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Kinetic adhesion of bacterial cells to sand: Cell surface properties and adhesion rate

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Abstract

Correlation between microbial surface thermodynamics using the extended DLVO (XDLVO) theory and kinetic adhesion of various bacterial cells to sand was investigated. Two experimental setups were utilized. Adhesion tests were conducted in batch reactors with slow agitation. Also, bacteria were circulated through small sand columns in a closed loop and the results were analyzed with a simple model which accounted for the rate of the adhesion phenomena (ω in h^{-1}) and adhesion percentage. Cells surface properties were derived from contact angle measurements. The wicking method was utilized to characterize the sand. Zeta potentials were measured for the sand and the cells. Kinetic of bacterial retention by the porous media was largely influenced by the electrostatic interactions which are correlated with ω from the model ($R^2 = 0.71$). Negative zeta potentials resulted in electrostatic repulsions occurring between the sand and the bacterial cells which in result delayed bacterial adhesion. While no correlation was found between the adhesion percentage and the total interaction energy calculated with the XDLVO theory the respective behavior of hydrophobic and hydrophilic bacteria as well as the importance of electrostatic interactions was evidenced. All the bacterial strains studied adhered more in the column experiments than in the adhesion tests, presumably due to enhanced collision efficiency and wedging in porous media, while filtration could be ignored except for the larger *Bacillus* strains. Approximate XDLVO calculations due to solid surface nanoscale roughness, retention in a secondary minimum and population heterogeneity are discussed. Our results obtained with a large variety of different physicochemical bacterial strains highlights the influence of both surface thermodynamics and porous media related effects as well as the limits of using the XDLVO theory for evaluating bacterial retention through porous media.

Keywords: Bacteria; Adhesion; Transport; Sand; XDLVO

1. Introduction

The transport and fate of microbial particles in subsurface environments is a significant stake in both bioremediation and drinking water contamination [1]. The consequences related to the displacement of bacterial cells in the soil can cause many environmental [2–7], agronomic [8] and health related problems [9]. Several contaminations of water sources with micro-organisms were most likely due to bacterial transport through the unsaturated zone [3]. However the displacement of micro-organisms in a soil profile is still poorly understood. Increasing experimental evidences suggest that bacterial transport is strongly influenced by cell characteristics [10]. Cell surface physicochemical properties and its biochemical composition seem to be closely related to the surface thermodynamics which are well known to play an important role in cell to solid surface interactions which will affect bacterial transport [11,12]. Marshall and colleagues [13] in a pioneering work were the first to use the classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory to describe microbial adhesion to solid surfaces. The DLVO theory has been widely used since that time to estimate the net interaction between bacteria cells and inert surfaces resulting from the addition of Lifshitz–van der Waals (LW) and electrostatic interactions (EL). Later Van Oss developed the extended the DLVO theory (XDLVO) by adding the Lewis acid–base interactions (AB) [14]. The polar Lewis acid–base or electron-acceptor/electron-donor interactions are responsible for all non-electrostatic, non-covalent, polar interactions occurring in water [15]. LW, AB and EL interactions can be influenced by cell modifications and properties such as the physicochemi-

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cal state [16], extracellular polymeric substance (EPS) [17–19], C/N ratio in nutrients [20], lipopolysaccharides (LPS) production [21,22], motility [23,24] or shape [25]. The surface of living cells is chemically complex and heterogeneous which complicates the physicochemical approach for the comprehension of bacterial adsorption phenomena on a solid surface. It has already been demonstrated that hydrophobicity, which can be described by AB interactions for biological compounds, is the driving force for bacterial deposition and is usually one order of magnitude greater than EL or LW interactions [26,27]. However contradictory reports show that correlation between the XDLVO theory and bacterial transport can either be good [28,29] or poor [30,31]. Although not investigated in this paper, physical factors (porous media, temperature, flow velocity) and chemical factors (ionic strength, ion species, pH) are also well known for their impact on bacterial adsorption mechanisms.

