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Development of the *Pseudomonas aeruginosa* mushroom morphology and cavity formation by iron-starvation: a mathematical modeling study

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Abstract

We present a mathematical model of mushroom-like architecture and cavity formation in *Pseudomonas aeruginosa* biofilms. We demonstrate that a proposed disparity in internal friction between the stalk and cap extracellular polymeric substances (EPS) leads to spatial variation in volumetric expansion sufficient to produce the mushroom morphology. The capability of diffusible signals to induce the formation of a fluid-filled cavity within the cap is then investigated. We assume that conversion of bacteria to the planktonic state within the cap occurs in response to the accumulation or depletion of some signal molecule. We (a) show that neither simple nutrient starvation nor signal production by one or more subpopulations of bacteria is sufficient to trigger localized cavity formation. We then (b) demonstrate various hypothetical scenarios that could result in localized cavity formation. Finally, we (c) model iron availability as a detachment signal and show simulation results demonstrating cavity formation by iron starvation. We conclude that iron availability is a plausible mechanism by which fluid-filled cavities form in the cap region of mushroom-like structures.

Keywords

Continuum modeling; Biofilm; Iron signaling

1. Introduction

Under flow cell conditions irrigated with FAB glucose media, *Pseudomonas aeruginosa* biofilms have been observed to generate complex mushroom-like architectural features [1]. These structures are characterized by a somewhat spherical cap atop a narrower base, often referred to as the stalk. As an important opportunistic pathogen and biofilm former, *P. aeruginosa* has become a model organism for the study of bacterial biofilms [2]. Thus, there has been a high level of interest in understanding the formation of this characteristic architectural feature.

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Figure 1 depicts a rendering of the *P. aeruginosa* biofilm life cycle [3, 4]. Initial attachment (stage 1) occurs first as a reversible tethering via the flagella, in which the bacteria assume an orientation with their long axes perpendicular to the substrate (Figure 1: stage 1 arrowhead) [5, 6]. The bacteria then commit to semi-permanent surface association, adopting a horizontal orientation [5, 6]. Once attached, cells demonstrate surface associated motility [5]. Some cells behave differently, producing mounds through clonal growth (stage 2). These mounds are referred to as microcolonies [1, 7]. Researchers distinguish between the cells that continue to explore the surface and those that form microcolonies as two distinct subpopulations [1]. The motile (cap-forming) subpopulation migrates to the top of the microcolonies formed by the non-motile (stalk-forming) subpopulation, generating a mushroom-like structure (stage 3). Mature biofilms undergo a process of detachment that initiates in the center of caps [2]. This results in the creation of a fluid-filled cavity (stage 4) inside the cap in which planktonic cells are visible [8]. The cavity becomes larger over time, eventually breaking open, allowing the planktonic cells to escape and presumably form biofilms elsewhere [8, 9]. This narrative is somewhat specific to these conditions. Under different conditions *P. aeruginosa* biofilms take on other morphological features [10]. Mushroom-like formations have not been observed elsewhere as far as we know.

We present a mathematical model of the *P. aeruginosa* biofilm life cycle beginning with the microcolony to macrocolony transition. With this model, we demonstrate hypothetical mechanisms for mushroom (Figure 1: stage 3) and cavity formation (Figure 1: stage 4). We focus on the latter stages of biofilm development because our purpose is to explore hypothetical mechanisms that might lead to these observed morphologies. We begin with the cap-forming subpopulation already atop the microcolonies. In particular, we ignore the earlier process whereby the cap-forming bacteria accumulate and adhere at these locations. We demonstrate through numerical simulations that an assumed disparity in internal friction between the stalk and cap extracellular polymeric substances (EPS) leads to spatial variation in expansion rate sufficient to produce the mushroom morphology. We then examine the capability of diffusible signals to cause the reported cavity phenomenon using numerical simulations. Under a signal-induced detachment paradigm, the breakdown of the surrounding matrix and conversion to the planktonic mode are assumed to be activities carried out by the bacteria in response to the accumulation or depletion of some trigger molecule. This is not the only mechanism for cavity formation that can be conjectured. See for example, Boles et al. [9]. However, we restrict the scope of the current work to an investigation of diffusible signals. We (a) show that neither simple nutrient starvation nor signal production by one or more subpopulations is sufficient to generate local extrema in signal concentration at the centers of the mushroom caps, and thus, is insufficient to trigger localized cavity formation. We then (b) demonstrate various hypothetical scenarios that do result in a local extremum in the cap. Finally, we (c) introduce iron availability as a concrete example and show simulation results demonstrating cavity formation by iron starvation.

Here, we review the relevant biological background. A review of other mathematical models of biofilm morphology is presented in the discussion section of this paper.

The work of Klausen et al. [1, 10] demonstrated that cell motility is critical to development of *P. aeruginosa* biofilms. It was initially proposed that type-IV pili-mediated surface motility (twitching) was responsible for aggregation of the motile subpopulation atop the non-motile microcolonies [1]. It is now thought, however, that while type-IV pili do play a role in cap formation, it may be limited to their action as an adhesin. Instead, flagellum-mediated motility (swarming) and flagella-associated chemotaxis appear to be critical for the mobilization of the cap-forming bacteria [11].

Despite these insights, why the bacteria aggregate on top of the microcolonies is still uncertain. The importance of the chemotaxis system [11] might suggest the presence of an attractant. It has been suggested that the tops of the microcolonies represent zones of high nutrient concentration [1]. In a similar vein, Yang et al. [12] showed that disrupting iron availability by preventing usage of the siderophore pyoverdine prevents cap formation. Pyoverdine has been shown to be produced solely by the stalk-forming subpopulation [12]. The authors hypothesize that the ferric-pyoverdine supplied by the stalk-forming subpopulation is necessary for the aggregation and development of the cap-forming subpopulation [12]. This disruption of cap formation is not strict however, since the addition of ferric-citrate, an alternative source of iron, restored cap-forming ability [12].

Iron metabolism plays a key role in *P. aeruginosa* biofilm formation [12, 13, 14]. Low iron levels have been shown to induce cell motility [15, 16] and inhibit biofilm formation [13], while sufficient iron availability (1 μ M) results in the characteristic mushroom-like structures [14]. An overabundance of iron (100 μ M) results in a nebulous biofilm morphology and distinct lack of extracellular DNA (eDNA) [14].

