MATHEMATICAL MODELLING OF QUORUM SENSING IN BIOFILMS

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ABSTRACT

MATHEMATICAL MODELLING OF QUORUM SENSING IN BIOFILMS

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Quorum sensing is a cell communication mechanism used to coordinate group behaviour based on population density. A mathematical model of quorum sensing in bacterial biofilms is developed, consisting of a nonlinear diffusion reaction system describing the effects of a growing biofilm on bacterial quorum sensing behaviour. In numerical experiments, the influence of the hydrodynamic environment and nutrient conditions on biofilm growth and quorum sensing behaviour are studied, and flow-facilitated inter-colony communication and spatiotemporal quorum sensing induction patterns are observed. The model is extended to include an impact of quorum sensing on biofilm growth, through the explicit description of EPS, the protective biomass layer surrounding bacterial biofilm cells. The circumstances under which quorum sensing-regulated EPS production is a beneficial strategy for cells are identified. Biofilm colonies that use this strategy have lower cell populations than non-quorum sensing colonies, but may secure nutrients in a space-limited environment and outcompete neighbouring colonies.
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Chapter 1

Introduction

Biofilms are microbial communities encased in a layer of extracellular polymeric substances (EPS), adhered to biotic or abiotic surfaces. In a biofilm, bacteria are protected by the EPS matrix from external stresses, and are able to flourish in any aqueous environment with adequate nutrients available. Quorum sensing is a type of cell-cell communication, used to coordinate gene expression and behaviour in groups. Bacteria cells produce and release signalling molecules (AHL), and when a critical environmental AHL concentration is reached, the cells become induced, and undergo changes in gene expressions.

The problem motivating this study is to understand how biofilm growth impacts quorum sensing induction of bacteria cells. Comprehension of the converse relation is also desirable, that is, how quorum sensing influences biofilm growth. This thesis is organized into a series of two related paper manuscripts. In the first paper, presented in Chapter 2, a convection-diffusion-reaction model of biofilm growth is combined with a model of quorum sensing in suspended bacteria cultures. Hydrodynamic conditions and nutrient resources are varied so that resulting spatial and temporal induction patterns may be identified. Communication behaviours amongst individual biofilm colonies and associated ecological implications will be analyzed.
The focus of the second paper, presented in Chapter 3, is resolving the problem of how quorum sensing in turn influences biofilm growth. The model is extended to explicitly describe EPS, a structural component of biofilms, and differential EPS production rates by induced and non-induced bacteria cells. A question of interest is whether or not quorum sensing-regulated EPS production provides a benefit to biofilm cells, and if so, under which environmental conditions. Competition between individual colonies will also be studied.

The first paper is a version of a manuscript submitted to the journal, “Canadian Applied Mathematics Quarterly”; this version has since been revised and shortened based on reviewer feedback. At the time of this thesis presentation, the second paper is a draft, prepared for future submission to a journal. Both papers have been prepared in collaboration with multiple authors. My contributions to the first research paper include revision and extension of simulation code, conduction of computer simulations, analysis of results, and the writing of most sections. My contributions to the second paper include the above tasks, as well as model development. My co-authors contributed to the model development, analysis, preparation of computer code for numerical solutions, simulation experiment design, and biological expertise.
Chapter 2

A mathematical model of quorum sensing in patchy biofilm communities with slow background flow

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Abstract

For an increasing number of bacterial species, regulation of gene expression by production of extracellular signalling molecules has been observed. This mechanism, generally called quorum sensing, is a type of cell communication used to coordinate behaviour in groups based on population density. We present a mathematical model of quorum sensing in bacterial biofilms, consisting of a nonlinear diffusion reaction
system which describes the effects of a growing biofilm on bacterial quorum sensing behaviour. The model includes production and spatial spreading of biomass, conversion of down-regulated biomass into up-regulated biomass as a consequence of the quorum sensing signalling molecule concentration, and convective and diffusive transport of nutrients and quorum sensing molecules. The biofilm structure is two-dimensional, and so it is possible to observe the effects of signals produced by one colony on neighbouring colonies. In numerical experiments we investigate how the hydrodynamic environment and nutrient conditions contribute to biofilm growth and quorum sensing behaviour, and interpret the ecological implications of our results. We discuss observed flow-facilitated inter-colony communication and spatiotemporal induction patterns for the various environmental conditions tested.

**Keywords:** biofilm; convective transport; mathematical model; quorum sensing

**Classification:** MSC2000:92D25

### 2.1 Introduction

#### 2.1.1 Bacterial biofilms

Biofilms are microbial communities encased in a layer of extracellular polymeric substances (EPS), adhered to biotic or abiotic surfaces. Biofilms are an extremely successful life form, and are able to flourish in any aqueous environment with adequate nutrients available. In fact, bacteria preferentially reside in biofilms, rather than in isolation as planktonic cells. In a biofilm, bacteria are protected by the EPS matrix from external stresses, and carry out a wide range of reactions which are rel-
evant in many disciplines, such as environmental engineering, food processing, and medicine (Lewandowski, 2007).

2.1.2 Quorum sensing in biofilms

Quorum sensing was originally described as a diffusion-reaction based cell-cell communication mechanism used by several bacterial taxa to coordinate gene expression and behaviour in groups, based on population densities (Fuqua et al., 1994). Bacteria cells constantly produce and release low amounts of signalling molecules, called autoinducers (e.g., acyl-homoserine lactones (AHL) in Gram-negative bacteria). When a critical environmental autoinducer concentration is reached, the bacteria are rapidly induced and undergo changes in gene expressions. In most bacterial autoinducer systems, the autoinducer synthase gene is up-regulated, initiating positive feedback, and the bacteria subsequently produce AHL molecules at an increased rate. As the extracellular autoinducer concentration reflects the cell density, the time at which this phenotypic change occurs is dependent on the cell density. Quorum sensing was originally described for and, until now, mainly investigated in homogeneous planktonic cultures. However, most bacteria live attached to surfaces in spatially heterogeneous biofilms, usually as assemblages of microcolonies, or “patchy” biofilm communities (Kierek-Pearson and Karatan, 2005, Costerton et al, 1995). Under these conditions, autoinducers reflect local, rather than global, cell densities. Redfield (2002) assumed alternatively that autoinducers are used by single cells for the measurement of the diffusible space, or more generally, the mass transfer properties of the environment. This concept, which does not involve cooperation, is called diffusion
sensing. Hense et al. (2007) unified both concepts with the idea of efficiency sensing and argued that induction of cells is influenced by both cell density and mass transfer limitations, as well as inhomogeneities of cell distribution. Although there are some experimental indications for the influence of mass transfer limitations (Dulla and Lindow, 2008, Kirisits et al., 2007, Shompole et al., 2003), this has never been analyzed systematically for spatially heterogeneous growing biofilms in complex matrices with varying nutrient conditions. In fact, most papers still assume that the purpose of autoinducers is for cell density assessment. The term “quorum sensing” originally referred to only the cell density measurement, and even today it is usually defined in this sense. However, the term is well established and indeed used to refer to general measurements of autoinducers, disregarding whether mass transfer limitation or cell distribution contribute or even dominate. We adopt this broad use of terminology and refer to pure cell density measurement as “quorum sensing in the strict sense”.

As a number of traits relevant for human, animal and plant health are regulated via autoinducers (Demuth and Lamont, 2006, Whitehead et al, 2001), a comprehensive understanding of quorum sensing systems is highly desirable.

### 2.1.3 Modelling of quorum sensing in biofilms

We will study how a hydrodynamic environment affects spatiotemporal quorum sensing induction patterns and flow-facilitated inter-colony communication in a developing biofilm. Specifically, we will model quorum sensing induction in patchy biofilms in a slow-flow hydrodynamic environment, and investigate the effect of environmental conditions such as bulk flow velocities and nutrient availability on the
onset of quorum sensing. We will consider Gram-negative bacteria with their typical autoinducer system, which involves acyl-homoserine lactones (AHLs) as the signalling molecule. The effect of nutrient availability on quorum sensing is qualitatively easy to predict: increased food availability promotes growth, and accelerated growth in turn accelerates quorum sensing. On the other hand, the contribution of bulk flow hydrodynamics is more difficult to estimate. Increased flow implies an increased convective supply of nutrients, implying growth is increased, and thus quorum sensing induction is accelerated. Increased flow velocities also imply increased convective transport of AHL in the aqueous phase. Convective AHL transport can have three effects on the onset of quorum sensing: (i) AHL molecules that are produced in a biofilm colony and diffuse from the colony into the aqueous phase are transported downstream (i.e., locally removed), which delays local upregulation. (ii) Downstream colonies receive AHL molecules from upstream. If the AHL concentration in the aqueous phase is higher than in the biofilm colonies, AHL will diffuse into the colony and can promote up-regulation, even when cell densities are relatively low. (iii) AHL is washed out from the channel by the flow field; these washed out AHL molecules have no effect on quorum sensing. Which of these three effects dominates, and how they balance each other, cannot be readily predicted, and must be investigated.

There are currently numerous mathematical models which can be found in the literature describing quorum sensing in both suspended bacteria and in biofilms, and for various applications. Ward et al. (2001), Dockery and Keener (2001), and Müller et al. (2006), proposed models of quorum sensing in a well-mixed population of bacteria cells, describing bacteria growth and AHL production in a manner
representative of a batch culture experiment. This model predicted a rapid switch in proportions of down- and up-regulated sub-populations, which is a characteristic feature of quorum sensing systems. Ward et al. (2003) and Chopp et al. (2002, 2003) studied models of quorum sensing activity in a growing one-dimensional biofilm, in which biofilm thickness varies in time, to identify the key biofilm and quorum sensing kinetics parameters responsible for induction. Other models detail the biochemistry of quorum sensing systems (Anguige et al., 2004, 2005, 2006). As fluid flow in the liquid phase of the biofilm has the potential to greatly impact both biofilm growth and quorum sensing (Purevdorj and Costerton, 2002), more recent models have incorporated hydrodynamic effects (Janakiraman et al., 2009, Vaughan et al., 2009).

Janakiraman et al. (2009) studied biofilm growth and quorum sensing in microfluidic chambers experimentally and with a one-dimensional mathematical biofilm thickness model, using very low flow rates (Reynolds number Re<< 1). They found that the flow rate greatly affects quorum sensing in the biofilm: at high flow rates, the biofilm thickness is smaller due to detachment, and the transport rate of AHL out of the biofilm channel can be so high that the AHL concentration does not reach the threshold required for induction. Purevdorj and Costerton (2002) remark on the importance of diffusive processes, in particular, that the washout of signal molecules from the bulk fluid surrounding microcolonies would increase the concentration gradient between the biofilm and the bulk liquid, driving the diffusive flux of signal molecules out of the biofilm. Their work indicates that diffusion through the biofilm in different hydrodynamic conditions is key to the AHL signal concentration in microcolonies. Wanner et al. (2006) also demonstrated that hydrodynamics are required
to accurately describe mass conversion and substrate degradation in biofilm systems, so including a flow field component will in turn lead to an accurate description of quorum sensing in our developing biofilm. Flow may have the potential to disturb quorum sensing, interrupting bacterial behaviour. This is relevant to the specific application of biofilm manipulation via quorum sensing control, which has been studied in the medical context (Anguige et al., 2004, Ward et al., 2001). With this evidence of the key roles a hydrodynamic environment plays, we will expand on the works of Janakiraman et al. (2009) and previous models by thoroughly investigating the contribution of flow to mass transfer and inter-colony communication in the simulation environment.

We will apply our model to a two-dimensional patchy biofilm population. There are limitations to one-dimensional or two-dimensional flat (slab-like) biofilm models. One-dimensional biofilm thickness models, predicting thickness or height in time, do not have a spatial structure. A flat, two-dimensional biofilm (e.g., Kirisits et al., 2007) may be described as a collection of cells in a rectangular grouping. The biofilm may grow thicker and horizontally expand in time, but cells are homogeneously distributed in this setup, and so microcolony behaviour cannot be monitored. In our “patchy” biofilm community, multiple microcolonies, or clusters of cells, are separately placed throughout a simulated environment. A limitation of one-dimensional or two-dimensional flat biofilm models is that induction will not be spatially resolved; once AHL reaches the threshold concentration for quorum sensing, the entire biofilm simultaneously becomes induced. In contrast, cells arranged in microcolonies do not react homogeneously, and will not necessarily undergo induction at the same time.
Furthermore, induction can occur for a lower number of cells in a patchy biofilm than in a homogeneous biofilm, because the cell density (and consequently, AHL concentrations) is higher in the small cell clusters (Müller et al., 2006). By using a two-dimensional patchy biofilm pattern, we are able to analyze multiple colonies, as well as the spatial variations in the sub-populations within the biofilm. In addition, the dissolved substrates will be spatially modelled, specifically, the depletion of carbon and accumulation of AHL. We are particularly interested in low flow velocities, which will enable us to focus on the contribution of flow to mass transfer reactions in our system, as opposed to Janakiraman et al. (2009), in which the system behaviour was largely controlled by detachment processes, and Chopp et al. (2009), in which the system is convection dominated.

To resolve the aforementioned limitations of existing models, and to enable ourselves to research in detail mass transfer and communication, we begin with a biofilm growth model based on the non-linear density dependent diffusion-reaction equation originally proposed in Eberl et al. (2001) for a single species biofilm with one limiting nutrient. In this paper, we include equations for the down- and up-regulated bacterial sub-populations, and consider convective transport for carbon and AHL, the dissolved substrates. Also, in contrast to the models which begin with a fully developed biofilm (e.g., Vaughan et al., 2009), we have a mechanistic biofilm growth model which starts with very few small bacteria colonies initially adhered to a substratum.

To the biofilm growth model of Eberl et al. (2001), we add the processes of a quorum sensing system in a molecularly founded way. In our quorum sensing model,
we take into account the dimerisation process for AHL, which appears for almost all investigated quorum sensing systems (Whitehead et al., 2001, Fuqua et al., 2002). When AHL binds to its receptor, this complex dimerizes, and becomes effective as a transcription factor. We model this non-linear AHL dose-response relationship in combination with the positive feedback loop of induction, describing accurately the switch-like behaviour in the upregulation of bacteria. The bacteria *Pseudomonas aeruginosa* is typically selected for biofilm and quorum sensing studies. Though this bacterium is highly relevant for quorum sensing, it has three quorum sensing systems, and so difficulties may arise in the definition of when quorum sensing is “on” or “off” in the bacteria cells. *Pseudomonas putida* is used in our study; with only one known quorum sensing system, this bacterium is practical for studying basic quorum sensing behaviour. The quantitative quorum sensing parameter values used in our numerical simulations were derived from laboratory experiments on planktonic *P.putida* cultures.

We exclude dead cells, additional microbial species, attachment/detachment, and high flow velocities in our model. Though these aspects may be present in a full biofilm and quorum sensing system, we choose to specifically model quorum sensing induction patterns in patchy biofilms in a slow-flow hydrodynamic environment. This is a creeping flow regime with Reynolds numbers smaller than one, but not so small that flow does not contribute to mass transfer. The slow flow regime was found to have significant effects in the Janakiraman et al. (2009) system. In fact, in biofilm systems, the dimensionless Peclet number which relates convective and diffusive contributions, is orders of magnitudes larger than the Reynolds number (Eberl et al., 2000, Eberl and
Sudarsan, 2008), indicating that mass transfer can be substantial even in slow flow regimes. Moreover, at the low flow velocities considered here, mechanical effects such as detachment of the biofilm matrix need not be considered - flow induced shear forces will have a negligible effect on the deformation of the biofilm (Eberl and Sudarsan, 2008). We may then focus on the contribution of flow to mass transfer reactions in our system. Secondly, we focus on narrow conduits, mimicking pore spaces in soils and fractured media. The numerical solution of the flow equations is the most computationally expensive part in biofilm modeling. However, with these assumptions that we have made, we are able to use a quick, analytical approximation to the hydrodynamic flow model, which was previously introduced in Eberl and Sudarsan (2008).

We have developed the first biofilm growth and quorum sensing mathematical model for two-dimensional patchy biofilm colonies with nutrient-dependent biomass growth and the consideration of fluid flow. Our two-dimensional flow model allows for the study of how AHL produced in one colony can have a non-local effect on neighbouring colonies, which is a consequence of the fact that AHL produced by the bacteria is transported by convection and diffusion in the aqueous phase. This is, in fact, the primary difference between the model presented here and previous models of quorum sensing in biofilms. As biofilms reside in aqueous habitats, the effects of the surrounding hydrodynamic environment play a critical role in growth and other processes. We will use our model to investigate the effects of environmental conditions such as bulk flow velocities and nutrient availability on the onset of quorum sensing.
2.1.4 Outline of Paper

In the following sections, we will derive our mathematical model, and describe the two different flow mechanisms we will use: one in which the volumetric flow rate is constant throughout the domain, and in the other, the flow varies as the channel fills with biomass. A series of simulations will be presented, in which we have conducted numerical experiments to compare the spatiotemporal effects of a growing biofilm on quorum sensing, under various environmental conditions, namely flow effects and nutrient availability.

2.2 Mathematical Model

2.2.1 Biofilm growth model

We formulate a mathematical model for biofilm development in terms of the dependent variable $m$, representing the local biomass density. As in Dockery and Klapper (2001) and Wood and Whitaker (1999), we use the mass balance for biomass as the starting point for the development of our biofilm model:

$$m_t + \nabla (Um) = G(C, m), \quad (2.1)$$

in which the function $G(C, m)$ is the net biomass production rate, which depends on the concentration of a growth limiting substrate $C$. The unknown vector-valued function $U$ is the velocity with which the biomass moves in the biofilm colony, caused by growth and spatial expansion of the biofilm. Equation (2.1) represents one equa-
tion for the unknown functions $m, U, C$. The growth limiting substrate $C$ will be described by a transport-reaction equation below. In order to obtain $U$, we consider the momentum equation for biomass,

$$(Um)_t + \nabla(UUm) = -\nabla P - f(m)mU,$$  \hspace{1cm} (2.2)

in which the new variable $P$ is the unknown biomass pressure. The last term in this equation describes forces moderating biomass movement, such as kinematic friction; see Kowlaczyk et al. (2004) and Wrzosek (2008), in which $f(m) = f = constant$ is suggested. In order to close this model, an additional relationship must be established. In Dockery and Klapper (2001), closure is achieved by introducing the assumption that in the biofilm, the biomass density is always at the maximum cell density, $m = M_{max} = const$, that is, the biofilm is assumed to be incompressible. Alternatively, Wood and Whitaker (1999) suggested to close the model algebraically by imposing a constitutive relationship $P = P(m)$, which recovers the traditional Wanner-Gujer (1986) biofilm model in the one-dimensional setting. However, the higher dimensional case remained open. We follow the Wood and Whitaker (1999) approach. As in Dockery and Klapper (2001), we neglect the inertial forces, and the momentum equation (2.2) reduces to the Darcy-like equation,

$$-\frac{1}{mf}\nabla P = -\frac{1}{mf}P'(m)\nabla m = U.$$
Substituting this into (2.1) gives for the biomass density the quasilinear diffusion-reaction equation,

\[
m_t = \nabla (D(m) \nabla m) + G(C, m),
\]

with the density-dependent diffusion coefficient,

\[
D(m) := \frac{1}{f} P'(m).
\]

\(D(m)\), or alternatively \(P(m)\) must be chosen such that (i) notable spatial expansion of the biofilm only takes place for large enough \(m\), and (ii) the biomass density \(m\) does not exceed the maximum possible value \(M_{\text{max}}\). One choice that is known to satisfy these conditions (Efendiev et al., 2009) is:

\[
D(m) = \delta \frac{m^a}{(M_{\text{max}} - m)^b}, \quad a, b > 1, \quad \delta > 0,
\]

which can be derived from power law assumptions \(P \sim m^\eta\) for \(m \approx 0\) and \(P \sim (M_{\text{max}} - m)^{-\lambda}\) for \(m \approx M_{\text{max}}\).

