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Modelling the Dynamics of *P. aeruginosa* in the Formation of Biofilms [†]

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Abstract: The accumulation, growth, and re-mobilization of pathogens on the pipe walls in drinking water distribution systems are processes that affect the risk of exposure at the tap. We present a model that uses the Buckingham Pi theory to embody the physics of *Pseudomonas aeruginosa* accumulation and move within the system. We apply it to model experimental data from a biofilm annular reactor operated in conditions that are commensurate with the flow in DWDS. By calibrating the model for this benchtop system, we intend to identify the most important physical parameters for use in a simpler, more prudent model, for application in large-scale DWDS.

Keywords: pseudomonas aeruginosa; planktonic; hydrodynamic layer; shear velocity; Brownian diffusion; particle size distributions; optimization technique

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1. Introduction

The transmission and growth of pathogens in drinking water distribution systems (DWDS) remains one of the primary risks in public health, and water companies spend considerable time and money in mitigating it [1,2]. Yet the underlying physical and biological processes that sustain and release pathogens within DWDS, and their interaction with mitigation strategies, such as disinfection residuals, are not fully understood. Here, we use *P. aeruginosa*, an opportunistic biofilm-forming pathogen, to quantify the dynamic transmission of pathogens in a biofilm reactor operated to mimic pipe flow conditions.

Typically, particle accumulation and mobilization rates to and from biofilm are not directly quantified but it can be estimated via numerical simulations of particle concentration [3]. Few studies have directly measured bacterial attachment, and there remains a lack of research on the initial contact of bacteria with the surface and reversible attachment under different environmental conditions. We aim to address these research studies here. Here, the Gaussian distribution of bacterial clumps with mean bacterial diameter (μ) and standard deviation (σ) is used to characterize the random distribution of bacteria within the range of 0.6–1.2 µm in the water system [4].

The primary objective of this study is to analyze, via the numerical simulation, the behavior of *P. aeruginosa* within a biofilm annular reactor (BAR), specifically focusing on understanding their transportation dynamics (Figure 1). This study used the Buckingham Pi theorem and mass balance principle to model concentrations of bacteria in bulk water and wall surfaces over time.

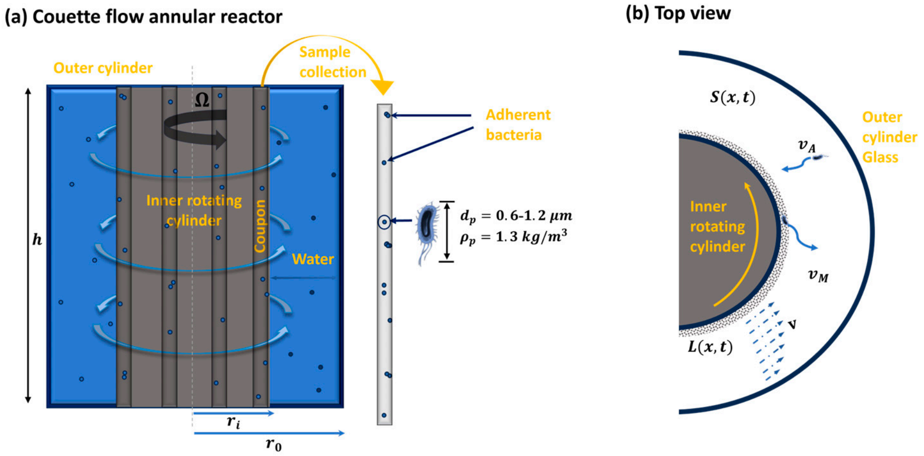

Figure 1. Schematic of the *P. aeruginosa* in the annular reactor: (**a**) the dynamic transmission of *P. aeruginosa* in the tap water and attachment on the coupon. Coupons provide a substrate for the initial attachment of the *P. aeruginosa*, allowing them to colonize and form biofilms; (**b**) top view to illustrate the generic phenomena of the two-phase bacteria model.

2. Methods

2.1. Experimental Design

Two experiments were conducted in a BAR in the laboratory at the University of Glasgow. Initially, 10^8 *P. aeruginosa cells*, were added into drinking water within the BAR in which drinking water biofilms had been grown for 14 weeks. During each step time ($\Delta t = 2$ h), a coupon (polycarbonate slide) was taken out for cell measurement (Figure 1a). The activity lasted 10 h with conditions listed in Table 1. The rotation speed of the BAR was 121 RPM in order to achieve a flow regime where the rotation Reynolds number (Re) equals 5915, indicating the onset of turbulent flow [5]. The shear stress exerted on the inner glass surface was 0.153 N·m^2.

