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Modelling bacterial biofilms in spatially heterogeneous environments

Patrick Sinclair
Biofilms are communities of one or more species of microorganism, such as bacteria, which have adhered both together and to a surface. Bacterial biofilms are extremely common in nature, with up to 80% of bacterial life on earth estimated to be found in a biofilm. However, they are a key concern in both medical and industrial scenarios, due to their increased resilience to chemical and physical treatments which have been found to be effective against free-floating bacteria. As such, further research into how bacterial biofilms grow and thereby develop these enhanced resistances is essential.

This thesis aims to further this research using various computational modelling techniques. Computational modelling has a number of advantages over traditional “wet-lab” experiments involving actual living bacteria, such as ease of replication and changing of system parameters. These models are of course an approximation of the “real-world” behaviour that bacteria would exhibit, but by reducing a biological system down to its most basic features, computational models aim to identify the key attributes of a biological system. These findings can then be used to complement laboratory experiments to help explain the observed outcomes.

Currently, most research involving computational models of bacterial biofilms is done under relatively idealised conditions, such as spatially uniform antibiotic concentrations (meaning the antibiotic is distributed completely evenly throughout the biofilm), or modelling biofilms which consist of only one bacterial species. These idealised conditions may not accurately capture the more complex conditions one would observe in the real world.

This thesis aims to address these shortcomings by implementing models which represent conditions more similar to what one might observe in a typical real-world scenario, such as spatial gradients of nutrients or antibiotics, or non-uniform flow fields. The thesis describes four distinct projects, linked together by this
common theme of spatial variation.

The first project presented here is a simple one-dimensional model of a bacterial population growing along a spatial antibiotic gradient, for antibiotics which are either more effective at targeting fast-growing bacteria or slow-growing bacteria. Initially the bacteria grow quickly, making them susceptible to the fast-growing antibiotic. However, as the nutrients run out, the overall growth rate of the bacteria decreases, meaning they are now more susceptible to the slow-growing antibiotics. This project highlights the differing outcomes for these two types of antibiotics, the results of which may help to inform future clinical treatment strategies.

Following this, the next project investigates the initial stages of biofilm formation on a surface. This chapter involves a pair of complementary models, a deterministic one, involving a system of differential equations; and a stochastic one, where the individual bacteria behave randomly, according to a given set of rates. By modifying these rates for certain actions which the bacteria undertake, the models suggest that under certain conditions biofilm formation can be highly predictable, but for other conditions it becomes noticeably unpredictable.

In the third project, the stochastic biofilm formation model is built upon to study the formation of a biofilm on a surface submerged in an aqueous environment, while the surface is releasing an antimicrobial chemical into the surrounding area. This model is intended to represent a marine biofouling growing on a ship hull which is coated in an antifouling coating. These coatings are a common technique used in the shipping industry to prevent biofilm growth on ship hulls, as the presence of biofilms increases the drag experienced by the ship, increasing fuel consumption. This model also introduces the concept of differing microbial species, each of which has a different level of resistance to the biocide. This is intended to represent the biodiversity found in a typical marine environment.

Finally, a model involving computational fluid dynamics is used to investigate the behaviour of a biofilm growing on a surface covered in “shark skin”-like grooves when the biofilm is exposed to an external flow field. These grooves are designed to reduce the overall drag experienced by the ship hulls to which they are applied, but they only work when they are unobstructed. As such, it is crucial to determine how the misalignment of these grooves affects the removal of biofilm from the surface due to the external flow, in order to optimise their drag-reducing performance.
Abstract

Biofilms are communities of one or more species of microorganism which have adhered both together and to a surface. Biofilms are ubiquitous in nature, with up to 80% of bacterial life on earth estimated to be found in a biofilm. Bacterial biofilms are far more resilient to both chemical and physical methods of removal than their planktonic counterparts, which presents numerous challenges in both clinical and industrial scenarios. Therefore, further research into the underlying mechanisms of how these biofilms develop and survive is essential. This thesis aims to do so via the implementation of various computational modelling techniques.

Currently, most computational modelling of biofilms is done under somewhat idealised conditions, such as uniform antibiotic concentrations and mono-species biofilms, which do not always reflect the complex conditions found in vivo. This thesis therefore also aims to address this problem by using computational models to understand how biofilms proliferate and resist methods of removal in spatially heterogeneous environments, such as chemical gradients of nutrients and antibiotics, or non-uniform flow fields. The thesis takes the form of three distinct projects, which are linked together by this common theme of spatial non-uniformity.

Presented first is an investigation into the coupling between nutrient availability and growth-dependent antibiotic susceptibility. This project uses a simple 1D Monte-Carlo model to simulate the advancement of a bacterial population along a spatial antibiotic concentration gradient. Bacterial replication consumes nutrients which in turn lowers the local growth rate, altering the antibiotic susceptibility. The results highlight the differing outcomes for antibiotics which target either slow-growing or fast-growing cells.

Following this, the next project investigates the initial stages of biofilm formation...
on a surface. This chapter involves a pair of complementary models, a
deterministic one, involving a system of differential equations; and a stochastic
one, where the individual bacteria are simulated using a modified $\tau$-leaping
algorithm, both again in 1D. By modifying the rates for certain actions
which the bacteria undertake, the models predict that under certain conditions
biofilm formation is highly predictable, but for other parameter regimes, biofilm
formation becomes more stochastic.

In the third project, the stochastic biofilm formation model described above
is extended to develop a model for the formation of biofilms on a surface
which leaches an antimicrobial compound into the surrounding environment,
similar to current antifouling coatings used to prevent marine biofouling in the
shipping industry. A key difference in this model is the inclusion of multiple
bacterial species, each with differing resistances to the applied biocide, intended
to represent the biodiversity found in a typical marine environment.

Finally, a computational fluid dynamics model is presented, which is used to
model the interaction between a micro-structured surface featuring shark skin-
like riblets and an enveloping biofilm, when exposed to an external flow field
of various incident flow angles. These riblets are a contemporary solution to
reducing hydrodynamic drag, e.g., on ship hulls, but are only effective when their
physical shape is unobstructed. Investigating how misaligned riblets can impede,
or even prevent, the sloughing of biofilm matter is therefore crucial to optimising
their performance.
Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified.

Parts of this work have been published in [1–3].

(Patrick Sinclair, January 18, 2023)
Acknowledgements

Thanks must of course be made to my supervisory team throughout this PhD, Professor Rosalind Allen, Dr Chris Brackley and Dr Martín Carballo-Pacheco, without whom this PhD would quite literally not have been possible. Their continued support and encouragement has been essential in not only the completion of this thesis, but also in my own professional development.

Gratitude should of course be shown to everyone at AkzoNobel who were involved in the production of this thesis. Their contributions, in the form of constructive conversations and the provision of technical insight, were invaluable in the development of a considerable portion of the work presented here.

Special thanks must be given to Professor Nick Cogan at FSU, whose assistance in the theoretical aspects of the work presented in Chapter 5 were fundamental to its completion. Also worthy of mention are all those I met in Tallahassee, who helped elevate my time there above more than just a simple academic secondment.

Finally, appreciation must be shown to all the friends I’ve made during my time in Edinburgh. Without whom this thesis would likely have been finished six months ago.
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Chapter 1

Introduction

1.1 An overview of biofilms

Bacteria are single-celled organisms which are ubiquitous in nature, comprising 15% of the planet’s total biomass [1], with an estimated 40-80% of the bacteria on Earth existing in what are known as “biofilms” [5]. A biofilm is typically defined as “a microbially derived sessile community characterised by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription” [6].

In simpler terms, a biofilm is an aggregate population of bacteria enveloped in a sticky goo which keeps them adhered to both one another and the surface upon which they are growing. This polymer matrix which surrounds the bacteria in the biofilm is commonly referred to as “extracellular polymeric substances”, or EPS [7], and can account for 50-90% of the total organic matter in the biofilm [6 8]. Fossil records suggest that bacteria have existed in biofilms for over 3 billion years [9 10], only 1.5 billion years fewer than the lifetime of the Earth itself [11], and far longer than the existence of mammals (185 million years [12]) or even plant life (500 million years [13]).

Bacteria found in biofilms tend to have higher resistance to external stressors than their planktonic (individual, free-floating) counterparts, stressors such as applied antimicrobial compounds [14 15], and mechanical stresses from external
fluid flow \cite{16,17}. However, this enhanced protection comes with the trade-off of reduced motility \cite{18,19} and lowered replication/metabolic rates \cite{20,21}.

1.2 Biofilm formation

A general prerequisite for biofilm formation is the presence of a solid surface on which to grow\footnote{This is not strictly required, with some biofilms observed growing on fluid-fluid interfaces \cite{22,25}, and biofilm-like aggregates can also be found suspended in liquid \cite{26,27}.}. If submerged in an aqueous environment such as the ocean, a lake or the human body, almost any surface will quickly become coated in a “conditioning layer” of various polymers found in the medium \cite{6,28}, which facilitates the attachment of bacteria to the surface. These surfaces do not need to be overtly rigid to be prone to biofilm formation - deformable surfaces such as the surfaces of plants and animals are also able to be colonised \cite{29,31}. Upon arriving to the surface, bacteria swim close and reversibly attach to it, typically via van der Waals interactions \cite{32} or loose mechanical attachment via their flagella \cite{33}. At this point in the formation process, the bacteria are still able to be washed from the surface \cite{34}.

Within several hours or days, depending on the bacterial species involved \cite{36,38}, the bacteria then transition to an irreversibly attached state and form a biofilm. The mechanisms governing this transition are still poorly understood \cite{39,41}, but in some cases it is thought that they are likely to involve cell-cell interactions (discussed in Section 1.3). In the final stage of the biofilm life cycle, the biofilm disperses, releasing planktonic cells that can later found new biofilms. A summary of this process is shown in Figure 1.1.

1.3 Quorum sensing

One phenomenon that has been implicated in biofilm formation is that of “quorum sensing” (QS) \cite{42,47}. Quorum sensing is a mechanism where, via the use of signalling molecules known as “autoinducers”, bacterial populations are able to monitor their own cell number density and regulate their gene expression accordingly. In the case of biofilm formation, it appears that the transition from planktonic to a sessile biofilm configuration occurs when the bacteria reaches
Figure 1.1 Overview of the biofilm formation process, taken from Lindemann, 2018 [35]. Planktonic bacteria migrate from the surrounding fluid environment. Initially these bacteria are reversibly attached and only a small population is present. Eventually this small population forms a bacterial monolayer and EPS is produced, resulting in the formation of a thin biofilm which is irreversibly attached to the surface, and is now resilient to washing and/or sloughing. Following this, the biofilm develops into a mature biofilm with complex 3D structures. The final stage in the biofilm lifecycle is dispersion, where individual bacteria can detach from the biofilm, becoming planktonic and returning to the surrounding environment. These bacteria can then go on to form new biofilms.

a “critical cell density” [47–49], i.e. it is a form of social interaction [42–47]. Upon reaching this critical density, the bacteria begin to express genes for the production of proteins and other substances which form the basis for EPS [50–52].

Other intracellular signalling mechanisms also play a key role. For example, signalling via the intracellular mediator cyclic di-GMP, which unlike quorum sensing, is not thought to act collectively. Cyclic di-GMP is not connected to the collective population and does not depend on bacteria density. Rather, individual cells detect cyclic di-GMP and engage in individualistic behaviour which prompts biofilm formation. e.g., an individual bacterium senses and attaches to a surface, but cannot determine if its neighbours are doing the same. [53–55].
A large body of work has demonstrated conclusively the key role for cyclic di-GMP signalling in triggering biofilm formation in *P. aeruginosa* and other bacteria [56–59]. Experimental work utilising gene knockout to prevent the production of cyclic di-GMP resulted in *P. aeruginosa* bacteria being unable to form any sort of biofilm, even after 72 hours - as opposed to wild-type *P. aeruginosa*, which is normally able to form robust biofilms in under 12 hours [60].

In short, the exact mechanisms involved in the regulation of the biofilm transition varies between species, and may involve many different QS autoinducers, molecular signallers and regulated genes [51, 61–63], although the involvement of QS appears to be a common motif [64, 65].

### 1.4 Chemical gradients in biofilms

As biofilms are intrinsically spatially structured, they will inevitably generate chemical gradients of nutrients and other substances [66–69], with these gradients having important implications for the bacteria contained within the biofilm. Bacteria located deeper inside the biofilm, away from the biofilm-fluid interface, have a lower exposure to oxygen and nutrients [70, 71], resulting in these bacteria exhibiting lower growth rates than their surface-growing equivalents [72–74]. This disparity in growth rates leads to the formation of the so-called “active layer”, wherein only the outer layer (typically 50-200 µm thick - corresponding to the depth of oxygen penetration) of a biofilm is metabolically active [75, 77–79]. In other words, the oxygen/nutrient gradient in the biofilm results in a corresponding gradient of growth rates. An example of this active layer is shown in Figure 1.2. It should be noted that the limited diffusion of oxygen and nutrients into the biofilm is due to their consumption by bacteria in the active layer, not due to the EPS limiting their diffusion [60].

However, some instances of other chemical gradients do arise due to the presence of the EPS matrix, in particular in the application of antimicrobial compounds to the biofilm. Reaction or sorption of these compounds with the EPS can establish a diffusion barrier, preventing the compound from reaching the bulk of the biofilm [81, 82]. Generally, the EPS matrix has a net negative charge [83], allowing it to

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2There are occasional cases where there are multiple active layers - in a case where the biofilm contains aerobic and anaerobic bacteria, or the location of the active layer is unconventional - such as membrane attached biofilms, where there may be two active layers on either side of the biofilm [76, 78].
Figure 1.2 Image highlighting regions of viable (metabolically active) and non-viable cells within an oral biofilm. Metabolic activity is highlighted via the use of a fluorescent dye in conjunction with confocal laser scanning microscopy. Image taken from Hope and Wilson, 2003 [80].

sequester positively charged compounds such as the commonly used antimicrobial chlorhexidine. Larger molecules such as human IgG antibody have also been found to be incapable of penetrating the EPS matrix [83]. However, this inhibited diffusion is not universal against all antibiotics. One study showed that when applied to biofilms of wild-type *Klebsiella pneumoniae*, the antibiotic ampicillin was unable to penetrate the biofilm to an appreciable degree, but other chemicals such as ciprofloxacin and a nonreactive tracer (chloride ion) were able to quickly penetrate the biofilms [85]. In fact, antimicrobials which do not appreciably interact with the molecules of the EPS matrix can often diffuse through biofilms as easily as through water [86].

The EPS matrix may also alter the effective concentration of antimicrobials throughout the biofilm in ways other than just acting as a simple diffusion barrier. The matrix can also quench the activity of antimicrobials diffusing throughout the biofilm [87] and various enzymes within the EPS matrix can inactivate or degrade certain antimicrobials [88-90]. The EPS matrix is also able to chelate[3] many metal ions such as lead (which are generally toxic to microbes [91, 92]), and react sacrificially with oxidative disinfectants such as chlorine [93, 94].

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[3]Chelation is the binding of ions and molecules to metal ions.
1.5 Evolutionary benefits of biofilm formation

Intuitively, one may be surprised by the prevalence of biofilms in nature. Since bacteria in biofilms have reduced reproductive fitness, arising from their lowered growth rates [95, 96], the existence of biofilms seems antithetical to the standard interpretation of Darwinian evolution. However, outside of the idealised laboratory environment, there are a litany of possible evolutionary advantages to the formation of biofilms, beyond the simple fact that unpopulated surfaces are a space to be occupied [97]. Some of these are similar to the benefits which macro-organisms experience when forming a community, such as commensalism - where a bacterial species may benefit from the presence of another, with neither one suffering detrimental effects [98-100]; or protection from predators (e.g., phagocytosis [101-103]). Other possible benefits include an enhanced level of resilience to mechanical stresses. Biofilms resist being sloughed from surfaces due to external flow fields, such as blood flow or water flowing though a pipe [16, 17, 104, 105], likely due to the viscoelastic properties of the EPS matrix [106-109]. Sessile bacteria are also more resilient to changes in pH [110,112], salinity [113, 114], pressure [115, 116] radiation, and ultraviolet light; although there are conflicting reports on some of these observations, suggesting that they may be species-dependent [117]. Crucially, bacterial biofilms are many times more resistant to antimicrobial therapies than their planktonic counterparts [14, 15, 118].

1.6 Enhanced antimicrobial resistance

Many studies have observed that bacteria which are contained in biofilms exhibit enhanced resistance to antimicrobials compared to bacteria which are not contained in biofilms. The factor by which the minimum inhibitory concentration (MIC - the lowest concentration of the antimicrobial required to inhibit the growth of the target organism [119]) is increased for bacteria within a biofilm ranges from ∼10-1000 [119, 121]. This increased resistance is generally considered to be the result of a variety of factors, such as the inhibited diffusion of the applied antimicrobial throughout the biofilm due to the EPS matrix [120, 122, 123], as discussed above.

The reduced growth rate of sessile bacteria within the biofilm is also a key factor
in their ability to resist applied antimicrobials. As the majority of antimicrobials target bacterial growth mechanisms such as cell wall, protein, or DNA synthesis, the rates of which increase with growth rate, the majority of antibiotics are less effective against slow-growing bacteria [95, 124–126]. Therefore, as it is generally only the outer layer of the biofilm which is metabolically active [127–129], even if antibiotics were able to penetrate the biofilm, they would still be unlikely to be efficacious against the dormant bacteria in the deeper parts of the biofilm.

Another important factor is the presence of “persisters” within the biofilm population. Persisters are dormant, non-growing cells which exhibit extremely high tolerances to applied antimicrobials [130], and have been observed to exist within biofilm populations [131, 132]. It should be noted here that tolerance to antibiotics is distinct from resistance. Resistance is the ability to grow in the presence of antibiotics, achieved via mechanisms such as modification of the drug site, or efflux pumps [133], quantified by the MIC. Whereas tolerance measures survival (not growth) in the presence of antibiotics. It is quantified by the time it takes to kill 99% of a population at concentrations much higher than the MIC [134, 135].

Highly tolerant persister cells can act as reservoirs for the biofilm population when non-persister cells are killed off, e.g., by antibiotics. While planktonic persister cells can be removed via the host’s immune response, in the case of biofilms, the EPS matrix protects the persisters [136, 137]. Once the surrounding antibiotic concentration has decreased to below the MIC, these persisters can then switch to a phase of rapid growth in order to repopulate the biofilm, continuing the infection [138, 139].

Persisters are not the only example of physiological variability within biofilms [140, 141]. Phenotypic heterogeneity within biofilms arises both as a response to the corresponding chemical heterogeneity [78, 142, 143], and also through mechanisms such as mutation and stochastic gene expression [144–146]. Biofilms generated from genetically homogeneous inocula can have populations composed of up to 10% variants only a few days later [147, 148], ergo, it is very likely that there is some level of natural selection at play within the biofilm which enhances the emergence of these variants. Multiple studies have verified the presence of variant subpopulations within bacterial biofilms [144, 145, 149, 150].

Phenotypic variants within biofilms often possess characteristics that may be advantageous in the biofilm environment. These variants may be more adept
at surface colonisation, exhibiting characteristics such as reduced motility [151]
and enhanced EPS production [146] [152] [153]; or they may exhibit a “division
of labour” stratagem, e.g., where some cells express the genes responsible for the
production of certain metabolites or enzymes while others do not [154] [153]. It
has also been proposed that this genetic/phenotypic variation within the biofilm
can act as an “insurance policy”, with the more diverse population typically
having a higher resilience to environmental challenges than a biofilm composed
of a singular phenotypic type [148] [156] [157].

Indeed, it is likely that multiple factors contribute to the enhanced resilience of
biofilms to applied antimicrobials. It should also be noted that enhanced levels of
tolerance to antimicrobial compounds appears to be intrinsic to the biofilm mode
of existence. Upon reaching the dispersal stage and becoming planktonic, the
bacteria lose their enhanced antimicrobial tolerances [158] [159], which further
suggests that the structure and composition of the biofilm do indeed impart
additional protections to its constituents.

1.7 Clinical and industrial implications of biofilm
formation

Biofilms are a concern in many clinical and industrial settings due to their
persistence and physical properties, which increases the difficulty of removing
them chemically. In clinical settings, biofilms can arise as contamination on
implanted devices, such as catheters, sutures, artificial hips or mechanical heart
valves [160] [161]; or as the result of various infections within the body, e.g., cystic
fibrosis [162] [163], periodontitis [164] [165] or native valve endocarditis [166] [167].
In total, bacterial biofilms are thought to be implicated in approximately 80% of
all infections found in the human body [168] [169].

In industrial settings, one particular issue caused by biofilms is their release
of harmful byproducts, which can cause spoilage of food products [170] [173] or
corrosion of the surface on which the biofilm is growing [174] [175]. Another main
area of concern arises from the mechanical properties of biofilms. As almost any
surface submerged in an aqueous environment is at risk of biofilm formation,
biofilms are frequently responsible for physical blockages in pipes and similar
structures [176] [177].
Biofilms are also responsible for one of the most pervasive problems in the shipping industry: marine biofouling [179–181]. The growth of bacterial biofilms on the exterior of a ship’s hull can significantly increase the drag experienced by the ship [182, 183]. One study found that a 1 mm thick layer of slime, which had developed on the side of a 23 m long hull over the course of 600 days, resulted in an 80% increase in the frictional resistance of the vessel [184]. The environmental and economic impact of this is summarised in Section 1.8.3.

1.8 Methods to combat biofilms

1.8.1 Mechanical methods

Various attempts have been made to curtail the growth of biofilms by mechanical means. As bacteria utilise magnetic isotopes such as $^{25}\text{Mg}$ in the production of the energy storage molecule ATP [185, 186], it was found that applying sufficiently high magnetic fields to bacterial populations of *E. coli* affects DNA replication and protein synthesis, thus suppressing biofilm formation [187–189]. Other studies found that bandaging wounds with dressings which generated weak electric fields could interrupt the electrostatic interactions required for bacteria to adhere to surfaces [190], as well as disrupt the electric current involved in interbacterial communication [191], therefore resulting in reduced levels of biofilm formation within the wounds [192]. Similarly, it was found that applying high voltage, pulsed electric fields to *P. aeruginosa* biofilms could cause permanent damage to the bacterial membranes, resulting in cell death [193].

In industrial settings, one method to remove established biofilms is by simply scraping them from the surface which they are growing on [194–198]. More sophisticated techniques such as ultrasound therapies have also been developed to enhance the removal of biofilm by inducing cavitation within the biofilm, thereby reducing its structural integrity and causing it to detach from the surface [199, 200]. Furthermore, ultrasound therapies have been shown to curtail biofilm growth and persistence in clinical contexts, such as in the treatment of wounds, but the underlying mechanisms of this phenomenon remain unclear [201, 202].

Ultraviolet (UV) light has also shown to be effective at reducing biofilm growth via damage to DNA and membranes [203, 204]. Contemporary research is being undertaken to investigate the viability of UV light for preventing marine
biofouling on ship hulls [205, 206]. Another solution which could be of use in preventing marine biofouling is that of surface wettability, wherein adjusting the material properties of the surface to increase its hydrophobicity may also reduce the ability of microbes to adhere to it [207, 208]. However, despite the growing catalogue of these mechanical methods, the majority of techniques to combat biofilm growth in use today are still chemical based [209].

1.8.2 Antibiotics

In clinical settings, chemical treatments for biofilm infections typically take the form of antibiotics - chemicals which kill bacteria or prevent their growth. There are multiple classes of antibiotics, but they can be grouped into two categories [210–212]: bacteriostatic antibiotics, which prevent cell growth; and bactericidal antibiotics, which cause cell death. Although the clinical relevance of this distinction is still debated [211, 212]. Some examples of bacteriostatic antibiotics are tetracyclines and macrolides, which inhibit the production of proteins within the bacteria by binding to ribosomes [213, 214]. Examples of bactericidal antibiotics include penicillins, which kill cells via inhibition of cell wall synthesis [215], and fluoroquinolones such as ciprofloxacin and levofloxacin, which cause cell death via damage to DNA [216].

The increasing prevalence of antibiotic/antimicrobial resistance (AMR) is a growing concern in the medical field. In 2019, there were an estimated 1.27 million deaths attributed to bacterial infections which had become genetically resistant to antibiotics [217], more than HIV/AIDS or malaria. This number has been predicted to rise to 10 million a year by 2050 [218]. This concern is compounded in the case of biofilms, where experiments [219, 220] and theory [221–224] have shown that the presence of an antimicrobial gradient can accelerate the evolution of resistance. Further theoretical work suggests that this depends on both the mutational pathway to resistance and the balance between the rates of bacterial migration and mutation [222, 224, 227]. A particular issue regarding the emergence of resistance within biofilms is the spread of resistant genes between adjacent bacteria. In a biofilm, the EPS immobilises the bacteria, facilitating greater rates of physical transference of DNA from one bacterium to another [228–230], via a mechanism known as “horizontal gene transfer” [231]. It is thought

\[\text{Similar spatial effects have also been observed in the emergence of resistance to cancer therapies [225, 226].}\]
that this process of physically transferring resistant genes from one bacterium to another may speed up the spread of resistance throughout the biofilm population.

1.8.3 Antifouling coatings

More intensive solutions for biofilm removal can be employed in industrial settings compared to clinical ones, such as scraping the biofilm from the surface, or harsh chemical treatments with toxic antimicrobial chemicals (biocides) [198, 236], as industrial surfaces are generally more resilient than the human body. However, it is often preferable to prevent biofilm formation in the first place. One particular example is the use of antifouling (AF) coatings in the shipping industry to inhibit marine biofouling.

AF coatings are paints which release a chemical biocide, intended to prevent the growth of micro- and macroorganisms on the coatings, and are often applied to the outer surface of ship hulls. In the case of the “self-polishing” or ablative class of AF paints, they consist of a biocidal compound encased in a polymer matrix. When exposed to seawater, the polymer matrix slowly dissolves, ensuring a controlled and constant biocide release rate [237, 239]. AF coatings are effective - they are estimated to reduce the fuel costs of the shipping industry by $60 billion each year, as well as lowering the yearly emissions of carbon dioxide and sulphur dioxide by 384 million and 3.6 million tonnes respectively [240]. However, they will inevitably fail and need to be reapplied every 1-5 years [238]. Furthermore, due to environmental concerns, an increasing number of biocidal compounds have either been banned or are being phased out of use [241].

Around 80% of the world’s trade by volume is transported via the shipping industry [242, 243], an industry which alone accounts for 7-8% of global greenhouse gases [244]. Understanding how AF paints affect microbial biofilms is therefore essential so as to design and utilise them with maximal effectiveness and minimal environmental harm.
1.9 Multispecies biofilms

Research on clinical and lab-grown biofilms has to date focused primarily on biofilms formed from a single bacterial species \cite{245,247}. While many, but not all, clinical biofilms are indeed dominated by a single bacterial species \cite{248-250,248,251,252}. In one instance, an oral biofilm was found to contain 100-200 different bacterial species \cite{253}, and others have been found to contain over 500 \cite{254}. In some cases these multispecies biofilms have been found to exhibit cooperative behaviour \cite{255-257}, such as the synergistic sharing of metabolites. This behaviour is likely not by coincidence, as co-evolution between species within the same biofilm has been observed, where the genome of one species will mutate to adapt to the presence of another one, thus reducing interspecies competition \cite{251,258,259}.

The presence of chemical gradients within biofilms also promotes the assembly of multispecies biofilms which exhibit commensalistic or mutualistic interactions. For example, in natural lakes or ocean environments, a biofilm exposed to an aqueous environment containing oxygen, sulphate and carbon sources has the potential to contain coexisting species of sulphate-reducing bacteria, sulphide-oxidising bacteria and aerobic heterotrophs - organisms which cannot produce organic substances from inorganic ones and must consume carbon compounds generated by other organisms \cite{260,261}. In the active layer, the heterotrophs consume oxygen, while in the anoxic core of the biofilm, the sulphate-reducing bacteria produce hydrogen sulphide from sulphate. Between these two regions, the sulphide-oxidising bacteria reduce the sulphide to sulphate \cite{66}. The combination of these three microbial “ecotypes” allows the multispecies biofilm community to maximise the usage of all nutrient sources and cycle nutrients where a monospecies biofilm could not.

In some cases, multispecies biofilms can enhance the population’s resistance to applied antimicrobials \cite{262}. In a biofilm comprised of the yeast *C. albicans* and the bacteria *S. aureus*, exopolysaccharides produced by *C. albicans* inhibited the penetration of antibacterial compounds such as vancomycin \cite{263,264}. Conversely, in a biofilm comprised of *C. albicans* and the bacteria *S. mutans*, polymers produced by *S. mutans* sequestered the antifungal agent fluconazole, providing additional protection to the *C. albicans* \cite{265}.
Just as phenotypic variation within monospecies biofilms can enhance antimicrobial resistance, as mentioned in Section 1.6, the presence of multiple species within a biofilm can also increase its overall resilience to an applied antimicrobial when compared to the corresponding monospecies biofilms. Either by synergistic nullification of the antimicrobial or due to the varying resistances of different species to the same antimicrobial. Multispecies biofilms are also relevant in marine biofouling, as the microbial biofilms that form are generally composed of many species, and prospective macrofoulers can differentiate between biofilms with different microbial species composition.

1.10 Previous biofilm models

Biofilm modelling has a rich history, with computational models of biofilms allowing researchers to easily investigate the basic principles governing the dynamics of biofilm formation, structure, composition and function. Spanning a decades-long history, these models cover a wide range of sophistication, computational intensity and underlying methodologies.

One such methodology, first developed in the 1970s and generally considered to be the progenitor of the field of biofilm modelling, is that of continuum models - an illustration of which is shown in Figure 1.3(a). These models do not examine the behaviours of individual bacteria, but rather utilise series of differential equations to model the concentrations of biomass in the system via one or more “density fields”, where the biofilm (bacteria + EPS) is approximated as a single fluid, with small localised stochastic variations in features such as growth rate or nutrient consumption smoothly averaged out over the group population.

In these types of models, growth and development of the biofilm is mediated via a corresponding series of conservation equations, such as the diffusion and subsequent consumption of nutrients from the surrounding bulk fluid into and throughout the biofilm; or conservation of momentum, used to model the effects of an external flow field on the shape and structure of the biofilm. The appeal of continuum models is that it is straightforward to incorporate governing laws taken from more mature fields of mathematical modelling, such as fluid dynamics, into the biofilm model. Furthermore,
due to their innately deterministic nature, they are only required to be simulated once for a given set of initial conditions, generally making them an efficient choice of model type. However, in order to fully capture the spatial heterogeneity of biofilms, these models need to be extended to higher dimensions, as shown in Figure 1.3(b). Doing so increases their mathematical and numerical complexity, which will in turn increase the associated computational cost required to solve them.

An alternative and popular approach to biofilm modelling is the more contemporary class of discrete models. First used to model biofilms in the 1990s, these models represent the bacteria as individual agents within the system. Discrete models are generally divided into two further sub-classes,
cellular automaton (CA) models, shown in Figure 1.3(c), where the agents in
the system are restricted to a grid/lattice of spatial coordinates, which also
describes their size/local concentration; or individual based (IB) models, shown
in Figure 1.3(d), where the agents can be located anywhere in space, and may have
a variety of sizes. These discrete agents can represent entities such as individual
bacteria or nutrient molecules [292], with their position and state being updated
each timestep according to a specified set of rules, which commonly depend
on the local conditions surrounding each agent. IB models are typically more
computationally intensive than CA ones, and are therefore generally used to
model biofilms on the lengthscales of micrometres to centimetres, whereas CA
models can be implemented to model larger scales [293].

A particular advantage which discrete models have over their continuum coun-
terparts is in the modelling of multispecies biofilms [294–296]. Where continuum
models require a complementary set of differential equations for each microbial
species in the system - quickly increasing the model’s complexity, discrete models
can incorporate multiple species by simply including additional rulesets for each
species. Furthermore, rules governing interactions such as attachment/detach-
ment of bacteria to/from the biofilm are also easily adjusted. Indeed, the rulesets
for discrete models are often able to be motivated from observed biological
principles, instead of via the analyses of physical and mathematical frameworks,
making these types of models more appealing to biologists [297]. However, care
must also be taken that in the absence of such frameworks, that the models do
not become overly driven by the aesthetics of the biofilm’s behaviour, with the
underlying ruleset having little grounding in reality [284].

