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Parameters Affecting Spore Recovery from Wipes Used in Biological Surface Sampling▽†

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The need for the precise and reliable collection of potential biothreat contaminants has motivated research in developing a better understanding of the variability in biological surface sampling methods. In this context, the objective of this work was to determine parameters affecting the efficiency of extracting *Bacillus anthracis* Sterne spores from commonly used wipe sampling materials and to describe performance using the interfacial energy concept. In addition, surface thermodynamics was applied to understand and predict surface sampling performance. Wipe materials were directly inoculated with known concentrations of *B. anthracis* spores and placed into extraction solutions, followed by sonication or vortexing. Experimental factors investigated included wipe material (polyester, cotton, and polyester-rayon), extraction solution (sterile deionized water [H_2O], deionized water with 0.04% Tween 80 [H_2O-T], phosphate-buffered saline [PBS], and PBS with 0.04% Tween 80 [PBST]), and physical dissociation method (vortexing or sonication). The most efficient extraction from wipes was observed for solutions containing the nonionic surfactant Tween 80. The increase in extraction efficiency due to surfactant addition was attributed to an attractive interfacial energy between Tween 80 and the centrifuge tube wall, which prevented spore adhesion. Extraction solution significantly impacted the extraction efficiency, as determined by statistical analysis ($P < 0.05$). Moreover, the extraction solution was the most important factor in extraction performance, followed by the wipe material. Polyester-rayon was the most efficient wipe material for releasing spores into solution by rank; however, no statistically significant difference between polyester-rayon and cotton was observed ($P > 0.05$). Vortexing provided higher spore recovery in H_2O and H_2O-T than sonication, when all three wipe materials and the reference control were considered ($P < 0.05$).

The successful collection of biological contaminants from surfaces is critical to gaining insight into the environmental conditions in which we live and work as well as to ensure public safety in times of biothreat incidents. Traditional methods for biological sample collection have focused on assessing bacterial contamination on surfaces relevant to environmental, clinical, and food safety settings, in which case swabs were the most common adsorptive materials used for sample collection (6, 20, 21, 23, 24, 29, 35, 38). Since the anthrax attacks in 2001, sampling methods using wipe and vacuum collection devices have been developed to meet the needs of a broader range of applications, including building characterization and clearance. Data accumulated from sampling of contaminated facilities in 2001 using HEPA vacuum, dry and premoistened swab, and wipe sampling methods demonstrated that sampling efficiency was dependent on surface sampling techniques and sample collection conditions (42, 47).

Overall recovery efficiency is sensitive to the applied experimental conditions due to a wide range of potential variables in surface sample collection methodologies, such as differences in extraction solution, adsorptive material, surface substrate, and surrogate biomaterial. The performance of sampling methodologies is typically studied by depositing known quantities of a particular microorganism on a surface, removal of this microorganism using an adsorptive material, and extraction of the microorganism from the adsorptive material (18, 22, 26, 27, 48). An effective extraction solution promotes the sufficient dispersal of the microorganism from the adsorptive material for quantification by direct plate count (7, 15, 41) or PCR (9, 10) to estimate the overall recovery efficiency. Common extraction solutions include phosphate-buffered saline (PBS) (37, 52) and water with and without the presence of a surfactant.

The impact of the extraction solution on the overall recovery efficiency is often convoluted with the removal efficiency, as extraction efficiency is not commonly reported, and if it is reported, the study authors may not report on the experimental controls used to estimate extraction efficiency. For example, Frawley et al. (22) reported overall recoveries of less than 10% for *Bacillus anthracis* sampling from plastic, wood, and cotton cloth surfaces using cotton wipes and less than 15% using polyester swabs for sampling of plastic, glass, desktop Formica, metal (tin plate), carpet, brick, and synthetic cloth surfaces. The authors did not discuss the effect of extraction solution and adsorptive sampling material on the extraction efficiency independently of the recovery steps, resulting in difficulty in interpreting removal and extraction performance (22). However, in an experiment by Rose et al. (41), recovery efficiencies

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of 41.7% and 43.6% were reported for cotton and macrofoam swabs, respectively, when *B. anthracis* Sterne spores were liquid deposited on stainless steel and PBS with 0.04% Tween 80 (PBST) was used as the extraction solution. Directly inoculated swabs were used as an experimental control, which resulted in an extraction efficiency of ~94% when they were processed in PBST (41). Since the extraction efficiency was significantly greater than the recovery efficiency, the authors concluded that the poor overall recovery efficiency could be explained by a substantial number of spores left on the stainless steel surface. Additionally, independent reporting of extraction efficiency facilitates comparisons of extraction conditions. Brown et al. (7) demonstrated that the ability of Butterfield buffer with 0.01% Tween 80 to extract *B. atrophaeus* spores from directly inoculated rayon swabs was about 76%. Recent extraction efficiency data reported by Lewandowski et al. (30) indicate that additional studies are needed to improve data analysis and enhance overall recovery.

Surfactants are common additives in extraction solutions, and variability in extraction efficiency may be largely dependent on physicochemical phenomena (e.g., adhesion and aggregation) between biomaterials and all surfaces (e.g., wipes and tubes) that surfactants can impact. Physicochemical factors have significant implications for understanding environmental and biological systems, such as adhesion and transport of microorganism in aqueous environments (11, 17, 34, 39), medical device development (12, 33, 45), and biofilm formation in the food industry (5, 44). Surface thermodynamics and its contribution to adhesion forces and interfacial energy are well-known parameters driving adhesion and aggregation between surfaces (49) and have been widely applied to understanding complex environmental and biological systems (33, 34, 39, 44). Surfactants can significantly alter physicochemical interactions, resulting in dispersal and enhanced transport of microbes in environmental systems (14, 16, 31, 46, 51). Understanding the physicochemical parameters governing this efficiency can potentially be of great value for the development of new extraction and sample collection methodologies.

