Mathematical Modeling Helps to Characterize Internally Triggered Biofilm Cell Dispersal

A Thesis presented to The University of Guelph

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ABSTRACT

MATHEMATICAL MODELING HELPS TO CHARACTERIZE INTERNALLY TRIGGERED BIOFILM CELL DISPERSAL

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University of Guelph, 2015  
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Cell dispersal (detachment) from mature biofilm is part of the developmental cycle of microbial biofilms. It can be externally or internally induced, leading to sloughing, erosion or seeding. We considered two potential triggers, which are studied independently in a two-dimensional setting. These triggers are quorum sensing and nutrient limitation. Quorum sensing is a cell-cell communication mechanism used to coordinate gene expression and behaviour in groups based on population densities; while the nutrient here is the growth limiting substrate. First, we develop a dynamic, spatially extended mathematical model that includes biofilm growth, production of quorum sensing molecules, cell dispersal triggered by quorum sensing, and re-attachment of cells. This is a highly non-linear system of diffusion-reaction equations with nonlinear diffusion effect in the diffusion coefficient of the sessile biomass, which highly degenerates as the biomass hits maximum. We study the model in computer simulations. The results show that dispersal can be discrete or continuous leading to hollow colonies. Furthermore, we study the well-posedness of the quorum sensing induced biofilm dispersal model in order to establish the existence and uniqueness of bounded non-negative solutions of the degenerate system. By considering smooth non-
degenerate auxiliary system we showed that the solution of the non-degenerate approximations converge to the solution of the degenerate problem. Finally, by using the same modeling approach we develop a mathematical model of nutrient limitation induced biofilm dispersal, having the same nonlinear effect in the diffusion coefficient of the sessile biomass. We study the model in computer simulations; and comment on the well-posedness of the model. Depending on parameter values, we observe a continuous, erosion-like biomass loss and hollow colonies resulting from the local nutrient limitation in the biofilm.
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Chapter 1

Introduction

1.1 Background

Bacterial biofilms are densely packed communities of bacterial cells that grow on living or inert surfaces and surround themselves with secreted extracellular polymeric substances (EPS), which protects them against hostile environmental factors such as antibiotics or mechanical washout. Biofilms are found in most natural and industrial aquatic systems and account for much of the overall microbial activity in these systems.

Biofilms have been found to play a very important role in many scientific and technological areas and as a consequence they are studied in several disciplines. Biofilms have been known to present some positive opportunities. The most studied example of beneficial biofilms is their use in wastewater treatment, groundwater protection and soil remediation. Recent studies show the use of bacterial biofilms as a catalyst to oxidize organic and inorganic matter and gener-
ate current [25, 26, 45]. On the contrary, biofilms can cause problems in industrial and medical settings mainly due to their increased resistance to environmental stress (e.g. nutritional and oxidative stress) and to host mediated responses (e.g. complement protein and phagocytes) [31]. Therefore, it is increasingly important to understand certain processes in biofilm development (e.g. formation and detachment) and factors that control these processes. In connection to industrial systems, biofilm research is usually aimed at the reduction or removal of unwanted biofilms.

Biofilm specialists have used the terms detachment and dispersal to describe the stages in biofilm development when individual cells or masses of bacterial cells separate from the biofilm and travel to other areas. Biofilm detachment is one of the mechanisms (or processes) that is known to limit the extent of biofilm accumulation on a substratum [23, 24]; and it is one of the least understood, in terms of variables that affect the process rates. It contributes to biological dispersal and biofilm rejuvenation. Dispersed (detached) cells can contribute to downstream colonization and thus result in pipe obstruction, bacterial infection and microbial contamination in industrial processing plants; in fact biofilm dispersal can lead to contamination of delicate components where a single bacterial cell is enough to cause a failure in computer chip [41]. This makes cell dispersal especially important in the industry as well as in medical and public health context and it is therefore a crucial focus in the study of biofilms.

The mechanism of quorum sensing was originally described as a diffusion-reaction based cell-cell communication mechanism used by several bacteria taxa
to coordinate gene expression and behavior in groups, based on population derivatives [18, 19, 36]. Bacterial cells produce and release small amounts of chemical signals referred to as autoinducers, e.g. *N-Acyl Homoserine Lactones* (AHL) [17] found in gram-negative bacteria. Once a threshold environmental autoinducer concentration level is reached, the bacteria are up-regulated and undergo alterations in gene expression, which synchronizes collective behavior. This up-regulation of cells typically also invokes signal production at an increased rate. The role of quorum sensing in dispersal events has been discovered and documented in some experimental studies, e.g. [6, 31, 39]. A very recent study [35] has shown how the mechanism of quorum sensing is adapted by different bacterial species for cell dispersal. Quorum sensing controlled dispersal is still a relatively new field in the study of biofilms but of general relevance as it occurs in a number of relevant species as shown in [35] with the assertion that this feature could be important for development of treatment strategies.

Limitation in nutrient concentration has been correlated to biofilm dispersal [21, 33]. It is readily apparent that in a nutrient limited system, bacterial cells deep within a biofilm would be more susceptible to starvation compared to bacterial cells at a location in direct contact with the bulk fluid. Therefore, from the perspective of natural selection, it makes sense that bacterial cells in the inner core of a biofilm would develop a means of escape. Due to the prevailing view among researchers on starvation induced dispersal, investigation have included effect of different nutrients [11], growth and detachment rates [34], nutrient starved under continuous flow condition in drip-flow reactor [21],
detachment induced by rapid change in nutrient concentration [37]. A common challenge that arises in the study of nutrient limitation induced dispersal is associated with switch from high-nutrient condition to low-nutrient condition in contrast to the other way round; thus, most studies on the role of starvation in biofilm dispersion have been done by running a system under high-nutrient loads to allow biofilm development and then abruptly changing conditions to a low-nutrient medium to induce starvation [22, 33, 37]. Unfortunately, there is insufficient data at this time to draw a firm conclusion of the nutrient limitation induced biofilm dispersal [10], this has increased the interest and study of nutrient limitation induced biofilm dispersal.

Over the years, mathematical biofilm models have been known to greatly contribute to our understanding of biofilm processes so far. Biofilm models are often called upon to elicit explanations of phenomenon occurring within biofilm-affected industrial equipment. Initially, biofilm models were analytical descriptions of flat “microbial films” used to study the industrially important processes of substrate diffusion [3], substrate uptake [4] and external mass transfer [42]. These early models were simple, steady state one dimensional simulations for the microscale trends in biofilm containing systems. Today, biofilm models are increasingly able to simulate microscale processes in a spatially, multi-dimensional, heterogeneous biofilm structures. A large number of mathematical modeling techniques have been proposed to model biofilms. Some of these models employ cellular automata [8, 28, 29, 30], individual based modeling or continuum methods [1, 2, 9, 12, 13] to investigate more complex interactions between biofilm
components and the surrounding environment. These models differ in the approach used to describe biomass movement and structure, but they all are coupled with diffusion-reaction models for growth controlling substrates. Models such as these are usually complex, mathematically difficult to analyze and often only amendable to computational simulations.

A variety of biofilm models have included detachment processes in some form, often highly simplified and mechanistic. The earliest detachment models were those based on concept of fluid shear [27, 32] in which the rate of detachment increases with the distance above the substratum [7, 38, 40]. A few other biofilm detachment models have made detachment rates dependent on the local concentration of a metabolic substrate or product. Apart from mechanical washout of biofilm, a model that describes biofilm detachment induced by chemical changes and food limitations is the stochastic cellular automation in [20, 21, 44].

Our model of interest is aimed at describing biofilm expansion, from production of new cells, cell loss (induced by quorum sensing and nutrient limitation respectively) and hollowing biofilm structure by reduced biofilm density in the interior of the biofilm communities. In order to achieve these aims, we will use as the basis for our study a single species density dependent diffusion-reaction biofilm model that was originally introduced in [15]. This is a multi-dimensional biofilm model. It encompasses two non-linear diffusion effects, namely degeneracy as in the porous medium equation where biomass vanishes, and a super-diffusion singularity as the biomass approaches an a priori known upper bound.
1.2 Research Objectives

Many experimental or modeling studies focus on biofilm growth, dispersal or quorum sensing induction. We are not aware of studies that focus on the interplay of these three aspects of biofilm system and how they affect biofilm structure, function and dynamics. Mechanical detachment has been known as a typical mechanism for biofilm detachment, and has attracted a considerable amount of study. We do not consider mechanical detachment in this study to reduce the scope of this thesis. In a mathematical modeling setup, it is easier to isolate and study a particular aspect of a system. For this reason, a modeling study can be a good first step that guides future work both theoretically and experimentally for non-mechanistic biofilm detachment. Therefore the objectives of this study is:

- To formulate a mathematical framework for non-mechanistic, internally triggered biofilm cell dispersal.

- To use the developed model to answer the following research question

  “How does biofilm dispersal triggered by cues such as quorum sensing and nutrient limitation affect biofilm size and structure?”

To achieve the above objectives, first we consider a biofilm consisting of bacteria that produce quorum sensing molecules and respond to quorum sensing, then we develop a dynamic deterministic continuum model of a quorum sensing induced biofilm dispersal suited to investigate a variety of conjectures about
how such a process at the biological level affects biofilm structure and/or function in a two-dimensional setting. The model is formulated to reflect the spatial heterogeneous structure of biofilms. This model will differ from other modeling techniques in the approach used to describe biomass movement and structure; and it will be studied both numerically and analytically.

Nutrient limitation has been empirically shown to trigger biofilm dispersal. We therefore implement the modeling approach above, leading to a system of reaction-diffusion equations to investigate the dynamics of a nutrient limitation induced biofilm dispersal in two dimensional setting; investigating the interplay of biofilm growth, dispersal and nutrient limitation.

### 1.3 Outline of this Thesis

The main contribution of this thesis is to develop a mathematical model that describes the interplay of biofilm growth, detachment and potential detachment triggers e.g. quorum sensing and nutrient limitation. The thesis is therefore arranged as follows:

Chapter 2, *A mathematical model of quorum sensing induced biofilm detachment*, presents a mathematical model of quorum sensing induced biofilm detachment. This is a system of non-linear, density-dependent diffusion-reaction equations for four dependent variables: sessile biomass, dispersed cells, nutrient concentration and quorum sensing molecule. The model describes the growth of the sessile biomass, detachment and reattachment of cells (which couples the model
for sessile biomass and the dispersed cells), nutrient consumption; and production of the quorum sensing molecule. Here the biofilm expansion is described by the density-dependent diffusion coefficient of the sessile biomass; this is a non-linear diffusion effect, a degeneracy as in the porous medium equation and super diffusion. The model is quantitatively studied using numerical simulation. The quorum sensing induction threshold and the maximum dispersal rate were varied and used to investigate different dispersal events that occur in such a biofilm system and their implications. This study was published in the *PloS ONE* [16]:


Chapter 3, *Mathematical analysis of a quorum sensing induced biofilm detachment model*, presents the well-posedness of the quorum sensing induced detachment model developed in Chapter 2. The non-linear diffusion effect of the sessile biomass contains a degeneracy that makes the model analytically difficult to solve. Here we prove the existence and uniqueness of a bounded non-negative solutions of the degenerate model. This is achieved by considering smooth non-degenerate auxiliary system, and passing the solution to the limit of the degenerate system. This chapter also presents some numerical simulation examples illustrating spatial effects of quorum sensing induced dispersal in the inner core of the biofilm colonies. This chapter is currently being finalized for submission to a journal and will be published as:
Chapter 4, *A mathematical model of nutrient limitation induced biofilm detachment*, extends upon the modeling concept of Chapter 2 to develop a model for nutrient limitation induced biofilm detachment. This is a system of non-linear diffusion-reaction equations for three dependent variables: sessile biomass, dispersed cells and nutrient concentration. The model is quantitatively studied in computer simulations to investigate biofilm cell detachment and its contribution to the biofilm and the industry. The detachment induction parameter and the maximum detachment rate were varied to investigate potential dispersal events and structural changes in the starved region of a biofilm colony. Similar to the model in Chapter 2, the nutrient limitation induced biofilm detachment model contains a degeneracy as in porous medium, so we prove the existence of bounded solutions, this we do by comparing the nutrient limitation induced biofilm detachment model with the single species prototype growth model, which was already shown in literature to have bounded solutions.

Chapter 5, *Conclusion and future work*, summarizes the findings of this dissertation and gives suggestions of how this research can be further extended.
Bibliography


Chapter 2

A Mathematical Model of Quorum Sensing Induced Biofilm Detachment

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Abstract

Cell dispersal (or detachment) is part of the developmental cycle of microbial biofilms. It can be externally or internally induced, and manifest itself in discrete sloughing events, whereby many cells disperse in an instance, or in continuous slower dispersal of single cells. One suggested trigger of cell dispersal is quorum sensing, a cell-cell communication mechanism used to coordinate gene expression and behavior in groups based on population densities.

To better understand the interplay of colony growth and cell dispersal, we develop a dynamic, spatially extended mathematical model that includes biofilm
growth, production of quorum sensing molecules, cell dispersal triggered by quorum sensing molecules, and re-attachment of cells. This is a highly nonlinear system of diffusion-reaction equations that we study in computer simulations.

Our results show that quorum sensing induced cell dispersal can be an efficient mechanism for bacteria to control the size of a biofilm colony, and at the same time enhance its downstream colonization potential. In fact we find that over the lifetime of a biofilm colony the majority of cells produced are lost into the aqueous phase, supporting the notion of biofilms as cell nurseries. We find that a single quorum sensing based mechanism can explain both, discrete dispersal events and continuous shedding of cells from a colony. Moreover, quorum sensing induced cell dispersal affects the structure and architecture of the biofilm, for example it might lead to the formation of hollow inner regions in a biofilm colony.

**keywords:** autoinducer, biofilm, cell dispersal, computer simulation, diffusion-reaction, quorum sensing

### 2.1 Introduction

Bacterial biofilms are microbial communities of same or different species that attach to surfaces, embedded in a self-produced extracellular polymeric matrix, which gives some protection to the sessile cells against hostile environmental factors such as antibiotics or mechanical washout. The adsorption and absorption properties and enhanced mechanical stability of biofilms make them advanta-
Geous to environmental engineers, e.g. in wastewater treatment, soil remediation and groundwater protection [86]. On the other hand, biofilms can be harmful, especially if they form on medical implants or natural surfaces in the human body, hence causing serious infections [29, 56, 63]. Biofilms can also lead to biocorrosion of drinking water pipes or industrial facilities [9] and contamination in food processing plants, causing food spoilage [37, 82].

Biofilm development can be divided into three distinct stages: (a) reversible initial attachment of cells to the surface (b) growth of the cells into a sessile biofilm colony and (c) dispersal, or detachment, of cells from the biofilm colony into the surrounding aqueous phase, which contributes to biological dispersal and biofilm rejuvenation.

Biofilm dispersal, such as erosion and sloughing, can be passive or active whereas seeding dispersal is always an active process [45]. The latter, also known as central hollowing refers to the release of large number of single cells from inside the biofilm colony [15, 48, 55]. Seeding dispersal can be internally triggered, e.g. by enzyme-mediated breakdown of the biofilm matrix [17], production of surfactants, which loosen cells from the biofilm [24], or externally triggered, e.g. changes in nutrient availability [44], production of free-radical species [6] and control by quorum sensing systems [68, 75, 89].

While many experimental studies of biofilm detachment and dispersal have been conducted and reported in the literature, this remains a challenging topic due to difficulties in biofilm detachment characterization [20, 90]. Mathematical modeling and simulation studies can provide a complementary view, as they
allow to distinguish between different detachment mechanisms.

Many bacteria have the ability to produce signaling molecules that play a part in inducing cell-cell communication [11]. This is normally referred to as quorum sensing. It is a system of stimulus and response, usually assumed to be correlated to local population density, but also affected by diffusive and convective transport of chemical signals [39, 81]. Bacterial cells produce and release small amounts of chemical signaling molecules referred to as autoinducers, e.g. *N-Acyl Homoserine Lactones* (AHL) [36] found in gram-negative bacteria. Once a threshold environmental autoinducer concentration level is reached, the bacteria undergo alterations in gene expression, which synchronizes collective behavior. This up-regulation of cells typically also invokes signal production at an increased rate.

The role of quorum sensing in dispersal events has been discovered and documented in some experimental studies, e.g. [10, 68, 75, 84]. Quorum sensing controlled dispersion is still a relatively new field in the study of biofilms but of general relevance as it occurs in a number of relevant species as shown in [75] with the assertion that this feature could be important for development of treatment strategies.

Many experimental or modeling studies focus on biofilm growth, dispersal or quorum sensing induction. We are not aware of studies that focus on the interplay of these three aspects of biofilms systems and how they affect biofilm structure, function and dynamics. In an experimental setting, it is difficult and sometimes challenging to separate the effects of different potential causes, e.g. to
distinguish between shear induced and quorum sensing induced dispersal. In a mathematical modeling setup, it is easier to isolate particular aspects of a system. For these reasons a modeling study can be a good first step that guides future work, both theoretically and experimentally. In this paper we will formulate a mathematical model for quorum sensing induced biofilm dispersal. We will carry out computer experiments to investigate potential effects of this phenomenon in biofilm growth and structure.

Mathematical models for bacterial biofilms over the years have greatly contributed to our understanding of biofilm processes so far. The first generation of biofilm models were continuum models with a focus on population and resource dynamics. These models are formulated under the assumption that a biofilm can be described as a one-dimensional homogeneous layer, cf [85, 87]. Newer models take the spatially heterogeneous structure of biofilms into account, and are formulated as spatially multi-dimensional models. A large number of mathematical modeling techniques have been proposed to model biofilms, consisting of stochastic individual based models e.g.[49, 50, 80], stochastic cellular automata models e.g.[19, 65, 67] and a variety of deterministic partial differential equation models e.g.[21, 28, 31].

These models differ in the approach used to describe biomass movement and structure, but they all are coupled with diffusion-reaction models for growth controlling substrates. Models such as these are usually complex, mathematically difficult to analyze and often only amendable to computational simulations.

A variety of biofilm models have included detachment processes in some
form, often highly simplified. In traditional one-dimensional models, the biofilm detachment rate is typically a function of the biofilm thickness [58, 87]; similarly in some 2D models the detachment rate is correlated with the biofilm geometry [94]. In other models detachment is correlated with the shear stress induced by the flowing bulk liquid in the biofilm system; e.g. [1, 30, 66, 78]. Apart from mechanical washout of biofilm, a model that describes biofilm detachment induced by chemical changes and food limitations is the stochastic cellular automation in [43, 44, 93].

Many modeling studies have investigated quorum sensing in planktonic populations and in biofilm systems, e.g [26, 28, 52, 61, 83, 88]. Most quorum sensing models in biofilms focus on up-regulation, only few include the effect of quorum sensing on the biofilm dynamics, structure, and function e.g.[4, 39]. Modeling studies have shown that quorum sensing can also induce inter-colony or non-local communication in biofilms [38, 83] and that gene regulation in a particular colony can be affected by the surrounding colonies via signal transport in the aqueous phase. In [41] it was suggested that both effects, local population size assessment (quorum sensing in the strict sense), and long range effects due to signal transport (e.g. diffusion sensing), can be unified in the concept of efficiency sensing.

An important first question is, which of the existing biofilm modeling frameworks to choose for our simulation study. One criterion to base our choice is the treatment of biomass. In many models the cell density is assumed to be always at maximum, e.g. [85, 27]. Biofilm expansion results from production of new cells,
and cell loss result to shrinkage of biofilm. Alternatively, some models treat the biomass density as a dependent variable, e.g. [31, 65], which allows them to describe biofilm colonies with strong biomass density gradient. More importantly for our purpose, such models will be able to describe hollowing biofilm structure by reduced biofilm density in the interior of such colonies.

Another criterion to distinguish between biofilm models is whether they are stochastic or deterministic. While often a single simulation of a stochastic model seems to be faster than that of a deterministic model, many such simulations are required to obtain reliable averages, which offset the computational speed advantage. Deterministic models, on the other hand have averaging properties built in.

Based on these considerations, the model that we will use as the basis for our study is the single species density dependent diffusion-reaction biofilm model that was originally introduced in [31]. It is a deterministic continuum model that treats biomass density as a dependent variable. It has been derived both via a spatially discrete master equation starting from the view point of a biofilm as a spatially structured population [47] and from equations for conservation of mass and momentum, starting from the view point of a biofilm as an incompressible fluid [38].

Since we are interested in the interplay of various colonies in a biofilm community, a two-dimensional representation of the biofilm instead of assuming the biofilm as a homogeneous layer seems appropriate. We will neglect the flow field in the aqueous phase and the shear induced detachment that it causes and
focus on quorum sensing induced dispersal alone. Since flow field calculations are in many instances the most time consuming step in biofilm simulations, this will simplify the modeling greatly. We will consider a hydrostatic environment, in which nutrients are transported to the biofilm from the aqueous phase by a diffusion gradient.

2.2 Method

2.2.1 Basic model assumptions

We develop a mathematical model that describes the dynamics of quorum sensing induced bacterial cell dispersal in growing biofilms. The local amount of sessile bacterial cells is expressed in terms of the local volume fraction they occupy in the biofilm [31]. The bacteria that engage in quorum sensing are assumed to switch from a down- to an up-regulated state when the local concentration of the quorum sensing molecule becomes large enough and vice versa. We do not explicitly distinguish between down- and up-regulated cells but implicitly: we assume that the autoinducer production rate is controlled by the local autoinducer concentration.

Dispersal of sessile cells is triggered as the local autoinducer concentration increases. The motility of dispersed cells and its dispersal from the biofilm into the aqueous phase is assumed to be governed essentially by Fickian diffusion, following [22], who studied the movement of motile bacteria in biofilms. In the
biofilm the diffusion coefficient of the dispersed bacteria is reduced due to the diffusive resistance of biofilm cells extracellular polymeric substances (EPS), which we subsume implicitly in biomass volume fraction. This is a common assumption in biofilm modeling, e.g. [27, 60, 65].

The quorum sensing signal molecules (autoinducers) are dissolved and are assumed to be transported by Fickian diffusion. They diffuse at a reduced rate in the biofilm compared to the aqueous phase, following [77]. The rate of production of signaling molecules is higher by one order of magnitude for up-regulated than for down-regulated cells.
### 2.2.2 Governing equations

Putting these aspects and assumptions together, the model describing the biofilm dispersal is formulated as a system of four partial differential equations. The dependent variables are $M$, $N$, $C$ and $A$. $M$ denotes the volume fraction occupied by sessile cells and subsumes the EPS. $N$ denotes the concentration of the motile bacterial cells, which are capable of moving into and in the liquid phase; we refer to these as 'dispersed cells'. $C$ denotes the concentrations of the growth controlling nutrient substrate. $A$ represents the concentration of the dissolved quorum sensing molecules. The governing equations read

$$\begin{align*}
\partial_t M &= \nabla \cdot (D_M(M) \nabla M) + \frac{\mu CM}{k_1 + C} - k_4 M - \frac{\eta_1 A^n M}{\tau^n + A^n} + \frac{\eta_2 MN}{k_5 + M}, \\
\partial_t N &= \nabla \cdot (d_N(M) \nabla N) + \frac{\mu CN}{k_1 + C} - k_4 N + \frac{\eta_1 A^n M}{\tau^n + A^n} - \frac{\eta_2 MN}{k_5 + M}, \\
\partial_t C &= \nabla \cdot (d_C(M) \nabla C) - \frac{\mu}{Y} \frac{M_\infty C}{k_1 + C} (M + N), \\
\partial_t A &= \nabla \cdot (d_A(M) \nabla A) + \gamma(C) \left[ \alpha + \beta \frac{A^n}{\tau^n + A^n} \right] M_\infty (M + N).
\end{align*}$$

Equations (2.1), (2.2) describe the growth and spatial movement of sessile and dispersed biomass, $M$ and $N$. They are directly coupled by the dispersal and re-attachment terms. The third equation describes the consumption of nutrients by $M$ and $N$ while the fourth equation describes the production of the quorum sensing molecules. These equations are defined in a domain $\Omega \subset \mathbb{R}^d, d \in \{2, 3\}$. The aqueous phase is the region without biomass present, $\Omega_1(t) = \{ x \in \Omega : M(t, x) = 0 \}$, and the biofilm phase is the region with biomass present,
\[ \Omega_2(t) = \{ x \in \Omega : M(t, x) > 0 \} \] (see Fig. 2.1). These regions change over time as the biofilm grows. They are separated by the biofilm/water interface, \( \Gamma(t) := \partial \Omega_2(t) \setminus \partial \Omega \). Neither \( \Omega_1(t) \) nor \( \Omega_2(t) \) need to be connected domains. In fact \( \Omega_2(t) \) will in general consist of several colonies that are separated from each other by water. The substratum, on which the biofilm grows is part of the boundary of the domain \( \Omega \). It is impermeable to substrate, autoinducer and biomass and it is not reactive.

The density-dependent diffusion coefficient in (2.1) that describes the biofilm expansion is formulated according to [31] and given by

\[ D_M(M) = \delta \frac{M^a}{(1 - M)^b}, \quad \text{where} \quad a, b > 1, \delta > 0. \] (2.5)

The equation degenerates for \( M = 0 \) where \( D(0) = 0 \). For \( 0 \approx M \ll 1 \) we have \( D(M) \approx \delta M^a \), i.e. the biofilm diffusion equation behaves like the porous medium equation. In particular this guarantees a finite speed of interface propagation. For \( M = 1 \) the diffusion coefficient attains a singularity and blows up. For \( 0 \ll M \approx 1 \) the equation behaves like a super-diffusion equation. In particular, the blow up of the diffusion coefficient guarantees that \( M < 1 \) if a Dirichlet condition is specified somewhere on the boundary of the domain. This means that the super-diffusion effect guarantees that the maximum possible cell density is never exceeded, independent of biomass production terms [34, 35]. Biomass spreading is much slower than the diffusion of the dissolved substrate [87], thus the biomass motility coefficient \( \delta [m^2 d^{-1}] \) is positive but much smaller than the diffusion coefficients of the \( N, C \) and \( A \) by several orders of magnitude.
The diffusion coefficients for $N$, $C$ and $A$ in (2.2)-(2.4) depend on $M$ as well, although in a non-critical way. For dissolved substances like nutrients and quorum sensing molecules, they are lower in the biofilm than in the aqueous phase [77]. We make the same assumption for dispersed cells. We make a linear ansatz that interpolates between the experimentally measurable values of diffusion in water ($M = 0$) and in a fully developed biofilm ($M = 1$), i.e.

\[
\begin{align*}
    d_N(M) &= d_N(0) + M(d_N(1) - d_N(0)), \\
    d_C(M) &= d_C(0) + M(d_C(1) - d_C(0)), \\
    d_A(M) &= d_A(0) + M(d_A(1) - d_A(0)).
\end{align*}
\]

(2.6)

Note that $d_{N,C,A}(0) > 0$, so that the diffusion coefficients are bounded between two finite values. Hence, diffusion is essentially Fickian, and non-degenerate. Diffusion coefficients are measured in $m^2d^{-1}$.

