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Laboratory results and mathematical modeling of spore surface interactions in stormwater runoff

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Abstract

Development of numerical models to predict stormwater-mediated transport of pathogenic spores in the environment depends on an understanding of adhesion forces that dictate detachment after rain events. Zeta potential values were measured in the laboratory for *Bacillus globigii* and *Bacillus thuringiensis kurstaki*, two common surrogates used to represent *Bacillus anthracis,* in synthetic baseline ultrapure water and laboratory prepared stormwater. Zeta potential curves were also determined for materials representative of urban infrastructure (concrete and asphalt). These data were used to predict the interaction energy between the spores and urban materials using Derjaguin-Landau-Verwey-Overbeek (DLVO) modeling. *B. globigii* and *B. thuringiensis kurstaki* sourced from Yakibou Inc., were found to have similar zeta potential curves, whereas spores sourced from the U.S. military's Dugway laboratory were found to diverge. In the ultrapure water, the modeling results use the laboratory data to demonstrate that the energy barriers between the spores and the urban materials were tunable through compression of the electrical double layer of the spores via changes of ionic strength and pH of the water. In the runoff water, charge neutralization dominated surface processes. The cations, metals, and natural organic matter (NOM) in the runoff water contributed to equalizing the zeta potential values for Dugway *B. globigii* and *B. thuringiensis kurstaki*, and drastically modified the surface of the concrete and asphalt. All DLVO energy curves using the runoff water were repulsive. The highest energy barrier predicted in this study was for Dugway *B. globigii* spores interacting with a concrete surface in runoff water, suggesting that this would be the most challenging combination to detach through water-based decontamination.

Keywords

Stormwater; DLVO; spores; zeta potential; fate and transport; natural organic matter

Introduction

Over 200 species of bacteria form spores which are capable of surviving in adverse environments for many years (Gopal et al. 2015). Spore-formers are highly prevalent in soils and the gastrointestinal tracts of warm-blooded animals (Griffin et al. 2014). Since spore-forming bacteria are resistant to extreme pressure, temperature, drought, famine, and biocides, they often present a challenge in disinfection efforts, such as hospital sterilization, and in food preservation (Rutala, Gergen, and Weber 1993, Weber et al. 2010, Magnusson, Christiansson, and Svensson 2007). Some species of spore-forming bacteria are particularly dangerous to humans, and contact can result in severe illness and even death. *Bacillus anthracis*, the causative agent of anthrax, is a global public health concern, both as a naturally occurring contaminant and as a biological weapon (Guh et al. 2010, D'Amelio et al. 2015, Sinclair et al. 2008, Weis et al. 2002, Meselson et al. 1994). Due to *B. anthracis*'s bioterrorism history, many researchers have studied its' dispersion in an aerosolized form and have evaluated the disinfection efficacy of decontaminants against *B. anthracis* spores (Wood et al. 2011, Szabo et al. 2017, Reshetin and Regens 2003).

During a contamination incident (*e.g.,* a terrorist attack), spores could be deposited over a large urban area, where they could persist for years. After the initial contamination event, spores can be redistributed even more broadly throughout the urban environment due to rain and wind, as well as by humans through vehicular and foot traffic. Developing numerical models that include the stormwater-driven fate and transport of spores can be useful to predict contamination extent as it evolves over time during emergency response and recovery, informing activities such as creating sampling maps, deciding where to stage waste, and developing strategic decontamination plans (Mikelonis et al. 2018). Conventional stormwater models have been used to predict water quality concentrations of common contaminants, such as solids or metals, at various points in a conveyance system (McPherson et al. 2005, Smith et al. 2007, Tiveron, Gholamreza-Kashi, and Joksimovic 2017, Aceves, Fuamba, and Angui 2017, Lee, Heaney, and Pack 2010). However, existing models have not been used to model the fate and transport of low probability, high consequence pathogens, such as *B. anthracis*. Therefore, laboratory and field studies are necessary to quantitatively represent these contaminants in stormwater models.

Since working with *B. anthracis* spores directly poses risks to laboratory workers, surrogates have been used for many different types of research experiments (Greenberg et al. 2010); however, a de-facto surrogate for stormwater-based transport studies does not exist. This paper presents a comparison of surface charge changes for two frequently used surrogates, *Bacillus globigii* and *Bacillus thuringiensis kurstaki*, in both an amended baseline ultrapure water and two stormwater runoff matrices. We also calculate the microorganisms' corresponding energies of interaction with two types of roadway materials to better understand the dominant environmental conditions influencing adhesion to urban impervious surfaces. A better understanding of these adhesion mechanisms will improve model representations and parameterizations of stormwater-driven transport processes related to spore-forming biological contaminants.

Selecting an appropriate surrogate for a specific research intention is not a straightforward task and often involves compromise. Both *B. globigii* and *B. thuringiensis kurstaki* have been used as a surrogate for *B. anthracis* in aerosol research (Van Cuyk et al. 2011, Layshock et al. 2012). In more recent literature, *B. thuringiensis kurstaki* has been recommended as the most representative aerosol surrogate due to its similar shape, hydrophobicity, and exosporium with the presence of a hairy nap

(Tufts et al. 2014). On the other hand, water-based experimental work (focused on drinking water treatment processes) has primarily used *B. globigii* as a surrogate (Szabo and Minamyer 2014, Szabo, Rice, and Bishop 2007). *B. globigii* is more resistant to chlorine than *B. anthracis* and is therefore considered a conservative surrogate for drinking water disinfection studies (Brazis et al. 1958). *B. globigii* is also an attractive surrogate for aqueous transport applications because it produces orange-colored colonies when cultured, which enables enumeration in environmental samples with high background levels of microorganisms. However, unlike *B. anthracis*, *B. globigii* does not have an exosporium with a hairy nap, which may impact how accurately it represents *B. anthracis* in transport processes.

