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Bacillus anthracis (BA) is a dangerous pathogen and a bioweapon of great concern. Because of the logistical and safety challenges associated with handling BA, finding a suitable surrogate is vital for disaster response and recovery planning. The results of this bench-scale study indicate that Bacillus globigii is a suitable surrogate for Bacillus anthracis Sterne (BAS) with respect to bioaerosol emission, but a poor surrogate in relation to UV inactivation and PAC adsorption. These results can be used to understand BAS as a surrogate for BA Ames because of its genetic and morphological similarities with BAS.

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Authors:

Leigh Durden[1], Kyle Eckhoff[1], Adam C. Burdsall[2], Sungmin Youn[3], Cindy Andújar-Gonzalez[1], Lubna Abu-Niaaj[4], Matthew Magnuson[2], and Willie F. Harper, Jr.*[1]

Affiliations:

[1]Air Force Institute of Technology, Engineering Management Program, Department of Systems Engineering and Management, 2950 Hobson Way, Wright-Patterson AFB, OH, US.

[2]US Environmental Protection Agency, National Homeland Security Research Center, Water Infrastructure Protection Division, Cincinnati, Ohio.

[3]Marshall University, Department of Civil Engineering, Huntington, West Virginia.

[4]Central State University, Department of Agricultural and Life Sciences, Wilberforce, Ohio.

Characterizing *Bacillus globigii* as a *Bacillus anthracis* surrogate for wastewater treatment studies and bioaerosol emissions

ABSTRACT

This study characterized *Bacillus globigii* (BG) as a *Bacillus anthracis* Sterne (BAS) surrogate for wastewater treatment-related studies of UV inactivation, adsorption onto powdered activated carbon (PAC), and bioaerosol emission. The inactivation of BG was faster than that of BAS in DI water (pseudo first-order rate constants of 0.065 and 0.016 min^{-1} respectively) and in PBS solution (0.030 and 0.005 min^{-1} respectively). BG was also removed more quickly than BAS by PAC adsorption in DI (0.07 and 0.05 min^{-1} respectively) and in PBS (0.09 and 0.04 min^{-1} respectively). In DI, BG aggregated more ($P < 0.05$) than BAS when the pH was 7 or greater but there were no statistically significant differences in NaCl solution. Spore aggregation was also studied with Extended Derjaguin-Landau-Verwey-Overbeek (xDLVO) models. Less than 1% of all spores were released as bioaerosols, and there was no significant difference ($P > 0.05$) in emission between BG and BAS. To the author's knowledge, this study is the first to demonstrate that BG is a suitable surrogate for BAS for bioaerosol emissions, but a poor surrogate for both UV inactivation and PAC adsorption. These results can be used to understand the ability of BAS to act as a surrogate for BA Ames because of its genetic and morphological similarities with BAS.

Keywords: *B. globigii*, *B. anthracis*, surrogacy, spores, wastewater, adsorption, inactivation, bioaerosol

1. INTRODUCTION

Identifying suitable microbial surrogates can help water resource recovery facilities (WRRFs) to prepare for scenarios that involve the intentional or inadvertent introduction of high consequence biocontaminants (HCBs) into the wastewater collection and treatment system. The US National Response Team's Quick Reference Guide for *Bacillus anthracis*, an HCB, reinforces the importance of proper management of wastewater during response activities[1]. HCBs include biological weapons, biohazardous or infectious waste, and medical waste; and they can originate from many anthropogenic sources[2,3,4]. HCBs are also of particular interest to the US Department of Defense (DOD), as well as local, state, federal, and tribal agencies with responsibility for response and recovery. Because of logistical and safety challenges associated with HCBs, finding suitable surrogates with which to perform response and recovery research and/or to develop appropriate guidelines will help nearby WRRFs prepare for handling such wastewater, one of many important aspects of response and recovery.