The objective of our work was to evaluate the impact of variables affecting the efficiency of extraction of *B. anthracis* Sterne spores from wipe materials and to assess the role of surface thermodynamics in characterizing extraction efficiency performance. Additionally, we determined the optimal wipe material and extraction method, defined here as the combination of extraction solution and physical dissociation method (PDM) for a given set of experimental conditions. The study was carried out using polyester-rayon, cotton, and polyester commercial wipes as adsorptive materials and both sterile deionized (DI) water and PBS, with and without surfactant (Tween 80), as extraction solutions. The extraction efficiency for spores applied directly to wipe materials for the examined extraction solutions was quantified, and the interaction between spores and all surfaces involved in this step, as well as the implications of this interaction for biological sampling, was evaluated.

MATERIALS AND METHODS

Bacterial spore preparation and culture methods. Suspensions of green fluorescent protein (GFP)-labeled *Bacillus anthracis* Sterne/pAFp8gfp provided by T. Hoover (40) were prepared by growing a uniform lawn of spores on a modified Schaeffer medium as described by Faille et al. (19). Sporulation medium was prepared by dissolving 8 g nutrient broth (Difco Bacto peptone; VGD, Inc.); 0.51 g $MgSO_4 \cdot 7H_2O$, 3×10^{-3} g $MnCl_2 \cdot 4H_2O$, 0.97 g KCl, 0.55×10^{-3} g $FeSO_4 \cdot 7H_2O$, and 0.2 g $CaCl_2 \cdot 2H_2O$ (chemicals from Sigma-Aldrich, Milwaukee, WI); and 1.5% agar in 1 liter sterile DI water (resistivity, 18 MΩ cm at 25°C; Milli-Q; Millipore Corp., Bedford, MA); and the pH was adjusted to 6.9. After ~5 days of growth at 32°C, plates were examined for spore formation by removing a small colony of growth and examining the growth with a phase microscope (Olympus AX-70; Olympus America Inc., Center Valley, PA). Once ~95% spore formation was noted, spores were harvested from the agar surface by placing 2 ml of sterile DI water on the agar and gently scraping the agar surface with a clean, sterile glass cell spreader. Spore suspensions were washed 7 times by centrifugation at $1,500 \times g$ for 2 min and resuspended in sterile DI water at $\sim 1 \times 10^8$ spores ml^{-1} for storage at 4°C. The spore suspension used in the study was prepared at $\sim 1 \times 10^6$ spores ml^{-1} in 95% ethanol (200 proof; Sigma-Aldrich, Milwaukee, WI). The suspension was confirmed to contain greater than 95% free spores by hemocytometer counts, and these were compared to colony counts from plating of a standard dilution series on Luria-Bertani (LB) agar (Fisher Scientific) before and after contact at 65°C for 25 min to inactivate any vegetative cells or germinated spores. Immediately prior to the experiment, working spore suspensions were prepared by diluting the stock suspension to $\sim 2 \times 10^5$ spores ml^{-1} in the extraction solution to be evaluated as described below in "Extraction procedure."

Wipe materials. Three types of wipe materials were evaluated: nonwoven polyester-rayon blend (Kendal Versalon; catalog no. 8042; Tyco Healthgroup LP, Mansfiled, MA), woven polyester (Value-Tek; catalog no. VTPNWIR-99; Phoenix, AZ), and woven cotton (One; catalog no. 9131; Hermitage Hospital Products, Niantic, CT). All wipes were ordered sterile and cut to 4 by 4 in. with appropriate sterile scissors, when needed, and folded to 2 by 2 in.

PDM. Two methods were used to enhance spore dissociation from the wipe material: vortexing and sonication. PDMs included vortexing at maximum speed for 2 min in 10-s bursts using a deluxe vortex mixer (catalog no. 02215370; Fisher Scientific, Pittsburg, PA) or sonication for 5 min at 42 kHz (model FS20; Fisher Scientific).

Extraction procedure. Wipe materials were premoistened with 1 ml of the extraction solution and subsequently inoculated with 100 μl of the working spore suspension ($\sim 2 \times 10^5$ spores ml^{-1}). For each adsorptive material, 3 to 5 replicate wipes were used. Extraction solutions evaluated included sterile DI water (H_2O), PBS, and both solutions plus 0.04% Tween 80 (H_2O-T and PBST, respectively). The solutions were chosen on the basis of their application as an extraction solution or wetting agent, described in the literature (8, 15, 37, 52). Immediately after spore deposition, wipes were added to a sterile 50-ml screw-top polypropylene tube (catalog no. 23-2262; Crystalgen, Plainview, NY) containing 30 ml of extraction solution. Following application of the physical dissociation method, 100-μl aliquots from each tube were spread in triplicate on LB agar and incubated at 32°C for 24 h. A reference control was run for each extraction solution studied. Reference controls were prepared by aliquoting 100 μl of the working spore suspension directly into a sterile polypropylene centrifuge tube containing 30 ml of the extraction solution without a wipe. The reference represents the maximum spore extraction that can be expected for a given method (i.e., combination of extraction solution with PDM).

Experimental design. The experimental design consisted of three factors ($K = 3$): (i) wipe material (nonwoven polyester-rayon, woven cotton, and woven polyester plus a reference control without a wipe), (ii) extraction solution (H_2O, PBS, H_2O-T, and PBST), and (iii) PDM (sonication and vortexing), as described in Table 1. The measured response variable for the experiment was the percentage of spores extracted from a given wipe material for a given extraction method.

Calculation of extraction efficiency. The raw data were the number of extracted spores per milliliter for a given wipe material and extraction method (extraction solution/PDM) and were converted to a percentage using the initial inoculated spore concentration, $(2.27 \pm 0.38) \times 10^4$ spores per milliliter ($n = 6$). Quantification of spores in the solution for the reference control relative to the initial inoculum represents the maximum expected extraction efficiency in a given solution. The difference between the reference control value and the known inoculation concentration describes the number of spores lost to the tube. Each experimental combination (wipe, extraction method) was analyzed with 9 to 15 replicates, and the result is expressed as the mean percentage of extracted spores, followed by the uncertainty, expressed as standard deviation (SD).