The growth controlling substrate \(C\) in (2.1) is governed by the transport-reaction equation:

\[
C_t + \nabla (UC) = \nabla (d \nabla c) - F(C, m).
\]

Commonly, Monod kinetics are assumed to describe growth of biomass:

\[
F(C, m) = \frac{\mu C m}{Y k_2 + C}, \quad G(C, m) = \frac{\mu C m}{\kappa_2 + C} - \kappa_4 m.
\]
Here, $\mu$ is the maximum growth rate of the species, $Y$ is the yield coefficient, $\kappa_2$ is the Monod half saturation concentration, and $\kappa_4$ is the cell death rate. The characteristic time-scale for spatial expansion of the biofilm is much larger than the characteristic time-scale for diffusion and consumption of substrate in the biofilm. Therefore, following Wanner et al. (2006), it is common practice to neglect the convective term.

For convenience, in the following section, and for the remainder of the paper, we will use the volume fraction occupied by biomass rather than the biomass density, that is, we use $M := m/M_{max}$ as a dependent variable. Then, summarizing the above, the biofilm growth model is:

\begin{align}
\partial_t M &= \nabla(D_M(M)\nabla M) + \frac{\kappa_3 CM}{\kappa_2 + C} - \kappa_4 M \quad (2.3) \\
\partial_t C &= \nabla(d\nabla C) - \frac{\kappa_1 CM}{\kappa_2 + C} \quad (2.4)
\end{align}

in which $\kappa_1 := \mu M_{max}/Y$, $\kappa_3 = \mu$, and the density dependent diffusion coefficient for biomass is formulated as $D_M(M) = \delta M^{a-b}_0 M^{a}(1 - M)^{-b}$. The spatial domain $\Omega$ in which our model is formulated is accordingly subdivided into two not necessarily connected subdomains, the aqueous phase $\Omega_1(t) := \{(x, y) \in \Omega : m(t, x, y) = 0\}$ and the solid biofilm phase $\Omega_2(t) := \{(x, y) : m(t, x, y) > 0\}$. These regions change as the biofilm grows.

The model (2.3), (2.4), which we derived here from the same starting point as the biofilm model of Dockery and Klapper (2001), is the density-dependent diffusion-reaction model for biofilm formation that was originally introduced in an $ad$ hoc
fashion in Eberl et al. (2001). More recently, this model was also derived from a semi-discrete master equation in Khassehkhan et al. (2009b), beginning with assumptions resembling those that are used in cellular automata models of biofilms, such as Picioreanu et al. (1998).

The diffusion-reaction biofilm model (2.3), (2.4) has been studied in a small series of papers, for analytical, numerical, and biological interest. In particular, an existence and uniqueness proof was given in Efendiev et al. (2009), in which it was also shown that the solution remains bounded by 1 (the maximum biomass density is not exceeded). Moreover, it was shown that there exists a $\eta > 0$, $\eta << 1$, such that the solution $M$ remains below $1 - \eta$, provided $M = 0$ somewhere on the boundary, and so $M$ will be separated from the singularity at $M = 1$. The model possesses a global attractor that was studied in Demaret and Efendiev (2008). In Eberl et al. (2001) and Eberl and Demaret (2007), it was shown in numerical simulations that this model is able to describe the formation of cluster-and-channel biofilms and the so-called “mushroom” biofilm architectures which can be observed under severe substrate limitations.

### 2.2.2 Quorum sensing model

We present a mathematical model for quorum sensing in a growing biofilm community in a narrow conduit which consists of several colonies. This model combines the diffusion-reaction biofilm model derived in Section 2.2.1 with the quorum sensing model for suspended populations of Müller et al. (2006). The dependent variables of our model are the down-regulated bacteria ($M_0$), up-regulated (quorum
sensing induced) bacteria ($M_1$), AHL ($AHL$), and growth limiting nutrient, the dissolved carbon substrate ($C$). Bacteria $M_0$ and $M_1$ are expressed as the fraction of the volume they locally occupy at each point in the domain (volume fraction). $AHL$ and $C$ are expressed as concentrations. The processes incorporated into our model are:

- production of new up- and down-regulated biomass, controlled by nutrient availability
- nutrient consumption by down- and up-regulated biomass
- spatial spreading of the biofilm for high local biomass volume fractions (i.e., for $M = M_0 + M_1 \approx 1$)
- conversion of down-regulated biomass into up-regulated biomass as a consequence of AHL concentration inducing a change in gene expression, and a constant rate of back-conversion
- natural cell death
- abiotic AHL decay
- differential AHL production rates of down-regulated and up-regulated cells
- diffusive transport of carbon substrate and AHL in the biofilm, and both convective and diffusive transport in the surrounding aqueous phase of the biofilm
The mathematical model is:

\[
\begin{align*}
\partial_t M_0 &= \nabla (D_M(M) \nabla M_0) + \frac{\kappa_3 C M_0}{\kappa_2 + C} - \kappa_4 M_0 - \kappa_5 \text{AHL}^n M_0 + \kappa_5 \tau^n M_1 \\
\partial_t M_1 &= \nabla (D_M(M) \nabla M_1) + \frac{\kappa_3 C M_1}{\kappa_2 + C} - \kappa_4 M_1 + \kappa_5 \text{AHL}^n M_0 - \kappa_5 \tau^n M_1 \\
\partial_t C &= \nabla (D_C(M) \nabla C) - \nabla (w C) - \frac{\kappa_1 C (M_0 + M_1)}{\kappa_2 + C} \\
\partial_t \text{AHL} &= \nabla (D_{\text{AHL}}(M) \nabla \text{AHL}) - \nabla (w \text{AHL}) - \sigma \text{AHL} + \alpha M_0 + (\alpha + \beta) M_1
\end{align*}
\]

The total volume fraction occupied by the biofilm is \( M = M_0 + M_1 \). The diffusion coefficient for biomass \( (D_M(M)) \) is described in Section 2.2.1. The diffusion coefficients for C and AHL are lower in the biofilm than in the surrounding aqueous phase (Stewart, 2003). We use \( D_C(M) = D_C(0) + M(D_C(1) - D_C(0)) \), \( D_{\text{AHL}}(M) = D_{\text{AHL}}(0) + M(D_{\text{AHL}}(1) - D_{\text{AHL}}(0)) \), where \( D_C(0) \) and \( D_{\text{AHL}}(0) \) are the diffusion coefficients in water, and \( D_C(1) \) and \( D_{\text{AHL}}(1) \) are the diffusion coefficients in a fully compressed biofilm.

The convective contribution to transport of \( C \) and \( \text{AHL} \) in the aqueous phase is controlled by the flow velocity vector \( w = (u, v) \), where \( u \) and \( v \) are the flow velocities in the \( x \)- and \( y \)- directions. The calculation of \( w \) is further described in Section 2.2.3.

In the reaction terms, \( \kappa_3 \) is the maximum specific growth rate of the bacterial biomass. The maximum specific substrate consumption rate is denoted by \( \kappa_1 = \kappa_3 M_{max}/Y \). Bacterial growth is described by Monod kinetics, where \( \kappa_2 \) is the half-saturation constant. \( \kappa_4 \) is the cell lysis rate. The parameter \( \kappa_5 \) is the quorum sensing
regulation rate — the rate at which down-regulated bacteria become up-regulated, and vice versa. The AHL production rate of down-regulated bacteria is $\alpha$, and the increased production rate of up-regulated bacteria is $\alpha + \beta$. AHL degrades abiotically at rate $\sigma$. $\tau$ is the threshold AHL concentration locally required for quorum sensing induction to occur. We use $n$ ($n > 1$) to describe the degree of polymerisation in the synthesis of AHL, equivalent to the Hill coefficient describing cooperative binding reactions in the Hill equation, acting as a switch function (Müller et al., 2006).

We non-dimensionalise our model using the following rescalings: $\tilde{x} = \frac{x}{L}, \tilde{t} = \frac{t}{\kappa_3}$, where $L$ is the flow channel length, and $\frac{1}{\kappa_3}$ is the characteristic time scale. The new dimensionless concentration variables are: $\tilde{C} = \frac{C}{C_{\text{bulk}}}, \tilde{A} = \frac{AHL}{\tau}$, where $C_{\text{bulk}}$ is the bulk substrate concentration (the amount of substrate $C$ supplied at the inflow boundary). The new reaction parameters are:

$$
\tilde{\kappa}_1 = \frac{M_{\text{max}}}{Y C_{\text{bulk}}}, \tilde{\kappa}_2 = \frac{\kappa_2}{C_{\text{bulk}}}, \tilde{\kappa}_3 = 1, \tilde{\kappa}_4 = \frac{\kappa_4}{\kappa_3}, \tilde{\kappa}_5 = \frac{\kappa_5 \tau^n}{\kappa_3}; \\
\tilde{\sigma} = \frac{\sigma}{\kappa_3}; \tilde{\alpha} = \frac{\alpha M_{\text{max}}}{\kappa_3 \tau}; \tilde{\beta} = \frac{\beta M_{\text{max}}}{\kappa_3 \tau};
$$

Finally, the diffusion coefficients become:

$$
\tilde{D}_C = \frac{D_C}{L^2 \kappa_3}; \tilde{D}_{AHL} = \frac{D_{AHL}}{L^2 \kappa_3}; \tilde{D}_M = \frac{D_M}{L^2 \kappa_3};
$$
and we obtain the non-dimensional system:

\[
\begin{align*}
\partial_t M_0 &= \tilde{\nabla}(\tilde{D}_M(M)\tilde{\nabla}M_0) + \frac{\tilde{\kappa}_3 \tilde{C} M_0}{\tilde{\kappa}_2 + \tilde{C}} - \tilde{\kappa}_4 M_0 - \tilde{\kappa}_5 A^n M_0 + \tilde{\kappa}_5 M_1 \\
\partial_t M_1 &= \tilde{\nabla}(\tilde{D}_M(M)\tilde{\nabla}M_1)) + \frac{\tilde{\kappa}_3 \tilde{C} M_1}{\tilde{\kappa}_2 + \tilde{C}} - \tilde{\kappa}_4 M_1 + \tilde{\kappa}_5 A^n M_0 - \tilde{\kappa}_5 M_1 \\
\partial_t \tilde{C} &= \tilde{\nabla}(\tilde{D}_C(M)\tilde{\nabla}\tilde{C})) - \tilde{\nabla}(w\tilde{C}) - \frac{\tilde{\kappa}_1 \tilde{C} M}{\tilde{\kappa}_2 + \tilde{C}} \\
\partial_t \tilde{A} &= \tilde{\nabla}(\tilde{D}_A(M)\tilde{\nabla}\tilde{A})) - \tilde{\nabla}(w\tilde{A}) - \tilde{\sigma} \tilde{A} + \tilde{\alpha} M_0 + (\tilde{\alpha} + \tilde{\beta}) M_1
\end{align*}
\]

Existence proofs for the solutions of similar biofilm models with multiple volume occupying substances have been presented in Demaret et al. (2008) and Khassekhan et al. (2009) for a purely diffusive setup, i.e., in the absence of convective transport of dissolved substrates in the aqueous phase. The same concept of the proof, which does not allow a statement on uniqueness of the model solution, may be applied to the quorum sensing and biofilm growth model introduced here. In contrast to the models studied in Demaret et al. (2008) and Khassekhan et al. (2009), we remark that here, by adding the equations for \( M_0 \) and \( M_1 \), the biofilm growth model (2.3), (2.4), can be recovered. With the arguments detailed in Efendiev et al. (2009), we know that \( M = M_0 + M_1 \) and \( C \) are unique and separated from the singularity \( M = 1 \). Therefore, the question of uniqueness of the solutions of the quorum sensing model reduces to the question of uniqueness of the semi-linear system:

\[
\begin{align*}
\partial_t M_1 &= \tilde{\nabla}(\tilde{D}_M(M)\tilde{\nabla}M_1) + \frac{\tilde{\kappa}_3 \tilde{C} M_1}{\tilde{\kappa}_2 + \tilde{C}} - (\tilde{\kappa}_4 + \tilde{\kappa}_5 - \tilde{\kappa}_5 A^n) M_1 + \tilde{\kappa}_5 A^n M \\
\partial_t \tilde{A} &= \tilde{\nabla}(\tilde{D}_A(M)\tilde{\nabla}\tilde{A})) - \tilde{\sigma} \tilde{A} + \tilde{\alpha} M + \tilde{\beta} M_1
\end{align*}
\]

in which we can treat $C$ and $M$ as known functions. Problems of this type have been extensively studied in the literature (e.g., Ladyzenskaja et al., 1988).

The parameters used in our simulations and their non-dimensional values are listed in Table 2.1. The biofilm parameters $\kappa_1-\kappa_4$ were chosen from the range of standard values in biofilm modelling literature (Wanner et al., 2006), the diffusion coefficient values $(d_M, a, b)$ were selected from Eberl and Sudarsan (2008), and the quorum sensing parameters $\kappa_5, \alpha, \beta, \tau, \gamma$ and $n$ were derived from experiments on the kinetics of suspended $P.\ putida$ IsoF cultures and the AHL molecule 3-oxo-C10-HSL.

Table 2.1: Constant model parameters used in our simulations and their non-dimensional values. Values for the parameters varied in our simulation experiments are given in the text. References: [46]= Wanner et al. (2006), [18]= Eberl and Sudarsan (2008), [24]=Hobbie and Roth (2007), $M=$measured experimentally, $S=$selected for our system.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Non-dimensional Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\kappa_3$</td>
<td>Maximum specific growth rate</td>
<td>1</td>
<td>[46]</td>
</tr>
<tr>
<td>$\kappa_4$</td>
<td>Lysis rate</td>
<td>0.067</td>
<td>[46]</td>
</tr>
<tr>
<td>$\kappa_5$</td>
<td>Quorum sensing up-regulation rate</td>
<td>2.5</td>
<td>M</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Abiotic degradation rate of AHL</td>
<td>0.022</td>
<td>M</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Constitutive production rate of AHL</td>
<td>13</td>
<td>M</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Induced production rate of AHL</td>
<td>130</td>
<td>M</td>
</tr>
<tr>
<td>$D_C(0), D_C(1)$</td>
<td>Diffusion coefficients for substrate</td>
<td>0.67, 0.54</td>
<td>[18]</td>
</tr>
<tr>
<td>$D_{AHL}(0), D_{AHL}(1)$</td>
<td>Diffusion coefficients for AHL</td>
<td>0.52, 0.26</td>
<td>[24]</td>
</tr>
<tr>
<td>$a$</td>
<td>Diffusion coefficient parameter</td>
<td>4.0</td>
<td>[18]</td>
</tr>
<tr>
<td>$b$</td>
<td>Diffusion coefficient parameter</td>
<td>4.0</td>
<td>[18]</td>
</tr>
<tr>
<td>$d_M$</td>
<td>Biomass motility coefficient</td>
<td>6.67e-09</td>
<td>[18]</td>
</tr>
<tr>
<td>$H/L$</td>
<td>Channel aspect ratio</td>
<td>0.1</td>
<td>S</td>
</tr>
</tbody>
</table>

The following boundary conditions are imposed on our domain $\Omega=[0,L] \times [0,H]$:

- For $M_0$ and $M_1$, no flux conditions everywhere ($n$ is the direction of the outward
normal):
\[ \partial_n M_0 = 0, \partial_n M_1 = 0 \text{ on } \partial \Omega \]

- For \( C \) and \( AHL \), no diffusive flux conditions everywhere except for on inflow, where we specify the bulk concentration:
\[ C = 1, \tilde{A} = 0 \text{ for } x = 0, \partial_n C = 0, \partial_n \tilde{A} = 0 \text{ everywhere else.} \]

Thus, no biomass leaves or enters the flow channel, and no AHL enters the flow channel from the upstream boundary. However, AHL and carbon in the dissolved liquid phase \( \Omega_1 \) may exit the system via convective transport. Initially, the substratum is inoculated by down-regulated cells; we prescribe the number of such inoculation sites, but their location and the biomass density is chosen randomly. So while our model is completely deterministic, the inoculation adds a stochastic element. This mimics the difficulty in controlling cell attachment sites in flow channel laboratory experiments.

### 2.2.3 Hydrodynamic component

Hydrodynamics have been incorporated via two alternative mechanisms for flow in the channel:

1. Regular: the volumetric flow rate in the channel remains constant throughout the simulation. Hydrodynamic conditions are specified in terms of the dimensionless Reynolds number for the empty flow channel

2. Bioclogging: the flow rate in the channel decreases due to the increased flow resistance from the growing biofilm
The flow in the aqueous phase is described by the incompressible Navier-Stokes equations. As an alternative to solving the complete flow equations numerically, we use the thin-film approximation as proposed in Eberl and Sudarsan (2008):

\[
0 = -\frac{1}{\rho} \frac{\partial p}{\partial x} + \nu \frac{\partial^2 u}{\partial y^2}, \quad 0 = \frac{\partial p}{\partial y}, \quad 0 = \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y}
\]

where \( p \) is the hydrodynamic pressure field; constants \( \rho \) and \( \nu \) are the density and kinematic viscosity of water. This approximation is valid in the liquid region of long, narrow conduits for slow flow regimes. Unlike the complete Navier-Stokes or the Stokes equations, this flow model can be solved analytically, which allows for fast computation. In order to drive the flow in the channel, we specify the volumetric flow rate in terms of the non-dimensional Reynolds number \( Re \) for the empty flow channel, given by:

\[
Re = \frac{HU}{\nu}
\]

where our characteristic height scale \( H \) is the channel height, and \( U \) is the maximum flow velocity of the unperturbed Poisseuille flow. According to Eberl and Sudarsan (2008), we have then the flow rate \( q \):

\[
q = \frac{2}{3} \nu Re,
\]

where \( \nu \) is the kinematic viscosity of the bulk liquid.
For the regular flow mechanism, we assume the flow rate to be constant for all time. An additional complexity was added to our regular flow model to account for the phenomenon of “bioclogging”, which refers to the change in porosity and hydraulic conductivity of a saturated porous medium due to microbial growth (Thullner et al., 2004). This is of particular importance for the study of biofilm processes in microbial soil ecology. Thullner et al. (2002a) demonstrated that a relatively small amount of biofilm can cause significant clogging effects. A growing biofilm changes the hydraulic conductivity of the porous medium, and so according to Darcy’s Law, the flow rate will change as well. The Darcy equations, used to relate flow velocities in porous media, express how the flow velocity $q$ is proportional to the pressure gradient $\nabla P$, where hydraulic conductivity is the proportionality factor $k$:

$$q = \frac{k}{\nu \rho} \nabla P$$

where $\nu, \rho$ are the kinematic viscosity and density of the bulk liquid respectively. The hydraulic conductivity $k$ varies in time with biofilm growth, as described above. A smaller hydraulic conductivity implies lower flow velocities.

Several empirical relations have been suggested to describe how hydraulic conductivity changes with porosity as the total amount of biomass increases (Thullner et al., 2004). Many of these relations require additional empirical model parameters, which we choose not to introduce. The Clement et al. (1996) model has been chosen here for simplicity; this model gives hydraulic conductivity $k$ as a function of porosity
\( h: \)

\[ k(h) = h^{19/6} \]

Note that \( 19/6 \approx 3; \) \( k(h) \) is approximately a cubic polynomial, similar to all other \( k - h \) relationships in Thullner et al. (2004).

For our simulations, we conceive our flow channel as a narrow conduit, such as a space between soil particles. Our pore space \( h \) is defined to be the smallest gap between the top of an individual biofilm colony and the top of the flow channel, scaled to our channel height. This minimum pore space \( h \) will change spatially and temporally as biomass grows and fills the channel.

We then scale the computed regular flow field velocities by the clogging function \( k(h) \). For the bioclogging conditions, our modified formula for the flow rate in terms of the Reynold’s number is,

\[ q = k(h)^{2/3} \nu Re. \]

When biomass is not present, \( h = 1.0 \) and \( k(h) = 1.0 \), so the flow velocities remain precisely the same as the regular flow mechanism. In time, total biomass increases, and \( k \to 0 \) as \( h \to 0 \), and the flow velocities will decrease.

Both the regular and bioclogging flow mechanisms for hydrodynamics will be used and compared in the subsequent simulations to investigate the effects of the flow field on our biofilm and quorum sensing system.
2.2.4 Computational aspects

The numerical solution of the biofilm model follows the approach that was developed and discussed in Eberl and Demaret (2007) and Eberl et al. (2009). The computational domain was discretised on a uniform rectangular grid of size 2000 x 200. We solve our system numerically using a semi-implicit finite difference-based finite volume scheme, formulated for the concentrations in the centers of the grid cells. The time-step size is variable; in each time step four sparse, banded diagonal linear algebraic systems are solved with the stabilized biconjugate gradient method (one system for each of $AHL$, $C$, $M_0$, and $M_1$).