Bacillus anthracis (BA) is one of the most likely HCBs to be used in an attack[5,6]. BA is a gram-positive, aerobic, non-motile, and rod-shaped bacterium, approximately about 1- 1.5 μm x 3 – 10 μm in size[7]. The chemical properties of the spore coat make it one of the most resistant and potent biological warfare agents[5,8]. Two plasmids, pXO1 and pX02, are responsible for its pathogenicity. BA is a threat in water, aerosols, and fomites[1]. Previous research has investigated *Bacillus globigii* (BG) as a BA surrogate during disinfection with chlorine, a common practice during both drinking water and wastewater treatment. Brazis et al., 1958[9] carried out bench top studies with hypochlorite over a range of pH and temperature conditions and found that BG was more resistant to disinfection than BA. Later studies confirmed that the required concentration-

time (CT) values of BG disinfection with hypochlorite were higher than those of BA Sterne (BAS), as well as the more virulent BA Ames strain[10,11]. BG is more resistant to chlorine and is therefore a conservative BA surrogate for inactivation with chlorine. However, there are no comparable studies for UV disinfection, which has become more common in both drinking water and wastewater plants due to concerns about safety and disinfection byproduct formation. There are also no previously published studies that have investigated BG as a BA surrogate for attachment to powdered activated carbon (PAC), which has been successfully used to capture biological materials from wastewater[12,13]. In addition, while bioaerosol emission from wastewater plants has been studied[14,15], no previous studies have investigated BG as a BA surrogate during aerosolization from wastewater.

The objectives of this study are to compare the fate of BG and BAS spores during processes relevant to wastewater treatment: 1) UV inactivation, 2) adsorption onto PAC, and 3) bioaerosol emission. Separate batch experiments were carried out for each objective. The BAS spore coat is more hydrophobic than that of BG[16], which may cause differences in the spore clustering[16,17]. Therefore, experiments were carried out in deionized water (DI) and in phosphate-buffered solution (PBS) in order to evaluate the possible effects since the ionic strength of wastewaters may vary[12,18]. To help understand the results mechanistically, differences in BAS and BG aggregation were experimentally evaluated as a function of pH and ionic strength through optical microscopy and theoretical XDLVO analysis. Finding a safe surrogate for BAS is valuable; if large amounts of BAS were used in large scale pilot- or full-scale studies, it could pose a significant risk to wastewater treatment plant workers and the general public.

2.0. MATERIALS AND METHODS

2.1. Experimental overview. Suspensions of BG and BAS were introduced into glass vessels during batch experiments. During all experiments, spore concentrations were determined using culture-dependent methods (described in Section 2.2 below). All experiments were completed in a Biological Safety Cabinet (model SG403, Baker Company SterilGuard II advance). Two-tailed, student t-tests performed in Microsoft Excel were used to determine statistical significance at the 95% confidence level ($\alpha = 0.05$).

2.2. Preparation of the spores. The original BG and BAS stocks used in these experiments were provided by the US Environmental Protection Agency (EPA). To prepare fresh spore stock solution, 1 mL of deionized (DI) water was added to 50 μL of spore stock solution. The mixture was homogenized using a Vortexer (Daigger Vortex Genie 2, Catalog Number 22220A, Daigger Scientific, Hamilton, NJ, USA) and then cultured onto nutrient agar plates. The plates were incubated at 35^{o}C for 7 days to induce bacterial growth and sporulation. At the end of the incubation, the colonies were scraped off the plates with a sterile cell scraper and transferred into sterile centrifuge tubes. The harvested spores were suspended in sterile DI water and centrifuged for 20 minutes at 4000 rpm (Relative Centrifugal Force, RCF = 3220) at a temperature of 4-8°C. Then, the supernatant was removed, and sterile DI water was added to resuspend the pellet for centrifugation. This process was repeated three times on the day of the harvest, one day after the harvest, and again three days later. Multiple washings and sonication steps ensured that any remaining vegetative cells were removed (i.e., <5% vegetative cells). The purity of the spores was confirmed by phase contrast microscopy (see Section 2.6).

