Quantitative Attachment and Detachment of Bacterial Spores from Fine Wires through Continuous and Pulsed DC Electrophoretic Deposition

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Supporting Information

1. INTRODUCTION

Concerns on bioterrorism1,2 have prompted efforts to discover, quantify, and compare neutralization methods such as heat,3,4 chemical,5 and other synergistic effects.6−8 The extreme stress-resistance of bacterial spores9 has provided the impetus to develop quantitative studies to more precisely define various neutralization mechanisms. Previous studies in defining spore neutralization have focused on heating under 100 °C in the absence of pressure or the combination of heat and pressure10,11 in which the temperature between 200 and 700 °C is still not available. The latter method is only useful if a well-defined spore population can be coated on the surface. Typically, this might be accomplished in a liquid suspension either naturally16−18 or by laboratory manipulation.19,20 Bacteria naturally have various adhesins to promote attachment to plastic, glass, and metal surfaces to form biofilms.16−18 In the laboratory, poly-L-lysine can be coated to impart a net positive charge on the surface to promote electrostatic interaction with the negatively charged exterior of most bacteria. However, neither mechanism can be utilized for the studies of spores, as spores are biological inert and often fail to attach to poly-L-lysine coated surfaces.21 We sought to develop an alternative approach to attach spores onto wires using physical forces.

Previously, dielectrophoresis has been utilized in the manipulation of bacterial spores.22 In the presence of an electric field gradient, a net force is imparted on the spore due to polarization, the magnitude of which is highly dependent on the material properties of the spore (including size),23 the characteristics of the fluid (including ionic strength and dielectric permittivity of the solvent and solute),24 and the field gradient.25−28 Dielectrophoresis is contrasted with electrophoresis which can take place if the cell has a net charge, and is directly proportional to the magnitude of the field.29,30 Most reported studies however modeled spore transport in liquids as...
being net neutral in charge, and only considered the dielectrophoretic effect. Actually, in aqueous dispersions, the glycoproteins and polysaccharides that comprise the exosporium (i.e., outer layer) are negatively charged. These negative charges arise from deprotonation of aldehydes (−RCOH), phosphodiester (−(RO)₂POOH), and carboxylic acids (−RCOOH), which have been detected by the infrared spectroscopy and zeta potential analysis.

Electrophoretic deposition (EPD) has been used in the past for a wide variety of bioparticles including bacteria, protein inclusion bodies, and yeast cells. While the generic process of EPD of bioparticles is similar, the exact rates of attachment to the wire may be complicated by side effects including bubble formation, surface chemistry, electrode curvature effects, and excretion of adhesive extracellular media. Bubble formation appears to be a significant problem for controlled and effective EPD. Two approaches have been reported to minimize this effect. AC-EPD has been found as a powerful method to mitigate the water electrolysis under some frequency conditions. Pulsed DC-EPD has also been shown to obtain dense bubble-free deposits at suitable pulse widths and duty cycles. From a practical standpoint, pulsed DC-EPD is a simpler approach that is easier to implement.

Besides the electrostatic interaction forces and the bubble formation effects on attachment, other forces may also contribute to the attachment of spores to surfaces. The Lifshitz–van der Waals force between the spore surface and the electrode surface can be described by the well-known Derjaguin–Landau–Verwey–Overbeek (DLVO) theory. The image force which arises due to the induced dipole effect can be attributed to the electrostatic force.

In this study, we will investigate electrophoretic attachment of uniform layers of spores to fine wires, and demonstrate reversible detachment. We demonstrate that, by applying a pulsed direct current (DC), we can quantitatively deposit and remove spores from the surface. While the Lifshitz–van der Waals force, the image force, and the electrostatic force all have an inverse square distance relationship, for the problem under consideration here, it has been shown that the dominant effect can be attributed to the electrostatic force.

