Environmental Science
Water Research & Technology

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Journal:	*Environmental Science: Water Research & Technology*
Manuscript ID	EW-ART-02-2021-000151.R2
Article Type:	Paper

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Wastewater treatment facilities are a significant source of bioaerosols, which pose a health hazard to operators and the general public. Despite many empirical studies on bioaerosol detection, quantification and attenuation, there is no quantitative framework that can be used to quantify or estimate bioaerosol generation. Based on this multi-scale study, correlations to various reactor operational parameters are established. This study is a significant step toward the development of predictive models for full scale systems.

The effect of mixing and free-floating carrier media on bioaerosol release from wastewater: a multiscale investigation with *Bacillus globigii*

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ABSTRACT

Aeration tanks in wastewater treatment plants (WWTPs) are significant sources of bioaerosols, which contain microbial contaminants and can travel miles from the site of origin, risking the health of operators and the general public. One potential mitigation strategy is to apply free-floating carrier media (FFCM) to suppress bioaerosol emission. This article presents a multiscale study on the effects of mixing and FFCM on bioaerosol release using *Bacillus globigii* spores in well-defined liquid media. Bioaerosol release, defined as percentage of spores aerosolized during a 30-minute sampling period, ranged from 6.09×10^{-7} % to 0.057%, depending upon the mixing mode and intensity. Bioaerosol release increased with the intensity of aeration (rotating speed in mechanical agitation and aeration rate in diffused aeration). A surface layer of polystyrene beads reduced bioaerosol released by > 92% in the bench-scale studies and >74% in the pilot-scale study. This study discovered strong correlations (R^2>0.82) between bioaerosol release and superficial gas velocity, Froude number, and volumetric gas flow per unit liquid volume per minute. The Reynolds number was found to be poorly correlated with bioaerosol release (R^2 <0.5). This study is a significant step toward the development of predictive models for full scale systems.

Keywords: aeration, Froude number, Reynolds number, *B. globigii* spores, Free-floating carrier media

1. INTRODUCTION

Wastewaters contain large numbers and varieties of viruses, drug-resistant bacteria, spore-forming microorganisms, and other pathogens (Metcalf and Eddy, 2003; Lodder and de Roda Husman, 2020). These microorganisms are aerosolized during wastewater treatment (Ding et al., 2016; Fracchia et al., 2006). Bioaerosols can be transported over length scales of several miles or more (Pepper and Gerba, 2015), and they can remain viable depending on factors such as temperature, relative humidity, oxygen content, water content, and UV radiation (Hinds, 1999; Pepper and Gerba, 2015; Brown and Mohr, 2016). Exposure to bioaerosols can cause respiratory diseases, acute toxic effects, and cancer (Kim et al., 2018; Núñez et al., 2016). Therefore, there is a need to study the release and control of bioaerosols from wastewater treatment processes.

Bioaerosol emissions have been reported near numerous wastewater treatment plants. Tian et al., 2020 used a real-time biosensor to detect bioaerosol concentrations present near a wastewater treatment plant in Cranfield, England. Gaviria-Figueroa et al., 2019 used quantitative Polymerase Chain Reaction (qPCR) to reveal the presence of antibiotic-resistant microorganisms, and they used atmospheric dispersion modeling to predict the downwind transport as a function of wind speed. Han et al., 2019 discovered that bioaerosol particles larger than 3.3 μm in diameter were emitted in spring and summer, while smaller particles were detected in autumn and winter. They also discovered the presence of numerous bacterial pathogens, including *Chryseobacterium*, *Stenotrophomonas*, and *Alcaligenes*. Several other studies have reported bioaerosol emissions near wastewater treatment plants (Carducci et al., 2000; Ding et al., 2016; Heinonen-Tanski et al., 2009; Korzeniewska et al., 2009; Lou et al., 2021; Michalkieweicz, 2018; Pascual et al., 2003; Pasalari et al, 2019; Uhrbrand et al, 2017; Wang et al., 2018, Wang et al., 2019a).

However, none of these previous studies have attempted to correlate the presence of bioaerosols with the operational parameters that govern their emission.