2.3. UV inactivation experiments. One hundred microliters of BAS or BG spore stock was vortexed for 20 to 30 seconds, and then combined with DI or PBS in a 50 mL volumetric flask to

achieve an initial concentration of approximately 10^4 colony forming units (CFU) per mL The suspension was mixed at 300 rpm (RCF = 18) for 2 minutes and then the 8 watt, 254 nm the low-pressure mercury lamp (Analytik Jena US, model UVS-28; UV Products, Upland, CA) was activated (see Figure A1, Panel A). The distance between the lamp and the flask was 3.6 inches. Samples were collected every five minutes to determine BAS or BG viability. Tubes were vortexed for 10 to 15 seconds before plating. One hundred microliters were spread with a L-shaped spreader on prepared nine-centimeter diameter nutrient agar plates (see Table A1). Bacterial viability was determined after overnight incubation at 35°C. Control experiments were carried out in the absence of UV light. The model used to retrieve observed first-order rate constants (k) was as follows[12,19]:

$$N(t) / N_o = 1 - (1- e^{-kt})^{Nc}$$

Viable spore concentration (i.e., N(t) and N_o) was in units of CFU/ml. The units of time (t) were minutes and those of k were min^{-1}. N_c is the number of critical targets that need to be disabled before inactivation occurs. N_c was set to one [20]. Curve fitting was carried out to retrieve the observed first-order rate constants (shown in Table A2) by minimizing the root mean square error[21].

For purposes of comparing rate constants, BG was characterized as a *poor* BAS surrogate if BAS was more difficult to inactivate than BG (i.e., lower rate constant), *conservative* if BG was more difficult to inactivate than BAS, and *suitable* if there was no statistically significant difference.

2.4. PAC adsorption experiments. BAS or BG spores (initial concentration ~ $3x10^6$ CFU/mL) were suspended in 300 mL (DI water or PBS) solution to give a typical initial concentration of

~10^3 CFU/mL. After mixing the suspension for 30 minutes with a magnetic stir bar, a 1mL sample was taken and plated in triplicate. Three hundred milligrams of PAC (74 – 177 μm in particle size, Cat No. M-2248, Cabot Corporation, Boston, MA) were added and mixed to keep the PAC suspended in the bulk liquid (see Figure A1, Panel B). Samples (1 ml) were collected in triplicate after 15, 30, 45, 60, and 120 minutes and plated for CFU determination. The point of zero charge for the PAC was 9.9, as experimentally determined using the drift method[22]. Because the pH was 8 during the PAC experiment, the positively charged PAC attracted the negatively charged spore. Control experiments were carried out in the absence of PAC. Curve fitting was carried out in order to retrieve the observed pseudo first-order rate constants (shown in Table A2) using the following pseudo first-order model[23].

$$\frac{dC}{dt} = \mathrm{k'(C - Ce)} \qquad (1)$$

where C is the concentration of CFU at time t; C_e is the concentration of CFU at equilibrium; k' is the observed pseudo first-order rate constant.

For purposes of comparing PAC removal, BG was characterized as a *poor* BAS surrogate if BAS was more difficult to remove than BG, *conservative* if BG was more difficult to remove than BAS, and *suitable* if there was no statistically significant difference.

2.5. Bioaerosol experiments. Bioaerosol experiments were carried out with diffused aeration as described previously[15]. Briefly, the bioreactor was a 250-mL wide-mouthed bottle (inner diameter: 6.3 cm) filled with 20 mL of sterile DI water, which was spiked with spores at the beginning of the experiment. The reactor was stirred for 30 minutes prior to bioaerosol sampling. The experiments were performed with an aeration rate of 0.5 L/min. Bioaerosols were captured for 30 minutes using a BioSampler (catalog #225-9595, SKC Inc., Eighty Four, PA, See Figure

