Short-Term Interactions Between *Staphylococcus aureus* and *Pseudomonas aeruginosa*

BEP Report

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Abstract

Staphylococcus aureus and *Pseudomonas aeruginosa* are two species of bacteria that are involved in numerous conditions, including lung infections and chronic wound infections [1]. The aim of this project was to study the short-term interactions that occur when *P. aeruginosa* first encounters an established *S. aureus* colony, which it then seeks to break apart whilst mixing with *S. aureus*. Limoli et al. [2] have studied these interactions using experiments, and have thus identified several key aspects involved in these interactions, such as the mechanisms that *P. aeruginosa* employs to approach the *S. aureus* colony. The means by which we intended to study interactions between *S. aureus* and *P. aeruginosa* is a model that was made by previous members of the Idema group and that was based on the experiments by Limoli et al. [2, 6]. In this report, we discuss this model and the biological background relevant to it. We also document the problems that we encountered while trying to run simulations using an existing implementation of this model.

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1 Introduction

S. aureus and *P. aeruginosa* are two species of bacteria that are well-known for the various and potentially life-threatening conditions that they may be involved in. While treating an infection in which only non-resistant strains of one of the two species are present can be relatively simple, the situation becomes much more complicated when both species are involved [2]. This alone makes understanding the interactions between *S. aureus* and *P. aeruginosa* a worthwhile endeavour. The main aim of this project was to verify and possibly enhance an existing model to study short-term interactions between these two species of bacteria. Interactions between *S. aureus* and *P. aeruginosa* are of interest for several reasons. Firstly, a better understanding of the interactions between *S. aureus* and *P. aeruginosa* may lead to better prospects for the prevention and treatment of infections in which both of these species of bacteria are involved. Secondly, both *S. aureus* and *P. aeruginosa* are relatively well-studied microorganisms [3, 4], which makes them well-suited as model organisms for somewhat generalistic models that may be later adapted to work well in other settings too.

The above-mentioned short-term interactions occur when a travelling swarm of *P. aeruginosa* encounters an established colony of *S. aureus*. Upon detecting the *S. aureus* colony, *P. aeruginosa* will move towards it and attempt to break it apart whilst mixing with *S. aureus* [2]. If successful, these interactions give rise to a state in which the *S. aureus* colony has been broken up and *S. aureus* and *P. aeruginosa* have become mixed. From this point onwards, *P. aeruginosa* and *S. aureus* continue to co-exist in this mixed condition. Since the interactions that give rise to this mixed state last for a comparatively short period of time, they are referred to as short-term interactions. We consider the short-term interactions to be completed once the *S. aureus* colony has been broken apart and the two species of bacteria have become mixed.

Even though hostility from *P. aeruginosa* towards *S. aureus* has been hypothesised to be the cause of these short-term interactions, the exact purpose behind them remains unknown [2]. What is known, however, is that once these short-term interactions have been completed and the two species of bacteria have become mixed, both *P. aeruginosa* and *S. aureus* are more resistant to antibiotics than they are separately [5]. Thus, the mixed state that these short-term interactions lead to may in fact be beneficial to both *S. aureus* and *P. aeruginosa*, while being detrimental to the host as it complicates treatment.

In this project, we studied the short-term interactions between *P. aeruginosa* and *S. aureus* using particle-based interactions. The particular model that was used in this project, was an expanded and possibly altered version of the model that was described in the Academic Internship report of Brian Analikwu [6].

In this model, the rod-shaped bacterium *P. aeruginosa* is represented by true spherocylinders, whereas the spherical *S. aureus* is represented by spheres. Since the aim of the model is to simulate the short-term interactions that typically occur when members of the species *P. aeruginosa* first encounter a colony of *S. aureus*, the spherocylinders are set to surround and attempt to break apart an already established colony of spherical particles. A very concise description of the model would be the following. The spheres that make up the *S. aureus* colony excrete particles that create a gradient which the spherocylinders follow. Consuming the excreted particles as they do, the spherocylinders follow the gradient until they arrive at its source, which is the *S. aureus* colony. They then continue to try and move forwards, which causes them to exert a force on the perimeter

of the colony that may eventually cause the colony to be broken up into smaller pieces. We shall discuss these interactions, along with they way in which they are modelled, in more detail in later sections of this report.