In addition to pyoverdine production, stalk-forming and cap-forming subpopulations differ in both eDNA and rhamnolipid production [12, 17, 18]. These metabolic differences can be explained by differences in quorum sensing system activation. *P. aeruginosa* has three major quorum sensing systems; las, pqs, and rhl, which sit in a quasi-hierarchy. Each quorum sensing system can regulate hundreds of genes, which include major virulence factors [19]. With regard to biofilm development, the pqs system controls eDNA production via cell lysis [17] and can also induce pyoverdine production [2]. The rhl system controls production of rhamnolipids, which are important for maintaining open “water channels” between the mushroom-like structures [20]. Pyoverdine, eDNA, and rhamnolipid production have all been shown to be spatially restricted to the stalks in a mature biofilm, indicating that the pqs and rhl systems may be activated in the stalk-forming subpopulation at some point in biofilm development, but not so in the cap-forming subpopulation [12, 14, 17, 18]. Mutation of the las and rhl systems results in reduced mushroom formation [17, 21]. Mutation of the pqs system results in no mushroom formation [17, 22], indicating that it is critical for the development of this morphology.

Differences in EPS composition may also be present between the stalk-and cap-forming subpopulations. *P. aeruginosa* biofilms include several different types of EPS including alginate, Pel polysaccharides, Psl polysaccharides, and extracellular DNA (eDNA) [17, 23, 24]. Ghafoor et al. [23] demonstrated that both Psl and alginate are necessary for mature biofilm development by showing that the lack of either resulted in diminished mushroom formation. Yang et al. [24] reported that both Psl and Pel polysaccharides are important for subpopulation interaction. eDNA has also been shown to be important in the formation of mushroom structures [11, 14]. The type-IV pili of the cap-forming bacteria are thought to bind to the eDNA expressed by the stalk [17]. In mushroom structures, high concentrations of eDNA are observed in the stalks and at the stalk-cap juncture with little to no eDNA present in the cap [14, 17]. Mutants deficient in eDNA production are deficient in cap formation [11]. While these studies demonstrate which components of the biofilm system are critical for the development of the mushroom-like morphology, it is still unclear how these components work to achieve this result. The studies outlined above make up our rationale for assuming a mechanical disparity between the EPS of the two subpopulations in the mathematical model presented below.

Cavity formation is one of the least understood aspects of the biofilm life cycle [9]. Fluid-filled cavities containing planktonic cells in the cap are clearly visible under a microscope in mature biofilms [9], but the mechanisms that lead to the transition of the bacteria within the

cap to a planktonic state are still unclear. It has been suggested that cavities are the result of the build up of metabolic waste products [25], critical depletion of nutrients [26], the result of spatially preferential autolysis [27], or a detachment resistant outer shell [9]. A number of chemical signals have been shown to cause detachment; most appear to work through a central regulatory system controlled by c-di-GMP, a regulator of matrix synthesis [2]. These signals include rhamnolipids, carbon-source fluctuations, and nitric oxide [2]. It has also been suggested that iron starvation may trigger rhamnolipid production and encourage the activation of twitching motility [2]. In addition, cis-2-decenoic acid has recently been implicated as a detachment-causing compound [25]. It may be that cavity formation is the result of high concentrations of one of these detachment-causing agents localized in the cap [9, 26]. Rhamnolipids, known to be important for preventing cell build up between mushroom structures [20], have been shown to induce cavity formation when added exogenously [9].

2. Model Development

Following [28], we develop a multidimensional continuum model of biofilm growth and development in a flow cell. In the flow cells used in experiments, fluid flow carries nutrients and waste products downstream. Near the biofilm advective fluid velocities are small. We therefore approximate this situation by implementing a boundary layer b_L a fixed distance from the biofilm-fluid interface Γ as represented in Figure 2 and ignore fluid flow. Figure 2 represents the computational domain with upper boundary $\Gamma + b_L$, lower boundary $y = 0$, left boundary $x = 0$, and right boundary $x = 0.02$ cm. On the upper boundary, the concentrations of all soluble species are fixed at their bulk values. For soluble species, no-flux conditions are enforced on the left, right, and lower boundaries. Growth-induced pressure P ($\text{g cm}^{-1} \text{s}^{-2}$) is restricted to $P_x = 0$ on the left and right boundaries, $P_y = 0$ on the lower boundary, and $P = 0$ on the biofilm-fluid interface Γ . The conditions for P on the left, right, and lower boundaries prevent biomass from passing through the edges of the domain. Table 1 lists all dependent variables, and Table 2 lists all model parameters.

Next we introduce three classifications of bacteria with bacterial densities B_1 , B_2 , and B_3 (g cm^{-3}). These classifications represent stalk-forming, cap-forming, and planktonic bacteria respectively, and are governed by

$$\frac{\partial}{\partial t} B_1 + \nabla \cdot (\vec{v} B_1) = \kappa_g \frac{\mu_s S}{K_s + S} B_1, \quad (1)$$

$$\frac{\partial}{\partial t} B_2 + \nabla \cdot (\vec{v} B_2) = \kappa_g \frac{\mu_s S}{K_s + S} B_2 - C B_2, \quad (2)$$

$$\frac{\partial}{\partial t} B_3 + \nabla \cdot (\vec{v} B_3) = \kappa_g \frac{\mu_s S}{K_s + S} B_3 - C B_2. \quad (3)$$

Here, $\vec{v}$ is the advective velocity within the biofilm due to biomass production, S (g cm^{-3}) is the local concentration of a single growth rate limiting nutrient (e.g. oxygen), and C is a conversion function that controls the transition to a planktonic state described at the end of this section. EPS is divided into two classifications: E_1 (g cm^{-3}) the eDNA containing EPS produced by the stalk-forming bacteria, and E_2 (g cm^{-3}) the EPS produced by the cap-forming bacteria. EPS densities are governed by

$$\frac{\partial}{\partial t} E_1 + \nabla \cdot (\vec{v} E_1) = \kappa_e \frac{\mu_s S}{K_s + S} B_1, \quad (4)$$

$$\frac{\partial}{\partial t} E_2 + \nabla \cdot (\vec{v} E_2) = \kappa_e \frac{\mu_s S}{K_s + S} B_2 - C E_2. \quad (5)$$

The function C , as in 2 and 3, describes the process whereby EPS is broken down, which allows cap-forming bacteria B_2 to convert into planktonic bacteria B_3 . For simplicity, we assume the same rate in all equations. The exact form of C is described later in this section.