Predicting the transport of bacterial cells or colloids in a porous media requires the attachment and detachment processes as well as the straining and liberation mechanisms to be modeled. Most studies [12,28] employ transport models based on the convection dispersion with various types of adhesion sites. Usually, one type is assumed to be instantaneously and reversibly in equilibrium with the aqueous concentration leading to the concept of retardation factor [28]. Adsorption on other sites is kinetic, and can be reversible or not [12]. The latter process is often referred as *deposition* and the coefficient controlling its rate is usually fitted together with other parameters (partition coefficients for equilibrium and kinetic site types) so that the model correctly reproduces the observed breakthrough curves [28]. The deposition coefficient can also be simply obtained experimentally from the fraction of micro-organisms recovered at the outlet of the soil column [32]. This method was used by Chen and Strevett [27]. As pointed out by Tufenkji et al. [33], the deposition factor may vary with time and space due to coverage of solid particle by attaching cells; which modifies the interactions between the cells and the porous medium. Chen and Strevett [27,28] and Chen et al. [34] reported laboratory experiments that clearly illustrate that bacterial cells transport through porous medium is largely controlled by adhesion (instantaneous and kinetic) and also that the deposition coefficient varies from a bacteria to another. In addition, they found that the total free energy of interaction evaluated by the DLVO was correlated with the deposition coefficient obtained from their column experiments.

In this study we are interested to further investigate how surface properties of a large number of bacteria influence attachment kinetics to sand. This work differs from previous column experiments in that a large variety of strains with different physicochemical properties (i.e. hydrophobicity and electrophoresis characteristics) were used. Our goal was to relate the rate of adhesion and the percentage of adhesion to Lifshitz–van der Waals, Lewis acid–base and electrostatic interactions. The impact of cell size and shape on retention in the porous media was also analyzed. In order to measure porous media related effects on bacterial adsorption, simple adhesion tests (batch with gentle agitation) and small column experiments were compared. Our goal is also to evaluate the possibility of using an experimental setup that requires less time and work than a traditional column experiment for evaluating bacterial cells deposition in a porous medium.

2. Materials and methods

2.1. Bacterial strains and solid phase

All the bacterial strains and their origins used in this work are listed in Table 1. Some of the strains listed are uncommon to soil environments such as bacteria from the *Streptococcus* and the *Lactococcus* families but their particular membrane properties (i.e. hydrophobicity and low zeta potential) were chosen in order to obtain a large variety of cell surface characteristics. All the *Streptococcus* strains were kindly given by the National Institute for Agricultural Research in Paris (INRA, France). The *L. lactis* NDCO2118 strain used in this study was isolated from frozen peas and was given by the National Collection of Dairy Organisms (France). Cell size measurements were performed with a Canon BX microscope. Results are listed in Table 1.

The bacteria were grown in half diluted Luria–Bertani (1/2LB) media or M17 with 10% lactose media for the lactic acid bacteria at 30 °C. The bacteria were first cultivated in 10 ml of their respective media for 6 h. About 0.1 ml of the first culture was used to inoculate 50 ml of 1/2LB or M17 and incubated for overnight culture.

The porous media used in this study was a sand called “sand of Fontainebleau” (south Paris, France) (Merck, grain size: Ø 230–310 μm). This sand is very homogeneous (over 99.6% silica) and is composed of quartz grains. Before use, the sand was thoroughly rinsed with milliQ water on a 40 μm filter (VWR international, 11 cm, type 417) then heat treated and oven dried for at least 2 h at 120 °C. For each experiment the sand was renewed in the columns.

2.2. Surface properties of cells and porous medium

Bacterial and sand surface thermodynamic properties can be described by their surface energy. According to the extended DLVO theory, bacterial and sand surface tension is mainly composed of an apolar component (i.e. Lifshitz–van der Waals: LW), a polar component (i.e. Lewis acid/base: AB) and an electrical component (EL) [35]:

$$\Delta G = \Delta G^{\mathrm{AB}} + \Delta G^{\mathrm{LW}} + \Delta G^{\mathrm{EL}}$$

Attraction between two surfaces occurs when ΔG is negative and repulsion occurs when ΔG is positive. Bacterial LW and AB components of surface energy were estimated by contact angle measurements following the method described by Grasso et al. [36]. The Dupré–Young equation relates the contact angle made by a drop of liquid (L) deposited on a flat solid (S), to the surface energy and the interfacial tension of the liquid and the flat solid:

$$(1 + \cos\theta)\gamma_{\mathrm{L}} = 2\left(\sqrt{\gamma_{\mathrm{S}}^{\mathrm{LW}}\gamma_{\mathrm{L}}^{\mathrm{LW}}} + \sqrt{\gamma_{\mathrm{S}}^{+}\gamma_{\mathrm{L}}^{-}} + \sqrt{\gamma_{\mathrm{S}}^{-}\gamma_{\mathrm{L}}^{+}}\right) \quad (1)$$