Modelling the development and growth of bacterial biofilms

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Abstract

When bacteria attach to a solid surface they form biofilms, which increases their chances of survival. These biofilms can be very useful for example in soil and water treatment; however they can also cause serious illness. In this thesis we study and model biofilms in both one and two dimensions, to increase our understanding of their growth and development. These models use a continuum approach where we assume that the biofilm is a viscous fluid that is free to grow, whilst sitting on a solid impermeable surface. The resulting equations where solved using various numerical techniques, including finite difference, level sets, conjugate gradient solvers and parallelised code. Comparisons between one and two dimensions are made, to understand whether predictions from one remain true in higher dimensions. A variety of anti-biofilm agents are also investigated to see what effects each of these have on biofilms and whether it is better to use a combination of these treatments.
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Chapter 1

Introduction

1.1 Background Information on Bacteria and Biofilms

Bacteria cells live in one of three states, either they are swimming freely within a fluid, in a reproductive structure that is used for dispersal of bacteria and surviving for extended periods of time in harsh conditions or they live in groups that are anchored to a solid surface. These three types are referred to as ‘planktonic’, ‘spores’ and ‘biofilms’ respectively [222] and it is this third type that will be studied in detail. However, to start with we will discuss planktonic cells and how they change their state to form biofilms.

There are many different types of bacteria and in their planktonic form they all swim through a fluid using slightly different techniques. Most types swim by the use of one or more flagella, which is a tail that they rotate to propel themselves forward. The bacterial species that have flagella can be grouped into four different types, depending upon how many flagella and their arrangement. These four different types, shown in Figure 1.1, are [101]:

- Monotrichous - only one flagella (e.g. Vibrio cholerae),
Figure 1.1: *The four different groups of bacteria classification according to the number and arrangement of flagella.*

- Lophotrichous - two or more all originating from the same end of the bacteria and pointing in the same direction (e.g. Pseudomonas putida),
- Peritrichous - multiple flagella all pointing in the same direction but originating from all around the cell (e.g. Escherichia coli),

- Amphitrichous - two, pointing in opposite directions (e.g. helicobacter muricola).

Other bacteria species use an axial filament, such as Spirochetes, that is oriented in a helix shape and twists to move the bacteria forward [26]. Although the flagella allow the bacteria to swim around to find nutrients, it consumes energy, thus remaining stationary in a biofilm is advantageous in this respect. When a cell finds a “suitable” solid surface, it will then attach itself, followed by repressing the motility operons (i.e. the flagella controls) [92, 91]. A “suitable” environment may have many different conditions, not all of which have to be satisfied at once. Examples of “suitable” locations are:

- there must be a sufficient amount of nutrients,

- sheltered, for example in a fast flowing river, otherwise the bacteria may get torn off by the shear forces,

- a pre-existing biofilm.

As bacteria approach a surface, their swimming speed decreases mainly due to hydro-dynamical forces [65], e.g. Brownian motion, however this may have the added benefit of allowing easier attachment. Bacteria can attach themselves to the surface in a variety of different ways that depend upon the particular species, the solid surface and the fluid. There are certain factors that are known to affect adhesion, which include the presence of particular surface proteins, extra-cellular polymers, the degree of cell surface hydrophobicity and electrostatic charge, cell size and the overall physiological status of the cell [213, 193]. In fact, it has been shown that some mutant bacteria, e.g. bacteria without flagella, are unable
to attach to surfaces, that the wild type can [91]. One particular method of adhesion is by the use of “type IV pili” [184], which is a fibrous tentacle like structure that is located on the side of a cell and adheres to the solid surface. These pili also allow the bacteria to move over the surface by stretching the pili out, letting it adhere and then retracting it, pulling the bacteria along and this is known as twitching mobility. In certain species the pili do other jobs as well, for example toxin secretion and the forming of mushroom shapes in Pseudomonas aeruginosa, excretion of DNA from bacteria cells in Neisseria gonorrhoeae [145, 99, 98]. Another method of adhesion is by cell-surface proteins called ‘adhesins’ or ‘flocculins’ that bind certain amino acids or sugars to the abiotic (i.e. non-living) surfaces [215]. Much work has been done on cell attachment to surfaces, as it plays a major role in the types of biofilms that can be formed [115].

Once a few bacteria have adhered to a surface they are then known as a biofilm. These bacteria then grow and divide to create a complex colony, which in nature can contain multiple different species all coexisting together [42, 209]. However, these different species may not live in harmony with one another, as they could well be competing for nutrients and space for example. Some bacteria, e.g. Pseudoalteromonas tunicata, actually produces various antibiotic chemicals, to kill other bacteria and give itself an advantage over them [164]. However, all the bacteria within the biofilm are held together by Extracellular Polymeric Substances (EPS) [7, 81], which are created by all of the cells. In young biofilms, the EPS is actually DNA released by cell lysis or by lysis of DNA-containing vesicles released from the bacteria, however as the biofilm matures other compounds are released [81]. These other compounds depend upon the species, as different ones may produce different types, but they all do the same job of holding the biofilm together. This is one of the defence mechanisms of biofilms, in that it takes more than one chemical to break down the different
EPS substances. Plus the EPS can reduce the concentration of many different chemicals by limiting diffusion, assuming the chemical is destroyed or used inside the biofilm, otherwise the concentration continuously increases. However this also means that a multitude of microenvironments within the biofilm can exist and can help to protect it from antibiotics [200]. The EPS allows complex structures to form, with only the bacteria at the bottom of the biofilm being attached to the solid surface, whilst all the others are attached to these cells by the EPS.

As the biofilm matures the cells grow and divide, creating a complex colony, with non uniform structures [197]. However this growth gradually decreases, starting from regions near the surface and moving outwards, due to the limited penetration of nutrients [194, 173, 165]. What is interesting; however is that as the biofilm matures the cells become differentiated (i.e. phenotypic variants). This means that although all the bacteria have the same DNA, they each have different genes switched on, and so will act slightly differently to each other [225, 224]. The processes that causes this differentiation among cells is still mainly unknown, although it has been shown that it is linked with nutrient levels and quorum sensing (a process where the bacteria are able to monitor their local population density, more information on this is given in Section 1.5.1). It is however understood that these variants help the biofilm to survive environmental changes. They also give the biofilm genetic variability and often each different type is located together within the biofilm. Some of these will then attack and kill other cells within the biofilm, to allow water channels (i.e. void channel that exist between the bacteria cells) to form and feed off the nutrients released by the dead cells [122, 226].

Other processes that affect biofilm development include;

- a sloughing process, where small pieces of the biofilm break off and travel
down stream, that may then attach to another surface and form a new biofilm [206]. This process appears to be enhanced under high shear stress, and under these conditions a patchy biofilm, as opposed to a highly heterogeneous one with many pores, will develop [111],

- individual bacteria cells escape from the biofilm to become planktonic and swim away [83].

Typically, biofilms will eventually cease to grow, as a volume balance is created through birth, death, cellular escape, sloughing and degradation of EPS. This will continue until a significant change in environmental conditions leads to the biofilm dying. Therefore the overall life cycle of bacteria, is them swimming through a fluid until a suitable surface is found. They will then adhere to the surface, producing a biofilm colony as well as dividing to produce new bacteria cells. Some of these new bacteria cells may then leave the biofilm to produce new colonies elsewhere.

1.2 Advantages of Biofilms for Bacteria

Biofilms have a number of properties that enhance survival of individual bacteria [221, 96]. For example if a bacteria cell is in a planktonic state then predation is easier by protozoas [127], and any anti-bacteria chemicals or bodies (i.e. white blood cells) within the fluid, can overwhelm it. There are also no other cells, locally, that can help to fight or break down the chemical, all of which means that a relatively low concentration is required to kill the planktonic cell. Within a biofilm however, the chemicals have to first diffuse into the biofilm before they can attack any individual cell. Also due to the nature of biofilms, there are going to be multiple cells all surrounding each other and this causes a decrease in the local chemical concentration. Cells may also be able to reduce the concentra-
tion further by releasing other chemicals that can neutralise the agent [39, 63]. Therefore, these biofilm methods can help to reduce the chemical concentration even close to the biofilm-fluid boundary and hence higher concentrations of anti-bacteria chemicals have to be used. However this may not be feasible due to the location of the biofilm, for example, if it is growing on living tissue then the chemicals may have detrimental effects if the concentration is too high. Limited penetration means that even if a high concentration can be used, then this may only kill the cells at the edge of the biofilm and not the ones deeper down. This is due to the fact that the dead cells still absorb the chemical, as they take up volume and are attached to the biofilm and in doing so cause the concentration to decreases from the outside, inwards. These factors enhance the survival potential of individual bacteria, although it should be noted that biofilms are predated upon by protozoas, as the bacteria are a relatively dense source of nutrition.

1.3 Where do Biofilms Grow?

Biofilms have the ability to grow in nearly any environment, as long as there are nutrients for food [171, 102, 204, 119] and a solid surface that they can attach to [211, 215, 145]. This has allowed biofilms to make bacteria very adaptable as they can grow in conditions ranging from rain forests to deserts, (they are known as “desert varnish”), from the bottom of the ocean to glaciers in the Antarctic, from living tissue to medical instruments [221]. They can grow in very extreme environments, which no other form of life can tolerate. For example, a class of bacteria called extremophiles can be found living on hot (60°C - 80°C) rocks under the Earth’s surface in high gas and liquid pressures [37, 72].

It is this wide range of locations that biofilms exist in, that contributes
towards the wide variety of animals and plants that live and have lived on the Earth. The reason being that biofilms generally form the bottom link in all food webs and hence nourish larger organisms. For example mosquito larva eat the algae, which in turn are consumed by dragonfly larva, which feed fish and these are eaten by birds, mammals, etc [117]. Biofilms can be useful though, for example they decontaminate soils and water supplies from accidental chemical release, whether this is from humans or nature, and hence are very important in maintaining the environment.

1.4 Biofilms are they Good or Bad?

Biofilms, like bacteria, are generally seen in a bad light even though they do a whole host of beneficial things. In this section we will discuss the effects biofilms have, both good and bad, on life, the environment and industry.

One industry where biofilms are used to great effect is in water treatment [70, 86, 237, 241, 203]. Here biofilms are encouraged to grow, in what are called bioreactors, which are large containers that are filled with water and contain porous material on which the biofilms can develop. The exact shape and design of these containers varies tremendously to obtain the best purification, which depends upon a whole range of things. For example the type of bacteria being used, the flow rate of the water in and out of the barrel and the quantity and type of contamination. Huge amount of research is still being carried out to find the optimum shape and internal workings of these containers, which can be quite complex. The water is purified by the biofilms as they consume the contaminates, as nutrients. This procedure is not without its problems, due to the fact the biofilms are continuously being feed. Therefore they grow to such a size that impedes water flow or worst contaminate the water themselves.
This means that from time to time, the biofilms have to be scraped off all the surfaces within the bioreactor, to regain optimum efficiency. It is not only waste water where biofilms are used in water treatment. Another example is in shrimp ponds, where biofilms are used to clean the water. This in turn improves the growth and survival rates of the shrimps [205].

In recent years, biofilms have been used to help clean up the environment, typically the soil, where contamination has occurred [46]. This is achieved by inserting porous blocks containing bacteria into the ground and as the contamination diffuses through these blocks, the bacteria consume it. This works on the same principle as the water treatment process and the advantage of using biofilms here is that they are natural cleaners. In fact it has been suggested that bacteria are required to help the formation of certain soil types [39]. However great care has to be taken when using this approach, as it is easy to introduce bacteria that do not naturally exist in that environment. Therefore in the long run this can cause more damage than the short term benefit, very similar to the introduction of African (Killer) Bees into North America [140].

In the new technology of fuel cells, which produce electricity from hydrogen based compounds and an oxidant, we are using biofilms to help increase their efficiency or power rating [152]. Biofilms are also involved in a number of symbiotic interactions, e.g. in plant roots [132]. Plants actively encourage this behaviour by secreting significant amounts of sugars, vitamins and amino acids for example. These are nutrients for the bacteria and hence they grow on the plant’s root hairs. These biofilms then help to facilitate the plant’s ability to absorb nutrients from the soil, hence grow faster than any competition not employing this tactic. On the other hand marine plant life, does not like bacteria growing on them, as this reduces its ability to produce food. Therefore the plant either produces chemicals that kill the bacteria as they settle on it, or
continuously sheds its outer layer of cells, hence washing them away [193].

It is not only plants that use bacteria animals do as well for example in their guts, or in special organs, e.g. for the emission of light in deep sea fish. We humans use bacteria throughout our bodies, in fact it has been estimated that about ninety percent of all the cells within us are not actually human [39]. These bacteria are often referred to as microbes and we have different species living throughout us, from the surface of our skin to our digestive tract [121]. Again here, just as with the plants we are mutually beneficial to each other. For example, the microbes within our digestive tract help us break down and absorb the nutrients from the food we eat. These microbes however must stay in the correct place within our bodies and not become too concentrated otherwise they cause illness. An example of this is Staphylococcus aureus, which is located on many people’s skin and causes us no problems. However if it gets inside us, then it can cause infections such as boils or pneumonia. The opposite is also true, where a particular person is deficient in a particular microbe or has killed them off due to antibiotic therapy, it can cause them problems. An example of this is when a person is lacking in Lactobacillus plantarum. This lives in the intestines and plays important roles in maintaining a healthy intestinal lining, protecting it from other bacteria, i.e. Salmonella enterica and Escherichia coli, encouraging the activity of macrophages (an immune system component that protects against invaders) and preventing diarrhoea. Also, biofilms consisting of “alien” bacteria to our bodies can cause a whole host of various illnesses, ranging in intensity from minor to major [60]. Examples of biofilms causing us harm include cavities in our teeth [204], peptic ulcers from Helicobacter pylori, Otitis Media (ear infection), endocarditis (infection of the heart and valves), and legionnaire’s disease [39]. Numerous studies are actually being done on Pseudomonas aeruginosa, which often affects people with cystic fibrosis, to help
patients with this deadly disease [126, 118, 242, 235, 228, 16, 186].

Biofilms also cause millions of pounds worth of damage each year in industry [39]. Bacteria can not only damage the surface they are living on, by their attachment method and/or the chemicals they excrete, but these chemicals could also affect any passing fluid, damage products, transport medium and the factory itself. For example biofilm contamination and fouling occur in almost all industrial water based processes [60], which include water treatment, water distribution and pulp and paper manufacturing. Biofilms are able to cause damage by plugging and corroding pipes both within a factory and in the transportation of products, e.g. water pipes. Biofilms are a ubiquitous problem in industry, as well as within your own home. Examples here include toilet bowls as the biofilms cause cosmetic damage, food storage and preparation to stop food poisoning and drains to allow water to flow away easily.

From the information given above, we can see that biofilms are both good and bad, depending upon where they exist, their concentration and the chemicals they are consuming and producing. It is also interesting to note that even biofilms that are being used for advantageous purposes can still be damaging if their population grows above certain thresholds. Hence it all depends upon their type, population and location.

1.5 Pseudomonas aeruginosa Biofilms

It is often assumed that a biofilm is a random collection of bacteria cells all held together by EPS; although this may be correct in early biofilm development. If we extend that view forwards, then we can imagine the biofilm becoming a thick clump of randomly distributed cells, as the EPS was not produced fast enough to spread the cells out. This however would lead to the starvation of cells in
the middle of the biofilm, due to the highly dense structure, leading to limited diffusion of nutrients and the lack of water channels into the biofilm.

However, the situation is not that simple. Some types of bacteria, e.g. Pseudomonas aeruginosa, have evolved a means of communication with each other, which enables them to adjust their behaviour according to the local population density. This process has come to be known as “quorum sensing” [7, 57, 221, 147, 18, 234, 191, 112, 146], and leads to mature rather than thin undifferentiated biofilms.

### 1.5.1 Quorum sensing

Each bacteria cell (in both the biofilm and planktonic states) produces a small amount of a chemical called N-acyl homoserine lactones (AHL) [147, 179], which can diffuse across cell membranes and in the surrounding environment. The cells are able to “detect” the local concentration of this chemical as it diffuses through the system and at a “critical concentration” a reaction happens between the AHL and other chemicals within the cell. This critical concentration can only be reached if there is sufficient local cell density. Hence, this process enables cells to detect its local population density without having to move or be in direct contact with them. The chemical reactions involved are very complex and are the focus of extensive study [47]. One of these reactions is between the AHL molecule and a protein called lasR, producing a complex that attaches itself to a lux box within the DNA structure of the cell, and in doing so activates certain genes. These genes increase the production rate of both AHL and EPS and any cell that has this occupied lux box we will referred to as up-regulated, otherwise it is referred to as down-regulated.

As the up-regulated cells produce AHL at a much faster rate, we can view this as an auto-inductive process. Therefore as the critical mass of cells is reached,
Figure 1.2: Describes the formation of biofilms, with their characteristics when quorum sensing is enabled and disabled.

A very quick transfer from down- to up-regulated cells occurs due to the rapid increase in the AHL concentration. Such behaviour is believed to be advantageous to the bacteria as this leads to a rapid population wide change in gene expression at a time when there is sufficient population density to make such expression worthwhile. Examples of this in Pseudomonas aeruginosa include EPS production and virulence expression, i.e. the release of toxins and enzymes that breakdown and destroy neighbouring cells or tissues [174]. The extra EPS produced allows the cells to be held at a greater distance apart, whilst keeping the biofilm structurally sound. The EPS produced by bacteria can take many different forms, for example Pseudomonas aeruginosa uses extra-cellular DNA in early biofilm development, before using exopolsaccharide and proteinaceous compounds [81]. This sparse nature of the biofilm allows for water channels to
form within it [39], further enhancing the penetration depth of nutrients. Figure 1.2 graphically demonstrates the differences mentioned between a biofilm that has quorum sensing enabled and disabled, with the cells located in a matrix formation in the bottom right hand figure.

This decrease in population density though does not affect the AHL concentration, for two reasons, one, the EPS helps keep the chemical within the biofilm and two, the up-regulated cells are producing more of it. Both of these reasons mean that the biofilm will not lose its up regulated state. Although these water channels could make it easier for anti-bacterial chemicals and bodies to penetrate the biofilm, it has several defence techniques to protect itself, which are discussed in the next section. Even with this system to expand the biofilms to allow water and nutrients to penetrate deep into it, it has been suggested that some bacteria species, including Pseudomonas aeruginosa, deep inside use anaerobic rather than normal aerobic respiration [178, 244]. This might be one of the reasons some bacteria are persistent, even after antibiotic treatment.

The above quorum sensing method is known as gram-negative and is used by gram-negative bacteria, e.g. Pseudomonas aeruginosa, Escherichia coli, Neisseria meningitidis, Proteus mirabilis and Salmonella enteritidis. However there are also a gram-positive method, which uses a slightly different regulatory pathway for quorum sensing, and one bacteria that uses it is called Streptococcus pneumoniae. This method uses for example “competence signalling peptide” (CSP) instead of AHL, as well as using quorum sensing for (horizontal) gene transfer between cells. In this thesis though we will be concentrating on gram-negative bacteria and therefore more information on gram-positive bacteria and their differences is given in Spoering et al. (2006, [188]).

We also find that some bacteria species have multiple quorum sensing systems, each of which uses a different diffusive chemical, so that different genes
are switched on at different population densities and/or as a backup system. Pseudomonas aeruginosa for example uses the chemical 2-heptyl-3-hydroxy-4-quinolone, known as the Pseudomonas quinolone signal (PQS), to switch on several virulence factors [188]. These different quorum sensing systems can be arranged in different fashions, and for Pseudomonas aeruginosa, this has a hierarchical structure with the AHL system, mentioned above, on top [167]. This means that the AHL systems help to regulate the others below it.

The quorum sensing regulation mechanism discussed above is common in gram negative bacteria, for example it is involved in surface adhesion e.g. Serratia liquefaciens [113, 169], communication between different species living in the same biofilm e.g. Vibrio parahaemolyticus, Vibrio cholerae and Vibrio harveyi [172, 169], communication with plant and animal host e.g. Pseudomonas aureofaciens [192, 129, 191] and virulence activities e.g. Pseudomonas aeruginosa and Escherichia coli [96, 177]. Although quorum sensing can affect a wide range of features, there are various chemicals that affect it, for example iron concentration has been shown to inhibit it [240].

### 1.6 Biofilm Defence Techniques

Biofilms can defend themselves in several different ways depending upon the bacteria species located within. In nature a biofilm can consist of many different types of bacteria, each of which could have a different defence mechanism. Therefore in combination, these will protect it against many anti-bacterial chemicals and agents, though resistance will be dependent on the concentration and duration of the treatment. However there are four main defence mechanisms that bacteria use to defend themselves [30, 39], which are described below and shown in Figure 1.3.
1. As discussed earlier, as nutrients diffuse into a biofilm they are consumed, which leads to a nutrient gradient and localized micro-environment. Diffusion limitations mean that cells deep within the biofilm are exposed to fewer agents. Moreover these cells will typically have a lower metabolic activity, which will further improve their resistance to many agents. Even as the outer cells die from these agents, a nutrient gradient will still exist, although to a less extent than before. This is due to the dead cells still taking up volume and therefore hindering the diffusion of the nutrients through the fluid inside the biofilm. Though the outer cells are vulnerable, the inner cells will continue to prevail due to their lower metabolic rate and the outer portion of these will be re-nourished by the absence of the outer cell layer. Consequently, continued re-application of the agent is required to eliminate the biofilm. Planktonic cells however will always be vulnerable.

2. The bacteria themselves carry genetic code for numerous protective stress responses. Therefore as the agent penetrates the biofilm, the cells activate the appropriate stress response, which increases the difficulty for the agent to kill them [202]. This in turn implies that either a stronger concentration of agents or a different type has to be used to overcome this stress response. Now, depending upon the location of the biofilm, a stronger concentration or indeed other agents may not be possible without damaging non bacteria cells within the local area. If this is the case, however, then the current concentration must be kept for a long period. Therefore a continuous small amount of cells die, which hopefully in the long run, will kill off the biofilm. This particular method gives the biofilm a good chance of survival in areas where agent concentrations cannot be large, for example on living tissue. The disadvantage, from the bacteria’s point of view, comes from when an
agent is introduced that they lack the appropriate defence response for and hence there is a negligible stress response. Again planktonic cells are less likely to use this approach, as the agent easily overwhelms the cells before an effective stress response can be activated.

3. Some bacteria species are able to release chemicals that neutralize the agents and therefore decrease its concentration. This is a very effective method as it decreases the overall effectiveness of the agents. As in the second method, this implies that either a stronger concentration or a different type of agent is required. Both of these methods have the advantage over the first, by decreasing the number of cells around the outside of the biofilm that are actually killed. This in turn leads to the biofilm keeping its structure and size intact, whilst giving the possibility of further expansion. Planktonic cells on their own, cannot create a strong enough neutralizing chemical and therefore are still overwhelmed.

4. Bacteria cells are able to produce protected persister cells, which as their name suggests oppose the agents in several ways. These can be from decreasing the agents concentration by breaking it down, meaning that a very high concentration of agent is required to kill it and by reducing the diffusion of the agents into the biofilm. The persister cells however convert back into a susceptible state after a period of time depending upon the local environmental conditions. For example if a planktonic cell were to create one, then the conversion time is short, whilst within a biofilm this is less rapid and more are produced. This method is very similar to the second one, but here only certain cells help stop the agent from working, rather than all of them. The advantage however is that these persister cells could be produced to fight one particular type of agent only. Therefore
they give a better, stronger defence to the whole biofilm.

Each of these defence mechanisms described have their weaknesses; however in combination they can cause numerous problems for anti-bacterial agents. It also shows that biofilms have far superior defences than planktonic cells.

1.7 Anti-bacterial Drugs

From the above section we have seen that bacteria in their biofilm state have a good number of defences and are therefore often very difficult to kill. Although
it has been stated and shown that cells have the same anti-bacterial resistance, independent of whether they are in their planktonic or biofilm states [189]. Traditionally we have drugs like “penicillin”, however many bacteria are now becoming immune to these [134, 181, 11] and hence there is considerable research being undertaken into new forms of drugs and treatments, both theoretically and experimentally [196, 48, 195, 59, 168, 114, 69, 22, 233, 80, 100, 234, 10, 125, 11, 227, 124, 173]. The work done on drugs, can be broken down into two main groups, being:

- **antibiotic**: The most common form of drugs which are aimed at killing or rendering the bacteria non-viable. They tend to be very effective against planktonic cells, but less so against biofilms, due to the different defence mechanisms mentioned above [67, 31, 63, 136, 45, 230, 181, 244, 160].

- **anti-quorum sensing**: These are agents that affect the quorum sensing system, for example soaking up AHLs (anti-AHL) or the cognate protein, lasR in Pseudomonas aeruginosa, (anti-lasR), so that the complex generation can be reduced or prevented, thereby restricting cell up-regulation. However, neither of these kill any of the bacteria, but by keeping them in a down-regulated state generally make them easier to kill. The reason is that the quorum sensing regulates a vast amount of processes, e.g. virulence factors and defence mechanisms, and hence by deactivating it these factors are also switched off. [1, 7, 201, 129, 214, 170, 36, 74, 73, 167, 16, 228, 166, 235, 185, 21].

Although the majority of work is being done on drugs to kill biofilms, there are investigations into how bacteria are able to exchange genes, specifically on antibiotic drug resistance [62]. Therefore if we are able to understand this process, then maybe we can develop different types of drugs, to stop this communi-
cation. This would increase the time it takes for bacteria to become immune or have resistance to any new antibiotic drugs. Certain chemicals have also been linked to the signalling of the sloughing process, and hence by increasing the concentration of these, we can actually disperse the biofilm, i.e. convert the bacteria cells back into a planktonic state [120, 17, 14]. Hence exposing new drugs to living bacteria will be significantly reduced and so likely to prolong the time taken for bacteria to develop resistance. It would also be hard for the bacteria to form a resistance against dispersal drugs, as we are using their own natural chemical signals against them. As well as artificially produced drugs affecting biofilms, certain metals and natural chemicals have been found to do the same. Example of these include nutrients (where the greater their concentration the higher the resistance [144]), iron (known to inhibit quorum sensing [240]) and arginine and nitrate (promotes the efficiency of antibiotic drugs [25]).

1.8 Background Work that has been done on Biofilms

1.8.1 Previous Work

A great deal of research, both theoretically and experimentally, has been carried out in the field of biofilms, since the 1970s. Our knowledge of this field is continuously expanding and below is an overview of both theoretical and experimental studies, which are relevant to the models derived and studied later.

Theoretical Approaches

One of the earliest theoretical approaches was done by Williamson et al., who looked at a one dimensional biofilm [141, 51]. However many of the models pub-
lished since can be described as falling into one of three categories, continuous, discrete or a hybrid of the two. Each of these different approaches has its own advantages and disadvantages, which are mentioned below.

- **Discrete/Stochastic Models**: These can be broken down into two groups
  - grid-based, which consist of either a cellular automata [75, 156] or monte carlo [68] approaches. In either of these the domain of interest is tessellated with the same cell shape, e.g. squares or hexagons, and these are either occupied by a bacterium or not [75]. Although in some models a bacterium is able to occupy multiple neighbouring shapes simultaneously, which allows it to grow in size, whilst maintaining the same tessellation shape [68]. Using this grid based approach the bacteria for example are able to move from one shape to the next, under a given set of rules that try to mimic their behaviour. A disadvantage with this approach, is that the results may be affected by the geometry of the tessellation.
  - individual-based models [153, 190, 105, 107], which assume that each bacterium is a certain shape, e.g. circular. These shapes can then move through the domain, again under a set of rules that try to mimic the bacteria’s behaviour, however they are not allowed to overlap as this would imply that multiple bacteria are occupying the same space at the same time [107, 5]. This is different from the grid-based approach, as here the shapes move as they represent the bacteria, where in the grid-based models the bacteria can move but must occupy a particular shape.

In either the grid or individual based models a certain set of rules are applied to each bacterium to mimic their behaviour, e.g. the movement
and division of cells over a time step. An advantage of this approach is that it is relatively easy to extend the rules to account for new mechanisms. The application of the rules are often probabilistic, e.g. whether a bacterium moves to a neighbouring shape in a single time step is dependent on a probability [75]. Such probabilities may be dependent on nutrients, space and other environmental factors. These approximations often appeal to biologists, as the rules can be motivated by clear biological principles, rather than a mathematical or physical framework of continuum approaches, which require more specialist understanding. Also, any error analysis is non trivial and without a mathematical framework there is a risk that the models become aesthetically driven, i.e. the person would like the results to look appealing, rather than scientifically correct [5].

Due to the statistical nature of these models no two simulations will be identical, as indeed with biofilms in the laboratory, and hence they are sometimes referred to as stochastic models. To get an overall view of what the model can predict, multiple simulations have to be run and averages taken, before any genuine insight can be drawn from the model. This can be considerably expensive computationally, as each run may take multiple days. These models also have difficulty in modelling continuous quantities, for example the nutrient and AHL concentrations reliably. This is due to fitting a continuous field over a discrete data set and hence can lack some of the complex biological structures [155].

- **Continuum Models:** In these models all of the biofilm components, e.g. cells, water and nutrients, are represent by a continuous variable [4, 3, 141, 212, 229]. These aim to predict an “average” biofilm state at each point over the entire biofilm and this usually leads to a system of ODEs and PDEs. This approach has been extensively employed by
engineers and scientists to model experiments or bioreactors [90, 210]. However, the down side is that the system of equations can be highly non-linear and therefore the analysis and computation is a non trivial process [59, 84]. Hence this leads into analysing and solving the equations by various numerical methods.

- **Hybrid Approach:** Hybrid models break the various system component into discrete (i.e. bacteria cells and water) and continuous (i.e. nutrients and AHL) items as appropriate [5, 158, 155, 156]. Once broken down, methods from that particular category are used, for example the bacteria are often modelled as discrete entities using either the grid or individual based models. Thus the approach maintains the intuitive treatment of bacteria as individuals, using rules for how they respond to the environment, whilst treating other features as a continuum for which ODEs or PDEs can often be solved effectively.

### 1.8.2 Previous Models and Applications

Using the above methods, many different mathematical models have been produced to model biofilms and the main examples of these are given below. As well as models, software for example BacSim, AQUASIM [107, 217, 109, 108, 216, 133] and COMSTAT [79] have been produced to help quantify the results from both models and experiments.

Biofilm structure has been modelled in various ways, using both discrete [154] and continuous [52] models in one, two and three dimensions. It has been shown that as the diffusion limitation increases, the biofilm becomes thicker and more heterogeneous, i.e. the biofilm produces finger like storks on its top. On the other hand the greater the shear force on the biofilm, the flatter, more homogeneous they are [212]. However these two and three dimensional models
tended to focus on explicit areas of biofilm development, for example Eberl et al. (2000, [52]) looked at how altering the biofilm’s shape affected the transfer of nutrients into it. This model lacked any growth in the biofilm and therefore was unable to model the relationship between the growth and the nutrients gradients. Picioreanu et al. (2004, [154] allowed the biofilm to grow in both two and three dimensions but was most interested is how multi species inter-reacted with each other and the different substrates used. The more general biofilms models have been mainly one dimensional and therefore lack the two or three dimensional nature and its effects, for example the nutrient gradients, which have been shown to affect the growth and development of the biofilm [154]. An example of this is Ward et al. (2008, [219]) who included growth, nutrient diffusion through the biofilm and anti-biofilm agents all in one dimension. This thesis will initially produce and investigate a general one dimensional model, very similar to Ward et al. (2008, [219]). This will then be expanded to address the three dimensional nature of biofilms by investigating a three dimensional model in two dimensions.

As mentioned earlier quorum sensing plays an important role for certain bacteria in their biofilm development, and hence a wide range of models have been developed. Some, e.g. Dockery et al. (2000, [47]) used a continuous model to describe the interactions of all the chemical concentrations, i.e. LasR, LasI, RsaL, 3-oxo-C12-HSL, to give a detailed description of the interaction between the components of the quorum sensing system. Chopp et al. (2002, [35]) took a simpler approach and modelled the quorum sensing, by modelling the AHL concentration. By doing this, they were able to show that all the bacteria throughout the biofilm must produce the AHL to agree with experimental data. This was later extended [34], to examine biofilm structure and environmental effects for Pseudomonas aeruginosa. Again they found that all the bacteria within the biofilm had to be producing AHL to match their results with the
experimental data. Their one-dimensional model also showed that the biofilm had to reach a critical height before the quorum sensing was switched on, and this was affected by the pH of the surrounding fluid. They predicted that quorum sensing could not be switched on above a pH of approximately eleven, due to the AHL being unstable in alkaline solutions and the biofilm only growing to a finite height [34]. However as biofilms are small the pH level will be roughly uniform throughout and therefore in most cases it does not need to be considered as another factor effecting the quorum sensing system. Kong et al. (2006, [103]) looked into the quorum sensing systems of agr and luxS, in Staphylococci as they regulate biofilm detachment and reduce cell to cell adhesion respectively. Ward et al. (2001, [220]) also expanded on the work done by Dockery et al. (2000, [47]) by deriving a new model for quorum sensing, which contained few parameters. From this model he was able to show that bistable kinetics, which Dockery et al. had used, was not necessary for rapid up regulation.

Drugs are another area where a considerable amount of research is taking place. Hence many different approaches have been taken in which to model these, from continuous multi-phase models [7], to stochastic transport models [48], fluid flow chambers [38], and persister cells [175]. Anguige et al. (2003, [7]) expanded upon the Ward et al. (2001, [220]) to investigate the effects of drugs on Pseudomonas aeruginosa, which is very similar to what will be presented in this thesis. However a limitation of their model, was that it only dealt in one dimension, something that will be addressed within this thesis. Although Cogan et al. (2004, [38]) investigated the effects of drugs on a two dimensional biofilm, it had a fixed geometry and therefore was unable to grow or decay depending upon the drug’s affect. The models presented here will allow the biofilm to grow or decay depending upon the strength and type of drug being investigated.

Many more models have been produced, to study various aspects of biofilms,
which include altruism [106], bioclogging [207], biofilm shear and detachment [44, 159, 83, 239, 85], bio-reactors [90, 137, 135, 94, 84], EPS [82], fluid flow over a biofilm [157, 13, 19, 161, 53, 183, 41], fuel cells [152], industrial applications [138], instability analysis to examine the Rayleigh number on Oxytactic bacteria [9], pattern formation [130] and biofilm structure [154, 52, 212].