The behavior of colloids in water treatment plants, ground waters, and surface waters has been well studied and may be drawn from for conceptual application to spores (Hermansson 1999, Ryan and Elimelech 1996, Elimelech, Gregory, and Jia 2013, Kim, Nason, and Lawler 2006). These, and many other researchers, have used the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of aqueous colloidal stability to predict the conditions under which particles adhere to each other and/or surfaces. DLVO theory is a summation of van der Waals interactions, typically attractive, and electrical double layer interactions (EDL), typically repulsive, to predict the energy of the interaction between two surfaces (Derjaguin 1940, Verwey, Overbeek, and Overbeek 1999).

Our study focuses on comparing how *B. globigii* and *B. thuringiensis kurstaki*'s zeta potential measurements change in different environmentally relevant stormwater conditions and understanding how these changes influence the DLVO predictions of spore adhesion to asphalt and concrete (*i.e.,* how do these spores behave in stormwater runoff and puddles on infrastructure such as roads and sidewalks). Stormwater quality varies substantially by location and is potentially affected by factors such as nearby land use, climatic zone, season, and the percentage of impervious cover in the drainage area (Pitt, Maestre, and Morquecho 2004). To produce a "typical" reproducible laboratory synthetic runoff water representative of cities in the United States, we included low levels of metals, two levels of soil humic acid (one representative of a more concentrated 'first flush', and a second representative of more prolonged runoff), a combination of naturally occurring salts, and used the median value of hardness (38 mg/L $CaCO_3$) from version 4.02 of the National Stormwater Quality Database (Pitt, Maestre, and Clary 2018) (Table 1). Spore surface properties can be affected by the growth media and processing (*e.g.,* sonicating), even for the same strain (White et al. 2014). The outer structures of *B. anthracis,* and its surrogates that include an exosporium, are a complicated landscape of glycoproteins (Lequette et al. 2011). For this research, both *B. globigii* and *B. thuringiensis kurstaki* from two different sources, Yakibou Inc. (Apex, North Carolina, USA) and the U.S. Army Dugway Proving Ground laboratory (Dugway, Utah, USA), were tested in the initial subset of ultrapure baseline experiments. Spores from Dugway were then subsequently used in experiments with the stormwater matrices. The spores produced by Dugway are milled (both *B. globigii* and *B. thuringiensis kurstaki)* and fluidized (only *B. globigii)*.

Materials and Methods

Preparation of Spores

The spores from Yakibou Inc. (Apex, North Carolina) were received in a 29% ethanol solution. *B. globigii* (ATCC #9372) had an as-received mean population (heat shock value) of 3.1 x 10^8 spores/mL. *B. thuringiensis kurstaki* (ATCC #33679) had an as-received mean population (heat shock value) of 2.2 x 10^8 spores/mL. Spores from the United States Army's Dugway Proving Ground (Dugway, Utah) were originally received as dry powders. The Dugway *B. globigii* was milled and fluidized during production whereas the *B. thuringiensis kurstaki* was only milled. The powders were resuspended in a 20% ethanol solution. Stock solutions of Dugway *B. globigii* had a mean population of 3.5 x 10^8 spores/mL, and *B. thuringiensis kurstaki* had a mean population of 7.0 x 10^8 spores/mL. Spores were stored at 4 ^{o} C, and populations were checked the week of experimentation by plating samples onto tryptic soy agar (BD DIFCO, Houston, TX), incubating at 35 ± 2 ˚C for 18 ± 3 hours, and enumerating colony forming units.

Water recipes

Four different waters were used during this research (Table 1). A Sartorius CP3246 (Goettingen, Germany) and an OHAUS HSR 011 Adventurer Pro (Parsippany, New Jersey, USA) analytical balances were used to weigh all dry chemicals. The potassium chloride (KCl), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), potassium nitrate (KNO_3), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), and sodium chloride (NaCl) were all ACS grade reagents.

Metals were included in the synthetic stormwater matrices, because stormwater in urban areas usually contain copper, zinc, lead, cadmium, chromium, and/or nickel leached from car parts. Copper and zinc were selected as representative metals because they demonstrate markedly different chemical behaviors regarding speciation and complexation with environmental chelators. Metals such as lead and nickel behave much like copper and complex strongly with organic matter, whereas cadmium and zinc predominantly exist as free divalent ions (Dean et al. 2005). The $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$ were 1,000 mg/L ACS grade ICP-MS standard solutions in 3% nitric acid. Solutions were prepared in Millipore 18.2 MΩ-cm ultrapure water.

NOM was included in the form of soil humic acid to represent build up and washoff of solids during stormwater runoff processes. The NOM used in this work was an Elliott soil humic acid standard IV (International Humic Acid Substances Society, St. Paul, Minnesota, USA). A stock solution of NOM was made by dissolving the humic acid in ultrapure water at a pH of 9.22 (adjusted using ACS grade NaOH) and stirring overnight. The stock solution's total organic carbon concentration was then measured using a Shimadzu Total Organic Carbon Analyzer (Kyoto, Japan) using glassware baked at 120 ^{o} C and a total carbon standard stock of 1000 mg/L potassium hydrogen phthalate diluted for standard points at 0, 40, 80, 120, 160, and 200 mg/L carbon. The instrument calibration had an R^2 value of 0.9984 and the stock NOM standard diluted to the target concentration for the synthetic stormwater was determined to have a concentration of 54.02 mg/L carbon.

Zeta Potential Measurements

Spore zeta potential measurements were performed using a ZEN3600 Malvern Zetasizer (Malvern, England) and the Malvern DTS1070 sample cell. Malvern DTS 1235-42 zeta potential standards were used each day of measurement to ensure the instrument was working properly. The standard's acceptable range (-42 mV +/- 4.2 mV) was achieved to proceed with measurement collection on the spore samples. Per recommendation of the manufacturer, the sample cells where flushed on initial use with ethanol once followed by five flushes with deionized water. A unique sample cell was used for the four different spore strains/sources, and in between each measurement point, the cell was flushed three times with deionized water and one time with the sample solution.