A1, Panel E) with the inlet connected via a 1/2"-ID tubing (Cole-Palmer, catalog #SKU 95802-23, Cole Parmer, Vernon Hills, IL, USA) to the top of the bioreactor and the outlet connected to a vacuum pump (catalog #15 32-101-G557X, GAST, Benton Harbor, MI). The collection liquid used was 20 mL sterile DI water. Samples were appropriately diluted or concentrated with centrifugation (when the bioaerosol count was low) and plated onto nutrient agar plates. CFU were determined after overnight incubation at 35°C. For purposes of comparing bioaerosol emission, BG was characterized as a *poor* BAS surrogate if BG emission was lower than that of BAS, *conservative* if BG emission was higher than that of BAS, and *suitable* if there was no statistically significant difference.

2.6. Aggregate imaging and counting. In this study, the differences in BAS and BG aggregation were experimentally evaluated as a function of pH and ionic strength. Ten microliters of bacteria spores of high concentration (~10^{11} CFU/mL) were added to 990µL of sterile DI or NaCl solution, mixed by manual inversion, and diluted until the optical density (OD600) fell within the range of 0.875-0.9 AU. 10µL of spores were suspended into 990µL of specific solutions including DI water and 0.15M NaCl solutions ranging in pH levels from 3-10. In these experiments, PBS was replaced with NaCl because the pH adjustment step diluted the PBS concentrations excessively. Three slides were prepared with 20x20 sticker grids added to the back side of each slide. 10µL of sample were added to each slide before adding a coverslip over the sample, samples were used for microscopic imaging (Zeiss, Axioskop, White Plains, NY). 40 images were taken on each slide. Grid squares for imaging were selected in pattern in order to ensure coverage of the entire slide and also so that no one grid was imaged twice. The spores present on each image were manually counted. Isolated spores, duplets, triplets, and any

aggregates of 4 or more spores were tallied. This process was used for all suspensions. Each experiment was done three times.

2.7. Extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) analysis. XDLVO theory was used to predict the relative favorability of aggregation and adsorption. The model includes van der Waals forces (attractive energy), repulsive electrostatic forces (electrostatic repulsion energy), and Lewis acid-base forces to calculate interaction energy as a function of the separation distances. XDLVO modeling was as described previously[24]. Model parameters were retrieved from published sources[25,26,27,28].

3. RESULTS AND DISCUSSION

3.1 UV Inactivation experiments. Figure 1 presents the inactivation profiles with the relative concentration (C/C_o) on the y-axis and time (in minutes) on the x-axis. The extent of BG inactivation was higher than that of BA in DI water, and the observed first order rate constants were 0.065 and 0.016 min^{-1} for BG and BA respectively. The differences in the inactivation extent and in the rate constants were statistically different ($P < 0.001$). Spore losses in controls carried out in DI and PBS were 7% and 8% respectively. BA was more resistant to UV inactivation; thus, BG was a poor BAS surrogate in DI water. The inactivation results in PBS also indicated that BG was a poor BAS surrogate. In both cases, the rate constants were lower in PBS than in DI; the observed first order rate constants in PBS were 0.030 and 0.005 min^{-1} for BG and BA, respectively.

In principle, aggregation can inhibit inactivation by protecting the spores located inside of the clusters. In this study, the differences in BAS and BG aggregation were experimentally evaluated

as a function of pH and ionic strength. In DI, BG aggregated more than BAS when the pH was 7 or greater ($P < 0.05$) (Figure A2); there were no statistically significant differences between spore aggregation in NaCl solution ($P > 0.05$). The percentages of dimers for both spores were higher in DI than in NaCl. XDLVO analysis was also used to study the tendencies of these single spores to cluster. The interaction energies for BAS were lower (i.e., more favorable for aggregation) than those of BG (Figure 2). This result is not consistent with the results shown in Figure A2. However, the XDLVO results also indicated that the spore interaction energies were lower in DI than they were at higher ionic strength, meaning that both spores should aggregate more favorably in DI; this is consistent with the results shown in Figure A2. Thus, XDLVO analysis partially corroborated the results of the aggregation study. This partial corroboration may result from limited XDLVO parameter values in the literature. More values and data are needed to computationally study the tendency to form trimers and quadruples.