Data analysis. As a framework for the analysis, four subjects were considered: (i) the effect of extraction method on the spore extraction and the most efficient extraction method, (ii) the effect of the wipe material on the spore extraction and the most efficient wipe, (iii) the most important factor of the two factors wipe material and extraction method affecting extraction efficiency, and (iv) potential

TABLE 1. Replicated full factorial experimental design

Wipe (factor 1)[a]	Cell entry[b] with the following extraction method (factor 2)					
	Sonication		Vortexing			
	H_2O	H_2O-T	H_2O	H_2O-T	PBS	PBST
PR	5, 15	3, 9	3, 9	4, 12	4, 12	3, 9
CO	5, 15	3, 9	3, 9	4, 12	4, 12	3, 9
PO	5, 15	3, 9	3, 9	4, 12	4, 12	3, 9
REF	5, 15	3, 9	3, 9	4, 12	4, 12	3, 9

[a] Abbreviations: PR, polyester-rayon; CO, cotton; PO, polyester; REF, reference.

[b] Cell entry means the number of wipes used in the experiment in which each wipe was run three times, total number of replicates for each combination. The extraction method entailed extraction solution plus PDM.

interactions between wipe material and extraction method affecting spore extraction. In order to address these issues, a variety of graphical and statistical analysis procedures were employed.

Spores released during extraction. The number of spores released from the wipe surface was determined by the number of extracted spores, per milliliter, in the presence of the wipe divided by the number of spores extracted in the absence of the wipe (reference control). This approach takes into consideration the potential interaction between spores and the centrifuge tube.

Contact angle measurement and surface tension calculation. Surface tension (γ) was utilized to calculate the interaction energy between two surfaces in a given solution. To obtain the surface tension of the three surfaces (spores, wipe, and Tween 80) involved in the study, contact angle measurements were performed. However, depending on the surface involved in the measurement, several methods for obtaining the contact angle were used, as detailed in the supplemental material. In summary, contact angle (θ) describes the angle resultant between the tangent line of a liquid and a solid when a liquid droplet contacts a flat surface (49). The magnitude of the incident angle of interaction is the result of a balance between adhesive and cohesive forces resulting from solid (S) and liquid (L) interfacial energy and can be expressed by the Young-Dupré equation (49), in which Lishitz-van der Waals (LW) and Lewis acid-base (AB) parameters are associated with apolar and polar forces, respectively. Furthermore, the Lewis acid-base contribution can be broken into two subcomponents: the electron-donating ($-$) and electron-accepting ($+$) components (equation 1).

$$(1 + \cos\theta)\gamma_L = 2(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}}) \quad (1)$$

The surface tension components of the liquid (γ_L^{LW}, γ_L^{-}, and γ_L^{+}) are known parameters obtained from the literature, while the components of the solid (γ_S^{LW}, γ_S^{-}, and γ_S^{+}) are parameters to be determined. Experimentally, to determine the surface tension components of the solid, three different liquids representing apolar and polar interactions are used for obtaining the contact angle required for solving equation 1 (49, 50). In the current study, the contact angles were acquired using DI water (polar), formamide, CH_3NO (polar), and diiodomethane (apolar); and their surface tension components were those reported by Aranberri-Askargorta et al. (3).

Interaction energy calculation. Spore hydrophobicity was expressed as the free energy of interaction (ΔG_{iwi}, in mJ/m^2) between two equal particles (i) immersed in a liquid (w). If the interaction between the two particles is stronger than the interaction of each particle with the liquid, the free energy of interaction is negative ($\Delta G_{iwi} < 0$) and the particle is considered hydrophobic. A stronger interaction between the particle and the liquid indicates positive free interaction energy ($\Delta G_{iwi} > 0$), which is considered hydrophilic. Interfacial energy (IF; ΔG_{iwi}^{IF}) can be obtained from the surface tension components of the particles (γ_i) and the liquid (γ_w), as shown on equation 2.

$$\Delta G_{iwi}^{IF} = -2(\sqrt{\gamma_i^{LW} - \gamma_w^{LW}})^2 + 4(\sqrt{\gamma_i^{+}\gamma_w^{-}} + \sqrt{\gamma_i^{-}\gamma_w^{+}} - \sqrt{\gamma_i^{+}\gamma_i^{-}} - \sqrt{\gamma_w^{+}\gamma_w^{-}}) \quad (2)$$

The attractive or repulsive interaction between two different particles (i and s) immersed in a liquid (w) was also determined. Surface tension parameters were used to calculate the attractive ($\Delta G_{iws} < 0$) or repulsive ($\Delta G_{iws} > 0$) force between different surfaces used in the study. The total interfacial surface energy (ΔG_{iws}^{IF}) between two different particles is a sum of the apolar (ΔG_{iws}^{LW}) and polar (ΔG_{iws}^{AB}) interfacial energies (Equations 3 and 4) (49).

$$\Delta G_{iws}^{LW} = -2(\sqrt{\gamma_i^{LW} - \gamma_w^{LW}})(\sqrt{\gamma_s^{LW} - \gamma_w^{LW}}) \quad (3)$$

$$\Delta G_{iws}^{AB} = 2[(\sqrt{\gamma_i^{+} - \gamma_s^{+}})(\sqrt{\gamma_i^{-} - \gamma_s^{-}})(\sqrt{\gamma_i^{+} - \gamma_w^{+}})(\sqrt{\gamma_i^{-} - \gamma_w^{-}})(\sqrt{\gamma_s^{+} - \gamma_w^{+}})(\sqrt{\gamma_s^{-} - \gamma_w^{-}})] \quad (4)$$

Surface characterization: electrophoretic mobility and effective diameter measurements. Effective diameters and electrophoretic mobility of *B. anthracis* spores in DI water and PBS buffer (pH 7.2) were obtained using a Zeta PALS apparatus (Brookhaven Instruments Corporation, Holtville, NY). Ten measurements were taken for each spore suspension, with each suspension being read in triplicate at 25°C. Effective diameters were measured after 1 h of spore contact with water or PBS. The Smoluchowski equation was used to determine the zeta potential values of both suspensions (25).

