Cross-diffusion in Biofilms

by

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ABSTRACT

CROSS-DIFFUSION IN BIOFILMS

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We propose a deterministic continuum model for mixed culture biofilms where movement of one species is affected by the presence of the other. Two derivations of this new model are presented. One derivation is based on the continuous time, discrete space master equation and the other one is based on the equations of conservation of mass and momentum. Starting from both viewpoints, we derive the same dual-species diffusion-reaction model for biofilms that comprises three non-standard diffusion effects: (i) degeneracy as the local biomass density vanishes, (ii) a super-diffusion singularity as the local biomass density approaches its *a priori* known maximum, and (iii) non-linear cross-diffusion. (i) describes the finite speed of propagation of the biofilm/water interface, (ii) describes volume filling effects, and (iii) describes the mixing of both biomass species. We present a numerical method for this highly nonlinear PDE model of biofilm that can tackle these three non-linear diffusion effects. To investigate the effect of the new model feature, we study the role of the cross-diffusion terms in numerical simulations of three biofilm models: competition, allelopathy, and a mixed system formed by an
aerobic and an anaerobic species. In all three systems we observe that accounting for cross-diffusion affects local biofilm structure, in particular the relative local distribution of biomass, but it does not affect overall lumped quantities such as the total amount of biomass in the system. As an application, our highly nonlinear density dependent cross-diffusion model is used in order to incorporate an experimental observation in models of disinfection of microbial biofilms. An extended reaction kinetics based on carbon consumption during disinfection is introduced. Our simulations show that the extended model captures the experimental observation, and suggest that the consumption of carbon substrates during inactivation due to antibiotics helps biofilms to survive and re-grow. Finally, as an extension of dual-species model, a generalized cross-diffusion model of \( k \) interacting species is derived considering the continuous time and discrete space master equation passing to the continuous limit. Moreover, a criterion for preserving the positivity of the solution of this type of generalized cross-diffusion model is presented.
Dedication

To my parents, wife and son.
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Chapter 1

Introduction

1.1 Cross-diffusion models

1.1.1 General cross-diffusion models

Diffusion is the passive movement of molecules or particles or species along a concentration gradient, or from regions of higher to regions of lower concentration. The classic theory of diffusion was founded more than one hundred years ago by the physiologist A. Fick [43]. According to Fick’s law, the rate of transport of matter in the $x$-direction across a unit normal area in a unit time, i.e., the flux $J_x$, is proportional to the gradient of the concentration of the matter. Thus, $J_x = -D \frac{\partial C}{\partial x}$, where $C$ is the concentration of matter and $D$ is the diffusivity. The minus sign indicates that diffusion occurs from high concentration to low concentration. Using this law, we can obtain Fick’s
equation of diffusion:

\[
\frac{\partial C}{\partial t} = -\frac{\partial J_x}{\partial x} = \frac{\partial}{\partial x}(D \frac{\partial C}{\partial x})
\]

Mathematical models with diffusion investigate a function of space and time, that describes concentrations, population densities, temperatures, charged particle densities etc. A biological process based on time and space, can be described by reaction-diffusion equations that is, with the help of partial differential equations.

A system of partial differential equations with reaction, self-diffusion and cross-diffusion is also a special kind of reaction-diffusion equation. Here, the reaction term expresses the local rate of growth or death of the species due to intra- and interspecific interactions. The term self-diffusion implies the movement of individuals from a higher to lower concentration region. Cross-diffusion expresses the population fluxes of one species due to the presence of the gradient of the other species. In fact, when two species merge, the cross-diffusion term has different sign than self-diffusion which phenomenon can be visualized in Figure (1.1). The value of the cross-diffusion coefficient may be positive, negative or zero. The term positive cross-diffusion coefficient denotes the movement of the species in the direction of lower concentration of another species and negative cross-diffusion coefficient denotes that one species tends to diffuse in the direction of higher concentration of another species. Different types of mathematical analysis of mathematical models
Figure 1.1: Observation of different diffusion gradient direction due to the presence of self- and cross-diffusion term in the system where $X$ and $Y$ are two distinct species.

with self-diffusion, cross-diffusion are found in many mathematical ecology fields and also in other natural sciences [4, 7, 8, 9, 12, 13, 48, 49, 54]. Most of them have considered Shigesada et al. [50] and Lotka-Volterra [36, 55] type models in the presence of cross-diffusion effects.

Shigesada, Kawasaki and Teramoto (SKT) proposed the following strongly coupled parabolic system [50]:

\[
\begin{align*}
\frac{\partial u}{\partial t} &= \Delta((D_1 + a_{11}u + a_{12}v)u) + e_1 \nabla.(u\nabla P) + u(a_1 - b_1u - c_1v) \\
\frac{\partial v}{\partial t} &= \Delta((D_2 + a_{21}u + a_{22}v)v) + e_2 \nabla.(v\nabla P) + v(a_2 - b_2u - c_2v)
\end{align*}
\]

(1.1)

on a bounded smooth domain $\Omega$ in $\mathbb{R}^d$, $d \geq 1$. The Neumann boundary conditions were considered. This mathematical model describes spatial seg-
regation of interacting species, where $u$ and $v$ represent the densities of two competing species. In the above model, $P$ is an environmental potential and for $i = 1, 2$ and $j = 1, 2$, $D_i \in \mathbb{R} \geq 0$, $a_{ij} \in \mathbb{R} > 0$ are diffusion coefficients, $e_i \in \mathbb{R}$ are transport coefficients, $a_i \in \mathbb{R} \geq 0$ are the intrinsic growth rates, and $b_i \in \mathbb{R} \geq 0$ are intra-specific, whereas $c_i \in \mathbb{R} \geq 0$ are interspecific competition coefficients. This model becomes the well known Lotka-Volterra competition-diffusion system when $a_{ij} = 0$ and $P$ is considered as constant. This type of model is studied frequently in the literature [3, 6, 27, 37, 45, 47]. For nonzero $a_{ij}$, model (1.1) is a cross diffusion parabolic system and this system has also been studied in the literature [33, 49, 59]. Most of the works investigate the conditions for existence and uniqueness of weak and global solutions and also discuss stability and instability of solutions. Recently, in [10], the authors use additional generating conditions to find exact solutions to the SKT model in the one dimensional case. The precise conditions are given for the existence of unstable equilibrium points for SKT-type systems [11, 32, 38, 53]. In [10], analysis of weak solutions of cross-diffusion systems is shown and in [47] global existence and uniform boundedness of solution is presented.

Shigesada et al. [50] derive a cross-diffusion model for the populations of two competitive species in heterogeneous environments. In the mathematical formulation, a non-linear dispersive force due to mutual interferences of individuals and an environmental potential function are introduced as a behavioral version of Morisita’s phenomenological theory of *Environmental*
density. The heterogeneity of the environment and the non-linear dispersive movements raise a spatial segregation of the populations of two similar and competing species and there is a possibility that this spatial segregation acts to stabilize the coexistence of two similar species, relaxing the interspecific competition [50]. Chattopadhyay et al. [6] has also presented a model for two competing species without showing any derivation. The derivation or the formulation of mathematical model for two spatially interacting populations in a continuous time and discrete in space under homogeneous environment has not been found so far in literature except [44]. The cross-diffusion models like Shigesada et al. [50] and Chattopadhyay et al. [6] that have been used in the literature to describe interacting species can be derived as special cases with the cross-diffusion model presented in [44]. In [44], no analytical analysis or simulation is carried out for the cross-diffusion model.

According to [27], no general theory is available till now that covers all possible cross-diffusion models, even in the simplest case of only two coupled partial differential equations. Therefore, every single model case has to be considered separately. Furthermore, cross-diffusion effects are not that well studied in literature. Our motivation is to derive cross-diffusion model from ecological viewpoint and develop some general theory for example positivity, boundedness and existence of the solutions of the system.
1.1.2 Cross-diffusion model for biofilm applications

A biofilm is an aggregate of microorganisms in which cells adhere to each other on surfaces and interfaces in aqueous environments. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Generally, biofilm EPS is composed of extracellular DNA, proteins, and polysaccharides. Biofilms can be prevalent in natural, industrial and hospital settings [26, 34]. This can be developed in spatially highly irregular morphological structures, where individual colonies are separated by voids and channels. These are substantially more resistant against conventional cleaning agents and antibiotics than suspended bacterial populations and are difficult to eradicate [40].

Biofilms play a very important role in many scientific and technological areas. Consequently, they are studied in many disciplines. Based on biofilm processes, environmental engineers have been using the beneficial properties of biofilms in order to develop remediation technologies for many years. For example, wastewater treatment, groundwater protection and soil remediation, where the sorption properties of microbial films play a major role in self-purification [40]. Recent research include the development of biofilm based fuel cells that are used for the production of energy [35, 39]. On the contrary, there are many harmful impacts of bacterial biofilms. Biofilms cause bacterial infections, biocorrosion of drinking water pipes or industrial facilities, biofouling of ships, food spoilage, etc. [16].

Biofilms are important because of microbial infections in the body.
fections of the oral soft tissues, teeth and dental implants, middle ear, gastrointestinal tract, urogenital tract, airway or lung tissue, eye, urinary tract protheses, peritoneal membrane and peritoneal dialysis catheters, indwelling catheters for hemodialysis and for chronic administration of chemotherapeutic agents, cardiac implants such as pacemakers, prosthetic heart valves, ventricular assist devices, and synthetic vascular grafts and stents, prostheses, internal fixation devices, percutaneous sutures and tracheal and ventilator tubing are caused by surface colonization by biofilms [52].

For devising medical treatment and for the prevention of biofilm-borne infections, the understanding of biofilm formation is important. Biofilm communities must be studied separately from planktonic bacteria since their behaviour is distinctive. Also, the experimental studies always suffer from the environmental conditions in the laboratory reactor and particular properties of the bacteria involved in formation of different biofilms based on different environments and conditions. Our motivation is that mathematical modelling of biofilm processes on a very general and basic level will help towards understanding them better [17].

First mathematical models for biofilms were derived based on the assumption that biofilms develop in homogeneous layers. These models serve well for the purpose of engineering applications on the macro-scale, i.e., on the reactor level [56]. One of this type of mathematical models for biofilm proposed by Wanner and Gujer in 1986 was the base of dynamic biofilm models for long period of time. This model describes the formation of biofilm
layer with uniform thickness in one direction perpendicular to substratum. The key concept of this model is the growth of biomass by a convective mechanism with the convection velocity directly related to the production of new biomass. However, laboratory experiments show that biofilms can grow in complicated non-homogeneous spatial architectures with voids and channels. Spatially heterogeneous biofilm morphologies cannot be predicted from the concept of one-dimensional models. Therefore, a variety of models for spatially heterogeneous biofilms has been proposed in recent years, ranging from stochastic individual based models to cellular automata models to deterministic continuum models [46]. The big challenge in this kind of biofilm modelling is to describe the spatial spreading mechanism for biomass which is responsible for spatial heterogeneities and determine the shape of biofilms.

In [17], a mathematical model for the development of spatially heterogeneous single species biofilm structures is presented. Unlike hybrid discrete/continuum models it is a continuum model, describing the interaction of nutrient availability and biomass production. Spatial biomass spreading is described by a nonlinear density-dependent diffusion mechanism. Some multi-species models [15, 18, 19, 20, 23, 29] have been developed from single species model [17]. In [40, 41] authors presented a dual species mathematical model for biofilms without having any cross-diffusion terms. More specifically, in [40] the authors formulated and analyzed a mathematical model of a biofilm formed by two bacterial species that compete for one substrate. One of the two species also degrades a second substrate. Such a biofilm
system can be found in biobarriers for the protection of groundwater from Trichloroethylene. The authors focused on the effect of parameter uncertainties. Due to the absence of cross-diffusion terms in their model, the impact of the presence of both species on each other has not been observed. This gap motivates us to derive a cross-diffusion mathematical model for biofilm development in terms of the local biomass density for two species. Our aim is to analyze the model; more specifically, to find positivity, boundedness and existence of the solution. We are also interested to develop an efficient numerical method or extend the existing numerical methods for single species biofilm model and analyse the effects of cross-diffusion on the solution of the system.

1.2 Objectives

1.2.1 Derivation of a cross-diffusion model for two interacting species from the ecological viewpoint

Taking the viewpoint of a biofilm as a spatially structured microbial population, we derive a cross-diffusion model for a mixed-culture biofilm. The starting point is the continuous time, discrete space master equation that is frequently used in Theoretical Ecology. The continuous model is obtained by passing to the continuous limit in space. This model is a multi-species generalisation of an existing single species density-dependent diffusion-reaction
model for biofilms.

1.2.2 Derivation of a cross-diffusion model for two interacting species based on conservation of mass and momentum

The starting point are the equations for conservation of mass and momentum that are frequently used in Fluid Mechanics. The cross-diffusion model is obtained by neglecting inertia terms (based on time scale arguments) and introducing an algebraic biomass-pressure relationship to close the model. The resulting cross-diffusion model is equivalent to the model derived in objective 1.2.1.

1.2.3 Numerical analysis of the model

From the numerical analysis viewpoint, there is a very limited number of studies on mathematical models for reaction-diffusion system with nonlinear cross-diffusion in the current literature. There are some studies on numerical methods for the Shigesada-Kawasaki-Teramoto model (1.1). Galiano et al. [24] show the existence of weak solutions to the parabolic system in any space dimension. In addition, the authors investigate the one-dimensional stationary problem analytically and discussed the notion of segregation. Finally, numerical results for the one-dimensional stationary problem underlining the effects of segregation of the species using a semi-implicit finite
difference method is presented. Galiano et al. [25] consider a non-linear implicit scheme based on a semi-discretization in time and show that the semi-discrete solutions converge to a non-negative solution of the continuous system in one space dimension. The proof of existence of strictly positive weak solutions to the semidiscrete problem is presented. Barret and Blowey [2] propose a fully discrete finite element scheme for a cross-diffusion system with a proof of convergence in order to show well-posedness of their approximation in space dimensions $d \leq 3$. The authors use an implicit scheme and present numerical examples in one space dimension. Andreianov et al. [1] present a convergent finite volume method to obtain approximate solutions to the reaction-diffusion system with cross-diffusion. An existence proof for a class of cross-diffusion systems is shown. The authors use standard two-point finite volume fluxes in combination with a nonlinear positivity-preserving approximation of the cross-diffusion coefficients. Also, an existence and uniqueness result for the studied model is presented. Furthermore, the authors provide a stability analysis to study pattern-formation phenomena, and perform two-dimensional numerical examples which exhibit formation of nonuniform spatial patterns. From the simulations, the authors find that experimental rates of convergence are slightly below second order. The authors also illustrate the accuracy and performance of their finite volume scheme. Murakawa [42] proposes a linear discrete-time scheme for general nonlinear cross-diffusion systems which is an extension of a linear scheme based on the nonlinear Chernoff formula for the degenerate parabolic equations, which was
proposed by Berger et al. [5]. The authors analyze stability and convergence of the linear scheme and also present numerical experiments in one and two space dimensions.

Our biofilm model shows degeneracy as the dependent variable vanishes, and a singularity as the dependent variable approaches its a priori known upper bound. The first property leads to a finite speed of interface propagation if the initial data have compact support, while the second one introduces counter-acting super diffusion. The squeezing property of this model leads to steep gradients at the interface. Moving interface problems of this kind are known to be problematic for traditional numerical methods. They can introduce non-physical and non-mathematical solutions, for example, with spurious oscillations around the interface, or numerical solutions that do not maintain non-negativity. In order to tackle this issue, a numerical method is proposed in [16] for the single species model. The main idea of this method is a non-local (in time) representation of the diffusion operator. It has also been shown that the proposed method is free of oscillations at the interface, that the discrete interface satisfies a discrete version of the continuous interface condition and that the effect of interface smearing is quantitatively small.

Our goal is to extend the existing numerical method or develop an efficient numerical method for our cross-diffusion models. We do this by treating cross-diffusion formally like convection terms.
1.2.4 Application to particular biofilm systems

We use the model and numerical method to study biofilm process in simulation experiments.

1.2.5 Derivation of a cross-diffusion model for $k$ interacting species from the ecological viewpoint

Taking the viewpoint of population as spatially structured, we derive a cross-diffusion model for $k$ interacting species. We use similar approach as in objective 1.2.1 to extend the model.

1.2.6 Boundedness and existence of solutions

We aim to establish existence, non-negativity, and boundedness of the solutions of the cross diffusion biofilm model in objective 1.2.5.

1.3 Thesis structure

This thesis is organised as follows.

Chapter 2 proposes a deterministic continuum model for mixed culture biofilms. A crucial aspect is that movement of one species is affected by the presence of the other. This leads to a degenerate cross-diffusion system that generalizes an earlier single species biofilm model. Two derivations of this new model are given. One, like cellular automata biofilm models,
starts from a discrete in space lattice differential equation where the spatial interaction is described by microscopic rules. The other one starts from the same continuous mass balances that are the basis of other deterministic biofilm models, but it gives up a simplifying assumption of these models that has recently been criticized as being to restrictive in terms of ecological structure. We show that both model derivations lead to the same PDE model, if corresponding closure assumptions are introduced. To investigate the role of cross-diffusion, we conduct numerical simulations of three biofilm systems: competition, allelopathy, and a mixed system formed by an aerobic and an anaerobic species. In all cases we find that accounting for cross-diffusion affects local distribution of biomass, but it does not affect overall lumped quantities such as the total amount of biomass in the system. This chapter is submitted as


Chapter 3 presents a numerical method for a highly nonlinear PDE model of biofilm response to antibiotics with three nonlinear diffusion effects: (i) porous medium degeneracy, (ii) super-diffusion singularity, (iii) nonlinear cross-diffusion. The scheme is based on a Finite Volume discretization in space and semi-implicit, non-local time integration. The resulting discretized system is implemented in Fortran and parallelized with OpenMP. The numer-
ical method is validated in a simulation study. This chapter was published as


Chapter 4 includes an experimental observation of recent laboratory experiments that showed increased metabolic activity, measured in terms of carbon dioxide production during periods of exposure of bacterial biofilms to antibiotics, a phenomenon that is not reflected by current biofilm models. In order to incorporate this observation in models of disinfection of microbial biofilms we introduce extended reaction kinetics based on carbon consumption during disinfection. We implement this extension in a 0-dimensional simplified reactor scale model (ODE) where carbon substrates and antibiotics are well mixed but biomass is attached to the wall and not washed out, and in our 2-dimensional density dependent cross-diffusion model (PDE), that considers spatial effects, such as substrate gradients and heterogeneous biofilm architectures. Our simulations show that the extended model captures the experimental observation, and suggest that the consumption of carbon substrates during inactivation due to antibiotics helps biofilms to survive and re-grow. Not accounting for this effect in a model might lead to false negatives, over-predicting the efficacy of antibiotic disinfection. This
Chapter 5 deals with the positivity property that is required for the solutions of mathematical models arising in biology. One of the first steps in analyzing ecological or biological models mathematically is to test whether solutions originating from non-negative initial data remain non-negative (as long as they exist). A general model of $k$ interacting species with self-diffusion, cross-diffusion and reaction terms is derived considering the continuous time and discrete space master equation by passing the continuous limit in space. This model consists of a system of partial differential equations with self diffusion, cross-diffusion and reaction terms. We also present a criterion for preserving the positivity of the solution of this type of generalized cross-diffusion model. This chapter will be submitted as

Rahman KA, Sonner S, Eberl HJ. "A generalized cross-diffusion model of spatially interacting populations and positivity of its solution", to be submitted

Chapter 6 wraps up this dissertation pointing out all its outcomes. It also highlights some open questions for future research motivation.
Bibliography


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Chapter 2

A Mixed Culture Biofilm Model with Cross-Diffusion


Abstract

We propose a deterministic continuum model for mixed culture biofilms. A crucial aspect is that movement of one species is affected by the presence of the other. This leads to a degenerate cross-diffusion system that generalizes an earlier single species biofilm model. Two derivations of this new model are given. One, like cellular automata biofilm models, starts from a discrete in space lattice differential equation where the spatial interaction is described by microscopic rules. The other one starts from the same continuous mass balances that are the basis of other determinisitic biofilm models, but it gives up a simplifying assumption of these models that has recently been criticized as being too restrictive in terms of ecological structure. We show that both model derivations lead to the same PDE model, if
corresponding closure assumptions are introduced. To investigate the role of cross-diffusion, we conduct numerical simulations of three biofilm systems: competition, allelopathy, and a mixed system formed by an aerobic and an anaerobic species. In all cases we find that accounting for cross-diffusion affects local distribution of biomass, but it does not affect overall lumped quantities such as the total amount of biomass in the system. **MSC:** 92D25 (primary), 35K65 (secondary)

### 2.1 Introduction

**Executive summary.** Natural or engineered biofilms are often mixed culture systems, in which several species interact. In this study we present a deterministic mixed culture biofilm model with cross-diffusion effects, which arise where the movement of one species is restricted by the presence of the other. The proposed cross-diffusion model provides a potential alternative to existing mixed-culture biofilm models, which have been criticized for overemphasizing or suppressing mixing of species in a colony.

**Biofilm background.** Bacterial biofilms are depositions of microbes on solid surfaces [40] or interfaces, such as air-liquid interfaces [37]. They form wherever the environmental conditions sustain microbial growth. Bacteria attach to the surface (a.k.a substratum in the biofilm literature), multiply and start producing extra cellular polymeric substances (EPS) that protect the community against mechanical washout. Biofilms can develop in spatially
complex morphologies [43]. In many instances they form cluster-and-channel architectures, in which neighboring colonies are separated by voids filled with bulk fluid.

Depending on the context, biofilms are harmful, neutral or beneficial. Examples for harmful biofilms are those associated with bacterial infections such as cystic fibrosis pneumonia, parodontitis, middle ear infections, and microbial depositions on implants [10]. In the food and beverage industry, biofilms impact food quality and safety [23]. In other industries, biofilms lead to biofouling and biocorrosion of materials and increase production costs [40, 78]. On the other hand, many environmental technologies are based on biofilm processes [39, 60]. The traditional examples stem from wastewater engineering, soil remediation or groundwater protection. These biofilms normally are mixed-culture biofilms, formed by several co-existing species that perform different tasks in the treatment process. More recently, biofilm based microbial fuel cells are being developed for the production of energy [42, 45].

In most biofilms, dissolved growth limiting substrates such as nutrients, oxygen, or antimicrobials, enter a colony through its interface with the surrounding aqueous phase, and then diffuse in the biofilm. The interplay of diffusion and consumption of nutrients leads to concentration gradients [68, 78]. While bacteria closer to the biofilm/water interface might live in a regime of abundance, nutrients might not reach the bacteria in the inner layers which consequently can experience a state of starvation. These heterogeneous growth conditions in a biofilm can lead to the formation of
microniches in biofilms, where species can survive together, which cannot coexist in a well-mixed setup, such as a laboratory chemostat. Examples are anaerobic pockets in the inner layers of otherwise aerobic biofilms, or simultaneous nitrification and denitrification in different regions of a biofilm [68]. Therefore life in biofilm communities differs greatly from life in a well mixed planktonic system [40].

The prevalence of biofilms together with their distinct features that set them apart from suspended communities prompted biofilm research as its own interdisciplinary field of research. From the early beginnings, mathematical modeling has made important contributions to this development. The models that have been developed and employed to study biofilm behavior differ greatly in terms of mathematical concepts, a priori modeling assumptions made, and purposes for which they were developed. Our focus is on mixed-culture biofilms, and the spatial interaction of species. We discuss here briefly two biofilm modeling concepts that have been developed for such systems: the deterministic and continuous Wanner-Gujer like biofilm models and the discrete and stochastic cellular automata based biofilm models.

**Wanner-Gujer type biofilm models.** The traditional Wanner-Gujer modeling framework for biofilms was largely developed in the 1980s and 1990s [78, 79]. It is originally a one-dimensional model, in which the biofilm is assumed to homogeneously cover the substratum. In the general multi-species, multi-substrate setting the model is a highly complex hybrid hyperbolic-elliptic free boundary value problem with nonlocal effects. The Wanner-
Gujer model was instrumental in understanding the interplay between substrate gradients and population dynamics in biofilms. It was developed as a dynamic model to assess biofilm performance, with well-developed biofilms in wastewater engineering applications in mind, but not to describe biofilm structure. It has been extended to the multi-dimensional setting in [2, 46].

The starting point for the development of the Wanner-Gujer model is a continuous mass balance with convective transport, see also Section 2.2.3 below. An assumption of the model is to constrain the volume fractions occupied by biomass to add up to unity. In the 1D case, this simplification allows to express the convective biomass transport velocity as a function of biomass growth, i.e. indirectly depending on substrate concentrations and biomass density. This assumption is a good approximation in many cases, such as well developed biofilms in nutrient rich environments with nonnegative net growth rates, for example in wastewater treatment. Indeed, it has been shown that under such conditions biofilm models that do not make this a priori assumption predict biofilms with almost constant biomass volume fraction approximately at unity as a model output [18]. On the other hand, it is well known that in some biofilms the biomass often does not attain everywhere the same volume fraction. An extreme case is the phenomenon of biofilm hollowing, where for example as a consequence of cell dispersal or lysis, hollow shell biofilms colonies form with internal regions with greatly reduced cell density, cf [29, 58, 62]. By construction, models making the assumption that all biomass volume fractions everywhere add to unity will
not be able to predict this phenomenon, but instead describe a shrinking or contracting biofilm, while a single-species model that give up this assumption was able to simulate this hollowing phenomenon in [22].

A second point of criticism, which is relevant only in the multi-species case that we are interested in, was recently pointed out and analyzed in [36]. The authors showed that the constraint that all volume fractions should add to unity introduces a ”restriction in ecological structure”, because the models imply a growth induced convective biomass velocity with which all species move, always away from the substratum and never downward. This puts a severe limitation on the ability of the model to predict the spatial relationship of different species in a biofilm. In order to predict spatial interaction and mixing of species in biofilms correctly, the authors conclude that ”some form of downward mobility, against the favorable substrate gradient direction, is needed” in Wanner-Gujer models, for example some form of diffusive transport. Although [36] only treated the one-dimensional case where movement perpendicular to the substratum is the only possibility, they point out that the same phenomenon can also be observed in the two-dimensional numerical simulations in [2] for lateral movement of biomass, parallel to the substratum. Indeed, the simulations of a heterotrophic/autotrophic biofilm in that paper show that initially segregated species will remain so over the course of the simulation, no mixing of species will take place, unless a colony was initially mixed. When neighboring colonies of different species expand, they will grow in the main growth direction along side each other but not
mix. Initially mixed colonies between such single species colonies will form a mixed band that separates these regions but no exchange will take place. In such a model with purely convective transport, the spatial interaction is by pushing and shoving, but not intermingling. This is at odds with microscopic studies, often based on confocal laser scanning microscopy (CLSM), that show that mixing of species in colonies occurs, if the growth conditions support this, as demonstrated for example in [1, 73]. To remedy this effect, in [36], the authors include, for illustration purposes, Fickian diffusion of biomass as an example for a mechanism that induces movement of biomass against the growth gradient. However they point out that the assumption of such a linear diffusion is not realistic. Indeed, Fickian diffusion, as the limit process of an unrestricted, unbiased random walk, assumes more or less free mobility, whereas in biofilms the biomass is often packed tightly, in particular under the additional assumption that all volume fractions add to one. In such a case the presence of one species will always pose a restriction on the movement of other species. In diffusive systems, such internal resistance to mixing often is expressed in terms of cross-diffusion effects in addition to self-diffusion.

**Cellular automaton like biofilm models.** An entirely different class of biofilm models, proposed since the late 1990s, are based on cellular automata. These are two- or three-dimensional models that have been primarily developed to better understand the sometimes very irregular biofilm architectures, rather than biofilm performance, i.e. they were formulated for a
different purpose than the Wanner-Gujer models. The underlying structure of these models is a discrete rectangular lattice, the cells of which can be occupied by biomass, or not. Biomass growth in the lattice cells is governed by an ordinary differential equation. If the biomass in a grid cell exceeds or approaches a maximum value, part of it is moved into another cell on the lattice according to some user defined, typically stochastic rule. Pioneering was the single species model in [55], which was soon followed by multi-species models, cf. [50, 71, 78]. In such cellular automaton models the user has full control over the mixing behavior of species by explicitly designing local rules of interaction accordingly. The biomass density, and hence the volume fraction occupied by biomass, are treated as dependent variables, and in contrast to Wanner-Gujer models normally not required to add to unity, although the spatial rules are designed such that unity is not exceeded. The stochasticity of the cellular automaton biofilm models introduces variability between replicates of a simulation [28], and therefore should necessitate several runs and statistical averaging in some sense, which can make these simulation models computationally quite expensive.

In [71], it was shown that also for cellular automata based biofilm models local rules can be designed that enforce the constraint that biomass densities add to unity in the interior cells of the colony. This mimics the convective pushing and shoving biomass transport mechanism of the Wanner-Gujer like models, with similar results, in particular vertical layering in multispecies applications as in [2]. On the other hand, the single species density diffusion-
reaction model in [18], in which the biomass volume fraction is a dependent variable, is a continuous deterministic model that in some sense shows more similarities with the cellular automaton models than with Wanner-Gujer like models; see [33] for a derivation of this model from a discrete lattice differential equation. An ad hoc extension of this model to mixed culture systems was proposed in [17, 32, 48], which however, appears to overpredict mixing and to obliterate biomass gradients too quickly, a criticism also faced by some multi-species cellular automata models [71]. This might be a consequence of the pure self-diffusion character of the model. An extension of this single species model to account for quorum sensing induced detachment showed that the model is able to describe biofilms colonies in which the biomass density is greatly induced, as in the case of biofilm hollowing [22].

**Objective of this study.** Our objective is to derive a deterministic, continuous, multi-dimensional mixed culture (more specific: binary) biofilm model, without the defining assumption of the Wanner-Gujer model that the biomass volume fractions must add to unity, and to show that such a model can address the criticism of Wanner-Gujer like models expressed in [36]. In particular we show that the model that we derive does not enforce segregation of species if colonies of different type merge, and if the local growth conditions permit coexistence. We will present two very different derivations in Sections 2.2.2 and 2.2.3 that ultimately lead to the same model. Like all other biofilm models, both will start from the principle of conservation of mass.

The first derivation, in Section 2.2.2, starts from a spatially discrete mas-
ter equation, in which spatial interaction is described by microscopic behavioral rules that describe the movement of biomass between sites on a lattice. This ansatz is related to cellular automaton biofilms models, but instead of explicitly defining discrete stochastic rules for spatial movement, we describe transfer rates between lattice cells as continuous functions that depend on the local biomass densities. A partial differential equation model is then obtained by refining the spatial discretization and passing to the continuous limit. Partial differential equations models of this type are frequently derived in theoretical ecology, and to describe cell movement such as chemotaxis, e.g. [4, 41, 51, 52, 53, 56, 67, 74, 76]. We tailor the approach here for biofilms. The key feature is that model closure must account for the fact that movement of biomass between grid cells can be hampered by the presence of the same or other species in neighboring sites.

The second ansatz, in Section 2.2.3, starts with the same convective transport equations for biomass that are the origin of the 1D Wanner-Gujer model [79] and its multi-dimensional extensions [2, 46], but we give up the constraint that the volume fractions occupied by biomass must add up to unity. This leads to an underdetermined system. This assumption is replaced by an algebraic model closure that ties local biomass densities and the forces that induce spatial expansion.

Both approaches lead to the same cross-diffusion model. Cross-diffusion is a phenomenon that occurs naturally in diffusive multi-species systems where the diffusive flux of one species depends also on the density of the other
species [64]. In the single-species case the cross-diffusion biofilm model that we derive here reduces to a density-dependent diffusion-reaction model like [18].