Spores were prepared for measurement by sonicating for one minute followed by vortexing for ten seconds and diluting the stock spore solutions in deionized water to a concentration of 10^6 spores/mL. A 2.5 M stock solution of KCl was used to adjust the ionic strength to either 10 mM or 100 mM. NaOH and HCl were used for pH adjustment (and accounted for in ionic strength calculations). A daily 3-point calibration was performed on the pH probe and all experiments achieved R^2 values greater than 0.95.

For obtaining the zeta potentials of roadway materials, concrete (Quikrete concrete, Home Depot, Durham, NC, USA) and asphalt (North Carolina Department of Transportation, Youngsville, North Carolina, USA) were pulverized using an angle grinder and a blade (SKil 9295-01 4.5 inch, Lowes, Raleigh, North Carolina, USA) inside a blasting chamber with a hand glove compartment. The generated particles were then sieved (using mesh opens of 1000 µm, 500 µm, 250 µm, and 106 µm), and the fraction of particles collected between the 250 µm and the 106 µm mesh were used for streaming potential measurements. These particles were soaked and rinsed with water prior to measurement to remove any finer particles and dust. Streaming potential measurements were collected on a SurPASS electrokinetic analyzer (Anton Paar, Ashland, Virgina, USA) using approximately 3 g of compacted pulverized sample in the cylindrical cell SurPASS sample holder accessory. A pressure ramp from 600 mbar to 200 mbar was employed for the test, and the pH of the electrolyte solution was adjusted by addition of 0.05 M or 0.1 M NaOH or 0.05 M or 0.1 M HCl (lower molarities were used for the stormwater matrices pH adjustment). At each pH condition, the sample was rinsed with the electrolyte solution for five minutes prior to determining the zeta potential.

DLVO Modeling Theoretical Calculations

DLVO calculations depend upon the ionic strength of the bulk solution, valency of ions, the distance between the particle and the surface, the Hamaker constant (a material property that describes the strength of interactions between surfaces and bulk solution), and the charge of the two interacting surfaces. DLVO theory is "extended" when it includes adjustments for characteristics such as surface roughness, steric interactions from features such as appendages, magnetic interaction, and/or short-range acid base reactions. Vegetative bacteria and spores have been considered inert particles for adhesion modeling purposes, although, not to our knowledge, in stormwater matrices (Chung et al. 2009, Chung, Yiacoumi, and Tsouris 2014, Chen et al. 2010).

Based on the experimentally measured zeta potentials of the spores and the roadway materials, the interaction energy (*i.e.*, DLVO energy) between them were modeled. Spore shape was approximated as a rod with the cylindrical radius of 0.6 µm, and van der Waals (vdW) attraction and electrical double layer (EDL) repulsion were calculated from cylinder-flat surface interaction equations provided by (Israelachvili 2011). The total interaction energy (J) as a function of the separation distance between the spore and the roadway material is the sum of vdW and EDL interactions. The vdW interaction energy was calculated using the following equation:

$$E_{vdW} = \frac{-A_H\sqrt{R}}{12\sqrt{2}D^{2/3}} \qquad \text{Eqn. (1)}$$

where, A_H is the Hamaker constant, R is the cylindrical radius, and D is the separation distance. The effective Hamaker constant (*i.e.*, a geometrical mean of the Hamaker constants of the two-interacting surfaces in a medium) was used (Elimelech et al., 1998). Previously reported Hamaker values for water, spore, and roadway materials were used in the calculations (Brown and Jaffé 2006, Lomboy et al. 2011, Morrison, Hedmark, and Hesp 1994, Israelachvili 2011). The effective Hamaker constants were 1.5E-20 J and 6.1E-21 J for spore-water-asphalt and spore-water-concrete scenarios, respectively.

For the EDL interaction, the linearized Poisson-Boltzmann equation is used instead of a numerical solution for the following reason. The linearized Poisson-Boltzmann equation can minimize the numerical burden on modeling while providing a reasonable approximation to exact solutions if the surface potential is less than |25.7 mV| (Benjamin and Lawler 2013). Most spore-roadway interaction scenarios considered and presented in this paper had low surface potential values that satisfied such condition. However, it should be noted that Dugway *B. globigii* spores in the low ionic strength baseline water had surface potentials higher than - 25.7 mV. Care must be taken interpreting this scenario. The EDL energy was obtained from following equation:

$$E_{EDL} = \kappa^{1/2} \sqrt{\frac{R}{2\pi}} Z e^{-\kappa D} \qquad \text{Eqn. (2)}$$

where, κ is the inverse of the Debye length, R is the cylindrical radius, and D is the separation distance, Z is the interaction constant defined as follow:

$$Z = 64\pi\, \varepsilon_0 \varepsilon \left(\frac{k_B T}{e}\right)^2 \tanh^2\left(\frac{ze\psi_0}{4kT}\right) \qquad \text{Eqn. (3)}$$

where, z is the electrolyte valency, ψ_0 is surface potential in mV, k is the Boltzmann constant, T is absolute temperature in Kelvin, e is elementary charge, ε_0 is permittivity in vacuum, ε is relative permittivity of water. EDL interaction between the spores and the flat surfaces were modeled with linear superposition approximation (LSA) with constant surface potential by using a geometrical mean of the interaction constants for the two-interacting surfaces.