3.2. PAC adsorption experiments. Figure 3 shows the results of the PAC adsorption experiments. The relative concentration is shown on the y-axis, and time in minutes is shown on the x-axis. BAS spores were more difficult to remove than BG spores. In DI water, an average of 49% (i.e. Ce/Co = 0.51) of BAS spores were removed while an average of 84% (i.e. Ce/Co = 0.16) of BG spores were removed (Figure 3). The observed first order rate constants in DI for BAS and BG adsorption were 0.05 and 0.07 min^{-1} respectively. The equilibrium concentrations (i.e., C_e) of BAS were significantly higher than those of BG ($P < 0.05$). In PBS, the average adsorptive removals were 34% and 91% for BAS and BG spores, respectively. The observed pseudo first order rate constants for adsorption were 0.04 min^{-1} and 0.09 min^{-1} for BA and BG respectively; and the kinetic differences in PBS were statically significant ($P < 0.05$). It was

more difficult to remove BAS than BG with PAC in both liquids. Thus, with respect to PAC adsorption, BG was a poor BAS surrogate.

At distances of 4 nm or less, the BAS XDLVO interaction energies with the PAC surface were lower than those of BG in both DI and PBS (Figure 4). These results did not complement the experimental results because they suggested that BA should adsorb more favorably to PAC. The discrepancies between the model outcomes and the experimental results may indicate that the XDLVO model parameters published in literature do not apply to the materials (e.g., PAC) or experimental conditions used in this study. The variability in the morphological properties of *Bacillus anthracis* clades has been discussed in literature[29]. However, to the author's knowledge, the range of effective values for XDLVO model parameters for the organisms (as well as those of PAC) has not been addressed in a previous study and may warrant further investigation.

3.3. Bioaerosol release experiments. Figure 5 shows the results of the aerosolization experiments. The x-axis shows the percentage of aerosolized spores (i.e., the CFU captured in an aerosol divided by the CFU initially present in the vessel). The average percentage of aerosolized BG and BAS spores were 0.30% and 0.48%, respectively, in DI, and 0.65% and 0.47%, respectively, in PBS. The emission of BG and BAS was not statistically different in DI water or PBS ($P > 0.05$), so BG is a suitable BAS surrogate for aerosolization. Previous work has recently demonstrated that the emission of BG bioaerosols from mixed vessels is driven by the intensity of mixing, and that superficial gas velocity and Froude number can be empirically correlated with spore emission[15]. It is likely that BAS emission may also be correlated to the same parameters because in general, bioaerosol emission is caused by splashing, mechanical forces, or

bubbles that burst at the air-water interface[14,30]. These causes may primarily be related to the properties of the liquid being aerosolized, not the microorganism contained within the aerosol. Thus, the statistical results for BG and BAS are consistent with the underlying physical phenomena of aerosolization. However, because of the potential importance of the bioaerosols containing high consequence contaminants, additional experiments are warranted that include a larger range of aerosol generation conditions, which can vary greatly between wastewater treatment plants.

3.4 Implications for BA Ames and future considerations. Overall, this study demonstrates that there is more than one aspect to selecting a suitable surrogate, meaning that one surrogate may not be appropriate for all processes of interest. In considering why this is for BG and BAS, it is useful to consider that BAS and BA Ames are more closely related to each other (genetically) than to BG[29,31,32]. BAS and BA Ames have similar surface architectures, including the exosporium and thin filaments which contain the collagen-like protein (BclA) and extend off the surface; BG does not have an exosporium, the protruding structures, or the BclA genes[29]. Because of the genetic and morphological similarities between BAS and BA Ames, BG may have similar surrogacy behavior with respect to both BAS and BA Ames, e.g., when BG is a poor surrogate for BAS, it is likely to also be a poor surrogate for BA Ames. It might be inferred that poor surrogacy has its origins in the physical differences between the organisms, especially when the process being surrogated is physical in nature, such as interactions with other organisms or with insoluble substances (e.g., PAC) in the water.