The reaction terms and their parameters in (2.1)-(2.4) have the following meaning:

- Growth of sessile and dispersed cells is controlled by the local availability of nutrients in (2.1), (2.2). This is described by standard Monod kinetics where $k_1 \ [gm^{-3}]$ is the half saturation concentration, and $\mu \ [d^{-1}]$ is the maximum growth rate. One can argue that the growth rate of the dispersed cells should be different than those of the sessile cells. This would require us to introduce additional model parameters. On the other hand, since dispersed cells diffuse out of the system quickly and, therefore, have only minor effect on the availability of substrate in the system and hence on
biofilm growth. Hence, for simplicity, we assume the same growth kinetics for sessile and dispersed cells.

- Cell lysis occurs at the rate of $k_4 \, [d^{-1}]$ in equation (2.1), (2.2). For simplicity we assume for both types of biomass the same lysis rate.

- Dispersal of cells from the biofilm is controlled by the local autoinducer concentration. It is negligible if $A$ is clearly below the switching threshold $\tau \, [nM]$ and attains the maximum dispersal rate $\eta_1 \, [d^{-1}]$, if $A \gg \tau$. The transition between both extreme stages is described by Hill kinetics with exponent $n \, [-]$. We assume that the dispersal rate is proportional to the autoinducer production rate by up-regulated cells, see below.

- Re-attachment of cells in the biofilm is controlled by the local biofilm density $M$. The re-attachment rate is proportional to $N$ for small $M$ (relative to $k_5 \, [gm^{-3}]$) and approximately constant for $M \gg k_5$, modeled by standard saturation kinetics. In the absence of quantitative data in the literature we assume that the maximum reattachment rate is not more than the maximum dispersal rate, but smaller. We take it as $\eta_2 = 0.5 \eta_1$. This reflects that some of the dispersed cells can re-attach to the biofilm, but that cells that are induced for planktonic life and detached would require a costly up- and down-regulation of many genes to become sessile again.

- Consumption of nutrients in (2.3) is proportional to the biomass growth rate in (2.1) and (2.2). The proportionality factor is the yield coefficient $Y$.
\( M_\infty \text{ [gm}^{-3}] \) is the maximum cell density. The compounded parameter \( \mu M_\infty /Y \) is the maximum consumption rate. Note that the maximum biomass cell density \( M_\infty \) does not explicitly occur in the biomass equation because it has been used for scaling when we stated the model in terms of biomass volume fraction instead of densities.

- In (2.4), autoinducers are produced at a base rate \( \alpha \text{ [nMd}^{-1}g^{-1}m^3] \) if the local autoinducer concentration is small (relative to induction threshold \( \tau \)) and increases to \( \alpha + \beta \) where \( \beta \) is also measured in \( \text{[nMd}^{-1}g^{-1}m^3] \), if it exceeds the induction threshold. The transition is described by a Hill function with exponent \( n \). In Fig. 2.2 we plot this function for degree of polymerization \( n = 2.5 \), as suggested in [36], which we will use in the simulations later on, whereas [53] used a slightly lower value of \( n = 2.2 \). This function describes a smooth transition between states of no increased autoinducer production (all cells down-regulated) and autoinducer production at maximum rate (all cells up-regulated). It accounts for individual variation between cells. In particular for higher induction threshold values \( \tau \) this implies that a significant amount of autoinducers is already produced at concentrations \( 0 \ll A < \tau \). A more pronounced switch between both states would be obtained for higher values of \( n \) than those found experimentally, e.g. [36, 53], see Fig. 2.2. According to experimental studies, the production of quorum sensing signal molecule can be affected by the nutrient, we have included this option in the model as \( \gamma(C) \), which is described in more detail in Appendix A.
The effect of the local autoinducer concentration $A$ on the autoinducer production rate and the dispersal rate is described by the Hill function $f(A) = A^n/(\tau^n + A^n)$. Plotted here for the degree of polymerization $n = 2.5$ that was obtained in [36] and is used in the simulations later on.

### 2.2.3 Computational realization

For our computer simulations we restrict ourselves to the two-dimensional setting with a rectangular computational domain $\Omega = [0, L] \times [0, H]$. The substratum, on which biofilm colonies form is the bottom boundary, $x_2 = 0$, see also Fig. 2.1. The substratum is assumed to be impermeable to biomass and dissolved substrate, so we pose homogeneous Neumann boundary conditions there i.e. $\partial_n M = \partial_n N = \partial_n C = \partial_n A = 0$, for $x_2 = 0$. We consider our rectangular computational domain as part of a larger biofilm reactor. At the lateral boundaries, where $x_1 = 0$ or $x_1 = L$, we assume a symmetry boundary condition, which allows us to view the domain as a part of a continuously repeating larger domain. Therefore, we pose here as well homogeneous Neumann conditions for
all dependent variables i.e. \( \partial_n M = \partial_n N = \partial_n C = \partial_n A = 0 \), for \( x_1 = 0 \) or \( x_1 = L \).

At the top boundary, \( x_2 = H \), we pose homogeneous Dirichlet conditions for the biofilm biomass \( M \). The degeneracy \( D(0) = 0 \) in (2.1) leads to a finite speed of interface propagation in the sense that initial data with compact support imply solutions with compact support. Therefore, as long as biomass does not reach the boundary of the domain, the model satisfies simultaneously homogeneous Dirichlet and Neumann conditions, which are combined in the no-flux conditions \( D(M) \nabla M = 0 \). Since our simulations will be terminated before biomass reaches the top of the domain, the choice of boundary conditions there is not critical.

For the nutrient \( C \), we pose at the top boundary, \( x_2 = H \), an inhomogeneous Dirichlet condition. \( C \) is set there to the bulk concentration value, which reflects that substrate is added to the system through this segment of the domain boundary. The dispersed cell density and the autoinducer concentration are set there to nil. This enforces a diffusion gradient from the biofilm in the interior of the domain to the boundary and mimics removal of quorum sensing molecules and dispersed cells into the surrounding bulk phase where they are negligible due to instantaneous dilution. Thus we have \( C = C_\infty, M = N = A = 0 \) at \( x_2 = H \).

Initially biofilm biomass is placed in small pockets with \( M > 0 \) at the substra-
tum only. The locations and initial sizes of these pockets will be chosen randomly or explicitly specified \textit{a priori}. Thus \( \partial \Omega_2(0) \cap \{ x_2 = 0 \} \neq \emptyset, \partial \Omega_2(0) \cap \partial \Omega \setminus \{ x_2 = 0 \} = \emptyset \) and \( \int_{\Omega_2(0)} dx \ll \int_{\Omega} dx \). \( \Omega_2(0) \) is typically not connected, i.e. several inoculation sites are usually considered and all have a boundary with \( x_2 = 0 \). We will
assume that initially no dispersing cells and no autoinducers are in the system, and that the concentration of nutrients is initially at bulk levels, i.e. $C = C_\infty$, $N = A = 0$ at $t = 0$.

Equations (2.1)-(2.4) are discretized on a regular grid using a cell centered finite difference-based finite volume scheme for space and semi-implicit time-integration, adapted from [32, 59, 60] to account for the new dependent variable $A, N$, which are treated in the same manner as $C$. In every time step, four linear algebraic systems are solved, one for each dependent variable. These linear systems are sparse and at least weakly diagonally dominant. They are efficiently solved with the stabilized biconjugate gradient method [69]. The linear solver is prepared for parallel execution on multi-core and shared memory multiprocessor architectures using OpenMP, as described in [59]. Simulations will be terminated when the biofilm reaches a set target size or when a set maximum simulation time is reached. For the visualization of simulation results we use the Kitware Paraview visualization package (spatially resolved plots) and gnuplot (lumped results).

For better interpretation of the computer simulations of the model, the following quantitative lumped measures will be used

- Biofilm size relative to the domain size

$$
\omega(t) := \frac{\int_{\Omega(t)} dx}{\int_{\Omega} dx}.
$$ (2.7)
• Average nutrient concentration in $\Omega_2$:

$$C_{avg}(t) := \frac{\int_{\Omega_2(t)} C(t, x)dx}{\int_{\Omega_2(t)} dx}.$$  \hspace{1cm} (2.8)

• Total sessile biomass in the biofilm:

$$M_{tot}(t) := \int_{\Omega} M(t, x)dx.$$  \hspace{1cm} (2.9)

• The total amount of dispersed cells:

$$N_{tot}(t) := \int_{\Omega} N(t, x)dx.$$  \hspace{1cm} (2.10)

• Average concentration of the quorum sensing molecules in $\Omega_2$, non-dimensionalized with respect to $\tau$:

$$A_{avg}(t) := \frac{\int_{\Omega_2(t)} A(t, x)dx}{\tau \int_{\Omega_2(t)} dx}.$$  \hspace{1cm} (2.11)

• Biomass loss $K(T)$: This is the relative difference between the net biomass gain and the produced sessile biomass over a period of time $T$ defined as follows

$$K(T) = \frac{\int_0^T \int_{\Omega} \left[ \mu \frac{C}{k_1 + C} \right] Mdxdt - [M_{tot}(T) - M_0]}{\int_0^T \int_{\Omega} \left[ \mu \frac{C}{k_1 + C} \right] Mdxdt},$$  \hspace{1cm} (2.12)

where $M_{tot}(T)$ is the amount of biomass in the system at $t = T$ and $M_0$ is the amount of biomass initially present in the system.
• The ratio of dispersed cells that are re-attached and the cells that are detached, at time \( t \):

\[
Z(t) = \frac{\eta_2}{\eta_1} \left[ \frac{\int_{\Omega} \left( \frac{M}{k_5 + M} \right) N\,dx}{\int_{\Omega} \left( \frac{A_n}{1 + A_n} \right) M\,dx} \right].
\] (2.13)

• A measure for the amount of dispersed cells (i.e. the diffusive flux) that left the domain over the time interval \([0, T]\)

\[
P(T) = \int_0^T \int_0^L \frac{\partial N}{\partial n} \bigg|_{y = H} \, dx_1 \, dt.
\] (2.14)

The default model parameters used in the simulations are summarized in Table 2.1. Parameter values that are varied in the simulations will be stated in the text where the simulation experiments are described.

### 2.3 Results

The objective of our numerical simulation experiments will be to better understand quorum sensing induced cell dispersal in biofilm. The primary parameters that we vary in these studies are the threshold parameter \( \tau \), which sets the scale for autoinducer induction; and the maximum dispersal rate \( \eta_1 \). These two parameters are the two most directly linked to this process.

We will first take a look at lumped results, integrated over the computational domain, and then at the local effects on biofilm structure.
Table 2.1: Parameter values used in the numerical simulations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter</th>
<th>Source</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu )</td>
<td>maximum specific growth rate</td>
<td>[87]</td>
<td>6.0</td>
<td>( d^{-1} )</td>
</tr>
<tr>
<td>( Y )</td>
<td>yield coefficient</td>
<td>[87]</td>
<td>0.63</td>
<td>-</td>
</tr>
<tr>
<td>( k_1 )</td>
<td>half saturation concentration (growth)</td>
<td>[87]</td>
<td>4.0</td>
<td>( gm^{-3} )</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>1st threshold concentration in ( \gamma_{1,2,3,4} )</td>
<td>assumed</td>
<td>0.05</td>
<td>( gm^{-3} )</td>
</tr>
<tr>
<td>( k_3 )</td>
<td>2nd threshold concentration in ( \gamma_{1,2,3,4} )</td>
<td>assumed</td>
<td>0.1</td>
<td>( gm^{-3} )</td>
</tr>
<tr>
<td>( k_4 )</td>
<td>lysis rate</td>
<td>[33]</td>
<td>0.4</td>
<td>( d^{-1} )</td>
</tr>
<tr>
<td>( k_5 )</td>
<td>half saturation density (re-attachment)</td>
<td>[87]</td>
<td>0.7</td>
<td>( gm^{-3} )</td>
</tr>
<tr>
<td>( M_\infty )</td>
<td>maximum cell density</td>
<td>[33]</td>
<td>10(^4)</td>
<td>( gm^{-3} )</td>
</tr>
<tr>
<td>( \eta_1 )</td>
<td>maximum dispersal rate</td>
<td>assumed</td>
<td>0.6 - 4.2</td>
<td>( d^{-1} )</td>
</tr>
<tr>
<td>( \eta_2 )</td>
<td>maximum re-attachment rate</td>
<td>assumed</td>
<td>0.3 - 2.1</td>
<td>( d^{-1} )</td>
</tr>
<tr>
<td>( \tau )</td>
<td>quorum sensing induction threshold</td>
<td>[36, 46]</td>
<td>10 - 70</td>
<td>( nM )</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>constitutive autoinducer production rate</td>
<td>[36]</td>
<td>0.5520</td>
<td>( d^{-1} )</td>
</tr>
<tr>
<td>( \beta )</td>
<td>induced autoinducer production rate</td>
<td>[36]</td>
<td>5.5200</td>
<td>( d^{-1} )</td>
</tr>
<tr>
<td>( n )</td>
<td>degree of polymerization</td>
<td>[36]</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>( d_A(0) )</td>
<td>diffusion coefficients of ( A ) (water)</td>
<td>[38]</td>
<td>7.8 \times 10^{-5}</td>
<td>( m^2d^{-1} )</td>
</tr>
<tr>
<td>( d_A(1) )</td>
<td>diffusion coefficients of ( A ) (biofilm)</td>
<td>[38]</td>
<td>3.9 \times 10^{-5}</td>
<td>( m^2d^{-1} )</td>
</tr>
<tr>
<td>( d_C(0) )</td>
<td>diffusion coefficients of ( C ) (water)</td>
<td>[33]</td>
<td>10(^{-4})</td>
<td>( m^2d^{-1} )</td>
</tr>
<tr>
<td>( d_C(1) )</td>
<td>diffusion coefficients for ( C ) (biofilm)</td>
<td>[33]</td>
<td>8 \times 10^{-5}</td>
<td>( m^2d^{-1} )</td>
</tr>
<tr>
<td>( d_N(0) )</td>
<td>diffusion coefficients of ( N ) (water)</td>
<td>assumed</td>
<td>10(^{-4})</td>
<td>( m^2d^{-1} )</td>
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<td>diffusion coefficients of ( N ) (biofilm)</td>
<td>assumed</td>
<td>2 \times 10^{-5}</td>
<td>( m^2d^{-1} )</td>
</tr>
<tr>
<td>( \delta )</td>
<td>biomass motility coefficient</td>
<td>[31]</td>
<td>10(^{-12})</td>
<td>( m^2d^{-1} )</td>
</tr>
<tr>
<td>( a )</td>
<td>biofilm diffusion exponent</td>
<td>[31]</td>
<td>4.0</td>
<td>-</td>
</tr>
<tr>
<td>( b )</td>
<td>biofilm diffusion exponent</td>
<td>[31]</td>
<td>4.0</td>
<td>-</td>
</tr>
<tr>
<td>( L )</td>
<td>system length</td>
<td>[33]</td>
<td>4 \times 10^{-3}</td>
<td>( m )</td>
</tr>
<tr>
<td>( H )</td>
<td>system height</td>
<td>assumed</td>
<td>1.6 \times 10^{-3}</td>
<td>( m )</td>
</tr>
<tr>
<td>( \epsilon )</td>
<td>asymptote controlling parameter for ( \gamma_3 ) and ( \gamma_4 )</td>
<td>assumed</td>
<td>10(^{-12})</td>
<td>-</td>
</tr>
</tbody>
</table>

2.3.1 Induction threshold \( \tau \) and erosion rate \( \eta_1 \) control discrete vs continuous dispersal patterns

This first simulation experiment investigates how quorum sensing induced dispersal affects the biofilm growth and dispersal events. Here we have considered
a situation where the nutrient concentration has no influence on the quorum sensing signal production, thus $\gamma(C) \equiv 1$. The parameter values listed in Table 2.1 were used. The quorum sensing threshold parameter was varied within one order of magnitude, $\tau = 10, 20, 30, 40, 50, 60, 70nM$. The maximum dispersal rate in these simulations was set to be $\eta_1 = 3.6^{-1}$, smaller than but in the same order of magnitude as the maximum growth rate. The simulations of the quorum sensing induced dispersal model are compared with the results of a non-quorum-sensing-producing biofilm ($\alpha = \beta = 0$). Lumped output parameters of the simulations are plotted in Fig. 2.3. In all cases we see that biofilm growth is sub-exponential, indicating nutrient limitations for growth.

For low values of $\tau$, the switching threshold is reached quickly leading to a rapid dispersal of the biomass before the biofilm can grow considerably as shown in Fig. 2.3a for $\tau \leq 30nM$. Both dispersed cells and autoinducers are removed quickly from the system. After the first dispersal event, the bacterial cells that are left behind in the biofilm are too few to keep the autoinducer concentration at levels that maintain dispersal, cf Fig. 2.3b and the quorum sensing signal concentration drops, see Fig. 2.3c. The biofilm population starts growing again. A newly increasing amount of biomass in the biofilm means a renewed increase in autoinducers, until these reach threshold and trigger the next dispersal event, and the pattern continues. Overall we see an almost periodic pattern of discrete dispersal events. The biofilm size did not shrink during the dispersal and loss of biomass events as shown in Fig. 2.3d, which implies that the biomass density in the biofilm colonies will reduce.
Figure 2.3: Temporal plots of simulations computed for a non-quorum sensing producing biofilm (Non-QS) and a quorum sensing producing biofilm. Here we used seven different quorum sensing threshold values $\tau = \{10, 20, 30, 40, 50, 60, 70\} nM$ and fixed maximum dispersal rate $\eta_1 = 3.6^{-1}$. Shown are (a) the total sessile biomass fraction $M_{tot}$ in the biofilm, (b) biomass loss $K(T)$ indicating the amount of biomass that actually dispersed, (c) the average autoinducer concentration $A_{avg}$ in $\Omega_2$, and (d) the biofilm size $\omega(t)$.

For higher values of $\tau$ ($\tau > 50 nM$), the biofilm develops into a stronger colony before the onset of dispersal. Release of cells from the biofilm into the
aqueous environment and removal from the system appears continuous and the
biofilm population reaches a plateau (see Fig. 2.3a) for $\tau \geq 50nM$. The higher the
threshold value is, the higher the level at which the biomass plateaus. The results
for $\tau = 40nM$ show a transition from the discrete sloughing-like dispersal event
to a continuous erosion-like dispersal event.

For all values of $\tau$, a substantial amount of the biomass that is produced in
the biofilm is dispersed and leaves the system. For low threshold values almost
all the produced biomass leaves, while even for high induction points still 90% of
the sessile biomass that is produced are lost.

A different growth behavior is observed when the biofilm produces no quorum sensing signal molecule (i.e. $\alpha = \beta = 0$), and thus does not induce dispersal. The biofilm growth for the Non-QS case was limited due to nutrient limitation as seen in Figs. 2.3a and 2.3d, albeit nutrient limitation is not sufficient enough to induce a leveling off of biomass production but that dispersal balances growth.

We also conducted simulations to investigate the effect of the maximum dispersal rate $\eta_1$ on the amount of cells dispersed and on the biofilm, which are reported in detail in Appendix A. The results obtained from this investigation reveals that the frequency of the dispersal event changes as $\eta_1$ changes. Lower dispersal rates led to a more continuous dispersal event. Increasing the dispersal rate resulted in a more rapid and discrete dispersal event, this is similar to what was observed for small induction threshold.
Figure 2.4: Temporal plots of results for constant dispersal with no influence of quorum sensing using dispersal rates $\eta_1 = 2.4d^{-1}$, compared to quorum sensing induced dispersal with $\tau = 50nM$. Shown are (a) the total amount of biomass in the biofilm $M_{\text{tot}}$, (b) the amount of suspended biomass that leaves the biofilm $P(T)$.

2.3.2 A quorum sensing controlled dispersal trigger allows the biofilm to mature before biomass loss

This simulation is carried out to investigate the effect of constant dispersal on biofilm with the aim of answering the question "If dispersal is important to biofilms, why don’t they shed cells continuously but rely on a mechanism like quorum sensing?". For these simulations, the biofilm consists of bacteria that produce quorum sensing signals. By "constant dispersal" we refer to the case where the dispersal rate in (2.1) is kept constant, i.e. does not depend on the autoinducer concentration. We call this also NonQS-induced. In the model, this is the limit case $\tau = 0$. This is compared with the situation whereby the dispersal is induced by quorum...
Figure 2.5: Temporal simulations results to investigate the re-attachment of bacterial cells after dispersal. Shown are (a) the ratio of dispersed cells that are re-attached and the cells that are detached $Z(t)$ using $\tau_L = 10nM, \tau_H = 70nM, \eta_{1L} = 0.6^{-1}, \eta_{1H} = 4.2^{-1}$; (b) amount of re-attached cells defined by $\eta_2 k_5 M$ and computed for different values of $k_5$ i.e. $k_5 = 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7gm^{-3}$ using the quorum sensing threshold value $\tau = 10nM$ and the maximum dispersal rate $\eta_1 = 3.6d^{-1}$.

sensing with $\tau = 50nM$. The maximum dispersal rate in these simulations is $\eta_1 = 2.4d^{-1}$. The other parameter values are listed in Table 2.1.

The results from these simulations are presented in Fig. 2.4 for the total sessile biomass fraction, and the amount of dispersed cells that leave the biofilm. We observe that quorum sensing induced dispersal allows the biofilm to grow before biomass loss is initiated, leading overall to a stronger biofilm, whereas continuous dispersal prevents notable growth of the biofilm.
2.3.3 Re-attachment of dispersed cells is negligible

Additional simulations were carried out to investigate the influence of the quorum sensing switching parameter $\tau$ and the maximum dispersal rate $\eta_1$ on re-attachment of bacterial cells to the biofilm. In these simulations we have considered four different scenarios consisting of high and low values of $\tau$ and $\eta_1$ respectively resulting from the following combinations: $\tau_H \eta_1 H$, $\tau_H \eta_1 L$, $\tau_L \eta_1 H$ and $\tau_L \eta_1 L$, where index $L$ represents 'Low value' and index $H$ represents 'High value'. The low and high values of $\tau$ are $\tau = 10 nM$ and $\tau = 70 nM$, respectively, while the low and high values of $\eta_1$ are $\eta_1 = 0.6 d^{-1}$ and $\eta_1 = 4.2 d^{-1}$, respectively. We assume here that the production of the signal molecule is not significantly influenced by the nutrient concentration, hence $\gamma(C) \equiv 1$. Every other parameter used for the simulation is as listed in Table 2.1.

By computing the ratio of the dispersed and re-attached cells $Z(t)$ as shown in Fig. 2.5a, we found that re-attachment is generally negligible compared to the amount of cells dispersed irrespective of the choice of $\tau$ and/or $\eta_1$.

Another parameter that controls re-attachment of cells is $k_5$ seen in equations (2.1), (2.2) describing the attraction of bacterial cells towards the biofilm. By setting $\tau = 10 nM$ and $\eta_1 = 3.6 d^{-1}$, we compare the amount of re-attached cells defined by $\eta_2 \frac{M}{k_5 + M}$ for different values of the parameter $k_5$, varied over one order of magnitude, $k_5 = 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 gm^{-3}$. We observe also here that re-attachment is negligible (see Fig. 2.5b).
\[ t = 2 \times 10^{-4} \]

\[ t = 5 \]

\[ t = 12 \]

\[ t = 14 \]

Figure 2.6: Simulation of biofilm growth for induction threshold \( \tau = 20nM \) and maximum dispersal rate \( \eta_1 = 3.6d^{-1} \). Color coded is the biomass density \( \dot{M} \), iso-lines of the autoinducer concentration \( A \) are plotted in grayscale.

### 2.3.4 Quorum sensing controlled dispersal can explain central hollowing

From the lumped output parameters in Figs. 2.3a and 2.3c we observe that during a discrete dispersal event and the following biomass growth period the biofilm size remains constant. This suggests that biomass loss due to dispersal does not lead to a shrinking of the colony but to a decrease in local cell density in the biofilm.

To investigate the effect of quorum sensing triggered dispersal on the spatial
Figure 2.7: Simulation of dispersed cells for induction threshold $\tau = 20nM$ and maximum dispersal rate $\eta_1 = 3.6d^{-1}$. Color coded is the biomass density $M$, iso-lines of the autoinducer concentration $A$ are plotted in grayscale. (This figure is not included in [19])

structure of the biofilm colonies and the local biomass distribution in more detail, we visualize two simulations, one in which discrete dispersal events are observed, with $\tau = 20nM$, $\eta_1 = 3.6^{-1}$ (Fig. 2.6), and one in which dispersal appears continuous, with $\tau = 60nM$, $\eta_1 = 3.6^{-1}$ (Fig. 2.8). We show for selected time instances the spatial distribution of the sessile biomass $M$ and iso-lines of the autoinducer concentration. In both cases we use the same initial distribution of biomass. Six colonies are randomly placed on the substratum. They differ in size, but the biomass density inside each colony is initially set to be 0.3.

In the case of the lower threshold value $\tau$ in Fig. 2.6, immediately after the simulation starts, $A$ is very small. At the next shown time instance $t = 5$ the biomass density inside the colonies has reached values close to maximum density
and expansion of the biofilm has started. The colonies have grown in size, a small
and a large colony that were initially placed close together have merged. Shortly
after, the first dispersal event was initiated, which leaves the colonies with lower
biomass density in the inner cores than in the outer rims. This structural change
is more conspicuous in the next snapshot at \( t = 12 \) taken shortly after the second
dispersal event. The colonies have still the same size as before, but in their inner
core the biomass density is substantially decreased. The amount of dispersed cells
in relation to the signal concentration can be seen in Figure 2.7. An exception is
the smaller colony in the middle of the domain, in which the biomass density is
larger than in the neighboring colonies. This is explained by lower autoinducer
concentrations there that have not triggered a discrete dispersal event. Due to
the smaller amount of biomass in the system overall and removal of the signal
from the system due to diffusion, the autoinducer concentration has dropped
again below the threshold value. At the last time instance that we show, \( t = 14 \),
the biomass inside the colonies has increased again, without a notable increase
in colony size. In the smallest colony in the center of the domain, in which no
dispersal event has taken place the biomass distribution is more homogeneous.