Results

Zeta potential measurements

In the baseline waters, all spore zeta potential values were increasingly positive with decreasing pH (Figure 1). Overall, increasing the ionic strength (I) from 10 mM to 100 mM reduced the magnitude of the zeta potentials. This is consistent with theory stating that the diffuse layers of charged surfaces are compressed with increased ionic strengths (Elimelech, Gregory, and Jia 2013). The Yakibou spores experienced isoelectric points (iep) near a pH value of two (Figure 1b). For both sources of *B. thuringiensis kurstaki* and the Yakibou *B. globigii*, zeta potential values clustered together across the range of pH values; however, for both ionic strengths, the Dugway *B. globigii* spores' zeta potentials diverged from the others. Dugway *B. globigii* had greater negative zeta potential values at all but one pH measurement near the iep (Figure 1a). From pH 4 to pH 12 Dugway spore zeta potentials also stayed relatively constant, indicating surface reactions were occurring between the electrolyte and the spore (*e.g.*, at an intermediate pH range most carboxyl groups on the spore surface are likely to be deprotonated, while most amino groups are protonated (Benjamin and Lawler 2013)).

Due to this clustering between both Yakibou spore species and the Dugway *B. thuringiensis kurstaki*, only *B. globigii* and *B. thuringiensis kurstaki* spores from Dugway were subsequently tested in synthetic stormwater matrices and compared to the I = 10 mM baseline water (Figure 2). In general, zeta potential values did not drastically change for the Dugway *B. thuringiensis kurstaki* in stormwater, although the ascent to the iep occurred more gradually in the stormwater runoff. Nonetheless, the Dugway *B. globigii* spores experienced a decrease in the magnitude of the zeta potentials across the entire range of pH values when exposed to the stormwater runoff (except at pH 3). For both Dugway *B. globigii* and *B. thuringiensis kurstaki*, there were little observable differences in zeta potential values between the first-flush level of NOM (10 mg/L Total Organic Carbon (TOC)) and the lower (prolonged runoff) level of NOM (1 mg/L TOC).

Conversely, the synthetic stormwater drastically changed the surface potential of the concrete and asphalt particles, indicating changing surface chemistry during the interactions (Figure 3). In the baseline water, the asphalt behaved as expected. The iep remained the same in a higher ionic strength solution, and the zeta potential's magnitude decreased with decreasing pH due to the compaction of the EDL. On the other hand, the iep changed at higher ionic strength for the concrete.. The iep dropped from a pH of 7.9 to a pH of 6.3 when the ionic strength was increased. Also, for I = 100 mM, the zeta potential values for the concrete samples were relatively similar between pH 6 and 10. The same trend occurred at I = 10 mM, but with a sign change. This static zeta potential magnitude over a range of pH is an indication of reactions between the electrolyte and the surface. These chemistry changes were even more drastic in the stormwater runoff. For both asphalt and concrete, zeta potential values did not change over the range of tested pH values. As the NOM concentration was increased from 1 mg/L to 10 mg/L TOC, the zeta potentials became more negative for both materials.

DLVO modeling

For all spore and baseline water types, DLVO modeling was performed at pH values of 5 and 7 to predict the energy of interaction between the spores and the roadway materials. Predictions were also calculated for concrete for all spore and baseline water types at pH 10. Over all combinations, Dugway *B. globigii* spores demonstrated the least attraction to the roadway materials, followed by Dugway *B. thuringiensis kurstaki*, Yakibou *B. thuringiensis kurstaki*, and Yakibou *B. globigii* (Figure 4). In line with the clustering of the spores' measured zeta potential values, Dugway *B. globigii* was consistently an outlier in its interaction energy with the roadway materials compared to the other spore types. Further, an energy barrier between Dugway *B. globigii* and asphalt existed at both pH conditions (5, 7) when I = 10 mM, but not for either pH condition at I = 100 mM. On the other hand, the pH value of the water, not just the ionic strength, controlled the existence of an energy barrier between Dugway *B. globigii* and concrete (Figure 5). Under both ionic strength conditions at pH 5 and at a high pH value with I = 10 mM, Dugway *B. globigii* spores interacting with concrete exhibited an energy barrier. However, the energy barrier was not predicted at the neutral pH conditions for either ionic strength.

DLVO modeling was also performed at multiple pH values (5 and 7 for asphalt; 5, 7, and 10 for concrete) using the zeta potential values for both stormwater matrices (Figure 6). Whereas the baseline water DLVO predictions produced energy barriers only during certain material-water combinations, an energy barrier persisted under all stormwater conditions for both Dugway *B. globigii* and *B. thuringiensis kurstaki*. In the absence of the stormwater matrix, the interaction between the Dugway spores and concrete is attractive (*i.e.*, negative interaction energy) at all separation distances; however, spore-concrete interactions became repulsive (*i.e.*, positive interaction energy) in the presence of the stormwater matrix (Figure 6a/b). If the barrier already existed in the baseline water, its magnitude increased in the stormwater matrices (Figure 6c/d). As zeta potential measurements indicated, the surface potentials of both materials became more negative due to the adsorption of NOM, divalent cations, and/or metals onto the surfaces of those materials. This suggests that Dugway spores will not easily attach nor detach from concrete or asphalt surfaces in stormwater runoff. These results are consistent with other studies that consider adsorption of NOM in aquatic systems and suggest that NOM plays an important role in understanding surface interactions between biological contaminants (*e.g*., spores) and flat surfaces (*e.g.,* roadway material) in stormwater runoff (Dai and Hozalski 2002, Pham, Mintz, and Nguyen 2009).