The different classifications of bacteria and EPS make up the particulate components of the biofilm model. To complete the volume occupied by the biofilm, we introduce a volume fraction ϕ that represents the water space between cells and EPS. It follows that

$$\phi + \frac{B_1}{\rho_B} + \frac{B_2}{\rho_B} + \frac{B_3}{\rho_B} + \frac{E_1}{\rho_E} + \frac{E_2}{\rho_E} = 1. \quad (6)$$

Following [29], we assume ϕ satisfies

$$\frac{\partial}{\partial t} \phi + \nabla \cdot (\vec{v} \phi) = \frac{\phi}{1 - \phi} \sum_{i=1}^3 \frac{\text{RHS}_{B_i}}{\rho_B} + \frac{\phi}{1 - \phi} \sum_{i=1}^2 \frac{\text{RHS}_{E_i}}{\rho_E} + r, \quad (7)$$

where r incorporates influences other than changes in population density. We define

$r = \frac{C}{(1 - \phi)\rho_E} E_2$, which corresponds to the dissolution of EPS into water fraction during the conversion process. Hence (7) becomes

$$\frac{\partial}{\partial t} \phi + \nabla \cdot (\vec{v} \phi) = \frac{\phi}{1 - \phi} \frac{\mu_s S}{K_s + S} \left[\left(\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_E} \right) (B_1 + B_2) + \frac{\kappa_g}{\rho_B} B_3 \right] + \frac{C}{(1 - \phi)\rho_E} E_2. \quad (8)$$

Under this equation, ϕ will increase during cavity formation, altering diffusion of soluble species within the biofilm (see, for example, (14) below).

Summing equations (1)–(5) divided by their associated mass densities with equation (8) we arrive at

$$\nabla \cdot \vec{v} = \frac{1}{1 - \phi} \frac{\mu_s S}{K_s + S} \left[\left(\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_E} \right) (B_1 + B_2) + \frac{\kappa_g}{\rho_B} B_3 \right], \quad (9)$$

which represents conservation of volume in the system. Due to the choice of r , E_2 does not appear on the right-hand side of (9), implying that EPS does not contribute to $\nabla \cdot \vec{v}$. We invoke the force balance equation used in [28, 30], commonly referred to as Darcy's law. This equation has the form

$$\vec{v} = -\lambda \nabla P, \quad (10)$$

where P represents the pressure due to biomass production and λ represents the inverse of the level of resistance to movement due to friction within the biofilm. Substituting into (9) gives

$$\nabla \cdot (-\lambda \nabla P) = \frac{1}{1-\phi} \frac{\mu_s S}{K_s + S} \left[\left(\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_E} \right) (B_1 + B_2) + \frac{\kappa_g}{\rho_B} B_3 \right], \quad (11)$$

which can be solved for P , ultimately giving $\vec{v}$. Note that if λ is constant, it scales out of the solution for $\vec{v}$, and so can be set to 1 without loss of generality [28]; this is common practice. *P. aeruginosa* EPS is made up of multiple constituents, some of which, like eDNA, have been shown to vary between stalk and cap [14, 17]. Different types or ratios of polymer components may lead to differences in frictional properties of the EPS as the result of differing degrees of crosslinking between polymers and/or steric entanglement. Under this assumption, we take λ to depend on E_1 as

$$\lambda(E_1) = e^{-\alpha E_1}. \quad (12)$$

Near points where E_1 is large, λ is small, making this area within the biofilm relatively high in friction. We require $\alpha \geq 0$, $E_1 \geq 0$ by definition, so that λ is bounded. The value of α used here was chosen so that λ differs by a factor of 10 when E_1 ranges from zero to its steady state value in the stalk. This specific form of λ is chosen for mathematical purposes. When solving the elliptic equation (11) it is desirable to prevent the first derivative terms from dominating the equation. This form of λ helps to keep these terms from becoming large, ensuring the stability of numerical solutions.

The function Γ describes the shape of the biofilm-fluid interface. The shape of this interface evolves according to

$$\frac{\partial}{\partial t} \Gamma = \vec{v} \cdot \vec{n}, \quad (13)$$

where $\vec{n}$ is the outward facing normal.

We now turn our attention to the soluble components of the model. As stated, S is assumed to be a single rate-limiting nutrient, which is consumed by the bacteria according to

$$\phi S_t = \nabla \cdot (\phi D_s \nabla S) - \frac{\mu_s S}{K_s + S} (B_1 + B_2 + B_3). \quad (14)$$

Advective transport of the soluble species is ignored on the grounds that diffusive transport dominates near the biofilm and that biofilm induced advection $\vec{v}$ operates on a significantly longer time scale than diffusion [28]. Nutrient concentration is fixed to S_0 on the boundary layer.

We take two approaches to analyze the ability of diffusible signals to induce cavity formation. First, we introduce a hypothetical signal molecule W that will be used to investigate the basic signaling scenarios of a signal produced by the stalk only, the cap only, and both the stalk and cap. In addition, W is used to investigate other purely hypothetical cases, such as a signal produced by the cap and consumed by the stalk. Second, we introduce equations that model the pyoverdine iron-acquisition system under the proposition that iron starvation may lead to cavity formation.

The concentration W of signal molecule is governed by

$$\phi W_t = \nabla \cdot (\phi D_w \nabla W) + f(B_1, B_2, S, W), \quad (15)$$

where the source function f is chosen to investigate several mechanisms by which a diffusible signal could induce cavity formation. Specifically, we consider

$f = \frac{\mu_s S}{K_s + S} B_1$, $f = \frac{\mu_s S}{K_s + S} B_2$, and $f = \frac{\mu_s S}{K_s + S} (B_1 + B_2)$. We shall refer to these as the stalk-forming, cap-forming, and stalk-and-cap-forming signal mechanisms. Also, we consider

$f = \frac{\mu_s S}{K_s + S} B_1 - \mu_w W B_2$ which represents a signal produced by the stalk and consumed by the

cap, and $f = \frac{\mu_s S}{K_s + S} B_2 - \mu_w W B_1$, which represents the opposite. We shall refer to these as the stalk-produced-cap-consumed and cap-produced-stalk-consumed signal mechanisms.