We contrast this with the result for the higher threshold value \( \tau = 60 \text{nM} \) in
Fig. 2.8. Immediately after the simulation starts, at \( t = 0.0002 \), the situation is the
same as in Fig. 2.6. At \( t = 5 \) The colonies have grown with a rather homogeneous
distribution of biomass. The small and large colony that were originally in close
proximity have merged. The signal concentration is still well below threshold
with a maximum value of \( A \approx 0.082 \tau \). Accounting for the different threshold
concentrations values, this is a value similar, but lower than at the same time in Fig. 2.6. At $t = 15$, the colonies have increased considerably in size, but in their inner regions the biomass density is much smaller than in the outer layers. The autoinducer concentration has increased as well, due to the larger amount of biomass. Although it remains clearly below the threshold value, substantial cell dispersal has started. Since the signal concentration are highest inside the
colonies, cells are lost there at a higher rate than in the outer layers. This is explained by Fig. 2.2, which shows that for such high threshold values even for signal concentrations clearly below $\tau$ the dispersal rate can be substantial. This pattern continues, as time progresses. For $t = 20$, we observe an increase in the size of the colonies, with an outer rim with high biomass density. The biomass density in the inner core decreases as the biofilm increases, leading to regions with fewer cells, i.e. hollowing structures, as reported in experimental studies, e.g. [23, 43].

We observe hollowing of biofilm colonies both for simulations with discrete and continuous cell dispersal, but there is a substantial difference. In the case of discrete dispersal events, the biomass density increases after the event, filling up the hollows. In the case of continuous dispersal loss, biomass is not replenished inside the colonies, rather the outer layers expand and the colony increases in size quicker, see also Fig. 2.3d. Hollowing occurs because the autoinducer concentration is highest in the inner layers of the colony, due to the maximum principle for diffusive systems, e.g. [74]. This implies that cells there up-regulate and disperse first.

### 2.3.5 Nutrient dependence of the autoinducer production rate has only minor effect on dispersal

In this simulation experiment, we investigated the influence of nutrient availability on the production of quorum sensing molecule, which was included in
our model (equation (2.4)) as an option controlled by the function $\gamma(C)$. The description of $\gamma(C)$ and the details of the simulation experiment and the results can be found in Appendix A. The effect of nutrient concentration on the production of the autoinducer signal molecule was investigated in two scenarios namely: biofilm and microfloc. For the biofilm case, high nutrient concentrations are observed only initially and decline quickly as the biofilm grows. In the case of a microfloc, there is a decreased autoinducer production during high concentration than when the nutrient concentration is low. In summary we observe that the influence of nutrient availability on the autoinducer production rate, as tested here, does not have much effect on cell dispersal.

2.4 Discussion

Modification of biofilm growth and dispersal. Our study presents, to our knowledge, the first theoretical model analyzing interaction between quorum sensing and dispersal in biofilms with respect to their effects on biofilm structure and population dynamics. The simulation results indicate that this interplay affects both the structure and thickness of existing biofilm colonies as well as the cell dispersal, i.e. the potential to colonize new habitats. The development of hollows, i.e. areas with very low cell densities within biofilms, and the potential to generate fluctuations of biofilm thickness, autoinducer concentration, cell dispersal and re-attachment are predicted by our model. The phenomenon of fluctuations in biofilms has been known before, but not necessarily discussed in the
context of quorum sensing, e.g. [44, 62]. Hollows caused by dispersion or other mechanisms are well-known in biofilms. The hollows within the colonies, which are predicted by our study, resemble the voids reported as a result of agr-QS induced detachment in an experimental study of *Staphylococcus aureus* colonies [95]. After detachment of the induced cells only a shell of non-induced cells remains, until the growing colony enters a new cycle of induction and detachment. On the biofilm scale, this can translate into waves of detachment and re-growth, which is connected with oscillations in biofilm mass and (effective) thickness. It was reported in [64] that such periodic detachment was mediated by quorum sensing induced surfactant production in *S. aureus* and speculated that this is a widespread mechanism in the bacterial world. Other released factors such as quorum sensing controlled exoprotease also have been found to promote detachment [16]. Generation of voids in biofilms or colonies caused by quorum sensing induced dispersal also occurs in other species, such as *Pseudomonas aeruginosa* [25]; [92] reported a rather continuous dispersal behavior in a *P. aeruginosa* biofilm.

Our study showed how cells principally can modify a number of critical traits such as biofilm thickness, biomass and fraction of dispersed cells by shifting QS threshold and dispersal rates. Even more, such shifts can switch dynamics of dispersal, biomass growth and autoinducer concentration between more continuously and discrete; the latter resulting in an oscillatory behavior. In case of such periodicity, amplitude and frequency can be changed via the same regulated parameters. In fact, experimental studies showed that cell dispersal rates
can be promoted by environmental factors like nutrient depletion [14, 44, 70]. Similarly, environmental stresses like starvation often result in a promotion of quorum sensing induction [41]. This can be achieved by regulation of autoinducer receptor or autoinducer synthase, which directly or indirectly corresponds to a shift of the threshold for induction [2, 3, 51, 54, 57, 73].

**Ecological relevance of biofilm and dispersal parameters.** Thickness and biomass have significant impact on the ecological functionality of biofilms. Growth in biofilms protects bacteria from environmental challenges such as antibiotica or other toxic substances, immune response in hosts, grazing stress from *protozoa*, and mechanical washout. Furthermore, it facilitates cooperation between cells. Most of these aspects are promoted with increasing biofilm thickness. Contrarily, competition for resources, such as nutrients, and waste accumulation impede growth of populations in biofilms of increasing thickness.

For colonization of new habitats bacteria in biofilms usually enter the planktonic state as single cells or in smaller groups with little EPS protection. The death rate in plankton is higher. Thus the decision between planktonic and biofilm states is a trade-off. It depends on a number of factors such as nutrient supply and pressure by competitors or predators, and therefore needs to be controlled by the cells, in order to promote fitness by keeping the biofilm at an optimal size. Our simulations indicate that biofilm dispersal tied to quorum sensing is a mechanism by which the biofilm could achieve this. Integration of nutrient or other stress aspects into the information carried by autoinducers probably allows the cells in a population for a coordinate response to environmental challenges,
optimized with respect to efficiency under the actual habitat conditions [41, 42].

It has been shown experimentally that the vast majority of cells produced in a biofilm will eventually detach and enter the aqueous phase [8, 12] and it was suggested that being a ”cell nursery”, i.e. a source of planktonic cells, is one of the functions of biofilms [13]. Our results are in agreement with this as we have seen that even maximum dispersal rates much smaller than maximum growth rates can lead to substantially more than 90% of cells detaching, both in a periodic or in a continuous dispersal mode. Moreover, experiments have indicated that dispersal of cells into the aqueous phase can occur at all stages of biofilm development, including small colonies that still grow or larger fully established colonies [6, 7, 12]; our simulations show that this is compatible with quorum sensing controlled dispersal, which regulated by parameters, can be observed for small and large colonies.

The outcome that the systems can change between continuous and oscillating behaviour by shifting one or a few controlled parameter values has some interesting ecological implications. Larger oscillatory detachment events will periodically move the biofilm thickness away from the optimal values with respect to the above mentioned fitness trade-off. On the other hand, such a coordinated detachment strategy might save costs for each involved cell, e.g. for the production of surfactants required for the removal from the biofilm matrix, and minimize loss by predators as known for mass events in eukaryotes. Although it is intriguing to assume that this degree of freedom is used by the cells to optimize behavior in a way dependent on the environmental conditions to optimize the
fitness, this has to be confirmed experimentally yet.

While detachment and dispersal are central features for the fitness and thus quite well investigated, relatively little is known about the dynamics and the underlying mechanisms. Although experimental studies with different species reported continuous and oscillatory dispersal behavior, to the best of our knowledge none investigated whether and under which conditions both can occur in the same species. Thus the model presented here gives some valuable indications. Although our model focuses on quorum sensing and dispersal, the outcome has an impact on a variety of other bacterial traits. Usually a bacterial quorum sensing system does not regulate just a single gene or phenotype, but up to several hundred genes and consequently a variety of phenotype aspects, including interaction with potential hosts [40, 71, 79, 91]

Thus, the strong effect of the interplay of quorum sensing and dispersal on the quorum sensing dynamics is directly connected with bacterial properties such as virulence in humans, animals or plants, but also to beneficial activities in other bacterial species. In summary, the interplay of quorum sensing and detachment under the influence of nutrients affects growth dynamics, structure and function of biofilms. Regulating this interplay adds a degree of freedom to bacterial biofilms in response to environmental conditions.

**Evolutionary advantage of QS regulated dispersal.** Although a thorough evolutionary analysis is beyond the scope of our study, it provides some hints to answer the question why QS control of dispersal might be advantageous. Beside the potential fitness benefits discussed above, i.e. keeping biofilm growth/thickness
and dispersal in an optimized balance, this control design enables a young colony or biofilm to focus first on growth, i.e. to maximize the protection rendered by attached growth in EPS matrices as fast as possible Fig. 2.4. Note that such a behavior could, in principle, also be reached by a more direct control of dispersal e.g. in dependency on nutrient depletion. Quorum sensing control allows for an integration of the specific information of each cell at its specific side in a spatially structured communication, enabling a response optimized rather with respect to the situation of each cell within the entire population than to the situation of isolated cells [42]. This is of special relevance in spatially structured populations, as e.g. cells in lower layers of the biofilm may be exposed to stronger nutrient stress, potentially resulting in an up-regulation of autoinducer production. As a result, the cell is enabled for a contextual interpretation of the state of the neighbouring cells relative to its own. Furthermore, QS control of dispersal promotes synchronization of response within the population, as seen e.g. in the oscillatory behavior.

**Treatment consequences.** Understanding the mechanisms that control biofilm dispersal and quorum sensing dynamics is of crucial interest from the human perspective, e.g. to develop and optimize treatment strategies, which consider or even exploit this interplay. As quorum sensing is a master regulator of virulence in most known pathogens, strategies for an efficient suppression are highly desirable and have been proposed as an alternative for antibiotics [4, 5]. On the one hand, promotion of detachment by other, non-quorum-sensing related inducers
would help to diminish or avoid virulence and, by limiting or even decreasing biofilm thickness, could promote effectiveness of antibiotic treatment. Detached, i.e. planktonic cells are assumed to be more vulnerable for antibiotica treatment, e.g. [14]. If a sufficiently high dose of antibiotics is supplied, a treatment which shifts the population towards the planktonic state will probably support an eradication of the infection [13].

To go even a step further, shifting the detachment towards an oscillating behavior might be another or an additional strategy, as thinner biofilms, which periodically emerge, combined with a larger fraction of cells in the planktonic state, could be more susceptible to antibiotics. Possible treatments include drugs that directly affect quorum sensing systems and/or detachment e.g. by blocking or mimicking autoinducers. Such treatment strategies are currently under development (see e.g. [72]). Our study indicates that in future more indirect treatments, e.g. via modulating nutritional or stress conditions, could aim at mechanisms which down-regulate quorum sensing threshold or up-regulate dispersal.

Antibiotic treatments might be associated with additional effects relevant for signaling, which are not regarded in our model, e.g. emergence of layers of dead cells possibly interfering with signal diffusion, and up-regulation of quorum sensing activity by low sublethal drug concentrations, which might occur in lower parts of a biofilm [18, 51]. The net effect of such new strategies should be estimated by a combination of experimental and mathematical modeling studies. Thus, a detailed knowledge about the interplay of quorum sensing, dispersal and nutrients is of high interest. Our study attempts to be a first step for such new
and promising strategies.

2.5 Conclusion

In summary, our in silico experiments suggest the following conclusions:

- Dispersal of cells from the biofilm into the aqueous environment balances growth of the biofilm and is important for downstream colonization. Coupling the dispersal rate to quorum sensing provides the opportunity for the biofilm colony to first invest in itself and to grow to a certain community size before shifting to a mode of producing cells for downstream proliferation.

- A single quorum sensing based mechanism can explain both, periodic dispersal in discrete events and continuous dispersal, depending on parameters. It can also, and again in dependence of parameters, explain cell dispersal from smaller and larger colonies. It also provides a potential mechanism for the biofilm to regulate dispersal independent of its size and to ensure a certain colony strength.

- Surface attached microcolonies of biofilms undergo internal changes during seeding dispersal. Quorum sensing induced cell dispersal may affect the structure and architecture of the biofilm and can leave behind ”hollow” shell-like structure, which are less dense with few cells inside. In this process of dispersal, only very few of the dispersed cells get re-attached to
the biofilm, hence we conclude that the re-attachment of dispersed bacterial cells is very negligible.

- Quorum sensing triggered seeding dispersal can lead to a substantial amount of cells produced in a biofilm under protected conditions to be shed into the environment. This supports the notion that biofilm act as cell nurseries in the facilitation of downstream colonization.

- Interfering with the quorum sensing mechanism and enhancing dispersal might make the biofilm more vulnerable to antibiotics; on the other hand suppressing quorum sensing might make the biofilm more susceptible to mechanical removal and slow down the biofilm’s potential for downstream colonization.
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Chapter 3

Mathematical Analysis of a Quorum Sensing Induced Biofilm Dispersal Model

Emerenini BO, Sonner S, Eberl HJ: Mathematical Analysis of a Quorum Sensing Induced Biofilm Dispersal Model (To be submitted)

Abstract

We analyze a mathematical model of quorum sensing induced biofilm dispersal. It is formulated as a system of non-linear, density-dependent, diffusion-reaction equations. The governing equation for the sessile biomass comprises two non-linear diffusion effects, a degeneracy as in the porous medium equation and fast diffusion. We prove the existence and uniqueness of bounded non-negative solutions of the degenerate problem by considering smooth regular approximations and passing to the limits. Moreover, we illustrate the behaviour of the solutions in numerical simulations.
3.1 Introduction

Biofilms are dense accumulations of microbial cells on biotic or abiotic surfaces (called substrata) in aqueous environments. Once the microbial cells become sessile, they produce extracellular polymeric substances that protect them against antibiotic attacks and mechanical washout [27]. Due to the sorption properties and enhanced mechanical stability of biofilms, they are beneficially used in wastewater treatment, soil remediation and groundwater protection [38]. On the other hand, biofilm formation and detachment can have very disadvantageous effects and lead to serious infections in the human body, biocorrosion of drinking water pipes or industrial facilities [12], contamination in food processing plants [25, 28], etc. Biofilm formation is characterized by the balance of attachment, growth and detachment (or dispersal) processes [23, 37]. Among these phenomena, there is a growing interest in the study of detachment [30]; detachment is the release of microbial cells from the biofilm into the aqueous environment. Biofilm dispersal (detachment) can be internally triggered, e.g. by enzyme-mediated breakdown of the biofilm matrix [6], production of surfactants which loosen cells from the biofilm [9]; or externally triggered, e.g. changes in nutrient availability [23], production of free-radical species [5] and control by quorum sensing systems [31, 33, 41]. Dispersed cells can contribute to downstream col-
onization and thus result in pipe obstructions, bacterial infection (biomedical implants), or increased microbial contamination in food processing plants [35]. This makes cell dispersal societally important in an industrial, but much more so in medical and public health context, and it is therefore crucial in the study of biofilms.

Mathematical models of biofilms have been studied for several decades and range from traditional one-dimensional models that describe biofilms as homogeneous layers to two- and three-dimensional biofilm models that account for the spatial heterogeneity of biofilm communities observed in laboratories [1, 7, 8, 29, 36, 42]. The model we analyze describes quorum sensing controlled biofilm detachment and is based on the interpretation of a biofilm as a continuous spatially structured microbial population. It is a system of partial differential equations for four dependent variables, namely: volume fraction of sessile biomass, the density of dispersed cells, concentration of a growth limiting substrate; and the concentration of the signaling molecule (also known as autoinducer). The model under study was originally introduced in [19], and extends the prototype growth model [13] for a single species biofilm. In fact, in the absence of quorum sensing molecules and dispersed cells, the model [19] reduces to the prototype growth model for the biomass and a single substrate. Such mono-species single-substrate biofilm models have been studied both analytically and numerically [14, 17, 13, 24]. The question of well-posedness of the model was not considered in [19] and will be addressed in this study.

The prototype biofilm growth model proposed in [13] consists of two reaction-
diffusion equations for the biomass density and the growth limiting substrate; as an extension we include the production of the quorum sensing molecule and dispersion of cells. The total biomass in the biofilm is split into two, namely: the sessile biomass and the suspended (dispersed) biomass. The diffusion coefficient for the sessile biomass shows two non-linear diffusion effects: (a) it degenerates like the porous medium equation for vanishing biomass densities and (b) the diffusion coefficient blows up if the local cell density approaches its maximum value. These effects ensure that the biofilm/water interface spreads at a finite speed and that the maximum biomass density is never exceeded. The biofilm expands spatially if the local cell density fills up the available volume while it does not spread notably if there is space locally available to accommodate new cells.

Some recent studies include more than one substrate (or component), for instance the model [21] which extends the prototype biofilm growth model and combines it with a model for the production of the quorum sensing molecule in patchy biofilm communities with slow background flow. The analytic aspects of the quorum sensing model [21] were addressed in [34]. The structure of the quorum sensing induced detachment model is different from the previous multi-component biofilm models [11, 24, 34, 16]. To prove existence and uniqueness of solutions and continuous dependence of solutions on initial data we use ideas applied in [18] for the mono-species model and in [2] for a scalar degenerate reaction-diffusion equation of porous-medium type.
### 3.2 Mathematical Model

We analyze the mathematical model of quorum sensing induced detachment in biofilms which was proposed in [19]. It is a single species model for a biofilm composed of sessile biomass and detached biomass. We denote the total biomass fraction of the sessile cells by $M$ and density of the detached cells by $N$. The bacterial growth depends on the growth limiting substrate, a nutrient whose concentration is denoted by $C$. The bacterial cells have the ability to produce quorum sensing signal molecules whose concentration is denoted by $A$. The model is formulated as a system of non-linear reaction-diffusion equations in a bounded spatial domain $\Omega \subset \mathbb{R}^m$, $n \in \{1, 2, 3\}$ with piecewise smooth boundary $\partial \Omega$. The spatial independent variable is denoted by $x \in \Omega$ and $t \geq 0$ denotes the time variable.

In dimensionless form, the four dependent variables $M, N, C$ and $A$ satisfy the parabolic system

\[
\begin{align*}
\partial_t M &= \nabla \cdot (D_M(M)\nabla M) + \frac{C}{k_1 + C} M - k_2 M - \eta_1 \left( \frac{A^m}{1 + A^m} \right) M \\
\partial_t N &= d_1 \Delta N + \frac{C}{k_1 + C} N - k_2 N + \eta_1 \left( \frac{A^m}{1 + A^m} \right) M \\
\partial_t C &= d_2 \Delta C - \frac{\sigma C}{k_1 + C} (M + N) \\
\partial_t A &= d_3 \Delta A - \lambda A + \left[ \alpha + \beta \frac{A^m}{1 + A^m} \right] (M + N)
\end{align*}
\]  

whereby we have used the following re-scaling: $\tilde{x} = \frac{x}{L}$, $\tilde{t} = t \mu$, $\tilde{C} = \frac{C}{C_\infty}$, $\tilde{A} =$
\[ \frac{A}{\tau}, \quad d_C = \frac{d_C}{\mu L^2}, d_A = \frac{d_A}{\mu L^2}, \quad D_M = \frac{D_M}{\mu L^2}, \quad d_N = \frac{d_N}{\mu L^2}, \quad k_1 = \frac{k_1}{C_\infty}, \quad \alpha = \frac{n M_\infty}{\tau \mu}, \quad \beta = \frac{\beta M_\infty}{\tau \mu}\]

where \( L \) is the length of the biofilm and \( \frac{1}{\mu} \) is the characteristic time scale.

The first and second equation of (3.1) describe the growth, spatial movement, decay and detachment of sessile and dispersed biomass, \( M \) and \( N \). These are directly coupled by the dispersal terms. We have intentionally omitted the re-attachment terms which were originally included in the model [19]. The simulation results in [19] show that only a negligible amount of dispersed cells gets re-attached, and hence the inclusion or exclusion of the re-attachment terms will not make a significant difference from an application point of view. The third equation in (3.1) describes diffusion and consumption of nutrients by \( M \) and \( N \), while the fourth equation describes the production, decay and diffusion of the quorum sensing molecules (i.e. autoinducers). We included an abiotic autoinducer decay rate \( \lambda > 0 \) in the fourth equation in (3.1) which is different from [19]. Such an abiotic decay rate has been used in a similar scenario [34] satisfying \( \lambda < \alpha + \beta \) which implies that the signal molecule production rate of the up-regulated cells is higher than the abiotic decay rate.

The biofilm is the region where sessile biomass is present, \( \Omega_2(t) := \{ x \in \Omega : M(t, x) > 0 \} \), whereas the surrounding aqueous phase is the region where sessile biomass is absent, \( \Omega_1(t) := \{ x \in \Omega : M(t, x) = 0 \} \), cf Figure 3.1. Since \( M \) changes with time due to growth and dispersal, also these two regions change. They are separated by the biofilm/water interface, \( \Gamma(t) := \partial \Omega_2(t) \setminus \partial \Omega \). Neither \( \Omega_1(t) \) nor \( \Omega_2(t) \) need to be connected domains. In fact, \( \Omega_2(t) \) will in general
Figure 3.1: Schematic of the biofilm system: The aqueous phase is the domain \( \Omega_1(t) = \{(x, y) \in \Omega : M(t; x, y) = 0\} \), the biofilm phase \( \Omega_2(t) = \{(x, y) \in \Omega : M(t; x, y) > 0\} \). These regions change over time as the biofilm grows. Biofilm colonies form attached to the substratum, which is a part of the boundary of the domain.

 consist of several colonies that are separated from each other by water-filled channels and voids. If a biofilm is contained in the inner region of \( \Omega \), away from its boundary, it is often called microbial floc (biofilm without substratum). This situation plays a major role in biological wastewater treatment.

The diffusion coefficient for the sessile biomass is density dependent and according to [13] given by

\[
D_M(M) = \delta \frac{M^a}{(1 - M)^b}, \quad \text{where } a, b > 1, \delta > 0.
\]  

(3.2)

The biomass diffusion coefficient \( D_M(M) \) vanishes when the total biomass approaches zero and blows up when the biomass density tends to its maximum value. The spatial expansion of the biofilm is driven by biomass accumulation. The polynomial degeneracy \( M^a \), well known from the porous medium equation,
guarantees that spatial spreading is negligible for low values of $M$ and yields the separation of biofilm and liquid phase.

For $0 \ll M \approx 1$, the equation shows a super-diffusion effect, the diffusion coefficient possesses a singularity at $M = 1$ and blows up. This ensures the maximal bound for the biomass density which is a physical limitation as the number of cells that fits into a unit volume is bounded [34]. In particular, the blow up of the diffusion coefficient guarantees that $M < 1$ if a homogeneous Dirichlet conditions are specified on some parts of the boundary of the domain.

Since the production of biomass depends on the availability of nutrients, the upper bound on $M$ cannot be guaranteed by the growth terms alone. The degeneracy $M^a$ alone does not yield this maximum bound for the cell density, while the singularity $(1 - M)^{-b}$ does not guarantee the separation of biofilm and liquid region by a sharp interface. Consequently, both non-linear diffusion effects are required to describe spatial expansion of the biofilm.

The diffusion coefficients $d_{N,C,A}$ of $N, C$ and $A$ as defined in [19] also depend on the cell density, albeit in a non-critical way; They are lower inside the biofilm than in the surrounding liquid region but well within one order of magnitude. In [19] the linearization ansatz

$$d_{N,C,A}(M) = d_{N,C,A}(0) - M(d_{N,C,A}(0) - d_{N,C,A}(1))$$

is made, where $d_{N,C,A}(0)$ denotes the diffusivity in water and $d_{N,C,A}(1)$ the diffusivity in a fully compressed biofilm for $N, C$ and $A$ respectively. Hence, the functions $d_{N,C,A}(M)$ are bounded from below and above by a positive constant.
and essentially behave like Fickian diffusion. In fact, in many cases, in particular for substrates of small molecular size such as oxygen, carbon, etc., $d_{N,C,A}(0) \approx d_{N,C,A}(1)$. Therefore, for simplicity we assume that the diffusion coefficients $d_{N,C,A}(M)$ are constants and write $d_N(M) = d_1, d_C(M) = d_2, d_A(M) = d_3$, where the constants $d_1, d_2, d_3$ are positive. We remark that the diffusion of the sessile biomass $M$ is non-Fickian whereas the diffusion of the dispersed biomass $N$ is Fickian, hence the dispersed cells will be treated like dissolved substrate in the analysis in this study. The following growth and decay processes are incorporated in the model.

- Growth of sessile and dispersed cells ($M$ and $N$) is controlled by the local availability of nutrients. This is described by standard Monod kinetics in the first and second equation of (3.1), where $k_1$ is the half saturation concentration. The growth kinetics is assumed to be the same for both sessile and dispersed biomass.

- Natural cell death occurs at the rate $k_2$ for $M$ and $N$. For simplicity, this value is assumed to be the same for both sessile and dispersed biomass.

- Dispersal, i.e. the transition of bacteria from the sessile state into the suspended motile state, is controlled by the local concentration of the quorum sensing molecule. For small concentrations of the quorum sensing molecule this transition is nearly zero but increases for large concentration values and levels off at a maximum rate. This transition between both states is described by a Hill function.
• Consumption of nutrient is determined by Monod kinetics at the rate $\sigma$ in the third equation in (3.1). This is proportional to the biomass growth rate.

• The signal molecules $A$ in the fourth equation of (3.1) are produced at a base rate $\alpha$ if the local signal concentration is small, and at the increased rate $\alpha + \beta$ if it exceeds unity. This transition is modelled by a Hill function with exponent $m$ describing a smooth transition between the production rate of down-regulated cells and the maximum production rate of up-regulated cells.