Discussion

The zeta potential data indicate that two different mechanisms, double layer compression and charge neutralization, influence the adhesion of spores to submerged urban surfaces. For both *B. globigii* and *B. thuringiensis kurstaki*, compression of the double layer was dependent on the I of the water, where a larger I lowered the magnitude of the zeta potential. On the other hand, charge neutralization (introduced in the runoff water through divalent cations, metals, and NOM) only affected the *B. globigii* zeta potentials. Further, the results showed that small amounts of NOM were as impactful as larger quantities; both the low “prolonged” washoff value of 1 mg/L TOC and the “first-flush” value of 10 mg/L TOC reduced the magnitude of the *B. globigii* zeta potentials the same degree. Also, this amount of zeta potential change was similar to that induced by increasing the ionic strength to 100 mM. Ultimately, the runoff water rendered the zeta potential differences indistinguishable for *B. globigii* and *B. thuringiensis kurstaki* (for the tested Dugway strains), whereas the baseline water adjustment (modifying only I) preserved variation between spore strains. Variations between spore strains is likely due to differences in surface functional groups. These vary by strain and most notably include those functional groups associated with the collagen-like BclA on the *B. thuringiensis* exosporium (Sylvestre, Couture-Tosi, and Mock 2002, Goodacre et al. 2000) and pydridin-2,6-dicarboxylic acid deposits on *B. globigii’s sport coat (Goodacre et al. 2000)*. This difference directly impacted the baseline water modeling results and demonstrated that there are multiple routes to modify adhesion in “clean” water (*i.e.*, ionic strengths, pH values, urban surface, or spore strain). Others have studied the surface properties of *B. anthracis* and found it to be the least electronegative when compared to *B. megaterium*, *B. cereus*, *B. globigii*, *B. subtilis*, and *B. thuringiensis* subsp. *israelensis* (White et al. 2014). The authors recommended that *B. thuringiensis kurstaki* was most representative as a surrogate for *B. anthracis* in tap water because it was the least electronegative. Organic matter is ubiquitous in the outdoor environment, and therefore runoff water holds the potential to equalize *B. thuringiensis kurstaki* and *B. globigii*’s zeta potential values. Although care should be taken when selecting a surrogate with a similar electronegativity to *B. anthracis* in “clean” tap water, these data document that *B. globigii* and *B. thuringiensis kurstaki* could be used interchangeably in stormwater (from an EDL perspective).

The DLVO modeling presented in this paper does not account for “extended” forces attributable to soft particle effects such as steric hinderance from the *B. thuringiensis kurstaki*’s hairy nap, the surface roughness of the concrete and asphalt, or Lewis acid-base interactions. Classic DLVO calculations have been shown to be consistent with atomic force microscopy measurements for *B. thuringiensis kurstaki* interacting with planar surfaces in aqueous solutions (Chung, Yiacoumi, and Tsouris 2014). If the nap, roughness, and acid-base interactions are accounted for, they are expected to lower the energy barrier (Wu et al. 2018, Chen et al. 2010). A harmonic oscillator model has also been implemented to describe how bacteria with fibrillar tethers couple to surfaces (Van Der Westen et al. 2018). Exploring these newer modeling techniques and accounting for extended forces not included in traditional DLVO modeling is an essential area of future investigations to better understand the fate and transport of high risk pathogens but require advanced imaging and analytical techniques to quantify the spacing and conformation of spore structures outside the scope of this paper and in many cases are very challenging to perform on these sample types without modifying the surfaces.

The classic DLVO modeling results predicted that the presence of an energy barrier was sensitive to pH and ionic strength conditions in the baseline water. Across tested combinations of water, urban material, and spore type, Dugway *B. globigii* consistently had the highest energy barrier (likely due to its processing), but with modifications of I and/or pH, adhesion or washoff can be promoted. While water characteristics caused subtle changes in the spore's surface properties, the modeling results suggested the dominant mechanism controlling adhesion was the charge neutralization reactions of concrete and asphalt urban materials in the runoff water. This is consistent with literature documenting that NOM alters the charge of metal oxide surfaces (Zhang et al. 2009). The modeling results predicted that under stormwater conditions, there was a barrier to attachment/detachment at all pH, spore, and material combinations. Since the runoff water did not notably change the *B. thuringiensis kurstaki* zeta potentials (and *B. globigii* became less, not more, electronegative), the presence of an energy barrier was attributable to the surface reactions with the concrete and asphalt. Because the highest predicted energy barrier existed between the Dugway *B. globigii* and concrete in stormwater runoff, we expect this would be the most difficult combination to remove through wetting by rain or by washing the surface with a hose if spores were already attached.

Conclusion

The laboratory data collected in this study demonstrated that the runoff water composition equalizes the surface charge differences between different surrogates for *B. anthracis* and drastically changes the surface charge characteristics of urban materials. From a surface charge perspective, these findings provide greater flexibility in selecting a surrogate microorganism to use in field and laboratory studies focused on stormwater transport since the NOM, salts, and metals found in runoff water coat the spores and therefore exert a more dominant control on spore adhesion processes than the surface properties of the spores themselves. The laboratory results are useful building blocks in the application of models that theorize how to remove pathogenic spores from the surface of materials. Classic DLVO modeling predicts that stormwater constituents could make the removal of spores from these surfaces by water more challenging and reinforce that surface differences between concrete and asphalt have a bigger impact on spore adhesion than variations in spore strain. Testing these predictions is an area of active research in the Homeland Security Research Program at the U.S. Environmental Protection Agency (EPA). Ongoing experiments are being performed measuring the washoff of both types of spores from concrete and asphalt coupons using a rainfall simulator. This study coupled with ongoing washoff experiments provides information to aid in modeling the spread of biological contamination during emergency response and recovery, which informs activities such as creating sampling maps, deciding where to stage waste, and developing strategic decontamination plans.

Disclaimer

This manuscript was subject to administrative review but does not necessarily reflect the view of the EPA. No official endorsement should be inferred, as the EPA does not endorse the purchase or sale of any commercial products or services.