Well-known operational parameters are relevant to both bioaerosol release and the mixing intensity observed during wastewater treatment. Both processes can be studied with the Reynolds number (Re), the Froude number (Fr), the volumetric gas flow per unit liquid volume per minute (vvm), and the superficial gas velocity (v_g). Re is the ratio of inertial forces to viscous forces and is used to characterize flow that is either laminar or turbulent (LaNasa and Upp, 2014). Turbulent flow is typical in wastewater treatment processes (Xu et al., 2014). Fr is the ratio of the flow inertia to the gravitational field; in the study of stirred wastewater treatment tanks, Fr governs the formation of surface vortices. In wastewater aeration tank design, both Re and Fr have been used to predict oxygen mass transfer through dimensional analysis (Andrews et al., 1988; Pittoors et al., 2014; Karpinska and Bridgeman, 2017). The vvm and v_g are ratios of gas flow to volume and surface area, respectively. These parameters have been used in the study of oxygen transfer efficiency (Metcalf and Eddy, 2003) and in bioreactor scale-up (Schmitz et al., 1987; Nauha et al., 2015).

WWTPs may attempt to suppress bioaerosol emission with the use of free-floating carrier media (FFCM). A wide variety of materials have been utilized as FFCM in an array of studies, including high-density polyethylene (HDPE) (Shreve and Brennan, 2019), high porosity elastic foams (Arias et al., 2009), gravel (El-Serehy et al., 2014), polyester fabric (Abou-Elela et al., 2014), clay (Stensel and Reiber, 1983), and polystyrene (Bourke 1999; Hung et al., 2010). These

studies have demonstrated that FFCM can reduce bioaerosol release. None of the previous studies have investigated the use of FFCM at different mixing intensities.

The objectives of this study are to: 1) evaluate the relationship between bioaerosol release and Re, Fr, vvm, and v_g, and 2) determine the effect of FFCM on bioaerosol release. This study was carried out with *Bacillus globigii* (BG) spores, which have been detected in wastewater bioaerosols (Bruni et al., 2019), are safe to work with (i.e. BSL 1) (Center for Research Information, Inc., 2004), and have a distinctive colony appearance (Szabo et al., 2007). The experiments simulated mechanical agitation and diffused aeration, two systems used in full-scale wastewater treatment. Aerosol emission is governed by laws of physics (Hinds, 1999), and as a matter of principle, physical phenomena may produce correlations involving systems of different sizes (Buckingham, 1915). It is possible to observe correlations involving dimensionless (e.g. Re and Fr) or normalized (e.g. vvm and v_g) parameters as a function of bioaerosol release percentages taken from bench and pilot scale experiments.

2.0. MATERIALS AND METHODS

2.1. Experimental overview. Suspensions of BG were introduced into wastewater vessels of different sizes and under different mixing conditions. Bioaerosol samples were collected and BG concentrations were determined using culture-dependent methods (described below). Experiments were carried out with and without FFCM. Relationships between bioaerosol capture and Re, Fr, vvm, and v_g were investigated. The effect of FFCM on bioaerosol capture was investigated. Two-tailed, student t-tests were used to determine statistical significance at the 95% confidence level ($\alpha = 0.05$).

2.2. Preparation of the BG. The original *B. globigii* stock used in these experiments was provided by the US EPA. To create a fresh BG stock solution, 1 mL of deionized (DI) water was added to 50 μL of the US EPA BG stock (obtained in-kind from EPA).solution. The mixture was homogenized using the Vortexer (Daigger Vortex Genie 2, Catalog Number 22220A, Daigger Scientific, Hamilton, NJ, USA) and then placed onto nutrient agar plates. The plates were incubated at 35°C for 7 days. 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 at a temperature of 4-8°C. Then, the supernatant was removed and fresh sterile DI water was added to suspend the pellet again for another iteration of centrifugation. This process was repeated five times: three times on the day of the harvest, once on the day after the harvest, and one other time three days later. The multiple washings are to ensure that the remaining vegetative cells are lyzed and removed, and the resulting spore stock is pure (i.e. <5% vegetative cells). Purity of the spores was confirmed by phase contrast microscopy.

2.3. Mechanical agitation experiments. For mechanical agitation experimentation, a 1.27-cm-long magnetic stir bar was used to maintain mixing and dispersion of *B. globigii* spores in the liquid medium (DI water). The bioreactor is 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 (described below in section 2.5). The experiments were performed with multiple replicates (12 -15 replicates) at each of the four tested rotating speeds: 900, 1000, 1100 and 1200 rpm. The high number of replicates was used to ensure that the phenomenon observed was repeatable and consistent.

