfaces

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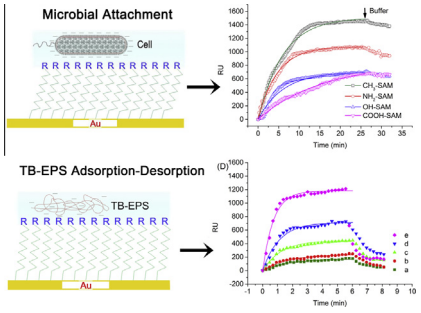
HIGHLIGHTS

- CH_3-SAM and NH_2-SAM surfaces encourage the cell adhesion.
- TB-EPS bound on hydrophobic surface was the fastest and most compactly.
- The neutral and hydrophilic surface was in favor of anti-biofouling.

GRAPHICAL ABSTRACT



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ABSTRACT

The attachment of microbial cells onto the surface is influenced by surface properties. In this study, in order to determine the bacterial attachment and adsorption behaviors of tightly bound extracellular polymeric substances (TB-EPS) on different surfaces, four self-assembled monolayers (SAMs) carrying methyl (CH_3-SAM), amino (NH_2-SAM), hydroxyl (OH-SAM), and carboxyl (COOH-SAM) terminal groups were modeled. The result indicated that the bacterial attachment and the adsorption/desorption rate of TB-EPS were dependent upon the surface properties. CH_3-SAM and NH_2-SAM surfaces encourage the cell attachment. The adsorption rate of TB-EPS on CH_3-SAM surface was the highest. The neutral and hydrophilic surface (OH-SAM) obtained the lowest cell attachment amount and TB-EPS affinity. The result indicated that the hydrophobic surface was much more favorable to the cell immobilization and TB-EPS deposition than hydrophilic surface. This work provided a foundation to control attachment of cells onto surfaces and the biofilm formation in wastewater treatment process.

1. Introduction

The primary attachment of cells onto surfaces is a critical stage in overall biofilm formation process, which is also considered the only reversible stage [1]. Then cells adsorbed on surfaces secret or produce extracellular polymeric substances (EPS), replicate and grow into thick biofilm [2]. The cells deposition onto surfaces is dependent upon the binding strength of cell and surface in the reversible stage. The surface property of surface plays an important role in the biofilm formation process [2]. A more cohesive attachment of cells onto surfaces could promote the biofilm formation faster and more stable. Thus, it is necessary to explore the attachment characteristics of microbial cell onto the different surfaces. Furthermore, EPS adhere onto the cell surface and alter the

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physicochemical characteristics of cell surface, consequently affecting the cell deposition onto surfaces, and thus affecting the biofilm formation [3–5].

Microbial EPS are mainly the high-molecular-weight polymers which derived from the secretions of microorganisms, the cellular lysis and macromolecules hydrolysis [6]. They are normally divided into two major fractions: soluble EPS (SEPS) and bound EPS [7,8]. The inner layer of bound EPS consists of tightly bound EPS (TB-EPS), whereas the outer layer consists of loosely bound EPS (LB-EPS) [9,10]. Compared with SEPS and LB-EPS, TB-EPS play a more important role in microbial aggregating, flocculating, and maintaining biofilm architecture [11,12]. Therefore, the adsorption–desorption behaviors and the binding strength of TB-EPS on different surfaces should be also elucidated.

Surface plasmon resonance (SPR) is widely used to study the interactions of biomacromolecules including protein and polysaccharide on sensor surfaces because of its notable features, including minimal requirement of reagent consumption, and offering free-label, real time, and rapid and simple detection as well as automated monitoring [13–15]. It also can be utilized to study bacterial attachment on self-assembled monolayer (SAM) surfaces [16]. Furthermore, SPR provide a new method to determine the kinetic parameters of association and dissociation process, and the affinity between two molecules [17]. In this work, the attachment of *Bacillus subtilis* on different organic surfaces was investigated. Four SAMs with different terminal functional groups carrying methyl (CH_3-SAM), amino (NH_2-SAM), hydroxyl (OH-SAM), and carboxyl (COOH-SAM) were prepared to model different surfaces. Our previous study indicated that the roles of pH and cation in TB-EPS adsorption were dependent on surface properties [18]. In the present study, the adsorption–desorption characteristics and affinity of TB-EPS on different surfaces were also determined.