In this study, we will investigate electrophoretic attachment of uniform layers of spores to fine wires, and demonstrate reversible detachment. We demonstrate that, by applying a pulsed direct current (DC), we can quantify incremental spore attachment with time by measuring the electrical current to the wire electrode. The advantages of this charging mode will be shown over the continuous charging mode. By using the measured current which can be directly related to the spore flux to the surface, we are further able to validate a transport model and use the model to directly determine the average spore surface charge and spore deposition efficiency. This methodology developed here is to our knowledge the first demonstration of direct quantitative attachment and detachment of spores from fine wires.

2. EXPERIMENTAL APPROACH TO SPORE ATTACHMENT AND DETACHMENT

2.1. Spore Deposition Cell. The spore deposition cell is shown schematically in Figure 1. It is composed of four compartments: an outer PTFE tubular shell with sealed base, a stainless steel cylinder as the outer electrode, a central wire as the deposition surface and inner electrode, and two PTFE plates to center the wire. The stainless steel outer electrode has an inner diameter of 16 mm and a height of 20 mm. The wire central deposition electrode, we use 76.2 μm platinum (Pt) (Omega Engineering, Inc.), since it is very stable in aqueous systems and will not be oxidized. The spore strain adopted in these studies is Bacillus subtilis (Bs) spores ATCC#6051 (see the optical microscopic image in Figure S1, Supporting Information), which were sporulated by growth in Diffco Sporulation Medium (DSM) at 30 °C for 48 h. A 250 mL DSM was prepared which included 2 g of Bacto nutrient broth, 2.5 mL of 10% KCl, 0.375 mL of 1 M NaOH, and 2.5 mL of 1.2% MgSO₄·7H₂O. The initial spore number concentration was regulated to be 8 × 10⁹ CFU/mL.

The power supply and current detection were performed with a 6430 sub-femtoamp remote sourcemeter from Keithley. Ultrasonication employed a Branson model 5510 ultrasonic cleaner. Optical microscopic images were taken by a Zeiss AxiosObserver microscope using a 40X objective with phase contrast illumination. Scanning electronic microscopic (SEM) images were taken of spores on wires fixed with 2% glutaldehyde, dehydrated through a series of alcohol, and sputter coated with platinum. Images were captured with a Hitachi S4700 FESEM in the Laboratory of Biological Ultrastructure at the University of Maryland. To validate our spore charging measurement approach, we employed zeta-potential analyses from a Zetasizer Nano ZS (Malvern Instruments, U.K.) equipped with a 633 nm laser and a palladium dip cell module. A 500 μL portion of sample suspension was mixed with 250 μL of 10 mM ammonium acetate aqueous solution prior to the measurement.

2.2. Spore Attachment in Continuous and Pulsed DC Charging Modes. The fine wire was stabilized inside the spore deposition cell vertically, and the spore suspension was added into the cell to immerse the wire up to a depth of 1 cm. The central wire was connected with the positive pole of the power supply, and the outer stainless steel cylinder was connected to the negative pole. A variety of EPD measurements were performed with varying voltages and on-times, and real-time current data were stored. Comparably, fine wires with different compositions and diameters were tested to find an optimal one. In the pulsed DC charging mode, a series of pulsed on-times and off-times were controlled to differentiate...
from the continuous charging mode, and the optimal pulsed charging condition for Bs spores ATCC#6051 was found.

2.3. Optical Microscopic and SEM Analyses. Wires coated with spores were observed under optical microscopy over glass slides and also by the naked eye. For the SEM analyses, samples were first fixed in 2% glutaraldehyde in buffer for 1 h at room temperature. Excess glutaraldehyde was removed in buffer by three washes of 10 min each. The samples were post fixed with 1–2% osmium tetroxide in the above buffer for at least 30 min, and then with double distilled water. After a series of dehydration processes in ethanol, the samples were treated with critical point drying with liquid carbon dioxide. Finally, samples were mounted to stubs and coated with gold/palladium alloy.