2.4. Diffused aeration experiments. Diffused aeration creates bubbles that rise to the top of the water column, and subsequent bubble-bursting results in aerosol release. Three sets of diffused aeration experiments at different experimental scales were performed in this study: bench scale study with a 250 mL reactor vessel (filled with 60 mL DI water), bench scale with 1 1 L reactor vessel (filled with 240 mL DI water), and in a pilot-scale activated sludge system (35.5 L reactor chamber, filled with 31.3 L synthetic wastewater) (see Figure S1 for schematics of the different systems). Table 1 summarizes the vessel dimensions and aeration conditions during each of the three experiments. Experiments were performed with or without FFCM. Three types of FFCM materials were initially tested: high-density polyethylene (HDPE) beads (Salem Specialty Ball Co., Inc., Canton, CT, USA), polyacetate beads (Salem Specialty Ball Co., Inc., Canton, CT, USA), and polystyrene beads (FloraCraft, Ludington, MI, USA). However, the former two were not pursued further after preliminary results showed no significant attenuation in bioaerosol emission. This study reports results obtained with polystyrene beads only.

The FFCM used in the pilot scale system was 19.1 mm (diameter) polystyrene beads. The aeration basin of the pilot-scale WWTP was divided into six identical chambers (each measured 35.5 L, see diagram in Figure S1) and filled with synthetic wastewater (Table S1). *B. globigii* spores were injected into the WWTP through the primary clarifier at the beginning of the experiment. The chambers were each aerated through a perforated line connected to an air pump. The aeration rate was 3.5 L/min. Bioaerosol collection started after 1 hour. The reactor spore concentration was monitored for 24 hours. The reactor spore concentration reached a steady state (0.8-2.4 x 10^5 CFU/mL) during the 7^{th} hour.

2.5. Bioaerosol capture and quantification. Bioaerosols from the above experiments were captured using a bioSampler (catalog #225-9595, SKC Inc., Eighty Four, PA) 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). Figure S1 shows the experimental set-up. The collection liquid used was 20 mL sterile DI water in bench scale experiments and 20 mL phosphate-buffered saline (PBS) buffer in the pilot scale. The path length between the bioaerosol sampler inlet and the liquid surface was 18 inches for the 250 mL reactor, 16 inches for the 1 L reactor, and 15 inches for the pilot plant (See Figure S1). Preliminary experiments comparing DI water and PBS collection liquids showed no significant difference in the capture or culture of aerosolized *B. globigii* spores (data not shown). During the bench scale experiments, the bioaerosol sampler was inside of a closed biosafety cabinet. During the pilot scale experiments, the bioaerosol sampler was exposed to indoor temperature at 1 atm.

Before the experiment started, the magnetic stir bar, the aeration frit, fittings, and the biosampler were thoroughly cleaned and the FFCM were sterilized by soaking them in 70% ethanol for at least 15 minutes and then thoroughly rinsing with sterile DI water. The reactor, its lid, all tubing and fitting connectors, and all parts of the biosampler were autoclaved. The vacuum pump, the ring stand, and the biosampler were squirted and wiped down with 70% ethanol before the start of the experiment. During the experiment, samples were collected for 30 minutes with the vacuum pump setting of 8.25 L/min. Then, the samples were properly diluted or concentrated with centrifugation (when the bioaerosol count was low) and plated onto nutrient agar plates. Colony forming unit (CFU) counts from these plates after overnight incubation at 35^{o}C were

recorded and compared with the starting spore concentration (CFU/mL) in the reactor. The bioaerosol capture ratio was calculated by the ratio of the BG spores captured in the biosampler and the total number of BG spores present in the water volume.

2.6. Parameter calculations. For mechanical agitation experiments, Re was calculated based on Halász et al., 2007:

$$Re = \frac{\Omega * a^2}{\upsilon} \qquad (1)$$

where Ω is the rotating speed (rad/s), a is the half-length of the stir bar (m), and υ is the kinematic viscosity of water at 20°C (1.00 x 10^{-6} m^2/s).

For diffused aeration experiments, Re was calculated based on Pittoors et al., 2014:

$$Re = \frac{Q_a}{D * \upsilon} \qquad (2)$$

where Q_a is the aeration rate (m^3/s), D is the diameter of the reactor bottle (m) and υ is the kinematic viscosity of water at 20°C (1.00 x 10^{-6} m^2/s).

For mechanical agitation experiments, Fr was calculated based on Halász et al., 2007:

$$Fr = \frac{\Omega * a}{\sqrt{g * R}} * \frac{d}{a} \qquad (3)$$

where Ω is the rotating speed (rad/s), a is the half-length of the stir bar (m), R is the radius of the reactor bottle, d is the cross-sectional diameter of the stir bar (m), and g is the standard gravitational acceleration (9.81 m/s^2).