Some of these mathematical models use a method known as multiphase, which uses either mixture theory or averaging methods, i.e. time or volume averaging [123]. For example with volume averaging the domain is broken into small volumes and the multiphase method investigates how much volume is occupied by each component or phase, within each of them (i.e. the small volumes). It assumes that all space is occupied within these small volumes and hence for example if \( \phi_b \) and \( \phi_w \) represents the bacteria cells and water volume fractions, respectively, then the method would assume that \( \phi_b + \phi_w = 1 \). Once all the components have been identified, statements are then developed for their conservation of mass and momentum such that, for example, they are able to move around the domain and convert from one phase to another. It is this particular method that will be used within this thesis and has already been used for example by Ward et al. (2001, [220]), Ward et al. (2003, [221]), Laspidou et al. (2007, [116]), Wood et al. (1998, [231], Anguige et al. (2004, [8]), Anguige et al. (2006, [7]) to model various aspects of biofilms. The method however is not limited to just modelling biofilms but has been applied to tumour growth by Ward et al. (1997, [223]), where the two phases consisted of live and dead cells and by Byrne et al. (2002, [28]) who assumed the domain consisted of live cells and water. This method has also been used to study other physical aspects, for example O’Dea et al. (2008, [139]) used it to investigate tissues growth within a perfusion bioreactor, by Blunt (2001, [23]) to investigate fluid flow within a porous media, by Galle et al. (2009, [66]) to investigate mono-layer
cell growth where surrounded cells could not divide, by Berning et al. (1991, [20]) to investigate the gas diffusion with in a PEM fuel cell.

1.8.3 Previous Experimental Work

There have been a wide range of experiments carried out on biofilms. The biggest problem that experimentalists face is that biofilm development can be very variable, implying that reliable and repeatable experiments are a significant challenge. There are also many different physical factors that are known to affect biofilm formation and hence it is difficult to present general models of biofilm development, based on experiments. These reasons may account for some of the contradicting reports on biofilm research and why there is sometimes a weak link between the experimental and theoretical work [77].

Another limitation that occurs, is whether to work with undefined mixed- or pure-culture biofilms, as the former yields difficult to interpret and reproducible results, whilst the latter does not represent the heterogeneity inherent of natural biofilms [198]. Therefore, which type of biofilm is used depends upon what is actually trying to be measured, and in some cases both will be used to compare the results.

Until recently, it was difficult to undertake experiments to obtain reliable data from biofilms, as the measurements are often destructive to it. As a result multiple biofilms need to be grown under the same conditions and successively measured and destroyed at different intervals. However, the validity of these results is difficult to assess due to variations in growth between the biofilms. Today, however there are multiple non invasive techniques [88], for example intensity of light (differential turbidity) [97], intensity of sound (ultrasonic frequency domain reflectometry) [110, 162], colour or wavelength analysis (bioluminesence, flurometry, spectroscopy) [199, 210, 76, 242, 203, 77], mechan-
ical resonance frequencies (quartz crystal microbalance) [142], light refraction indices (surface plasmon resonance) [198], friction (pressure drops) [95, 151], scanning microscopy [150, 55, 238], and optical input signals that are modified into acoustic output signals (photoacoustic spectroscopy) [104]. Although there is a wealth of different measurement techniques, due to the chaotic nature of biofilms and the infancy of some of these techniques, producing consistent measurements and results are difficult.

As well as these non invasive techniques, some researchers have turned to programs like COMSTAT, mentioned above, to analyse the results collected from multiple experiments carried out under the same conditions. Hence this gives them qualitative results of their experiments that should be reproducible.

1.9 Overview of the Models presented in this Thesis

The overall aim is to gain a deeper insight into the role of transport mechanisms (both advective and diffusive) in biofilm growth and development. This is achieved by presenting several models in the forthcoming chapters, which are aimed at providing a general framework in identifying most of the key components of Pseudomonas aeruginosa’s biofilm development in one and two dimensions. It is this use of general rather than specific models, particularly in two dimensions, that makes this work different from others and allows us to easily investigate a wide range of situations. This includes how nutrient diffusion effects the birth and death of cells, how AHL diffusion and accumulation affect the quorum sensing process, what affect various drugs have to the general development of biofilms. Therefore we start in Chapter 2, by deriving and investigate two one dimensional models, the first incorporating the birth and death of bac-
teria, with nutrient diffusing through it from an infinite source located above the biofilm. Whilst the second expands this one dimensional model to incorporate the quorum sensing process. This is followed in Chapter 3 with a two dimensional model, sitting in a static water environment, which includes all of the above features. It assumes, due to the static water, that there are no external forces, whilst dealing with the ones, inside the biofilm. A small extension to this model is investigated, where the nutrients are assumed to diffuse through the still water that surrounds the biofilm, rather than having a constant concentration around the biofilm/fluid boundary. Chapter 4, then expands upon the model presented in Chapter 3 to include antibiotics and anti-quorum sensing drugs, so that we can investigate their effects both separately and combined.

In each of the models we use a continuum approach, which leads to complicated systems of interconnected partial differential equations and hence numerical approaches are required to obtain their solution. In particular computer programs were produced in Fortran 90 and we used a finite difference approach to convert the differential equations into difference equations. These were then solved on a fixed rectangular mesh and depending upon the type of equation and whether it could be decoupled, various numerical solvers were applied. These include:

- upwind schemes: these are used on the biofilm component equations as they can be decoupled from the rest of the system,
- a backward difference scheme: this was used to solve the bacteria’s advective velocity field in the the one dimension models, i.e. Model 1 and 2,
- a central difference scheme in conjunction with Thomas’s tridiagonal matrix solver: this is used to solve the nutrient and AHL concentration equa-
tions in the one dimensional models as it is fast and efficient,

- trapezoidal rule: this is used to solve the integral water velocity equation over the length of the biofilm in the one dimensional models,

- least square conjugate gradient solver: in the two dimensional models the advective velocity, water velocity and water pressure equations did not decouple from each other and due to their two dimensional nature formed square indefinite (i.e. not positive or negative definite) matrices. Therefore least squares conjugate gradient solver was used, as it is efficient at solving a wide variety of indefinite matrix equations and is largely based on the conjugate gradient solver. Furthermore this method was used to solve both the AHL and nutrient equations in the two dimensional models, as the extra dimension meant that the central difference scheme applied to them, no longer produced tridiagonal matrices.

The majority of the numerical methods applied to the two dimensional models were also parallelised, such that they could make full use of the multi-core architecture inside the computer. This allowed the program to have an almost linear speed increase with the number of the cores, as the majority of time was spent solving matrix equations. Further speed increases were achieved by only solving the equations at points within the biofilm and compressing the matrices such that only the non-zero values and their locations were stored. For more information and a greater understanding of the numerical methods used and their parallelisation see Appendix A and C. Also as a fixed mesh grid was employed and we allowed the biofilm to grow, this meant that we had to track its moving boundary. This was achieved by using the level set technique, which uses the characteristic nature of hyperbolic equations to track a moving boundary. In particular the initial shape of the biofilm is given and then all points within the
domain are given the closest distance between themselves and the initial shape of the biofilm. This is achieved by using the fast marching process, which is detailed in Section B.2. This distance is also signed, which implies whether the point is inside or outside of the biofilm and we will assume negative distances mean inside. Therefore at all the points inside the biofilm have a negative distance to the biofilm’s boundary, all points with a positive distance are outside the biofilm and most importantly all points with a zero value lie on the biofilm boundary. Then when the level set equation is applied to this signed distance the zero valued line moves with the biofilm growth, as it forms a characteristic of the hyperbolic equation. Hence this allows us to tell where the biofilm boundary is at anytime and more information on this method is given in Appendix B.

An overall conclusion to the results produced from the various models is given in Chapter 5, with ideas on further work.
Chapter 2

Modelling One Dimensional Biofilm Growth

In this chapter we will investigate the early stages of biofilm development through the production and simulation of two different models. The first deals with very early biofilm development, where we assume that the biofilm consists of only live and dead cells plus water. Furthermore we assume that the water volume fraction is approximately constant throughout the biofilm and that the quorum sensing system is disabled. This model is very similar to Ward et al. (1997, [223]), although Ward is investigating tumour growth rather than bacteria growth. The two are similar in many aspects, which include dealing with cells that are dividing and dying, an advective velocity moving the cells through their domain and a nutrient diffusing through the system. There are also differences in that Ward only deals with live and dead cells, whilst we include them plus water in our biofilm model, plus we use different equations relating to the how fast the cells divide, die and consume nutrients. The second extends the first to investigate a slightly later stage of development, where the quorum sensing system, as described in Section 1.5.1, is about to be switch on. This leads to
the water volume fraction being a function of the EPS rather than a constant. Both models are one dimensional and their basic schematic is shown in Figure 2.1.

2.1 Model 1: Early Biofilm Development

2.1.1 Introduction to the Model

This particular model is very basic, and assumes that the bacteria divide (i.e. one cell divides to give two cells) and die, whilst a nutrient flows through the biofilm and is used up by the living cells within. This nutrient then generates growth, which creates biofilm volume and hence expansion upwards. Figure 2.1 is a schematic of the biofilm domain to be modelled.

2.1.2 Model Derivation

In this model the biofilm is assumed to consist only of live and dead cells plus water. Due to the large number of cells that are present within a biofilm we consider the volume fraction of these types, which allows for more manageable values. The live and dead cell volume fractions are then referred to as $b(z,t)$
and \( p(z,t) \) respectively, where \( z > 0 \) is the coordinate above the solid surface located at \( z = 0 \), and \( t \) represents time. The bacteria need nutrients from the water to survive and these diffuse through the biofilm from the top. We assume all space is occupied by live and dead cells and water, \( w(z,t) \), hence

\[
b + p + w = 1. \tag{2.1}
\]

The biofilm expands as the bacteria cells divide and grow forcing the neighbouring cells to move to accommodate the new volume. This advective movement is described by a velocity field \( v(z,t) \). The height of the biofilm \( H(t) \) moves at a rate given by the local velocity field, hence

\[
\frac{dH}{dt} = v(H(t),t). \tag{2.2}
\]

Let \( c(z,t) \) be the nutrient concentration, and we assume that nutrient transfer in and out of cells is sufficiently rapid, such that the internal and external concentrations are equal. From the information given above we can now write down some equations governing \( b(z,t) \), \( p(z,t) \), \( w(z,t) \), \( c(z,t) \):

\[
\frac{\partial b}{\partial t} = -\frac{\partial}{\partial z} J_b + (k_b(c) - k_d(c))b, \tag{2.3}
\]

\[
\frac{\partial p}{\partial t} = -\frac{\partial}{\partial z} J_p + k_d(c)b, \tag{2.4}
\]

\[
\frac{\partial w}{\partial t} = -\frac{\partial}{\partial z} J_w - k_b(c)b, \tag{2.5}
\]

\[
\frac{\partial c}{\partial t} = -\frac{\partial}{\partial z} J_c - k_n(c)b, \tag{2.6}
\]

where \( J_b(z,t), J_p(z,t), J_w(z,t), J_c(z,t) \) are the fluxes of \( b(z,t) \), \( p(z,t) \), \( w(z,t) \), \( c(z,t) \) respectively.

The fluxes describe advective motion of cells and nutrients, the latter being
also transported by diffusion. Diffusion, here, is modelled using Fick’s Law, i.e. 
\[ J_{\text{diff}} = -D \frac{dc}{dz}, \]
where \( J_{\text{diff}} \) is the diffusive flux and \( D \) the diffusion constant. Therefore

\[
\begin{align*}
J_b &= vb, \\
J_p &= vp, \\
J_w &= uw, \\
J_c &= v(b + p)c + uw c - D \frac{dc}{dz},
\end{align*}
\]

where \( u(z) \) is the velocity of the water that is generated by cell movement and \( D \) is the nutrient diffusion coefficient. Equations (2.7) - (2.9) state that the live and dead cells, plus water only move through the domain due to advection or the underlying fluid flow in the water’s case, i.e. they do not diffuse. Equation (2.10) though states that the nutrients will be advected through the system by the movement of the live and dead cells, i.e. \( v(b + p)c \), as well as by the movement of the water, i.e. \( uw c \). However, unlike the cells and the water, it will also diffuse through the system, as there is no interconnection between its particles or molecules and this is represented by the \( D \frac{dc}{dz} \) term. From the conservation of mass of \( b(z,t) \), \( p(z,t) \) and \( w(z,t) \), equations (2.3), (2.4), (2.5) imply that

\[
\frac{\partial}{\partial z}(J_b + J_p + J_w) = 0,
\]

hence,

\[
\frac{\partial}{\partial z}(v(b + p) + uw) = 0.
\]
We assume that material cannot cross the substrate boundary at \( z = 0 \), hence \( v(0, t) = u(0, t) = 0 \) and integrating (2.12) thus yields

\[
v(b + p) + uw = 0. \tag{2.13}
\]

Combining (2.1) and (2.13) gives

\[
u = \frac{v(b + p)}{w} = \frac{v(1 - w)}{w}, \tag{2.14}
\]

reducing the nutrient flux term to

\[
J_c = -D_c \frac{\partial c}{\partial z}. \tag{2.15}
\]

Equations (2.3), (2.4) and (2.5) become upon substituting (2.7), (2.8) and (2.10)

\[
\frac{\partial b}{\partial t} + \frac{\partial}{\partial z}(vb) = (k_b(c) - k_d(c))b, \tag{2.16}
\]
\[
\frac{\partial p}{\partial t} + \frac{\partial}{\partial z}(vp) = k_d(c)b, \tag{2.17}
\]
\[
\frac{\partial c}{\partial t} - D_c \frac{\partial^2 c}{\partial z^2} = -k_n(c)b. \tag{2.18}
\]

A useful formula is now derived by adding equations (2.16) and (2.17) to give

\[
\frac{\partial}{\partial t}(b + p) + \frac{\partial}{\partial z}(v(b + p)) = k_b(c)b, \tag{2.19}
\]

and can be simplified from (2.1) to give

\[
\frac{\partial}{\partial t}(1 - w) + \frac{\partial}{\partial z}(v(1 - w)) = k_b(c)b. \tag{2.20}
\]

Equation (2.16) - (2.18) and (2.20) are four partial differential equations describing the unknown variables \( b, p, w, c \) and \( v \), hence the system needs an-
other equation for closure. For simplicity we adopt the constitutive relation
\( w(z, t) = w_0 \) (a constant), i.e. the water volume fraction is fixed throughout
the biofilm, and is a suitable assumption in the early stages of biofilm growth.
Hence from equation (2.20) we obtain

\[
\frac{\partial v}{\partial z} = \frac{k_b(c)b}{1 - w_0}, \tag{2.21}
\]

Also as \( w(z, t) \) is now a constant we can decouple either \( b(z, t) \) or \( p(z, t) \) from
the system of equations by rearranging equation (2.1) into the form

\[
p = 1 - w_0 - b. \tag{2.22}
\]

Therefore the system of equations reduces to the following set

\[
\begin{align*}
\frac{\partial b}{\partial t} + \frac{\partial}{\partial z}(vb) &= (k_b(c) - k_d(c))b, \tag{2.23} \\
\frac{\partial c}{\partial t} &= D_c \frac{\partial^2 c}{\partial z^2} - k_n(c)b, \tag{2.24} \\
\frac{\partial v}{\partial z} &= \frac{k_b(c)b}{1 - w_0}, \tag{2.25} \\
\frac{dH(t)}{dt} &= v(H(t), t), \tag{2.26}
\end{align*}
\]

where we can assume

\[
\begin{align*}
k_b(c) &= \frac{A_{bd}c}{1 + c}, \tag{2.27} \\
k_d(c) &= B \left( 1 - \frac{\sigma c}{1 + c} \right), \tag{2.28} \\
k_n(c) &= Fc, \tag{2.29}
\end{align*}
\]

where \( A_{bd}, B, F \) and \( \sigma \) are all non negative constants. In particular \( A_{bd} \) and \( B \)
represent the birth and death rate for a particular nutrient concentration and
\( \sigma \) is a non-dimensional scaling constant that affects the curvature of the death
rate as it changes with nutrient concentration. Lastly \( F \) is the rate at which a fixed bacteria volume fraction will consume nutrients. It has been assumed that the birth, \( k_b \), and death, \( k_d \), rates are monotonically increasing and decreasing, respectively, saturating functions of the nutrient. We have assumed that \( k_n \) is a monotonically increasing function of \( c \), reflecting the more food that is available the greater amount eaten, as expected. The current function of \( k_n \) is the simplest function with that behaviour.

### 2.1.3 Boundary and Initial Conditions

Equations (2.23) - (2.25) form a system of non-linear partial differential equations for \( b, v \) and \( c \) together with the surface coordinate \( z = H(t) \), which moves according to (2.26). The advective velocity equation, (2.25), is an ODE and we assume that any bacteria in contact with the solid surface on \( z = 0 \) have adhered to it and therefore we let \( v = 0 \) at \( z = 0 \). Equation (2.23) is a first order hyperbolic differential equation and therefore has characteristics defined by \( dz/dt = v \). However we just let \( v = 0 \) on \( z = 0 \) and at \( z = H(t) \) we have \( v = dH/dt \) from equation (2.26), hence \( z \equiv 0 \) and \( z = H(t) \) are characteristics of equation (2.23). The method of characteristics state, that you only need an initial condition to solve along a characteristic. Also by definition no characteristics are entering or leaving at \( z = 0 \) and \( z = H(t) \), assuming the advective velocity remains finite, which seems reasonable as we are modelling living creatures, we only need an initial condition to solve equation (2.23). Equation (2.24) forms a parabolic differential equation and therefore requires two boundary and an initial condition. We assume that no nutrients can penetrate the solid surface at \( z = 0 \) and that they have a fixed concentration on the boundary \( z = H(t) \). Therefore collating this information gives us the following boundary and initial
conditions. On the boundary \( z = 0 \) we have

\[
v = 0, \quad \frac{\partial c}{\partial z} = 0.
\]  

(2.30)

At the top of the biofilm \( z = H(t) \)

\[
c(H(t), t) = c_0,
\]  

(2.31)

where \( c_0 \) is a positive constant. Initially we assume that all the bacteria cells are alive, the nutrient concentration is given by the function \( c_I(z) \) and the height of the biofilm is given by \( H_0 \), therefore

\[
b(z, 0) = 1 - w_0, \quad c(z, 0) = c_I(z) \quad H(0) = H_0.
\]  

(2.32)

### 2.1.4 Non-Dimensionalisation

In order to reduce the number of parameters and to perhaps systematically simplify the model we non-dimensionalise equations (2.23) - (2.32). All hatted variables from this point onwards will refer to dimensionless variables. Let

\[
t = \frac{\hat{t}}{A_{bd}}, \quad z = H_0 \hat{z}, \quad v = H_0 A_{bd} \hat{v}, \quad c = c_0 \hat{c} \quad H = H_0 \hat{H}.
\]  

(2.33)

Here we have rescaled time with the maximum birth rate and space with the initial height of the biofilm. We also note that the \( b(z, t), w_0 \) and \( \sigma \) are all dimensionless. If we substitute these equations into the nutrient equation, i.e. (2.24), then we obtain

\[
\beta t \frac{\partial c}{\partial t} = \frac{\partial^2 c}{\partial z^2} - \beta_0 b \hat{c},
\]  

(2.34)
where $\beta_t = H_0^2 A_{bd}/D_c$ and represents the ratio of nutrient consumption to the diffusion of nutrients over the biofilm, and $\beta_6 = \frac{F H_0^2}{D_c}$, which represents the ratio of consumption to diffusion over the length of the biofilm. However $\beta_t$ is the ratio of rate of cell birth to the rate at which nutrients can diffuse over the length of the biofilm, which is small as the birth rate is in hours whilst the nutrient diffusion is in seconds. Therefore $\beta_t << 1$ and we can assume that the nutrients are quasi-steady. Furthermore if we expand out the partial differentials in equations (2.23) - (2.29) and substitute in equations (2.33), as well as assuming the quasi-steady form for $c(z, t)$, we get

$$\frac{\partial b}{\partial \hat{t}} + \hat{v} \frac{\partial b}{\partial \hat{z}} = (\hat{k}_b(\hat{c}) - \hat{k}_d(\hat{c})) b - \hat{k}_b(\hat{c}) \frac{b^2}{1 - w_0}, \quad (2.35)$$

$$\frac{\partial^2 \hat{c}}{\partial \hat{z}^2} = \beta_6 b \hat{c}, \quad (2.36)$$

$$\frac{\partial \hat{v}}{\partial \hat{z}} = \frac{\hat{k}_b(\hat{c})}{1 - w_0} b, \quad (2.37)$$

$$\frac{\partial \hat{H}}{\partial \hat{t}} = \hat{v}(\hat{H}(\hat{t}), \hat{t}), \quad (2.38)$$

where

$$\hat{k}_b(\hat{c}) = \frac{\hat{c}}{1 + \hat{c}}, \quad (2.39)$$

$$\hat{k}_d(\hat{c}) = D \left(1 - \frac{\sigma_{bd} \hat{c}}{1 + c_{bd} \hat{c}}\right), \quad \text{where} \quad D = \frac{B}{A_{bd}}, \quad (2.40)$$

$$\beta_6 = \frac{F H_0^2}{D_c}. \quad (2.41)$$

Here $D$ represents the ratio of the death to birth rate of cells. Now all that remains is to substitute the non-dimensional variables into the initial and boundary conditions given in equations (2.30) - (2.32). The boundary conditions on $\hat{z} = 0$ are

$$v(\hat{z}) = 0, \quad \frac{\partial \hat{c}}{\partial \hat{z}} = 0. \quad (2.42)$$
The boundary conditions on $\hat{z} = \hat{H}(\hat{t})$ are

$$\hat{c}(\hat{H}(\hat{t}), \hat{t}) = 1.$$ \hfill (2.43)

The initial conditions are

$$b(\hat{z}, 0) = 1 - w_0, \quad \hat{H}(0) = 1,$$

where the nutrient condition has been dropped due to its quasi-steady behaviour. The hats are now dropped in the rest of the model, as we will only be referring to the non-dimensional values.

### 2.1.5 Numerical Methods

The model consists of coupled non-linear partial differential equations, which appear not to be solvable analytically, hence we resort to approximating them numerically. We used a finite difference approach [33], with a fixed rectangular mesh grid, whilst using equation (2.38) to track the moving boundary. In particular an upwind scheme [64] was used for the bacteria volume fraction, whilst a central difference with Thomas’ method [61] was used for solving the nutrients. The velocity equation transformed into an integral equation, which was solved using the trapezoidal rule [61]. More information on these various numerical techniques and why they were chosen are given in Appendix A.

These various finite difference approaches converted the system of PDE equations into first order difference equations, which were solved using a computer program written in Fortran [54, 187]. Figure 2.2 illustrates the order in which the different variables were solved, within the program.

A code validation check was also done on the Fortran code, by comparing the calculated height of the biofilm for various space steps sizes of the mesh grid.
Define the initial data

Update ‘b’ using the upwind method

Update ‘v’ using a backwards finite difference stencil

Calculate ‘c’ using Thomas’ method

Update the height of the biofilm

Time = Time + dt

Loop

Figure 2.2: A flow diagram of how Model 1 was solved and what methods were employed for the different variables.

<table>
<thead>
<tr>
<th>Initial values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_0$ = 0.8</td>
<td>$w_0$ = 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_6$ = 100</td>
<td>$\alpha_{bd}$ = 200 Est</td>
</tr>
<tr>
<td>$D$ = 1 Est</td>
<td>$\sigma$ = 1 Est</td>
</tr>
</tbody>
</table>

Table 2.1: The initial and parameter values used in model 1’s results.
Table 2.2: This shows the calculated height of the biofilm for various values of \( \delta x \) (normalised such that the height at \( \delta x = 0.01 \) equals 1), and \( \delta t = 0.0001 \), as well as their absolute error compared to the height calculated when \( \delta x = 0.01 \).

The results from this are shown in Table 2.2 and we see that all the errors are sufficiently small compared to the order of \( \delta x \). This is the maximum theoretical error and hence implies that the Fortran code is consistent.

### 2.1.6 Results

Table 2.1 gives the initial and parameter values used to obtain the results shown in this section. We assumed that the fixed water volume fraction within the biofilm, \( w_0 \), to be 0.2, as in early development all the bacteria are very closely packed together due to negligible EPS. The parameters linked to the birth and death rates, are estimated based upon the fact that the higher the nutrient concentration the more births and less deaths, whilst the equilibrium point occurred when \( c(z, t) = 0.1 \). The other parameter, \( \beta_6 \), was obtained from the reference given, although it was scaled. The scaling was done such that the initial distribution of nutrients just penetrated the whole biofilm.

Figure 2.3 shows how the height of the biofilm changes with time and initially the biofilm has a slightly faster than linear expansion rate, before the growth becomes linear from approximately \( t = 2 \). This change in growth rate is due to the biofilm initially being very small and compact, allowing the nutrients to
Figure 2.3: The evolution of the biofilm’s height, using the initial and parameter values given in Table 2.1.

Figure 2.4: Evolution of the bacteria volume fraction throughout the biofilm, between times $t = 2.5$ and $t = 20$ in steps of $t = 2.5$, moving from left to right, and using the initial and parameter values given in Table 2.1.
Figure 2.5: Evolution of the nutrient concentration within the biofilm, between times $t = 0$ and $t = 20$ in steps of $t = 2.5$, moving from left to right, and using the initial and parameter values given in Table 2.1.

Figure 2.6: Evolution of the advective velocity of the biofilm, between times $t = 0$ and $t = 20$ in steps of $t = 2.5$, moving from left to right, and using the initial and parameter values given in Table 2.1.
fully penetrate it, as shown in Figure 2.5. Therefore all of the cells within the biofilm are dividing causing the biofilm to initially expand at a faster rate. This is shown by the first line in Figure 2.6, since it has no section that is approximately zero, i.e. all cells within the biofilm are dividing, creating an upwards velocity. Shortly afterwards however, the nutrients are no longer able to fully penetrate the biofilm, leading to cell death near the bottom and hence a decrease in the bacteria volume fraction, as shown in Figure 2.4. Plus only a certain range of cells within the biofilm, defined by the penetration depth, have sufficient nutrients to divide, which leads to the linear growth rate observed. This is shown by the straight line in Figure 2.3 and the constant maximum velocity seen from $t = 2.5$ onwards in Figure 2.6. However as the biofilm is still growing the nutrient penetration depth moves with it, creating a travelling wave effect, which is seen in all figures. This long term linear growth rate is in broad agreement with observations made by Bujler et al. (1998, [27]). These results also correlate with previously published work of Ward et al. (2003, [221]) and Anguige et al. (2006, [7]), as their results also showed these travelling wave propagating in the direction of increasing $z$. The travelling wave solutions of a similar model was investigated by Ward et al. (1997, [223]), where if chosen $k_n = \beta_6 k_b$, we find in the limit of $D \to 0$, the wave speed $U$ is approximately

$$U \sim \sqrt{\frac{2 \int_0^1 k_b(c)dc}{\beta_6}}.$$  \hspace{1cm} (2.45)

The results also showed that the highest volume fraction of bacteria cells existed at the top of the biofilm and decreases as you move deeper into it. This profile of live bacteria cells with the biofilm, is in broad agreement with Webb et al. (2003, [226]). In terms of the advective velocity of the biofilm, we see that this is approximately zero over the majority of the biofilm, due to the lack of
bacteria dividing, which correlates well with Anguige et al. (2006, [7]).

2.2 Model 2: Biofilm Growth with Quorum Sensing Enabled

2.2.1 Introduction to the Model

In this model we investigate the effect quorum sensing has on biofilm growth and development. Based on the discussion in Section 1.5.1, we will model live cells as being either down- or up-regulated, as well as model the EPS volume fraction and AHL concentration. Hence we expect this model to describe the processes of a biofilm after its very early development.

2.2.2 Model Derivation

The quorum sensing process involves the switching on and off of genes within the cells and therefore the bacteria consist of two sub-populations, which we term down- and up-regulated cells. As in Model 1, we are dealing with cell volume fractions within the biofilm and hence in this model we let \( b_d(z,t) \) and \( b_u(z,t) \) represent down- and up-regulated volume fractions respectively. Note that \( b = b_d + b_u \) where \( b \) refers to the total bacteria volume fraction, as used in Model 1. This gave us the following two equations

\[
\frac{\partial b_d}{\partial t} + \frac{\partial}{\partial z}(v b_d) = (k_{bd}(c) - k_{dd}(c))b_d - \alpha_1 Ab_d + \alpha_5 b_u + (2 - \gamma)k_{bu}(c)b_u, \tag{2.46}
\]

\[
\frac{\partial b_u}{\partial t} + \frac{\partial}{\partial z}(v b_u) = ((\gamma - 1)k_{bu}(c) - k_{du}(c))b_u + \alpha_1 Ab_d - \alpha_5 b_u, \tag{2.47}
\]
where all the $\alpha_i$’s are constants and $\alpha_1$ represents the rate of up regulation and $\alpha_5$ the rate of down regulation and therefore is less than $\alpha_1$. The $k_{ij}(c)$’s represent the birth and death functions which will be defined later. The parameter $\gamma$ is used to give us the average number of up-regulated cells following cell division. Hence $\gamma \in [0, 2]$, such that for $\gamma = 0$ an up-regulated cell divides to produce two down-regulated cells and for $\gamma = 2$ it produces two up-regulated cells. This allows us to look at all the combinations without major changes to the equations each time. We assumed that a cell becomes up regulated mutually proportional to the AHL concentration, $A(z, t)$, and the volume fraction of down-regulated cells, i.e. $\alpha_1 A b_d$, whilst also allowing the up-regulated cells to down regulate at a slower rate proportional to their volume fraction.

The nutrient concentration equation remains unchanged from the first model, i.e.

$$\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial z^2} - k_n(c)(b_d + b_u).$$

$$\text{(2.48)}$$

In this model, we decided to use a level set approach rather than explicitly defining the height, by the rate of its change being equal to the local velocity field, i.e. $\frac{dH}{dt} = v(H, t)$. Although this is overkill in a one dimensional environment, this is the approach employed in the higher dimensional studies in the following Chapters. The level set function $\phi(z, t)$ is defined so that the top of the biofilm is located initially at $\phi = 0$ and evolves according to

$$\frac{\partial \phi}{\partial t} = -v \frac{\partial \phi}{\partial z},$$

$$\text{(2.49)}$$

and using $\frac{dH}{dt} = v(H, t)$ we have the characteristic $\phi(H, t) = 0$ for all time. Thus solving the PDE for $\phi(z, t)$ in this way means we do not explicitly track the moving boundary $z = H(t)$. More details on this method can be found in
Appendix B.

In Model 1 we imposed the constitutive relation $b + p + w = 1$, here we extend this to account for the volume fraction of EPS $E(z,t)$. We have also assumed that any dead cells are converted into water, on a faster time scale than cell division, allowing us to slightly simplify the model. This assumption has negligible impact on the model, as the dead cells consume volume, limiting the water flow. Therefore we have

$$1 = b_d + b_u + E + w. \quad (2.50)$$

The AHL concentration is produced by all the cells, but at a considerably faster rate by the up-regulated ones. It is assumed that down-regulated cells become up regulated by a simple reaction with the AHL molecules and we simply apply the law of mass action. We also assume that the AHL molecules are present in the water and treated similarly to the nutrients, but unlike them the AHL breaks down at a constant rate. Putting all of this information together we get

$$\frac{\partial A}{\partial t} = D_A \frac{\partial^2 A}{\partial z^2} - k_A A + \alpha_2 b_u - \alpha_3 b_d A + \alpha_4 b_d, \quad (2.51)$$

where $k_A$, $\alpha_i$’s and $D_A$ are all constants. In particular $k_A$ represents the rate of degradation, $\alpha_2$ and $\alpha_4$ are the rate of production of AHL from up- and down-regulated cells respectively and $\alpha_3$ the rate at which AHL is used, up regulating the bacteria. Also since AHL production in up-regulated cells is assumed to be much higher than that of down-regulated cells, then $\alpha_4 \ll \alpha_2$.

The EPS is produced by living cells at a rate dependent on nutrients and whether they are down- or up-regulated, it naturally decays and being attached
to cells it drifts along with them, hence

\[
\frac{\partial E}{\partial t} + \frac{\partial}{\partial z} (vE) = (\alpha_{su}b_u + \alpha_{sd}b_d)c - \alpha_{10}E, \quad (2.52)
\]

where \(\alpha_{si}\) are the production rate of EPS by the down cells and up-regulated cells, and \(\alpha_{10}\) is the rate at which the EPS degrades.

The equation for the biofilm velocity again is obtained in the same way as for Model 1, by adding equations (2.17), (2.46), (2.47) and (2.52) to give

\[
\frac{\partial}{\partial t} (b_d + b_u + E) + \frac{\partial}{\partial z} (v(b_d + b_u + E)) = (k_{bd}(c) - k_{dd}(c))b_d + (k_{bu}(c) - k_{du}(c))b_u + (\alpha_{su}b_u + \alpha_{sd}b_d)c - \alpha_{10}E.
\quad (2.53)
\]

We will also assume that any small change in the EPS, will affect the water volume fraction using the simple constitutive relation

\[
w = w_0 + \alpha E, \quad (2.54)
\]

where \(0 < w_0 < 1\) is a constant and \(\alpha\) is a non negative proportionality constants. Hence this assumption implies that as EPS is produced it generates space, which is infiltrated by water. This causes a local expansion forcing apart the neighbouring particles to accommodate the volume of the infiltrating water. Also the constant \(w_0\) can be interpreted as the maximum packing density of the bacteria cells.