The code was prepared for parallel execution on multi-core/multi-processor platforms as described in Muhammad and Eberl (2009). Simulations were carried out on a SGI ALTIX450 with 32 dual core itanium processors; in most simulations 12 cores were used per compute job.

2.3 Results

2.3.1 Simulation Experiment Design

In simulation experiments, we measure the effects of environmental conditions (nutrient concentrations and channel hydrodynamics) on the onset of quorum sensing induction in the biofilm system, and how production of autoinducer molecules in one colony affects induction in neighbouring colonies. One measurement of these effects is the “switching time”, which we define as the time when the AHL concentration first reaches the threshold value for induction somewhere in the domain. We
also investigate the spatial distribution of up- and down-regulated cells in the flow channel, and biomass growth under these various environmental conditions.

A typical computer simulation of biofilm growth in a long, narrow channel with aspect ratio $\epsilon = H/L = 0.1$ and slow flow ($\text{Re}=10^{-3}$) is shown in Figure 2.1. The bottom surface, or substratum, is initially inoculated with twenty randomly chosen small colonies of down-regulated cells with density $0.2 < M_0 < 0.4$. The growth period begins with biomass in the inoculated colonies growing, but not expanding. As the total biomass ($M_0 + M_1$) locally approaches 1.0, the maximum cell density, the colonies expand. The individual colonies initially grow at the same rate, but in later timesteps the downstream colonies will experience slower growth due to substrate depletion by the upstream colonies, which are first to receive and utilize the incoming nutrients. In time, some neighbouring colonies begin to merge. The shading of the biofilm colonies in Figure 2.1 represents the fraction of down-regulated cells in the biofilm ($\frac{M_0}{M_0 + M_1}$), where red colonies are completely down-regulated, and blue colonies are fully up-regulated.

Isolines of AHL concentration are equidistantly distributed between 0 and the maximum AHL value (reported in each subfigure); the maximum AHL concentration changes in time as it accumulates in the system. Initially (Figure 2.1 (a),(b)), AHL concentrations increase in the main flow direction. The colonies produce AHL molecules, which diffuse into the liquid region, and are then transported downstream by convection and diffusion. In later time steps (Figure 2.1 (c),(d)), the maximum AHL concentration is found in the middle of the channel. This is due to both the accumulation of AHL that has been transported via convection from the upstream
Figure 2.1: Development of a biofilm in a flow channel using the bioclogging flow mechanism. Flow is from left to right; food is supplied from the upstream (left) boundary. AHL molecules produced by large, upstream colonies are transported through the channel and contribute to up-regulation downstream, facilitating inter-colony communication. Biofilm colonies are coloured to indicate the fraction of the cells that are downregulated ($\frac{M_0}{M_0 + M_1}$); from completely down-regulated (red) to fully up-regulated (blue). AHL is coloured by isolines from zero (black) to maximum AHL (white). In (a) and (b), the biofilm grows, but it is still down-regulated. Some downstream and mid-channel colonies become induced in (c), and shortly afterwards, the entire biofilm is up-regulated (d).
region, and the increased AHL produced by up-regulated colonies in the mid-channel.

In regards to the biofilm composition, we observe that the down-regulated biofilm grows in time (Figure 2.1 (a),(b)). The smaller, downstream colonies begin to upregulate, or “switch” before the larger upstream colonies in Figure 2.1 (c). The switch occurs rapidly, and has passed in Figure 2.1 (d), where upregulation is present in nearly the entire biofilm. The observation that the smaller, downstream colonies are first to undergo the switch to up-regulation demonstrates the effects of mass transfer. AHL molecules produced by the large, upstream colonies are transported downstream, and once there, contribute to upregulation. Thus, inter-colony communication has been facilitated by convective transport. In the convection dominated system of Vaughan et al. (2009), we also observe important inter-colony interactions resulting from convective processes.

The concentration of the bulk substrate, the carbon nutrient $C$, decreases in the flow direction (left to right) due to consumption by biomass (carbon concentration isolines are not shown). As the biofilm grows, the demand for carbon increases. The minimum carbon concentration in the domain is tracked to determine when and where carbon limitations occur. The minimum carbon concentration values are found in the proximity of the furthest downstream colonies, as these colonies have to consume only what remains after consumption by the upstream colonies closest to the nutrient source at the inflow boundary. After $t=5.0$, the minimum concentration is 90.2% of the bulk concentration on inflow. Figure 2.2(a) displays the rapid drop in minimum $C$ concentration; after $t=7.3$ the minimum $C$ concentration has declined to 10.2%. However, $C$ is not limited overall (for example, upon inflow), so the biofilm may
continue to grow locally at almost the maximal rate.

To analyze effects averaged across the computational domain, we will use the parameter $\omega$ to represent the fraction of the domain occupied by the biofilm (occupancy):

$$\omega(t) := \frac{1}{LH} \int_{\Omega_2(t)} dxdy$$

which is a simple measure of biofilm size. The total down-regulated and up-regulated biomass in the system are the volume fractions $M_{0\text{total}}$ and $M_{1\text{total}}$:

$$M_{0\text{total}}(t) := \int_{\Omega} M_0(t, x, y) dxdy,$$

$$M_{1\text{total}}(t) := \int_{\Omega} M_1(t, x, y) dxdy$$

$M_{0\text{total}}$ and $M_{1\text{total}}$ are measures of biofilm composition. Finally, the total AHL in the system is given by:

$$AHL_{\text{total}}(t) := \int_{\Omega} AHL(t, x, y) dxdy.$$

The occupancy plot in Figure 2.2(b) for our simulation in Figure 2.1 is flat in the beginning timesteps ($0 < t < 1$), representing the initial phase of bacteria cell population growth, prior to biofilm expansion. After $t = 1$, bacteria population growth and biofilm expansion is maximal in time, until nutrient demand begins to exceed the inflow of bulk substrate. Growth continues, but at a lower rate. Figure 2.2(c)
Figure 2.2: For the typical simulation in Figure 2.1, (a) the minimum carbon substrate concentration in the channel, (b) the fraction of the domain occupied by biofilm (occupancy), (c) biofilm composition (the total $M_0,M_1$ biomass), (d) average AHL in the domain, and (e) average flow velocity for the bioclogging mechanism.
is a biofilm composition plot, showing the total down-regulated and up-regulated biomass in the system. We initially see only growth of down-regulated biomass, until the switch to upregulation occurs, after which the biofilm composition becomes dominated by up-regulated biomass. This switch corresponds to the rapid increase in average AHL concentration, shown in Figure 2.2(d). A non-dimensional AHL concentration of 1.0 is required for upregulation, after which AHL concentrations rapidly increase, due to positive feedback in the quorum sensing system.

The bioclogging flow mechanism was implemented in this example. The decrease in flow velocity in time due to biomass accumulation in the channel can be seen in Figure 2.2(e). This plot shows how the average flow velocity, which we have specified in terms of Re, is scaled in time by the bioclogging function $k(h)$ - by the end of the simulation, the flow velocity has decreased by several orders of magnitude. The effects of bioclogging and regular flow on biofilm growth and quorum sensing will be discussed in Sections 2.3.2 and 2.3.3.

Three simulation experiments were conducted to investigate the influence of environmental conditions and biofilm kinetics on autoinducer induction in the growing biofilm. In these three experiments, we varied (i) the flow velocity, specified in terms of the dimensionless Reynolds number (Re) (ii) nutrient availability $C_{\text{bulk}}$, and (iii) up-regulation rate $\kappa_5$.

**2.3.2 Influence of hydrodynamics**

The Reynolds number $Re$ was varied over several orders of magnitude, between $10^{-5} \leq Re \leq 10^{-1}$. The bulk substrate concentration on inflow was kept
constant throughout the experiment, at an abundant $C_{\text{bulk}}$ value. The corresponding effective Reynolds number $Re^*$ for thin-film flows is obtained as $Re^* = \epsilon Re$ (Eberl and Sudarsan, 2008), where $\epsilon$ is the channel aspect ratio $\epsilon = H/L = 0.1$. The effective Reynolds numbers for our simulation experiments are then: $10^{-6} \leq Re^* \leq 10^{-2}$. To estimate the relative contributions of convective and diffusive mass transport, we will calculate the dimensionless effective Peclet number:

$$Pe^* = \frac{\text{convection}}{\text{diffusion}} = Re^* Sc,$$

where $Sc$ is the Schmitt number ($Sc = \nu/D$), the ratio of the fluid momentum diffusivity and substrate mass diffusivity (Eberl and Sudarsan, 2008). For carbon and AHL in water, $\nu \approx 0.0864 \text{ m}^2\text{d}^{-1}$, $D_C \approx 10^{-4} \text{ m}^2\text{d}^{-1}$, and $D_{AHL} \approx 7.76^{-5} \text{ m}^2\text{d}^{-1}$, yielding $Sc \approx 10^3$ for both dissolved substrates, with $Sc$ slightly larger for AHL. The effective Peclet numbers for our simulations are then $Pe^* = 10.0, 0.1, \text{ and } 0.001$ for $Re^* = 10^{-2}, 10^{-4}, \text{ and } 10^{-6}$ respectively. So, our system is convection dominated for $Re=10^{-1}$, mildly diffusion dominated for $Re=10^{-3}$, and clearly diffusion dominated for $Re=10^{-5}$. Flow has two primary roles in our model: one is the contribution to biomass growth through nutrient delivery, and the second is the contribution to AHL mass transfer. These roles have a counteracting effect on quorum sensing induction: an increase in growth will lead to increased AHL production, implying earlier upregulation, though this is not guaranteed to occur when there is a simultaneous increase in AHL washout from the downstream boundary. Because of these counteracting roles, we do not know a priori the net effect flow will have on our system.
Figure 2.3: The fraction of the domain occupied by biofilm (occupancy) for the flow velocity experiments, using the (a) regular and (b) bioclogging flow mechanisms. Higher flow velocities result in greater occupancies and a longer period of maximum growth. Numerical values of the final occupancy for these regimes are given in Table 2.2.

The occupancy curves for the flow velocity experiments are plotted using a logarithmic scale in Figure 2.3, using the regular (2.3a) and bioclogging (2.3b) flow mechanisms. For an initial time period, the growth curves fall on the same line, indicating that the biofilm is growing at the maximum rate. When growth becomes limited, the curves will deviate from the line of maximum growth. We find that growth becomes limited earlier for the smaller Re, $Re=10^{-5}$ and $10^{-3}$, than for $Re=10^{-1}$, and that higher flow velocities result in greater occupancies. This trend is present for both flow mechanisms; the occupancy values for the regimes are given in Table 2.2. The differences in the total ($M_0 + M_1$) and upregulated ($M_1$) occupancies are listed in Table 2.2, for the various flow rates at $t = 9.7$, the approximate end of the simulation. Some simulation experiments were longer in duration than others due to the imposed stopping criteria, for example, attainment of the maximum specified biofilm height. Interestingly, despite the fact that the biofilms are smaller for the slower flows, the
proportion of up-regulated biomass is greater for \( \text{Re}=10^{-3} \) and \( 10^{-5} \) than for \( \text{Re}=10^{-1} \). When the flow velocity decreases, less AHL is removed via convective transport, allowing a greater proportion of bacteria to become up-regulated.

In each case, the regular flow mechanism allowed for greater biomass growth (measured by the total occupancy) than the bioclogging mechanism, although the difference between the final occupancies of the regular and bioclogging flow simulations decreases as \( \text{Re} \) decreases. Greater biomass growth with regular flow is due to the fact that the regular flow mechanism delivers nutrients at a fixed rate throughout the simulation experiment, whereas the delivery rate decreases in time with bioclogging flow. Figure 2.4 is a plot of the regular and bioclogging flow velocities in time. The differences between the two mechanisms emerge at approximately \( t = 5 \) in each simulation. We note that the drop in flow velocities for the bioclogging case occurs at a late stage of biofilm growth, in fact, past the period of maximal growth. The difference in velocity at the end of the simulation is approximately two orders of magnitude in each case. However, the differences in total occupancy are smaller for lower initial velocities - the biofilm was 20% larger with initial \( \text{Re}=10^{-1} \), 20% larger with initial \( \text{Re}=10^{-3} \), and 0.2% larger with \( \text{Re}=10^{-5} \).

To study the effects of flow on quorum sensing behaviour in our biofilm system, we measured the maximum AHL concentration in the channel in time for various flow velocities, using both flow mechanisms. The maximum AHL concentrations in time are plotted in Figure 2.5, in which we observe the rapid increase in concentrations corresponding to induction, representative of the positive feedback in a quorum sensing system. The AHL concentration values at \( t = 9.7 \) are given in Table
Table 2.2: A comparison of the total occupancy \((M_0 + M_1)\), proportion of up-regulated biomass \((M_1/(M_0 + M_1))\), and maximum AHL concentrations in the channel, for both flow mechanisms, at \(t = 9.7\). The regular flow mechanism allowed for greater biomass growth for all \(Re\).

<table>
<thead>
<tr>
<th>(Re)</th>
<th>Mechanism</th>
<th>Total occupancy</th>
<th>Proportion of upregulated biomass</th>
<th>Maximum AHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^{-1})</td>
<td>Regular</td>
<td>0.4737</td>
<td>0.001451</td>
<td>0.1519</td>
</tr>
<tr>
<td></td>
<td>Bioclogging</td>
<td>0.3950</td>
<td>0.1067</td>
<td>1.401</td>
</tr>
<tr>
<td>(10^{-3})</td>
<td>Regular</td>
<td>0.2493</td>
<td>0.1350</td>
<td>1.183</td>
</tr>
<tr>
<td></td>
<td>Bioclogging</td>
<td>0.2062</td>
<td>0.9686</td>
<td>6.264</td>
</tr>
<tr>
<td>(10^{-5})</td>
<td>Regular</td>
<td>0.1876</td>
<td>0.9647</td>
<td>5.896</td>
</tr>
<tr>
<td></td>
<td>Bioclogging</td>
<td>0.1872</td>
<td>0.9650</td>
<td>5.911</td>
</tr>
</tbody>
</table>

Figure 2.4: A comparison of the regular and bioclogging flow velocities in time.

2.2. For higher flow velocities, in particular \(Re= 10^{-1}\) (both flows) and \(Re= 10^{-3}\) (regular flow), convective AHL washout is increased, and so less AHL accumulates in the domain. As convective transport decreases in time in the bioclogging case, more AHL remains in the system, more cells are up-regulated (see Table 2.2), and so the maximum AHL for bioclogging is ultimately greater than for regular flow. The influence of flow on the maximum AHL increases with increasing velocity - for the regular flow case, the maximum AHL is five times greater for \(Re= 10^{-5}\) than for \(Re= 10^{-3}\), and ten times greater for \(Re= 10^{-3}\) than for \(Re= 10^{-1}\). In the bioclogging
Figure 2.5: The maximum AHL concentration in the channel in time for different $Re$ using the two flow mechanisms, regular (a) and bioclogging (b). For lower flow velocities, and for the bioclogging case, convective transport of AHL is decreased, and so more AHL may accumulate in the channel.

case, there are less significant differences between the maximum AHL values for the different $Re$ tested. In fact, the largest maximum AHL value for the bioclogging case was observed for $Re=10^{-3}$. At the lowest flow velocities tested in the bioclogging case (approximately $Re=10^{-5}$ and $=10^{-7}$ at $t=9.7$), the convective forces are nearly negligible. Because convective removal is so similar for $Re=10^{-3}$ and $Re=10^{-5}$ with bioclogging, the maximum AHL in the channel is greatest when $Re=10^{-3}$, as the biofilm is larger in this case. The trends in the relation between maximum AHL concentration and flow velocity are consistent with the up-regulated biomass occupancy.

To determine the temporal effects of flow on quorum sensing behaviour in our biofilm system, we measured the switching time in each simulation - the time at which AHL concentrations first reach the threshold concentration for induction at some point within the biofilm domain. Figure 2.6 shows the switching time for simulations using $10^{-5} \leq Re \leq 10^{-1}$, and both flow mechanisms. Note that for the
convection dominated regime $\text{Re} = 10^{-1}$, using the regular flow mechanism, switching does not occur in the simulation time. These results coincide with Janakiraman et al. 2009, in that flow rate can be set so high that AHL is washed out of the channel to the point that it cannot accumulate to the induction threshold concentration, even though biomass growth is greatest for the high flow velocities. However, each of the remaining simulations undergo initial switching at approximately $t = 9.0$, indicating that there is not a clear influence of the flow velocity on the switching time of the biofilm for either flow mechanism.

We now consider the spatial impacts of flow on quorum sensing induction. In Figure 2.1(c), we observe that flow induced convective AHL transport has a great local impact on induction - large upstream colonies experience a delay in induction, or no induction at all. In contrast, smaller downstream colonies experience an accelerated induction due to a supply of AHL from upstream (i.e., intercolony communication in our patchy biofilm community). In all experiments conducted where induction
occurs, upregulation is initiated at the base of the biofilm (corresponding to \( y = 1 \) in our domain of 2000x200 grid cells), and the faster growing upstream colonies are upregulated last. However, the spatial distribution of upregulated biomass varies with flow. The \( x- \) and \( y- \) co-ordinates of the regions of the biofilm and surrounding liquid where AHL concentrations first reach the threshold for induction, or equivalently, the spatial distribution of upregulated biomass at the switching time, are recorded in Table 2.3. The times given in Table 2.3 correspond to the switching times plotted in Figure 2.6. For the lower flow velocities, initial induction occurs further upstream than for the higher flow velocities. At low flow velocities, the removal of AHL by convection is to some extent balanced by the AHL produced by local colonies, allowing for the effect of initial switching to be reached further upstream. For \( Re = 10^{-1} \), the largest colony is located in the entrance region, and it is the last colony to participate in this initial switching period. In the case of the slowest flow, \( Re = 10^{-5} \), the large upstream colony clearly experiences much faster growth than the other microcolonies, and in turn, produces more AHL. Low flow velocities imply that most nutrients are utilized upstream, and there is less AHL removal by convection, therefore we see initial switching closer to the channel entry. In the fastest flow case, convective forces clearly dominate, and the downstream colonies are the first to upregulate. A visualisation of the biofilm and surrounding liquid with AHL concentrations higher than the threshold, just shortly after the switching time, is shown in Figure 2.7. The \( x- \)coordinates of initial induction, listed in Table 2.3, are depicted in Figure 2.7 as vertical lines (recall that induction is initiated at the base of the biofilm). The bioclogging mechanism is used for this comparison, which illustrates the upstream
extent to which initial induction may occur for the varying flow velocities. In the 
Re= $10^{-5}$ bioclogging simulation, initial induction occurs simultaneously at grid cells 
$1 \leq y \leq 4$, at and a few grid cells directly above the base of the biofilm. For Re= $10^{-1}$ 
and Re= $10^{-3}$, induction occurs further upstream in the bioclogging case than for 
regular flow (in fact, induction does not occur for Re= $10^{-1}$, regular flow). This is 
again the result of decreased AHL removal by convection. Initial induction occurs 
at almost precisely the same location for Re= $10^{-5}$ in the regular and bioclogging 
cases. This similarity is again due to the fact that at these very low flow velocities, 
Re= $10^{-5}$ (regular flow), and Re= $10^{-6}$ (approximate velocity for bioclogging at 
t=8.5), the convective forces are nearly negligible.

Table 2.3: The co-ordinates of the domain (2000 x 200 grid cells) where AHL concentrations first reach the threshold for induction for the flow velocity experiments.