Iron has been demonstrated to be an important signal in biofilm development [13, 14]. As such, we explore the possibility that iron deficit might lead to cavity formation. Due to the nature of iron uptake by *P. aeruginosa*, this system requires its own set of equations. In low-iron environments like FAB media, often used to study biofilm formation [31], the stalk-forming subpopulation of *P. aeruginosa* produces pyoverdine [12]. Pyoverdine reacts readily with the Fe^{2+} present in the media [32], forming ferric-pyoverdine, which can be taken up by the bacteria as an additional source of iron [12]. Diffusion, production, and consumption of the components of this iron-acquisition system are given by

$$\phi I_t = \nabla \cdot (\phi D_I \nabla I) - \mu_I I (B_1 + B_2 + B_3) - r_I Y I, \quad (16)$$

$$\phi Y_t = \nabla \cdot (\phi D_Y \nabla Y) + \kappa_Y \frac{\mu_s S}{K_s + S} B_1 - r_Y Y I, \quad (17)$$

$$\phi F_t = \nabla \cdot (\phi D_F \nabla F) - \mu_F F (B_1 + B_2 + B_3) + r_F Y I. \quad (18)$$

Here, I , Y , and F represent the mass densities (g cm^{-3}) of Fe^{2+} , pyoverdine, and ferric-pyoverdine respectively. The coefficients r_I , r_Y , and r_F represent the molar reaction rate for the Fe^{2+} -pyoverdine reaction, scaled by the molar mass of each component. The concentrations on the upper boundary are fixed at $I = I_0$, and $Y = F = 0$.

Dissolution of EPS and conversion to a planktonic mode in equations (2), (3), (5), and (8) are controlled by the function C , which is taken to be zero unless some threshold condition is met for the signal molecule. Formally, this is expressed as

$$C(\xi) = \begin{cases} 0, & \text{if } \xi \geq C_0 \\ r_c, & \text{if } \xi < C_0. \end{cases} \quad (19)$$

for an investigation-dependent definition of the signal ξ . Here we use C to demonstrate the ability of iron starvation to induce cavity formation, and so take $\xi = I + F$. Values of C_0 near the bulk concentration of Fe^{2+} work well; see Table 2.

3. Results

There is a large disparity in time scales between the reaction-diffusion of soluble species and the growth of bacteria [28]. We take advantage of this disparity and treat the particulate components of the model as quasi-static while solving for the steady state concentrations of

the soluble compounds. We then solve for the instantaneous pressure field P and the resulting advective velocity $\mathbf{v}$. Once the velocity has been found, we can solve for the change in particulate components and update the biofilm-fluid interface Γ over time.

The entire system of equations can be solved using standard numerical techniques, albeit with some slight modifications. The steady-state concentrations of the soluble species, described by equations (14) to (18), are solved with a Crank-Nicolson scheme modified with a successive approximation approach to handle the nonlinearities. A similar method is used to solve equation (11), but since this equation is only valid within the biofilm, the method was modified to handle the irregular domain. The hyperbolic equations (1)–(5) and (8) are solved using a characteristic based approach [33, 34]. Equation (13) is solved using the level set method. The solution was implemented in FORTRAN and run on a 2.53 GHz Intel® i5 processor. The solution took approximately 2 hrs to run.

The computations were carried out on a regular grid with spacing corresponding to $1\ \mu\text{m}$ and dimensions $200\ \mu\text{m} \times 200\ \mu\text{m}$. The boundary layer, as represented in Figure 2, was set at $40\ \mu\text{m}$. Simulations were run with the following initial conditions. Two hemispherical microcolonies of radius $17\ \mu\text{m}$ were placed at locations of 50 and $150\ \mu\text{m}$. B_1 and E_1 were

set to $\kappa_g(1 - \phi)/(\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_E})$ and $\kappa_e(1 - \phi)/(\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_E})$, respectively, for $y \leq 12\ \mu\text{m}$ (the stalk) and 0 elsewhere. B_2 was set to $(1 - \phi)\rho_B$ for $y > 12\ \mu\text{m}$ (the cap) and 0 elsewhere. E_2 and B_3 were set to 0 everywhere, and ϕ was set to 1.0 outside the biofilm and 0.5 inside. Table 2 gives values for other model parameters.

The rationale for the above choice of boundary layer is as follows. We consider a flow cell with chamber dimensions $4\ \text{cm}$ long, $0.4\ \text{cm}$ wide, and $0.1\ \text{cm}$ deep, treated as flow between two parallel plates (i.e. uniform in the transverse direction). This gives rise to the two dimensional advection-diffusion equation for nutrient concentration S , $\tilde{S}_t + u\tilde{S}_{\tilde{x}} + v\tilde{S}_{\tilde{y}} = D_S(\tilde{S}_{\tilde{x}\tilde{x}} + \tilde{S}_{\tilde{y}\tilde{y}})$ in a rectangular domain. Here, u and v are the horizontal and vertical flow velocities. We consider the action of the biofilm as a boundary condition on the lower boundary. We scale the advection-diffusion equation using $\tilde{S} = \bar{S}S$, $\tilde{x} = Lx$, and $\tilde{y} = dy$ with $L = 4\ \text{cm}$ and $d = 0.1\ \text{cm}$. Here, L and d are the length and depth of the channel respectively, $\bar{S}$ is the characteristic value of S . Between parallel plates flow velocities are parabolic, $u = a(y^2 - dy)$ and $v = 0$, where the constant a depends on the volumetric flow rate $3\ \text{ml/hr}$. Therefore, the steady state concentration satisfies the non-dimensional equation

$\frac{d^2 u}{D_S L} S_x = \frac{d^2}{L^2} S_{xx} + S_{yy}$. The size of the boundary layer is approximated by determining the distance from the boundary at which the coefficient of S_x dominates that of S_{yy} . That is, for

what value of y does $\frac{d^2 u}{D_S L}$ dominate 1? Although this is subjective, we note that at $y = 0.004\ \text{cm}$, $\frac{d^2 u}{D_S L} \sim O(4)$ which we deem sufficient to justify a boundary layer of $40\ \mu\text{m}$. We verified this result by solving the advection-diffusion problem numerically and investigating at what distance the concentration was nearly unaffected by the source (not shown).

Nutrient and signal concentrations resulting from numerical simulations are presented as level sets in the following sections. Because we are interested in demonstrating local minima or maxima, the value of each contour is unimportant. Therefore, for clarity, we show the level sets without the accompanying values.