Subsequently in Section 2.3, we will apply the so derived quasilinear cross-diffusion-reaction modeling framework to three biofilm systems, describing different types of interactions between the species involved. We will assume in all three cases that spatial behavior of the different biomass fractions is determined by the same effects, and leave algebraically more involved types of interaction for a future study. The simplest example, in Section 2.3.1 is competition for a shared substrate. In this case the growth rates for both species depend on the concentration of a common nutrient. In Section 2.3.2 we will extend the competition model to a biofilm allelopathy model. Here, when the shared resource becomes limited, one species produces a second substrate that is inhibitory for the other species. Consequently, under nutrient limitations the environment is much more habitable for one species than for the other, in which case we expect slower mixing effects. The third biofilm system that we will apply the new modeling framework to in Section 2.3.3 is a mixed culture system of an aerobic biofilm former and a facultative anaerobic species that can grow both under aerobic and anaerobic conditions. These three models will be studied in numerical simulations. To assess the role and importance of cross-diffusion effects the simulation results are compared against simulations of a related earlier model that neglects cross-diffusion.

Limitation of the scope of the study vis-a-vis other biofilm mod-
els in the literature. For the sake of completeness we mention that in recent years several other, more or less related, multi-dimensional deterministic growth models have been added to the biofilm modeling literature, typically formulated as fluid models, for example [3, 8, 11, 7, 14, 27, 35, 44, 63, 75, 80, 84, 85]. These focus on biofilms of a single bacterial species and are not directly applicable to our investigation. Some of these and other models also include mechanical effects such as biofilm deformation due to external shear forces. This is primarily relevant for systems with a dominant flow in the aqueous environment (which is not the situation that we investigate) and happens often at time scales much faster than bacterial growth (which is the time scale that we are interested in). It is not clear whether, and if so which, such mechanical deformation has an effect on spatial mixed-culture interactions and biofilm performance. Therefore, we do not consider this aspect of biofilm dynamics here. Another class of biofilm models in the literature are discrete, stochastic individual based models, including several for multispecies systems [38, 54, 83, 82]. Like cellular automata models, the user has full control over the mixing behavior of species by designing local rules of interaction accordingly. These models can become computationally quite expensive due to their stochastic nature that requires repeated simulations and averaging, in particular if they are combined with continuum models for the transport of dissolved substrates. The relationship between such individual models and continuum models is not well understood; therefore we consider them a parallel model development, the discussion of which is beyond the
2.2 Derivation of a dual species biofilm model

2.2.1 An underlying single-species biofilm model

We recall the single-species prototype biofilm model of [18], in which the spatio-temporal evolution of biomass is described by the quasilinear diffusion-reaction equation

\[ M_t = \nabla \left( D(M) \nabla M \right) + KM, \quad t > 0, \quad x \in \Omega \subset \mathbb{R}^d, \quad d \in \{1, 2, 3\}. \quad (2.1) \]

The dependent variable biomass density \( M \) is normalized with respect to the maximum attainable cell density, i.e. can be understood as the local volume fraction occupied by the biofilm. As in most biofilm models [2, 46, 50, 55, 78, 79] the EPS is subsumed in the active biomass density, essentially assuming that the ratio of EPS to bacterial biomass is approximately constant throughout. Other assumptions can be made in applications that require a separate treatment of EPS [25]. In (2.1), \( K \) denotes the bacterial growth rate, which typically will depend on one or more growth controlling substances, such as nutrients.

The density dependent diffusion coefficient \( D(M) \) has the form

\[ D(M) = \delta \frac{M^a}{(1 - M)^b}, \quad a, b > 1, \delta > 0. \quad (2.2) \]
Hence, (2.1) degenerates for $M = 0$. This effect, as in the porous medium equation, leads to a finite speed of interface propagation. For $M = 1$ the diffusion coefficient attains a singularity. This super-diffusion singularity assures that indeed the solutions of (2.1) are bounded by the maximum cell density, i.e. that $M \leq 1$. It was shown in [20, 21] that indeed the solutions remain separated from unity for all $t > 0$ if a homogeneous Dirichlet condition is specified somewhere on the boundary of the domain, while homogeneous Neumann conditions everywhere can lead to $M = 1$ almost everywhere in finite time. It has been shown in numerous numerical simulations, that regions with $M \approx 0$ and $M \approx 1$ can be found in very close proximity [16, 18]. Near the biofilm/water interface very sharp biomass gradients with blow-up can occur, which have been investigated, e.g. in [30].

The actual biofilm is the region $\Omega_2(t) = \{x \in \Omega : M(t, x) > 0\} \subset \Omega$. The aqueous phase is then $\Omega_1(t) = \{x \in \Omega : M(t, x) = 0\} = \Omega \setminus \Omega_2(t)$. Both regions are separated by the biofilm/water interface $\Gamma(t) = \partial \Omega_2(t) \setminus \partial \Omega$, see Figure 2.1. Note that neither $\Omega_1$ nor $\Omega_2$ must be connected. In fact, for most biofilm systems $\Omega_2(t)$ will consist of several subdomains, each of which represents a biofilm colony. The substratum is part of the boundary of $\Omega$. Normally every biofilm colony will share some part of its boundary with the substratum. Biomass growth and spatial expansion can lead to merging of neighboring colonies into a new one. The biofilm interface $\Gamma(t)$ is implicitly defined as a property of the weak solutions of (2.1); no additional equations must be provided to determine it.
Figure 2.1: Schematic of the computational domain: The aqueous phase $\Omega_1(t)$ is the region where $M = 0$ (white), the actual biofilm phase $\Omega_2(t)$ is the region where $M > 0$ (shaded area). The substratum (black), i.e. the surface on which the biofilm grows is part of the boundary of $\Omega$. In the simulations lateron we will consider the two-dimensional case with a rectangular domain, i.e. $x = (x_1, x_2) \in \Omega := [0, L] \times [0, H]$.

In [33], it was shown how this single species biofilm model can be derived as the macroscopic limit of a microscopic continuous in time, discrete in space master equation, in which the movement of biomass from one site into a neighboring site is governed by microscopic behavioral rules that account for the biomass already present in both sites. This modeling approach follows a strategy often employed in spatially structured populations or directed cell movement like chemotaxis [4, 41, 51, 53, 67, 74, 76]. In the biofilm context, this is essentially a volume filling problem, since the amount of biomass that can be locally accommodated is limited.

On the other hand, in [26], the single species biofilm model was derived starting from the same continuous mass and momentum balance as [13],
a multi-dimensional extension of the single-species variant Wanner-Gujer model. This leads to an underdetermined problem which was closed by assuming an algebraic relationship between the inherent biomass pressure that is responsible for spatial expansion and the biomass density, an approach related to the one suggested earlier by [81]. More specifically power law nonlinearities are assumed to account for the fact that spatial expansion is negligible for low biomass densities and that the biomass pressure gradient that enforces spatial extension is strong enough to guarantee that the a priori known maximum biomass density is not exceeded. Again, this is essentially a volume filling problem.

In the two subsequent Sections 2.2.2 and 2.2.3 we will extend these two model derivations to dual species biofilm systems, i.e. microbial communities that are described by the local volume fraction $X$ occupied by one species and $Y$ occupied by a second species.

2.2.2 Derivation of a dual-species model using a spatially discrete lattice differential equation for structured populations

Our first derivation will be based on a continuous in time, discrete in space master equation that is frequently used in theoretical ecology and other areas of mathematical biology for single species communities, to derive population level macroscopic partial differential equation models from microscopic be-
behavioral rules [4, 41, 51, 53, 56, 67, 74, 76]. The model domain is discretized by a uniform lattice. The populations are described in terms of the population density in the lattice sites. In the biofilm modeling context, this ansatz resembles closest the cellular automaton models like [6, 50, 55]. Movement of biomass between lattice sites is described by local microscopic behavioral rules. Instead of discrete rules as in cellular automata models, we express them in terms of continuous functions that depend on local biomass densities. A macroscopic PDE model is then obtained by refining the spatial discretization and passing to the macroscopic, continuous limit. For general dual-species systems with spatially interacting populations, this was worked out in [52]. Here we tailor this approach to the biofilm context. A simpler dual species model following the same master equation approach was previously derived in [57], where it was implicitly assumed that movement of individuals is not restricted by the presence of others, and that interaction between species takes place in form of reactions only, in which case one obtains a semi-linear model with Fickian diffusion. In our study we account for the effect that the presence of other cells has on the movement of individuals. In a biofilm, where cells are often packed tightly, this is an important aspect.

For simplicity of the derivation and notation, we explain this approach in a one-dimensional setting. The 2D or 3D case follows with the same arguments but more elaborate algebraic manipulations in a straightforward manner. In the 1D case, the lattice consists of a chain of ordered grid cells. The $i$th grid cell has the two neighbors $i \pm 1$. We denote by $X_i$ and $Y_i$ the population
densities of the two species in the \( i \)th site on the lattice. The transfer rate of biomass from the \( i \)th grid cell into its neighbor cells for the species \( X \) and \( Y \) are denoted by \( \tau_i^\pm \) and \( \eta_i^\pm \). In the biofilm context, we must account for the fact that a site's capacity to accommodate bacteria is limited. We, therefore, can normalize the density with respect to the maximum density, i.e. we interpret it as the volume fraction of site \( i \) occupied by the population. Thus, \( 0 \leq X_i, 0 \leq Y_i \) and \( X_i + Y_i \leq 1 \) are mandatory.

The spatially discrete, continuous in time master equation describes the population change of a particular species in a particular site, by balancing the amount of biomass which leaves the site to move into neighboring locations, and the amount arriving from neighboring sites. For two species it reads

\[
\begin{align*}
\frac{dX_i}{dt} &= \tau_{i-1}^+ X_{i-1} + \tau_{i+1}^- X_{i+1} - (\tau_i^+ + \tau_i^-)X_i + K_i X_i \\
\frac{dY_i}{dt} &= \eta_{i-1}^+ Y_{i-1} + \eta_{i+1}^- Y_{i+1} - (\eta_i^+ + \eta_i^-)Y_i + \tilde{K}_i Y_i
\end{align*}
\tag{2.3}
\]

where \( K_i \) and \( \tilde{K}_i \) are the net biomass production rates of both species in grid cell \( i \). The transfer rates \( \tau_i^\pm, \eta_i^\pm \) depend on the biomass of either type in the current site \( i \) (which we will also call the departure site) and in the target site \( i \pm 1 \) (which we will also call the arrival sites). The transfer rates are compounded from the incentive of bacteria to leave the departure site and the incentive to move into the arrival site. In a biofilm system, if locally the biomass density is small then there is no incentive to move into a neighboring site because new biomass can be accommodated locally. If
the departure site is crowded then the incentive to leave is increased. On the other hand, if the target site is crowded, the incentive to move there is small, whereas it is biggest when the target site is empty. This suggests a multiplicative formulation

\[
\begin{align*}
\tau_i^\pm &= \alpha q_1(X_i, Y_i) p_1(X_{i\pm1}, Y_{i\pm1}), \\
\eta_i^\pm &= \beta q_2(X_i, Y_i) p_2(X_{i\pm1}, Y_{i\pm1}),
\end{align*}
\]

(2.4)

where the non-negative functions \( q_1, p_1 \) control the local movements of species \( X \) from one site on the lattice to a neighboring site and are taken to be continuous. Similarly \( q_2, p_2 \) control the local movements of species \( Y \) between sites. The functions \( q_{1,2}(X_i, Y_i) \) are measures of the incentive for species \( X \) and \( Y \) to leave lattice site \( i \). The functions \( p_{1,2}(X_i, Y_i) \) represent how favorable the lattice site \( i \) is for incoming individuals of species \( X \) and \( Y \).

If a neighbor cell, say \( i + 1 \), is not able to accommodate more biomass, then \( p_{1,2}(X_{i+1}, Y_{i+1}) = 0 \) and, hence, \( \tau_i^+ = 0, \eta_i^+ = 0 \). The functions \( p_{1,2} \) are monotonically decreasing, whereas the functions \( q_{1,2} \) are monotonically increasing.

In biofilm systems, volume filling plays an important role. An underlying principle of virtually all 2D/3D biofilm models that are based on the interpretation of biofilms as spatially structured populations, regardless whether they are cellular automata, agent based, or PDE models, is that biofilms do not expand notably if locally new biomass can be incorporated, but start spreading when the space locally available becomes limited, in order to guar-
antee that the local biomass density is limited by the maximum cell density. To account for this, we assume for simplicity in this first study that the functions $p_{1,2}$ and $q_{1,2}$ depend on the overall cell density only, namely $X + Y$. Thus, we have

$$p_{1,2}(X, Y) = p_{1,2}(X + Y), \quad q_{1,2}(X, Y) = q_{1,2}(X + Y). \quad (2.5)$$

This assumption can be easily relaxed in subsequent investigations, and it can be considered for example that the movement of bacteria of one species depends differently on both species, but this will increase algebraic complexity considerably.

In order to make the transition from a spatially discrete to a continuous model, we first introduce two continuous functions $X(t, x)$ and $Y(t, x)$ that interpolate the grid functions, $X(t, x_i) = X_i(t), Y(t, x_i) = Y_i(t)$. We formally expand these two functions about $x_i$ in terms of the variable $h$, which is the distance between two neighboring sites. We also approximate $q_{1,2}(X_{i\pm 1} + Y_{i\pm 1})$ and $p_{1,2}(X_{i\pm 1} + Y_{i\pm 1})$ by their Taylor polynomials about $X_i + Y_i$. Then substituting $X_i(t), Y_i(t), q_{1,2}(X_{i\pm 1} + Y_{i\pm 1})$ and $p_{1,2}(X_{i\pm 1} + Y_{i\pm 1})$, dropping $O(h^3)$ terms, passing to the continuous limit, $h \to 0$, and rearranging the order of terms we obtain (see [52] for calculations in more detail)

$$\begin{align*}
\frac{\partial X}{\partial t} &= \alpha_0 \frac{\partial}{\partial x} \left( p_1 q_1 \frac{\partial X}{\partial x} + X (p_1 q_1' - q_1 p_1') \frac{\partial X}{\partial x} + X (p_1 q_1' - q_1 p_1') \frac{\partial Y}{\partial x} \right) + KX, \\
\frac{\partial Y}{\partial t} &= \beta_0 \frac{\partial}{\partial x} \left( p_2 q_2 \frac{\partial Y}{\partial x} + Y (p_2 q_2' - q_2 p_2') \frac{\partial X}{\partial x} + Y (p_2 q_2' - q_2 p_2') \frac{\partial Y}{\partial x} \right) + KY
\end{align*}$$

(2.6)
Here the functions $p_{1,2}, q_{1,2}$ are evaluated in $X + Y$, and $p'_{1,2}, q'_{1,2}$ are their derivatives. The factors $\alpha$ and $\beta$ measure how fast biomass moves between neighbouring sites. They depend on the size of the lattice cells $h$ and, in the usual manner, scale such that \( \lim_{h \to 0} \alpha h^2 = \alpha_0 > 0 \) and \( \lim_{h \to 0} \beta h^2 = \beta_0 > 0 \), where $\alpha_0$ and $\beta_0$ are constants [53, 56, 67]. The functions $p_{1,2}, q_{1,2}$ and their derivatives can be assumed to be given. Therefore, equations (2.6) are cross-diffusion equations.

The same procedure for two- or three-dimensional lattices, leads to the cross-diffusion system

\[
\begin{aligned}
X_t &= \alpha_0 \nabla (D_{11}(X, Y) \nabla X + D_{12}(X, Y) \nabla Y) + KX \\
Y_t &= \beta_0 \nabla (D_{21}(X, Y) \nabla X + D_{22}(X, Y) \nabla Y) + \tilde{K}Y 
\end{aligned}
\]  

(2.7)

where

\[
\begin{aligned}
D_{11}(X, Y) &= p_1 q_1 + X (p_1 q'_1 - q_1 p'_1), & D_{12}(X, Y) &= X (p_1 q'_1 - q_1 p'_1), \\
D_{21}(X, Y) &= Y (p_2 q'_2 - q_2 p'_2), & D_{22}(X, Y) &= p_2 q_2 + Y (p_2 q'_2 - q_2 p'_2).
\end{aligned}
\]

A further simplification can be made, if it can be assumed that the spatial spreading of both species is the same. Then we have $p := p_1 = p_2$, $q := q_1 = q_2$, and we obtain for the diffusion coefficients the simpler

\[
\begin{aligned}
D_{11}(X, Y) &= pq + X (pq' - qp'), & D_{12}(X, Y) &= X (pq' - qp'), \\
D_{21}(X, Y) &= Y (pq' - qp'), & D_{22}(X, Y) &= pq + Y (pq' - qp').
\end{aligned}
\]  

(2.8)
Also this assumption can be relaxed in further investigations to extend to systems where both species show different spatial behavior. Note that $p, q$ depend on the total biomass density $X + Y$ only, but the $D_{ij}$ explicitly depend on $X$ and $Y$. To close the model, the functions $p, q$, or equivalently $D_{ij}$, still need to be chosen. This is postponed to Section 2.2.4.

2.2.3 Derivation from continuous mass balances

An alternative ansatz to derive a mixed-culture model is to start from the same continuous mass balances that underly the 1D Wanner-Gujer model [79] and its multi-dimensional extensions [2, 46]. If $X$ and $Y$ denote the local biomass volume fractions, the mass balances read

$$
\begin{align*}
X_t + \nabla(uX) &= G_1 \\
Y_t + \nabla(uY) &= G_2
\end{align*}
$$

(2.9)

where $G_{1,2}$ are the net biomass production rates for both species, which may depend on $X$ and $Y$ and on further quantities, such as nutrient concentrations. Note that the volume fractions $X, Y$ are equivalent to the biomass densities $X \cdot X_\infty, Y \cdot Y_\infty$, where the constants $X_\infty, Y_\infty$ are the maximum biomass densities which may occur in $G_1, G_2$ as parameters. The a priori unknown vector function $u$ is the velocity with which the biomass fractions move in the biofilm, caused, e.g., by growth and spatial expansion of the biofilm. As in [2, 46, 79] we assume here that both species move with the
same biomass velocity. Equations (2.9) are two equations for the three unknown functions $X, Y, u$. Additional assumptions must be introduced to close this model.

In the one-dimensional Wanner-Gujer model [79] and its 2D/3D extensions [2, 46], closure is achieved by constraining the volume fractions to unity, i.e. by introducing the assumption $X + Y \equiv 1$, i.e. it is assumed that inside the biofilm structure all volume is filled by biomass. Then, adding both equations in (2.9) one obtains immediately

$$\nabla u = G_1 + G_2.$$  \hspace{1cm} (2.10)

In the one dimensional case $u$ reduces to a scalar function and the $\nabla$ operator to the usual derivative. One obtains then the velocity by integration, $u(t, x) = \int_0^x (G_1 + G_2) dz$, i.e. $u$ is determined by the biomass densities and substrate concentrations. In the multi-dimensional case, $u$ is a vector and the assumption $X + Y \equiv 1$ does not suffice to determine $u$ in (2.10) and to close the model. To this end, with the same arguments as in [13] for the single species case, [2] assume that $u$ is governed essentially by a Darcy equation, $u = -\lambda \nabla P$, where $P$ is the biomass pressure. The assumption of a Darcy equation as a phenomenological model will in fact automatically follow from a biomass momentum equation if we assume an additional linear viscous loss term in the momentum equation which accounts for the resistance to movement of cells due to the close packing of cells within the biofilm. Equivalently,
[46] assume that $u$ is governed by potential flow. The pressure $P$ can then be computed from (2.10) as solution of the elliptic problem $-\Delta P = G_1 + G_2$ which closes the model for [2, 46].

As discussed in the introduction above, the $a$ priori assumption $X + Y \equiv 1$, which is central to the 1D Wanner-Gujer model [79] and its multidimensional extensions [2, 46], may be too restrictive in some applications. We present here an alternate model closure without this assumption. The momentum balance is

\[
\begin{align*}
(uX)_t + \nabla(uuX) &= -\nabla P_1 - f_{XX} X^2 u - f_{XY} XY u \\
(uY)_t + \nabla(uuY) &= -\nabla P_2 - f_{YY} Y^2 u
\end{align*}
\]

(2.11)

where $P_1$ and $P_2$ denote the pressure acting on $X$ and $Y$, respectively. The terms with $f_{ij}$, $i, j \in \{X, Y\}$ describe the momentum sink for species $i$ due to friction with species $j$. It is assumed here that these loss terms are proportional to both interacting species and the magnitude of the velocity $u$. With the same arguments as in [2, 13, 46], we neglect the inertial terms in (2.11), which then reduces (2.11) to the Darcy like equations for each species

\[
\begin{align*}
0 &= -\nabla P_1 - f_{XX} X^2 u - f_{XY} XY u, \\
0 &= -\nabla P_2 - f_{YY} Y^2 u.
\end{align*}
\]

(2.12)

This is a linear system for the moments $uX, uY$. To close our model we follow an approach similar to [81], and make an algebraic closure ansatz.
To this end, we assume that the pressure $P_{1,2}$ that drives the movement of bacteria builds up as locally biomass accumulates and we assume for the total pressure $P$ an algebraic relationship $P = P(X + Y)$. For the dual species system, we assume that this total pressure is distributed between both species proportionally, relative to their current density,

$$P_1(X, Y) = \frac{X}{X + Y} P(X + Y), \quad P_2(X, Y) = \frac{Y}{X + Y} P(X + Y). \quad (2.13)$$

The gradients of the pressure functions $P_{1,2}$ in (2.12) can then be expressed in terms of $P$ and its derivative $P'$ as

$$\nabla P_1 = \frac{\partial P_1}{\partial X} \nabla X + \frac{\partial P_1}{\partial Y} \nabla Y, \quad \nabla P_2 = \frac{\partial P_2}{\partial X} \nabla X + \frac{\partial P_2}{\partial Y} \nabla Y,$$

with

$$\begin{cases} \frac{\partial P_1}{\partial X} = \frac{P(X+Y)}{X+Y} + X \left( \frac{P'(X+Y)}{X+Y} - \frac{P(X+Y)}{(X+Y)^2} \right), \\
\frac{\partial P_1}{\partial Y} = X \left( \frac{P'(X+Y)}{X+Y} - \frac{P(X+Y)}{(X+Y)^2} \right), \\
\frac{\partial P_2}{\partial X} = Y \left( \frac{P'(X+Y)}{X+Y} - \frac{P(X+Y)}{(X+Y)^2} \right), \\
\frac{\partial P_2}{\partial Y} = \frac{P(X+Y)}{X+Y} + Y \left( \frac{P'(X+Y)}{X+Y} - \frac{P(X+Y)}{(X+Y)^2} \right). \end{cases} \quad (2.14)$$

Solving (2.12) for $uX, uY$ and substituting these expressions into the mass balance (2.9) gives a cross-diffusion system for $X, Y$ with self- and cross-diffusion coefficients depending on $P$ and $P'$.

A simpler model is obtained, if, as in the previous section, we consider again the special case where the spatial behavior of both biomass fractions is the same, i.e. we consider the special case $f_{XX} = f_{XY} = f_{YX} = f_{YY} =: f$. 50
Then (2.12) becomes

\[
\begin{cases}
0 &= -\nabla P_1 - f(X + Y)uX, \\
0 &= -\nabla P_2 - f(X + Y)uY.
\end{cases}
\] (2.15)

and we have

\[
\begin{cases}
uX &= -\frac{1}{f(X+Y)} \left( \frac{\partial P_1}{\partial x} \nabla X + \frac{\partial P_1}{\partial y} \nabla Y \right), \\
uY &= -\frac{1}{f(X+Y)} \left( \frac{\partial P_2}{\partial x} \nabla X + \frac{\partial P_1}{\partial y} \nabla Y \right). 
\end{cases}
\] (2.16)

Substituting (2.16) into (2.9), we can rewrite the mass balances in the form

\[
\begin{cases}
X_t &= \nabla(D_{11}(X,Y)\nabla X + D_{12}(X,Y)\nabla Y) + G_1(X,Y), \\
Y_t &= \nabla(D_{21}(X,Y)\nabla X + D_{22}(X,Y)\nabla Y) + G_2(X,Y),
\end{cases}
\] (2.17)

where

\[
\begin{align*}
D_{11}(X,Y) &= \frac{1}{f(X+Y)} \frac{\partial P_1}{\partial x}, & D_{12}(X,Y) &= \frac{1}{f(X+Y)} \frac{\partial P_1}{\partial y}, \\
D_{12}(X,Y) &= \frac{1}{f(X+Y)} \frac{\partial P_2}{\partial x}, & D_{22}(X,Y) &= \frac{1}{f(X+Y)} \frac{\partial P_2}{\partial y}.
\end{align*}
\]

Considering the special form of the pressure derivatives in (2.14), we note that (2.17), which has been derived from a continuous mass and momentum balance, has the same form as (2.7), which has been derived from a lattice differential equation with local behavioral rules. In both cases we can find
functions $\Phi(X + Y)$ and $\Psi(X + Y)$ such that

$$
\begin{align*}
D_{11}(X, Y) &= \Phi(X + Y) + X\Psi(X + Y), \\
D_{12}(X, Y) &= X\Psi(X + Y), \\
D_{12}(X, Y) &= Y\Psi(X + Y), \\
D_{22}(X, Y) &= \Phi(X + Y) + Y\Psi(X + Y).
\end{align*}
$$

(2.18)

In the case of (2.7) the functions $\Phi$ and $\Psi$ depend on $p(X + Y), q(X + Y)$ and their derivatives, and in case (2.17) on $P(X + Y)$ and $P'(X + Y)$.

In summary, it is important to remark that the cross-diffusion terms arise naturally when we subsume the effect of the interaction between the species modeled by the viscous momentum loss terms in the species transport equation (2.12) into the diffusion operator for biomass movement.

### 2.2.4 Choice of cross-diffusion coefficients for biofilms

It remains to determine the self- and cross-diffusion coefficients $D_{ij}(X, Y)$, $i, j \in \{1, 2\}$, or alternatively the function $\Phi$ and $\Psi$ in (2.18). We use the results from the single-species model in Section 2.2.1. In [33], the diffusion coefficient $D(M)$ for the single species model (2.1) was obtained in the form

$$
D(M) = p(M)q(M) + M\left(p(M)q'(M) - q(M)p(M)\right),
$$

(2.19)

where functions $p(M), q(M)$ play the same role as in Section 2.2.2, i.e. they describe the incentive of biomass to leave the departure site and move into
the arrival site. Introducing

\[ \Phi(M) := p(M)q(M), \quad \Psi(M) := p(M)q'(M) - q(M)p(M), \]

equation (2.19) is rewritten as

\[ D(M) = \Phi(M) + M\Psi(M). \quad (2.20) \]

Note the similarity of this equation with the self-diffusion coefficient \( D_{11}(X, Y) \), \( D_{22}(X, Y) \) in (2.8) and (2.14). After additional calculations, [33] established the further relationship between \( D(M) \) and \( \Phi(M) \)

\[ \Phi(M) = \left( 1 - \int_0^M D(m)dm \right) \frac{\int_0^M D(m)dm}{M}. \quad (2.21) \]

For general exponents \( a \) and \( b \) the integral takes the form

\[ \int D(m)dm = \frac{M^{a+1}}{a+1} 2F_1(a+1, b; a+2, M), \]

where \( 2F_1 \) is the hypergeometric function. For specific integer choices of exponents \( a, b \) these integrals can be expressed in terms of elementary functions [66]. The expression for \( \Psi(M) \) follows then from (2.20) and (2.21).

We use these results to derive \( D_{ij}(X, Y) \). In particular, we use the assumptions that a dual-species biofilm model for two species with volume
fractions $X$ and $Y$ must satisfy two simple properties: (i) If one species is absent in a colony, then the cross-diffusion model becomes the single species biofilm model (2.19). (ii) If both species are identical then the total biomass $M := X + Y$ should behave like the solution of the single species model (2.19).

It is easily verified that these two properties are satisfied for the choices

$$
\begin{align*}
D_{11}(X, Y) &= \Phi(X + Y) + X\Psi(X + Y), \quad D_{12}(X, Y) = X\Psi(X + Y), \\
D_{21}(X, Y) &= Y\Psi(X + Y), \quad D_{22}(X, Y) = \Phi(X + Y) + Y\Psi(X + Y),
\end{align*}
$$

where $\Phi$ and $\Psi$ are defined by (2.20) and (2.21) as in the single species case.

The relationship between $\Phi$ and $\Psi$ with the incentive function $p, q$ is then given by (2.8), the relationship with the biomass pressure function $P$ by (2.14).

### 2.2.5 Qualitative comparison with an earlier diffusion-reaction multi-species biofilm model without cross-diffusion effects

The cross-diffusion model that we just derived in two different ways is different from the earlier multi-species diffusion-reaction biofilm model without cross-diffusion that was used in [32, 48], but is also based on the single species
In our current notation the earlier model would be written as

\[
\begin{align*}
X_t & = \nabla (D(X + Y)\nabla X) + G_1(X, Y), \\
Y_t & = \nabla (D(X + Y)\nabla Y) + G_2(X, Y),
\end{align*}
\] (2.23)

with \(D(M)\) as in (2.2).

To gain an intuitive understanding of the qualitative effect of the cross-diffusion terms we consider, in a one dimensional setting, the case of two colonies, one of species \(X\) and one of species \(Y\), that move toward each other and eventually merge. The cross diffusion model with \(\Phi = pq, \Psi = pq' - qp'\), reads

\[
\begin{align*}
X_t & = \frac{\partial}{\partial x} \left( (pq + X(pq' - qp')) \frac{\partial X}{\partial x} + X (pq' - qp') \frac{\partial Y}{\partial x} \right) + G_1, \\
Y_t & = \frac{\partial}{\partial x} \left( Y (pq' - qp') \frac{\partial X}{\partial x} + (pq + Y(pq' - qp')) \frac{\partial Y}{\partial x} \right) + G_2,
\end{align*}
\] (2.24)

whereas the corresponding dual species model without cross-diffusion reads

\[
\begin{align*}
X_t & = \frac{\partial}{\partial x} \left( (pq + (X + Y)(pq' - qp')) \frac{\partial X}{\partial x} \right) + G_1, \\
Y_t & = \frac{\partial}{\partial x} \left( (pq + (X + Y)(pq' - qp')) \frac{\partial Y}{\partial x} \right) + G_2.
\end{align*}
\] (2.25)

The fluxes for \(Y\) are then in the case with cross-diffusion

\[
J^{CD}_Y = Y \left( p(X + Y)q'(X + Y) - q(X + Y)p'(X + Y) \right) \frac{\partial X}{\partial x} \\
+ \left( p(X + Y)q(X + Y) + Y(p(X + Y)q'(X + Y)) \right) \frac{\partial Y}{\partial x} \\
- \left( q(X + Y)p'(X + Y) \right) \frac{\partial Y}{\partial x}
\] (2.26)
and in the case without cross-diffusion

\[
J_{Y}^{\text{NoCD}} = (p(X + Y)q(X + Y) + (X + Y)(p(X + Y)q'(X + Y))) \frac{\partial Y}{\partial x}
- ((X + Y)q(X + Y)p'(X + Y)) \frac{\partial Y}{\partial x}.
\] (2.27)

Recalling that the functions \( p \) and \( q \) are monotonic with \( q(0) = 0 \), \( q'(M) \geq 0 \), \( p(1) = 0 \), \( p'(M) \leq 0 \), we find for \( X + Y > 0 \)

\[
p(X+Y)q(X+Y) \geq 0, \quad p(X+Y)q'(X+Y) - q(X+Y)p'(X+Y) \geq 0. \quad (2.28)
\]

Before the two colonies merge both are described by a single species model, i.e. the cross diffusion terms are not active. Assume that the interface of the colony of species \( X \) is moving in positive \( x \)-direction, while the one of the \( Y \) colony moves in negative \( x \)-direction. When they start merging we have that \( \partial X/\partial x < 0 \) and \( \partial Y/\partial x > 0 \). Using this and that \( X \geq 0, Y \geq 0 \) we find \( J_{Y}^{\text{NoCD}} \geq J_{Y}^{\text{CD}} \geq 0 \). Hence, neglecting cross-diffusion overestimates mixing, or, equivalently, cross-diffusion delays mixing.