<table>
<thead>
<tr>
<th>Re</th>
<th>Mechanism</th>
<th>x co-ordinates</th>
<th>y co-ordinates</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>Regular</td>
<td>-</td>
<td>1</td>
<td>9.647</td>
</tr>
<tr>
<td></td>
<td>Bioclogging</td>
<td>1363-1366</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>Regular</td>
<td>1344-1349</td>
<td>1</td>
<td>9.585</td>
</tr>
<tr>
<td></td>
<td>Bioclogging</td>
<td>1305-1309</td>
<td>1</td>
<td>8.759</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>Regular</td>
<td>1283-1300</td>
<td>1-4</td>
<td>8.531</td>
</tr>
<tr>
<td></td>
<td>Bioclogging</td>
<td>1284-1299</td>
<td>1-3</td>
<td>8.525</td>
</tr>
</tbody>
</table>

Figure 2.8 depicts the spatial differences between simulations of (8a) a 
convection-dominated system (Re= $10^{-1}$) and (8b,c) diffusion-dominated systems 
(Re= $10^{-3}, 10^{-5}$) for t = 9.0, for the regular flow mechanism. t = 9.0 is the ap-
proximate switching time for all flow regimes with the current $C_{bulk}$ value, but here 
induction is not observed for the convection-dominated case. The maximum AHL con-
centration is shown in each subfigure. Though the biofilm is much larger in 2.8(a),
Figure 2.7: The spatial distribution of upregulated biomass just shortly after the respective switching times for three different Re: Re= $10^{-1}$ (a), Re= $10^{-3}$ (b), and Re= $10^{-5}$ (c). Shown are the areas of the biofilm (red) and surrounding bulk liquid (black) where AHL concentrations first reach the threshold level for quorum sensing induction. The green band corresponds to the x-coordinates of the initial induction values listed in Table 2.3. For lower flow velocities, initial induction occurs further upstream than for the higher flow velocities, demonstrating the balance between diffusive and convective transport of dissolved substrate at low flow velocities.

The forces of convection (seen by the AHL isolines) wash out AHL, and it cannot accumulate to the threshold. In Figure 2.8(b,c), the highest fraction of upregulated cells is found in the medium-sized colonies in the centre of the domain, demonstrating the balance between AHL production by local colonies and AHL received from the upstream region by convective transport. These results contrast those produced by systems that are highly convection dominated (e.g., Vaughan et al., 2009), in which the highest AHL colonies are only found at the outflow boundary of the domain. In Figure 8, note the size of the upstream colonies relative to the downstream colonies for Re=$10^{-1}$ and Re=$10^{-3},10^{-5}$. The colonies are approximately equal in size for Re=$10^{-1}$, the convection case. The upstream colonies near the nutrient source are much larger than the downstream colonies for Re=$10^{-3}$ and $10^{-5}$, the diffusion dominated cases. As a result, biomass growth and AHL production are increased up-
Figure 2.8: Differences between simulations of a convection-dominated system (a) and diffusion-dominated systems (b) and (c) at time $t=9.0$. In (b) and (c), the highest fraction of upregulated cells is found in the medium-sized colonies in the centre of the domain, demonstrating the balance between AHL production by local colonies and AHL received from the upstream region by convective transport.

stream. Along with the decreased convective AHL removal, this contributes to where the highest AHL concentrations are found.

From these experiments on the effects of flow velocity on biofilm growth and quorum sensing behaviour, we conclude that the flow velocity alone cannot be used to predict the induction time for the biofilm. However, hydrodynamics did have significant spatial impacts: for high flow velocities, convective transport of AHL was the dominant process, leading to increased biofilm growth but also increased AHL washout downstream. For low flow velocities, convective and diffusive AHL transport were balanced, allowing initial induction to occur further upstream, and for a greater accumulation of upregulated biomass and AHL in the mid-channel. The results produced using the regular and bioclogging mechanisms were qualitatively similar. However, when the bioclogging mechanism was used, convective AHL removal
from the system was decreased, which led to a higher retention of AHL in the system, and greater proportions of up-regulated biomass. Overall, bioclogging flow had a greater impact on quorum sensing than on growth - quorum sensing activity was increased, even though the biofilm was slightly smaller.

2.3.3 Nutrient availability

In a second experiment, the bulk substrate concentration $C_{\text{bulk}}$ was varied over one order of magnitude, with a flow velocity of $Re=10^{-3}$. Because our model is non-dimensionalised with respect to the bulk concentration value, this was achieved by varying the half saturation constant $\kappa_2$ in the range $1/100 \leq \kappa_2 \leq 1/25$. The non-dimensional maximum substrate consumption rate parameter, $\kappa_1$, also varies with $C_{\text{bulk}}$, in the range $158 \leq \kappa_1 \leq 635$. In each simulation, $Re=10^{-3}$. Figure 2.9 shows the occupancy for various nutrient supplies on inflow, using both the regular and the bioclogging flow mechanisms. For higher bulk substrate concentrations, a greater amount of biomass is produced, and substrate limitations are observed later in time. This direct relation between nutrient supply and growth holds for both flow mechanisms. Table 2.4 compares the differences in the occupancy and maximum AHL concentrations between the two flow mechanisms for these nutrient conditions, at $t = 12.39$, which is the length of the shortest simulation ($C_{\text{bulk}} = 100$). In each case, the regular flow mechanism allowed for greater biomass growth. This is an effect of flow on biomass growth - the regular flow mechanism delivers nutrients at a fixed rate throughout the simulation experiment, whereas with bioclogging, flow velocity decreases in time, and so nutrient supply to the biofilm decreases in time as well.
However, the proportion of up-regulated biomass is higher for the bioclogging case, which we also observed in the hydrodynamic experiments. Similarly, although total occupancy is less when the bioclogging flow mechanism is used, lower flow velocities in the channel implies higher AHL concentrations, and therefore a greater proportion of biomass in the biofilm colonies may upregulate.

Table 2.4: A comparison of the occupancy, proportion of upregulated biomass $M_1/(M_0 + M_1)$, and maximum AHL using both flow mechanisms, at $t = 12.39$. The regular flow mechanism allowed for greater biomass growth under all nutrient conditions.

<table>
<thead>
<tr>
<th>$C_{\text{bulk}}$</th>
<th>Mechanism</th>
<th>Total Occupancy</th>
<th>Proportion of upregulated biomass</th>
<th>Maximum AHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Regular</td>
<td>0.1809</td>
<td>0.7549</td>
<td>3.072</td>
</tr>
<tr>
<td></td>
<td>Bioclogging</td>
<td>0.1146</td>
<td>0.9864</td>
<td>7.235</td>
</tr>
<tr>
<td>50</td>
<td>Regular</td>
<td>0.09638</td>
<td>0.01676</td>
<td>0.2865</td>
</tr>
<tr>
<td></td>
<td>Bioclogging</td>
<td>0.06626</td>
<td>0.1431</td>
<td>0.7090</td>
</tr>
<tr>
<td>25</td>
<td>Regular</td>
<td>0.04961</td>
<td>0.002477</td>
<td>0.1274</td>
</tr>
<tr>
<td></td>
<td>Bioclogging</td>
<td>0.03769</td>
<td>0.006707</td>
<td>0.1799</td>
</tr>
</tbody>
</table>

Figure 2.10 shows the maximum AHL concentration in the channel in time.
for the various bulk substrate concentrations, using both flow mechanisms. For higher bulk substrate concentrations, increased AHL concentrations are found, and induction occurs at an earlier time. When growth is not limited by nutrient supply, the biofilm colonies grow rapidly. AHL production is increased, and so the threshold for induction is consequently obtained earlier. There is a difference in AHL concentrations between the two flow mechanisms - higher maximum AHL values and earlier induction times are obtained for the bioclogging case. Because flow is decreasing in time for the bioclogging case, less AHL is washed out of the channel by convective transport in the liquid phase. So from Figures 2.9 and 2.10, we see that for all nutrient conditions, flow will contribute to biofilm growth but impede quorum sensing induction. Note that for $C_{\text{bulk}} = 50$, at the end of the simulation ($t = 15.0$), AHL does not reach the threshold for induction with regular flow, but it does surpass the threshold for the bioclogging flow case (maximum AHL concentrations at $t=15.0$ are 0.5076 and 4.462 respectively) - indicating that for some range of bulk substrate concentrations, the flow regime chosen could lead us to draw different conclusions about quorum sensing behaviour in our biofilm system.

Figure 2.11 further demonstrates the distinct relationship between bulk substrate concentration and the switching time (the time at which AHL concentrations first reach the threshold at some point in the domain); here it is also clear that switching occurs earlier for the bioclogging flow. Switching does not occur for $C_{\text{bulk}} = 50$ (regular flow) or $C_{\text{bulk}} = 25$ (either flow), as in these cases the nutrients are severely limited, and the biofilm remains so small that insufficient AHL is produced for accumulation to the threshold level.
Figure 2.10: The maximum AHL concentration in the channel in time for various nutrient supplies on inflow (Cbulk) using the two flow mechanisms. Higher maximum AHL values are obtained with the bioclogging flow mechanism; in this case flow decreases in time, so less AHL is washed out by convective transport.

Figure 2.11: Switching time: to study the induction of quorum sensing under various nutrient conditions (bulk substrate C concentration on inflow, C_{bulk}), the time at which AHL concentrations first reach the threshold concentration in the domain is measured. Higher C_{bulk} values directly lead to earlier switching times.
Figure 2.12: Differences between simulations of a high-nutrient supply system (a) and a low-nutrient supply system (b) at time $t=15.0$, $Re = 10^{-3}$, using the bioclogging flow mechanism.

In Figure 2.12, the biofilm has been plotted at $t=9.0$, $Re=10^{-3}$, with the bioclogging flow mechanism for the case of a high substrate supply ($C_{bulk}=100$) and a low substrate supply ($C_{bulk}=25$). A different initial inoculation was used than that for the biofilm in Section 3a, in which colonies were inoculated on both the top and bottom boundaries. The upstream and downstream colonies are able to grow larger when substrate availability is increased. In Figure 2.12(a), sufficient nutrient supply results in upregulation in the biofilm after a period of time. In contrast, the biofilm in Figure 2.12(b) is unable to grow large enough and produce enough AHL to upregulate anywhere in the domain under the severely limited nutrient supply.

Figure 2.13 illustrates the state of the biofilms at $C_{bulk}=100$ and $C_{bulk}=50$, for $Re = 10^{-3}$ (bioclogging), at their switching times, $t = 10.0$ and $t = 13.0$ respectively, to analyze the spatial induction patterns. Just as we observed in the hydrodynamics experiments, the medium-sized colonies in the mid-channel are first to upregulate. This again is the result of the balance between local AHL production and AHL received from upstream colonies via convective transport for the chosen
Figure 2.13: The spatial distribution of biomass at the respective switching times for (a) $C_{bulk} = 100$ and (b) $C_{bulk} = 50$, $Re = 10^{-3}$, using the bioclogging flow mechanism. Initial induction occurs in the mid-channel, and the upstream colonies are last to upregulate.

flow regime.

From these experiments on bulk substrate concentrations, we conclude that a clear temporal relation exists: increased nutrient supply resulted in increased biofilm growth and earlier quorum sensing induction times. Induction occurs first in the mid-channel, and upstream colonies are last to upregulate. Also, it is possible to limit nutrients to the point where the biofilm does not grow large enough for quorum sensing to occur in the experimental timescale. The bioclogging flow mechanism led to earlier switching times and increased AHL concentrations in the domain.

2.3.4 Influence of quorum sensing regulation rate $\kappa_5$

Among the experimentally determined quorum sensing parameters, the quorum sensing up-regulation rate ($\kappa_5$) had the most uncertainty. We varied $\kappa_5$ between 1.0 and 5.0 (results not shown), and confirmed our intuitive expectations - a higher quorum sensing up-regulation rate led to earlier switching times.
2.4 Ecological Implications

Flow in the liquid surrounding the biofilm clearly influences autoinduction, which is in accordance with the results of Kirisits et al. (2007). Our results indicate that flow has an impact on both the spatial and temporal induction patterns. The flow velocity, in the range we have chosen, has a significant local impact - the exact area where upregulation is initiated depended on the specific values of flow velocity. The other important factor analyzed in our experiments was the dissolved growth limiting nutrient. Interestingly, the combination of increased flow velocity and nutrients resulted in an increased colony growth upstream, but also resulted in an accelerated induction downstream. So under the chosen conditions, smaller downstream colonies were earlier upregulated than the larger upstream colonies. This result is in conflict with the idea of quorum sensing in the strict sense, in which cell density is monitored by bacteria to determine whether density is sufficient for induction. In the idea of diffusion sensing, individual cells measure the autoinducer concentration produced by themselves, to detect mass transfer limitations, that is, the amount of their AHL produced and lost due to both diffusion and convection (Redfield, 2002). However, we have groups of cells producing AHL, and there are clearly interactions between the colonies. Our results are therefore best interpreted using the efficiency sensing concept - colonies sense a combination of spatial distribution, cell density, and mass transfer limitations (Hense et al., 2007). In summary, the nutrient supply and the presence of flow play a role in determining when cells switch from a single cell to a more collective behaviour. The combination of nutrients and flow (via direct impact
on AHL transfer and indirect impact on AHL production) controls the intracolonial induction and intercolonial interactions. In contrast to plankton or biofilms in the absence of flow, biofilms under flow conditions act highly heterogeneously and interactively. There is additional value for cells to participate in collective behaviour, i.e., benefits to living in a biofilm, and the environmental conditions influence when these benefits are recognized and the collective behaviour emerges.

The question arises whether the influence of flow disturbs the autoinducer regulation of behaviour, or if flow is an integral part of the regulation - ultimately, can flow be used to interrupt bacterial behaviour? Autoinducers are thought to generate collective, cooperate behaviour, for example, in the release of effectors such as exoenzymes and antibiotica (Hense et al., 2007). With the presence of flow, downstream cells lose their effectors, but obtain them from upstream cells. In contrast, large upstream colonies enforce cooperative behaviour by promoting induction of downstream cells, but themselves do not necessarily participate in the induced cooperative behaviour, depending on the flow velocity, nutrient conditions, and growth stage. This seems to contravene the reliability of the autoinducer signalling process. In the absence of flow, enforcement of cooperation is attained by the positive feedback mechanism of autoinducer signalling. Within the colonies, enforcement can stabilize cooperativity - so under flow conditions, high enforcement (positive feedback) will ensure cooperation within the colony. With flow, this enforcement is of only limited utility beyond the boundaries of each colony. However, the number of non-cooperative cells or entire colonies is limited and decreases over time, and thus may not be very relevant for the ecological function of the sensing mechanism. Furthermore, most
cells may obtain a major fraction of sensed autoinducers from their neighbouring cells within the colony, where flow likely does not relevantly disturb cooperation.

Significantly higher flow velocities may restrict induction to be caused by autoinducers produced within the colony itself, but high flow velocities can also diminish nutrient limitations for downstream cells, consequently promoting growth and induction. Thus, although flow does alter the autoinducer regulation system, the goal of manipulating bacteria through quorum sensing control would be best reached through utilization of flow to affect nutrient supply. Note that in our model, nutrients affected biofilm growth. Nutrient limitations have also been known to affect the quorum sensing system, particularly autoinducer production. If nutrient-dependent AHL production were taken into account, this process would add an additional complexity to our model.

It should also be mentioned that microcolony, or “patchy” structures, are representative of the first stage of biofilm development (Parsek and Greenberg, 2005, Demuth and Lamont, 2006). According to experimental and theoretical considerations, microcolonies are the structures where quorum sensing primarily occurs, so the investigation of quorum sensing using this structure is realistic. However, in time, microcolonies grow and may eventually merge. In such a homogeneous thick biofilm, flow would less disturb inter-colony communications, resulting in a more homogeneous response by the biofilm as a whole.
2.5 Conclusion

The model of biofilm growth and quorum sensing that was presented here is the first mathematical model for two-dimensional patchy biofilm colonies with flow and nutrient-dependent growth included. Our two-dimensional flow model allows for the study of how AHL produced in one colony can have a non-local effect on neighbouring colonies, which is a consequence of the fact that AHL produced by the bacteria is transported by convection and diffusion in the aqueous phase. Nutrient-dependent biomass growth, a realistic process, was included to enhance our understanding of quorum sensing systems under flow conditions. Two flow mechanisms were tested, regular flow, and bioclogging, in which flow velocities decreased with biomass accumulation in the channel. We focused on slow flowing systems in narrow conduits, mimicking in particular the situation in pore spaces in soils and fractured media. Moreover, while faster flow conditions would imply a quick washout of AHL molecules, the flow range chosen here is in the range where the effects of AHL production and convective transport intricately interact. Environmental conditions such as substrate availability and mass transfer clearly show an effect on local autoinduction.

The key findings from the numerical simulations are:

- the observation of inter-colony communication: production of AHL from upstream colonies caused induction of downstream colonies. The exact location where initial induction occurred depended on the combined effect of nutrients and flow conditions.

- mass transfer processes influence quorum sensing: induction does not occur
first for the largest colonies. Our induction patterns exemplify the efficiency sensing concept, in which colonies sense a combination of spatial distribution, cell density, and mass transfer limitations.

- increased bulk substrate concentration directly resulted in increased biofilm growth and earlier induction times

- increased flow velocities resulted in greater biofilm growth, but not in induction time variation

- hydrodynamics had significant spatial impacts. For high flow velocities, convective transport of AHL was dominant over diffusion, leading to increased biofilm growth but also increased AHL washout downstream. For low flow velocities, convective and diffusive AHL transport were balanced, allowing initial induction to occur further upstream, and a greater accumulation of upregulated biomass and AHL in the mid-channel.

- the regular and bioclogging flow mechanisms produced results that were qualitatively very similar. Within each simulation, however, there were differences in the biofilm occupancy, proportion of upregulated biomass, and total AHL between the two flow mechanisms. Bioclogging flow led to increased quorum sensing activity in the biofilm, that is, more upregulated cells and higher concentrations of AHL, even though the biofilms were smaller with this mechanism. Overall, bioclogging flow had a greater impact on quorum sensing than on biofilm growth.
• both the flow velocity and the mechanism driving the flow impact quorum sensing. In future simulations, the flow mechanism should be chosen to best represent the simulated biofilm environment. Our simulated environment is a narrow conduit, such as a soil pore, and so bioclogging flow is the more realistic representation.

Our model is particularly suited for describing biofilm development, which is when many processes regulated by quorum sensing are known to occur. The model presented here, or extensions of it, will allow us to investigate how the control of environmental conditions can be used to control biofilm processes, in particular those which are governed by quorum sensing.
Bibliography


Chapter 3

A mathematical model of quorum sensing regulated EPS production in biofilm communities

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Abstract

We mathematically model quorum sensing and EPS production in a growing biofilm under various environmental conditions, to study how a developing biofilm impacts quorum sensing, and how the biofilm structure is affected by quorum sensing-regulated EPS production. We investigate circumstances when using quorum-sensing regulated EPS production is a beneficial strategy for biofilm cells. We find that biofilms that use quorum-sensing regulated EPS production do not attain higher cell populations than non-quorum sensing biofilms, but occupy a greater volume in their environment.
The associated benefits are thick, protective EPS layers, and ability to outcompete other colonies by clogging a space-limited environment.

**Keywords:** biofilm; EPS; mathematical model; quorum sensing

**Classification:** MSC2000:92D25

### 3.1 Introduction

#### 3.1.1 Biofilms, quorum sensing, and EPS

Biofilms are microbial communities encased in a layer of extracellular polymeric substances (EPS), adhered to biotic or abiotic surfaces. Biofilms are extremely successful, and are able to flourish in any aqueous environment with adequate nutrients available. Bacteria preferentially reside in biofilms, rather than in isolation as planktonic cells. In a biofilm, bacteria are protected by the EPS matrix from external stresses, and carry out a wide range of reactions which are relevant in many disciplines, such as environmental engineering, food processing, and medicine (Lewandowski, 2007). Quorum sensing is a cell-cell communication mechanism used by several bacterial taxa to coordinate gene expression and behaviour in groups, based on population densities (Fuqua et al., 1994). Bacteria cells constantly produce and release low amounts of signalling molecules, called autoinducers (e.g., acyl-homoserine lactones (AHL) in Gram-negative bacteria). When a critical environmental autoinducer concentration is reached, the bacteria are rapidly induced and undergo changes in gene expression. In most bacterial autoinducer systems, the autoinducer synthase gene is upregulated, initiating positive feedback, and the bacteria subsequently pro-
duce AHL molecules at an increased rate. As a number of traits in bacterial biofilms relevant for human and plant health are regulated via autoinducers (Demuth and Lamont, 2006, Whitehead et al, 2001), a comprehensive understanding of quorum sensing systems is highly desirable. We will consider Gram-negative bacteria with their typical autoinducer system, which involves acyl-homoserine lactones (AHLs) as the signalling molecule.