It remains to specify initial and boundary values for the biomass fraction $M$ and concentrations of detached cells $N$, nutrient $C$ and quorum sensing molecule $A$ to complement the model. This will be made precise in the following section.
3.3 Analysis of the quorum sensing detachment model

Preliminaries

For technical reasons we study the model in the auxiliary form

\[
\begin{align*}
\partial_t M &= \nabla(D_M(M)\nabla M) + \frac{C}{k_1 + C} M - k_2 M - \eta_1 \left( \frac{|A|^m}{1 + |A|^m} \right) M, \\
\partial_t N &= d_1 \Delta N + \frac{C}{k_1 + C} N - k_2 N + \eta_1 \left( \frac{|A|^m}{1 + |A|^m} \right) M, \\
\partial_t C &= d_2 \Delta C - \frac{\sigma C}{k_1 + C} (M + N), \\
\partial_t A &= d_3 \Delta A - \lambda |A| + \left[ \alpha + \beta \frac{|A|^m}{1 + |A|^m} \right] (M + N),
\end{align*}
\]

(3.3)

with initial and boundary conditions

\[
\begin{align*}
M|_{\partial \Omega} &= 0, & N|_{\partial \Omega} &= 0, & C|_{\partial \Omega} &= C_\infty, & A|_{\partial \Omega} &= 0, \\
M|_{t=0} &= M_0, & N|_{t=0} &= N_0, & C|_{t=0} &= C_0, & A|_{t=0} &= A_0,
\end{align*}
\]

(3.4)

where \(C_\infty\) is a positive constant, \(M_0, N_0, C_0, A_0\) are non-negative and in \(L^\infty(\Omega)\).

Moreover, we assume that

\[
\|M_0\|_{L^\infty(\Omega)} < 1 - \rho,
\]

(3.5)
for some $\rho \in (0, 1)$.

We point out that non-negative solutions of (3.1) solve (3.3) and vice versa, i.e. after the non-negativity is shown, the absolute value $|.|$ can be removed from (3.3) to obtain (3.1). Constant Dirichlet boundary conditions are imposed on the nutrient concentration $C$ reflecting a constant unlimited nutrient supply at the boundary of the considered domain, while homogeneous Dirichlet boundary conditions are assumed for $N$ and $A$ to enforce the removal of dispersed cells and quorum sensing signal molecules. Similarly, homogeneous Dirichlet boundary conditions are assumed for the biomass fraction $M$. This describes the situation of a growing biofilm in the interior of the considered domain, away from the boundary. These specific boundary conditions are primarily chosen for convenience with respect to the analysis, albeit boundary conditions of mixed type are often considered more appropriate in applications. Typically, Dirichlet boundary conditions are prescribed on some parts of the boundary while Neumann or Robin boundary conditions are specified on the other parts. In particular, the substratum on which the biofilm grows is impermeable for all dependent variables, and can be modelled by homogenous Neumann boundary conditions.

Here and in the sequel, we use the following notations, $Q_T := (0, T] \times \Omega$ for $T > 0$, $Q = \mathbb{R}^+ \times \Omega$ and

$$\Phi(M) := \int_0^M D(s)ds = \int_0^M d \frac{s^a}{(1-s)^b}ds \text{ for } 0 \leq M < 1.$$ 

**Definition 1.** We call $(M, N, C, A)$ a solution of system (3.3) with the initial and
boundary data (3.4), if for any $T > 0$ the functions

$$M, N, C, A \in C([0, T]; L^1(\Omega)) \cap L^\infty(Q_T)$$

and satisfy (3.3) in distributional sense.

More precisely, if $M$ is a solution of system (3.3), then

$$\int_\Omega (M\varphi) |_{s=t} - \int_\Omega M_0\varphi |_{s=0} - \int_{Q_t} (M\partial_s \varphi + \Phi(M)\Delta \varphi) = \int_{Q_t} g(M, N, C, A) \varphi,$$

for all $t \in [0, T]$ and $\varphi \in C^2(Q_T)$ such that $\varphi \geq 0$ in $Q_T$ and $\varphi|_{\partial\Omega} = 0$. Similarly, for $C$ we have

$$\int_\Omega (C\varphi) |_{s=t} - \int_\Omega C_0\varphi |_{s=0} - \int_{Q_t} (C\partial_s \varphi + C\Delta \varphi) + \int_0^t \int_{\partial\Omega} C_\infty \partial_n \varphi = \int_{Q_t} f_2(M, N, C, A) \varphi,$$

for all $t \in [0, T]$ and $\varphi \in C^2(Q_T)$ such that $\varphi \geq 0$ in $Q_T$ and $\varphi|_{\partial\Omega} = 0$. As usual, $\partial_n$ denotes the outward normal derivative at the boundary. The identities for $N$ and $A$ follow accordingly.

### 3.3.1 Existence for smooth initial data

We consider smooth non-degenerate approximations for system (3.3) and show that their solutions converge to the solution of the degenerate problem (3.3). The ideas are based on the proof developed for scalar degenerate reaction-diffusion equations of porous medium type in [3], the solution theory in [18] for the single species biofilm model and the ideas applied in [11, 24, 34] and [16] for multi-
species biofilm models.

For $\epsilon > 0$ small, we define

$$D_\epsilon(M) := \begin{cases} 
  d\epsilon^a & \text{if } M < 0, \\
  d\frac{(M+\epsilon)^a}{(1-M)^\alpha} & \text{if } 0 \leq M \leq 1 - \epsilon, \\
  d\frac{1}{\epsilon^a} & \text{if } M \geq 1 - \epsilon.
\end{cases}$$

(3.6)

$$\Phi_\epsilon(M) := \int_0^M D_\epsilon(s)ds,$$

and denote the solutions of the regular auxiliary systems

$$\begin{align*}
  \partial_t M^\epsilon &= \nabla \cdot (D_\epsilon(M^\epsilon)\nabla M^\epsilon) + g(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon), \\
  \partial_t N^\epsilon &= d_1 \Delta N^\epsilon + f_1(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon), \\
  \partial_t C^\epsilon &= d_2 \Delta C^\epsilon + f_2(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon), \\
  \partial_t A^\epsilon &= d_3 \Delta A^\epsilon + f_3(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon).
\end{align*}$$

(3.7)

by $(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)$.

**Lemma 1.** Let the boundary and initial data be non-negative and smooth, $M_0 \in C_0^\infty, N_0, A_0 \in C^\infty, C_0 \in C^\infty(\overline{\Omega})$ such that $C_0|_{\partial\Omega} = 1$ and $\|M_0\|_{L^\infty(\Omega)} < 1 - \rho$. Then, there exist unique solutions $(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon) \in C^{1,2}(\overline{Q_T})$ of (3.3), that are non-negative and uniformly bounded w.r.t. $\epsilon > 0$ in $L^\infty(Q_T)$.

**Proof.** By the classical theory for quasilinear parabolic equations there exist unique solutions $(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon) \in C^{1,2}(\overline{Q_T})$ of (3.3). We use comparison theorems for quasilinear parabolic equations (e.g., see [3]) for each equation separately to show the non negativity and uniform boundedness of all components.

All components of the solution take non-negative values at the boundary,
and the initial data are non-negative. Moreover, we observe that
\[ g(0, N, C, A) = 0 = f_2(M, N, 0, A), \]
and, hence, zero is a subsolution for \( M^\epsilon \) and \( C^\epsilon \). Since \( M^\epsilon \) is non-negative and
\[ f_1(M, 0, C, A) \geq 0 \quad \text{for } M \geq 0, \]
we conclude the non-negativity of \( N^\epsilon \). Finally, we observe that
\[ f_3(M, N, C, 0) \geq 0 \quad \text{for } M \geq 0, N \geq 0, \]
which implies that zero is a subsolution for \( A^\epsilon \). To show the uniform boundedness of \( M^\epsilon \) we introduce the barrier function \( M_\theta = 1 + \theta \), where \( \theta \) is a solution of the elliptic problem
\[ \Delta \theta(x) = -1, \quad x \in \Omega \]
\[ \theta|_{\partial \Omega}(x) = 0. \]
(3.8)
The maximum principle implies that \( \theta \geq 0 \) in \( \Omega \), and
\[ 1 \leq M_\theta(x) \leq 1 + R_1, \quad x \in \Omega \]
for some constant \( R_1 \geq 0 \). Moreover, we observe that
\[ M_0 = M^\epsilon|_{t=0} \leq M_\theta|_{t=0}, \quad 0 = M^\epsilon|_{\partial \Omega} \leq M_\theta|_{\partial \Omega} \quad \text{and} \]
\[
\partial_t M_\theta - \nabla \cdot (D_\epsilon(M_\theta)\nabla M_\theta) - \frac{C^\epsilon}{k_1 + C^\epsilon} \theta + k_2 \theta + \eta_1 \left( \frac{|A^\epsilon|^n}{1 + |A^\epsilon|^n} \right) M_\theta \\
= \frac{d}{\epsilon^b} \frac{C^\epsilon}{k_1 + C^\epsilon} \theta + k_2 \theta + \eta_1 \left( \frac{|A^\epsilon|^n}{1 + |A^\epsilon|^n} \right) M_\theta \\
\geq \frac{d}{\epsilon^b} \theta + k_2 \theta \geq \frac{d}{\epsilon^b} - (1 + R_1) \\
\geq 0 = \partial_t M^\epsilon - \nabla \cdot (D_\epsilon(M^\epsilon)\nabla M^\epsilon) - g(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon),
\]

for all sufficiently small \( \epsilon > 0 \). Consequently, there exists \( \epsilon'_0 > 0 \) such that the solutions \( M^\epsilon \) are uniformly bounded for all \( 0 < \epsilon \leq \epsilon'_0 \).

Next, we show the uniform boundedness of the nutrient concentration \( C^\epsilon \) by defining \( C_{\text{max}} := \max\{||C_0||_{L^\infty(\Omega)}, ||C_\infty||_{L^\infty(\partial\Omega)}\} \). Then

\[
\partial_t C_{\text{max}} - d_2 \Delta C_{\text{max}} + \sigma \frac{C_{\text{max}}}{k_1 + C_{\text{max}}} (M^\epsilon + N^\epsilon) = \sigma \frac{C_{\text{max}}}{k_1 + C_{\text{max}}} (M^\epsilon + N^\epsilon) \geq 0
\]

where we have used the non-negativity of the sessile biomass \( M_\epsilon \) and the dispersed cells \( N_\epsilon \). This shows that \( C_{\text{max}} \) is an upper solution for \( C^\epsilon \). To prove the uniform boundedness of \( N^\epsilon \) we denote by \( \hat{N} \) the solution of the initial value problem

\[
\partial_t \hat{N} = d_1 \Delta \hat{N} + \hat{N} - k_2 \hat{N} + \eta_1(1 + R_1), \\
\hat{N}|_{\partial\Omega} = 0, \\
\hat{N}|_{t=0} = N_0,
\]

where \( 1 + R_1 \) is the upper bound for the sessile biomass fraction. We observe that \( \hat{N} \) is non-negative, satisfies \( \hat{N} \in L^\infty(Q_T) \) and

\[
\partial_t \hat{N} - d_1 \Delta \hat{N} - \frac{C^\epsilon}{k_1 + C^\epsilon} \hat{N} + k_2 \hat{N} - \eta_1 \frac{|A^\epsilon|^m}{1 + |A^\epsilon|^m} M^\epsilon \\
\geq \partial_t \hat{N} - d_1 \Delta \hat{N} - \hat{N} + k_2 \hat{N} - \eta_1(1 + R_1) \\
= 0 = \partial_t N^\epsilon - d_1 \Delta N^\epsilon - \frac{C^\epsilon}{k_1 + C^\epsilon} N^\epsilon + k_2 N^\epsilon - \eta_1 \frac{|A^\epsilon|^m}{1 + |A^\epsilon|^m} M^\epsilon.
\]
Consequently, $\tilde{N}$ is an upper solution for the dispersed cells $N^\epsilon$.

Finally, we proof the uniform boundedness of the quorum sensing signal molecule concentration $A$ by showing that there exists a constant $A_{\text{max}}$ which is an upper solution for $A^\epsilon$. It satisfies $A_{\text{max}}|_{\partial \Omega} \geq 0 = A^\epsilon|_{\partial \Omega}$, $A_{\text{max}}|_{t=0} \geq A_0 = A^\epsilon|_{t=0}$ and

$$
\partial_t A_{\text{max}} - d_A \Delta A_{\text{max}} + \lambda A_{\text{max}} = \left[ \alpha + \beta \frac{|A_{\text{max}}|^m}{1 + |A_{\text{max}}|^m} \right] (M^\epsilon + N^\epsilon)
$$

$$
= \lambda A_{\text{max}} - \left[ \alpha + \beta \frac{|A_{\text{max}}|^m}{1 + |A_{\text{max}}|^m} \right] (M^\epsilon + N^\epsilon)
$$

$$
\geq \lambda A_{\text{max}} - (\alpha + \beta)(|M^\epsilon| + |N^\epsilon|)
$$

$$
\geq \lambda A_{\text{max}} - (\alpha + \beta)(1 + R_1 + \tilde{N})
$$

$$
\geq 0
$$

(3.10)

where we use the boundedness of $M^\epsilon$ and $N^\epsilon$; and $\frac{|A_{\text{max}}|^m}{1 + |A_{\text{max}}|^m} \leq 1$; this shows that $A_{\text{max}}$ is an upper solution for $A^\epsilon$. 

In the following Lemma, we improve the upper bound on the sessile biomass density. In particular, we show that the singularity for $M = 1$ is not attained.

**Lemma 2.** *Under the hypothesis of Lemma 1, there exist $\delta > 0$ and $\epsilon_0 > 0$ such that $M^\epsilon \leq 1 - \delta$ in $Q_T$ for all $\epsilon < \epsilon_0$.*

**Proof.** In order to improve the upper estimate on $M$ we construct a suitable barrier function and consider the elliptic problem

$$
\Delta \theta(x) = -c_1, \quad x \in \Omega,
$$

$$
\theta|_{\partial \Omega} = c_2, \quad x \in \partial \Omega.
$$

(3.11)

The constants $c_1$ and $c_2$ are defined by

$$
c_1 := \|g(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)\|_{L^\infty(Q_T)}
$$

$$
c_2 := \|\Phi_0(M_0)\|_{L^\infty(Q_T)}
$$

(3.12)

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for \( \epsilon < \epsilon_0 \), and \( \Phi_\epsilon(M_0) := \int_0^{M_0} \frac{(s+\epsilon)^n}{(1-\epsilon^n)} ds \) for \( 0 \leq M_0 < 1 - \epsilon \). We remark that for sufficiently small \( \epsilon' > \epsilon_1 := \min\{\delta, \epsilon_0\} \), the constants \( c_1 \) and \( c_2 \) can be chosen uniformly for all \( 0 < \epsilon < \epsilon_1 \). Moreover, the solution \( \theta \) of (3.11) is bounded on \( \Omega \), and by the maximum principle follows \( \theta \geq c_2 \) in \( \Omega \).

For \( \epsilon < \epsilon_1 \) we define \( Z_\epsilon := \Phi^{-1}_\epsilon(\theta) \) and observe that

\[
\partial_t Z_\epsilon - \Delta(\Phi_\epsilon(Z_\epsilon)) = c_1 = \|g(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)\|_{L^\infty(Q_T)} \geq \partial_t M^\epsilon - \Delta(\Phi_\epsilon(M^\epsilon))
\]

in \( Q_T \). Moreover, the boundary conditions imply

\[
Z_\epsilon|_{\partial\Omega} = \Phi^{-1}_\epsilon(\theta)|_{\partial\Omega} = \Phi^{-1}_\epsilon(c_2) \geq M^\epsilon|_{\partial\Omega} = 0,
\]

and the initial data satisfies

\[
Z_\epsilon|_{t=0} = \Phi^{-1}_\epsilon(\theta)|_{t=0} \geq \Phi^{-1}_\epsilon(c_2) \geq \Phi^{-1}_\epsilon(\Phi_\epsilon(M_0)) = M_0,
\]

where we used the monotonicity of the function \( \Phi^{-1}_\epsilon \). Consequently, the function \( Z_\epsilon \) is an upper solution for the total biomass \( M^\epsilon \). Using the fact that \( \theta \) is bounded in \( \Omega \) and that \( \Phi^{-1}_\epsilon \) converges pointwise to infinity in the interval \((0,1)\), we conclude that there exist \( 0 < \epsilon_0 \leq \epsilon_1 \) and \( \delta \in (0,1) \) such that \( M^\epsilon \leq Z_\epsilon = \Phi^{-1}_\epsilon(\theta) < 1 - \delta \) for all \( \epsilon < \epsilon_0 \).

\[ \square \]

**Lemma 3.** Under the hypotheses of Lemma 1, the approximate solutions \( M^\epsilon \) satisfy

\[
\int_{Q_T} D_\epsilon(M^\epsilon) \left( \partial_t M^\epsilon \right)^2 + \sup_{t \in [0,T]} \int_{\Omega} |\nabla \Phi_\epsilon(M^\epsilon)|^2 \leq c
\]

\[
\int_{Q_T} \left( |\partial_t N^\epsilon|^2 + |\partial_t C^\epsilon|^2 + |\partial_t A^\epsilon|^2 \right) + \sup_{t \in [0,T]} \int_{\Omega} \left( |\nabla N^\epsilon|^2 + |\nabla C^\epsilon|^2 + |\nabla A^\epsilon|^2 \right) \leq c,
\]

for some constant \( c > 0 \).
Proof. We first multiply the second equation in (3.7) by $\partial_s N^\epsilon$ and integrate,

$$
\int_\tau^t \int_\Omega |\partial_s N^\epsilon|^2 = -\frac{d_1}{2} \int_\tau^t \int_\Omega |\nabla N^\epsilon|^2 + \int_\tau^t \int_\Omega \partial_s N^\epsilon f_1(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon),
$$

where $0 \leq \tau \leq t \leq T$. Using Young’s inequality leads to the estimate

$$
\int_\tau^t \int_\Omega |\partial_s N^\epsilon|^2 + \frac{d_1}{2} \int_\Omega |\nabla N^\epsilon|^2 |_{s=t}
= \frac{d_1}{2} \int_\Omega |\nabla N^\epsilon|^2 |_{s=\tau} + \int_\tau^t \int_\Omega \partial_s N^\epsilon f_1(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)
\leq \frac{d_1}{2} \int_\Omega |\nabla N^\epsilon|^2 |_{s=\tau} + \xi \int_\tau^t \int_\Omega |\partial_s N^\epsilon|^2 + C\xi \int_\tau^t \int_\Omega |f_1(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)|^2,
$$

for small $\xi > 0$, and some constant $C\xi$. Setting $\tau = 0$ we obtain

$$
(1 - \xi) \int_{Q_t} |\partial_s N^\epsilon|^2 + \frac{d_1}{2} \int_\Omega |\nabla N^\epsilon|^2 |_{s=t}
\leq \frac{d_1}{2} \int_\Omega |\nabla N_0|^2 + C\xi \int_{Q_t} |f_1(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)|^2.
$$

for small $\xi > 0$, and some constant $C\xi$. Lemma 1 and the continuity of $f_1$ now imply that

$$
\int_{Q_T} |\partial_t N^\epsilon|^2 \leq C', \quad \sup_{t \in [0, T]} \int_\Omega |\nabla N^\epsilon|^2 \leq C',
$$

(3.13)

for some constant $C' \geq 0$. The corresponding bounds for the solutions $C^\epsilon$ and $A^\epsilon$ can be obtained in the same way. To derive the estimates for the biomass fraction $M^\epsilon$ let

$$
G(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon) := \int_0^{M^\epsilon} D_\epsilon(\zeta) g(\zeta, N^\epsilon, C^\epsilon, A^\epsilon) d\zeta.
$$
Multiplying the first equation of (3.7) by $\partial_s(\Phi_\epsilon(M^\epsilon))$ and integrating we obtain

$$
\int_\tau^t \int_\Omega D_\epsilon(M^\epsilon) \left( \partial_s M^\epsilon \right)^2 = \int_\tau^t \int_\Omega \partial_s(\Phi_\epsilon(M^\epsilon)) \partial_s M^\epsilon
$$

$$
= \int_\tau^t \int_\Omega \nabla \cdot (D_\epsilon(M^\epsilon) \nabla M^\epsilon) \partial_s(\Phi_\epsilon(M^\epsilon)) + \int_\tau^t \int_\Omega \partial_s(\Phi_\epsilon(M^\epsilon)) g(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)
$$

$$
= -\frac{1}{2} \int_\tau^t \int_\Omega \partial_s|D_\epsilon(M^\epsilon)\nabla M^\epsilon|\|^2 + \int_\tau^t \int_\Omega \partial_s(\Phi_\epsilon(M^\epsilon)) g(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)
$$

and consequently,

$$
\int_\tau^t \int_\Omega D_\epsilon(M^\epsilon) \left( \partial_s M^\epsilon \right)^2 + \frac{1}{2} \int_\Omega |D_\epsilon(M^\epsilon) \nabla M^\epsilon|\|^2|_{s=t}
$$

$$
= \frac{1}{2} \int_\Omega |D_\epsilon(M^\epsilon) \nabla M^\epsilon|\|^2|_{s=t} + \int_\Omega G(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)|_{s=t} - \int_\Omega G(M^\epsilon, N^\epsilon, C^\epsilon, A^\epsilon)|_{s=t}
$$

$$
+ \int_\tau^t \int_0^\| M^\epsilon \|_D(\zeta) (\partial_s N \partial_N g(\zeta, N^\epsilon, C^\epsilon, A^\epsilon) + \partial_s C \partial_C g(\zeta, N^\epsilon, C^\epsilon, A^\epsilon)
$$

$$
+ \partial_s A \partial_A g(\zeta, N^\epsilon, C^\epsilon, A^\epsilon))
$$

where $\partial_N, \partial_C, \partial_A$ denote the partial derivatives w.r.t. the variables $N, C, A$, respectively. Lemma 1 and Lemma 2 implies that there exists $\epsilon_0 > 0$ and $\delta \in (0, 1)$ such that the approximate solutions $M^\epsilon$ satisfy $M^\epsilon < 1 - \delta$ in $Q_T$ for all $\epsilon < \epsilon_0$, where $\delta$ is independent of $\epsilon$. Consequently, $D_\epsilon(M^\epsilon)$ is positive and uniformly bounded from above by a constant, which is independent of $\epsilon$,

$$
d^\epsilon \leq D_\epsilon(M^\epsilon(t, x)) = \frac{(M^\epsilon(t, x) + \epsilon)^a}{(1 - M^\epsilon(t, x))^b} \leq \frac{(1 - \delta + \epsilon)^a}{(1 - (1 - \delta))^b} \leq \frac{d}{\delta^b}, \quad (t, x) \in Q_T,
$$

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for all $\epsilon < \epsilon_1 := \min\{\delta, \epsilon_0\}$. The last integral can therefore be estimated by

$$
\int_{\tau}^{t} \int_{\Omega} D_{\epsilon}(\zeta)(\partial_s N \partial_2 g(\zeta, N^\epsilon, C^\epsilon, A^\epsilon) + \partial_s C \partial_3 g(\zeta, N^\epsilon, C^\epsilon, A^\epsilon) + \partial_s A \partial_4 g(\zeta, N^\epsilon, C^\epsilon, A^\epsilon))
\leq \frac{d}{\delta^6} \int_{\tau}^{t} \int_{\Omega} \left( |\partial_s N|^2 + |\partial_s C|^2 + |\partial_s A|^2 \right) + \int_{0}^{M^\epsilon} \sum_{i=2}^{4} |\partial_i g(\zeta, N^\epsilon, C^\epsilon, A^\epsilon)|^2

$$

By the above estimates (3.13), Lemma 1, Lemma 2 and setting $\tau = 0$, we conclude that

$$
\int_{Q_T} D_{\epsilon}(M^\epsilon) (\partial_t M^\epsilon)^2 \leq C', \quad \sup_{t \in [0,T]} \int_{\Omega} |D_{\epsilon}(M^\epsilon) \nabla M^\epsilon|^2 \leq C',
$$

for some constant $C' \geq 0$. \hfill \Box

**Lemma 4.** Under the hypotheses of Lemma 1 and 2 the approximate solutions of (3.7) converge to a solution of the degenerate system (3.3) as $\epsilon$ tends to zero.

**Proof.** By Lemma 1 the solutions $M^\epsilon$ are uniformly bounded by $1 - \delta$, and the following estimate for the diffusion coefficient was shown in the proof of Lemma 3,

$$
d e^\alpha \leq D_{\epsilon}(M^\epsilon(t, x)) \leq \frac{d}{\delta^6}, \quad (t, x) \in Q_T,
$$

for all $\epsilon < \epsilon_1$. Moreover, if $\epsilon < \epsilon_1$ we observe that

$$
D(M^\epsilon) \leq D_{\epsilon}(M^\epsilon) \leq \frac{d}{\delta^6},
$$

and consequently,

$$
\Phi(M^\epsilon(t, x)) \leq \Phi_{\epsilon}(M^\epsilon(t, x)) \leq (1 - \delta)\frac{d}{\delta^6}, \quad (t, x) \in Q_T.
$$
Lemma 1 and Lemma 3 further imply that
\[
\int_{Q_T} \left( \partial_t (\Phi(M^\epsilon)) \right)^2 = \int_{Q_T} \left( D(M^\epsilon) \partial_t M^\epsilon \right)^2 \leq \int_{Q_T} \left( D_\epsilon(M^\epsilon) \partial_t M^\epsilon \right)^2 \\
\leq \|D_\epsilon(M^\epsilon)\|_{L^\infty(Q_T)} \int_{Q_T} D_\epsilon(M^\epsilon) \left( \partial_t M^\epsilon \right)^2 \leq c \frac{d}{\delta^6},
\]
for some constant \( c \geq 0 \). This shows that the family \( \Psi^\epsilon := \Phi(M^\epsilon), \epsilon < \epsilon_1 \), is uniformly bounded in \( W = \{ u \in L^\infty(0,T; H^1(\Omega)) \mid \partial_t u \in L^2(0,T; L^2(\Omega)) \} \), which is compactly embedded into \( C([0,T]; L^2(\Omega)) \) by Aubin-Lions’ Lemma (e.g., see [4], Theorem II.1.5). Consequently, there exists \( \Psi \in C([0,T]; L^2(\Omega)) \) and a sequence \( \epsilon_n \) tending to zero as \( n \to \infty \) such that \( \Psi^{\epsilon_n} \to \Psi \) in \( C([0,T]; L^2(\Omega)) \). This implies that \( M^{\epsilon_n} = \Phi^{-1}(\Psi^{\epsilon_n}) \to M \) and \( \Phi(M^{\epsilon_n}) \to \Phi(M) \) in \( C([0,T]; L^2(\Omega)) \).