3.1. Biofilm Morphology

Figure 3 shows simulation results for the evolution of biofilm morphology over time $t = 0$ to $t = 35.1$ hrs. Yang et al. [14] show no mushrooms at day 2 and fully formed mushrooms at day 4. Klausen et al. [1] shows time series images indicating migration onto microcolonies after 22 hrs and development of a partially formed cap after 44 hrs. In our simulations, a mushroom-shaped morphology develops from the combination of accelerated expansion in the cap and high concentration of E_1 (increased friction) in the stalk. Simulation results indicate that both are necessary to produce the mushroom-like morphology (data not shown). Without differential friction coefficients between the stalk and cap, biomass production in the cap transmits force to the stalk, causing a general spreading effect. This results in somewhat hemispherical colonies. Without accelerated expansion in the cap, the cap volume does not grow fast enough relative to the volume of the stalk and so does not result in a mushroom-like architecture.

3.2. Investigation of General Signals

To analyze the potential for diffusible signals to generate cavity formation, we investigate the level sets of the associated concentration fields in a mature biofilm. In order for a diffusible signal to cause cavity formation in the center of the cap, the concentration of the signal must show either a local minimum or maximum in this location. We assume that all cap-forming bacteria behave the same (i.e. we do not consider the resistant shell hypothesis [9]). We therefore seek a radial gradient, indicating a local extremum. These simulations are conducted for a single time point, to determine the instantaneous signal distribution. The morphology of the biofilm used in these cases is taken from Figure 3 at time $t = 32.2$ hrs. Figure 4 shows the level sets for the nutrient S (A), and the level sets of signal W produced under different conditions (B,C,D). Panel (B) shows the level sets for the stalk-forming signal mechanism. Panel (C) shows the level sets for the cap-forming signal mechanism. Panel (D) shows the level sets for the stalk-and-cap-forming signal mechanism. None of these level sets show a radial gradient in the mature biofilm, making it unlikely that these signals are capable of causing cavity formation. This is largely because the no-flux condition on the lower boundary tends to have a flattening effect on the concentration distribution. A signal produced by the cap only (panel (C)) diffuses out, but is impeded by the no-flux lower boundary, causing the concentration to build up. Hence, despite the localized production, this signal fails to generate a local maximum in the cap.

Figure 5 (A) and (D) again show the level sets for the nutrient S and the signal W for the stalk-and-cap-forming signal mechanism. Since neither shows a radial gradient centered on the cap, we investigated whether some combination of S and W is capable of causing cavity formation. In their review, Stewart and Franklin [35] discuss how combinations of the gradients of nutrients and waste products can result in a spatially heterogeneous environment. In this spirit, we consider the scenario in which detachment initiates if the pair of inequalities $S < S^*$ and $W > W^*$ are satisfied for some critical values of S^* and W^* . For the sake of completeness, we consider all four variations on the directions of the inequalities. Figure 5 (B,C,E,F) represent the cases with values of S^* and W^* chosen for illustration. The shaded overlap regions represent the regions where the associated pair of inequalities is satisfied. None of these 4 cases demonstrate a radial gradient, which would be represented by a circular shaded region in the cap. Further, changing the values of S^* and W^* changes only the location and width of the shaded region, and cannot cause a radial gradient in the cap. Similar results are generated when W is generated by the stalk-forming or by the cap-forming signal mechanisms.

To further investigate the possibility of generating a radial gradient, we examined the function $S + W$ for the case in which W generated by the stalk-and-cap-forming signal

mechanism $f = \frac{\mu_s S}{K_s + S} (B_1 + B_2)$. Figure 6 (C) represents the level sets of this function, with W scaled so that neither dominates the other. Figure 6 (A) and (B) are the level sets for S or W . Since many detachment inducing signal molecules act on the central regulatory protein c-di-GMP [2], it may be that multiple signals working in concert can lead to cap centered cavity formation. The function $S + W$ represents such a situation, supposing that both the nutrient S and the bacterially produced signal W increase intracellular c-di-GMP. Figure 6 (C) shows that this mechanism is capable of generating a local minimum centered in the cap. This means that bacteria in the center of the cap would have relatively low levels of c-di-GMP. Since a low level of c-di-GMP decreases matrix production and induces a planktonic state [2], this scenario would result in cavity formation in the cap.

The work of Yang et al. [12] demonstrated that the cap-forming subpopulation can depend nutritionally on the stalk-forming subpopulation in some cases. We analyzed this situation in the form of a general signal produced by one subpopulation and consumed by the other. Figure 7 (A) represents the stalk-produced-cap-consumed signal mechanism in which a

signal is produced by B_1 and consumed by B_2 ; $f = \frac{\mu_s S}{K_s + S} B_1 - \mu_w W B_2$. This scenario does not lead to a radial gradient in the cap. The boundary condition that $W = 0$ on the upper boundary results in a concentration that decreases with y . This implies that fluid flow in the bulk removes the signal W faster than it can build up around the cap sufficiently for consumption to create any kind of local minimum in concentration. Figure 7 (B) represents the cap-produced-stalk-consumed signal mechanism in which a signal is produced by B_2 and

consumed by B_1 ; $f = \frac{\mu_s S}{K_s + S} B_2 - \mu_w W B_1$. This scenario is capable of generating a local maximum centered in the cap, if the consumption rate μ_w is high enough, because consumption by the stalk counters the effects of the no flux condition on W on the lower boundary.

3.3. Investigation of Iron as a Signal Molecule

In the previous section we investigate the ability of hypothetical signal mechanisms to induce cavity formation. Our results suggest that a signal based on the combination $S + W$ and cap-produced-stalk-consumed signal mechanisms are capable of generating the required radial gradient in signal concentration, indicative of a local minimum or maximum centered in the cap. However, we are not aware of a bacterially produced signal molecule that encourages the sessile state. Nor are we aware of a signal molecule produced by the cap-forming subpopulation and consumed by the stalk-forming subpopulation. The pyoverdine iron-acquisition system bears some resemblance to these concepts. Thus we investigate next whether iron availability can generate a signal for cavity formation. As in the previous section we use the biofilm morphology from Figure 3 at time $t = 32.2$ hrs. Figure 8 illustrates the level sets of components of the iron-aquisition system. Figure 8 (A) shows the level sets for Fe^{2+} . Fe^{2+} concentration decreases with depth because it is consumed by the bacteria and reaction with pyoverdine. Figure 8 (B) shows the level sets for pyoverdine. The concentration of pyoverdine increases with depth because it is produced by the stalk-forming subpopulation and consumed by reaction with Fe^{2+} . Figure 8 (C) shows the level sets for ferric-pyoverdine, produced by the Fe^{2+} -pyoverdine reaction and consumed by both subpopulations. Ferric-pyoverdine also increases with depth. Figure 8 (D) shows the total iron availability. Total iron shows a radial gradient centered on the cap, indicating a local minimum in iron availability.