EPS refers to organic molecules such as polysaccharides, proteins, and lipids, which form the outermost surface layer of biofilms. Bacteria cells produce EPS, which plays an important role in maintaining the structural integrity of the biofilm (Wingender et al., 1999). The EPS matrix provides several functional purposes for the biofilm, such as protecting bacteria from environmental threats, providing mechanical stability, and degrading macromolecules to be used by the cells (Flemming et al., 2007). EPS is thought to indirectly store nutrients, which could later be converted to an available form and used as an energy source during low nutrient conditions (Sutherland, 1999, Wolfaardt et al., 1999); this has been investigated in theoretical studies (Laspidou & Rittmann, 2002), and shown experimentally (Wang et al., 2007).

3.1.2 Modelling of biofilms and quorum sensing

Initial mathematical models of quorum sensing describe the phenomena in suspended bacteria cultures (Ward et al., 2001, Dockery and Keener, 2001, Müller et al., 2006). These models focus on predicting the rapid switch in proportions of down- and upregulated sub-populations of bacteria in a batch culture, which is the characteristic positive-feedback feature of quorum sensing systems. Ward et al. (2003)
and Chopp et al. (2002, 2003) extended the work of early models to study quorum sensing in a growing biofilm, identifying key physical kinetics parameters required for induction. More recent models include the effects of hydrodynamics (Janakiraman et al. 2009, Vaughan et al. 2009, Frederick et al. 2010), as well as modelling growth in two dimensions (Frederick et al. 2010).

A variety of applications motivate development of specific quorum sensing and biofilm models. For example, Chopp et al. (2002, 2003) determine the critical depth the biofilm must grow to, as a function of pH, in order for induction to occur. The models of Anguige et al. (2004, 2005, 2006) detail biochemical pathways in quorum sensing systems, also describing anti-quorum sensing treatments for applications in the medical field. Frederick et al. (2010) investigate the role of convective and diffusive transport of signal molecules in inter-colony communication within biofilm communities.

These models share a common element: autoinducer molecules (e.g., AHL) are produced by downregulated bacteria, and AHL production is greatly enhanced when the characteristic switch (change from low to high quorum sensing activity) rapidly occurs throughout the biofilm.

Much research has been conducted to understand when biofilms partake in quorum sensing activity, for example, determining population thresholds (Dockery and Keener, 2001, Ward et al 2001), critical biofilm depth (Chopp et al. 2002, 2003), and the influence of the hydrodynamic and nutritional environment (Kirisits et al. 2007, Frederick et al. 2010, Janakiraman et al. 2009). There have, however, been few studies that look at the reverse effect - the effect of quorum sensing induction
on biofilms. Once biofilm cells are upregulated, AHL is produced at an increased rate, but the question of whether the biofilm behaves differently, grows differently, or undergoes some other functional change, remains unanswered.

We expand on the works of Anguige et al. (2004, 2005, 2006), which model the effects of anti-quorum sensing therapies on bacteria growth and biofilm development. Ward (2008) analyzes the effectiveness of the modelled anti-quorum sensing therapies by comparing growth rates of the biofilms, and states that quorum sensing activity may be detected by EPS production and associated enhanced biofilm growth. Ward (2008) assumes that EPS is regulated by quorum sensing, based on the findings of Davies et al. (1998), and allows for significantly enhanced EPS production by upregulated cells. With our model, we will study in detail how the process of quorum sensing-regulated EPS production impacts biofilm growth and development in a two-dimensional patchy biofilm community under various environmental conditions. Our objective is to understand the relationship between quorum sensing, biofilm growth, and EPS production, and investigate the benefits a biofilm receives by using quorum sensing-regulated EPS production.

To validate the claim that quorum sensing controls EPS production, and to what degree, we turn to the experimental literature. In many studies, quorum sensing has been found to impact the quality of EPS. For example, Sakuragi and Kolter (2007) showed differences in biofilm appearance with and without expression of the pelA gene, which is essential for the production of the EPS matrix; wild-type biofilms are wrinkled, but mutants are flat. In a later study, Friedman and Kolter (2004) research the quorum sensing-regulated expression of the pelA enzyme, and show that
Pel-genes are required for EPS production. In fact, Pel mutants have no EPS. Shrout et al. (2006) found many factors which affect the quality of the EPS matrix to be regulated by quorum sensing in the early development stage, such as channel production within the biofilm, swarming activity, and lipid production. In contrast, cases have been found in which quorum sensing down-regulates structural integrity via changes to the EPS (Edwards et al., 2009). Also, many studies have shown the connection between quorum sensing and mucosity (Morohoshi et al., 2007, Marketon et al., 2003, von Bodman et al., 2005). Quorum sensing regulates components of EPS (e.g., EPS II, polysaccharides) which contribute to the mucosity, thus impacting the biofilm matrix. These studies support the idea that the amount of EPS production per cell is influenced by quorum sensing, but do not show to what degree.

There are some examples of bacteria species, mostly plant pathogens, in which a quantitative increase of EPS production by quorum sensing regulation has been demonstrated. In a study by Quinones, Dulla, and Lindow (2005), quorum sensing was found to regulate alginate production in *P. syringae*. Alginate is an important component of EPS, and without quorum sensing, alginate levels were 70% lower. However, the impact on biofilm thickness is not described, so conclusions cannot be drawn regarding whether overall EPS is significantly reduced by the drop in alginate levels.

Koutsoudis et al. (2006) conclude that the amount of EPS production per cell in a *Pantoea stewartii* biofilm is increased by quorum sensing, though the degree of production is not given. Similarly, Gao et al. (2009) claim that quorum sensing upregulated EPS production in the plant pathogen *Erwinia amylovora*, but do not
provide quantitative data. However, images are shown, from which the upregulated EPS may be estimated as a factor five to ten increase. This is supported by the experiments of Beck von Bodman et al. (1998), in which an approximately ten-fold increase of EPS production in a *Pantoea stewartii* biofilm upon QS induction was discovered. The ten-fold increased production factor is orders of magnitudes different than the value used in the Ward (2008) model.

Though many studies have established connections between quorum sensing activity and qualitative changes in EPS or other structural components, there are very few quantitative studies which investigate the amount of EPS produced through quorum sensing regulation. We choose to use the direct values for change in EPS production as reported in Beck von Bodman (1998) as an estimate for the difference in downregulated and upregulated cell production rates in our system.

### 3.1.3 Aim of study

In previous research, we developed a two-dimensional model of quorum sensing in patchy biofilm communities to study how the hydrodynamic environment and nutrient conditions contribute to biofilm growth, spatiotemporal quorum sensing induction patterns, and flow-facilitated intercolony communication (Frederick et al. 2010).

In this paper, we will extend this model to include a response from the biofilm once quorum sensing has been induced. The upregulated cells not only produce AHL at increased rates, but produce EPS at an increased rate as well. We wish to investigate two main research questions with our model:
1. *How does quorum sensing-regulated EPS production impact the growing biofilm?*

   What does the biofilm look like over time - how are cells and EPS distributed?

2. *When does quorum sensing-regulated EPS production provide an advantage to the biofilm?* Specifically, which biofilms succeed when the nutrient conditions and EPS consumption process are altered?

   Answers to these questions will be sought through numerical experiments which simulate the growth of biofilms in microfluidic chambers.

### 3.2 Mathematical Model and Simulation Design

#### 3.2.1 Model

We present a mathematical model for quorum sensing in a growing biofilm community in a narrow conduit which consists of several colonies. The dependent variables of our model are the downregulated bacteria ($M_0$), upregulated (quorum sensing induced) bacteria ($M_1$), non-quorum sensing bacteria ($M_2$), EPS ($EPS$), AHL ($AHL$), and growth limiting carbon nutrient ($C$). Bacteria ($M_0$, $M_1$, $M_2$), and EPS are expressed as the fraction of the volume they locally occupy at each point in the domain (volume fraction). $AHL$ and $C$ are dissolved, and are expressed as concentrations. The total amount of EPS is accounted for; we do not distinguish between EPS produced by $M_0$, $M_1$, or $M_2$.

The processes incorporated into our model are:

- production of new particulate substances (bacteria and EPS, referred to as
biomass), controlled by nutrient availability

- nutrient consumption by bacterial biomass

- spatial spreading of the biofilm only for high local biomass volume fractions (i.e., for $M_0 + M_1 + M_2 + EPS \approx 1$)

- conversion of downregulated biomass into upregulated biomass as a consequence of AHL concentration inducing a change in gene expression, and a constant rate of back-conversion

- natural cell death

- abiotic AHL decay

- differential AHL and EPS production rates of downregulated and upregulated cells

- diffusive transport of carbon substrate and AHL in the biofilm, and both convective and diffusive transport in the surrounding aqueous phase of the biofilm

- when carbon becomes limited, EPS may be used as a food source

This model is a major extension of our earlier studies (Frederick et al., 2010), differing in several ways. Previously, EPS was assumed to be implicitly included with the bacteria cells. We have introduced EPS in a separate equation, and now EPS contributes explicitly to total biomass $M_0 + M_1 + M_2 + EPS$. Our previous study modelled only downregulated and upregulated cells ($M_0, M_1$). We have now introduced a new bacteria species ($M_2$), which behaves in the same manner as the
downregulated cell $M_0$ (with respect to parameters for growth, consumption, and EPS production). However, $M_2$ cells do not carry the quorum sensing gene, and therefore are considered *non-quorum sensing* biofilm cells. Other assumptions made in our model include:

- upregulated cells produce ten times as much EPS as downregulated cells
- upregulated cells $M_1$ will consume twice as much as $M_0$
- growth rates for all bacteria cells are equivalent
- non-quorum sensing cells ($M_2$) have the same reaction kinetics as downregulated cells ($M_0$); specifically, consumption and EPS production rates are equivalent.

The mathematical model for biofilm growth, quorum sensing, and EPS production is:

\[
\begin{align*}
\partial_t M_0 &= \nabla(D_M(M)\nabla M_0) + \frac{\kappa_3 C M_0}{\kappa_2 + C} - \kappa_4 M_0 - \kappa_5 \text{AHL}^n M_0 + \kappa_5 \tau^n M_1 \\
\partial_t M_1 &= \nabla(D_M(M)\nabla M_1) + \frac{\kappa_3 C M_1}{\kappa_2 + C} - \kappa_4 M_1 + \kappa_5 \text{AHL}^n M_0 - \kappa_5 \tau^n M_1 \\
\partial_t M_2 &= \nabla(D_M(M)\nabla M_2) + \frac{\kappa_3 C M_2}{\kappa_2 + C} - \kappa_4 M_2 \\
\partial_t C &= \nabla(D_C(M)\nabla C) - \nabla(wC) - \frac{\kappa_{10} C M_0}{\kappa_2 + C} - \frac{\kappa_{11} C M_1}{\kappa_2 + C} - \frac{\kappa_{12} C M_2}{\kappa_2 + C} + \frac{\delta \kappa_6 \text{EPS}}{\kappa_6 + C} \\
\partial_t \text{AHL} &= \nabla(D_{\text{AHL}}(M)\nabla \text{AHL}) - \nabla(w\text{AHL}) - \sigma \text{AHL} + \alpha M_0 + (\alpha + \beta) M_1 \\
\partial_t \text{EPS} &= \nabla(D_M(M)\nabla \text{EPS}) + \frac{\gamma_0 C M_0}{\kappa_2 + C} + \frac{\gamma_1 C M_1}{\kappa_2 + C} + \frac{\gamma_2 C M_2}{\kappa_2 + C} - \frac{\delta \kappa_6 \text{EPS}}{\kappa_6 + C}
\end{align*}
\]

The total volume fraction occupied by the biofilm is $M$, where $M = M_0 + M_1 + M_2 + \text{EPS}$. The two-dimensional computational domain $\Omega$ consists of a liquid
phase with no biomass, $\Omega_1(t) = (x, y) \in \Omega : M(t, x, y) = 0$, and the solid biofilm phase, $\Omega_2(t) = (x, y) \in \Omega : M(t, x, y) > 0$. These regions change as the biofilm grows.

The diffusion coefficient for biomass ($D_M(M)$) is density dependent, and is formulated as $D_M(M) = d_M \frac{M^a}{(1-M)^b}$ (Eberl et al., 2001). Exponents $a > 1$ and $b > 1$ ensure biofilm expansion when $M$ approaches 1 (implying all available space is filled by biomass), and little or no expansion provided $M$ is small. This choice of diffusion coefficient ensures a separation of the biofilm and its surrounding aqueous phase, and that the maximum cell density will not be exceeded. The diffusion coefficients for $C$ and AHL are lower in the biofilm than in the surrounding aqueous phase (Stewart, 2003). We let $D_C(M) = D_C(0) + M(D_C(1) - D_C(0))$, $D_{AHL}(M) = D_{AHL}(0) + M(D_{AHL}(1) - D_{AHL}(0))$, where $D_C(0)$ and $D_{AHL}(0)$ are the diffusion coefficients in water, and $D_C(1)$ and $D_{AHL}(1)$ are the diffusion coefficients in a fully compressed biofilm.

In the reaction terms, $\kappa_3$ is the maximum specific growth rate of bacterial biomass. The maximum specific substrate consumption rates are denoted by $\kappa_{0,1,2} = \kappa_3 M_{\text{max}}/Y_{0,1,2}$, where $M_{\text{max}}$ is the maximum cell density, and $Y_{0,1,2}$ are the yield coefficients. Bacterial growth is described by Monod kinetics, where $\kappa_2$ is the half-saturation constant. $\kappa_4$ is the cell lysis rate. The parameter $\kappa_5$ is the quorum sensing regulation rate - the rate at which downregulated bacteria become upregulated, and vice versa. The AHL production rate of downregulated bacteria is $\alpha$, and the increased production rate of upregulated bacteria is $\alpha + \beta$. AHL degrades abiotically at rate $\sigma$. $\tau$ is the threshold AHL concentration locally required for quorum sensing induction to occur. We use $n$ ($n > 1$) to describe the degree of polymerisation in the synthesis
of AHL, equivalent to the Hill coefficient describing cooperative binding reactions in the Hill equation, acting as a switch function (Müller et al., 2006).

EPS serves as an additional source of carbon when carbon concentrations are low; this process is represented by an inhibition Monod term, in which EPS is transformed into carbon at rate \( \delta \), with Monod constant \( \kappa_6 \). The EPS production rates are \( \gamma_0, \gamma_1, \) and \( \gamma_2 \) for \( M_0, M_1, \) and \( M_2 \) respectively. The EPS production rates incorporate maximum cell density, EPS density \( (EPS_{max}) \), and EPS yield \( Y_{EPS} \) for these reactions: \( \gamma_{0,1,2} = M_{max} \ast Y_{0,1,2(EPS)} \ast \kappa_3 \). Reaction kinetics and parameter values used in our model to describe EPS production by non-induced bacteria cells and conversion of EPS to carbon substrate were developed by Horn et al. (2001) in a model of biofilm growth and EPS production. In simulations excluding the EPS consumption process, we let \( \delta = 0 \).

The convective contribution to transport of \( C \) and \( AHL \) in the aqueous phase is controlled by the flow velocity vector \( w = (u, v) \), where \( u \) and \( v \) are the flow velocities in the \( x \)- and \( y \)-directions. The flow in the aqueous phase is described by the incompressible Navier-Stokes equations. As an alternative to solving the complete flow equations numerically, we use the thin-film approximation as proposed in Eberl and Sudarsan (2008):

\[
0 = -\frac{1}{\rho} \frac{\partial p}{\partial x} + \nu \frac{\partial^2 u}{\partial y^2}, \quad 0 = \frac{\partial p}{\partial y}, \quad 0 = \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y}
\]

where \( p \) is the hydrodynamic pressure field; constants \( \rho \) and \( \nu \) are the density and kinematic viscosity of water. This approximation is valid in the liquid region of long,
narrow conduits for slow flow regimes, and can be solved analytically, which allows for fast computation. In order to drive the flow in the channel, we specify the volumetric flow rate in terms of the non-dimensional Reynolds number $Re$ for the empty flow channel, given by:

$$Re = \frac{HU}{\nu}$$

where the characteristic height scale $H$ is the channel height, and $U$ is the maximum flow velocity of the unperturbed Poiseuille flow. The flow rate $q$ is then:

$$q = \frac{2}{3} \nu Re,$$

where $\nu$ is the kinematic viscosity of the bulk liquid.

The model is non-dimensionalized with the choices: $\tilde{x} = \frac{x}{L}, \tilde{t} = t\kappa_3$, where $L$ is the flow channel length, and $\frac{1}{\kappa_3}$ is the characteristic time scale for $M_0$. The new dimensionless concentration variables are: $\tilde{C} = \frac{C}{C_{bulk}}, A = \frac{AHL}{\tau}$, where $C_{bulk}$ is the bulk substrate concentration (the amount of substrate $C$ supplied at the inflow
boundary). The new reaction parameters are:

\[ \tilde{\kappa}_{10} = \frac{M_{\text{max}}}{Y_0 C_{\text{bulk}}}; \quad \tilde{\kappa}_{11} = \frac{M_{\text{max}}}{Y_1 C_{\text{bulk}}}; \quad \tilde{\kappa}_{12} = \frac{M_{\text{max}}}{Y_2 C_{\text{bulk}}}; \]

\[ \tilde{\kappa}_2 = \frac{\kappa_2}{C_{\text{bulk}}}; \quad \tilde{\kappa}_3 = 1; \quad \tilde{\kappa}_4 = \frac{\kappa_4}{\kappa_3}; \quad \tilde{\kappa}_5 = \frac{\kappa_5 \tau}{\kappa_3}; \quad \tilde{\kappa}_6 = \frac{\kappa_6}{C_{\text{bulk}}}; \]

\[ \tilde{\delta} = \frac{\delta}{\kappa_3}; \quad \tilde{\delta} = \frac{\delta E \text{P} S_{\text{max}}}{\kappa_3 C_{\text{bulk}}}; \quad \tilde{\gamma}_0 = \frac{Y_0 E \text{P} S \kappa_3 M_{\text{max}}}{\kappa_3 E \text{P} S_{\text{max}}}; \quad \tilde{\gamma}_1 = \frac{Y_1 E \text{P} S \kappa_3 M_{\text{max}}}{\kappa_3 E \text{P} S_{\text{max}}}; \quad \tilde{\gamma}_2 = \frac{Y_2 E \text{P} S \kappa_3 M_{\text{max}}}{\kappa_3 E \text{P} S_{\text{max}}}; \]

\[ \tilde{\sigma} = \frac{\sigma}{\kappa_3}; \quad \tilde{\alpha} = \frac{\alpha M_{\text{max}}}{\kappa_3 \tau}; \quad \tilde{\beta} = \frac{\beta M_{\text{max}}}{\kappa_3 \tau}; \]

The diffusion coefficients become:

\[ \tilde{D}_C = \frac{D_C}{L^2 \kappa_3}; \quad \tilde{D}_{\text{AHL}} = \frac{D_{\text{AHL}}}{L^2 \kappa_3}; \quad \tilde{D}_M = \frac{D_M}{L^2 \kappa_3}; \]

The non-dimensionalized equations are then:

\[
\begin{align*}
\partial_t M_0 & = \tilde{\nabla}(\tilde{D}_M(M)\tilde{\nabla}M_0)) + \frac{\tilde{\kappa}_3 \tilde{C} M_0}{\tilde{\kappa}_2 + \tilde{C}} - \tilde{\kappa}_4 M_0 - \tilde{\kappa}_5 \tilde{A} M_0 + \tilde{\kappa}_5 M_1 \\
\partial_t M_1 & = \tilde{\nabla}(\tilde{D}_M(M)\tilde{\nabla}M_1)) + \frac{\tilde{\kappa}_3 \tilde{C} M_1}{\tilde{\kappa}_2 + \tilde{C}} - \tilde{\kappa}_4 M_1 + \tilde{\kappa}_5 \tilde{A} M_0 - \tilde{\kappa}_5 M_1 \\
\partial_t M_2 & = \tilde{\nabla}(\tilde{D}_M(M)\tilde{\nabla}M_2)) + \frac{\tilde{\kappa}_3 \tilde{C} M_2}{\tilde{\kappa}_2 + \tilde{C}} - \tilde{\kappa}_4 M_2 \\
\partial_t \tilde{C} & = \tilde{\nabla}(\tilde{D}_C(M)\tilde{\nabla}\tilde{C})) - \tilde{\nabla}(w \tilde{C}) - \frac{\tilde{\kappa}_{10} \tilde{C} M_0}{\tilde{\kappa}_2 + \tilde{C}} - \frac{\tilde{\kappa}_{11} \tilde{C} M_1}{\tilde{\kappa}_2 + \tilde{C}} - \frac{\tilde{\kappa}_{12} \tilde{C} M_2}{\tilde{\kappa}_2 + \tilde{C}} + \frac{\tilde{\delta}_5 \tilde{\kappa}_6 \tilde{E}}{\tilde{\kappa}_6 + \tilde{C}} \\
\partial_t \tilde{A} & = \tilde{\nabla}(\tilde{D}_A(M)\tilde{\nabla}\tilde{A})) - \tilde{\nabla}(w \tilde{A}) - \tilde{\sigma} \tilde{A} + \tilde{\alpha} M_0 + \tilde{\beta} M_1 \\
\partial_t \tilde{E} & = \tilde{\nabla}(\tilde{D}_M(M)\tilde{\nabla}\tilde{E}) + \frac{\tilde{\gamma}_0 \tilde{C} M_0}{\tilde{\kappa}_2 + \tilde{C}} + \frac{\tilde{\gamma}_1 \tilde{C} M_1}{\tilde{\kappa}_2 + \tilde{C}} + \frac{\tilde{\gamma}_2 \tilde{C} M_2}{\tilde{\kappa}_2 + \tilde{C}} - \frac{\tilde{\delta}_5 \tilde{\kappa}_6 \tilde{E}}{\tilde{\kappa}_6 + \tilde{C}}
\end{align*}
\]
The parameters used in our simulations and their non-dimensional values are listed in Table 3.1. The biofilm parameters $\kappa_1$-$\kappa_4$ were chosen from the range of standard values in biofilm modelling literature (Wanner et al., 2006, Horn et al., 2001), the diffusion coefficient values ($d_M, a, b$) were selected from Eberl and Sudarsan (2008), and the quorum sensing parameters $\kappa_5, \alpha, \beta, \tau, \gamma$ and $n$ were derived from experiments on the kinetics of suspended $P.\ putida$ IsoF cultures and the AHL molecule 3-oxo-C10-HSL (Fekete et al., 2009).