\subsection{2.2.6 Numerical method}

The cross-diffusion biofilm model shows three nonlinear diffusion effects: (i) a porous medium degeneracy for \( X + Y = 0 \), (ii) a super-diffusion singularity for \( X + Y = 1 \), and (iii) cross-diffusion. Each of these effects comes with its own numerical difficulties. Degeneracy (i) induces a finite speed of interface
propagation with biomass gradient blowup at interface. Common problems of numerical methods for this type of problems include superficial oscillations at the interface in the numerical solution, or artificial smearing of the sharp interface. The super-diffusion singularity (ii) induces high stiffness and arithmetic difficulties for nonlinear solvers close to the singularity. Cross-diffusion (iii) leads to the loss of a maximum principle for the underlying continuous model, which is reflected in the numerical realization by difficulties to maintain the positivity of solutions. A semi-implicit numerical method has been developed in [16, 47, 48] in the context of single-species biofilm models and the dual-species model without cross-diffusion, including (i), (ii). It was extended in [59] to include cross-diffusion terms, by formally writing them as convective transport terms which allows one to design a positivity preserving method. The method was implemented in Fortran, and prepared for parallel execution on shared memory and multi-core architectures with OpenMP. In [59] a grid refinement study has been carried out for an application of the model to antibiotic treatment of biofilms, which demonstrated the suitability of the method for this type of cross-diffusion problem. This is the method that we use in the subsequent sections.
2.3 Biofilm models and Applications

2.3.1 Competition for a shared resource

Governing equations and simulation setup

A basic interaction between two bacterial species is the competition for a shared growth limiting substrate, for example a carbon source. This requires to include the substrate, \( C \), as an additional dependent variable. The substrate is assumed to be dissolved and to diffuse both, in the aqueous phase and in the biofilm. It is consumed by the bacteria. Assuming Monod kinetics for substrate uptake and growth, the dual-species biofilm competition model reads

\[
\begin{align*}
X_t &= \nabla(D_{11}(X,Y)\nabla X + D_{12}(X,Y)\nabla Y) + \mu_1 \frac{C}{\kappa_1+C} X - k_1 X, \\
Y_t &= \nabla(D_{21}(X,Y)\nabla X + D_{22}(X,Y)\nabla Y) + \mu_2 \frac{C}{\kappa_2+C} Y - k_2 Y, \\
C_t &= \nabla(D_C(X+Y)\nabla C) - \frac{\mu_1 X}{Y_1} \frac{C}{\kappa_1+C} X - \frac{\mu_2 Y}{Y_2} \frac{C}{\kappa_2+C} Y.
\end{align*}
\] (2.29)

Here, \( X \) and \( Y \) are the volume fractions occupied by biomass of the two species. The biomass densities are then \( X \cdot X^\infty \) and \( Y \cdot Y^\infty \), where the parameters \( X^\infty \) and \( Y^\infty \) are the maximum cell densities. As is common in biofilm modeling, all masses are expressed in terms of chemical oxygen demand, i.e. in gCOD, and EPS is implicitly subsumed in the bacterial biomass.

The self- and cross-diffusion coefficients \( D_{ij}, i, j \in \{1, 2\} \) here and in the
subsequent sections are as introduced above. We derive $\Phi$ and $\Psi$ from (2.2) with exponents $a = b = 4$ and diffusion constant $\delta = 10^{-12}m^2/d$. These values have been used in the single-species degenerate diffusion-reaction biofilm model in [18, 19, 65]. The diffusion coefficient for the dissolved substrate $D_C(M)$ depends on the overall local biomass density $M := X + Y$. Diffusion coefficients of dissolved substrates can be substantially smaller in the biofilm colony than in the surrounding aqueous phase [5, 68]. We make the linearization ansatz

$$D_C(M) = D_C(0) + M (D_C(1) - D_C(0))$$

where $D_C(0)$ is the experimentally measurable diffusion coefficient of the substrate in the aqueous phase and $D_C(1)$ in a fully developed biofilm. The ratio $\delta_C := D_C(1)/D_C(0)$ depends typically on the size of the molecules. In [5], it is reported that for small molecules, like oxygen $\delta_C \approx 1$, while for larger molecules such as antibiotics one can have $\delta_C \approx 0.5$. In any case we have $0 < D_C(1) \leq D_C(M) \leq D_C(0) < \infty$. Thus the diffusion coefficient is bounded from below and above by known positive numbers. Hence, despite it depending on $M$, the additional substrate equation behaves essentially Fickian.

In (2.29), $\mu_1$ and $\mu_2$ are the maximum specific growth rates for species $X$ and $Y$, respectively. Similarly, $k_1$ and $k_2$ are the corresponding lysis rates, $\kappa_1$ and $\kappa_2$ the half saturation concentrations, $\Upsilon_1$ and $\Upsilon_2$ are yield coefficients.
All parameters are non-negative.

In our simulations, we restrict ourselves to the two dimensional case, \( d = 2 \) and consider a rectangular domain, \( \Omega := [0, L] \times [0, H] \subset \mathbb{R}^2 \). We use the notation \( x = (x_1, x_2) \) and denote by \( \partial_n \) the derivative on the boundary in outer normal direction. The substratum is the bottom boundary, \( x_2 = 0 \). It is impermeable to biomass, wherefore we pose the no-flux condition \( \partial_n X = \partial_n Y = 0 \) there. 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; also here we have \( \partial_n X = \partial_n Y = 0 \). At the top boundary, \( x_2 = H \), we pose homogeneous Dirichlet conditions, \( X = Y = 0 \). The porous medium degeneracy of the underlying equation leads to a finite speed of interface propagation in the sense that initial data with compact support imply solutions with compact support. As long as biomass does not reach the boundary of the domain, the model satisfies simultaneously homogeneous Dirichlet and Neumann conditions. Our simulations will be terminated before biomass reaches the top of the domain, thus the choice of boundary conditions here is not critical.

For the substrate \( C \), the boundary conditions at the bottom and lateral boundaries are also homogeneous Neumann conditions, \( \partial_n C = 0 \), with the same argumentation as for biomass fractions. On the top boundary, \( x_2 = H \), we pose an inhomogeneous Dirichlet condition with \( C = C_\infty > 0 \), which reflects that substrate is replenished through this segment of the domain boundary.
Biomass is initially placed in small pockets at the substratum only. Depending on the simulation experiments, the locations and initial sizes of these pockets can be chosen randomly, or can be 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. The amount of biomass of type \( X \) or \( Y \) initially varies between such pockets. Specifics are given below where the simulation experiments are described. For \( C \) we assume that initially the bulk concentration value is attained, \( C = C^\infty \).

The first purpose of the simulations presented in this section is to describe the solutions of the cross-diffusion biofilm model. The second objective is to investigate the differences of the solutions of the cross-diffusion biofilm model with the simpler model (2.23) that neglects mixing resistance due to cross-diffusion.

**Numerical experiments**

We consider four different cases of interaction between the two species, expressed in terms of the reaction parameters:

(i) Growth rate and half saturation concentration of species \( Y \) are bigger than that of species \( X \), \textit{i.e.} \( \mu_2 > \mu_1 \) and \( \kappa_2 > \kappa_1 \)

(ii) Both growth rates are equal and half saturation concentration of species \( Y \) is bigger than that of species \( X \), \textit{i.e.} \( \mu_2 = \mu_1 \) and \( \kappa_2 > \kappa_1 \)
Table 2.1: Model parameters that were changed in the simulations of the competition model.

<table>
<thead>
<tr>
<th>parameter</th>
<th>case (i)</th>
<th>case (ii)</th>
<th>case (iii)</th>
<th>case (iv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_2 &gt; \mu_1$</td>
<td>$\mu_2 = \mu_1$</td>
<td>$\mu_2 &gt; \mu_1$</td>
<td>$\mu_2 = \mu_1$</td>
<td></td>
</tr>
<tr>
<td>$\kappa_2 &gt; \kappa_1$</td>
<td>$\kappa_2 &gt; \kappa_1$</td>
<td>$\kappa_2 = \kappa_1$</td>
<td>$\kappa_2 = \kappa_1$</td>
<td></td>
</tr>
<tr>
<td>$\mu_1$</td>
<td>$6 \text{ d}^{-1}$</td>
<td>$6 \text{ d}^{-1}$</td>
<td>$6 \text{ d}^{-1}$</td>
<td>$6 \text{ d}^{-1}$</td>
</tr>
<tr>
<td>$\mu_2$</td>
<td>$9 \text{ d}^{-1}$</td>
<td>$6 \text{ d}^{-1}$</td>
<td>$9 \text{ d}^{-1}$</td>
<td>$6 \text{ d}^{-1}$</td>
</tr>
<tr>
<td>$\kappa_1$</td>
<td>$0.4 \text{ gm}^{-3}$</td>
<td>$0.4 \text{ gm}^{-3}$</td>
<td>$0.4 \text{ gm}^{-3}$</td>
<td>$0.4 \text{ gm}^{-3}$</td>
</tr>
<tr>
<td>$\kappa_2$</td>
<td>$0.8 \text{ gm}^{-3}$</td>
<td>$0.8 \text{ gm}^{-3}$</td>
<td>$0.4 \text{ gm}^{-3}$</td>
<td>$0.4 \text{ gm}^{-3}$</td>
</tr>
</tbody>
</table>

(iii) Both half saturation concentrations are equal and growth rate of species $Y$ is bigger than that of species $X$, i.e. $\mu_2 > \mu_1$ and $\kappa_2 = \kappa_1$

(iv) Both growth and half saturation concentration for both of the species are same, i.e. $\mu_2 = \mu_1$ and $\kappa_2 = \kappa_1$

The reaction parameters for these four cases are summarized in Table 2.1. The remaining model parameters, which are kept the same for all simulations are given in Table 2.2. Biofilm growth parameters were chosen and adapted from [78]. In case (iv), both species behave the same and $X + Y$ is a solution of the single-species prototype biofilm model of [18]. In (ii) species $X$ has a growth advantage due to a lower half saturation concentration, in (iii) species $Y$ has a growth advantage due to higher growth rate.

Besides visualising the 2D simulations we report the following lumped output parameters

$$\text{biofilm size} \quad \omega(t) = \int_{\Omega_2(t)} dx$$
Table 2.2: Fixed model parameters in the simulations of the competition model.

<table>
<thead>
<tr>
<th>parameter</th>
<th>symbol</th>
<th>value</th>
<th>unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk substrate concentration</td>
<td>$C^\infty$</td>
<td>40</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Lysis rate</td>
<td>$k_1$, $k_2$</td>
<td>0.4</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Maximum biomass density</td>
<td>$X^\infty$, $Y^\infty$</td>
<td>10000</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Yield coefficient</td>
<td>$\Upsilon_1$, $\Upsilon_2$</td>
<td>0.63</td>
<td>-</td>
</tr>
<tr>
<td>Substrate diffusion coefficient in water</td>
<td>$D_C(0)$</td>
<td>$10^{-4}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
<tr>
<td>Substrate diffusion coefficient in biofilm</td>
<td>$D_C(1)$</td>
<td>$0.8 \cdot 10^{-4}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
</tbody>
</table>

The simulations stop when the biofilm size reaches a specified maximum value.

**Merging of two biofilm colonies.** In the first simulation experiment, we place initially two semi-spherical biofilm colonies of identical size on the substratum in a square domain of size $[0, L] \times [0, H]$ with $L = H = 10^{-3}$mm, discretized by $200 \times 200$ grid cells.

In order to focus solely on cross-diffusion effects, we first keep the reaction parameters for both species the same, i.e. case (iv) above. This prevents that spatial mixing effects are overshadowed or otherwise affected by different reaction rates of both species. We compare three scenarios: (a) The colonies consist initially of different species. (b) Both colonies are initially mixed. (c) One colony consists of one species only, the other one is mixed. We simulate
Figure 2.2: Biofilm formation and interaction between two different species of biofilm with(without) cross-diffusion (CD) at different selected times. The colour coding refers to the relative local fraction of the biomass for species $X$, $R = \frac{X}{X+Y}$, the contour lines indicate the substrate concentration $C$. Time $t$ is non-dimensionalised with respect to growth rate $\mu_1$, substrate concentration $C$ is non-dimensionalised relative to the bulk concentration $C^\infty$.

The growth of the colonies and observe the biomass mixing behavior when both colonies merge.

(a) The colonies consist initially of different species. The simulations are shown in Figure 2.2 (left three columns). Initially, the left colony contains only biomass of species $X$, whereas the right colony consists only of species $Y$. First both colonies grow and expand and grow towards each other. The two neighboring colonies start merging at $t = 2.20$. The newly formed
colony continues growing as more substrate is utilized. The two populations penetrate each other and an overlapping region forms, starting at $t = 2.40$.

Both species are present in this overlapping region, with clear biomass gradient from higher densities of $X$ in the region that was originally occupied by this species into the direction of the region that was originally occupied by the other species and *vice versa*. The overlapping region is increasing gradually at $t = 2.60, 2.80, 3.00$ and the biomass density gradients that indicate mixing stretch out to become less steep. At $t = 3.80$, the outer layers of the colony, closest to the lateral boundaries appear still to be entirely occupied by the original inhabitants, but eventually, at $t = 5.72$, the entire biofilm colony is occupied by both species, albeit at different levels of mixing, with strong biomass density gradients. Since both biomass types follow the same reaction kinetics and since the initial distribution of biomass was symmetric, a symmetric pattern is maintained throughout.

The corresponding results for scenarios (b) and (c) are summarized in the Appendix A. In (b) where initially the colonies were mixed they remained so throughout the simulations. In (c) where one colony was mixed and one was single-species, we observe a biomass gradient upon merging.

**Random selection of inoculation sites on the substratum.** In laboratory experiments the inoculation sites are not easy to control. To account for this we conduct a series of simulations where the initial inoculation sites are chosen randomly. The simulation domain is $\Omega = [0, L] \times [0, H]$ with
Figure 2.3: Biofilm formation with (top row) and without (bottom row) cross-diffusion with randomly inoculated substratum by 20 colonies of each species. Shown are the results for three time steps of case (iv) with $\mu_2 = \mu_1$ and $\kappa_2 = \kappa_1$. The colour coding refers to the relative fraction of the biomass for species $X$, $R = \frac{X}{X+Y}$ and the contour for substrate, $C$.

$L = 4 \cdot 10^{-3}m$, $H = 10^{-3}m$, discretized by $800 \times 200$ grid cells. We consider two distinct scenarios, one with sparse inoculation where initially four colonies of each type are placed on the substratum, the other one with a denser biomass coverage, where initially 20 colonies of each type are placed on the substratum. At inoculation each colony consists only of a single species. Each such simulation was repeated 20 times with different randomly chosen initial data.

In Figure 2.3, we visualize one simulation of the case (iv) with reaction parameters, as defined in Table 2.1. The results for the cases (i)-(iii) are included in the Appendix A. Shown is the biofilm at three time steps. At $t = 6$ the biofilm colonies are still small. While already some merging between
colonies has taken place, individual colonies are still distinguishable. At $t = 10$, $t = 14$, full merging has taken place. The biofilm appears as a relatively homogeneous layer, in which, however, the biomass distributions in quite heterogeneous. The species mixed but clear biomass gradients are observable.

In case (iv), where no species has a growth advantage, both species are represented at the same level. The results in the Appendix A show: In case (i), where the outcome is not a priori predictable, the system is overall dominated by species $Y$ but pockets of species $X$ survive close to the boundaries, which were initially inoculated by species $X$ only. In case (ii) species $X$ has a growth advantage due to a smaller half saturation concentration, in case (iii) species $Y$ has a growth advantage due to an increased growth rate. The species with the growth advantage eventually dominates in the system but in both cases we can find small niches where the other species is locally dominant. This happens where in the initial biomass distribution a cluster of the slower growing species is found.

The simulations with initial four colonies of each simulation show qualitatively the same results but with less compact biofilms. Cross-diffusion maintains the spatial heterogeneity in the initial data throughout the simulation (data not shown).

**Comparison of the cross-diffusion model with the model without cross-diffusion.** We compare the results of the cross-diffusion model with
the results of the earlier dual-species biofilm model without cross-diffusion effects in Section 2.2.5. To this end we repeat the simulations of Sections 2.3.1, 2.3.1. The latter simulations were carried out with the numerical method detailed in [47, 48].

In Figure 2.2 we plot the simulation results of this model in the rightmost column at the same time steps at which the neighboring results of the cross-diffusion model are shown for case (a) where initially both colonies were occupied by different species. In absence of one species both models reduce to the prototype single species model of Section 2.2.1. Therefore, up to $t = 2.20$ when the two colonies begin to merge both models produce the same results. This changes after merging begins. The model without cross-diffusion leads to much faster mixing, as can be seen from the results at time $t = 2.8$ where in the cross-diffusion model a strong biomass gradient is observed and mixing is restricted to a small overlapping region, whereas in the model without cross diffusion the biomass in the merged colony is stratified. While the biomass distribution within merging colonies is different in both models, the shape of the biofilm colony is in both cases the same.

In Figure 2.3 (bottom row) the results of the model without cross-diffusion are plotted for representative simulations with a random initial inoculation of 20 colonies of each species. These results are compared with those in Figure 2.3 (top row) that show the corresponding simulations of the cross-diffusion model, however with a different randomly generated set of initial data. In the case of the non-cross-diffusion model we can see differences of
biomass between merged colonies, but in each newly formed large colony the biomass distribution is rather homogeneous. The biomass gradients observed with the cross-diffusion model are obliterated in the model without cross diffusion. This implies strongly that cross-diffusion delays mixing, or stated in other words: neglecting cross-diffusion, which occurs in the modeling in a straightforward manner, overestimates mixing.

Our simulations show that models with and without cross diffusion lead to biofilms with locally different biomass distributions, even in cases where the overall biofilm structure is the same. This raises the question whether the differences in local biomass distribution imply differences in the ecology within the biofilm, which may have implications for biofilm performance, e.g. in environmental engineering applications. To investigate this question we compare the lumped parameters biofilm size \( \omega(t) \), and total biomass of either type, \( X_{\text{total}}(t) \) and \( Y_{\text{total}}(t) \) computed with both models. These parameters have been identified in a previous study as being relatively sensitive to reaction parameters [48], and, therefore, seem good indicators to measure the effect introduced by neglecting cross-diffusion on overall population behavior. In Figure 2.4 we depict \( \omega(t), X_{\text{total}}(t), Y_{\text{total}}(t) \) for the case (i), where both species have different growth parameters \( \mu_1 < \mu_2 \) and \( \kappa_1 < \kappa_2 \). We carry the simulations out for initially four colonies of each species (bottom panel) and initially 20 colonies of each species (top panel). Each simulation is repeated 20 times. The lumped output parameters are averaged over these 20 simulations and plotted with error bars. Species \( Y \) grows faster than species
Figure 2.4: Comparison of biofilm size and total biomass of both species with (without) cross-diffusion for case (i) $\mu_2 > \mu_1$ and $\kappa_2 > \kappa_1$ with 20 colonies of either species initially (top) and 4 colonies initially (bottom). Time is non-dimensionalised with respect to growth rate $\mu_1$.

$X$ and attains higher amounts of biomass. Comparing the lumped results for the model with cross-diffusion against the results of the model without cross-diffusion, we see that the difference in spatial distribution of biomass does not translate into differences in biofilm size and total biomass fractions.

Qualitatively the same results are found for the remaining cases (i)-(iii), with different values of growth rates and half saturation concentrations; the
results can be found in the Appendix A. This strongly implies that the differences in spatial biomass distributions in the colonies do not affect overall global behavior of the biofilm. In particular it does not affect the total biomass found for both species.

Discussion

The cross-diffusion model predicts that merging biofilm colonies of different species slowly penetrate each other, leading to clear biomass gradients within the colony and overlapping regions in which both species are present, as shown also in some CLSM studies, e.g. [1, 73]. This is in contrast to Wanner-Gujer like models, such as [2, 46], which by virtue of the spatial operator always keep the two populations separated (see also [36]). In colonies, in which only one species is present, e.g in colonies that start from one species before they mix with colonies of another species, the cross-diffusion biofilm model reduces to the single-species prototype biofilm model and predicts biofilm growth accordingly. Compared to a purely diffusive model, cross-diffusion leads to slower mixing, because the density gradients of one species pose a constraint on the movement of the other. This leads to different local distributions of biomass in the models with and without cross-diffusion. For the simple competition model, however, the heterogeneity of the biomass distribution did not notably affect lumped parameters such as biofilm size or the amount of biomass of both species, which were shown to be the same for both models. In the simple competition model both species respond in
the same way to the environmental conditions, i.e. if substrate is available in abundance, then it is available in abundance for both species; if it is limited, it is limited for both species. This raises the question whether the observation that lumped parameters are not affected if cross-diffusion is neglected is also valid for more complex interaction between both species. This will be investigated for a more involved type of interaction in the subsequent section.

2.3.2 A biofilm system with allelopathy

Governing equations and simulation setup

In some microbial competition systems, one species obtains a growth advantage by producing a substrate that is detrimental to the other. Such an allelopathic interaction has been suggested in [24] as a potential mechanism behind the Listeria overgrowth, a phenomenon that can lead to false negatives in food safety studies when the pathogenic biofilm former *Listeria monocytogenes* is overgrown by non pathogenic Listeria species. To account for allelopathy, (2.29) is extended by including a differential equation for the concentration of the inhibitory substance, $P$, which is produced by species $X$ when the substrate $C$ becomes limited and it affects the growth of species $
The extended allelopathic biofilm model becomes

\[
\begin{align*}
X_t &= \nabla(D_{11}(X,Y)\nabla X + D_{12}(X,Y)\nabla Y) + \mu_1 \frac{C}{\kappa_1 + C} X - k_1 X, \\
Y_t &= \nabla(D_{21}(X,Y)\nabla X + D_{22}(X,Y)\nabla Y) + \mu_2 \frac{C}{\kappa_2 + C} Y - k_2 Y - \rho_2 P Y, \\
C_t &= \nabla(D_C(X + Y)\nabla C) - \frac{\mu_1 X^\infty}{\kappa_1 + C} X - \frac{\mu_2 Y^\infty}{\kappa_2 + C} Y, \\
P_t &= \nabla(D_P(X + Y)\nabla P) + \rho_1 \frac{\kappa_1}{\kappa_1 + C} X - k_3 P,
\end{align*}
\]

(2.31)

where the diffusion coefficient \( D_P \) is defined similar to \( D_C \) in (2.30). The parameter \( \rho_1 \) is the maximum production rate for \( P \). Standard inhibition kinetics is used to describe the dependency of \( P \) production on the availability of substrate \( C \). The parameter \( \rho_2 \) in (2.31) is the rate of inactivation of species \( Y \) per unit mass of \( P \). The parameter \( k_3 \) in (2.31) is an abiotic decay rate of \( P \).

The computational domain is \( \Omega = [0, L] \times [0, H] \) with \( L = 4 \cdot 10^{-3}m \), \( H = 10^{-3}m \), discretized by a grid of size \( 800 \times 200 \) cells. The boundary and initial conditions for \( X, Y, C \) are the same as for the pure competition model in Section 2.3.1. For the \( P \) we pose homogeneous Dirichlet conditions at the substratum and at the lateral boundaries, following the same line of argumentation that we employed for \( X, Y, C \). At the top boundary, \( x_2 = H \) homogeneous Dirichlet conditions are postulated, i.e. \( P = 0 \) there. This mimics that the product diffuses from the biofilm into the bulk phase, where its concentration is assumed to be negligible due to dilution.
Table 2.3: Model parameters used in the allelopathy model in Section 2.3.2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domain length</td>
<td>$L$</td>
<td>$4 \cdot 10^{-3}$</td>
<td>m</td>
</tr>
<tr>
<td>Domain height</td>
<td>$H$</td>
<td>$10^{-3}$</td>
<td>m</td>
</tr>
<tr>
<td>Bulk substrate concentration</td>
<td>$C^\infty$</td>
<td>30</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Maximum specific growth rates</td>
<td>$\mu_1, \mu_2$</td>
<td>6</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Half saturation concentration</td>
<td>$\kappa_1, \kappa_2$</td>
<td>4</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Lysis rate</td>
<td>$k_1, k_2$</td>
<td>0.4</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Abiotic decay rate for product</td>
<td>$k_3$</td>
<td>0</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Toxin production rate</td>
<td>$\rho_1$</td>
<td>60</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Maximum biomass density</td>
<td>$X^\infty, Y^\infty$</td>
<td>10000</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Yield coefficient</td>
<td>$\Upsilon_1, \Upsilon_2$</td>
<td>0.63</td>
<td>–</td>
</tr>
<tr>
<td>Substrate diffusion coefficient in water</td>
<td>$D_C(0)$</td>
<td>$10^{-4}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
<tr>
<td>Substrate diffusion coefficient in biofilm</td>
<td>$D_C(1)$</td>
<td>$0.8 \cdot 10^{-3}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
<tr>
<td>Toxin diffusion coefficient in water</td>
<td>$D_C(0)$</td>
<td>$7.76 \cdot 10^{-5}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
<tr>
<td>Toxin diffusion coefficient in biofilm</td>
<td>$D_C(1)$</td>
<td>$3.88 \cdot 10^{-5}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
</tbody>
</table>

**Numerical experiments**

The model parameters used in all simulations of the allelopathy biofilm model are summarized in Table 2.3. The growth parameters used are the default values of the International Water Association’s Biofilm Modeling Task Group [78] Benchmark Problem 1, the parameters of the allelopathy sub-model were adapted accordingly, based on [24]. From first exploratory simulations (data not shown) we concluded that the initial distribution of biomass, i.e. the inoculation of the substratum, plays a much bigger role than in the case of the pure competition model above. Our simulation experiments were designed with this in mind. In all simulations we initially inoculated the substratum with 5 colonies of the toxin producing species $X$ and 20 colonies of species $Y$ that is controlled by toxins. All colonies have initially equal size. We consider
three different setups: [i] The 5 colonies of species X are all located on one side of the domain, interspersed between the colonies of species Y. [ii] The 20 colonies of species Y are uniformly distributed across the substratum, amongst which the 5 colonies of species X are regularly interspersed. [iii] Random selection of inoculation sites of both species. In case [iii], where the initial data are randomly selected, we carry out 20 simulations for each, the model with and without cross-diffusion effects. We performed the simulations with the cross-diffusion model and for comparison with the model without cross-diffusion.

In Figure 2.5 we plot snapshots at $t = 4$ and $t = 20$ for the simulation of cases [i],[ii], and a randomly selected representative of case [iii]. The earlier time step is included primarily to give the reader an impression about the initial distribution of biomass. In cases [i], [ii] this is before merging of neighboring colonies starts. In case [iii], where the inoculation sites were randomly selected first colonies have started to merge that were initially close together. At this time the biofilm is still small enough that no notable production of $P$ has taken place, i.e. the model behaves essentially like the single species model. At $t = 20$, the biofilm has grown considerably in all three cases, which leads to local substrate depletion. In case [i] where the toxin producer X was located on one end of the substratum, it has completely taken over the biofilm there by production of the toxin that strongly inhibits the growth of the other species. The rest of the biofilm is entirely dominated by $Y$; here initially no toxin producers were located and toxin has not yet
Figure 2.5: Snapshots of the simulations of the cross-diffusion model of the allelopathic biofilm system for the cases [i], [ii], [iii] in Figures (a), (b), (c), at $t = 4$ (left) and $t = 20$ (right). Yellow-green color-coded is the fraction $R = \frac{X}{X+Y}$ of biomass $X$ relative to the overall biomass; greyscale contour lines indicate the substrate concentration $C$, rainbow colored contour lines indicate the product $P$. Figure (d)-(f) show the corresponding results for the model without cross diffusion for cases [i], [ii], [iii]. Time $t$ is non-dimensionalised with respect to growth rate $\mu_1$, substrate concentration $C$ is non-dimensionalised relative to the bulk concentration $C^\infty$. 

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diffused into this region. The transition between the $X$ and $Y$ dominated regions is marked by biomass gradients that describe slow mixing. In case [ii] the toxin producers $X$ were initially regularly interspersed between the colonies of species $Y$. Substrate limitations prompt the production of toxin $P$ all across the substratum. The fewer colonies of $X$ grow faster and larger due to their growth advantage under substrate limitations and start to dominate over and become bigger than the colonies of species $Y$ which initially outnumbered $X$. Also here the local transitions between $X$ and $Y$ dominated colonies show clear biomass gradients. In case [iii], where the initial data were chosen randomly, the left end of the domain was dominated by $X$, whereas $X$ was absent initially at the right end of the domain. Consequently, at $t = 20$ we find a large $X$ colony on one end, and a large $Y$ colony at the other end, which has not yet been reached by toxin $P$. In the middle we find one large colony in which $X$ and $Y$ are present, although $X$ developed better than $Y$ due to its growth advantage. It depends largely on the initial distribution of biomass which species dominates locally; there are regions in which both species are present at the same time. Again we observe distinct biomass gradient in the colony.

The corresponding results for the model without cross-diffusion effects are shown in Figure 2.5(d)-(f). While we observe biomass gradients in case [i] and in the larger merged colony in case [iii], and to some extent also in case [ii], these are less pronounced than in the results for the model with cross-diffusion effects, implying faster mixing.
The $X$ dominated regions are inhospitable for $Y$, therefore mixing of species is to some extent obliterated by faster decline of $Y$ in these regions via reactions. Consequently, the biomass density gradients are steeper (less mixing) than in the pure competition model above. This holds for both, the cross-diffusion model and the model without cross-diffusion. However, the biomass gradients are nevertheless more pronounced than in pure convective models, such such [2, 46], where complete segregation is enforced.

We plot the lumped parameters biofilm size $\omega(t)$ and the total biomass fraction $X_{total}(t)$ and $Y_{total}(t)$ in Figure 2.6. In case [ii] where we enforced a uniform distribution of the inoculum, we observe that initially both biomass types grew. Species $Y$, which was initially stronger represented, faster than species $X$. Eventually, biomass $Y$ declines after species $X$ starts producing $P$ due to substrate limitations. The differences between the predictions of the models with and without cross-diffusion are small and for practical purposes negligible. For species $X$ they are slightly more pronounced than for species $Y$. In case [i], where the two types of biomass were initially separated, the model behavior is quite different. Both species grow over the entire simulation period; the growth phase of species $X$ accelerates throughout, while the growth of species $Y$ eventually slows down, when the substrate limitations prompt the production of toxin $P$ by $X$. The differences in the global output parameters between the models with and without cross-diffusion are more pronounced than in case [ii]. These differences between cases [ii] and [i] are also rendered by the biofilm size $\omega(t)$. In case [i] the difference between
Figure 2.6: Lumped output parameters for the cases [i], [ii], [iii] of the allelopathic biofilm model: biofilm size (left column) and total biomass fraction (right columns) for the models with and without cross-diffusion. Time is non-dimensionalised with respect to growth rate $\mu_1$. 
the two models is larger than in case [ii] where they are negligible.

For case [iii], Figure 2.6 shows the mean values of both biomass fraction and the biofilm size, averaged over all 20 simulations. The mean values behave very similar as in case [ii]. Species $Y$ grows initially faster but eventually declines due to production of toxin $P$ by species $X$ when substrate becomes limited. The differences between the solution of the models with and without cross-diffusion are small and well within the uncertainty due to random inoculation.

**Discussion**

In this system the initial distribution of biomass plays a much larger role for the overall behavior of the biofilm than in the pure competition model, because the reactions can lead locally to detrimental environments for one species. Whereas the model without cross-diffusion leads to faster mixing and smoothing of biomass gradients than the cross-diffusion model when colonies of different type begin merging and penetrating each other. As such, cross-diffusion effects are important to described the biofilm structure and biomass distribution locally.