2.4. Spore Detachment. A very important aspect of this work was to develop the methodology to detach spores from the wire. To accomplish this, the wire loaded with spores was placed in a clean cell with distilled water and the wire oppositely biased to be the cathode. Simultaneously, the cell was immersed into an ultrasonic bath. The separate effects of opposite biasing and ultrasonication were compared. The treated wire was inspected by optical microscopic and SEM analyses to be shown later. The treated wire loaded with spores was immersed into an ultrasonic bath. The untreated spore was directly evaluated in a force balance:

\[ F_v = -6\eta \pi R u \]  
(5)

where \( \eta \) is the viscosity of water (1.002 \( x \times 10^{-3} \) Pa s) at room temperature and \( u \) is the velocity of spores. It should be noted that the Bs spores are not strictly spherical; thus, \( R \) in both eqs 3 and 5 is an effective radius (a spherical spore with this effective radius is defined to have the same volume as a real spore). From previous reports of spore volumes, \( R \) the effective \( R \) value for Bs spores is 0.336 \( \mu \)m. The spore motion can then be directly evaluated in a force balance:

\[ \Delta F = m \frac{d^2r}{dt^2} = F_{\text{DEP}} + F_{\text{EP}} + F_v \]  
(6)

Since the inertia term is relatively small (i.e., the spore response time to any voltage perturbation is fast relative to the transit time), we can assume steady state (\( \Delta F = 0 \)). The resulting governing equation becomes

\[ \frac{dr}{dt} = A \frac{r}{r^2} + \frac{B}{r^3} \]  
(7)

where \( A = \Delta U q/(0.101 R) \), \( B = 8.26 \times 10^{-9}(\Delta U) R^2 \).

The above equation describes the spore velocity at any radial location in the cell between two cylindrical electrodes and thus can be directly related to the experimental current data, since it is the motion of spores that contributes to the current formation. The time for spores with an initial distance \( r + \Delta r \) away from the center to move a differential distance of \( \Delta r \) is from eq 7:

\[ \Delta t = \frac{r^2 - (r + \Delta r)^2}{2A} + \frac{B}{2A^2} \ln \frac{A^2(r + \Delta r)^2 + AB}{A^2r^2 + AB} \]  
(8)

In this time interval, the total charge quantity passing through this differential distance \( \Delta r \) is

\[ \Delta Q = q' C \Delta V = q' C t [l (r + \Delta r)^2 - r^2] \]  
(9)

where \( C \) is the spore number concentration in the cell, \( l \) is the immersed depth of Pt wire inside the liquid (0.01m), and \( \Delta V \) is the differential volume element around the wire. It should be noted that the charge \( q' \) is the effective overall charge after being shielded by counterions in the vicinity of the spore surface (i.e., the diffuse double layer). The importance of this is that the mobility of a spore is influenced by the thickness of the double layer that is dragged along with the spore. The current can be expressed as

\[ I = \lim_{\Delta t \to 0} \frac{\Delta Q}{\Delta t} = \frac{2q' \pi C l (A^2 + B)}{r^2} \]  
(10)

Then, substituting for \( A \) and \( B \) above, the initial current observed can be expressed as

\[ I_0 = 0.622 C \Delta U \frac{q' + 3.58 \times 10^{-7} C \Delta U R^2 q'}{R} \]  
(11)

Equation 11 is a simplified illustration of the current corresponding to the spore deposition rate but which does...
not include possible effects due to particle−particle and particle−electrode interactions. Near the electrode, the spore transport may be complicated by electrohydrodynamic flows as well as electroosmotic flows due to the electric forces on charges from the electrode polarization layer and the equilibrium diffuse double layer near the spore.56,57 However, since eq 11 describes the initial current when \( t = 0 \), the influence of those flows can be neglected. In the remainder of the paper, we analyze our experimental results in the context of the model resulting in eq 11.