Since iron availability demonstrates a radial gradient, we combine our model of the pyoverdine system with our model of biofilm growth to demonstrate cavity formation by

this mechanism. Figure 9 illustrates the results of this simulation. The rate at which cap-forming bacteria convert to the planktonic state, C , is taken to be a function of total iron, $I + F$. Panels (A,B,C,D) show the evolution of the different bacterial subpopulations: stalk-forming B_1 (red), cap-forming B_2 (green), and planktonic B_3 (blue). The cap-forming subpopulation expands until the macrocolony reaches a critical size, driving $I + F$ below the threshold C_0 . At this point (C), cavity formation initiates. Panels (E,F,G,H) show the EPS distribution E_1 (red) and E_2 (green). No EPS is present in the cap at the initial time; EPS is produced in the cap as the simulation progresses and is finally broken down in the center during the conversion process. Panels (I,J,K,L) show the distribution of the water fraction ϕ (blue: 0.5, white: 1.0), which shows an increase in the cavity, representing a fluid filled space inside the cap. Panels (M,N,O,P) show the level sets for $I + F$. A depression in total iron concentration in the cap is present at very early time points (M) due to the initially high density of B_2 , but the concentration is still well above the critical value C_0 . Columns, panels (A,E,I,M), panels (B,F,J,N), panels (C,G,K,O), and panels (D,H,L,P), correspond to time $t = 0$, $t = 20$, $t = 32.3$, and $t = 35.1$ hrs., respectively.

4. Discussion

Mathematical models of the kind presented here serve to generate and investigate hypotheses which can aid in our efforts to develop a deeper understanding of complex systems. Our ability to faithfully reproduce biofilm growth through modeling can increase our confidence that our understanding is accurate, as well as point out gaps [36]. Here, we introduce insights drawn from our modeling efforts. An increased level of friction amongst EPS constituents in the stalk (possibly due to eDNA) and accelerated expansion of the cap relative to the stalk may be important to the formation of the characteristic mushroom morphology. In addition, iron limitation is a plausible trigger for cavity formation inside the cap.

We analyzed the steady state distribution of several hypothetical diffusible signals to see if one or more of them might demonstrate the ability to cause the formation of cap-centered cavities. We found that limitation or accumulation of a simple nutrient or signal molecule is insufficient to cause cavity formation. This is largely a consequence of the signal molecule building up near the lower boundary. Simple combinations of signals however, are capable of generating concentration profiles that could cause conversion to a planktonic state in the center of the mushroom caps. We demonstrate two purely hypothetical examples, a signal based on the additive effect of nutrient and a bacterially produced signal molecule, and a signal molecule produced by the cap and consumed by the stalk. The pyoverdine iron acquisition system is an explicit example closely related to the former of these hypothetical mechanisms. Modeling of this system demonstrates that it is capable of increasing total iron availability for bacteria in the biofilm. As the biofilm grows however, a radial gradient develops in the cap, indicating iron starvation at this location.

Simulations demonstrate that increased friction coefficient in the stalk and an accelerated expansion of the cap relative to the stalk are sufficient to generate a mushroom-like morphology. Accelerated expansion in the cap can be accomplished in a number of ways. Owing to the differential expression of rhamnolipids [18], pyoverdine [12], and other metabolic processes, it may be that the base growth rate κ_g is decreased for the stalk-forming subpopulation. Here we take a different approach - accelerated expansion of the cap is brought about by choice of initial conditions. We argue that the motile subpopulation do not produce EPS while in their motile state, and thus are likely to pack at a density of $(1 - \phi)\rho_B$ when collecting on top of the stalk-forming bacteria. Next, we consider the behavior of B_2 . Since $C = 0$ early in the simulation, equation (2) can be written as

$\frac{\partial}{\partial t} B_2 + \vec{v} \cdot \nabla B_2 = \kappa_g \frac{\mu_s S}{K_s + S} B_2 - (\nabla \cdot \vec{v}) B_2$. The right-hand side of this equation represents the evolution of B_2 along the characteristics determined by $\vec{v}$. The first term on the right-hand side represents change due to local bacterial growth, and the second term represents change in the density due to expansion or contraction. If we consider a characteristic originating from the cap, then early in the simulation $B_1 = B_3 = 0$ and by use of equation (9) we can write

$$\frac{\partial}{\partial t} B_2 + \vec{v} \cdot \nabla B_2 = \frac{\mu_s S}{K_s + S} \left[\kappa_g B_2 - \frac{1}{1 - \phi} \left(\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_E} \right) B_2^2 \right]. \quad (20)$$

From this equation we can see that B_2 in the cap behaves logistically along the

characteristics with fixed points 0 (unstable) and $\kappa_g(1 - \phi) / (\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_E})$ (stable). Because $(1 - \phi)\rho_B$ is greater than the stable fixed point, B_2 drops during the simulation, as expected. We

can conclude that B_2 drops because $\frac{1}{1 - \phi} (\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_E}) B_2 > \kappa_g$. In other words, volumetric expansion of the cap is accelerated because EPS production drives the bacteria apart faster than they grow. This mechanism has the added feature that the expansion of the cap slows as B_2 drops, preventing the caps from growing too large.

The disparity in friction coefficient between the stalk and cap is enforced through equation (10). This equation represents the force balance equation for the particulate components of the biofilm, describing how the bacteria push each other as biomass is produced. The equation bears a superficial resemblance to Darcy's law, and is often referred to as such. It is not being used, however, to describe the motion of a fluid through a porous media, and therefore does not invoke the associated assumptions. Klapper [28, 30] gives a justification for the use of this equation, saying that it represents a balance of osmotic swelling with friction. Another argument for its use is to invoke Stokes drag (I. Klapper, personal communication, November 2, 2010). The effect of defining λ as in (12) influences the growth of the biofilm only in the transition regions between the stalk and the cap. Since the stalk EPS concentration E_1 is nearly piecewise constant, λ is as well. Locally, where λ is constant λ scales out of the velocity. We note that taking λ constant leads to biofilm growth in which no mushroom-like morphology develops.