In the allelopathic system investigated here, the different species mix slower than in the pure competition model. In some instances we observe even after merging larger regions that are almost exclusively inhabited by species $X$ neighboring regions that are almost exclusively inhibited by species $Y$, with only small overlapping regions. In these cases living conditions are
locally inhospitable for one species which affects the ability to proliferate there. Such systems where local growth conditions affect mixing of bacteria in a biofilm have been observed in experiments, cf [1, 49]. Whereas spatial biofilm models can be designed to enforce such separation of species, our simulations show that it can be obtained as the interplay of cross-diffusion and reaction, with the same model of spatial biomass interaction that can describe mixing colonies, as seen in the previous example for a pure competition biofilm.

However, our results suggest also that the cross-diffusion effects can be neglected if the objective of a study is to assess the overall, global behavior of the biofilm rather than detailed local information, in particular due to the uncertainties that are introduced by initial inoculation of the substratum, which is very difficult to control even in a laboratory setup. The differences between the models with and without cross-diffusion are more pronounced than for the simpler competition model. However, they are small compared to the uncertainty introduced by the stochastic effects of inoculation.

2.3.3 Mixed-culture system of an aerobic and a facultative anaerobic species

As a third example we study a biofilm system formed by a traditional aerobic biofilm former and a facultative anaerobic biofilm former. An experimental study for a system of this kind is documented in [31] for *Pseudomonas putida*
and *Listeria monocytogenes*.

We assume that both species compete for oxygen as the only potentially growth limiting substrate. To the extent that oxygen becomes limited, species $Y$ switches into an anaerobic mode of growth. The governing equations read

\[
\begin{align*}
X_t &= \nabla(D_{11}(X,Y)\nabla X + D_{12}(X,Y)\nabla Y) + \mu_1 \frac{C}{\kappa_1+C} X - k_1 X, \\
Y_t &= \nabla(D_{21}(X,Y)\nabla X + D_{22}(X,Y)\nabla Y) + \mu_2 \frac{C}{\kappa_2+C} Y + \mu_3 \frac{\kappa_2}{\kappa_2+C} Y - k_2 Y, \\
C_t &= \nabla(D_C(X+Y)\nabla C) - \frac{\mu_1 X^\infty}{\kappa_1+C} X - \frac{\mu_2 Y^\infty}{\kappa_2+C} Y.
\end{align*}
\]

(2.32)

Here, $X$ and $Y$ are again the volume fractions occupied by biomass of the two species and $X^\infty$, $Y^\infty$ are defined as in Section 2.3.1. The growth rate of the aerobic grower $X$ is assumed to be higher than that of $Y$. In (2.32) the dependent variable $C$ denotes the oxygen concentration. The growth of species $X$ is described by Monod kinetics. For species $Y$ we implement both aerobic growth modeled by Monod kinetics, and anaerobic growth modeled by inhibition kinetics. An additional minor extension to the model was introduced, prompted by the observation that some biofilms, for example *Listeria monocytogenes* have a preferred growth direction along the substratum, forming layers rather than semi-spherical or mushroom shaped colonies. To account for this preferential direction in our simulations, diffusion of species $Y$ is assumed 10 times as fast in $x_1$ direction than in $x_2$ direction. The other parameters used in the simulation are given in Table 2.4. For the strong
Table 2.4: Model parameters used in the model of Section 2.3.3. The parameters for X are taken from [78], those for Y were chosen relative to those.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domain length</td>
<td>$L$</td>
<td>$10^{-3}$</td>
<td>m</td>
</tr>
<tr>
<td>Domain height</td>
<td>$H$</td>
<td>$10^{-3}$</td>
<td>m</td>
</tr>
<tr>
<td>Bulk substrate concentration</td>
<td>$C_{\infty}$</td>
<td>10</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Maximum specific growth rate $X$</td>
<td>$\mu_1$</td>
<td>6</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Maximum specific growth rate $Y$ (aerob)</td>
<td>$\mu_2$</td>
<td>1.2</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Maximum specific growth rate $Y$ (anaerob)</td>
<td>$\mu_3$</td>
<td>1.2</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Half saturation concentration $X$</td>
<td>$\kappa_1$</td>
<td>0.2</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Half saturation concentration $Y$</td>
<td>$\kappa_2$</td>
<td>0.2</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Lysis rate $X$</td>
<td>$k_1$</td>
<td>0.3</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Lysis rate $Y$</td>
<td>$k_2$</td>
<td>0</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Maximum biomass density $X,Y$</td>
<td>$X_{\infty},Y_{\infty}$</td>
<td>10000</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Yield coefficient</td>
<td>$\Upsilon_1,\Upsilon_2$</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Substrate diffusion coefficient in water</td>
<td>$D_C(0)$</td>
<td>$2 \cdot 10^{-4}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
<tr>
<td>Substrate diffusion coefficient in biofilm</td>
<td>$D_C(1)$</td>
<td>$1.6 \cdot 10^{-3}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
</tbody>
</table>

aerobic biofilm former we chose parameters in the typical range, as can be found in the Biofilm Modelling Benchmark Problems in [78]; the parameters for Y we have adjusted relative to those for X. The parameters were chosen such that oxygen limitations can be expected, based on a sufficiently large biofilm growth number (see [18] for more details regarding this dimensionless G-number that correlates growth parameters to substrate supply parameters). The model is simulated in a square domain on a $200 \times 200$ grid, with the same boundary conditions as in 2.3.1.

As initial data we choose initially three semi-spherical colonies of species $X$ on the substratum and three (smaller) semi-spherical colonies of $Y$. These six colonies initially did not overlap. Simulation snapshots are shown in
Figure 2.8. First, as long as the oxygen concentration $C$ is high enough species $X$ develops much faster; the colonies for $Y$, as intended spread across the substratum. Eventually these colonies of $Y$ invade the $X$ colonies. The increase in biofilm leads to a depletion of oxygen in the inner layers close to the substratum. Species $Y$ goes over into an anaerobic mode of growth, i.e. the inhibition kinetics term dominates over the Monod kinetics term. Species $X$ continues to grow in the leading front towards the oxygen source at the top, but due to lack of oxygen no notable amount of new aerobic biomass is produced closer to the substratum; in fact, locally $X$ starts to decline in the lower layers and the biomass density remains below unity. Thus biofilm growth close to the substratum is fully dominated by species $Y$. Eventually, a two-layered biofilm forms: a base layer in which $X$ and $Y$ are mixed with $Y$ strongly dominating, on top of which cluster/mushroom type colonies of $X$ tower. This qualitatively resembles the biofilms in the CLSM images of a *Pseudomonad putida* and *Listeria monocytogenes* biofilm, which are also included in Figure 2.8e,f.

In Figure 2.8 we compare the lumped output parameters biofilms size and biomass of both species for the model with cross-diffusion compared to corresponding simulations of the model without cross diffusion. The curves obtained for both species agree very well between the two models. Note that due to our choice of parameters for $Y$, this species grows at constant rate which is reflected in an exponential increase of biomass. Only for large time, slight differences between the model with and without cross-diffusion become
Figure 2.7: Top two rows: Snapshots at different times of the simulations of the cross-diffusion model of an aerobic and a facultative anaerobic species (2.32). Green-red color-coded is the fraction \( Z = \frac{X}{X+Y} \) of biomass \( X \) relative to the overall biomass; greyscale contour lines indicate the substrate concentration \( C \). Time \( t \) is non-dimensionalised with respect to growth rate \( \mu_1 \), oxygen concentration \( C \) is non-dimensionalised relative to the bulk concentration \( C^\infty \). Bottom row: CLSM micrographs (different angles) of a young *Pseudomonas putida* (green) and *Listeria monocytogenes* (red) mixed-culture biofilm from experiments described in [31]; micrographs courtesy of Greg Kepka (Lakehead University).
Figure 2.8: **Left:** Biomass of species $X$ and $Y$ in the simulation of (2.32) that was visualized in (2.7); shown are the data for the model with and without cross-diffusion. Time is non-dimensionalised with respect to growth rate $\mu_1$. **Right:** Snapshot of biomass density $M := X + Y$ in the biofilm at time $t = 19.5\mu$ noticeable, which are likely due to known boundary condition effects (see [15]) for large biofilm colonies in such a system. As in the previous examples, if one is only interested in the overall fate of the biofilm, cross-diffusion terms can be neglected since the model with and without cross-diffusion give similar results.

Also included in Figure 2.8 is for $t = 19.5\mu_1$, at a time where oxygen limitations in the inner layers have become severe, a plot of the biomass density $M = X + Y$, to illustrate that the model allows deviations from the *a priori* assumption of Wanner-Gujer like models that enforce $X + Y = 1$. In this particular case the density in the aerobic colonies is somewhat reduced as a consequence of oxygen limitation, whereas the facultative anaerobic layer has $X + Y \approx 1$ throughout.
2.4 Summary, Conclusion, Outlook

In recent years a slew of multi-dimensional deterministic continuum models of biofilms have been introduced in the literature, focusing on various aspects of biofilm formation, dynamics and response to environmental stimuli. While many among them consider multi-component biofilms, including inactive bacteria and EPS, only very few are concerned with mixed culture biofilms systems that involve more than one bacterial species, based on the seminal one-dimensional Wanner-Gujer model. A key assumption of these models is that the volume fractions occupied by the different species always add to unity. The limitations of this assumption, in particular for multi-species systems has been recently pointed out and discussed, and it was shown that colonies that are initially not mixed will not mix but remain segregated. On the other hand, several stochastic, discrete mixed culture biofilm models have been proposed that focus on biofilm structure, which do not make this \textit{a priori} assumption. In these models the user has full control over the amount of mixing that should take place when colonies of different species merge, by formulating local interaction rules. In these models the volume fraction occupied by the different biomass fractions does not add to unity but can vary between 0 and 1. These multi-species models have sometimes been criticized for overemphasizing mixing.

We derived a multi-species biofilm model that combines aspects of both model classes. It is a deterministic continuum model but does not \textit{a priori}
impose that bacterial volume fractions must add to unity. It is a cross-diffusion extension of a previously developed degenerate density-dependent diffusion-reaction single-species biofilm model. We give two derivations of the multi-species model, one from a discrete lattice differential equation similar to cellular automaton models, other from the same continuous mass balances as the Wanner-Gujer model.

Numerical simulations show that when two colonies of different species merge, pronounced spatial biomass gradients will be observed that lead to spatially heterogeneous distribution of biomass within the resulting larger colony, as have been observed in microscopy experiments. This is in contrast to Wanner-Gujer like deterministic models that prevent mixing. By comparison of the dual species model with an earlier multi-species model that neglects cross-diffusion, we found that cross-diffusion terms are crucial to not over estimate mixing of species in colonies; neglecting cross-diffusion effects can obliterate, or at least dampen biomass gradients. This observation positions the proposed cross-diffusion model as a potential alternative besides these existing models that have been criticized for displaying too much or not enough mixing of species. This is in particular a crucial point if one is interested in local effects within biofilm colonies. Examples for this arise in wastewater engineering, where often different processes can happen concurrently in layers and niches of a biofilm, in which different species co-habit. In membrane aerated biofilm systems, this could be aerobic and anoxic processes in different regions of the biofilm [72, 77], or simultaneous sulfate
reduction and nitrogen removal [70]. Wastewater biofilms typically consist of more than two species, which requires an extension of our dual species derivation. The model presented here, however, an important first step in this direction.

However, if one is primarily interested in global biofilm behavior and lumped parameters such as amount of biomass and biofilm size, all three of our examples suggest that the diffusion model with and without cross-diffusion effects lead to very similar results. Therefore in such studies it is acceptable to use a mathematically and numerically simpler biofilm model, in which spatial mixing is simplified by neglecting the cross-diffusion terms. This is often the case, e.g. in biofilm control or disinfection studies which focus on the question whether a biofilm community will survive rather than the distribution of cells in the biofilm, or in engineering systems where the focus is on community wide biofilm performance rather than on detailed information about cell distribution in the colony.

Being a deterministic continuum model, it should be straightforward to couple the model with a flow model to compute the convective contribution to substrate transport in the aqueous phase, which we neglected so far. If the hydrodynamic conditions are such that this needs to be modeled by the full incompressible Navier-Stokes equations, then the flow field calculations become the computationally most expensive part. In simulations with stochastic discrete models, this means that the flow field has to be recomputed with every realization of the stochastic process simulated, which
presumably makes these models inefficient, so that deterministic models will be preferable.

In the examples that we included we made two important assumptions: the spatial behavior of both species in the biofilm is the same, and volume filling depends only on the total local biomass, but not on the particular species. The modeling framework, however, by derivation is general enough to relax both assumptions in a straightforward manner and to investigate systems in which these assumptions do not apply, and which will lead to algebraically more involved models. This will be the subject of future work. Similarly, we derived and studied the model here for binary biofilms consisting of two bacterial species. It can be extended to three and more species in a straightforward manner, which however increases computational complexity. This is beyond the focus of this first study that introduces the framework in the simplest case.
Bibliography


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Chapter 3

Numerical treatment of a cross-diffusion model of biofilm exposure to antimicrobials

_Springers Lecture Notes in Computer Science (LNCS), Parallel Processing and Applied Mathematics_, 2014, pp. 134-144.

Abstract

We present a numerical method for a highly nonlinear PDE model of biofilm response to antibiotics with three nonlinear diffusion effects: (i) porous medium degeneracy, (ii) super-diffusion singularity, (iii) nonlinear cross-diffusion. The scheme is based on a Finite Volume discretization in space and semi-implicit, non-local time integration. The resulting discretized system is implemented in Fortran and parallelized with OpenMP. The numerical method is validated in a simulation study.
3.1 Introduction

Bacterial biofilms are microbial layers on biotic and abiotic surfaces. Bacteria attach to the surface and start producing extracellular polymeric substances (EPS) in which they are themselves embedded and which protect them against washout and antimicrobials. This protection makes biofilm eradication difficult which causes major problems in the treatment of bacterial infection and in disinfection of medical and industrial surfaces [2]. The mathematical model of biofilm exposure to biocides in our study is a highly nonlinear diffusion reaction system, based on the prototype single-species biofilm model of [5]. In [6] this model was extended to a model of biofilm response to biocides. This is extended in [14] by including cross-diffusion effects describing mixing and separation of active and inactive biomass in more detail. The model shows several non-standard diffusion effects that make numerical treatment difficult: (i) porous medium degeneracy, (ii) super-diffusion singularity, (iii) nonlinear cross-diffusion. We propose a numerical method based on a Finite Volume discretization in space and semi-implicit, non-local time integration. This method is a cross-diffusion extension of the method studied in [12, 13] for models of the type [6], and is easy to parallelize.
3.2 Mathematical model

The biofilm model is formulated in terms of the dependent variables volume fractions occupied by active biomass $X$ and by inert biomass $Y$, and the concentrations of nutrient $C$ and biocide $B$. It reads

$$
X_t = \nabla (D_{11}(X,Y)\nabla X + D_{12}(X,Y)\nabla Y) + \mu \frac{CX}{\kappa_1 + C} - \xi_1 X - \xi_2 \frac{BX}{\kappa_2 + B}, \quad (3.1)
$$

$$
Y_t = \nabla (D_{21}(X,Y)\nabla X + D_{22}(X,Y)\nabla Y) + \xi_2 \frac{BX}{\kappa_2 + B}, \quad (3.2)
$$

$$
C_t = \nabla (D_C(X+Y)\nabla C) - \frac{\mu X^\infty}{\Gamma_1} \frac{CX}{\kappa_1 + C}, \quad (3.3)
$$

$$
B_t = \nabla (D_B(X+Y)\nabla B) - \frac{\xi_2 X^\infty}{\Gamma_2} \frac{BX}{\kappa_2 + B}. \quad (3.4)
$$

All parameters are positive; see Table 3.1 for their definition. Growth of active biomass is due to nutrient uptake. Biocide is degraded during inactivation of active biomass. Spatial expansion of the biofilm is described by the interaction of three nonlinear diffusion effects: (i) a porous medium degeneracy that ensures that the biofilm does not expand as long as there is locally space available to accommodate newly produced biomass, (ii) a super-diffusion singularity that ensures that the maximum biomass density is obeyed, and (iii) cross-diffusion that describes the mixing of both biomass fractions. Following [14], the biomass
diffusion coefficients are with $M := X + Y$ given by

$$\begin{cases} D_{11}(X,Y) = \Phi(M) + X\Psi(M), & D_{12}(M) = X\Psi(M), \\
D_{21}(X,Y) = Y\Psi(M), & D_{22}(X,Y) = \Phi(M) + Y\Psi(M), \end{cases}$$

(3.5)

where the functions $\Phi$ and $\Psi$ are defined using the density-dependent diffusion coefficient $D(M)$ of a single species biofilm model through

$$D(M) = \delta \frac{M^4}{(1 - M)^4} = \Phi(M) + M\Psi(M)$$

(3.6)

$$\Phi(M) = \left(1 - \int_0^M D(m)dm\right)\int_0^M D(m)dm = \frac{18M^2 + 30M - 13}{3(1 - M)^3} - \frac{13}{3}.$$ 

(3.7)

The integral can be expressed as

$$\int_0^M D(m)dm = M + 4\log(1 - M) + \frac{-18M^2 + 30M - 13}{3(1 - M)^3} - \frac{13}{3}.$$ 

(3.8)

The biofilm phase proper is the region where $M(t) > 0$, the aqueous phase is the region where $X(t) = Y(t) = 0$. Both regions are connected by a moving interface. Diffusion of substrates $B, C$ is slower in the biofilm than in the aqueous phase. Linear interpolation between a fully compressed biofilm with $M = 1$ and the aqueous phase with $M = 0$ gives

$$D_{B,C}(M) = D_{B,C}^0 \cdot (1 + M(\rho_{C,B} - 1)).$$

(3.9)
For the biomass fractions we pose homogeneous Neumann conditions on $\partial \Omega$. For the dissolved substrates we pose Dirichlet conditions on some part of the boundary, from where substrates and biocides are added, and homogeneous Neumann conditions everywhere else. The initial data have compact support: Active biomass is located in small pockets/colonies somewhere at the boundary, everywhere else $X = 0$ initially. We shall assume that initially no inert biomass is in the system $Y = 0$. The initial data for the substrates are $C = C_0 > 0$ and $B = B_0 \geq 0$.

The special form of the biomass cross-diffusivities, in particular $D_{12}(0, \cdot) = D_{21}(\cdot, 0) = 0$ maintains non-negativity of $X$ and $Y$. Adding (3.1) and (3.2) we find that $M = X + Y$ is bounded from above by the solution of the prototype single species biofilm model [5], implying with [7] that the solution of the model might come close to its upper bound but never actually attains the super-diffusion singularity, i.e. $X + Y < 1$, at least as long as the interface is separated from the boundary somewhere. A finite speed of interface propagation follows with standard results on degenerate parabolic equations [17]. Nevertheless, regions with $X + Y = 0$ and $X + Y \approx 1$ can be in very close proximity. While the solutions are continuous, the biomass density gradients can blow up at the interface [9].

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3.3 Numerical method

Each of the three non-Fickian diffusion effects (i), (ii), (iii) mentioned in the Introduction has its own numerical challenges. To deal with (i) and (ii), a semi-implicit numerical method has been developed in [4, 12, 13], which we extend here to include (iii). A key property of the underlying method is that after semi-implicit discretization it requires the repeated solution of linear systems with at least weakly diagonally dominant M-matrices. Direct discretization of cross-diffusion terms spoils this property. Instead, in the semi-implicit framework used here, the cross-diffusion terms are treated as advection terms. We use upwinding for its discretization, which preserves the M-matrix properties [8, 11]. Beside it being only first order, the main disadvantage commonly associated with this method is that it can induce strong artificial numerical diffusion. In our model, where the transition from the biofilm to the aqueous phase is described by a steep interface, interface smearing due to numerical diffusion is an important concern. However, due to the specific form of the diffusion coefficients, we have close to the interface $M \ll 1$, thus $D_{ij} < \delta M^a$, and therefore we can hope that these effects are small.

Equations (3.1), (3.2) can be written as convection-diffusion-reaction
equations

\[ X_t = \nabla (D_{11}(X,Y) \nabla X - w_X X) + R_1(C, B)X, \quad (3.10) \]

\[ Y_t = \nabla (D_{22}(X,Y) \nabla Y - w_Y Y) + R_2(X), \quad (3.11) \]

with "cross-diffusion velocities"

\[ w_X := -\Psi(X + Y) \nabla Y, \quad w_Y := -\Psi(X + Y) \nabla X. \]

We first discretize in time, using a non-local (in time) representation of nonlinearities as suggested in [3, 10] for ODE problems, then in space. We use the shorthand notation for dependent variables and nonlinearities,

\[ X^k := X(t^k, \cdot), \quad w_X^k := -\Psi(X^k + Y^k) \nabla Y^k, \quad R_1^k := R_1(C^k, B^k), \quad etc. \]

Following [4, 12, 13] we obtain the semi-implicit time discretization of (3.10), (3.11)

\[ \frac{X^{k+1} - X^k}{\tau^k} = \nabla \left( D_{11}(X^k, Y^k) \nabla X^{k+1} - w_X^k X^{k+1} \right) + \frac{R_1^k X^{k+1}}{\tau^k} \quad (3.12) \]

\[ \frac{Y^{k+1} - Y^k}{\tau^k} = \nabla \left( D_{22}(X^k, Y^k) \nabla Y^{k+1} - w_Y^k Y^{k+1} \right) + \frac{R_2^k}{\tau^k} \quad (3.13) \]
where $\tau^k := t^{k+1} - t^k$. For the spatial discretization, we use a Finite Volume scheme on a regular grid of size $n \times m$ for the rectangular domain $L \times H$. The dependent variables are evaluated in the center of the grid cells and the diffusive and convective fluxes at the grid cell edges. Thus, for $i = 1, ..., n, j = 1, ..., m, k = 0, 1, 2, ...$ with $\Delta x := L/n = H/m$,

$$X_{i,j}^k \approx X \left( t^k, (i - 1/2) \Delta x, (j - 1/2) \Delta x \right).$$

For the self-diffusive fluxes in (3.10) we use, as in [4, 12, 13], the standard second order approximation. For the flux between cells $(i,j)$ and $(i+1,j)$ we have

$$J_{i+1/2,j}^{k+1} = \frac{1}{2} \left( D(X_{i+1,j}^k, Y_{i+1,j}^k) + D(X_{i,j}^k, Y_{i,j}^k) \right) \frac{X_{i+1,j}^{k+1} - X_{i,j}^{k+1}}{\Delta x},$$

and accordingly for the fluxes across the remaining edges of cell $(i,j)$

$$J_{i-1/2,j}^{k+1} = \frac{1}{2} \left( D(X_{i-1,j}^k, Y_{i-1,j}^k) + D(X_{i,j}^k, Y_{i,j}^k) \right) \frac{X_{i-1,j}^{k+1} - X_{i,j}^{k+1}}{\Delta x},$$

$$J_{i,j+1/2}^{k+1} = \frac{1}{2} \left( D(X_{i,j+1}^k, Y_{i,j+1}^k) + D(X_{i,j}^k, Y_{i,j}^k) \right) \frac{X_{i,j+1}^{k+1} - X_{i,j}^{k+1}}{\Delta x},$$

$$J_{i,j-1/2}^{k+1} = \frac{1}{2} \left( D(X_{i,j-1}^k, Y_{i,j-1}^k) + D(X_{i,j}^k, Y_{i,j}^k) \right) \frac{X_{i,j-1}^{k+1} - X_{i,j}^{k+1}}{\Delta x}.$$

New in the present model are the cross-diffusion terms, which we represented in (3.10) as convective terms. At the edge between cells $(i,j)$ and $(i+1,j)$ the velocity component $u$ in $x$-direction of the velocity vector
\( w_X \) is calculated as

\[
u_{i+1/2,j}^k = \frac{1}{2} \left( \Psi(X_{i+1,j}^k + Y_{i+1,j}^k) + \Psi(X_{i,j}^k + Y_{i,j}^k) \right) \frac{Y_{i+1,j}^k - Y_{i,j}^k}{\Delta x}
\]

and accordingly at the edge between cells \((i-1,j)\) and \((i,j)\)

\[
u_{i-1/2,j}^k = \frac{1}{2} \left( \Psi(X_{i-1,j}^k + Y_{i-1,j}^k) + \Psi(X_{i,j}^k + Y_{i,j}^k) \right) \frac{Y_{i,j}^k - Y_{i-1,j}^k}{\Delta x}.
\]

At the edges between the cells \((i,j)\) and \((i,j+1)\) and \((i,j)\) and \((i,j-1)\), the velocity components in \(y\)-direction of \(w_X\) are

\[
u_{i,j+1/2}^k = \frac{1}{2} \left( \Psi(X_{i,j+1}^k + Y_{i,j+1}^k) + \Psi(X_{i,j}^k + Y_{i,j}^k) \right) \frac{Y_{i,j+1}^k - Y_{i,j}^k}{\Delta x},
\]

\[
u_{i,j-1/2}^k = \frac{1}{2} \left( \Psi(X_{i,j-1}^k + Y_{i,j-1}^k) + \Psi(X_{i,j}^k + Y_{i,j}^k) \right) \frac{Y_{i,j}^k - Y_{i,j-1}^k}{\Delta x}.
\]

Simple first order upwinding leads to the approximation of the cross-diffusion flux \(F_{i+1/2,j}^k\) at the edge shared by the cells \((i,j)\) and \((i+1,j)\),

\[
F_{i+1/2,j}^{k+1} = \begin{cases} 
    u_{i+1/2,j}^k X_{i,j}^{k+1} & \text{if } u_{i+1/2,j}^k \geq 0, \\
    u_{i+1/2,j}^k X_{i+1,j}^{k+1} & \text{if } u_{i+1/2,j}^k < 0,
\end{cases}
\]

and similarly at the remaining cell edges

\[
F_{i-1/2,j}^{k+1} = \begin{cases} 
    u_{i-1/2,j}^k X_{i-1,j}^{k+1} & \text{if } u_{i-1/2,j}^k \geq 0, \\
    u_{i-1/2,j}^k X_{i,j}^{k+1} & \text{if } u_{i-1/2,j}^k < 0,
\end{cases}
\]
Putting all of the above together, we arrive for grid cells in the interior of the domain, i.e. for cells with $1 < i < n$, $1 < j < m$ at

$$\frac{X_{i,j}^{k+1} - X_{i,j}^k}{\tau^k} = \frac{J_{i+1/2,j}^{k+1} - J_{i-1/2,j}^{k+1}}{\Delta x} + \frac{J_{i,j+1/2}^{k+1} - J_{i,j-1/2}^{k+1}}{\Delta x}$$

$$- \left( \frac{F_{i+1/2,j}^{k+1} - F_{i-1/2,j}^{k+1}}{\Delta x} + \frac{F_{i,j+1/2}^{k+1} - F_{i,j-1/2}^{k+1}}{\Delta x} \right) + R_{i,j}^{k+1} X_{i,j}^{k+1}$$

For grid cells that share at least one of their edges with a boundary of the domain, this formula accesses non-existing cells outside the domain. These are eliminated in the usual manner. For example, for the homogeneous Neumann boundary condition $\partial_n X = 0$ at $x = 0$, we eliminate $X_{0,j}^{k}$ using $\frac{1}{\Delta x} (X_{0,j}^{k} - X_{1,j}^{k}) = 0$. Finally, we introduce the lexicographical grid ordering

$$\pi : \{1, ..., n\} \times \{1, ..., m\} \to \{1, ..., nm\}, (i, j) \mapsto (i - 1)m + j$$

and the vector notation $X = (X_1, ..., X_{nm})^T$ with $X_{\pi(i,j)}^k = X_{i,j}^k$. This allows us to re-arrange and re-write (3.14) in the compact matrix vector
\[
(I - \tau^k \mathcal{D}_X^k + \tau^k \mathcal{F}_X^k - \tau^k \mathcal{R}_X^k) \mathcal{X}^{k+1} = \mathcal{X}^k
\]  
(3.15)

where \( I \) is the \( nm \times nm \) identity matrix, the matrix \( \mathcal{D}_X^k \) contains the contributions of self-diffusion, the matrix \( \mathcal{F}_X^k \) contains the cross-diffusion contributions, and the diagonal matrix \( \mathcal{R}_X^k \) contains the reaction terms.

**Remark 3.3.1.** These matrices depend only on the dependent variables of the previous time-step and have the following properties, which can easily be verified by straightforward calculations:

\( \mathcal{D}_X^k \) is sparse with non-positive diagonal entries and non-negative off-diagonal entries if the biomass densities \( X_{i,j}^k \) and \( Y_{i,j}^k \) are non-negative and \( X_{i,j}^k + Y_{i,j}^k < 1 \)

\( \mathcal{F}_X^k \) is sparse with non-negative diagonal entries and non-positive off-diagonal entries if the biomass densities \( X_{i,j}^k \) and \( Y_{i,j}^k \) are non-negative and \( X_{i,j}^k + Y_{i,j}^k < 1 \).

We obtain for \( Y \) in a similar manner a linear system of the form

\[
(I - \tau^k \mathcal{D}_Y^k + \tau^k \mathcal{F}_Y^k) \mathcal{Y}^{k+1} = \mathcal{Y}^k + r^k,
\]  
(3.16)

where vector \( r^k \) contains the contributions of \( R_2^k \), and for \( C \) and \( B \)
without cross-diffusion

\[
(I - \tau^k D_C^k - \tau^k R_C^k) C^{k+1} = C^k + b_1 \quad (3.17)
\]

\[
(I - \tau^k D_B^k - \tau^k R_B^k) B^{k+1} = B^k + b_2 \quad (3.18)
\]

where vectors \( b_1, b_2 \) contain the contributions of the Dirichlet boundary conditions. Note that the matrices \( D, F, R \) are different in (3.16) - (3.18) than in (3.15), but have the same properties as stated above.

In the biological/physical context \( X \) and \( Y \) as volume fractions need to be non-negative. In the continuous model (3.1), (3.2) this is ensured by the specific form of the cross-diffusion terms with \( D_{12}(0, \cdot) = D_{21}(\cdot, 0) = 0 \). That the numerical method inherits this property shows the following result, which also implies the absence of spurious oscillations at the steep biofilm/water interface, which are often observed in numerical solutions of such interface propagation problems.

**Proposition 3.3.2.** If \( \tau^k \) is small enough and \( X^k \geq 0, Y^k \geq 0, C^k \geq 0, B^k \geq 0 \) then \( X^{k+1} \geq 0, Y^{k+1} \geq 0, C^{k+1} \geq 0, B^{k+1} \geq 0 \).

**Proof.** The matrices \( D^k \) are weakly diagonal dominant with non-positive diagonal entries and non-negative off-diagonal entries and the matrices \( F^k \) are diagonally dominant with non-negative diagonal entries and non-positive off-diagonal entries. The diagonal matrices \( R^k \) in (3.17), (3.18) are non-negative. Thus the system matrices of (3.16), (3.17), (3.18) are
M-matrices for every choice $\tau^k > 0$. The signs of the entries of $\mathcal{R}_X^k$ in (3.15) depend on $C^k$ and $B^k$. Let $K$ be the maximum entry of this matrix. Then for the system matrix of (3.15) to be an M-matrix it suffices if $\tau^k < 1/K$. By hypothesis $X^k \geq 0, Y^k \geq 0, C^k \geq 0, B^k \geq 0$, and $r^k$ in (3.16), $b_{1,2}$ in (3.17), (3.18) are non-negative by definition. Since the inverse of M-matrices are non-negative, it follows $X^{k+1} \geq 0, Y^{k+1} \geq 0, C^{k+1} \geq 0$ and $B^{k+1} \geq 0$.

**Remark 3.3.3.** $K$ in the above proof is the characteristic time scale for biofilm growth. Hence the constraint $\tau < 1/K$ is not critical for applications.