Equation (2.54) along with (2.50) allows us to simplify equation (2.53) to
give
\[
\frac{\partial v}{\partial z} = \frac{(k_{bd}(c) - k_{dd}(c))b_d + (k_{bu}(c) - k_{du}(c))b_u}{1 - w_0} \\
+ \frac{(1 + \alpha)}{1 - w_0} ((\alpha_{8u}b_u + \alpha_{8d}b_d)c - \alpha_{16}E).
\]

(2.55)

The equation for the water’s velocity is obtained from the water’s volume fraction equation, being
\[
\frac{\partial (w + w_0)}{\partial t} + \frac{\partial}{\partial z} (u(w + w_0)) = -(k_{bu} + k_{du})b_u - (k_{bd} + k_{dd})b_d,
\]

(2.56)

which on substitution of \( w = w_0 + \alpha E \) gives
\[
\alpha \frac{\partial E}{\partial t} + 2w_0 \frac{\partial u}{\partial z} + \alpha \frac{\partial}{\partial z} (uE) = -(k_{bu} + k_{du})b_u - (k_{bd} + k_{dd})b_d.
\]

(2.57)

Now if we add and subtract \( \alpha \frac{\partial}{\partial z} (vE) \) on the left hand side of the equation and substitute equation (2.52) gives
\[
2w_0 \frac{\partial u}{\partial z} + \alpha \frac{\partial}{\partial z} ((u - v)E) = -\alpha((\alpha_{8u}b_u + \alpha_{8d}b_d)c - k_E E) \\
-(k_{bd}(c) - k_{dd}(c))b_d \\
-(k_{bu}(c) - k_{du}(c))b_u.
\]

(2.58)

Now we can integrate both sides with respect to \( z \) and rearrange to give \( u \),
\[
u = \frac{\alpha v E}{2w_0 + \alpha E} \\
- \frac{1}{2w_0 + \alpha E} \int_{0}^{z} \alpha((\alpha_{8u}b_u + \alpha_{8d}b_d)c - k_E E) \\
+ (k_{bu} - k_{du})b_u + (k_{bd} - k_{dd})b_d dz' \]

(2.59)
Therefore the full system of equations are:

\[
\frac{\partial b_d}{\partial t} + \frac{\partial}{\partial z}(vb_d) = (k_{bd}(c) - k_{dd}(c))b_d - \alpha_1 Ab_d + \alpha_5 b_u + (2 - \gamma)k_{bu}(c)b_u, \quad (2.60)
\]

\[
\frac{\partial b_u}{\partial t} + \frac{\partial}{\partial z}(vb_u) = ((\gamma - 1)k_{ba}(c) - k_{du}(c))b_u + \alpha_1 Ab_d - \alpha_3 b_u, \quad (2.61)
\]

\[
\frac{\partial A}{\partial t} = D_A \frac{\partial^2 A}{\partial z^2} - k_A A + \alpha_2 b_u - \alpha_3 b_d A + \alpha_4 b_d, \quad (2.62)
\]

\[
\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial z^2} - k_n(c)(b_d + b_u), \quad (2.63)
\]

\[
\frac{\partial \phi}{\partial t} = -v \frac{\partial \phi}{\partial z}, \quad (2.64)
\]

\[
\frac{dH}{dt} = v(H, t), \quad (2.65)
\]

\[
\frac{\partial E}{\partial t} + \frac{\partial}{\partial z}(vE) = (\alpha_{8u} b_u + \alpha_{8d} b_d)c - \alpha_{10} E, \quad (2.66)
\]

\[
\frac{\partial v}{\partial z} = \frac{(k_{bd}(c) - k_{dd}(c))b_d + (k_{ba}(c) - k_{du}(c))b_u}{1 - w_0} + \frac{(1 + \alpha)}{1 - w_0} ((\alpha_{8u} b_u + \alpha_{8d} b_d)c - \alpha_{10} E), \quad (2.67)
\]

\[
u = \frac{\alpha v E}{2 w_0 + \alpha E} - \frac{1}{2 w_0 + \alpha E} \int_0^z \alpha ((\alpha_{8a} b_u + \alpha_{8d} b_d)c - k_E E) \\
+ (k_{bu} - k_{du}) b_u + (k_{bd} - k_{dd}) b_d \, dz', \quad (2.68)
\]

where

\[
k_{bd}(c) = \frac{A_{bd} c}{c_0 + c}, \quad (2.69)
\]

\[
k_{ba}(c) = \frac{A_{ba} c}{1 + c}, \quad (2.70)
\]

\[
k_{dd}(c) = B_{dd} \left(1 - \frac{\sigma c}{1 + c}\right), \quad (2.71)
\]

\[
k_{du}(c) = B_{du} \left(1 - \frac{\sigma c}{1 + c}\right), \quad (2.72)
\]

\[
k_n(c) = \frac{c}{c_1 + c}. \quad (2.73)
\]
These sub-equations for the birth, $k_b$, and death, $k_d$, rate came directly from Model 1 and were used for the same reasons. A different form of nutrient consumption, $k_n$, was used to account for the fact that the bacteria can only consume up to a fixed amount of nutrients, independent of the local concentration.

### 2.2.3 Boundary and Initial Conditions

To complete the system of equations (2.60) - (2.68), we need to apply boundary and initial conditions to them. From Model 1, we know that equations for the biofilm components (i.e. the bacteria cells and EPS) only require an initial condition, whilst the nutrients require an initial and two boundary conditions.

The AHL concentration is given as a parabolic differential equation and therefore the same conditions as the nutrients and just like them we assume that they cannot penetrate the solid surface at $z = 0$. Above the biofilm we assume a Robin boundary condition, namely

$$\frac{\partial A(H,t)}{\partial z} = -\frac{Q}{D_A} A, \quad (2.74)$$

where $Q$ is a positive constant and $D_A$ is defined as above. Therefore the full set of boundary conditions on the solid impermeable surface at $z = 0$ are

$$\frac{\partial A(0,t)}{\partial z} = \frac{\partial c(0,t)}{\partial z} = v(0) = 0. \quad (2.75)$$

At the top of the biofilm, $\phi(z,t) = 0$, we have

$$\frac{\partial A(H,t)}{\partial z} = -\frac{Q}{D_A} A, \quad c(H,t) = c_0. \quad (2.76)$$
Initially we assume the biofilm has a height $H_0$ and consists only of down-regulated bacteria with no AHL or EPS present. Hence

\begin{align}
  b_u(z, 0) &= E(z, 0) = 0, \quad b_d(z, 0) = 1 - w_0, \quad A(z, 0) = 0, \quad (2.77) \\
  c(z, 0) &= c_I(z) \quad \phi(z, 0) = \frac{z}{H_0} - 1, \quad (2.78)
\end{align}

where $c_I(z)$ are the initial distribution of nutrients throughout the biofilm.

### 2.2.4 Non-Dimensionalisation

We rescale the system of equations (2.60) - (2.73) using

\begin{align}
  A &= A_0 \hat{A}, \quad c = c_0 \hat{c}, \quad z = H_0 \hat{z}, \quad v = H_0 A_{bd} \hat{v}, \quad (2.79) \\
  t &= \frac{\hat{t}}{A_{bd}}, \quad u = H_0 A_{bd} \hat{u}, \quad (2.80)
\end{align}

where all hatted variables are dimensionless. We note at this point that $b_d, b_u, E, w_0$ and $\alpha$ are all dimensionless variables to start with. Substituting these rescaling into equations (2.60) - (2.73) gives

\begin{align}
  \frac{\partial b_d}{\partial \hat{t}} + \frac{\partial}{\partial \hat{z}}(\hat{v} b_d) &= (\hat{k}_{bd}(\hat{c}) - \hat{k}_{dd}(\hat{c})) b_u - \frac{\alpha_1 A_0}{A_{bd}} \hat{A} b_d + \frac{\alpha_5}{A_{bd}} b_u + (2 - \gamma) \hat{k}_{bu}(\hat{c}) b_u, \quad (2.81) \\
  \frac{\partial b_u}{\partial \hat{t}} + \frac{\partial}{\partial \hat{z}}(\hat{v} b_u) &= ((\gamma - 1) \hat{k}_{bu}(\hat{c}) - \hat{k}_{du}(\hat{c})) b_u + \frac{\alpha_1 A_0}{A_{bd}} \hat{A} b_d - \frac{\alpha_5}{A_{bd}} b_u, \quad (2.82) \\
  \frac{\partial E}{\partial \hat{t}} + \frac{\partial}{\partial \hat{z}}(\hat{v} E) &= \frac{\alpha_8 c_0}{A_{bd}} b_u \hat{c} + \frac{\alpha_8 c_0}{A_{bd}} b_d \hat{c} - \frac{\alpha_{10}}{A_{bd}} E, \quad (2.83) \\
  \frac{d \hat{v}}{d \hat{z}} &= \frac{(\hat{k}_{bd}(c) - \hat{k}_{dd}(c)) b_d + (\hat{k}_{bu}(c) - \hat{k}_{du}(c)) b_u}{1 - w_0} + \frac{1 + \alpha}{1 - w_0} \left( \frac{\alpha_8 c_0}{A_{bd}} b_u \hat{c} + \frac{\alpha_8 c_0}{A_{bd}} b_d \hat{c} - \frac{\alpha_{10}}{A_{bd}} E \right), \quad (2.84)
\end{align}
\[ \hat{u} = \frac{\alpha \hat{v} E}{2w_0 + \alpha E} \]
\[ - \frac{1}{H_0(2w_0 + \alpha E)} \int_0^z \alpha \left( \frac{\alpha_{su} c_0}{A_{bd}} b_u \hat{c} + \frac{\alpha_{sd} c_0}{A_{bd}} b_d \hat{c} - \frac{\alpha_{10}}{A_{bd}} E \right) \]
\[ + (\hat{k}_{bu} - \hat{k}_{du}) b_u + (\hat{k}_{bd} - \hat{k}_{dd}) b_d \, dz'. \]

However we assume the AHL and nutrient concentrations are quasi steady for the same reason as given in Model 1, see equation (2.34) and parameter \( \beta_t \).

Hence
\[ \frac{\partial^2 \hat{A}}{\partial z^2} = \frac{H_0^2 k_A}{D_A} \hat{A} - \frac{\alpha_2 H_0}{D_A A_0} b_u + \frac{\alpha_3 H_0^2}{D_A} b_d \hat{A} - \frac{\alpha_4 H_0}{D_A A_0} b_d, \]
\[ \frac{\partial^2 \hat{c}}{\partial z^2} = \frac{H_0^2}{D_c c_0} (b_d + b_u) \hat{c} - \frac{\hat{c}}{c_1 + \hat{c}}. \]

These equations can be simplified further by defining the following parameters:

\[ A_0 = \frac{D_A}{\alpha_4 H_0^2}, \quad \beta_1 = \frac{\alpha_1 A_0}{A_{bd}}, \quad \beta_2 = \frac{\alpha_5}{A_{bd}}, \quad \beta_3_u = \frac{\alpha_{8u} c_0}{A_{bd}}, \quad \beta_3_d = \frac{\alpha_{8d} c_0}{A_{bd}}, \quad \beta_4 = \frac{\alpha_{10}}{A_{bd}}, \]
\[ \beta_5 = \frac{H_0^2 k_A}{D_A}, \quad \beta_6 = \frac{H_0^2}{D_c c_0}, \quad \beta_17 = \frac{\alpha_2}{\alpha_4}, \quad \beta_18 = 1, \quad \beta_19 = \frac{\alpha_3 H_0^2}{D_A}. \]

In particular

- \( A_0 \) represents the ratio of AHL diffusion over the length of the biofilm to its production rate by down-regulated cells,
- \( \beta_1 \) is the ratio of the rate of up regulation to the birth rate of cells for a particular AHL concentration,
- \( \beta_2 \) is the ratio of down regulation to the birth rate,
- \( \beta_3_u \) is the ratio of EPS production to the birth rate for a particular nutrient concentration,
- \( \beta_4 \) is the ratio of the rate of EPS degradation to the birth rate,
• $\beta_5$ is the ratio of AHL degradation to diffusion over the length of the biofilm,

• $\beta_6$ is the ratio of nutrient consumption to the diffusion of nutrients over the length of the biofilm,

• $\beta_{17}$ is the ratio of production by up- to down-regulated cells,

• $\beta_{19}$ is the ratio of AHL used in up regulation to its diffusion across the length of the biofilm.

Hence, on dropping the hats, we get the full non-dimensional system.

\[ \frac{\partial^2 A}{\partial z^2} = \beta_5 A - \beta_{17} b_u - \beta_{18} b_d + \beta_{19} b_d A, \]  
\[ \frac{\partial b_d}{\partial t} + \frac{\partial}{\partial z}(v b_d) = (k_{bd}(c) - k_{dd}(c)) b_d - \beta_1 A b_d + \beta_2 b_u 
+ (2 - \gamma) k_{bu}(c)b_u, \]  
\[ \frac{\partial b_u}{\partial t} + \frac{\partial}{\partial z}(v b_u) = ((\gamma - 1)k_{bu}(c) - k_{du}(c)) b_u + \beta_1 A b_d - \beta_2 b_u, \]  
\[ \frac{\partial^2 c}{\partial z^2} = \beta_6 (b_d + b_u) \frac{\hat{c}}{c_1 + \hat{c}}, \]  
\[ \frac{\partial E}{\partial t} + \frac{\partial}{\partial z}(v E) = (\beta_{3u} b_u + \beta_{3d} b_d) c - \beta_4 E, \]  
\[ \frac{\partial \phi}{\partial t} + v \frac{\partial \phi}{\partial z} = 0, \]
with

\[ \begin{align*}
  k_{bd}(c) &= \frac{c}{1+c}, \quad k_{bu}(c) = \frac{A_{bud}c}{1+c}, \\
  k_{dd}(c) &= D_{ddd} \left(1 - \frac{\sigma c_{bd} c}{1 + c_{ch} c}\right), \quad k_{du}(c) = D_{dud} \left(1 - \frac{\sigma c_{bd} c}{1 + c_{ch} c}\right),
\end{align*} \tag{2.97} \]

where \( A_{bud} = \frac{A_{bu}}{A_{bd}}, \quad D_{ddd} = \frac{B_{dd}}{A_{bd}} \) and \( D_{dud} = \frac{B_{du}}{A_{bd}} \). The \( z = 0 \) boundary conditions are

\[ \frac{\partial A(0, t)}{\partial z} = \frac{\partial c(0, t)}{\partial z} = v(0) = 0, \tag{2.98} \]

with the conditions at \( \phi = 0 \) or \( z = h \) being

\[ \frac{\partial A(H, t)}{\partial z} = -\beta_s A, \quad c(H, t) = 1. \tag{2.99} \]

The initial conditions are

\[ b_u(z, 0) = E(z, 0) = 0, \quad b_d(z, 0) = 1 - w_0, \quad \phi(z, 0) = z - 1, \tag{2.100} \]

where \( \phi \), the level set variable is dimensionless.

### 2.2.5 Numerical Methods

Just as with the first model (see Section 2.1.5), we have a system of coupled non-linear PDEs and hence they were solved in the same manner with the various finite difference approaches. As stated earlier, the level set approach [182] was used here to track the moving boundary. More details about the method and its application in one and two dimensions is given in Appendix B.

The difference equations produced by the finite difference methods, were still solved using Fortran and Figure 2.7 shows how and what order the different
Figure 2.7: A flow diagram of how Model 2 was solved and what methods were employed for the different variables.

variables were dealt with. For more information on the different numerical techniques used see Appendix A.

A code validation check was also done, by comparing the calculated height of the biofilm for various space steps sizes of the mesh grid. The results from this are shown in Table 2.3 and we see that all the errors are sufficiently small compared to the order of $\delta x$. This is the maximum theoretical error and hence
Space step size ($\delta x$) | Calculate height of the biofilm | Absolute Error, compared to height when $\delta x = 0.01$
---|---|---
0.00125 | 0.999743 | $2.57 \times 10^{-4}$
0.0025 | 0.999696 | $3.04 \times 10^{-4}$
0.005 | 0.999363 | $6.37 \times 10^{-4}$
0.01 | 1.000000 | 0.0
0.02 | 1.000541 | $5.41 \times 10^{-4}$
0.03 | 1.000855 | $8.55 \times 10^{-4}$
0.04 | 1.000253 | $2.53 \times 10^{-4}$

Table 2.3: This shows the calculated height of the biofilm for various values of $\delta x$ (normalised such that the height at $\delta x = 0.01$ equals 1) and $\delta t = 0.0001$, as well as their absolute error compared to the height calculated when $\delta x = 0.01$.

implies that the Fortran code is consistent.

### 2.2.6 Results

Table 2.4 gives the initial data and parameter values used in this section. Parameters carried forward from the first model, remain the same; whilst the rest are obtained from their respective references. Again, some of these may have been scaled so that we did not have extremely large or small values, both of which could have affected the stability of the numerical methods employed.

Figure 2.8 gives a comparison of the different heights the biofilm would achieve with quorum sensing turned on and off. To turn the quorum sensing off, we changed $\beta_{17} = \beta_{18} = 0$, so that no AHL was produced and therefore all cells remained in their down-regulated state. We observed that when quorum sensing was enabled, the biofilm is able to grow at a substantially faster rate, which was observed in Anguige et al. (2006, [7]) and experimentally by Davier et al. (1998, [43]). A reason for this increased growth rate, is due to the increased production of EPS by the up-regulated bacteria cells. The extra EPS produced may also allow sufficient nutrients to penetrate through the biofilm to more bacteria cells. This in turn would cause more cell division and hence also increase the growth rate.
Figure 2.10 shows the volume fraction of up-regulated cells, which we see fall off dramatically near the top of the biofilm although the total bacteria cells increase in this region. The reason is that near the surface there is a high nutrient and relatively low AHL concentrations, the latter meaning there is locally a relatively low fraction of up-regulated cells and hence low EPS, as shown in Figure 2.11. The high nutrients imply that there is relatively high cell division and therefore a high number of bacteria cells. This increase in the total bacteria volume fraction, near the top of the biofilm, was also observed in Anguige et al. (2006, [7]) and experimentally in Mohle et al. (2007, [131]). Also after a short period of time the bacteria settle down into a travelling wave profile, as seen in Figures 2.9 and 2.10. This is due to the nutrients only being able to diffuse a fixed distance into the biofilm, which is the same as in Model 1. Therefore we see the bacteria dying off towards the bottom of the biofilm, whilst the most rapid growth is near the top, which broadly agrees with Webb et al. (2003, [226]). It takes a while for the bacteria to settle down into this travelling wave solution due to the high density of cells initially. The last point
Figure 2.8: Comparison between the heights of the biofilm over time when quorum sensing is enabled, the top line, and disabled, the bottom line. When enabled we used $\beta_{17} = 5$ and $\beta_{18} = 1$, which sets the production rates of AHL for the up- and down-regulated bacteria respectively, whilst both of these were set to zero, when the quorum sensing was disabled. All the other parameters are given in Table 2.4.

to note is the increased density as we approach $z = 0$, which happens as it takes a while for the EPS to build up at the bottom due to lack of nutrients.

The EPS also shows a travelling wave solution after a short period of time, as shown in Figure 2.11, as it is produced by the bacteria cells. However unlike the bacteria, the EPS volume fraction remains constant near the bottom of the biofilm, due to our assumption that there was no degradation, i.e. $\beta_4 = 0$. The AHL concentration, Figure 2.12, initially starts out very small, before jumping up very rapidly, due to its auto-inductive nature and that the cells are still reasonably clumped together. After a while the concentration drops as the cells density drops and the AHL degrades, however there is still sufficient for the vast majority of cells to stay up-regulated.

Figure 2.13 shows that the nutrient profiles are qualitatively similar to those
Figure 2.9: Evolution of the bacteria’s volume fraction throughout the biofilm, between times $t = 2.5$ and $t = 20$ in steps of $t = 2.5$, moving from left to right and using the initial and parameter values given in Table 2.4.

Figure 2.10: Evolution of the up-regulated bacteria’s volume fraction throughout the biofilm, between times $t = 2.5$ and $t = 20$ in steps of $t = 2.5$ moving from left to right and using the initial and parameter values given in Table 2.4.
produced by Model 1, both of which show a travelling wave formation. Figure 2.14 shows the advective velocity, which generally increases as we move away from the bottom of the biofilm. The reason being that the velocity at a point \( z^* \) is the sum of the movement of cells below it, i.e. \( 0 < z < z^* \). Hence, where nutrients are sufficient more cells below \( z^* \) are dividing and therefore the speed increases as you approach the top of the biofilm. This region of sufficient nutrients however moves as the biofilm grows, creating the travelling wave pattern. In the early development of the biofilm, we see that the velocity goes negative for a short while; this is caused by the production of the EPS, which takes up volume and therefore pushes some of the cells towards the bottom of the biofilm. However after a period of time the EPS reaches it maximum volume fraction, as shown in Figure 2.11 by the lines converging at \( z = 0 \). Hence the EPS does not increase its volume size, which stops the cells being forced backwards towards the bottom of the biofilm. Furthermore cell death occurs near the bottom of the biofilm, due to insufficient nutrients penetrating the entire length of it. This allows more room there which is why the magnitude of the negative velocity decreases. Therefore after a period of time stability occurs around the bottom of the biofilm and afterwards we obtain this consistent velocity profile shown in Figure 2.14. Figure 2.11 shows that EPS is being continuously produced by cells and we have assumed that it traps water. Hence as the EPS volume fraction increases, so does the water volume fraction, imply water must continuously enter the biofilm. This is shown by the negative values in Figure 2.15. The small amount of positive water flow, near the bottom of the biofilm, in its early development is due to the small amount of bacteria division within the region.

Although we have added the quorum sensing process into this model, the results still show all of the same characteristics as Model 1, for example the
Figure 2.11: Evolution of the EPS’s volume fraction throughout the biofilm, between times $t = 2.5$ and $t = 20$ in steps of $t = 2.5$ moving from left to right and using the initial and parameter values given in Table 2.4.

Figure 2.12: Evolution of the AHL concentration within the biofilm, between times $t = 0$ and $t = 20$ in steps of $t = 2.5$ moving from left to right and using the initial and parameter values given in Table 2.4.
Figure 2.13: Evolution of the nutrient concentration within the biofilm, between times \( t = 0 \) and \( t = 20 \) in steps of \( t = 2.5 \) moving from left to right and using the initial and parameter values given in Table 2.4.

Figure 2.14: Evolution of the advective velocity of the biofilm, between times \( t = 0 \) and \( t = 20 \) in steps of \( t = 2.5 \) moving from left to right, and using the initial and parameter values given in Table 2.4.
Figure 2.15: Evolution of the water velocity through the biofilm, between times $t = 0$ and $t = 20$ in steps of $t = 2.5$ moving from left to right and using the initial and parameter values given in Table 2.4.

travelling waves. Moreover, these results still give a good correlation with the work presented by Anguige et al. (2006, [7]) and Ward et al. (2003, [221]). Examples of these correlations from Anguige et al. include the EPS volume fraction dropping off near the top of the biofilm, although they also have a drop off near the bottom of the biofilm as well. The reason for this difference is that they have a higher rate of EPS production, which with the greater nutrient concentration, gives you this greater EPS volume fraction near the top of the biofilm.

We also investigated how $\gamma$, which represents the statistical average number of up-regulated cells produced when one of them divides affects the ratio of up-regulated to total number of bacteria cells, i.e.

$$\gamma = \frac{b_u}{b_d + b_d}. \quad (2.101)$$
Three different values of $\gamma$, i.e. $\gamma = 0, 1$ and 2, were investigated however they made no difference to the ratio of up-regulated cells to the total population, as there is always sufficient AHL to convert any down-regulated cells produced back up. The fact that $\gamma$ makes little difference to the amount of up-regulated cells within the biofilm is in broad agreement with Ward et al. (2001, [220]) and Anguige et al. (2004, [8]). Overall though, we see that all the bacteria in the biofilm switch very quickly from one state to another, which is in correlation with other research including Dockery and Keener, (2001, [47]).

2.3 Conclusions

In the first model we investigated very early biofilm development, where it only consisted of live and dead cells plus water. We found that all cells within the biofilm grow and divide; expanding the biofilm, until the point where insufficient nutrients were able to fully penetrate the biofilm. At this point the biofilm continues to grow linearly with a high cell density near the top where the nutrient concentration is at its highest, whilst decreasing as we move further down. This growth caused a travelling wave pattern to form, which propagates forward with the increasing height of the biofilm and the limited nutrient penetration, which is to be expected from prior work by Anguige et al. (2006, [7]).

For the second model we investigated the biofilm development, particularly for Pseudomonas aeruginosa, from the point where quorum sensing was about to be switched on. This was done by expanding the first model to include the quorum sensing process, which meant splitting up the bacteria cells into down- and up-regulated, as well as modelling the EPS volume fractions and AHL concentration. The results show that quorum sensing causes dramatic increases in the biofilm’s growth rate. The reason for this was due to the increased
production rate of EPS by the up-regulated cells. Also all of the figures still show travelling wave solutions, which again originate from the nutrient gradients, which is in correlation with Ward et al. (2003 [221]).
Chapter 3

Model 3: A Three Dimensional Model of Biofilm Growth, in Still Water

3.1 Introduction to the Model

In this chapter we investigate a three dimensional biofilm model, although it will only be solve in two dimensions, to increase our understanding of how the diffusion of nutrients and other chemicals (i.e. AHL) effects the growth of the biofilm. It also allows us to check whether biofilms do grow at a faster rate vertically than horizontally, which is assumed to be true in the one dimensional models. This three dimensional model should also display the different shape characteristics that biofilm’s form, which cannot be seen in one dimension.

This model expands on the approach used in Model 2 so that it can be extended into higher dimensions. Plus, the advective velocity field is generated by assuming the following:

- by balancing the forces between the different biofilm components,
the bacteria cells and EPS are physically restricted to a maximum volume fraction,

- considering the drag forces between the bacteria cells, EPS and water,

- there are no external forces on the biofilm.

We will also follow Anguige et al. (2006, [7]), initially, and relax the assumption that the internal and external concentrations of nutrients and AHLs are automatically in an equilibrated state. Figure 3.1 gives a schematic of the biofilm to be modelled in this chapter.

### 3.2 Model Derivation

As with the one dimensional models we have a volume fraction of down-, $b_d$, and up-regulated, $b_u$, bacteria cells. Both of the down- and up-regulated bacteria produce EPS, $E$, with the latter at elevated rates as mentioned in Section 1.5.1. We assume that the remaining space is occupied by water, with volume fraction, $w$, such that as a cell dies it is instantly, on the time scale of interest, converted into water. Hence, we have

$$b_d + b_u + E + w = 1. \quad (3.1)$$
As before we assume that the biofilm grows at an advection velocity $v$, whilst the water flows at velocity $u$. Following the work of the one dimensional case, we produce the following three dimensional equations, where $A_i$ and $c_i$ are the internal AHL and nutrient concentrations, respectively,

\[
\frac{\partial b_d}{\partial t} + \nabla \cdot (v b_d) = (k_{bd}(c) - k_{dd}(c))b_d - \alpha_1 A_ib_d + \alpha_5 b_u
\]

\[
+ (2 - \gamma)k_{bu}(c)b_u,
\] (3.2)

\[
\frac{\partial b_d}{\partial t} + \nabla \cdot (v b_u) = ((\gamma - 1)k_{bd}(c) - k_{dd}(c))b_u + \alpha_1 A_ib_d - \alpha_5 b_u,
\] (3.3)

\[
\frac{\partial E}{\partial t} + \nabla \cdot (v E) = (\alpha_{8u}b_u + \alpha_{8d}b_d)c_i - \alpha_{10}E,
\] (3.4)

\[
\frac{\partial w}{\partial t} + \nabla \cdot (u w) = -(k_{bd}(c) - k_{dd}(c))b_d - (k_{bu}(c) - k_{du}(c))b_u.
\] (3.5)

To track the moving boundary of the biofilm we used a level set approach. However unlike in Model 2, we required the conservative form of the level set equation, i.e.

\[
\frac{\partial \phi}{\partial t} + \nabla \cdot (v \phi) = \phi \nabla \cdot v,
\] (3.6)

where $\phi(x, t)$ is the level set variable, and we assume that it is negative at all points inside the biofilm and positive outside. Under most circumstances the right hand side of the equation is zero, however we will model the bacteria as a compressible viscous fluid and therefore $\nabla \cdot v \neq 0$. The reason the conservative form was used, is it allows the biofilm to move along the solid surface, even though the boundary condition there stated that the advective velocity was zero. This can occur as the conservative form deals with the derivatives of the velocity field, rather than point values, as in the standard version. Hence, for example if the local advective velocity field is negative just above the solid surface, which implies the bacteria cells are moving down towards the surface.

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then the conservative equations allows the cells to expand the biofilm along the surface. However for this to be achieved the conservative equation has to be dealt with in its current form, as if it is expanded out, then it degenerates into the standard equation. The reason being, that when applying finite difference methods to the conservative equation, extra terms are generated compared to the standard equation. These extra terms come from the derivatives, and it is these that allow the biofilm to move along the solid surface. More information on the level set method and how the conservative equations allows the biofilm to expand along the solid surface is given in Appendix B.

We assume that the AHL and nutrient molecules can diffuse at different rates through the water and bacteria cells (but not the EPS) and hence they have different concentration inside and out of the cells. This difference in concentrations was measured by Pearson et al. (1999, [148]). We let $A_o$ and $A_i$ be the AHL concentration, and $c_o$ and $c_i$ be the nutrient concentration, in the water and bacteria cells respectively. As in the previous model, the AHL concentration is produced at a considerably faster rate by the up- compared to the down-regulated cells. It is also used at a rate proportional to the rate of up regulation and it breaks down at a constant rate. We therefore obtain the following set of equations, which are of a similar nature to Model 2.

\[
\frac{\partial}{\partial t}(bA_i) + \nabla \cdot (vA_i) = D_{A_i} \nabla \cdot (b \nabla A_i) - \lambda A_i b + \alpha_2 b_u + \alpha_4 b_d + b(k_{A_i} A_o - k_{A_o} A_i),
\]

\[
\frac{\partial}{\partial t}(wA_o) + \nabla \cdot (u wA_o) = D_{A_o} \nabla \cdot (w \nabla A_o) - \lambda A_o w - \alpha_3 b_d A_o - b(k_{A_i} A_o - k_{A_o} A_i),
\]

recalling that $b = b_d + b_u$ and $\lambda$ represents the degradation rate of the AHL.

In Pearson et al. (1999, [148]), the equilibrium time was about five minutes,
which is rapid compared to the growth timescales. We therefore assume, following Anguige et al. (2006, [7]), that the terms involving \( k_{A_i} A_i \) and \( k_{A_o} A_o \) are much larger than the rest, hence we consider expansions of \( A_i \) and \( A_o \) of the form

\[
A_o \sim A_o^0 + \frac{A_o^1}{k_{A_o}},
\]

(3.9)

\[
A_i \sim A_i^0 + \frac{A_i^1}{k_{A_i}},
\]

(3.10)
as \( k_{A_o} \to \infty \) and \( k_{A_i} \to \infty \). On substitution into equations (3.7) and (3.8), we obtain

\[
\frac{\partial}{\partial t} \left( b(A_i^0 + \frac{A_i^1}{k_{A_i}}) \right) + \nabla \cdot \left( v b (A_i^0 + \frac{A_i^1}{k_{A_i}}) \right) = D_{A_i} \nabla \cdot \left( b \nabla (A_i^0 + \frac{A_i^1}{k_{A_i}}) \right) - \lambda (A_i^0 + \frac{A_i^1}{k_{A_i}}) b + \alpha_2 b u + \alpha_4 b d
\]

(3.11)

\[
\frac{\partial}{\partial t} \left( w(A_o^0 + \frac{A_o^1}{k_{A_o}}) \right) + \nabla \cdot \left( u w (A_o^0 + \frac{A_o^1}{k_{A_o}}) \right) = D_{A_o} \nabla \cdot \left( w \nabla (A_o^0 + \frac{A_o^1}{k_{A_o}}) \right) - \lambda (A_o^0 + \frac{A_o^1}{k_{A_o}}) w - \alpha_3 b d (A_o^0 + \frac{A_o^1}{k_{A_o}})
\]

(3.12)

From the leading order terms we get

\[
0 = \frac{k_{A_i}}{k_{A_o}} A_o^0 - A_i^0,
\]

(3.13)

assuming \( k_{A_i}/k_{A_o} = O(1) \) and for the correction terms we have

\[
\frac{\partial}{\partial t} (b A_i^0) + \nabla \cdot (v b A_i^0) = D_{A_i} \nabla \cdot (b \nabla A_i^0) - \lambda A_i^0 b + \alpha_2 b u + \alpha_4 b d + b (A_o^0 - \frac{k_{A_o}}{k_{A_i}} A_i^1),
\]

(3.14)
\[
\frac{\partial}{\partial t}(wA_o^0) + \nabla \cdot (uwA_o^0) = D_{A_o} \nabla \cdot (w\nabla A_o^0) - \lambda A_o^0 w - \alpha_3 b_d A_o^0 \\
- b(A_o^1 - \frac{k_{A_o}}{k_{A_i}} A_i^1).
\] (3.15)

Adding equations (3.14) and (3.15) and substituting in equation (3.13) gives

\[
\frac{\partial}{\partial t}(bk_A A_o + wA_o) + \nabla \cdot (vb_k A_o + uw A_o) = D_{A_i} \nabla \cdot (b\nabla k_A A_o) + D_{A_o} \nabla \cdot (w\nabla A_o) \\
- \lambda (k_A A_o b + A_o w) + \alpha_2 b_u \\
+ \alpha_4 b_d - \alpha_3 b_d A_o,
\] (3.16)

where \( k_A = \frac{k_{A_o}}{k_{A_i}} \). Typically, over the relevant distances, diffusion transport through the biofilm will occur on a much faster timescale, which occurs in minutes, compared to the growth, which happens in hours. Hence a quasi-steady assumption is adopted, which was derived via the non-dimensionalisation in Chapter 2, therefore

\[
0 = D_{A_i} \nabla \cdot (b\nabla k_A A) + D_{A_o} \nabla \cdot (w\nabla A) - \lambda A(k_A b + w) \\
+ \alpha_2 b_u + \alpha_4 b_d - \alpha_3 b_d A,
\] (3.17)

where \( A = A_o \) for notational simplicity. In a similar fashion, we can arrive at an equation describing the nutrient concentration, where \( c = c_o = c_i/k_c \)

\[
0 = D_{c_i} \nabla \cdot (b\nabla k_c c) + D_{c_o} \nabla \cdot (w\nabla c) - \alpha_9 (b_d + b_u) \frac{c}{c_1 + c}.
\] (3.18)

We now turn our attention to formulating a set of equations to model the water and biofilm’s advective velocities. Adding equations (3.2), (3.3), (3.4) and (3.5) gives

\[
\frac{\partial}{\partial t}(b_d + b_u + E + w) + \nabla \cdot (v(b_d + b_u + E) + uw) = (\alpha_8 u b_u + \alpha_8 d b_d) c - \alpha_{10} E,
\] (3.19)
and using equation (3.1) we obtain the equation for conservation of mass,

$$\nabla \cdot (\mathbf{v}(1-w) + \mathbf{u}w) = (\alpha_{8u}b_u + \alpha_{8d}b_d)c - \alpha_{10}E. \quad (3.20)$$

This means there are five equations, (i.e. (3.1), (3.2), (3.3), (3.4) and (3.20)) and $4+2d$ variables, where $d$ is the dimension of interest (leaving $A$ and $c$ to one side for the moment), leaving us with a significantly under determined system.