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Table

Table 1. Water Recipes

Water Type	Component	Concentration (μM)
Ultrapure Amended I = 10 mM	KCl	10,000
Ultrapure Amended I = 100 mM	KCl	100,000
Stormwater Base I = 10 mM (Pitt, Maestre, and Clary 2018)	$MgSO_4 \cdot 7H_2O$	60.98
	KCl	1,677.85
	KNO_3	891.09
	$NaNO_3$	1,058.82
	$CaCl_2 \cdot 2H_2O$	319.73
	NaCl	5,172.41
	$CuSO_4 \cdot 5H_2O$	0.64
	$ZnSO_4 \cdot 7H_2O$	0.41
	Elliott Soil Humic Acid	1 mg/L TOC*
First Flush Stormwater I = 10 mM	Stormwater Base Recipe but Elliott Soil Humic Acid = 10 mg/L as TOC	

**I = Ionic Strength; TOC = Total Organic Carbon*

Figures

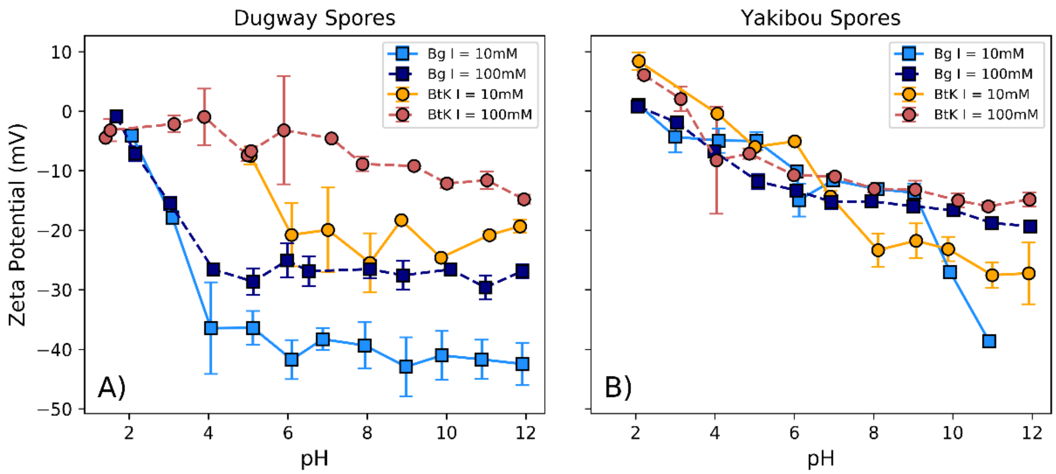

Figure 1. Zeta potential curves for *B. globigii* (Bg) and *B. thuringiensis kurstaki* (BtK) spores sourced from A) Dugway and B) Yakibou laboratories in different ionic strength solutions (baseline waters). Error bars represent one standard deviation for repeat measurements.

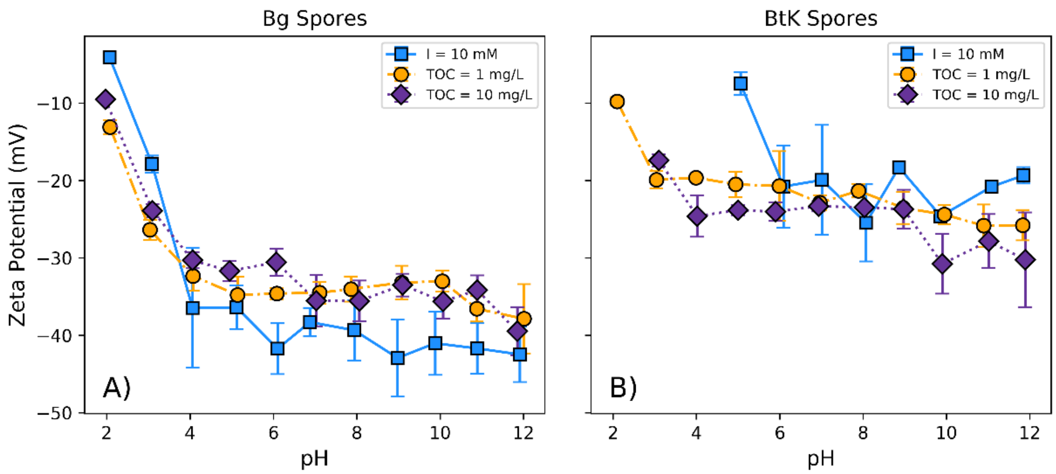

Figure 2. Zeta potential curves for A) *B. globigii* and B) *B. thuringiensis kurstaki* spores from Dugway U.S. Army Laboratory in stormwater matrices (including Elliott soil humic acid) and lower ionic strength baseline water. Error bars represent one standard deviation for repeat measurements.

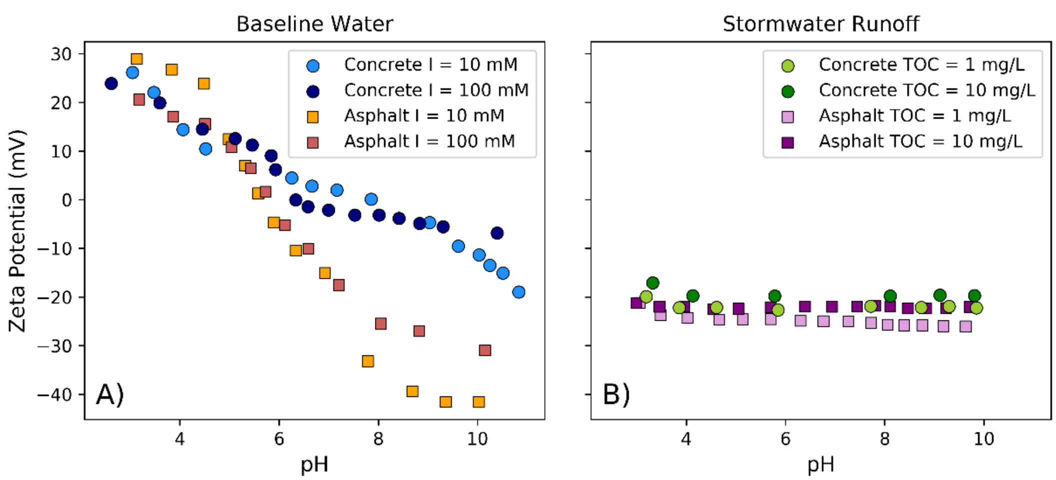

Figure 3. Zeta potential curves for concrete and asphalt particles exposed to A) ultrapure water with salts (baseline water at two different ionic strengths) and B) two synthetic stormwater runoff matrices (a 'first flush' [TOC = 10 mg/L], and a prolonged runoff [TOC = 1 mg/L]).