For UV inactivation, the results suggest the poor surrogacy may result less from interaction between organisms (e.g., aggregation), and more from better shielding of the interior of the spore by the exosporium of BAS. UV light induces photochemical reactions that damage nucleic acid and photothermal effects that cause membrane disruption and protein unfolding[33]. The relative impact of these two mechanisms may be different in future studies. For instance, using a different light source with different wavelengths (e.g., light emitting diodes or pulsed UV lamps instead of a mercury lamp) could produce results that are unlike those presented in this study. Additionally, the reactor configuration and lamp placement could impact the findings due to the changes in reflectance and incident power[23,34,35]. Future studies should employ the experimental conditions that include other operational situations of interest.

BG was a poor surrogate for BAS with respect to PAC adsorption and UV inactivation in both DI (ionic strength = 0.0001 M) and PBS media (ionic strength = 0.15 M). Therefore, ionic strength did not affect these two surrogacy assessments. This finding is relevant to real wastewater because ionic strength varies greatly in full scale systems. The ionic strength of influent domestic wastewater is typically between 0.003 to 0.10 M[12,18] while the ionic strength of digestor recycle streams can be as high as 0.17 M[18]. Many municipal wastewater treatment plants also receive significant industrial discharges, which can have ionic strength levels that exceed 1 M[36,37,38]. Since ionic strength can impact the surface properties of Bacillus spores, future studies may investigate the effects of the higher ionic strength conditions (e.g. > 1 M) present when a large volumetric fraction of the influent is received from industrial sources.

4.0. CONCLUSIONS

This study characterized BG as a BAS surrogate during wastewater treatment and bioaerosol emission batch tests. Observed pseudo first-order rate constants showed that BG UV inactivation and PAC adsorption were faster than that of BA in both DI water and PBS solution, meaning that BG was a poor BAS surrogate for both processes. There was no difference in aerosol emission between BG and BA in DI water or PBS solutions, thus BG was a suitable BAS surrogate for bioaerosol emission under the conditions tested. Aggregation batch tests showed that spore clustering was greater for BG in DI water when the pH was 7 or greater but no statistically significant differences were observed in NaCl solution. Interaction energy calculations partially corroborated the aggregation and adsorption experimental results. Overall, this article demonstrates that there is more than one aspect to selecting a suitable surrogate.

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Author contributions:

Leigh Durden: Formal analysis; Investigation; Writing - original draft; Writing - review & editing

Kyle Eckhoff: Formal analysis; Investigation; Writing - original draft

Adam C. Burdsall: Formal analysis; Investigation; Writing - original draft

Sungmin Youn: Formal analysis; Investigation; Writing - original draft

Cindy Andujar-Gonzalez: Formal analysis; Investigation; Writing - original draft

Lubna Abu-Niaaj: Formal analysis; Investigation; Writing - original draft

Matthew Magnuson: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing - original draft; Writing - review & editing

Willie F. Harper: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Writing - original draft; Writing - review & editing.