The numerical method requires in every time-step to solve the linear systems (3.15)-(3.18). We use BiCGSTAB [16] from the free Fortran source code library SPARSKIT [15], which we prepared for parallel execution on shared memory computers using OpenMP [12]. This is the computationally most expensive part, and the only part of the code that is parallelized. BiCGSTAB requires in each iteration two matrix-vector products, four inner products with reduction, and a number of vector additions and scalar-vector multiplications. We compiled and tested the code with the Intel, Portland Group and GNU Fortran compilers on a SGI ALTIX 450, a SGI ALTIXUV, a custom workstation and a Toshiba Tecra Laptop. The simulations reported here were conducted on the ALTIXUV, where the number of OpenMP threads was adjusted to grid
size. The parallel behaviour of the underlying method, using the same linear algebra routines, for a related model was previously documented in [12].

### 3.4 A typical simulation and grid refinement

We test the numerical method in a case study. The parameters for biofilm growth are taken from the Benchmark Problem 1 of [18], disinfection parameters are adapted from [1]. They are summarized in Table 3.1.

Initially, three semi-spherical biofilm colonies are placed on the bottom boundary, two of them centred, and a smaller one at the left corner. Sub-
Figure 3.1: Simulation of biofilm growth and exposure to biocides. Shown is the biofilm region $X + Y > 0$ for selected $t$. Biocide is added after the biofilm reaches a certain size. The colour represents active biomass, relative to the overall biomass, $Z = \frac{X}{X+Y}$.

Initially the biofilm grows due to steady substrate supply. The colonies start expanding when the local biomass density approaches the maximum cell density $M \approx 1$. Eventually the neighbouring colonies in the center merge to form a single, larger colony. In the absence of biocides and inactive biomass, the model behaves like the single-species/single-substrate biofilm model of [5]. At $t \approx 6.9$ biocides are added from the top. After the biofilm reaches a given height, biocide is added from the top. The simulation stops when the biofilm reaches a given size (area or height). The results are shown in Figure 3.1.
top and diffuse toward the biofilm. Inactivation begins. Inert biomass is observed primarily in the outer layers. In the inner layer new biomass continues to be produced. Despite the disinfection rate being significantly larger than the growth rate, this leads to a further expansion of the biofilm in the presence of biocide. Eventually the larger double colony merges with the smaller colony. Throughout the disinfection period, a distinct gradient of inactive biomass relative to the total biomass is observed, from the biofilm/water interface inward. For the parameters used in this simulation, adding the biocide does not prevent the biofilm from growing further or even lead to its decay. This is due to rapid degradation of biocides in the outer layers of the biofilm during inactivation and slow diffusion of biocides into the biofilm.

Due to the nonlinearities, theoretical convergence analysis is difficult. Instead we conduct a computational study on grids of different resolutions, with \( n = m = 2^k, k = 5, ..., 11 \). The setup is as in Figure 3.1, but the colonies are initially bigger, to shorten the phase where no expansion takes place and where cross-diffusion effects do not play a role; see [4, 13] for convergence studies of models without cross-diffusion. In Figure 3.2 we plot the time course of the following parameters of interest: biofilm size, total active biomass, total inactive biomass, and minimum substrate concentration. We observe grid size effects for the coarsest resolutions, in particular for the measure of biofilm size and the total amount of inactive biomass. Biofilm size is included here also.
Figure 3.2: Output parameters for various grid resolutions: (a) biofilm, (b) total active biomass, (c) total inactive biomass, (c) minimum substrate concentration as indicator for the convergence of the biofilm interface. The location of the interface can be accurate at most with $\Delta x$ which explains the strong differences for the coarsest grids. Disinfection starts when the biofilm reaches a given height. Hence, the onset of disinfection is also closely linked to the accuracy of the interface location. Also here we see big difference for the coarsest grids but note convergence as the grid is refined. Total active biomass and minimum substrate concentration in the system agree well for all grid resolution over the course of the sim-
Table 3.2: Results of the grid refinement study: least square norms for the differences of solutions for grids with $2^k \times 2^k$ and $2^{k-1} \times 2^{k-1}$ cell resolution at the final time step.

<table>
<thead>
<tr>
<th>$k$</th>
<th>$X$</th>
<th>$Y$</th>
<th>$C$</th>
<th>$B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>$0.1690 \cdot 10^{-2}$</td>
<td>$0.2473 \cdot 10^{-3}$</td>
<td>$0.3817 \cdot 10^{-3}$</td>
<td>$0.2520 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>7</td>
<td>$0.5110 \cdot 10^{-3}$</td>
<td>$0.9279 \cdot 10^{-4}$</td>
<td>$0.7629 \cdot 10^{-4}$</td>
<td>$0.5579 \cdot 10^{-4}$</td>
</tr>
<tr>
<td>8</td>
<td>$0.2361 \cdot 10^{-3}$</td>
<td>$0.4853 \cdot 10^{-4}$</td>
<td>$0.2730 \cdot 10^{-4}$</td>
<td>$0.2145 \cdot 10^{-4}$</td>
</tr>
<tr>
<td>9</td>
<td>$0.5970 \cdot 10^{-4}$</td>
<td>$0.1427 \cdot 10^{-4}$</td>
<td>$0.4607 \cdot 10^{-5}$</td>
<td>$0.3420 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>10</td>
<td>$0.2669 \cdot 10^{-4}$</td>
<td>$0.6714 \cdot 10^{-5}$</td>
<td>$0.1749 \cdot 10^{-5}$</td>
<td>$0.1252 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>11</td>
<td>$0.9344 \cdot 10^{-5}$</td>
<td>$0.2428 \cdot 10^{-5}$</td>
<td>$0.4369 \cdot 10^{-6}$</td>
<td>$0.3019 \cdot 10^{-6}$</td>
</tr>
</tbody>
</table>

ulation. For practical purposes, a resolution with $n = m = 256$ appears as a good accuracy/cost trade-off. To also account for spatial accuracy explicitly, we compute the difference of the solutions of two subsequent grid resolutions for the last simulation time step, see Table 3.2. We note a steady decrease in error when refining the grid, indicating convergence of the method. To compute these data the simulation results had to be interpolated between grids. They are subject to interpolation errors and cannot be used to extract the convergence rate. Moreover, these data also are sensitive to the interface location being accurate at most within $\Delta x$.

In each time step (3.15)-(3.18) are solved. The system matrices change with each instance. Therefore, the number of BiCGSTAB iterations, and hence cost per timestep, is variable over the duration of the simulation (data not shown).
3.5 Conclusion

We present a numerical method for a highly nonlinear PDE model of biofilm response to biocides with three nonlinear diffusion effects: (i) porous medium degeneracy, (ii) super-diffusion singularity, (iii) nonlinear cross-diffusion. The scheme extends a previous methods for problems with properties (i) and (ii). The new cross-diffusion terms are treated formally as convective terms with density dependent velocity. The method is based on a Finite Volume discretization in space and semi-implicit, non-local time integration. It preserves positivity. In simulations we showed its convergence with respect to grid refinement.
Bibliography


Chapter 4

New reaction kinetics for models of disinfection of microbial biofilms by antibiotics: interpretation of an experimental observation

Abstract

Recent laboratory experiments showed increased metabolic activity, measured in terms of carbon dioxide production during periods of exposure of bacterial biofilms to antibiotics, a phenomenon that is not reflected by current biofilm models. In order to incorporate this observation in models of disinfection of microbial biofilms we introduce extended reaction kinetics based on carbon consumption during disinfection. We implement this extension in a 0-dimensional simplified reactor scale model (ODE) where carbon substrates and antibiotics are well mixed but biomass is attached to the wall and not washed out, and in a highly nonlinear 2-dimensional density dependent cross-diffusion model
(PDE), that considers spatial effects, such as substrate gradients and heterogeneous biofilm architectures. Our simulations show that the extended model captures the experimental observation, and suggest that the consumption of carbon substrates during inactivation due to antibiotics helps biofilms to survive and re-grow. Not accounting for this effect in a model might lead to false negatives, over-predicting the efficacy of antibiotic disinfection.

4.1 Introduction

Biofilms are complex three-dimensional aggregates of microorganisms in which cells adhere to each other on surfaces and interfaces in moist environments [39] where the required substances such as nutrients, oxygen etc are available for microbial growth. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS are composed of extracellular DNA, proteins, and polysaccharides. The EPS helps the microorganism to protect them against harmful environmental conditions. Biofilms can develop in spatially highly irregular morphological structures, where individual colonies are separated by voids and channels.

Biofilms play a very important role in many scientific and technological areas. Biofilms can be prevalent in natural, industrial and hospital settings [12, 19]. The degradation of toxic substances in soil and water,
the commercial production of chemicals, and the generation of electricity are the current applications of biofilms [18, 20]. Based on biofilm processes, environmental engineers have been using the beneficial properties of biofilm in order to develop remediation technologies for many years e.g. in wastewater treatment, groundwater protection and soil remediation [16, 28]. On the other hand, there are many harmful impacts of bacterial biofilms. Biofilms cause biofouling and microbially-induced corrosion in industry, microbial infections in the body that cause harm to millions of humans and animals annually and also can affect the quality and yield of crops [22]. Therefore, their pervasive effects on drinking water quality, power generation, energy efficiency, human and animal health, and product quality could be extremely costly to industry and harmful to health [22].

It is more difficult to control microbial biofilms by antibiotics, chemical disinfectants or other biocides than free-floating, planktonic populations [8]. According to [17],[32] several reasons are claimed responsible for this, including (i) diffusive and reactive penetration barriers, (ii) adaptive stress response below a reactive layer, (iii) presence of persister cells, and (iv) nutrient limitation and slow growth below the active layer.

Diffusion is the main transport mechanism for antimicrobial agents to move into and inside the biofilm. At the same time there are also some reactive processes present that might influence the transport rate significantly. When antibiotics approach the biofilm, they interact with top
layer cells/bacteria near the biofilm/liquid interface. Hence, the antibiotics are depleted in order to kill the top layer cells. But the bacteria inside the deeper layers of biofilm still remain unharmed and they are protected and keep growing as long as nutrients are available. Thus, attack by antibiotics doesn’t ensure removal/killing of the whole biofilm. Furthermore, slow growing microorganisms are known to be less susceptible to antibiotics [4].

The antibiotic treatment of biofilms is a complex phenomenon. It comprises various mechanisms of biological, chemical, and physical processes. Indeed, mathematical modeling plays an important role, as a powerful tool, in investigating these mechanisms. One can find a variety of different mathematical models in the literature describing disinfection of biofilms by antibiotics or other disinfectants [8]. These modeling variations are presented based on some basic assumptions on mechanisms of bacterial tolerance to antibiotics. [4] outlined the models considering the assumptions that include (i) biofilms to be spatially homogeneous, flat layer where diffusion is considered to be in one direction, perpendicular to the substratum and the biofilm/liquid interface [5, 29, 30, 35, 31, 34, 36], (ii) fixed biofilm morphology where the assumption has been made that the biofilm structure does not change in the time interval under consideration [3, 5, 29, 35, 31, 36], (iii) steady state assumptions for antimicrobial agents where it is assumed that the concentration field of dissolved substrates is in a pseudo-steady state
[5, 36], (iv) hydrostatic environment where biofilms form in aqueous environment with the hydrodynamic flow field carrying nutrients and substrates and assumed that the concentration of dissolved substrates are constant in the aqueous phase [5, 30, 31, 36]. The review article [2] tried to distinguish models according to the mechanisms that induce bacterial tolerance to disinfection. The mechanisms are genotypic, physical, physiological and phenotypic. Most mathematical models tend to address one mechanisms at a time and the most mathematically challenging models address physical and physiological aspects simultaneously since physiological effects typically stem from physical transport of dissolved substrates and biocides [2]. We also refer to [14] for an extensive review on mathematical description of biofilm disinfection.

The biofilm growth and disinfection model presented in [4] is formulated as a density-dependent diffusion-reaction system for the dependent variables active biomass, inert biomass, substrate concentration and disinfectant concentration. This system, as a system of evolution equations, describes the growth of biofilm and its simultaneous disinfection by the action of antibiotics. This type of biofilm model was first introduced in [8] for single species and was studied in a small series of papers analytically, numerically and in applications.

The disinfection process defined by reaction kinetics is another key part of the modeling. First order reaction kinetics is the most frequently used one in the disinfection of biofilm modeling literature [7, 10, 29, 30, 31,
This reaction kinetics is always assumed proportional to the local disinfectant concentration and the local active biomass.

Otini et al. [23] observed in laboratory experiment that carbon dioxide has been produced at an increasing rate during periods of exposure of bacterial biofilms to antibiotics in a high dosage regime (see Figure 4.1). The cause of the production of carbon dioxide is metabolic activity and carbon consumption. The experimental observations suggest, therefore, that exposure to antibiotics causes an increase in substrate utilization.

The main purpose of our work is to capture this experimental observations and explain/discuss this experimental new outcome using mathematical models available in the current literature. In fact, no existing mathematical model describes this particular experimental scenario in a straightforward way. This is why, to incorporate this experimental observations in models of disinfection of microbial biofilms, we use a cross-diffusion extension of the model presented in [4] and introduce a new reaction kinetics which is potentially limited by the local antibiotics concentration and by the local substrate concentration. In addition, we consider double saturation kinetics described by Monod reactions. We implement this extension in an ODE system (a 0-dimensional simplified reactor scale model) and in a PDE system (a highly nonlinear 2-dimensional density dependent cross-diffusion model). In the ODE system we consider antibiotics and carbon substrates are well mixed and biomass is attached to the substratum and not washed out whereas the
Figure 4.1: Illustration of an experiment of biofilm exposure to antibiotics. Biofilm activity is measured in terms of CO2 production. Initially biofilm activity increases and attains a plateau. At $t \approx 50h$ the biofilm is exposed to antibiotics for a short period of about 5 hours. At the begin of exposure CO2 production sharply spikes and then quickly decreases. Two different types of outcome have been observed: (a) after exposure biofilm activity decreases continuously and remains almost at zero level [top panel], (b) biofilm activity decreases to a very small level and after some time increases again to attain a plateau phase [bottom panel]. Experimental data were provided by Otini Kroukamp and Lindsay Jackson (Ryerson University).
PDE system considers spatial effects that include heterogeneous structures of biofilms and substrate gradients. We also extended the model here by adding new dependent variable, carbon dioxide concentration as a product, for both ODE and PDE system.

4.2 Model assumptions

We summarize here the model assumptions that are common to both models:

- The two main processes that we consider are biomass growth and inactivation of active biomass by antibiotics. Biomass is assumed to be sessile, colonizing a nonreactive surface; it is not washed out from the system. We assume that oxygen is available in abundance and does not limit process rates.

- Biomass growth depends on the local availability of a carbon substrate. We assume simple saturation kinetics, i.e. biomass production occurs at an approximately constant rate if the carbon nutrient is available in abundance. If it becomes limited, the biomass production rate becomes proportional to the nutrients available. During biomass growth carbon substrates are consumed [22].

- Exposure to antibiotics causes inactivation of active biomass. The inactivation rate is potentially limited by the local antibiotics con-
centration and by the local carbon concentration. We assume double saturation kinetics, i.e. if both concentrations are high, the inactivation rate is approximately constant, if one or both concentrations become low, the inactivation rate is proportional to that particular concentration [23].

- During inactivation, carbon is utilised at a rate proportional to the inactivation rate. This assumption is introduced here, based on new experimental data [23]; all other assumptions are established in the biofilm modelling literature.

- When carbon substrate is utilised, carbon dioxide is produced [23].

- Natural cell death occurs at a constant rate [4].

- Carbon and antibiotics are externally supplied to the system.

- As it is common in most biofilm modelling studies, EPS is not explicitly considered but implicitly lumped into the biomass [22, 38].

For the 0-dimensional description in Section 4.3 we make the following additional simplifying assumptions, which allow us to focus entirely on the role of the extended inactivation kinetics.

- The system is completely mixed. We neglect substrate gradients and assume that the concentration in the biofilm is the same as
in the bulk phase. This assumption has been made also, e.g. in the Freter model of growth with wall attachment [13], however, we do not put a limitation on the amount of biomass that can accumulate on the substratum.

The two-dimensional description in Section 4.4 that accounts for a spatial resolution of growth and inactivation in the biofilm colony is further based on the following standard assumptions from the biofilm modelling literature.

- We distinguish between the aqueous phase where no biomass is present and the actual biofilm which is implicitly defined as the region in which biomass is present. The biofilm region changes as more and more biomass is produced and the biofilm grows. Spatial expansion takes place when the local biomass density approaches an *a priori* known maximum density; as long as the biomass density remains clearly below this threshold, no notable biofilm expansion takes place [22].

- Active and inactive biomass are treated as particulate substances that occupy volume in the biofilm [8, 38].

- The carbon nutrient and the antibiotics are dissolved in the aqueous phase. They are transported in the aqueous and in the biofilm phase by Fickian diffusion, where the diffusion coefficients in the
biofilm phase are reduced compared to the diffusion coefficients in water [1, 33].

- We neglect the flowfield in the aqueous phase and its contribution to convective transport of dissolved substrates. Instead, carbon substrate and antibiotics are supplied to the biofilm by diffusion only, and carbon dioxide is removed by diffusion only. This assumption mimics a system in which the biofilm lies entirely in concentration boundary layers and where substrates are supplied from the bulk phase, or a biofilm system in a hydrostatic environment to which substrates are supplied or removed through a membrane. This also circumvents the computationally costly simulation of the governing equations of fluid mechanics and allows to focus our study on the role and effect of the new inactivation kinetics.

Based on the above assumptions, the models will be formulated in terms of the dependent variables densities of active and inactive biomass, carbon nutrient concentration, antibiotics concentration, and carbon-dioxide concentration. The latter is included as a measure of metabolic activity. A Petersen matrix that describes the reactions between the system components is given in Table 4.1. The parameters and variable are explained in Sections 4.3.
Table 4.1: Stoichiometric matrix of the reaction kinetics; the functions $f(C)$ and $g(A)$ describe the dependency of the growth and inactivation rates on carbon and antibiotics concentration. These are the Monod saturation functions $f(C) = \frac{C}{\beta_1 + C}$, $g(A) = \frac{A}{\beta_2 + A}$.

<table>
<thead>
<tr>
<th>#</th>
<th>process</th>
<th>active biomass</th>
<th>inactive biomass</th>
<th>carbon</th>
<th>antibiotics</th>
<th>carbon dioxide</th>
<th>process rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>growth</td>
<td>X</td>
<td>Y</td>
<td>C</td>
<td>A</td>
<td>P</td>
<td>$\rho_1$</td>
</tr>
<tr>
<td>2</td>
<td>cell death</td>
<td>-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>$k_2$</td>
</tr>
<tr>
<td>3</td>
<td>inactivation</td>
<td>-1</td>
<td>+1</td>
<td>$-\frac{\gamma_1}{\beta_1}$</td>
<td>$-\frac{\gamma_2}{\beta_1}$</td>
<td>$\xi$</td>
<td>$k_1 f(C) g(A) X$</td>
</tr>
</tbody>
</table>

4.3 A simple zero-dimensional biofilm model

4.3.1 Governing equations

In our first model we neglect all spatial effects and focus only on the inactivation kinetics. Following the model assumptions described in the previous Section 4.2, it is formulated in terms of the dependent variables active biomass density $X$, inactive biomass density $Y$, carbon concentration $C$, antibiotics concentration $A$, and carbon dioxide concentration $P$ as a product. We assume a completely mixed reactor with wall attachment.

The dissolved substrates $C$, $A$, $P$ are added to the system at a rate $q$ and at concentrations $C_\infty, A_\infty, P_\infty$ that may depend on time. The latter
assumption is in particular important for antibiotics, to mimic various dosing strategies. We will assume \( P_\infty \equiv 0 \). The dissolved substrates are removed from the reactor at the same constant dilution rate \( q \) and at the current reactor concentrations \( C, A, P \). Biomass fractions \( X \) and \( Y \) are neither added nor washed out from the system. They are assumed to be completely sessile.

Considering the assumptions of Section 4.2 and Table 4.1 the model reads

\[
\begin{align*}
\frac{dX}{dt} &= \mu f(C)X - k_1 g(A)f(C)X - k_2 X \\
\frac{dY}{dt} &= k_1 g(A)f(C)X \\
\frac{dC}{dt} &= -\mu f(C)X - \gamma_1 g(A)f(C)X - qC + qC_\infty(t) \\
\frac{dA}{dt} &= -\gamma_2 g(A)f(C)X - qA + qA_\infty(t) \\
\frac{dP}{dt} &= \xi \left( \frac{\mu}{\Upsilon} f(C)X + \gamma_1 g(A)f(C)X \right) - qP + qP_\infty(t)
\end{align*}
\]

with initial data \( A(0) = A_0, C(0) = C_0, P(0) = P_0, X(0) = X_0, Y(0) = Y_0 \). Typically we will assume \( A_0 = P_0 = Y_0 = 0, X_0 > 0, C_0 = C_\infty(0) \). In (4.1), the functions \( f(C) \) and \( g(A) \) describe the dependency of growth and inactivation on \( C \) and \( A \). We use standard Monod kinetics, i.e.

\[
f(C) := \frac{C}{\beta_1 + C}, \quad g(A) := \frac{A}{\beta_2 + A},
\]

where \( \beta_1 \) and \( \beta_2 \) are the half saturation concentrations.
In (4.1a), the parameter $\mu$ is the maximum specific growth rate for biomass growth, $k_1$ is the maximum inactivation rate, $k_2$ is the natural cell death rate. Biomass that is inactivated in (4.1a) is converted 1:1 into inactive biomass in (4.1b). The growth of active biomass in (4.1a) incurs consumption of substrate in (4.1c) at a rate proportional to the growth rate. Here $\Upsilon$ is a yield coefficient. Furthermore, biofilm response to antibiotics in (4.1a) incurs depletion of carbon in (4.1c) at a rate proportional to the inactivation rate with a maximum depletion rate $\gamma_1$. The last two terms in (4.1c) are removal of dissolved substrate from the system and addition of fresh substrate at bulk concentration $C_\infty(t)$. Disinfection of active biomass in (4.1a) leads to depletion of antibiotics at a rate proportional to the inactivation rate in (4.1d); parameter $\gamma_2$ here is the maximum antibiotics depletion rate due to inactivation. The last two terms in (4.1d) are removal of antibiotics from the system and addition of antibiotics at bulk concentration $A_\infty(t)$. Finally, in equation (4.1e) the conversion of carbon to carbon dioxide is described. All reaction that lead to a decrease in $C$ lead proportionally to an increase in $P$ with proportionality constant $\xi$. The last two terms in (4.1e) denote removal of carbon dioxide with dilution rate $q$ and supply of carbon dioxide to the system at flux $qP_\infty(t)$. Normally, we will assume that no carbon dioxide is added, i.e. $P_\infty(t) \equiv 0$.

The parameter $\gamma_1$ is non-negative and all other parameters are positive. The case $\gamma_1 = 0$ corresponds to the reaction kinetics that has been used
e.g. [26, 39]. Permitting also $\gamma_2 = 0$ would model an antibiotic that is not degraded during inactivation as in [26]. The parameter choices $\beta_{1,2} = 0$ or $\beta_{1,2} \to \infty$ describe the limiting cases of 1st and 0th order kinetics, as e.g. in [10] for antibiotics. We do not consider these cases.

Note that inactive biomass $Y$ and the carbon dioxide product $P$ do not appear in any of the equations for $X, C, A$. Therefore, system (4.1) can be simplified by eliminating (4.1b) and (4.1e). One obtains then

\[
\begin{align*}
\frac{dX}{dt} &= \mu f(C)X - k_1 g(A)f(C)X - k_2 X \\
\frac{dA}{dt} &= -\gamma_2 g(A)f(C)X - qA + qA_\infty \\
\frac{dC}{dt} &= -\frac{\mu}{Y} f(C)X - \gamma_1 g(A)f(C)X - qC + qC_\infty
\end{align*}
\]

This is the system we will consider from now on. Conclusions about the system (4.1) can be easily recovered from the system (4.3). $Y(t)$ can be obtained by integration of (4.1b) if $X, A, C$ are known. $P(t)$ is closely coupled to $C$ and can be computed from it:

**Lemma 4.3.1.** The solution components $C$ and $P$ of (4.1a)-(4.1e) sat-
isfy the relationship

\[ P(t) = e^{-qt} \left( q \int_0^t (P_\infty(\tau) + \xi C_\infty(\tau)) e^{q\tau} d\tau + P(0) + \xi C(0) \right) - \xi C(t) \]  

(4.4)

Proof. Adding (4.1c) and (4.1e) gives

\[ \frac{d}{dt} (\xi C + P) = q (\xi C_\infty + P_\infty - \xi C - P). \]

Let \( c(t) := \xi C(t) + P(t) \) and \( c_\infty(t) = \xi C_\infty(t) + P_\infty(t) \) then

\[ \frac{dc}{dt} = q(c_\infty(t) - c) \]

whence

\[ c(t) = e^{-qt} \left( q \int_0^t c_\infty(\tau)e^{q\tau} d\tau + c(0) \right). \]

The assertion follows with \( P(t) = c(t) - \xi C(t). \)

Remark 4.3.2. In many instances one will have \( C_\infty(t) = \text{const}, \)
\( P_\infty(t) = P(0) = 0. \) Then (4.4) simplifies greatly to

\[ P(t) = \xi C_\infty \left( 1 - e^{-qt} \right) + \xi C(0)e^{-qt} - \xi C(t) \]

Proposition 4.3.3. The initial value problem of (4.3a)-(4.3c) with non-negative initial data and non-negative functions \( X_\infty(t), C_\infty(t), A_\infty(t) \)

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possesses a unique, non-negative solution that exists for all $t > 0$.

**Proof.** Positive invariance of the non-negative cone follows from the tangent criterion with standard arguments [37]. In this cone the system satisfies a Lipschitz condition, which implies existence of a unique solution of the initial value problem.

$X(t)$ is bounded from above by the solution of $d\tilde{x}/dt = (\mu - k_2)\tilde{x}$ with $\tilde{x}(0) = X(0)$, and from below by the solution of $d\tilde{x}/dt = (-k_1 - k_2)\tilde{x}$ with $\tilde{x}(0) = X(0)$. Thus

$$X(0)e^{(-k_1-k_2)t} \leq X(t) \leq X(0)e^{(\mu-k_2)t}. \quad (4.5)$$

$C(t)$ is bounded from above by the solution of $d\tilde{c}/dt = q(C_\infty(t) - \tilde{c})$ with $\tilde{c}(0) = C(0)$. It is bounded from below by the solution of $d\tilde{c}/dt = -(\mu/\Upsilon + \gamma_1)X(0)e^{(\mu-k_2)t} - q\tilde{c} + qC_\infty(t)$ with $\tilde{c}(0) = C(0)$, i.e.

$$e^{-qt}\left(\int_0^t qC_\infty(\tau)e^{\gamma\tau}d\tau + C(0)\right) \geq C(t)$$

$$\geq e^{-qt}\left(\int_0^t \left[qC_\infty(\tau) - (\frac{\mu}{\Upsilon} + \gamma_1)X(0)e^{(\mu-k_2)\tau}\right] e^{\gamma\tau} d\tau + C(0)\right) \quad (4.6)$$

$A(t)$ is bounded from above by the solution of $d\tilde{a}/dt = q(A_\infty(t) - \tilde{a})$ with $\tilde{a}(0) = A(0)$. It is bounded from below by the solution of $d\tilde{a}/dt =$
\[-\gamma_2 X(0) e^{(\mu - k_2)t} - q\tilde{a} + qA_\infty(t)\] with \(\tilde{a}(0) = A(0)\), i.e.

\[
e^{-qt} \left( \int_0^t qA_\infty(\tau)e^{q\tau} d\tau + A(0) \right) \geq A(t)
\]

\[
\geq e^{-qt} \left( \int_0^t [qA_\infty(\tau) - \gamma_2 X(0)e^{(\mu - k_2)\tau}] e^{q\tau} d\tau + A(0) \right). \quad (4.7)
\]

The estimates (4.5), (4.6), (4.7) guarantee that the solution of the initial value problem exists for all \(t > 0\).

**Remark 4.3.4.** In many instances one will have \(C_\infty(t) = C_0 = \text{const}\) and \(C(0) = C_0\). Then estimate (4.6) simplifies greatly to

\[
C_0 \geq C(t) \geq C_0 - \frac{(\mu + \gamma_1)X(0)}{\mu - k_2 + q} (e^{(\mu - k_2)t} - e^{-qt})
\]

**Proposition 4.3.5.** If \(A_\infty = \text{const}, C_\infty = \text{const.}\), then (4.3) has an active biomass free equilibrium \(x_0 = (X, A, C)_{ABE} = (0, A_\infty, C_\infty)\), which is asymptotically stable iff

\[
\mu f(C_\infty) - k_1 g(A_\infty) f(C_\infty) - k_2 - q < 0.
\]
Proof. The Jacobian of the system in this equilibrium is

\[
J_0(0, A_\infty, C_\infty) = \begin{pmatrix}
\mu f(C_\infty) - k_1 g(A_\infty) f(C_\infty) - k_2 - q & 0 & 0 \\
-\gamma_2 g(A_\infty) f(C_\infty) & -q & 0 \\
-\mu^* f(C_\infty) - \gamma_1 g(A_\infty) f(C_\infty) & 0 & -q
\end{pmatrix}
\]  

(4.8)

and has eigenvalues

\[
\lambda_1 = -q \\
\lambda_2 = -q \\
\lambda_3 = \mu f(C_\infty) - k_1 g(A_\infty) f(C_\infty) - k_2 - q
\]

(4.9)

Eigenvalues \(\lambda_1, \lambda_2\) are always negative. Thus, asymptotic stability of the equilibrium is decided by eigenvalue \(\lambda_3\). \(\square\)

Remark 4.3.6. The above stability condition for the active biomass free equilibrium does not depend on the new parameter \(\gamma_1\), i.e, is independent of carbon consumption during exposure to antibiotics.

### 4.3.2 Simulation experiment for the ODE system

To investigate the effect of including carbon consumption during inactivation with antibiotics, we conduct a numerical simulation experiment. We consider (4.3) with constant inflow concentration of carbon, \(C_\infty = \text{const}\), and no carbon-dioxide on inflow, \(P_\infty = 0\). The inflow con-
Table 4.2: Summary of parameters used in the simulations of model (4.3a)-(4.3c); \(^1\) this parameter can be chosen arbitrarily, it introduces a scaling of \(P\); we choose it here such that \(C\) and \(P\) become comparable.