While continuum models typically demonstrate deterministic behaviour, discrete
models are customarily more stochastic in nature. This is due to factors such as
diffusion and cell movement obeying random walks, or the placement of
daughter cells surrounding the mother cell following cell replication also being
randomly selected [275]. While this feature of discrete models can indeed be
desirable, due to the inherent stochasticity of in vivo biofilms [293], this
innate stochasticity also has its downsides. Namely, before conclusions can
be confidently drawn from the models, the stochasticity must be averaged out
by performing replicate simulations with identical initial conditions, thereby
increasing the overall computational cost.

5Such as John Conway’s famous Game of Life, the first example of a CA model [291].
Hybrid models are also common, where the bacteria and corresponding biofilm structure are modelled as discrete agents, but other system components/dynamics are modelled via a continuum manner, such as the transport and consumption of nutrients throughout the biofilm behaving according to a standard reaction-diffusion equation, rather than as a discrete random walk [298–300]. It has been found that using finite-difference methods to model the nutrient field in this manner can increase both computational efficiency and the fidelity of model outcomes [298]. Furthermore, as external flow fields primarily target the slime of the biofilm, rather than the individual cells [301], treating the EPS matrix as a continuum field allows for the effects of flow on the topography of the biofilm-fluid interface to be included in the model [297, 302] - a feature which is decidedly non-trivial to incorporate in purely discrete models [303].

Amongst many examples, the usage of computational models has helped further the understanding the role of preformed cellular aggregates on biofilm growth [304], the role of fluid flow on the shape of the mature biofilm [305, 306], the role of cooperation between bacteria in biofilm formation [307, 308], how bacteria orient themselves vertically within biofilms [309], and what factors influence a biofilm’s shape and structure [310–312]. However, the majority of the work undertaken in the modelling of biofilms to date has focused on well-developed biofilms, few theoretical models exist for the early stages of biofilm establishment [313].

1.11 Thesis overview

This thesis aims to build on the aforementioned rich body of work of using computational models to understand biofilm growth and development, both by adapting previously established model architectures and by utilising contemporary tools to develop novel models. The work presented here takes the form of several relatively distinct projects, united under the common theme of biofilm initiation and growth in spatially heterogeneous environments, including both antibiotic concentration gradients and non-uniform flow fields. It begins in Chapter 2 with a simple 1D lattice-based model of a bacterial population growing in a spatial gradient of growth rate-dependent antibiotics. This aims to emulate the conditions typically found in biofilms of both chemical gradients and disparate growth rates within the population.

Chapter 3 builds on this model architecture to develop a model for the stochastic
formation of biofilms on surfaces, with the aim of determining which system parameters play a key role in the unpredictability of biofilm nucleation. Chapter 4 then extends this model to study marine biofilms growing on AF surfaces, by incorporating multiple microbial species into the model, as well as a spatial gradient of a biocidal compound originating from the surface.

Chapter 5 presents the development of an entirely new model, using computational fluid dynamics to investigate the effects of varying flow angle on the drag and biofilm sloughing properties of a shark skin-like riblet surface, a novel approach to reducing drag in the shipping industry. Finally, some concluding remarks on the thesis as a whole are presented in Chapter 6.
Chapter 2

Growth rate-dependent drug susceptibility

2.1 Introduction

Spatial gradients of environmental parameters are known to affect both the ecology and evolution of populations consisting of both macro- and microorganisms [314–323]. One such scenario is where a population wave is halted in its spatial expansion into an increasingly deleterious environment, to which the population must develop resistance in order to further advance. Through the use of a simple stochastic, lattice-based computational model, this chapter aims to investigate a specific example of this scenario, namely the expansion of a bacterial population wave along an increasing antibiotic concentration gradient. In this context, a population wave can be interpreted as a growing bacterial colony (or biofilm), expanding into an uncolonised territory in order to increase the nutrient availability of the population.

As the population wave expands along the concentration gradient of the inhibitory substance, individuals within the population experience changes in the abundances of both nutrients and the inhibitor. At the wave’s tip, individuals are exposed to higher concentrations of the inhibitor, and due to the lower population density, nutrient availability is also higher. In contrast, for the bulk of the population behind the tip, the environment is less deleterious due to the lower inhibitor concentration, but the higher population density limits growth due to
reduced nutrient availability.

This scenario is of particular interest if there exists a coupling between an organism’s susceptibility to the inhibitor and the nutrient availability. In the case of bacteria, it has been shown that the nutrient conditions can strongly influence their susceptibility to antibiotics, as briefly mentioned previously in Section 1.6. For most antibiotics, *E. coli* that are growing quickly due to rich nutrient availability are more susceptible than their slow-growing counterparts in nutrient poor media [126, 324–327]. For other antibiotics however, the inverse is true, with slow-growing *E. coli* being more susceptible [124, 126, 325, 326].

Previous work by Greulich et al. [124, 328] suggests that antibiotics with the same molecular target (e.g., ribosomes) can have differing effectiveness between fast- or slow-growing bacteria depending on molecular parameters such as target-binding and transport rates. For *P. aeruginosa* growing in an established biofilm, experimental work has shown that antibiotics such as ciprofloxacin, tetracycline and tobramycin are more effective against the fast-growing subpopulation at the biofilm’s surface [73], whereas membrane-targeting antimicrobials such as the peptide colistin, ethylenediaminetetraacetic acid (EDTA) and sodium dodecyl
sulfate (SDS) are more effective against the slow-to-non-growing subpopulation found deeper inside the biofilm [73, 329, 330]. An example of this growth rate dependency is shown in Figure 2.1 where, depending on the type of antibiotic applied to the pictured *P. aeruginosa* biofilms, either the fast growing outer layer or slow growing inner bulk experience cell death.

In the context of an advancing bacterial population wave, growth-dependent susceptibility implies that the fast-growing bacteria at the wavefront may exhibit differing susceptibility than the slow growing bacteria in the bulk of the population, *i.e.* there may be a coupling between the ability of the population to colonise along an antimicrobial gradient and nutrient limitation within the advancing population wave. The implications of such a coupling are investigated here by modelling the advancement of a population wave exposed to gradients of either bacteriostatic (inhibit bacterial growth) or bactericidal (cause bacterial death) antibiotics, under conditions where either fast-growing or slow-growing bacteria are more susceptible to the antibiotic.

The results obtained from the model presented here suggest that there are significantly different outcomes in these two cases. For antibiotics where the fast-growing bacteria are more susceptible, the population wave cannot expand past a relatively low antibiotic concentration, and if the applied antibiotic is bactericidal, a large proportion of the population remains alive behind the wavefront. For antibiotics where the slow-growing bacteria are more susceptible, the population is able to spread much further, but leaves a wake of dead bacteria behind it.

The work undertaken here reveals basic principles which may have implications for developing treatment strategies for bacterial infections growing in spatially structure environments, such as biofilms.

It should be noted that while this model can be interpreted as a biofilm growing on a surface, it is not explicitly intended to be interpreted as such (unlike the models presented in the other chapters), but rather as a more general model of a bacterial population growing in a spatially heterogeneous environment.
2.2 Methodology

![Diagram of interconnected microhabitats](image)

**Figure 2.2** (a) Illustration of the model. It consists of a chain of interconnected microhabitats, labelled by an index \( i = 0 \) to \( L - 1 \). The concentration of antibiotic can be different in each microhabitat (and is fixed throughout the simulation). As the bacteria multiply, the nutrient is consumed, which alters the local growth rate of the bacteria. (b) Growth-dependent susceptibility as implemented in the model. The minimal inhibitory concentration (MIC) is plotted versus the Monod growth function \( g(s) \) (Equation 2.2), which depends on the nutrient concentration \( s \), for the three types of antibiotics studied: fast-growth targeting antibiotics (FGTA), growth-independent antibiotics (GIA) and slow-growth targeting antibiotics (SGTA). The arrows represent the direction in which the MIC changes with nutrient consumption - consuming nutrient during bacterial replication decreases the local value of \( g(s) \). For FGTA the MIC increases as \( g(s) \) decreases, whereas the inverse is true for SGTA.

The model here is inspired by previous work by Greulich et al. [223, 323], which aimed to study the influence of antibiotic gradients on the evolution of resistance. The model presented here differs from that of Greulich’s in two key ways. Firstly, no mutations are present, the efficacy of the applied antibiotics varies only with local growth rate. Secondly, rather than utilising a “carrying capacity” to limit bacterial growth, where the growth rate decreases as the local population density approaches a specified upper limit, it is instead constrained via a finite supply of nutrient: replication consumes nutrient, once the supply is depleted, no further growth is possible.
The form of the model is that of a series of $L$ linearly interconnected “microhabitats”, as shown in Figure 2.2(a). In Greulich’s model, this was intended to represent connected chambers in a microfluidic experiment \[219, 223\], here it is intended as a generic representation of a spatially expanding population. Each of these microhabitats contain a specified concentration of antibiotic and nutrient. The system is initialised with $N_0$ bacteria in the leftmost microhabitat, these bacteria can then replicate, consuming a unit of nutrient in the process; migrate between adjacent microhabitats, or die, in the case where bactericidal antibiotics are applied.

The number of bacteria $N_i$, and the nutrient concentration $s_i$, in each microhabitat $i \in [0, L)$ changes over time. The antibiotic concentration $c_i$ and the rate at which bacteria can migrate between microhabitats $m$, are fixed at the start of the simulations and remain constant throughout, i.e. the effects of diffusion on the antibiotic gradient are neglected here.

All bacteria in a given microhabitat have the same local growth rate, $b_i$, which is dependent on the nutrient concentration and antibiotic concentration in the microhabitat,

$$b_i = \phi(c_i, \beta_i) \cdot g(s_i). \quad (2.1)$$

Here $g(s_i)$ is the Monod function, which couples the nutrient concentration to the growth rate \[331\]:

$$g(s_i) = \frac{s_i}{s_i + K}, \quad (2.2)$$

where $K$ is the Monod constant - the value of the nutrient concentration at which bacterial growth is half-maximal. Here it was set to $K = 33$ nutrient concentration units, discussed further in Section 2.2.1 $g(s)$ increases with $s$, and saturates at values of $s \gg K$.

$\phi(c_i, \beta_i)$ is the pharmacodynamic function. This incorporates the inhibitory effects of the antibiotic concentration on the bacterial growth rate. $\beta_i$ is the minimum inhibitory concentration (MIC), with the subscript $i$ denoting the fact that this MIC applies specifically to the bacteria in microhabitat $i$. It is via $\beta_i$ that growth-dependence of antibiotic susceptibility is incorporated into the model, due
to the MIC being a function of the local nutrient concentration, $\beta_i(s_i)$.

In the following work, two contrasting classes of antibiotics were modelled, fast-growth targeting antibiotics (FGTA), which fast-growing bacteria are more susceptible to, and slow-growth targeting antibiotics (SGTA), which slow-growing bacteria are more susceptible to. Based on the findings of [124], a linear relation between $s$ and $\beta$ was assumed. For the FGTA, the MIC decreases with growth rate:

$$\beta_{\text{FGTA}}^i(s_i) = 10 - \frac{9g(s_i)}{g_{\text{max}}}, \quad (2.3)$$

while for the SGTA the MIC increases with growth rate

$$\beta_{\text{SGTA}}^i(s_i) = 1 + \frac{9g(s_i)}{g_{\text{max}}}, \quad (2.4)$$

see figure 2.2(b). $g_{\text{max}}$ is the maximal value of $g$, when no nutrient has been consumed. Also modelled is the situation where the MIC is independent of growth rate, termed “growth-independent antibiotics”, or GIA; set here to $\beta^{\text{GIA}} = 5$.

Both bacteriostatic and bactericidal versions of the growth-targeting antibiotics were modelled, via the use of differing pharmacodynamic functions. For bacteriostatic antibiotics, a quadratic form was assumed [223],

$$\phi(c_i, \beta_i) = \begin{cases} 
1 - \left( \frac{c_i}{\beta_i} \right)^2 & \text{if } \frac{c_i}{\beta_i} < 1, \\
0 & \text{if } \frac{c_i}{\beta_i} \geq 1.
\end{cases} \quad (2.5)$$

For a bactericidal antibiotic, the general pharmacodynamic function proposed by Regoes et al. [332],

$$\phi(c, \beta) = \psi_{\text{max}} - \frac{(\psi_{\text{max}} - \psi_{\text{min}})(c/\beta)^\kappa}{(c/\beta)^\kappa - \psi_{\text{min}}/\psi_{\text{max}}} \quad (2.6)$$

was used. $\psi_{\text{max}}$ corresponds to the growth rate of bacteria in the absence of antibiotics and $\psi_{\text{min}} = \psi_{\text{max}} - E_{\text{max}}$ is the net minimum growth rate, with $E_{\text{max}}$ signifying the maximum antibiotic-mediated death rate. If $\psi_{\text{min}} < 0$, i.e. the bacteria are dying faster than they can replicate, then $\psi_{\text{min}}$ corresponds to
the maximum possible death rate which the bacteria may experience. $\kappa$ is the Hill coefficient \cite{Hill}, which measures how strongly the death rate changes in response to changes in the concentration of the antibiotic around the MIC, \textit{i.e.} the steepness of the sigmoid. The values of $\kappa$ are obtained via experimental methods and are antibiotic dependent. For all simulations performed here, $\kappa = 2$, which is both consistent with experimental data and increases the efficiency of numerically evaluating the function. The other parameters had values of $\psi_{\text{max}} = 1$ and $\psi_{\text{min}} = -5$,

$$
\phi(c_i, \beta_i) = 1 - \frac{6 (c_i/\beta_i)^2}{5 + (c_i/\beta_i)^2}.
$$

(2.7)

If the local antibiotic concentration is less than the MIC of the bacteria in the microhabitat, $c_i < \beta_i$, Equation (2.7) is used as input to Equation (2.1) to compute the local growth rate. For higher concentrations of the bactericidal antibiotic, $c_i > \beta_i$, $\phi$ becomes negative and bacteria do not grow but instead die, at rate $d_i = -\phi(c_i, \beta_i)$. Upon dying, bacteria are simply removed from the system - dead cells do not occupy any physical space.

### 2.2.1 Model parameters

The model here aims to be generic in its nature, rather than represent particular antibiotic-bacteria species combinations. Nonetheless, it is important to consider the units of time, space, antibiotic and nutrient concentrations. All simulations are initialised with a nutrient concentration $s_{\text{max}} = 500$ in all microhabitats, and an initial inoculum of $N_0 = 100$ bacteria in the first microhabitat ($N_i = 0$ elsewhere).

The units of time can be taken to be hours. This is consistent with the choice of maximal growth rate, $b_{\text{max}} \approx 1 \text{ h}^{-1}$, which is a typical growth rate for \textit{E. coli} on minimal lab media \cite{Ecoli_Growth} and the volume of a microhabitat is $\sim 1 \text{ \mu l}$, or alternatively $1 \times 1 \times 1 \text{ mm}$. Once again following the previous work of Greulich et al. \cite{Greulich_2018}, the number of microhabitats in the model, $L$, was set to $L = 500$. Also following Greulich et al., the migration rate was set to $m = 0.1$ microhabitats per hour ($\text{mh h}^{-1}$). This was chosen to be relatively small compared to the maximal growth rate, which corresponds to a fairly low effective bacterial diffusion constant, similar to that of the microfluidic devices used by Zhang et al. \cite{Zhang_2018}.
The units of nutrient concentration are defined in terms of the yield, or the amount of nutrient required to create a single bacterial cell. Thus the chosen value of $s_{\text{max}} = 500$ implies that the maximal bacteria density which may be reached in the model is 500 bacteria per microhabitat, or $\sim 5 \times 10^5$ cells ml$^{-1}$. For E. coli growing on glucose minimal media, the Monod constant is approximately $1 \, \mu$M, or equivalently $6 \times 10^{17}$ molecules per litre [335, 336]. The yield, i.e. the amount of glucose consumed to make one E. coli bacterium, is approximately $1.8 \times 10^{10}$ molecules [335, 336]. With a microhabitat volume of $1 \, \mu$l, $K$ translates to $3.3 \times 10^7$ bacterial yields per litre, or $K = 33$ bacterial yields per microlitre.

As the MIC functions $\beta_j^{\text{FGTA}}$ and $\beta_j^{\text{SGTA}}$ (Equations (2.3) and (2.4)) have minimal values of 1, the antibiotic concentration can be considered to be measured in units of the MIC. For FGTA, this minimal value occurs when the nutrient availability is at its highest, and the inverse for SGTA.

### Table 2.1 Parameters used in the computational model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V$</td>
<td>Microhabitat volume</td>
<td>$1 , \mu$l</td>
</tr>
<tr>
<td>$\delta z$</td>
<td>Microhabitat thickness</td>
<td>$1 , \text{mm}$</td>
</tr>
<tr>
<td>$L$</td>
<td>No. of microhabitats</td>
<td>500</td>
</tr>
<tr>
<td>$b_{\text{max}}$</td>
<td>Maximum growth rate</td>
<td>$\approx 1 , \text{h}^{-1}$</td>
</tr>
<tr>
<td>$m$</td>
<td>Migration rate</td>
<td>$0.1 , \text{h}^{-1}$</td>
</tr>
<tr>
<td>$s_{\text{max}}$</td>
<td>Initial nutrient concentration</td>
<td>$500 , \text{mh}^{-1}$</td>
</tr>
<tr>
<td>$K$</td>
<td>Monod constant</td>
<td>33</td>
</tr>
<tr>
<td>$c_L$</td>
<td>Maximum antibiotic concentration</td>
<td>$\approx 10.6$</td>
</tr>
<tr>
<td>$\beta_{\text{max}}$</td>
<td>Maximum MIC</td>
<td>10</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Antibiotic gradient steepness</td>
<td>$4.9 \times 10^{-3} , \text{mh}^{-1}$</td>
</tr>
</tbody>
</table>

#### 2.2.2 Model algorithm

The model was simulated using the Monte Carlo algorithm introduced by Greulich et al. [223], which is comparable to the well-known Gillespie algorithm [337, 338]. For this particular model, the Monte Carlo algorithm is faster than the standard Gillespie algorithm, due to the small number of calculations required to iterate each step, and the fraction of steps in which an event happens is relatively high (typically $>25\%$).
1. A bacterium is selected at random from the current system population, $N_{tot}$.

2. The rates for birth ($b_i$), migration ($m$) and death ($d_i$, in the case of bactericidal antibiotics) were calculated for the selected bacterium.

3. A random number, $r$, is drawn from a uniform distribution between 0 and $R_{max}$, where $R_{max} > b_i + m + d_i$. A value of $R_{max} = 1.2$ was used for the bacteriostatic antibiotic and $R_{max} = 5.2$ for the bactericidal antibiotic, due to the larger size of the death rates resulting from the bactericidal pharmacodynamic function.

4. $r$ is then used to select either a migration event, replication event, a death event or no event, with probability proportional to the rates for these events.

5. Once the event has been carried out, the time elapsed in the system ($t$) is updated by $\Delta t = 1/N_{tot}R_{max}$.

2.3 Results

2.3.1 Expansion in a uniform antibiotic concentration

Firstly, the manner in which a bacterial population invades a region with a spatially uniform bacteriostatic antibiotic concentration was studied, i.e. $c_i = c$ for $i \in [0, L)$. In the continuum limit and in the absence of growth-dependent susceptibility, the model is expected to map onto the Fisher-KPP equation \[223, 339–342,\]

$$\frac{\partial n}{\partial t} = D \frac{\partial^2 n}{\partial x^2} + b_{max} n \left( 1 - \frac{n}{n_{max}} \right),$$

(2.8)

where $D$ is an effective diffusion constant arising from the migration between microhabitats, $n_{max}$ is the carrying capacity, $n$ is the population density ($N_i/n_{max}$), and $b_{max}$ is the maximal growth rate.

The Fisher-KPP equation has solutions of travelling waves \[343\] with speed

$$v = 2\sqrt{Db_{max}n_{max}}.$$
Figure 2.3 Population waves for a uniform concentration of a bacteriostatic antibiotic. Panels (a-i) show snapshots of the population density sampled every 300 time units, with the different columns representing FGTA, GIA and SGTA (left to right) at antibiotic concentrations $c = 0.5$, $c = 1.0$ and $c = 5.0$ (top to bottom). Successive samples are represented chromatically, with light green corresponding to earlier times, darker green to later times. Panels (j) and (k) show the average speed of expansion of the population wave as a function of antibiotic concentration for the three antibiotic types. The dependence of wave speed on concentration is quadratic (k, inset), consistent with expectations from Fisher-KPP wave theory. The wave travels faster in the SGTA case than the FGTA case, but the curves all collapse onto one another when the antibiotic concentration is scaled by the value of the MIC for maximal nutrient concentration, $\beta_{\text{max}} \equiv \beta(s_{\text{max}})$ (j, k).
In the model, $b_{\text{max}}$ is scaled by the pharmacodynamic function $\phi(c, \beta)$ which, in the case of bacteriostatic antibiotics, obeys Equation 2.5. Therefore, the speed of these Fisher-KPP waves is expected to scale with

$$v \propto \sqrt{1 - \left(\frac{c}{\beta}\right)^2},$$

provided that the antibiotic concentration is below the MIC ($c < \beta$). For antibiotic concentrations above the MIC, the bacteria are unable to grow, therefore there is no wave propagation.

Figure 2.3(b, e, h) illustrates that for growth-independent antibiotics (GIA), the population does indeed expand as a travelling wave for $c < \beta_{\text{GIA}} = 5$, with the speed scaling as expected, Figure 2.3(j, k). Growth-dependent susceptibility introduces a quantitative but not qualitative change to the results. For both FGTA and SGTA, the simulations show that the population expands as a travelling wave, but the growth-dependent susceptibility alters the range of antibiotic concentrations over which expansion can occur. For FGTA, population expansion happens only for $c < 1$, Figure 2.3(a, d, g), whereas for SGTA, expansion is observed even for $c = 5$ Figure 2.3(c, f, i). Thus, for a uniform antibiotic concentration, the fast-growth targeting antibiotic is much more effective at preventing bacterial proliferation.

This can be explained by noting that the governing dynamics of Fisher-KPP arise at the leading edge of the wave front [342, 344], i.e. these are “pulled waves” [342, 344, 345]. The leading edge of the wave front is where the population density is at its lowest, and the nutrient concentration is at its highest. Therefore, for FGTA, as the MIC decreases with increasing nutrient concentration, this implies that bacteria at the edge of the wave front are maximally inhibited by the antibiotic. In contrast, the MIC for SGTA increases with increasing nutrient concentration, meaning that bacteria at the tip of the population are minimally inhibited.

This suggests that the observed dynamics of growth-dependent susceptibility should correlate with Fisher-KPP theory, but with the wave speed controlled by the value of the MIC at the wave edge. Figure 2.3(j, k) confirms this hypothesis. Scaling the antibiotic concentration by $\beta_{\text{FGTA}}(s_{\text{max}}) = 1$ for the FGTA and by $\beta_{\text{SGTA}}(s_{\text{max}}) = 10$ for the SGTA, causes the three sets of simulation
data to collapse onto a common curve. Therefore, in this model, the significant quantitative differences observed between FGTA and SGTA can be explained by the contrasting antibiotic susceptibilities of bacteria at the nutrient-rich tip of the travelling population wave.

### 2.3.2 Expansion in a non-uniform antibiotic concentration

For a bacterial population expanding into a region containing a spatial gradient of antibiotic, it is expected that the population’s expansion will slow and eventually halt once the population reaches a region with an antibiotic concentration equivalent to its MIC \[223\]. Here, once again following Greulich et al. \[223\], a monotonically increasing exponential antibiotic gradient is assumed, given by

\[
\begin{align*}
    c_i &= \exp(\alpha i) - 1,
\end{align*}
\]

where \(\alpha\) represents the steepness of the gradient. Once again, bacteriostatic antibiotics are utilised in these simulations.

This antibiotic profile is shown in Figure 2.4(a), with a value of \(\alpha = 0.0049\) (purple curve) which results in a relatively long decay length of around \(\sim 1/\alpha \simeq 2\) m. This lengthscale is similar to that of the large gradient plate used by Baym et al. \[220\], but was also chosen such that the highest concentration of antibiotic was slightly greater than the maximal MIC value: \(c_{L-1} \approx 10.6\). Using steeper gradients produces qualitatively similar results, albeit with the population wave halted at a shorter distance along the microhabitat chain.

Figure 2.4(b) shows the positions of the population wavefronts vs time. Looking firstly at the GIA (orange line), after some delay the progress of the population is indeed inhibited by the presence of the antibiotic gradient. Figure 2.4(d) shows the wave profile for GIA, each line represents successive time samples. Once the concentration reaches a sufficiently high level such that growth is no longer possible, further population expansion is possible only through diffusion. Figure 2.4(g) further illustrates the cause of the wave’s impedance: the growth rate \(b_i\) decreases in front of the wave due to the increasing antibiotic concentration. Additionally, the growth rate decreases behind the wavefront due to nutrient depletion.
Figure 2.4 Population waves in a gradient of a bacteriostatic antibiotic. (a) Profile of the antibiotic concentration for $\alpha = 0.0049$ (studied here), and for $\alpha = 0.02$. (b) Position of the advancing population wavefront over time for the three antibiotic types. The waves advance at a constant speed before stopping when the antibiotic concentration prevents bacterial growth. The SGTA is the least effective at curtailing the spatial advancement of the population, followed by GIA then FGTA. (c-e) Snapshots of the bacterial population density sampled every 300 time units, for the three antibiotic types. Sample time is represented by the shade of green, ranging from light (early) to dark (late). The wave spreads further in the SGTA than the FGTA case. (f-h) Spatial profiles of the local bacterial growth rate ($b_i$), sampled every 300 time units for the three antibiotic types. Sample time is again represented chromatically, ranging from blue (early) to orange (late). As the simulation progresses, the growth rate decreases behind the wavefront, illustrated by the orange curves approaching 0 in the leftmost microhabitats at later sample times.
This behaviour is modified with the presence of growth-dependent susceptibility. For the bacteria exposed to FGTA, antibiotic susceptibility is at its highest at the very tip of the advancing wave, due to the increased access to the nutrient. This in turn lowers the growth rate at the tip of the wave, Figure 2.4(f), which results in the wave’s advancement being inhibited at a lower antibiotic concentration than the GIA, Figure 2.4(b). Contrasting results are obtained for SGTA. There, antibiotic susceptibility is at its lowest at the wavefront and at its highest in the trailing bulk due to limited nutrient availability, Figure 2.4(h). This allows for the population exposed to SGTA to proliferate further than those exposed to FGTA and GIA, as shown in Figure 2.4(b).

Overall, the simulations performed here illustrate that growth-dependent susceptibility can have important effects on bacterial populations proliferating through spatial antibiotic gradients. For antibiotics more effective at curtailing fast-growing bacteria (FGTA), strong inhibition is exhibited at the tip of the population wavefront, which in turn strongly reduces the population’s ability to colonise along the gradient. However, in the opposite case, where the antibiotic is more effective against slow-growing bacteria (SGTA), the antibiotic gradient fails to inhibit growth at the population wavefront, allowing colonisation up to much higher antibiotic concentrations than FGTA.

### 2.3.3 Bactericidal antibiotics

Following the above simulations, the models were then modified to incorporate bactericidal antibiotics, which cause bacterial cell death rather than only preventing cell growth. To do so, the pharmacodynamic function as shown in Equation 2.7 was implemented, with the bacteria experiencing a death rate equal to $d = -\phi$ when $\phi < 0$. The results of which are shown in Figure 2.5.

For the bacteria exposed to FGTA, Figure 2.5(a-c) shows several snapshots of the advancing population wave, taken at $t = 500, 1000, 2000$. Live bacteria are shown in green, dead in red. The bacteria at the tip of the wave die, while the bulk of the bacteria remain alive, but are mainly non-growing due to nutrient depletion, Figure 2.5(g).
Figure 2.5  Population dynamics in a gradient of a bactericidal antibiotic (with $\alpha = 0.0049$). Panels (a-f) show snapshots of the population densities of live (green) and dead (red) bacteria, sampled at $t = 500, 1000$ and $2000$ time units, for the FGTA (a-c) and SGTA (d-f). For the FGTA (a-c), dead bacteria appear at the wave edge once the wave’s advance halts. For the SGTA (d-f), a “striped” population structure emerges, with alternating blocks of live and dead bacteria, and a large “dead zone” of dead bacteria behind the wave front. Panels (g-h) show snapshots of the local growth rate $b_i$ for the FGTA and SGTA respectively (in panel (g), the red line overlays the other lines). Panels (i, j) track the percentage of the population which is alive (green lines, left-hand y-axis) and the total population size (alive and dead; purple line, right-hand y-axis), for the FGTA (i) and SGTA (j). Although the total population is larger for the SGTA, the absolute number of live bacteria is similar for the two antibiotics. Therefore there is a trade-off between having a small, yet active population (FGTA), or a larger, but more inert one (SGTA).

Turning now to the SGTA, as in the case for the bacteriostatic SGTA, the
population advances further into the antibiotic gradient than the FGTA. However, the population composition within the wave differs greatly from the FGTA here. For the SGTA, a “striped” population structure is observed, as shown in Figure 2.5(d-f). As in, bacteria at the tip of the wave are live and growing, followed by a zone of dead bacteria and finally a region of live but non-growing bacteria at the rear of the wave.

2.3.4 Continuum model

Figure 2.5 reveals a discretisation effect in the model. For the SGTA simulations, the repeating peaks in the distribution of dead bacteria throughout the microhabitats arise due to the nutrient concentration being limited to discrete values, coupled with the small number of bacteria. i.e. in the model, there is a brief crossover between growth and death, which occurs in microhabitat $i^*$, where $\phi_i(c_i, \beta_i) = 0$. From Equations (2.7) and (2.9), $i^*$ can be calculated to have a value of $i^* = \alpha^{-1} \ln \left[ 2 + 9\left(\frac{g(s_i)}{g_{\text{max}}}\right) \right]$.

The nutrient concentration is measured in discrete units of the yield. Starting at the back of the population wave, when the nutrient is depleted ($s_i = 0$), $i^* = \alpha^{-1} \ln \left[ 2 + 9(g(0)/g_{\text{max}}) \right] = 141$. This is the index of the microhabitat at which the population begins to die. Beyond this point, the nutrient concentration is higher and therefore the bacterial susceptibility is lower. As such, the next microhabitat where $\phi_i(c_i, \beta_i) = 0$ is when $s_i = 1$. This shifts $i^*$ to $i^* = \alpha^{-1} \ln \left[ 2 + 9(g(1)/g_{\text{max}}) \right] = 168$. $i^*$ continues to increase in this manner, with $i^*(s_i = 3) = 191$, and so on. This causes the nutrient concentration in the model to undergo a series of discrete steps close to the wave edge, each of which shifts the threshold between growth and death, and causes a peak in the distribution of the number of dead bacteria. The largest size of the first peak can be attributed to live bacteria migrating from the “live zone” into the “dead zone”.

To verify that the key observations of the SGTA were robust, namely that the expanding population wave leaves a trail of dead bacteria in its wake, a continuum model for the bactericidal antibiotic was also simulated. This continuum model consists of the following equations for the population density $n(x, t)$ and nutrient concentration $s(x, t)$,
\[
\frac{\partial n}{\partial t} = \begin{cases} 
D \frac{\partial^2 n}{\partial x^2} + n[\phi(c, \beta) + \epsilon] & \text{if } \phi(c, \beta) \geq 0, \\
D \frac{\partial^2 n}{\partial x^2} + n\phi(c, \beta) & \text{if } \phi(c, \beta) < 0.
\end{cases}
\] (2.10)

and

\[
\frac{\partial s}{\partial t} = \begin{cases} 
-n[\phi(c, \beta)g(s) + \epsilon] & \text{if } \phi(c, \beta) \geq 0, \\
0 & \text{if } \phi(c, \beta) < 0,
\end{cases}
\] (2.11)

where \( D \) is a diffusion constant, related to the migration rate \( m \) in the discrete model. Mapping a discrete 1D diffusion model with a lattice size of \( \delta x \) and migration rate \( m \) results in \( D = m(\delta x)^2 \). In the discrete model the microhabitat size is taken to be \( \delta x = 1 \) mm and \( m = 0.1 \) h\(^{-1}\), which gives a value of \( D = 0.01 \) mm\(^2\)h\(^{-1}\) \( \approx 3 \) \( \mu \)m\(^2\)s\(^{-1}\). \( \epsilon \) is a random variable with mean zero, uniformly distributed in the range \( \pm 0.1 \cdot \phi_{\text{max}}(c, \beta) \).

The continuum model was solved using a simple Forwards-Time Central-Space finite difference method, with \( \delta t = 0.01 \) and \( \delta x = 0.1 \) mm on a spatial grid of 5000 elements, with zero-flux boundary conditions at both ends.