Moreover, by Lemma 3 the approximate solutions \( N^\epsilon, C^\epsilon \) and \( A^\epsilon \) are uniformly bounded in \( W \), which implies that there exist \( N, C, A \in C([0,T]; L^2(\Omega)) \) and a sequence \( \epsilon_n \) tending to zero as \( n \to \infty \) such that
\[
N^{\epsilon_n} \to N, \quad C^{\epsilon_n} \to C, \quad A^{\epsilon_n} \to A \quad \text{in} \quad C([0,T]; L^2(\Omega)).
\]

We can now pass to the limit \( \epsilon \to 0 \) in the distributional formulation of the degenerate system (3.1) using the uniform boundedness of the approximate solutions and the continuous embedding \( C([0,T]; L^2(\Omega)) \hookrightarrow C([0,T]; L^1(\Omega)) \), and conclude that the limits \( M, N, C, A \) are solutions of the degenerate problem.

\[\square\]

3.3.2 Uniqueness and well-posedness for general initial data

**Lemma 5.** Let the hypotheses of Lemma 1 be satisfied. If \((M, N, C, A)\) and \((\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A})\) are solutions corresponding to initial data \((M_0, N_0, C_0, A_0)\) and \((\tilde{M}_0, \tilde{N}_0, \tilde{C}_0, \tilde{A}_0)\),
respectively, then

\begin{align}
\|M(T) - \tilde{M}(T)\|_{L^1(\Omega)} - \|M_0 - \tilde{M}_0\|_{L^1(\Omega)} & \leq \int_0^T \int_\Omega |g_0(t, x)| \, dx \, dt, \\
\|N(T) - \tilde{N}(T)\|_{L^1(\Omega)} - \|N_0 - \tilde{N}_0\|_{L^1(\Omega)} & \leq \int_0^T \int_\Omega |h_1(t, x)| \, dx \, dt, \\
\|C(T) - \tilde{C}(T)\|_{L^1(\Omega)} - \|C_0 - \tilde{C}_0\|_{L^1(\Omega)} & \leq \int_0^T \int_\Omega |h_2(t, x)| \, dx \, dt, \\
\|A(T) - \tilde{A}(T)\|_{L^1(\Omega)} - \|A_0 - \tilde{A}_0\|_{L^1(\Omega)} & \leq \int_0^T \int_\Omega |h_3(t, x)| \, dx \, dt,
\end{align}

(3.14)

where the functions \(g_0\) and \(h_i\) are defined as

\begin{align}
g_0(t, x) & := g(M(t, x), N(t, x), C(t, x), A(t, x)) - g(\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A}), \\
h_i(t, x) & := f_i(M(t, x), N(t, x), C(t, x), A(t, x)) - f_i(\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A}),
\end{align}

(3.15)

for \(i = 1, 2, 3\), where \(g(\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A}) := g(\tilde{M}(t, x), \tilde{N}(t, x), \tilde{C}(t, x), \tilde{A}(t, x))\) and

\(f_i(\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A}) := f_i(\tilde{M}(t, x), \tilde{N}(t, x), \tilde{C}(t, x), \tilde{A}(t, x))\)

**Proof.** The estimates immediately follow from Lemma 3.3 in [18] \(\square\)

**Lemma 6.** Let \(\mathbb{B}\) be a bounded subset of \(\mathbb{R}^4_+\). Then for all \((M, N, C, A), (\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A}) \in \mathbb{B}\) we have

\begin{equation}
|g(M, N, C, A) - g(\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A})| + \sum_{i=1}^3 |f_i(M, N, C, A) - f_i(\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A})| \\
\leq c \left( |M - \tilde{M}| + |N - \tilde{N}| + |C - \tilde{C}| + |A - \tilde{A}| \right),
\end{equation}

(3.16)

for some constant \(c \geq 0\).

**Proof.** Let \((M, N, C, A), (\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A}) \in \mathbb{B}\).
For the function \( f_2 \) we obtain,

\[
|f_2(M, N, C, A) - f_2(\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A})| = -\sigma \left| \left( \frac{C}{k_1 + C} \right) (M + N) - \left( \frac{\tilde{C}}{k_1 + \tilde{C}} \right) (\tilde{M} + \tilde{N}) \right| \\
\leq r_1 \left( |M - \tilde{M}| + |N - \tilde{N}| \right),
\]

(3.17)

for some constant \( r_1 \geq 0 \). To show that the functions \( f_1, f_3 \) and \( g \) satisfy (??) we observe, that

\[
A_1^m X_1 - A_2^m X_2 = A_1^m(X_1 - X_2) + X_2(A_1^m - A_2^m) \\
= A_1^m(X_1 - X_2) + \nu X_2(A_1 - A_2) \int_0^1 (s A_1 + (1 - s) A_2)^{n-1} ds,
\]

(3.18)

which implies that \( |A_1^m X_1 - A_2^m X_2| \leq r |(X_1 - X_2) + X_2(A_1 - A_2)| \) for some constants \( \nu \) and \( r \geq 0 \) and \((X_1, A_1), (X_2, A_2)\) in bounded subsets of \( \mathbb{R}_+^2 \).

Applying this to \( f_1 \), we obtain

\[
|f_1(M, N, C, A) - f_1(\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A})| \\
= \left| \left[ \left( \frac{C}{k_1 + C} - k_2 \right) N + \eta_1 \left( \frac{A_1^m}{1 + A_1^m} \right) M \right] - \left[ \left( \frac{\tilde{C}}{k_1 + \tilde{C} - k_2} \right) \tilde{N} + \eta_1 \left( \frac{\tilde{A}_1^m}{1 + \tilde{A}_1^m} \right) \tilde{M} \right] \right| \\
\leq |1 - k_2||N - \tilde{N}| + |\eta_1||M - \tilde{M} + |M(A - \tilde{A})|\right| \\
= r_2 (|N - \tilde{N}| + |M - \tilde{M}| + |A - \tilde{A}|)
\]

(3.19)

for some constant \( r_2 \geq 0 \). Similarly, for \( f_3 \) we obtain
\(|f_3(M, N, C, A) - f_3(\widetilde{M}, \widetilde{N}, \widetilde{C}, \widetilde{A})| \\
= \left| -\lambda A + \left( \alpha + \beta \frac{A^m}{1 + A^m} \right) (M + N) - \left( -\lambda \widetilde{A} + \left( \alpha + \beta \frac{A^m}{1 + A^m} \right) (\widetilde{M} + \widetilde{N}) \right) \right| \\
\leq \lambda |A - \widetilde{A}| + \alpha \left[ |M - \widetilde{M}| + |N - \widetilde{N}| \right] \\
+ \beta \left[ |M - \widetilde{M}| + |M||A - \widetilde{A}| \right] + \beta \left[ |N - \widetilde{N}| + |\widetilde{N}||A - \widetilde{A}| \right] \\
= r_3(|A - \widetilde{A}| + |M - \widetilde{M}| + |N - \widetilde{N}|) \\
(3.20)

for some constant \(r_3 \geq 0\). Finally, for \(g\) we obtain

\(|g(M, N, C, A) - g(\widetilde{M}, \widetilde{N}, \widetilde{C}, \widetilde{A})| \\
= \left| \left( \frac{C}{k_1 + C} - k_2 \right) M - \eta_1 \left( \frac{A^m}{1 + A^m} \right) M \right| - \left[ \left( \frac{\widetilde{C}}{k_1 + C} - k_2 \right) \widetilde{M} - \eta_1 \left( \frac{A^m}{1 + A^m} \right) \widetilde{M} \right] \right| \\
\leq |1 - k_2||M - \widetilde{M}| + \eta_1 \left[ |M - \widetilde{M}| + |\widetilde{M}||A - \widetilde{A}| \right] \\
= r_4|M - \widetilde{M}| + r_2|A - \widetilde{A}| \\
(3.21)

for some constant \(r_4 \geq 0\). Combining equations (B.18), (B.20), (3.20) and (3.21) gives

\(|g(M, N, C, A) - g(\widetilde{M}, \widetilde{N}, \widetilde{C}, \widetilde{A})| + \sum_{i=1}^{3} |f_i(M, N, C, A) - f_i(\widetilde{M}, \widetilde{N}, \widetilde{C}, \widetilde{A})| \\
\leq r'(|M - \widetilde{M}| + |N - \widetilde{N}| + |C - \widetilde{C}| + |A - \widetilde{A}|) \\
(3.22)

for some constant \(r' \geq 0\).

\[\square\]

**Theorem 7.** For every \(T > 0\) and initial data \((M_0, N_0, C_0, A_0)\) in \(L^\infty(\Omega)\) such
That

\[ M_0 \geq 0, \quad \| M_0 \|_{L^\infty(\Omega)} < 1 - \rho, \quad N_0 \geq 0, \quad A_0 \geq 0, \quad C_0 \geq 0 \]

for some \( \rho \in (0, 1) \), there exists a unique solution of model (3.3). The solutions \( M, N, C, A \) are non-negative, belong to \( L^\infty(Q_T) \) and \( M \) satisfies \( \| M \|_{L^\infty(Q_T)} < 1 - \delta \) for some \( \delta \in (0, 1) \).

**Proof.** We first assume the initial data are smooth and satisfy the hypotheses of Lemma 1.

If \((M, N, C, A)\) and \((\tilde{M}, \tilde{N}, \tilde{C}, \tilde{A})\) are solutions corresponding to initial data \((M_0, N_0, C_0, A_0)\) and \((\tilde{M}_0, \tilde{N}_0, \tilde{C}_0, \tilde{A}_0)\), respectively, then Lemma 5 and the estimate of Lemma 6 imply that

\[
F(T) - F(0) \leq c \int_0^T F(s) ds,
\]

where

\[
F(t) := \| M(t) - \tilde{M}(t) \|_{L^1(\Omega)} + \| N(t) - \tilde{N}(t) \|_{L^1(\Omega)} + \| C(t) - \tilde{C}(t) \|_{L^1(\Omega)} + \| A(t) - \tilde{A}(t) \|_{L^1(\Omega)}.
\]

By Gronwall’s Lemma we conclude that

\[
F(T) \leq F(0) e^{cT}, \tag{3.23}
\]

for some constant \( c \geq 0 \), which implies the uniqueness of solutions corresponding to smooth initial data. For general initial data, let \( M^n_0, N^n_0, C^n_0, A^n_0 \) be an approximating sequence satisfying the hypothesis of Lemma 1 such that

\[
\| M^n_0 - M_0 \|_{L^1(\Omega)} + \| N^n_0 - N_0 \|_{L^1(\Omega)} + \| C^n_0 - C_0 \|_{L^1(\Omega)} + \| A^n_0 - A_0 \|_{L^1(\Omega)} \to 0 \text{ as } n \to \infty.
\]

The Lipschitz-continuity of solutions in \( L^1(\Omega) \)-norm (3.23) implies that the cor-
responding solutions $M^n, N^n, C^n, A^n$ satisfy

$$\sup_{t \in [0, T]} \left\{ \| M^n(t) - M^m(t) \|_{L^1(\Omega)} + \| N^n(t) - N^m(t) \|_{L^1(\Omega)} 
+ \| C^n(t) - C^m(t) \|_{L^1(\Omega)} + \| A^n(t) - A^m(t) \|_{L^1(\Omega)} \right\}$$

$$\leq C_T \left( \| M^n_0 - M^m_0 \|_{L^1(\Omega)} + \| N^n_0 - N^m_0 \|_{L^1(\Omega)} + \| C^n_0 - C^m_0 \|_{L^1(\Omega)} + \| A^n_0 - A^m_0 \|_{L^1(\Omega)} \right).$$

(3.24)

for some constant $C_T \geq 0$ and all $n, m \in \mathbb{N}$. Consequently, $M^n, N^n, C^n, A^n$ form a Cauchy sequence in $C([0, T]; L^1(\Omega))$ and converge to the unique solution $M, N, C, A$ of (3.3).

3.4 Numerical Simulation

In the previous section we established the well-posedness of the quorum sensing induced biofilm detachment model, however, we are currently unable to describe the solutions of the model qualitatively based on rigorous analytical arguments. Hence, we illustrate the model behaviour in computer simulations. The model (3.1) is discretized on a regular grid using a cell centered finite difference based finite volume scheme for space and a semi-implicit time integration adapted from [14, 26] to account for the new dependent variables $A$ and $N$ which are treated in the same manner as $C$. In every time step, this requires the solution of a sparse linear system for each dependent variable. By construction, the system matrices are at least weakly diagonally dominant, and the systems are solved with the stabilized biconjugate gradient method [32]. The linear solver is prepared for parallel execution on multi-core and shared memory multiprocessor.
architectures using OpenMP as described in [26]. Simulations are terminated when the biofilm (or the microbial floc) reaches a set target size, or when a set maximum simulation time is reached. The ecological and physical parameters used are dimensionless and are derived from sources presented in Table 3.1. In the numerical experiments, we investigate the model behaviour under different boundary conditions reflecting biofilms or microbial flocs with particular emphasis on the internal structure of the colonies. We define the following output parameters:

- Relative Variation: This is the standard deviation of the sessile biomass density from its mean in the biofilm,

\[
R(t) := \left[ \int_{\Omega_2} \left( M(t, x) - \frac{1}{|\Omega_2|} \int_{\Omega_2} M(t, y) dy \right)^2 dx \right]^{\frac{1}{2}} \tag{3.25}
\]

- Relative biofilm (floc) size: This is the size of the biofilm relative to the domain size,

\[
\omega(t) := \frac{1}{|\Omega|} \int_{\Omega_2(t)} dx \tag{3.26}
\]

- Sessile biomass in the biofilm:

\[
M_{tot}(t) := \int_{\Omega} M(t, x) dx \tag{3.27}
\]

- Dispersed cells:

\[
N_{tot}(t) := \int_{\Omega} N(t, x) dx \tag{3.28}
\]
• Average signal molecule concentration in the biofilm:

\[ A_{\text{avg}}(t) := \frac{\int_{\Omega_2(t)} A(t, x) dx}{\tau \int_{\Omega_2(t)} dx} \]  

(3.29)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
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<td>-</td>
</tr>
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<td>( \beta )</td>
<td>induced autoinducer production rate</td>
<td>varied</td>
<td>-</td>
</tr>
<tr>
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<td>degree of polymerization</td>
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<td>assumed</td>
</tr>
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<td>constant diffusion coefficients for ( C )</td>
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<td>( d_3 )</td>
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<td>( \delta )</td>
<td>biomass motility coefficient</td>
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<td>biofilm diffusion exponent</td>
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</tr>
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<td>( H )</td>
<td>system height</td>
<td>1.0</td>
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</tr>
</tbody>
</table>

3.4.1 Microbial floc

In the first simulation experiments, we consider a biofilm without substratum i.e. a microbial floc. We restrict ourselves to a two-dimensional setting with rectangular computational domain \( \Omega = [0, 1] \times [0, 1] \). The boundary conditions
used are the Dirichlet boundary conditions (3.4). One spherically shaped floc is placed in the centre of the domain which at time $t = 0$ contains sessile cells of biomass density $M_0 = 0.1$ while everywhere else is $M_0 \equiv 0$. Initially, no signal molecule $A$ and no dispersed cells $N$ are assumed to be in the system, thus $A_0 \equiv 0$ and $N_0 \equiv 0$. The nutrient concentration in the interior at time $t = 0$ is everywhere at the same level as the concentration at the boundary, $C_0 = C_\infty$.

For the visualization presented in Figure 3.2 we used an induction parameter $\alpha = 30.7$ and a maximum dispersal rate $\eta_1 = 0.6$, every other parameter is as listed in Table 3.1. Immediately after the simulation starts, the floc is very small in size and the concentration of the signal molecule $A$ is very low. At the snapshots $t = 5$, $t = 10$ and $t = 15$, the original spherical shape of the floc is still visible, the nutrient is not strongly growth limiting and the floc expands. As the floc grows within the rectangular domain, it looses its spherical shape. Some parts of the floc expand towards the sides of the domain rather than towards the diagonal corners of the domain, which is likely due to closeness to nutrients which are added through the boundaries; this is also in analogy to biofilms which have been found to grow in compact, homogenous layers when nutrients are nowhere severely limited. With the increase in biomass density in the system, the signal concentration also increases. The autoinducer concentration attains its highest values inside the floc and decreases from there towards the floc/water interface and the boundary of the domain where the signal concentration is kept at $A = 0$; this relates to the maximum principle. At the next snapshot $t = 20,$
we observe a visible decrease in biomass density in the centre of the floc, thus creating a hollow in the floc. The last snapshot taken at $t = 25$ shows that the microbial floc has started growing again both internally and on the surface; and consequently the signal concentration has started rising.

The 2-D structural representation of the biofilm shown in Figure 3.2 reveals
that the bacterial cells leave from the inner core of the microfloc, but it does not illustrate the extent of the dispersal effect on the biofilm structure. Hence, we present a 1-D spatial representation of a typical biofilm dispersal event in Figure 3.3. The sessile biomass density and the concentration of the quorum sensing molecule are shown at selected times $t = 0.0002, 5, 10, 15, 20, 25$. From the top view of the microfloc, we observe that at time $t = 0.0002, t = 5$, and $t = 10$ the microfloc is still in its initial growth phase, the sessile biomass density has increased leading to spatial spreading. The signal molecule concentration also rises until it is strong enough to induce cell dispersal as seen at the next selected time $t = 15$. The next two snapshots at times $t = 20$ and $t = 25$ show that the dispersal of cells from the microfloc creates voids in the floc and its depth increases over time (as seen from the top view).

We have seen in Figures 3.2 and 3.3 that cell dispersal occurs from the inner core of the microfloc thereby creating hollowing structures, as reported in experimental studies, e.g. [10, 22]. Furthermore, we will investigate the general extent of the hollow effect over a longer period of time through the lumped quantities $M_{\text{tot}}, N_{\text{tot}}, A_{\text{tot}}$ and $R$ defined in equations (3.25)-(3.29).

The temporal plots are shown in Figure 3.4 where the quorum sensing induction parameter is varied as $\alpha \in \{92.0, 46.0, 30.7, 23.0, 18.4, 15.3, 13.1\}$. For $\alpha \geq 30.7$, we observe a rapid removal of biomass and signal molecule (see Figure 3.4c,e) due to the Dirichlet boundary conditions prescribed on the boundary. After the first dispersal event, the bacterial cells that are left behind in the floc are too few to maintain the signal concentration at a level to maintain cell dis-
Figure 3.3: 1-D Spatial representation of the development and dispersal of bacterial cells from the microbial floc. The snapshots are taken at different computational time $t$, with an induction parameter $\alpha = 30.7$ and a dispersal rate of 0.6
persal, so the biomass as well as the autoinducer concentration in the floc start increasing until the next dispersal event is induced. This pattern continues, thus producing an almost periodic pattern of discrete dispersal events (see Figure 3.4 a,c,e). For $\alpha < 30.7$, the floc grows larger with higher levels of biomass density and autoinducer concentration before dispersal is induced. Here, the cell dispersal appears continuous and the floc biomass density reaches a plateau. For the entire simulation, the microbial floc did not shrink which indicates that most of the dispersed cells are from the inner core of the floc (see Figure 3.4b).

The relative variation of the biomass density defined in equation (3.25) is the standard deviation of the sessile biomass density from its mean in the microfloc. This is evaluated and plotted in Figure 3.4d. So far, we established that cell dispersal occurs in the inner core of the biofilm, it creates voids (hollows) whose depth increases over time when viewed from the top and the floc does not shrink as a result of dispersal. The importance of the variable $R$ is to investigate these correlated results over time. When the standard deviation is close to zero, it means that the hollow in the floc is small (or narrow); on the other hand, if the standard deviation is large, it means that the hollow is big (or wide). The plots in Figure 3.4d show that the size of the hollow decreases over time as the standard deviation from the mean decreases. This is more obvious in smaller flocs (i.e. where $\alpha \geq 30.7$). Hence, after the voids are created by cell dispersal, the floc grows both inside and outside which leads to "shrinking" of the inner core even though the floc outwardly does not shrink. For bigger flocs (i.e. where $\alpha < 30.7$), the dispersal occurs rather continuous which does not give the floc a good opportu-
Figure 3.4: Temporal plots of simulations computed for a non-quorum sensing producing microfloc (Non-QS) and a quorum sensing producing microfloc using seven different constitutive autoinducer production rate $\alpha = \{92.0, 46.0, 30.7, 23.0, 18.4, 15.3, 13.1\}$ and fixed maximum dispersal rate $\eta_1 = 0.6$. Shown are (a) the total sessile biomass fraction $M_{tot}$ in the floc, (b) the floc size $\Omega$ (c) dispersed cells $N_{tot}$, (d) relative variation $R(t)$, and (e) signal concentration $A_{tot}$.

Community to grow more especially in the inner core, hence the created hollow becomes constant as $R$ approaches a plateau.
In the simulation discussed above, we have prescribed homogeneous Dirichlet boundary conditions for the autoinducer $A$. We compare the results with the situation where homogeneous Neumann boundary conditions are imposed for the autoinducer. This is presented on the left panel of Figure 3.5 using an induction threshold $\alpha = 18.4$ and maximum dispersal rate $\eta_1 = 0.6$. The Neumann boundary condition models the case where the signal molecule cannot leave the domain and therefore accumulates faster. As a consequence, the onset of quorum sensing occurs much earlier than with Dirichlet boundary conditions. As a result of unhindered accumulation of signal molecules in the system, very high autoinducer concentrations are attained, hence quick up-regulation and cell dispersal occurs which reduces the density of sessile biomass in the floc. Whereas in the case of Dirichlet boundary conditions, the accumulation of the signal molecule is slower due to its early removal through the boundaries, hence causing a delay in up-regulation and cell dispersal.

So far, we presented simulation experiments for a microfloc. We will compare this situation with a biofilm. Besides, we will investigate the effect of quorum sensing induced dispersal on merged colonies which was not shown in the previous study [19]. More specifically, we will analyze the behaviour of merging colonies in terms of biomass growth, cell dispersal and hollow structures.
Figure 3.5: Comparison of the sessile biomass $M_{tot}$ and the dispersed cells $N_{tot}$ under different boundary conditions for the signal molecule $A$: Homogenous Dirichlet conditions and Neumann conditions for the signal molecule $A$. The left panel is for a microbial floc while the right panel is for a biofilm.

### 3.4.2 Microbial Biofilm

The simulated biofilm community consists of bacterial cells accumulating on a surface (substratum) surrounded by an aquatic region. The substratum is inoculated by two colonies of small pockets of down regulated sessile cells with biomass density $M = 0.1$ in each colony. The substratum forms the bottom boundary of the domain $\Omega$ and is impermeable to biomass, substrate and signal molecule. This is described by homogeneous Neumann boundary conditions imposed on the dependent variables $M, N, C, A$. These can be understood as symmetry boundary conditions and permit to interpret our domain as one half of a continuously repeating segment of an infinite domain. At the top boundary, we impose homogenous Dirichlet conditions for the biomass $M$ and for the nutrient $C$ an inhomogeneous Dirichlet condition. The nutrient concentration is set here
to the bulk concentration value, which reflects that the growth limiting substrate is added to the system through this segment of the boundary. The dispersed cells $N$ and the autoinducer concentration $A$ are set there to zero. This enforces a diffusion gradient from the biofilm in the interior of the domain to the boundary and mimics removal of quorum sensing molecule and dispersed cells into the surrounding bulk phase, where they are negligible due to instantaneous dilution. The same boundary conditions are imposed on the left and right boundaries of the domain. Initially, no dispersed cells and no autoinducers are in the system, and the concentration of nutrients is at bulk level, i.e. $C_0 = C_\infty$, $N_0 = A_0 = 0$.

To investigate the effect of quorum sensing triggered dispersal on the spatial structure of the biofilm colony in more detail, we visualize the development, growth and cell dispersal of the biofilm shown in Figure 3.6. We used an induction parameter $\alpha = 23.0$ and a maximum dispersal rate $\eta_1 = 3.6$, which mimics the continuous dispersal event described in [19]. All other parameter values used are in Table 3.1. We show the spatial distribution of the sessile biomass $M$ and the iso-lines of the autoinducer concentration $A$ within the first dispersal event (cycle); the selected time instances that illustrate the biofilm growth and dispersal in response to autoinducer concentration.

After the simulation starts, the biomass $M$ starts growing and colonies gradually expanding as shown in the snapshot at $t = 0.002$ and $t = 5$, but due to the removal of signal molecules from three out of four boundary segments of the domain, accumulation of signal molecules is not strong enough to induce dispersal. Expansion starts locally when and where the biomass density $M$ approaches
Figure 3.6: 2-D structural representation of the microbial biofilm growth for induction parameter $\alpha = 30.7$ and maximum dispersal rate $\eta_1 = 0.6$ for selected time instances $t$. Color coded is the biomass density $M$, iso-lines of the autoinducer concentration $A$ are plotted in grayscale.

its maximum value. As long as the nutrient substrate is not severely limited, the total biomass density $M \approx 1$ in the biofilm.

At the next shown time instance $t = 10$, the biomass density $M$ inside the colonies has increased. The colonies which were initially placed apart are now
drawing close and then merge, albeit the biomass density inside the colonies still remains below the maximum biomass density. The autoinducer concentration has risen, though not strong enough to induce cell dispersal. Moreover, the merged colonies now act as one colony, whereby the iso-lines of the signal concentration are no longer separated. The autoinducer concentration in the middle of the merged colony has increased and few cells started dispersing as shown at the time instance $t = 11$.

At the next snapshot $t = 12$, we notice a huge biomass loss resulting in a significant decrease in the biomass density of the merged colony. An important observation is the void region of the colonies, seen at the centre of the merged colonies and not at the centre of the individual colonies as in the study [19]. At the snapshot at $t = 13$, the biomass density in the merged colony has started to increase again.