A number of other mathematical models have been developed to investigate development of complex morphology in biofilms. Hermanowicz [37] and Picioreanu et al. [38] developed models of biofilm growth wherein a disparity between bacterial growth rate and nutrient diffusion rate creates a substantial growth advantage for cells on the peaks of small perturbations in the biofilm-fluid interface. Dockery and Klapper [39] introduced another mechanism by which mushroom-like towers may be formed. In their model, mass production competes with a decay process. When the biofilm reaches a critical thickness the decay process wins out at the bottom, resulting in a net loss of volume at the bottom and a net gain at the top. This causes an instability in the shape of the biofilm interface resulting in mushroom-like structures. Alpkvist et al. [40] assumed an advective velocity that is proportional to the nutrient gradient. The constant of proportionality was carefully chosen so that conservation of mass was preserved. The model resulted in tall mushroom or finger-like structures. Picioreanu et al. [41] attempted to reproduce the experimental results of Klausen et al. [1, 10]. They demonstrated that cell motility could reproduce a flat biofilm like those grown under citrate [10]. They encountered difficulty however, when trying to recreate mushroom structures as observed in biofilms grown under glucose. The authors observed that cell aggregation via motility resulted in tall, thin vertical strands, and they resorted to a

nutrient driven detachment-reattachment process whereby bacteria on the top of microcolonies detached at a lower rate. Interestingly, the implication of flagellum-mediated motility in cap-forming bacterial relocation has suggested that swimming motility of this type may in fact play a role [11].

The model presented here differs from these previous models in that it relies on the mechanics of the biofilm rather than nutrient gradients to generate the mushroom morphology. The disparity in friction coefficient between the stalk and cap represents a novel hypothesis. The investigation of mechanisms by which cavity formation might occur is relatively new in the mathematical modeling of biofilms. While detachment has been considered in a number of models [29, 37, 42, 43], it has been approached as cell erosion or sloughing. Cavity formation has been addressed by a small number of other modeling studies [26, 44]. Hunt et al. [26] used a simple nutrient limitation scheme. Their 3D simulations demonstrated hemispherical gaps beneath broad canopies which were tethered to the substratum by small pieces of biofilm. In our model, such a scheme would cause conversion in regions defined by the level sets represented in Figure 5 (A). The aspect ratio of the mushroom formations presented here favors the vertical, making the broad level sets in Figure 5 (A) unlikely to produce cavities. Instead, in our model, this mechanism would likely result in the entire lower portion of the biofilm converting to a planktonic state. While this does occur in biofilm systems [45], it is unclear whether or not it is related to cavity formation as discussed here. Fagerlind et al. [44] used a cellular-automata model and demonstrate similar results to Hunt et al. [26] when considering a nutrient limitation scheme. Fagerlind et al. [44] also demonstrate the formation of centralized cavities by means of competition between biomass production and endogenous metabolism, assuming that cells lyse if the ratio between the two processes drops too low. Our results offer an alternative hypothesis based on iron signaling.

Our simulation results demonstrating cavity formation by iron-availability suggest that this mechanism may be a central component to a complete *Pseudomonas aeruginosa* biofilm model. The model presented here represents stages 3 and 4 of biofilm development as depicted in Figure 1. We envision modeling stages 1 and 2 as follows. Division into motile and non-motile subpopulations follows from stochastic variation in phenotypic state. The non-motile subpopulation grows as microcolonies until quorum sensing activation and iron limitation induces rhamnolipid and pyoverdine production. The resulting increase in iron availability causes a conversion to a sessile state on the part of the motile subpopulation. This conversion is frustrated near the substratum between microcolonies due to rhamnolipid build up, causing an apparent aggregation on the top of the microcolonies.

Perhaps somewhat contrary to the hypotheses presented here, Boles et al. [9] demonstrated that rhamnolipids may induce cavity formation. The authors observed a hyper-detaching mutant that forms cavities significantly earlier than wild-type and that induces the same effect in wild-type cells when grown in a biofilm together. The mutant was shown to overproduce rhamnolipids. The authors also demonstrated that exogenously added rhamnolipids induce cavity formation in established biofilms [9]. If it is reasonable to model rhamnolipids as a diffusible molecule and if one assumes rhamnolipids do not interact with EPS, then our model suggests that accumulation in the cap is unlikely. The fact that rhamnolipids induce cavity formation when added exogenously suggests that such a localized gradient is not present. Boles et al. proposed a model of detachment wherein a detachment-resistant subpopulation makes up the outer portions of the mushroom formations. As this hypothesis suggests, there are a number of mechanisms that might lead to cavity formation. We have investigated only a handful here, with the hope that this preliminary study will drive further research. To unify the hypotheses proffered here with

the results of Boles et al. [9], we suggest that rhamnolipids, which some evidence suggests are associated with c-di-GMP [2, 46], may sensitize bacteria to other signals.

5. Summary

We have presented a mathematical model of *Pseudomonas aeruginosa* biofilm growth and development under glucose FAB media, focusing on the latter 2 stages of this process (Figure 1). This model includes an investigation into possible mechanisms by which fluid-filled cavities within the cap structure form. We raise the hypothesis that iron starvation may be responsible for this phenomenon and demonstrate, through our mathematical model, the plausibility of this hypothesis. The key assumptions made in this work are

- Cap-forming bacteria aggregate atop stalk-forming microcolonies at a relatively high density,
- EPS produced by stalk-forming bacteria differs in internal friction from EPS produced by the cap,
- Cavity formation results from local extrema of some diffusible signal(s).

Numerical simulations based on a model that incorporates these assumptions support the following conclusions:

- Mechanical causes, rather than nutrient gradients, may be responsible for the characteristic mushroom-like morphology of *P. aeruginosa* biofilms,
- Neither simple nutrient starvation nor signal accumulation are likely to cause cavity formation,
- Iron starvation may cause cavity formation.

The ideas presented here suggest several new directions in both modeling and experimental research on biofilm development.

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Figure 1.

Rendering of *Pseudomonas aeruginosa* biofilm life cycle, represented in four stages. Stage 1 represents initial attachment. Stage 2 represents microcolony formation. Stage 3 represents formation of the mushroom-like architecture, referred to as macrocolonies. Stage 4 represents formation of fluid-filled cavities in which planktonic cells are visible. These cavities subsequently rupture, allowing for the dispersal of planktonic cells.

Figure 2. Model domain showing upper boundary located a distance of b_L from the biofilm-fluid interface Γ

Figure 3.