4. RESULTS AND DISCUSSION

4.1. Spore Attachment in the Continuous DC Mode.
To exemplify the spore attachment in the continuous DC mode, a biased voltage of 20 V and a spore number concentration of \( 8 \times 10^9 \) CFU/mL were adopted. The EPD conditions employed were chosen on the basis of prior work that showed that bacterial spores can withstand DC electric fields as high as 1500 V/cm and still maintain viability.31 Before we proceeded with direct measurement of the current in evaluating spore deposition, we evaluated the temporal variation of current for pure water and the spore suspension in Figure S2 (Supporting Information). We found that steady state could be achieved on the order of 100 s, and that the measured current due to charge migration in the spore containing suspension was about an order of magnitude larger than that contributed to by pure water. This allows us to ignore the influence of the solvent in subsequent current measurements during EPD. Images of spore attachment are shown in Figure 2 for a deposition time of 20 s. The visual images (Figure 2A and B) show that, after DC biasing, an obvious white spore coating appeared in the immersed portion of the wire. Further optical microscopic images (Figure 2D and E) reflect the spore deposition in that region (the dark region represented the coverage of spores). SEM images (Figure 2G and H) show an evenly distributed and dense coating on the wire and more directly demonstrated the attachment of spores. The enlarged SEM images in Figure 3A and B also exhibit the structures of attached Bs spores on surfaces.

Nominally, we should expect that increasing the charging time would cause incremental increases in spore deposition to the surface. However, such a relationship was not linear especially after a relatively long time, and when a higher voltage was applied (Figure S3, Supporting Information). In those cases, the current−time curves show fluctuations which could be correlated to the generation of visible gas bubbles formed from water electrolysis58 (Figure S4C, Supporting Information).

Figure 2. Parts A−C show Pt wires before coating, after coating, and after detaching. Parts D−F show optical microscopic images of Pt wires before coating, after coating, and after detaching. In both parts B and E, the immersed parts of the wire inside the spore suspension were marked. Parts G−I show SEM images of Pt wires before coating, after coating, and after detaching. The charging condition for spore attachment is 20 s at 20 V. The spore detaching condition is 10−15 min at reversed 60 V, combined with the ultrasonication.

Figure 3. SEM images of Bs spores ATCC#6051 attached on Pt wires.
tion). As a result, spore deposits became highly uneven along the wire surface, and subsequently were detached from the wire at high voltages, or long charging times (Figure S4A and B, Supporting Information). This prompted us to evaluate a pulsed deposition mode that will be described later.

4.2. Spore Detachment. To properly evaluate our eventual exposure studies (heat, chemicals), an efficient and quantitative method to detach the spores is necessary so that standard assays can be employed. We found that either reversed biasing the wire at $-60$ V for 5 min or ultrasonicallyating for 30 min allowed partial detachment of spores from the wire (Figure S5, Supporting Information). While some reports indicate that ultrasonication is known to harm spores\textsuperscript{59,60} and repeated electric pulse cycling can also induce spore inactivation,\textsuperscript{61,62} we operated at much shorter ultrasonication time and bias voltage which are $\sim100\times$ less than those studies. Similarly, pH changes which occur during deposition (Figure S6, Supporting Information) were evaluated for viability by exposing spores to a range of pH (5–10) (Figure S7, Supporting Information) and were found to have no effect. We found that the combination of reverse bias and ultrasonication would detach the spores completely.

The photographic, optical microscopic, and SEM images shown in Figure 2C, F, and I, respectively, demonstrate that all the Bs spores were removed from the surface after reversed biasing for 10–15 min at $-60$ V in an ultrasonic bath. Once the spores on the surface were removed into liquid, they could be enumerated by the plate counting method to determine the number of spores deposited on the wire. It should be noted that the reverse-bias charging time and voltage here are larger than those for spore attachment, which presumably accounts for the force required to overcome the binding energy between the spore and wire surface. Together, these results indicate that the combination of reversed bias and ultrasonication is an effective means to completely remove spores deposited on the surface.