RESULTS

Wipe material and extraction method performances. Figure 1 summarizes the efficiency of extraction of *B. anthracis* as a function of the different extraction solutions and physical dissociation methods used for three different wipe materials and the reference (no wipe). The percentage of extracted spores was calculated on the basis of the initial inoculated spore concentration. Across all wipe materials there was variability associated with the extraction method (extraction solution and PDM). PBS resulted in the poorest performance. Extraction efficiencies in PBS ranged from 3.1% (SD, 2.2%) to 9.8% (SD, 3.3%), while those in the other solutions ranged from 39.8% (SD, 16.9%) to 100.7% (SD 9.8%) (Fig. 1). In addition, extraction performance varied significantly across the wipe materials, indicating an impact of the wipe material on the release of spores from the wipe into the extraction solution (Fig. 1).

Statistical analysis of the extraction performance of the three wipes across the six extraction methods showed that it varied significantly ($P < 0.05$). A pairwise comparison between polyester-rayon and cotton wipes presented no statistically signifi-

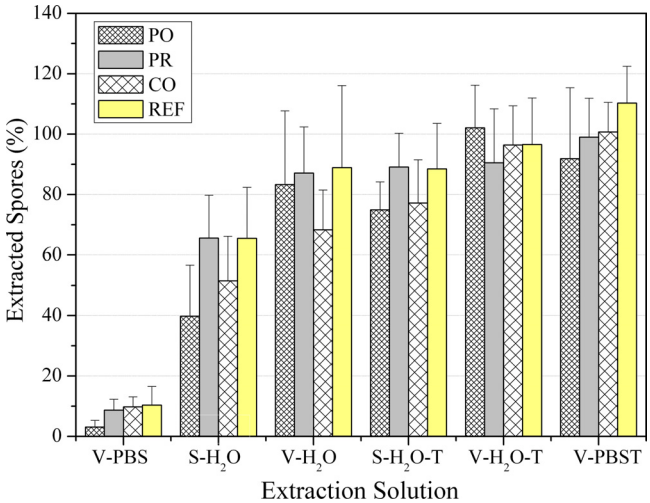

FIG. 1. Extracted spore mean values as a function of different extraction methods (solution and PDM) shown in a histogram. The mean values, including the reference value, are relative to the known inoculated concentration, $(2.27 \pm 0.38) \times 10^4$ spores ml^{-1} ($n = 6$), and uncertainty values (bars) are the standard deviations of the replication experiment ($n = 9$ to 15) for sonication (S), vortexing (V) of wipes in solutions, sterile DI water (H_2O), H_2O-T, PBS, and PBST. Wipe materials are nonwoven polyester-rayon (PR), woven cotton (CO), woven polyester (PO), and a reference control (REF; no wipe added).

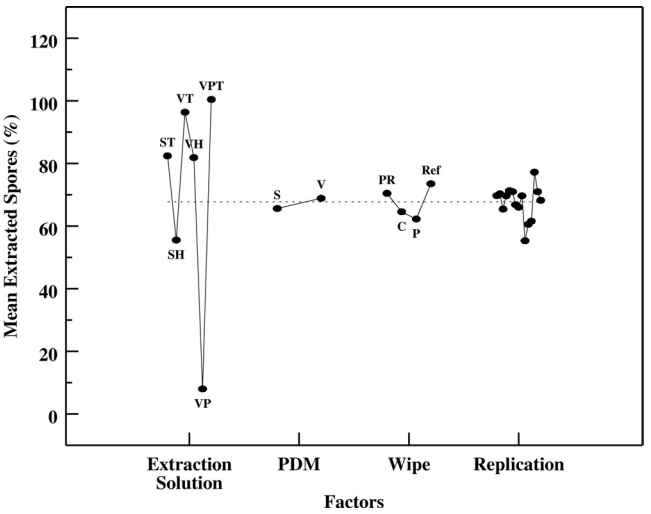

FIG. 2. Graphical representation of the mean value of extracted spores as a function of the experimental factors impacting extraction efficiency according to the extraction solution, which included DI water (H), water plus 0.04% Tween 80 (T), PBS (P), and PBS plus 0.04% Tween 80 (PT); PDM, which included sonication (S) and vortexing (V); and wipe material, which included polyester-rayon (PR), cotton (C), polyester (P), and a reference (Ref) for spore extraction in the absence of wipe. In the replication factor, 15 replicates were involved.

cant difference in wipe performance across all six methods ($P > 0.05$) (see Table S1 in the supplemental material). In contrast, the performance of cotton and polyester was statistically significantly different across all six methods ($P < 0.05$). However, this difference was caused by the extraction efficiency in PBS using vortexing. Any pairwise comparison involving polyester resulted in a statistically significant difference in wipe performance across all six extraction methods (see Table S1 in the supplemental material). A rank analysis showed that polyester-rayon was the most efficient wipe material across all six extraction methods and that polyester was the least efficient (data not shown).

Addition of Tween 80 to the extraction solution dramatically increased wipe extraction efficiency (Fig. 1). Comparisons of surfactant-free solutions (H_2O and PBS) with solutions containing surfactant (H_2O-T and PBST) demonstrated the increase in performance. Statistical analysis between the six extraction methods over all three wipes showed a significant difference ($P < 0.05$) between sample populations (see Table S2 in the supplemental material). Pairwise comparisons for extraction methods across all wipe materials showed no statistically significant difference between sonication with H_2O-T and vortexing with H_2O or vortexing with H_2O-T and vortexing with PBST ($P > 0.05$). In addition, cotton was shown to be more susceptible to the extraction conditions than polyester-rayon, as only 2 pairings out of 15 parings revealed no statistically significant difference between extraction methods, while for polyester-rayon, 6 pairings revealed no statistically significant difference (see Table S2 in the supplemental material).

Statistical analysis for comparisons between vortexing (H_2O and H_2O-T) and sonication (H_2O and H_2O-T) revealed statistically significant differences across all three wipes, with vortexing providing the highest extraction efficiency (data not shown). PBST and H_2O-T using vortexing yielded the highest extraction efficiency for all three wipe materials, with no statistically significant difference between sample means. However, by rank analysis PBST was the best extraction solution over all wipes.