To complete the model, we need to introduce constitutive relations to resolve the velocity field. We adapt the general approach used in a multiphase model of tumour growth [28] whereby force balance between the phases (i.e. the bacteria cells, EPS and water) are resolved. We assume there are no external forces, hence

$$\begin{align*}
0 &= \nabla \cdot (b\sigma_b + E\sigma_E) + \mathbf{F}_b + \mathbf{F}_E, \quad \text{where } b = b_d + b_u \quad (3.21) \\
0 &= \nabla \cdot (w\sigma_w) + \mathbf{F}_w. \quad (3.22)
\end{align*}$$

Here $\sigma_i$ denotes the stress tensor of the forces exerted on phase $i$ and we combine the stresses on the bacteria cells and EPS, assuming the drag between these phases is very large. Also $\mathbf{F}_b$ and $\mathbf{F}_E$ denote the force of the water on the bacteria and EPS respectively, whilst $\mathbf{F}_w$ is the force of the bacteria and EPS on the water. As there are no external forces we must have $\mathbf{F}_b + \mathbf{F}_E + \mathbf{F}_w = 0$, which gives us

$$0 = \nabla \cdot (b\sigma_b + E\sigma_E + w\sigma_w). \quad (3.23)$$

Our system of equations is now (3.1), (3.2), (3.3), (3.4), (3.20), (3.21) and (3.22) representing the variables $b_d, b_u, E, w, \mathbf{v}, \mathbf{u}, \sigma_b, \sigma_E, \sigma_w, \mathbf{F}_b, \mathbf{F}_E$ and $\mathbf{F}_w$. This system of equations is well determined and all that remains is to propose
suitable expressions for $\sigma_i$ and $F_i$.

We assume that cell and water phases, on the timescale of interest, behave in a similar fashion and hence can be treated as a compressible viscous and incompressible inviscid fluids, respectively. This assumption is used as cells, EPS and water are able to flow past one another. However the cells and EPS generate as much drag on themselves as they do the water, and hence we have included the viscosity terms. Although the water is viscous, its effects are small compared to the drag generated between it and the solid phase, i.e. the bacteria cells and EPS, and hence we neglect the viscosity terms.

The water though travels through narrow channels, whilst not sticking to the bacteria or EPS and hence flows freely without friction. Therefore,

$$
\begin{align*}
\sigma_b &= -p_b I + \mu_b (\nabla \mathbf{v} + \nabla \mathbf{v}^T) + \lambda_b (\nabla \cdot \mathbf{v}) I, \\
\sigma_E &= -p_E I + \mu_E (\nabla \mathbf{v} + \nabla \mathbf{v}^T) + \lambda_E (\nabla \cdot \mathbf{v}) I, \\
\sigma_w &= -p_w I, \\
p_b &= p_w + \Sigma_b, \\
p_E &= p_w + \Sigma_E,
\end{align*}
$$

(3.24) (3.25) (3.26) (3.27) (3.28)

where $\mu_i$ and $\lambda_i$ are the shear and bulk viscosity coefficients and $I$ is the identity tensor. The shear viscosity is cause by neighbouring particles (either bacteria, EPS or water) moving pass each other at different velocities, whilst the bulk viscosity can be viewed as the additional frictional forces caused by the fluids compressibility. $\Sigma_i$ refers to the pressure difference between the water and phase $i$ and are defined in equations (3.49) and (3.50). Also, unlike the standard Navier-Stokes formulation, $\nabla \cdot \mathbf{v} \neq 0$, as the bacteria can have a varying “density” through the biofilm.

We also assume that the forces on the various elements are proportional to
the pressure and drag [3], which results in Darcy’s law, hence

\begin{align}
\mathbf{F}_b &= p_w \nabla b - k_b (v - u), \quad (3.29) \\
\mathbf{F}_E &= p_w \nabla E - k_E (v - u), \quad (3.30) \\
\mathbf{F}_w &= p_w \nabla w - (k_b + k_E) (u - v). \quad (3.31)
\end{align}

We note these equations sum to zero, as there are no external forces. The \( p_w \nabla x \) describe the amount of force pushing the bacteria cells, EPS and water volumes to equilibrate within the local environment, such that we get an even distribution, given sufficient pressure.

Substituting equations (3.24) - (3.31) into equations (3.21) and (3.22) gives

\begin{align}
0 &= \nabla \cdot \left( -(p_b b + p_E E) \mathbf{I} + \hat{\mu} (\nabla v + \nabla v^T) + \hat{\lambda} (\nabla \cdot v) \mathbf{I} \right) \\
&\quad + \hat{k} (u - v) + p_w \nabla (b + E), \quad (3.32) \\
0 &= \nabla \cdot (-p_w w \mathbf{I}) - \hat{k} (u - v) + p_w \nabla w, \quad (3.33)
\end{align}

where \( \hat{\mu} = \mu_b b + \mu_E E, \hat{\lambda} = \lambda_b b + \lambda_E E \) and \( \hat{k} = k_b b + k_E E \).

The resulting system of equations consist of (3.1), (3.2), (3.3), (3.4), (3.20), (3.32) and (3.33), which is sufficient to close the system. Using (3.33) we can write

\begin{equation}
\mathbf{u} = \mathbf{v} + \frac{\nabla \cdot (-p_w w \mathbf{I}) + p_w \nabla w}{\hat{k}}, \quad (3.34)
\end{equation}

which is used to reduce the order of the system by \( d \), the dimension of the model. Substituting \( \mathbf{u} \) and equations (3.27) and (3.28) into (3.20) and (3.32) we obtain

\begin{align}
(\alpha_{s_b} b_u + \alpha_{s_d} b_d) c - \alpha_{10} E &= \nabla \cdot \left( \mathbf{v} + \frac{w}{k} (\nabla \cdot (-p_w w \mathbf{I}) + p_w \nabla w) \right), \quad (3.35) \\
0 &= \nabla \cdot (-p_w + b \Sigma_b + E \Sigma_E) \mathbf{I}
\end{align}
\[
+\hat{\mu}(\nabla \mathbf{v} + \nabla \mathbf{v}^T) + \hat{\lambda}(\nabla \cdot \mathbf{v})\mathbf{I}. \quad (3.36)
\]

Below is the complete set of model equations:

\[
b_d + b_u + E + w = 1, \quad (3.37)
\]

\[
\frac{\partial b_d}{\partial t} + \nabla \cdot (v b_d) = (k_{bd}(c) - k_{dd}(c))b_d - \alpha_1 k_A b_d + \alpha_3 b_u + (2 - \gamma)k_{bu}(c)b_u, \quad (3.38)
\]

\[
\frac{\partial b_u}{\partial t} + \nabla \cdot (v b_u) = ((\gamma - 1)k_{bu}(c) - k_{du}(c))b_u + \alpha_1 k_A b_d - \alpha_5 b_u, \quad (3.39)
\]

\[
\frac{\partial E}{\partial t} + \nabla \cdot (v E) = k_c(\alpha_8 b_u + \alpha_8 b_d)c - \alpha_{10} E; \quad (3.40)
\]

\[
0 = D_A \nabla \cdot (b \nabla k_A A) + D_A \nabla \cdot (w \nabla A) - \lambda A(k_A b + w) + \alpha_2 b_u + \alpha_4 b_d - \alpha_3 b_d A(3.41)
\]

\[
0 = D_c \nabla \cdot (b \nabla k_c c) + D_c \nabla \cdot (w \nabla c) - \alpha_9(b_d + b_u) \frac{c}{c_1 + c}, \quad (3.42)
\]

\[
(\alpha_8 b_u + \alpha_8 b_d)c - \alpha_{10} E = \nabla \cdot \left(\mathbf{v} + \frac{w}{k}(\nabla \cdot (-p_w w \mathbf{I}) + p_w \nabla w)\right), \quad (3.43)
\]

\[
0 = \nabla \cdot (-p_w + b \Sigma_b + E \Sigma E))\mathbf{I}
+ \hat{\mu}(\nabla \mathbf{v} + \nabla \mathbf{v}^T) + \hat{\lambda}(\nabla \cdot \mathbf{v})\mathbf{I}, \quad (3.44)
\]

\[
\frac{\partial \phi}{\partial t} + \nabla \cdot (v \phi) = \phi \nabla \cdot \mathbf{v}, \quad (3.45)
\]

which are \(7 + d\) equations for the \(7 + d\) variables, i.e. where \(d\) is the dimension of the system. The various functions are as follows.

\[
\hat{\mu} = \mu_b b + \mu_E E, \quad (3.46)
\]

\[
\hat{\lambda} = \lambda_b b + \lambda_E E, \quad (3.47)
\]

\[
\hat{k} = k_b b + k_E E, \quad (3.48)
\]
\[
\Sigma_b = \begin{cases} 
0 & b + \alpha E \leq B_c \\
\left( \frac{b + \alpha E - B_c}{B_m - b - \alpha E} \right)^2 & \text{otherwise,}
\end{cases}
\]

\[
\Sigma_E = \begin{cases} 
0 & b + \alpha E \leq B_c \\
\left( \frac{b + \alpha E - B_c}{B_m - b - \alpha E} \right)^2 & \text{otherwise,}
\end{cases}
\]

\[
k_{bd}(c) = \frac{A_{bd} c}{c_1 + c},
\]

\[
k_{bu}(c) = \frac{A_{bu} c}{c_1 + c},
\]

\[
k_{dd}(c) = D_{dd} \left( 1 - \frac{\sigma c}{1 + c} \right),
\]

\[
k_{du}(c) = D_{du} \left( 1 - \frac{\sigma c}{1 + c} \right).
\]

The pressure functions \(\Sigma_b\) and \(\Sigma_E\), allow the bacteria and EPS to sit in equilibrium until they reach a certain density, \(B_c\). After this point their pressure increases rapidly to infinity as \(b + \alpha E \to B_m\), i.e. the solid phase will be forced to expand out as it approaches a fraction of \(B_m\), as shown in Figure 3.2. The parameter \(\alpha\) represents the same ideas as in Model 2, i.e. the amount of extra volume that is generated by the EPS that can be infiltrated by water. These pressure functions are different to what Byrne et al. (2002, [28]) used, as they included attraction between the cells where we only had expansion.

### 3.3 Boundary and Initial Conditions

The boundary conditions for this model are very similar to the one dimensional cases mentioned in Chapter 2. As the biofilm is sitting on a solid, impermeable surface at \(y = 0\), this implies that the \(y\) derivates for the AHL and nutrient concentrations are equal to zero. For the velocities themselves, we assume a no-slip condition, which implies that the biofilm is firmly adhered to the surface.
Figure 3.2: A plot showing how the pressure functions $\Sigma_b$ and $\Sigma_E$ change with respect to $b + \alpha E$.

This gives

$$\frac{\partial A}{\partial y} = \frac{\partial c}{\partial y} = \frac{\partial p_w}{\partial y} = v(x, 0, t) = 0.$$

(3.55)

In the production of the model we assumed that there were no external forces acting on the biofilm, and for this to be true we require the fluid/biofilm boundary to be stress free. This implies that on $\phi(x, y, t) = 0$ we have

$$0 = (b\sigma_b + E\sigma_E) \cdot n,$$

(3.56)

$$0 = (w\sigma_w) \cdot n = p_w,$$

(3.57)

where

$$n = \frac{\left(\frac{\partial \phi}{\partial x}, \frac{\partial \phi}{\partial y}\right)}{\left(\left|\frac{\partial \phi}{\partial x}, \frac{\partial \phi}{\partial y}\right|\right)},$$

(3.58)

is the unit normal vector pointing outside the biofilm.

We assume that nutrients are well mixed within the surrounding fluid and
that there is a continuous supply of them. This means that there would be a fixed concentration for all time around the entire edge of the biofilm, i.e. on $\phi(x, y, t) = 0$ we have

$$c(x, y, t) = c_0.$$  \hfill (3.59)

Furthermore we assume that the AHL molecules diffuse through the water on a much faster time scale than the bacteria’s birth rate. Therefore on the biofilm/fluid boundary they will rapidly diffuse into the surrounding fluid, sufficiently far away and diluted that there concentration is approximately zero. Hence we have on $\phi(x, y, t) = 0$ that

$$A = 0.$$ \hfill (3.60)

The initial conditions used for this model, are identical to those used in the one dimensional case for the biofilm components, i.e. the biofilm only consists of down-regulated cells and water, to allow for comparisons to be made. The initial shape of the biofilm is given by a level set function $\phi_I$, such that

$$\phi_I(x, y, t) < 0 \text{ represents inside the biofilm,}$$
$$\phi_I(x, y, t) = 0 \text{ represents the edge of the biofilm,}$$
$$\phi_I(x, y, t) > 0 \text{ represents outside the biofilm.}$$

In terms of equations, this means

$$b_d(x, y, 0) = \begin{cases} b_0 & \phi(x, y, 0) \leq 0, \\ 0 & \phi(x, y, 0) > 0, \end{cases} \hfill (3.61)$$

$$b_u(x, y, 0) = 0, \hfill (3.62)$$

$$E(x, y, 0) = 0, \hfill (3.63)$$

$$\phi(x, y, 0) = \phi_I(x, y). \hfill (3.64)$$
3.4 Non-Dimensionalisation

We again non-dimensionalised the above system of equations, (3.37) - (3.44), to produce a system of equations with fewer parameters. We will denote dimensionless quantities with hats and define

\[
t = \frac{\hat{t}}{A_{bd}}, \quad x = H_0 \hat{x}, \quad y = H_0 \hat{y}, \quad A = A_0 \hat{A}, \quad c = c_0 \hat{c},
\]

\[
p_w = p_w \hat{p}_w, \quad \hat{v} = H_0 A_{bd} \hat{\hat{v}}, \quad \hat{u} = H_0 A_{bd} \hat{\hat{u}},
\]

where \( H_0 \) is the initial height of the biofilm and \( A_{bd} \) is the rate at which bacteria cells divide. As before \( b_d, b_u, E \) and \( w \) are all dimensionless variables to start with. Substituting equations (3.65) into our system of equations gives:

\[
b_d + b_u + E + w = 1,
\]

\[
\frac{\partial b_d}{\partial t} + \nabla \cdot (\hat{v} b_d) = (\hat{k}_{bd}(c) - \hat{k}_{dd}(c))b_d - \frac{\alpha_1 k AA_0}{A_{bd}} \hat{A} b_d + \frac{\alpha_5}{A_{bd}} b_u + (2 - \gamma) \hat{k}_{bu}(c) b_u,
\]

\[
\frac{\partial b_u}{\partial t} + \nabla \cdot (\hat{v} b_u) = ((\gamma - 1) \hat{k}_{bu}(c) - \hat{k}_{du}(c)) b_u + \frac{\alpha_1 K AA_0}{A_{bd}} A_{bd} - \frac{\alpha_5}{A_{bd}} b_u,
\]

\[
\frac{\partial E}{\partial t} + \nabla \cdot (\hat{v} E) = \frac{\alpha_{8u} c_0 k c}{A_{bd}} b_u c + \frac{\alpha_{8d} c_0 k c}{A_{bd}} b_d c - \frac{\alpha_{10}}{A_{bd}} E,
\]

\[
0 = \nabla \cdot (k_c b \nabla \hat{\hat{c}}) + D_c \nabla \cdot (w \nabla \hat{\hat{c}}) - H_0^2 \frac{\lambda}{D_{\hat{c}}} \hat{A}(k_c b + w)
\]

\[
0 = \nabla \cdot (k_c b \nabla \hat{\hat{c}}) + D_c \nabla \cdot (w \nabla \hat{\hat{c}}) - \frac{H_0^2 \lambda}{D_{\hat{c}}} \hat{\hat{c}}(k_c b + w) - \frac{\alpha_9 H_0^2}{D_{\hat{c}}} b_c + \frac{\alpha_9 H_0^2}{D_{\hat{c}}} b_c + \frac{\alpha_9 H_0^2}{D_{\hat{c}}} b_c,
\]

\[
\frac{\alpha_{8u} c_0}{A_{bd}} b_u c + \frac{\alpha_{8d} c_0}{A_{bd}} b_d c - \frac{\alpha_{10}}{A_{bd}} E = \nabla \cdot \left( \hat{\hat{v}} - \frac{p_{wu}}{A_{bd} H_0^2} \frac{w}{k} \nabla p_w \right).
\]
\[
\n\nabla \cdot (b \hat{\Sigma}_b + E \hat{\Sigma}_E) I = \nabla \cdot (-p_w I + \frac{\hat{\mu} A_{bd}}{p_{wo}} (\nabla \hat{\nu} + \nabla \hat{\nu}^T) + \frac{\lambda A_{bd}}{p_{wo}} (\nabla \cdot \hat{\nu}) I),
\]

where \(D_A = D_{A_o}/D_{A_i}\) and \(D_c = D_{c_o}/D_{c_i}\). These equations can be further simplified by defining the following non-dimensional parameters:

\[
\beta_1 = \frac{\alpha_1 A_0 k_A}{A_{bd}}, \quad \beta_2 = \frac{\alpha_5}{A_{bd}}, \quad \beta_3 = \frac{\alpha_{8u} c_0 k_c}{A_{bd}}, \quad \beta_3 = \frac{\alpha_{8d} c_0 k_c}{A_{bd}},
\]

\[
\beta_4 = \frac{\alpha_{10}}{A_{bd}}, \quad \beta_5 = \frac{H_0^2 \lambda}{D_{A_i}}, \quad \beta_6 = \frac{\alpha_9 H_0^2}{D_{c}}, \quad \beta_13 = \frac{A_{bd}}{p_{wo}}, \quad \beta_14 = \frac{A_{bd} H_0^2}{p_{wo}}.
\]

Substituting these parameters into equations (3.66) - (3.73) and dropping the hats for clarity gives:

\[
b_d + b_u + E + w = 1, \quad (3.75)
\]

\[
\frac{\partial b_d}{\partial t} + \nabla \cdot (v b_d) = (k_{bd}(c) - k_{dd}(c)) b_d - \beta_1 A b_d + \beta_2 b_u + (2 - \gamma) k_{bu}(c) b_u, \quad (3.76)
\]

\[
\frac{\partial b_u}{\partial t} + \nabla \cdot (v b_u) = ((\gamma - 1) k_{bu}(c) - k_{du}(c)) b_u + \beta_1 A b_d - \beta_2 b_u, \quad (3.77)
\]

\[
\frac{\partial E}{\partial t} + \nabla \cdot (v E) = (\beta_3 b_u + \beta_3 b_d) c - \beta_4 E, \quad (3.78)
\]

\[
0 = \nabla \cdot (k_A b \nabla A) + D_A \nabla \cdot (w \nabla A) - \beta_5 A (k_A b + w) + \beta_17 b_u + \beta_{18} b_d - \beta_{19} b_d A, \quad (3.79)
\]

\[
0 = \nabla \cdot (k_c b \nabla c) + D_c \nabla \cdot (w \nabla c) - \beta_6 b \frac{c}{c_1 + c}, \quad (3.80)
\]

\[
(\beta_3 b_u + \beta_3 d b_d) c - \beta_4 E = \nabla \cdot \left( v - \frac{w}{\beta_{20} k} \nabla p_w \right), \quad (3.81)
\]
\[ \nabla \cdot (b \Sigma_b + E \Sigma_E) \mathbf{I} = \nabla \cdot (-p_w \mathbf{I} + \beta_{13} \mu (\nabla \mathbf{v} + \nabla \mathbf{v}^T) + \beta_{13} \lambda (\nabla \cdot \mathbf{v}) \mathbf{I}), \quad (3.82) \]

\[ \frac{\partial \phi}{\partial t} + \nabla \cdot (\mathbf{v} \phi) = \phi \nabla \cdot \mathbf{v}, \quad (3.83) \]

where

\[ \mu = \mu_b b + \mu_E E, \quad (3.84) \]

\[ \lambda = \lambda_b b + \lambda_E E, \quad (3.85) \]

\[ k = k_b b + k_E E, \quad (3.86) \]

\[ \Sigma_b = \begin{cases} 0 & b + \alpha E \leq B_c \\ (\frac{b + \alpha E - B_c}{B_m - b - \alpha E})^2 & \text{otherwise}, \end{cases} \quad (3.87) \]

\[ \Sigma_E = \begin{cases} 0 & b + \alpha E \leq B_c \\ (\frac{b + \alpha E - B_c}{B_m - b - \alpha E})^2 & \text{otherwise}, \end{cases} \quad (3.88) \]

\[ k_{bd}(c) = \frac{c}{c_1 + c}, \quad (3.89) \]

\[ k_{bu}(c) = \frac{A_{bud} c}{c_1 + c}, \quad (3.90) \]

\[ k_{dd}(c) = D_{ddd} \left( 1 - \frac{\sigma c}{1 + c} \right), \quad (3.91) \]

\[ k_{du}(c) = D_{dud} \left( 1 - \frac{\sigma c}{1 + c} \right). \quad (3.92) \]

The full set of initial and boundary conditions upon non-dimensionalising become

- Solid surface boundary conditions, i.e. \( y = 0 \):

\[ \frac{\partial A}{\partial y} = \frac{\partial c}{\partial y} = \frac{\partial p_w}{\partial y} = \mathbf{v}(x, 0, t) = 0. \quad (3.93) \]
• moving biofilm boundary conditions, i.e. \( \phi(x, y, t) = 0 \):

\[
0 = (-b \Sigma_b + E \Sigma_E) I + \beta_{13} \mu (\nabla v + \nabla v^T) + \beta_{13} \lambda (\nabla \cdot v) I \cdot n,
\]

(3.94)

\[
0 = p_w,
\]

(3.95)

\[ c = 1, \]

(3.96)

\[ A = 0. \]

(3.97)

• Initial conditions:

\[
b_d(x, y, 0) = \begin{cases} 
  b_0 & \phi(x, y, 0) \leq 0, \\
  0 & \phi(x, y, 0) > 0,
\end{cases}
\]

(3.98)

\[
b_u(x, y, 0) = 0,
\]

(3.99)

\[ E(x, y, 0) = 0, \]

(3.100)

\[ \phi(x, y, 0) = \phi_f. \]

(3.101)

We now have a full system of non-dimensional equations (3.75) - (3.83) coupled with the initial and boundary conditions (3.93) - (3.101).

### 3.4.1 One Dimensional System

In this section we apply the above model to a one dimensional biofilm, as shown in Figure 2.1. Hence

\[
b_d + b_u + E + w = 1, 
\]

(3.102)

\[
\frac{\partial b_d}{\partial t} + \frac{\partial (vb_d)}{\partial y} = (k_{bd}(c) - k_{dd}(c))b_d - \beta_1 A b_d + \beta_2 b_u + (2 - \gamma)k_{ba}(c)b_u,
\]

(3.103)
\[
\begin{align*}
\frac{\partial b_u}{\partial t} + \frac{\partial (v b_u)}{\partial y} &= ((\gamma - 1)k_{ba}(c) - k_{da}(c))b_u + \beta_1 A b_d - \beta_2 b_u, \\
\frac{\partial E}{\partial t} + \frac{\partial (v E)}{\partial y} &= (\beta_3 b_u + \beta_3 d b)d - \beta_4 E, \\
0 &= \frac{\partial}{\partial y} (k_{A} b \frac{\partial A}{\partial y}) + D_A \frac{\partial A}{\partial y} (w \frac{\partial A}{\partial y}) - \beta_5 A(k_{A} b + w) + \beta_{17} b_u + \beta_{18} b_d - \beta_{19} b_d A, \\
0 &= \frac{\partial}{\partial y} (k_{C} b \frac{\partial C}{\partial y}) + D_C \frac{\partial C}{\partial y} (w \frac{\partial C}{\partial y}) - \beta_6 b c c + \beta_{20} k \frac{\partial p}{\partial y}, \\
(\beta_3 b_u + \beta_3 d b)d - \beta_4 E &= \frac{\partial}{\partial y} \left( v - \frac{w}{\beta_{20} k} \frac{\partial p}{\partial y} \right), \\
\frac{\partial}{\partial y} (b \Sigma_b + E \Sigma_E) &= \frac{\partial}{\partial y} (-p_w + 2\beta_3 \mu \frac{\partial v}{\partial y}) + \beta_{13} \lambda \frac{\partial v}{\partial y}, \\
\frac{\partial \phi}{\partial t} + \frac{\partial (v \phi)}{\partial y} &= \phi \frac{\partial v}{\partial y},
\end{align*}
\]

where

\[
\begin{align*}
\mu &= \mu_b b + \mu_E E, \\
\lambda &= \lambda_b b + \lambda_E E, \\
k &= k_b b + k_E E, \\
\Sigma_b &= \begin{cases} 
0 & b + \alpha E \leq B_c \\
\left( \frac{b + \alpha E - B_c}{B_m - b - \alpha E} \right)^2 & \text{otherwise}, 
\end{cases} \\
\Sigma_E &= \begin{cases} 
0 & b + \alpha E \leq B_c \\
\left( \frac{b + \alpha E - B_c}{B_m - b - \alpha E} \right)^2 & \text{otherwise}, 
\end{cases} \\
k_{bd}(c) &= \frac{c}{c_1 + c}, \\
k_{ba}(c) &= \frac{A_{bud} c}{c_1 + c}.
\end{align*}
\]
\[ k_{dd}(c) = D_{dd} \left( 1 - \frac{\sigma c}{1 + c} \right), \quad (3.118) \]
\[ k_{du}(c) = D_{du} \left( 1 - \frac{\sigma c}{1 + c} \right) . \quad (3.119) \]

The initial and boundary conditions become

- Solid surface boundary conditions, i.e. \( y = 0 \):

\[ \frac{\partial A}{\partial y} = \frac{\partial c}{\partial y} = \frac{\partial p_w}{\partial y} = v(0, t) = 0. \quad (3.120) \]

- Moving biofilm boundary conditions, i.e. \( \phi(y, t) = 0 \):

\[ 0 = \left( -(b \Sigma_b + E \Sigma_E) + \beta_{13}(2\mu + \lambda) \frac{\partial v}{\partial y} \right) \quad (3.121) \]
\[ 0 = p_w, \quad (3.122) \]
\[ c = 1, \quad (3.123) \]
\[ A = 0. \quad (3.124) \]

- Initial conditions:

\[ b_d(y, 0) = \begin{cases} b_0 & \phi(y, 0) \leq 0, \\ 0 & \phi(y, 0) > 0, \end{cases} \quad (3.125) \]
\[ b_u(y, 0) = 0, \quad (3.126) \]
\[ E(y, 0) = 0, \quad (3.127) \]
\[ \phi(y, 0) = \phi_I. \quad (3.128) \]

We now have a full system of one dimensional equations, i.e. (3.102) - (3.110) coupled with the initial and boundary conditions (3.120) - (3.128).
3.5 Analysis of the Model

One characteristic of the quorum sensing process is its bi-stable states, where either all of the cells are down-regulated or the vast majority are up-regulated [47, 221, 220]. This change occurs rapidly due to the auto-inductive nature of the AHL production and hence the biofilms flips from one state to the next. With some analysis, this change of state can be shown to hold true in the current model, if we make the following assumptions:

- that the biofilm components are spatially independent,
- that on a small timescale the down- and up-regulated cells are in equilibrium,
- that \( \beta_1 \) and \( \beta_2 \) are very large compared to the birth and death rates of the down- and up-regulated cells,
- that the EPS volume fraction is small, which seems reasonable from the results shown in Section 3.7.1,
- that the down-regulated cells produce negligible AHL.

Taking these assumptions on board, then equations (3.75) - (3.77) and (3.79) become

\[
\begin{align*}
    w &= 1 - b_d - b_u, \\
    0 &= -\beta_1 A b_d + \beta_2 b_u, \\
    0 &= \beta_1 A b_d - \beta_2 b_u, \\
    0 &= -\beta_5 A (k_A b + w) + \beta_{17} b_u + \beta_{18} b_d - \beta_{19} b_d A.
\end{align*}
\]

However we also known that the total bacteria volume fraction, \( b^* \), is the sum of the down- and up-regulated cells. Therefore re-writing and manipulating these
Figure 3.3: Plots showing how the AHL concentration, left, and the up-regulated bacteria volume fraction, right, change with the total bacteria volume fraction plotted on a logarithmic scale.

equations, we can produce formulas for the up-regulated cell volume fraction and AHL concentration in terms of the parameter $b^*$, i.e.

\[
\begin{align*}
    b_d &= b^* - b_u, \\
    b_u &= \frac{\beta_1\beta_{17}b - \beta_5\beta_2(k_{A}b + 1) - \beta_{19}\beta_2b}{\beta_1\beta_{17} - \beta_2\beta_{19}} \\
    A &= \frac{\beta_1\beta_{17}b - \beta_5\beta_2(k_{A}b + 1) - \beta_{19}\beta_2b}{\beta_1\beta_{5}((k_{A} - 1)b + 1)}
\end{align*}
\]

These results are shown in Figure 3.3 using the values in Table 3.2.

The sharp rises in the up-regulated cells and the AHL concentration, in Figure 3.3, shows that the biofilm will quickly flip from one state to another, with just a small change in the total bacteria volume fraction. In fact with the parameter values used from Table 3.2, we see that this occurs around the 0.00001 mark.

### 3.6 Numerical Solution

Although a small amount of analysis can be done on this model, to fully understand it, a numerical approach is required. As with the previous models this one was
Initialise variables

Update 'b_d', 'b_u' and 'E' using the upwind method

Calculate ‘c’ coefficient matrix and vector and solved, using parallel CGLS

Calculate ‘A’ coefficient matrix and vector and solved, using parallel CGLS

Calculate ‘v_x’, ‘v_y’ and ‘p_w’ coefficient matrix and vector and solved, using parallel CGLS

Update level set, with upwinding inside and Fast Marching outside the biofilm

Time = Time + dt

Loop

Figure 3.4: A flow diagram of how Model 3 was solved in two dimensions and what methods were employed for the different variables. The variables v_x and v_y represent the advective velocity in the x and y directions respectively.
Table 3.1: This shows the calculated height of the biofilm, along $x = 0$, for various values of $\delta x$ (normalised such that the height at $\delta x = 0.001$ equals 1) with an adaptive $\delta t$, as well as their absolute error compared to the height calculated when $\delta x = 0.001$.

<table>
<thead>
<tr>
<th>Space step size ($\delta x$)</th>
<th>Calculate height of the biofilm</th>
<th>Absolute Error, compared to height when $\delta x = 0.001$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000125</td>
<td>0.999997</td>
<td>3.38 $\times 10^{-6}$</td>
</tr>
<tr>
<td>0.000025</td>
<td>0.99997</td>
<td>3.39 $\times 10^{-6}$</td>
</tr>
<tr>
<td>0.00005</td>
<td>0.99997</td>
<td>3.32 $\times 10^{-6}$</td>
</tr>
<tr>
<td>0.0001</td>
<td>1.000000</td>
<td>0.0</td>
</tr>
<tr>
<td>0.0002</td>
<td>1.000006</td>
<td>6.43 $\times 10^{-6}$</td>
</tr>
<tr>
<td>0.0003</td>
<td>1.000007</td>
<td>6.64 $\times 10^{-6}$</td>
</tr>
<tr>
<td>0.0004</td>
<td>1.000008</td>
<td>8.46 $\times 10^{-6}$</td>
</tr>
</tbody>
</table>

was solved by writing a computer program in Fortran and using finite difference methods. Whilst the biofilm components, i.e. bacteria cells and EPS, were still solved using an upwind method, all of the other variables were solved using the conjugate gradient least squares (CGLS) method. The reason for this alteration was due to the extra dimension changing their structure, such that they no longer formed tridiagonal or integral equations, but indefinite, sparse matrices. These matrices however were very large and therefore to solve them in a realistic time frame, the CGLS method was parallelised. Another difference that occurred, between this model and Model 2, was in equation (3.80) due to its non-linear nature, even after applying a finite difference scheme. This was overcome by assuming the value of $c$ in the denominator at a particular point, was equal to its value at the previous time step. The equation was then solved using the parallel CGLS method and the new values obtained were put back into the denominator and re-calculated. This re-calculation was done until the difference between them was of the same order as the errors within the finite difference scheme, i.e. $\delta x^2$ where $\delta x$ was the grid spacing. By using this method we were able to solve the non-linear equation, using the same techniques as those developed for its linear counterparts. For more details on these different methods, see Appendix 91.
A.2 and C. A level set approach had to be used to track the biofilm’s moving boundary, as we were solving the model in two dimensions. This also meant that the Fast Marching method was required, which was used to initialise the level set, such that each grid point contained the shortest distance to the outside edge of the biofilm. More information on both of these methods are given in Appendix B. Figure 3.4 shows how and what order the different variables were solved.

A code validation check was done on the Fortran code, by comparing the calculated height of the biofilm along the $x = 0$ line for various space steps sizes of the mesh grid. The results from this are shown in Table 3.1 and we see that all the errors are sufficiently small compared to the order of $\delta x$, which is the maximum theoretical error, implying consistence within the Fortran code.

3.7 Results

3.7.1 The Standard Results and Comparisons between One and Two Dimensions

In this section, we will be looking at the full two dimensional results, as well as comparing these with those obtained from the one dimensional version of this model. The parameter values and initial conditions used for the following results are given in Table 3.2. The parameters linked to the birth and death rates, were taken from the previous models (see Table 2.4). The parameters $\alpha$, $B_c$ and $B_m$ were estimated, such that the bacterial and EPS pressures would increase gradually over a wide range. Also any small increases in EPS would have a larger impact on these pressures. All of the other parameters were obtained from the references given, although many were then scaled accordingly. The scaling were chosen, such that most of the values were of the $O(0.01 - 100)$
Table 3.2: The initial and parameter values used in the standard results of Model 3.

to prevent numerical complications, e.g. stability limitations. More information on this is given in Appendix A. The comparative sizes between these re-scaled parameters were retained, such that qualitative differences between one and two dimensional simulations could be highlighted, rather than accurate quantitative predictions of biofilm growth.