Figure 2.6 shows the results of the continuum simulations, for the FGTA and SGTA antibiotics. These results have the same qualitative features as those of the discrete model: the population wave advances further into the antibiotic gradient for the SGTA than for the FGTA, but for the SGTA a wake of dead bacteria trails behind the wave tip.

It should be noted that the noise term in Equations (2.10) and (2.11) is necessary to cause this behaviour. This is due to the “tipping points” observed in the model when the local nutrient concentration is such that the pharmacodynamic function \( \phi = 0 \). For bactericidal antibiotics, when the nutrient concentration reaches this threshold, bacterial death begins, which is the cause of the striped population distribution. In a purely deterministic model, once the system approaches the tipping point, it remains there but does not pass it, due to the almost-zero growth rate. The noise term serves to nudge the population over this threshold. While the form of the noise used in the model equations is somewhat arbitrary, it is to be expected that there would be various sources of stochasticity present in any \textit{in vivo} scenario.

Returning to the discrete model, the results for the FGTA and SGTA can be summarised by tracking the total population size of the bacteria, as well as the
Figure 2.6  Population dynamics in a gradient of a bactericidal antibiotic, for the continuum model defined by Equations (2.10-2.11), with $D = 3 \mu m^2/s$ and $\alpha = 0.0049$. Snapshots of the population densities of live (green) and dead (red) bacteria are shown, sampled at $t = 500$, $t = 1000$ and $t = 2000$ time units (left to right), for the FGTA and SGTA cases (top and bottom rows respectively).

percentage of the population which is alive. Figure 2.5(i) shows that for FGTA, the population size remains small, as the population does not advance far into the gradient. However, a large percentage of the total cell count remains alive.

The SGTA system exhibits contrasting behaviour to this. As can be seen in Figure 2.5(j), the total cell count is much larger than the FGTA-exposed population, but the majority of these bacteria are dead. These observations suggest a trade-off, choosing an FGTA would be optimal where the total bacterial population is the key issue, e.g., to reduce biofilm thicknesses in order to prevent inflammation, the release of toxins, or clogging. Whereas in situations where the aim is to maximise the percentage of the population which is killed, likely to be an important factor in controlling the propagation and eradication of infections, an SGTA could be the preferred choice.
2.3.5 Alternative pharmacodynamic functions

The choice of pharmacodynamic function, $\phi(c, \beta)$, used here was somewhat arbitrary. The threshold-like form of the ones used in the simulations, as seen in Equation (2.5) and Equation (2.7) is relatively appropriate for antibiotics such as ciprofloxacin, rifampicin and streptomycin at sub-MIC concentrations [124, 332]. However, antibiotics such as tetracycline and chloramphenicol exhibit smoother, Langmuir-like pharmacodynamic functions. For these functions, general forms such as

$$\phi(c, \beta) = \frac{1}{1 + \frac{c}{\beta}}$$  \hspace{1cm} (2.12)$$

have been found to provide a good fit to data [124].

To determine the influence of the form of the pharmacodynamic functions, the above experiments were repeated using Equation (2.12), but maintaining the

Figure 2.7 Population waves for a gradient of bacteriostatic antibiotic with a Langmuir-like pharmacodynamic function. Simulations were performed with $\alpha = 0.0049$ and the function $\phi(c, \beta)$ of Equation (2.12). Panels (a) and (b) show population density snapshots sampled every 300 time units (with every 4th sample in black), for FGTA and SGTA antibiotics; panels (c) and (d) show corresponding profiles of the local growth rate $b_i$. 
same functions for $\beta$ as before, Equations (2.3) and (2.4), for FGTA and SGTA respectively.

Qualitatively similar results are obtained from this model, as shown in Figure 2.7, where the expansion of FGTA exposed bacteria is again inhibited sooner than the SGTA exposed bacteria, which are able to expand much further into the gradient. In general, antibiotics which exhibit Langmuir-like pharmacodynamic functions are less effective at inhibiting bacterial expansion than their threshold-like counterparts, regardless of growth-dependent susceptibility.

A key point to note here is that the form of the pharmacodynamic curve may be coupled to the growth-dependence drug susceptibility. e.g., the models of Greulich et al. \cite{124} suggests that ribosome-targeting antibiotics such as tetracycline, with smoothly decreasing, Langmuir-like pharmacodynamic functions are intrinsically linked to the FGTA scenario. Whereas a threshold-like pharmacodynamic function is more a property of the SGTA scenario.

![Figure 2.8](image.png)

*Figure 2.8* (a)-(b) Population density and (c)-(d) growth rate profiles, for (left): an FGTA growth-dependent susceptibility combined with a Langmuir-like pharmacodynamic function (mimicking tetracycline) and (right): an SGTA growth-dependent susceptibility combined with a quadratic pharmacodynamic function (mimicking streptomycin). In all cases, $\alpha = 0.0049$.

Therefore, a direct comparison of the population dynamics in gradients such as streptomycin (SGTA, quadratic pharmacodynamic function) vs tetracycline
(FGTA, Langmuir) may be decidedly non-trivial. Simulations of this scenario are shown in Figure 2.8. Here the difference in the spatial profiles between FGTA and SGTA is much more subtle than in previous simulations where both antibiotics had the same pharmacodynamic function.

### 2.4 Discussion

Large gaps still remain in the overall understanding of antibiotics and their underlying functionality in realistic situations such as spatially heterogeneous environments. Further research is still required in order to develop the kind of quantitative models that are needed to further said understanding, allowing for the design of better treatment strategies [346–350] and the development of methods to counteract the emergence of antimicrobial resistance [351, 352].

One gap in particular concerns how the interplay between the action of antibiotics and the physiology of the target bacteria affects the form and growth-rate dependence of the pharmacodynamic function. Another gap relates to the impact of spatial heterogeneity. It is currently unclear how the spatial structure of infections affect their susceptibility to antibiotic therapies and their propensity for evolving resistant mutants [73, 219, 222–224, 353, 354]. The work undertaken here aimed to investigate a combination of these two effects. Namely, it aimed to show how growth-rate dependent susceptibility can affect how well a bacterial population can proliferate along a spatial antibiotic gradient.

It was shown that fast-growth targeting bacteriostatic antibiotics are overall superior at preventing the spatial expansion of bacterial populations than their slow-growth targeting counterparts. However, for bactericidal antibiotics a trade-off exists. The application of bactericidal FGTA results in a smaller population, but one that is mainly alive, whereas bactericidal SGTA allow for a larger overall population, in which the majority of the cells are dead. Either one of these situations may be more desirable depending on the clinical scenario.

This work connects with a previously established body of theoretical research on the field of travelling waves [342, 355] as well as contemporary research on their application to bacterial systems [223, 345, 356, 357]. The results presented here can also be interpreted in the context of Fisher-KPP wave theory, by considering the fact that Fisher-KPP waves are “pulled waves”, i.e. their dynamics are
governed by the actions at the wave’s tip. In the model developed here, growth-dependent susceptibility results in discrepancies between the MIC of FGTA and SGTA at the bacterial population wave’s tip, due to the abundance of the nutrient. This leads to differing wave speeds and the overall extent to which the populations are able to expand along an antibiotic gradient.

The model developed here is simplistic in nature, but the data generated could influence future treatment strategies for biofilm infections. Growth rate-dependency has been observed before in some experimental cases. Pamp et al. observed that when applied to a P. aeruginosa biofilm, some antibiotics such as ciprofloxacin, tetracycline and tobramycin were more effective against the fast-growing bacteria at the surface of the biofilm, whereas other antibiotics like colistin, EDTA and SDS had better results against the slow/non-growing population in the bulk of the biofilm. This is in agreement with the results of the above model, which suggest that FGTA may be more effective at the biofilm’s surface, whereas SGTA could perform better against the slow-growing biofilm bulk, as shown in Figure 2.5.

Several assumptions and simplifications were made in the design of this model. Spatial structure was reduced to a 1D chain of microhabitats, exposed to either a constant or fixed exponential gradient of antibiotic concentration. Features that might influence the distribution of antibiotic or the spread of bacteria such as the biofilm structure, chemotaxis and fluid flow were neglected. This is in contrast to in vivo infections, which are subject to gradients in higher spatial dimensions. As such, it could be of interest to extend the model to more complex geometries.

Bacterial physiology has also been considerably simplified. Crucially, insights on growth rate-dependent susceptibility taken from experiments where growth rate is determined by nutrient richness are transferred to a situation where the growth rate is determined by nutrient concentration. i.e. it is assumed here that a slow growth rate as a result of poor nutrient availability has the same effect on antibiotic susceptibility as a slow growth rate as a result of poor nutrient with high availability. Currently there appears to be little work to test this hypothesis, but work by Neidhart and Magasanik suggests that the RNA:protein ratio achieved by E. coli in the stationary phase in glucose media is the same as that achieved during steady-state growth in a poor nutrient media. As the RNA:protein ratio is indicative of ribosome abundance, this suggests that this hypothesis should be reasonable for ribosome-targeting antibiotics, however
further data on this feature would be extremely beneficial.

Additionally, it was assumed that the bacterial response to the applied antibiotic is determined purely by the nutrient and antibiotic concentrations. In reality, a more heterogeneous response amongst individual bacteria would be expected. e.g., for a given antibiotic concentration, some may be dying while others are still replicating [351, 367]. This could also be an interesting addition to the model.

Regarding the coupling between growth rate and MIC, a simple linear relationship was assumed here. This is appropriate for some antibiotics, but is fairly unrealistic for others [124]. The conclusions reached remain the same when non-linear relationships between MIC and growth rate are used, as shown in Figure 2, but can become unclear when comparing antibiotics with differing pharmacodynamic functions, as in Figure 2. It was also assumed that the MIC is dependent solely on the nutrient-dependent component of the growth rate $g(s)$. Were it to depend on the full growth rate $g \cdot \phi$, some interesting feedback mechanisms could be observed.

The link between growth rate-dependent susceptibility and shape of the pharmacodynamic function has also been ignored. As antibiotics with differing growth rate-dependent susceptibilities could likely have significantly differing pharmacodynamic functions, experimentally testing the predictions of the model regarding whether FGTA are better than SGTA at impeding the progress of an advancing bacterial population wave may be decidedly non-trivial. However, it should be more simple to test and verify the appearance of the “striped” population structure for bactericidal SGTA. If this phenomenon is able to be confirmed, it could be well be of practical importance for furthering the understanding of how biofilms respond to antibiotics.

This understanding, and particularly quantitative data on the subject, is key for optimising antibiotic treatment plans and preventing the emergence of antimicrobial resistance. The results shown here suggest that growth-dependent susceptibility and spatial heterogeneity of antibiotic concentrations may play important roles in the efficacy of applied antibiotics. Antibiotics which target fast-growing cells are predicted to be more effective at preventing the spatial advancement of a bacterial population compared to antibiotics which target slow-growing cells. However, if the aim is to treat an established population, e.g., a biofilm, then bactericidal slow-growth targeting antibiotics are predicted to be the more effective choice in order to kill the slow-growing bulk.
Chapter 3

Stochastic nucleation of biofilms on surfaces

3.1 Introduction

The classic picture of biofilm initiation begins with the reversible attachment of planktonic bacteria to a surface, which may be mediated by bacterial surface appendages such as flagella and pili. Surface-attached bacteria then proliferate to form microcolonies, and undergo a transition to a different physiological state in which they begin to produce the components of the biofilm extracellular matrix, which facilitates irreversible attachment to the surface [368]. There are many examples of the involvement of collective signalling via quorum sensing mediating this transition [369–372]. The transition to the biofilm phenotype can also involve biophysical factors such as cell surface motility (which may have collective aspects) [313, 373–375], physical interactions among cells [309] and surface sensing [376].

Presented in this chapter is a simple theoretical model for quorum sensing mediated biofilm initiation. Quorum sensing is a well-studied, density-dependent mode of bacterial signalling. In many bacterial species, autoinducer signalling molecules are secreted into the environment and the local concentration of these autoinducers provides a way for bacteria to sense the local bacterial density. Once the autoinducer concentration reaches a critical threshold, a gene regulatory response is initiated [42, 47]. This quorum sensing response can affect a host
of different behaviours, including motility, conjugation - the process of bacteria transferring genetic material to one another via direct contact, competence - the ability of bacteria to uptake extracellular DNA from their surrounding environment, sporulation, virulence and biofilm formation [377–381].

In this chapter, the population dynamics of quorum sensing mediated biofilm formation were investigated using stochastic simulations and simple analytic theory of a coarse-grained microhabitat model. This model predicts a stochastic transition from a loosely attached layer of bacteria near the surface to a growing biofilm. This transition is governed by two key parameters: the threshold population density for the collective transition, and the rate at which bacteria immigrate into the system relative to the rate at which they detach from the surface.

3.2 Model overview

3.2.1 Microhabitat model

Similarly to the work presented in Chapter 2 and other previous works [1, 223, 323], the model here takes the form of a 1D series of microhabitats oriented along the \( \hat{z} \)-axis, extending outwards normal to the surface which the biofilm is growing on, as shown in Figure 3.1(a). These microhabitats have lateral area \( A \) and thickness \( \delta z = 1 \mu m \) - roughly the same thickness as a monolayer of bacteria. This model differs from the one presented in Chapter 2 as the number of microhabitats is not fixed, but rather increases as the biofilm expands.

The system is initialised with a single, empty microhabitat adjacent to the surface. Bacteria immigrate into this microhabitat from the external aqueous environment with a rate \( r_{im} \), detach from it with a rate \( r_{det} \) and replicate within it with a replication rate constrained by a logistic growth term,

\[
r_{rep} = g \left( 1 - \frac{N}{K} \right),
\]

where \( g \) is the maximum growth rate of the bacteria, \( N \) is the current population

\[
A \text{ does not have a specific value } \text{per se}, \text{ but is rather used as a rationalisation of a scaling factor in later simulations.}
\]
Figure 3.1  (a) Model for biofilm initiation. The system is initialised with a single, empty microhabitat. Bacteria (green circles) can immigrate into, proliferate within or detach from this microhabitat, shown on the LHS of (a). Once a critical population size is reached, the first microhabitat transitions to biofilm (green) and a second microhabitat is added, shown on the RHS of (a). This new microhabitat now becomes the one which bacteria may immigrate into or detach from. Bacteria may now also migrate between adjacent microhabitats.  
(b)-(d): Stochastic simulation trajectories for different parameter values. The total population size \(N_{\text{tot}}\) is plotted relative to carrying capacity \(K\), as a function of dimensionless time \(gt\). In each plot, each line represents a replicate simulation with identical initial conditions. Parameter values for \(r_{\text{im}}/gK\) and \(N^*/K\) are indicated above each plot, the other parameter values are \(r_{\text{mig}}/g = 0.8\), \(r_{\text{det}}/g = 0.5\), \(K = 1000\), \(g = 0.083\).

The total population size \(N_{\text{tot}}\) is plotted relative to carrying capacity \(K\), as a function of dimensionless time \(gt\). In each plot, each line represents a replicate simulation with identical initial conditions. Parameter values for \(r_{\text{im}}/gK\) and \(N^*/K\) are indicated above each plot, the other parameter values are \(r_{\text{mig}}/g = 0.8\), \(r_{\text{det}}/g = 0.5\), \(K = 1000\), \(g = 0.083\).

To model a quorum sensing mediated transition from the planktonic to the biofilm phenotype, when the population density reaches a critical value \(N^*/K\), the microhabitat irreversibly transitions to a “biofilm” state, from which bacteria...
can no longer detach. When this transition occurs, a new empty microhabitat is added to the system, adjacent to the previous one, as shown in Figure 3.1(a). For simplicity, microhabitats cannot revert to the planktonic state once they have been established as biofilm. When there are >1 microhabitats in the system, bacteria can migrate between adjacent microhabitats with a rate $r_{\text{mig}}$. In the inner microhabitats, bacteria can still replicate as before, but there is no immigration or detachment.

In this model, the carrying capacity and biofilm formation threshold population scale with microhabitat volume ($K \propto A \times \delta z$ and $N^* \propto A \times \delta z$), the migration rate scales with microhabitat thickness ($r_{\text{mig}} \propto \delta z$), and the immigration rate scales with microhabitat area ($r_{\text{im}} \propto A$). The detachment rate $r_{\text{det}}$ and maximum growth rate $g$ are independent of microhabitat dimensions. The timescale used in the analysis of the results here is in units of $1/g$.

### 3.2.2 Stochastic model

Stochastic simulations of this model were performed using a modified $\tau$-leaping algorithm [382], which takes account of the stochasticity of individual immigration, migration, replication, and detachment events. $\tau$-leaping [383] is an approximated version of the famous Gillespie stochastic simulation algorithm (SSA) [337, 338], which is commonly used to simulate well-mixed biochemical systems. This modified version differs from regular $\tau$-leaping by dynamically updating the timestep to ensure that negative populations do not occur.

SSA is essentially an exact numerical solution for these systems, but as it tracks every reaction event and channel, it is impractical for more realistic or complex systems [384, 385]. Each bacterium in this model is treated as an individual entity, meaning a reaction has be calculated for each of these bacteria, resulting in the regular SSA becoming inefficient to simulate this system. Hence $\tau$-leaping was implemented, which is better suited to model systems involving many reaction channels.

In this algorithm, the bacteria are modelled as discrete agents and all events are taken to be Poisson processes with mean waiting time $\mu = r \times \tau$, where $\tau$ is the size of the timestep being simulated, and $r$ is the occurrence rate of the chosen event.
The general overview of the algorithm procedure is as follows:

1. A timestep $\tau$ is selected. In this model, an initial value of $\tau = 0.1$ hours is used.

2. Each bacterium in the system is iterated over. For each bacterium, the number of replications ($b_{i,j}$) or deaths ($d_{i,j}$) it will undergo is determined by sampling from a Poisson distribution which has a mean of $\mu_{\text{rep}} = r_{i,j} \times \tau$. Here $r_{i,j}$ is the replication rate of bacterium $j$ in microhabitat $i$, which is calculated based on the local population density, $N_i/K$. Death arises due to the presence of the logistic growth limiting term $(1 - N_i/K)$ in the expression for the replication rate, and therefore only occurs when $N_i > K$. This is the only source of death in the model - bacteria cannot die if $N_i < K$.

To prevent negative populations arising, if a bacterium is designated to die more than once in a single timestep, the timestep is halved in size ($\tau \rightarrow \tau/2$) and the algorithm returns to step 2, proceeding with the new reduced timestep. This serves to reduce the mean of the corresponding Poisson distribution, which in turn reduces the likelihood of these events happening more than once. There is no limit to the number of times that $\tau$ can be halved.

3. Within this iteration, the number of times each bacterium will migrate to an adjacent microhabitat ($m$) are sampled from a Poisson distribution with $\mu_{\text{mig}} = r_{\text{mig}} \times \tau$. As bacteria in the surface/edge microhabitats can only migrate in one direction, these allocations are instead sampled from a Poisson distribution with mean $\mu^*_{\text{mig}} = 0.5 \times r_{\text{mig}} \times \tau$. To prevent bacteria “skipping over” microhabitats, if a bacterium is designated to migrate more than once in a single timestep, the timestep is halved in size ($\tau \rightarrow \tau/2$) and the algorithm again returns to step 2, proceeding with the new reduced timestep.

4. Finally, the detachment allocations ($z$) for the bacteria in the edge microhabitat are sampled from a Poisson distribution with $\mu_{\text{det}} = r_{\text{det}} \times \tau$. Similarly to the death and migration allocations, if a bacterium is designated to detach more than once in a single timestep, the timestep is halved in size ($\tau \rightarrow \tau/2$), and the algorithm returns to step 2.

5. The events calculated in the previous steps are then carried out. Specifically, bacterium $j$ in microhabitat $i$ is replicated $b_{i,j}$ times. If the bacterium is
in the edge microhabitat then it will detach if $z_j = 1$ and not if $z_j = 0$. For migration, if $m_{i,j} > 0$ then microbe $j$ in microhabitat $i$ is sent to either microhabitat $i + 1$ or $i - 1$ with a 50/50 probability (unless in the surface/edge microhabitats). Detachment events are assumed to have priority over migration events and detached bacteria are simply removed from the system. Bacteria designated to both replicate and migrate in the same timestep will first replicate, then the mother cell will migrate.

6. The number of bacteria which immigrate into the edge microhabitat is then sampled from a Poisson distribution with $\mu = r_{im} \times \tau$.

7. If the number of bacteria in the biofilm edge microhabitat has reached the threshold for biofilm formation ($N_i \geq N^*$), then another microhabitat is added to the system, in series with the current edge microhabitat. This new microhabitat is now allocated as the new edge microhabitat and is the one which bacteria will immigrate into in the next timestep.

8. The time elapsed in the simulation is updated to $t \rightarrow t + \tau$. If $\tau$ has been adjusted, then for the next timestep, $\tau$ returns to its original value of 0.1 hours.

Figure 3.1(b-d) shows stochastic trajectories of the total population size $N$ for several parameter sets. Depending on the parameter sets used, perceptibly different outcomes can be observed. For some parameter sets, a two-step biofilm initiation is observed, in which a “pre-biofilm” forms initially, before it transitions to a growing biofilm, Figure 3.1(b). For some other parameter sets, the biofilm grows almost linearly from the start, Figure 3.1(c), whereas for others, no biofilm observation is observed for the duration of the simulations, Figure 3.1(d). In this model, all bacteria in the population are identical, representing an idealised monospecies biofilm. In the next chapter, the model is updated to include bacteria of differing species, with corresponding differing growth rates.

3.2.3 Deterministic model

To better understand the results of the stochastic simulations, a deterministic version of the model was developed by a co-supervisor of this project, Dr Chris Brackley. For a system of $M$ microhabitats, the number of bacteria in the $i^{th}$ microhabitat, $N_i$, is described by the master equation
\[
\frac{dN_i}{dt} = gN_i \left(1 - \frac{N_i}{K}\right) + \left[\frac{r_{\text{mig}}}{2} [(1 - \delta_{iM})N_{i+1} + (1 - \delta_{i1})N_{i-1} - (2 - \delta_{i1} - \delta_{iM})N_i] - \delta_{iM} [r_{\text{im}} - N_i r_{\text{det}}]} \right], \quad (3.2)
\]

where \(\delta_{i,j}\) is the Kronecker delta, \(\delta_{i,j} = 1\) if \(i = j\), 0 otherwise. These are used to ensure that immigration and detachment only occur in the edge microhabitats, and that migration occurs at the correct rates. The dynamics of increasing numbers of microhabitats is incorporated into this model by starting with \(M = 1\) and \(N_1 = 0\) at \(t = 0\) and increasing \(M\) by 1 whenever \(N_{M}/K = N^*/K\). \(i.e.\) whenever the outermost, or \(M^{th}\) microhabitat population reaches the biofilm threshold.

\section*{3.3 Results}

\subsection*{3.3.1 Deterministic model}

One microhabitat

At the start of biofilm initiation, when there is only one microhabitat, \(M = 1\), Equation (3.2) reduces to a single equation for \(N_1\),

\[
\frac{dN_1}{dt} = gN_1 \left(1 - \frac{N_1}{K}\right) + r_{\text{im}} - N_1 r_{\text{det}}, \quad (3.3)
\]

Setting \(dN_1/dt = 0\) gives the steady state population for a system consisting of one microhabitat, with fixed points

\[
\frac{N_{1,M=1}^\pm}{K} = \frac{1}{2} \left(1 - \frac{r_{\text{det}}}{g}\right) \left[1 \pm \sqrt{1 + \frac{4r_{\text{im}}}{gK} \left(1 - \frac{r_{\text{det}}}{g}\right)^{-2}}\right]. \quad (3.4)
\]
$M = 1$ is present in the subscript as a reminder that the solution will change once further microhabitats are added.

The dimensionless parameter combinations $r_{\text{det}}/g$ and $r_{\text{im}}/gK$ emerge naturally from Equation (3.4). Of these, $r_{\text{det}}/g$ is independent of the microhabitat dimensions while $r_{\text{im}}/gK$ scales with the inverse of the microhabitat depth, $1/\delta z$. The nature of these fixed points can be inferred from a plot of $f_{1,M=1}(N_1) \equiv dN_1/dt$: the larger fixed point is stable and always $\geq 0$, while the smaller one is unstable. 0 itself is only a stable fixed point if $r_{\text{det}}/g > 0$ and $r_{\text{im}} = 0$. Hereon, the superscript “fp” will be used to refer to the positive, stable, fixed point, e.g., $N_{1, M=1}^{\text{fp}}$. These equations can be made dimensionless by considering the ratios $N_1/K$, $r_{\text{det}}/g$ and $r_{\text{im}}/gK$. Figure 3.2 shows how $N_{1, M=1}^{\text{fp}}$ depends on these parameters.

![Figure 3.2](image)

_Figure 3.2_ Plot showing how the value of the largest fixed point for the single microhabitat case $N_{1, M=1}^{\text{fp}}$ (scaled by the carrying capacity $K$), varies with the dimensionless parameters $r_{\text{im}}/gK$ and $r_{\text{det}}/g$, as given by Equation (3.4).

If $r_{\text{det}}/g = r_{\text{im}}/gK$ (or equivalently, $r_{\text{det}}K/r_{\text{im}} = 1$), then $N_{1, M=1}^{\text{fp}} / K = 1$, i.e. the population grows to the carrying capacity. This leads to two regimes: if $r_{\text{det}}K/r_{\text{im}} < 1$ then $N_{1, M=1}^{\text{fp}} / K > 1$, and growth is augmented by immigration. If $r_{\text{det}}K/r_{\text{im}} > 1$ then growth is limited by detachment ($N_{1, M=1}^{\text{fp}} / K < 1$).
Two further parameter regimes can be considered, depending on the relative size of the stable fixed point and the biofilm threshold $N^*$. For an initial condition $M = 1$ and $N_1(t=0) = 0$, when $N_{1,M=1}^{fp} < N^*$, the system will grow only until $N_1(t) \rightarrow N_{1,M=1}^{fp}$. Thus the population reaches a steady state, but a biofilm will never become fully established. Alternatively, when $N_{1,M=1}^{fp} > N^*$, the system will grow until $N_1(t) = N^*$, at which point the first microhabitat will transition to the biofilm state, and a second microhabitat will be generated.

**Two microhabitats**

Focusing on the dynamics in the biofilm establishment regime ($N_{1,M=1}^{fp} > N^*$), once the second microhabitat has been generated ($M = 2$), the system is now governed by two equations, obtained from Equation (3.2) with $i = \{1,2\}$,

$$\frac{dN_1}{dt} = f_1(N_1, N_2), \quad \frac{dN_2}{dt} = f_2(N_1, N_2),$$

(3.5)

where

$$f_1(N_1, N_2) = gN_1 \left(1 - \frac{N_1}{K}\right) + \frac{r_{\text{mig}}}{2} [N_2 - N_1],$$

(3.6)

$$f_2(N_1, N_2) = gN_2 \left(1 - \frac{N_2}{K}\right) + \frac{r_{\text{mig}}}{2} [N_1 - N_2] + [r_{\text{im}} - N_2 r_{\text{det}}].$$

(3.7)

Considering again the fixed points ($dN_1/dt = 0, dN_2/dt = 0$), two non-linear equations coupled through the migration terms are obtained. For a given set of parameters, these can be inspected graphically as the intersection of the two curves, Figure 3.3(a-b), or solved numerically. As before, it can be inferred that the negative (or zero) fixed point is unstable in favour of the positive one, denoted by $(N_{1,M=2}^{fp}, N_{2,M=2}^{fp})$ in the ($N_1, N_2$) plane.

Interestingly, the population size in the outermost microhabitat $N_{2,M=2}^{fp}$ can be larger or smaller than $N_{1,M=1}^{fp}$ depending on the value of the ratio $r_{\text{det}} K / r_{\text{im}}$, which measures the relative importance of detachment and immigration in the outer microhabitat. If detachment dominates, migration tends to be from the inner to the outer microhabitat, whereas if immigration dominates, they tend to migrate from the outer to the inner microhabitat. Thus, as the biofilm grows, its outer edge may become either more or less dense (Figure 3.4(b)).

A special case arises when $r_{\text{mig}} = 0$, where the microhabitats are decoupled from
Figure 3.3  

(a) Plot showing the functions \( f_1(N_1, N_2) = 0 \) (dot-dash line, Equation (3.6)) and \( f_2(N_1, N_2) = 0 \) (solid lines, Equation (3.7)) for different values of \( r_{im} \). The intersection of these lines gives the fixed point (both equations equal zero); there is a second intersection but these have negative values of \( N_1 \) or \( N_2 \), meaning that fixed point is unstable. Note that \( f_1(N_1, N_2) \) is independent of \( r_{im} \) and \( r_{det} \). Other parameter values are \( r_{det}/g = 0.75 \), \( r_{mig}/g = 0.9 \), \( g = 1 \) and \( K = 1 \). (b) Similar plot showing \( f_1(N_1, N_2) = 0 \) (dot-dash line) and \( f_2(N_1, N_2) = 0 \) (solid), but for different values of \( r_{det} \). Here \( r_{im}/gK = 0 \).75 and the other parameters are as in panel (a). (c) Plot showing the function \( f_1, M=2(N_1, N_2) = 0 \) for different values of \( r_{mig}/g \). Note that the equations become decoupled when \( r_{mig} = 0 \). (d) Plots showing the values at the fixed point for the two-microhabitat system (blue lines) as a function of \( r_{mig} \) for the case \( r_{det}K/r_{im} < 1 \), which were found numerically from the intersections of curves as in panels (a-b). Also shown is the value at the fixed point for the corresponding one-microhabitat case (which is independent of \( r_{mig} \); red dashed line, Equation (3.4)). Note that \( N_{1,M=1}^{fp} \geq N_{2,M=2}^{fp} \). (e) A similar plot shows the case where \( r_{det}K/r_{im} > 1 \). Here \( N_{1,M=1}^{fp} \leq N_{2,M=2}^{fp} \).  

Each other, as shown by the orange line in Figure 3.3(c). In this case, the inner microhabitat fills to capacity, and the outer one behaves exactly as the \( M = 1 \) case, i.e. \( N_{1,M=2}^{fp}/K = 1 \) and \( N_{2,M=2}^{fp} = N_{1,M=1}^{fp} \). For \( r_{mig} > 0 \) the behaviour depends on the ratio \( r_{det}K/r_{im} \), with \( N_{2,M=2}^{fp} \) either increasing or decreasing with \( r_{mig} \), Figure 3.3(d, e). Additionally, there are once again two regimes which are dependent on the value of \( r_{det}K/r_{im} \) - the values of the fixed points decrease as the ratio \( r_{det}K/r_{im} \) increases.
For the immigration dominated regime \((r_{\text{det}} K/r_{\text{im}} < 1)\), \(r_{\text{mig}} > 0\) results in 
\(N_{fp,2,M=2} < N_{fp,1,M=1}\), compare red dashed and blue solid lines in Figure 3.3(d). This means that choosing \(N^*\) such that 
\(N_{fp,1,M=1}^* > N^* > N_{fp,2,M=2}^*\) gives a system which will grow to generate a second microhabitat, but will then reach steady state before \(N_2\) grows above \(N^*\), meaning it will fail to generate a third microhabitat.

This leads to the surprising result that a population whose growth is immigration dominated can in fact grow to a steady state population size consisting of two microhabitats, but not grow any further, \(i.e.\) the system forms a biofilm of finite thickness. The small region of parameter space which allows this is shown as the region between the solid and dashed lines in Figure 3.4(a). However, it should be noted that this requires a biofilm formation threshold larger than the carrying capacity \((N^* > K)\). Namely, it requires a scenario where the environment is inhospitable enough such that it cannot sustain a large enough population to trigger biofilm formation.

A detachment limited population \((r_{\text{det}} K/r_{\text{im}} > 1)\) will either never manage to reach the biofilm formation threshold in the first microhabitat \((N_{fp,1,M=1} < N^*)\), or will exhibit sustained growth after this threshold is reached \((N_{fp,1,M=1}^* > N^*)\). In the latter case, the inner microhabitats feed the outer ones, boosting the steady state population size.

**Multiple microhabitats**

For the case of three or more microhabitats, determining the fixed points amounts to simultaneously solving a set of three or more non-linear equations. Nevertheless, one can again consider the \(r_{\text{mig}} = 0\) case where the equations decouple. The internal microhabitats would have a fixed point \(N_{fp,i<\!\!<\!\!M=1} = K\) while the outermost microhabitat will behave according to Equation (3.4). One might imagine that, as with the case of two microhabitats, increasing \(r_{\text{mig}}\) will lead to an increase or a decrease of the fixed point value depending on the ratio \(r_{\text{im}}/K r_{\text{det}}\). This can be confirmed by direct numerical solution of a given system with a fixed number of equations, \(i.e.\) solving Equation (3.2) with fixed \(M\). These numerical results are shown in Figure 3.4(b). The value of \(N_{fp,M}^*\) initially depends on \(M\), but plateaus at large \(M\).