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REFERENCES

1. US National Response Team, *NRT Quick Reference Guide for Bacillus anthracis (causes Anthrax)*, Final 2022-02-16, Washington, DC, 2022.
2. U.S. EPA, *Progress on water sector decontamination recommendation and proposed strategic plan*, Report 817-R-15-001, Washington, DC, 2015.
3. B. Levy and V. Sidel, *Biological Agents and Infectious Diseases in War and Terrorism. Encyclopedia of Environmental Health*, 2nd ed, Elsevier Publishers; 2011. Reference module in earth systems and environmental sciences, 351-358.
4. WERF, *Collaborative workshop on handling, management, and treatment of bio-contaminated wastewater by water resource recovery facilities*, Report WERF7W15, Alexandria, VA, 2016.
5. A. Driks, The *Bacillus anthracis* spore, *Mol. Aspects Med.*, 2009, **30**, 368-373.
6. L. Rose and E. Rice, Inactivation of bacterial biothreat agents in water, A review, *J Water Health*, 2014, **12**, 618-633.
7. R. Spencer, Bacillus anthracis, *J Clin Pathol*, 2003, **56**, 82–187.
8. A.M. Mikelonis, K. Ratliff and S. Youn, Laboratory results and mathematical modeling of spore surface interactions in stormwater runoff, *J Contam Hydrol*, 2020, **235**, 103707.
9. A.R. Brazis, J. Leslie, P. Kabler and R. Woodward, The inactivation of spores of *Bacillus globigii* and *Bacillus anthracis* by free available chlorine, *Appl Microbiol*, 1958, **6**, 338-342.
10. E. Rice, N.J. Adcock, M. Sivaganesan and L. Rose, Inactivation of spores of *Bacillus anthracis* Sterne, *Bacillus cereus*, and *Bacillus thuringiensis* subspecies *israelensis* by chlorination, *Appl Environ Microbiol*, 2005, **71**, 5587-5589.

11. M. Sivaganesan, N. J. Adcock and E. Rice, Inactivation of *Bacillus globigii* by chlorination: A hierarchical Bayesian model, *J Water Supply Res T*, 2006, **55**, 33-43.
12. Metcalf and Eddy, *Wastewater Engineering Treatment and Reuse*, 4th ed, McGraw Hill Publishers, New York, NY, 2003.
13. A. Bonille-Petriciolet, D. Mendoza-Castillo and H. Reynel-Avila, *Adsorption Processes for Water Treatment and Purification*, 1st ed, Springer Publishers, New York, NY, 2017.
14. A.C. Burdsall, Y. Xing, C.W. Cooper and W.F. Harper, Bioaerosol emissions from activated sludge basins: characterization, release, and attenuation, *Sci Total Environ*, 2021, **753**, 141852.
15. Y. Xing, A. Burdsall, A., A. Owens, M. Magnuson and W.F. Harper, The effect of mixing and free-floating carrier media on bioaerosol release from wastewater: a multi-scale investigation with *Bacillus globigii*, *Environ Sci Water Res Technol*, 2021, **7**, 1240-1249.
16. C. White, J. Popovici, D. Lytle and E. Rice, Endospore surface properties of commonly used *Bacillus anthracis* surrogates vary in aqueous solution, *Anton Van Lee*, 2014, **106**, 243-251.
17. C.P. White, J. Popovici, D.A. Lytle, N.J. Adcock, N. J. and E. Rice, Effect of pH on the Electrophoretic Mobility of Spores of *Bacillus anthracis* and Its Surrogates in Aqueous Solutions, *Appl Environ Microbiol*, 2012, **78**, 8470-8473.
18. T. Prot, L. Korving and M.C.M. van Loosdrecht, Ionic strength of the liquid phase of different sludge streams in a wastewater treatment plant, *Water Sci Technol*, 2022, **85**, 1920-1935.

19. R. Severin, M. Suidan and R.S. Engelbrecht, Kinetic modeling of UV disinfection of water, *Water Res*, 1983, **17**, 1669-1678.

20. T. Tran, L. Racz, M. Grimaila, M. Miller and W.F. Harper, Comparison of continuous versus pulsed ultraviolet light emitting diode use for the inactivation of *Bacillus globigii* spores, *Water Sci Technol*, 2014, **70**, 1473-1480.

21. P.C. Hansen, V. Pereyra and G. Scherer, *Least Squares Data Fitting with Applications*, Illustrated Ed, Johns Hopkins University Press, Baltimore, MD, 2013.

22. H. Wang, W. Wang, S. Zhou and X. Gao, Adsorption mechanism of Cr(VI) on woody-activated carbons, *Heliyon*, 2023, **9**, e13267.