For diffused aeration experiments, Fr was calculated based on Pittoors et al., 2014:

$$Fr = \frac{Q_g}{\sqrt{D^5 * g}} \quad (4)$$

where Q_g is the aeration rate (m^3/s), D is the diameter of the reactor bottle (m), and g is the standard gravitational acceleration (9.81 m/s^2).

For diffused aeration experiments, vvm was calculated as follows:

$$vvm = Q_g/V \quad (5)$$

where Q_g is the aeration rate (m^3/min), and V is the diameter of the reactor liquid volume (m^3).

For diffused aeration experiments, ν_g was calculated as follows:

$$\nu_g = Q_g/A \quad (6)$$

where Q_g is the aeration rate (m^3/s), and A is the cross- sectional area of the bioreactor (m^2).

3. RESULTS AND DISCUSSION

3.1 Bioaerosol release in the mechanical agitation experiments.

Bioaerosol release increased with rotating speed. At 900 rpm, the number of aerosolized spores was approximately 5.1 x 10^{-5} % of the number of spores in the reactor. This number rose steadily as the rotating speed increased: 3.65 x 10^{-4} % at 1000 rpm, 3.53 x 10^{-3} % at 1100 rpm and 1.19 x 10^{-2} % at 1200 rpm (Figure 1a). The bioaerosol release differences between the different rotating speeds were statistically significant ($p<0.05$, see Table S2 for p values). The relationships between the bioaerosol release against rotating speed, Re, and the Fr revealed an exponential-function relationship with $R^2 > 0.98$ (Figure 1b-d). The flow in the bioreactor was turbulent ($Re > 1000$) at all rotational speeds. No laminar-to-turbulent flow transition was observed. However, a notable increase in the aerosolization percentage was observed during the transition

from subcritical flow (*Fr* < 1.0), in which the fluid motion is influenced predominantly by gravitational forces, to supercritical flow (*Fr* > 1.0), where the fluid motion is controlled by inertial forces. These results underscored the potential usefulness of the Fr as a metric for understanding bioaerosol release during mechanical agitation.

3.2. Bioaerosol release in diffused aeration experiments.

3.2.1 Bench scale studies. The aeration rate influenced bioaerosol release in the 250 mL reactor vessel study (Figure 2). Bioaerosol release at the 0.486 L/min aeration rate was 3.05 x 10^{-3} % and at 0.854 L/min it was 5.7 x 10^{-2} %, nearly four times higher than the bioaerosol release from the mechanical agitation experiment at 1200 rpm. The use of 0.3 or 0.5 g polystyrene beads (2.25 mm diameter) as FFCM significantly reduced ($p<0.05$, Table S2) bioaerosol release by > 92% for both aeration rates tested. The water surface was completely covered by both 0.5 g and 0.3 g polystyrene beads, but the layer was thinner for the 0.3 g experiment. However, there was no statistically significant difference in the attenuation that was observed. These results were consistent with the findings of Hung et al. (2010), who reduced bioaerosol emissions by up to 90% from a laboratory scale bioreactor by using polystyrene beads of various sizes (i.e., 1.9, 2.9, 3.4, 4.8 cm).

In the larger (i.e. 1 L) vessel study, FFCM caused bioaerosol release to be reduced by 87%-94% for the smaller polystyrene beads (4.2 mm in diameter) and by 80-86% for the larger polystyrene beads (19.1 mm diameter). The water surface was completely covered in both experiments. The percentage of spores aerosolized was positively correlated to aeration rate, rising from 5.63 x 10^{-3} % at 0.5 L/min to 1.73 x 10^{-2} % at 1.0 L/min (Figure 3b), consistent with previous observations

by Wang et al. (2019b), who reported that higher aeration rates led to higher bioaerosol release from the reaction tank of an indoor wastewater treatment facility. Smaller polystyrene beads appeared to perform better than the larger beads, even though the differences were statistically significant ($p < 0.05$, Table S2) only at an aeration rate of 1.0 L/min.

3.2.2. Pilot scale study. Bioaerosol capture data showed bioaerosol release of approximately 1.5 x 10^{-6} % and an averaged reduction of 74.3% by the polystyrene beads (19.1 mm diameter) (Figure 4). FFCM completely covered the water surface. Thus, the bioaerosol attenuation effect of polystyrene bead FFCM was verified by experiments at all scales tested in this study. The bioaerosol release was 4-5 orders of magnitude lower than the bioaerosol release observed in bench-scale studies, even though the aeration rate was more than three times that of the highest aeration rate tested in the bench-scale experiments (see Table 1). The aeration rate correlates well with bioaerosol release for a given reactor, but aeration rate cannot be used to predict bioaerosol release across reactor scales, underscoring the need for multiscale parameters.