Figure 3.4(a) shows a phase diagram in the \((N^*, r_{\text{det}})\) plane, illustrating the regions of parameter space corresponding to biofilm establishment vs non-
Figure 3.4 Deterministic model. (a) Phase diagram for $\frac{r_{im}}{gK} = 0.8$ and $\frac{r_{mig}}{g} = 0.8$ (with $K = 1$ and $g = 1$). The solid blue line separates the regime where the population does not reach the biofilm threshold in the first microhabitat and the regime where the biofilm grows. In the region between the dashed and solid blue lines, the first microhabitat will be able to transition to biofilm, but growth will halt in the second. (b) Steady-state population size in the outermost microhabitat for different numbers of microhabitats $M$. Grey points: $\frac{r_{det}}{g} = 0.6$; pink points: $\frac{r_{det}}{g} = 1.1$; for round points, $\frac{r_{mig}}{g} = 0.8$; for triangles, $\frac{r_{mig}}{g} = 0.1$. In all cases $\frac{r_{im}}{gK} = 0.8$. Connecting lines are included as a guide for the eye. (c-d) Total population size $N_{tot}(t) = \sum_{i=1}^{M} N_{i}(t)$ obtained from numerical solution of Equation (3.2) with increasing numbers of microhabitats. The parameter values correspond to the coloured crosses in panel (a). In (c) $\frac{r_{mig}}{g} = 0.8$, $\frac{r_{im}}{gK} = 0.8$ and $N^*/K = 1.17$. From top to bottom the different curves show $\frac{r_{det}}{g} = 0.4$, 0.5 and 0.6. Points are shown at the times where a new microhabitat is introduced. In (d) $\frac{r_{mig}}{g} = 0.8$, $\frac{r_{im}}{gK} = 0.8$ and $N^*/K = 0.7$. The top curve has $\frac{r_{det}}{g} = 1.1$ and the bottom curve has $\frac{r_{det}}{g} = 1.3$. establishment, separated by the solid line. Note that this line always goes through the point $(N^*/K = 1, \frac{r_{det}K}{r_{im}} = 1)$, but otherwise depends on the dimensionless ratios $\frac{r_{im}}{gK}$ and $\frac{r_{det}}{g}$. This may explain some of the observed
outcomes in Figure 3.1(b-d), particularly that some parameter sets result in runaway growth, while others do not.

For the immigration dominated regime \((r_{\text{det}}K/r_{\text{im}} < 1)\), the value of \(N^p_M\) decreases with increasing \(M\), shown by the grey points in Figure 3.4(b). Similarly to the two microhabitat scenario, there is a very small range of possible \(N^*\) values where \(N^p_{3,M=3} > N^* > N^p_{4,M=4}\), i.e. the biofilm would grow to the third microhabitat before halting. This continues for larger \(M\), with an ever decreasing range of possible \(N^*\) values.

To study the dynamics of the sustained growth regime, Equation (3.2) was integrated numerically. Population trajectories are obtained by starting with the \(i = 1\) equation, integrating until \(N_1 = N^*\), adding the \(i = 2\) equation, continuing to integrate both equations until \(N_2 = N^*\), and so on. Figure 3.4(c-d) shows trajectories for the total population size obtained for different parameter sets, the immigration dominated regime (Figure 3.4(c)) and the detachment limited one (Figure 3.4(d)).

As discussed above, for \(r_{\text{det}}K/r_{\text{im}} < 1\), depending on the value of \(N^*\) relative to the fixed points, either the biofilm will never establish, it will establish but be limited to a finite size, or it will continue to grow indefinitely. There are only two possible regimes for \(r_{\text{det}}K/r_{\text{im}} > 1\): non-establishment or continued growth, since the values of the fixed points increase with \(M\) - shown by the pink curves in Figure 3.4(b).

### 3.3.2 Stochastic model

#### Role of stochasticity

The above deterministic analysis can help provide a better understanding of the stochastic simulations, such as those shown in Figure 3.1(b-d). Firstly, the existence of the different growth regimes explains why for some parameters the stochastic system grows, but for others it does not. Figure 3.5 shows a heatmap from the stochastic simulations for the time taken for the system to form a biofilm \(t_1\), defined as the time taken for the first microhabitat to reach the biofilm transition threshold \(N^*/K\), as a function of the parameter combinations \(N^*/K\) and \(r_{\text{det}}K/r_{\text{im}}\). Where no colour is shown, the simulations did not form a biofilm within the maximum simulation duration (1000 hours \(\simeq 83g^{-1}\)). The pink line...
Figure 3.5  Stochastic initiation dynamics. Centre: Heatmap showing $t_1$, the mean lag time before biofilm initiation (i.e. until the first microhabitat reaches $N^*$) in our stochastic simulations, as a function of $N^*/K$ and $r_{det}K/r_{im}$. The colourbar for the values of $t_1$ is located directly to the right of the heatmap. Here $r_{im}/gK = 0.8$ and $r_{mig}/g = 0.8$, with $K = 1000$ and $g = 0.083$ hr$^{-1}$, while $r_{det}$ and $N^*$ were varied. Here the dimensionless time $gt$ is used. The white region in the upper right quadrant denotes parameters where the biofilm does not initiate within the maximum simulation time of $\sim 83g^{-1}$. The dashed pink line shows the deterministic phase boundary (as in Figure 3.4(a)). Panels (a-d) show lag time distributions for the corresponding parameter sets indicated in the heatmap. Distributions (a) and (b) are close to exponential (insets show the same plots on a log scale) with coefficients of variation (CV) 0.639 and 0.703 respectively. Distributions (c) and (d) are closer to Gaussian, with CV values of 0.045 and 0.065. 25 replicate simulations were performed for each square in the heatmap, 500 for each of the surrounding histograms.

shows the prediction from the deterministic theory for the boundary between biofilm initiation and non-initiation. Interestingly, Figure 3.5 shows stochastic biofilm growth in the region of parameter space beyond the deterministic phase boundary.

To better understand this, trajectories of biofilm growth from the stochastic simulations were compared to those predicted by the deterministic model, shown in Figure 3.6. In the region of parameter space where growth is predicted deterministically, the biofilm grows from the start and there is a good, quantitative agreement between the stochastic and deterministic results, Figure
Comparison between stochastic (left-hand panels) and deterministic (right-hand panels) biofilm growth trajectories. (a, b) Parameters in the deterministic growth regime; $N^*/K = 0.7$. (c, d) Parameters in the stochastic growth regime; $N^*/K = 1.17$. The colours indicated in the deterministic plot legends also correspond to the parameter sets used in the stochastic simulations, i.e. the purple deterministic line is the same parameter set as the purple stochastic lines, and so on. In all cases, $r_{mig}/g = 0.8$, $r_{det}/g = 0.5$, $K = 1000$ and the immigration rate is varied: $r_{im}/gK = 0.65$, 0.725 and 0.783. The red dashed lines indicate the biofilm transition threshold $N^*/K$.

However, for some parameter sets where there is no growth in the deterministic model, the stochastic simulations show a lag time followed by a transition to growth, Figure 3.6(c,d).

The mean lag time varies between replicate simulations. Parameter values which lead to a longer mean lag time also exhibit more lag time variability: compare the blue and green sets of lines in Figure 3.6(c). In fact, the distribution of lag times before the threshold $N^*$ is reached is close to exponential for these simulations, as shown in the histograms of $t_1$ in Figure 3.5(a, b). In contrast, the lag time distribution is narrower and approximately Gaussian for parameter sets in the
deterministic growth regime, Figure 3.5(c, d).

These results point to the following scenario: for parameters where the deterministic fixed point population size in the first microhabitat is only slightly smaller than the biofilm formation threshold $N^*$, a small fluctuation in population size can push the system over the threshold, generating a second microhabitat. The system obeys “one-way” dynamics - once new microhabitats form, they cannot be removed, this model cannot represent the “dispersal” stage of the biofilm lifecycle. Furthermore, the model does not contain a limiting term which is dependent on biofilm thickness. As such, these fluctuations trigger sustained biofilm growth.

If $N_{1,M=1}^{fp} < N^*$, this results in a first passage\footnote{In a stochastic system, the first passage time is the time taken for a state variable to reach a certain value.} stochastic process with a metastable potential minimum at $N_{1,M=1}^{fp}$, and the lag time observed in Figure 3.5(a, b) is the time taken to escape this minimum and reach the absorbing state at $N^*$. Computing the stochastic waiting time distribution (Figure 3.5(a, b)) amounts to a first passage time process, in which growth, immigration and detachment all play a role, although their relative importance depends on the parameters.

**Increasing system size**

To investigate the influence of stochastic fluctuations on the transition to biofilm, the lateral area, $A$, of the system was increased, with the intention that doing so would reduce the influence of these fluctuations. Assuming that there are no other environmental changes, such as to the availability of nutrients, increasing the lateral area of the system will also increase $K$, $N^*$ and $r_{im}$. It was observed that increasing $A$ in this manner suppressed biofilm formation, as it reduced the relative significance of these population fluctuations.

Figure 3.7(a) shows the population trajectories of multiple replicate simulations for a given parameter set and a system size of $K = 10^3$. Here, some of the simulations are able to establish a biofilm, but upon increasing the system size such that $K = 10^4$, none of the replicate simulations are able to reach $N^*$, Figure 3.7(b). Even when the duration of the simulations is increased by a factor of 10, as shown in Figure 3.7(c), all of the runs remain unable to establish a biofilm.
Figure 3.7 Increasing the system size/lateral area \(A\) of the microhabitats serves to increase \(N^*, K\) and \(r_{im}\) and also reduces the influence of the stochastic noise. These plots each show the population over time for 100 runs with parameters \(r_{im}/gK = 0.7\), \(r_{det}/g = 0.5\), \(r_{mig}/g = 0.8\) and \(N^*/K = 1.17\). The carrying capacity has a value of (a) \(K = 10^3\) and (b-c) \(K = 10^4\). In (a), the lower carrying capacity allows for fluctuations to drive the formation of a second biofilm, whereas in (b) no such fluctuations occur. Even when the duration of the simulation is increased by a factor of 10, as shown in (c), all of the runs remain unable to reach the second microhabitat.

3.3.3 Stochastic vs. deterministic model dynamics

Some more comparisons of the stochastic and deterministic models are shown in Figure 3.8. The parameter set shown here is in the immigration dominated regime, complementing that of Figure 3.4(c), and once again has \(N^* > K\). The dot-dashed pink lines show the results of the deterministic model, while the blue lines show the corresponding stochastic simulations. For certain values of \(r_{im}/gK\), the deterministic and stochastic models show good agreement with one another, Figure 3.8(a, d).
Figure 3.8  Comparison between stochastic and deterministic biofilm growth dynamics for parameter sets in the immigration-dominated regime. Each plot shows the population size as a function of time for 100 runs of the stochastic model, together with the prediction of the deterministic model highlighted by the pink dot-dashed line. The value of $r_{im}/gK$ is varied, with (a) $r_{im}/gK = 0.6$, (b) $r_{im}/gK = 0.75$, (c) $r_{im}/gK = 0.8$ and (d) $r_{im}/gK = 0.9$. The other parameters were kept at constant values of $K = 10000$, $g = 0.083$, $r_{det}/g = 0.5$, $r_{mig}/g = 0.8$ and $N^*/K = 1.17$. Additionally, where the deterministic model predicted a finite number of microhabitats, the stochastic model exhibits runaway growth (c).

However, for other values of $r_{im}/gK$, such as those shown in Figure 3.8(b, c), there is now some clear discrepancy between the two models, with the stochastic model exhibiting biofilm growth even when the deterministic model does not. Additionally, the regime in which a biofilm of finite thickness forms, which is predicted by the deterministic model, Figure 3.8(c), is not observed in the stochastic simulations.

Figure 3.9 shows another comparison between the deterministic and stochastic model, this time with two parameter sets complementing that of Figure 3.4(d). Once again, for the parameter set which has a steady state population close to
the biofilm formation threshold, the stochastic model exhibits growth where the deterministic one does not.

3.4 Conclusion

This chapter presented a coarse-grained microhabitat model for quorum sensing mediated biofilm initiation, where a transition to the biofilm state occurs when the population density close to the surface reaches a critical threshold density. The model accounts for immigration, detachment, replication and migration within the biofilm - migration only occurring once multiple microhabitats have been established. The work presented here points to different modes of biofilm initiation under different parameter regimes. In favourable conditions, biofilm growth can initiate immediately. However, under less favourable conditions, a loosely attached layer of bacteria first forms at the surface, which may or may not undergo a stochastic transition to biofilm growth following a lag time.

In this model, the boundary between deterministic and stochastic biofilm initiation depends on whether the biofilm threshold density, $N^*/K$, is smaller or larger than the deterministic steady-state population density. Quorum sensing is
generally studied in liquid cultures, where quorum sensing regulated genes become activated at cell densities of $\sim 10^9 \text{ ml}^{-1}$, although this can vary \[380\]. For surfaces which are able to sustain a densely-packed monolayer of cells \textit{e.g.}, nutrient agar, the steady-state population density is clearly well above this quorum sensing threshold.

However, this may not be the case in conditions where nutrient availability is poor, for example, a marine environment. Therefore, greater stochasticity in biofilm formation may be predicted under poor nutrient conditions, which corresponds to a small value of $K$. Indeed, understanding biofilm initiation is particularly important in marine environments, where biofouling on ship hulls and marine installations is a major concern \[387\]. This scenario is examined in greater detail in Chapter 4 where this model is expanded upon to represent a multispecies marine biofilm growing on the exterior of a ship hull which is leaching biocidal compounds.

Other previous theoretical models for biofilm initiation have focused on \textit{P. aeruginosa}, where motile cells explore the surface before committing to surface attachment via the production of EPS. The motile cells can leave trails of EPS that influence the motion of other cells \[313, 374\], leading to collective phenomena. Although these models are conceptually very different from the ones presented here, both involve collective effects and a transition from reversible to irreversible attachment.

The models demonstrated in this chapter are of course highly idealised. Quorum sensing may not be involved in biofilm initiation for all bacterial species, and even where it is, the process is far more complex than that represented here - \textit{e.g.}, a biofilm may initiate even under conditions of low population densities \[51, 371\]. The use of discrete microhabitats, while conceptually simple and computationally efficient, omits important biological information pertaining to the spatial structure of biofilms \[304, 388\], and the multispecies aspect of many natural biofilms is also neglected here. Nevertheless, this work suggests that biofilm initiation can be an intrinsically stochastic process - with potentially important implications for the ability to predict and control biofilm infections and industrial biofouling processes.

Laboratory biofilm growth experiments are notoriously difficult to reproduce quantitatively, and some studies have attempted to quantify biofilm growth variability \[389, 390\]. However, such quantification is challenging due to the
technical difficulty in achieving well-controlled biofilm growth experiments, due to factors such as inevitable spatial heterogeneity within flow devices, the corresponding feedback between flow patterns and biofilm growth, and differences in the state of the inoculating microbes [390]. This work suggests that, although challenging, investigation of intrinsic biofilm stochasticity may prove to be fruitful.
Chapter 4

Assembly of multispecies biofilms on antifouling surfaces

4.1 Introduction

This chapter extends the work presented in the previous chapter to address a common scenario relating to industrial biofilm formation; that of a biofilm forming on an antifouling (AF) coating submerged in a marine environment, a process known as marine biofouling, which typically occurs in several stages. Firstly, within a few seconds of a surface being submerged in the marine environment, it becomes covered by a conditioning layer of dissolved proteins and other organic detritus. Following this, the surface can be colonised by microbes in a matter of hours, resulting in the formation of a biofilm. Finally, in the macrofouling stage, larger marine invertebrates such as barnacles or mussels attach [179]. While macrofouling is the major contributor to drag and ship hull degradation [391], the microbial biofilm facilitates the attachment of the larger fauna [392, 393] and also contributes to the drag experienced by the vessel, as discussed earlier in Section [1.7]. AF coatings work to prevent the formation of these marine biofilms by leaching biocidal compounds, which are encased in the coatings via the usage of a polymer matrix, into the surrounding marine environment, thereby preventing the growth and proliferation of microbial populations on the exterior of the ship’s hull.

The model developed here, undertaken in collaboration with the paint and
performance coatings company AkzoNobel, is the first computational model for the colonisation of an AF surface by a multispecies microbial community. The model predicts biofilm formation dynamics and provides insight into the microbial biodiversity of the biofilm. The latter feature is of particular interest to marine biofouling, as it has been observed that prospective macrofoulers can also differentiate between biofilms with different microbial species composition. The simulations suggest that biofilm formation on the AF surface can be highly stochastic, with an exponential distribution of the time until a biofilm forms. The lifetimes of AF coatings are generally hard to predict, motivating further research into the initial formation dynamics of marine biofouling.

4.2 Methodology

4.2.1 Model overview

This model builds on the stochastic model described in Chapter 3, but with the key additions of differing microbial species and the presence of a spatial biocide gradient. Otherwise the core model dynamics remain the same, including the modelling of quorum sensing mediated biofilm formation via a threshold population density used as a trigger for the transition from a planktonic population to a biofilm configuration.

This model is intended to represent the type of biofilm that could be found in a marine environment, particularly on the hull of a ship coated in a biocide-leaching paint. Different microbial species stochastically enter the system from the well-mixed external marine environment. Rather than being assigned a taxon, species are differentiated only by their level of susceptibility to the biocide, which is represented by a numeric value corresponding to their minimum inhibitory concentration (MIC), i.e. the minimum concentration of biocide required to inhibit growth or kill the microbe. These resistance values are chosen from a log-normal distribution, as explained in section 4.2.2. In some sense, this model can be viewed as an ecotype model, where “ecotype” refers here to the level of biocide susceptibility.

\[1\] To the best of our knowledge.
Figure 4.1  (a) Diagram of the model. Microbes enter the system from the well-mixed marine environment into the edge microhabitat. From here they can replicate, die, migrate between adjacent microhabitats (provided the number of microhabitats is $> 1$), or detach from the system. Once the population of the edge microhabitat reaches the transition threshold, it is designated as “biofilm” and another microhabitat is added to the system, which then becomes the edge microhabitat. This creates an expanding series of microhabitats, representing the growth of a marine biofilm. Each microhabitat $i$ contains a concentration of biocide, $c_i$, which decreases exponentially with increasing microhabitats, as the edge moves further from the surface. (b) Plot of the biocide gradient throughout the microhabitats. It has a maximum value of 5 ppm, decreasing exponentially in successive microhabitats. The model has a “thickness limit” of 40 microhabitats for computational feasibility, meaning that the minimum biocide concentration possible in the system is $c_{min} \approx 3.36$ ppm. (c) Growth curves as a function of $c$ for microbes with $\beta$ values between 1 and 10.

Once again, the model takes the form of a 1D series of “microhabitats”, labelled with index $i \in [0, L)$, where $L$ is the maximum number of microhabitats the system can support, which was chosen for computational feasibility, rather than to represent any thickness-limiting behaviour of in vivo biofilms. The microhabitats here are taken to have size $X \times Y \times Z = 0.5 \text{mm} \times 0.5 \text{mm} \times 1 \mu\text{m} = 2.5 \times 10^{-4}$ mm$^3$. The innermost microhabitat ($i = 0$), is immediately adjacent to the AF surface, with subsequent microhabitats extending into the marine environment, as shown in Figure 4.1(a). Each microhabitat contains a differing concentration
of biocide, starting with $c_{\text{max}}$ in the innermost microhabitat and monotonically decreasing in subsequent microhabitats. This represents the spatial concentration gradient arising from the diffusion of biocide away from the AF surface. Within each microhabitat, the total population density of microbes of all species is tracked over time. New microbes enter the system via immigration from the external marine environment into the edge microhabitat.

When the system is initialised, microbes immigrate into the first microhabitat (adjacent to the AF surface) and form a loosely-attached layer, proliferating or dying at rates that depend on the local biocide concentration and their susceptibility. The dependence of microbial growth/death rates on biocide concentration are illustrated in Figure 4.1(c) and discussed further in Section 4.2.2.

As in Chapter 3, biofilm formation is modelled via a simplified quorum sensing mediated transition to the biofilm state. When the population density in a microhabitat reaches a threshold value $N^*/K$, where $N^*$ is the threshold number of microbes, and $K$ is the carrying capacity of the microhabitat, the microhabitat transitions irreversibly from a planktonic to a biofilm state. At this point, a new microhabitat is added to the system; this new microhabitat becomes the one which immigrating microbes will now enter into. This process continues, with new microhabitats being added when the population in the outermost microhabitat reaches the threshold density, such that the growing biofilm expands outwards. Therefore, as in Chapter 3, the number of microhabitats, $M$, increases as the simulation progresses.

The model is simulated via a stochastic agent-based approach which tracks the number of microbes of each biocide-susceptibility level in each microhabitat. The key model parameters are: the maximal biocide concentration $c_{\text{max}}$, the steepness of the biocide gradient $\alpha$, the mean and standard deviation of the log-normal biocide susceptibility distribution of the immigrating microbes $\mu$ and $\sigma$ (which control the mean MIC value for immigrants and the percentage of immigrants with MIC above $c_{\text{max}}$), the microbial immigration rate $r_{\text{imm}}$, the maximum rate of microbial growth $r_{\text{max}}$, which also controls the maximal rate of biocide killing, the carrying capacity $K$ of a microhabitat (which depends on the microhabitat thickness $\delta z$ and lateral area $A$), the population size $N^*$ at which a microhabitat transitions to the biofilm state (which also depends on $\delta z$ and $A$), the detachment rate $r_{\text{det}}$ of microbes from the outer microhabitat, the rate $r_{\text{mig}}$ of migration of microbes within the biofilm, and the biocide-independent microbial mortality.
Presented below are more detailed descriptions of the model components, and discussions of the parameter values.

### 4.2.2 Model components

#### Biocide gradient

In this model, the biocide concentration profile takes the form of an exponential, decaying from the ship surface into the marine environment, with a steepness $\alpha$. The biocide concentration in microhabitat $i$ is given by

$$c_i = c_{\text{max}} e^{-\alpha(i + \frac{1}{2})\delta z},$$  \hspace{1cm} (4.1)

where $(i + \frac{1}{2})\delta z$ represents the midpoint of the $i$-th microhabitat.

In this work, $\alpha$ has a value of 0.01 $\mu$m$^{-1}$, $c_{\text{max}} = 5$ ppm, and the thickness of each microhabitat is $\delta z = 1$ $\mu$m. The maximal biocide concentration was based on experimental data provided by AkzoNobel.

The exponential form of the biocide gradients is consistent with the biocide diffusing away from the surface and then being removed at a uniform rate, e.g., via degradation. In this case, the biocide concentration can be expressed via the following reaction-diffusion equation

$$\frac{\partial c(z)}{\partial t} = D \frac{\partial^2 c(z)}{\partial z^2} - \zeta c(z)$$  \hspace{1cm} (4.2)

where $D$ is the biocide diffusion constant in water and $\zeta$ the biocide removal rate. Equation (4.2) has a steady-state solution of $c = C e^{-\alpha z}$. The lengthscale of the biocide concentration decay is controlled via the ratio of the removal rate and the diffusion constant, $\alpha = \sqrt{\zeta/D}$, not by the properties of the paint.

However the properties of the paint do determine the value of the constant, $C$. As the biocide release rate $\xi$ must match the flux at the surface $-(\partial c/\partial z)_{z=0}$, this implies that $\alpha C = \xi$. Hence, $C = \xi/\alpha$. 

Species diversity

In this model, differing microbial species are represented via a numeric “ecotype”, characterised by the value of their MIC. The species diversity of the ocean microbial community is modelled via the MIC values of immigrating microbes being randomly sampled from a log-normal distribution \[397\],

\[
\beta(x, \mu, \sigma) = \frac{1}{x\sigma\sqrt{2\pi}} \exp\left(-\frac{(\ln x - \mu)^2}{2\sigma^2}\right). \tag{4.3}
\]

Replicated microbes have the same MIC value as the mother cell, since it is assumed that no mutation is present. The MIC distribution is configured such that highly resistant microbes are rare.

A biocide was selected from data provided by the industrial partners AkzoNobel which detailed the population size as a function of time (kill curves) for marine microbial populations exposed to various biocides. To determine the average level of resistance to this biocide, the conditions under which the AkzoNobel data was obtained was simulated. A single microhabitat was created with an initial population of 600,000 microbes and a biocide concentration of 10 ppm. The system was then simulated for a time period of 24 hours. This was repeated for a range of microbial populations, with MIC values in a range of 1-10 ppm, shown in Figure 4.2(a). In these simulations, it was assumed that all microbes had the same MIC, i.e. there was no species diversity.

From these simulations, plots of population size vs time were obtained, and the half-life of the population was then obtained and plotted as a function of the MIC, shown in Figure 4.2(b). This allowed for the determination of an MIC which corresponded to a population half-life of around 3 hours, similar to that seen in the AkzoNobel data.

This MIC values was then used as the average value of the MIC distribution described above, and \( \mu \) and \( \sigma \) in Equation (4.3) were adjusted such that the MIC distribution had a mean value corresponding to the MIC obtained in these population dynamics simulations and a specified percentage of the immigrating population were resistant to the maximal biocide concentration (i.e. their MIC \( > c_{\text{max}} \)).
Figure 4.2  (a) The killing dynamics for populations of microbes exposed to a concentration of 10 ppm of biocide for a range of MICs between 1 – 10 ppm. Only those MICs which caused the population to halve in size within 24 hours are shown. Increasing MIC is represented by darker lines. (b) The time taken for the number of live microbes to reduce by half, as a function of MIC. The simulated data is shown in blue, with the fitted curve in orange.

Growth and death rates

The effect of the applied biocide on the microbial growth, or death, rate was once again modelled via the use of a pharmacodynamic function, which depends on the ratio of the microbe’s MIC to the concentration of the applied biocide. In this model, similar to that in Chapter 2, the function had the form

\[ \phi(c_i, \beta_{i,j}) = r_{max} \left( 1 - \frac{6 \left( \frac{c_i}{\beta_{i,j}} \right)^2}{5 + \left( \frac{c_i}{\beta_{i,j}} \right)^2} \right), \]  

which is related to the general function proposed by Regoes at al. for bacterial response to antibiotics with parameters \( \kappa = 2, \psi_{\text{max}} = 1 \) and \( \psi_{\text{min}} = -5 \). In Equation (4.4), \( \beta_{i,j} \) is the MIC of microbe \( j \) in microhabitat \( i \), \( c_i \) is the concentration of the applied biocide in microhabitat \( i \), and \( r_{\text{max}} \) is a scaling factor representing the maximal growth rate of marine microbes.

Furthermore, in this model all microbes experience a uniform death rate, \( d_{\text{uniform}} \) which is based on \textit{in vivo} observations of marine microbes and is
independent of the value of $\phi(c, \beta)$. This might represent, for example, lysis by bacteriophages.

There are two scenarios which may occur in Equation (4.4), depending on the value of $\phi(c, \beta)$. For the case where $\phi(c_i, \beta_{i,j}) > 0$, the net growth and death rates are given by

$$g_{i,j} = \phi(c_i, \beta_{i,j}) \left(1 - \frac{N_i}{K}\right),$$  
(4.5)

$$d_{i,j} = d_{\text{uniform}},$$  
(4.6)

where $N_i$ is the number of microbes in microhabitat $i$ and $K$ is the microhabitat’s carrying capacity. In the other case, where $\phi(c_i, \beta_{i,j}) \leq 0$, the microbes cannot grow and the growth/death rates are instead given by

$$g_{i,j} = 0,$$  
(4.7)

$$d_{i,j} = |\phi(c, \beta_{i,j})| + d_{\text{uniform}}.$$  
(4.8)

Some examples of how Equation (4.4) varies with $c$ and $\beta$ are shown in Figure 4.1(c). For smaller values of $\beta$, relatively low values of $c$ are sufficient to induce microbial death, as illustrated by the blue curve. However, higher $\beta$ values allow for growth at even the maximal value of $c$, as shown by the red curve.

### 4.2.3 Model parameters

Care was taken to ensure the values of the parameters used correspond well to literature values wherever possible. Due to the lack of consistent data for certain aspects of the model, such as the physical properties of biofilms, this was not always possible. Therefore, values for the biofilm transition density and the detachment rate have instead been determined by adjusting them such that in the absence of an applied biocide, they produce biofilm thicknesses in accordance with observed values.
Carrying capacity

In this model, population growth in each microhabitat is limited by a logistic growth term. This depends on the “carrying capacity”, $K$, of the microhabitat, which is the maximum number of microbes that a microhabitat sized volume can sustain [400]. The choice of $K$ for this model was based upon microbial densities of marine biofilms observed in nature. For ease of comparison, these values are also given in terms of microhabitat volume (mhv) when relevant, which is equivalent to: $1 \text{ mhv} = 0.5 \text{ mm} \times 0.5 \text{ mm} \times 1 \mu\text{m} = 2.5 \times 10^{-4} \text{ mm}^3$.

Microfouling communities cultivated in the White Sea were found to have a cell density of $10^7 \text{ cells ml}^{-1}$. $1 \text{ ml} = 10^3 \text{ mm}^3$, therefore the White Sea cell density $= 10^4 \text{ cells mm}^3 = 2.5 \text{ cells mhv}^{-1}$ [401].

A different study into the growth of biofilms on fouling-release coatings was performed by Dobretsov et al. by immersing several slides with fouling-release coatings into the Banda Rawdha Marina in Oman [402]. This was a more short-term study, taking place over the course of 10 days. Here, cell densities were observed in the range $2.62 \times 10^4 \text{ cells in a volume of } 1.28 \times 10^{-2} \text{ mm}^3$ (511 cells mhv$^{-1}$) to $1.95 \times 10^4 \text{ cells in a volume of } 8.06 \times 10^{-3} \text{ mm}^3$ (605 cells mhv$^{-1}$). There is clearly a high degree of variability in the possible values for the carrying capacity, so for simplicity $K$ was set to a value of $K = 550 \text{ cells mhv}^{-1}$.

Maximum growth rate

Marine microbes tend to have growth rates around an order of magnitude lower than their enteric counterparts. While a bacterium such as $E. \text{ coli}$ has a growth rate of around 0.3 to 1.6 h$^{-1}$ [403], marine microbes tend to have lower growth rates, in the range of 0.04 to 0.1 h$^{-1}$ [404–406]. Therefore the maximal growth rate parameter $r_{\text{max}}$ was given a value of 2 day$^{-1}$, or $r_{\text{max}} = 0.083 \text{ h}^{-1}$.

Immigration rate

The rate at which microbes enter the system is based upon an experimental study by Fletcher and Loeb [407] which investigated how the properties of a substratum affected the microbial attachment rate. In their study, the number of microbes which attached to a surface of area $1 \times 10^2 \mu\text{m}^2$ submerged in seawater with a
microbial concentration of $2.5-5 \times 10^9$ microbes ml$^{-1}$ over a period of 2 hours was measured. Their findings correspond to an immigration rate of 20 h$^{-1}$.