<table>
<thead>
<tr>
<th>parameter</th>
<th>symbol</th>
<th>value</th>
<th>unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum density of substrate</td>
<td>(C_\infty)</td>
<td>10.0</td>
<td>gm(^{-3})</td>
</tr>
<tr>
<td>Maximum density of antibiotics</td>
<td>(A_\infty)</td>
<td>1.0</td>
<td>gm(^{-3})</td>
</tr>
<tr>
<td>Maximum density of carbon dioxide</td>
<td>(P_\infty)</td>
<td>0.0</td>
<td>gm(^{-3})</td>
</tr>
<tr>
<td>Maximum specific growth rate for active biomass</td>
<td>(\mu)</td>
<td>6</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>Maximum disinfection rate for active biomass</td>
<td>(k_1)</td>
<td>16.0</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>Maximum carbon uptake rate due to disinfection</td>
<td>(\gamma_1)</td>
<td>varied</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>Maximum degradation rate for antibiotic</td>
<td>(\gamma_2)</td>
<td>100.0</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>Half saturation concentration for substrate</td>
<td>(\beta_1)</td>
<td>4.0</td>
<td>gm(^{-3})</td>
</tr>
<tr>
<td>Half saturation concentration for antibiotics</td>
<td>(\beta_2)</td>
<td>0.1</td>
<td>gm(^{-3})</td>
</tr>
<tr>
<td>Lysis rate for active biomass</td>
<td>(k_2)</td>
<td>0.4</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>Flow rate</td>
<td>(q)</td>
<td>2.0</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>Yield coefficient</td>
<td>(\Upsilon)</td>
<td>0.063</td>
<td>–</td>
</tr>
<tr>
<td>Conversion constant(^1)</td>
<td>(\xi)</td>
<td>1.0</td>
<td>–</td>
</tr>
</tbody>
</table>
centration of antibiotics, $A_{\infty}(t)$ is taken to mimic periodic exposure. We assume it to be $T_p$-periodic, where in each time interval $[iT_p, (i+1)T_p)$ the inflow concentration takes the constant value $A_m$ for a duration of $t_p$ and then 0; here $t_p < T_p$. We express this in terms of the rectangular window function $\Pi_{a,b}(t)$

$$A_{\infty}(t) = A_m \cdot \Pi_{iT_p,iT_p+t_p}(t), \quad \text{if } iT_p \leq t < (i+1)T_p. \quad (4.10)$$

With our simulation experiment we pursue two goals: (i) We want to explore whether the new term $-\gamma_1 g(A) f(C) X$ that was introduced in (4.3b) to account for increased substrate utilization during antibiotic exposure explains the characteristic patterns that were observed in the carbon dioxide measurements in [23]. (ii) If this is confirmed, we will investigate the effect that $\gamma_1$ has on the long term behavior of the system, i.e. to which extent this parameter effects the solution both quantitatively and qualitatively.

To this end we simulate the model with six different values for $\gamma_1$, leaving the other model parameters unchanged, as given in Table 4.2. The case $\gamma_1 = 0$ is included in our simulations, for which our reaction kinetics reduce to standard reaction kinetics that have been used elsewhere [10, 26, 39]. In our simulations we choose for the time between the onset of two subsequent exposure events, i.e. the time-period of the system, $T_p = 4$, and the length of the exposure period as $t_p = 0.1$. 

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Figure 4.2: Active biomass $X$ and carbon dioxide $P$ for different values 
$\gamma_1 = 0$, 40, 80, 120, 160, 200/d.
We assume that initially no inactive biomass, no antibiotics and no carbon dioxide is in the system. The initial data are

\[ X(0) = 0.5, \quad Y(0) = 0, \quad C(0) = C_{\infty}, \quad A(0) = 0.0, \quad P(0) = 0.0. \]

The two most important quantities of interest are active biomass \( X \) to observe the effect of antibiotic exposure on the bacteria, and the product carbon dioxide \( P \) for qualitative comparison with measured data. These are plotted in Figure 4.2. The simulations can be grouped into two categories.

For the smaller values \( \gamma_1 = 0, 40, 80 \), the active biomass is depleted after the second exposure interval. In these three cases, during the first exposure period \( t < t_p \), the active biomass declines quickly and is almost completely eradicated. In the subsequent no-exposure period \( t_p < t < T_p \), the active biomass recovers and begins to grow substantially toward the end of the interval, but we observe in all cases \( X(T_p) < X(0) \), i.e. after one full treatment period the active biomass in the system is smaller than it was initially. A second exposure interval follows, \( T_p < t < T_p + t_p \), during which again the active biomass is rapidly diminished. Since the initial amount in this interval was smaller than for the preceding interval, also the amount of biomass at the end of the interval is smaller. Too small, in fact, to notably bounce back and subsequent exposure intervals keep the amount of active biomass close to zero. In these three
simulations, \( \gamma_1 \) does not have a qualitative effect, but clear quantitative effects are observed, as it affects the level which the biomass reaches immediately before the second exposure interval. The behavior of the carbon dioxide concentration reflects the observations made for the active biomass. During the initial exposure period \( 0 < t < t_p \), \( P \) increases quickly, due to carbon consumption in growth and disinfection. In the subsequent recovery and growth period with no antibiotics, \( t_p < t < T_p \), the carbon dioxide concentration drops again initially, due to washout from the system and the extremely slow growth due to the small amount of bacteria. Toward the end of this no-exposure interval, when bacterial growth accelerates, also carbon dioxide production increases. As the second exposure period begins at \( t = T_p \), carbon dioxide production spikes and declines again after the exposure interval is over at \( t = T_p + t_p \). From here on the carbon dioxide concentration declines and remains at near 0 levels due to the lack of bacterial growth and hence substrate consumption.

The second group of data are the results for the larger values \( \gamma_1 = 120, 160, 200 \). First the system undergoes a transient period. Initially active biomass declines during the first exposure interval, \( 0 < t < t_p \). In the subsequent no-exposure interval \( t_p < t < T_p \) biomass growth resumes; the dependency of inactivation of carbon and the lower carbon values due to higher \( \gamma_1 \) values together lead to higher biomass amounts at the beginning of this no-exposure interval and therefore higher amount
of biomass at the end of this period. In fact we observe $X(T_p) > X(0)$, i.e. at the end of the first treatment period more active biomass is in the system as before. This qualitative patterns repeats over then next 2 or three exposure intervals and eventually, a periodic pattern for active biomass is attained. The loss of biomass during exposure to antibiotics is compensated by growth in the subsequent no-exposure period. The periodic solution itself appears little affected by the value of $\gamma_1$ after the initial transient period. This suggests that during the exposure periods the system becomes entirely substrate limited. The pattern of carbon dioxide $P$ is again closely aligned with the growth curves. At the beginning of the exposure intervals, when the active biomass drops, the carbon dioxide concentration jumps rapidly and remains at elevated level first and then dips down. This can again be explained by nearly complete substrate exhaustion. When the growth period resumes, carbon dioxide production resumes and $P$ levels of at the end of the no-exposure interval at the same value as it was before the preceding exposure event. This leveling off, together with the sub-exponential growth of biomass suggests again that the system is strongly substrate limited.

The importance of substrate limitations for the observed behavior is also supported by Figure 4.3, where the substrate concentration $C$ is plotted for all six different values of $\gamma_1$. In the three cases $\gamma_1 = 120, 160, 200$ the substrate concentration stays at low levels. During the exposure periods the substrate concentration declines rapidly to very low level. At the
Figure 4.3: Substrate concentration $C$ (top) and antibiotics concentration $A$ (bottom) for $\gamma_1 = 0, 40, 80, 120, 160, 200/day$. 
onset of the subsequent growth period it increases due to the smaller amount of biomass and hence lower growth activity. As the biofilm grows, the substrate levels off again at pre-exposure values. On the other hand, for lower values of $\gamma_1 = 0, 40, 80$ the substrate concentration dips down during the initial exposure period due to carbon consumption in inactivation and simultaneous growth. In the subsequent no-exposure period, the carbon concentration is replenished, because more substrate is externally added than is consumed in growth. Eventually, when the active biomass is fully diminished, the carbon concentration attains bulk concentration levels. Also shown in Figure 4.3 is the antibiotics concentration which in all cases spikes during the exposure periods and then declines due to washout.

In summary, we find that the carbon dioxide curves observed for larger values $\gamma_1$ resemble closely those observed in the experiments of [23], while those for smaller values of $\gamma_1$ do not. This suggests that during biofilm exposure to antibiotics indeed an increased consumption of carbon substrate takes place.

Comparing the results for the smaller values of $\gamma_1 \leq 80$ and the results for the larger values, $\gamma_1 \geq 120$ we observe two entirely different scenarios. In the former case, the active biomass is predicted to be entirely suppressed, whereas in the latter case it bounces back after each exposure interval; its overall growth and the levels that active biomass reaches is restricted due to substrate limitation, not due to antibiotic
activity. Since substrate supply is kept at a fixed level also the amount of bacteria that can be sustained is limited. While during periods of antibiotic exposure the biomass temporarily decreases, it grows back in the subsequent no-exposure periods. The substrate in the system is too low to allow growth at maximum growth rate.

This simple simulation experiment suggests that neglecting the role of carbon utilization during antibiotic disinfection in models of biofilm exposure to antibiotics, i.e the assumption can lead to false negatives. The model can falsely predict the absence of active biomass after few exposure intervals, when – if this effect is included – in fact the biomass is able to survive and is only temporarily affected by antibiotics but returns to pre-exposure levels afterwards, where the amount of biomass is limited by nutrient availability, but not by antibiotics.

4.4 Two-dimensional setting

4.4.1 Governing Equations

In the reactor scale 0-dimensional ODE model in Section 4.3 we neglected all spatial effects in order to focus on the reaction kinetics only. In order to account for heterogeneous conditions in biofilms due to substrate gradients, we cast the disinfection model as a two-dimensional biofilm model on the actual biofilm scale. We use the density dependent
diffusion-reaction biofilm framework that was originally introduced in [6] for a single species, single substrate biofilm. This model concept was previously used to study biofilm exposure to antibiotics in [4, 7, 8]. In [27] a major extension of this model was presented, where it was shown that it can be derived both from the continuous time, discrete space master equation and from the equations of conservation of mass and momentum. This lead to the introduction of cross-diffusion terms that were absent in earlier versions of the model. For our current study we revise and extend this model to include the effect of carbon consumption during inactivation and to account for carbon dioxide as a product of biofilm activity.

The amount of biomass that can be accommodated in a unit volume is limited, i.e. there is a maximum attainable biomass density $X_\infty$. In this model framework, biomass densities are described in terms of the volume fractions they occupy, i.e. they are bounded between 0 and 1. We denote these volume fractions of active and inactive biomass by $X(t, x)$ and $Y(t, x)$. The biomass densities are then $X_\infty X(t, x)$ and $X_\infty Y(t, x)$. The dissolved substrates carbon, antibiotics and carbon dioxide are described in terms of their concentrations $C(t, x), A(t, x), P(t, x)$.

The model is formulated for the rectangular domain $\Omega = [0, L] \times [0, H]$. 
It reads

\begin{align}
X_t & = \nabla(D_{11}(X,Y)\nabla X + D_{12}(X,Y)\nabla Y) + \mu \frac{CX}{\beta_1 + C} \\
& - k_1 \frac{A}{\beta_2 + A \beta_1 + C} - k_2 X \quad (4.11a) \\
Y_t & = \nabla(D_{21}(X,Y)\nabla X + D_{22}(X,Y)\nabla Y) + k_1 \frac{A}{\beta_2 + A \beta_1 + C} X \quad (4.11b) \\
C_t & = \nabla(d_C(M)\nabla C) - \frac{\mu X_\infty}{Y} \frac{C}{\beta_1 + C} X - \gamma_1 \frac{A}{\beta_2 + A \beta_1 + C} X \quad (4.11c) \\
A_t & = \nabla(d_A(M)\nabla A) - \gamma_2 \frac{A}{\beta_2 + A \beta_1 + C} X \quad (4.11d) \\
P_t & = \nabla(d_P(M)\nabla P) + \xi \left( \frac{\mu X_\infty}{Y} \frac{C}{\beta_1 + C} X + \gamma_1 \frac{A}{\beta_2 + A \beta_1 + C} X \right) \quad (4.11e) 
\end{align}

with

\[ M = X + Y. \]

The reaction terms and their parameters have the same meaning as in Section 4.3 and Table 4.1.

The proper biofilm region is implicitly defined as the region where the total biomass \( M \) is positive, i.e. the set \( \Omega_2(t) := \{ x \in \Omega : M(t, x) > 0 \} \).

The aqueous phase is then \( \Omega_1(t) := \{ x \in \Omega : M(t, x) \equiv 0 \} \). Both regions are separated by the biofilm/water interface \( \Gamma(t) := (\partial \Omega_1(t) \cap \partial \Omega_2(t)) \setminus \partial \Omega \). Both regions \( \Omega_1(t) \) and \( \Omega_2(t) \) change due to biofilm growth and expansion.
As in [26, 27], the self- and cross-diffusion coefficients $D_{ij}(X,Y)$, $i = 1, 2, j = 1, 2$ for active and inactive biomass are chosen as

\[
\begin{align*}
D_{11}(X,Y) &= \Phi(M) + X\Psi(M), & D_{12}(X,Y) &= X\Psi(M), \\
D_{21}(X,Y) &= Y\Psi(M), & D_{22}(X,Y) &= \Phi(M) + Y\Psi(M),
\end{align*}
\] (4.12)

where functions $\Phi$ is defined by

\[
\Phi(M) = \left(1 - \int_0^M D(s)ds\right) \frac{\int_0^M D(s)ds}{M}.
\] (4.13)

Here $D(M)$ is the density dependent diffusion coefficient of the single species model [6], see also [15, 11]

\[
D(M) = \delta \frac{M^a}{(1 - M)^b}, \quad \delta > 0.
\] (4.14)

This diffusion coefficient combines two non-Fickian effects. For $D(0) = 0$ the model degenerates like the porous medium equation, for $M = 1$ it attains a super-diffusion singularity. For small $M$ no major expansion of the biofilm takes place, where the biofilm/water interface spreads with finite speed. For large $M$ expansion starts. The super-diffusion singularity ensures that the maximum cell density is not exceeded. We have

\[
\int D(s)ds = \delta \frac{M^{a+1}}{a+1} F_1(a+1, b; a+2, M),
\]
where \( a \) and \( b \) are general exponents and \( _2 F_1 \) is the hypergeometric function. For specific integer choices of exponents \( a, b \) these integrals can be expressed in terms of elementary functions. The function \( \Psi(M) \) follows then from the equation \( D(M) = \Phi(M) + M\Psi(M) \) and (4.13).

In the absence of antibiotics and inert biomass this model reduces to the original prototype biofilm model of [6].

The diffusion coefficients of the dissolved substrates \( d_C(M), d_A(M), d_P(M) \) depend on the overall total biomass density \( M \) as well, but in a non-critical manner. They are bounded from above and below by positive values, namely by the diffusion coefficients in water, \( M = 0 \), and in a fully compressed biofilm, \( M = 1 \). The exact dependency of the diffusion coefficients on \( M \) is not known. Therefore, we make a linearization ansatz and interpolate between the two bounding values. This gives

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

Note that \( d_{C,A,P}(1) > 0 \) and \( d_{C,A,P}(1) \) and \( d_{C,A,P}(0) \) are within one order of magnitude. Solving the mesoscopic biofilm model such as (4.11) for an entire biofilm reactor is still out of reach. Therefore, the domain \( \Omega \) is only a section of a much larger system. The boundary conditions must be chosen to reflect this. For biofilm models this is still a challenging
problem to which no satisfactory solution exists in most cases. We use boundary conditions that are commonly used in biofilm modelling. In particular, we assume the bottom boundary to be part of the substratum on which the biofilm grows. We assume it to be impermeable to biomass, dissolved carbon and antibiotics, i.e.

$$\frac{\partial}{\partial n} X = \frac{\partial}{\partial n} Y = \frac{\partial}{\partial n} C = \frac{\partial}{\partial n} A = 0, \quad x_2 = 0$$

The top boundary, $x_2 = H$, is the boundary through which nutrient and antibiotics are added to the system. We do this by prescribing Dirichlet conditions there,

$$C = C_\infty, \quad A = A_{in} \cdot \Pi_{iT,T+iT_p}(t), \quad \text{if} \quad iT_p \leq t < (i+1)T_p, \quad x_2 = H$$

where $t_p$ and $T_p$ are defined as in (4.10), i.e. again we consider a short period of exposure to antibiotics, followed by a prolonged recovery and growth period without antibiotics, and then repeat this pattern.

For the biomass fractions we assume non-homogeneous Neumann conditions also at the top boundary, but this is not critical. As long as the biofilm interface does not reach the top boundary, and simulations will be terminated before that, homogeneous Neumann and homogenous Dirichlet conditions are satisfied simultaneously. We have thus

$$\frac{\partial}{\partial n} X = \frac{\partial}{\partial n} Y = 0, \quad x_2 = H$$
The situation is different for the product carbon dioxide. In experiments, it is removed from the system through the substratum on which the biofilm grows. To enforce a diffusion gradient of $P$ out of the system there, we pose homogenous Dirichlet conditions. On the top boundary we impose homogeneous Neumann conditions to ensure that the product does not leave the domain across this boundary. Thus, we have

$$P = 0, \quad x_2 = 0, \quad \partial_n P = 0, \quad x_2 = H.$$  

At the lateral boundaries, $x_1 = 0$ and $x_1 = L$, we assume symmetric reflecting boundary conditions which allow us to view $\Omega$ as a part of a longer system, i.e. we have

$$\partial_n X = \partial_n Y = \partial_n C = \partial_n A = \partial_n P = 0, \quad x_1 = 0, x_1 = L$$

We assume that initially there is no inactive biomass, no antibiotics and no carbon dioxide in the system, i.e. we have the initial conditions

$$Y(0, \cdot) = A(0, \cdot) = P(0, \cdot) = 0.$$  

The initial conditions for $X$ and $C$ are the results of a simulation of the prototype growth model. To this end the substratum was inoculated at random locations along the substratum. The biofilm region initially, $\Omega_2(0)$ thus consists of several colonies on the substratum.
The model (4.11) has three non-standard diffusion effects: (i) as $M = 0$, the model degenerates which indicates the finite speed of propagation of water/biofilm interface and gradient blow up at this interface, (ii) as $M = 1$, the model shows super-diffusion that describes volume filling effects, and (iii) the presence of non-linear cross-diffusion i.e $D_{12}(M)$ and $D_{21}(M)$ which describes the mixing of both active and inactive biomass as we assume that both biomass are moved together in our model. Each of these effects carries its own numerical challenge. In order to tackle the diffusion effects (i) and (ii), a semi-implicit Finite Volume Method has been developed in [9, 21, 22] for the numerical simulations of single-species biofilm models and dual-species model without cross-diffusion. To deal with cross-diffusional effects (iii), this method was extended in [26]. It requires to solve in each time step a sparse linear system for each of the five dependent variables. This is the computationally most expensive part of the solution procedure and parallelised for execution on shared memory and multi-core computers with OpenMP.

4.4.2 Numerical experiment

For our numerical simulations, we discretise the rectangular domain by a uniform grid of 800 × 200 cells. As in the case of the 0-dimensional model in Section 4.3, we vary the new parameter $\gamma_1$ to explore how accounting for substrate consumption during antibiotic exposure affects
Table 4.3: Model parameters used in the antibiotic model simulations.

<table>
<thead>
<tr>
<th>parameter</th>
<th>symbol</th>
<th>value</th>
<th>unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domain length</td>
<td>$L$</td>
<td>$4 \cdot 10^{-3}$</td>
<td>m</td>
</tr>
<tr>
<td>Domain height</td>
<td>$H$</td>
<td>$10^{-3}$</td>
<td>m</td>
</tr>
<tr>
<td>Bulk substrate concentration</td>
<td>$C_\infty$</td>
<td>30</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Relative max antibiotics concentration</td>
<td>$A_\infty$</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Maximum specific growth rate for active biomass</td>
<td>$\mu$</td>
<td>6</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Maximum disinfection rate for active biomass</td>
<td>$k_1$</td>
<td>15</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Maximum degradation rate for antibiotic $A$</td>
<td>$\gamma_2$</td>
<td>15</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Half saturation concentration for substrate $C$</td>
<td>$\beta_1$</td>
<td>4.0</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Half saturation concentration for antibiotics $A$</td>
<td>$\beta_2$</td>
<td>0.1</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Lysis rate for active biomass</td>
<td>$k_2$</td>
<td>0.4</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>Maximum biomass density for species $X$</td>
<td>$X_\infty$</td>
<td>10000</td>
<td>gm$^{-3}$</td>
</tr>
<tr>
<td>Yield coefficient for species $X$</td>
<td>$\Upsilon$</td>
<td>0.63</td>
<td>–</td>
</tr>
<tr>
<td>Substrate diffusion coefficient in water</td>
<td>$d_C(0)$</td>
<td>$10^{-4}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
<tr>
<td>Substrate biofilm/water diffusivity ratio</td>
<td>$\rho_C$</td>
<td>0.9</td>
<td>–</td>
</tr>
<tr>
<td>Antibiotics diffusion coefficient in water</td>
<td>$d_A(0)$</td>
<td>$0.7 \cdot 10^{-4}$</td>
<td>m$^2$d$^{-1}$</td>
</tr>
<tr>
<td>Antibiotics biofilm/water diffusivity ratio</td>
<td>$\rho_A$</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>$CO_2$ diffusion coefficient in water</td>
<td>$d_P(0)$</td>
<td>0.9</td>
<td>m$^2$d$^{-1}$</td>
</tr>
<tr>
<td>$CO_2$ biofilm/water diffusivity ratio</td>
<td>$\rho_P$</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

The model solution compared to the case of reaction kinetics that ignore this effect. All other parameters values are adapted from the standard biofilm modeling literature and are unchanged during the experiment; they are shown in Table 4.3. The values for the maximum substrate consumption rate due to response to antibiotics that we report the results for are $\gamma_1 = 0, 79362.00, 158731.20, 238095.00 \text{[g/(m}^3\text{d)}]}. The larger two of these values are higher than the maximum substrate degradation rate due to growth of $\mu X_\infty/\Upsilon = 95238.10 \text{[g/(m}^3\text{d)}]$, the smallest positive value lies below this value. $\gamma_1 = 0$ corresponds to the traditional reaction kinetics as used, e.g. in [26, 27].

In addition to 2D visualizations we report the following output param-
eters that can be computed from the simulation results.

total volume fraction occupied by \( X \)

\[
X_{\text{total}}(t) = \int_{\Omega} X \, dx
\]

average nutrient concentration \( C \)

\[
C_{\text{avg}}(t) = \frac{\int_{\Omega} C \, dx}{\int_{\Omega} dx}
\]

average antibiotics concentration \( A \)

\[
A_{\text{avg}}(t) = \frac{\int_{\Omega} A \, dx}{\int_{\Omega} dx}
\]

average product concentration \( P \)

\[
P_{\text{avg}}(t) = \frac{\int_{\Omega} P \, dx}{\int_{\Omega} dx}
\]

These quantities are plotted in Figure 4.4. The active biomass declines for all \( \gamma_1 \) rapidly during the first exposure event, after which it enters a growth phase. At the end of this growth phase the amount of active biomass is smaller than it was before the exposure event for the two smallest values of \( \gamma_1 \) \((X_{\text{total}}(T_P) < X_{\text{total}}(0))\), and larger for the two larger values of \( \gamma_1 \). During the first treatment interval, \( 0 < t < T_p \), the active biomass in the system is the larger the higher the \( \gamma_1 \) values are. For \( \gamma_1 = 0 \), the active biomass drops to near 0 during the second exposure interval and remains there for the rest of the simulation, i.e. the biofilm is completely controlled. For the second lowest \( \gamma_1 \) value, full suppression of active biomass is observed after the third exposure event. For the second highest \( \gamma_1 \) value the maximum and minimum amount of biomass in each cycle decreases after each exposure event and overall the biomass declines. For the largest \( \gamma_1 \) value, the active biomass increases from cycle
Figure 4.4: Active biomass, substrate, antibiotic and \( CO_2 \) for four different values of \( \gamma_1 \).
to cycle. Note that unlike the 0D model no periodic solution is attained. This is an artifact of the Dirichlet boundary conditions for the nutrient substrate. In Section 4.3 the amount of substrate supplied to the reactor was kept constant, which determines the amount of active biomass than can be sustained. The Dirichlet conditions for substrate in the 2D simulations on the other hand imply by virtue of the maximum principle that the more substrate is supplied to the domain the larger the biofilm, due to an increased diffusive flux into the system as a consequence of a stronger substrate sink. The higher amount of active biomass in the system goes hand in hand with lower substrate concentration values. In the case of the highest $\gamma_1$ values. During the exposure events we observe a rapid dip that is caused by increased substrate consumption during inactivation. The higher $\gamma_1$, the larger the dip. Naturally, this is reflected in spikes in the carbon dioxide concentration $P$, that are the larger the larger $\gamma_1$. After the spike due to antibiotic inactivation the amount of carbon dioxide drops to a minimum, due to the decreased growth activity because of smaller amounts of active biomass due to inactivation. As biomass resumes growth, the amount of carbon dioxide increases before the next exposure event. Also between to exposure events $P$ is larger for higher $\gamma_1$ values because of higher growth activity.

In summary, the 2D simulations confirm the observation of the 0D simulations in Section 4.3. In particular they show that if one includes consumption of substrates during inactivation by antibiotics in the model,
spikes in carbon dioxide production with a subsequent steep dip are observed, as in the experiments [23]. Also in these 2D simulations neglecting this process can lead to false negatives.

Having confirmed that the lumped parameters of the 2D simulation agree with the 0D simulations and the experimental observations qualitatively, investigate the effects of varying $\gamma_1$ on spatial effects in the 2D
Figure 4.6: 2D simulation for $\gamma_1 = 0$ at different times (cont’d); (a), (b) are taken shortly before the second exposure event, (c), (d) after this event, (e) during the growth period between the second and third event, (f) shortly before the third event. Shown is the amount of active biomass relative to total biomass, $R = \frac{X}{X + Y}$ (blue-red field data), the contour for antibiotics $A$ (black-white isolines) and carbon dioxide, $P$ (yellow-green isolines).
simulation. We visualize the simulations for $\gamma_1 = 0$ in Figures 4.5, 4.6 and for $\gamma_1 = 238095.1$ in Figures 4.7, 4.8.

For $\gamma_1 = 0$ in Figures 4.5 we observe that initially the biofilm is entirely dominated by active biomass but as exposure to antibiotics starts and active biomass is inactivated, it is composed equally of active and inactive biomass at $t = 0.10$ and dominated by inactive biomass at $t = 0.20$ with an approximately equal balance between both biomass types in the inner layers of the larger colonies. Inactivation continues and eventually at $t = 0.40$ and $t = 0.50$ the biofilm is predominantly inactive everywhere. Carbon dioxide production sets in immediately. Initially both production of new biomass and inactivation increase the levels of $P$, with most production in the larger colonies. Carbon dioxide production then declines as more and more active biomass in inactivated and no notable amount of carbon dioxide is produced at later times due to the lack of active biomass. In Figures 4.6 we observe that during the no-exposure period following the first inactivation event active biomass slowly regrows, but the biofilms remains dominated by inactive biomass. The biggest share of active biomass is found in the center of the largest colony. During the next exposure event, active biomass is further decimated and remains so. As there is not much active biomass left, no carbon dioxide production is observed.

For $\gamma_1 = 238095.00$ we begin with visualizing again the first exposure interval, cf. Figure 4.7. The biofilm starts being completely dominated
Figure 4.7: 2D simulation for $\gamma_1 = 238095.00$ at different times (cont’d) during the first exposure event. Shown is the amount of active biomass relative to total biomass, $R = \frac{X}{X+Y}$ (blue-red field data), the contour for antibiotics $A$ (black-white isolines) and carbon dioxide, $P$ (yellow-green isolines).
Figure 4.8: 2D simulation for $\gamma_1 = 238095.00$ at different times (cont’d); (a), (b) are taken shortly before the second exposure event, (c), (d) after this event, (e) during the growth period between the second and third event, (f) shortly before the third event. Shown the the amount of active biomass relative to total biomass, $R = \frac{X}{X+Y}$ (blue-red field data), the contour for antibiotics $A$ (black-white isolines) and carbon dioxide, $P$ (yellow-green isolines).
by active biomass. After it has been exposed for a short while, at $t = 0.10$ it is still primarily active but a thin layer dominated by inactive biomass forms. This inactive layers grows thicker as antibiotics diffuse into the biofilm from the bulk liquid, kill of the bacteria in the outer layer first in which step they are degraded themselves and they fail to penetrate into the inner layers closer to the substratum. As the inactive dominated layer increases the inner layers that are dominated by active biomass shrink. Both layers are separated by a relatively thin layer in which active and inactive biomass are balanced. Despite the presence of an active biomass dominated region no notable expansion of the biofilm is observed indicating that substrate is limited, which not only suppresses growth but protects the remaining active biomass from inactivation. This is also supported by the decrease in microbial activity, as indicated by the smaller carbon dioxide concentrations. Although by the end of the exposure period most of the biofilm is dominated by inactive biomass, a notable active core remains to initiate an immediate growth period after exposure to antibiotics stops.

The visualization of the simulation is continued in Figure 4.8. The first two panels, $t = 3.7, 3.9$ show the biofilm at the end of the first treatment cycle, before the second exposure period sets in. The biofilm structure has grown considerable. Most active biomass is produced in the inner layers that were sheltered from antibiotics during the first exposure event, and at the topmost layers, where nutrient conditions are most favourable.
to growth. Small regions dominated by inactive biomass remain primarily in peripheral layers of the larger colonies, and in a smaller colony. Since nutrients are transported by Fickian diffusion these smaller colony lies in a region of low substrate availability which prevents strong growth. After the second exposure event, at $t = 4.3, 4.5$, we observe again a strongly layered biofilm with a thick predominantly inactive outer layer and inner layer close to the substratum in which a considerable amount of biomass is active. As the growth period extends, new active biomass is produced again which leads to a growth of the biofilm structure which is occupied both by active and inactive biomass throughout. The biomass growth activity leads to increase carbon dioxide concentrations, in particular in and around the larger colonies.

The difference between the results with $\gamma_1 = 0$ and $\gamma_1 = 238095$ are striking. During the first exposure period, in the latter case disinfection lowers the carbon concentration. This slows down metabolic activity of the bacteria which are less susceptible to antibiotics and therefore inactivated less. In the inner layers, where substrate concentration are lowest this enables the formation of an active pocket. While decreased substrate availability also slows down production of new biomass, this is of minor consequence, since the maximum inactivation rate is substantially larger than the maximum growth rate, i.e. active biomass gain by growth is small compared to active biomass loss by inactivation. Hence, degradation of carbon during antibiotic exposure, helps the biofilm to protect
itself and to ensure survival of a larger population of active cells, which helps in the re-population of the colony and can guarantee survival or at least delay eradication.

4.5 Conclusion

In a laboratory experiment antibiotics are exposed to the biofilms. The observation from the experiment came out that carbon dioxide, a product, has been produced during the disinfection process (Figure 4.1). It is suggested that this is happening due to the consumption of carbon substrate at the time of metabolic activity. The experimental observation also note that carbon consumption is also high when antibiotics are exposed. We use mathematical models of disinfection of microbial biofilms that can help to interpret this experimental phenomenon and outcomes. As the existing models reaction kinetics fail to portray this specific scenario, we introduce a new reaction kinetics that is controlled by the local substrate concentration and by the local antibiotics concentration. We implement this extension through both the ODE- and PDE-systems. To capture the product carbon dioxide profile, we also add it as a new dependent variable in the system. Simulations are carried out using both system. The model without spatial effects shows that if carbon consumption during disinfection process is not considered, it may lead to the false negatives, i.e neglecting the assumption
of carbon utilization could predict that no active biomass survives after few intervals of exposure of antibiotics. On the other hand, carbon consumption during disinfection process helps active biomass to survive even after few intervals of exposure of antibiotics. We also present the simulation results using the model that includes spatial effects account for heterogenous condition in biofilms due to substrate gradients. This 2D model also reflects the results obtained from 0D model. More specifically, the 2D simulations show that if the model includes consumption of carbon substrate inactivation by antibiotics, spikes in carbon dioxide production with a subsequent steep dip are observed, as in the laboratory experiment results. In this case, during the disinfection process, utilization of carbon helps biofilm to protect itself from the action of antibiotics exposure and ultimately guarantees their survival and repopulation in the colony.
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Chapter 5

A generalized cross-diffusion model of spatially interacting populations and positivity of its solutions

Abstract

We derive a generalized model for $k$ interacting species by considering a continuous time and discrete space master equation passing to the continuous limit. It leads to a system of partial differential equations comprising self-diffusion, cross-diffusion and reaction terms. We present a positivity criterion for this generalized cross-diffusion model that allows to explicitly characterize the class of models preserving the non-negativity of solutions.
5.1 Introduction

Cross-diffusion models are used in mathematical ecology and other natural sciences to describe certain time and space dependent physical, chemical and biological processes. Different types of mathematical analysis of cross-diffusion models are found in scientific literature [2, 4, 5, 6, 7, 8, 24, 25, 30]. Most of them have considered Shigesada et al [27] and Lotka-Volterra [18, 31] types models in the presence of cross-diffusion effects. Shigesada et al [27] derive a cross-diffusion model for the populations of two competitive species in heterogeneous environments. In the mathematical formulation, a non-linear dispersive force due to mutual interferences of individuals and an environmental potential function are introduced as a behavioral version of Morisita’s phenomenological theory of \textit{Environmental density}. The heterogeneity of the environment and the non-linear dispersive movements raise a spatial segregation of the populations of two similar and competing species and there is a possibility that this spatial segregation acts to stabilize the coexistence of two similar species, relaxing the interspecific competition [27]. Chattopadhyay et al [3] has also presented a model for two competing species without showing any derivation.