The results obtained for spore extraction indicated that the extraction efficiency step is composed of many variables that could affect the efficiency of sample processing to some degree. In order to evaluate the impact of each variable, a sensitivity analysis was performed (Fig. 2). The mean value of the extracted spores as a function of factors affecting extraction showed that the extraction solution is the most important factor driving extraction efficiency, followed by the wipe material.

Surface thermodynamics. Surface tension components (γ_S^{LW}, γ_S^-, and γ_S^+) for *B. anthracis* spores measured in different solutions are presented in Table 2. Although the spores were prepared in different solutions, they had similar surface tension components, suggesting that the spore surface energy was not altered by the composition of the solution. All spores were hydrophilic ($\Delta G_{iwi} > 0$) (Table 3). The zeta potential values indicated that all spores had a net negative surface charge in

TABLE 2. Surface thermodynamics for polyester, cotton, polyester-rayon, polypropylene, and *B. anthracis* Sterne

Material[a]	Zeta potential (mV)	Effective diam (nm)	Contact angle[b] (degree)			Surface tension[b] (mJ/m^2)		
			Water	Diiodomethane	Formamide	γ^{LW}	γ^+	γ^-
PO			122[c]	77 (7)	43 (4)	38.1	0	5.8
CO			54 (17)	0	87 (2)	14.1	22.2	10.7
PR			88 (1)	38 (2)	0	50.8	1.6	0.1
PP[d]	−58.5[d]					25.7[e]	0[e]	0[e]
B. anthracis[f]	−32.5 (0.8)[g]	1,376.5 (56.5)[g]	12 (0)	16 (1)	55 (3)	31.5	2.9	57.0
B. anthracis[h]	−13.3 (5.6)[g]	1,354.1 (45.4)[g]	14 (1)	17 (5)	32 (4)	43.1	0.5	55.0
B. anthracis[i]	−29.7 (2.5)[g]		11 (2)	17 (2)	38 (3)	40.4	0.8	55.9

[a] Abbreviations: PR, polyester-rayon; CO, cotton; PO, polyester; PP, polypropylene; REF, reference.
[b] Contact angle data report the mean value for 3 to 6 replications for each condition. The standard deviations are shown in parentheses. Values for the surface tension components (γ^{LW}, γ^+, and γ^-) were calculated by equation 1.
[c] Obtained from the literature due to the lack of wettability by capillary adsorption (53).
[d] Zeta potential components of polypropylene were obtained from the literature (32).
[e] Surface tension components of polypropylene were obtained from the literature (49).
[f] Measured in DI water,
[g] The number in parentheses is the standard error.
[h] Measured in PBS buffer.
[i] Measured in 0.04% Tween 80, in the case of zeta potential and effective diameter.

TABLE 3. Calculated interfacial energies ΔG_{iwi} and ΔG_{iws} for surfaces i and s immersed in solution w

Surface i	Surface s	Solution w	ΔG_{iwi} (mJ m^{-2})	ΔG_{iws} (mJ m^{-2})	Interaction[a]
B. anthracis	*B. anthracis*	H_2O	31.68		Repulsive
B. anthracis	*B. anthracis*	PBS	33.76		Repulsive
B. anthracis	*B. anthracis*	H_2O-T	34.46		Repulsive
B. anthracis	Polypropylene	H_2O		−9.25	Attractive
B. anthracis	Polyester	H_2O		4.34	Repulsive
B. anthracis	Cotton	H_2O		−8.49	Attractive
B. anthracis	Polyester-rayon	H_2O		−16.87	Attractive

[a] $\Delta G < 0$ is attractive; $\Delta G > 0$ is repulsive.

water, PBS, and water with Tween 80. No change in spore diameter in water or PBS that could suggest potential spore aggregation in PBS was observed (Table 2).

Wipe surface tension components (γ_S^{LW}, γ_S^-, and γ_S^+) are presented in Table 2. The wipes had very different surface tensions, which is a result of the difference in surface chemistry involved (polyester, rayon, and cellulose). The surface tension components for Tween 80 were determined for both sides of the molecule due to the hydrophobic (tail) and hydrophilic (head) character of the molecule (see Table S3 in the supplemental material). The surface tensions of the head and tail ends of the molecule were very distinct, which would be expected.

The interfacial energy between spores and wipe materials (ΔG_{iws}) immersed in water was attractive ($\Delta G_{iws} < 0$) for cotton and polyester-rayon but repulsive for polyester ($\Delta G_{iws} > 0$), as shown on Table 3. In addition, an interaction between polypropylene (centrifuge tube wall) and spores in water was attractive, suggesting that spores dispersed in water will potentially adhere to the tube wall.

The interfacial energy between Tween 80 molecules and all surfaces (wipe materials, spores, and centrifuge tube wall) immersed in water is presented in Table 4. The Tween 80 hydrophilic head was attractive ($\Delta G_{iws} < 0$) for polyester, polyester-rayon, and polypropylene, while the interaction with cotton or spores was repulsive ($\Delta G_{iws} > 0$). In contrast, the Tween 80 hydrophobic tail was attractive for all wipes and polypropylene but repulsive for spores. In addition, a comparison between interaction energies of surfaces with the hydrophilic or hydrophobic side of Tween 80 showed a stronger attractive interaction ($\Delta G_{iws} < 0$) with the hydrophobic side, suggesting that the surfaces involved in the study (spores, wipes, and centrifuge tube wall) will interact with the hydrophobic side of the Tween 80 molecule once it is immersed in water. Figure 3 summarizes the possible energetic interactions involving wipes, spores, and polypropylene tube when the system is immersed in water and water-surfactant on the basis of the obtained interfacial energy data (ΔG).