To rationalise the scaling required to convert the findings of Fletcher and Loeb into a value suitable for this model, the following calculations were performed. Consider a square patch of surface of side $L$ and area $A = L^2$. The volume of a cube above it is $V = L^3$. If a microbe has length $a$, then we assume it occupies a volume $V_b = a^3$. Now consider a single microbe within the volume $V$. The time taken for it to diffuse out of this volume by travelling a distance $L$ is

$$\tau \propto \frac{L^2}{D},$$

(4.9)

where $D$ is the microbe’s diffusion coefficient. If the volume $V$ is then split into multiple sub-volumes $V_b$, which are the same size as the microbe’s volume, $V_m = a^3$, then the number of sub-volumes in the overall volume is

$$N_s = \frac{V}{V_b} = \frac{L^3}{a^3}.$$  

(4.10)

This results in a grid of $N_s^{1/3} \times N_s^{1/3} \times N_s^{1/3}$ sub-volumes in the volume $V$. The number of these sub-volumes which are adjacent to the surface is

$$N_s^2 = \frac{L^2}{a^2}.$$  

(4.11)

The time taken for a microbe to diffuse out of one of these sub-volumes (a distance $a$) is therefore

$$\tau_m = \frac{a^2}{D}.$$  

(4.12)

Ergo, within the time $\tau$, the number of sub-volumes that can be explored is given by

$$N_{\text{explored}} = \frac{\tau}{\tau_m} = \frac{L^2}{D} \cdot \frac{D}{a^2} = \frac{L^2}{a^2}.$$  

(4.13)

If the microbe explores 1 sub-volume, the probability that it is at the surface is given by
If the microbe explores $N_{\text{explored}}$ sub-volumes, the number of times it will hit the surface on average, i.e. the probability that the microbe will hit the surface within a time $\tau$ is

$$p_{\text{hit}} = N_{\text{explored}} \frac{N_s^{\frac{2}{3}}}{N_s} = \frac{L^2}{a^2} \frac{L^2/a^2}{L^3/a^3} = \frac{L}{a},$$  \hspace{1cm} (4.15)$$

and the corresponding rate at which a single microbe hits the surface is therefore given by

$$r_1 = \frac{L}{\tau a}. \hspace{1cm} (4.16)$$

If instead of a single microbe, there is a microbial density $\rho$, the total number of microbes in the volume $V$ is given by $N_m = \rho L^3$. The overall surface collision rate is therefore

$$r \propto \frac{\rho L^4}{\tau a}. \hspace{1cm} (4.17)$$

Substituting in $\tau = L^2/D$, $D = a^2/\tau_b$ and $L^2 = A$, results in an expression for the overall immigration rate,

$$r_{\text{im}} \propto \frac{\rho A a}{\tau_b}. \hspace{1cm} (4.18)$$

This expression scales linearly with $\rho$, $A$ and $a$, as expected. As the model microhabitats have an area of 0.25 mm$^2$ (2.5$\times$10$^5$ µm$^2$), and the surrounding seawater is assumed to contain a concentration of microbes 10$^6$ ml$^{-1}$, this increases the immigration rate relative to that of Fletcher and Loeb, by a factor of 2.5$\times$10$^3$ due to the difference in area, but a decrease by a factor of 2.5$\times$10$^3$ due to the difference in microbial concentration compared to the Fletcher and Loeb study, resulting in an immigration rate which is conveniently the same as...
that of Fletcher and Loeb’s observations, \( i.e. r_{lm} = 20 \, \text{h}^{-1}. \)

**Migration rate**

Motivated by the lack of literature values for cell movements within biofilms, Rice et al., 2003 [408] performed experiments involving \( P. \) aeruginosa tagged with a fluorescent green protein, cultivated in a 3-10 \( \mu \text{m} \) thick biofilm. The motion of the individual cells in the biofilm was then tracked via confocal microscopy and were found to move with a typical velocity of \( 1 \, \mu \text{m} \, \text{h}^{-1} \). With a microhabitat thickness of \( \delta z = 1 \, \mu \text{m} \), the rate at which microbes will migrate in or out of a microhabitat is \( r_{mig} \approx 1 \, \text{h}^{-1} \).

This initial value of \( r_{mig} \) resulted in undesirable behaviour in the simulations. When the first microhabitat population reached \( N^* \), the model exhibited a noticeable lag time before the second microhabitat was filled. This was similar to the results shown in Figure 3.4(b), where the steady state population decreased when an additional microhabitat was added to the system. Rather than an observable phenomenon, this was deemed to be an artefact of the model, where the innermost microhabitat tended act as a “sink” for the second microhabitat’s population, as microbes that migrated from the outer microhabitat to the more deleterious inner one then tended to die. Therefore, the decision was made to lower the migration rate to \( r_{mig} = 0.1 \, \text{h}^{-1} \), which greatly reduced the impact of this artefact.

**Threshold density and detachment**

To replicate the biofilm growth rate observed on a control surface by Dobretsov and Thomason [402], trial simulations were performed without any antimicrobial present in the system. In these simulations, the resulting biofilms were found to be an order of magnitude thicker than the observed ones. It was decided that the model required an additional factor in order to reduce the overall biofilm growth rate to a realistic value. Thus, the concept of a detachment rate was introduced to the model.

The “detachment” process removes microbes from the outermost microhabitat, before it has transitioned to biofilm. Initially this rate was intended to only represent the detachment of microbes from the surface or from the edge of the
biofilm, but due to the variability in reported literature values for microbial detachment rates (ranging between $10^0$-$10^{-4}$ hr$^{-1}$ [409, 410]) and the fact that the interpretation of the attachment rate in this model also includes the effect of microbial detachment (see the **Immigration rate** subsection above), this detachment rate instead aims to include all sources of detachment that a marine biofilm might encounter, such as sloughing or grazing [411, 412].

Due to the lack of relevant literature values for both the detachment rate and the threshold density required for quorum sensing, these parameters were reverse engineered by running a range of biocide-free simulations with differing parameter values. From these trial simulations, a pair of parameters was selected which resulted in a biofilm thickness in accordance with the observed values of Dobretsov and Thomason, as shown in Figure 4.3.

![Figure 4.3](image)

**Figure 4.3** *Heatmap showing the resultant biofilm thickness for parameter pairs of $N^*/K$ and $r_{det}/r_{max}$. The green annotated region shows parameter pairs which produce a biofilm thickness within observed values.*

Based on these simulations, values of $N^* = 0.75 \cdot K$ and $r_{det} = 0.22 \cdot r_{max}$ were used, highlighted by the blue square in Figure 4.3.
4.2.4 Simulation algorithm

As in Chapter 3, the model was simulated via the modified $\tau$-leaping algorithm \cite{382}, which takes account of the stochasticity of individual immigration, migration, birth, death and detachment events. As mentioned previously, $\tau$-leaping \cite{383} is an approximate version of the famous Gillespie stochastic simulation algorithm (SSA) \cite{337, 338}, commonly used to simulate well-mixed biochemical systems.

The algorithm used here differs slightly from the one used in Chapter 3 due to the inclusion of microbial death, both via the uniform death rate and the biocidal effects of the AF surface, as well as the fact that multiple different species of microbes are tracked in this model, each of which have their own growth and death rates. The general overview of the algorithm procedure is as follows:

1. A timestep $\tau$ is selected. In this model, an initial value of $\tau = 0.2$ hours is used. This timestep is larger than the one used in Chapter 3 due to the longer simulation times in the runs performed in this chapter.

2. Each microbe in the system is iterated over and the number of births/deaths each microbe will experience ($b_{i,j}/d_{i,j}$) is determined by sampling from a Poisson distribution with mean $\mu_{rep} = r_{i,j} \times \tau$. Here the indices $i$ and $j$ refer to microbe $j$ in microhabitat $i$.

3. In order to prevent negative populations arising (a microbe cannot die twice), if any of the values in $d$ are $>1$, then the algorithm returns to step 2 and restarts the process, now with $\tau \rightarrow \tau/2$. This is also done if a microbe is designated to both replicate and die in the same timestep.

4. The migration allocations for each microbe ($m$) are sampled from a Poisson distribution with $\mu_{mig} = r_{mig} \times \tau$. As microbes in the inner/outer edge microhabitats can only migrate in one direction, these allocations are instead sampled from a Poisson distribution with $\mu_{mig}^* = 0.5 \times r_{mig} \times \tau$.

5. Finally, the detachment allocations ($z$) for the microbes in the edge microhabitat are sampled from a Poisson distribution with $\mu_{det} = r_{det} \times \tau$. Migration and detachment allocations are handled in a similar way to the death allocations; if a microbe is designated to migrate or detach more than once, $\tau \rightarrow \tau/2$ and the algorithm returns to step 2.
6. The events calculated in the previous steps are then carried out, with microbe $j$ in microhabitat $i$ either replicating $b_{i,j}$ times, or dying $d_{i,j}$ times. If the microbe is in the edge microhabitat then it will detach according to $z_j$. For migration, if $m_{i,j} > 0$ then microbe $j$ in microhabitat $i$ is sent to either microhabitat $i + 1$ or $i - 1$ with a 50/50 probability. Detachment events have priority over migration events. Microbes which are designated to both replicate and migrate in the same timestep will first replicate, then the mother cell will migrate. Dead or detached microbes are simply removed from the system.

7. The number of microbes which immigrate into the edge microhabitat is then sampled from a Poisson distribution with $\mu_{\text{imm}} = r_{\text{imm}} \times \tau$. The MIC values of these microbes are randomly sampled from the lognormal distribution described in Section 4.2.2.

8. If the number of microbes in the biofilm edge microhabitat has reached the threshold for biofilm formation ($N_i \geq N^*$), then another microhabitat is added to the system, in series with the current edge microhabitat. This new microhabitat is now allocated as the new edge microhabitat and is the one which microbes will immigrate into in the next timestep.

9. The time elapsed in the simulation is updated to $t \rightarrow t + \tau$. For the next timestep, $\tau$ returns to its original value of 0.2 hours.

**Table of parameters**

Presented here are the parameters used in the model, their values and the rationale for said values, with sources where available. It should be noted that according to the theory presented in Chapter 3, the model here is in the regime where the steady state population size in the first microhabitat is not enough to trigger the transition to the biofilm phase. Therefore, stochasticity in the biofilm formation process is to be expected.
Table 4.1  System parameters used in the multispecies simulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta z$</td>
<td>Microhabitat thickness</td>
<td>1 $\mu$m</td>
<td>Approx. width of one microbial layer</td>
</tr>
<tr>
<td>$A$</td>
<td>Microhabitat lateral area</td>
<td>$2.5 \times 10^5$ $\mu$m$^2$</td>
<td></td>
</tr>
<tr>
<td>$r_{\text{max}}$</td>
<td>Maximum growth rate, controls biocide kill rate</td>
<td>0.083 h$^{-1}$ (varied in Fig. 4.8)</td>
<td>growth rates observed for marine microbes [404–406]</td>
</tr>
<tr>
<td>$d_{\text{uniform}}$</td>
<td>Uniform death rate</td>
<td>0.018 h$^{-1}$</td>
<td>Ocean mortality [398, 399]</td>
</tr>
<tr>
<td>$K$</td>
<td>Carrying capacity of microhabitat</td>
<td>550 microbes (2.2 $\times 10^6$ mm$^{-3}$)</td>
<td>Marine biofilm density on fouling-release coatings [402]</td>
</tr>
<tr>
<td>$N^*$</td>
<td>Population threshold for biofilm transition</td>
<td>0.75$\times K$</td>
<td>Adjusted to biofilm growth rate [402]</td>
</tr>
<tr>
<td>$\text{MIC}_{\text{avg}}$</td>
<td>Average biocide MIC</td>
<td>3.179 ppm</td>
<td>Adjusted to fix overall killing rate</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Scale parameter: mean of the normally distributed natural logarithm of MIC distribution</td>
<td>2.48 (Figs 4.4, 4.6, 4.5; varied in Figure 4.8)</td>
<td>Set to achieve desired $\text{MIC}<em>{\text{avg}}$ and $p</em>{\text{res}}$</td>
</tr>
</tbody>
</table>

(Cont.)
<table>
<thead>
<tr>
<th>parameter</th>
<th>definition</th>
<th>value</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma$</td>
<td>Shape parameter: standard deviation of the normally distributed natural logarithm of MIC distribution</td>
<td>0.71 (Figs 4.4, 4.6, 4.5); varied in Fig 4.8</td>
<td>Set to achieve desired MIC$<em>{avg}$ and pc$</em>{res}$</td>
</tr>
<tr>
<td>pc$_{res}$</td>
<td>% of immigrants with MIC &gt; $c_{max}$</td>
<td>16% (Figs 4.4, 4.6, 4.5); varied in Figure 4.8</td>
<td>Guessed: no data available</td>
</tr>
<tr>
<td>$c_{max}$</td>
<td>Maximum biocide concentration</td>
<td>5 ppm (varied in Figs 4.7, 4.8)</td>
<td>Assumed to be controlled by biocide solubility in seawater, e.g., 4.7 ppm for Kalthon930 [413]</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Biocide gradient parameter</td>
<td>0.01 $\mu$m$^{-1}$</td>
<td>Consistent with diffusion/degradation</td>
</tr>
<tr>
<td>$r_{imm}$</td>
<td>Immigration rate</td>
<td>20 h$^{-1}$ (varied in Fig. 4.8)</td>
<td>Scaling of values reported by [407]</td>
</tr>
<tr>
<td>$r_{mig}$</td>
<td>Migration rate</td>
<td>0.1 h$^{-1}$</td>
<td>Scaling of values for P. aeruginosa biofilms [408]</td>
</tr>
<tr>
<td>$r_{det}$</td>
<td>Detachment rate</td>
<td>$0.22 \times r_{max}$ (varied in Fig. 4.8)</td>
<td>Adjusted to biofilm growth rate [402]</td>
</tr>
<tr>
<td>$t_{max}$</td>
<td>Maximum simulation time</td>
<td>6 months (Figs 4.4, 4.6, 4.5); 1 year (Figs 4.7, 4.8)</td>
<td>Computational feasibility</td>
</tr>
<tr>
<td>$L_{max}$</td>
<td>Maximum biofilm thickness</td>
<td>40 microhabitats</td>
<td>Computational feasibility</td>
</tr>
</tbody>
</table>
4.3 Results

4.3.1 Microbial colonisation of an AF surface

Figure 4.4 An example of a simulation run in which a biofilm is established. The population composition vs time $t$ is represented in 3 different ways. In all cases, the colours represent the resistance levels (MIC, in ppm) of microbes within the population (see colour scale). For each time point, a vertical bar shows the state of the population; these bars are stacked adjacent to each other to show dynamical changes. This run stopped when the biofilm reached the thickness limit of 40 microhabitats. The green dashed lines represent times at which new microhabitats were added to the system. For clarity, only the first 3 such events are shown. (a) Total population size and composition. Here, the bar height represents the total population size. The colours show the resistance levels within the population; individual bars for each microhabitat are stacked such that the lower part of each bar represents the region of the biofilm close to the surface while the upper part represents the region further from the surface. (b) Same plot as in (a), but with a log scale on the vertical axis. (c) Relative population composition. Here the colours represent the resistance levels present in the population, as fractions of the total population.

Figure 4.4 shows the results of a typical simulation run in which the AF surface becomes colonised. In Figure 4.4(a) the dynamics of biofilm development are represented as a series of vertical bars, corresponding to the biofilm population at increasing times. The height of each bar corresponds to the total biofilm population size, illustrating the overall growth dynamics of the biofilm. Within each bar, the colours show the composition of the population in terms of biocide susceptibility, from purple (low MIC, susceptible) to orange (high MIC, resistant). To account for the spatial structure of the biofilm, each vertical bar consists of a stack of smaller bars, each corresponding to one microhabitat. Thus, the lower
part of each bar represents the region of the biofilm close to the surface while the upper part represents the region further from the surface. The dashed green lines highlight the times at which the first 3 additional microhabitats were added to the system.

Figure 4.4(b) shows the same information as Figure 4.4(a), but with a log scale on the vertical axis, allowing the early-time dynamics to be more clearly seen. Figure 4.4(c) focuses on changes in the microbial community composition as the biofilm develops. Here, the vertical height of the bars is scaled by the population size, and within each bar the colours are ordered by MIC value. This gives a view of changes in the relative abundance of different biocide susceptibility levels within the total population, i.e. each bar shows the proportion of each species within the biofilm at time $t$ - information on spatial structure is lost in Figure 4.4(c).

In these simulations, biofilm formation happens as follows. First, the initially empty surface acquires a loosely attached layer of microbes, corresponding to a single microhabitat with a population density below the biofilm threshold. Microbes arrive in this layer by immigration, but as the biocide concentration is high close to the surface, the majority of them rapidly die. Some marginally resistant immigrants are able to replicate, but for the chosen parameter set, even a fully resistant microbe would not be able to establish a population size above the biofilm threshold, resulting in the loosely attached layer being maintained for some time.

During this time, the overall population fluctuates due to random immigration, proliferation of more resistant microbes and microbial death. Eventually, one of these population fluctuations pushes the total population size above the biofilm formation threshold $N^*$. At this point, the first microhabitat transitions to the biofilm state and a second microhabitat is added. This triggers the second stage of biofilm development, in which biofilm growth is inevitable. Although the second microhabitat may spend a short time in the loosely attached state$^2$, its lower biocide concentration means that it soon transitions to the biofilm state. Subsequent microhabitats are rapidly added, such that the biofilm grows approximately linearly in time.

In Figure 4.4 the first, loosely attached, stage of biofilm formation is characterised

$^2$In simulations with a higher migration rate, the high death rate in the first microhabitat causes a net migration of microbes inwards from the second microhabitat, suppressing population growth in the second microhabitat.
by biocide-susceptible microorganisms (denoted by the dark colours in Figure 4.4(b) and (c) at early times), but the transition to the second stage of sustained growth coincides with the arrival of a more biocide-resistant microbe (orange colour in Figure 4.4), which later dominates the biofilm community, seen clearly in Figure 4.4(c). It is possible that the immigration of this microbe provided the population fluctuation that triggered the transition to biofilm formation.

Furthermore, Figure 4.4(c) shows that as the biofilm grows, marginally resistant microbes begin to have a more significant presence in the community compared to earlier times. This suggests a “shielding” effect: the more resistant microbial type populates the inner parts of the biofilm, as shown in Figure 4.4(a), where the biocide concentration is high, allowing for less resistant microbes to contribute to population growth in the outer parts - the outer layer of darker colours in Figure 4.4(a) and (b).

### 4.3.2 Biodiversity of the biofilm community

![Graphs showing biodiversity metrics](image)

Figure 4.5 (a) The number of species in the system $S$, (b) the Shannon index $H$, and (c) the Shannon equitability $E$ over time, averaged over the runs which exhibited growth (63/400). While $S$ increases over time, $H$ and $E$ both decrease. This suggests that the biofilm is dominated by a few resistant species, which in turn allow for less resistant microbes to enter the system by proliferating into a more favourable environment.

To further understand changes in community composition during biofilm development, the dynamics of three quantitative measures of community structure were investigated. The number of species $S$ measures how many distinct microbial types (with distinct biocide MIC values) are present in the simulation at any time. The Shannon index $H = - \sum_i p_i \ln p_i$ measures biodiversity, taking account of
the relative abundances $p_i$ of the species in the system \([414]\), $p_i = n_i/N$ where $n_i$ is the number of members of species $i$, $N$ is the total population size. $H$ increases when more species are present, or when their abundances are more evenly distributed. The Shannon equitability $E = H / \ln S$ measures the evenness of the distribution of species abundances, a value of 1 corresponds to all species being equally abundant, whereas a value close to 0 means that one (or a small number of) species is dominant. Figure 4.5 shows the dynamical changes in $S$, $H$ and $E$ during biofilm development, averaged over 63 replicate simulation runs where a biofilm was able to be established.

As seen in Figure 4.5(a), on average the number of distinct microbial species, $S$, within the biofilm community increases over time. This is consistent with the previous observations discussed in Figure 4.4, with the number of immigrating species which are viable for growth increasing over time, due to the biofilm expanding along the decreasing biocide gradient.

However, both the Shannon index $H$ and the Shannon equitability $E$ decrease as the biofilm grows, Figure 4.5(b, c). This is also consistent with the picture which emerges from Figure 4.4(c), in which the microbial species distribution remains highly skewed, even at late times. In other words, the biofilm community generally remains dominated by the most biocide-resistance microbial type, even when the biofilm has become thick enough to considerably reduce the biocide concentration at the growing edge. This is indicative of a priority effect: biocide-resistant organisms that are able to establish early in biofilm development, when the biofilm is thin, maintain their dominance at later times - even when biocide-resistance is no longer as advantageous.

### 4.3.3 Colonisation of the AF surface is stochastic

Repeating the simulations with the same parameter set as in Figure 4.4 results in noticeably different outcomes in replicate simulation runs, perhaps mimicking the unpredictable nature of AF paint failure \([394–396]\). Out of 625 replicate simulation runs, 100 (16%) formed biofilm within the 6 months of simulated time (biofilm formation being defined by whether the first microhabitat is able to reach the biofilm transition density, $N^*/K$).

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\(^{3}\text{Priority effects are a concept from ecology, which refer to the effects of the order/timing of the arrival of species on the development of the species composition at later times.}\)
Figure 4.6 Another selection of runs, highlighting the stochastic nature of biofilm formation and illustrating how the species composition changes over time. Each column is a different replicate simulation, with the bottom row showing the corresponding population fraction plots. Replicate A shows an example of a run which reached the “thickness limit” and stopped early. Around 60% of the runs in this parameter set which exhibited growth reached this limit.

Figure 4.6 shows the results of three replicate simulations in which a biofilm was able to be formed. These simulations vary strongly in the duration of the first, loosely-attached, stage of colonisation. Because sustained biofilm growth begins at different times, the final biomass of the biofilm is also different in the 3 pictured runs, despite the similar rates of sustained growth. The three replicate runs also exhibit quite different community compositions.

Replicate A shows a similar pattern to the run shown in Figure 4.4 in which a somewhat resistant microbe appears at around the time of the biofilm transition and later makes up a significant fraction of the microbial community, while coexisting with other less resistant species. Examining the species composition of the replicate B population, it appears to be less resistant overall than replicate A, and has a greater diversity of species. In contrast, replicate C contains a highly biocide-resistant species which almost completely dominates the community, with less resistant microbes being confined to the outer layer of the biofilm. The fact that replicate simulations with the same parameter sets show such qualitatively differing outcomes (biofilm vs no biofilm, differing growth dynamics and community species composition), demonstrates that in this model, biofouling of the AF surface is indeed a highly stochastic process.
4.3.4 Simulations can predict probability of biofilm establishment on AF surfaces

From an industrial point of view, the waiting time before a biofilm can be formed or established on a submerged AF surface is a useful metric in the testing and development of AF coatings, as well as for establishing in-service paint performance expectations. To investigate in greater detail the factors which influenced the average time taken for a biofilm to be established, sets of 2000 replicate simulations were performed, each run stopping at the point when a biofilm was formed (up to a maximum simulated time of 1 year). For each simulation, the “biofilm formation time” $t_f$, i.e. the time at which biofilm became established, was recorded. Once again, biofilm formation was defined as when the first microhabitat was able to reach the threshold population density, $N^*/K$. This time could vary greatly between simulation runs, as seen in Figure 4.6.

![Figure 4.7](image-url) (a) Survival function constructed from the histogram of biofilm formation times from the set of simulations with $c_{\text{max}} = 4.7$. The fitted survival probability, $p_s$, is shown in orange. (b) The survival probability $p_s$ vs time for a population with 14% resistance at a $c_{\text{max}} = 5$, for a variety of $c_{\text{max}}$ vals. (c) The expected biofilm formation time, $t_f$ for a surface exposed to a population with 14% resistance at a $c_{\text{max}} = 5$. $t_f$ is calculated by taking the reciprocals of the characteristic length of the fitted slopes as shown in (b). $t_f$ increases exponentially with $c_{\text{max}}$.

Histograms of the biofilm formation time from these replicate simulations are strikingly exponential in form, as seen in Figure 4.7(a). In statistical physics, exponential waiting time distributions are typical of Poisson processes\[4\]. In other words, in these simulations biofilm formation can initiate at any time, and the probability of this happening within a given time interval is the same no
matter how old the surface, or what its history is. Therefore, the timing of biofilm establishment in a particular simulation cannot be predicted - it is controlled by a stochastic process which is history-independent. The exponential waiting time distribution also implies that even if the average time to biofilm establishment is long, there will also be some instances of early biofilm formation.

To quantify the mean waiting time before biofilm formation, $t_f$, the histograms of the biofilm formation times were normalised and converted to a “survival function”. The survival function $S(t)$ is defined as the complementary cumulative distribution function for the formation probability,

$$S(t) = 1 - \sum_{i=0}^{t} \frac{n_i}{N},$$

(4.19)

where $n_i$ is the number of runs in which a biofilm has established within time $t = i$ and $N$ is the total number of runs. The survival probability $p_s(t)$, and $t_f$ are then calculated by fitting

$$p_s(t) = e^{-\frac{t}{t_f}},$$

(4.20)

to the $S(t)$ curves. Some examples of these fits are shown in Figure 4.7(b). The exponential function (Figure 4.7(b), dashed line) is an excellent fit to the simulation data (Figure 4.7(b), solid line). An example of $t_f$ as a function of the surface biocide concentration $c_{max}$ is shown in Figure 4.7(c). As $c_{max}$ increases, the exponential function decreases more slowly with time - $t_f$ increases.

To investigate what factors control the time to biofilm establishment in these simulations, the mean time until biofilm formation $t_f$ was measured as a function of key model parameters (using sets of 2000 replicate simulations for each parameter value), the results of which are shown in Figure 4.8. Note that all y-axes are shown in a log scale.

As expected, and shown already in Figure 4.7(c), $t_f$ increases as the surface biocide concentration does, Figure 4.8(a). This dependence is exponential, suggesting that a small change in biocide concentration can have a large impact on biofilm establishment. $t_f$ also decreases upon increasing the abundance of biocide-resistant organisms, Figure 4.8(b), or the immigration rate, Figure 4.8(c). This is consistent with a picture in which the immigration of biocide-resistant organisms
Figure 4.8 The mean biofilm formation time $t_f$ as a function of various model parameters. Values for $t_f$ are obtained from fits to the survival time distribution as detailed in the text; an error can be obtained from the fit, but error bars are be smaller than the points. $t_f$ vs. (a) $c_{\text{max}}$, (b) % resistant, (c) $r_{\text{imm}}$, (d) $r_{\text{det}}$, (e) $N^*/K$, (f) $r_{\text{max}}$, all shown with a log-scale on the y-axis. For the most part, $t_f$ scales with the model parameters as expected, however an interesting phenomenon is observed in (f). Initially the formation time increases with $r_{\text{max}}$, as fast-growing microbes are more susceptible to the biocide. However once $r_{\text{max}}$ reaches a sufficiently high value, marginally resistant microbes are now able to outcompete the detachment rate, reducing the time for a biofilm to be established.

plays a key role in the colonisation process.

Increasing the rate $r_{\text{det}}$ at which loosely attached organisms detach from the outer edge of the biofilm increases the average time of biofilm formation, Figure 4.8(d). Increasing the threshold density $N^*/K$ has a similar effect, Figure 4.8(e). From an experimental point of view, while the detachment rate could possibly be increased by modifying the wettability or similar properties of the AF surface, it is unclear how $N^*/K$ could be altered.

Interestingly, $t_f$ depends non-monotonically on the $r_{\text{max}}$ parameter, shown in Figure 4.8(f). $r_{\text{max}}$ controls both the the maximum growth rate for organisms with an MIC greater than the local biocide concentration and the biocide killing rate for organisms whose MIC is less than the local biocide concentration, Equation (4.4). This suggests the existence of qualitatively different parameter regimes
within the model. For low values of $r_{\text{max}}$, the community in the first microhabitat may be dominated by immigrants with low MIC values which are eventually killed by the biocide. These susceptible microbes die more slowly at low $r_{\text{max}}$, resulting in a higher steady-state population of susceptible microbes, making it easier for moderately resistant microbes to reach the threshold density.

Increasing $r_{\text{max}}$ speeds up the rate at which these biocide-sensitive immigrants are killed, decreasing the population density of the first microhabitat and making it harder for a biocide-resistant immigrant to trigger biofilm formation, increasing the mean time taken for a biofilm to form. At even higher values of $r_{\text{max}}$, the growth rate of resistant organisms is increased enough to outcompete other deleterious rates in the system, making biofilm establishment more likely.

4.4 Discussion

4.4.1 Stochastic microbial colonisation of an AF surface

A striking result of the simulations presented here is that colonisation of the AF surface can be inherently stochastic, with identical initial conditions leading to diverse biofilm formation trajectories. In this model, biofilm formation occurs in two stages: initial formation of a loosely-attached layer of microbes, followed by biofilm growth once the population reaches a threshold density. The biofilm community tends to be dominated by a single, more biocide-resistant, microbial species, even once the biofilm becomes thick enough that microbes at the growing edge are exposed to considerably lower biocide concentrations - an example of a priority effect. However, it is also observed that biocide-resistant microbes can shield the community from the biocide, meaning that species with lower levels of biocide resistance can join the community once it has been established.

For the parameter set used here, a random fluctuation in the population size is needed to reach the threshold density for biofilm growth, even for resistant microbes, i.e. the model is in the stochastic regime identified in the simpler model in Chapter 3. The waiting times until biofilm formation occurs follow an exponential distribution, suggesting that it can be modelled as a Poisson process, which is inherently unpredictable. In other words, the probability that a biofilm can establish on an AF surface is independent of the surface’s history. Investigating the parameter dependence of the average biofilm formation time,
it was found that it depends exponentially on the biocide concentration, the immigration rate and the detachment rate. This supports a hypothesis in which a key factor in the triggering of biofilm growth is the immigration of microbes which have sufficient biocide resistance to be able to grow in proximity to the AF surface.

For other parameter choices, the model is expected to exhibit different behaviour. In particular, if the microhabitat closest to the AF surface was able to support a microbial population greater than the threshold density, then the arrival of a resistant immigrant would immediately trigger biofilm growth. In that regime, the biofilm establishment time would simply be controlled by the rate of immigration of sufficiently resistant microbes, and parameters controlling growth behaviour close to the surface (e.g., $r_{det}$) would not be expected to play a role. $t_f$ would then also be expected to depend linearly on the immigration rate, rather than exhibiting the exponential dependence seen in the simulations presented above.

### 4.4.2 Biocide concentration profile

For simplicity, it was assumed that the biocide concentration decreases exponentially with distance away from the AF paint surface, and this spatial gradient remains constant in time. An exponential profile is consistent with diffusion of the biocide combined with its removal at a fixed rate, perhaps due to chemical degradation in the seawater. In reality, the concentration profile of biocide around a moving ship coated in AF paint will be determined not only by diffusion and chemical degradation, but also by the fluid flow. The resulting convection-diffusion problem is non-trivial, even in an idealised scenario of a planar surface with laminar flow in the parallel direction, as here biocide will accumulate along the flow lines. The inclusion of turbulent flow would complicate things even further. Another omission is a feedback mechanism between biofilm growth and the biocide concentration profile, since the presence of the biofilm (and the biomass of any cells which died while adhered to the surface) might impede either the release of biocide or its diffusion away from the surface.
4.4.3 Distribution of biocide susceptibilities

The distribution of MIC values for the microbes in the ocean (the immigrating microbes in the model) was assumed to follow a log-normal distribution. This assumption was based on more general measurements of microbial MIC distributions [397], there appears to be little-to-no investigation into biocide susceptibility distributions for specifically marine microorganisms. These distributions might also be expected to vary in different geographical regions or different bodies of water (e.g., estuaries compared to the open ocean).

This model suggests that biocide-resistant immigrants appear to be important in triggering the onset of biofouling. If the biocide-susceptibility distributions of marine organisms were known at different geographical locations, one may be able to identify particularly troublesome “hot spots” for biocide-resistant organisms along a ship’s planned route. It should also be noted that biocide-susceptibility is not the only trait relevant to biofilm formation on an AF surface. One such example of another potentially interesting trait to model could be that of a “stickiness”, or EPS production, trait. This could cause the detachment rate to vary with time or population density.

4.4.4 Biocide killing

To incorporate microbial growth and biocide killing, a pharmacodynamic function as proposed by Regoes et al. [332] was used to model the response of microbial populations to an applied biocide. This function is convenient as it allows for microbes to be characterised by a single number - their MIC value. All other parameters are assumed to be the same for all microbial species present. Furthermore, the pharmacodynamic function also uses a single parameter, $r_{\text{max}}$, to describe both the maximal growth rate and the maximal rate of biocide-mediated killing. While this may be true for cells such as *E. coli* exposed to cell-wall targeting antibiotics [327], in reality one would expect different marine microbial species to exhibit qualitatively different growth and death dynamics, both in the presence and absence of biocide. It would be of interest to measure growth and killing curves for marine organisms and incorporate this data into future computational models.

Additionally, biocide-susceptibility might well change when microbes transition
from the loosely-attached planktonic phase to the biofilm phase [417]. For the simulations presented here, this should not change the results significantly, since the biocide mostly plays a role in the first microhabitat, before the microbial population transitions to the biofilm phase. However, it may be an important factor in other parameter regimes. This model also currently does not include a fitness cost for biocide resistance, which may explain the strength of the observed priority effects - biocide-resistant organisms which establish early continue to dominate the biofilm in the later stages of growth, even far from the surface where the biocide concentration is low. An interesting investigation could be to incorporate a fitness cost for resistance, such as resistance lowering both death and growth rates, and determine whether this affects the overall species composition.

The fate of dead biomass could also be a relevant factor to consider in future work. Here, dead cells are simply removed from the system, implicitly freeing up space for new microbes due to the implementation of a carrying capacity term. Depending on whether or not the biocide causes lysis, dead microbes could remain within the biofilm or even provide structural elements such as DNA which could strengthen the mechanical properties of the biofilm. These factors would likely have a quantitative, but not qualitative, effect on the results.