3.2.3 Bioaerosol release correlations in diffused aeration experiments. For all aeration experiments, the Re values were smaller than 1000, indicating laminar flow (Table 2). Fr values were less than 0.01, indicating subcritical flow and that gravitational force dominated fluid motion (Table 2). Plotting bioaerosol release (without FFCM) against these parameters revealed strong correlations ($R^2>0.82$) with Fr, vvm, and ν_g, with the correlation with ν_g being the strongest (Figure 5, a, c and d). The strong correlation did not exist between bioaerosol release and the Re ($R^2<0.5$) (Figure 5b). The air requirement for good mixing is 20 to 30 m^3/min per 1000 m^3 to achieve a spiral roll aeration pattern and for the grid system, the air requirement is

10-15 m^3/min per 1000 ft^3 (Metcalf and Eddy, 2003). These design values were used to calculate the Re, Fr, vvm, and v_g values associated with realistic operating conditions (Figure 6, Table 2). The conditions used in this current study overlapped with the design values for vvm, Fr, and v_g, but not for Re. This shows that the parameter values observed in the current study are relevant to full-scale operation. Consequently, the current results may serve as a guide for future studies that may wish to estimate bioaerosol release and the human exposure at full-scale wastewater treatment plants.

It is useful to revisit the effect of liquid media on spores. Synthetic wastewater was used during the pilot scale experiments while DI water was used during the bench scale experiments. The results shown in Figure 5 and 6 showed that pilot scale bioaerosol release (i.e. percentage of spores aerosolized) was smaller than that of the bench scale experiments but it is unlikely that the liquid media was a meaningful factor in causing these differences. Ionic strength affects spore clumping, and aggregates are less likely to be aerosolized and captured by a biosampler. Clumping is less favorable in synthetic wastewater because it has a higher ionic strength than DI. Thus, in principle, one may expect a higher bioaerosol release percentage in synthetic wastewater than in DI when the operational parameters (e.g. Fr, vvm, and v_g) are the same. However, in the current study, the pilot scale aerosolization percentages were lower than those of the bench scale experiments because the operational parameters were smaller.

3.3. Comparison of the mechanical agitation and diffused aeration data. Figure 7 shows the comparison of bioaerosol release data from the two different mixing regimes. These two data subgroups did not share overlapping Fr or Re values. Diffused aeration experiments generated

more aerosolized spores at lower Fr and Re values, while mechanical agitation generated fewer aerosolized spores at higher Fr and Re values. Diffused aeration and mechanical agitation generate mixing patterns that are different and as expected, the relationships between Re, Fr, and bioaerosol release were different. However, good correlations between bioaerosol release and Fr exist in both mixing schemes.

4.0. CONCLUSIONS

This study determined the percentage of *B. globigii* spores aerosolized with well-defined systems under two different commonly-used mixing strategies: mechanical agitation and diffused aeration. The percentage of spores aerosolized in these experiments ranged from 6.09×10^{-7} % (diffused aeration, pilot scale) to 5.71×10^{-2} % (diffused aeration, bench scale, 250 mL vessel) during a 30-minute sampling period. Bioaerosol release was positively correlated with rotating speed in the agitation experiment and with the aeration rates in the diffused aeration experiments. For the mechanical mixing experiments, bioaerosol release was well-correlated with Re and Fr while for the diffused aeration experiments the bioaerosol release was best correlated with the v_g. To the author's knowledge, this study is the first to correlate well-established, quantitative operational parameters to the emission of bioaerosols from wastewater tanks. Lastly, the application of proper FFCM was able to reduce the emission of aerosolized spores.

5.0 ACKNOWLEDGEMENTS

This research was supported in part by an appointment to the Postgraduate Research Participation Program at the U.S. Air Force Institute of Technology (AFIT), administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the

U.S. Department of Energy and AFIT. The data that support the findings of this study are available upon request.

6.0 DISCLAIMER

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Air Force Institute of Technology, the United States Air Force, the Department of Defense, or the United States government. The U.S. Environmental Protection Agency through its Office of Research and Development partially funded and collaborated in the research described here under Interagency Agreement DW-057-92440901-4. It has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents necessarily reflect the views of the Agency. Any mention of trade names, products, or services does not imply an endorsement by the EPA or the U.S. Government. EPA does not endorse any commercial products, services, or enterprises.

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