Table 1. Parametric values of the water system.

Parameters	Value
Water density (ρ_{14^o})	999.2 kg/m^3
Dynamic Viscosity (μ_{14^o})	1.168×10^{-3} kg/m·s
Temperature (T)	287.15 K

2.2. Model for Bacteria Accumulation and Mobilization

This model was formulated by taking into account two phases within a system: (i) the bulk water (bacterial concentration in the water) and (ii) the - pipe-surface (bacterial mass attached to the surface), as shown in Figure 1. The two-phase bacteria balance equations of *P. aeruginosa* are derived as follows:

$$V\frac{dS(x,t)}{dt} = Q(S_{in}(x,t) - S_{out}(x,t)) - JA + MA\left(\frac{W_M}{W_L}\right)^{0.96}, \quad (1)$$

$$A\frac{dL(x,t)}{dt} = JA - MA\left(\frac{W_M}{W_L}\right)^{0.96}, \quad (2)$$

where $S(x,t)$ and $L(x,t)$ represent the concentration of bacteria based on their size (x) in the bulk water and wall surface at time t. The parameters $J(= v_A S)$ and $M(= v_M L)$ denote the bacteria flux and bacteria reflux, respectively. W_M/W_L is the mass-to-mobility ratio. V and A denote the volume and surface area of the reactor, respectively.

2.3. Determined the Bacteria Accumulation and Mobilization Velocity

The accumulation velocity (v_A) depends on the hydraulic condition, which is characterized by the Particle Reynolds number $\left(u_* d_p \rho / \mu\right)$, Brownian diffusion number $\left(K_B T / u_*^2 d_p^3 \rho\right)$, and Rouse number (w/ku_*). Similarly, the re-mobilization velocity (v_M) depends on how easily these adhesive bacteria can be stirred up by the surrounding environment and is a function of the Stokes number $\left(\rho_p d_p^2 u_* / \rho h_d \nu\right)$, the intermolecular force $\left(I_f / \mu d_p h_d u_*\right)$, and the excess shear stress ratio $\left(u_*^2 \rho / g d_p (\rho_p - \rho)\right)$. The Buckingham Pi theorem is used to derive the analytical expression of accumulation and mobilization velocity as follows:

$$v_J\left(\frac{1}{u_*}\right) = a\left(\frac{u_* d_p \rho}{\mu}\right)^{\alpha 1}\left(\frac{K_B T}{u_*^2 d_p^3 \rho}\right)^{\alpha 2}\left(\frac{w}{k u_*}\right)^{\alpha 3}, \tag{3}$$

$$v_M\left(\frac{d_p}{u_*}\right) = b\left(\frac{\rho_p d_p^2 u_*}{\rho h_d \nu}\right)^{\beta 1}\left(\frac{I_f}{\mu d_p h_d u_*}\right)^{\beta 2}\left(\frac{u_*^2 \rho}{g d_p (\rho_p - \rho)}\right)^{\beta 3}. \tag{4}$$

Here, ν, u_*, w, h_d, g, K_B, and I_f are the kinematic viscosity, shear velocity, settling velocity, hydaulic daimeter, gravity, Boltzmann constant, and the Hamaker's constant of *E. coli* in water [6]. The coefficients $\alpha_{i \in [1 \ldots 3]}$ and $\beta_{j \in [1 \ldots 3]}$ represent six dependent coefficients associated with six dimensionless groups that are important for the accumulation and mobilization velocity of *P. aeruginosa*. Using optimization techniques (fminsearch), we determined the value of $\alpha_1 = 1.47$, $\alpha_2 = 0.126$, $\alpha_3 = -0.23$, $\beta_1 = -0.041$, $\beta_2 = 0.327$, and $\beta_3 = 1.66$. These coefficients were chosen to best fit our two-phase bacterial balance model to the experimental data.

3. Results and Discussion

Figure 2a shows the dynamics of the accumulation and re-mobilization of *P. aeruginosa* over time under specific hydraulic conditions. The overall average accumulation velocity of *P. aeruginosa* toward the surface is estimated to be 0.0037 m/h.