4.4.5 Density-dependent transition to the biofilm state

A major assumption of this model is that the loosely-attached community at the surface transitions to biofilm in a density-dependent manner. This is intended to represent a quorum sensing mechanism, justified by observations that quorum sensing is implicated in biofilm initiation in a variety of microorganisms, as first discussed in Section 1.3. However, even if quorum sensing is indeed involved, it is not clear whether a collective transition to biofilm would occur when the total microbial density reaches a threshold value, or whether distinct microbial species would transition when their individual sub-populations reaches this threshold. Other factors, such as microbial surface sensing and motility on the surface prior to full attachment via the production of EPS, have also been ignored here [34].
4.4.6 Conclusion

These simulations raise several interesting implications for the design of AF paint. Firstly, they suggest that biofilm formation on an AF surface may in some cases be inherently unpredictable, since the underlying process of immigration of resistant microbes and their transition to the biofilm state, are stochastic. However, these simulations identify key parameters that can, on average, increase the time until a biofilm is able to be established. In particular, for the parameter regime studied here, the biocide concentration is a key factor, upon which $t_f$ depends exponentially. Furthermore, these simulations imply that the immigration of biocide-resistant organisms plays a key role in marine biofouling.

The model presented here is clearly a crude approximation, with the intention that it should be greatly improved upon as more information emerges on how microbes initiate biofilm formation. Nevertheless, it is hoped that this model raises interesting questions that may stimulate further investigation, in particular about the stochastic nature of biofilm initiation.
Chapter 5

The influence of drag-reducing riblets on biofilm sloughing

5.1 Introduction

The drag experienced by vessels in the shipping industry, and the associated economic and environmental costs, have been discussed in previous chapters. However, this drag is not caused solely by marine biofouling. Depending on the shape and size of the vessel, it is estimated that up to 90% of the total fuel consumption is spent overcoming the hydrodynamic drag caused by the interactions between the ship hull and the surrounding ocean [418]. Reducing this drag is therefore a high priority in the industry.

One approach is via the use of microstructures known as riblets, an example of which is shown in Figure 5.1(a). These riblets are sometimes referred to “artificial sharkskin” due to their resemblance to the grooved topography of shark scales, which can be seen in Figure 5.1(b). First proposed in the 1980s [422–425], the structure of these riblets typically consists of some form of triangular grooves embossed onto a surface. One riblet design of triangular-shaped riblets with trapezoidal grooves (the same geometry as used in the work presented below), applied in the form of a bonded film, obtained a maximal drag reduction of 8.2% [426]. A paint-based approach, which was intended for a more feasible and reproducible application process, utilising a similar riblet structure obtained a drag reduction of 5.2% [427].
The drag reducing properties of riblets are well-documented \[424, 428, 429\]. In turbulent flows, streamwise vortices which rotate along the streamwise axis are generated. Riblets serve to lower the drag experienced by a surface by causing these vortices to be “lifted off” the surface such that the vortices only interact with the riblet tips, rather than the surface as a whole, which reduces the effects of drag and shear stresses \[430\], see Figure 5.1(c-d).

The geometry of the riblet structure is key, with sharp tips being an important feature for riblet structures such as the triangular ones discussed above. Experimental work by Walsh \[431\] found that rounded riblet tips resulted in up to a 40% decrease in riblet performance. The riblets must also be small, typically on
the order of micrometres, as overly large riblets result in a drag increase, rather than reduction [432].

Riblet structures are only effective at reducing drag if their physical shape is unobstructed. This is particularly relevant when considering another source of drag in the shipping industry, as examined in Chapter 4 - marine biofouling. Marine biofouling involves the initial formation of a biofilm on the exterior of the ship hull submerged in the marine environment, which in turn facilitates the attachment of larger macrofoulers [179, 436, 437]. Both the marine biofilm and these macrofoulers contribute to the drag experienced by the vessel. This is especially concerning in the case of riblet-embossed surfaces, as a marine biofilm would envelope the riblets, removing their drag-reducing properties.

The project presented in this chapter aims to investigate the interplay between riblets and the removal of marine biofouling via fluid flow, *i.e.* sloughing. It examines how biofilm is sloughed from a riblet-coated surface and how the relative orientation of the riblets with respect to the flow influences the sloughing process. To this end, a computational fluid dynamics (CFD) model was developed, consisting of a riblet-embossed surface covered in a uniform layer of biofilm - represented as a viscous fluid. A flow was then applied to the system, originating at the top of the computational domain and oriented parallel to the surface. This was intended to emulate lid-driven flow, similar to what riblets would experience in a real-world scenario when applied to a ship’s hull. The total biofilm coverage on the riblet surface and the resulting drag were then tracked over time. Several flow angles relative to the alignment of the riblets were investigated in order to explore the effects of misaligned riblets, which can arise because of errors during the application process, deformation over time, or changing flow profiles as a result of increasing velocity [438, 440].

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1It has been observed that textured surfaces (*e.g.*, riblets) can inhibit the settlement of certain macrofoulers such as barnacles, which do not necessarily require a biofilm [438, 439]. It is thought that this is why sharks, which have textured skin, remain free of barnacles, while marine organisms with smoother skin, such as whales, do not [435].
5.2 Background theory

5.2.1 Governing equations

The system was modelled using the Navier Stokes equations for two fluids, representing biofilm and the surrounding water. The two fluids are assumed to be incompressible, immiscible, isothermal, Newtonian fluids, and are distinguished from one another via the use of a volume fraction field, \( \alpha \). This field has a value of 1 in regions of the domain where the fluid is purely biofilm, and a value of 0 for regions where the fluid is purely water. Intermediate values of \( \alpha \) occur only at the biofilm-water interface. The viscosity and density of each fluid depend on the value of \( \alpha \), discussed further in Section 5.2.2.

Presented here is a brief introduction to the Navier-Stokes equations, beginning with a single fluid for simplicity. In the case of a single fluid, the Navier-Stokes equations can be thought of as two conservation laws, conservation of momentum and conservation of mass.

**Conservation of mass**

![Figure 5.2: The continuity equation states that for an incompressible fluid, the rate of mass entering a volume is the same as the rate of mass exiting a volume of equal size. In this example, \( v_2 > v_1 \), as \( A_2 < A_1 \), and according to the continuity equation, \( A_1 v_1 = A_2 v_2 \). Image taken from APlusPhysics.](image)

Firstly, the conservation of mass, also referred to as the continuity equation,

\[
\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}) = 0, 
\] (5.1)
where $\rho$ is the density of the fluid, $u$ its velocity, and $t$ representing time. This equation states that the change in density over time is equal to the difference between the flux into and the flux out of the system, a diagram of the concept is shown in Figure 5.2. The divergence represents the sum of changes in density in each direction due to mass flowing in or out of that region (the majority of derivations done in this section are shown in 2D for brevity, the extension to 3D is straightforward)

$$
\rho \nabla \cdot u = \rho \frac{\partial u_x}{\partial x} + \rho \frac{\partial u_y}{\partial y}.
$$

(5.2)

To model a fluid as incompressible, its density is constrained to be constant in space and time, i.e.

$$
\frac{\partial \rho}{\partial t} = 0, \quad \nabla \rho = 0.
$$

(5.3)

Expanding out Equation (5.1) using the chain rule and incorporating the expressions from Equation (5.3) results in

$$
\frac{\partial \rho}{\partial t} + u \cdot \nabla \rho + \rho \nabla \cdot u = 0,
$$

(5.4)

which leads to the first of the Navier-Stokes equations, the famous incompressibility condition:

$$
\nabla \cdot u = 0.
$$

(5.5)

**Conservation of momentum**

The second of the Navier-Stokes equations represents the conservation of momentum, and can also be thought of as a manifestation of Newton’s Second Law. It is given by
\[\frac{D\rho}{Dt} = -\nabla p + \nabla \cdot \tau + F,\]  
with \(\frac{D\rho}{Dt}\) representing the material derivative, \(\nabla p\) the pressure gradient experienced by the system, \(\nabla \cdot \tau\) is the deviatoric tensor and \(F\) is a catch-all term representing the total external forces acting on the system. These terms are explained in further detail below.

The LHS of Equation (5.6) represents mass \(\times\) acceleration (mass and density are often considered to be interchangeable in fluid dynamics when dealing with incompressible fluids), and the RHS represents the total forces experienced by the fluid.

The material derivative is defined as

\[
\frac{D}{Dt} = \frac{\partial}{\partial t} + u \cdot \nabla.
\]

This incorporates the effects of an external velocity field on the rate of change of a field. In other words, the time derivative of a field which varies in both space and time. For example, consider a fluid flowing towards a nozzle. As the system narrows, the velocity of a fluid particle increases as it approaches the nozzle. But if only a single \((x, y)\) point in the system was observed, this change in acceleration would not be noticed, as the same particle is no longer being followed throughout.

To understand this mathematically, the time derivative of a field \(\phi\) can be written as\(^2\)

\[
\frac{d\phi(t, x, y)}{dt} = \frac{\partial \phi}{\partial t} + \left( \frac{\partial \phi}{\partial x} \right) \left( \frac{\partial x}{\partial t} \right) + \left( \frac{\partial \phi}{\partial y} \right) \left( \frac{\partial y}{\partial t} \right)
= \frac{\partial \phi}{\partial t} + u_x \left( \frac{\partial \phi}{\partial x} \right) + u_y \left( \frac{\partial \phi}{\partial y} \right)
= \frac{\partial \phi}{\partial t} + u \cdot \nabla \phi
\]

The \(\frac{\partial}{\partial t}\) term represents how the field of interest varies over time at a fixed point in

\(^2\)Again, in 2D for brevity.
space, and the additional \( \mathbf{u} \cdot \nabla \) term includes the variation of the field following a particle through space. In layman’s terms, considering only the time derivative is analogous to looking at a single point in a river, whereas the material derivative is more akin to following a floating twig as it travels downstream guided by the river’s current.

Moving now to the RHS of (5.6), the first two terms \((-\nabla p + \nabla \cdot \mathbf{\tau})\) represent the internal forces experienced by the fluid, such as particles bumping into or dragging on one another, while \(F\) represents a catch-all term for all external forces, \(e.g.,\) gravity.

An important internal force which the system experiences is stress. Stress describes the force acting on an object per unit area, \(i.e.\) the magnitude of a deforming force applied to a body, divided by the cross-sectional area (the area of one surface of a volume element which the external medium is applying the force to),

\[
\sigma = \frac{F}{A_0}. \tag{5.9}
\]

One must consider contributions to the stress in directions both parallel and perpendicular to the force-normal plane. This results in one stress component normal to the area (normal stresses, \(\sigma\)), and two stress components parallel to it (shear stresses, \(\tau\)). For a 3D material, the stress tensor must therefore have 9 components,

\[
\begin{bmatrix}
\sigma_{xx} & \tau_{xy} & \tau_{xz} \\
\tau_{yx} & \sigma_{yy} & \tau_{yz} \\
\tau_{zx} & \tau_{zy} & \sigma_{zz}
\end{bmatrix} . \tag{5.10}
\]

Here \(\sigma_{xx}\) is the stress in the \(\hat{x}\)-direction due to force in the \(\hat{x}\)-direction, and \(\tau_{xy}\) is the stress in the \(\hat{x}\)-direction due to force in the \(\hat{y}\)-direction.

The internal forces in the fluid are given by the Cauchy stress tensor, \(\sigma = -pI + \mathbf{\tau}\), with \(I\) representing the identity matrix. To find the total sum of the forces within the fluid, the divergence of the Cauchy stress tensor is taken, leading to the familiar expression \(\nabla \cdot \sigma = -\nabla p + \nabla \cdot \mathbf{\tau}\). Intuitively, the divergence of the stress tensor can be thought of in a similar manner to the continuity equation, Equation (5.1). Rather than being a source or sink of mass, here it is a source or
sink of momentum.

Returning now to Equation (5.6), the $\nabla \cdot \tau$ term is known as the stress deviator tensor. Its contribution to the motion of the fluid represents the friction and shear stresses due to viscosity. If one imagines a fluid as consisting of numerous thin sheets stacked on top of one another, as shown in Figure 5.3, viscosity is analogous to the coefficient of friction between the sheets that exchange momentum between regions moving at different velocities.

![Figure 5.3](image)

**Figure 5.3** If one imagines a fluid as material consisting of numerous thin sheets stacked on top of one another, then viscosity is the friction between these layers. Image taken from Quora [442].

In order for the Navier-Stokes equations to be implemented, an expression for $\tau$ must be assumed, which will depend on the type of fluid involved, which in this case is that of incompressible Newtonian fluids. By definition, for Newtonian fluids, the stress is proportional to the velocity gradient in the direction of the stress (rate of deformation). *i.e.*

$$\tau_{i,j} = \mu \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right), \tag{5.11}$$

where $\mu$ is a constant representing the viscosity. Examining now the divergence of the stress tensor using the expression in (5.11),

---

$^3$Again in 2D, for ease of notation.
\[ \nabla \cdot \tau = \mu \nabla \cdot (\nabla u + \nabla u^T) \]

\[ = \mu \nabla \cdot \left( \frac{\partial u}{\partial x} \left( \frac{\partial u_x}{\partial x} + \frac{\partial u_y}{\partial x} \right) \right) \]

(5.12)

Evaluating the \( \hat{x} \)-component of the above equation,

\[ (\nabla \cdot \tau)_x = \mu \left[ \frac{\partial}{\partial x} \left( 2 \frac{\partial u_x}{\partial x} + \frac{\partial u_x}{\partial y} \right) + \frac{\partial}{\partial x} \left( \frac{\partial u_x}{\partial x} + \frac{\partial u_y}{\partial x} \right) \right] \]

\[ = \mu \left[ \frac{\partial^2 u_x}{\partial x^2} + \frac{\partial^2 u_x}{\partial y^2} + \frac{\partial^2 u_y}{\partial x \partial y} \right] \]

\[ = \mu \left[ \frac{\partial^2 u_x}{\partial x^2} + \frac{\partial^2 u_x}{\partial y^2} + \frac{\partial}{\partial x} \left( \frac{\partial u_x}{\partial x} + \frac{\partial u_y}{\partial y} \right) \right] \]

(5.13)

where the last term in Equation (5.13) is equal to 0, from the incompressibility condition given by Equation (5.5). Repeating for the other Cartesian components results in:

\[ \nabla \cdot \tau = \mu \nabla^2 u. \]

(5.14)

This result can also be arrived at more succinctly by noting that \( \nabla \cdot (\nabla u) = \nabla^2 u \)

and \( \nabla \cdot (\nabla u)^T = \nabla (\nabla \cdot u) = 0. \)

This form of the stress tensor, \( \mu(\nabla u + \nabla u^T) \), is a somewhat heuristic model. It can be rationalised by considering that the stress is a function of the momentum transfer between the laminar layers, and depends only on the stratification of the flow profile, therefore making it proportional to the velocity gradient. However this transference of momentum must be symmetric, hence the inclusion of the transpose term.

The other internal forces term, \( \nabla p \), represents the pressure gradient within the
system, since particles tend to move from areas of high to low pressure. It should be noted that for incompressible fluids, where the pressure does not affect the density, the behaviour of the fluid depends only on the gradient of pressure, not on its actual numerical value. As seen later in the construction of the boundary conditions of the system, this allows for the outlet pressure to be set to the intuitively nonphysical value of 0. The $\nabla p$ term also incorporates the pressures arising from the fluid pressing on itself, and prevents the fluid from shrinking.

Combining the requisite equations from above leads to the second Navier-Stokes equation for an incompressible Newtonian fluid,

$$\rho \left( \frac{\partial u}{\partial t} + u \cdot \nabla u \right) = -\nabla p + \mu \nabla^2 u + F.$$  \hspace{1cm} (5.15)

### 5.2.2 Multiphase modelling using interFoam

The equations used in the model are found in the OpenFOAM solver *interFoam* (discussed further in Section 5.3.2). These equations are based on the Navier-Stokes equations mentioned above, modified to model two incompressible, isothermal, immiscible fluids.

The continuity equation, Equation (5.1), is unmodified in *interFoam*, while $F$, the external forces term in the momentum equation, Equation (5.15), has now been expanded upon to give

$$\rho \left( \frac{\partial u}{\partial t} + u \cdot \nabla u \right) = -\nabla p + \mu \nabla^2 u + F_\sigma,$$  \hspace{1cm} (5.16)

where $F_\sigma$ represents the surface tension between the two fluids. Gravitational forces were ignored. In order to model the flow and interaction of multiple fluids, or “phases”, the “volume of fluid” (VOF) method was utilised. This involves the introduction of a scalar field $\alpha$, which denotes the volume fraction of each fluid at each point in the system. As mentioned earlier, a value of $\alpha = 1$ indicates that the fluid is purely biofilm, whereas $\alpha = 0$ is purely water. The only region of the system where $\alpha$ takes an intermediate value is at the interface between the fluids.

The density also therefore varies in space, depending on the volume fraction,
\[
\rho(x) = \alpha(x)\rho_1 + (1 - \alpha(x))\rho_2,
\]  
(5.17)

where \( \rho_1 \) is the density of the biofilm and \( \rho_2 \) the density of the surrounding fluid. A similar expression is used for the fluids’ viscosity.

As mentioned above, \( F_\sigma \) represents the surface tension of the biofilm-water interface,

\[
F_\sigma = \sigma \kappa \nabla \alpha,
\]  
(5.18)

where \( \sigma \) is the surface tension constant and \( \kappa \) is the curvature of the interface, given by the divergence of the unit vector normal to the interface (\( \hat{n} \)) [443, 444]. It can be approximated as

\[
\kappa = -\nabla \cdot \hat{n} \approx -\nabla \cdot \left( \frac{\nabla \alpha}{|\nabla \alpha|} \right).
\]  
(5.19)

The surface tension is an important consideration in multiphase modelling. The physical origin of this term arises due to the imbalance of inter-molecular forces at the fluid-fluid interface. Inside a fluid, cohesive intermolecular forces balance, resulting in each molecule experiencing a net force of 0. However, at the interface, molecules of different types, which may not have cohesive interactions, are in contact. The resulting force imbalance causes the molecules to be pulled inward towards the centre of the fluid [445]. This results in an internal pressure in the fluid, so it configures itself to minimise the surface area of the interface, which explains why bubbles adopt a spherical shape.

In this work, the surface tension is modelled as a “continuum surface force” [444]. In this method of surface tension modelling, the fluid interface is defined as varying continuously over a finite “transition length” between the two phases. At each point in the fluid interface, a “force density” is calculated, which has a value proportional to the curvature of the surface at that point. This allows the surface tension to be interpreted as a continuum effect across the interface, rather than as a boundary value problem. This means that the transition from one fluid to another is now smooth and continuous, rather than discontinuous, resulting in smoother and more stable solutions.
Finally, the interface evolves via

\[ \frac{\partial \alpha}{\partial t} + u \cdot (\nabla \alpha) = 0, \quad (5.20) \]

which may be interpreted as the conservation of the volume fraction along the path of a fluid packet, much like the continuity equation conserves mass.

### 5.2.3 Couette flow

In most cases, it is not possible to solve the Navier-Stokes equations analytically. However, one instance which is both analytically solvable and relevant to this project is that of lid-driven Couette flow.

Consider a viscous incompressible fluid sandwiched between two parallel plates which are separated by a distance \( h \). The top plate moves at a constant relative velocity of \( \mathbf{u} = (u, v, w) = (V, 0, 0) \), \( \frac{\partial u}{\partial t} = 0 \) and there is no pressure gradient \( (\frac{\partial p}{\partial x} = 0) \).

From before, as shown in Equation (5.1), the continuity equation,

\[ \nabla \cdot \mathbf{u} = 0 \]
\[ \Rightarrow \frac{\partial u}{\partial x} = 0, \]

as \( \mathbf{u} = (V, 0, 0) \), this implies that \( u \) varies only in \( \hat{y} \), \( u = u(y) \).

Due to the velocity having only a non-zero \( \hat{x} \) component, solely this component of the momentum equation, Equation (5.15) needs to be considered,

\[ \frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} = -\frac{1}{\rho} \frac{\partial p}{\partial x} + \mu \left( \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right), \]

which reduces to

\[ \frac{\partial^2 u}{\partial y^2} = 0 \Rightarrow u = c_1 y + c_2. \]
Including the boundary conditions \( u(y = h) = V \) and \( u(y = 0) = 0 \), produces the Couette flow velocity profile:

\[
 u = \frac{V}{h} y. \tag{5.21}
\]

Attention is drawn to this particular flow profile, as it is the one used in the model presented in this chapter, with the intention to emulate the experimental setup of the Couette flow cylinder used by Benschop et al. Couette flow cylinders consist of two concentric cylinders, with a viscous fluid between the exterior of the inner cylinder and the interior of the outer cylinder. By rotating the cylinders in opposing directions, a velocity gradient is established, resulting in the emergence of a flow profile.

As such, flow across the simulated riblets arises due to lid-driven Couette flow. The flow angle relative to the riblets can be adjusted to model misaligned riblets. For example, flow parallel to the riblets has a flow angle of 0°, whereas severely misaligned riblets experience flow perpendicular to their alignment, i.e. a flow angle of 90°. These flow angles are illustrated later in Figure 5.10.

### 5.2.4 Drag calculation

Drag is typically defined as the force acting in the opposite direction to the relative motion of any object moving with respect to a surrounding fluid. As discussed above in Section 5.1, the riblets’ primary function is to reduce the hydrodynamic drag experienced by the vessel which they are applied to. In the context of the simulations presented in this chapter, computing the drag on the riblet surface is decidedly non-trivial due to the contributions of both the riblet surface itself and also the moving biofilm surface. As such, the following method to compute the total drag on the riblet surface, taken from Batchelor, 2000, was suggested by our collaborator on this project, Professor Nick Cogan.

In general, the drag experienced by a system on a surface \( \Gamma \) is defined as \( \int_{\Gamma} \sigma \cdot \eta \, ds \), where \( \Gamma \) is a surface, as shown in Figure 5.4, and \( \eta \) is the outward normal of that surface. \( \sigma \) is the Cauchy rate of strain tensor and as discussed above in Section 5.2.1 has the form
Figure 5.4 Diagram highlighting the domain components used in the drag calculations here, flow is from left to right in this instance. This diagram represents a 2D scenario, the extension to 3D is straightforward. The entire domain is represented by $\Omega$, surfaces denoted by $\Gamma$. $\Gamma_r$ is the riblet surface, and $\Gamma_b$ is the biofilm-water interface.

$$\sigma = \mu(\nabla u + \nabla u^T) - pI,$$

(5.22)

where $\mu$ represents the dynamic viscosity of the fluid, $p$ the pressure and $I$ the identity matrix.

For a simple example, consider a single creeping fluid flowing over an object. Creeping flow, also known as Stokes flow, is a type of fluid flow where viscous forces dominate over advective inertial forces \[448\], i.e. the Reynolds number is low ($\text{Re} \ll 1$). This is typical in scenarios with high viscosities, low fluid velocities, or over very small lengthscales. In the case of Stokes flow,

$$\nabla \cdot u = 0$$

$$\nabla \cdot \sigma = 0,$$

Again in 2D for brevity (this method is extended to 3D when used to calculate the drag on the simulated 3D systems), integrating $\nabla \cdot \sigma$ over the entire domain $\Omega$, and using the divergence theorem results in
\[ 0 = \int_\Omega \nabla \cdot \sigma \, dv = \int_\Gamma \sigma \cdot \eta \, ds \]
\[ = \int_{\Gamma_r} \sigma \cdot \eta \, ds + \int_{\Gamma_b} \sigma \cdot \eta \, ds \]
\[ + \int_{\Gamma_{p1}} \sigma \cdot \eta \, ds + \int_{\Gamma_{p2}} \sigma \cdot \eta \, ds \]
\[ + \int_{\Gamma_{p3}} \sigma \cdot \eta \, ds, \]  
\hspace{1cm} (5.23)

where \( \Gamma_r \) is the riblet surface, \( \Gamma_b \) is the fluid-fluid interface and \( \Gamma_{p1..3} \) are the domain boundaries. \( \Gamma = \Gamma_r + \Gamma_b + \Gamma_{p1..3} \), see Figure 5.4. Therefore the force of interest here, \( i.e. \) the total drag force acting on both the riblet surface and the creeping fluid interface, \( F \) is given by

\[ F = \int_{\Gamma_r} \sigma \cdot \eta \, ds + \int_{\Gamma_b} \sigma \cdot \eta \, ds = -\int_{\Gamma_{p1}} \sigma \cdot \eta \, ds - \int_{\Gamma_{p2}} \sigma \cdot \eta \, ds - \int_{\Gamma_{p3}} \sigma \cdot \eta \, ds. \]  
\hspace{1cm} (5.24)

However, the riblets and biofilm are experiencing Navier-Stokes flow, rather than Stokes flow. So if the corresponding conservations,

\[ \nabla \cdot \vec{u} = 0 \]
\[ \frac{\partial \vec{u}}{\partial t} + (\vec{u} \cdot \nabla)\vec{u} = \nabla \cdot \sigma, \]

are inserted into Equation (5.23), this leads to
\[ 0 = \int_{\Omega} \frac{\partial u}{\partial t} + (u \cdot \nabla)u - \nabla \cdot \sigma \, dV = \int_{\Omega} \frac{\partial u}{\partial t} + (u \cdot \nabla)u \, dV - \int_{\Gamma} \sigma \cdot \eta \, ds \]

\[ = \int_{\Omega} \frac{\partial u}{\partial t} + (u \cdot \nabla)u \, dV - \int_{\Gamma_r} \sigma \cdot \eta \, ds - \int_{\Gamma_b} \sigma \cdot \eta \, ds - \int_{\Gamma_{p1}} \sigma \cdot \eta \, ds - \int_{\Gamma_{p2}} \sigma \cdot \eta \, ds - \int_{\Gamma_{p3}} \sigma \cdot \eta \, ds. \]

(5.25)

The resultant drag vector experienced by the riblet surface is therefore

\[ F = \int_{\Gamma_r} \sigma \cdot \eta \, ds + \int_{\Gamma_b} \sigma \cdot \eta \, ds = \int_{\Omega} \frac{\partial u}{\partial t} + (u \cdot \nabla)u \, dV - \int_{\Gamma_{p1}} \sigma \cdot \eta \, ds - \int_{\Gamma_{p2}} \sigma \cdot \eta \, ds - \int_{\Gamma_{p3}} \sigma \cdot \eta \, ds, \]

(5.26)

and the total drag on the system, i.e. on the riblet surface and on the biofilm itself, is then given as the magnitude of this force vector, \(|F|\).

### 5.3 Methodology

#### 5.3.1 System geometry

The general system geometry was based on the setup of previous experimental work undertaken by Benschop et al., 2018 [446], which compared the sloughing of biofilms on smooth and ribletted surfaces. The computational domain used here consisted of a surface with 4 riblets, enclosed in a volume measuring 577 µm × 200 µm × 577 µm (X × Y × Z). The size and shape of the riblets were kept constant throughout all simulations, and the specific dimensions were based on previous work which investigated the optimal design for a drag reducing riblet [449, 450], which were also the dimensions used by Benschop et al., 2018 [446].
The system is initialised with a layer of biofilm covering the riblet surface, an example of which is shown in Figure 5.5. The biofilm is modelled as a viscous Newtonian fluid, with a density equal to that of water, but with a viscosity $10^3$ times greater. Bacterial growth in the biofilm is neglected due to the large difference in timescales, with the duration of the simulations being on the order of seconds, while bacterial growth occurs on timescales of hours or days.

![Figure 5.5](image)

Figure 5.5  (a) Overview of the riblet system. (b) The initial configuration of the biofilm deposit. The image shows a biofilm with initial thickness $28 \mu m$.

### 5.3.2 OpenFOAM

To simulate the model, the computational fluid dynamics software package OpenFOAM (Open Source Field Operation and Manipulation) [451] was utilised. It uses finite volume methods [452] to simulate systems of partial differential equations (PDEs), by discretising the computational domain into sub-volumes known as “cells” and integrating the system of PDEs over this cell volume.

For terms without spatial derivatives, the integral provides an average value associated with the cell centre, and terms that do contain spatial derivatives can be converted into an equivalent flux term using the divergence theorem. The fluxes can then be computed by interpolating values from the surrounding cell centres. The advantage of finite volume methods is that the cells can be whatever shape is required, allowing for the modelling of complex system geometries.

Practically speaking, OpenFOAM is a collection of C++ libraries, which uses a text-based interface in the form of several dictionary files to set up and construct models, referred to as “cases”. The initial file structure of an OpenFOAM case consists of three directories;

- **system**, which contains information such as the run time of the simulation, sampling intervals, system geometry parameters, numerical algorithm choices, methods for parallel decomposition and suchlike.
• constant, which contains files that dictate the physical properties of the system, such as the densities/viscosities of the fluids involved, turbulence properties and values for other external fields, such as gravity.

• 0, which contains the boundary and initial conditions of the vector/scalar fields involved in the simulation. In this case it contains the velocity field $U$, the pressure $p_{rgh}$ and the volume fraction $\alpha_{biofilm}$, which dictates whether a region of fluid is either biofilm or water.

The dynamics of the system being simulated are governed by the appropriate choice of “solver”, which is specified in system/controlDict. As the model here is a multiphase flow of two incompressible, isothermal, immiscible fluids, the solver interFoam was used, as discussed earlier in Section 5.2.2.

5.3.3 Geometry creation via blockMesh

Generally, the first step in the setup of an OpenFOAM model is the creation of the system geometry, including the finite volume cell mesh. The cell mesh is the discretisation of the system into the “finite volumes” which are used to solve the equations describing the dynamics of the system. There are numerous ways to do this, depending on the complexity of the geometry being modelled. For basic geometries such as the prism riblets presented in this chapter, the built-in OpenFOAM utility blockMesh is sufficient.

All of the information required for blockMesh to create the system geometry and cell mesh is contained within the system/blockMeshDict file. The general methodology for constructing a geometry via blockMesh is as follows.

1. Define the vertices of the system. These are contained in a vertices list in the format ($x$ $y$ $z$). The order in which they are defined is also their index, which will become important later on.

2. Divide the total cell mesh in the system into sub-sections called “blocks”. The number, positioning and shape of these blocks depends on the system geometry being modelled, the most basic system consists of one block representing the entire computational domain. The configuration of the blocks used here to create the riblet system is shown in Figure 5.6.
Figure 5.6  Sketch of the vertices layout used to generate the riblet system via blockMesh. The pictured pattern of points can be tessellated to create systems of \( N \) riblets. The indices \( n_b \), \( n_m \) and \( n_t \) refer to the number of vertices in the bottom, middle and top rows respectively, and depend on the number of riblets in the system. \( n_b = 6N - (N - 1) \), \( n_m = 2n_b - N \), \( n_t = 3n_b - 2N \). The solid lines represent the boundary of the riblet surface, the dashed lines are the blocks used to construct the mesh. This configuration is repeated further along the \( \hat{z} \)-axis (into the page) to generate the back face of the system.

Typically blockMesh only uses hexahedra for its blocks. Other shapes can be constructed by collapsing vertices on top of one another, but it is best to avoid doing this if possible, since this technique can produce errors when refining the mesh at later stages, due to the fact that OpenFOAM can only automatically refine cells which are also hexahedra. Blocks are defined with the following syntax:

```
hex (v_0 v_1 v_2 v_3 v_4 v_5 v_6 v_7) (n_x n_y n_z) simpleGrading (g_x g_y g_z).
```

Here \( v_0 \)…\( v_7 \) represent the vertices. The order in which they are declared here is important as this defines the orientation of the local coordinate system, which is used to arrange the number and grading of the cells in the block. The line from \( v_0 \) to \( v_1 \) defines the \( \hat{x} \) axis, then from \( v_1 \) to \( v_2 \) defines the \( \hat{y} \) axis. Once one side of the hexahedron has been defined in the \( \hat{x} - \hat{y} \) plane, moving to the next plane beginning with \( v_4 \) defines the \( \hat{z} \) axis, as shown in Figure 5.7.

\( n_x \) represents the number of cells in each direction in the blocks, based on the local coordinate system, and \( g_x \) represents the expansion ratios. For cells at the end of the block to be twice the width of the cells at the start of the block, \( g_x \) would be set to 2, for example. To generate cells of uniform size, these values are set to 1.

3. Block faces which boundary conditions are applied to are referred to as
“patches” in OpenFOAM. These patches must be listed in the boundary list, where the block faces comprising each boundary must be named and grouped together. e.g., all the patches in the left wall, all the patches in the outlet, floor etc.