4.3. Spore Attachment in the Pulsed DC Mode. The fluctuation in the deposition current observed (Figure S3, Supporting Information) coincided with visible bubble formation (Figure S4C, Supporting Information). To mitigate this effect, we shifted our efforts to a pulsed DC mode, based on results of Besra et al.\textsuperscript{50} who showed that, using a pulsed voltage strategy, an extension of an application used to deposit metal films could lead to a minimization of bubble formation and conformal deposits of small particles. We have adopted this strategy in order to generate a uniform and densely packed spore coating, by applying multiple electric pulses instead of a continuous DC bias. We have found that this leads to reproducible spore deposits by enabling consistent current measurements. In Figure 4, the measured temporal currents during deposition of Bs spores (ATCC#6051) at a voltage of 5 V are shown, with an on-time of 30 s followed by an off-time of 30 s. Over eight cycles of this experiment, the measured current was consistent between cycles, which implies that the ionic strength of the liquid was stable. The SEM images (Figure S) reflect the increase of Bs spore attachment on the surface with the pulse number. After charging for two pulses, only partial coverage was achieved; however, these deposits were uniform. A monolayer of Bs spores could be formed after four pulses, and multiple layers of spores were found after seven pulses. Optical microscopic images (Figure S8, Supporting Information) also show that the Bs spore deposits increased as the pulses accumulated. After enumerating all the spores detached

Figure 4. Current–time relationship in the pulsed DC charging mode for Bs spores (ATCC#6051). Both the charging time and the pulse-off time were 30 s, and the applied on-time voltage was 5 V. In our design, Bs spores were deposited to the surface for up to eight cycles.

Figure 5. SEM images of Bs spore coating (ATCC#6051) on Pt wires after different biased DC pulses. Parts A, B, and C show the cases after 2, 4, and 7 pulses, respectively. Each DC pulse is set to last 30 s and stop for another 30 s before the next pulse. The enlarged SEM images are inserted which exhibit the different spore deposition densities on the surfaces.
from the surface, a linear relationship between the spore deposits and applied pulse number was found (Figure 6A). By measuring four times, which are reidealized spore deposits (from the current spores. The spore attachment efficiency was calculated by the ratio of measured spore deposits (from the detached spores) over the idealized spore deposits (from the current-time data). Data were measured four times, which are reflected in the error bars.

Figure 6. (A) *B. subtilis* spore deposits (ATCC#6051) on the Pt wire after different pulses. The black points are for the spore deposits obtained from the current-time measurement, while the red points are from spore deposits counted from the detached spores. (B) Relationship between spore attachment efficiency and number of pulses for *B. subtilis* spores. The spore attachment efficiency was calculated by the ratio of measured spore deposits (from the detached spore counting), over the idealized spore deposits (from the current-time data). Data were measured four times, which are reflected in the error bars.

It should be noted that, in this pulsed DC-EPD process up to eight cycles, the pH value of the spore suspension did not vary much (Figure S10, Supporting Information) which had no effect on the deposition rate and spore viability.

4.4. Calculations of the Spore Surface Charge and Deposition Efficiency. In principle, a measurement of the current is also a measure of spore transport to the wire (but not necessarily stick to), if one knows the average charge on a spore. From eq 11, the average charge on a spore can be measured by a prior knowledge of the spore concentration in the suspension and the initial current. A series of initial currents of the spore suspensions were collected under different applied potentials and spore concentrations, which were further subtracted from the initial current of a solution without spores and defined as the effective initial current of spores (*I₀*) and tabulated in Table 1. The corresponding surface charge (∆q) per spore is evaluated from eq 11.

![Image](image-url)  

Figure S11, Supporting Information)

However, we still need a relationship between ∆q and ∆q’ to determine the fraction of the current associated with the diffuse double layer (Figure S11, Supporting Information). One approach is to find the electrical potential at the shear plane (i.e., the outer radius of solution ions that are carried along with the spore). The Helmholtz–Smoluchowski equation describes the relationship between the shear plane potential (ζ) and the mobility of spores (μ):  

\[
\mu = \frac{e \gamma}{k_B T} \frac{1}{\eta}
\]

By equating the friction force (eq 5) to the electrophoretic force (eq 2) and also omitting the dielectrophoretic force (eq 3) (note: it has been found that the dielectrophoretic force is much smaller than the electrophoretic force), we can obtain

\[
\zeta = \frac{q}{6 \pi \eta a R_c}
\]