TABLE 4. Calculated interfacial energy ΔG_{iws} between surface i and surface s (Tween 80 molecules) immersed in water (solution w)

Surface i	ΔG_{iws} (mJ m^{-2}) for Tween 80 (surface s) films[a]		Interaction for head/tail[b]
	Head group	Tail group	
B. anthracis	21.5	6.99	Repulsive/repulsive
Polyester	−17.98	−53.99	Attractive/attractive
Cotton	4.17	−7.15	Repulsive/attractive
Polyester-rayon	−44.91	−71.05	Attractive/attractive
Polypropylene	−36.6	−75.28	Attractive/attractive

[a] Interfacial energy calculations for surfaces with Tween 80 moieties exposed at the interface.
[b] $\Delta G < 0$ is attractive; $\Delta G > 0$ is repulsive.

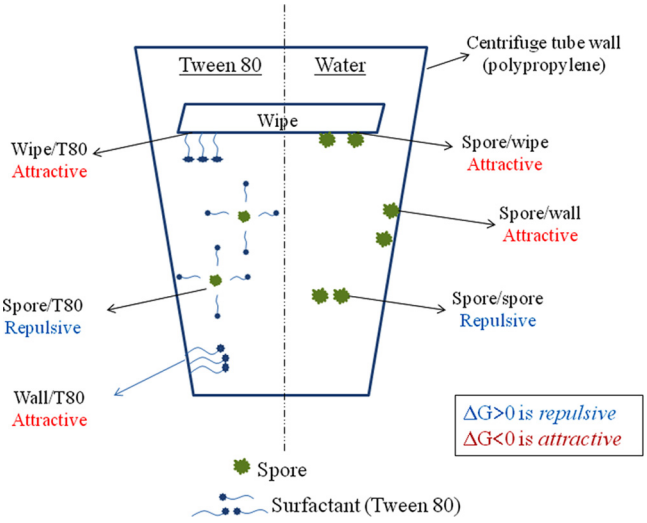

FIG. 3. Representation of the interaction energy (ΔG) between *B. anthracis* spores and all surfaces (wipe, centrifuge tube wall, and Tween 80 molecules) involved in the extraction step when they are immersed in water or surfactant solution (Tween 80).

DISCUSSION

Efficient extraction processes provide optimal spore release from the wipe material surfaces while imposing minimal losses to other surfaces during the extraction process. The objective of this work was to determine the parameters affecting spore extraction processing and to apply surface thermodynamics to explain the observed results. Surface thermodynamics are useful to predict sampling extraction performance on the basis of the surface energy of the microorganism and its interaction with the surrounding media (e.g., solutions, tubes, and sampling materials).

In this work, the extraction solution had the greatest impact on sample processing performance. Extraction efficiencies were dramatically lower when PBS was used as the extraction solution (Fig. 1). Evaluation of the reference control sample in PBS revealed that only 10.4% (SD, 6.1%) of spores would be available for detection in solution. The limited extraction observed in PBS was consistent with colloid stability theory, which has been used to predict and understand particle aggregation as a function of ionic strength (49). Colloidal stability theory predicts the interaction energy between two charged surfaces as a function of the separation distance (4, 36, 49). By increasing ionic strength, the long-range repulsion between two surfaces decreases due to electrostatic interaction, resulting in adhesion or aggregation of the involved surfaces in most of the cases (1, 28, 49). Both polypropylene (centrifuge tube) and spores presented negative surface charge values charac-

teristic of a repulsive interaction between surfaces. However, the high salt content in PBS decreased the repulsive interaction by increasing spore adherence to the polypropylene tube, resulting in fewer spores available in solution. The ability of a microorganism to be dispersed in solution depends on the physicochemical properties (hydrophobicity or hydrophilicity) of the cell surface, as it varies among different genus and species (13), as well as on the characteristics of the solution. Thus, recovery efficiencies will be dependent on the organism sampled during the collection (2, 37, 43, 52). In contrast, solutions containing Tween 80 provided superior extraction efficiency. Extraction efficiencies in Tween 80 were consistent with several reports in the literature (27, 41). The strong attraction between Tween 80 and the polypropylene tube resulted in Tween 80 film formation on the centrifuge tube wall. In addition, the interaction between Tween 80 and polypropylene (-75.28 mJ/m^2) was stronger than the interaction between spores and polypropylene (-9.25 mJ/m^2) (Tables 3 and 4). Thus, the competition between spores and Tween 80 to adhere to the tube wall was in favor of the surfactant. As a result, the surfactant coating the tube wall limited spore attachment and increased the number of spores in solution available for quantification.

During extraction processing, the sampled spore population would be distributed between the solution and wipe material surface, either entrapped or adsorbed. The efficiency of wipe material release or retention of spores from its surface could be partially explained by the wipe surface thermodynamics. Polyester retained more spores than the other wipe materials across all extraction methods. In addition, polyester was the only wipe material evaluated with repulsive interaction energies for spores in water. In contrast, polyester-rayon released the highest number of spores across all extraction methods. Interaction of spores with the complex fiber structure of the wipe makes separation of the contribution of surface thermodynamics from spore entrapment challenging. Rose et al. (41), using scanning electron microscopy (SEM), observed irregularities in polyester fiber structure compared with the structures of cotton, polyester-rayon, and macrofoam that resulted in polyester retaining more spores than the other wipe materials.

Interaction energies between wipes and Tween 80 were more attractive for all three wipes (Table 4, tail group), suggesting that the spores did not adhere directly to the wipe material in solutions containing Tween 80. Moreover, the interaction energy between polyester-rayon or polyester and Tween 80 was more attractive than the interaction between spores and Tween 80 (Table 4). Therefore, the lower recovery obtained for polyester (Fig. 1) indicates that entrapment or another factor may be governing the release of spores from the polyester surface. In contrast, cotton was more attracted to spores (-8.49 mJ/m^2) than Tween 80 (-7.15 mJ/m^2; tail group) (Tables 3 and 4), indicating that the spores were likely associated with the cotton surface even when Tween 80 was present in the solution. The performance of cotton compared to that of the other wipe materials (Fig. 1) may also be due to cotton fiber degradation during processing, as cotton produced a slightly yellowish hue in the extraction solution during the extraction procedure.