The evolution of the biofilm’s outer edge is shown Figure 3.5, from $t = 0$ to 3 in half time steps. Since the boundary and initial conditions are symmetric about $x = 0$, we obtain symmetric solutions and therefore this is a prediction made by the model, rather than something artificially engineered. Also the small “wobbles” observed in the figure relate to a combination of small numerical and graphical plotting errors. However we observe from the graph that there is a greater amount of growth in the vertical than the horizontal direction, which
Figure 3.5: Evolution of the level set $\phi(x,y,t) = 0$, representing the growth of the biofilm for $t = 0$ to 3 in half time steps, using the initial and parameter values given in Table 3.2.

implies that some predictions can be obtained from one dimensional results. However Figures 3.6 and 3.7, shows that the biofilm does grow differently in two compared to one dimension and therefore care has to be taken with those predictions. The reason for this difference in growth between vertically and horizontally is linked with the velocity field, shown in Figure 3.15, which shows that at nearly any point there is at least a small upwards component. In fact the closer a point is to the centre of the biofilm, the greater this vertical component is, and therefore we observe more growth in this direction. We notice as well, that the biofilm is growing along the solid surface at the bottom, although at a much slower rate than the rest of the biofilm. This leads to the biofilm forming a mushroom shape, with a relatively small stork, which has been observed in other models and experiments [81, 99, 163, 15, 71, 24, 56]. The reason why the
Figure 3.6: Plots comparing solutions of the model in one (red) and two (green) dimensions, along the x = 0 axis, over time at t=0.5, 1, 2 and 3, using the initial and parameter values given in Table 3.2. In particular we have the heights of the biofilms in the top left against time, the AHL concentration in the top right, the nutrient concentrations in the middle left, the EPS volume fraction in the middle right, the net birth rate in the bottom left and b + αE volume fraction in the bottom right.
biofilm can move along the solid surface, even though the advective velocity is identically zero, is due to the conservative level set equation. The difference between the conservative version and the standard equation, is that the former uses derivatives of the advective velocity, whilst the latter only takes a point value. Therefore when the velocity field just above the solid surface is negative, i.e. the bacteria are moving towards the solid surface, the conservative equation allows them to touch the surface expanding the biofilm along it. More information on this is given in Appendix B.

Figures 3.6 and 3.7 show comparisons between results obtained in one and two dimensions, along the $x = 0$ axis, shown in red and green respectively. Some of the variables, for example the AHL and nutrient concentrations, $b + \alpha E$ and
the EPS and water volume fraction, all have the same qualitative shape. The biofilm’s height initial are very similar before diverging and by the time \( t = 3 \), we see a notable difference. There are several reasons for this, the first of which is the nutrient concentration. Initially the nutrients are able to fully penetrate the biofilm, whilst still having a sufficient amount, i.e. greater than 0.1, for the net birth rate to be positive in both one and two dimensions. This leads to the similar growth rates between the two, but they start to diverge as the nutrients in the one dimensional case stop fully penetrating the biofilm, whilst continuing to do so in two dimensions. The other reason for the difference in biofilm heights is the completely different advective velocity profiles, shown in the bottom left hand corner of Figure 3.7. Once the biofilm has settled down, we see that in one dimension the velocity profile will go negative initially before increasing. The reason for this, is that unlike in two dimensions, we have a non negligible bacteria pressure, which pushes some of them back towards the bottom of the biofilm. This is due to the \( b + \alpha E \) density decreases in that direction, as shown by the middle, right hand graph in Figure 3.6. Also there is cell death occurring towards the bottom of the biofilm, which helps with the negative velocity, as it creates further space for the more densely packed bacteria to move into, due to the bacterial pressure. However as the nutrients increase and the bacteria start dividing, the velocity starts increasing again, further expanding the biofilm. In two dimensions however, we have a very rapid growth near the bottom of the biofilm before it levels off. This is due to water entering the hollow core, as shown by the arrows in Figure 3.17, which pushes bacteria cells and EPS away due to the conservation of mass. The bacteria and EPS pressure around the edge of the hollow core, shown in Figures 3.8 - 3.12, halt the increasing water volume, whilst having insufficient nutrients to divide and hence no growth occurs. We then reach a point where there is sufficient nutrient for the bacteria cells to
divide, which in turn further increase the advective velocity.

We also notice that near $t = 2.5$ the growth rate of the two dimensional biofilm increases, which is due to the nutrients having a greater penetration depth from the top of the biofilm. From the data we do see that the nutrients are able to penetrate a further 0.2 into the biofilm between $t = 2$ and 3, although this is hard to see from the nutrient graphs, see Figure 3.14. We also still have all the one dimensional variables, shown in Figures 3.6 and 3.7, tending to travelling wave solutions, as seen in the previous models. The two dimensions lines shown in Figures 3.6 and 3.7 also show these travelling wave, although they are not always as distinctive as their one dimensional counterparts. However overall the results in one and two dimensions are qualitatively similar and we have no contrasting results.

The most interesting aspects of Figures 3.8 - 3.12 is the hollow core that develops in the middle of the biofilm, which corresponds with the fluid void at the bottom of the biofilms in the one dimensional case. There we had the bacteria dying off near the bottom of the biofilm, as it grew, due to limited nutrient penetration. Figure 3.14 shows a similar result, although this time they are able to penetrate the biofilm from all sides, but there is still insufficient nutrients reaching the core. Therefore in two dimensions the cells die off in a region, located in the bottom middle of the biofilm, as shown by Figure 3.10. This hollow will grow in size, with the biofilm, as the nutrients can only penetrate to a limited depth, as predicted by the one dimensional models. It will also remain as the lack of nutrients prevent reoccupation by live cells and no further EPS will be produced there, as both live cells and nutrients are required for its production. This hollow region has been observed and mentioned various times in connection with Pseudomonas aeruginosa and other bacteria [225, 224, 17, 226, 176, 208].
Figure 3.8: Evolution of the total bacteria volume fraction ($b = b_d + b_u$) for time, $t=0, 1, 2$ and $3$, using the initial and parameter values given in Table 3.2.

Figure 3.9: Evolution of the up-regulated bacteria volume fraction for time, $t=0, 1, 2$ and $3$, using the initial and parameter values given in Table 3.2.
Figure 3.10: Evolution of the net birth rate for time, \( t=0, 1, 2 \) and 3, using the initial and parameter values given in Table 3.2.

Figure 3.11: Evolution of the EPS volume fraction for time, \( t=0, 1, 2 \) and 3, using the initial and parameter values given in Table 3.2.
From Figure 3.9 we see that the up-regulated cells are dominant inside the biofilm, but decline near the edge, in correlation with the AHL concentration, Figure 3.13 and the prediction shown in Model 2. Around the hollow core at \( t = 2.5 \), there is a region of relatively high density of up-regulated cells, shown in yellow, which is caused by the low nutrient levels restricting the production of EPS. By \( t = 3 \) this region has decreased as more EPS has been produced and some of the cells have died as the hollow region expands. There are a couple of points of high EPS at the top of the biofilm at \( t = 3 \), shown in yellow, which have occurred due to a slightly lower bacteria density there. This is due to neither the bacteria nor EPS pressure building up sufficiently to even out the distribution, leaving the two spots that we see. We can also see a good correlation between the net birth rate and nutrient concentration, Figures 3.10 and 3.14 respectively, which we would expect as the cells need the nutrient to reproduce. There is also a link, although not as strong, between the net birth rate and the velocity field.
Figure 3.15, as the arrows are generally more longer in regions of high birth rate.

Both Figures 3.13 and 3.14 have the same characteristics as the one dimensional models. Its interesting though that the AHL concentration does not show a dip where the hollow is, in fact the concentration is at its highest there. This is due to it being able to diffuse through the water and is not consumed within the hollow as there are very few live cells. The concentration also drops off as we approach the edge of the biofilm, however from Figure 3.9 we see that there is still sufficient to convert the cells into an up-regulated state until just off the boundary. The nutrients are able to penetrate the biofilm further from the sides than from the top, approximately 2.5 and 1.5 respectively. We also see a greater birth rate along the sides of the biofilm as well, which is unsurprising as the two of them are linked together. Along the solid surface however we see a very short penetration depth of nutrients, which is due to a higher constant density of bacteria cells from the edge all the way to the hollow core.

The initial conditions in this simulation have the bacteria cells more densely packed than “natural”, i.e $b + \alpha E > B_c$, which leads to the high velocity field and bacteria pressure at $t = 0$, in Figures 3.15 and 3.16. Once the biofilm has grown and settled down, we see that growth occurs in all directions, creating a mushroom shape. We also notice that a component of the majority of the arrows are pointing upwards, as we would expect by the more rapid growth in this direction. Figure 3.15 explains why the growth along the solid surface is slower than elsewhere, in that the arrows only just start pointing downwards by $t = 2$, whilst growth elsewhere has occurred since $t = 0$. Also the velocity field is very similar to both the nutrient concentration and net birth rate, in that the arrows are longer around the outside, whilst being much smaller internally. Therefore, as we would expect, the higher the nutrients, the more births that occur, creating higher advective velocities. The bacterial and EPS pressure is
Figure 3.13: Evolution of the AHL concentration for time, $t=0, 1, 2$ and 3, using the initial and parameter values given in Table 3.2.

Figure 3.14: Evolution of the nutrient concentration for time, $t=0, 1, 2$ and 3, using the initial and parameter values given in Table 3.2.
Figure 3.15: Evolution of the advective velocity field for time $t=0$, 1, 2 and 3, with the size of the arrows representing the velocity’s magnitude, using the initial and parameter values given in Table 3.2. The line around the outside represents the edge of the biofilm.

Figure 3.16: Evolution of the bacterial and EPS pressures for time $t=0$, 1, 2 and 3, using the initial and parameter values given in Table 3.2.
Figure 3.17: Evolution of the water velocity field for time \( t=0, 1, 2 \) and \( 3 \), with the size of the arrows representing the velocity’s magnitude, using the initial and parameter values given in Table 3.2. The line around the outside represents the edge of the biofilm.

Figure 3.18: Evolution of the water pressure difference between inside and outside the biofilm, for time \( t=0, 1, 2 \) and \( 3 \), using the initial and parameter values given in Table 3.2.
also similar, in that it is higher around the edge of the biofilm than internally. This is to be expected, as the cells are dividing more rapidly here and hence a greater pressure is needed for them to exist at their “natural” density, i.e. \( b + \alpha E \leq B_c \). Although a conservative level set formulation was used, to enable the biofilm to expand along the solid surface, we notice that there are hot spots, shown in red on Figure 3.16, at the edge of the biofilm, on the solid surface. This is due to the no slip condition imposed on the boundary, which restricts the expansion inducing a localised accumulation of cells near the surface and the biofilm’s edge. Over the rest of the biofilm though the pressure remains approximately constant from \( t = 1 \) onwards.

We see that initially the water is flowing out of the biofilm as initially the cells are all densely packed and the nutrients are able to fully penetrate. Hence all the cells at this time are dividing, pushing the water out of the biofilm. However very quickly as the biofilm starts to mature, a slightly negative pressure is created internally, pulling the water back in, as shown in Figures 3.17 and 3.18. In fact we see the fastest water velocity near the bottom of the biofilm, as there is a “large” pressure gradient there. Also, after \( t = 0 \) we see that water is continuously coming in at the top, which is required to fill the space between the bacteria cells and EPS as they move upwards. This also accounts for the lower flow rate seen in the middle of the biofilm, as the net birth rate is negative there, implying that the cells are converting into water. Plus by \( t = 3 \), we see that some of the water coming in at the bottom is moved around and back out the side, as there is sufficient water coming in from the top of the biofilm. We also see that the maximum water pressure increases with time, which is necessary to pull more water into the biofilm from the surrounding area, as it grows in size.
Figure 3.19: Low nutrient evolution of the level set $\phi(x, y, t) = 0$, representing the growth of the biofilm for $t = 0$ to 3 in half time steps. These results were obtained with $c_0 = 0.5$, whilst the rest of the initial and parameter values given in Table 3.2 remain unchanged.

Figure 3.20: Low nutrient graphs of bacteria volume fraction in the top left ($b_d + b_u$), net birth rate in the top right ($k_b - k_d$), nutrient concentration in the bottom left ($c$) and advective velocity field in the bottom right ($v$), at $t = 3$, using $c_0 = 0.5$, whilst all the other parameters remain unchanged from Table 3.2.
3.7.2 Growth in a Low Nutrient Environment

The results below used the same parameter set as given in Table 3.2, for the standard results above. However to study the effects a low nutrient environment has on a biofilm’s development, we change the nutrient concentration on the biofilm/fluid boundary from $c_0 = 1$ to $c_0 = 0.5$. We would expect the biofilm’s structure to remain qualitatively the same but for it to grow and develop slower due to the lack of nutrients.

Comparing Figures 3.19 and 3.5, we can clearly see that in the low nutrient environment the biofilm is smaller in size at each time step shown. The main reason for this, is that the net birth rate (top right hand graph in Figure 3.20), has only a narrow region of positive growth, compared to Figure 3.10. The reason being that a sufficient amount of nutrients are no longer able to penetrate deep into the biofilm, due to the external concentration being halved. However even with this smaller penetration depth, we still have approximately the same growth rate around the edge of the biofilm and the bacteria density is slightly higher. The reason for the higher density is due to less EPS pushing the bacteria further apart, as nutrients are required in its production. One interesting aspect though is the velocity field, which shows that most of the cells are moving upwards rather than side ways especially from the biofilm’s core. The reason for this is the comparatively high bacteria volume on the sides of the biofilm restricting the movement of cells from the core in those directions. The overall picture of the biofilm though has not changed and we see the same structures appearing, for example the hollow in the centre of the biofilm.

3.7.3 Quorum Sensing Mutant

The results below use the same parameter set as given for the standard results above in Table 3.2. The only change was to set $\beta_1 = 0$, to stop the bacteria cells
from up regulating and hence disabling the quorum sensing process. From experimental work carried out by Heydorn et al. (2002, [77]) we would expect very little structural difference between these results and those of the wild type, i.e. the standard results.

For the disabled quorum sensing case in Model 2, see Figure 2.8, we saw that the biofilm grew considerably slower, however this appears not to be the case in the two dimensional model. If we compare Figures 3.21 and 3.5, we see that the mutant initially grows more slowly before increasing in speed vertically, such that the two biofilms are approximately the same height at $t = 3$. Figure 3.26 shows that to start with the biofilm grows in a similar, although slower, manner to the wild type. However the $t = 3$ graph shows a similar characteristic to what was seen in the low nutrient case, i.e. Figure 3.20, where the bacteria
cells deep within the biofilm are moving vertically upwards. This is due to the densely packed bacteria on either side of the biofilm, whilst they are slightly less dense for a small region just above the hollow. Hence there is less resistance to movement in this direction and we obtain a velocity field with a greater vertical component. Also the bacteria cells are more densely packed, Figure 3.23, in general than in the wild type, Figure 3.8, which also helps to accelerate the rate of biofilm growth. Although the biofilm is growing sideways as well, this movement upwards from the centre causes the difference we see between the two directions.

Figure 3.22: Comparison of the heights of the biofilms in the top left ($\phi = 0$), the nutrient concentrations in the top right ($c$), the bacteria pressure in the bottom left ($p_b$) and water volume fraction in the bottom right ($w$), for time, $t=0.5, 1, 2$ and $3$, between one dimension in red and two dimensions in green taken along the $x = 0$ axis. For these results the initial and parameter values are given in Table 3.2, except $\beta_1 = 0$. 

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Figure 3.23: Evolution of the quorum sensing mutant’s total bacteria volume fraction ($b = b_d + b_u$) for time, $t=0, 1, 2$ and $3$, with $\beta_1 = 0$, whilst all the other parameter values remained unchanged from Table 3.2.

Figure 3.24: Evolution of the quorum sensing mutant’s net birth rate for time, $t=0, 1, 2$ and $3$, with $\beta_1 = 0$, whilst all the other parameter values remained unchanged from Table 3.2.
Figure 3.25: Evolution of the quorum sensing mutant’s nutrient concentration for time, $t=0, 1, 2$ and $3$, with $\beta_1 = 0$, whilst all the other parameter values remained unchanged from Table 3.2.

Figure 3.26: Evolution of the quorum sensing mutant’s velocity field for time, $t=0, 1, 2$ and $3$, with $\beta_1 = 0$, whilst all the other parameter values remained unchanged from Table 3.2.
The results shown in Figure 3.22 are qualitatively similar to those of the standard results, shown in Figures 3.6 and 3.7. For example the biofilm’s height between one and two dimensions remains the same initially before the one dimensional’s height slows down and they diverge. The reason for the decreased growth rate in one dimension is the same as before, in that the nutrients cannot fully penetrate the biofilm, which leads to cells dying near the bottom. Plus the bacteria density is high, and hence its pressure forces cells backwards towards the bottom of the biofilm, implying that not all the cell division creates an expansion. In two dimensions we see that the bacteria pressure increases more significantly near the top of the biofilm, than in the standard results, which leads to the biofilm increasing in size faster than we would otherwise expect. Plus we see that the water volume fraction quickly rising from approximately 0.5 to 0.95 at $t = 3$, which as mentioned above, causes an increase in the advective velocity near the bottom of the biofilm.

We notice from Figure 3.23 that the hollow core of the mutant is taller than the wild type, as the nutrients are not able to penetrate as deep in the vertical direction. Horizontally though the hollow remains approximately the same size, compared to the wild type, however the biofilm is thinner and hence proportionally it is larger as well. These results though show that the biofilm is structurally similar to the wild type shown in the standard results section above, which broadly correlates with the experiments done by Heydorn et al. (2002, [77]). There is disagreement with other experiments, e.g. Davier et al. (1998, [43]), but this can be explain by the reasons given above. Another explanation of the similarity between the wild and mutant types could also be explained by the small value of $\beta_{3u}$, which determines the production of EPS by the up-regulated cells. If EPS was produced at a much faster rate by the up-regulated cells in the wild type, we would see a faster growing biofilm than the mutant, due to
the extra EPS pressure.

3.7.4 Small, Initial Biofilm

In the standard results, see Section 3.7.1, we saw that the one and two dimensional results diverged quite quickly. Therefore in this section we will investigate a small, wide biofilm which should better resemble its one dimensional counterpart. Hence this allows us to check whether, in the limit as the biofilm becomes infinity wide, that the results in two dimensions are the same as those in one dimension. It will also allow the nutrients to fully penetrate the biofilm for a longer period of time and therefore increase the growth rate. Hence we would expect to see a biofilm that has grown larger relative to its initial size, compared to before, although not necessarily horizontally. For this comparison we have left all the parameters identical to those of Table 3.2 and just halved the height of the initial biofilm.

Figure 3.27 compares the results obtained from both one and two dimensions, shown in red and green lines respectively. As expected the two biofilms do grow more similarly, as this time they have the same height until approximately $t = 1.5$, whereas with the results shown in Figure 3.6, they had diverged by $t = 1$. However many of the characteristics seen with the two dimensional biofilms, shown in Figures 3.6 and 3.7 can still be seen with this smaller biofilm. Examples of these include the fact that the vertical velocity is never negative, as occurs in one dimension and the bacteria pressure is always negligible.

Comparing the full two dimensional results, Figure 3.28 shows that the biofilm has now grown approximately five and a half times larger in height, compared to the four times we saw with the standard results, in Section 3.7.1, in the same time period. However it has not even doubled in width by $t = 3$, which was easily achieved by the standard results, although this has more than
Figure 3.27: Comparison of the heights of the biofilms on the top left ($\phi = 0$), the nutrient concentrations on the top right ($c$), $b + \alpha E$ volume fraction on the middle left, the bacteria pressure on the middle right ($p_b$), the vertical advective velocity on the bottom left ($v$) and water volume fraction solutions on the bottom right ($w$) for time, $t=0.5, 1, 2, 3$ and $4$, between one dimension, in red, and two dimension, in green taken along the $x = 0$ axis. For these results all of the initial and parameter values remained unchanged from Table 3.2, only the initial height of the biofilm was halved.
Figure 3.28: Evolution of the level set $\phi(x, y, t) = 0$, representing the growth of the biofilm for $t = 0$ to 4 in half time steps, with half the initial height. The biofilm size has also been rescaled, such that the initial height is equal to one. Apart from the smaller initial height all the other initial and parameter values remained unchanged from Table 3.2.

Figure 3.29: Graphs of bacteria volume fraction on the top left $(b_d + b_u)$, net birth rate on the top right $(k_b - k_d)$, nutrient concentration on the bottom left $(c)$ and advective velocity field on the bottom right $(v)$, at $t = 3$, with half the initial height. Apart from the smaller initial height all the other initial and parameter values remained unchanged from Table 3.2.
occurred by \( t = 4 \). This would imply that by decreasing the initial height, the biofilm is able to recover its height but at the expense of width, over the same time interval. However given a bit more time and the horizontal growth quickly catches up as well. Although as the long term growth of the biofilm is approximately linear and the smaller biofilm is growing slightly slower, this implies that given the same time period there will always be a difference in size between the two biofilms. If different time intervals are given to each biofilm though, such that the smaller biofilm is given more time, then they would both reach approximately the same size.

Figure 3.29, shows that at \( t = 3 \) the nutrients are failing to reach the central regions at adequate survival concentrations and therefore cells are beginning to die there. Hence a hollow, as seen in the standard results, is starting to form in this region. The velocity profile shows that the biofilm is still growing slightly faster upwards than outwards, however we are getting a greater downwards speed near the solid surface. Although at \( t = 3 \) the biofilm has hardly moved along the solid surface, unlike the standard results but has started doing so by \( t = 4 \). Also comparing Figures 3.15 and 3.29 at \( t = 3 \), shows that they both have approximately the same vertical velocity at the top of the biofilm. This demonstrates that the growth of the biofilm has tended towards a long term growth pattern.

Overall we have seen that by decreasing the initial height of the biofilm, the nutrients were able to penetrate the biofilm for longer, giving an increase in growth rate. However we observe that the qualitative distribution of the variables are similar in both cases.
3.8 The Effect of a Remote Nutrient Source

The simulations so far are of biofilms grown in a well mixed nutrient medium. However in this section we will assume that it is sitting in a tank of shallow, still water where there is negligible evaporation in the time scale of interest. The nutrients are then fed continuously and evenly into the water from the top and are unable to penetrate any of the walls. The nutrients are then able to diffuse through the water and into the biofilm where they are consumed as before. This is the expected situation if we consider oxygen as our nutrient. Figure 3.30 shows a schematic of the spatial set up for this simulation.

From this information we obtain the following new non-dimensional equations for the nutrients, outside of the biofilm, whilst still using equation (3.80) for inside. Plus the new boundary conditions are:

\[
\nabla^2 c = 0 \quad \text{outside the biofilm,} \\
\n\nabla \cdot (k_c b \nabla c) + D_c \nabla \cdot (w \nabla c) - \beta_b \frac{c}{0.1 + c} = 0 \quad \text{inside the biofilm,} \\
\nc = 1 \quad \text{at the top of the domain,} \\
\frac{\partial c}{\partial y} = 0 \quad \text{on the solid surface,} \\
\frac{\partial c}{\partial x} = 0 \quad \text{on the LHS and RHS of the domain.}
\]

All of the other equations remain unchanged and are given by (3.75) - (3.79) and (3.81) - (3.83) coupled with the initial and boundary conditions (3.93) - (3.101).

3.8.1 Results

To allow a comparison to be made between these results and those of the standard set in Section 3.7.1, all of the parameters in Table 3.2 were used in this
Figures 3.30: A schematic of the extension to Model 3 being considered.

Simulation. The only parameters not included in the table, are in relationship to the tank size, which was set at a height of 4, with the left and right walls set at $-6$ and $6$ respectively.

Figure 3.31: Evolution of the level set $\phi(x, y, t) = 0$, representing the growth of the biofilm for $t = 0$ to 2.5 in half time steps. The initial and parameter values for these results are given in Table 3.2, whilst the left, right and top boundary walls were to $-6$, 6 and 4 respectively.
Figure 3.31 shows the biofilm accelerating in speed towards the top of the tank, where the highest nutrient concentration exists. We did not see this in the standard results, due to the uniform distribution of nutrients around the edge of the biofilm. The reason is, as the biofilm grows higher, cells at the top are receiving more nutrients and thus grow and divide quicker.

The velocity field, in Figure 3.32, also shows that most movement occurs in the vertical direction and hence further increases the movement in this direction compared to horizontally. The distributions of bacteria cells and EPS are also shown in Figure 3.32 where the majority of the active biomass is located around the edges and towards the top of the biofilm. Plus as the highest nutrient concentration occurs above the biofilm, we end up with this finger rather than mushroom shape, which correlates with the results obtained by Duddu et al. (2008, [49]).

Figure 3.32: Graphs of bacteria volume fraction on the top left ($b_d + b_w$), net birth rate on the top right ($k_b - k_d$), EPS volume fraction on the bottom left ($E$) and advective velocity field on the bottom right ($v$), at $t = 2.5$. The initial and parameter values for these results are given in Table 3.2, whilst the left, right and top boundary walls were to $-6, 6$ and $4$ respectively.
Figure 3.33: Evolution of the nutrient concentration for time, $t=0$, 1, 2 and 2.5. The initial and parameter values for these results are given in Table 3.2, whilst the left, right and top boundary walls were to $-6$, 6 and 4 respectively.

Figure 3.33 shows how the growing biofilm affects the surrounding nutrient concentration. Whilst the biofilm is small we observe an approximately even distribution of nutrients around the edge. However as the biofilm grows less nutrients are able to diffuse through the water on either side of it and hence further restricts growth near the bottom. Another slight variation that we have not seen before is the increased nutrient concentration in the middle of the biofilm at $t=2.5$. This is due to the lack of bacteria in the centre and hence any nutrients that have diffused into that region are only slowly consumed. On either side however we still have a region of highly active biomass, which is consuming the nutrients and hence they have a decreased concentration in that area. Therefore we see the observed effect of higher nutrient concentration inside the biofilm than around the outside. The simulation was stopped at $t=2.5$, otherwise the biofilm would have grown large enough to reach the top of the tank, resulting in a programme crash.
3.9 Transport of Large Molecules

When modelling the transport of drugs, we assumed that advection was negligible, i.e. they moved through the biofilm by diffusion alone. This is a valid assumption for small molecules; however the larger they are the more effect advection has on their movement. Therefore in this section, we will examine the differences between the penetration of molecules under advection and diffusion against diffusion alone. To simplify this comparison, we took a ‘static’ biofilm, which came from the standard results of Model 3 (see Section 3.7.1), at time $t = 1$.

Using this information, we assume that we have a chemical, $s$, that moves through the biofilm due to advection and diffusion, whilst being consumed by the bacteria cells at a rate $\lambda$. Hence

$$\frac{\partial s}{\partial t} + \nabla \cdot (u s) = D \nabla^2 s - \lambda b s, \quad (3.133)$$

where $u$ is the fluid velocity inside the biofilm, $D$ is the diffusion coefficient and $b$ is the bacteria cell volume fraction. To simplify the problem even further, we looked at the steady state case, i.e. we set $\partial s / \partial t = 0$, with boundary conditions of

- $s = s_0$ on the fluid/biofilm interface,
- $\frac{\partial s}{\partial y} = 0$ on the solid surface, at the bottom of the biofilm.

If we now non-dimensionalise equation (3.133), using the standard formulae, i.e.

$$x = H_0 \hat{x}, \quad y = H_0 \hat{y}, \quad u = H_0 A_{bd} \hat{u}, \quad s = s_0 \hat{s}, \quad (3.134)$$

where hatted variables are non-dimensional and the parameters $H_0$ and $A_{bd}$ are
Figure 3.34: Comparison between a chemical under advection and diffusion, shown on the left, and diffusion alone, shown on the right. These results were obtained using the results of the standard biofilm at $t = 1$, shown in Section 3.7.1, with $D = 0.000001$ and $\lambda = 5000$. Plus in the left hand graph $A_{bd} = 500$, whilst in the right hand graph $A_{bd} = 0$.

defined as above. Therefore after some rearrangement and dropping the hats, we obtain

$$
\frac{A_{bd}H_0^2}{D} \nabla \cdot (us) = \nabla^2 s - \frac{H_0^2 \lambda}{D} b_s,
$$

with $s = 1$ on the biofilm/fluid interface and $\frac{\partial s}{\partial y} = 0$ on the solid surface.

By letting the left hand side equal zero, we cut out the advection term, leaving the chemical to only diffuse through the biofilm. Solving these two equations, inside the static biofilm described above, we obtain the results shown in Figure 3.34, with $D = 0.000001$ and $\lambda = 5000$.

The penetration of large molecules as shown in Figure 3.34, is about one and a half times in the advection and diffusion case compared to diffusion only case. Hence it it probably true that if considering large molecules that fluid advection should to be considered, when the Peclet number is of order one, i.e. $A_{bd}H_0^2/D = O(1)$ in this model.
3.10 Conclusions

In this chapter we developed a three dimensional biofilm model, which is solved in two dimensions, for Pseudomonas aeruginosa that incorporated quorum sensing, force balances and drag forces between the different biofilm components, whilst assuming that there were no external forces. To accomplish this, we modelled the biofilm components, i.e. the bacteria and EPS, as a viscous fluid, with non viscous water flowing through it. All of the results obtained, showed a biofilm growing out both vertically and horizontally creating a mushroom shape, which has been observed in various models and experiments [81, 99, 163, 15]. The biofilms also grew along the solid surface, which was allowed due to the use of the conservative level set equation. However this growth was at a considerable slower rate and we did see a small pressure build up at the biofilm’s edge on the surface, implying that the no-slip condition was restricting the growth. Hence if we were to remove this base from the general biofilm’s shape, then our model predicts that it would grow into an elliptical ball. This might explain why the other models and experiments mentioned produced their mushroom shaped biofilms via nutrient diffusion driven instabilities.

The biofilm’s growth behaviour presented in the one and two dimensional simulations of Model 3, share many common features with those presented in Chapter 2. For example the biofilm mainly consisted of up-regulated cells, the down-regulated cells only formed around the edge, where the AHL concentration was low. Therefore the extension of the model, allowing partitioning of the internal and external AHL and nutrient concentrations has made little difference to the qualitative results. The results also showed that with the given initial biofilm shape, that they grew faster in two than one dimension. This was mainly due to a less steep nutrient gradient and hence it could penetrate deeper, which allowed more of the bacteria cells to divide, creating a larger advective velocity.
However it was shown with the Small, Initial Biofilm, see Section 3.7.4, that the long flat two dimensional biofilm would grow in a similar manner as a one dimensional biofilm. One contrasting feature between the one and two dimensional simulations is in the more mature biofilms, the velocities in the one dimension version can become negative. This was due to a greater bacteria pressure, which pushed the cells towards the bottom of the biofilm once the nutrients could not fully penetrate. The cells were pushed downwards due to the lower cell volume fraction, caused by cell death following the lack of nutrients. This was not observed in two dimensions, as the bacteria pressure was much smaller and hence near the bottom of the biofilm it was the influx of water that dominated the velocity profile. Other variables were similar in their nature, for example the AHL concentration, which was still greatest at the centre of the biofilm, before decreasing to zero as it approaches biofilm/fluid interface. In one dimension the solutions still tended to a travelling wave solution as the biofilm increased in size. As the biofilm matured and increased in size, in the two dimensional case, we would expect travelling wave solutions to exist along the $x = 0$, similar to their one dimensional counterpart. The start of some of these travelling waves were observed for example in the AHL and nutrient concentrations.

In the one dimensional biofilm, we had the bacteria dying off behind the live zone of the biofilm, due to limited nutrient penetration. In two dimensions, we still had a limited nutrient penetration depth, causing a dead zone to appear. However the manifestation of this dead zone formed a hollow bacteria free core within the structure, which was observed in all our results, although this process was just starting with the small, initial biofilm at $t = 3$ [225, 224, 17, 226, 176, 208].

We also looked at development in a low nutrient environment, which produced a structurally similar biofilm. However as there was less nutrients, it could
not able to penetrate as deep. Hence, as expected, a smaller biofilm with a larger hollow section was observed, compared to the “standard” nutrient environment biofilm. The simulation of a quorum sensing negative mutant, which produced AHLs and EPS at a much reduced rate, produced results qualitatively similar to the wild type, but being marginally narrower. This structural similarity was observed by Heydorn et al. (2002, [77]), when they carried out experiments looking at the biofilms of different mutants. However this similarity is due to the low production rate and volume fraction of EPS within the wild type, i.e. “standard results” biofilm of Section 3.7.1. Furthermore the relative lack of EPS in the mutant, is likely to make it more vulnerable to shear forces, and thereby easier to displace.

We also investigated the effect of changing the initial biofilm’s shape by observing what occurred with a biofilm that had half the initial height to that used in the “standard” simulation. In this case we saw that the biofilm grew quicker and produced a smaller biofilm that was proportionally narrower over the same time interval, compared to the “standard” simulation. The reason behind the increased growth rate was that the nutrients could fully penetrate the biofilm for an extended period of time, allowing all cells to divide rather than just the ones around the outside. However after leaving the biofilm to continue developing for a short period of time, it had fully recovered to the state that the standard biofilm was in at $t = 3$.

An extension to the basic model was investigated where we changed the way the nutrients entered the fluid surrounding the biofilm. Previously we had assumed that there was a constant nutrient concentration in the fluid; this assumption was dropped. Instead we assumed that there was a constant concentration at the top of a tank, and let the nutrients diffuse through the still water and into the biofilm, sitting on the bottom. The rest of the model and parameters
remained unchanged, but the shape and growth dynamics were different to the “standard” simulation. Here a narrower biofilm developed with growth apparently accelerating towards the water surface at the top of the tank. Furthermore the active biomass was largely concentrated near the upper surface of the biofilm and very much thinner around the side walls, compared to what we had previously seen. Alpkvist et al. (2006, [5]) investigated nutrient driven instability leading to finger formations. Although this has not been formally investigated with the current model, the set up of this simulation with the predicted, enhanced upwards growth, leads to the proposition that instability induced finger formation is possible here.
Chapter 4

Modelling Anti-biofilm Agents

4.1 Background

Bacteria in biofilms tend to be more resistant to removal by anti-bacterial agents than in either of their other two states. This is typically due to

- diffusion limitations of anti-biofilm agents through the biofilm and hence they only affect the outer edges,

- change in sensitivity due to reduced metabolic activity from low levels of nutrients deep within the biofilm,

- phenotypic shifts in biofilm forming bacteria in which they have a greater intrinsic resistance to the agent, e.g. persister cells in Candida albicans biofilms [114].

These defences do not work, when bacteria cells are in their planktonic state as their effect at limiting the diffusion or lowering the nutrient concentrations, for example, are negligible. This is in comparison to the concentration of the anti-bacteria agents or chemicals in the local environment. Further information on bacteria defence mechanisms in discussed in Section 1.6.
In Pseudomonas aeruginosa, EPS production enhances the growth of biofilms, leading to a biofilm that is more resistant to externally applied agents. Since production of EPS appears to be regulated by quorum sensing in this bacterium, biofilm control may be achieved by interfering with this mechanism. Hence in this chapter we will investigate the drugs mentioned in Section 1.7, and see how each one of them affects the growth and development of the biofilm that was modelled in Chapter 3. These drugs were:

- **Antibiotics**, which just kill the bacteria cells. These include the antibiotic, which act directly on cells such as penicillin, antineoplastic and doxorubicin, and surfactants [87], which “dissolve” the EPS disintegrating the biofilm. The problem is that it maybe impossible to deliver these agents in a way that is safe and effective.