In the spherical coordinates, the potential as a function of radial distance from the shear plane can be evaluated, from the simplified Poisson–Boltzmann equation:

\[
\zeta = \frac{q}{4 \pi \varepsilon_0 a R_c} \exp(-\kappa R_c)
\]

where *R_c* is the radius of the shear plane and *κ* is the inverse Debye length (1.0384 × 10⁻⁶ m⁻¹) in water at room temperature. By combining eqs 13 and 14, we obtain

\[
R_c = 0.35 \times 10^{-6} \text{ m, thus slightly larger than the radius of one *B. subtilis* spore.}
\]

### Note: Table 1. Surface Charges and Numbers of Charges for *B. subtilis* Spores (ATCC#6051)

<table>
<thead>
<tr>
<th>concentration (CFU/mL)</th>
<th>voltage (V)</th>
<th>current (mA)</th>
<th>surface charge (C)</th>
<th>number of charges</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.77 × 10⁶</td>
<td>5</td>
<td>0.0002 ± 0.0002</td>
<td>(5.36 ± 5.36) × 10⁻¹⁴</td>
<td>(3.35 ± 3.35) × 10⁵</td>
</tr>
<tr>
<td>8.59 × 10⁷</td>
<td>5</td>
<td>0.0031 ± 0.0008</td>
<td>(6.20 ± 0.81) × 10⁻¹⁴</td>
<td>(3.88 ± 0.51) × 10⁵</td>
</tr>
<tr>
<td>7.67 × 10⁸</td>
<td>5</td>
<td>0.1615 ± 0.0006</td>
<td>(4.81 ± 0.01) × 10⁻¹⁴</td>
<td>(3.00 ± 0.01) × 10⁵</td>
</tr>
<tr>
<td>10</td>
<td>0.4459 ± 0.0311</td>
<td>(5.61 ± 0.20) × 10⁻¹⁴</td>
<td>(3.51 ± 0.12) × 10⁵</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.8509 ± 0.1080</td>
<td>(5.47 ± 0.35) × 10⁻¹⁴</td>
<td>(3.42 ± 0.22) × 10⁵</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.4584 ± 0.2005</td>
<td>(5.85 ± 0.40) × 10⁻¹⁴</td>
<td>(3.65 ± 0.25) × 10⁵</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1.7991 ± 0.2294</td>
<td>(5.63 ± 0.36) × 10⁻¹⁴</td>
<td>(3.52 ± 0.23) × 10⁵</td>
<td></td>
</tr>
<tr>
<td>1.53 × 10⁹</td>
<td>5</td>
<td>0.1774 ± 0.0008</td>
<td>(3.55 ± 0.01) × 10⁻¹⁴</td>
<td>(2.22 ± 0.00) × 10⁵</td>
</tr>
<tr>
<td>50</td>
<td>0.5162 ± 0.0179</td>
<td>(4.28 ± 0.07) × 10⁻¹⁴</td>
<td>(2.67 ± 0.05) × 10⁵</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1.1060 ± 0.0450</td>
<td>(4.53 ± 0.09) × 10⁻¹⁴</td>
<td>(2.83 ± 0.05) × 10⁵</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.4610 ± 0.1474</td>
<td>(4.15 ± 0.21) × 10⁻¹⁴</td>
<td>(2.59 ± 0.13) × 10⁵</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3.1724 ± 0.2643</td>
<td>(4.74 ± 0.20) × 10⁻¹⁴</td>
<td>(2.96 ± 0.12) × 10⁵</td>
<td></td>
</tr>
</tbody>
</table>

*Note: the average value of surface charge is 5.01 × 10⁻¹⁴ C, and the average number of charges on the spore surface is 3.13 × 10⁵.*
spore \((0.336 \times 10^{-6} \text{ m})\). The quantity of charges between \(R\) and \(R_c\) can be calculated:

\[
q - q' = - \int_{R}^{R_c} \rho^* 4 \pi r^2 \, dr
\]  

(15)

where \(\rho^*\) is the charge density in the diffuse layer which can be expressed from the Poisson equation: 

\[
\rho^* = (-q_{f} \frac{k^2}{4 \pi}) \exp(-kr)
\]  