Furthermore, we investigated the behaviour of the biofilm if cell dispersal occurs before merging of the colonies. The simulation setup here is the same as in Figure 3.6 except that the induction parameter is set to $\alpha = 92.0$ which relates to a small biofilm. Here, the biofilm grows less and merging of the colonies is delayed. Similar to the results in Figure 3.6, we notice that at the first two time instances $t = 0.002$ and $t = 5$, the colonies are still in the growth phase (see Figure 3.7). At the next two snapshots $t = 10$ and $t = 11$, we observe a significant biomass loss leading to hollow structures. After the dispersal event, the biofilm starts growing again but the colonies do not merge (see figure 3.7f). This could be due to mass transfer, which makes the signal concentration to
Figure 3.7: 2-D structural representation of the microbial biofilm growth for induction parameter $\alpha = 92.0$ and maximum dispersal rate $\eta_1 = 0.6$ for selected time instances $t$. Color coded is the biomass density $M$, iso-lines of the autoinducer concentration $A$ are plotted in grayscale.

attain its highest values at the sides of the colony close to the neighbouring colony, and hence more cells disperse from the sides and so inhibits merging. Furthermore, the extent of the hollow effect is investigated through the lump quantities $M_{tot}, N_{tot}, A_{tot}, \Omega$ and $R$ shown in Figure 3.8. The values of $\alpha$ were varied as $\alpha = 92.0, 46.0, 30.7, 23.0, 18.4, 15.3, 13.1$. For $\alpha \geq 30.7$, we notice
Figure 3.8: Temporal plots of simulations computed for a non-quorum sensing producing biofilm (Non-QS) and a quorum sensing producing biofilm using seven different constitutive autoinducer production rate $\alpha = \{92.0, 46.0, 30.7, 23.0, 18.4, 15.3, 13.1\}$ and fixed maximum dispersal rate $\eta_1 = 0.6$. Shown are (a) the total sessile biomass fraction $M_{\text{tot}}$ in the floc, (b) the floc size $\Omega$ (c) dispersed cells $N_{\text{tot}}$, (d) relative variation $R(t)$, and (e) signal concentration.

a rapid removal of biomass and signal molecules due to the Dirichlet boundary conditions prescribed on all the boundary segments except the substratum; we
observe discrete dispersal events similar to the case of microbial flocs, thus producing an almost periodic pattern (see Figure 3.8a,c), while for $\alpha < 30.7$ the biofilm grows bigger with increase in biomass density and autoinducer concentration before the onset of dispersal. Here, the cell dispersal appears continuous and the population reaches a plateau. During the dispersal process, the biofilm does not shrink, suggesting a hollow within the colony for all values of $\alpha$. Thus we investigate the extent of hollow by computing the relative variation $R$, which is the standard deviation of the bacterial biomass density from the mean (see Figure 3.8d). Small values of $R$ indicate small hollows while large values of $R$ indicate large deviation of the bacterial density from the mean which implies large hollows. We observe that generally, in smaller biofilms (e.g. $\alpha < 30.7$) smaller hollows are created after dispersal and vice versa. The size of the hollows is not steady but rather decreases as new cells are produced during the biofilm intermittent growth phase. It is interesting that the size of the void in the biofilm colony is not a constant feature.

We compare these results with a simulation whereby homogeneous Neumann conditions are imposed for $M, N, C, A$ on all boundaries except for the top boundary as in [19]. This comparison is shown on the right panel of Figure 3.5 using an induction threshold $\alpha = 18.4$ and maximum dispersal rate $\eta_1 = 0.6$. Compared to the Dirichlet conditions, we notice an increased signal production at the onset of the simulation which reflects the physical interpretation of Neumann boundary conditions. Quick up-regulation and cell dispersal occurs due to the increased production of the signal molecule. Whereas in the Dirichlet case,
the signal concentration at the onset of the simulation decreases leading to a delay in up-regulation and cell dispersal.

3.5 Conclusion

We proved the well-posedness of the quorum sensing controlled detachment model. The existence results in the previous section are formulated assuming homogeneous Dirichlet boundary conditions for the sessile biomass, the dispersed cells and the signal molecules. This situation depicts biofilms without substratum, which are commonly called microbial flocs. Albeit, in most applications, it is more appropriate to prescribe Neumann boundary conditions on some parts of the boundary to ensure impermeability of cells or substrate components, for instance, for biofilms growing on an impermeable substratum; while homogenous and constant Dirichlet boundary values are prescribed on other parts (e.g. top boundary) enforcing the removal of bacterial cells and signal molecules; and the replenishment of nutrients. The well-posedness proof can be extended to these more more appropriate situations as long as homogeneous Dirichlet boundary conditions are imposed for the sessile biomass on one part of the boundary.

The simulation results in this study reveal (i) microflocs and biofilms do not shrink as a result of dispersal, albeit the voids resulting from quorum sensing induced dispersal can get smaller as new cells are formed in the inner core of the biofilm. (ii) Hollowing in biofilms can be associated with quorum sensing induced dispersal in biofilms. (iii) Hollowing is a dynamic feature, thus chang-
ing in size and depth over time (iv)quorum sensing controlled cell dispersal in biofilms or flocs does not only balance growth but has the potential to balance the hollow sizes.
Bibliography


Chapter 4

A Mathematical Model of Nutrient Limitation Induced Biofilm Detachment

Abstract

The release of bacterial cells into the aquatic phase of a biofilm system has been known to be an integral part of a biofilm life cycle. This phenomenon has been known to be triggered by several cues, which are still under serious research. Nutrient-limitation (starvation) has been experimentally shown to be one of the cues to trigger dispersal of cells from biofilms. For a better understanding of the interplay of colony growth and cell dispersal in a nutrient starved biofilm environment, we develop a dynamic, spatially extended mathematical model that includes biofilm growth and starvation induced cell dispersal. The model developed in this study is a highly non-linear system of partial differential equations used in describing the diffusion and reactions occurring in a nutrient-limited
biofilm system. The model is numerically solved and simulated. The results from this study reveal continuous dispersal events with evidence of hollow structures, and that the size of the hollow changes as new bacterial cells are being produced. We further comment on the existence and uniqueness of the developed model.

**Key words:** Nutrient limitation, cell dispersal, model, hollow, diffusion-reaction

### 4.1 Introduction

It was long assumed that bacterial cells were loners that floated through their single-celled existence without need of companionship. That simple, independent life style makes it easy to study them in test tubes. But it turns out that bacteria are actually social creatures, which congregate and chemically work together to stay alive. More often than not, they exist in complex communities called biofilms. Most bacteria live in biofilms. Biofilms grow on surfaces usually referred to as substratum and can be found in almost any solid-liquid interface, including inner surfaces of pipes in industrial facilities [10], on rocks in streams [24] and in human body in association with medical implants [31].

The detachment of cells from the biofilm colony into the surrounding aqueous phase is an important phenomenon in biofilms, which contributes to biological dispersal and biofilm rejuvenation [32, 33, 41]. The dispersal (detachment) of cells from a biofilm is not restricted to any particular time/stage in the biofilm development but may occur continuously at low levels over the course of biofilm
formation [3, 23, 40, 41], such cell dispersal may be in response to environmental changes; due to cell lysis or the removal of intact, viable cells. The removal of intact viable cells from a biofilm can be passive, active or both; which can be characterized as sloughing, erosion or seeding [20]. Seeding dispersal can be internally triggered by some effectors developed by the biofilm, example of such effectors are enzymes, surfactants and bacteriophage, which degrade the biofilm matrix and thus mediate changes in biofilm resulting from production and dispersal of cells e.g cell death, formation of hollow microcolony, etc [50].

Seeding dispersal refers to the rapid release of a large number of single cells or small clusters of cells from the biofilm; this can also be triggered by cues and signals such as Nitric Oxide signaling [5], Cyclic di-GMP signaling [35], quorum sensing [20]. Some experimental studies have shown that seeding dispersal in biofilm can be induced by nutrient limitation in the biofilm [25, 28, 42].

Starvation in biofilm resulting from nutrient limitation can weaken the Extracellular Polymeric Substances (EPS) matrix and induce dispersal [34, 12]. Similarly nutrient limitation can lead to an increase in detachment rates [43], whereas in some cases it could induce an environmental stress, which in some instances may arise from a breakdown in C-di-CMP and thereby positively regulate motility and dispersal [4, 45]. Other studies such as [28] have investigated the role of nutrient starvation in biofilm detachment by hypothesizing that localized nutrient depletion in a biofilm induces starvation in some cells and that those cells detach when the starvation persists for a sufficient period of time. By contrast, some studies have shown that bacterial cells are more tightly packed when starved but
display detachment and drifting behaviour during nutrient abundance [29].

There are many experimental and modeling studies that focus on biofilm growth resulting from nutrient consumption, biofilm dispersal and architectural effects. In this study we investigate the interplay of nutrient concentration, biofilm growth and cell dispersal and how these affect the structure, function and general dynamics in biofilm. We will formulate a mathematical model for a nutrient limitation induced biofilm cell dispersal, which will be numerically solved. We will carry out computer simulation experiments to investigate potential effects of this phenomenon on biofilm growth and structure and explore whether it offers any advantage to the biofilm to control the release of bacterial cells into the liquid phase.

Mathematical models of biofilm have been studied in several ways ranging from 1-dimensional models describing biofilms as homogenous flat layers, to higher dimensional models, which accounts for the spatial heterogeneity of biofilm communities. The first detailed mathematical model of biofilm growth proposed by [48] was based on a one dimensional description of the biofilm, in which only gradients perpendicular to biofilm-liquid interface were taken into considerations. This model has been a good tool for understanding several biofilm processes in a quantitative manner [49], more especially in biofilm engineering [46].

Over the years, multi dimensional models have been proposed and formulated with considerations on the spatially heterogeneous structure of the biofilm. A large number of mathematical modeling techniques have been proposed to
model biofilms consisting of stochastic individual based models, stochastic cellular automata models and a variety of deterministic partial differential equation models; some examples in the literature as [1, 6, 9]. The common differences in these models are found in the approach used to describe biomass movement and the biofilm structure. These models are usually complex and are coupled with diffusion reaction models for growth controlling substrates and are mathematically difficult to analyze, hence the use of computational simulation experiments is oftentimes employed.

Mathematical models of detachment processes have been included in a variety of models albeit in different forms. In the traditional one-dimensional models, the biofilm detachment rate is typically a function of the biofilm thickness [2, 7, 8, 33], whereas in some two-dimensional models, the detachment rate is correlated with the biofilm geometry [51]. Apart from mechanical washout of biofilm, models that describe biofilm detachment induced by chemical changes, and food limitations is the stochastic cellular automation in [47, 26, 27] while others have been correlated with shears stress induced by the flowing bulk liquid in the biofilm system e.g. [14, 38, 52].

In our choice of biofilm modeling framework to use, we are faced with two criteria; one criterion to distinguish between biofilm models in their treatment of biomass, for instance the traditional one-dimensional model [48] and its multi-dimensional extension such as [11] assumes that in the biofilm, cells are always at maximum cell density; where production of new cells lead to the expansion of the biofilm proportional to the amount of net biomass growth and cell loss
corresponding to biofilm shrinkage. Mathematical models with this type of assumptions, by construction, are not suited to describe both colonies with strong biomass density gradient such as the hollow biofilm structures that have been reported in the experimental literature as consequence of cell dispersal. Alternatively, some models have treated the biomass density as dependent variables e.g. [15]; whereby the biomass density attains a constant value inside the colony of growing well developed biofilm, hence such models can describe hollowing biofilm structures by reducing biofilm density in the interior of such biofilm colonies. Another criterion to distinguish between biofilm models is based on whether they are stochastic or deterministic. While frequently a single simulation of stochastic model seems to be faster than a simulation of a deterministic model, many such simulations are required to obtain reliable averages, which offset the computational speed advantage. Deterministic models, on the other hand have averaging properties built in.

Based on the above considerations, we will study the nutrient biofilm dispersal of a single-species biofilm, here we will use the single-species density dependent diffusion reaction model that was originally introduced in [15] as a basis for our model. This type of model is usually referred to as deterministic continuum model that treats biofilm density as a dependent variable. This will reflect the interplay of various colonies in a biofilm community rather than assuming the biofilm as a homogeneous layer.
4.2 Model development

The prototype of the model we address here is deterministic biofilm growth model, which was first proposed in [15]. The model describes the growth of a bacterial biofilm community consisting of only one species and is formulated as a highly non-linear reaction-diffusion system of the volume fraction occupied by biomass $M$ and the concentration of the nutrient substrate $C$,

\[
\begin{align*}
\partial_t M &= \nabla(D_M(M)\nabla M) + \frac{\mu C}{k_1 + C} M - k_4 M, \\
\partial_t C &= d_c \Delta C - \frac{k_2 C}{k_1 + C} M,
\end{align*}
\]

where $\mu$ is the maximum specific growth rate, $k_1$ is the Monod half saturation constant relative to the nutrient and $k_2$ is the maximum specific consumption rate. The primary challenge is to model the spatial spreading mechanism of biomass. Expansion of the biofilm occurs locally where and when the biomass density approaches maximal possible cell density. While the substrate concentration satisfies a standard semi-linear parabolic equation, spatial expansion of the biofilm is described according to [15] and given by

\[
D_M(M) = \delta \frac{M^a}{(1 - M)^b}, \quad \text{where} \quad a, b > 1, \delta > 0.
\]

The diffusion coefficient for the sessile biomass degenerates for $M = 0$ where $D(0) = 0$. For $0 \approx M << 1$ we have $D(M) \approx \delta M^a$, i.e. the biofilm diffusion
equation. This type of diffusion guarantees a finite speed of interface propagation. For $M = 1$, the diffusion coefficient attains a singularity and blows up. For $0 << M \approx 1$ the equation behaves like super-diffusion. The blow up of the diffusion coefficient guarantees that $M < 1$ if a Dirichlet condition is specified somewhere on the boundary of the domain. This implies that the super-diffusion effect guarantees that the maximum possible cell density is never exceeded, independent of biomass production terms [18, 19]. The non-linear diffusion effects are necessary to reflect the experimentally observed characteristic growth behaviour of biofilm and the highly irregular structure, which causes difficulties in the mathematical analysis. The mono-species mono-substrate model (4.1) was mathematically analyzed in [19] and the well-posedness of the problem was established.

The diffusion coefficients of the dissolved nutrient $C$ in (4.1) is lower in the biofilm than in the aqueous phase, and depend on $M$ as well, though in a non-critical way. We make a linear ansatz that interpolates between the experimentally measurable values of diffusion in water ($M = 0$) and in a well-developed biofilm ($M = 1$) i.e.

$$d_C(M) = d_C(0) + M(d_C(1) - d_C(0)). \quad (4.3)$$

The diffusion coefficient of $C$ in the aqueous phase satisfy $d_N(C) > 0$, and is bounded by finite values. The diffusion is essentially Fickian and does not degenerate like in the porous media.

Due to the need to include further biofilm processes, the prototype model has
been extended to reaction diffusion systems involving several types of biomass and dissolved substrates e.g. [30, 20, 22]. Essentially, the structure or the prototype model differs from the multi-species, multi-substrate models, and the analytical results for the prototype model could not be carried over to the more involved multi-species, multi-substrate case.

4.2.1 Governing equation

Based on the prototype model [19] and the modeling approach for a biofilm dispersal in [20], we formulate the following model describing the nutrient-controlled biofilm cell dispersal as a system of three partial differential equations for the dependent variable $M, N, C$. It reads

$$
\begin{align*}
\partial_t M &= \nabla (D_M(M) \nabla M) + \frac{\mu C}{k_1 + C} M - \eta_1 \left( \frac{k_2^n}{k_2^n + C^n} \right) M, \quad (4.4) \\
\partial_t N &= \nabla (d_N(M) \nabla N) + \frac{\mu C}{k_1 + C} N + \eta_1 \left( \frac{k_2^n}{k_2^n + C^n} \right) M, \quad (4.5) \\
\partial_t C &= \nabla (d_C(M) \nabla C) - \frac{\mu}{\gamma} \frac{M \infty C}{k_1 + C} (M + N), \quad (4.6)
\end{align*}
$$

where equations (4.4) and (4.5) describe the growth and spatial movement of the sessile biomass $M$ and the dispersed biomass $N$. These two equations are directly coupled by the dispersal term. The equation (4.6) describes the consumption of nutrient $C$ by $M$ and $N$. We have assumed that the dispersed cells are washed out and that the death rate of the bacterial cells is negligible.
The diffusion coefficient of the dispersed cells $N$ is assumed to depend on $M$ as well just like in the case of the dissolved nutrient, therefore we make a similar linear ansatz that interpolates between the experimentally measurable values of diffusion in water ($M = 0$) and in a well-developed biofilm ($M = 1$) i.e.

$$d_N(M) = d_N(0) + M(d_N(1) - d_N(0)).$$ \hspace{1cm} (4.7)

The diffusion coefficient of $N$ in the aqueous phase satisfies $d_N(0) > 0$, so that it is bounded by finite values. The diffusion is essentially Fickian and does not degenerate like in the porous media.

**Remark on Existence of bounded solutions:** By the model formulation in the section above, the diffusion coefficient of the sessile biomass $M$ degenerates like in porous medium and attains singularity at $M = 1$. In the attempt to prove the existence of bounded solutions we compare the model (4.4)-(4.6) with the single species prototype growth model [19], which was shown to have bounded solutions. In the prototype model [19], the decay term had a constant rate (say $\gamma$) whereas in the model presented in this study, it is a decreasing function $f$, which depends on the substrate i.e. $f(C) := \frac{k_2}{k_2 + C}$$. We know that since $0 \leq C \leq C_{\text{max}}$, we have $f(0) \geq f(C) > f(C_{\text{Max}})$. Therefore, the prototype single species model with a decay rate $\gamma \cdot f(C_{\text{max}})$ provides an upper bound on the solution $M$ of the current model (4.4)-(4.6). With this in mind, we have $M < 1$, hence the solution is bounded away from singularity and the usual theory of parabolic equations applies. We present the proof in Appendix B.
Table 4.1: Parameter values

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Source</th>
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<tbody>
<tr>
<td>$\mu$</td>
<td>maximum specific growth rate</td>
<td>6.0</td>
<td>$d^{-1}$</td>
<td>[49]</td>
</tr>
<tr>
<td>$Y$</td>
<td>yield coefficient</td>
<td>0.63</td>
<td>-</td>
<td>[49]</td>
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<tr>
<td>$C_\infty$</td>
<td>bulk nutrient concentration</td>
<td>30</td>
<td>$gm^{-3}$</td>
<td>[49]</td>
</tr>
<tr>
<td>$k_4$</td>
<td>lysis rate</td>
<td>0.02</td>
<td>$d^{-1}$</td>
<td>assumed</td>
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<tr>
<td>$k_1$</td>
<td>half saturation concentration (growth)</td>
<td>0.4</td>
<td>$gm^{-3}$</td>
<td>assumed</td>
</tr>
<tr>
<td>$k_2$</td>
<td>half saturation concentration (dispersal)</td>
<td>0.4</td>
<td>$gm^{-3}$</td>
<td>assumed</td>
</tr>
<tr>
<td>$\eta_1$</td>
<td>maximum dispersal rate</td>
<td>varied</td>
<td>$d^{-1}$</td>
<td>[20]</td>
</tr>
<tr>
<td>$d_C(0)$</td>
<td>diffusion coefficients of $C$ (water)</td>
<td>$10^{-4}$</td>
<td>$m^2d^{-1}$</td>
<td>[17]</td>
</tr>
<tr>
<td>$d_C(1)$</td>
<td>diffusion coefficients for $C$ (biofilm)</td>
<td>$8 \times 10^{-5}$</td>
<td>$m^2d^{-1}$</td>
<td>[17]</td>
</tr>
<tr>
<td>$d_N(0)$</td>
<td>diffusion coefficients of $N$ (water)</td>
<td>$10^{-4}$</td>
<td>$m^2d^{-1}$</td>
<td>[20]</td>
</tr>
<tr>
<td>$d_N(1)$</td>
<td>diffusion coefficients of $N$ (biofilm)</td>
<td>$2 \times 10^{-5}$</td>
<td>$m^2d^{-1}$</td>
<td>[20]</td>
</tr>
<tr>
<td>$\delta$</td>
<td>biomass motility coefficient</td>
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<td>$m^2d^{-1}$</td>
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<td>$a$</td>
<td>biofilm diffusion exponent</td>
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<td>[15]</td>
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<td>m</td>
<td>assumed</td>
</tr>
<tr>
<td>$H$</td>
<td>system height</td>
<td>$1.6 \times 10^{-3}$</td>
<td>m</td>
<td>[20]</td>
</tr>
</tbody>
</table>

4.2.2 Computational Realization

The mathematical model (4.4)-(4.6) is discretized on a regular rectangular computational domain $\Omega = [0, L] \times [0, H]$. The rectangular computational domain here is considered as part of a larger biofilm reactor. The substratum, on which biofilm colonies form is the bottom boundary, $x_2 = 0$, see also Figure 4.1. The substratum is assumed to be impermeable to biomass and dissolved substrate so we pose homogeneous Neumann boundary conditions there

$$\partial_n M = \partial_n N = \partial_n C = 0, \quad \text{for} \quad x_2 = 0.$$
At the lateral boundaries, where \( x_1 = 0 \) or \( x_1 = L \), we assume a symmetry boundary condition, which allow us to view the domain as part of a continuously repeating larger domain. Hence, we pose the homogenous Neumann boundary condition as well for all dependent variables, i.e. \( \partial_n M = \partial_n N = \partial_n C = 0 \), for \( x_1 = 0 \) for \( x_1 = L \). At the top boundary, \( x_2 = H \), we pose a homogenous Dirichlet boundary conditions for the sessile and dispersed biomass \( M, N \).

The degeneracy \( D(0) = 0 \) in (4.4) leads to a finite speed of interface propagation in the sense that initial data with compact support imply solutions with compact support. Therefore, as long as biomass does not reach the boundary of the domain, the model satisfies simultaneously homogenous Dirichlet and Neumann conditions, which are combined in the no-flux condition \( D(M)\nabla M = 0 \). Since our simulations will be terminated before biomass reaches the top of the domain, the choice of boundary condition there is not critical.

The dispersed cell density is set to zero at the top boundary, this enforces a diffusion gradient from the biofilm in the interior of the domain to the boundary and mimics removal of the dispersed cells into the surrounding bulk phase.

At the top boundary \( x_2 = H \), we pose an inhomogeneous Dirichlet boundary condition for the nutrient \( C \) by setting the nutrient concentration at the top boundary to the bulk concentration value, which reflects that substrate is added to the system through this segment of the domain boundary. Thus, for the top boundary \( x_2 = H \) we have \( C = C_\infty, \quad M = 0, N = 0 \) at \( x_2 = H \).

Initially biofilm biomass is placed in small pockets with \( M > 0 \) at the sub-
The computational domain $\Omega$ consists of the aqueous phase $\Omega_1(t) = \{(x, y) \in \Omega : M(t; x, y) = 0\}$ and the biofilm phase $\Omega_2(t) = \{(x, y) \in \Omega : M(t; x, y) > 0\}$. These regions change over time as the biofilm grows. Biofilm colonies form attached to the substratum, which is a part of the boundary of the domain. The locations and initial sizes of these pockets will be explicitly specified a priori. Thus $\partial \Omega_2(0) \cap \{x_2 = 0\} \neq \emptyset$, $\partial \Omega_2(0) \cap \partial \Omega \setminus \{x_2 = 0\} = \emptyset$ and $\int_{\Omega_2(0)} dx \ll \int_{\Omega} dx$, $\Omega_2(0)$ is typically not connected, i.e. several inoculation sites are usually considered and all have a boundary with $x_2 = 0$. We will assume that initially no dispersing cells are in the system, and that the concentration of nutrients is initially at bulk levels, i.e. $C = C_\infty$, $N = 0$ at $t = 0$.

Equations (4.4)-(4.6) are discretized on a regular grid using a cell centered finite difference-based finite volume scheme for space and semi-implicit time-integration, adapted from [16, 36, 37] to account for the new dependent variable $N$, which is treated in the same manner as $C$. In every time step, three linear
algebraic systems are solved, one for each dependent variable. These linear systems are sparse and at least weakly diagonally dominant. They are solved with the stabilized biconjugate gradient method [39]. The linear solver is prepared for parallel execution on multi-core and shared memory multiprocessor architectures using OpenMP, as described in [36]. Simulations will be terminated when the biofilm reaches a set target size or when a set maximum simulation time is reached. For the visualization of simulation results we use the Kitware Paraview visualization package (spatially resolved plots) and gnuplot (lumped results).

For better interpretation of the computer simulations of the model, the following quantitative lumped measures will be used

- Biofilm size relative to the domain size

\[
\omega(t) := \frac{\int_{\Omega_2(t)} dx}{\int_{\Omega} dx}. \tag{4.8}
\]

- Average nutrient concentration in \( \Omega_2 \):

\[
C_{\text{avg}}(t) := \frac{\int_{\Omega_2(t)} C(t, x) dx}{C_\infty \int_{\Omega_2(t)} dx}. \tag{4.9}
\]

- Total sessile biomass in the biofilm:

\[
M_{\text{tot}}(t) := \int_{\Omega} M(t, x) dx. \tag{4.10}
\]

- Total amount of dispersed cells:

\[
N_{\text{tot}}(t) := \int_{\Omega} N(t, x) dx. \tag{4.11}
\]
• Relative Variation: This is calculated as the standard deviation of the sessile biomass density from the mean sessile biomass density in the biofilm,

\[ R(t) := \left[ \int_{\Omega_2} \left( M(t, x) - \int_{\Omega_2} M(t, y) \, dy \right)^2 \, dx \right]^{\frac{1}{2}}. \tag{4.12} \]

• Biomass loss \( K(T) \): This is the relative difference between the net biomass gain and the produced sessile biomass over a period of time \( T \) defined as follows

\[ K(T) = \frac{\int_0^T \int_{\Omega} \left[ \mu \frac{C}{k_1 + C} \right] M \, dx \, dt - \left[ M_{\text{tot}}(T) - M_0 \right]}{\int_0^T \int_{\Omega} \left[ \mu \frac{C}{k_1 + C} \right] M \, dx \, dt}. \tag{4.13} \]

The default model parameters used in the simulations are summarized in Table 4.1. Other parameter values will be given in the text where the simulation experiments are described.

### 4.3 Results

The model simulations presented in this study are not intended to provide quantitative matches to the experimental data, rather the simulations are offered as illustrations of general biofilm structures or behaviours that arise from nutrient-dependent biofilm dispersal. The primary parameters that were varied in this study are the dispersal induction parameter \( k_2 \) and the maximum dispersal rate \( \eta_1 \). These parameters are most directly linked to the dispersal process. Recall
from the simulation setup that the nutrient substrate is added to the biofilm from
the top boundary, we therefore investigate a situation whereby the local concentra-
tion of the nutrients in the biofilm reduces as a result of consumption.

We will start the result description and discussion by first taking a look at
lumped parameters, integrated over the computational domain and then at the
local effects on biofilm structure.

4.3.1 Prediction of cell dispersal for different values of $k_2$
and $\eta_1$

In this simulation experiment, we predict nutrient limitation induced biofilm dis-
persal depending on the dispersal induction parameter $k_2$ and the maximum dis-
persal rate $\eta_1$. These effects will be investigated using the lumped output param-
eters defined in equations (4.8)-(4.11).