Shigesada, Kawasaki and Teramoto The derivation or the formulation of mathematical model for two spatially interacting populations in a continuous time and discrete in space under homogeneous environment
has not been found so far in literature except [19]. The cross-diffusion models like Shigesada et al [27] and Chattopadhyay et al [3] that have been used in the literature to describe interacting species can be derived as special cases with the cross-diffusion model presented in [19]. In [19], no analytical analysis or simulation is carried out for the cross-diffusion model. According to [14], no general theory is available till now that covers all possible cross-diffusion models, even in the simplest case of only two coupled partial differential equations. Therefore, every single model case has to be considered separately. Furthermore, cross-diffusion effects are not that well studied in literature.

In this paper our main focus is to derive a generalized cross-diffusion model that includes spatial interactions among an arbitrary number of species. We use the approach of [22] for dual species model to derive the $k$ species model. The main feature of this class of models is that the diffusion matrix is non-symmetric, generally not positive definite and the non-diagonal matrix elements (the cross-diffusion terms) are allowed to be large [4]. We also like to investigate whether the solutions for our generalized cross-diffusion model starting from arbitrary non-negative initial data stay non-negative as long as they exist. An explicit characterization of the class of parabolic systems that preserve the positivity of solutions is important since it provides the modeller with a tool, which is easy to verify, to approach the question of the positive invariance of the model. For scalar parabolic equations the non-negativity of solutions
emanating from nonnegative initial data is a direct consequence of the maximum principle (see [17, 20]). However, for the cross-diffusion system of equations the maximum principle is not valid. Furthermore, for the cross-diffusion models with constant diffusion coefficients the positivity of solutions does not hold even if the nonnegative initial data is chosen.

This paper also presents some well known applications of the derivation such as Shigesada, Kawasaki and Teramoto cross-diffusion model, Chattopadhyay and Chatterjee cross-diffusion model, predator-Prey model, Keller-Segel model for chemotaxis, biofilm model etc. It is also shown that these models satisfy the assumptions of the positivity criterion.

5.2 A generalized cross-diffusion model for $k$ interacting species

A one-dimensional spatial lattice is a finite real line divided into a finite number of sections. It is created as an equidistant discretization of an interval. We consider the variables $u_i^j$, where $i = 1, \ldots, k$ and $j = 1, \ldots, m$, as the population densities for the $i$th species in the $j$th site on the lattice. The transfer functions that species $u_i$ moves from the $j$th grid cell into the neighbor cell $j \pm 1$ are denoted by $r_i^{j\pm}$. Since the site’s capacity to accommodate population density is limited, we
normalize the density with respect to the maximum density, i.e. we interpret it as the volume fraction of site $j$ occupied by the population. Thus, the conditions $0 \leq u_j^i < 1$ and $\sum_{i=1}^{n} u_j^i \leq 1$ are mandatory.

The master equation describes the population change of a particular species in a particular site, by balancing the density of populations which leaves the site to move into neighboring locations, and the density which arrives from neighboring sites. The difference between these densities is the population change of the species in the site. The master equation for $i$th species reads

$$\frac{\partial u_j^i}{\partial t} = \tau_i^{(j-1)+} u_i^{j-1} + \tau_i^{(j+1)-} u_i^{j+1} - (\tau_i^{j+} + \tau_i^{j-}) u_i^j + k_i^j u_i^j$$

(5.1)

where $k_i^j$ is the net $i$th species growth rate in grid cell $j$ and

$$\tau_i^{j\pm} = \alpha_i q_i(u_1^j, \ldots, u_k^j)p_i(u_1^{j\pm1}, \ldots, u_k^{j\pm1}).$$

(5.2)

Here, the constants $\alpha_i$ are scaling factors that depend on the length- and time-scale, i.e. the distance between two sites. The non-negative functions $q_i, p_i$ are transfer functions which control the local movements of a species from one site on the lattice to a neighboring site. They are taken to be continuous. In general, the transfer rates $\tau_i^{j\pm}$ can depend on the density of all populations in sites $j$ and $j \pm 1$. The transfer function $q_i(u_1^j, \ldots, u_k^j)$ is a measure of the desire of $i$th species $u_i^j$, that is currently
in site \( j \), to leave the cell; \( p_i(u_1^{j \pm 1}, \ldots, u_k^{j \pm 1}) \) represents the favorableness of the cell population \( u_i^j \) for the incoming individuals \( u_i^{j \pm 1} \) from sites \( j \pm 1 \).

In order to make the transition from a spatially discrete to a continuous model, we first introduce continuous functions \( u_i(t, x) \) that interpolate the grid functions \( u_i^j(t) \), i.e., \( u_i(t, x^j) = u_i^j(t) \). Assuming sufficient smoothness, we formally expand these functions around \( x^j \) in terms of the variable \( h \), which is the distance between two neighboring cells. We get

\[
u_i(t, x^{j \pm 1}) = u_i(t, x^j) \pm h \frac{\partial u_i(t, x^j)}{\partial x} + \frac{h^2}{2} \frac{\partial^2 u_i(t, x^j)}{\partial x^2} + \mathcal{O}(h^3) \quad (5.3)
\]

Shigesada, Kawasaki and Teramoto We also approximate the transfer functions \( q_i(u_1(t, x^{j \pm 1}), \ldots, u_k(t, x^{j \pm 1})) \) and \( p_i(u_1(t, x^{j \pm 1}), \ldots, u_k(t, x^{j \pm 1})) \) second order Taylor polynomials about
\[ u_i(t, x^j), \]

\[ q_i(u_1(t, x^{j\pm1}), \ldots, u_k(t, x^{j\pm1})) \approx q_i(u_1(t, x^j), \ldots, u_k(t, x^j)) \]

\[ + \sum_{l=1}^{k} \frac{\partial q_i}{\partial u_l}(u_1(t, x^j), \ldots, u_k(t, x^j))[u_l(t, x^{j\pm1}) - u_l(t, x^j)] \]

\[ + \frac{1}{2} \sum_{l=1}^{k} \sum_{m=1}^{k} \left( \frac{\partial^2 q_i}{\partial u_l \partial u_k}(u_1(t, x^j), \ldots, u_k(t, x^j)) \right) \]

\[ \left[ u_l(t, x^{j\pm1}) - u_l(t, x^j) \right] \left[ u_m(t, x^{j\pm1}) - u_m(t, x^j) \right] \],

\[ p_i(u_1(t, x^{j\pm1}), \ldots, u_k(t, x^{j\pm1})) \approx p_i(u_1(t, x^j), \ldots, u_k(t, x^j)) \]

\[ + \sum_{l=1}^{k} \frac{\partial p_i}{\partial u_l}(u_1(t, x^j), \ldots, u_k(t, x^j))[u_l(t, x^{j\pm1}) - u_l(t, x^j)] \]

\[ + \frac{1}{2} \sum_{l=1}^{k} \sum_{m=1}^{k} \left( \frac{\partial^2 p_i}{\partial u_l \partial u_k}(u_1(t, x^j), \ldots, u_k(t, x^j)) \right) \]

\[ \left[ u_l(t, x^{j\pm1}) - u_l(t, x^j) \right] \left[ u_m(t, x^{j\pm1}) - u_m(t, x^j) \right] \].

Now, we write \( \hat{u} = (u_1(t, x^j), \ldots, u_k(t, x^j)) \) and substituting \( \tau_i^{j\pm} \) and the approximations for \( u_i(t, x^{j\pm1}), q_i(u_1(t, x^{j\pm1}), \ldots, u_k(t, x^{j\pm1})) \) and
\[ p_i(u_1(t, x^{j\pm 1}), \ldots, u_k(t, x^{j\pm 1})) \] into (5.1). We obtain

\[
\frac{\partial u_i(t, x^j)}{\partial t} = \alpha_i h^2 \left[ u_i(t, x^j) p_i(\hat{u}) \sum_{l=1}^{k} \frac{\partial q_i}{\partial u_l} \frac{\partial^2 u_l(t, x^j)}{\partial x^2} + p_i(\hat{u}) q_i(\hat{u}) \frac{\partial^2 u_i(t, x^j)}{\partial x^2} \right. \\
+ u_i(t, x^j) p_i(\hat{u}) \sum_{l=1}^{k} \sum_{m=1}^{k} \frac{\partial^2 q_i}{\partial u_l \partial u_m} (\hat{u}) \frac{\partial u_l(t, x^j)}{\partial x} \frac{\partial u_m(t, x^j)}{\partial x} \\
+ 2 p_i(\hat{u}) \frac{\partial u_i(t, x^j)}{\partial x} \sum_{l=1}^{k} \frac{\partial q_i}{\partial u_l} (\hat{u}) \frac{\partial u_l(t, x^j)}{\partial x} \\
- u_i(t, x^j) q_i(\hat{u}) \sum_{l=1}^{k} \frac{\partial p_i}{\partial u_l} (\hat{u}) \frac{\partial^2 u_l(t, x^j)}{\partial x^2} \\
- u_i(t, x^j) q_i(\hat{u}) \sum_{l=1}^{k} \sum_{m=1}^{k} \frac{\partial^2 p_i}{\partial u_l \partial u_m} (\hat{u}) \frac{\partial u_l(t, x^j)}{\partial x} \frac{\partial u_m(t, x^j)}{\partial x} \left. \right] \\
+ k_i(t, x^j) u_i(t, x^j) + \mathcal{O}(h^3)
\]

After dropping all \( \mathcal{O}(h^3) \) terms, passing to the continuous limit, \( h \to 0 \), and rearranging the order of terms we obtain the general system of equations as

\[
\frac{\partial u_i}{\partial t} = \alpha_{i0} \frac{\partial}{\partial x} \left( \sum_{l=1}^{k} d_{il}(u) \frac{\partial u_l}{\partial x} \right) + k_i u_i, \quad i = 1, \ldots, k, \quad (5.4)
\]

where \( u = (u_1, \ldots, u_k) \), \( \lim_{h \to 0} \alpha_i h^2 = \alpha_{i0} > 0 \), and \( d(u) = (d_{il}(u))_{1 \leq i, l \leq k} \) is the diffusion matrix with components
\[ d_{ii}(u) = u_i \left( p_i(u) \frac{\partial q_i}{\partial u_i} - q_i(u) \frac{\partial p_i}{\partial u_i} \right) + p_i(u) q_i(u) \quad \text{if } i = l, \]
\[ d_{il}(u) = u_i \left( p_i(u) \frac{\partial q_i}{\partial u_l} - q_i(u) \frac{\partial p_i}{\partial u_l} \right) \quad \text{if } i \neq l. \]

The same procedure applied on a two- or three-dimensional spatial lattice leads to the cross diffusion system

\[ \frac{\partial u_i}{\partial t} = \alpha_{0i} \nabla \left( \sum_{l=1}^{k} d_{il}(u) \nabla u_l \right) + k_i u_i, \quad i = 1, \ldots, k. \quad (5.5) \]

In particular, for two species, i.e. \( k = 2 \), we write \( u_1 = u, u_2 = v \), \( p_1 = p, p_2 = q, q_1 = s, q_2 = r \), and the model takes the form

\[
\begin{align*}
    u_t & = \alpha_{01} \nabla (d_{11}(u,v) \nabla u + d_{12}(u,v) \nabla v) + k_1 u \\
    v_t & = \alpha_{02} \nabla (d_{21}(u,v) \nabla u + d_{22}(u,v) \nabla v) + k_2 v,
\end{align*}
\]

where

\begin{align*}
    d_{11}(u,v) & = pq + u(p \frac{\partial q}{\partial u} - q \frac{\partial p}{\partial u}) \\
    d_{12}(u,v) & = u(p \frac{\partial q}{\partial v} - q \frac{\partial p}{\partial v}) \\
    d_{21}(u,v) & = v(s \frac{\partial r}{\partial u} - r \frac{\partial s}{\partial u}) \\
    d_{22}(u,v) & = sr + v(s \frac{\partial r}{\partial v} - r \frac{\partial s}{\partial v}).
\end{align*}
5.3 A positivity criterion

In this section we derive explicit necessary and sufficient conditions for the non-negativity of solutions for multi-species cross-diffusion systems of the form

$$\frac{\partial}{\partial t} u = \nabla \cdot (D(u) \cdot \nabla u) + f(u) \quad \Omega \times (0, T),$$

$$u|_{\partial \Omega} = 0 \quad \partial \Omega \times [0, T],$$

$$u|_{t=0} = u_0 \quad \Omega \times \{0\},$$

where $$u = (u_1, \ldots, u_k) : \Omega \times [0, T] \to \mathbb{R}^k$$, $$k \in \mathbb{N}$$, is a vector-valued function of the spatial variable $$x \in \Omega$$ and time $$t \in [0, T]$$. Here, $$T > 0$$ and $$\Omega \subset \mathbb{R}^n$$, $$n \in \mathbb{N}$$, denotes a bounded domain with boundary $$\partial \Omega$$.

Here and in the sequel, the partial derivatives $$\partial_t$$ and $$\partial_{x_l}$$, $$1 \leq l \leq n$$, as well as the Laplace operator $$\Delta = \Delta_x$$ and the gradient $$\nabla = \nabla_x$$ are applied componentwise to the vector-valued function $$u$$.

We will formulate explicit conditions on the matrix $$D$$ and the interaction function $$f$$ such that the solutions of system (5.6) preserve positivity.

Let $$L^p(\Omega; \mathbb{R}^k)$$, where $$1 \leq p \leq \infty$$, be the space of vector-valued functions $$u : \Omega \to \mathbb{R}^k$$ such that the components satisfy $$u_i \in L^p(\Omega)$$, $$1 \leq i \leq k$$. The scalar product in the Hilbert space $$L^2(\Omega; \mathbb{R}^k)$$ is defined by

$$\langle u, v \rangle_{L^2(\Omega; \mathbb{R}^k)} := \sum_{i=1}^k \langle u_i, v_i \rangle_{L^2(\Omega)} \quad u, v \in L^2(\Omega; \mathbb{R}^k).$$
For vectors $y \in \mathbb{R}^k$ we write $y \geq 0$ if the inequality is satisfied componentwise, i.e.,

$$y_i \geq 0 \quad \text{for all } 1 \leq i \leq k,$$

and denote all non-negative vectors by $\mathbb{R}_+^k := \{ y \in \mathbb{R}^k \mid y \geq 0 \}$.

**Definition 5.3.1.** The **positive cone** in $L^2(\Omega; \mathbb{R}^k)$ is the set

$$K^+ := \{ u \in L^2(\Omega; \mathbb{R}^k) \mid u \geq 0 \text{ a.e. in } \Omega \}.$$

Moreover, we say that system (5.6) fulfils the **positivity property** if for every initial data $u_0 \in K^+$ the corresponding solution $u(\cdot, \cdot; u_0) : \Omega \times [0, t_{max}] \to \mathbb{R}^k$ satisfies

$$u(\cdot, t; u_0) \in K^+ \quad \text{for } t \in [0, t_{max}],$$

where $t_{max} > 0$ and $[0, t_{max}]$ denotes the maximal existence interval of the solution.

The following theorem characterizes the class of cross-diffusion systems (5.6) that satisfy the positivity property.
Under our assumptions the system (5.6) can be expressed as

\[ \partial_t u = D(u) \cdot \Delta u + \sum_{i=1}^{k} \partial_l D(u) \begin{pmatrix} \nabla u_l \cdot \nabla u_1 \\ \vdots \\ \nabla u_l \cdot \nabla u_k \end{pmatrix} + f(u), \]

where \( \partial_l D(u) = (\partial_l D_{ij}(u))_{1 \leq i,j \leq k} \) for \( 1 \leq l \leq n \).

**Theorem 5.3.2.** We assume the diffusion matrix \( D(u) = (D_{ij}(u))_{1 \leq i,j \leq k} \) is density-dependent with continuously differentiable coefficient functions \( D_{ij} : \mathbb{R}^k \to \mathbb{R} \) and the diagonal elements of the matrix \( D(u) \) are strictly positive,

\[ D_{ii}(u) \geq \mu_i \quad \text{for all} \quad i = 1, \ldots, k, \quad (5.7) \]

where the constant \( \mu_i > 0 \). We also assume that the interaction function \( f = (f_1, \ldots, f_k) \) is continuously differentiable,

\[ f \in C^1(\mathbb{R}^k; \mathbb{R}^k). \quad (5.8) \]

We also assume that for every initial data \( u_0 \in K^+ \) there exists a unique solution of system (5.6), and the solution and its derivatives with respect
to \( x \) satisfy \( L^\infty \)-estimates,

\[
  u(\cdot, t; u_0), \quad \partial_x u(\cdot, t; u_0) \in L^\infty(\Omega; \mathbb{R}^k) \quad \text{for} \quad t \in [0, t_{\max}], \quad 1 \leq l \leq n.
\]

(5.9)

Let the initial data \( u_0 \in K^+ \) satisfy the compatibility conditions. Then, system (5.6) satisfies the positivity property if and only if the coefficient functions of the matrix \( D \) fulfill

\[
    D_{ij}(y) = 0 \quad \text{for all} \quad y \in \mathbb{R}^k_+ \text{ such that } y_i = 0,
\]

where \( i \neq j, 1 \leq i, j \leq k \), and the interaction function \( f \) satisfies

\[
    f_i(y) = 0 \quad \text{for all} \quad y \in \mathbb{R}_+^k \text{ such that } y_i = 0,
\]

where \( 1 \leq i \leq k \).

The conditions on the functions \( D_{ij} \) imply that the diffusion matrix can
be written as

\[
D(u) = \begin{pmatrix}
  D_{11}(u) & u_1d_{12}(u) & u_1d_{13}(u) & \cdots & u_1d_{1k}(u) \\
  u_2d_{21}(u) & D_{22}(u) & u_2d_{23}(u) & \cdots & u_2d_{2k}(u) \\
  \vdots & \vdots & \vdots & \ddots & \vdots \\
  u_kd_{k1}(u) & u_kd_{k2}(u) & u_kd_{k3}(u) & \cdots & D_{kk}(u)
\end{pmatrix}
\]

with bounded functions \(d_{ij}(u), i \neq j, 1 \leq i, j \leq k\).

**Proof. Necessity:** We assume the solution \(u = u(\cdot, \cdot; u_0) : \Omega \times [0, t_{\text{max}}[ \rightarrow \mathbb{R}^k\) corresponding to initial data \(u_0 \in K^+\) remains non-negative for \(t > 0\) and prove the necessity of the stated conditions.

In the following we make formal calculations, for their validity we refer to [16]. Taking smooth initial data \(u_0\) and an arbitrary function \(v \in K^+\), which is orthogonal to \(u_0\) in \(L^2(\Omega; \mathbb{R}^k)\), we obtain

\[
\left\langle \partial_t u |_{t=0}, v \right\rangle_{L^2(\Omega; \mathbb{R}^k)} = \left\langle \lim_{t \to 0^+} \frac{u(\cdot, t; u_0) - u_0}{t}, v \right\rangle_{L^2(\Omega; \mathbb{R}^k)}
\]

\[
= \lim_{t \to 0^+} \left\langle \frac{u(\cdot, t; u_0)}{t}, v \right\rangle_{L^2(\Omega; \mathbb{R}^k)} - \lim_{t \to 0^+} \left\langle \frac{u_0}{t}, v \right\rangle_{L^2(\Omega; \mathbb{R}^k)}
\]

\[
= \lim_{t \to 0^+} \left\langle \frac{u(\cdot, t; u_0)}{t}, v \right\rangle_{L^2(\Omega; \mathbb{R}^k)} \geq 0,
\]

where we used the orthogonality of \(u_0\) and \(v\) as well as the hypothesis \(u(\cdot, t; u_0) \in K^+\) for \(t > 0\), and \(t \to 0^+\) denotes the right derivative. We
remark that for the particular initial data $u_0$ that we will choose below there always exists an orthogonal element $v \in K^+$. On the other hand, since $u$ is the solution of system (5.6) corresponding to initial data $u_0$, we observe

$$\langle \partial_t u|_{t=0}, v \rangle_{L^2(\Omega;\mathbb{R}^k)} = \langle D(u_0) \cdot \Delta u_0 + \sum_{l=1}^k \partial_l D(u_0) \left( \begin{array}{c} \nabla u_l \cdot \nabla u_1 \\ \vdots \\ \nabla u_l \cdot \nabla u_k \end{array} \right), v \rangle_{L^2(\Omega;\mathbb{R}^k)}$$

$$+ \langle f(u_0), v \rangle_{L^2(\Omega;\mathbb{R}^k)} \geq 0,$$

(5.10)

where $\partial_l D(u_0) = (\partial_l D_{ij}(u_0))_{1 \leq i,j \leq k}$. In particular, for fixed $i \in \{1, \ldots, k\}$ choosing the functions $u_0 = (\tilde{u}_1, \ldots, 0, \ldots, \tilde{u}_k)$ and $v = (0, \ldots, \tilde{v}, \ldots, 0)$ with $u_0, v \in K^+$ leads to the scalar inequality

$$\langle \sum_{j=1,j\neq i}^k D_{ij}(u_0) \Delta \tilde{u}_j + \sum_{l=1,l\neq i}^k \sum_{j=1,j\neq i}^k \partial_l D_{ij}(u_0) \nabla \tilde{u}_l \cdot \nabla \tilde{u}_j + f_i(u_0), \tilde{v} \rangle_{L^2(\Omega)} \geq 0.$$

Since this inequality holds for arbitrary non-negative $\tilde{v} \in L^2(\Omega)$, we obtain the pointwise estimate

$$\sum_{j=1,j\neq i}^k D_{ij}(u_0) \Delta \tilde{u}_j + \sum_{l=1,l\neq i}^k \sum_{j=1,j\neq i}^k \partial_l D_{ij}(u_0) \nabla \tilde{u}_l \cdot \nabla \tilde{u}_j + f_i(u_0) \geq 0 \quad \text{a.e. in } \Omega.$$
This implies that

$$D_{ij}(\tilde{u}_1, \ldots, 0, \ldots, \tilde{u}_k) = 0 \quad \tilde{u}_j \geq 0, \ j \neq i,$$

for all $1 \leq j \leq k$, and the functions $D_{ij}$ can be written as

$$D_{ij}(u) = u_id_{ij}(u), \quad 1 \leq i, j \leq k, \ j \neq i.$$

Consequently, for $j \neq i, l \neq i$ we obtain $\partial_l D_{ij}(u) = u_i \partial_l d_{ij}(u)$, and it follows that $\partial_l D_{ij}(u_0) = 0$. From Inequality (5.11) we now deduce that the components of the interaction term satisfy

$$f_i(\tilde{u}_1, \ldots, 0, \ldots, \tilde{u}_k) \geq 0 \quad \tilde{u}_j \geq 0, \ j \neq i,$$

for all $1 \leq i, j \leq k$.

**Sufficiency:** We show that the stated conditions on $D$ and $f$ ensure that the solution $u = u(\cdot, \cdot; u_0)$ corresponding to initial data $u_0 \in K^+$ remains non-negative. First, we assume that the conditions on the coefficient functions $D_{ij}$ and the interaction functions $f_i$ are satisfied for all $y \in \mathbb{R}^k$ such that $y_i = 0, 1 \leq i \leq k$. The system of equations takes the form

$$\partial_t u_i = \nabla (D_{ii}(u) \nabla u_i + \sum_{j=1, j \neq i}^k u_id_{ij}(u) \nabla u_j) + f_i(u), \quad 1 \leq i \leq k,$$

where $\nabla$ denotes the gradient operator.
where the functions $d_{ij} : \mathbb{R}^k \to \mathbb{R}$ are defined by

$$d_{ij}(y) := \int_0^1 \partial_i D_{ij}(y_1, \ldots, s y_i, \ldots, y_k) ds, \quad y \in \mathbb{R}^k.$$  

For a function $u \in L^2(\Omega)$ we denote its positive and negative part by $u_+ := \max\{u, 0\}$ and $u_- := \max\{-u, 0\}$, respectively, and obtain the representation $u = u_+ - u_-$. Its absolute value is given by $|u| = u_+ + u_-$. By the definition immediately follows $u_- u_+ = 0$. Furthermore, if $u \in H^1(\Omega)$, then also its positive and negative part, $u_+, u_- \in H^1(\Omega)$, and

$$\partial_{x_l} u_- = \begin{cases} -\partial_{x_l} u & u < 0 \\ 0 & u \geq 0 \end{cases} \quad \partial_{x_l} u_+ = \begin{cases} \partial_{x_l} u & u > 0 \\ 0 & u \leq 0 \end{cases}$$

for all $1 \leq l \leq n$ (cf. [13]). This implies

$$(\partial_{x_l} u_+) u_- = u_+ \partial_{x_l} u_- = (\partial_{x_l} u_+) \partial_{x_m} u_- = 0 \quad 1 \leq l, m \leq n.$$  

In order to prove the positivity of the solution $u$ corresponding to initial data $u_0 \in K^+$ we show that $(u_0)_- = 0$ implies that $u_- := (u_i(\cdot, t; u_0))_- = 0$ for $t > 0$ and all $1 \leq i \leq k$. Multiplying the $i$-th
equation by the negative part $u_-$ and integrating over $\Omega$ yields

$$
\langle \partial_t u_i, u_i- \rangle_{L^2(\Omega)} = \langle \nabla(D_{ii}(u) \nabla u_i), u_i- \rangle_{L^2(\Omega)} + \sum_{j=1, j\neq i}^k \langle \nabla(u_j d_{ij}(u) \nabla u_j), u_i- \rangle_{L^2(\Omega)} + \langle f_i(u), u_i- \rangle_{L^2(\Omega)}.
$$

We observe that the left-hand side of the equation can be written as

$$
\langle \partial_t u_i, u_i- \rangle_{L^2(\Omega)} = -\langle \partial_t u_i-, u_i- \rangle_{L^2(\Omega)} = -\frac{1}{2} \partial_t \| u_i- \|^2_{L^2(\Omega)}.
$$

Taking into account the homogeneous Dirichlet boundary conditions we obtain for the diffusion terms

$$
\langle \nabla(D_{ii}(u) \nabla u_i), u_i- \rangle_{L^2(\Omega)} = -\langle D_{ii}(u) \nabla u_i, \nabla u_i- \rangle_{L^2(\Omega; \mathbb{R}^n)} = \langle D_{ii}(u) \nabla u_i-, \nabla u_i- \rangle_{L^2(\Omega; \mathbb{R}^n)},
$$

$$
\sum_{j=1, j\neq i}^k \langle \nabla(u_j d_{ij}(u) \nabla u_j), u_i- \rangle_{L^2(\Omega)} = -\sum_{j=1, j\neq i}^k \langle u_j d_{ij}(u) \nabla u_j, \nabla u_i- \rangle_{L^2(\Omega; \mathbb{R}^n)} = \sum_{j=1, j\neq i}^k \langle u_i- d_{ij}(u) \nabla u_j, \nabla u_i- \rangle_{L^2(\Omega; \mathbb{R}^n)}.
$$

We estimate the last term by

$$
\left| \sum_{j=1, j\neq i}^k \langle u_i- d_{ij}(u) \nabla u_j, \nabla u_i- \rangle_{L^2(\Omega; \mathbb{R}^n)} \right| \leq c_1 \sum_{l=1}^n \langle |\partial_{x_l} u_i-|, u_i- \rangle_{L^2(\Omega)},
$$

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for some constant \( c_1 \geq 0 \), where we used the hypothesis (5.9).

To estimate the interaction term we use that \( f \in C^1(\mathbb{R}^k; \mathbb{R}^k) \), which leads to

\[
\begin{align*}
 f_i(u_1, \ldots, u_k) &= f_i(u_1, \ldots, 0_i, \ldots, u_k) \\
 &+ u_i \int_0^1 \partial_i f_i(u_1, \ldots, su_i, \ldots, u_k) ds \\
 &= f_i(u_1, \ldots, 0_i, \ldots, u_k) + u_i F_i(u_1, \ldots, u_k),
\end{align*}
\]

where the function \( F_i : \mathbb{R}^k \to \mathbb{R} \) is bounded. This representation yields

\[
\langle f_i(u), u_i- \rangle_{L^2(\Omega)} = \langle f_i(u_1, \ldots, 0_i, \ldots, u_k), u_i- \rangle_{L^2(\Omega)} \\
+ \langle u_i F_i(u_1, \ldots, u_k), u_i- \rangle_{L^2(\Omega)} \\
= \langle f_i(u_1, \ldots, 0_i, \ldots, u_k), u_i- \rangle_{L^2(\Omega)} \\
- \langle F_i(u_1, \ldots, u_k)u_i-, u_i- \rangle_{L^2(\Omega)}.
\]

Summing up all terms we obtain

\[
\begin{align*}
\frac{1}{2} \partial_t \| u_i- \|^2_{L^2(\Omega)} + \langle D_{ii}(u) \nabla u_i-, \nabla u_i- \rangle_{L^2(\Omega; \mathbb{R}^n)} \\
\leq c_1 \sum_{l=1}^n \langle |\partial_{x_l} u_i-|, u_i- \rangle_{L^2(\Omega)} + c_2 \| u_i- \|^2_{L^2(\Omega)} \\
- \langle f_i(u_1, \ldots, 0_i, \ldots, u_k), u_i- \rangle_{L^2(\Omega)},
\end{align*}
\]

for some constants \( c_1, c_2 \geq 0 \).
To estimate the mixed terms we use Young’s inequality; for every \( \epsilon > 0 \) there exists a constant \( C_\epsilon \geq 0 \) such that

\[
\sum_{l=1}^{n} \langle |\partial_x u_i|, u_i \rangle_{L^2(\Omega)} \leq \epsilon \| \nabla u_i \|_{L^2(\Omega; \mathbb{R}^n)}^2 + C_\epsilon \| u_i \|_{L^2(\Omega)}^2.
\]

If we choose \( \epsilon > 0 \) sufficiently small and take hypothesis (5.7) into account, it follows that

\[
\partial_t \| u_i \|_{L^2(\Omega)}^2 \leq c_3 \| u_i \|_{L^2(\Omega)}^2 - 2 \langle f_i(u_1, \ldots, 0, \ldots, u_k), u_i \rangle_{L^2(\Omega)},
\]

for some constant \( c_3 \geq 0 \). Since in the beginning we assumed that \( f_i(y) \geq 0 \) for all \( y \in \mathbb{R}^k \) such that \( y_i = 0, 1 \leq i \leq k \), we obtain the estimate

\[
\partial_t \| u_i \|_{L^2(\Omega)}^2 \leq c_3 \| u_i \|_{L^2(\Omega)}^2.
\]

By Gronwall’s Lemma and the initial condition \( (u_0)_{i-} = 0 \) we conclude \( \| u_i \|_{L^2(\Omega)} = 0 \).