(16)

Finally, the spore surface charge can be found:

\[
q = \frac{q_{f}}{0.9964}
\]  

(17)

Equation 17 implies that the contribution of charges between the spore surface and the sheet layer is negligible. Evaluation of eq 11 gives a value of \(q = 5.01 \times 10^{-14} \text{ C}\). Accordingly, the average number of negative charges on the spore surface is 3.13 \(10^{15}\) (Table 1). The result above is based on the assumption that the surface charge (or zeta-potential) of spores in suspension does not vary during deposition, which is essentially what we observed experimentally (Figure S12, Supporting Information).

The most commonly used method to detect the surface charges of particles is through a measurement of the zeta-potential.\(^{65}\) The zeta-potential measured for \(Bs\) spores yielded values of \(-33 \text{ mV}\), which accordingly gave a surface charge density of \(0.0384 \text{ C/m}^2\). On the basis of the enlarged SEM images (Figure 3), it is reasonable to assume the spore as a prolate ellipsoid with a semilength axis \(c\) and a semiaxis axis \(a\), from which the spore surface area can be estimated. It has been reported that, for \(Bs\) spores, \(c = 0.535 \mu\text{m}\) and \(a = 0.24 \mu\text{m}\).\(^{55}\) Together with the charge density data, the surface charges on the spores can be determined and are listed in Table 2. The table shows that the charge values obtained from our method and the zeta-potential assay are quite similar. Alternatively, Douglas\(^{65}\) detected the surface charges of \(Bs\) spores through a mobility test in liquid (Table 2). These results suggest that our approach gives results that are consistent with other reported results, with the added benefit of being considerably simpler in terms of the sophistication of the instrumentation needed, and for our purposes the in situ nature of the measurement.

With a known shielded charge \(q'\), the net charge accumulation from our current measurement can be directly used to estimate the deposition rate. Accordingly, the deposited spore dose \(N_{\text{current}}\) on the wire could be determined as (Figure 4)

\[
N_{\text{current}} = \int_0^t dN_{\text{current}} = \frac{1}{q} \int_0^t I(t) \, dt
\]  

(18)

In our pulsed DC-EPD model, the relationship between spore deposits based on the current measurement and pulses is shown in Figure 6A. \(N_{\text{current}}\) for just one pulse is in the range of \(10^{11}\) spores, which implies the formation of multiple spore layers. However, one densely packed monolayer of \(Bs\) spores \((6.1 \times 10^9)\) actually required four pulses. This leads to a somewhat surprising result that the deposition efficiency is only \(\sim 10^{-6}\) (Figure 6B). Thus, it appears that many spores apparently make it to the surface and become dislodged, possibly due to nonvisible gas generation at the electrode.\(^{66}\) In spite of this low efficiency, the measured deposited spore amount is proportional to the calculated deposits based on the current (Figure 6B), consistent with the results of Hamaker.\(^{67,68}\) On the basis of this model together with the verification from experiments, our result demonstrates that one is able to in reasonable short deposition time create a near conformal monolayer of spores and subsequently detach them.

### 5. CONCLUSION

We show in this study the ability to controllably deposit spores electrotheropically to fine wires using a pulsed voltage method which prevents electrolysis. Attached spores can be totally removed by a combination of reversing the electrical polarity and ultrasonicating the wire. We are also able to use this method to quantitatively measure the surface charge on spores and the deposition rate. The method is generic and should be applicable to the deposition of any biological material (e.g., spores, bacteria, viruses) onto metallic surfaces.

#### ASSOCIATED CONTENT

3 Supporting Information

Optical microscopic image of \(Bs\) spores; optical microscopic images of \(Bs\) spores in the continuous and pulsed DC-EPD modes; temporal current in the continuous DC-EPD mode; pH–time relationships during pulsed DC-EPD mode and reversal charging; dense spore packing model on a surface; scheme of the diffuse double layer of the spore surface; zeta-potential–pH relationship in spore suspension. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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