In the first part of this simulation, we varied the dispersal induction param-
eter as $k_2 = \{0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35\} \text{gm}^{-3}$. Here, we used a max-
imum dispersal rate $\eta_1 = 3.6d^{-1}$ and every other parameter used is as listed on
Table 4.1. The lumped output parameters of this simulation are plotted in Figure
4.2.

By comparing the results for varied values of $k_2$, we observe that small values
of $k_2$ induce more bacterial cells to disperse from the the biofilm whereas large
values of $k_2$ induces less dispersed cells as seen in Figures 4.2a and 4.2c. it is
also obvious from Figure 4.2d that the dispersal is as a result of changes in the
nutrient concentration in the biofilm. In other words, small values of $k_2$ lead to high nutrient limitation in the biofilm and as a consequence, the biofilm did not grow (see Figure 4.2a and 4.2b). Generally, for all values of $k_2$, the biomass density increases continuously until it reaches a plateau (see Figure 4.2a). The dispersal of bacterial cells from the biofilm is quick and continuous and as such the biofilm could not really establish (more especially for high values of $k_2$). The biofilm size is always growing and never shrinking despite the cell dispersal, this suggests that bacterial cells leave from the biofilm. From the study [21], it was established that when bacterial cells leave from the biofilm, it can lead to spatially heterogeneous biomass distribution; we tested this finding [21] with our model by computing the standard deviation of the sessile biomass density from the mean biomass density in the biofilm. This is evaluated and plotted in Figure 4.2e as $R(T)$. Standard deviations that are close to zero indicate that more cells are within the inner core of the biofilm, while the standard deviations far from zero indicate that fewer cells are within the inner core of the biofilm. The result presented in 4.2e generally show that the standard deviation is tending to zero (though not quickly) and this indicates that as cells leave the biofilm colonies, new cells start occupying the inner core.

In the second part of this simulation experiment, we varied the maximum dispersal rate $\eta_1 = \{0.6, 1.2, 1.8, 2.4, 3.0, 3.6, 4.2\} d^{-1}$ and set the value of the dispersal induction parameter $k_2 = 0.05 gm^{-3}$. Every other parameter used is as listed on Table 4.1. Here, similar growth pattern is observed in the biofilm for different choices of $\eta_1$ albeit at different levels.

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For small values of $\eta_1$, more sessile biomass were produced but does not lead to more dispersal whereas large values of $\eta_1$ leads to more dispersal of cells as seen in Figure 4.3a and 4.3c. These results show that the dispersal event in a nutrient limited biofilm does not solely depend on the nutrient concentration but also on the dispersal rate. Similar to the first part of this simulation, we observe that the biofilm is not shrinking (see Figure 4.2b), which suggests hollowing, due to the maximum principle for parabolic equations applied to the nutrient concentration. We therefore computed the relative variation defined in (4.12) to investigate the size of the hollow. The result presented in Figure 4.3e shows that hollow size is not constant, it changes depending on new cells are being produced and the amount of cells dispersing from the biofilm.

Nutrient limitation (starvation) has been experimentally shown to induce biofilm detachment. It was shown by [28, 42] that cells may detach to escape unfavourable conditions when nutrients are scarce. In the study by [13] starvation induces an increase in polysaccharides lyase production, and so increases the metabolic breakdown and induction of biofilm dispersal.

From the results presented in Figure 4.2c and 4.3c, we saw that in overall, about 80% of the produced cells leave the biofilm [42]. However, nutrient limitation in the biofilm is not sufficient enough to induce a leveling off of biomass production but can possibly balance growth as seen in Figures 4.2a, 4.2b, 4.3a, 4.3b. Nutrient induced dispersed cells have been shown to exhibit different behavior and gene expression [42], which suggests that dispersed cells may not re-attach to the original biofilm, hence may be washed out or colonize down
Figure 4.2: Temporal plots of simulations computed for a growing biofilm using seven different values of the parameter $k_2 = \{0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35\} \text{gm}^{-3}$ using a fixed maximum dispersal rate $\eta_1 = 3.6^{-1}$. Shown are (a) sessile biomass fraction $M_{\text{tot}}$ in the biofilm, (b) the biofilm size $\omega(t)$ (c) the biomass loss, and (d) the average nutrient concentration in $\Omega_2$.

stream as speculated by [20], which justifies the exclusion of re-attachment in the model (4.4)-(4.6). Despite the findings here and the support from several ex-
Figure 4.3: Temporal plots of simulations computed for a growing biofilm using seven different values of the maximum dispersal rate $\eta = \{0.6, 1.2, 1.8, 2.4, 3.0, 3.6, 4.2\} \text{d}^{-1}$ using $k = 0.2 \text{gm}^{-3}$. Shown are (a) the total sessile biomass fraction $M_{\text{tot}}$ in the biofilm, (b) the biofilm size $\omega(t)$, (c) the total dispersed cells $N_{\text{tot}}$, and (d) the average autoinducer concentration $A_{\text{avg}}$ in $\Omega_2$.
Experimental studies, we want to point out that not all nutrient limitation can lead to biofilm dispersal, it depends on the type of nutrient and bacterial species. In some cases, high nutrient condition will induce biofilm dispersal [29]. Nutrient-induced biofilm dispersal is still an open area of study in biofilm research, hence more experimental and computation studies are needed to understand and isolate the factors that control biofilm dispersal.

### 4.3.2 Nutrient induced dispersal can lead to hollowing

From the result of the lumped parameters presented in Figure 4.2, we observed that the dispersal of cells from the biofilm appears continuous; and this does not affect the biofilm size by shrinking (see Figure 4.2b,e), which gives a clue that cell loss is from within the biofilm.

To investigate the impact of nutrient limitation induced biofilm cell dispersal on the spatial heterogeneity of the biofilm colonies, we therefore carry out a single simulation experiment. Here we have used a dispersal induction parameter $k_2 = 0.5gm^{-3}$ and dispersal rate $\eta_1 = 3.6^{-1}$. For selected time instances, we show the spatial distribution of the sessile biomass $M$ and the iso-lines of the nutrient concentration in the biofilm. The biofilm substratum is inoculated with four colonies having the same initial biomass density $0.1gm^{-3}$. At the time instance $t = 0.002$ to $t = 10$, the biofilm is still in its growing stage, the nutrient concentration has dropped considerably enough, more especially inside the biofilm colonies. The first dispersal event was observed in the next time instance.
Figure 4.4: Simulation of biofilm growth for a growing biofilm without lysis using the value of the parameter $k_2 = 0.5g m^{-3}$ and a maximum disperal rate $\eta_1 = 3.6^{-1}$. Color coded is the biomass density $M$, iso-lines of the nutrient concentration $C$ are plotted in greyscale.

$t = 14$ showing fewer cells in the centre of the colonies than towards the bulk fluid interface, which indicates hollowing. The hollowing appears to be spatially organized with two key features: (a) hollowing only occurred in cell colonies that had reached a sufficient size, though in this study we do not set any specific size for hollowing. (b) Voids were always centrally located at the substratum. These features are qualitatively consistent with the conjecture that cell detach-
ment from biofilm depends on nutrient availability. Hollowing occurred at the substratum in the centre of larger colonies (Figure 4.4 at $t = 12, 14, 16$). The hollow regions in the biofilm correspond to areas of limited nutrient reflected by the nutrient concentration. The simulation indicated that the transition from high to low nutrient availability occurred over a short time interval (see Figure 4.4 at $t = 10$ and at $t = 12$).

Some experimental and modeling studies such as [20, 44, 50] have observed hollowing in biofilm colonies similar to that described here. The study by [50] showed that hollowing was associated with the activation of a bacteriophage. At a certain stage in biofilm development, the phage is activated in the centre of some cell colonies and this causes some cells to lyse. This thins and degrades the biofilm EPS matrix. Some of the cells that survive from the phage are released through a pore to the bulk aqueous phase while some are trapped inside the hollow where they become motile inside the biofilm colony. The hollowing observed by [50] results from a combination of cell lysis, EPS degradation and cell dispersal; but the results from this study show that hollowing can be as a result of nutrient limitation induced cell dispersal from the biofilm.

**Conclusion**

In this study we have investigated the dispersal of cells from a single species biofilm induced by nutrient limitation in the system. We have investigated this phenomenon in a 2-dimensional setting. Experimental observations have been
made in different studies [25, 28, 42] for a variety of bacteria species, showing nutrient limitation as a potential trigger to cell dispersal from the biofilm. In summary, our simulation experiments suggest the following conclusions:

• Nutrient induced biofilm cell dispersal has the potential to balance biofilm growth. The nutrient-dependent dispersal term, which includes the dispersal induction parameter could be a good instrument to control the biofilm growth (size).

• Nutrient limitation induced biofilm dispersal occur in large biofilms. As the biomass density of a biofilm increases, the cells buried inside the biofilm get distanced from the nutrient source and hence finds a way to escape.

• Bacterial biofilm undergo structural changes during nutrient induced biofilm dispersal; this dispersal can create hollows in the inner core of the micro-colonies.

• The size of the hollow created by nutrient induced cell dispersal is not constant. Since the biofilm is always growing and producing new cells, it may not be easy to control the hollowing arising from nutrient induced biofilm dispersal.
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Chapter 5

Conclusion and Future Work

5.1 Conclusion

The main objective of this dissertation is centered around the conjecture "mathematical modeling helps to characterize internally triggered biofilm cell dispersal". The main findings are as follows:

- The modeling framework of quorum sensing induced cell dispersal can be adapted to describe the interplay of biofilm growth, cell dispersal and quorum sensing. While we have developed the model for quorum sensing induced, in principle the modeling techniques should be applicable to other biofilm detachment triggers as already shown for nutrient limitation induced biofilm dispersal.

- The biofilm structure and thickness may be affected by the interplay of biofilm growth, cell dispersal and quorum sensing. Therefore, the biofilm structure and thickness may be controlled by manipulating the variables
that relates to growth, dispersal and quorum sensing in the biofilm.

- A single quorum sensing based mechanism can explain both periodic dispersal in discrete and continuous dispersal events depending on parameters. It can also, and again depending on parameters, explain cell dispersal from smaller and larger colonies. Bacterial cells can modify a number of critical traits such as biofilm thickness by shifting the induction parameter threshold and dispersal rate.

- Cell dispersal induced by quorum sensing and nutrient limitation respectively can lead to hollowing in biofilm colonies. While the biofilm does not shrink from cell dispersal, the hollow regions in the inner core of the colonies “shrink” through the growing of new cells within the inner core of the colonies. In other words, the size of hollows in biofilm colonies reduces as the biofilm grows.

- The biomass spreading and the separation of biofilm and liquid region by a sharp interface can be guaranteed by regularizing the solution of the sessile biomass for a non-negative initial data.

- Large biomass loss can result from quorum sensing and nutrient induced dispersal respectively, thereby facilitating downstream colonization and making the biofilm act as cell nursery.
5.2 Contribution to Biofilm Research

Mathematical models are valuable tools in the investigation of biofilm phenomena. There are numerous models currently being used in biofilm research, but most of them focus on biofilm growth, detachment or quorum sensing induction. We are not aware of studies that focus on the interplay of these three aspects of biofilm systems and how they affect biofilm structure, function and dynamics. This thesis represents an original study, which considers biological mechanisms of detachment and potential environmental cues that trigger them. It predicts hollow colonies and structural heterogeneity ensuing from detachment. The mathematical model is founded on real experimentally related parameters and assumptions; it provides potential biological explanations contributing to biofilm sloughing/detachment. The simulation results obtained demonstrate that the model can predict many of the same phenomena observed experimentally and that the model can be adapted to test a variety of postulated biofilm mechanisms. In this type of mathematical modeling setup; it should be possible to isolate and study a particular aspect of a system.

5.3 Future Work

The strength of the mathematical models developed in this study lies in their relative simplicity. They do not attempt to include all conventional biofilm processes for the sake of completeness. However, this does not imply that other processes
not included in the models above do not contribute significantly to biofilm dynamics, only that they were not required for the conjectures considered in this thesis. Having said that, there is room for improvement to the existing models, which can be made in two ways: adding additional processes to the model and improving the implementation of the existing processes. We therefore suggest the following extensions and improvement.

- **Fluid flow and hydrodynamics**: Hydrodynamics usually have prominent impact on biofilm structures and detachment. Increasing liquid velocities or flow rates imply increasing shear forces at the biofilm surfaces, which could conceivably increase detachment and thinner more homogeneous structures. In the model presented in this thesis, we found out that only a negligible amount of cells re-attach to the biofilm, which is due to boundary conditions; however by the inclusion of fluid flow, the cells that leave the biofilm can be easily accounted for as re-attaching down stream or re-attached to the biofilm. However, accounting for fluid flow around irregular structures could be mechanically complicated and very little is still known about mechanical properties of biofilms.

- **Surface Erosion**: The model in its current form has no mechanism for cell dispersal from the surface of the biofilm. The erosion of bacterial cells from the surface of biofilm is already well established experimentally.

- **Combining two or more processes**: Single triggers of biofilm detachment are usually investigated. It will be interesting to combine two or more
triggers into a single system. This could be a good tool to investigate the eradication of biofilm.

- In Chapter 2, the dispersal term is formulated as a Hill function, which is closely related to the Holling Type 3 function. By this formulation, the induction threshold controls the dispersal event: discrete events in small biofilms and erosion-like events in larger biofilms. It will be worthwhile to define this function differently e.g. Holling Type 2 function, which is somehow hyperbolic, we can use this formulation to study the characterization of quorum sensing induced dispersal in small biofilms.

- This thesis focuses on a single species biofilm. We can extend the quorum sensing induced and the nutrient limitation induced biofilm dispersal models to a multi-species biofilm e.g. 2 species. In the case of the quorum sensing induced biofilm dispersal, we do not explicitly distinguish between up- and down-regulated cells. We can extend the model by explicitly distinguishing the up- and down-regulated cells. This we can do by replacing the dependent variable for sessile biomass by three new dependent variables to account for up- and down regulated quorum sensing bacterial cells; and non-quorum sensing bacterial cells, here we can vary the growth rates and the signal production rates. Studies have shown that these three distinct group of cells can interact and affect biofilm activity, therefore extending the quorum sensing induced dispersal model in this way will help to investigate the interplay of up- and down-regulated cells, biofilm growth and
dispersal in a quorum sensing and non-quorum sensing biofilms.

- Research studies have shown that one of the efficient ways to enhance cell dispersal is the breaking down of the EPS matrix. Another study [1] has presented quorum sensing regulated EPS production in biofilm communities. The results shown that low EPS producing biofilm generally obtain high population whereas in Chapter 2 of this thesis we have it that quorum sensing concentration is high when the biomass density is high. We can extend the model in Chapter 2 by introducing one dependent variable to account for EPS production and further couple the system by the dependence of cell dispersal on EPS breakdown and quorum sensing induction in biofilm. With this extension, we can answer the open question ”can the dual property of quorum sensing as an EPS regulator and cell dispersal trigger lead to eradication of biofilms?”

- In Chapter 4, the nutrient limitation induced biofilm is formulated for a heterogeneous biofilm. We propose an extension of the model to a homogenous biofilm, this will incorporate the effect of flow and biofilm areal density, which was not included in any part of this thesis.

- Domain with irregular boundaries: We have considered rectangular domains with smooth boundaries, which typify a section of a reactor. As an improvement, the model can be implemented on domains with irregular boundaries such as curvilinear surfaces and tooth cavities. This may involve some aspect of grid generation to account for the irregularities. This
implementation will involve refining the grids and may increase the computational time for running the simulation experiments.
Bibliography

Appendix A

Supplementary Material for the Quorum sensing induced biofilm detachment model (Chapter 2)

This supplementary material was published as a supplementary information to the paper

Figure A.1: Temporal plots of simulations of a non-quorum sensing biofilm (Non-QS) and a quorum sensing biofilm using seven different dispersal rates $\eta_1 = \{0.6, 1.2, 1.8, 2.4, 3.0, 3.6, 4.2 d^{-1}\}$ and fixed quorum sensing threshold $\tau = 10 nM$. Shown are (a) the total amount of sessile biomass in the biofilm, $M_{tot}(t)$, (b) biomass loss $K(T)$ indicating the amount of biomass that actually dispersed, (c) the average autoinducer concentration $A_{avg}(t)$ in $\Omega_2$, (d) the biofilm size $\omega(t)$.

### A.1 Influence of the quorum sensing induced dispersal parameter $\eta_1$

We conducted simulations to investigate the effect of the maximum dispersal rate $\eta_1$ on the amount of cells dispersed and on the biofilm. For low values of
$\eta_1$, we observe a continuous biomass loss and that the amount of biomass in the biofilm, as well as the autoinducer concentration levels off, as shown in Figure A.1a,c. The higher the plateau values are, smaller the maximum erosion rate $\eta_1$ will be. Smaller maximum erosion rates lead to larger biofilms, cf Figure A.1d.

The lower the dispersal rate is, the more continuous is cell dispersal, even for small value of $\tau$. For $\tau \geq 50$, the dispersal starts later when the colony is already big. For higher values of $\eta_1$, the dispersal events are rapid and discrete as we have seen before for small induction thresholds. Again, after each dispersal event, too few bacterial cells are left behind to produce enough autoinducers to maintain dispersal; the signal concentration falls back below threshold, cf Figure A.1c and the biofilm starts growing again. When the amount of biomass becomes strong enough for the concentration of the quorum sensing signal molecule to reach threshold, the next dispersal event is triggered, etc. The frequency of the dispersal events changes as $\eta_1$ changes, but the amplitude appears to be insensitive to this parameter. In all cases the vast majority of biomass produced in the biofilm is lost, cf Figure A.1b.

### A.2 Biofilm Structure

Here we have carried out more simulation experiment to investigate the impact of biofilm cell dispersal on the structure of the biofilm for a higher threshold value $\tau = 60nM$ as shown in Figure A.2 with a maximum dispersal rate $\eta_1 = 3.6/d$. We have considered a situation where the nutrient concentration has no influence
Figure A.2: Simulation of biofilm growth for induction threshold $\tau = 60\text{nM}$ and maximum dispersal rate $\eta_1 = 3.6d^{-1}$. Color coded is the biomass density $M$, iso-lines of the autoinducer concentration $A$ are plotted in grayscale.

on the quorum sensing signal production, thus $\gamma_1(C) = 1$. We show for selected time instances the spatial distribution of the sessile biomass $M$ and iso-lines of the autoinducer concentration.

Immediately after the simulation starts, at $t = 0.0002$, the biofilm colonies are still very small, which indicates no significant growth has taken place yet.
At $t = 5$ the colonies have grown with a rather homogeneous distribution of biomass. The small and large colony that were originally in close proximity have merged. The signal concentration is still well below threshold with a maximum value of $A \approx 0.082\tau$. At $t = 15$, the colonies have increased considerably in size, but in their inner regions the biomass density is much smaller than in the outer layers. The autoinducer concentration has increased as well, due to the larger amount of biomass. Although it is remains clearly below the threshold value, substantial cell dispersal has started. Since the signal concentration are highest inside the colonies, cells are lost there at a higher rate than in the outer layers. This is explained by the Hill function which was included in the model for the autoinducer concentration $A$. This shows that for such high threshold values even for signal concentrations clearly below $\tau$ the dispersal rate can be substantial. This patterns continues, as time progresses. For $t = 20, 25, 40$, we observe an increase in the size of the colonies, with an outer rim with high biomass density. The biomass density in the inner core decreases as the biofilm increases, leading to regions with fewer cells, i.e. hollowing structures, as reported in experimental studies, e.g. [1, 2].
A.3 Influence of nutrient availability on the production of quorum sensing signal and cell dispersal

For the simulation results shown in Figure A.3, we fix $\tau = 120nM$ and $\eta_1 = 0.6d^{-1}$ while every other parameter used is listed on Table 1. The choice of parameters in this simulation enables the investigation under limited substrate concentration for a biofilm with all the boundary conditions defined and described in the previous sections.

On the other hand to enable the investigation under increased availability of nutrients we have considered a microfloc, i.e. a biofilm without substratum. We prescribe a non-homogeneous Dirichlet boundary condition for the substrate on all the boundaries to ensure a constant supply of nutrients to the flocs across all domain boundaries. Moreover, we pose a homogeneous Neumann boundary condition on all the boundaries of the microfloc system to ensure that the signal molecules does not leave the system and is not diluted, i.e. $\partial_nA = 0$. For the biomass, we pose a homogeneous Dirichlet boundary condition on all the boundaries for the biomass i.e. $M = N = 0$, which allows the bacterial cells to leave from any of the boundaries. For the microfloc, we fix the signal threshold concentration $\tau = 50nM$ and the maximum dispersal rate $3.6d^{-1}$ while every other parameter used is as listed in Table 1. Lumped output parameters for these simulations are presented in Figures A.3 and A.4.
We first focus on the biofilm (Figure A.3). High nutrient concentrations are observed only initially and decline quickly as the biofilm grows. The concentration of the nutrient in the biofilm is not affected by the production of the quorum sensing signal molecule production rate which depends on the nutrient. Once the nutrient concentration drops below the concentration value $k_2$ we see a clear separation of curves for the total biomass density, biofilm size, fraction of dispersed cells and total amount of dispersed cells. The curves obtained for $\gamma_2(C)$ and $\gamma_4(C)$, which are sensitive to low concentration values are grouped together, as well as the curves for $\gamma_1(C)$ and $\gamma_3(C)$ which are not sensitive to low substrate concentrations. As long as the substrate concentration is above $k_2$ we do not detect a notable difference between the four curves.

For larger nutrient concentration values Figure A.4, referring to the microbial floc, the curves of $\gamma_1$ and $\gamma_2$ (insensitive to high concentrations) are grouped together and those of $\gamma_3$ and $\gamma_4$ (sensitive to high concentrations). In the latter cases the autoinducer concentrations are lower than in the former cases. In general, we observe that the different concentration levels of the quorum sensing signal does not translate to cell dispersal. As the nutrient concentration $C$ drops the curves regroup and for small concentration values we see again the curves of $\gamma_2$ and $\gamma_4$ grouped together and those of $\gamma_1$ and $\gamma_3$. In the latter cases the autoinducer concentration is higher, thus leading to more dispersal and a smaller biofilm remaining.
Figure A.3: Temporal plots of simulations of a biofilm using different $\gamma_i(C)$, using $\tau = 120 nM$, $\eta_1 = 0.6d^{-1}$. Shown are (a) total sessile biomass fraction $M_{tot}$ in the biofilm, (b) average concentration of the nutrients $C_{avg}$ in $\Omega_2$, (c) average autoinducer concentration $A_{avg}$ in $\Omega_2$, (d) biofilm size $\omega$, (e) total dispersed cells $N_{tot}$, (f) biomass loss $K(T)$ indicating the fraction of the produced biomass that are actually dispersed from the biofilm.
Figure A.4: Temporal plots of simulations of a microfloc using different $\gamma_i(C)$, using $\tau = 50nM$, $\eta_1 = 3.6d^{-1}$. Shown are (a) total sessile biomass fraction $M_{tot}$ in the biofilm, (b) average concentration of the nutrients $C_{avg}$ in $\Omega_2$, (c) average autoinducer concentration $A_{avg}$ in $\Omega_2$, (d) biofilm size $\omega$, (e) total dispersed cells $N_{tot}$, (f) biomass loss $K(T)$ indicating the fraction of the produced biomass that are actually dispersed from the microfloc. The curves of $\gamma_1$ and $\gamma_2$ (insensitive to high nutrient concentrations) are grouped together and appear as one curve; and so are those of $\gamma_3$ and $\gamma_4$ (sensitive to high nutrient concentrations).
Bibliography


Appendix B

Mathematical Analysis of Nutrient Limitation Induced Biofilm Dispersal Model that was presented in Chapter 4

B.1 Well-posedness of the Nutrient Limitation Induced Biofilm Dispersal Model

B.1.1 Preamble

In Chapter 4 of this thesis, we developed the nutrient limitation induced biofilm dispersal model, it reads
\partial_t M = \nabla (D_M(M) \nabla M) + \frac{\mu C}{k_1 + C} M - \eta_1 \left( \frac{k_2^n}{k_2^n + C^n} \right) M \quad \text{(B.1)}
\partial_t N = \nabla (d_N(M) \nabla N) + \frac{\mu C}{k_1 + C} N + \eta_1 \left( \frac{k_2^n}{k_2^n + C^n} \right) M \quad \text{(B.2)}
\partial_t C = \nabla (d_C(M) \nabla C) - \frac{\mu}{Y} \frac{M_{\infty} C}{k_1 + C} (M + N) \quad \text{(B.3)}

where the dependent variables $M, N, C$ represents the sessile biomass fraction, dispersed biomass and nutrient concentration respectively. The model (B.1) - (B.3) comprises two non-linear effects: a porous medium type degeneracy, and super-diffusion; this makes the model essentially difficult to solve analytically. In this Appendix, we present the ideas for proving the existence and uniqueness of bounded non-negative solution of the degenerate problem (B.1) - (B.3). This idea is gotten from [6]. We consider smooth non-degenerate approximations for the degenerate problem and show that their solutions converge to the solution of the degenerate problem. These ideas are based on the proof developed for scalar degenerate reaction-diffusion equations of porous medium type in [2], the solution theory in [3, 8, 9] for single species biofilm model and the ideas applied in [4, 6]
For technical reasons we study the model in the dimensionless auxiliary form

$$
\partial_t M = \nabla(D_M(M) \nabla M) + \frac{C}{k_1 + C} M - \eta_1 \left( \frac{k_2^n}{k_2^n + |C|^n} \right) M
$$

$$
\partial_t N = d_1 \Delta N + \frac{C}{k_1 + C} N + \eta_1 \left( \frac{k_2^n}{k_2^n + |C|^n} \right) M
$$

$$
\partial_t C = d_2 \Delta C - \frac{\sigma C}{k_1 + C} (M + N)
$$

with initial and boundary conditions

$$
M|_{\partial \Omega} = 0 \quad N|_{\partial \Omega} = 0 \quad C|_{\partial \Omega} = C_\infty
$$

$$
M|_{t=0} = M_0 \quad N|_{t=0} = N_0 \quad C|_{t=0} = C_0,
$$

where $C_\infty$ is a positive constant, $M_0, N_0, C_0$ are non-negative and in $L^\infty(\Omega)$. Moreover, we assume that

$$
\|M_0\|_{L^\infty(\Omega)} < 1 - \rho
$$

for some $\rho \in (0, 1)$.

**Remark:** The absolute value of $C$ in the dispersal term is taken just for convenience, and so non-negative solutions of (B.4) solves (B.1)- (B.3) and vice versa.