- **Anti-quorum sensing**, which is possibly a new strategy in treating biofilms that can slow the growth rate and lower the EPS, making it easier for removal. Examples of these include RNAIII-inhibiting peptide, which helps to prevent drug-resistant Staphylococcus epidermidis biofilm formation [12] and furanones that can inhibit various bacteria including salmonella [89] and Pseudomonas aeruginosa [236]. There are actually two different anti-quorum sensing strategies that we will be looking at:
  
  - anti-AHL, which degrades sequestering AHL molecules and thus decreases the rate of up regulation,
  
  - anti-lasR, which inhibits the up regulation rate, by removing the lasR complex. This complex is what binds with the AHL molecules, in order to up-regulate a cell.
4.2 Mathematical Modelling

Each of these drugs will now be modelled based upon the work done by Anguige et al. (2006, [7]) and Ward (2008, [218]). Both the antibiotic and anti-lasR drugs mentioned above directly affect the down- and up-regulated cell’s volume fraction, previously defined by equation (3.38) and (3.39) in Section 3.2. We know that many antibiotic, $Q$, drugs are most effective when bacteria cells are actively dividing and it is assumed that the kill rate is proportional to the birth rate. The reason is that cells are in their most vulnerable state at this time, due to the division of the DNA and weaker cell walls [7]. The anti-lasR drugs on the other hand inhibit up regulation by reducing the amount of lasR the AHL can react with. This occurs at a rate proportional to the anti-lasR concentration, $R$, and the amount lost during the reaction, $k_R$, with the lasR complex [218]. Hence the modified form of the down- and up-regulated bacteria volume fraction become,

$$\frac{\partial b_d}{\partial t} + \nabla \cdot (vb_d) = (k_{bd}(c) - k_{dd}(c))b_d - \left( \frac{\alpha_1 A}{1 + k_R R} \right) b_d + \alpha_5 b_u$$

$$+(2 - \gamma)k_b b_u - k_Q k_{bd}(c)Q b_d,$$

$$\quad \quad (4.1)$$

$$\frac{\partial b_u}{\partial t} + \nabla \cdot (vb_u) = ((\gamma - 1)k_{bd}(c) - k_{dd}(c))b_u + \left( \frac{\alpha_1 A}{1 + k_R R} \right) b_d - \alpha_5 b_u$$

$$-k_Q k_{bu}Q b_u.$$  

$$\quad \quad (4.2)$$

The parameter $k_Q$ represents the amount of drug lost during the reaction between the antibiotics and the bacteria cells. Moving onto the AHL concentration, we have that both the anti-AHL, $N$, and anti-lasR drugs will affect it. The first by reacting with it in the water phase, as it is assumed that the anti-AHL drugs are unable to penetrate the cell membranes. The second simply reduces the up regulation rate and hence decreases the amount of AHL used in a particular time interval. Therefore equation (3.41) in Section 3.2, which defines the AHL
concentration becomes

$$0 = D_A \nabla \cdot (b \nabla k_A A) + D_A \nabla \cdot (w \nabla A) - \lambda A (k_A b + w) + \alpha_2 b_u + \alpha_4 b_d - \left( \frac{\alpha_3 b_d}{1 + k_R R} \right) A - k_N w N A,$$

(4.3)

where $k_N$ is the constant representing how effective the anti-AHL drug is.

We have seen how the different drugs affect the various biofilm components, which just leaves us to model their transportation and kinetics through the biofilm. We make the same assumptions that we used for the AHL and nutrient concentrations in Model 3, in that the drugs inside and outside the bacteria equilibrate rapidly. Therefore $Drug_{\text{outside}} \approx k_{Drug_{\text{inside}}}$, where $k_{Drug_i}$ is the partition coefficient and $i$ represents either the antibiotics or anti-lasR drugs. Hence we obtain, in a similar fashion to that described in Section 3.2, the following equations,

$$0 = D_Q \nabla \cdot (b \nabla k_Q Q) + D_Q \nabla \cdot (w \nabla Q) - M_Q k_Q k_b Q,$$

(4.4)

$$0 = D_R \nabla \cdot (b \nabla k_R R) + D_R \nabla \cdot (w \nabla R) - \frac{M_R k_R b R}{1 + k_R R},$$

(4.5)

The constant $M_i$ is a measure of the drug’s effectiveness, in which more potent drugs will have a correspondingly smaller $M$. This leaves the anti-AHL drug, which as stated earlier cannot diffuse through the bacteria cells, i.e. $D_{N_i} = 0$, and thus we obtain the slightly simpler equation

$$0 = D_{N_u} \nabla \cdot (w \nabla N) - M_N k_N w N A,$$

(4.6)

where $M_N$ is defined as above.

For each of the drugs, we assumed the same boundary conditions as for the nutrients in Model 3, i.e. they are unable to penetrate the solid surface at the
bottom of the biofilm and they have a fixed concentration at all points around the biofilm/fluid boundary. Therefore we have

\[ y = 0 : \quad \frac{\partial Q}{\partial y} = \frac{\partial R}{\partial y} = \frac{\partial N}{\partial y} = 0, \]

\[ \phi = 0 : \quad Q = Q_0 Q_e, \quad R = R_0 R_e, \quad N = N_0 N_e, \]

where \( Q_0, R_0 \) and \( N_0 \) are dimensionless constants and \( Q_e, R_e, \) and \( N_e \) are external drug concentrations.

### 4.3 Non-Dimensionalisation

We now non-dimensionalise our system of equations, i.e. (4.1) - (4.7), using the following substitutions,

\[
t = \frac{t}{A_{bd}}, \quad x = H_0 \hat{x}, \quad v = H_0 A_{bd} \hat{v}, \quad A = A_0 \hat{A},
\]

\[
Q = Q_0 \hat{Q}, \quad R = R_0 \hat{R}, \quad N = N_0 \hat{N},
\]

where the hatted variables are non-dimensional and \( A_{bd}, H_0, A_0, Q_0, R_0 \) and \( N_0 \) are defined as before. Therefore substituting these non-dimensional variables into our equations and dropping the hats we obtain

\[
\frac{\partial b_d}{\partial t} + \nabla \cdot (\nu b_d) = \left( k_{bd}(c) - k_{dd}(c) \right) b_d - \left( \frac{\beta_1 A}{1 + \beta_{40} R} \right) b_d + \beta_2 b_u
\]

\[ + (2 - \gamma) k_{ba}(c) b_u - \beta_{41} Q b_d, \]  

(4.8)

\[
\frac{\partial b_u}{\partial t} + \nabla \cdot (\nu b_u) = \left( (\gamma - 1) k_{bu}(c) - k_{du}(c) \right) b_u + \left( \frac{\beta_1 A}{1 + \beta_{40} R} \right) b_d - \beta_2 b_u
\]

\[ - \beta_{41} Q b_u, \]  

(4.9)

\[
0 = \nabla \cdot (b \nabla k_A A) + D_A \nabla \cdot (w \nabla A) - \beta_5 A (k_A b + w) + \beta_{17} b_u
\]

\[ + \beta_{18} b_d - \left( \frac{\beta_{19} b_d}{1 + \beta_{40} R} \right) A - \beta_{42} w N A, \]  

(4.10)

\[
0 = \nabla \cdot (b \nabla k_Q Q) + D_Q \nabla \cdot (w \nabla Q) - \beta_{43} k_b Q,
\]

(4.11)

\[
0 = \nabla \cdot (b \nabla k_R R) + D_R \nabla \cdot (w \nabla R) - \left( \frac{\beta_{44} b R}{1 + \beta_{40} R} \right), \]  

(4.12)
\[0 = \nabla \cdot (w \nabla N) - \beta_{45} w A N,\]  
\[b_d + b_u + E + w = 1,\]  
\[\frac{\partial E}{\partial t} + \nabla \cdot (v E) = (\beta_{3u} b_u + \beta_{3d} b_d) c - \beta_4 E,\]  
\[0 = \nabla \cdot (k_c \nabla c) + D_c \nabla \cdot (w \nabla c) - \beta_{6c} \frac{c}{c_1 + c},\]  
\[\nabla \cdot (b \Sigma_b + E \Sigma_E) I = \nabla \cdot (-p_a I + \beta_{13} \mu (\nabla v + \nabla v^T) + \beta_{13} \lambda (\nabla \cdot v) I),\]  
\[\frac{\partial \phi}{\partial t} + \nabla \cdot (v \phi) = \phi \nabla \cdot v,\]  

where

\[\beta_{40} = k_{R} R_0, \quad \beta_{41} = \frac{k_{Q} Q_0}{A_{bd}}, \quad \beta_{42} = \frac{k_{N} N_0 H_0^2}{D_{A_i}} ,\]  
\[\beta_{43} = \frac{M_{Q} k_{Q} A_{bd} H_0^2}{D_{Q_i}}, \quad \beta_{44} = \frac{M_{R} k_{R} H_0^2}{D_{R_i}}, \quad \beta_{45} = \frac{M_{N} k_{N} H_0^2 A_0}{D_{N_o}} ,\]  
\[\mu = \mu_b + \mu_E E,\]  
\[\lambda = \lambda_b + \lambda_E E,\]  
\[k = k_b + k_E E,\]  
\[\Sigma_b = \begin{cases} 0 & b + \alpha E \leq B_c \\ \left(\frac{b + \alpha E - B_c}{B_m - b - \alpha E}\right)^2 & \text{otherwise}, \end{cases}\]  
\[\Sigma_E = \begin{cases} 0 & b + \alpha E \leq B_c \\ \left(\frac{b + \alpha E - B_c}{B_m - b - \alpha E}\right)^2 & \text{otherwise}, \end{cases}\]  
\[k_{bd}(c) = \frac{c}{c_1 + c},\]  
\[k_{bu}(c) = \frac{A_{bud} c}{c_1 + c},\]  
\[k_{dd}(c) = D_{dd}(1 - \frac{\sigma_{bd} c}{1 + c_0 c}),\]  
\[k_{du}(c) = D_{du}(1 - \frac{\sigma_{bd} c}{1 + c_0 c}).\]
We also have to update the volume conservation equation, i.e. (3.81), as both the down- and up-regulated bacteria equations have now been changed. This equation therefore becomes

\[ \nabla \cdot \left( \mathbf{v} - \frac{w}{\beta_{20}k} \nabla p_w \right) = (\beta_{3u}b_u + \beta_{3d}b_d)c - \beta_4E - \beta_{41}k_bQ(b_d + b_u). \]  

(4.19)

The only thing left is the boundary conditions, which become

- \( y = 0 \):
  \[ \frac{\partial A}{\partial y} = \frac{\partial c}{\partial y} = \frac{\partial p_w}{\partial y} = \frac{\partial Q}{\partial y} = \frac{\partial R}{\partial y} = \frac{\partial N}{\partial y} = v(x, 0, t) = 0. \]
  (4.20)

- \( \phi(x, y, t) = 0 \):
  \[ 0 = \left( -(b\Sigma_b + E\Sigma_E)I + \beta_{13}\mu(\nabla \mathbf{v} + \nabla \mathbf{v}^T) \right) + \beta_{13} \hat{\lambda} (\nabla \cdot \mathbf{v}) I \cdot \mathbf{n}, \]
  (4.21)
  \[ 0 = p_w, \]
  (4.22)
  \[ c = 1, \]
  (4.23)
  \[ A = 0 \]
  (4.24)
  \[ Q = Q_e, \]
  (4.25)
  \[ R = R_e, \]
  (4.26)
  \[ N = N_e. \]
  (4.27)

- Initial conditions:
  \[ b_d(x, y, 0) = \begin{cases} 
  b_0 & \phi(x, y, 0) \leq 0, \\
  0 & \phi(x, y, 0) > 0, 
  \end{cases} \]
  (4.28)
\[ b_u(x, y, 0) = 0, \quad (4.29) \]
\[ E(x, y, 0) = 0, \quad (4.30) \]
\[ \phi(x, y, 0) = \phi_I. \quad (4.31) \]

We now have a full system of non-dimensional equations (4.8) - (4.18) and (4.19) coupled with the initial and boundary conditions (4.20) - (4.31).

4.4 Numerical Methods

To solve the system of equations defined above, we used the same methods and techniques as those developed for Model 3. Equation (4.12) was the most difficult to deal with due to its non-linear nature, even after applying a finite difference scheme. This was overcome by assuming the value of \( R \) in the denominator at a particular point, was equal to its value at the previous time step. The equation was then solved using the parallel CGLS method and the new values obtained were put back into the denominator and re-calculated. This re-calculation was done until the difference between the re-calculations was of the same order as the errors within the finite difference scheme. By using this method we were able to solve the non-linear equation, using the same techniques as those already developed for the linear equations. The others were solved separately using the parallel CGLS method and Figure 4.1 gives a graphic representation of this.

<table>
<thead>
<tr>
<th>Parameter values</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta_{40} ) = 10</td>
</tr>
<tr>
<td>( \beta_{42} ) = 1.1</td>
</tr>
<tr>
<td>( \beta_{44} ) = 2500</td>
</tr>
<tr>
<td>( k_Q ) = 4</td>
</tr>
<tr>
<td>( D_R ) = 1</td>
</tr>
<tr>
<td>( \beta_{41} ) = 1</td>
</tr>
<tr>
<td>( \beta_{43} ) = 250</td>
</tr>
<tr>
<td>( \beta_{45} ) = 100</td>
</tr>
<tr>
<td>( k_R ) = 1</td>
</tr>
</tbody>
</table>

Table 4.1: The parameter values used in the drug equations of Model 3.
More information on the finite different schemes, the level set methods and the parallel CGLS method are given in Appendix A.2, B and C. Figure 3.4 shows how and what order the different variables were solved and in what order.

Figure 4.1: A flow diagram of how drug equations incorporated into Model 3 were solved and what methods were employed for the different variables.
Figure 4.2: The initial variation of biofilm components, i.e. the volume fractions of the total bacteria on the top left ($b_d + b_u$), up-regulated bacteria on the top right ($b_u$), EPS on the bottom left ($E$) and water on the bottom right ($w$), used in the drug results, taken from the standard results of Model 3, Section 3.7.1, at time $t = 0.5$.

### 4.5 Effects of Anti-Bacterial Drugs on the “standard” Biofilm

The standard biofilm represents the wild type Pseudomonas aeruginosa, which was simulated in the standard results of Model 3 (see Section 3.7.1). The initial state of the biofilm for these simulations were taken from these standard results at time $t = 0.5$, so that the biofilm had started to develop and the quorum sensing was enabled and had been for a period of time. Figure 4.2 gives a graphical representation of these initial conditions, and it allowed us to investigate how these different drugs affects a “mature” biofilm that is still developing, rather than one just starting out as in Model 3 at $t = 0$. The new parameter values for the drug equations, that were used in the following results are shown in Ta-
ble 4.1, whilst all the rest remain unchanged as given in Table 3.2. All of the parameters were obtained from the references given, although many were then scaled accordingly, for the same reasons as given in Section 3.7.1.

We will look at each drug individually at various concentrations before investigating the effects of multiple drugs have on the biofilm’s growth and development.

### 4.5.1 Antibiotic Drugs

In this section we will investigate the effects of antibiotic drugs on biofilm development, explicitly at three different concentrations that differ by tenfold increases.

Figure 4.4 shows how the bacteria cells inside the biofilm are affected by the drugs and in correlation with Figure 4.3 we see that \( Q_e = 0.1 \) has little effect on the distribution. We note that the hollow core is slightly smaller, but this is explained by the biofilm not being as large and hence nutrient starvation in the centre is smaller. With a tenfold increase in the drug concentration, i.e. \( Q_e = 1 \), the bacteria volume fraction has decreased, which in turn decreases the biofilm’s advective velocity and bacteria pressure, causing a slower growth rate, as seen in Figure 4.3. A further tenfold increase to \( Q_e = 10 \), kills nearly all the bacteria cells very quickly, in fact the fourth graph of Figure 4.4 shows the volume fraction at \( t = 1 \) instead of \( t = 3 \) like the other three graphs. However we have assumed that the EPS does not disintegrate and therefore Figure 4.3 shows that the biofilm remains static in size, even though all the bacteria cells have been killed. Figure 4.5 shows how the drugs at different external concentrations are able to diffuse through the biofilm and as we would expect they look very similar to the nutrient graphs shown in Figure 3.14. We also notice that all the drug concentrations are able to fully diffuse through the biofilm and that with
Figure 4.3: Evolution of the level set $\phi(x,y,t) = 0$, representing the growth of the biofilm for $t = 0.5$, 1, 2 and 3, with $Q_e = 0$, 0.1, 1 and 10, on the top left, top right, bottom left, bottom right, respectively. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

Figure 4.4: The total bacteria volume fraction $b = b_d + b_u$, with $Q_e = 0$, 0.1 and 1 at $t = 3$ and $Q_e = 10$ at $t = 1$, shown in the top left, top right, bottom left and bottom right respectively. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.
Figure 4.5: The anti-bacteria drug concentration, with $Q_e = 0.1$ and 1 at $t = 3$ and $Q_e = 10$ at $t = 1$, shown in the top left, top right and bottom graphs respectively. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

$Q_e = 1$ there is still sufficient in the centre of the biofilm to kill off some of the bacteria.

If we now compare the effects of antibiotic drugs in both one and two dimensional biofilms, so that we can understand whether any predictions made from one dimension results are still valid in two dimensions. Figure 4.6 shows how the heights of a biofilm, in both one and two dimensions, change with time, given various external drug concentrations. As above we see that in both dimensions the higher the external drug concentration is, the slower the biofilm grows, until $Q_e = 10$ and then no growth occurs in either. We also notice that independent of the drug concentration the two dimensional biofilm always grows faster than its one dimensional counterpart. This occurs for the same reasons as given in Section 3.7.1, namely nutrients are able to penetrate further into two than a one dimensional biofilm, which allows for more cell growth throughout. This extra
Figure 4.6: A comparison of biofilm heights between one and two dimensions with various antibiotic concentrations, as shown in the key. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

Figure 4.7: Comparison of a high, i.e. $Q_e = 1$, (left) and low, i.e. $Q_e = 0.1$ (right) antibiotic drug concentrations, throughout the biofilm at time $t=1$, 2 and 3 between one and two dimensions. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.
growth means that we have a faster growing biofilm, however the antibiotics are also able to further penetrate the biofilm in two dimensions as well. The extra growth from the nutrients though is able to compensate for that, and hence the biofilm still grows faster in two than in one dimension. Furthermore the higher the antibiotic concentration the less able the extra growth in two dimensions is able to compensate. Therefore the heights of the biofilm in one and two dimensions converge towards each other.

Figure 4.7 shows how different concentrations of antibiotics vary through the biofilm and we see, that there is a good correlation between the two dimensions independent of the concentration. For example in the left hand graph we notice that the concentration remains high throughout the biofilm, whilst it drops off to zero on the right hand side. However we still see differences in the size of the biofilms, shown by the green and red lines being in different positions at the maximum concentration on each graph. Therefore these figures show that the biofilm should be modelled in at least two dimensions, as done above, to give more realistic predications upon the drugs effect. For example whether they cause irregular structures to form, which is likely to lead to more vulnerable biofilms.

Overall these results show that the higher the drug concentration the more of the biofilm is killed off, which is in broad agreement with Agarwal et al. (2008, [2]) and Anguiano-Beltran et al. (2007, [6]).

4.5.2 Anti-lasR

We will now look at the first of the anti-quorum sensing drugs, at two different concentrations to see their effect on the biofilm’s development. We would expect that the higher the concentration the more the biofilm should resemble the quorum sensing mutant described in Section 3.7.3.
Figure 4.8: *Evolution of the level set $\phi(x, y, t) = 0$, representing the growth of the biofilm for $t = 0.5, 1, 2$ and 3, with $R_e = 0, 0.5$ and 50, shown in the top left, top right and bottom respectively. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.*

Figure 4.9: *The up-regulated bacteria volume fraction, with $R_e = 0, 0.5$ and 50 at $t = 3$, shown in the top left, top right and bottom respectively. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.*
Figure 4.10: The EPS volume fraction, with $R_e = 0, 0.5$ and $50$ at $t = 3$, shown in the top left, top right and bottom respectively. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

Figure 4.11: The down-regulating drug concentration, with $R_e = 0.5$ and $50$, on the left and right respectively, at $t = 3$. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.
Figure 4.12: A comparison between the up-regulated volume fractions in one (red) and two (green) dimensions, when $R_e = 0.5$ and $N_e = 0.285$ on the left and right, respectively. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

Figure 4.13: Comparison of a high, i.e. $R_e = 50$, (left) and low, i.e. $R_e = 0.5$, (right) anti-lasR drug concentrations, throughout the biofilm at time $t = 1, 2$ and $3$ between one and two dimensions. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

The standard parameters are such that, not much EPS is produced and is spread thinly through the biofilm. Therefore the anti-quorum sensing drugs, which inhibit up regulation of the cells and hence EPS production, show only minimal effect on the biofilm’s size and shape, as shown in Figure 4.8. However Figure 4.9 shows that these drugs do affect the volume of up-regulated cells. With the low concentration, i.e. $R_e = 0.5$, the drug is only able to penetrate
a short distance into the biofilm, as shown by Figure 4.11 and therefore the up-regulated cell’s volume only drops around the outside. Internally the biofilm is very similar to the drug free biofilm and therefore some EPS is still being produced and the total cell volume fraction remains identical, as the drugs only inhibit up regulation. As the cell volume fraction remains unchanged, we would expect the biofilm to grow in a similar fashion, although with less EPS, as shown in Figure 4.10. This reduction in EPS is why we end up with a taller biofilm, due to the situation being similar to the quorum sensing mutant (see Section 3.7.3), which produced a tall, thin biofilm. If we increase the drug concentration to $R_e = 50$ then it is able to fully penetrate the biofilm and hence we end up with very little up-regulated cells, as shown in Figures 4.9 and 4.11. We now have almost no EPS being produced, which decreases the bacteria and EPS pressures, causing a reduction in the biofilm’s growth.

Comparing the effect of anti-lasR and -AHL drugs on the up-regulated cells in one and two dimensions, in Figure 4.12, shows that the one dimension results overestimate the volume fraction compared to the two dimensions, even if the biofilms are approximately the same height. Plus Figure 4.13 shows how different anti-lasR concentrations vary through the biofilm and we see, as with the antibiotics, that there is a good correlation between the two dimensions independent of the concentration. However we still see differences in the size of the biofilms, shown by the green and red lines being in different positions at the maximum concentration on each graph. Therefore these figures also show that the biofilm should be modelled in at least two dimensions, to give a more realistic predications upon the effect of drugs.
4.5.3 Anti-AHL Drugs

In this section, we will investigate the second anti-quorum sensing drug, again looking at two different concentrations. Just as with the anti-lasR drugs, we would expect to see that the higher the concentration of anti-AHL the more the development of the biofilm should correlate with the quorum sensing mutant discussed in Section 3.7.3.

We see from Figure 4.17 that “moderate” drug concentration of \( N_e = 0.285 \) does affect the levels of AHL within the biofilm, however there is still sufficient to up-regulate the majority of the bacteria cells. Therefore when we look at Figures 4.14 and 4.16, we see little difference in the biofilm’s size or components compared to the drug free case. If however we increase the drug concentration to 28.5, then we are able to destroy nearly all the AHL molecules and hence the volume fraction of up-regulated cells has decreased vasty, as shown in Figures 4.16 and 4.17. However for the same reasons as with the anti-lasR drugs the overall biofilm size does not change very much, as shown in Figure 4.14, i.e. both drugs just decrease the total volume fraction of up-regulated cells.

These results are very similar in nature to those of the anti-lasR shown above, which is to be expected as both of them should have the same resulting effect. In these simulations, \( N_e = 28.5 \) was found to be effective whilst \( N_e = 0.285 \) was not. The parameters are such that \( \beta_{45}/\beta_{42} \sim 100 \) and it can be shown that at \( N_e = 28.5 \), the ratio of molecules of N and the maximum A (approximately 0.05 in dimensionless terms) is about \( 6D_{Ao}/D_{No} \). This indicates that we need to introduce an anti-AHL drug at a concentration of N that significantly exceeds that of AHL in order to be effective. However by increasing the diffusion rate of the anti-AHL drug, e.g. by using smaller molecules, a lower effective external concentration can be used.
Figure 4.14: Evolution of the level set $\phi(x, y, t) = 0$, representing the growth of the biofilm for $t = 0.5, 1, 2$ and $3$, with $N_e = 0$, $0.285$ and $28.5$, shown on the top left, top right and bottom respectively. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

Figure 4.15: The anti-quorum sensing drug concentration, with $N_e = 0.285$ and $28.5$ at $t = 3$. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.
Figure 4.16: The up-regulated bacteria volume fraction, with $N_e = 0$, 0.285 and 28.5 at $t = 3$. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

Figure 4.17: The AHL concentration, with $N_e = 0$, 0.285 and 28.5 at $t = 3$. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.
4.5.4 A Combination of Drugs

Figure 4.18: Evolution of the level set $\phi(x, y, t) = 0$, representing the growth of the biofilm for $t = 0.5, 1, 2$ and $3$, with no drugs on the top left, only antibiotic drugs with $Q_e = 1$ on the top right, a combination of antibiotic and anti-lasR drugs with $Q_e = 1$ and $R_e = 50$ on the bottom left and a combination of antibiotic and anti-AHL drugs with $Q_e = 1$ and $N_e = 28.5$ on the bottom right. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

We have now combined the antibiotic with either of the anti-lasR or anti-AHL drugs to investigate the effects of using multiple drugs at once. Therefore all of the figures in this section show a drug free system, a biofilm only affected by antibiotics at the medium concentration of $Q_e = 1$ and then the results of these two different combinations. These combinations used the medium concentration of antibiotics and the high concentration of anti-quorum sensing drugs i.e. $R_e = 50$ and $N_e = 28.5$.

It is clear from Figure 4.18 that either of these combinations all but stops the biofilm from growing, something that was not achieved by the antibiotic drugs on their own. Figures 4.19 and 4.20 show that these combination of drugs also
Figure 4.19: The total bacteria volume fraction ($b = b_d + b_u$) at $t = 3$, with no drugs on the top left, only antibiotic drugs with $Q_e = 1$ on the top right, a combination of antibiotic and anti-lasR drugs with $Q_e = 1$ and $R_e = 50$ on the bottom left and a combination of antibiotic and anti-AHL drugs with $Q_e = 1$ and $N_e = 28.5$ on the bottom right. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.

Figure 4.20: The up-regulated cell’s volume fraction at $t = 3$, with no drugs on the top left, only antibiotic drugs with $Q_e = 1$ on the top right, a combination of antibiotic and anti-lasR drugs with $Q_e = 1$ and $R_e = 50$ on the bottom left and a combination of antibiotic and anti-AHL drugs with $Q_e = 1$ and $N_e = 28.5$ on the bottom right. The initial conditions for these results are shown in Figure 4.2, whilst the remaining parameters are given in Tables 3.2 and 4.1.
changes the internal structure of the biofilm from a large “sparse” array of cells to a thick clump of down-regulated cells. In fact this was not something we have previously observed with any of the drugs when they were used separately.

These results show that when using a combination of these drugs, we are able to change the internal structure of the biofilm, as well as stopping its growth. This was something that the antibiotic drugs alone were not able to achieve. This implies that we could actually kill the biofilm off by using a lower concentration of antibiotics when combining them with one of these other substances.

4.6 Affect of Drugs on Biofilms with a High EPS production and volume fraction

Both the anti-lasR and -AHL drugs did not greatly effect the “standard biofilm’s” growth, although they were able to down-regulate the biofilm. We would expect however that these drugs would have a greater effect on biofilms which have a higher production rate or volume fraction of EPS. The reason is that either of these changes would increase the rate at which the biofilm grew and hence applying the drugs would reduce some of this increase, depending upon the external concentration.

Figure 4.21 shows how a drug free biofilm with both high production rate (i.e. $\beta_{3u} = 4$) and volume fraction of EPS (i.e. $\alpha = 2$) grows compares with the “standard biofilm” (i.e. $\beta_{3u} = 0.04$ and $\alpha = 20$) described in Section 3.7.1 and one subjected to anti-lasR drugs. As we would expect the drug free biofilm grows much faster than the “standard” biofilm due to the extra production of EPS. However when the drug free biofilm is applied with anti-lasR drugs, as shown in the bottom graph of Figure 4.21, we can significantly reduce this growth rate. Figure 4.23 and 4.24 show that the anti-lasR drugs were able to all but stop the
Figure 4.21: Evolution of the level set $\phi(x,y,t) = 0$, representing the growth of the biofilm for $t = 0, 0.5, 1$ and $2$ for the top two and $t = 0.5, 1$ and $2$ for the bottom graph. The top left biofilm is drug free with $\beta_{3u} = 4$ and $\alpha = 2$, the top right is the “standard” biofilm with $\beta_{3u} = 0.04$ and $\alpha = 20$, whilst the bottom biofilm has anti-lasR drugs with $R_e = 50$, applied to the drug free biofilm from $t = 0.5$ onwards. All the other parameters stayed the same as giving in Tables 3.2 and 4.1.

Figure 4.22: The bacteria cell volume fraction at $t = 2$, with the drug free biofilm on the left and one with anti-lasR drugs, $R_e = 50$, on the right. Both the biofilms had $\beta_{3u} = 4$ and $\alpha = 2$ whilst all the other parameters stayed the same as giving in Tables 3.2 and 4.1.
up regulation of the cells and by doing so considerably reduce the EPS volume fraction throughout the biofilm. This leads to the cells being more densely packed, as shown in Figure 4.22, as well as a significantly smaller biofilm. These results show that when there is significant quorum sensing mediated EPS production, then anti-quorum sensing treatments at sufficient levels will restrict biofilm growth. This makes it more likely that diffusion limited treatments, such as antibiotics, will be more effective at their removal.
4.7 Conclusions

In this chapter we adapted Model 3 to include three different types of drugs, an antibiotic and two different anti-quorum sensing drugs. We then investigated their effects individually on the biofilm, before looking at two different combinations to see if this had any benefits. The results for each drug showed, that the higher the drug concentration the more effect it had on biofilm development, which is in correlation with both Anguige et al., (2006, [7]) and Ward et al, (2008, [219]). Of the three different types investigated, the antibiotics had the most effect on the biofilm, as they were the only one that killed bacteria cells. In fact under the highest concentration investigated; we were able to completely kill it. However, both the anti-lasR and anti-AHL drugs are not designed to kill the biofilm but to reduce quorum sensing activity, which for the paradigm bacteria, Pseudomonas aeruginosa, results in down regulation of EPS production. The results showed that both drugs, given sufficient external concentrations, were able to disable the quorum sensing process in the modelled biofilm. Lower concentrations were only able to affect the quorum sensing around the edge of the biofilm, leaving the bacteria in the centre, in an up-regulated state.

After investigating all of the drugs individually, we looked at the effects of combining either the anti-lasR or anti-AHL drugs with the antibiotics, had on the biofilm’s development. Both of these combinations were able to all but stop the biofilm growing, which was not achieved by the antibiotics alone. These combinations also changed the internal structure from a large “sparse” array of cells to a thick clump of down-regulated bacteria, which was not achieved with any one particular drug. A further investigation of drug combinations was undertaken, to see what effect they had on biofilms with higher production rates and volume fractions of EPS. These results showed that in the case of high levels of EPS production, mediated by quorum sensing, anti-quorum sensing
drugs can be highly effective at restricting growth and hence more vulnerable to antibiotics. Furthermore, decreasing the amount of EPS within a biofilm may make it more vulnerable for removal via shear stress from fluid flow. Therefore, a combination of anti-quorum sensing drugs and “modest” levels of antibiotics can be much more effective than using greater amount of antibiotics alone.

The results show that the use of anti-quorum sensing drugs on bacteria that employ quorum sensing to regulate biofilm development can be an effective means of controlling growth, though antibiotics are still required for their complete removal. However it is probable that many bacteria do not regulate biofilm growth by quorum sensing, but it does regulate the bacteria pathogenicity in an infection scenario. Consequently by down regulating the quorum sensing, the biofilm will become less harmful to the host and easier for the immune system to deal with.
Chapter 5

Overall Conclusions

In this thesis we studied a number of models that expanded on existing work [221, 7, 7, 220, 38]. In particular we coupled the models of Anguige et al. (2006, [7]), with a viscous flow description of biofilm structure to simulate growth and development in two dimensions. A lot of work has been done in one dimension [154, 219, 219, 35, 175] and multi-dimensional models tend to be very simplistic [52, 154]. The simplest of the two and three dimensional models is obtained by producing explicit models that can only be used to investigate a small area of biofilm development. Thus one of the aims of this thesis was to find out more about how results from one dimensional scenarios relate to non flat biofilms. We were also interested in producing broad two and three dimensional models that can be used to investigate various aspects that may affect biofilm development.

We produced and investigated four different models, which looked into the growth and development of biofilms, in particular for the pathogen Pseudomonas aeruginosa. In Chapter 2 we presented results for two, one dimensional models, that largely agreed with results published in other papers [226, 7]. These models allowed the bacteria to divide and die, as well as consume nutrients, with the latter incorporating a quorum sensing system. The results showed that after a certain height the nutrients were not able to fully penetrate the biofilm, due to their consumption by the bacteria. This lead to a layer of constant thickness of
live cells, which existed at the top and moved upwards with the growing biofilm. This constant thickness of live cells, implied that the biofilm grew linearly and lead to travelling wave solutions. However, these one dimensional models did not generalise to higher dimensions. Therefore in Chapters 3 - 4 we extended the model of Anguige by modelling the biofilm as a viscous-porous structure, treating the solid and fluid components as compressible viscous and incompressible inviscid fluids, respectively. A Darcy like flow for the biofilm fluid was derived and we assume that the forces balance between the solid and fluid components. We assumed that there was no external force on the biofilm and included a quorum sensing system, thereby making the model relevant to bacteria such as Pseudomonas aeruginosa. We investigated a number of scenarios with this model in two dimensions and the results suggested that in general the biofilms tended to have enhanced growth. This was caused by the nutrients penetrating deeper into the biofilm and therefore creating a larger region of active biomass. Hence a conclusion drawn here is that one dimensional results tend to under predict the actual growth potential of a biofilm and therefore two or three dimensional models are required. The limited nutrient penetration also lead to a hollow region forming, consisting of mainly fluid, in the centre of the biofilm, which had been experimentally observed [225, 224, 17, 226, 176, 208]. The results also gave some indication into the biofilms shape, mainly a mushroom in our case, with active biomass located all around the edge. An extension to this model was applied where nutrient diffused through an external fluid, from a fixed source above the biofilm. This produced a long thin biofilm with the majority of active biomass at its top. Therefore the two dimension models, not only gave information on the shape of the biofilm, but upon where the majority of the active biomass is likely to be located. In Chapter 4, various treatment strategies were investigated based upon the model produced in Chapter 3. Here we assumed that each of the
drugs had a fixed external concentration and could diffuse through the biofilm. The drugs then affected the relevant parts proportional to their concentration and being consumed in the process. The two dimensional nature of the model meant, that like the nutrients, it produced more realistic penetration depths for each of the drugs, compared to using one dimensional models. The results showed that “high” concentrations of antibiotics were required to remove the biofilm, although lower concentrations did affect it. However the model also showed that the use of anti-quorum sensing drugs, particularly on bacteria that employ quorum sensing to regulate its development can be an effective means of controlling its growth. Antibiotics though where still required for complete removal, but a lower concentration could be used to that same effect. This is particularly important if the biofilm is growing on living tissue, for example.