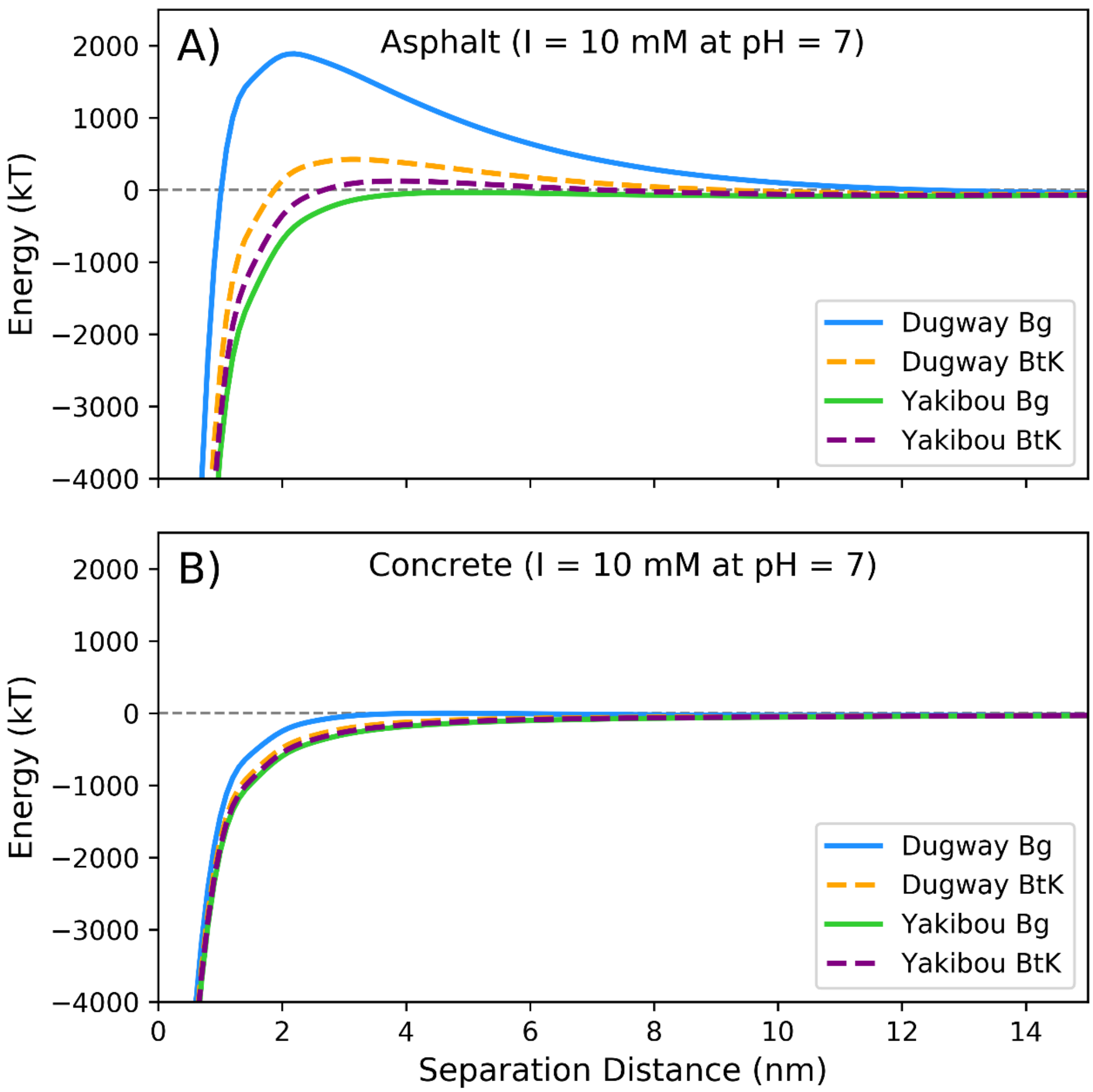

Figure 4. DLVO modeling predictions for all spore types interacting with A) asphalt and B) concrete urban materials in baseline water (I = 10 mM) and at pH 7.