2. Materials and methods

2.1. SAMs preparation

In order to model different organic surfaces, four SAMs with different terminal functional groups (CH_3-SAM, NH_2-SAM, OH-SAM, and COOH-SAM) were prepared on the gold substrates. 11-mercaptoundecanoic acid [$HS(CH_2)_{10}COOH$], 11-mercapto-1-undecanol [$HS(CH_2)_{11}OH$], N-dodecyl mercaptan [$HS(CH_2)_{11}CH_3$], and 11-amino-1-undecanethiol [$HS(CH_2)_{11}NH_2$] were purchased from Sigma–Aldrich (Shanghai, CHA). CH_3-SAMs of and OH-SAM were formed by immersion of gold sensors in 1 mM ethanol solutions of the appropriate thiol for 24 h. NH_2-SAM was prepared using 1 mM 11-amino-1-undecanethiol in an aqueous solution with 1% v/v NH_4OH while COOH-SAM was formed using 1 mM 11-mercaptoundecanoic acid in an ethanol solution with 1% v/v CH_3COOH [19]. SAMs was rinsed in ethanol or water, and dried in a stream of N_2 after incubation in corresponding thiol solutions. The surface contact angles and X-ray photo electron spectroscopy of SAM sensors have been described previously [18].

2.2. Bacterial attachment measurement by SPR

B. subtilis (ATCC 6633) was purchased from Guangdong Microbiology Culture Center, China, and selected to perform bacterial attachment experiment. The bacterium was grown according to protocols described in a previous publication [20]. The freeze-dried culture was suspended in LB-Lennox media (5 g/L yeast extract, 10 g/L tryptone, 5 g/L NaCl) for 24 h at 30 °C, and then a 1 mL aliquot was incubated in 250 mL LB-Lennox medium in 500 mL flasks (40 h, 30 °C, 120 rpm).

The bacterial initial attachment on model organic surfaces was observed by an SPR (Navi 200, BioNavis, Inc., FI) with dual-channel detection. The bacterial suspension was centrifuged at 5000*g* for 20 min at 4 °C to remove growth medium. The cell pellet was washed 3 times and finally suspended in 0.9% NaCl (pH ≈ 7.0). The optical density (OD) of the suspension (OD_{600}) was adjusted to 1 for SPR analysis [16]. Bacterial attachment measurements were performed on the SPR device at 20 °C. The SAMs surface was flushed with buffer (0.9% NaCl) for ~5 min, and then the flow rate was adjusted to 25 μL/min until the baseline was stabilized for ~20 min. Every sample was pumped at a flow rate of 25 μL/min for 25 min for determining bacterial attachment on four SAM surfaces, followed by buffer injection again to remove unbound or loosely bound bacteria. The attachment measurements were duplicated. The kinetics for the attachment process may be described by pseudo first-order equation (Eq. (1)) [21,22]:

$$\Delta R = \Delta R_{eq}\left(1 - e^{-tk}\right), \tag{1}$$

where ΔR is the SPR signal (response units, RU) of bacteria adhered on SAM surfaces versus the attachment time (t, min). ΔR_{eq} is the SPR signal of adhered bacteria at equilibrium, and k is the pseudo-first-order attachment rate constant (1/min).

2.3. Bacterial surface characterization

The hydrophobicity and zeta potential of the bacteria were evaluated using the method depicted in our previous study [12]. The OD_{546} of microbial cell suspension was adjusted to 0.1 using 0.9% NaCl, and the zeta potential was estimated with Zetasizer Nano ZS (Malvern, UK). Microbial adhesion to hydrocarbon test with n-dodecane-water system was used to determine the bacteria hydrophobicity. All determinations were triplicates. The cell pellet was lyophilized to perform FT-IR spectrophotometry analysis (Cary 630, Agilent, USA).

2.4. TB-EPS adsorption assay

Sludge was sampled from a sequencing biofilm batch reactor. The TB-EPS extraction and characterization protocols were described in Supporting information. The initial acquired TB-EPS solution was diluted to 1, 2, 5, 10, and 20, in order to determine kinetic parameters. Adsorption experiments of TB-EPS were performed by the SPR device. The flow cell was closed bonding to the SAM surface after the SAMs chip was docked inside the SPR device. The SAMs surface was flushed with the PBS at 100 μL/min flow rate for 2–3 min, then the flow rate was adjusted to 20 μL/min until the baseline was stabilized for ~10 min. Every sample was injected at a flow rate of 20 μL/min for 6 min for measuring TB-EPS adsorption on SAM surface, followed by PBS injection again. All measurements were duplicated and carried out at 25 °C.

TB-EPS interacting with R-SAMs, forming the complex (TB-EPS:R-SAM) at the sensor surface, ideally the SPR signal (ΔR) versus time (t) is given by Eq. (2) [23]:

$$\Delta R = \Delta R_{eq}\left(1 - e^{-tk_{obs}}\right), \tag{2}$$

$$k_{obs} = k_a[C] + k_d, \tag{3}$$

where, ΔR_{eq} was the SPR signal of adsorption equilibrium, k_{obs} was the apparent rate constant (1/s). [C] is the TB-EPS concentration (g/L), and k_a is the second-order adsorption rate constant (L/(g s)) and k_d (1/s) is the first-order desorption rate constant. According to Eq. (3), k_a as the slope and k_d as the intercept can be obtained