23. M. Benjamin and D. Lawler, *Water Quality Engineering*, 1st ed., John Wiley and Sons, Hoboken, NJ, 2013.

24. A. Harimawan, S. Zhong, C. Lim and Y-P Ting, Adhesion of *B. subtilis* spores and vegetative cells onto stainless steel – DLVO theories and AFM spectroscopy, *J Colloid Interface Sci*, 2013, **405**, 233-241.

25. J. Ryan, Effects of ionic strength and flow rate on colloid release: Relating kinetics to intersurface potential energy, *J Colloid Interface Sci*, 1994, **164**, 21-34.

26. H. Schwegmann, J. Ruppert and F. Frimmel, Influence of the pH-value on the photocatalytic disinfection of bacteria with TiO2-explnanation by DLVO and XDLVO theory, Water Res, 2013, **47**, 1503-1511.

27. W. Zhang and X. Zhang, Adsorption of MS2 on oxide nanoparticles affects chlorine disinfection and solar inactivation, *Water Res*, 2015, **69**, 59-67.

28. C.V. Chrysikopoulos and V.I. Syngouna, Attachment of bacteriophages MS2 and ΦX174 onto kaolinite and montmorillonite: Extended-DLVO interactions, *Colloids Surf B*, 2012, **92**, 74-83.

29. D. Greenberg, J. Busch, P. Kein and D. Wagner, Identifying experimental surrogates for *Bacillus anthracis* spores: A review, *Investig Genet*, 2010, **1**, 1-12.

30. D.C. Blanchard and L. Syzdek, Mechanism for the water-to-air transfer and concentration of bacteria, *Science*, 1970, **170**, 626-628.

31. C. Ash, J. Farrow, S. Wallbanks and M.D. Collins, Phylogenetic heterogeneity of the genus Bacillus revealed by comparative analysis of small subunit ribosomal RNA sequences, *Lett Appl Microbiol*, 1991, **13**, 202-206.

32. P. Keim, A.M. Klevytska, L.B. Price, J.M. Schupp, G. Zinser, K.L. Smith, M.E. Hugh-Jones, R. Okinaka, K.K. Hill and P.J. Jackson, Molecular diversity in *Bacillus anthracis, J Appl Microbiol*, 1999, **87**, 215-217.

33. A. Wekhof, F.J. Trompeter and O. Franken, Pulse UV-disintegration (PUVD): a new sterilization mechanism for packaging and broad medical-hospital applications, In: Proceedings of the First International Conference on Ultraviolet Technologies, 2001, Washington, DC, USA.

34. R. Sommer, A. Cabaj and T. Haider, Microbicidal effect of reflected UV radiation in devices for water disinfection, *Water Sci Technol*, **34**, 173-177.

35. X. Liu, X. Shang, Q. Cai and J. Hu, Lab- and pilot- scale evaluation of bacterial inactivation and reactivation influenced by UV-LEDs arrangements in continuous flow water disinfection reactors, *J Water Process Eng*, 2023, **55**, 104093.

36. D. Shaffer, C. Arias, M. Ben-Sasson, S. Romero-Vargas, N. Yip and M. Elimelech, Desalination and reuse of high-salinity shale gas produced water: drivers, technologies, and future directions, *Environ Sci Technol*, 2013, **47**, 9569-9583.
37. A. Butkovskyi, H. Bruning, S. Kools, H. Rijnaarts and A. Van Wezel, Organic pollutants in shale gas flowback and produced waters: Identification, potential ecological impact, and implications for treatment strategies, *Environ Sci Technol*, 2017, **51**, 4740-4754.
38. X. Ou, H. Daly, X. Fan, S.K. Beaumont, S. Chansai, A. Garforth, S. Xu and C. Hardacre, High-ionic-strength wastewater treatment via catalytic wet oxidation over a MnCeOx catalyst, *ACS Catal*, 2022, **12**, 7598-7608.