These faces are defined anticlockwise to the face normal vector pointing out of the system. For example, in the block shown in Figure 5.7 in order to define the roof boundary, one must create an entry roof and insert the vertices list (4 5 6 7). The initial vertex used does not matter so long as they are in the correct order. Thus, (7 4 5 6) is also a valid permutation.

![Diagram of a block generated via blockMesh](image.png)

**Figure 5.7** Overview of a block generated via blockMesh. The ordering of the indices in the block definition dictates the local co-ordinate system of the block. Starting from index 0, moving anticlockwise around the hexahedron, with respect to the outward-pointing face normal vector, defines the $\hat{x} - \hat{y}$ plane. Defining the face above the first one in a similar manner then defines the $\hat{z}$ axis. $\hat{x}$, $\hat{y}$ and $\hat{z}$ are represented by $\hat{x}_1$, $\hat{x}_2$ and $\hat{x}_3$ respectively in the diagram. The models presented in this chapter are orientated such that the $\hat{z}$-axis is pointing into the page. Image taken from OpenFOAM [453].

A section of the mesh used in the simulations is shown in Figure 5.8(a). The mesh was configured such that it was more refined, meaning the cells were smaller, close to the riblet peaks, and coarser towards the top of the computational domain and in the troughs between riblets. This was done in an attempt to maximise both computational efficiency and accurately representing the flow profile across the riblets.

Furthermore, to better track the changing topography of the biofilm-water interface, dynamic mesh refinement was implemented. i.e. at each timestep, cells which had a value of $\alpha$ between 0.001 and 0.999 were halved in size, as were a
buffer layer of 2 cells around them. An example of this is shown in Figure 5.8(c). Cells were halved in size from their original size twice at most, and the mesh could only be refined up to a limit of 800,000 cells.

The timestep was also dynamically resized in order to keep the Courant number below 0.95. The Courant number $C$ is a dimensionless value which essentially represents the duration a particle, or fluid packet, stays in one cell in the mesh. It is given by $C = \frac{u \Delta t}{\Delta x}$, where $u$ is the magnitude of the velocity in the cell, $\Delta t$ is the size of the time step and $\Delta x$ is the length of a mesh element. If $C > 1$, then the particle can “skip” mesh elements between timesteps, creating instabilities in the simulation.
An initial biofilm deposit of 28 µm is shown in Figure 5.8(b). It should be noted that the exact thickness of the initial deposit is dependent on the arrangement of the cells - a cell cannot be “half-filled” with biofilm, they are initialised with values of α = 0 or 1. This means that the true biofilm thickness depends on the \( \hat{y} \)-position of the roof of the highest cell with \( \alpha = 1 \).

### 5.3.4 Boundary conditions

When defining the groups of boundary block faces (patches) in `blockMeshDict`, one must also specify the patch type. The type of patch dictates the types of boundary conditions that can be used on that patch, as well as providing additional information to the solvers about the system. The most common patch types, which are also the ones used in this project, are

- **patch** - generic type containing no geometric or topological info about the mesh, *e.g.*, an inlet or outlet.

- **wall** - used for patches which coincide with a solid wall, required for some physical modelling, especially when turbulence is involved.

- **empty** - for solutions in 2D/1D, this type is used on the patch whose plane is normal to the dimension for which no solution is required. *e.g.*, for modelling 2D, one would set the patches normal to the \( \hat{z} \)-axis (the ones facing the viewer) to be empty. Any patches which are not defined in the `boundary` list are automatically designated as this type, including the internal faces of intersecting blocks.

Here the boundary conditions for the fields used in the OpenFOAM simulations, velocity \( \mathbf{u} \), pressure \( p \), and biofilm volume fraction \( \alpha \), are discussed in detail. The boundary conditions were varied between simulations, with either the rear, right, or both walls acting as the outlet of the system, depending on the flow angle being simulated. This is illustrated further in Figure 5.9.

**Velocity: U**

Four different boundary conditions were used on different surfaces for the velocity field, \( \mathbf{u} \), as detailed in Table 5.1. For the riblet surface, denoted by `lowerWall`
in the OpenFOAM files, the no-slip boundary condition noSlip was used. This boundary condition is a common approximation used in fluid dynamics models, which assumes that any fluid in contact with a solid boundary has zero velocity relative to the boundary. As the fluid velocity has a prescribed value at the boundary, this is an example of a Dirichlet boundary condition. The physical origin of the no-slip boundary arises from the assumption that at the solid boundary, the adhesive forces between the fluid and the boundary are stronger than the cohesive forces between the fluid molecules. As such, the fluid at the boundary is not dragged along the surface by the bulk of the fluid. This is of course an approximation, and the no-slip condition is rarely completely true in real-world scenarios [454].

In order to emulate lid driven Couette flow, as used in the experimental work of Benschop et al. [446] and discussed previously in section [5.2.3] the upper wall (upperWall) of the system was subject to the boundary condition fixedValue, referring to a constant velocity vector field. To model differing flow angles, the $\hat{x}$ and $\hat{z}$ components of this vector were adjusted accordingly. For flow parallel to
the riblets, the flow was purely in the \( \hat{z} \)-direction, while perpendicular flow was purely in the \( \hat{x} \)-direction.

The setup of the Couette cylinder used by Benschop et al. involved a cylinder separation of 1 cm and a shear velocity between the two cylinder plates of 9 ms\(^{-1}\). Assuming a linear velocity gradient, and a velocity of 0 on the cylinder surface (no-slip condition), this results in a simulated lid velocity of magnitude

\[
\frac{u_{\text{max}}}{y_{\text{sim}}} = \frac{u_{\text{exp}}}{y_{\text{exp}}} \Rightarrow u_{\text{max}} = \frac{y_{\text{sim}} u_{\text{exp}}}{y_{\text{exp}}}
\]

\[
\frac{u_{\text{max}}}{200 \times 10^{-6}} = 1 \times 10^{-2} \times 9 \, \text{ms}^{-1} = 0.18 \, \text{ms}^{-1}.
\]

(5.27)

The outlet walls were subject to the boundary condition \texttt{inletOutlet}. This is a special boundary condition which varies depending on the direction of the flow incident on the patch. For flows which point out of the system, the \texttt{zeroGradient} condition is applied to them, which states that the gradient of the field normal to the wall is equal to zero. \texttt{zeroGradient} is an example of a Neumann boundary condition, where the derivative of the solution has a prescribed value, rather than the solution itself. This effectively says that the flow is fully developed at the boundary, and the presence of the boundary therefore does not affect it. Any flow velocity vectors which are redirected back into the system through the \texttt{inletOutlet} patch have a fixed velocity of \( U = 0 \) applied to them in order to prevent backwash.

The remaining walls were subject to the \texttt{zeroGradient} boundary condition. The choice of which walls were designated as outlets depended on the flow angle. For parallel flows, the patch \texttt{backWall} was chosen, while for perpendicular flow, \texttt{rightWall} was chosen. For off-angle flows, both \texttt{backWall} and \texttt{rightWall} were designated as outlets.
Table 5.1  *Velocity boundary conditions.*

<table>
<thead>
<tr>
<th>Boundary name</th>
<th>Boundary condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>frontWall</td>
<td>zeroGradient</td>
<td>Gradient of the field is 0 in the direction perpendicular to the boundary.</td>
</tr>
<tr>
<td>backWall</td>
<td>inletOutlet/zeroGradient</td>
<td>B.C. depends on flow direction being simulated. inletOutlet has the same conditions as zeroGradient for flow leaving the system but sets the velocity to 0 for flow entering the system (backwash).</td>
</tr>
<tr>
<td>rightWall</td>
<td>zeroGradient/inletOutlet</td>
<td>B.C. depends on flow direction being simulated.</td>
</tr>
<tr>
<td>leftWall</td>
<td>zeroGradient</td>
<td></td>
</tr>
<tr>
<td>upperWall</td>
<td>fixedValue</td>
<td>The upper wall has a constant uniform value for the velocity, to represent the lid-driven flow found in a Couette cylinder.</td>
</tr>
<tr>
<td>lowerWall</td>
<td>noSlip</td>
<td>Velocity is 0 at the boundary.</td>
</tr>
</tbody>
</table>

**Pressure: $p_{rgh}$**

By default, *interFoam* uses the dynamic pressure

$$p_{rgh} = p - \rho gh, \quad (5.28)$$

as its internal variable, where $g$ is the acceleration due to gravity and $h$ is the value of the $\hat{y}$-coordinate at that point. However, as $g$ is set to 0 throughout these simulations, $p_{rgh}$ reduces to the static pressure $p$.

The pressure boundary conditions used in this model were somewhat more straightforward than the velocity boundary conditions. A general approach for boundary conditions in “pipe-like” fluid dynamics systems is to specify the
velocity at one end and the pressure at the other, thereby creating a boundary-value problem rather than just an initial-value problem.

This argument is based on Bernoulli’s principle, which states that for incompressible flow, the energy per unit volume remains constant. Therefore, the energy per unit volume at the inlet is the same as the outlet. In terms of velocity and pressure,

\[ p_i + \rho v_i^2 = p_o + \rho v_o^2, \]  

(5.29)

where the subscripts \( i \) and \( o \) denote “inlet” and “outlet” respectively. Therefore, by specifying \( v_i \) and \( p_o \), the system can “fill in the blanks” in order to compute \( v_o \) and \( p_i \).

The pressure boundary conditions are listed in Table 5.2. For simplicity, a common outlet pressure boundary condition, and the one used here, is to set the outlet pressure to the nonphysical value of 0 Pa. As discussed above, for incompressible flows it is only the pressure gradient \( \nabla p \) which contributes to the dynamics, rather than the absolute value, so the choice of absolute pressure at the outlet is arbitrary.

The pressure boundary conditions for the remaining surfaces - the riblet surface, side walls, front and upper wall, were set to zeroGradient, and similarly to the velocity boundary conditions, were varied depending on the flow angle. Unfortunately, it was only realised towards the end of the project that for off-angle flows such as 45°, where both the rear and a side wall were set to outlets, that this interfered with the pressure contribution of the drag calculation, affecting the comparability of results between simulations with 1 or 2 outlets. This is discussed in greater detail in Section 5.4.5.
Table 5.2  Pressure boundary conditions.

<table>
<thead>
<tr>
<th>Boundary name</th>
<th>Boundary condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>frontWall</td>
<td>zeroGradient</td>
<td>Gradient of the field is 0 in the direction perpendicular to the boundary.</td>
</tr>
<tr>
<td>backWall</td>
<td>fixedValue/zeroGradient</td>
<td>B.C. depends on flow direction being simulated. For outlet, the pressure is set to 0.</td>
</tr>
<tr>
<td>rightWall</td>
<td>zeroGradient/fixedValue</td>
<td>B.C. depends on flow direction being simulated.</td>
</tr>
<tr>
<td>leftWall</td>
<td>zeroGradient</td>
<td></td>
</tr>
<tr>
<td>upperWall</td>
<td>zeroGradient</td>
<td></td>
</tr>
<tr>
<td>lowerWall</td>
<td>zeroGradient</td>
<td></td>
</tr>
</tbody>
</table>

**Volume fraction: alpha.biofilm**

Finally, as this is a multiphase flow system, it is necessary to specify boundary conditions for the volume fraction $\alpha$, which is denoted in OpenFOAM by `alpha.biofilm`. This field takes values between 0 and 1, with 1 signifying a region which is purely biofilm, 0 purely water. Its boundary conditions are summarised in Table 5.3.

The interaction between the riblet surface and the biofilm was mediated via the contact angle of the surface, which was set via the boundary condition `constantAlphaContactAngle`. Varying this parameter serves to increase or decrease the surface’s wettability. The contact angle of the riblet surface is an important factor in industrial applications, as it affects the adhesion of biofilm to the surface. Previous experimental work has been undertaken to investigate the performance of hydrophobic riblets [455–457].

The remaining boundary conditions for $\alpha$ were set to `inletOutlet` with an inlet value of 0. Much like the outlet velocity boundary condition, this allowed the biofilm to leave the system via any of the boundaries, but also prevented any backwash, *i.e.* any biofilm which leaves the system cannot re-enter it.
Table 5.3  Biofilm phase fractions boundary conditions.

<table>
<thead>
<tr>
<th>Boundary name</th>
<th>Boundary condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>frontWall</td>
<td>inletOutlet</td>
<td>Gradient of the field is 0 in the direction perpendicular to the boundary.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The value for $\alpha$ reentering the system is set to 0 to prevent backwash.</td>
</tr>
<tr>
<td>backWall</td>
<td>inletOutlet</td>
<td></td>
</tr>
<tr>
<td>rightWall</td>
<td>inletOutlet</td>
<td></td>
</tr>
<tr>
<td>leftWall</td>
<td>inletOutlet</td>
<td></td>
</tr>
<tr>
<td>upperWall</td>
<td>inletOutlet</td>
<td></td>
</tr>
<tr>
<td>lowerWall</td>
<td>constantAlphaContactAngle</td>
<td>The value of the contact angle between the biofilm interface and the riblet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surface is kept at a constant value of 90°.</td>
</tr>
</tbody>
</table>

5.3.5 Parameters

The system parameters are summarised in Table 5.4. As discussed above in Section 5.3.1, the geometry of the riblets was taken from previous experimental work by Benschop et al. [446], as was the value of the lid velocity $u_{\text{max}}$. The contact angle of the riblet surface was set to 90° in order to minimise interactions between the biofilm and the riblet surface.

Due to the large variance in the literature values for the mechanical properties of biofilms [458, 459], the biofilm modelled here was approximated to have density equal to that of water, but with a kinematic viscosity $10^3$ times greater. The initial biofilm thicknesses were chosen to be the equivalent of $\frac{1}{3}$ or $\frac{2}{3}$ the height of the riblets, or to completely envelop them.
Table 5.4  System parameters.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$h$</td>
<td>riblet height</td>
<td>42 $\mu$m</td>
</tr>
<tr>
<td>$s$</td>
<td>riblet spacing</td>
<td>92 $\mu$m</td>
</tr>
<tr>
<td>$\theta$</td>
<td>riblet tip angle</td>
<td>42°</td>
</tr>
<tr>
<td>$N$</td>
<td>no. of riblets</td>
<td>4</td>
</tr>
<tr>
<td>$W$</td>
<td>domain width</td>
<td>577 $\mu$m</td>
</tr>
<tr>
<td>$H$</td>
<td>domain height</td>
<td>200 $\mu$m</td>
</tr>
<tr>
<td>$Z$</td>
<td>domain depth</td>
<td>577 $\mu$m</td>
</tr>
<tr>
<td>$u_{\text{max}}$</td>
<td>lid velocity</td>
<td>0.18 m s$^{-1}$</td>
</tr>
<tr>
<td>$p_{\text{out}}$</td>
<td>outlet pressure</td>
<td>0 Pa</td>
</tr>
<tr>
<td>$\nu_b$</td>
<td>biofilm kinematic viscosity</td>
<td>$10^{-3}$ m$^2$s$^{-1}$</td>
</tr>
<tr>
<td>$\rho_b$</td>
<td>biofilm density</td>
<td>$10^3$ kg m$^{-3}$</td>
</tr>
<tr>
<td>$\nu_w$</td>
<td>water kinematic viscosity</td>
<td>$10^{-6}$ m$^2$s$^{-1}$</td>
</tr>
<tr>
<td>$\rho_w$</td>
<td>water density</td>
<td>$10^3$ kg m$^{-3}$</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>surface tension</td>
<td>$10^{-3}$ N m$^{-1}$</td>
</tr>
<tr>
<td>$\theta_c$</td>
<td>riblet surface contact angle</td>
<td>90°</td>
</tr>
<tr>
<td>$h_{\text{bf1}}$</td>
<td>biofilm thickness (thin)</td>
<td>14 $\mu$m</td>
</tr>
<tr>
<td>$h_{\text{bf2}}$</td>
<td>biofilm thickness (medium)</td>
<td>28 $\mu$m</td>
</tr>
<tr>
<td>$h_{\text{bf3}}$</td>
<td>biofilm thickness (thick)</td>
<td>50 $\mu$m</td>
</tr>
</tbody>
</table>

5.3.6  Post-processing

OpenFOAM

Once the simulations were completed, the raw data then had to be post-processed in order to compute the additional $\frac{\partial \phi}{\partial t}$ and $\nabla \cdot \mathbf{u}$ terms required to compute the drag, as described by Equation (5.26). OpenFOAM has several built-in command line post-processing tools which are relevant to this project, and it also comes bundled with the GUI program ParaView, which allows for the visualisation and post-processing of OpenFOAM data.

$\frac{\partial \phi}{\partial t}$ was calculated using central finite-difference methods via an in-house python script which saved the calculated field in the same format as the other OpenFOAM vector fields, allowing for later importation into ParaView. In order to use finite-difference methods, the dynamically refined mesh - which varied between timesteps - had to be interpolated onto a new mesh which remained
constant in time. This was achieved using the built-in OpenFOAM utility mapFields. The interpolated mesh was constructed such that it was more refined than the original mesh, in an attempt to capture some of the benefits of the dynamic refinement, as shown in Figure 5.8.

This interpolated mesh was also used as the basis for the velocity gradient $\nabla u$ calculation, which was computed using the OpenFOAM post-processing command

$$\texttt{postProcess -func \"grad(U)\"}.$$  

Once these additional fields had been created, they were then imported into the GUI program ParaView for further processing.

NB: the velocity vector field is denoted by $U$ internally in OpenFOAM. To maintain parity with the notation used elsewhere in this chapter, $u$ is used instead of $U$ when discussing equations involving the velocity.

**ParaView**

To compute the volume and surface integrals for the drag calculation in Equation (5.26), ParaView was used to compute the cell volumes, areas, and surface normals. For the volume integral, the cell volumes were extracted via the Cell Size filter. The output of this filter was a .csv file for each timestep which contained the values of each required field ($u$, $p$, $\frac{\partial u}{\partial t}$ and $\nabla u$) in each cell, along with the corresponding cells’ volume.

To select the relevant boundaries for the surface integral, i.e. all but the riblet surface, the Extract Blocks filter was used. The Cell Size filter was then used in order to calculate the cells’ area. Finally, the cell normals were calculated via the Generate Surface Normals filter.

### 5.3.7 Computation of drag integrals

The total drag experienced by the system, i.e. the sum of the drag on both the riblet surface and the biofilm surface, is given by the sum of the volume and surface integrals as shown in Equation (5.26). The methods by which these integrals were computed are discussed in detail below.
Volume integral

By testing the software using Poiseuille flow simulations (see Appendix A.1), the layout of the $\nabla u$ matrix generated by the OpenFOAM post-processing utility grad($u$) was deduced to be

$$
\nabla u = \begin{bmatrix}
\frac{\partial u}{\partial x} & \frac{\partial v}{\partial x} & \frac{\partial w}{\partial x} \\
\frac{\partial u}{\partial y} & \frac{\partial v}{\partial y} & \frac{\partial w}{\partial y} \\
\frac{\partial u}{\partial z} & \frac{\partial v}{\partial z} & \frac{\partial w}{\partial z}
\end{bmatrix} = \begin{bmatrix}
\nabla U_0 & \nabla U_1 & \nabla U_2 \\
\nabla U_3 & \nabla U_4 & \nabla U_5 \\
\nabla U_6 & \nabla U_7 & \nabla U_8
\end{bmatrix}.
$$

(5.30)

$\nabla U_i$ is the $i$th component of the grad($u$) array.

In order to calculate the integrand, the identity

$$
(u \cdot \nabla)u = \begin{bmatrix}
u u_x + v u_y + w u_z \\
u v_x + v v_y + w v_z \\
u w_x + v w_y + w w_z
\end{bmatrix},
$$

(5.31)

is required. Here $u$, $v$, $w$ are the $\hat{x}$, $\hat{y}$, $\hat{z}$ components of $u$, and $u_x$ is shorthand for $\frac{\partial u}{\partial x}$.

The RHS of Equation (5.31) was obtained by taking the transpose of the $\nabla u$ matrix, then multiplying the columns of this matrix by the respective entry in $u$; i.e. multiplying the first column by $u$, the second by $v$ etc. If $u$ is in a standard NumPy array $U$, and $\nabla u$ is in a 2D NumPy array gradU then the resulting matrix $r$ is given by

$$
r = U \ast \text{gradU.T}
$$

The rows of this matrix are then summed to get the desired vector:

$$
U_{\text{gradU}} = \text{np.sum}(r, \text{axis}=1)
$$

The volume integral is then calculated by taking the quantity described in Equation (5.31), adding it to the corresponding cell value of $\frac{\partial u}{\partial t}$, scaling it by the
cell volume and then summing this resultant value over all cells in the domain. However, as the numerical value of the volume integral was several orders of magnitude smaller than the contributions of the surface integrals, and there were significant computational costs associated with its calculation, the contributions of the volume integral were ultimately omitted from the final drag calculations.

**Surface integral**

For the surface integrals in Equation (5.26), $\sigma$ as described by Equation (5.22), is calculated via

\[
sigma = \mu*(\text{grad}U + \text{grad}U.T) - p*\text{np.identity}(3)
\]

and the integrand via

\[
\text{integrand} = -(\sigma@\text{norm}_v)\text{area},
\]

where $\text{norm}_v$ is the cell normal vector and $\text{area}$ is the area of the cell. As before, these are scaled and summed together in order to obtain the surface integral.

### 5.3.8 Biofilm sloughing

Biofilm sloughing was simply quantified by monitoring the total volume of biofilm present in the system over time. As the biofilm fluid was removed from the system upon reaching the outlet, this then served to decrease the total volume of biofilm remaining in the system. To compute the value of the total biofilm volume, the *Integrate Variables* filter was used to integrate the volume fraction $\alpha$ over the entire computational domain. The volume of biofilm was computed at each timestep, allowing for the change in the total volume of biofilm in the system over time to be recorded, which corresponds to the sloughing rate.
5.4 Results

![Figure 5.10](image)

Figure 5.10 Snapshots of the simulations for flow angles of 0°, 45° and 90° flow angles, shown at times of t = 5, 10 and 15 s. The t = 5 s snapshots show the “budding” effect that is observed in all of the runs, where in the initial few seconds of the simulation, the biofilm reconfigures itself from the initial flat sheet distribution, to a more energetically favourable “globule”. The top row has a flow angle of 0° (into the page), the middle row a flow angle of 45°, and the bottom row a flow angle of 90° (right to left). All simulations pictured here have an initial biofilm thickness of 28 μm and are sampled at times of 5, 10 and 15 seconds. As can be seen, increasing the flow angle decreases the biofilm removal rate. At t = 15 s, all of the biofilm in the parallel (0°) flow case has been removed, with more biofilm remaining on the riblets at this time for larger incident flow angles.

A total of 12 3D simulations were performed to explore the effects of biofilm thickness and flow angle. These simulations explored three initial thicknesses of biofilm: 14 μm, 28 μm and 50 μm (the general initial conditions of which are shown in Figure 5.5), for each of four flow angles with respect to the riblet alignment: 0° (parallel), 45°, 67.5° and 90° (perpendicular). Each run was simulated for a duration of 20 seconds. For all flow angles and initial biofilm thicknesses, the total volume of biofilm in the system and the combined drag on the riblet and biofilm surfaces were recorded over time.

A phenomenon which was observed in all of the runs was that in the initial few seconds of the simulation, the biofilm exhibited “budding” behaviour, where the
initial flat biofilm deposit (as shown in Figure 5.5) reshaped itself into a more energetically favourable, curved configuration, as can be seen in the $t = 5$ s column in Figure 5.10. This budding is not due to the effects of the external flow field, as it is also observed in simulations where there is no flow; rather it is due to the effects of the surface tension of the biofilm-water interface and the biofilm-riblet contact angle.

### 5.4.1 Flow parallel to riblets

The first case examined is that of flow parallel to the riblets. This is expected to be the standard case, since riblets are intended to be applied to a ship hull oriented in the direction parallel to the flow field which the hull will experience. The drag on the riblet surface as a function of time, the total volume of biofilm in the system, and the total volume of biofilm normalised by its initial volume (which can also be interpreted as the percentage of the original biofilm remaining) over time for the riblet surface exposed to parallel flow are shown in Figure 5.11.

**Drag**

In Figure 5.11(a), the first 1 s of the drag curves are not plotted due to the large amount of noise generated by the budding. Following this initial interval, the drag increases as the surface area of the biofilm incident on the flow field increases. As the biofilm is pushed along the riblet surface towards the outlet, it is removed from the system. This corresponds to sloughing, and reduces the total drag experienced by the system. For parallel ($0^\circ$) flow, increasing biofilm thickness appears to have relatively minimal effects on the drag curves, with all three initial thicknesses achieving a similar maximum drag value/increase, shown in Figure 5.11(a, b).

**Biofilm volume**

Following a small initial decrease in the volume of biofilm in the system, the total biofilm volume in the system then exhibits a “lag time”, where it remains roughly constant for the following few seconds of the simulation, as can be seen in Figure 5.11(c, d). This effect is again due to the initial budding phase of the simulations, where the biofilm reconfigures itself into a more energetically favourable shape,
Flow angle = 0°

Initial biofilm thickness

Figure 5.11 Results for parallel (0°) flow. (a) Drag as a function of time for the three initial biofilm thicknesses. The dashed black line represents the baseline drag on the surface, i.e. the drag experienced by the surface when no biofilm is present. The first 1 s of the simulations have not been plotted, as there is an appreciable amount of noise during the initial “budding” phase of the biofilm removal. The drag initially increases as the biofilm deposit redistributes itself into a configuration with a larger surface area incident to the flow. As the biofilm begins to be sloughed from the surface, the drag decreases. (b) The percentage increase in the drag on the system compared to the baseline drag. At its peak, the drag on the system is more than doubled due to the effects of the biofilm being reshaped in the sloughing process. (c) The total biofilm volume in the system over time. For all initial thicknesses, there is an initial “lag time”, where the total biofilm volume remains almost constant, due to the budding effects. For parallel flow, all three initial biofilm thicknesses are completely sloughed at approximately the same time. (d) The total biofilm volume in the system over time, normalised by the initial biofilm volume. Here it can be seen that the thickest initial biofilm deposit has a marginally faster sloughing rate, but all three initial deposits are still fully sloughed at approximately the same time.

and in doing so pulls the biofilm towards the centre of the system - away from the outlet. As such, the biofilm then has to be pushed back towards the outlet before it can be sloughed, resulting in the observed plateaus in biofilm volume.

Figure 5.11(c) shows that the system becomes completely biofilm free after around 12 s, with biofilms of all three initial thicknesses having been fully removed from the system at approximately the same time. Some slight differences in
the sloughing rates are more visible in the normalised biofilm curves, Figure 5.11(d), where it can be seen that the thickest initial biofilm deposit has the fastest sloughing rate, i.e. the highest rate of biofilm removed per unit time.

### 5.4.2 45° flow

Considered next is the case where the riblets are misaligned with the flow by an angle of 45°.

![Figure 5.12](image-url) **Results for 45° flow.** (a) Drag over time for the three initial biofilm thicknesses. The dashed black line represents the baseline drag on the surface. As in Figure 5.11, the first 1 s of the simulations have not been plotted. Here the three initial biofilm thicknesses begin to exhibit differing behaviour, with the time taken to reach the baseline drag value now varying between thicknesses. It should also be noted that while the baseline drag value is higher than that of parallel flow, the peak drag experienced by the system appears to be lower. (b) The percentage increase in drag due to the biofilm. Here the drag increase due to the biofilm is around half as large as that for the parallel flow, shown in Figure 5.11(b). (c) The total biofilm volume in the system over time. For 45° flow, the three initial biofilm thicknesses now more clearly exhibit a difference in sloughing rate. (d) The total biofilm volume in the system over time, normalised by the initial biofilm volume. Compared to the parallel flow results shown in Figure 5.11(d), here there is a clear discrepancy in the sloughing rates, with thinner biofilm deposits taking longer to be fully sloughed.
Drag

To mimic the effects of misaligned riblets, the flow angle was then increased to 45°. This had the effect of increasing the baseline drag on the riblets - the total drag experienced when the surface is free of biofilm, but also decreased the peak drag experienced by the surface, as shown in Figure 5.12(a). Comparing Figure 5.11(b) to Figure 5.12(b) it can be seen that the percentage drag increase due to the biofilm is around 60% for 45° flow, compared to around 120% for 0° flow. This can be explained by the riblets interfering with the sloughing of the biofilm, both by preventing it from maximising its surface area incident to the flow as much as the parallel flow runs and by partially sheltering the biofilm from the incident flow, seen in Figure 5.10. The riblet misalignment therefore increases the baseline drag, but appears to actually lower the peak drag.

Biofilm volume

For the 45° flow angle, some differences appear in the overall sloughing rate in Figure 5.12(c-d), when compared to the parallel flow results in Figure 5.11(c-d). The differences in sloughing rates between the thick and thin biofilm deposits are now more exaggerated, with the thinner initial deposits now more clearly taking longer to be fully sloughed than the thicker ones. Furthermore, the sloughing rates are overall lower here than in the parallel flow runs, with all of the initial biofilm deposits now taking longer to be fully sloughed. This suggests that misaligned riblets may indeed prevent biofilm sloughing.

5.4.3 67.5° flow

Considered next is a somewhat more misaligned flow, with a 67.5° angle between flow direction and riblet alignment.

Drag

The trend of an increasing baseline drag value continues upon increasing the incident flow angle to 67.5°, as shown in Figure 5.13(a), and the peak percentage increase in drag also continues to be lower than previous flow angles.
Flow angle = 67°

Figure 5.13  Results for 67.5° flow. (a) Drag over time for the three initial biofilm thicknesses. The dashed black line represents the baseline drag on the surface when no biofilm is present. Similarly to the 0° → 45° results, moving from 45° → 67.5° increases the baseline drag, but decreases the peak drag on the system. Unlike the previous results, here the drag curves do not reach the baseline values within the simulated timeframe. (c) Total biofilm volume in the system over time. As expected from the drag results, unlike for the previous flow angles, here the biofilm is not entirely sloughed from the riblet surface by the end of the simulation, with all three initial thicknesses approaching a common non-zero value. (d) The total biofilm volume in the system over time, normalised by the initial biofilm volume. The difference in sloughing rates between the three initial deposits are now even more emphasised than before, with almost half of the thinnest initial deposit remaining at the end of the simulation.

Biofilm volume

Interestingly, the sloughing rates have now decreased such that none of the initial biofilm deposits have been fully sloughed by the end of the simulated timeframe. As such, the drag experienced by the system remains higher than the baseline value. Furthermore, the three initial biofilm volumes appear to converge towards a common value approaching the end of the simulations, Figure 5.13(c). This further strengthens the argument that severely misaligned riblets, or riblets experiencing a flow which is transverse to their alignment, can in fact protect the biofilm from being sloughed by allowing biofilm deposits to “shelter” within the riblet troughs.
5.4.4 Flow perpendicular to riblets

Finally, simulations were performed for a flow angle of 90°, perpendicular to the riblets. This represents the absolute worst-case scenario in terms of riblet misalignment.

![Figure 5.14](image)

**Figure 5.14** Results for perpendicular (90°) flow. (a) Drag over time for the three initial biofilm thicknesses. The dashed black line represents the baseline drag on the surface. Compared to previous flow angles, there is a marked increase in the baseline drag experienced by the system. (b) The percentage increase in drag caused by the biofilm, compared to the baseline drag value. (c) Total biofilm volume in the system over time. As in the 67.5° flow results, Figure 5.13, none of the three initial biofilm deposits are able to be fully sloughed from the riblet surface. (d) The total biofilm volume in the system over time, normalised by the initial biofilm volume.

**Drag**

For the perpendicular flow case, there is a considerable increase in the baseline drag, the dashed black line in Figure 5.14(a), when compared to previous flow angles. The baseline drag for 90° flow is 81% higher than the baseline drag of the parallel flow runs - the dashed black line in Figure 5.11(a). There is also only a relatively marginal percentage increase in drag relative to the baseline value when the biofilm is present on the surface.
As can be seen in Figure 5.14(a), there is a sharp increase in the baseline drag when the flow angle is increased to 90°, much larger than in the previous 0° → 45° → 67.5° transitions. Unfortunately, this discrepancy is due to an issue with the boundary conditions for the off-angle flows, which is discussed later in Section 5.4.5.