Our biofilm model is on a mesoscopic scale, and so the computational domain is considered a small portion, or open subdomain, existing within a larger reactor. The boundary conditions we choose describe both the reactor type and the operating conditions in which the experiment is conducted, and connect the computational domain to the outside physical environment. Establishing boundary conditions for the mesoscopic scale is a difficult issue in biofilm modelling, and so we must make some assumptions. Our computational domain is representative of a microfluidics chamber which receives fluid at the left (inflow) boundary from a large, well mixed reactor. The inflow boundary acts as a hydrophilic membrane, allowing a specified amount of carbon and AHL into the channel, but preventing biomass from leaving. The outflow boundary permits carbon and AHL to be washed out with the fluid flow.

Specifically, the following boundary conditions are imposed on our domain $\Omega=[0,L]x[0,H]$:

- For $M_0, M_1, M_2$, and $EPS$, no flux conditions everywhere ($n$ is the direction of the outward normal):
Table 3.1: Model parameters used in our simulations and their non-dimensional values in the high and low nutrient cases. References: H=Horn et al. (2001), M=measured experimentally in Fekete et al. (2009), W=Wanner et al. (2006)

<table>
<thead>
<tr>
<th>Param</th>
<th>Description</th>
<th>Source</th>
<th>Value (high nutrient)</th>
<th>Value (low nutrient)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\kappa_{10}$</td>
<td>Rate of C consumption by $M_0$</td>
<td>W</td>
<td>462</td>
<td>1846</td>
</tr>
<tr>
<td>$\kappa_{11}$</td>
<td>Rate of C consumption by $M_1$</td>
<td>W</td>
<td>923</td>
<td>3692</td>
</tr>
<tr>
<td>$\kappa_{12}$</td>
<td>Rate of C consumption by $M_2$</td>
<td>W</td>
<td>462</td>
<td>1846</td>
</tr>
<tr>
<td>$\kappa_2$</td>
<td>Monod half sat. const.</td>
<td>W</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>$\kappa_3$</td>
<td>Max specific growth rate of bacteria</td>
<td>H</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$\kappa_4$</td>
<td>Lysis rate</td>
<td>W</td>
<td>0.2083</td>
<td>0.2083</td>
</tr>
<tr>
<td>$\kappa_5$</td>
<td>QS up-regulation rate</td>
<td>M</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>$\kappa_6$</td>
<td>Monod half sat. const.</td>
<td>H</td>
<td>0.005</td>
<td>0.08</td>
</tr>
<tr>
<td>$\delta$</td>
<td>EPS conversion to C rate (C equation)</td>
<td>H</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>$\hat{\delta}$</td>
<td>EPS conversion to C rate (EPS equation)</td>
<td>H</td>
<td>5.6</td>
<td>22.4</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Abiotic degradation rate of AHL</td>
<td>M</td>
<td>0.1109</td>
<td>0.1109</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Constitutive production rate of AHL</td>
<td>M</td>
<td>65.7</td>
<td>65.7</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Induced production rate of AHL</td>
<td>M</td>
<td>657</td>
<td>657</td>
</tr>
<tr>
<td>$n$</td>
<td>Degree of polymerisation</td>
<td>M</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>$\gamma_0$</td>
<td>$M_0$ EPS production rate</td>
<td>H</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>$\gamma_1$</td>
<td>$M_1$ EPS production rate</td>
<td>H</td>
<td>8.4</td>
<td>8.4</td>
</tr>
<tr>
<td>$\gamma_2$</td>
<td>$M_2$ EPS production rate</td>
<td>H</td>
<td>0.84</td>
<td>0.84</td>
</tr>
</tbody>
</table>

$$\partial_n M_0 = 0, \partial_n M_1 = 0, \partial_n M_2 = 0, \partial_n EPS = 0 \text{ on } \partial\Omega$$

- For C and AHL, no diffusive flux conditions everywhere except for on inflow, where we specify the bulk concentration:
\( C = 1, A = 0 \) for \( x = 0, \partial_n C = 0, \partial_n A = 0 \) everywhere else.

In summary, no biomass leaves or enters the flow channel. Carbon is supplied into the channel from the upstream boundary. No AHL enters the flow channel upstream, however, AHL and carbon in the dissolved liquid phase \( \Omega_1 \) may exit the system via convective transport.

### 3.2.2 Simulation setup and computation

We will use our model to investigate how quorum sensing impacts a growing biofilm, and when quorum sensing-regulated EPS production provides a benefit to the biofilm. Three different types of biofilms will be studied: quorum sensing (\( M_0, M_1 \) cells only), non-quorum sensing (\( M_2 \) cells only), and mixed (\( M_0, M_1, M_2 \) cells). Three nutrient conditions are tested: high, low, and feast-famine (high during feast periods, and no nutrients supplied during famine times: \( 4 \leq t \leq 6, 8 \leq t \leq 10, 14 \leq t \leq 16 \)).

The parameter values used for the high and low cases are given in Table 3.1. Simulations are performed with and without the biological process of EPS consumption; the parameters \( \delta \) and \( \hat{\delta} \) are set equal to zero when EPS consumption is excluded.

The majority of our simulations use a setup representative of a microfluidic chamber, sometimes used in biofilm experiments (e.g., Janakiraman et al., 2009), in which a surface is inoculated with several biofilm colonies, and nutrients are supplied at the inflow boundary of the channel. We will also simulate biofilms growing on a nutrient-rich substratum, in which nutrients are supplied via the bottom surface of the channel (the boundary condition \( C = 1 \) is imposed on \( y = 0, \) with \( \partial_n C = 0 \) elsewhere).
summary of the simulation experiments is given in Table 3.2. Our simulations will give us qualitative information about quorum sensing and biofilm systems. Numerical results, including time, are described using non-dimensional measures, and should not be deemed as quantitative conclusions.

Table 3.2: Summary of the simulation experiments. Three types of biofilms are studied: quorum sensing ($M_0, M_1$), non-quorum sensing ($M_2$), and mixed ($M_0, M_1, M_2$). Three different nutrient scenarios are used, high (H), low (L), and feast-famine (FF), with the nutrient source located at either the left inflow boundary or the bottom boundary. These biofilms are tested with and without the EPS consumption process. In total, 24 simulations were conducted.

<table>
<thead>
<tr>
<th>Biofilm Type</th>
<th>Nutrient Source</th>
<th>Nutrient Case</th>
<th>EPS consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_0, M_1$</td>
<td>Inflow</td>
<td>H,L,FF</td>
<td>yes, no</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>H</td>
<td>yes, no</td>
</tr>
<tr>
<td>$M_2$</td>
<td>Inflow</td>
<td>H,L,FF</td>
<td>yes, no</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>H</td>
<td>yes, no</td>
</tr>
<tr>
<td>$M_0, M_1, M_2$</td>
<td>Inflow</td>
<td>H,L,FF</td>
<td>yes, no</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>H</td>
<td>yes, no</td>
</tr>
</tbody>
</table>

The numerical solution of the biofilm model follows the approach that was developed and discussed in Eberl and Demaret (2007) and Eberl et al. (2009). The computational domain was discretised on a uniform rectangular grid of size 2000 x 200. We solve our system numerically using a semi-implicit finite difference-based finite volume scheme, formulated for the concentrations in the centers of the grid cells. The time-step size is variable; in each time step six sparse, banded diagonal linear algebraic systems are solved with the stabilized biconjugate gradient method (one system for each of $M_0, M_1, M_2, C, AHL,$ and $EPS$).

The code was prepared for parallel execution on multi-core/multi-processor platforms as described in Muhammad and Eberl (2009). Simulations were carried
out on a SGI ALTIX450 with 32 dual core itanium processors; in most simulations 12 cores were used per compute job.

3.2.3 Analysis

To interpret the results of computer simulations of our model, we will provide two-dimensional visualisations of the simulations, and use the following quantitative measures. The volume fraction of the domain occupied by the biofilm (cells and EPS), or the occupancy, is a simple measure of biofilm size. The occupancy is an average across the computational domain, and is given by:

$$\text{occupancy}(t) := \frac{1}{LH} \int_{\Omega_{z}(t)} dx \, dy.$$ 

The total downregulated cell biomass in the system is the volume fraction $M_{0\text{total}}$:

$$M_{0\text{total}}(t) := \int_{\Omega} M_{0}(t, x, y) \, dx \, dy.$$ 

The total upregulated ($M_{1}$) and non-quorum sensing cell ($M_{2}$) biomass are computed similarly. The total cell biomass is therefore:

$$M(t) = M_{0\text{total}} + M_{1\text{total}} + M_{2\text{total}}.$$
and the total EPS in the system is given by:

\[ EPS_{\text{total}}(t) := \int_{\Omega} EPS(t, x, y) \, dx \, dy. \]

Consumption of the carbon substrate by each of the cell species is measured as well, for example,

\[ M_{0\text{con}}(t) = \int_{\Omega} \frac{\kappa_{10}C(t, x, y)M_0(t, x, y)}{\kappa_2 + C(t, x, y)} \, dx \, dy \]

where \( M_0 \) has the consumption rate \( \kappa_{10} = \kappa_3 M_{\text{max}}/Y_0 \). Consumption by \( M_1 \) and \( M_2 \) are calculated accordingly. The occupancy and total cell and EPS biomass measures will be used to compare the growth and composition of the biofilm over time.

The amount of AHL in the system, averaged across the computational domain, is given by:

\[ AHL_{\text{average}}(t) := \frac{1}{LH} \int_{\Omega_2(t)} AHL \, dx \, dy. \]

AHL is dissolved, and is found in both the liquid and solid regions of the domain.
3.3 Results

3.3.1 Example simulation of a QS biofilm under high nutrient conditions

To simulate biofilm growth in a long, narrow channel, we begin by inoculating the bottom surface of the channel with twenty downregulated biofilm microcolonies with densities ranging from $0.2 < M_0 < 0.4$, placed randomly along the channel. No $M_2$ cells are present in the channel, so a quorum sensing biofilm will develop. The flow velocity is $Re = 10^{-4}$ for all simulations. A high supply of substrate enters the channel from the left (inflow) boundary, and the process of EPS consumption is excluded. Note that initially, biomass consists of cells only; EPS production begins immediately upon the start of the simulation.

The growth period begins with biomass in the inoculated colonies growing and spatially spreading when the total biomass ($M_0 + M_1 + EPS$) locally approaches the maximum density, 1.0. In time, some neighbouring colonies begin to merge. Figure 3.1(a) depicts the biofilm at $t = 8.0$, before induction occurs. The colour scale in Figure 3.1 represents the fraction of downregulated cells in the biofilm ($M_0/(M_0 + M_1)$); red colonies are completely downregulated, and blue colonies are fully upregulated. In Figure 3.1(a), the colonies consist almost entirely of $M_0$ cells.

Isolines of AHL concentration are distributed between 0 and the maximum AHL value at the given time. AHL accumulates over time in the channel as the colonies grow and produce AHL. The maximum concentration changes over time, and so the AHL isoline colour scale is given in each subfigure of Figure 3.1 along with the maximum AHL value. Molecules produced by the colonies diffuse into the
(a) $t=8.0$. $M_0, M_1$ cells and AHL isolines, before quorum sensing induction occurs. Max AHL=0.61.

(b) $t=10.0$. $M_0, M_1$ cells and AHL isolines, downstream colonies have upregulated. Max AHL=3.40.

(c) $t=10.5$. $M_0, M_1$ cells and AHL isolines, after induction has occurred. Max AHL=4.65.

(d) Colour scale. Fraction of $M_0$ cells.

Figure 3.1: Example simulation of a quorum sensing biofilm in the case of high nutrient conditions, and no EPS consumption. The colour scale in the subfigures represents the fraction of downregulated cells in the biofilm ($M_0/(M_0 + M_1)$). The change in colour depicts induction occurring throughout the biofilm. AHL concentration lines are shown in black and white, distributed between zero and the maximum AHL value at each time. A non-dimensional value of AHL=1.0 is required for upregulation.
Figure 3.2: Example simulation of a QS biofilm in the case of high nutrient conditions, and no EPS consumption. The colour scale in the subfigures represents the fraction of cells biomass in the biofilm \( \left( \frac{M_0 + M_1}{M_0 + M_1 + EPS} \right) \), showing mass composition of the biofilm (the proportions of cellular and EPS biomass).

A non-dimensional AHL concentration of 1.0 is required for local upregulation. As the average AHL concentration approaches 1.0, the switch to quorum sensing is occurring. After the switch, AHL concentrations rapidly increase due to positive feedback in the quorum sensing system — upregulated cells produce AHL at ten times the downregulated rate, leading to large increases in AHL concentrations, and further upregulation of cells throughout the domain.

The upstream colonies in Figures 3.1(b,c) are larger than the mid-channel and downstream colonies, because they are situated near the nutrient source, and so they are first to receive and utilize the incoming nutrients. Although the downstream
colonies are the smallest, they begin to upregulate first, indicated by the colour change in Figure 3.1(b). The effect of mass transfer — AHL molecules produced by the large, upstream colonies are transported downstream, contributing to upregulation there — is an observation of inter-colony communication. In our previous studies of the impact of a growing biofilm on quorum sensing in a hydrodynamic environment (Frederick et al., 2010), similar spatial induction patterns were found. Convective and diffusive transport of AHL facilitate the inter-colony communication; downstream colonies are first to upregulate, followed by the upstream colonies.

The biofilm at $t = 10.5$ (Figure 3.1(c)) is fully upregulated, with the exception of the portion of the large, merged colonies closest to the inflow boundary, which still contains approximately 40-50% $M_0$ cells. After induction, EPS production increases by a factor of ten. The biofilm grows and expands rapidly, until the flow channel becomes clogged with biomass (a maximum predetermined biofilm height is obtained), stopping the simulation. In our earlier study, the simulations also ended shortly after induction was obtained. Now, because of the rapid growth phase following quorum-sensing induced EPS production, the observation period for a fully induced biofilm before channel clogging occurs will be even more brief.

In Figure 3.2, the biofilm is shown again at $t = 8.0$ and 10.5, with the colour scale representing the proportion of cellular and EPS biomass (the fraction $(M_0 + M_1)/(M_0 + M_1 + EPS)$). The concentration isolines of the carbon nutrient $C$ are shown, where the concentrations are normalized with respect to the value of the incoming carbon concentration (the non-dimensional parameter $C_{bulk}$, where $C_{bulk} = 1$). Carbon concentrations decrease in the flow direction, due to consumption
by biomass. At $t = 10.0$ (Figure 3.2(b)), mid-channel and downstream colonies experience severe substrate limitations. By $t = 10.5$ (Figure 3.2(b)), only the most upstream part of the large, merged colony does not experience limitations. In Figure 3.2(b), the biofilm has just undergone induction, and the biomass composition by mass is approximately 75-85% cells, and the remainder is EPS. Following induction, EPS production rates are upregulated, resulting in a change in biofilm composition to 55-75% cells, 25-45% EPS. The portion of the large, merged, upstream colony with the highest fraction of upregulated cells experienced the greatest increase in volume - in order for colonies to have increased growth due to upregulated EPS production rates, upregulated cells and adequate nutrients are required. So although the downstream colonies are first to upregulate, these colonies lack the nutrients needed to expand. The upregulated bacteria cells in the downstream region are genetically programmed to produce EPS at induced rates, but require sufficient carbon substrate to carry out this reaction.

In Figure 3.3, the spatially averaged measures introduced in Section 3.2.3 are plotted, describing biofilm growth, composition, and consumption in time. In Figure 3.3(a), the biofilm occupancy in time is plotted on a logarithmic scale, and shows that cellular and EPS biomass growth is maximal. A decrease in the slope of the occupancy curve indicates continued growth, but at a lower rate. This is generally observed in later timesteps, when the nutrient demand begins to exceed the inflow of bulk substrate. Figure 3.3(b) shows the total $M_0, M_1$, and EPS biomass constituting the biofilm in time. Initially, only downregulated cells grow, until the switch to upregulation occurs around $t = 9.0$. Subsequently, $M_0$ cells are upregulated,
Figure 3.3: Spatially averaged results for the example simulation of a quorum sensing biofilm with high nutrient supply, excluding EPS consumption.
and the total $M_0$ biomass in the biofilm declines as $M_1$ cell representation increases. $M_1$ produces EPS at the induced rate, so EPS biomass increases after the switch as well. The effects of quorum sensing on carbon consumption are seen in Figure 3.3(c), which shows consumption by the $M_0$ and $M_1$ cells in time. Initially, $M_0$ cells are the primary consumers of carbon, until induction occurs, after which we see the rise in consumption by the $M_1$ cells.

Figures 3.3(d) and (e) demonstrate the composition of the biofilm as it grows in time. Figure 3.3(d) shows the fraction of downregulated bacteria, of all bacteria in the microcolonies ($M_0/(M_0 + M_1)$). Initially, only $M_0$ cells are present in the biofilm, until induction occurs, and cells switch to the upregulated state. Upon completion of the simulation at $t = 10.5$, the biofilm has not fully upregulated, and $M_0$ cells comprise approximately 35% of the bacterial biomass; we know from Figure 3.1(c) that these downregulated cells remain in the large, merged upstream colony.

Figure 3.3(e) shows the fraction of bacterial biomass of all biomass in the biofilm ($M_0 + M_1)/(M_0 + M_1 + M_2)$). The proportion of bacteria to EPS within the biofilm changes in time. The simulation experiment began with an inoculation of $M_0$ cells only, and so the fraction of bacteria cells initially declines due to production of EPS. By $t = 2.0$, the biofilm is composed of approximately 85% cells, and 15% EPS. The decline in proportion of bacteria after $t = 9.0$ corresponds to upregulation, when $M_1$ bacteria produce EPS at the increased rate.

