INITIAL MONOLAYER FORMATION IN MARINE BIOFILMS

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Abstract

Studies of biofilm formation are essential for understanding and control of processes in natural aquatic systems and for industrial application. Experiments with expanding mercury electrode/seawater interface offer an approach to studies of initial monolayer in biofilm formation in seawater. Chronoamperometry of dissolved oxygen was used as a measuring technic. Dynamics of film formation is measured as a time which is necessary to reach a full coverage of the electrode by adsorption of dissolved biomolecules and/or adhesion of cells suspended in aqueous electrolyte solution. Phytoplankton organism *D. tertiolecta*, a free-living bacteria strain and hydrophobic *Acinetobacter* sp. were chosen as model organisms. Dextran and albumin were chosen as dissolved biomolecules, and triton T-X-100 as non-ionic detergent. We have demonstrated direct competition of adsorption of dissolved biomolecules and adhesion of cells in the initial monolayer formation at a freshly exposed surface and a high rate of such processes.

Key-words: bacteria, electrochemistry

Introduction

Zobell in 1943 alerted us to the importance of the attached microbial community in the sea, but until now it is not clear completely how cells sense surfaces nor the relative importance of biochemistry and physico-chemical interaction behind the mechanism of adhesion.

A biofilm consists of cells immobilized at a substratum and frequently embedded in an organic polymer matrix of microbial origin (1, 2). In marine environment marine biofilms cause loss of performance on ships, increase fuel consumption, and corrosion on a ship surface. It has been accepted generally that the first step in the formation of a biofilm on a clean surface is the adsorption of an organic layer onto the surface from the aqueous milieu (2). Adsorbed organic films affect adsorption of other dissolved compounds as well as microbial colonization and subsequent growth on surfaces (3). In mixed population (bacteria and diatoms) each organism had a chance to attached sequentially. In nature, both population, in theory, would have the opportunity to attach simultaneously (2).

Mixtures of dissolved molecules and suspended microbial cells are typical for natural aquatic environment such as seawater. The interaction between the various components in biofilm systems occurs via transport and interfacial transfer processes. For dissolved components it occurs via molecular diffusion and volumetric displacement (including cell motility) for particulate components (3). From applied and fundamental points of view, the transport step is important since insufficient transport can be the limiting factor in biofilm formation (4).

Methods and material

Adsorption of dissolved organic molecules and adhesion of phytoplankton cells at the mercury electrode/seawater interface, which we use as a model, results in coverage of the electrode with organic material that displaces counter ions and water molecules from the interface (Fig. 1). Chronoamperometry of dissolved oxygen at renewable mercury electrode (5, 6) is used as a measuring technique. The experiments were performed under conditions of maximum attraction at positively charged and hydrophobic interface, and enhanced transport to the interface by convective streaming.

Fig. 1. Schematic presentation of interaction of a cell and a biomolecule with positively charged mercury electrode in aqueous solution and the current-time transient (attachment signal).

Phytoplankton organism *Dunaliella tertiolecta* (8-10 μm) has been chosen as model organism because it forms stable suspensions, has fluid cell wall and adhesion of individual cells to the electrode results in well defined electrical attachment signals. Their duration is 0.06-0.2 s, and amplitudes 0.6-2.2 μA (Fig. 2.). Biomolecules dextran and albumin, and nonionic detergent Triton-X-100 were chosen as soluble molecules in concentration range 0.05-500 mg/l. Measurements were performed in seawater and in 0.1 M NaCl.

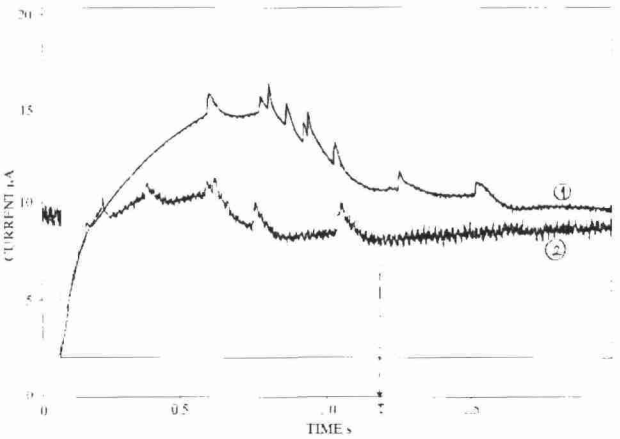

Fig. 2. Current-time curves of oxygen reduction in suspension of *D. tertiolecta* ($5x10^7$/l) alone (curve 1) and in mixture with 15 mg/l dextran sulphate M = 500.000 (curve 2) in 0.1 M NaCl at -400 mV. The spikes are attachment signals of individual cells.

Results and discussion

Dynamics of film formation is measured as a film formation time (τ) (Fig. 2.) which is necessary to reach a full monolayer coverage of the expanding mercury electrode in the time scale 10-2000 ms. Figure 3 shows dependence of film formation in presence of dissolved molecules (dextran sulphate M = 500.000), and in mixture with *D. tertiolecta* cells. Adsorption of dissolved dextran molecules causes a decrease of the current of streaming maximum of oxygen reduction, of attachment signals frequency of cells (Fig. 2.), and a film formation time (τ) (Fig. 3.).

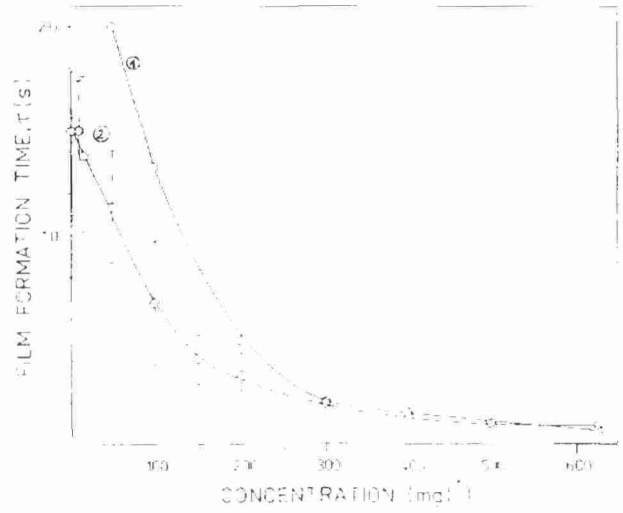

Fig. 3. Dependence of film formation time (τ) on concentration of dextran sulphate M = 500.000 alone (curve 1) and in a mixture with *D. tertiolecta* $5x10^7$/l (curve 2).