Initial Adhesion of *Methanosarcina barkeri* to Support Materials

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Introduction

Microbial adhesion to surface is known to play an important role in a wide variety of situations. In applications such as fermentation, wastewater treatment, water purification, etc, this microbial adhesion phenomenon is positively utilized and useful. Contrastingly, it causes various difficulties in other fields, such as food contamination, medical device pollution, ship fouling, pipeline corrosion, dental decay, etc.

Anaerobic digestion has been widely used in wastewater treatment because of the low consumption of energy, low level of sludge production, and recovery of methane gas as a bio-energy resource. In this process, acetate is the precursor for about 70% of the methane produced during the anaerobic digestion of complex organic compounds[1], and it was found that the decarboxylation of acetate is the rate-limiting step in anaerobic digestion[2]. Therefore, primary attention should generally focus on achieving the most favorable conditions to ensure proper methanogenesis. Under favorable conditions, the multitude of other microbes within the anaerobic consortium will also function well[3]. Therefore, to achieve highly efficient anaerobic treatment, it is necessary to immobilize the acetate-utilizing methanogens in the fermenter. However, the basic mechanisms of immobilization are poorly understood. Adhesion is strongly dependent on the surface characteristics of the microbial cells. Surface physico-chemical properties can be treated as an indicator of the adhesion properties of microbial cells.

In this study, we examined experimentally the effect of the electrostatic and hydrophobic properties of acetate-utilizing methanogens on the immobilization of support materials. *Methanosaeta* and *Methanosarcina* species are the only known methanogens that are capable of acetate catabolism. *Methanosarcina* species are able to convert acetate to methane at a faster rate than *Methanosaeta* species[4]. A pure culture of *Methanosarcina barkeri* was used in the current experiments to investigate the factors that control the immobilization of methanogens.

Materials and methods

Strain and growth conditions

Methanosarcina barkeri (JCM 10043) was purchased from the Japan Collection of Microorganisms and grown anaerobically without shaking at 37 °C within a 120-ml pressure culture bottle containing 40 ml of DSM Medium 120[5]. The bottle was sealed with a butyl rubber stopper and an aluminum crimp seal. The pH of the medium was 6.8, and methanol was used as substrate. To ensure anaerobic conditions, the headspace of the bottle was purged with an oxygen-free 80% N_2–20% CO_2 gas mixture. Methane production was monitored by TCD gas chromatograph (GC-8APT; Shimazu, Kyoto, Japan). The absorbance of the culture (A_{660}) was measured at 660 nm using a spectrophotometer (Mini Photo 10; Sanshin, Yokohama, Japan). Cells were harvested by centrifugation at 10,000 rpm at 4 °C for 10 min and washed in triplicate using 0.9w/v% sterile NaCl aqueous solution. The washed cells were resuspended in the sterile solution to evaluate the physico-chemical properties of the microbial cells.

Support materials

A hydrophilic vinyl polymer particle with a treated surface was used as support material. Three types of support materials were tested: an anion-exchange resin with quaternary ammonium groups (SuperQ-650M; Tosoh, Tokyo, Japan), a cation-exchange resin with sulfopropyl groups (SP-650M), and a hydrophobic resin with butyl group (Butyl-650M). The size of these support materials was 40–90 μm.

Measurements of electrophoretic mobility

The electrophoretic mobility (EPM) of microbial cells was measured using an electrophoretic light-scattering spectrophotometer (ELS-800; Otsuka Electronics, Osaka, Japan). In this procedure, the migration velocity of microbial cells within the electric field of an electrophoresis cell is determined by measuring the Doppler effect using a laser beam. The washed microbial cells were resuspended in the sterile NaCl aqueous solution at the desired concentration and vortexed for 3 min. All measurements were carried out in triplicate.

Measurements of hydrophobicity

The surface hydrophobicity of microbial cells was determined by microbial adhesion to hydrocarbon (MATH) assay[6]. 0.4 ml of hydrocarbon (n-hexadecane) was added to a test tube containing 2.4 ml of the washed cell suspension resuspended in a PUM buffer. The mixtures were vortexed uniformly for 2 min. The solution was then allowed to stand for 15 min to ensure complete separation of the two phases. The percentage of cells adhering to hydrocarbon F, which was used as a measure of cell-surface hydrophobicity, was calculated using the following equation: $F = (1 - A_t/A_0) \times 100$, where A_0 is the initial absorbance of the microbial suspension before mixing, and A_t is the absorbance after mixing.

Microbial adhesion test

The microbial adhesion test of support materials was carried out as follows. The washed cells were resuspended in 100 mol/m^3 of sterile NaCl aqueous solution. The support materials were added to 3.0 ml of cell suspension in a 30-ml test tube, and the test tube was shaken on a reciprocating shaker. The test tube was then allowed to stand for 2 min to completely settle the support materials. The degree of microbial adhesion to support materials (W) was calculated using the following equation: $W = V(C_0 - C_t)/M$, where V is the volume of cell suspension, C_0 is the initial dry weight of cells in the solution, C_t is the dry weight of cells after shaking, and M is the volume of support materials. The dry weight of cells (C) can be calculated using the following equation: $C = 1.28 \, A_{660}$.