This example simulation of a quorum sensing biofilm under high nutrient conditions provides insight into the connection between quorum sensing, growth, and EPS production in a biofilm. Biofilm colonies grow and merge over time, and quorum
sensing is induced first in downstream colonies, and soon after, upstream colonies are induced. Upregulated colonies situated in nutrient-rich areas produce EPS at the induced rate, grow rapidly, and consume high levels of nutrients. These colonies are composed of almost equivalent amounts of cellular and EPS biomass, versus upregulated colonies in nutrient-deprived locations, which are smaller in size and contain a greater proportion of cellular biomass. In the next sections, we will investigate the responses of biofilms to quorum sensing under various nutrient conditions, the process of EPS consumption, and simulation environments.

3.3.2 Simulations without EPS consumption process

Quorum sensing and non-quorum sensing biofilms

In this section, results of the simulations excluding the process of EPS consumption are presented. We begin by describing a quorum sensing (QS) biofilm ($M_0, M_1$ cells), under high, low, and feast-famine nutrient conditions. Simulations finish once a maximum biofilm height is reached (80% of the channel height). The nutrient supply directly influences growth rates, and so the simulations finish earliest for the high nutrient case, followed by the low nutrient case, and finally the feast-famine case. In Figure 3.4, the QS biofilms at the end of the simulations are plotted to show the size and composition of the microcolonies. Isolines of AHL are shown in black and white; note that the maximum AHL concentration is different in each subfigure. The colour scale represents the proportion of downregulated cells, of all bacteria cells ($M_0/(M_0 + M_1)$).
In the high nutrient case (Fig.3.4a), AHL concentrations are the greatest (maximum: 4.65). A large, merged colony is present at the upstream boundary, and the downstream colonies are smaller in size. The most upstream portion of the large, merged, colony is not fully upregulated, approximately 40-50% of the cells are $M_0$. The biofilm in the low nutrient case (Fig.3.4b) contains a large, merged colony in the upstream region as well, though smaller than that which grew in the high nutrient case. The upstream colony is first to receive and utilize any incoming nutrients, leaving very little, if any, carbon for the downstream colonies to grow and expand. In the low nutrient case, the maximum AHL concentration at the end of the simulation is well below the induction threshold of 1.0, and so the biofilm is comprised of downregulated cells only. Consequently, QS-induced EPS production cannot occur. Although AHL is produced by the large, merged, upstream colony, AHL is transported out of the channel by convection and diffusion, and so AHL does not accumulate to the threshold level. The biofilm in the feast-famine conditions (Fig.3.4c) appears quite similar to the QS biofilm with respect to colony sizes and composition under high nutrient conditions, though the imposed starvation periods delayed growth of this biofilm. AHL concentrations are slightly lower than those in the high nutrient case, but have passed the induction threshold.

Figure 3.5 shows the spatially averaged results for these three biofilm simulations, and numerical values for the occupancy, total bacteria, and total consumption are given in Table 3.3. The occupancy over time of the three QS biofilm simulations is shown in Fig. 3.5a; note that occupancy is inclusive of both cells and EPS. The final occupancy is greatest for the high nutrient case, followed by feast-famine, and
(a) **High nutrient case,** $t=10.5$. The biofilm is almost entirely upregulated.

(b) **Low nutrient case,** $t=13.0$. The biofilm did not upregulate.

(c) **Feast-famine nutrient case,** $t=18.0$. The biofilm is almost entirely upregulated.

(d) **Colour scale.** Fraction of $M_0$ cells.

Figure 3.4: Size and extent of upregulation for QS biofilms under the three nutrient scenarios, at the end of each simulation. EPS consumption was excluded. The same initial inoculation of microcolonies was used. Isolines of AHL are shown in black and white, note that the maximum AHL concentration differs in each subfigure. The colour represents the fraction of downregulated ($M_0$ cells) in the biofilm. The biofilm colonies in the low nutrient case (b) are quite small, except for the furthest upstream colony. The biofilm in the feast-famine case (b) attains induction and a size comparable to the high nutrient case (a), but at a much later time.
Lastly the low nutrient case. In the low nutrient case, deviation from the line of maximal growth occurs at an early stage of development ($t < 5.0$). The famine periods ($4.0 \leq t \leq 6.0, 8.0 \leq t \leq 10.0, 14.0 \leq t \leq 16.0$) are apparent. The occupancy remains constant during the famine periods, indicating that the biofilm is neither growing nor shrinking. A lag period is present between the return of the “feast” nutrient supply ($t = 6.0, 10.0, 16.0$), and subsequent rise in occupancy, indicative of the time required for maximum cell and EPS density to increase to the point of colony expansion. The total cell biomass (Fig.3.5c) in time further demonstrates the direct relationship between nutrient supply and cell growth. In famine periods, all populations decline, and grow exponentially when the food supply returns. Consumption of the carbon nutrient in time is shown in Fig.3.5e. The sharp rise in carbon consumption in the high nutrient and feast-famine cases corresponds to QS induction, because upregulated cells consume nutrients at twice the rate of downregulated cells. The composition of the biofilm over time is shown in Fig.3.5g. After the initial time period in which EPS production begins, the three QS biofilms are composed of approximately 85% cells, 15% EPS. The proportion of EPS rapidly increases when induction occurs ($t = 9.0$ for the high nutrient case, $t = 16.0$ for the feast-famine case). Induction does not occur under low nutrient conditions, and so the proportion of cells remains approximately 85% throughout the entire simulation. Declines in the proportion of cellular biomass in the feast-famine condition correspond to loss of bacteria cells in the famine time periods.

The same initial inoculation was used to grow a non-quorum sensing (non-QS) biofilm ($M_2$ cells only) under the three nutrient conditions. The spatially aver-
Figure 3.5: Spatially averaged results for QS and non-QS biofilms under various nutrient conditions, EPS consumption excluded. Qualitative trends in occupancy, cell biomass growth, and consumption are similar for QS and non-QS biofilms. Occupancy is higher for QS biofilms, but total cell biomass is higher for non-QS biofilms. Increased C consumption by upregulated cells is seen in (e), and the increase in proportion of EPS biomass in the biofilm is seen in (g).
Table 3.3: Simulation experiment results for QS ($M_0, M_1$) and non-QS ($M_2$) biofilms. These biofilms were tested under three different nutrient scenarios, with and without the EPS consumption process. The reported values were taken at $t = 10.57$ for the high nutrient case, $t = 12.86$ for low nutrient, and $t = 17.52$ for feast-famine (FF).

<table>
<thead>
<tr>
<th>Biofilm Type</th>
<th>Nutrient Case</th>
<th>EPS Con.</th>
<th>Occupancy</th>
<th>Total Cell Biomass</th>
<th>Total Con.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_0, M_1$</td>
<td>High</td>
<td>yes</td>
<td>0.2005</td>
<td>1748</td>
<td>8.15E+04</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>yes</td>
<td>0.0794</td>
<td>1450</td>
<td>5.84E+04</td>
</tr>
<tr>
<td></td>
<td>FF</td>
<td>yes</td>
<td>0.1769</td>
<td>1367</td>
<td>3.96E+04</td>
</tr>
<tr>
<td>$M_2$</td>
<td>High</td>
<td>yes</td>
<td>0.1466</td>
<td>1936</td>
<td>1.46E+04</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>yes</td>
<td>0.0794</td>
<td>1452</td>
<td>7.53E+04</td>
</tr>
<tr>
<td></td>
<td>FF</td>
<td>yes</td>
<td>0.1194</td>
<td>1589</td>
<td>0.740E+04</td>
</tr>
<tr>
<td>$M_0, M_1$</td>
<td>High</td>
<td>no</td>
<td>0.2940</td>
<td>1733</td>
<td>12.6E+04</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>no</td>
<td>0.1575</td>
<td>1414</td>
<td>12.6E+04</td>
</tr>
<tr>
<td></td>
<td>FF</td>
<td>no</td>
<td>0.3059</td>
<td>1348</td>
<td>9.51E+04</td>
</tr>
<tr>
<td>$M_2$</td>
<td>High</td>
<td>no</td>
<td>0.2003</td>
<td>1931</td>
<td>1.85E+04</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>no</td>
<td>0.1564</td>
<td>1415</td>
<td>16.2E+04</td>
</tr>
<tr>
<td></td>
<td>FF</td>
<td>no</td>
<td>0.1899</td>
<td>1581</td>
<td>1.20E+04</td>
</tr>
</tbody>
</table>

Aged results for occupancy, cell biomass, consumption, and composition are shown in Figure 3.5, alongside the plots for the QS biofilms, and the numerical values are summarized in Table 3.3. The non-QS biofilms display similar patterns for biomass growth as the QS biofilm: under high nutrient conditions, occupancy and cell biomass are greatest. However, because $M_2$ cells do no change behaviour with upregulation, consumption and composition of biomass do not vary in later timesteps.

The differences between simulations of QS and non-QS biofilms are apparent by the values recorded in the summary table (Table 3.3). QS biofilms which upregulated (high nutrient and feast-famine cases) had higher occupancies than the non-QS biofilms. In contrast, the non-QS biofilms had higher bacteria cell counts (see the "Total Bacteria" column in Table 3.3). So, the greater QS biofilm occupancies are
not due to additional bacteria cells, but are due to the presence of EPS produced at
the induced rates. Although QS biofilms have fewer bacteria cells, the total nutrient
consumption over the entire simulation period is greater than the consumption by QS
biofilms. In summary, QS biofilms occupy more volume in the channel and consume
a greater amount of nutrients than non-QS biofilms, but non-QS biofilm have higher
cell populations.

Mixed biofilms

The following results pertain to the set of simulations of mixed biofilms under
the high nutrient case. Mixed biofilms contain both QS cells ($M_0, M_1$) and non-QS
cells ($M_2$) in the channel. To set up the simulation, the substratum was inoculated
with twenty randomly placed colonies - ten downregulated QS ($M_0$) colonies, and ten
non-QS ($M_2$) colonies. An example of growth of a mixed biofilm over time is shown
in Figure 3.6. Isolines of AHL are shown in black and white; again, note that the
maximum AHL concentration differs in each subfigure, and a non-dimensional AHL
concentration of 1.0 is required for upregulation. Figure 3.6a shows the biofilm at
t = 6.0, an early development stage. The colour represents the fraction of quorum
sensing ($M_0, M_1$) cells in the biofilm, relative to all cells ($(M_0 + M_1)/(M_0 + M_1 + M_2)$).
The quorum sensing colonies are red, the non-quorum sensing colonies are blue. Some
colonies, which were placed close to each other in the random inoculation, have merged
by this time, these are yellow-green in colour. The microcolonies are approximately
equal in size, as carbon supply is sufficient for maximal growth at this time. AHL
concentrations are very low. QS induction has not yet occurred, and C is not limited,
so EPS is produced at equivalent rates by all the QS and non-QS colonies. At $t = 9.0$ (Fig. 3.6b), merging of QS and non-QS colonies is prevalent in the mid-channel, though colonies at the upstream and downstream extent of the biofilm remain exclusively QS or non-QS. AHL concentrations are still considerably lower than the induction threshold, indicating cells in the QS and merged colonies are $M_0$, not yet upregulated to $M_1$.

Merging of colonies continues until $t = 10.5$ (Fig. 3.6c), shortly before the simulation completes due to maximum channel height being obtained. AHL concentrations have not accumulated to the threshold concentration, and so QS induction did not occur for this mixed biofilm simulation. This implies that the mixed biofilm is growing as a non-QS biofilm (in regards to EPS production, cell growth, and consumption). Note that in this example, the colony closest to the inflow boundary is a QS colony, and several of the furthest downstream colonies, where nutrient deficiencies are highest, are non-QS colonies.

Because the resulting QS and non-QS cell numbers may be impacted by the stochastic distribution of cells in the initial inoculation, in particular whether a QS or non-QS colony is located closest to the upstream boundary, this mixed biofilm experiment was repeated using different initial distributions, for a total of ten simulations. The total QS and non-QS biomass averaged over all ten simulations is shown in Figure 3.7a, and the QS and non-QS cellular biomass for each of the individual simulations is shown in Figures 3.7b,c. The average of the final biomass in each individual simulation is recorded in Table 3.4, in which the results are separated into mixed biofilms that induced and biofilms that did not induce. Of these ten simulations, induction
Figure 3.6: Simulation of the growth of a mixed biofilm under high nutrient conditions, without the process of EPS consumption. The biofilm is shown at three different time steps throughout the simulation, with contour lines showing the AHL concentration gradient. Colonies are coloured to represent the fraction of cells which are QS ($M_0, M_1$) or non-QS ($M_2$): $((M_0 + M_1)/(M_0 + M_1 + M_2))$. In this example, the QS colonies do not upregulate.
Table 3.4: Cell biomass (“Total $M_0, M_1$” and “Total $M_2$”) and consumption (“Total Con.”) in the 20 mixed biofilm simulations. These biofilms were tested under the high nutrient case, with and without the EPS consumption process. The total cell biomass and EPS consumption values are taken as the average of the values at the end of each individual simulation. The values reported are separated into the biofilms that did and did not reach induction; the number of corresponding simulations are given in brackets.

<table>
<thead>
<tr>
<th>Biofilm</th>
<th>EPS Con.</th>
<th>Induced</th>
<th>Total $M_0, M_1$</th>
<th>Total $M_2$</th>
<th>Total Con. (QS)</th>
<th>Total Con. (Non-QS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_0, M_1, M_2$</td>
<td>no</td>
<td>yes (1)</td>
<td>920</td>
<td>2080</td>
<td>1.75E+04</td>
<td>2.14E+04</td>
</tr>
<tr>
<td>$M_0, M_1, M_2$</td>
<td>no</td>
<td>no (9)</td>
<td>1124</td>
<td>1339</td>
<td>4.84E+04</td>
<td>3.97E+04</td>
</tr>
<tr>
<td>$M_0, M_1, M_2$</td>
<td>yes</td>
<td>yes (3)</td>
<td>1453</td>
<td>809</td>
<td>5.94E+04</td>
<td>1.25E+04</td>
</tr>
<tr>
<td>$M_0, M_1, M_2$</td>
<td>yes</td>
<td>no (7)</td>
<td>1120</td>
<td>1407</td>
<td>2.55E+04</td>
<td>3.11E+04</td>
</tr>
</tbody>
</table>

only occurred once. In the simulation in which the biofilm induced, QS cells outnumbered the non-QS cells. Interestingly, the furthest upstream colony in the induced simulation was a non-QS colony, while QS colonies were situated in the mid-channel and downstream. Though colonies in these regions tend to be small, adequate AHL was produced and accumulated for upregulation to occur. In this one case, total consumption by QS cells is nearly five times the amount of carbon consumed by non-QS cells, as upregulated QS cells have twice the nutrient demand. The simulation which reached induction is noticeable in the consumption plot (Fig.3.7e) by the line which has a sharp increase in slope near the end of the simulation, corresponding to increased consumption by induced cells. In Fig.3.7b, the QS cell population declined in one simulation in the second half of the simulation, in contrast to the non-QS cell population in Fig.3.7c, which do not suffer declines in time.
Figure 3.7: Consumption and biomass of QS and non-QS colonies in mixed biofilms. High nutrient case, EPS consumption excluded. Figures (a) and (d) show the average biomass and consumption, and standard deviation, for the ten simulations; total cell biomass (b,c) and consumption (e,f) for each individual simulation are shown as well. On average, non-QS ($M_2$) cells outnumber the QS ($M_0, M_1$) cells, and consume a greater amount of nutrients. Of the ten simulations, quorum sensing induction only occurred once.
3.3.3 Simulations with EPS consumption process

An additional complexity was considered in our biofilm simulations to account for the utilization of EPS by bacteria cells as a secondary source of the carbon nutrient, when limitations occur. The experiments of Section 3.3.2 were repeated, but with consideration of this biological process. In the early stages of biofilm development ($t \leq 5.0$), the results are expected to be quite similar, as carbon limitations are generally present only in the second half of the simulation. Spatial gradients of EPS and carbon distribution may potentially lead to different trends in biofilm growth and total cell biomass.

Quorum sensing and non-QS biofilms

In this set of simulations, QS biofilms were grown separately in the channel, again using the same initial distribution of colonies as Section 3.3.2, under high, low, and feast-famine nutrient conditions. “Biofilm composition” refers to the percentage by mass of cells and EPS comprising the biofilm. Figure 3.8 shows the biofilm composition by cellular biomass over time for each of these simulations. The high and feast-famine nutrient conditions for the QS biofilm demonstrate how biofilm composition changes in time with QS-induced EPS production and EPS consumption included. In all simulations, the proportion of cells in the biofilm lowers from 100% to 85-90%, representing the initial stages of EPS production by bacteria cells. When nutrient deficiencies begin to occur, around $t = 7.0$ for the high and low nutrient cases, and during the famine periods ($4.0 \leq t \leq 6.0, 8.0 \leq t \leq 10.0, 12.0 \leq t \leq 14.0$),
Figure 3.8: The fraction of QS and non-QS biofilms that is cellular biomass, under various nutrient conditions, for the EPS consumption case. The biofilm is composed of both cellular and EPS biomass.

The EPS biomass is depleted by the cells which require additional available nutrients, and the proportion of cells in the biofilm rises. During famine periods, EPS is completely depleted, until the feast periods restart, and the cells have adequate nutrients to grow and produce EPS again. After induction occurs (Fig.3.8a), at approximately $t = 9.0$ for the high nutrient case, $t = 14.0$ for the feast famine case, the fraction of cell biomass in the biofilm begins to decline, with EPS being produced at upregulated rates.

The numerical values for occupancy, total bacteria, and total consumption at the end of these six biofilm simulations are listed in Table 3.3. The trends are similar to the simulations in Section 3.3.2 for the biofilms in which EPS is not consumed. The greatest occupancy, of all simulations, in fact, is found for the QS biofilm under feast-famine conditions, followed by the high nutrient condition, and finally for low nutrient supply. The occupancy is lower for the biofilms which use EPS as a nutrient source. Occupancy includes EPS, and so when EPS is lost through consumption, lower occupancies result.
The non-QS biofilm is again found to have a higher total bacteria cell count and lower total carbon consumption than for induced QS biofilms, as was also found for biofilms which did not consume EPS. Upregulated cells have a high nutrient demand due to the requirement for induced EPS production, and if nutrient supply is insufficient, non-QS biofilm cells will reach higher populations than QS cells.

**Mixed biofilms**

Ten additional simulations of mixed biofilms ($M_0, M_1, M_2$) under the high nutrient condition were performed, with the EPS consumption process included. Figure 3.9 shows the development of one mixed biofilm at $t = 6.0$ (an early development stage) and $t = 13.0$ (the end of the simulation). In Fig.3.9a,b, the colour scale represents the fraction of QS cells, of all cells in the biofilm. At $t = 6.0$, the individual QS and non-QS colonies are apparent, note that the furthest upstream colony is a QS colony. AHL concentrations are well below the threshold for induction. By $t = 13.0$, the switch to QS has occurred, resulting in increased growth and expansion of QS colonies from high EPS production rates. The large, merged upstream colony is mixed, but dominated by QS cells: $M_0, M_1 > 75\%, M_2 < 25\%$.

The fraction of cells and EPS in the biofilm at these times are plotted in Figure 3.9d,e, with the nutrient concentrations. The nutrient levels are sufficient at $t = 6.0$ (Fig.3.9d), and the composition of the biofilm is uniformly 85% cells, 15% EPS. Nutrient supply is plentiful, and induction has not occurred, so the $M_0$ and $M_2$ cells in the biofilm are producing equivalent amounts of EPS. At $t = 13.0$, the biofilm composition is quite different. Carbon is severely limited in the mid-
Figure 3.9: An example of a mixed biofilm under high nutrient conditions, EPS consumption case, before and after induction. Subfigures (a,b) show the distribution of QS and non-QS cells, (d,e) show the composition of the biofilm by EPS and cellular biomass.
channel and downstream. Although the downstream cells have upregulated, they lack the nutrients required for production of EPS at the induced rate, and the nutrient deficiencies have caused bacteria cells to consume any available EPS. As a result, the biofilm is composed only of cells in these nutrient deficient regions. The upmost upstream portion of the large, merged colony consists of both cells and EPS. In this area, upregulated cells have access to the nutrients required to produce EPS at the induced rate, and do not need to consume the local EPS for additional nutrients.