The models presented in this thesis, produced systems of coupled non-linear partial differential equations of mixed types and therefore had to be solved using numerical techniques. The equations were solved using finite difference methods on a fixed rectangular mesh, which allowed for consistent stencils to be used over the whole numerical domain. This gave simplicity to the numerics and was therefore used in favour of either finite elements or finite volume approaches. The finite difference method converted the partial differential equations into difference equations which were solved using a computer program written in Fortran 90, as it was relatively faster than MATLAB and “easier” to parallelise than C. A disadvantage of using the fixed mesh though, was implementing the moving boundary conditions, as they rarely coincided with the mesh points. A level set approach was therefore used to track the moving boundary, which gave a distance to the boundary at each mesh point. This allowed the boundary conditions to be interpolated to the boundary, using the neighbouring mesh points. Therefore the same stencils to those used inside the biofilm, could be used at the
boundary allowing for greater simplicity that could not otherwise be achieved. In the majority of cases the finite difference stencils produced a system of linear difference equations, which in one dimension formed a tridiagonal matrix. This was relatively easy to solve using Thomas’ method; however this structure was lost when moving to the more complicated two dimensional models. In this case the matrices had very little structure and therefore a conjugate gradient least squares (CGLS) method was used. This had the advantage of being able to solve any square matrix system of equations and hence gave the simplest approach. The CGLS was an iterative method and therefore in some case took longer to solve the required matrix problem, than for example a direct LU factorisation. A further disadvantage of the fixed mesh grid, was that the size of these matrices grew with the biofilm and could become extremely large. Therefore a matrix compression was used, which allow only the non-zero values to be stored. This not only decreased the amount of storage the program required but increased the speed of the CGLS method, as no manipulation was done on the zero values within the matrix. Although this helped increased the speed at which the computer program ran, further increases in speed were required. This was achieved by parallelising the CGLS method, such that it could run on a multi-cored shared memory computer. In doing so, the program scaled almost linearly with the number of cores, due to the amount of time spent solving the various matrix equations at each time step. Therefore these various approaches allowed the numerics to remain as simple as possible, whilst solving the system of equations in a suitable time period.

There is plenty of scope for further work in both the modelling and computational aspects of this study, for example Model 3 could be extended to include an adaptive multi-grid method. Although this would increase the complexity of the numerical stencils, it should provide more detail in regions of active biomass
and around the biofilm/fluid interface. Furthermore it should also decrease the
time taken to calculate all the variables at each time step, allowing for longer
time simulations to be run in a more satisfactory time frame. In Section 1.6
we mentioned various defence techniques used by bacteria against anti-biofilm
agents, some of which were not implemented in Chapter 4. These could be im-
plemented by splitting the bacteria into sub-populations, with each one having
a specific trait, for example down- or up-regulated, persisters and bacteria that
release chemicals to neutralize the anti-biofilm agents. By incorporating these,
with the ability to switch them on or off, would give a broader picture of how
some bacteria defences work against various anti-biofilm agents. Furthermore
in Section 3.9 we showed the importance advection plays in the transport of
large molecular agents and hence that could be included in any further mod-
els. Model 3 also assumed that the water surrounding the biofilm was static
therefore investigations could be carried out to see what affects, a fluid flowing
passed the biofilm would have on its development. An possible model for this
is given in Appendix D, where the biofilm was assumed to be growing in a pipe
with water flowing from left to right under a Stoke’s flow regime. However the
boundary condition for the model require more work to guarantee in the limit
as the water flow tends to zero, that the model equations degenerate into Model
3.

In this thesis we have began to investigate continuum models that can de-
scribe the growth and development of biofilms in two and three dimensions.
Therefore this work could act as a starting point for continued investigations,
which in conjunction with the ever increasing speeds of computers, could allow
for full three dimensional simulations to be obtained. It is the hope that this
work will contribute towards more effective measures in controlling biofilms in
a medical and industrial settings.
Appendix A

Numerical Methods

The systems of equations derived for each model consist of coupled non-linear partial differential equations of mixed types, which offer little to analytical progress. Hence their study and approximate solutions are obtained by the use of numerical methods. In this section the numerical methods used will be discussed, including their advantages and disadvantages.

To solve these systems of non-linear partial differential equations derived in Chapters 2 - 4, finite difference methods were employed [33]. On top of which a level-set approach was used to handle the moving boundary. This allowed for a fixed rectangular mesh grid to be implemented, with space steps of $\delta x$ in all directions and a maximum time step of $\delta t_{max}$. A maximum time step is given, as some of the numerical methods used require the time step to change for their stability requirement to hold. Using the finite difference and fixed rectangular grids gave simplicity to the numerics and hence was used in favour of either finite elements or finite volume approaches.

The finite difference stencils, converted the systems of PDE equations into difference equations, which were solved using a computer program written in Fortran 90 [54, 187]. Fortran was used as it is faster relative to MATLAB,
whilst also being “easier” to parallelise than say C [93]. It also has the added bonus of being explicitly built to run mathematical programs.

A.1 Methods for the One Dimensional Models

In the one dimensional case, the biofilm lies in the region $0 \leq z \leq H(t)$, where $H(t)$ is the height of the biofilm at time $t$, and we analysed all the variables only over this domain. This allowed for a computationally efficient program.

A.1.1 Hyperbolic equations

The hyperbolic equations that describe the biofilm components, were solved using an explicit upwind method [64], which has an accuracy of $O(\delta x) + O(\delta t)$. The upwinding process means that we use a backwards or forwards finite difference scheme for the spatial derivative, depending upon the sign of the local velocity. For example, consider the following one dimensional linear wave equation,

$$\frac{\partial w}{\partial t} = -v \frac{\partial w}{\partial x}. \quad (A.1)$$

Applying a finite difference, upwind scheme we obtain

$$\frac{w_{i}^{n+1} - w_{i}^{n}}{\delta t} = \begin{cases} -v_{i} \frac{w_{i}^{n} - w_{i-1}^{n}}{\delta x} & v_{i} \geq 0, \\ -v_{i} \frac{w_{i+1}^{n} - w_{i}^{n}}{\delta x} & v_{i} < 0. \end{cases} \quad (A.2)$$

The upwind scheme though is generally only stable if the following Courant-Friedrichs-Lewy (CFL) [232, 149] condition is satisfied

$$\left| \frac{v \delta t}{\delta x} \right| \leq 1. \quad (A.3)$$
Hence this was checked after the velocities were calculated at each time step and the value of $\delta t$ adjusted so that the equation (A.3) held. This condition gives stability, as it guarantees that the numerical domain of dependence at least covers the analytical domain of dependence, which is enclosed by the characteristics equations. If this condition failed, then the program automatically decreased $\delta t$ and restarted the time step. After a few time steps, the program checked if $\delta t < \delta t_{\text{max}}$, and only then did the program calculate a new value for $\delta t = \text{max}(\delta t_{\text{max}}, \frac{\delta x}{v_{\text{max}}})$. This meant that the CFL condition should hold at the next time, whilst allowing the program to propagate the simulation forward as fast as possible.

### A.1.2 Elliptic equations

The AHL and nutrient concentrations are elliptic equations and therefore have to be solved with an implicit method. Hence a central finite difference scheme [33] was picked, which convert the equations for the AHL and nutrient concentrations into a system of linear equations. This system formed a tridiagonal matrix, which is straightforward to solve by using Thomas’ method [61].

### A.1.3 Advective velocity equation in one dimension

The advective velocity $v$, e.g. equations (2.37) and (2.94), was solved using a backwards finite difference stencil. As the right hand side of the equations were independent of $v$, the stencil could be iterated through the space domain, solving the equation. The water velocity $u$, e.g. equation (2.95), was given as an integral, with the right hand side being independent of $u$. Therefore this was solved using the trapezoidal rule [61], as follows

$$u_{i+1}^n = \int_0^{(i+1)\delta x} f(...)d\hat{x},$$

(A.4)
\[
\int_0^{i\delta x} f(...) d\hat{x} + \int_{i\delta x}^{(i+1)\delta x} f(...) d\hat{x}, \quad (A.5)
\]

\[
\approx v^n_i + \frac{\delta x}{2} (f(i\delta x) + f((i+1)\delta x)). \quad (A.6)
\]

**A.1.4 Moving boundary**

The moving boundary was taken into account using a level set approach, see Appendix B for more details, though it would have been much simpler to scale \(H(t)\) into the space coordinate and solve on the unit interval. However the \(H(t)\) scaling only works in one dimension and therefore as two dimensional biofilms were going to be investigated, a level set approach was used so that experience could be gained using a relatively simple example. The moving boundary nevertheless did cause a slight problem when the biofilm crossed a mesh point. Two different approaches were used to overcome this problem:

- **In Model 1**, when the biofilm crossed a mesh point, the biofilm components were extrapolated to this new point at the previous time step. These extrapolated values were then used in the current time step, when solving the biofilm components at this new mesh point.

- **In Model 2**, however this no longer produced reliable results, as the extrapolated values were sometimes outside the range, zero to one. Therefore we started out, as before, by doing a linear extrapolation and checking if the values produced were in the required range, and if so, they were used in the same manner as for Model 1. Otherwise successively more accurate Taylor series expansions, were used until we got to \(\frac{\partial \phi_0}{\partial z^m}\) or values within the range zero to one was obtained. All of these derivates were calculated using finite different stencils as given in [33].

Although the second method was harder to implement, it was found that the values produced were biologically relevant, as well as producing more accurate
and stable results.

A.2 Methods for the Two Dimensional Models

Many of the techniques and methods used for the one dimensional models could automatically be transferred, into two dimensions. An example of this is the biofilm components, which were still solved using the upwind method, as the format of the equations remained identical, i.e. for

\[
\frac{\partial w}{\partial t} = -v_x \frac{\partial w}{\partial x} - v_y \frac{\partial w}{\partial y}
\] (A.7)

we get

\[
\frac{w_{i,j}^{n+1} - w_{i,j}^n}{\delta t} = \begin{cases} 
    -v_{x_i} \frac{w_{i,j}^n - w_{i-1,j}^n}{\delta x} - v_{y_i} \frac{w_{i,j}^n - w_{i,j-1}^n}{\delta y} & v_{x_i} \geq 0 \& v_{y_i} \geq 0, \\
    -v_{x_i} \frac{w_{i,j}^n - w_{i-1,j}^n}{\delta x} - v_{y_i} \frac{w_{i,j+1}^n - w_{i,j}^n}{\delta y} & v_{x_i} \geq 0 \& v_{y_i} < 0, \\
    -v_{x_i} \frac{w_{i,j}^n - w_{i-1,j}^n}{\delta x} - v_{y_i} \frac{w_{i,j}^n - w_{i,j-1}^n}{\delta y} & v_{x_i} < 0 \& v_{y_i} \geq 0, \\
    -v_{x_i} \frac{w_{i,j}^n - w_{i-1,j}^n}{\delta x} - v_{y_i} \frac{w_{i,j+1}^n - w_{i,j}^n}{\delta y} & v_{x_i} < 0 \& v_{y_i} \geq 0.
\end{cases}
\] (A.8)

However the stability condition (i.e. the CFL) also change from one to two dimensions, which became

\[
\left| \frac{v_x \delta t}{\delta x} \right| + \left| \frac{v_y \delta t}{\delta x} \right| \leq 1.
\] (A.9)

Special attention was also needed, when the biofilm grew over a mesh point, to make sure the extrapolated values were biologically relevant. The same trick of using more accurate Taylor series, as in Model 2, did not work this time as in two dimensions there was a good chance of having insufficient points to calculate the derivatives. Therefore to get around this problem, with a method that would
always work, the following restrictions were set:

- no biofilm component, i.e. the bacteria cells and EPS, can be outside the range zero to one,

- the sum of the bacteria cells and EPS volume fractions cannot be more than $B_m$, defined in equation (3.87).

These restrictions gave biologically relevant values and stability to the program, as if the water component became small then division by small numbers occurred. This caused the advective velocities to hugely increase, which in turn decreased $\delta t$. The simulation then never recovers due to the small size of $\delta t$ and the number of time steps required to set everything back into equilibrium. Hence, if one of these restrictions were not met, then the values were set equal to the ones given by the closest neighbour just inside the biofilm. Then just as in the one dimensional case these extrapolated values were plugged into their respective equations. This is a valid approximation as it has an error of $O(\delta x)$, which is the same as the upwinding scheme. Also as the models produced very complex equations that took a long time to solve, computational efficiency was very important. This was achieved in Model 3 by only solving all the equations at mesh points inside the biofilm, as this was the only area of interest.

The AHL and nutrient concentration were solved with the same central difference stencil scheme, as in the one dimensional case. However this time it formed a five point stencil, instead of three, due to the two dimensions and therefore no longer formed a tridiagonal matrix. Hence a different method was needed to solve these equations that would be robust, fast and hopefully be able to solve the system of equations produced by the velocity and pressure equations. After testing several methods we came to the conclusion that the Conjugate Gradient Least Squares method (CGLS) [40], a variation of the Conjugate Gradient (CG)
algorithm [40], was the best for our purposes. The CGLS is effective for non symmetric indefinite matrices, which are properties that limit the application of the standard CG algorithm. Let’s consider the following matrix problem

\[ A\mathbf{x} = \mathbf{b}, \]  
\[ \text{(A.10)} \]

where \( A \) is a square, non symmetric, indefinite, coefficient matrix, \( \mathbf{x} \) is the solution vector and \( \mathbf{b} \) is the right hand side vector. Now multiply through by \( A^T \), gives

\[ A^T A\mathbf{x} = A^T \mathbf{b}, \]  
\[ \text{(A.11)} \]

and let \( \hat{A} = A^T A \) and \( \hat{\mathbf{b}} = A^T \mathbf{b} \). Therefore we have

\[ \hat{A}\mathbf{x} = \hat{\mathbf{b}}, \]  
\[ \text{(A.12)} \]

such that the \( \hat{A} \) is a square, symmetric, positive definite matrix. Although this maybe a standard result, no proof could be found and hence one is now given.

**Proposition:** The matrix \( A^T A \) is a symmetric, positive definite matrix, whilst assuming that \( A \) is square and non-singular.

**Proof:** The definition of a symmetric is that it is equal to its transpose, which if we take the transpose of \( A^T A \), we obtain

\[ (A^T A)^T = A^T (A^T)^T, \]  
\[ = A^T A. \]  
\[ \text{(A.13, A.14)} \]
Hence we have proved the first part. Now a matrix $M$ is said to be positive definite if for any non zero vector $x$,

$$x^T M x > 0.$$  \hfill (A.15)

Therefore we need to prove that

$$x^T A^T A x > 0,$$  \hfill (A.16)

for any square non singular matrix $A$. As $A$ is non singular then there exists a LU factorization with row pivoting, such that

$$A = PLU,$$  \hfill (A.17)

where $P$ is the permutation matrix, and $L$ and $U$ are non singular lower and upper triangular matrices respectively. Hence we have

$$x^T A^T A x = x^T U^T L^T P^T P L U x,$$  \hfill (A.18)

but as $P$ is a permutation matrix, its inverse is $P^T$ [61]. Therefore

$$x^T A^T A x = x^T U^T L^T L U x.$$  \hfill (A.19)

Let $y = U x$ and $z = L y$ then

$$x^T A^T A x = y^T L^T L y,$$  \hfill (A.20)

$$= z^T z,$$  \hfill (A.21)

$$= \sum_{i=1}^{n} z_i^2,$$  \hfill (A.22)

$$\geq 0.$$  \hfill (A.23)
\[ p_0 = r_0 = b - Ax_0; \]

\[
\begin{align*}
\text{for } k &= 0, 1, 2, ... \\
n_k &= Ap_k; \\
\alpha_k &= (r_k, r_k)/(p_k, q_k); \\
x_{k+1} &= x_k + \alpha_k p_k \\
r_{k+1} &= r_k - \alpha_k q_k \\
\text{if } (r_k, r_k) &\leq tol^2 \text{ then exit loop;}
\end{align*}
\]

\[
\begin{align*}
\beta_k &= (r_{k+1}, r_{k+1})/(r_k, r_k); \\
p_{k+1} &= r_{k+1} + \beta_k p_k; \\
\text{loop.}
\end{align*}
\]

\[ r_0 = b - Ax_0; \]

\[
\begin{align*}
\text{for } k &= 0, 1, 2, ... \\
n_k &= Ap_k; \\
\alpha_k &= (s_k, s_k)/(q_k, q_k); \\
x_{k+1} &= x_k + \alpha_k p_k; \\
r_{k+1} &= r_k - \alpha_k q_k \\
\text{if } (r_k, r_k) &\leq tol^2 \text{ then exit loop;}
\end{align*}
\]

\[
\begin{align*}
\beta_k &= (s_{k+1}, s_{k+1})/(s_k, s_k); \\
p_{k+1} &= s_{k+1} + \beta_k p_k; \\
\text{loop.}
\end{align*}
\]

<table>
<thead>
<tr>
<th>CG algorithm</th>
<th>CGLS algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p_0 = r_0 = b - Ax_0; )</td>
<td>( r_0 = b - Ax_0; )</td>
</tr>
<tr>
<td>for ( k = 0, 1, 2, ... )</td>
<td>for ( k = 0, 1, 2, ... )</td>
</tr>
<tr>
<td>( q_k = Ap_k; )</td>
<td>( q_k = Ap_k; )</td>
</tr>
<tr>
<td>( \alpha_k = (r_k, r_k)/(p_k, q_k); )</td>
<td>( \alpha_k = (s_k, s_k)/(q_k, q_k); )</td>
</tr>
<tr>
<td>( x_{k+1} = x_k + \alpha_k p_k )</td>
<td>( x_{k+1} = x_k + \alpha_k p_k )</td>
</tr>
<tr>
<td>( r_{k+1} = r_k - \alpha_k q_k )</td>
<td>( r_{k+1} = r_k - \alpha_k q_k )</td>
</tr>
<tr>
<td>if ((r_k, r_k) \leq tol^2 ) then exit loop;</td>
<td>if ((r_k, r_k) \leq tol^2 ) then exit loop;</td>
</tr>
<tr>
<td>( \beta_k = (r_{k+1}, r_{k+1})/(r_k, r_k); )</td>
<td>( \beta_k = (s_{k+1}, s_{k+1})/(s_k, s_k); )</td>
</tr>
<tr>
<td>( p_{k+1} = r_{k+1} + \beta_k p_k );</td>
<td>( p_{k+1} = s_{k+1} + \beta_k p_k );</td>
</tr>
<tr>
<td>loop.</td>
<td>loop.</td>
</tr>
</tbody>
</table>

Table A.1: CG and CGLS algorithms.

However \( z^Tz = 0 \) if and only if \( z = 0 \). The only way for \( z = 0 \) is if \( Ly = 0 \), which is only true if \( y = 0 \) since \( L \) is non singular triangular matrix. Similarly, the only way \( y = 0 \) is if \( Ux = 0 \) but then \( x = 0 \) must be true, by the same argument. Hence \( x^TA^TAx = 0 \) if and only if \( x = 0 \) and therefore we have \( x^TA^TAx > 0 \) for all non zero \( x \). \( \square \)

The CG algorithm often turns out to be numerically unstable when applied to equation A.12, as it first has to calculate \( A = A^TA \). The CGLS algorithm, however, does not directly do this multiplication and instead for improved stability, it introduces a new vector, \( s = A^Tr \), where \( r \) is the residual vector. It then uses this new vector \( s \) to produce the set of orthogonal vectors required to solve equation (A.11). The full CG and CGLS algorithm [40], are shown in Table A.1, where \( tol \) is the required tolerance:

**A.2.1 Dealing with Model 3’s Boundary Conditions**

The moving boundary conditions in two dimensions were applied in a variety of ways depending upon the variable and local shape of the biofilm. Due to the fact that the biofilm boundary hardly ever landed exactly on a particular grid
point, such that the level set variable was zero there, meant that the conditions were very difficult to implement. That compounded with the boundary moving with respect to time, implied that the program had to dynamically pick how to apply these conditions at all the relevant points. Application of the boundary conditions on the moving boundary is therefore highly non trivial and warrants a detailed discussion.

In Model 3 the boundary conditions for the AHL and nutrient concentrations were dealt with, by applying them to any points just inside the biofilm that had a neighbouring point north, east, south or west of it that was outside of the biofilm. This is graphically represented in Figure A.1. All the figures presented in this section have the same format, where the grey area represents inside the biofilm; the crosses are mesh points and the red ones show where the boundary conditions are being applied.

The more interesting boundary conditions were for the water pressure and

Figure A.1: The red ‘plus’ signs represent the points where the boundary conditions for the AHL and nutrient concentrations were applied in Model 3, whilst the grey area represents inside the biofilm and the black crosses as other mesh points.
advective velocities of the biofilm, these being

\[ 0 = (- (b \Sigma_b + E \Sigma_E) I + \beta_{13} \mu (\nabla v + \nabla v^T)) + \beta_{13} \lambda (\nabla \cdot v) I \cdot n, \]  
(A.24)

\[ 0 = p_w. \]  
(A.25)

Of these the water pressure was the easiest, which was applied to points just outside of the biofilm, that had neighbours north, east, south or west that were inside. There were two reasons for this change in approach, the first was due to the need to solve the volume conservation equation at all mesh points inside the biofilm, to make sure we always account for all the biofilm components. The volume conservation equation was one of the three coupled equations used for solving the advective velocities and internal water pressure and hence the reason for discussing it. The second and most important, however, was due to consistency, as it allowed us to use the same finite difference stencil at all points inside the biofilm, irrespective of biofilm’s boundary. Furthermore, as the biofilm is growing, these mesh points just outside the biofilm at time \( t \) could be inside at time \( t + \delta t \). Therefore the velocity profile, just outside the biofilm is required.

The boundary condition stated that the water pressure was zero on the boundary. Hence the nearest neighbour values of \( p_w \) outside the biofilm were extrapolated based on the assumption

\[ \frac{\partial p_w}{\partial x} \text{ just outside} - \frac{\partial p_w}{\partial x} \text{ just inside} = 0, \]  
(A.26)

\[ \text{or } \frac{\partial p_w}{\partial y} \text{ just outside} - \frac{\partial p_w}{\partial y} \text{ just inside} = 0, \]  
(A.27)

depending upon whether the shortest distance to the edge of the biofilm was in the \( x \) or \( y \) direction. The application of a finite difference scheme to these
\[ \begin{align*}
\frac{p_w^* - p_{w_{i,j}}}{\theta dx} - \frac{p_{w_{i,j+1}} - p_{w_{i,j}}}{(1-\theta)dx} &= 0 \\
(1-\theta)p_{w_{i,j}} + \theta p_{w_{i,j+1}} &= 0
\end{align*} \]

and

\[ \begin{align*}
\frac{p_{w_{i,j}} - p_{w_{i,j-1}}}{\theta dx} - \frac{p_{w_{i,j+1}} - p_{w_{i,j}}}{(1-\theta)dx} &= 0 \\
(1-\theta)p_{w_{i,j}} + \theta p_{w_{i,j-1}} &= 0
\end{align*} \]

where

\[ \theta = \frac{|\phi_{i,j}|}{|\phi_{i,j}| + |\phi_{i,j+1}|} \]

and

\[ \theta = \frac{|\phi_{i,j}|}{|\phi_{i,j}| + |\phi_{i,j-1}|} \]

\[ \begin{align*}
\frac{p_w^* - p_{w_{i,j}}}{\theta dx} - \frac{p_{w_{i,j+1}} - p_{w_{i,j}}}{(1-\theta)dx} &= 0 \\
(1-\theta)p_{w_{i,j}} + \theta p_{w_{i,j+1}} &= 0
\end{align*} \]

and

\[ \begin{align*}
\frac{p_{w_{i,j}} - p_{w_{i,j-1}}}{\theta dx} - \frac{p_{w_{i,j+1}} - p_{w_{i,j}}}{(1-\theta)dx} &= 0 \\
(1-\theta)p_{w_{i,j}} + \theta p_{w_{i,j-1}} &= 0
\end{align*} \]

where

\[ \theta = \frac{|\phi_{i,j}|}{|\phi_{i,j}| + |\phi_{i,j+1}|} \]

and

\[ \theta = \frac{|\phi_{i,j}|}{|\phi_{i,j}| + |\phi_{i,j-1}|} \]

Figure A.2: Schematic demonstrating how water pressure’s boundary condition was implemented, where \( p_{w*} \) is the value on the boundary, equal to zero.

Equations and imposing \( p_w = p_{w*} = 0 \) on the boundary, is shown in Figure A.2.

The variable \( \theta \) equals the fractional distance between the points \((i, j)\) and \( \phi = 0 \), such that \( \theta = 0 \) if \( \phi(i, j, t) = 0 \) or \( \theta = 1 \) if \( \phi(\text{nearest neighbour}) = 0 \).

The advective velocities were dealt with in a similar fashion, but this time to any point just outside the biofilm, that had a point north, south, east, west, north east, north west, south east or south west inside the biofilm. The reason for the extra four directions, was due to the fact that the velocities used mixed derivatives, which have a finite difference stencil that uses the ‘diagonal’ points (i.e. NE, NW, ...). Hence the boundary conditions had to be applied at these
Figure A.3: Schematic demonstrating how the values of $\Sigma_b$, $\Sigma_E$, $\mu$ and $\lambda$ are interpolated to the boundary, in conjunction with equations (A.28) and (A.29), with $\theta dx$ or $\theta dy$ represents the distance to the edge of the biofilm.

points also. There were two problems when it came to dealing with these, one was calculating values for $\Sigma_b$, $\Sigma_E$, $\mu$ and $\lambda$ and the other was the derivates of the velocities. We dealt with calculating the values of $\Sigma_b$, $\Sigma_E$, $\mu$ and $\lambda$ by using linear interpolation, as described in equations (A.28) and (A.29),

$$(1 - \theta)\Sigma_{b_{i,j}} + \theta\Sigma_{b_{inside}} = \Sigma_{b_{on\ the\ boundary}}, \quad (A.28)$$

where $\theta = \frac{|\phi_{i,j}|}{|\phi_{i,j}| + |\phi_{inside}|}, \quad (A.29)$

where ‘inside’ refers to the point that has the minimum value of $\phi$ in all of these eight directions.

The derivatives were dealt with by finding the minimum value of $\phi$ in these eight directions and then using a forward or backward stencil in that direction. In the other direction, we looked to see which points are inside or just outside the biofilm and from that information decided whether to take a central, forward or backward stencil. Figure A.4 illustrates a number of scenarios and the stencils used. The scenarios not shown in Figure A.4, are either mirrored or rotated versions of those illustrated. As usual the grey area shows inside the biofilm and the red cross where the boundary conditions are being applied. The blue rectangles group together the points that form the finite difference stencil, and when these encompass three points, only the outer two are used in a central
Figure A.4: Schematic demonstrating how the advective velocity derivative stencils are formed in different cases, at the boundary in Model 3. The red plus showing the point at which the boundary conditions are being applied, and the blue rectangles group together the points that form the finite difference stencil, and when these encompass three points, only the outer two are used in a central difference scheme.
Appendix B

Level Set Methods

Level set methods are used to track moving boundaries, with a wide variety of applications, including ocean waves, burning flames and material boundaries [182]. This method works by using a first order partial differential equation of the function $\phi(x, t)$, that incorporates intrinsic properties of the system, such as the curvature at the interface or a velocity field, to propagate the moving boundary. In brief, the method typically starts with a signed distance function, $\phi(x, 0)$, which is the shortest distance between any point and the boundary edge. The sign indicates whether a point is inside or outside the boundary. In what follows we will use the convention that a negative distance corresponds to being inside the biofilm. On applying the distance function initially, the level set value of zero corresponds to the boundary. The moving boundary is then a characteristic of a first order PDE level set equation, and hence the boundary can be estimated at any future time.

There are several advantages of using this method, which include:

- Complex moving boundary events are described, for example the development of singularities, weak solutions, shock formations, entropy conditions and topological changes.
• Accurate ways of computing delicate quantities, for example the ability to deal with high order advective schemes, compute the local curvature in two and three dimensions, track sharp corners and cusps and handle topological changes occurring from the merger and separation of objects.

• Robust schemes exist for solving the level set PDE, with the error estimation being controllable by the choice of numerical method employed.

A disadvantage of the level set methods is the fact that they can require a considerable amount of thought and perception into the underlying characteristics within the object being studied, to produce the required PDE equation. Due to the nature of biofilms however, the boundary only moves in the direction of the velocity field of the solid components, generated by the movement, birth and death of the bacteria. Therefore the level set method produced all of the advantages above, without having any drawbacks.

### B.1 Applying Level Set Methods to Biofilms

The level set equation for propagating a moving boundary with velocity \( \mathbf{v} \) are

\[
\frac{\partial \phi}{\partial t} + \mathbf{v} \cdot \nabla \phi = 0 \quad \text{standard, non conservative equation,} \quad (B.1)
\]

\[
\frac{\partial \phi}{\partial t} + \nabla \cdot (\mathbf{v} \phi) = \phi \nabla \cdot \mathbf{v} \quad \text{conservative equation.} \quad (B.2)
\]

In the one dimensional models we used equation (B.1), due to its simplicity and the need to only track a single moving point. However for the two dimensional models, to make sure that the biofilm is not pinned by the no slip boundary condition and that the biofilm mass is not lost, the conservative form was used. The conservative equation allowed the biofilm to expand along the solid surface by using the derivatives of the advective velocity field rather than just a point
value as in the standard equation. Therefore when the velocity field just above
the solid surface was negative, i.e. the bacteria cells were moving towards it, the
conservative equation allowed them to touch the surface and hence expand the
width of the biofilm. This can be explicitly seen if we apply a finite difference
scheme to these equations, at a point on the solid surface and rearrange for $\phi^{k+1}_{i,j}$
they become:

\[
\phi^{k+1}_{i,j} = \phi^k_{i,j} - \delta t \left( v_{x_{(i+1),j}} \frac{\phi^k_{(i+1),j} - \phi^k_{(i-1),j}}{2\delta x} + v_{y_{i,j}} \frac{\phi^k_{i,(j+1)} - \phi^k_{i,j}}{\delta y} \right),
\]

(B.3)

\[
\phi^{k+1}_{i,j} = \phi^k_{i,j} - \delta t \left( v_{x_{(i+1),j}} \frac{\phi^k_{(i+1),j} - \phi^k_{(i-1),j}}{2\delta x} + \frac{v_{y_{i,(j+1)}} \phi^k_{i,(j+1)} - v_{y_{i,j}} \phi^k_{i,j}}{\delta y} \right)
+ \phi^k_{i,j} \delta t \left( \frac{v_{x_{i,(j+1)}} - v_{x_{i,(j-1)}}}{2\delta x} + \frac{v_{y_{i,(j+1)}} - v_{y_{i,j}}}{\delta y} \right),
\]

(B.4)

where $(i,j)$ represent the points location in space and $k$ is the time step. These
can be further simplified as $v = 0$ on the solid surface, i.e. $j = 0$, and therefore
these equations become

\[
\phi^{k+1}_{i,j} = \phi^k_{i,j},
\]

(B.5)

\[
\phi^{k+1}_{i,j} = \phi^k_{i,j} - \delta t \left( \frac{v_{y_{i,(j+1)}} \phi^k_{i,(j+1)} - v_{y_{i,j}} \phi^k_{i,j}}{\delta y} \right) + \phi^k_{i,j} \delta t \left( \frac{v_{y_{i,(j+1)}}}{\delta y} \right),
\]

(B.6)

\[
= \phi^k_{i,j} - v_{y_{i,(j+1)}} \delta t \left( \frac{\phi^k_{i,(j+1)} - \phi^k_{i,j}}{\delta y} \right).
\]

(B.7)

Hence with the standard equation the biofilm cannot move along the solid sur-
face. However with the conservative equation $\phi^{k+1}_{i,j}$ would decrease in value
compared to $\phi^k_{i,j}$, i.e. the point $(i,j)$ would either move deeper into the biofilm
or come close to the edge of the biofilm, if $v_y$ just above the solid surface and
the derivative of $\phi$ both have the same sign. This can occur for example if $v_y$
was negative, i.e. the bacteria are moving down towards the solid surface, and the point just above the solid surface is inside the biofilm, i.e. \( \phi_{i,(j+1)}^k < 0 \) whilst the point on the solid surface is outside, i.e. \( \phi_{i,j}^k > 0 \). This particular example is shown in Figure B.1. Furthermore it should be noted that in most applications, equation (B.2) has a zero right hand side due to the velocity field having a zero divergence; however this is not true in our case due to the compressibility of the bacteria volume fraction. Both of these equations were solved using the upwind method [64], making it straight forward to solve at each time step. The signed distance function used for the initial condition is the solution of the Eikonal equation \( |\nabla \phi| = 1 \) [182]. In one dimension this reduces to

\[
\phi(x, t) = x - H_0, \tag{B.8}
\]

where \( H_0 \) is the initial height of the biofilm. In two dimensions, a simple for-
mulation cannot be written down, but an approximate solution is achieved with the Fast Marching technique [182], described below.

**B.2 The Fast Marching Technique**

In more than one dimension the Fast Marching technique is used, as an approximation solver of the Eikonal equation, $|\nabla \phi| = 1$, with $\phi = 0$ on the boundary. To use this technique, you initially create a numerical mesh, with points spaced a distance $\delta x$ and $\delta y$ in the ‘x’ and ‘y’ directions respectively. To simplify the process, we will assume that $\delta y = \delta x$, i.e. we have a square mesh. You proceed by selecting a point or points with a known distance to the boundary. For example, consider the left diagram in Figure B.2, in which the red point is a known distance ‘L’ to the boundary, and we now give trial values to all of its neighbouring points, e.g. ‘$A’ = ‘L’ + \delta x$.