Results and discussion

Electrostatic properties of microbial cell

Fig. 1 shows changes in the EPM of *M. barkeri* as a function of ionic strength. The EPM decreased with increasing ionic strength of the cell suspension, and gradually approached the non-zero constant value (-1.45×10^{-8} m^2/V/s). This result indicates that *M. barkeri* is negatively charged at any ionic strength. The majority of microbial cells are generally negatively charged because the carboxyl group and phosphoric acid group at the microbial surface are dissociated at neutral pH.

The EPM of soft particles such as microbes can be expressed as follows[7]:

$$u = \frac{\varepsilon_r \varepsilon_0}{\eta} \frac{\psi_0 / \kappa_m + \psi_{DON} / \lambda}{1/\kappa_m + 1/\lambda} + \frac{ZeN}{\eta \lambda^2} \tag{1}$$

where u is the electrophoretic mobility, ε_r is the relative permittivity of the medium, ε_0 is the permittivity of a vacuum, η is the viscosity of the medium, ψ_{DON} is the Donnan potential of the polymer layer, ψ_0 is the potential at the boundary between the surface region and the solution, κ_m is the Debye–Hückel parameter of the polymer layer, λ is the softness parameter, which has dimensions of reciprocal length, Z is the valency of the charged groups within the polymers, e is the electron charge, and N is the density of the charged group.

The EPM of *M. barkeri* was fitted using the Ohshima equation with two unknown parameters: ZeN and λ. The parameter ZeN represents the spatial charge density in the polyelectrolyte region, and the parameter $1/\lambda$ has a length that can be regarded as the "softness" parameter of the microbial surface. Fig. 1 shows the theoretical curve calculated using the following parameter: $ZeN = -1.15 \times 10^6$ C/m^3, $1/\lambda = 3.35 \times 10^{-9}$ m.
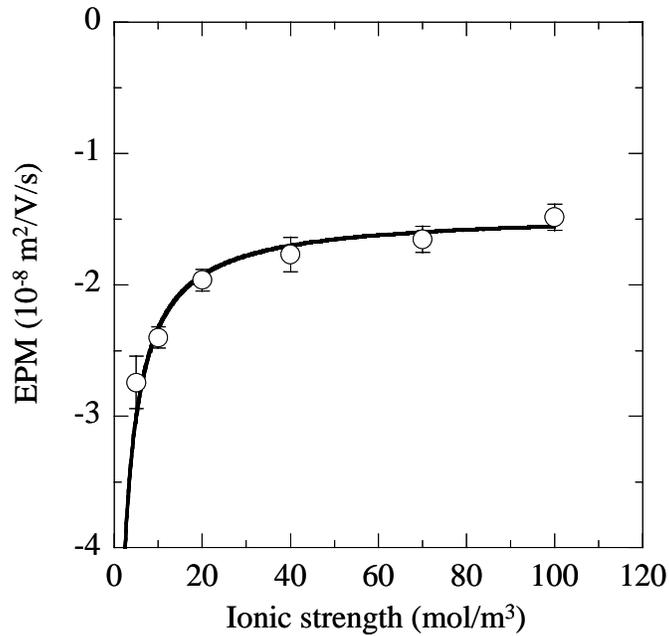

Fig. 1 Change in the EPM of *M. barkeri* as a function of ionic strength.

Fig. 2 shows the surface potential of *M. barkeri* calculated using the Ohshima and Smoluchowski equations as a function of ionic strength. At high ionic strength, the two surface potential values are surprisingly different. For example, when the ionic strength of the cell suspension is 100 mol/m^3, the surface potentials calculated using the Ohshima and Smoluchowski equations are –0.77 mV and –19.0 mV, respectively. Thus, to accurately evaluate surface potential it is necessary to consider the effect of charged polymers at the surfaces of microbial cells.
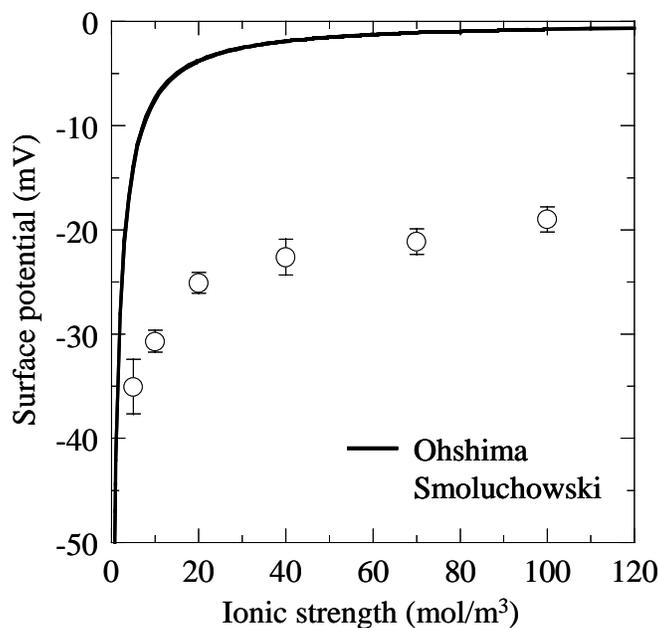
Fig. 2 Surface potential of *M. barkeri* calculated using Ohshima and Smoluchowski equations.