The EPS consumption process allows for more pronounced spatial differences in biofilm composition to be seen, in comparison to simulations which exclude EPS consumption. In regions of high nutrient availability, the biofilm comprises bacteria cells and EPS - high levels of EPS, if bacteria cells are upregulated - and where nutrients are lacking, the biofilm is composed entirely of cells, with no protective layer of EPS. In this example, QS cells outnumbered non-QS cells in the mixed biofilm at the end of the simulation, and induction occurred. Of the ten repetitions of this experiment, induction occurred three times, which is more frequent than the one case of induction obtained in the simulations excluding EPS consumption. The average QS and non-QS biomass for these simulations is given in Table 3.4, separated into the biofilms which did and did not attain induction. When induction was obtained, 80% more QS biomass was present than non-QS biomass, and consumption was almost five times as high. In contrast, in cases where induction was not obtained, the biofilm contained 26% more non-QS cells than QS cells, similar to what was observed in the mixed biofilms excluding the EPS consumption process. Of the three simulations which induced, two contained QS colonies at the upstream boundary, and one con-
tained a non-QS colony. Of the seven simulations which did not induce, four had QS colonies at the upstream boundary, and three had non-QS colonies. This indicates that placement of a QS colony closest to the nutrient source does not necessarily imply whether induction will occur or not. In the ten mixed simulations, the QS cell populations do not decline, but in two simulations, non-QS cell populations decrease near the end of the experiment. In fact, induction of QS colonies was obtained in the two simulations in which declines of non-QS cell populations were observed, implying that rises in QS populations could have a detrimental effect on non-QS cell populations.

3.3.4 Bottom-feeding biofilms

Three additional simulations were conducted to study biofilm development in an environment where the source of incoming nutrients is the bottom boundary of the channel. This experimental setup is representative of biofilms which grow naturally on a nutrient-rich substratum, such as plant root nodules, rather than relying on nutrients to be flushed into a channel, as our flow chamber setup in the previous sections modelled. We expect microcolony growth to be uniform, as colonies receive equivalent amounts of nutrients regardless of their location along the channel. Flow is included in the simulations, which could impact nutrient distributions, i.e. if excess carbon present is transported through the channel via convection and diffusion. This setup may provide insight into the development of QS, non-QS, and mixed biofilms under ideal growth conditions.

Figure 3.10 shows the growth of QS and non-QS biofilms, without the EPS
Figure 3.10: Bottom-feeding biofilms: QS and non-QS biofilms grown with nutrient supplied from the channel bottom. Biofilm colony growth is uniform when food is supplied from the bottom boundary, and AHL concentration gradients are not present. The QS biofilm (a,c) grows quicker than the non-QS biofilm (b,d,e), due to QS-induced EPS production.
consumption process, at an early stage of development \((t = 5.5, \text{Figs.3.10a,b})\), and late stage of development \((t = 9.0, \text{Figs.3.10d,e})\). The simulations finish when the biofilm grows to a maximum height in the channel. The QS biofilm reaches the maximum height around \(t = 9.0\), and the non-QS biofilm reaches the maximum height later \((t = 11.0)\). The colour scale represents the fraction of cells in the biofilm, showing composition of cellular and EPS biomass. Carbon concentration isolines are shown for the later development stages.

In Figs.3.10a,b, both the QS and non-QS biofilms are quite small. No carbon limitations are present, and the AHL concentration in Fig.3.10b is approximately 0.30 throughout the entire channel; AHL concentration gradients are not present. The biofilms are composed of 85% bacteria cells, 15% EPS. By \(t = 9.0\), induction has occurred for the QS biofilm (Fig. 3.10c). AHL concentrations are very high, \(\text{AHL}=26.0\), again with no spatial concentration gradient in the channel. Slight carbon deficiencies are experienced by the top of the colonies, which are located furthest from the nutrient source. The large, merged upstream colonies, typical of flow chamber simulations, are absent when nutrients are supplied from the channel bottom. As anticipated, colony development is uniform throughout the channel. Because the upregulated cells in the biofilm of Fig. 3.10c are producing EPS at the induced rate, the composition of this biofilm is 50% cells, 50% EPS. The non-QS biofilm at \(t = 9.0\) (Fig.3.10d), is much smaller than the induced QS biofilm, as EPS is produced at the downregulated rate. The proportion of cells remains at 85%.

The QS biofilm simulation completes when the maximum height is obtained, around \(t = 9.0\). The non-QS biofilm continues to grow until it too obtains the
maximum height in the channel \((t = 11.0, \text{Fig.3.10e})\). The biofilm colonies have merged into one large colony in the centre of the domain, and remain composed of 85% cellular biomass, 15% EPS biomass.

Figure 3.11 shows the growth of a mixed biofilm on a nutrient-rich channel bottom, at \(t = 7.0\), before QS induction, and \(t = 9.0\), shortly before completion of the simulation. Figs.3.11a,b show the proportion of QS and non-QS cells in the biofilm. At \(t = 7.0\) (Fig.3.11a), the AHL concentration in the channel is 0.60, so the QS cells are downregulated. Only three QS and non-QS colonies have merged at this point. The composition of the biofilm (Fig.3.11d) is uniformly 85%. By \(t = 9.0\), QS induction has occurred, and AHL concentration is 19.0 throughout the channel. The largest merged colonies consist of either 50% QS cells, 50% non-QS cells, or QS cells exclusively (e.g., the large, merged QS colony at the downstream boundary).

The proportion of cells and EPS differs depending on whether \(M_1\) or \(M_2\) cells are present (Fig.3.11e). The biofilm contains greater amounts of EPS (up to 50%) where upregulated QS cells are found, and remain between 75-85% where the non-QS cell colonies are growing. The total cell biomass for all the simulations is given in Table 3.5. The simulations were conducted with and without the EPS consumption process; only those without EPS consumption are recorded in the table. Because nutrient limitations were not generally observed with this simulation setup, with the exception of the highest portions of the biofilm colonies in the late development stages, the influence of the EPS consumption process was minimal.

The total cell biomass at the end of each simulation is given; the simulation times differ based on the induction time of QS and mixed biofilms; after induction
Figure 3.11: Bottom-feeding biofilms: mixed biofilms grown with food supply on the bottom of the channel, before and after induction. Figures (a) and (b) show the fraction of QS and non-QS cells, and Figures (d) and (e) show the fraction of cellular and EPS biomass in the biofilm composition. QS and non-QS cell populations are equivalent upon completion of the simulation, despite the fact that QS colonies occupy more space.
Table 3.5: Cell populations at the end of simulation of QS, non-QS, and mixed biofilms grown on a nutrient-rich substratum, without the EPS consumption process. The occupancy and total biomass values at the end of each respective simulation are reported. The non-QS biofilm takes longer to grow to the maximum channel height, but contains considerably more bacterial biomass than the QS biofilm, which underwent enhanced EPS production. In the mixed biofilm, QS and non-QS cell populations are equivalent.

<table>
<thead>
<tr>
<th>Biofilm</th>
<th>Time</th>
<th>Occupancy</th>
<th>Total ( M_0, M_1 ) Biomass</th>
<th>Total ( M_2 ) Biomass</th>
<th>Total EPS biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>( M_0, M_1 )</td>
<td>8.73</td>
<td>0.4356</td>
<td>1033</td>
<td></td>
<td>1503</td>
</tr>
<tr>
<td>( M_2 )</td>
<td>10.88</td>
<td>0.4668</td>
<td>5208</td>
<td>931</td>
<td></td>
</tr>
<tr>
<td>( M_0, M_1, M_2 )</td>
<td>9.23</td>
<td>0.3701</td>
<td>753</td>
<td>754</td>
<td>1162</td>
</tr>
</tbody>
</table>

Figure 3.12: Bottom feeding biofilm: spatially averaged results of QS, non-QS, and mixed biofilms grown on a nutrient-rich substratum, without the EPS consumption process.

those biofilms quickly reach the maximum height. At the end of the simulation, the non-QS biofilms contain five times as many bacteria cells as the QS biofilms. The QS biofilms consist of 61% more EPS biomass than the non-QS biofilms. Because QS biofilms increase their EPS production rates upon induction, their growth is enhanced, but cell populations do not reach the levels of non-QS biofilms. In the mixed biofilm, \( M_1 \) and \( M_2 \) cell populations are equivalent at the end of the simulation, even though the QS colonies were observed to occupy more space than non-QS colonies (Fig.3.11).
Figure 3.12a shows the occupancy for these three simulations. The non-QS biofilm grows exponentially throughout the entire simulation, as nutrient supply is sufficient. The exponential growth rate increases when QS induction begins and initializes enhanced EPS production in the QS and mixed biofilms. The total biomass, inclusive of cells and EPS, is shown in Fig.3.12b. The growth rate increases first for the QS biofilm, as all cells upregulate around $t = 6.0$. Induction is obtained around $t = 8.0$ for the mixed biofilm, which is second to undergo an increased rate of biomass growth. Though growth is the slowest for the non-QS biofilm, when it completes growing at $t = 10.88$, it contains the greatest levels of cellular biomass.

The changes in biofilm composition averaged across the domain are shown in Fig.3.12c. The non-QS biofilm maintains a constant ratio of bacteria cells and EPS. The fraction of cells declines in the QS and mixed biofilms when induction occurs.

### 3.4 Discussion

The first question that motivated our study was: *how does QS-regulated EPS production impact the growing biofilm? In particular, what does the biofilm look like over time, with respect to distribution and composition of cells and EPS?* The simulations that help us answer this question are: QS biofilms under high and feast-famine nutrient conditions (Sections 3.3.2,3.3.3), mixed biofilms in which induction occurred (Sections 3.3.2,3.3.3), bottom-feeding QS and mixed biofilms (Section 3.3.4).

We found that colony growth was so greatly enhanced following QS induction, due to upregulated EPS production rates, that upregulated biofilms rapidly fill
the channel with biomass. When bacteria cells upregulate in a region of the domain where nutrient supply is abundant, upregulated cells have access to the nutrients required for EPS production at the maximum rate (ten times the downregulated rate). If cells are upregulated, but located in a region of nutrient scarcity, upregulated EPS production reactions cannot occur, and so colonies do not undergo enhanced expansion. This was frequently observed in the simulations - a large, upregulated upstream colony has full access to available nutrients, grows and fills the channel, and the upregulated downstream colonies remain small. When food is supplied from the bottom boundary, biofilm colony growth is homogeneous, and we do not observe spatial variations in colony size or gradients in AHL concentrations. Before QS induction, biofilm composition is generally 85% cellular biomass, 15% EPS. After induction, the composition changes to approximately 50% cells, 50% EPS. This composition differs greatly if the EPS consumption process is modelled. In places of nutrient deficiencies, EPS may be completely consumed by the bacteria cells, to the extent that the biofilm consists only of cells (generally in the mid- to downstream region), and only the portion of the biofilm closest to the inflow boundary contains a protective layer of EPS.

We were also interested in investigating, when does QS-regulated EPS production provide a benefit to the biofilm? Several factors are considered in determining whether one biofilm was more successful than another - occupancy, total cell biomass, and consumption. QS biofilms have higher occupancies than non-QS biofilms; similarly, QS colonies in a mixed biofilm occupy a greater amount of space than non-QS colonies once induction has occurred. The high occupancies are a result of increased
levels of EPS. In situations when high EPS is beneficial to a biofilm, for example, protection of bacteria cells from environmental hazards, such as detachment, antibiotics, or grazers, the use of QS-regulated EPS production is considered a beneficial strategy. The non-QS cells which have merged with upregulated QS colonies (in mixed simulations) therefore experience benefits from the thick EPS layer as well.

However, in nearly all simulations, non-QS cells outnumbered the QS cells. This occurred in strictly QS and non-QS biofilms, mixed biofilms when food was supplied from either the inflow or bottom boundaries, with and without the EPS consumption process. These findings show that QS-regulated EPS production does not provide a benefit to the biofilm in regards to achieving a high cell population, which is one potential objective of the bacteria cells residing in a biofilm. Upregulated bacteria cells have a high nutrient demand, and if this demand is not met, growth of upregulated cells cannot occur. Another cause of low upregulated cell populations in QS biofilm is the high production rate of EPS in upregulated colonies - the expanding upregulated colonies have high proportions of EPS, and quickly clog the channel. In contrast, non-QS biofilm colonies take much longer to grow to the point that the channel is clogged, but the biofilm is composed primarily of cells.

In the mixed biofilm simulations in which nutrients were supplied from the inflow boundary, induction occurred in only four of the twenty simulations. The introduction of non-QS cells into a domain with QS bacteria may therefore be considered a strategy for the prevention of quorum sensing. Three of the four mixed simulations in which induction was obtained occurred when EPS consumption was permitted. In two of these cases, non-QS cell populations declined as QS cell populations grew. Re-
source requirements are considerably higher for QS cells. If adequate nutrient supply can be secured, either by locating a colony near the nutrient source or utilizing EPS as a secondary food source, then it is possible for QS cell populations to be greater than non-QS cell populations. In a mixed environment, clogging of the channel by QS-induced colonies may prevent non-QS colonies from receiving nutrients, causing their populations to suffer declines. When EPS may be consumed, non-QS cells could benefit from the additional nutrients produced by upregulated QS colonies.

We found that overall, QS-regulated EPS production rarely provides a benefit to a biofilm with the objective of achieving a high cell population. However, maximizing offspring generation is not necessarily the best strategy under all conditions. QS-regulated EPS production would be beneficial if the objective of the bacteria cells in the biofilm is to clog the channel as quickly as possible. An upregulated biofilm colony located near the nutrient source (i.e., the inflow boundary) may use QS to increase its volume, clog the channel, and secure its supply of nutrients while starving downstream colonies. This is a competitive advantage for a colony, whether it is located in a QS biofilm (indicating intra-species competition), or in a mixed biofilm (inter-species competition). In any space-limited environment, including environments in which nutrients are supplied on the bottom boundary, channel clogging may be considered a beneficial strategy for bacteria cells to use. In the study by Koutsoudis (2006), it was shown that biofilms developing in plants stems used QS to clog the xylem (plant vessel used for water and nutrient transportation), securing nutrients for themselves. Channel clogging occurs earlier for QS-induced bacteria. Non-QS biofilms do reach the point of channel clogging, but at a later time, and with
a higher cell population - implying that the nutrients must be divided amongst more individuals. Individual colonies in patchy biofilm communities have an antagonistic interaction (some colonies benefit at the expense of others) through utilization of an exploitative competition strategy - the upstream colonies reduce nutrient resources in the environment, depleting the resource required by the downstream colonies. If cells wish to out-compete another cell population in a biofilm, of their own species or a different species, QS-controlled EPS production can provide an advantage. The drawback is that nutrient supply must be abundant, as upregulated cells have a high nutrient demand. Also, QS colonies competing intraspecifically within their environment take a risk that their signal molecules will be transported via diffusion and convection, upregulate colonies located further downstream, and induce downstream bacteria into the state of high growth and consumption.

The tabulated results showed that on a population level, QS biofilms suffered lower cell populations. However, the spatial distributions in the 2D visualisations that were qualitatively analyzed further demonstrated that for individual colonies, clogging the channel with biomass may be a beneficial strategy. Individual cells within microcolonies are genetically related, and have the ultimate objective of maintaining their own genes and reproducing in the future. To optimize survival of their genes in the colonies, and ensure survival of their offspring, bacteria cells would be interested in suppressing other colonies. In the event of a structural reorganization or detachment/reattachment, the upstream colony has an advantage, in that their genetically similar offspring will continue to succeed.

QS-regulated EPS production may also provide a benefit to the biofilm if the
The objective of the bacteria is to maximize EPS because of its protective properties, for example, mechanical stability and protection from grazers (Flemming et al., 2007). However, the high nutrient demand of upregulated bacteria cells comes at the price of cell populations. The question arises of why EPS production would be associated with quorum sensing, that is, why would cells wait until upregulation to produce EPS at higher rates, versus always producing EPS at enhanced levels? A minimum thickness of an EPS layer is required for effective protection of cells from a hazard, such as antibiotics. If a cell population is small, much energy would be expended to produce a layer of EPS which may not provide adequate protection. Coupling EPS production with quorum sensing ensures that a sufficient colony size is obtained such that the cost to produce EPS is returned by the benefits of a protective layer.

### 3.5 Conclusions and further work

In this study, we developed a mathematical model of quorum sensing in biofilms which incorporated an effect of quorum sensing on biofilm growth: upregulated cells produce EPS at an increased rate. Through simulations, we investigated quorum sensing, non-quorum sensing, and mixed biofilms under various nutrient regimes and the biological process of EPS consumption. Our results indicated that upregulated biofilm colonies rapidly filled their space-limited environment with biomass, and upregulated biofilms consist of relatively equal proportions of cellular and EPS biomass. When EPS is consumed, however, extreme nutrient deficiencies result in biofilms comprised purely of cells, with little to no EPS present. In comparing
quorum sensing to non-quorum sensing biofilms, or individual colonies in mixed environments, it was found that non-quorum sensing biofilms generally obtained higher cell populations, and upregulated quorum sensing biofilms obtained greater occupancies. In mixed simulations, non-quorum sensing colonies frequently prevented quorum sensing induction. A biofilm will benefit from using quorum sensing-induced EPS production if bacteria cells have the objective of acquiring a thick, protective layer of EPS, or if they wish to clog their environment with biomass as a means of securing nutrient supply and outcompeting other colonies in the channel, of their own or a different species.

We found that quorum sensing-upregulated biofilms consumed a greater amount of nutrients than non-quorum sensing biofilms, despite the higher cell populations of non-quorum sensing biofilms. This observation may be practical for wastewater and environmental engineering applications, in which biofilms are used to purify water or remove contaminants by consuming substrate. A small population of upregulated bacteria may be more effective than a larger population of non-quorum sensing bacteria for the removal of substrate.

Limitations of our model and simulation setup include the narrow simulation space. We chose to model biofilm growth in narrow channels, and because upregulated biofilm colonies expand rapidly, the observation period of a quorum-sensing induced biofilm is brief. Other experimental setups that allow for long-term biofilm development could be of interest; our model is intended to describe only the initial stages of biofilm growth in a reactor system representative of space-restricted environments with fluid flow, such as soil pores.
We were successful in using our model to observe connections between biofilm growth, quorum sensing, and EPS production. Our study could be extended to test biofilms that use quorum-sensing induced EPS production under the scenarios which we speculated biofilms would benefit from high levels of EPS, for example, when grazers or antibiotics are present. Future work in biofilm and quorum sensing modelling is required to continue to investigate how biofilms respond to quorum sensing induction.
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Chapter 4

Conclusion and Future Work

The objective of this study was to understand how biofilm growth impacts quorum sensing, and also how quorum sensing may influence biofilm growth. The first paper detailed the development of the convection-diffusion-reaction model describing biofilm growth, signal molecule production, and induction of bacteria cells, in a hydrodynamic environment. Numerical simulations demonstrated relations between nutrient availability and induction times, as well as the phenomenon of inter-colony communication. In the second study, production of EPS was explicitly modelled. Upregulated cells increase their rate of EPS production, leading to increased biofilm colony growth under ideal nutrient conditions. Though biofilms that employ a quorum sensing-regulated EPS production strategy will have lower cell populations, their thick layer of EPS will provide protection from potential environmental hazards, and the competitive ability to secure nutrient resources for themselves and restrict nutrients from being available to neighbouring colonies. This research may be improved by revisiting the assumptions made for the described reactor setup and the associated boundary conditions for the model. Currently, the model is intended to describe biofilm growth within a long, narrow channel. In a space-limited environment, the observation period for a quorum sensing-induced biofilm is brief; changing the reactor
setup may allow for the study of biofilm development on a longer time scale. Because this model includes the two-way relationship between biofilm growth and quorum sensing, this model may be used to further investigate the problem of biofilm control through manipulation of environmental conditions that govern quorum sensing. In the second study, it was suggested that biofilms that use quorum sensing-regulated EPS production would have an advantage in the presence of grazing predators or antibiotics over non-quorum sensing biofilms which produce EPS at a standard rate; this speculation may be validated in future work.