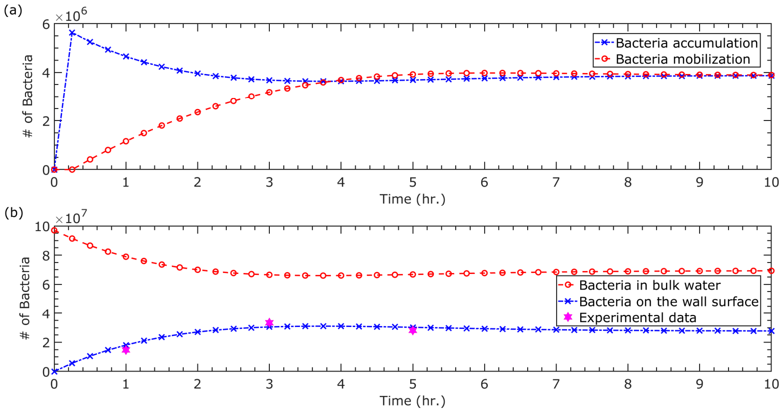

Figure 2. The two-phase bacteria balance model for the dynamic transmission of *P. aeruginosa* with time illustrates (**a**) the number of accumulated and mobilized bacteria in the system; (**b**) a comparison of theoretical and experimental data.

The exponential coefficient $\alpha_1 = 1.47$ reflects the nature of *P. aeruginosa* transmission in the water system. In this experiment, bacteria are deposited and removed as individuals with a size range of 0.6 μm $< d_p <$ 1.2 μm, rather than in clumps, and they tend to follow streamlines toward the surface due to lower particle Reynolds numbers ($Re_p < 0.015$). The short residence time of *P. aeruginosa* near the inner wall, reflected in the coefficient $\alpha_3 = -0.23$, diminishes the impact of Brownian motion on the bacteria. Consequently, this leads to particle–surface interaction with a value of $\alpha_2 = 0.126$.

In Figure 2a, it can be noted that the initial attachment of *P. aeruginosa* is predominantly reversible (weak attachment), as bacteria can detach depending on nutrient availability, surface roughness, and hydraulic stress. The negative value of $\beta_1(-0.041$ in Equation (4)) for the Stokes number likely correlates with the streamlined path of the bacteria. The value of $\beta_2 = 0.327$ and $\beta_3 = 1.66$ corresponds to intermolecular forces between *P. aeruginosa* and a surface and the excess shear stress, respectively. Weaker adhesive interactions $\left(\text{expressed as } \mathrm{I_f}/\left(\mu d_p h_d u_*\right) < 0.1\right)$ and a higher hydrodynamic force $\left(\text{applied shear stress}(\tau_a) > \text{critical shear stress}(\tau_*)\right)$ suggest the likelihood of bacteria being detached. This condition ensures the re-mobilization of weakly adherent bacteria as shown in Figure 2a.

Figure 2b reflects the dynamic nature of *P. aeruginosa*'s behavior in response to simulated environmental conditions. It is shown that under consistent hydraulic conditions and water chemistry, the concentration of bacteria in the bulk water and surface stabilizes over time.

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Conflicts of Interest: The authors declare no competing interests.

References

1. Potable Water Quality and Bacteria in Water Distribution Systems. Available online: https://www.wcs-group.co.uk/wcs-blog/bacteria-in-water-distribution-systems (accessed on 27 March 2024).
2. Erdei-Tombor, P.; Kiskó, G.; Taczman-Brückner, A. Biofilm Formation in Water Distribution Systems. *Processes* **2024**, *12*, 280. [CrossRef]
3. Carniello, V.; Peterson, B.W.; van der Mei, H.C.; Busscher, H.J. Physico-chemistry from initial bacterial adhesion to surface-programmed biofilm growth. *Adv. Colloid Interface Sci.* **2018**, *261*, 1–14. [CrossRef] [PubMed]
4. Tonner, P.D.; Darnell, C.L.; Engelhardt, B.E.; Schmid, A.K. Detecting differential growth of microbial populations with Gaussian process regression. *Genome Res.* **2017**, *27*, 320–333. [CrossRef] [PubMed]
5. Childs, P. Rotating Cylinders, Annuli, and Spheres. In *Rotating Flow*, 1st ed.; Elsevier Science & Technology Books: Shanghai, China, 2011; pp. 177–247.
6. Janjaroen, D.; Ling, F.; Monroy, G.; Derlon, N.; Mogenroth, E.; Boppart, S.A.; Liu, W.T.; Nguyen, T.H. Roles of ionic strength and biofilm roughness on adhesion kinetics of Escherichia coli onto groundwater biofilm grown on PVC surfaces. *Water Res.* **2013**, *47*, 2531–2542. [CrossRef] [PubMed]