In summary, the impact of different parameters (e.g., extraction solution and wipe material) on spore extraction efficiency was evaluated, and it was found that the extraction solution used followed by the wipe material used was critical to enhancing wipe extraction efficiency. The best combination for optimal spore extraction was polyester-rayon wipe, PBST, and vortexing. The potential use of surface thermodynamics to understand and predict surface sampling methods on the basis of the microorganism surface characteristic and its interaction with the surrounding environment was presented as a new approach for improving biological surface sampling and understanding extraction efficiency. Future work should focus on developing an understanding of the role of extraction solution on effectively capturing bacterial populations for follow-on enumeration while preserving viability, a critical factor to be explored for other organisms.

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REFERENCES

1. **Abu-Lail, N. I., and T. A. Camesano.** 2003. Role of ionic strength on the relationship of biopolymer conformation, DLVO contributions, and steric interactions to bioadhesion of Pseudomonas putida KT2442. Biomacromolecules **4:**1000–1012.
2. **Angelotti, R., J. L. Wilson, W. Litsky, and W. G. Walter.** 1964. Comparative evaluation of the cotton swab and Rodac methods for the recovery of Bacillus subtilis spore contamination from stainless steel surfaces. Health Lab. Sci. **1:**289–296.
3. **Aranberri-Askargorta, I., T. Lampke, and A. Bismarck.** 2003. Wetting behavior of flax fibers as reinforcement for polypropylene. J. Colloid Interface Sci. **263:**580–589.
4. **Bostrom, M., D. R. M. Williams, and B. W. Ninham.** 2001. Specific ion effects: why DLVO theory fails for biology and colloid systems. Phys. Rev. Lett. **87:**168103.
5. **Bower, C. K., J. McGuire, and M. A. Daeschel.** 1996. The adhesion and detachment of bacteria and spores on food-contact surfaces. Trends Food Sci. Technol. **7:**152–157.
6. **Boxman, I., et al.** 2009. Norovirus on swabs taken from hands illustrate route of transmission: a case study. J. Food Prot. **72:**1753–1755.
7. **Brown, G. S., et al.** 2007. Evaluation of a wipe surface sample method for collection of Bacillus spores from nonporous surfaces. Appl. Environ. Microbiol. **73:**706–710.
8. **Brown, G. S., et al.** 2007. Evaluation of rayon swab surface sample collection method for Bacillus spores from nonporous surfaces. J. Appl. Microbiol. **103:**1074–1080.
9. **Buttner, M. P., et al.** 2004. Evaluation of the biological sampling kit (BiSKit) for large-area surface sampling. Appl. Environ. Microbiol. **70:**7040–7045.
10. **Buttner, M. P., P. Cruz-Perez, and L. D. Stetzenbach.** 2001. Enhanced detection of surface-associated bacteria in indoor environments by quantitative PCR. Appl. Environ. Microbiol. **67:**2564–2570.
11. **Chen, G., and K. A. Strevett.** 2003. Microbial surface thermodynamics and interactions in aqueous media. J. Colloid Interface Sci. **261:**283–290.
12. **Cunliffe, D., C. A. Smart, C. Alexander, and E. N. Vulfson.** 1999. Bacterial adhesion at synthetic surfaces. Appl. Environ. Microbiol. **65:**4995–5002.
13. **Daffonchio, D., J. Thaveesri, and W. Verstraete.** 1995. Contact-angle measurement and cell hydrophobicity of granular sludge from upflow anaerobic sludge bed reactors. Appl. Environ. Microbiol. **61:**3676–3680.