It remains to justify our initial assumptions on the functions \( f_i \) and \( D_{ij} \), \( 1 \leq i, j \leq k, j \neq i \). To this end we consider the modified system

\[
\partial_t \hat{u} = \nabla \cdot (\hat{D}(\hat{u}) \cdot \nabla \hat{u}) + \hat{f}(\hat{u}) \quad \Omega \times (0, T),
\]

\[
\hat{u} \big|_{\partial \Omega} = 0 \quad \partial \Omega \times [0, T],
\]

\[
\hat{u} \big|_{t=0} = u_0 \quad \Omega \times \{0\}.
\]
where the function $\hat{f} : \mathbb{R}^k \rightarrow \mathbb{R}^k$ is given by

$$\hat{f}_i(y) = f_i(|y_1|, \ldots, 0, \ldots, |y_k|) + y_i F_i(y) \quad y \in \mathbb{R}^k,$$

and the function $F_i$ was defined as

$$F_i(y_1, \ldots, y_k) := \int_0^1 \partial_i f_i(y_1, \ldots, s y_i, \ldots, y_k) ds \quad y \in \mathbb{R}^k.$$  

The modified diffusion matrix is given by

$$\hat{D}_{ij}(y_1, \ldots, y_k) := D_{ij}(|y_1|, \ldots, 0, \ldots, |y_k|) + y_i d_{ij}(y) \quad y \in \mathbb{R}^k,$$

for $1 \leq i, j \leq k, j \neq i$. Following the same arguments we conclude that the solution $\hat{u}$ of the modified system remains non-negative. However, if the function $\hat{u}$ is non-negative we can remove the absolute values, and $\hat{u}$ is a solution of the original system

$$\partial_t u = \nabla \cdot (D(u) \cdot \nabla u) + f(u) \quad \Omega \times (0, T),$$

$$u|_{\partial \Omega} = 0 \quad \partial \Omega \times [0, T],$$

$$u|_{t=0} = u_0 \quad \Omega \times \{0\}.$$

By the uniqueness of solutions corresponding to initial data $u_0$ follows that $u = \hat{u}$, which implies that $u(\cdot, t; u_0) \in K^+$ for $t > 0$, and concludes the proof of the theorem. \[\square\]
Remark 5.3.3. The diffusion matrix $D(u) = (D_{ij}(u))_{1 \leq i,j \leq k}, u = (u_1, \ldots, u_k)$ in (5.5) is density dependent with continuous differentiable coefficient functions $D_{ij}(u) : \mathbb{R}^k \rightarrow \mathbb{R}$ and the diagonal elements are strictly positive i.e. $D_{ii}(u) > 0, i = 1, \ldots, k$ since the population movements are controlled by $\frac{\partial p_i}{\partial u_i} < 0, \frac{\partial q_i}{\partial u_i} > 0$. Also, the growth terms $f_i(u_i) = k_i u_i$ vanish as $u_i = 0$ for $i = 1, \ldots, k$. Hence, the generalized cross-diffusion system (5.5) for $k$ interacting species derived in section 5.2 satisfies the assumptions considered in the Theorem 5.3.2.

5.4 Applications

In this section we present some applications of the derivation and show that they satisfy the conditions of the Theorem 5.3.2.

5.4.1 Shigesada, Kawasaki and Teramoto cross-diffusion model

Shigesada, Kawasaki and Teramoto (SKT) proposed the following strongly coupled parabolic system [27]:

\[
\begin{aligned}
\frac{\partial u}{\partial t} &= \Delta((D_1 + D_{11}u + D_{12}v)u) + e_1 \nabla.(u\nabla P) + u(a_1 - b_1u - c_1v) \\
\frac{\partial v}{\partial t} &= \Delta((D_2 + D_{21}u + D_{22}v)v) + e_2 \nabla.(v\nabla P) + v(a_2 - b_2u - c_2v)
\end{aligned}
\]  

(5.12)
on a bounded smooth domain $\Omega$ in $\mathbb{R}^d$, $d \geq 1$. This mathematical model describes spatial segregation of interacting species, where $u$ and $v$ represent the densities of two competing species. Here, $P$ is an environmental potential and for $i = 1, 2$ and $j = 1, 2$, $D_i \in \mathbb{R} \geq 0, D_{ij} \in \mathbb{R} > 0$ are diffusion coefficients, $e_i \in \mathbb{R}$ are transport coefficients, $a_i \in \mathbb{R} \geq 0$ are the intrinsic growth rates, and $b_i \in \mathbb{R} \geq 0$ are intra-specific, whereas $c_i \in \mathbb{R} \geq 0$ are interspecific competition coefficients.

In [19], Shigesada, Kawasaki and Teramoto model is shown as a special case of the dual species cross-diffusion model (5.6). Since the diagonal elements of diffusion matrix are strictly positive and the reaction terms $f_1(u, v) = u(a_1 - b_1 u - c_1 v)$ and $f_2(u, v) = v(a_2 - b_2 u - c_2 v)$ vanish as $u = 0, v = 0$, the SKT model satisfies the conditions of the Theorem 5.3.2.

5.4.2 Chattopadhyay and Chatterjee cross-diffusion model

As in [3], the Chattopadhyay and Chatterjee model reads

\[
\begin{align*}
 u_t &= D_{11} \frac{\partial^2 u}{\partial x^2} + D_{12}(u) \frac{\partial v}{\partial x} + u(a_1 - b_1 u - c_1 v) \\
 v_t &= D_{22} \frac{\partial^2 v}{\partial x^2} + v(a_2 - b_2 u - c_2 v)
\end{align*}
\]  

(5.13)
where \( D_{12} = D_1' \frac{u}{\epsilon + u}, D_2' = \text{const.} \) Here, species \( v \) spreads by Fickian diffusion with diffusion coefficient \( D_{22} \), while the other species, \( u \), is modeled by a cross-diffusion term, \( D_{12}(u) \), which vanishes if \( u \) vanishes. Species \( u \) also spreads by Fickian diffusion with diffusion coefficient \( D_{11} \). In this model, \( a_i \in \mathbb{R} \geq 0 \) are the intrinsic growth rates, and \( b_i \in \mathbb{R} \geq 0 \) are intra-specific, whereas \( c_i \in \mathbb{R} \geq 0 \) are interspecific competition coefficients.

The Chattopadhyay and Chatterjee model is a special case of the cross-diffusion model (5.6) as shown in [19]. This model also satisfies the conditions of the Theorem 5.3.2 as the diagonal elements of its diffusion matrix are strictly positive and the reaction terms \( f_1(u, v) = u(a_1 - b_1 u - c_1 v) \) and \( f_2(u, v) = v(a_2 - b_2 u - c_2 v) \) vanish as \( u = 0, v = 0 \).

### 5.4.3 Keller-Segal model for Chemotaxis

According to Murray et. al. [29], the general Keller Segel (KS) model of chemotaxis reads

\[
\begin{align*}
    u_t &= \frac{\partial}{\partial x} \left( D_u \frac{\partial u}{\partial x} - \chi_0 u \frac{\partial v}{\partial x} \right) \\
    v_t &= D_v \frac{\partial^2 v}{\partial x^2} + l_1 u - l_2 v
\end{align*}
\]

(5.14)

where \( u \) denotes the cell (or organism) density on a given domain \( \Omega \subset \mathbb{R}^n \) and \( v \) describes the concentration of the chemical signal. The cell dynamics derive from population kinetics and movement, the latter comprising
a diffusive flux modelling undirected (random) cell migration and an
advective flux with velocity dependent on the gradient of the signal,
modelling the contribution of chemotaxis. \( D_u \) describes the diffusivity
of the cells (sometimes also called motility) while \( \chi_0 \) is the chemotactic
sensitivity. \( l_1u \) and \( l_2v \) are kinetics that describes production and
degradation of the chemical signal.

In order to recover Keller Segel model (5.14) from the general cross-
diffusion model (5.5), the following equalities must hold:

\[
\begin{align*}
D_u &= pq + u(pq_u - qp_u) & (5.15) \\
\chi_0 &= (pq_v - qp_v) & (5.16) \\
D_v &= sr + v(sr_v - rs_v) & (5.17) \\
sr_u &= rs_u & (5.18)
\end{align*}
\]

where \( \alpha_0 \) and \( \beta_0 \) are included in the diffusion coefficients \( D_{ij} \). Since the
functions \( s(u, v) \) and \( r(u, v) \) have the same properties as the case of
Fickian diffusion [19]. Therefore, one possible solution is \( s = r = \sqrt{D_v} \).

**Proposition 5.4.1.** The Keller Segel model can be recovered from the
general cross-diffusion model (5.5) assuming the transfer functions can
be separated into independent functions of \( u \) and \( v \).

**Proof.** At first, we consider \( p(u, v) = f(u)g(v) \) and \( q(u, v) = h(u)j(v) \).
Then, we have the following two equations that are required to be sat-
isfied form (5.15) and (5.16) respectively.

\[ D_u = g(v)j(v)(f(u)h(u) + u(f(u)h'(u) - h(u)f'(u))) \quad (5.19) \]
\[ \chi_0 = f(u)h(u)(g(v)j'(v) - j(v)g'(v)) \quad (5.20) \]

This implies that the following must hold for arbitrary constants \( \lambda_5 \) and \( \lambda_6 \):

\[ g(v)j'(v) - j(v)g'(v) = \lambda_5 \quad (5.21) \]
\[ g(v)j(v) = \lambda_6 \quad (5.22) \]
\[ f(u)h(u) = \frac{\chi_0}{\lambda_5} \quad (5.23) \]
\[ f(u)h(u) + u(f(u)h'(u) - h(u)f'(u)) = \frac{D_u}{\lambda_6} \quad (5.24) \]

The functions \( g(v) \) and \( j(v) \) are solved first. Expressions for \( g(v) \) and \( g'(v) \) are obtained from (5.22) and substituted in (5.21), giving the ordinary differential equation \( j' = \frac{\lambda_5}{2\lambda_6}j \). The functions can then be solved and are given as

\[ j(v) = c_4 e^{\frac{\lambda_5 v}{2\lambda_6}} \quad (5.25) \]
\[ g(v) = \frac{\lambda_6}{c_4} e^{-\frac{\lambda_5 v}{2\lambda_6}} \quad (5.26) \]

where \( c_4 \) is an arbitrary constant.

Now, we find the functions \( f(u) \) and \( h(u) \). Expressions for \( f(u) \) and \( f'(u) \)
are obtained from (5.23) and substituted in (5.24), yielding the ordinary
differential equation \( \frac{dh}{du} = h\left(\frac{D_u \lambda_5 - \chi_0 \lambda_6}{2 \chi_0 \lambda_6}\right) \). From this, \( h(u) \) is solved, after which \( f(u) \) is solved from (5.23). Finally, we have the following expressions for \( h(u) \) and \( f(u) \),

\[
\begin{align*}
  h(u) &= c_5 e^{\frac{D_u \lambda_5 - \chi_0 \lambda_6 \ln u}{2 \chi_0 \lambda_6}} \\
  f(u) &= \frac{\chi_0}{c_5 \lambda_5} e^{-\frac{(D_u \lambda_5 - \chi_0 \lambda_6 \ln u)}{2 \chi_0 \lambda_6}}
\end{align*}
\]

(5.27) (5.28)

Thus, the expressions of transfer functions are

\[
\begin{align*}
  p(u, v) &= \frac{\chi_0 \lambda_6}{c_4 c_5 \lambda_5} e^{-\frac{(D_u \lambda_5 - \chi_0 \lambda_6 \ln u)}{2 \chi_0 \lambda_6}} e^{-\frac{\lambda_5}{2 \lambda_6}} v \\
  q(u, v) &= c_4 c_5 e^{\frac{D_u \lambda_5 - \chi_0 \lambda_6 \ln u}{2 \chi_0 \lambda_6}} e^{\frac{\lambda_5}{2 \lambda_6}} v \\
  s(u, v) &= \sqrt{D_v} \\
  r(u, v) &= \sqrt{D_v}
\end{align*}
\]

(5.29) (5.30) (5.31) (5.32)

Thus, it shows that Keller Segel model for chemotaxis is a special case of the cross-diffusion model (5.5). Also, the diagonal elements \( D_u \) and \( D_v \) of the diffusion matrix are strictly positive and the reaction terms \( f_1(u, v) = 0 \) and \( f_2(u, v) = l_1 u - l_2 v \) vanish as \( u = 0, v = 0 \). Hence, this model fulfills the conditions of the Theorem 5.3.2.
5.4.4 Predator-Prey model

This model presents predator-prey interaction, where species \( u(x, t) \) is the prey population and \( v(x, t) \) is the predator population density at time \( t \). We consider a predator-prey interaction model with self and cross-diffusion that can be read as

\[
\begin{align*}
\frac{\partial u}{\partial t} &= \nabla (D_{11}(u, v) \nabla u + D_{12}(u, v) \nabla v) + ug(u) - vp(u) \\
\frac{\partial v}{\partial t} &= \nabla (D_{21}(u, v) \nabla u + D_{22}(u, v) \nabla v) - vq(u) + cvp(u)
\end{align*}
\]

(5.33)

where \( c > 0 \). The function \( g(u) \) represents the specific growth rate of the prey in the absence of any predator and the function \( p(u) \) is the predator response function for the predator with respect to that particular prey. \( q(u) \) is the death rate. \( D_{11}, D_{22} \) are the self-diffusion coefficients and \( D_{12}, D_{21} \) are the cross-diffusion coefficients of prey and predator respectively.

From the predator prey model (5.33) and the general cross-diffusion model (5.5), we have the following equalities:

\[
\begin{align*}
D_{11}(u, v) &= pq + u(pq_u - qp_u) \\
D_{12}(u, v) &= v(pq_v - qp_v) \\
D_{21}(u, v) &= u(sr_u - rs_u) \\
D_{22}(u, v) &= sr + v(sr_v - rs_v)
\end{align*}
\]

(5.34) (5.35) (5.36) (5.37)
where $\alpha_0$ and $\beta_0$ are included in the diffusion coefficients $D_{ij}$. Using these above relations, it can easily be shown predator prey model (5.33) as a special case of the general cross-diffusion model (5.5). The functions $p(u, v)$ and $q(u, v)$ relate to the prey seeking locations with low predator density ($\frac{\partial p}{\partial v} < 0, \frac{\partial q}{\partial u} > 0, \frac{\partial p}{\partial u} < 0, \frac{\partial q}{\partial v} > 0$). Also, the functions $r(u, v)$ and $s(u, v)$ relate to the predator seeking locations with high prey density ($\frac{\partial s}{\partial u} < 0, \frac{\partial r}{\partial u} > 0, \frac{\partial s}{\partial v} < 0, \frac{\partial r}{\partial v} > 0$). Hence, the diagonal elements of this model are strictly positive. Also, the reaction terms $f_1(u, v) = ug(u) - vp(u)$ and $f_2(u, v) = cvp(u) - vq(u)$ vanish as $u = 0, v = 0$. Thus, this model satisfies the conditions of the Theorem 5.3.2.

### 5.4.5 Biofilm model

We consider the biofilm model of two bacterial species where species compete for a shared growth limiting substrate, for example a carbon source. The model is formulated in terms of dependent variables $X, Y$ and $C$. Here, $X$ and $Y$ are the volume fractions occupied by biomass of the two species. $C$ is the concentration of substrate. The substrate is assumed to be dissolved and to diffuse both, in the aqueous phase and in the biofilm. Assuming Monod kinetics for substrate uptake and
growth, the dual-species biofilm competition model reads

\[
\begin{align*}
X_t &= \nabla(D_{11}(X,Y)\nabla X + D_{12}(X,Y)\nabla Y) + \mu_1 \frac{C}{\kappa_1 + C} X - k_1 X, \\
Y_t &= \nabla(D_{21}(X,Y)\nabla X + D_{22}(X,Y)\nabla Y) + \mu_2 \frac{C}{\kappa_2 + C} Y - k_2 Y, \\
C_t &= \nabla(D_C(X + Y)\nabla C) - \frac{\mu_1 X^\infty}{\Upsilon_1} \frac{C}{\kappa_1 + C} X - \frac{\mu_2 Y^\infty}{\Upsilon_2} \frac{C}{\kappa_2 + C} Y.
\end{align*}
\]  

(5.38)

The biomass densities are then \(X \cdot X^\infty\) and \(Y \cdot Y^\infty\), where the parameters \(X^\infty\) and \(Y^\infty\) are the maximum cell densities. As is common in biofilm modeling, all masses are expressed in terms of chemical oxygen demand, i.e.

\(\text{COD}\), and EPS is implicitly subsumed in the bacterial biomass. \(\mu_1\) and \(\mu_2\) are the maximum specific growth rates for species \(X\) and \(Y\), respectively. Similarly, \(k_1\) and \(k_2\) are the corresponding lysis rates, \(\kappa_1\) and \(\kappa_2\) the half saturation concentrations, \(\Upsilon_1\) and \(\Upsilon_2\) are yield coefficients. All parameters are non-negative.

The self- and cross-diffusion coefficients \(D_{ij}\), \(i, j \in \{1, 2\}\) have the following structure (see [22] for a derivation and more details):

\[
\begin{align*}
D_{11}(X,Y) &= \Phi(X + Y) + X\Psi(X + Y), \\
D_{12}(X,Y) &= X\Psi(X + Y), \\
D_{21}(X,Y) &= Y\Psi(X + Y), \\
D_{22}(X,Y) &= \Phi(X + Y) + Y\Psi(X + Y).
\end{align*}
\]

where the functions \(\Phi\) and \(\Psi\) stem from the algebraic closure of the
pressure-biomass relationship, see [22]. The relationship between the density-dependent diffusion coefficient \( D(M), M = X + Y \) and the functions \( \Phi(M) \) and \( \Psi(M) \) are given by

\[
D(M) = \Phi(M) + M\Psi(M) \quad (5.39)
\]

and, after some calculations, by

\[
\Phi(M) = \left( 1 - \int_0^M D(s)ds \right) \frac{\int_0^M D(s)ds}{M} \quad (5.40)
\]

For the density-dependent diffusion coefficient \( D(M) \) we use, as in [11, 12],

\[
D(M) = \delta \frac{M^\alpha}{(1 - M)^\beta}, \delta > 0, \alpha, \beta > 1. \quad (5.41)
\]

The diffusion coefficient for the dissolved substrate \( D_C(M) \) depends on the overall local biomass density \( M \). Diffusion coefficients of dissolved substrates can be substantially smaller in the biofilm colony than in the surrounding aqueous phase [1, 26]. We make the linearization ansatz

\[
D_C(M) = D_C(0) + M (D_C(1) - D_C(0)) \quad (5.42)
\]

where \( D_C(0) \) is the experimentally measurable diffusion coefficient of the substrate in the aqueous phase and \( D_C(1) \) in a fully developed
biofilm. The ratio $\delta_C := D_C(1)/D_C(0)$ depends typically on the size of the molecules. In [1], it is reported that for small molecules, like oxygen $\delta_C \approx 1$, while for larger molecules such as antibiotics one can have $\delta_C \approx 0.5$. In any case we have $0 < D_C(0) \leq D_C(M) \leq D_C(1) < \infty$. Thus the diffusion coefficient is bounded from below and above by known positive numbers. Hence, despite it depending on $M$, the additional substrate equation behaves essentially Fickian.

Since the diffusion coefficient vanishes for $M = 0$, diagonal elements of the diffusion matrix are not strictly positive i.e. 0. Thus, this model fails to satisfy the positivity criterion.

Following the regularization technique used for single species biofilm model in [12], we can approximate this system by replacing the diffusion function $D(M)$ by a regular function $D_\epsilon(M)$, for $\epsilon > 0$ with no degeneracies. The regularized diffusion function for any $\epsilon < 1, a, b > 1, \delta > 0$ can be defined as

$$D_\epsilon(M) = \begin{cases} \frac{\delta}{\epsilon^a} & \text{if } M > 1 - \epsilon, \\ \frac{\delta(M+\epsilon)^a}{(1-M)^b} & \text{if } M \leq 1 - \epsilon, \end{cases}$$

Regularized diffusion function leads to regularize the functions $\Phi(M)$ and $\Psi(M)$ i.e, $\Phi_\epsilon(M)$ and $\Psi_\epsilon(M)$. Thus, this competition model can be regularized where diffusion doesn’t degenerate to 0. The diagonal elements of diffusion matrix of the regularized biofilm model are
strictly positive. Also, the reaction terms $f_1(X, Y) = \mu_1 \frac{C}{\kappa_1 + C} X - k_1 X$, $f_2(X, Y) = \mu_2 \frac{C}{\kappa_2 + C} Y - k_2 Y$ and $f_3(X, Y) = -\frac{\mu_1 X}{Y_1} \frac{C}{\kappa_1 + C} X - \frac{\mu_2 Y}{Y_2} \frac{C}{\kappa_2 + C} Y$ vanish as $X = 0, Y = 0$. Hence, the regularized competition model satisfies the conditions of the Theorem 5.3.2.

5.4.6 Aggregation model

We consider the population density of one species $u(x, t)$ and the other species $v(x, t)$ where both species aggregate separately without any interaction with each other. The dual species aggregation model can be formulated as

$$\frac{\partial u}{\partial t} = \nabla (D_{11}(u, v) \nabla u + D_{12}(u, v) \nabla v) + g_1(u)$$
$$\frac{\partial v}{\partial t} = \nabla (D_{21}(u, v) \nabla u + D_{22}(u, v) \nabla v) + g_2(v)$$

(5.43)

where $g_1(u)$ and $g_2(v)$ represent the net growth of the type logistic with a threshold. $D_{11}, D_{22}$ are the self-diffusion coefficients which control density dependent movement of each species separately. As there is no interaction between species, the cross-diffusion coefficients of aggregation model are absent, i.e., $D_{12} = 0, D_{21} = 0$.

From the aggregation model (5.43) and the general cross-diffusion model
(5.5), we have the following equalities:

\[ D_{11}(u, v) = pq + u(pq_u - qp_u) \]  
\[ 0 = v(pq_v - qp_v) \]  
\[ 0 = u(sr_u - rs_u) \]  
\[ D_{22}(u, v) = sr + v(sr_v - rs_v) \]

where \( \alpha_0 \) and \( \beta_0 \) are included in the diffusion coefficients \( D_{ij} \). Using these above relations, it can easily be shown aggregation model (5.43) as a special case of the general cross-diffusion model (5.5). The functions \( p(u, v) \) and \( q(u, v) \) relate to the species \( u(x, t) \) seeking locations with high species \( u(x, t) \) density \( (\frac{\partial p}{\partial u} < 0, \frac{\partial q}{\partial u} > 0) \). Also, the functions \( r(u, v) \) and \( s(u, v) \) relate to the species \( v(x, t) \) seeking locations with high species \( v(x, t) \) density \( (\frac{\partial r}{\partial v} < 0, \frac{\partial s}{\partial v} > 0) \). The diagonal elements \( D_{11}, D_{22} \) of the diffusion matrix are strictly positive if \( pq > pq_u + qp_u \) and \( rs > sr_v + rs_v \). Also, the net growth terms \( g_1(u) = 0, g_2(v) = 0 \) if \( u = 0, v = 0 \). Thus, this model satisfies the conditions of the Theorem 5.3.2.

5.5 Conclusion

We derived generalized cross-diffusion models for an arbitrary number of interacting species. Moreover, to characterize the class of generalized cross-diffusion systems preserving positivity we formulate explicit neces-
sary and sufficient conditions on the diffusion matrix $D$ and interaction function $f$ that can easily be verified in different applications.
Bibliography


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Chapter 6

Conclusion and Future Work

6.1 Conclusion

The outcomes of this dissertation are as follows:

- Two derivations of the dual species cross-diffusion model are presented. One derivation is based on the continuous time, discrete space master equation and the other one is based on the equations of conservation of mass and momentum.

- A numerical method for our highly non-linear cross-diffusion PDE model of biofilm with three diffusion effects (i. prorous medium degeneracy, ii. super-diffusion singularity, iii. non-linear cross-diffusion) has been presented. The method is based on a Finite Volume discretization in space and semi-implicit, non-local time
integration. We treat new cross-diffusion term in our model as a convective term with density dependent velocity. The resulting discretized system is implemented in Fortran and parallelized in OpenMP. This method preserves positivity. Moreover, we validate the method with a grid refinement simulation study.

- This dissertation finds that cross-diffusion terms are crucial to not over estimate mixing of species in colonies; neglecting cross-diffusion effect can obliterate, or at least dampen biomass gradients. This is particularly important point if local mixing behaviour is the key part of someone’s interest. Otherwise, cross-diffusion does not affect in global biofilm behaviour and lumped parameters such as amount of total biomass and biofilm size. Thus, the observations regarding local mixing behaviour positions the proposed cross-diffusion model as a potential alternative besides the existing models that have been criticised for displaying too much or not enough mixing of species.

- Our cross-diffusion model with newly introduced reaction kinetics is used to interpret laboratory experiment results when antibiotics are exposed to the biofilms. The experimental observations found that a product, carbon dioxide, has been produced during disinfection process due to the consumption of carbon substrate because of metabolic activity increase. The study captures the experimental
observations and shows that if carbon consumption during disinfection process is not considered, it may lead the result to false negatives. Thus, during the disinfection process, utilization of carbon helps biofilm to protect itself from the action of antibiotics exposure and ultimately guarantees their survival and repopulation in the colony.

- The derivation of a generalized cross-diffusion model for \( k \) interacting species is presented. Considering solutions to originate from a non-negative initial data, a positivity criterion for the solutions of this generalized cross-diffusion system is also formulated.

We study how the solution behaves due to the presence of cross-diffusion in the model. Cross-diffusion has potential impact locally but not globally. In addition, it slows down the interaction process between species.

### 6.2 Future Work

Followings are some open and interesting questions that should be investigated in future:

1. An underlying principle of virtually all 2D/3D biofilm models is that biofilms do not expand notably if locally new biomass can be incorporated. The local biomass density is limited by the maximum
cell density. They start spreading when the space locally available becomes limited. In our cross-diffusion model presented in Chapter 2, we consider for simplicity the functions $p_{1,2}$ and $q_{1,2}$ depend on the total biomass density i.e $X + Y$. Therefore, we consider $p_{1,2}(X, Y) = p_{1,2}(X + Y)$, $q_{1,2}(X, Y) = q_{1,2}(X + Y)$. By relaxing this assumption, one can consider for example that the movement of bacteria of one species depends differently on both species. In fact, this will lead more algebraic complexity.

2. In Chapter 2, it is noted that the functions $p_{1,2}$ and $q_{1,2}$ control the local movements of species. For more simplification, we assume that the spatial spreading of both species are the same i.e $p_1 = p_2, q_1 = q_2$. This assumption can be relaxed in future investigations to extend to systems where both species show different spatial behaviour i.e. $p_1 \neq p_2, q_1 \neq q_2$.

3. In Chapter 4, we introduce new reaction kinetics with the existing model of biofilms of disinfection in order to capture the experimental observations. We consider a constant inflow of carbon substrate all the time. Another interesting investigation can be done just by adding more carbon substrate during the exposure of antibiotics in addition to the constant flow of carbon substrate.

4. In Chapter 5, we only present a criterion for preserving the positivity of the solution of the generalized cross-diffusion model. A
further task for the future is to establish existence and boundedness of the solutions of this cross-diffusion model.
Appendix A

A Mixed Culture Biofilm Model with Cross-Diffusion

A.1 Additional simulation results for the competition model

In Figure A.1 we show additional results for the merging of two colonies. This corresponds to scenarios (b), (c) as described in the main text in Section 3.1.2.1.

(b) Both colonies are initially mixed. We now have initially in both colonies biomass of both types X and Y at the same levels, i.e. no biomass gradients within each colony. The results are shown in Figure A.1 (top row) for $t = 1.80, 3.80, 5.72$. The equal distribution of biomass
is maintained in the colony throughout, before and after merging. Cross-diffusion does not introduce biomass gradients. Since the reaction kinetics is the same for both species, the biofilm shape and size is the same as in case (a) above. Merging starts at $t = 2.20$. The maximum biofilm size at which simulation is terminated is reached at $t = 5.72$ (data not shown).

(c) One colony consists of one species only, the other one of both species. The left colony is initially mixed with both species represented at equal levels, as in (b), and the right colony only contains species $X$ as in (a). The results are shown in Figure A.1 (bottom row). Again, since the reaction kinetics are the same for both species, the size and shape of the colonies are the same as in the previous cases. Both colonies merge at $t = 2.2$ and the final biofilm size is reached at $t = 5.72$ (data not shown). When the colonies start merging, a biomass gradient emerges and mixing fronts penetrate the colonies, as in (a), where the left half remains relatively well mixed while the right half remains dominated by species $X$.

In Figure A.2 the simulation results of the competition model are shown for the cases (i)-(iii) with random inoculation of the substratum, as described in the main text in Section 3.1.2.2, where the growth parameters of both species are distinct. Shown is the biofilm, originating from initially 20 randomly colonies of either species at three different time instances. The results are qualitatively the same as in case (iv) in the main
Figure A.1: Simulation of biofilm formation and interaction in cases (b) (top), where initially both colonies are equally mixed, and (c) (bottom) where initially one colony is mixed and the other one consists of species $X$ only. The color coding refers to the relative fraction of the biomass for species $X$, $R = \frac{X}{X+Y}$ and the contour for substrate, $C$. 
Figure A.2: Biofilm formation according to the cross-diffusion model with randomly inoculated substratum by 20 colonies of each species. Shown are the results for three time steps and case (i) (first row) with \( \mu_2 > \mu_1 \) and \( \kappa_2 > \kappa_1 \), case (ii) (second row) with \( \mu_2 = \mu_1 \) and \( \kappa_2 > \kappa_1 \), case (iii) (third row) with \( \mu_2 > \mu_1 \) and \( \kappa_2 = \kappa_1 \). The colour coding refers to the relative fraction of the biomass for species \( X \), \( R = \frac{X}{X+Y} \) and the contour for substrate, \( C \).
text, differences are explained by the different reaction kinetics giving a growth advantage to one of the species. In all cases we see observe biomass gradients in regions where colonies of different species merge. Case (iii) is dominated by species X but small pockets for species Y are obtained. Case (ii) is mostly dominated by species Y but regions of coexistence (in light colors) form. Similarly, case (i) is dominated by X with larger regions of coexistence. Figure A.3 shows the corresponding data for the model without cross diffusion. While the ratio of both biomass densities within each colony stratifies, there are differences between colonies.

Figures A.4, A.5, A.6 give a comparison of the lumped output parameters biofilm size and total biomass of either species for the biofilm competition model in Section 3.1 of the main text, where also the model equations and parameters are given. Shown are the results for the model with and without cross-diffusion. These are the three cases (ii)-(iv), which differ with respect to growth parameters of both species. In all cases we report the data for initially 20 and 4 randomly placed colonies of either species. 20 replicates of each simulation were run. Plotted are the averaged values with error bars. In all cases we observe that the differences in lumped data between both models are small.
Figure A.3: Biofilm formation according to model without cross-diffusion with randomly inoculated substratum by 20 colonies of each species. Shown are the results for three time steps and case (i) (first row) with \( \mu_2 > \mu_1 \) and \( \kappa_2 > \kappa_1 \), case (ii) (second row) with \( \mu_2 = \mu_1 \) and \( \kappa_2 > \kappa_1 \), case (iii) (third row) with \( \mu_2 > \mu_1 \) and \( \kappa_2 = \kappa_1 \). The colour coding refers to the relative fraction of the biomass for species \( X \), \( R = \frac{X}{X+Y} \) and the contour for substrate, \( C \).
Figure A.4: Comparison of biomass occupancy and total biomass of both species with (without) cross-diffusion for case (ii) $\mu_2 = \mu_1$ and $\kappa_2 > \kappa_1$ with 20 colonies of either species initially (top) and 4 colonies initially (bottom).
Figure A.5: Comparison of biomass occupancy and total biomass of both species with (without) cross-diffusion for case (iii) $\mu_2 > \mu_1$ and $\kappa_2 = \kappa_1$ with 20 colonies of either species initially (top) and 4 colonies initially (bottom).
Figure A.6: Comparison of biomass occupancy and total biomass of both species with (without) cross-diffusion for case (iv) $\mu_2 = \mu_1$ and $\kappa_2 = \kappa_1$ with 20 colonies of either species initially (top) and 4 colonies initially (bottom).