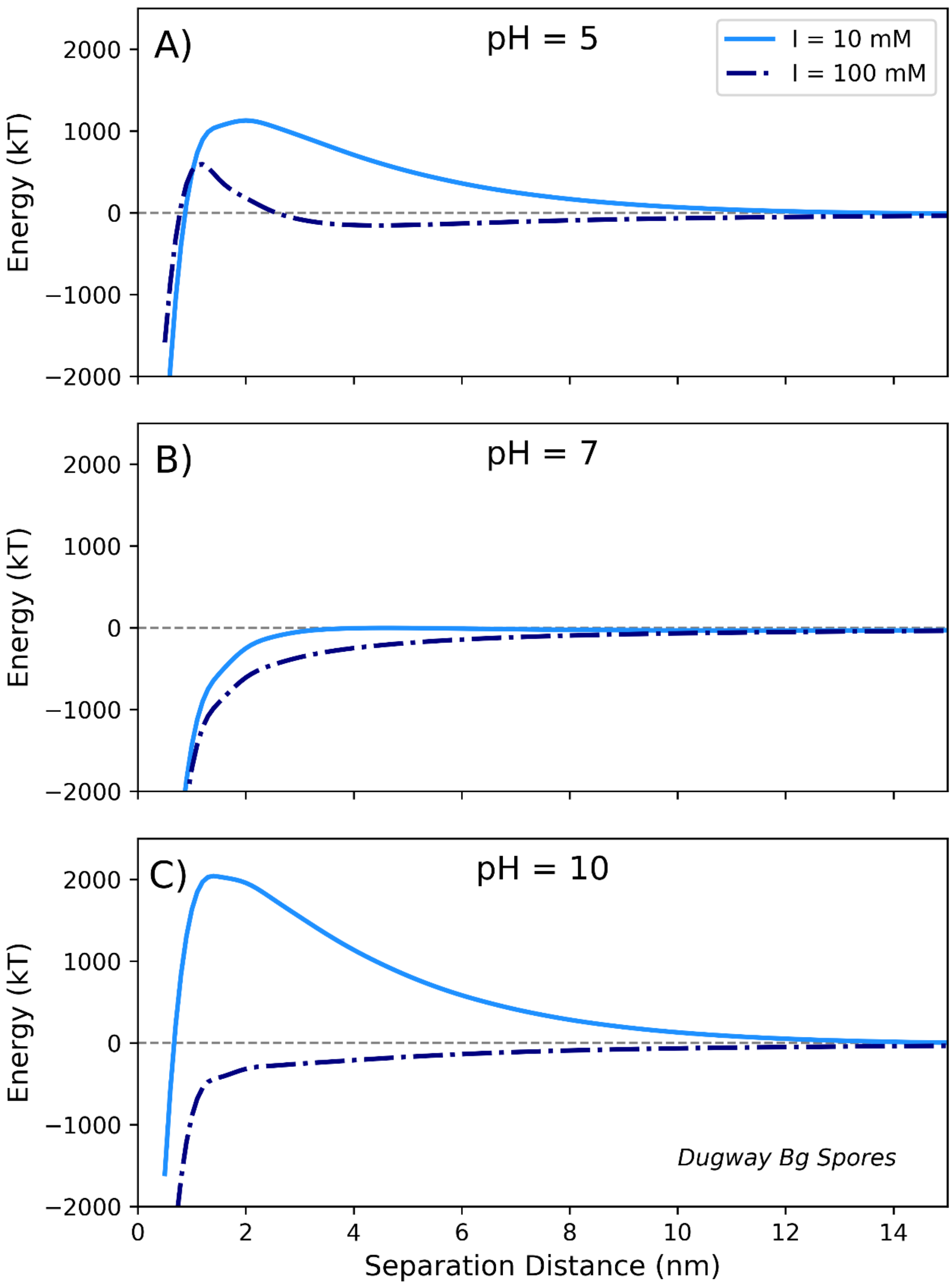

Figure 5. Concrete DLVO modeling predictions with Dugway *B. globigii* Spores in baseline water recipes at A) pH = 5, B) pH = 7, and C) pH = 10.

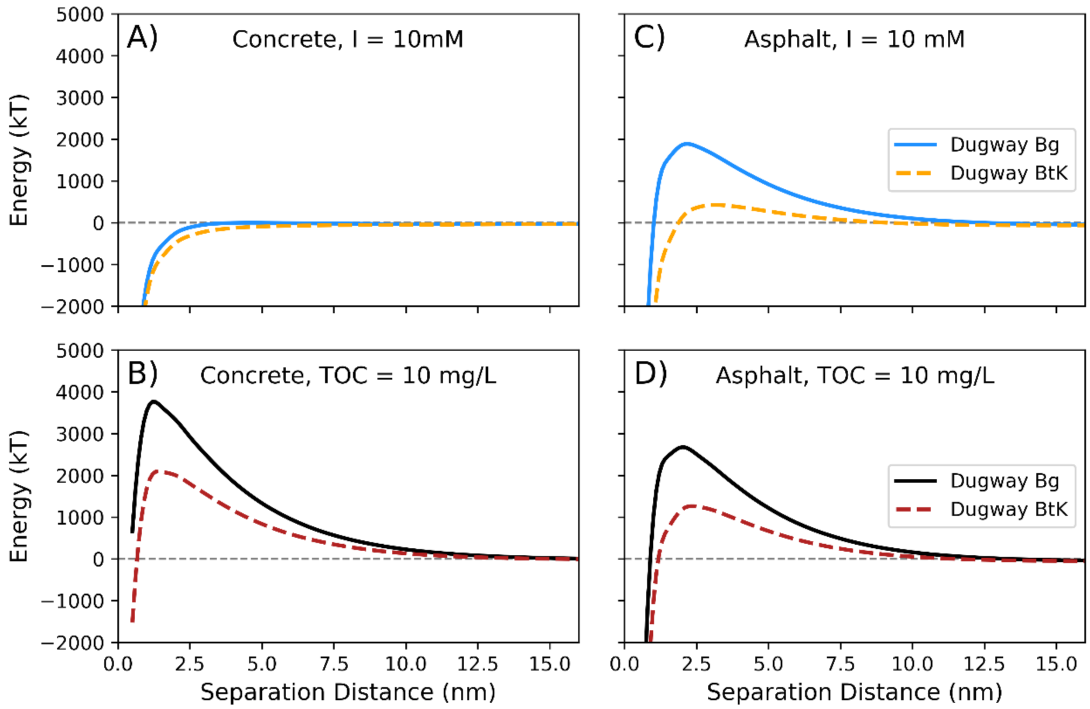

Figure 6. Concrete and Asphalt pH = 7 DLVO modeling predictions for Dugway *B. globigii* and *B. thuringiensis kurstaki* in baseline (a & c) and 'first flush' (higher TOC) runoff water conditions (b & d).

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