Biofilm volume

![Diagram of biofilm volume over time](image)

**Figure 5.15** Volume of biofilm in the system over time for perpendicular flow (left to right of the system diagrams (“a”-“d”)), with an initial biofilm thickness of 14 µm. The attached screenshots illustrate some of the features observed in the plot. The plateaus, (“a” to “b”) are caused by packets of biofilm moving from the front of the biofilm to the back, without any biofilm actually leaving the system. The large drop seen at point “c” is due to the packet now reaching the outlet and beginning to exit the system. At “d”, the large packet has now almost completely left the system, resulting in a more steady flow of biofilm removal.

Figures 5.14(c, d) show that the biofilm volume as a function of time appears to undergo a series of discrete drops when exposed to perpendicular flow. To better understand this, Figure 5.15 examines the normalised biofilm over time curve for an initial thickness of 14 µm, alongside some snapshots of the biofilm distribution at various points along these drops. The centre plot is the biofilm volume as a function of time curve for an initial biofilm thickness of 14 µm exposed to perpendicular flow, taken from Figure 5.14(d). The surrounding screenshots show the source of these plateaus - namely, “packets” of biofilm which are moved from the front of the system towards the back, before being sloughed from the system. During the time that these packets are moving towards the outlet, no biofilm is removed, hence the biofilm volume as a function of time exhibits a
transient plateau. Upon reaching the outlet, this packet is sloughed almost all at once, resulting in the sudden drop in biofilm volume.

![Graphs and diagrams](image)

**Figure 5.16** (a) Plot of the volume of biofilm in the system for perpendicular flow, simulated for an additional 10 s. The volume of biofilm in the system appears to plateau towards a common non-zero value, suggesting that extremely misaligned riblets can indeed protect the biofilm from being fully sloughed. (b) The same biofilm over time curves, normalised by the initial biofilm volume. The attached simulation snapshots (lower row of figures), labelled by initial biofilm thickness and sampled at \( t = 30 \) s, support this idea. The leftmost two riblets have a similar volume of biofilm sheltered in front of them. Extended simulations are required to verify if all initial biofilm thicknesses converge to the same non-zero biomass volume remaining in the system when it reaches steady-state.

It was apparent from the 90° results that, similarly to the 67° results, the riblets were sheltering small deposits of biofilm, as evidenced by the common non-zero volume of biofilm which remained at the end of the simulations. This could be an important observation, as it may imply that misaligned riblets could contribute to marine biofouling. To further investigate this sheltering effect, the 90° flows were simulated for an additional 10 s, for a total of 30 s, as shown in Figure 5.16(a, b). Below the plots in Figure 5.16 are snapshots showing the biofilm configuration at the end of the extended simulations, highlighting how severely misaligned riblets can prevent biofilm sloughing. It can be seen that even for these longer simulations, the biofilm is still not fully removed from the domain.

The configuration of the biofilm deposits shown in the snapshots of Figure 5.16...
Figure 5.17  (a-c) Biofilm distribution at $t = 60$ s for 14, 28, and 50 $\mu$m initial biofilm thicknesses respectively, simulated in 2D. Flow is directed from the left to the right of the diagram. (d) Drag on the system over time for the three initial biofilm thicknesses. (e) Total biomass in the system over time, (f) total biomass in the system over time, normalised by the initial biofilm volume. All three simulations plateau to a constant “steady-state” biomass volume, suggesting that severely misaligned riblets may indeed protect biofilms from being fully sloughed from the surface.

as well as the fact that the biofilm volume vs time curves were still slightly decreasing, suggested that the biofilm deposits had still not yet reached a steady-state configuration. By simplifying the model to 2D, this greatly lowered the computational cost of the simulations, therefore allowing for longer simulation times to be investigated. All other system parameters were left unchanged.

Simplifying the system to 2D also served to reduce the budding effects seen in the 3D simulations, as there is now no compression along the $z$-axis (into the page). The 2D systems were simulated for a total of $t = 60$ s, and the domain size was unchanged in order to maximise comparability with the 3D systems. To simulate the system in 2D, OpenFOAM was instructed not to simulate the model in the
which is done by changing the patch type of the front and rear domain walls from \textit{wall} to \textit{empty}, as discussed previously in Section 5.3.4.

Figure 5.17(a-c) shows the configurations of the biofilm at the end of the 2D simulations, for initial biofilm thicknesses of 14, 28 and 50 µm respectively. Based on the fact that biofilm remains in these simulations even at long times, and the clear plateaus in the biofilm volume vs time curves, Figure 5.17(d), there appears to be a steady-state configuration for the biofilm where the riblets shelter it from being sloughed by the external flow field.

As opposed to the 3D simulations, the 2D simulations do not approach a common final volume of biofilm; rather it appears that thicker initial deposits also result in thicker final ones. This could be due to the lack of compression in the \( \hat{z} \)-axis that the biofilm experiences - in its absence, the biofilm globule is limited by how much it can coalesce and increase its surface area relative to the flow.

5.4.5 Boundary condition and drag calculation error

![Graphs showing drag increase and drag values vs flow angle](image)

\textbf{Figure 5.18} (a) The percentage increase in baseline drag compared to parallel flow vs incident flow angle. While the drag increase at a flow angle of 90° is consistent with previous computational work [46], there is a discontinuity going from the off-angle flows to 90°. (b) The values of the baseline drag vs drag angle for systems with two outlets (blue) and the original 90° one outlet system (red). There is a significant difference between the drag results for systems with one or two outlets.

Upon completion of the original three flow angle runs, 0°, 45° and 90°, it was noticed that there was a considerable discontinuity in the baseline drag values
between 45° and 90°, when compared to the values between 0° and 45°; as shown in Figure 5.18. To discern whether there was indeed a non-linear relationship between baseline drag and flow angle, the 67.5° simulations were performed, with the hope that these drag values would be more of an intermediate value between 45° and 90°. However, this was not the case, with the difference between 45° and 67.5° being comparable to the difference between 0° and 45°, and a large discrepancy still remaining between 67.5° and 90°.

It was proposed that these discrepancies could be explained by the differing boundary conditions used between the straight-on (0° and 90°) and off-angle (45° and 67.5°) flows, in particular the pressure conditions due to the differing number of outlets. The 0° and 90° flows were directly incident on a domain wall, which allowed for the conventional choice of boundary conditions - setting the incident wall to be the system outlet with a pressure value of \( p = 0 \). But for 45° flow, where the flow was incident directly on the corner of the system domain, the appropriate choice of boundary conditions was less obvious. Ultimately the decision was made to designate both the back and right walls as outlets, with pressure \( p = 0 \).

While this choice of boundary conditions produced qualitatively sound results, in terms of flow through the system and the behaviour of the biofilm, they did not allow for the production of quantitatively sound ones. The percentage increase in drag for the one-outlet systems, \((0° \rightarrow 90°)\) shows agreement with prior computational work performed by Li et al. [461], which investigated the drag on a riblet-embossed surface as a function of flow angle. However, Li et al. predict a relatively linear relationship between drag increase and flow angle, which contradicts the results presented in Figure 5.18.

As discussed previously in Section 5.3.4, the specific numerical value of the pressure at the outlet is generally inconsequential when modelling incompressible flows, as it is typically only the gradient of the pressure which appears in the equations governing the system dynamics. However, the method used here to compute the drag on the system, Equation (5.26), is dependent on the actual values of the pressure, due to the \(-pI\) term in Equation (5.22). Therefore, the off-angle flows deviate from the expected outcomes due to the additional surface where \( p = 0 \).

Typically in computational fluid dynamics, outlet boundary conditions are chosen such that they have minimal impact on the upstream flow and are ideally placed as far away as possible from the region of interest in order to achieve this -
the flow should be fully developed by the time it reaches the outlet. This is why computational fluid dynamics systems commonly take the form of long pipes or wind tunnels. However, here the domain size was kept relatively constrained, primarily for the sloughing of biofilm to be easily recorded, as well as for computational feasibility. Both of these factors resulted in the outlet being located directly adjacent to the region of interest.

As biofilm sloughing was quantified here by the total volume of biofilm in the system over time, this required the outlet to be placed in proximity to the riblet structure, such that biofilm could be removed from the system by being moved through the outlet. One potential solution could have been to use a method to quantify sloughing which did not require the biofilm to be removed from the system, which would allow for the outlet to be placed further away from the region of interest.

For example, in the post-processing stage, it could have been possible to create a slice across the system, orthogonal to the flow direction, and compute the mass flux through this slice. Or alternatively, create a sub-volume in the system and quantify sloughing via the rate at which the biofilm leaves this sub-volume. However, this would not allow for the effects of biofilm coverage on the drag experienced by the surface to be investigated, as the biofilm would still remain on the surface.

The other factor which necessitated the outlet being placed in such close proximity was computational feasibility. Reducing the cell sizes by dynamically refining the mesh also served to severely reduce the size of the timestep, resulting in considerable simulation run times, several weeks in most cases. The initial mesh configuration used in the simulations was relatively ad hoc in its setup, with little to no testing done to optimise its cell distribution.

As such, due to the incorporation of dynamic mesh refinement, the base mesh could have been made considerably coarser, which would have lowered overall simulation times, therefore allowing for a larger computational domain. This would allow for the outlet to be placed further away from the region of interest, but still has the same shortcoming as the slice method discussed above, where the relation between biofilm coverage and drag would not be able to be investigated, since the biofilm would not be removed from the system.

Alternatively, the riblets themselves could be rotated, rather than adjusting the relative angle of the flow velocity vector. This would allow for the domain
boundaries to be kept consistent throughout all the simulations, and therefore permit the conventional one-outlet setup to be used. This approach was considered initially, however it was ultimately abandoned. Partially due to the difficulty in constructing these meshes using the rudimentary blockMesh utility, but also due to the fact that the rotated riblets would have different surface areas for each angle - which would increase the complexity of comparing drag between different flow angles. There are alternative methods available to create the mesh, such as via CAD software, but these were not utilised due to a lack of familiarity. In this scenario, the drag could potentially have been normalised to be presented per unit area, by dividing the drag by the area of the riblet surface. The value of which could have been calculated via ParaView by integrating the areas of all cells on the riblet surface.

Attempts were made to salvage the off-angle results in the post-processing stage, simplifying Equation (5.26) by removing the problematic integrands corresponding to the back and right walls for all runs. However, this proved fruitless, with the discrepancies still remaining. While the method for computing the drag appears to be solid, at least in terms of agreement with the methods of Li et al. [461], it is apparent that the appropriate use of boundary conditions is critical to its implementation.

5.5 Discussion

Presented in this chapter was the development of a computational fluid dynamics (CFD) model for the sloughing of a biofilm established on a drag-reducing riblet-embossed surface. The model verifies previous computational and experimental work showing that increasing the incident flow angle results in an increase of the overall drag experienced by the surface [423, 461], and also provides insight into how misaligned riblets can prevent the sloughing of established biofilms. Also presented was the development of a computational method for calculating the total drag on a fixed surface partially covered by a moving viscous fluid, produced in conjunction with Professor Nick Cogan, a collaborator on the project. This method could potentially have future industrial uses, and should be straightforward to implement in similar CFD simulations in the future.

Several key omissions were made in the construction of this model. In particular, turbulence was neglected. While it is true that the intended functionality of these
riblets is to lower drag via the reduction of turbulence, a back-of-the-envelope calculation can be performed to compute the thickness of the laminar boundary layer, via the use of the “99% method” [462], where the thickness of the boundary layer is defined as the distance from the surface to the point where the velocity is 99% of the freestream velocity. This method gives a value for the thickness of the laminar boundary layer as

$$\delta(x) \approx 5 \times \sqrt{\frac{\nu x}{u_0}} = 5 \times \sqrt{\frac{1 \times 10^{-6} \cdot 288.5 \times 10^{-6}}{0.18}} \approx 200 \mu m, \quad (5.32)$$

where $\nu$ is the kinematic viscosity of the surrounding fluid, $u_0$ is the freestream velocity, and $x$ is the distance downstream from the start of the boundary layer - approximated here as the midpoint of the riblet surface. The result of this calculation shows that the thickness of the laminar boundary layer is approximately the same height as the computational domain. Furthermore, the aim of this project was mainly to investigate the interplay between flow angle and biofilm sloughing, rather than to perform an in-depth analysis of the development of flow around the riblets, of which there is already a substantial body of work [422–424, 425, 427–429]. As such, omitting turbulence was considered to be a justified simplification to the model.

Another key simplification of the model was neglecting the viscoelastic properties of the biofilm. While there is a relatively high variance in the measured mechanical properties of biofilms [458, 459], many biofilms have been observed to exhibit viscoelastic behaviour [106–109]. In this work, viscoelastic effects were omitted due to a lack of support in OpenFOAM for modelling multiphase viscoelastic flows at the time when the model was constructed. The third-party package rheoTool [463], which allows for the modelling of multiphase viscoelastic fluids with OpenFOAM, was discovered towards the end of the project, which may allow for the inclusion of a viscoelastic biofilm in future work. But for the work presented in this chapter, modelling the biofilm as a viscous fluid was still deemed to be useful enough as a first approximation. Similarly, other physical properties of the biofilm could have been varied, such as its density or surface tension.

Yet another potential shortcoming of the biofilm model was the budding effect which was present in all of the simulations. This budding appears to be unrealistic, since biofilms are typically found in more sheet-like configurations [464, 467].
One possible solution could be implement periodic boundary conditions on the side walls, such that the lateral forces on the biofilm are balanced and compression cannot occur in these directions. However, this would complicate the modelling of any off-angle flows, where the side walls also act as an outlet. This budding could also be reduced by varying certain parameter values such as the strength of the surface tension or riblet contact angle.

The contact angle is one parameter in particular which lends itself to further investigation. While the riblet geometry used was based on “optimal” values [449, 450], the riblet surface contact angle was set to a value of 90° simply to minimise surface-biofilm interactions. The wettability of the surface is a property which a manufacturer can control, and previous work has determined that increasing the hydrophobicity of the riblet surface can enhance the drag-reducing quality of the riblets [455, 457]. As the contact angle of the surface is a parameter which can be easily set in OpenFOAM, investigating the effects of surface wettability on biofilm sloughing could be a relatively simple follow-on project. This was a focus of Benschop et al.’s experimental work [446], but computational models would allow for a much greater range of surface energies to be investigated, including both static and dynamic contact angles, which could inform manufacturers on which coating properties to pursue.

On the subject of riblet geometry, work is still ongoing in the field to investigate various different riblet geometries and configurations. While the triangular riblets with trapezoidal grooves used here have been found to be a good compromise

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4As opposed to static contact angles, which depend only on the fluid and the surface, dynamic contact angles depend on whether the fluid is advancing (wetting) or receding (dewetting). The difference between the advancing and receding contact angles is known as contact angle hysteresis, and is a measure of the roughness of the surface.
between drag reduction and reproducible application \[420, 419\], other riblet geometries have been tested for their drag-reducing properties, such as the blade or scalloped riblets shown in Figure \[5.19\]. These geometries can actually exhibit higher drag reductions than the trapezoidal ones used in this chapter \[457\], but may also result in lower rates of biofilm sloughing.
Chapter 6

Conclusions

Essentially, all models are wrong, but some are useful.

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George E. P. Box

This thesis has focused on the modelling of the formation and growth of biofilms in a variety of spatially heterogeneous environments. Each chapter presented here described a distinct project, investigating a different scenario in which a biofilm might grow, united under a common motif of spatial non-uniformity.

In Chapter 2 I developed a simple model exploring the coupling of growth rate dependency and spatial structure on the efficacy of an applied antibiotic, for antibiotics which were either more effective against slow-growing or fast-growing antibiotics. This model built upon a previous 1D lattice model that had been used to study the influence of antibiotic gradients on the evolution of resistance, developed by Greulich et al., 2012 [223]. In my work, this model framework was instead used to study the effects of nutrient availability on antibiotic susceptibility. My simulations predicted that antibiotics which are more effective against fast-growing cells exhibit qualitatively different spatial behaviours than antibiotics which are more effective against slow-growing cells, particularly for the case of bactericidal antibiotics.

The results of this work could potentially be used to inform more effective antibiotic treatment strategies, as they predict that antibiotics which target fast-growing cells would be more effective to prevent the spatial advancement of biofilms.
of a bacterial population, whereas a bactericidal antibiotic which targets slow-growth could be more effective at eradicating an established population, \textit{e.g.}, a biofilm. While the pharmacodynamic curves of differing antibiotics are not always directly comparable, the computational models could be experimentally verified using a bacterial population growing along an agar plate containing a fixed spatial antibiotic concentration gradient.

Of all the chapters in the thesis, I feel that Chapter 2 presents the most “complete” picture. While the model itself is considerably rudimentary, taking the form of a simple 1D lattice, I believe it still incorporates everything it needs to in order to sufficiently describe the system and its dynamics. More realistic components could certainly have been included in the model, such as diffusion of the antibiotic or nutrients, the evolution of antibiotic resistance, or perhaps utilising a 2D or agent-based model in order to capture the spatial structure of the growing bacterial population, but ultimately I do not believe these additions would fundamentally alter the key findings of the model.

Following this, Chapter 3 presented two models for the very early stages of biofilm formation on a surface: a stochastic lattice-based one, similar in some respects to the model presented in Chapter 2 and a deterministic one developed together with Dr Chris Brackley. These models aimed to investigate the stochastic nature of the initial stages of biofilm formation, and what factors influence whether early biofilm formation is stochastic or deterministic. According to the predictions of these models, a biofilm can initiate almost immediately under favourable conditions. However, in less favourable conditions, a loosely attached layer of bacteria will first aggregate on the surface, this population then may or may not transition to a biofilm following a stochastic lag time. The models that we developed in Chapter 3 were intentionally simple in their construction, as they were originally created to be used as a simpler “test case” during the development of the model presented in Chapter 4. However, the results generated were interesting enough to be given their own chapter, since they provide some stimulating insight into possible fundamental principles underlying early biofilm formation.

Due to their simplicity, the models of Chapter 3 were somewhat limited in their scope, meaning that there remained many potential additions that could be made to them. These models highlight the stochasticity and inherent unpredictability of biofilm formation, but neglect many important features that could introduce qualitative changes to the models’ behaviour. For example, the quorum sensing-
mediated transition to a biofilm state is modelled as a simple binary switch. In reality this would likely be a more continuous transition over time, it may have several stages, and for some species it might have other control mechanisms. The transition to a biofilm state would likely also influence other properties of the bacterial population, namely the production of EPS which could in turn alter the overall detachment rate. One other potential improvement to this project could be to change the 1D lattice based model to a 2D/3D agent based model, where the individual bacteria are modelled as discrete entities. This could provide a more detailed description of the processes involved in initial biofilm formation. But all in all, I think the models presented in Chapter 3 present an interesting, if somewhat limited, insight into the initial stages of biofilm formation.

Chapter 4 presented another 1D lattice based model, extending the one in the previous chapter to model the more specific scenario of the formation of a marine microbial biofilm on an antifouling (AF) surface. This chapter introduced both a spatial biocide gradient - emulating the biocidal compound leaching from the AF surface and diffusing into the surrounding ocean; and the concept of a microbial “ecotype”, which represented each microbial species’ innate resistance to the biocide. This model further served to highlight the stochasticity of biofilm formation and development, not just in formation time, but also in its community composition, with replicate simulations producing qualitatively different results.

While Chapter 3 was focused primarily on the initial formation of the biofilm, Chapter 4 also examined the growth and development of the biofilm after it had formed. Although the model did track the dynamics of the species/ecotype composition over time, there were many other interesting aspects of biofilm growth which it did not include or capture. I believe out of all the projects presented in this thesis, the model of Chapter 4 has the largest scope for improvement or extension. Being limited to 1D, the model is unable to capture the complex spatial structure of mature biofilms - this would also likely contribute to the drag experienced by the AF surface, which was an avenue of interest that motivated the construction of the model. While interesting observations were made regarding the overall species composition, the addition of another spatial dimension would also allow to investigate the distribution of these ecotypes in more detail, determining whether the ecotypes arrange themselves in “pockets” or a stratified manner.

The properties of the microbes, and the biofilm itself, could also have been
explored further. The enhanced resistance to the biocide that was assumed in some ecotypes did not have a corresponding trade-off associated with it. In other words, one might expect that higher levels of resistance lowers the death rate, but also lowers the growth rate. Another addition to the model which could have caused qualitative changes to its behaviour would have been some sort of feedback mechanism between the biofilm and the biocide gradient, where the nascent biofilm could absorb or denature the biocide, affecting the gradient. This could affect the final community compositions, as the initial population could reduce the effects of the biocide, allowing for a wider variety of species to populate the surface.

Chapter 5 described a significantly different form of model, moving away from the simple 1D lattice architecture to a more complex 3D finite volume computational fluid dynamics model. This chapter focused on the industrially relevant scenario of a biofilm growing on drag-reducing riblet surface, similar to the previous experimental work of Benschop et al. [446], and investigated how the incident flow angle can affect the process of biofilm sloughing. The work presented in this chapter could potentially have numerous industrial use-cases, in optimising the design or orientation of riblets in order to maximise biofilm sloughing and reduce drag. A particularly notable outcome of this chapter was the development of a novel computational method to compute the drag experienced by the riblet surface over time as the viscous biofilm fluid moves across it. This method is versatile and simple to implement, and should be able to be utilised regardless of riblet geometry or domain size, which is also highly desirable from an industrial modelling standpoint.

In terms of improvements to the model presented in Chapter 5, one clear candidate would be the implementation of correct, or at the very least, more consistent boundary conditions between differing flow angles, as discussed in Section 5.4.5. While the method itself appears sound, the drag results for the off-angle flows were somewhat marred by the inappropriate boundary conditions. Nevertheless, while there are indeed some improvements which could be made to the model, I consider the erroneous drag results to be the only real “failure” of this chapter. Another issue, which unfortunately seemed to be largely unavoidable, was the computational expense of these large-scale fluid dynamics calculations. However, I believe there was still a fair amount of headroom to lower the simulation times, which would have potentially allowed for repeat simulations to have been performed.
Similarly to Chapter 4, I think the work presented in Chapter 5 likely had scope to form a thesis in and of itself. While the Chapter 5 model produced some compelling results, there were several aspects in which it resembled a prototype more than a finished product, such as in the omission of viscoelasticity. Furthermore, there remained several avenues of interest which could have been explored with the model, such as simulating other riblet geometries, varying the physical properties of the biofilm or other properties of the riblet surface - such as its wettability. But overall I feel a decent piece of research was achieved here, which also laid a solid foundation for future work.

In conclusion, I think this thesis exhibits a common feature of modelling studies. Namely, it raises more questions than it answers. A key aspect of modelling is to reduce a system down to its most fundamental components, which in turn oftentimes leads to conversations about which of these components are most influential, and which omitted components should perhaps also be included to better capture the system’s in vivo behaviour. As such, I don’t believe it was a coincidence that I generally found the Discussion sections of this thesis to be the most enjoyable to write. While I am of the opinion that the results presented here are interesting in their own right, I feel that the overarching achievement of this thesis is the construction of several foundational models, which can be built further upon in future works. As such, I find it only fitting to end with the old adage, “further research needed”.
Appendix A

OpenFOAM velocity gradient

During the process of determining how to compute the integrals mentioned in Section 5.3.6, it was discovered that there were three different utilities available for computing the velocity gradient, $\nabla \mathbf{u}$. ParaView contained two methods, Compute Derivatives and Gradient of Unstructured Dataset, and OpenFOAM also had an inbuilt post-processing command line utility to calculate the velocity gradient, `postProcess -func "grad(U)"`. To determine which of these was the most accurate, a simple test case involving plane-Poiseuille flow was constructed.

A.1 Poiseuille flow

Similarly to Couette flow, as described in section 5.2.3, Poiseuille flow is a flow profile which allows for the Navier-Stokes equations to be solved analytically [469]. Unlike Couette flow, Poiseuille flow is pressure-driven, rather than velocity-driven.

Consider flow of an incompressible, viscous Newtonian fluid between two solid boundaries which are located at $(0, \pm h, 0)$. The flow is driven by a uniform pressure gradient $(\nabla p = (-P, 0, 0))$, and the solid boundaries have a no-slip boundary condition $(\mathbf{u}(y = \pm h) = 0)$. The components of the velocity vector are given as $\mathbf{u} = (u, v, w)$.

The system is experiencing steady flow, so

$$\frac{\partial u}{\partial t} = 0, \quad \frac{\partial p}{\partial t} = 0. \quad (A.1)$$
The form of the pressure gradient and these boundary conditions imply that the velocity only varies in $\hat{y}$, i.e. $u = u(y)$, which reduces the incompressibility condition to

$$\nabla \cdot u = 0 \implies \frac{\partial v}{\partial y} = 0. \quad (A.2)$$

Combining this lack of spatial variance in $\hat{y}$ with the no-slip boundary conditions ($v(y = \pm h) = 0$), and it may be inferred that $v = 0$.

Although this is a 2D case, for completeness let us examine flow in the $\hat{z}$-direction. Again, the only variation is in the $\hat{y}$ direction, so the Navier-Stokes momentum equation reduces to

$$v \frac{\partial w}{\partial y} = \frac{\partial^2 w}{\partial y^2} \implies \frac{\partial^2 w}{\partial y^2} = 0 \implies w = c_1 y + c_2. \quad (A.3)$$

From the boundary conditions $w(y = -h) = w(y = h) = 0 \implies c_1 = c_2 = 0$, therefore $w = 0$. Ergo, the components of the velocity are $u = (u(y), 0, 0)$.

Turning now to the $\hat{x}$-component of the velocity,

$$0 = \frac{1}{\rho} \frac{\partial p}{\partial x} + \nu \frac{\partial^2 u}{\partial y^2} \implies \frac{\partial u}{\partial y} = -\frac{1}{\nu \rho} \left( \frac{\partial p}{\partial x} \right) y + k_1 \quad (A.4)$$

$$\implies u_x = -\frac{1}{2\mu} \left( \frac{\partial p}{\partial x} \right) y^2 + k_1 y + k_2.$$
Here the kinematic viscosity has been converted to the dynamic viscosity, \( \mu = \nu \rho \)

Inserting the boundary conditions for \( u \) at \( y = \pm h \) results in

\[
0 = -\frac{1}{2\mu} \left( \frac{\partial p}{\partial x} \right) h^2 + k_1 h + k_2 = -\frac{1}{2\mu} \left( \frac{\partial p}{\partial x} \right) h^2 - k_1 h + k_2. \tag{A.5}
\]

Therefore \( k_1 = 0 \) and \( k_2 = \frac{h^2}{2\mu} \left( \frac{\partial p}{\partial x} \right) \). Combining these terms for the components of the velocity vector results in

\[
\mathbf{u} = \left( \frac{1}{2\mu} \left( \frac{\partial p}{\partial x} \right) (h^2 - y^2), \ 0, \ 0 \right). \tag{A.6}
\]

As can be seen, for an incompressible viscous fluid flowing between two solid boundaries with no-slip conditions, in the presence of a constant pressure gradient, a parabolic velocity profile is obtained, with components purely in the \( \hat{x} \)-direction.

![Poiseuille flow velocity profile](image)

**Figure A.1** Poiseuille flow profile simulated using OpenFOAM. The \( \hat{x} \)-component of the velocity exhibits a parabolic form along the \( \hat{y} \)-axis, whereas the other two velocity components have constant values of 0.
A.2 Gradient verification

Once the Poiseuille flow profile had developed, the three aforementioned utilities: Compute Derivatives, Gradient of Unstructured Dataset and postProcess -func "grad(\mathbf{U})", were used to generate \( \nabla \mathbf{u} \). As only the \( \hat{x} \)-component of the Poiseuille flow velocity vector has a non-zero value, which varies only in \( \hat{y} \), the gradient of the velocity has a simple analytic solution,

\[
\nabla \mathbf{u} = \begin{bmatrix}
\frac{\partial u}{\partial x} & \frac{\partial v}{\partial x} & \frac{\partial w}{\partial x} \\
\frac{\partial u}{\partial y} & \frac{\partial v}{\partial y} & \frac{\partial w}{\partial y} \\
\frac{\partial u}{\partial z} & \frac{\partial v}{\partial z} & \frac{\partial w}{\partial z}
\end{bmatrix} = \begin{bmatrix}
0 & 0 & 0 \\
-\frac{\nabla p}{2\mu} (H - 2y) & 0 & 0 \\
0 & 0 & 0
\end{bmatrix}.
\] (A.7)

As the pressure is linear in \( \hat{x} \), it can be expressed as

\[
\nabla p = \frac{p_2 - p_1}{L},
\] (A.8)

where \( p_1 \) and \( p_2 \) are the pressures at the inlet and outlet of the system respectively, and \( L \) is the system’s length.

The system constructed for the simulated plane Poiseuille flow was a 2D channel with \( L = 2 \text{ m}, H = 0.1 \text{ m}, \mu = 10 \text{ Pa s}, p_1 = 101,325 \text{ Pa} \) (atmospheric pressure) and \( p_2 = 0 \text{ Pa} \). This gives expected values of \( \frac{\partial u}{\partial y} = \mp 253.31 \text{ s}^{-1} \) at the upper/lower boundaries of the system. The three \( \nabla \mathbf{u} \) utilities were then used to compute \( \nabla \mathbf{u} \) at the outlet of the system, the results of which are shown in Figure A.2.

As can be seen, the three methods produce fairly consistent results for the most part. However, at the wall boundaries, the ParaView methods produce results that are inconsistent with the expected outcome. Therefore, the inbuilt OpenFOAM post-processing function \( \text{grad}(\mathbf{U}) \) was the one selected to compute the results presented in Chapter 5.

One thing to note is that the \( \nabla \mathbf{u} \) matrix is indexed differently between the OpenFOAM function compared to the ParaView ones. In the ParaView results, \( \frac{\partial u}{\partial y} \) is located at index 1, whereas in the OpenFOAM version it is located at index 3. This transposed indexing was confirmed by rerunning the above simulations but with a vertically aligned tube instead of a horizontal one, which resulted in
The $\frac{\partial u}{\partial y}$ component of the $\nabla u$ tensor, sampled at the outlet of a system experiencing plane Poiseuille flow. Three methods for calculating $\nabla u$ are shown: the inbuilt OpenFOAM function, grad(U), and two ParaView methods; “Gradient of Unstructured Dataset” (GOUDS) and “Compute Derivatives” (CompDerivs). The $\hat{x}$-axis is the index of the cells at the outlet, cells 0 and 31 correspond to the cells adjacent to the upper/lower boundaries. It appears that the ParaView methods produce some unintended edge effects.

$\frac{\partial v}{\partial x}$ becoming the non-zero component. In the ParaView results, this term was located at index 3, whereas for the OpenFOAM function it was located at index 1.
Appendix B

OpenFOAM mesh refinement

It was observed that the drag calculation method presented in Chapter 5 Section 5.2.4 resulted in a slight discrepancy between the LHS and RHS of Equation (5.26). This was diagnosed by performing simulations with no biofilm present on the riblet surface, which simplifies Equation (5.26) to equating the surface integral of the riblet surface to the sum of the volume integral and the remaining surface integrals.

Upon repeating these simulations with a more refined mesh, as shown in Figure B.1, this discrepancy was reduced in size. It was therefore attributed to a numerical error in the integration, and was not deemed to be significant enough to invalidate this method for drag calculation. It was also observed that an overly refined mesh increased this discrepancy, again likely due to numerical noise or instabilities. Due to the increased computational cost of simulating the more refined meshes, the original mesh was used as the base mesh in the simulations presented in Chapter 5.
Figure B.1 Percentage difference of the LHS and RHS of the numeric calculation of Equation (5.26). The $\hat{x}$-axis shows the relative refinement of the mesh, 1.0 representing the original mesh, 2.0 representing a mesh which is twice as refined as the original one. The discrepancy between the LHS and RHS can be reduced by further refinement of the mesh, but not eliminated. There also appears to be a point at which further refinement increases the size of this discrepancy, most likely due to numerical noise or instabilities.
Appendix C

Published work

Papers have been published based on the work presented in Chapter 2 of this thesis, Sinclair et al., 2019 [1]; Chapter 3, Sinclair et al., 2022 [2]; and Chapter 4, Sinclair et al., 2022 [3].

A paper based on Chapter 5 is also in development.
Bibliography


[94] L. Yuan, F. A. Sadiq, N. Wang, Z. Yang, and G. He, “Recent advances in understanding the control of disinfectant-resistant biofilms by hurdle


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J. Hou, D. H. Veeregowda, B. van de Belt-Gritter, H. J. Busscher, and H. C. van der Mei, “Extracellular polymeric matrix production and relaxation under fluid shear and mechanical pressure in staphylococcus


P. Oger and S. K. Farrand, “Co-evolution of the agrocinopine opines and the agrocinopine-mediated control of trar, the quorum-sensing activator of...


