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Interaction forces between spores and planar surfaces in aqueous solutions

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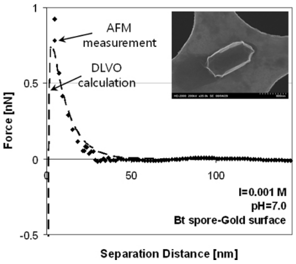
HIGHLIGHTS

- Bacterial spore interactions with planar surfaces were studied in aqueous solutions.
- AFM measured adhesive forces of the spore are compared with modeling results.
- Classical DLVO theory can explain the behavior of spores in aqueous environments.
- Surface roughness must be considered for accurate prediction of the adhesive force.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacterial spore interactions with planar surfaces in aquatic environments, including adhesive forces and force–distance profiles, are influenced by the geometry and physicochemical properties of the system. The characteristics of spores of *Bacillus thuringiensis* (*Bt*) are determined using electron microscopy and electrokinetic measurements. The average size of the spores is 1.57 μm long and 0.86 μm wide, and the zeta potential values are negative for the solutions used in this work. The zeta potentials of the spores and mica surfaces used in the experiments are measured as a function of pH and ionic strength. The Derjaguin, Landau, Verwey and Overbeek (DLVO) theory is employed to predict the interaction force between the spores and planar surfaces as a function of the separation distance, and a force balance is used to explain the adhesive force. Theoretical estimations are compared to experimental measurements obtained from atomic force microscopy (AFM). The DLVO-based calculations are consistent with AFM force measurements, while the calculated adhesive force shows some deviations from the measurements. The deviations can be minimized by considering the roughness of the *Bt* spore and substrate surfaces. Results are important in the understanding of spore interactions with environmental surfaces in aquatic systems.

1. Introduction

The behavior of microbial colloids in aquatic environments has been the focus of several studies because biocolloids are contaminants in groundwater or subsurface systems and they can also facilitate the transport of other contaminants, such as heavy metals. Transport of bacterial spores was investigated by laboratory-scale soil column experiments and field-scale experiments under saturated or unsaturated conditions [1–4]. The factors affecting the process of spore or bacterial transport were also investigated [5,6]. An advection-dispersion model of microbial colloids was tested in a filtration process [7]. These studies have applications in groundwater contamination, subsurface bioremediation, subsurface ecology, water-treatment system design, and risk assessment of pathogen

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contamination. Microbial interactions are important in understanding how the microorganisms adhere to such common surfaces as those of soil particles, metal, or glass in natural and engineered environments and how they are redispersed in aquatic environments.

Bacterial spores are a dormant form of microorganisms during their life cycle. Spores have a coat, their most outer layer, as a structural support and a barrier against external conditions [8]. Inside the coat, the core contains DNA, ribosome, and enzymes, as well as protein, calcium, and dipicolinic acid, which provide resistance to external stimuli such as heat, oxidizing agents, or UV radiation. The properties of the spores allow them to travel through either atmospheric or aquatic environments for a relatively long time and distance compared to the transport of their active form. This behavior can explain a global transport or dispersion of microbial colloids related to microbial ecology. Microbial colloids can also be used as a tracer. Biocolloids, including bacterial spores, for example, can be used as a tracer for sewage dispersion [9].

There are many mechanisms contributing to the interaction force between two surfaces. The main interaction force components between two objects, such as a colloidal-size particle and a planar surface, are the van der Waals force, electrostatic interaction, acid–base interaction, and steric forces [10,11]. Electrostatic interaction occurs when the interacting bodies are electrically charged. Traditionally, the electrostatic force in an aqueous solution is calculated by using the electrical double layer theory. The electrostatic force may be a significant component for objects with a large surface area to volume ratio. The double layer is formed by the depletion of coions and accumulation of counterions, as a consequence of the surface charge. Near the surface exposed to the liquid, two layers are formed. Hydrated counterions adsorb onto the surface, building an inner layer called Stern layer, while the outer layer is called the diffuse layer. The Lifshitz–van der Waals force arises from the interactive forces between the permanent or instantaneous dipoles in molecules and, for macroscopic bodies, it is calculated using the Derjaguin approximation. While the van der Waals force appears between molecules in both polar and nonpolar systems, the acid–base interaction plays a role only in polar systems. Combination of these forces determines the total interaction force between a particle and a planar surface. The contribution of each component varies with the properties of the medium and the particle/planar-surface system such as type of materials, shape, and charge density.