Hydrophobic properties of microbial cell

Fig. 3 shows microbial adhesion to hydrocarbons as a function of the initial absorbance of the cell suspension. *Escherichia coli* (JM 109) was used as a control. *M. barkeri* showed an affinity to n-hexadecane: the adhesion to n-hexadecane was greater than 60% when the initial absorbance of the cell suspension was low. The degree of adhesion decreased with increasing initial absorbance because microbial cells were sufficiently unable to adhere to the hydrocarbon. *E. coli* did not show any affinity to n-hexadecane. We speculate that *M. barkeri* is relatively hydrophobic compared with *E. coli*.
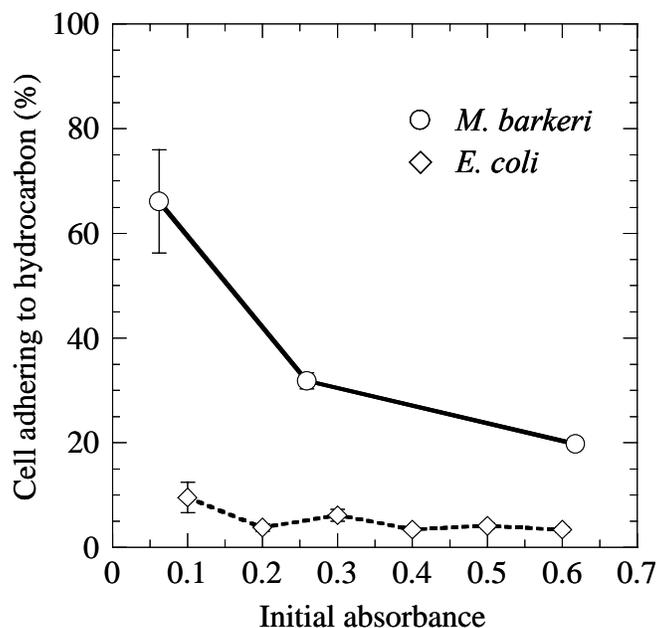

Fig. 3 Microbial adhesion to n-hexadecane as a function of the initial absorbance of the cell suspension.

Microbial adhesion to support materials

Fig. 4 shows the adhesion of *M. barkeri* to support materials as a function of the equilibrium concentration of the cell suspension. *M. barkeri* showed a stronger adhesion to the anion-exchange resin than to the hydrophobic resin, and did not adhere well to the cation-exchange resin. The cells were suspended in 100 mol/m^3 sterile NaCl aqueous solution. At this concentration, the surface potential of microbial cells was –0.77 mV. This result indicates that the Coulomb force interacts only weakly between the cell and support material. The surface of the microbial cells contains functional anionic groups such as the carboxyl group and the phosphoric acid group. It appears that the ion-exchange reaction between anions of the resin surface and anionic functional groups of the cell surface occurs when the cell approaches the anion-exchange resin surface. Furthermore, the cell is relatively hydrophobic. It appears that the cells increasingly adhere to the hydrophobic resin with increasing cell concentration because the hydrophobic interaction is a short-range force. The anion-exchange resin is most effective in immobilizing *M. barkeri* within the fermenter.
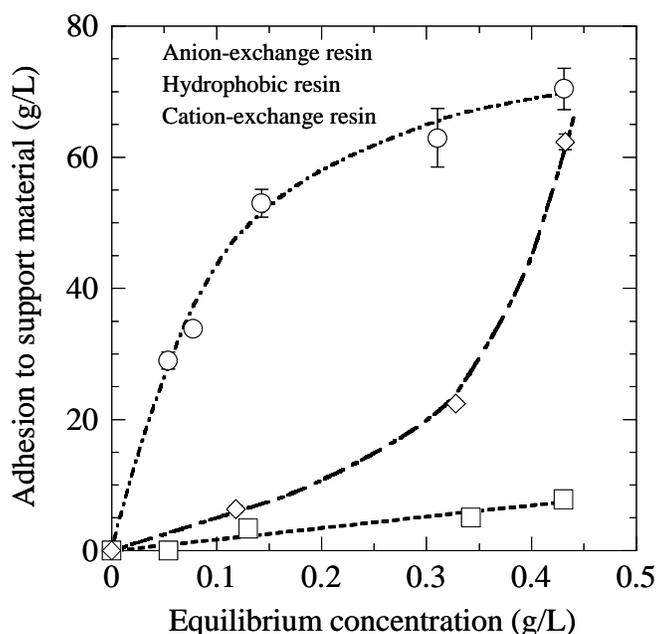

Fig. 4 Adhesion of *M. barkeri* to support materials as a function of the equilibrium concentration.

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