Evolution of the biofilm-fluid interface for two hemispherical microcolonies with initial

conditions $B_1 = \kappa_g(1 - \phi) / (\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_F})$ and $E_1 = \kappa_e(1 - \phi) / (\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_F})$ for $y \leq 12 \mu\text{m}$, and $B_2 = (1 - \phi)\rho_B$ and $E_2 = 0$ for $y > 12 \mu\text{m}$. These contours represent biofilm growth from $t = 0$ to $t = 35.1$ hrs. Each contour represents 5 hrs of growth.

Figure 4.

Level sets for nutrient S (A), level sets for the signal molecule W under the stalk-forming signal mechanism (B), under the cap-forming signal mechanism (C), and under the stalk-and-cap-forming signal mechanism (D)

Figure 5.

Level sets for nutrient S (A) and signal W under stalk-and-cap-forming signal mechanism (D). The shading in the remaining panels where the concentrations S and W are above and below S^* and W^* . In the dark purple regions, $S > S^*$ & $W > W^*$ (B), $S < S^*$ & $W > W^*$ (C), $S > S^*$ & $W < W^*$ (E), and $S < S^*$ & $W < W^*$ (F).

Figure 6.

Level sets of nutrient S (A), level sets of the signal W under the stalk-and-cap-forming signal mechanism (B), and level sets of a scaled addition of S and W which exhibits a radial gradient centered on the cap (C)

Figure 7.
 Level sets of the signal W under the stalk-produced-cap-consumed signal mechanism (A)
 and under the cap-produced-cap-consumed signal mechanism (B)

Figure 8. Level sets of components of the pyoverdine iron-acquisition system. Exogenous Fe^{2+} I (A), stalk-produced pyoverdine Y (B), ferric-pyoverdine F (C), and total iron $I + F$ (D).

Figure 9.

Simulation results with initial condition (A,E,I,M) demonstrating mushroom morphology development and cavity formation due to iron limitation. Stalk-forming (red), cap-forming (green), and planktonic (blue) subpopulations are spatially distinct (A,B,C,D). EPS E_1 (red) and E_2 (green) distributions are shown in panels (E,F,G,H). Panels (I,J,K,L) show water volume fraction ϕ on a scale from 0.5 (blue) to 1.0 (white). Level sets of total iron concentration show a radial gradient centered in the cap when the cap reaches a critical size (M,N,O,P). Columns correspond to time $t = 0$, $t = 20$, $t = 32.3$, and $t = 35.1$ hrs., respectively.

Table 1

Description of dependent variables

Variable	Description
B_1	Concentration of stalk-forming bacteria (g cm^{-3})
B_2	Concentration of cap-forming bacteria (g cm^{-3})
B_3	Concentration of planktonic bacteria (g cm^{-3})
E_1	Concentration of EPS produced by stalk-forming bacteria (g cm^{-3})
E_2	Concentration of EPS produced by cap-forming bacteria (g cm^{-3})
ϕ	Water volume fraction (unitless)
$\vec{v}$	Growth induced advective velocity (cm s^{-1})
P	Growth induced pressure ($\text{g cm}^{-1} \text{s}^{-2}$)
λ	Local biofilm friction ($\text{g}^{-1} \text{cm}^3 \text{s}$)
Γ	Location of biofilm-fluid interface (cm)
S	Concentration of a single growth rate limiting nutrient (g cm^{-3})
W	Concentration of detachment inducing signal molecule (g cm^{-3})
I	Concentration of Fe^{2+} (g cm^{-3})
Y	Concentration of pyoverdine (g cm^{-3})
F	Concentration of ferric-pyoverdine (g cm^{-3})
C	Detachment rate (s^{-1})

Table 2

Description of model parameters

Parameter	Description	Value
S_0	Nutrient boundary value (g cm^{-3})	$1 \cdot 10^{-6}$
I_0	Fe^{2+} boundary value (g cm^{-3})	$4.0174 \cdot 10^{-8}$
C_0	Critical value for conversion due to Fe^{2+} limitation (g cm^{-3})	$4.01 \cdot 10^{-8}$
μ_S	Nutrient consumption rate (s^{-1})	0.4 [47]
$\mu_F, \mu_{F'}$	Iron & ferric-pyoverdine consumption rates ($\text{cm}^3 \text{g}^{-1} \text{s}^{-1}$)	1.11
μ_W	Signal consumption rate ($\text{cm}^3 \text{g}^{-1} \text{s}^{-1}$)	69.4
K_S	Nutrient consumption half-saturation constant (g cm^{-3})	$1 \cdot 10^{-6}$ [47]
κ_g	Nutrient to biomass conversion factor (unitless)	0.725
κ_e	Nutrient to EPS conversion factor (unitless)	$10\kappa_g \frac{\rho_E}{\rho_B}$
κ_Y	Nutrient to pyoverdine conversion factor (unitless)	2.5
ρ_B	Bacterial mass density (g cm^{-3})	0.2 [43]
ρ_E	EPS mass density (g cm^{-3})	0.033 [43]
D_S	Diffusion constant for nutrient ($\text{cm}^2 \text{s}^{-1}$)	$2.97 \cdot 10^{-6}$
D_W	Diffusion constant for signal ($\text{cm}^2 \text{s}^{-1}$)	$1 \cdot 10^{-6}$
D_I	Diffusion constant for Fe^{2+} ($\text{cm}^2 \text{s}^{-1}$)	$6.4 \cdot 10^{-6}$ [48]
D_Y, D_F	Diffusion constant for pyoverdine and ferric-pyoverdine ($\text{cm}^2 \text{s}^{-1}$)	$0.1D_I$
r_I	Fe^{2+} -pyoverdine reaction rate: Fe^{2+} equation ($\text{cm}^3 \text{g}^{-1} \text{s}^{-1}$)	26.44 [32]
r_Y	Fe^{2+} -pyoverdine reaction rate: pyoverdine equation ($\text{cm}^3 \text{g}^{-1} \text{s}^{-1}$)	646.46 [32]
r_F	Fe^{2+} -pyoverdine reaction rate: ferric-pyoverdine equation ($\text{cm}^3 \text{g}^{-1} \text{s}^{-1}$)	672.90 [32]
r_C	Rate of conversion to planktonic state (s^{-1})	$5.56 \cdot 10^{-4}$
b_L	Distance from biofilm-fluid interface to upper boundary (cm)	0.004
α	EPS friction coefficient ($\text{cm}^3 \text{g}^{-1}$)	$-\ln(0.1) \left(\frac{\kappa_g}{\rho_B} + \frac{\kappa_e}{\rho_E} \right) / (\kappa_e(1 - \phi))$