Adhesion of a spore or a bacterial cell onto surfaces has been previously studied [12,13]. The magnitude of the adhesive force and the effects of the material properties on the adhesion have been investigated; however, the components of the total force have not been fully examined. In this study, the forces between a *Bacillus thuringiensis* (*Bt*) spore and planar surfaces of mica and gold are investigated. Within the *Bacillus* genus, *Bt* is a member of the *Bacillus cereus* group, which includes the pathogens *B. cereus* and *Bacillus anthracis* that are potentially dangerous to human health. *B. thuringiensis* is a reasonable representative of the *B. cereus* group and an excellent substitute of the dangerous *B. anthracis*. Mica and gold surfaces are also good model surfaces in terms of surface charge. Mica is an example of a surface that can be found in natural systems and is known to have a high surface charge in aquatic environments. Gold is chosen as an electrically conductive material, representing metal surfaces found in engineered environments. The aim of the research is to provide a better understanding of the interaction forces between spores and model surfaces in aquatic environments.

Quantifying these interactions will enable the development of effective removal techniques for biological particles, including pathogenic *Bacillus* spores, from contaminated surfaces in aquatic systems.

2. Experimental methods

2.1. Spore characterization: Size and zeta potential measurements

The size of *Bt* spores was measured using electron microscopy. Images of the spores were taken by scanning electron microscopy and scanning/transmission microscopy, and the images showed that the size of the spores varies. More than 50 spore sizes were acquired from at least five different images and averaged. The zeta potential of *Bt* spores and mica particles was measured by a zetameter (ZetaPlus, Brookhaven Instruments Corporation, Holtsville, NY) in solutions of varying pH and ionic strength. For such solutions, the measurements were repeated 15 to 25 times and averaged values were obtained. Three pH values (4.5, 7.0, and 9.5) adjusted by addition of HCl or NaOH solution and three ionic strengths (0.0001, 0.001, and 0.01 M) of sodium chloride were considered resulting in nine different conditions from the three by three combinations of the variables considered.

2.2. AFM force measurements

B. thuringiensis spores were purchased from Raven Labs (Omaha, Nebraska). Details of the preparation of the spore AFM probes can be found elsewhere [14]. In brief, drops of spore suspension were air-dried on a filter paper, and the spores were transferred onto a micro-cantilever. Epoxy glue was used to fix a single spore on a tipless AFM cantilever. Some types of spores including *Bt* spores come with exosporium, while other types do not. It has been reported that the presence of the exosporium plays a significant role in the spore adhesive force [15]. The spring constant of the spore probe was measured after mounting of the spore, with typical values close to 0.3 N/m. The *Bt* spore probes prepared, as well as various substrates such as mica and gold, were used for measurements with a multi-mode atomic force microscope (Veeco, Plainview, NY) to yield the interaction force between a spore and a substrate. Two types of forces can be measured by the AFM force mode between two objects; adhesive force and force–distance profile. The force–distance profile is obtained as the AFM probe approaches the substrate, and the adhesive force is measured from the maximum deflection of the cantilever when the probe retracts from the substrate. A number of 1024 data points were recorded for each force–distance curve, as the probe approached toward and retracted from the substrate.

3. Theoretical considerations

3.1. Adhesive forces

The adhesive force can be modeled with the assumption that the particle is attached onto the planar surface at a distance of an intermolecular spacing from the surface. In previous studies by the authors, the adhesive force between a spherical particle and a planar surface in atmospheric environments was modeled as the addition of van der Waals, capillary, and electrostatic forces [14,16]. In an aquatic system, however, the capillary force that is due to water condensation between the surfaces does not apply. Therefore, in theoretical considerations, the adhesive force between a particle and a flat surface has been assumed to consist of van der Waals and electrostatic interactions.

The van der Waals force has been mathematically expressed by Hamaker and Lifschitz by the integration of all interactions between atoms or molecules of the objects [10]. For aqueous systems, the Hamaker constant needs to be adjusted to take into account the presence of water molecules in between the interacting