14. **Dewhurst, E., D. M. Rawson, and G. C. Steele.** 1986. The use of a model system to compare the efficiency of ultrasound and agitation in the recovery of Bacillus subtilis spores from polymer surfaces. J. Appl. Bacteriol. **61:**357–363.
15. **Edmonds, J. M., et al.** 2009. Surface sampling of spores in dry-deposition aerosols. Appl. Environ. Microbiol. **75:**39–44.
16. **Eginton, P. J., J. Holah, D. G. Allison, P. S. Handley, and P. Gilbert.** 1998. Changes in the strength of attachment of micro-organisms to surfaces following treatment with disinfectants and cleansing agents. Lett. Appl. Microbiol. **27:**101–105.
17. **Elimelech, M., J. Y. Chen, and Z. A. Kuznar.** 2003. Particle deposition onto solid surfaces with micropatterned charge heterogeneity: the "hydrodynamic bump" effect. Langmuir **19:**6594–6597.
18. **Estill, C. F., et al.** 2009. Recovery efficiency and limit of detection of aerosolized Bacillus anthracis Sterne from environmental surface samples. Appl. Environ. Microbiol. **75:**4297–4306.
19. **Faille, C., V. Lebret, F. Gavini, and J. F. Maingonnat.** 1997. Injury and lethality of heat treatment of Bacillus cereus spores suspended in buffer and in poultry meat. J. Food Prot. **60:**544–547.
20. **Favero, M. S., J. J. Mcdade, J. A. Robertse, R. K. Hoffman, and R. W. Edwards.** 1968. Microbiological sampling of surfaces. J. Appl. Bacteriol. **31:**336–343.
21. **Fernandez, D., E. M. Rodriguez, G. H. Arroyo, N. L. Padola, and A. E. Parma.** 2009. Seasonal variation of Shiga toxin-encoding genes (stx) and detection of E. coli O157 in dairy cattle from Argentina. J. Appl. Microbiol. **106:**1260–1267.
22. **Frawley, D. A., et al.** 2008. Recovery efficiencies of anthrax spores and ricin from nonporous or nonabsorbent and porous or absorbent surfaces by a variety of sampling methods. J. Forensic Sci. **53:**1102–1107.
23. **Gaydos, C. A., and T. C. Quinn.** 2005. Urine nucleic acid amplification tests for the diagnosis of sexually transmitted infections in clinical practice. Curr. Opin. Infect. Dis. **18:**55–66.
24. **Gunduz, T., et al.** 2008. The prevalence of intestinal parasites and nasal S. aureus carriage among food handlers. J. Environ. Health **70:**64–65.
25. **Hiemenz, P., and R. Rajagopalan.** 1997. Principles of colloid and surface chemistry. Marcel Dekker, New York, NY.
26. **Hodges, L. R., L. J. Rose, H. O'Connell, and M. J. Arduino.** 2010. National validation study of a swab protocol for the recovery of Bacillus anthracis spores from surfaces. J. Microbiol. Methods **81:**141–146.
27. **Hodges, L. R., L. J. Rose, A. Peterson, J. Noble-Wang, and M. J. Arduino.** 2006. Evaluation of a macrofoam swab protocol for the recovery of Bacillus anthracis spores from a steel surface. Appl. Environ. Microbiol. **72:**4429–4430.
28. **Israelachvilli, J.** 1992. Intermolecular and surface forces. Academic Press, Inc., San Diego, CA.
29. **Kirschner, L. E., and J. R. Puleo.** 1979. Wipe-rinse technique for quantitating microbial contamination on large surfaces. Appl. Environ. Microbiol. **38:**466–470.
30. **Lewandowski, R., K. Kozlowska, M. Szpakowska, M. Stepinska, and E. A. Trafny.** 2010. Use of a foam spatula for sampling surfaces after bioaerosol deposition. Appl. Environ. Microbiol. **76:**688–694.
31. **Li, Q., and B. E. Logan.** 1999. Enhancing bacterial transport for bioaugmentation of aquifers using low ionic strength solutions and surfactants. Water Res. **33:**1090–1100.
32. **Lokhande, H. T., V. S. Thakar, and S. R. Shukla.** 1984. Electrokinetic properties of acrylic acid-grafted and methacrylic acid-grafted polypropylene during chemically-initiated graft-copolymerization. J. Appl. Polym. Sci. **29:** 2989–2996.
33. **MacKintosh, E. E., J. D. Patel, R. E. Marchant, and J. M. Anderson.** 2006. Effects of biomaterial surface chemistry on the adhesion and biofilm formation of Staphylococcus epidermidis in vitro. J. Biomed. Mater. Res. A **78A:** 836–842.
34. **Mceldowney, S., and M. Fletcher.** 1986. Effect of growth-conditions and surface characteristics of aquatic bacteria on their attachment to solid-surfaces. J. Gen. Microbiol. **132:**513–523.
35. **Moore, G., and C. Griffith.** 2002. A comparison of surface sampling methods for detecting coliforms on food contact surfaces. Food Microbiol. **19:**65–73.
36. **Morrow, J. B., R. Stratton, H. H. Yang, B. F. Smets, and D. Grasso.** 2005. Macro- and manoscale observations of adhesive behavior for several E. coli strains (O157:H7 and environmental isolates) on mineral surfaces. Environ. Sci. Technol. **39:**6395–6404.
37. **Nellen, J., P. Rettberg, G. Horneck, and W. R. Streit.** 2006. Planetary protection—approaching uncultivable microorganisms. Adv. Space Res. **38:** 1266–1270.
38. **Petersen, N. J., D. E. Collins, and J. H. Marshall.** 1974. Evaluation of skin cleansing procedures using wipe rinse technique. Health Lab. Sci. **11:**182–187.
39. **Redman, J. A., S. L. Walker, and M. Elimelech.** 2004. Bacterial adhesion and transport in porous media: role of the secondary energy minimum. Environ. Sci. Technol. **38:**1777–1785.
40. **Ribot, W. J., et al.** 2006. Anthrax lethal toxin impairs innate immune functions of alveolar macrophages and facilitates Bacillus anthracis survival. Infect. Immun. **74:**5029–5034.
41. **Rose, L., B. Jensen, A. Peterson, S. N. Banerjee, and M. J. Arduino.** 2004. Swab materials and Bacillus anthracis spore recovery from nonporous surfaces. Emerg. Infect. Dis. **10:**1023–1029.
42. **Sanderson, W. T., et al.** 2004. Bacillus anthracis contamination and inhalational anthrax in a mail processing and distribution center. J. Appl. Microbiol. **96:**1048–1056.
43. **Scherer, K., et al.** 2009. Application of a swab sampling method for the detection of norovirus and rotavirus on artificially contaminated food and environmental surfaces. Food Environ. Virol. **1:**42–49.
44. **Shi, X. M., and X. N. Zhu.** 2009. Biofilm formation and food safety in food industries. Trends Food Sci. Technol. **20:**407–413.
45. **Speranza, G., et al.** 2004. Role of chemical interactions in bacterial adhesion to polymer surfaces. Biomaterials **25:**2029–2037.
46. **Stelmack, P. L., M. R. Gray, and M. A. Pickard.** 1999. Bacterial adhesion to soil contaminants in the presence of surfactants. Appl. Environ. Microbiol. **65:**163–168.
47. **Teshale, E. H., et al.** 2002. Environmental sampling for spores of Bacillus anthracis. Emerging Infect. Dis. **8:**1083–1087.
48. **Valentine, N. B., et al.** 2008. Evaluation of sampling tools for environmental sampling of bacterial endospores from porous and nonporous surfaces. J. Appl. Microbiol. **105:**1107–1113.
49. **Van Oss, C. J.** 1994. Interfacial forces in aqueous media. Marcel Dekker, Inc., New York, NY.
50. **Van Oss, C. J., M. K. Chaudhury, and R. J. Good.** 1988. Interfacial Lifshitz-vanderwaals and polar interactions in macroscopic systems. Chem. Rev. **88:**927–941.
51. **Volkering, F., A. M. Breure, and W. H. Rulkens.** 1997. Microbiological aspects of surfactant use for biological soil remediation. Biodegradation **8:**401–417.
52. **Vorst, K. L., E. C. Todd, and E. T. Rysert.** 2004. Improved quantitative recovery of Listeria monocytogenes from stainless steel surfaces using a one-ply composite tissue. J. Food Prot. **67:**2212–2217.
53. **Zhu, L., A. Perwuelz, M. Lewandowski, and C. Campagne.** 2006. Wetting behavior of thermally bonded polyester nonwoven fabrics: the importance of porosity. J. Appl. Polym. Sci. **102:**387–394.