We impose constant Dirichlet boundary conditions on the nutrient concentration $C$ reflecting a constant unlimited nutrient supply at the boundary of the considered domain; while homogeneous Dirichlet boundary conditions is
assumed for $N$ to enforce the removal of motile (or suspended) cells from the system. In the same way, we prescribe homogeneous Dirichlet boundary conditions for the biomass fraction $M$. This describes the situation of a growing biofilm.

We point out that these specific boundary conditions are primarily chosen for convenience with respect to this analysis, albeit boundary conditions of mixed type are often considered more appropriate in applications. Typically, Dirichlet boundary conditions are prescribed on some parts of the boundary while Neumann or Robin boundary conditions are specified on the other parts. In particular, the substratum on which the biofilm grows is impermeable for all dependent variables, and can be modeled by homogenous Neumann boundary conditions.

Here and in the sequel, we use the following notations, $Q_T := \Omega \times (0, T]$ for $T > 0$, $Q = \Omega \times \mathbb{R}^+$ and

$$\Phi(M) := \int_0^M D(s)ds = \int_0^M \frac{s^a}{(1 - s)^b}ds \quad \text{for } 0 \leq M < 1.$$

**Definition 2.** We call $(M, N, C)$ a solution of system (B.4) with the initial and boundary data (B.5), if for any $T > 0$ the functions

$$M, N, C \in C([0, T]; L^1(\Omega)) \cap L^\infty(Q_T)$$

and satisfy (B.4) in distributional sense.

To be more precise, if $(M, N, C)$ is a solution according to Definition [2],

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then the equality

\[
\int_{\Omega} M(x, T)\varphi(x)dx - \int_{\Omega} M_0(x)\varphi(x)dx + d \int_{Q_T} D(M(x, t))\nabla M(x, t) \cdot \nabla \varphi(x)dtdx \\
= \int_{Q_T} \left( \frac{C}{k_1 + C} M - \eta \frac{k_2^n}{k_2^n + |C|^n} M \right) \varphi(x)dtdx
\]

(B.7)

holds for all test functions \( \varphi \in C_0^\infty(\Omega) \) and almost every \( T > 0 \). The determining equations for the other components of the solution are defined in a similar way as follows:

\[
\int_{\Omega} N(x, T)\varphi(x)dx - \int_{\Omega} N_0(x)\varphi(x)dx + d_1 \int_{Q_T} \nabla N(x, t) \cdot \nabla \varphi(x)dtdx \\
= \int_{Q_T} \left( \frac{C}{k_1 + C} N + \eta \frac{k_2^n}{k_2^n + |C|^n} M \right) \varphi(x)dtdx
\]

(B.8)

\[
\int_{\Omega} C(x, T)\varphi(x)dx - \int_{\Omega} C_0(x)\varphi(x)dx + d_2 \int_{Q_T} \nabla C(x, t) \cdot \nabla \varphi(x)dtdx \\
= - \int_{Q_T} \left( \frac{\sigma C}{k_1 + C} (M + N) \right) \varphi(x)dtdx
\]

(B.9)

Let \( \epsilon \) be the regularization parameter. For \( \epsilon > 0 \) small, we define

\[
D_\epsilon(M) := \begin{cases} 
\epsilon^a & \text{if } M < 0, \\
\frac{(M+\epsilon)^a}{(1-M)^b} & \text{if } 0 \leq M \leq 1 - \epsilon, \\
\frac{1}{\epsilon} & \text{if } M \geq 1 - \epsilon.
\end{cases}
\]

(B.10)

\[
\Phi_\epsilon(M) := \int_0^M D_\epsilon(s)ds
\]
and the non-degenerate (regularized) system

\[ \begin{align*}
\partial_t M^\epsilon &= \nabla \cdot (D_\epsilon(M^\epsilon)\nabla M^\epsilon) + g(M^\epsilon, N^\epsilon, C^\epsilon), \\
\partial_t N^\epsilon &= d_1 \Delta N^\epsilon + f_1(M^\epsilon, N^\epsilon, C^\epsilon), \\
\partial_t C^\epsilon &= d_2 \Delta C^\epsilon + f_2(M^\epsilon, N^\epsilon, C^\epsilon).
\end{align*} \]  

(B.11)

and the regularized solutions denoted by \((M^\epsilon, N^\epsilon, C^\epsilon)\).

### B.1.2 Existence and Uniqueness

**Proposition 8.** Let the boundary and initial data be non-negative and smooth, \(M_0 \in C_0^\infty, N_0 \in C^\infty, C_0 \in C^\infty(\bar{\Omega})\) such that \(C_0|_{\partial \Omega} = 1\) and \(\|M_0\|_{L^\infty(\Omega)} < 1 - \rho\). Then, there exist unique solutions \((M^\epsilon, N^\epsilon, C^\epsilon) \in C^{1,2}(\bar{Q_T})\) of (B.11), that are non-negative and uniformly bounded w.r.t. \(\epsilon > 0\) in \(L^\infty(Q_T)\). Moreover, there exist \(0 < \delta < 1\) and \(\epsilon_0 > 0\) such that \(M^\epsilon \leq 1 - \delta\) in \(Q_T\) for all \(\epsilon < \epsilon_0\).

**Proof.** By the classical theory for quasilinear parabolic equations there exist unique solutions \((M^\epsilon, N^\epsilon, C^\epsilon) \in C^{1,2}(\bar{Q_T})\) of (B.4). We use comparison theorems for quasilinear parabolic equations (e.g., see [1]) for each equation separately to show the non-negativity and uniform boundedness of all components. First of all we observe that the functions \(g, f_1, f_2\) satisfy

\[ f_1(M, 0, C) \geq 0, \quad f_2(M, N, 0) = 0, \quad g(0, N, C) = 0. \]

All components of the solution take non-negative values on the boundary \(\partial \Omega\) and the initial data are non-negative in \(\Omega\). This implies that the constant zero is a subsolution for \(M^\epsilon, N^\epsilon\) and \(C^\epsilon\), which follows by comparison with zero. In order to establish an upper estimate on \(M\) we construct a suitable barrier function and consider the elliptic problem.
\[ \Delta \theta(x) = -c_1, \quad x \in \Omega, \]
\[ \theta|_{\partial \Omega}(x) = c_2, \quad x \in \partial \Omega. \]

where the constants \(c_1\) and \(c_2\) are defined by

\[ c_1 := \sup_{0 < \epsilon < \epsilon_0} \| g(M^\epsilon, N^\epsilon, C^\epsilon) \|_{L^\infty(Q_T)} \]
\[ c_2 := \sup_{0 < \epsilon < \epsilon_0} \| \Phi_\epsilon(M_0) \|_{L^\infty(Q_T)} \]

and \(\Phi_\epsilon(M_0) := \int_0^{M_0} \frac{(s+\epsilon)^a}{(1-\epsilon)^b} \, ds\) for \(0 \leq M_0 < 1 - \epsilon\). Moreover, the solution \(\theta\) of (B.12) is bounded on \(\Omega\), and by the maximum principle follows \(\theta \geq c_2\) in \(\Omega\).

For \(\epsilon < \epsilon_1\) we define \(Z_\epsilon := \Phi_\epsilon^{-1}(\theta)\) and observe that

\[ \partial_t Z_\epsilon - \Delta(\Phi_\epsilon(Z_\epsilon)) = c_1 = \sup_{0 < \epsilon < \epsilon_1} \| g(M^\epsilon, N^\epsilon, C^\epsilon) \|_{L^\infty(Q_T)} \geq \partial_t M^\epsilon - \Delta(\Phi_\epsilon(M^\epsilon)) \]

in \(Q_T\). Moreover, the boundary conditions imply

\[ Z_\epsilon|_{\partial \Omega} = \Phi_\epsilon^{-1}(\theta)|_{\partial \Omega} = \Phi_\epsilon^{-1}(c_2) \geq M^\epsilon|_{\partial \Omega} = 0, \]

and the initial data satisfies

\[ Z_\epsilon|_{t=0} = \Phi_\epsilon^{-1}(\theta)|_{t=0} \geq \Phi_\epsilon^{-1}(c_2) \geq \Phi_\epsilon^{-1}(\Phi_\epsilon(M_0)) = M_0, \]

where we used the monotonicity of the function \(\Phi_\epsilon^{-1}\). Consequently, the function \(Z_\epsilon\) is an upper solution for \(M^\epsilon\). Using the fact that \(\theta\) is bounded in \(\Omega\) and that \(\Phi_\epsilon^{-1}\) converges pointwise to infinity in the interval \((0, 1)\), we conclude that there exist \(0 < \epsilon_0 \leq \epsilon_1\) and \(\delta \in (0, 1)\) such that \(M^\epsilon \leq Z_\epsilon = \Phi_\epsilon^{-1}(\theta) < 1 - \delta\) for all \(\epsilon < \epsilon_0\). Furthermore, we show the uniform boundedness of \(C^\epsilon\) by defining

\[ C_{\text{max}} := \max\{||C_0||_{L^\infty(\Omega)}, ||C_\infty||_{L^\infty(\partial \Omega)}\}. \]

Then

\[ \partial_t C_{\text{max}} - d_2 \Delta C_{\text{max}} + \sigma \frac{C_{\text{max}}}{k_1 + C_{\text{max}}}(M^\epsilon + N^\epsilon) = \sigma \frac{C_{\text{max}}}{k_1 + C_{\text{max}}}(M^\epsilon + N^\epsilon) \geq 0 \]
where we have used the non-negativity of the sessile biomass $M$ and the dispersed cells $N$. This shows that $C_{\text{max}}$ is an upper solution for $C^\epsilon$. To prove the uniform boundedness of $N^\epsilon$ we denote by $\hat{N}$ the solution of the initial value problem

$$
\partial_t \hat{N} = d_1 \Delta \hat{N} + \hat{N} - k_2 \hat{N} + \eta_1 (1 + R_1),
$$

$$
\hat{N}|_{\partial \Omega} = 0,
$$

$$
\hat{N}|_{t=0} = N_0,
$$

where $1 + R_1$ is the upper bound for the sessile biomass fraction. We observe that $\hat{N}$ is non-negative, satisfies $\hat{N} \in L^\infty(Q_T)$ and

$$
\partial_t \hat{N} - d_1 \Delta \hat{N} - \frac{C^\epsilon}{k_1 + C^\epsilon} \hat{N} + k_2 \hat{N} - \eta_1 \frac{k_2}{1 + |C^\epsilon|} M^\epsilon \\
\geq \partial_t \hat{N} - d_1 \Delta \hat{N} - \hat{N} + k_2 \hat{N} - \eta_1 (1 + R_1) \\
= 0 = \partial_t N^\epsilon - d_1 \Delta N^\epsilon - \frac{C^\epsilon}{k_1 + C^\epsilon} N^\epsilon + k_2 N^\epsilon - \eta_1 \frac{k_2}{1 + |C^\epsilon|} M^\epsilon.
$$

Consequently, $\hat{N}$ is an upper solution for the dispersed cells $N^\epsilon$.

**Proposition 9.** Under the hypotheses of Proposition [8] the approximate solutions $M^\epsilon$ satisfy

$$
\int_{Q_T} D_\epsilon(M^\epsilon) \left( \partial_t M^\epsilon \right)^2 + \sup_{t \in [0,T]} \int_\Omega |\nabla \Phi_\epsilon(M^\epsilon)|^2 \leq c
$$

$$
\int_{Q_T} \left( |\partial_t N^\epsilon|^2 + |\partial_t C^\epsilon|^2 \right) + \sup_{t \in [0,T]} \int_\Omega \left( |\nabla N^\epsilon|^2 + |\nabla C^\epsilon|^2 \right) \leq c,
$$

for some constant $c > 0$.

**Proof.** We first multiply the second equation in (B.11) by $\partial_s N^\epsilon$ and integrate,

$$
\int_\tau^t \int_\Omega |\partial_s N^\epsilon|^2 = -\frac{d_1}{2} \int_\tau^t \int_\Omega \partial_s |\nabla N^\epsilon|^2 + \int_\tau^t \int_\Omega \partial_s N^\epsilon f_1(M^\epsilon, N^\epsilon, C^\epsilon),
$$
where $0 \leq \tau \leq t \leq T$. Using Young’s inequality leads to the estimate cf[6]

$$
\int_\tau^t \int_\Omega |\partial_s N^\epsilon|^2 + \frac{d_1}{2} \int_\Omega |\nabla N^\epsilon|^2 \bigg|_{s=t} = \frac{d_1}{2} \int_\Omega |\nabla N^\epsilon|^2 + \int_\tau^t \int_\Omega |\partial_s N^\epsilon f_1(M^\epsilon, N^\epsilon, C^\epsilon)|^2,
$$

$$
\leq \frac{d_1}{2} \int_\Omega |\nabla N^\epsilon|^2 \bigg|_{s=t} + \xi \int_\tau^t \int_\Omega |\partial_s N^\epsilon|^2 + C_\xi \int_\tau^t \int_\Omega |f_1(M^\epsilon, N^\epsilon, C^\epsilon)|^2,
$$

and, setting $\tau = 0$ we obtain

$$
(1 - \xi) \int_{Q_\tau} |\partial_s N^\epsilon|^2 + \frac{d_1}{2} \int_\Omega |\nabla N^\epsilon|^2 \bigg|_{s=t} \leq \frac{d_1}{2} \int_\Omega |\nabla N_0|^2 + C_\xi \int_{Q_t} |f_1(M^\epsilon, N^\epsilon, C^\epsilon)|^2.
$$

for small $\zeta > 0$, and some constant $C_\zeta$. By the continuity of $f_1$ we deduce

$$
\int_{Q_\tau} |\partial_s N^\epsilon|^2 \leq c', \quad \sup_{t \in [0,T]} \int_{Q_T} |\nabla N^\epsilon|^2 \leq c', \quad (B.14)
$$

for some constant $c' \geq 0$. The corresponding bounds for the solutions $C^\epsilon$ can be obtained in the same way. The estimate for $M^\epsilon$ follows immediately from [6]

\[ \square \]

**Proposition 10.** Under the hypotheses of Proposition [8] the solutions of (B.11) converge to a solution of the degenerate system (B.4) as $\epsilon$ tends to zero.

**Proof.** By Proposition [8] the solutions $M^\epsilon$ are uniformly bounded by $1 - \delta$, and the following estimate for the diffusion coefficient of $M$ was shown in [6], thus

$$
\epsilon^a \leq D_\epsilon(M^\epsilon(t, x)) \leq \frac{1}{\delta^b}, \quad (t, x) \in Q_T,
$$

for all $\epsilon < \epsilon_1$. Moreover, if $\epsilon < \epsilon_1$ we observe that

$$
D(M^\epsilon) \leq D_\epsilon(M^\epsilon) \leq \frac{1}{\delta^b},
$$

and consequently,

$$
\Phi(M^\epsilon(t, x)) \leq \Phi_\epsilon(M^\epsilon(t, x)) \leq \frac{1 - \delta}{\delta^b}, \quad (t, x) \in Q_T.
$$
Proposition [8] and Proposition [9] further imply that
\[
\int_{Q_T} \left( \partial_t (\Phi(M^\epsilon)) \right)^2 = \int_{Q_T} \left( D(M^\epsilon) \partial_t M^\epsilon \right)^2 \leq \int_{Q_T} \left( D_\epsilon(M^\epsilon) \partial_t M^\epsilon \right)^2 \\
\leq \|D_\epsilon(M^\epsilon)\|_{L^\infty(Q_T)} \int_{Q_T} D_\epsilon(M^\epsilon) \left( \partial_t M^\epsilon \right)^2 \leq \frac{c}{\delta^5},
\]
for some constant \( c \geq 0 \). This shows that the family \( \Psi^\epsilon := \Phi(M^\epsilon), \epsilon < \epsilon_1 \), is uniformly bounded in \( W = \{ u \in L^\infty(0, T; H^1(\Omega)) \mid \partial_t u \in L^2(0, T; L^2(\Omega)) \} \), which is compactly embedded into \( C([0, T]; L^2(\Omega)) \) by Aubin-Lions’ Lemma (e.g., see [7], Theorem II.1.5). Consequently, there exists \( \Psi \in C([0, T]; L^2(\Omega)) \) and a sequence \( \epsilon_n \) tending to zero as \( n \to \infty \) such that \( \Psi^{\epsilon_n} \to \Psi \) in \( C([0, T]; L^2(\Omega)) \). This implies that \( M^{\epsilon_n} = \Phi^{-1}(\Psi^{\epsilon_n}) \to M \) and \( \Phi(M^{\epsilon_n}) \to \Phi(M) \) in \( C([0, T]; L^2(\Omega)) \).

Moreover, by proposition [9] the solutions \( N^\epsilon \) and \( C^\epsilon \) are uniformly bounded in \( W \), which implies that there exist \( N, C \in C([0, T]; L^2(\Omega)) \) and a sequence \( \epsilon_n \) tending to zero as \( n \to \infty \) such that
\[
N^{\epsilon_n} \to N, \ C^{\epsilon_n} \to C \quad \text{in} \ C([0, T]; L^2(\Omega)).
\]

We can now pass to the limit \( \epsilon \to 0 \) in the distributional formulation of the degenerate system (B.4) using the uniform boundedness of the approximate solutions and the continuous embedding \( C([0, T]; L^2(\Omega)) \hookrightarrow C([0, T]; L^1(\Omega)) \), and conclude that the limits \( M, N, C \) are solutions of the degenerate problem.

\[ \square \]

**Proposition 11.** Let the hypotheses of Proposition [8] be satisfied. If \((M, N, C)\) and \((\bar{M}, \bar{N}, \bar{C})\) are solutions corresponding to initial data \((M_0, N_0, C_0)\) and \((\bar{M}_0, \bar{N}_0, \bar{C}_0)\),
respectively, then

\[\|M(T) - \tilde{M}(T)\|_{L^1(\Omega)} - \|M_0 - \tilde{M}_0\|_{L^1(\Omega)} \leq \int_0^T \int_\Omega |g_0(t, x)| \, dx \, dt,\]
\[\|N(T) - \tilde{N}(T)\|_{L^1(\Omega)} - \|N_0 - \tilde{N}_0\|_{L^1(\Omega)} \leq \int_0^T \int_\Omega |h_1(t, x)| \, dx \, dt,\]
\[\|C(T) - \tilde{C}(T)\|_{L^1(\Omega)} - \|C_0 - \tilde{C}_0\|_{L^1(\Omega)} \leq \int_0^T \int_\Omega |h_2(t, x)| \, dx \, dt,\]  \hspace{1cm} (B.15)

where the functions \(g_0\) and \(h_i\) are defined as

\[g_0(t, x) := g(M(t, x), N(t, x), C(t, x)) - g(\tilde{M}(t, x), \tilde{N}(t, x), \tilde{C}(t, x))\]
\[h_i(t, x) := f_i(M(t, x), N(t, x), C(t, x)) - f_i(\tilde{M}(t, x), \tilde{N}(t, x), \tilde{C}(t, x))\]  \hspace{1cm} (B.16)

for \(i = 1, 2, 3\).

**Proof.** This immediately follows from the Lemma 3.3 in [5]. \(\square\)

**Proposition 12.** Let \((M, N, C)\) and \((\tilde{M}, \tilde{N}, \tilde{C})\) be solutions of (B.4) corresponding to initial data \((M_0, N_0, C_0)\) and \((\tilde{M}_0, \tilde{N}_0, \tilde{C}_0)\) respectively, satisfying the assumptions in (B.5) and Lipschitz continuity of solutions, then functions \(g\) and \(f_i\) satisfy

\[\|g(M, N, C) - g(\tilde{M}, \tilde{N}, \tilde{C})\| + \sum_{i=1}^{3} \|f_i(M, N, C) - f_i(\tilde{M}, \tilde{N}, \tilde{C})\| \leq c \left( \|M - \tilde{M}\| + \|N - \tilde{N}\| + \|C - \tilde{C}\| \right)\]  \hspace{1cm} (B.17)

**Proof.** This is just algebraic manipulations and calculus, it follows immediately from [6] Let \(B\) be a bounded subset of \(\mathbb{R}_+^3\) and \((M, N, C), (\tilde{M}, \tilde{N}, \tilde{C}) \in \Omega.\)
The function $f_2$ satisfies the (B.17) and we obtain,

$$
\|f_2(M, N, C) - f_2(\tilde{M}, \tilde{N}, \tilde{C})\|
= -\sigma \left\| \left( \frac{C}{k_1 + C} \right) (M + N) - \left( \frac{\tilde{C}}{k_1 + \tilde{C}} \right) (\tilde{M} + \tilde{N}) \right\|
\leq r_1 \left( \|M - \tilde{M}\| + \|N - \tilde{N}\| \right)
$$

(B.18)

where $r_1 \geq 0$. To show that the functions $f_1, f_3$ and $g$ satisfy (B.17) we observe, that

$$
C_1^n X_1 - C_2^n X_2 = C_1^n (X_1 - X_2) + X_2 (C_1^n - C_2^n)
= C_1^n (X_1 - X_2) + \nu X_2 (C_1 - C_2) \int_0^1 (sC_1 + (1 - s)C_2)^{n-1} ds
$$

(B.19)

which implies that $|C_1^n X_1 - C_2^n X_2| \leq r|X_1 - X_2 + X_2 (C_1 - C_2)|$ for some constants $\nu$ and $r \geq 0$ and $(X_1, C_1), (X_2, C_2)$ in bounded subsets of $\mathbb{R}_+^4$.

Applying this to $f_1$ and $g$ respectively we have

$$
\|f_1(M, N, C) - f_1(\tilde{M}, \tilde{N}, \tilde{C})\|
= \left\| \left( \frac{C}{k_1 + C} - k_4 \right) N + \eta_1 \left( \frac{k_2^n}{k_2^n + C^n} \right) M \right\|
- \left\| \left( \frac{\tilde{C}}{k_1 + \tilde{C}} - k_4 \right) \tilde{N} + \eta_1 \left( \frac{k_2^n}{k_2^n + \tilde{C}^n} \right) \tilde{M} \right\|
\leq |1 - k_4| \|N - \tilde{N}\| + |\eta_1| \left( \|M - \tilde{M}\| \right)
\leq r_2(\|N - \tilde{N}\| + \|M - \tilde{M}\|)
$$

(B.20)
where $r_2 \geq 0$. Similarly, $g$ satisfies (B.17), thus we obtain

$$
\|g(M, N, C) - g(\tilde{M}, \tilde{N}, \tilde{C})\|
= \left\| \left( \frac{C}{k_1 + C} - k_4 \right) M - \eta_1 \left( \frac{k_2^n}{k_2^n + C} \right) M \right\|
- \left\| \left( \frac{\tilde{C}}{k_1 + \tilde{C}} - k_4 \right) \tilde{M} - \eta_1 \left( \frac{k_2^n}{k_2^n + \tilde{C}} \right) \tilde{M} \right\|
\leq |1 - k_4||M - \tilde{M}| + |\eta_1| \left[ \|M - \tilde{M}\| + \|M\||A - \tilde{A}\| \right]
= r_4\|M - \tilde{M}\|
$$

(B.21)

where $r_4 \geq 0$. Combining equations (B.18), (B.20) and (B.21) gives

$$
\|g(M, N, C) - g(\tilde{M}, \tilde{N}, \tilde{C})\| + \sum_{i=1}^{3} \|f_i(M, N, C) - f_i(\tilde{M}, \tilde{N}, \tilde{C})\|
\leq r'(\|M - \tilde{M}\| + \|N - \tilde{N}\| + \|C - \tilde{C}\|)
$$

(B.22)

where $r' \geq 0$. This concludes the proof in that $g, f_1, f_2$ satisfy (B.17). \qed

**Theorem 13.** For every $T > 0$ and initial data $(M_0, N_0, C_0)$ in $L^\infty(\Omega)$ such that

$$M_0 \geq 0, \quad \|M_0\|_{L^\infty(\Omega)} < 1 - \rho, \quad N_0 \geq 0, \quad C_0 \geq 0$$

for some $\rho \in (0, 1)$, there exists a unique solution of model (B.4). The solutions $M, N, C$ are non-negative in $L^\infty(Q_T)$ and $M$ satisfies $\|M\|_{L^\infty(Q_T)} < 1 - \delta$ for some $\delta \in (0, 1)$.

**Proof.** First, we assume the initial data is smooth and satisfies the hypothesis of Proposition [8] and Proposition [12]. If $(M, N, C)$ and $(\tilde{M}, \tilde{N}, \tilde{C})$ are solutions corresponding to initial data $(M_0, N_0, C_0)$ and $(\tilde{M}_0, \tilde{N}_0, \tilde{C}_0)$, respectively, then Proposition [11] and the estimate of Proposition [12] imply that

$$F(T) - F(0) \leq c \int_0^T F(s)ds,$$
where

\[ F(t) := \|M(t) - \tilde{M}(t)\|_{L^1(\Omega)} + \|N(t) - \tilde{N}(t)\|_{L^1(\Omega)} + \|C(t) - \tilde{C}(t)\|_{L^1(\Omega)}. \]

By Gronwall’s lemma we conclude

\[ F(T) \leq F(0) e^{cT}, \tag{B.23} \]

for some constant \( c \geq 0 \), which implies uniqueness of solutions corresponding to smooth initial data. For general initial data, let \( M^n_0, N^n_0, C^n_0 \) be a sequence satisfying the hypothesis of Proposition [10] such that

\[ \|M^n_0 - M_0\|_{L^1(\Omega)} + \|N^n_0 - N_0\|_{L^1(\Omega)} + \|C^n_0 - C_0\|_{L^1(\Omega)} \text{ as } n \to \infty. \]

The Lipschitz-continuity of solutions \( L^1(\Omega) \)-norm (B.23) implies that the corresponding solutions \( M^n, N^n, C^n \) satisfy

\[
\sup_{t \in [0,T]} \left\{ \|M^n(t) - M^m(t)\|_{L^1(\Omega)} + \|N^n(t) - N^m(t)\|_{L^1(\Omega)} \\
+ \|C^n(t) - C^m(t)\|_{L^1(\Omega)} \right\} 
\leq C_T (\|M^n_0 - M^m_0\|_{L^1(\Omega)} + \|N^n_0 - N^m_0\|_{L^1(\Omega)} + \|C^n_0 - C^m_0\|_{L^1(\Omega)}).
\]

for some constant \( C_T \geq 0 \) and all \( n, m \in \mathbb{N} \). Consequently, \( M^n, N^n, C^n \) form a Cauchy sequence in \( C([0, T]; L^1(\Omega)) \) converging to \( M, N, C \), which is the unique solution of (B.4). \qed
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