Now choose the smallest of these values, say ‘$A’$, and we fix the trial value at that point to be its actual distance. This is followed by calculating trial

Figure B.2: These diagrams give a graphical description of how the Fast Marching technique progresses. Initially, from the left, we take a single point (in red) with a known distance, ‘L’, and calculate the trial distances to all of its neighbours. We take the trial point with the shortest trial distance and fixing it, say ‘$A’ as shown in the centre and update the trial distances for all of it neighbours. The process then starts again, by taking the trial point with the shortest trial distance and fixing it, say ‘$D’ this time, and updating. Therefore by continuing this process all points within the mesh end up with a fixed distance that is approximately the shortest distance to the boundary.
distances, for its neighbouring points, in this case points ‘E’, ‘F’ and ‘G’, as shown in the middle diagram of Figure B.2. We now repeat this process for the next smallest value, say ‘D’, fixing its value. We then process as before updating its neighbours ‘H’, ‘G’ and ‘I’, as shown in the right diagram of Figure B.2. We note that the trial distance at point ‘G’ is updated a second time however the minimum value between ‘A’ + \( \delta y \) and ‘D’ + \( \delta x \) is chosen. Continuing for a few more iterations, an example situation that may arise is illustrated in Figure B.3, where the fixed distance values advance in a propagating wave moving across all the points in the domain. We continue this process until all points have been set a value, which will correspond to the shortest distance to the boundary.

Applying this technique to the biofilm models, we start by locating all the points such that the biofilm/fluid boundary lies at a distance less than \( \delta x \) away from it, as shown in Figure B.4. These points then have their distance set, either encoded in the program or by calculating their minimum distance to a known function, representing the biofilm’s boundary.

After we have given a fixed distance to all of the points, close to the boundary, shown in red in Figure B.4, we give positive trial distances, the sign is added later, to all of their neighbours. These trial distances are added into a “binary tree” [180], which is a “tree-like” data structure, with each node (point), having a maximum of two children, typically referred to as left and right. Due to its structure traversing through the binary tree and dealing with it is generally relatively easier than just a standard list of numbers. This structure is a well established computational technique used in the Fast Marching method. The blue crosses illustrate the points that have been given trial distance, as shown in Figure B.5, and are the ones that the Fast Marching method starts with.

When all the blue points have been added to the tree, the point at the top, which by construction has the shortest distance to the boundary, is fixed and
removed, say point ‘A’ in Figure B.6. The neighbouring points of ‘A’ either have their trial value updated, for example point ‘B’ or a trial value given to them and added into the binary tree, for example point ‘C’, giving Figure B.6. When fixing the value, we have to check whether the point is inside or outside the biofilm, as the sign of the distance is not stored in the binary tree. If the point is inside the biofilm, as ‘A’ is, then the distance is multiplied by minus one.

Figure B.3: The Fast Marching technique, after a number of iterations, where the red points have a fixed distance, the blue have a trial distance and the black have not yet been set.

Figure B.4: The Fast Marching technique applied to a biofilm, where the red crosses have their distance set and black have not yet been set.
We then continue on in the same fashion, until all points in the domain have been set, i.e. there are no entries left in the binary tree. In the implementation of this method, we are not interested in points that are outside and beyond a fixed distance ‘D’ say, from the edge of the biofilm and hence, they are not added to the binary tree. This saves on computational time and decreases the amount of memory needed when calculating all of these distances. Figure B.7 shows the end result, with the black crosses representing points whose distance was not calculated for being too far from the boundary. As the advective velocity field is not defined outside of the biofilm, direct use of either equations (B.1) or (B.2) was not possible. Hence the Fast Marching method was used to update these points at every time step. For increased numerical stability, the solutions of (B.1) and (B.2) were reinitialised every hundred time steps, so that numerical errors in the upwind scheme were not allowed to swamp the results. Therefore $\phi(x, t)$ reasonably well approximates the position of the moving boundary.
Figure B.6: The Fast Marching technique, applied to a biofilm, where the first trial point, ‘A’ in this case, has been fixed. The trial distance at point ‘B’ has been updated, whilst ‘C’ has been given a trial distance and added to the binary tree.

Figure B.7: The points (in red) where a distance has been set using the Fast Marching technique.
Appendix C

Parallelisation of Numerical Methods

The models presented in Chapters 2 - 4 are non-linear, highly-coupled PDE systems and their numerical solution results in the application of iterative schemes. These require the solving of linear systems of equations, which involve, in particular for two dimensional simulations, very large sparse matrices. To optimise the solution procedure in terms of computational time, it was necessary to adapt the numerical methods to work in parallel. There are two different types of parallel processing approaches implemented in the programming language Fortran, these being:

- A cluster, which is a group of computers that are connected via a network. This uses a technique called Message Passing Interface (MPI) [143], where each computer runs small pieces of the same program and communicates with the others by sending messages across the network.

- A shared memory computer with multiple processors. This uses Open Multi-Processing (OpenMP) [32], which works on a single computer that has multiple processors all accessing the same memory. Certain parts of
### Advantages and Disadvantages of the MPI and OpenMP Parallel Approaches Used within Fortran

<table>
<thead>
<tr>
<th>Pros</th>
<th>MPI</th>
<th>OpenMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Runs on either multi-cored and/or multiple computers.</td>
<td>• Easier to program and debug, as the commands are compiler directive, rather than explicit commands individually written into the code.</td>
<td></td>
</tr>
<tr>
<td>• Can be used for</td>
<td>• Directives can be added gradually, so that only parts of the program become parallelised.</td>
<td></td>
</tr>
<tr>
<td>* task parallelism, where a list is given and then distributed to computers in the cluster as they become free,</td>
<td>• The program can still run in serial, i.e. on one processor in a single computer.</td>
<td></td>
</tr>
<tr>
<td>* data parallelism, where data is split between all of the computers and then manipulated.</td>
<td>• Hardly any modification to the code.</td>
<td></td>
</tr>
<tr>
<td>• Each process has its own local variables and hence data corruption is less likely to occur.</td>
<td>• The code is easier to understand and hence maintain.</td>
<td></td>
</tr>
<tr>
<td>• A cluster of computers is generally less expensive than one large shared memory computer.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cons</td>
<td>• The user needs to write code that manages the parallelisation, rather than done by the compiler.</td>
<td>• Can only be run on shared memory computers,</td>
</tr>
<tr>
<td></td>
<td>• Can be harder to debug.</td>
<td>• Mostly used for loop parallelisation.</td>
</tr>
<tr>
<td></td>
<td>• Performance is limited by the network communication between the computers.</td>
<td>• Limited by memory bandwidth when many processors are used.</td>
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<tr>
<td></td>
<td></td>
<td>• Cannot be used for task parallelism.</td>
</tr>
</tbody>
</table>

Table C.1: *Advantages and disadvantages of the MPI and OpenMP parallel approaches used within Fortran.*

The program are then parallelised, which breaks it up into multiple threads, each of which runs on a separate processor or processor core. The shared memory means that no information has to be transferred, as each processor has access to all of the data, all of the time.

The term thread, refers to the number of concurrent calculations occurring, which in general is equal to the number of processors within the computer. The advantages and disadvantages of each of the approaches, are outlined in Table C.1.
<table>
<thead>
<tr>
<th>Number of Computers/Processors</th>
<th>MPI (s)</th>
<th>OpenMP (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>123</td>
<td>3.7</td>
</tr>
<tr>
<td>4</td>
<td>296</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>460</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>620</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table C.2: A comparison of the times, in seconds, to solve a sparse system of 10,201 equations using both MPI and OpenMP versions of a CGLS algorithm.

Both parallelisation approaches were tested to solve example sparse matrix equations, the results of which are shown in Table C.2. However, due to the amount of network communications required to solve the system of equations with MPI, the speed up was significantly hindered. In fact from Table C.2 we see that the time increases with the number of computers. OpenMP on the other hand, scaled well with different numbers of processors. Hence attention quickly focused on this method and the optimisation of the parallel CGLS algorithm.

C.1 Parallelisation of the CGLS Algorithm

The CGLS algorithm involves three types of vector calculations which concerns its parallelisation,

- vector dot products,
- vector addition,
- matrix-vector products,

each of which are discussed in detail below.
C.1.1 Dot Products

In the CGLS algorithm, dot-products are used to obtain the vector norms. These dot-products can be parallelise as follows,

\[ ||x||_2^2 = x \cdot x, \]
\[ = \sum_{i=1}^{n} x_i^2, \]  \hspace{1cm} (C.1)  
\[ = \sum_{i=1}^{d} x_i^2 + \sum_{i=d+1}^{2d} x_i^2 + \ldots + \sum_{i=n-2d+1}^{n-d} x_i^2 + \sum_{i=n-d+1}^{n} x_i^2, \]  \hspace{1cm} (C.2)  

where \( d = \lfloor n/T \rfloor \), implies taking the floor value of the result, \( n \) is the vector size and \( T \) is the total number of threads. Hence the optimisation of the dot-product is done by splitting the summation up, so that each thread calculates part of it. The summed parts are then totalled by a single processor to give the final answer.

C.1.2 Vector Addition

Vector addition is done similarly, the two vectors are split into \( d \) portions and each thread then adds its particular portion together and returns the result in the solution vector. The OpenMP approach is the most efficient, as the full result can be accessed in memory by all the processors, once the calculation is complete. This is illustrated, for two threads in Figure C.1, where Thread 0 does the top half and Thread 1 does the bottom half of the vector addition.

C.1.3 Matrix-Vector Products

To optimise the matrix-vector multiplication, we require that each thread independently produces a portion of the solution vector. This was found to eliminate the following:
Figure C.1: Schematic of vector addition using two threads.

Figure C.2: Schematic of a matrix-vector product, using two threads.

- time lost, due to one thread having to wait for a partial answer from another, before continuing,

- data corruption, as the threads are independent and hence cannot update a value that is being manipulated by another.

This is achieved by splitting the matrix into $T$ rows, where $T$ is the total number of threads. For example if we had two threads then the matrix equation $A\mathbf{y} = \mathbf{z}$, would be split up as illustrated in Figure C.2. The shared memory architecture, ensures all threads have access to vector $\mathbf{y}$ in order to calculate $\mathbf{z}$. The CGLS algorithm also requires us to multiply the transpose of the matrix by a vector and this can be achieved in exactly the same way. However a much more efficient way, is to split the matrix into $T$ rows and $T$ columns, naming these sub-matrices 1, 2, 3, ... from the top, reading left to right, as illustrated in the bottom diagram of Figure C.3. This time, Thread $p$, would calculate its portion of the
Figure C.3: Schematic of how the matrix is split for both two and four threads, plus which sub-matrices are used by which thread for doing $A y = z$ and $A^T y = z$.

- matrix equation, $A y = z$, by multiplying the sub-matrices $T_p + 1$ to $(T + 1)p$,
- transpose matrix equation, $A^T y = z$, by multiplying the sub-matrices $p + 1, p + 1 + T, p + 1 + 2T, \ldots, p + 1 + (T - 1)T$,

to the vector $y$, for Thread $p = 0, 1, \ldots$. By applying these different ranges to each equation, as well as having the matrix in shared memory, means that each thread can independently calculate either result. Plus this eliminates the need to manipulate the matrix in any fashion, apart from interchanging the row and column coordinates. Figure C.3 shows two examples of matrix-vector multiplication for the two and four threads cases. As an example, focussing on Thread 0 in the two threaded case shown in Figure C.3, i.e. $T = 2$ and $p = 0$, we observe that

- it performs calculations of $A y = z$ only with sub-matrices 1 and 2,
- it performs calculations of $A^T y = z$ only with sub-matrices 1 and 3.
In the two thread case, it is clear that Thread 1, would have to deal with the other two sub-matrices for $Ay = z$ and $A^T y = z$ respectively, although these values can be obtained from the ranges given above. For the four threaded case, looking at equations $Ay = z$ and $A^T y = z$ respectively, we see that:

- Thread 0 deals with sub-matrices 1 to 4 or sub-matrices 1, 5, 9 and 13,
- Thread 1 deals with sub-matrices 5 to 8 or sub-matrices 2, 6, 10 and 14,
- Thread 2 deals with sub-matrices 9 to 12 or sub-matrices 3, 7, 11 and 15,
- Thread 3 deals with sub-matrices 13 to 16 or sub-matrices 4, 8, 12 and 16.

Splitting the matrix up into these sub-matrices is not computationally intensive, as it is accomplished while the matrix is generated. This approach easily expands to any number of processors.

### C.2 Efficient Storage of the Matrix

The implicit algorithms applied to the finite difference partial differential equations, produce sparse matrices, which are inefficient if stored in their full form. Therefore it is sensible to only store the non zero values with their row and column coordinates, which can be done in multiple ways [243]. As we are required to transpose the matrix, the method that we selected, stores the non zero values with their row and column coordinates, neither of which were compressed, as illustrated in Figure C.4. For example the highlighted element 12 in the matrix, becomes the sixth number down in the non zero vector, with its location being the sixth item in the row and column vectors. Not only did this decrease the amount of computer memory needed to store the matrix, but improved the efficiency of the matrix-vector products, as only the non zero values had to be multiplied. Another advantage, and the main reason for picking this method,
is that to transpose the matrix, we only need to interchange the row and column vectors. We note that this is a very efficient method, as no calculation or memory manipulation is necessary.

However in the matrix-vector products, we required sub-matrices to be produced, for efficiency. Therefore to add these into the compressed matrix form, we add an extra dimension to the non zero elements, row and column vectors. The first dimension, of each still stores its relevant value, whilst the second stores which sub-matrix it belonged to. Using this approach, each thread does not have to go through all the non zero values picking out only the ones relevant to itself enhancing the programs efficiency. Figure C.5, uses the same example, as above, to illustrate how this works for two threads, i.e. $T = 2$. As above, the element 12 has been highlighted, and we can see that it is located in the fourth sub-matrix, as shown by the schematic on the top right. Therefore this element goes into the fourth column of the non zero elements, row and column coordinate, as shown. However the information contained and meaning for the non zero elements, row and column coordinate “vectors” remain exactly the same. In fact the same trick of interchanging the row and column coordinates still produces the transposed matrix.
The matrix, split into the required four sub-matrices, numbered as before

\[
\begin{bmatrix}
1 & 0 & 3 & 0 \\
5 & 6 & 0 & 0 \\
0 & 10 & 0 & 12 \\
13 & 0 & 15 & 16 \\
\end{bmatrix}
\]

= 

\[
\begin{bmatrix}
1 & 0 & 3 & 0 \\
5 & 6 & 0 & 0 \\
0 & 10 & 0 & 12 \\
13 & 0 & 15 & 16 \\
\end{bmatrix}
\]

Figure C.5: Schematic of the matrix compression used in conjunction with sub-matrices for two threads.

Therefore this produces a compressed storage method for the matrix, whilst also allowing each thread to efficiently and independently, calculate a portion of either the matrix-vector or transposed matrix-vector products.
Appendix D

Model 4: Biofilm growth in a Stokes Flow

D.1 Introduction to the Model

This is an extension of Model 3 to the situation where the biofilm is growing on the inside wall of a pipe or flow chamber with water flowing slowly over it, as illustrated in Figure D.1. We require the water to flow slowly, as under the presence of a strong flow we would anticipate the biofilm to be smeared to a negligible height downstream along the surface of the vessel. The reason is in Model 3 the solid components of the biofilm were modelled as a viscous material. This is reasonable as bacteria do not tend to form firm bonds with neighbouring cells and the EPS in its natural state forms a loose mesh of fibres. Under significant stress these EPS fibres are likely to become entangled and tightly entwined to form EPS cables, which will have more elastic properties and generate greater biofilm rigidity. This situation is not described by the current model, and hence under strong flow the biofilm would be smeared to negligible height. Our interest in this chapter is to investigate biofilm development in the
presence of an intermediate flow, in which growth and deformation are occurring at approximately the same rate. Hence in our model we will assume that the flow over the biofilm is very slow, with low Reynold’s number and described by a Stoke’s flow model [78]. Consequently we would expect the flow upstream and “far” downstream to be Poiseulle [50]. Nutrients will be delivered in the external fluid and its concentration is assumed to be fixed at the inlet, i.e. the left hand side in Figure D.1. This set up creates external forces on the biofilm, which affect its moving boundary and complicates the model and its solution considerably.

### D.2 Model Derivation

Due to the fact that we are only adding water flowing around the outside of the biofilm, equations (3.75) - (3.83) are still valid and hence will be used to model the internal structure of the biofilm. Turning our attention to the fluid flow, for simplicity we assume that water flow around the biofilm is steady. This means that we will not have to deal with turbulence within the flow. This assumption
implies that the Reynold’s number is low and hence we are able to model the fluid flow via Stokes flow. The governing equations for Stokes flow are:

\[
\begin{align*}
\sigma_f &= -p_f I + \mu_f (\nabla u_f + \nabla u_f^T), \\
\nabla \cdot u_f &= 0, \\
\nabla p_f &= \mu_f \nabla^2 u_f,
\end{align*}
\] (D.1-3)

where \(u_f\) is the water velocity, \(p_f\) is the water pressure, \(\sigma_f\) is the fluid’s stress tensor and \(\mu_f\) is the shear viscosity coefficients. For numerical purposes, this system was rearranged into a more amenable format using standard identities in vector calculus. Taking the divergence of equation (D.3), we obtain

\[
\nabla^2 p_f = \mu_w \nabla \cdot (\nabla^2 u_f).
\]

Since for a vector quantity \(\mathbf{F}\), \(\nabla^2 \mathbf{F} = \nabla (\nabla \cdot \mathbf{F}) - \nabla \times (\nabla \times \mathbf{F})\), and that the divergence of a curl is zero, then it follows from equation (D.3) that the system can be rewritten as

\[
\begin{align*}
\nabla^2 p_f &= 0, \\
\nabla p_f &= \mu_f \nabla^2 u_f.
\end{align*}
\] (D.4-5)

This leaves us with describing the nutrient concentration, which diffuse through and are carried by the external fluid flow, with a quasi-steady assumption, such that

\[
\mathbf{u}_f \cdot \nabla c = D_{c_0} \nabla^2 c,
\] (D.6)

where \(D_{c_0}\) is the diffusion coefficient.

We now have a model that describes the fluid flow and nutrient concentration outside of the biofilm, whilst Model 3’s equations take care of the internal workings of the biofilm. This just leaves us to deal with the initial and boundary
D.3 Initial and Boundary Conditions

The initial conditions the same as those used in the standard simulation of Model 3, plus additional ones for the new model components. The same boundary conditions at the solid surface will remain, with the addition of \( u_f = 0 \), i.e. a non slip condition. Therefore the full list of conditions carried over from Model 3 are:

- solid surface boundary conditions, i.e. \( y = 0 \):

\[
\frac{\partial A}{\partial y} = \frac{\partial c}{\partial y} = \frac{\partial p_w}{\partial y} = v(x, 0, t) = u_f(x, 0, t) = 0. \tag{D.7}
\]

- moving biofilm boundary conditions, i.e. \( \phi(x, y, t) = 0 \):

\[
A = 0. \tag{D.8}
\]

- Initial conditions:

\[
\begin{align*}
    b_d(x, y, 0) &= \begin{cases} 
        b_0 & \phi(x, y, 0) \leq 0, \\
        0 & \phi(x, y, 0) > 0,
    \end{cases} \tag{D.9} \\
    b_u(x, y, 0) &= 0, \tag{D.10} \\
    E(x, y, 0) &= 0, \tag{D.11} \\
    \phi(x, y, 0) &= \phi_f. \tag{D.12}
\end{align*}
\]

We assume the flow is occurring in a narrow channel \( y \in (0, 2Y) \), and impose no slip conditions on the boundaries. Upstream from the biofilm, the flow is
fully established and we expect there to be no variation in the \( x \) direction, i.e. 
\[ p \sim p(y) \text{ and } u \sim (u_x(y), u_y(y)). \]
Thus at the inlet, we can solve the system (D.2) and (D.3) to obtain the classic solutions for Poiseuille flow,

\[
\begin{align*}
  u_x &= \frac{yu_{\text{max}}(2Y - y)}{Y^2}, \quad (D.13) \\
  u_y &= 0, \quad (D.14) \\
  \frac{\partial p_f}{\partial x} &= -\frac{2\mu_f u_{\text{max}}}{Y^2}, \quad (D.15)
\end{align*}
\]

where \( u_{\text{max}} \) is the maximum velocity at the inlet, corresponding to \( y = Y \).

Downstream from the biofilm in the vicinity of the outlet, \( x = X \), we assume that the fluid flow has re-established itself after flowing over the biofilm. Hence the same boundary conditions are used at the inlet and outlet for the water velocities and the pressures. We have

\[
\begin{align*}
  p_f &= 0 \quad \text{at } x = -X, \text{ without lose of generality}, \quad (D.16) \\
  \frac{\partial p_f}{\partial x} &= -\frac{2\mu_f u_{\text{max}}}{Y^2} \quad \text{at } x = X. \quad (D.17)
\end{align*}
\]

On the solid surface at \( y = 0 \) we impose the following boundary condition for the pressure,

\[
\frac{\partial p_f}{\partial y} = 0. \quad (D.18)
\]

At the fluid/biofilm interface we need three equations that link the fluid velocities and pressures outside the biofilm with the advective velocities and water pressure inside the biofilm. One of these equations is the mass balance between the amount of water flowing in and out of the biofilm. The other two are equilibrium conditions, one acting in the normal and the other in the tangential direction. We assume that the stresses normal to the interface are in equilibrium.
and the tangential shear is described using an extended Beavers-Joseph condition [19]. This empirically derived condition is often used in models for flow over porous media and states that the tangential component of the surface stresses are equal to the jump in the flow rate of the fluid phases. Hence, we impose

$$u_f \cdot n = w u_b \cdot n,$$

(D.19)

$$n \cdot (b \sigma_b + E \sigma_E + w \sigma_w) \cdot n - n \cdot \sigma_f \cdot n = 0,$$

(D.20)

$$t \cdot (b \sigma_b + E \sigma_E + w \sigma_w) \cdot n - t \cdot \sigma_f \cdot n - \frac{\alpha_{BJ}}{w} (u_f - w u_b) \cdot t = 0,$$

(D.21)

where $n$ and $t$ are the unit normal and tangential vectors, respectively, and are calculated as follows

$$n = \frac{\left( \frac{\partial \phi}{\partial x}, \frac{\partial \phi}{\partial y} \right)}{\sqrt{\left( \frac{\partial \phi}{\partial x} \right)^2 + \left( \frac{\partial \phi}{\partial y} \right)^2}},$$

(D.22)

$$t = \frac{\left( \frac{\partial \phi}{\partial y}, -\frac{\partial \phi}{\partial x} \right)}{\sqrt{\left( \frac{\partial \phi}{\partial x} \right)^2 + \left( \frac{\partial \phi}{\partial y} \right)^2}}.$$

(D.23)

The subscripts “f” and “b” refer to in the fluid and biofilm respectively and $\alpha_{BJ}$ is the Beavers Joseph constant. From above we have that $\sigma_i$’s are:

$$\sigma_b = -p_b I + \mu_b (\nabla v + \nabla v^T) + \lambda_b (\nabla \cdot v) I,$$

(D.24)

$$\sigma_E = -p_E I + \mu_E (\nabla v + \nabla v^T) + \lambda_E (\nabla \cdot v) I,$$

(D.25)

$$\sigma_w = -p_w I,$$

(D.26)

$$\sigma_f = -p_f I + \mu_f (\nabla u_f + \nabla u_f^T).$$

(D.27)

For the nutrients, we have that they flow in at the inlet at a fixed concentration and freely escape at the outlet. We assume zero flux through the boundary
walls of the pipe and hence

\[ c = c_0 \text{ inlet,} \quad (D.28) \]
\[ \frac{\partial c}{\partial x} = 0 \text{ outlet,} \quad (D.29) \]
\[ \frac{\partial c}{\partial y} = 0 \text{ \( y = 0 \) and \( y = 2Y \).} \quad (D.30) \]

We now have a full set of boundary and initial conditions for our model.

### D.4 Non Dimensionalisation

The variables pertaining to the biofilm components are rescaled in the same manner, as used in Model 3, i.e. equations (3.65). For the external flow we used

\[ \mathbf{u}_f = H_0 A_{bd} \hat{\mathbf{u}}_f, \quad p_f = p_{wu} \hat{p}_f, \quad \mu_f = \mu_0 \hat{\mu}_f, \quad (D.31) \]

where \( H_0 \) is the scaling height of the biofilm. The hatted variables described above are non dimensional and we substituting these equations into (D.4), (D.5) and (D.6), to obtain

\[ p_{wu} \nabla^2 p_f = 0, \quad (D.32) \]
\[ \frac{p_{wu}}{A_{bd} \mu_0} \nabla p_f = \hat{\mu}_f \nabla^2 \hat{\mathbf{u}}_f, \quad (D.33) \]
\[ \frac{H_0^2 A_{bd}}{D_{ca}} \hat{\mathbf{u}}_f \cdot \nabla c = \nabla^2 c. \quad (D.34) \]

These can be further simplified by letting

\[ \beta_{23} = \frac{H_0^2 A_{bd}}{D_{ca}}, \quad (D.35) \]

which refers to the ratio of advection to diffusion over the height of the biofilm. Substituting these parameters along with \( \beta_{13} \), defined in equations (3.74), into
Figure D.2: A schematic of the symmetry, with a biofilm growing on the top and bottom of the pipe.

(D.32) - (D.34) gives

\[ \nabla^2 p_f = 0, \]  
\[ \nabla p_f = \beta_{13} \hat{u}_f \nabla^2 \hat{u}_f, \]  
\[ \beta_{23} \hat{u}_f \cdot \nabla c = \nabla^2 c. \]

Furthermore if we assume that a biofilm is growing symmetrically on the bottom and top of the pipe, as shown in Figure D.2, then we only need to solve the equations over half the domain. Therefore substituting equation (D.31) into (D.13) - (D.15) and (D.28) - (D.30) and implying symmetrical boundary conditions at \( y = Y \), leads to the following non-dimensional boundary conditions,

\[ u_x = \frac{y \hat{u}_{max} (2 \hat{Y} - y)}{\hat{Y}^2} \text{ at the inlet and outlet}, \]  
\[ u_y = 0 \text{ at the inlet and outlet}, \]  
\[ p_f = 0 \text{ at the inlet}, \]  
\[ \frac{\partial p_f}{\partial x} = -\frac{A_{bf} \mu_0}{\rho w_0} \frac{2 \mu_f \hat{u}_{max}}{\hat{Y}^2} = -\frac{2 \mu_f \beta_{13} \hat{u}_{max}}{\delta Y^2} \text{ at the outlet}, \]
\( u_x = u_y = \frac{\partial p_f}{\partial y} = 0 \) at \( y = 0 \),  
(D.43)  
\( \frac{\partial u_x}{\partial y} = u_y = \frac{\partial p_f}{\partial y} = 0 \) at \( y = \hat{Y} \),  
(D.44)  
\( \hat{c} = 1 \) at the inlet,  
(D.45)  
\( \frac{\partial \hat{c}}{\partial \hat{x}} = 0 \) at the outlet,  
(D.46)  
\( \frac{\partial \hat{c}}{\partial \hat{y}} = 0 \) at the \( y = 0 \) and \( \hat{Y} \).  
(D.47)  
where \( u_{\text{max}} = H_0 A_{bd} \hat{u}_{\text{max}} \) and \( Y = H_0 \hat{Y} \). This leaves us with the fluid/biofilm boundary conditions, which upon substituting equations (3.24) - (3.28) and (D.1) into equations (D.19) and (D.21) and simplifying them gives

\[
\hat{u}_f \cdot n = w \hat{u}_b \cdot n, \quad \text{(D.48)}
\]

\[
0 = n \cdot (\hat{\sigma}_b) \cdot n - n \cdot (\hat{\sigma}_f) \cdot n, \quad \text{(D.49)}
\]

\[
0 = t \cdot (\hat{\sigma}_b) \cdot n - t \cdot (\hat{\sigma}_f) \cdot n
- \alpha_{bf} \frac{\beta_{20} k}{\beta_{13} w} (u_f - w u_b) \cdot t, \quad \text{(D.50)}
\]

where

\[
\hat{\sigma}_b = -\frac{1}{\beta_{13}} (p_w + b \Sigma_b + E \Sigma_E) I + \mu (\nabla \mathbf{v} + \nabla \mathbf{v}^T) + \lambda (\nabla \cdot \mathbf{v}) I, \quad \text{(D.51)}
\]

\[
\hat{\sigma}_f = -\frac{1}{\beta_{13}} p_f I + \mu_f (\nabla u_f + \nabla u_f^T). \quad \text{(D.52)}
\]

We have now non dimensionalised the complete system of equations and for summary purposes these are listed below:

- The equations for inside the biofilm:

\[
b_d + b_u + E + w = 1, \quad \text{(D.53)}
\]

\[
\frac{\partial b_d}{\partial t} + \nabla \cdot (v b_d) = (k_{bd}(c) - k_{dd}(c))b_d - \beta_1 A b_d + \beta_2 b_u 
+ (2 - \gamma) k_{bu}(c) b_u, \quad \text{(D.54)}
\]

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\[
\frac{\partial b_u}{\partial t} + \nabla \cdot (v b_u) = ((\gamma - 1)k_{bu}(c) - k_{du}(c))b_u + \beta_1 A b_d - \beta_2 b_u, \tag{D.55}
\]
\[
\frac{\partial E}{\partial t} + \nabla \cdot (v E) = (\beta_3 u b_u + \beta_3 d b_d) c - \beta_4 E, \tag{D.56}
\]
\[
0 = \nabla \cdot (k_A b \nabla A) + D_A \nabla \cdot (w \nabla A) - \beta_5 A (k_A b + w) + \beta_17 b_u + \beta_18 b_d - \beta_19 b_d A, \tag{D.57}
\]
\[
0 = \nabla \cdot (k_c b \nabla c) + D_c \nabla \cdot (w \nabla c) - \beta_6 b c, \tag{D.58}
\]
\[
(\beta_3 u b_u + \beta_3 d b_d) c - \beta_4 E = \nabla \cdot \left( v - \frac{w}{\beta_3 k} \nabla p_w \right), \tag{D.59}
\]
\[
\nabla \cdot (b \Sigma_b + E \Sigma_E) I = \nabla \cdot (-p_w I + \beta_13 \mu (\nabla v + \nabla v^T)) + \beta_13 \lambda (\nabla \cdot v) I, \tag{D.60}
\]
\[
\frac{\partial \phi}{\partial t} + \nabla \cdot (v \phi) = \phi \nabla \cdot v, \tag{D.61}
\]

where \(\mu, \lambda, k, \Sigma_b, \Sigma_E, k_{bd}, k_{bu}, k_{dd}\) and \(k_{du}\) are given by equations (3.84) - (3.84).

- The equations for outside the biofilm are:

\[
\nabla^2 p_f = 0, \tag{D.62}
\]
\[
\frac{\delta}{\beta_13} \nabla p_f = \mu_f \nabla^2 \hat{u}_f, \tag{D.63}
\]
\[
\beta_{23} \hat{u}_f \cdot \nabla c = \nabla^2 c. \tag{D.64}
\]

- The initial conditions are:

\[
b_d(x, y, 0) = \begin{cases} 
  b_0 & \phi(x, y, 0) \leq 0, \\
  0 & \phi(x, y, 0) > 0,
\end{cases} \tag{D.65}
\]
\[
b_u(x, y, 0) = 0, \tag{D.66}
\]
\[ E(x, y, 0) = 0, \quad \text{(D.67)} \]
\[ \phi(x, y, 0) = \phi_f. \quad \text{(D.68)} \]

- The boundary conditions at the inlet are:

\[ u_x = \frac{y\hat{u}_{\text{max}}(2\hat{Y} - y)}{\hat{Y}^2}, \quad \text{(D.69)} \]
\[ u_y = 0, \quad \text{(D.70)} \]
\[ p_f = 0 \quad \text{(D.71)} \]
\[ c = 1. \quad \text{(D.72)} \]

- The boundary conditions at \( y = \hat{Y} \) are:

\[ \frac{\partial u_x}{\partial y} = 0, \quad \text{(D.73)} \]
\[ u_y = 0, \quad \text{(D.74)} \]
\[ \frac{\partial p_f}{\partial y} = 0, \quad \text{(D.75)} \]
\[ \frac{\partial c}{\partial y} = 0. \quad \text{(D.76)} \]

- The boundary conditions at \( y = 0 \) are:

\[ u_x = u_y = v_x = v_y = 0, \quad \text{(D.77)} \]
\[ \frac{\partial A}{\partial y} = \frac{\partial c}{\partial y} = \frac{\partial p_w}{\partial y} = \frac{\partial p_f}{\partial y} = 0. \quad \text{(D.78)} \]

- The boundary conditions at the outlet are:

\[ u_x = \frac{y\hat{u}_{\text{max}}(2\hat{Y} - y)}{\hat{Y}^2}, \quad \text{(D.79)} \]
\[ u_y = 0, \quad (D.80) \]
\[ \frac{\partial p_f}{\partial x} = \frac{-2\mu_f\beta_{13}u_{\text{max}}}{Y^2}, \quad (D.81) \]
\[ \frac{\partial c}{\partial x} = 0. \quad (D.82) \]

- The fluid/biofilm boundary conditions are:

\[ \hat{u}_f \cdot n = w\hat{u}_b \cdot n, \quad (D.83) \]
\[ 0 = n \cdot (\hat{\sigma}_b) \cdot n - n \cdot (\hat{\sigma}_f) \cdot n, \quad (D.84) \]
\[ 0 = t \cdot (\hat{\sigma}_b) \cdot n - t \cdot (\hat{\sigma}_f) \cdot n \]
\[ -\alpha_{BJ} \frac{\beta_{20}k}{\beta_{13}w} (u_f - wu_b) \cdot t, \quad (D.85) \]

where

\[ \hat{\sigma}_b = -\frac{1}{\beta_{13}}(p_w + b\Sigma_b + E\Sigma_E)I + \mu(\nabla v + \nabla v^T) + \lambda(\nabla \cdot v)I \quad (D.86) \]
\[ \hat{\sigma}_f = -\frac{1}{\beta_{13}}p_f I + \mu_f(\nabla u_f + \nabla u_{f}^T). \quad (D.87) \]

We now have a full system of non-dimensional equation, however more work is required to guarantee that in the limit as the water flow tends to zero, the model degenerate into Model 3 (i.e. the static water case).
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