Because the motions of members of the two species of bacteria essentially occur in only two dimensions in this model, the spheres are replaced by circles and the spherocylinders by their two-dimensional counterparts [6]. As a result, the outcome of such a simulation can be directly compared to microscopical observations that have been made of short-term interactions between *S. aureus* and *P. aeruginosa* by Limoli et al. [2]. And, as can be read about in more detail in Analikwu's internship report, the results of the model that he worked on are in many ways already quite similar to observations that have been made in actual experiments [6].

However, as in every model, simplifications have been made, and one may wonder whether significant improvements can be made to the model by adding some factors that were previously omitted. For instance, one force that was not taken into consideration in Analikwu's model, is the static friction(-like) force between the bacteria and the surface on which their interactions take place. This surface may, for instance, be lung tissue, to which the bacteria can cling with their pili or by other means [21]. If this resistance force proves to be significant enough to be taken into account, then the model should be expanded by introducing a threshold force that the *P. aeruginosa* bacteria would need to exert on members of the *S. aureus* colony in order to get them to move in the first place. And, if members of the colony are brought into motion, they might try to reattach themselves to the substrate, which too would make it more difficult for *P. aeruginosa* to keep pushing them. Since such additions to the model will contribute to the amount of time that the simulation takes to complete, it is important to properly consider whether such additions to the model are worthwhile. Doing so was one of the goals of this project.

Another goal of this project was properly testing the existing model and its implementation against the outcomes of the experiments carried out by Limoli et al. [2]. For even though, as stated before, the results that Analikwu [6] obtained resemble those obtained by Limoli et al. [2], it remains important to quantitatively check how well the results of the simulations actually match the results of the experiments. Carrying out such a rigorous check is also one of the suggestions that Analikwu himself made in his internship report [6].

Summarising the above, the three most prominent goals of this project were the following:

- Study short-term interactions between *P. aeruginosa* and *S. aureus*, preferably in a setting that has some clinical relevance.
- Research whether certain additions to the existing model would be necessary.
- Implement worthwhile additions to the model (both on paper and in C++) and determine their impact on the model predictions.

In the next subsection, we shall take a closer look at the goals listed above. Then, we shall move on the Literature Study, in which some further biological background for the interactions is provided. In the Model section, we consider the model that was described by Analikwu [6], before moving on to the simulations that we attempted to carry out using this model and its implementation by Analikwu [6] in the Simulation section. Finally, we shall discuss the results of this project and the conclusions that may be drawn from them, in the Results and in the Conclusion and Discussion sections, respectively.

1.1 Goals

The first goal that we intended to achieve is testing the results of Analikwu's model [6] against those of experiments with live bacteria. For this test, we intended to use the data that are available from the experiments that were carried out by Limoli et el. [2] and which serve as an inspiration for the model [6]. For while qualitative comparisons between the outcomes of the simulations and the experiments were already made by Analikwu and included in his internship report, a quantitative verification remains to be made [6]. What such a check would have entailed, was performing a simulation using an unaltered version of Analikwu's implementation of his model, and then checking whether certain quantities, such as the velocities of *P. aeruginosa* bacteria and the maximal and average penetration depth of *P. aeruginosa* into the *S. aureus* colony found in the simulation corresponded with those found in the experiments that were carried out by Limoli et al. [2].

Once this first check of the unaltered model and its implementation had been carried out, a second check would have been performed using a slightly changed version of the implementation. The change that would have been made to the implementation concerns the starting positions of *P. aeruginosa* in the simulation. Currently, *P. aeruginosa* is randomly distributed in the domain outside of the *S. aureus* colony at the start of the simulation. In the experiments of Limoli et al., however, *P. aeruginosa* started out closely grouped together, rather than randomly distributed [2]. The starting position of *P. aeruginosa* in the experiments corresponds with a typical mode of movement of *P. aeruginosa* that is known as swarming [2]. Swarming is a form of bacterial movement in which numerous bacteria all move in one direction while remaining close to one another [8]. We shall discuss swarming, along with some other forms of bacterial motility, in more detail in the Literature Study section of this report. What is important for now, however, is that Limoli et al. found in their experiments that when a swarm of *P. aeruginosa* first detects the presence of a *S. aureus* colony, *P. aeruginosa* switches from group motion to individual motion [2]. This means that the individual *P. aeruginosa* bacteria break apart from the swarm as they each begin to follow the gradient of chemo-attractant towards the *S. aureus* colony [2]. The *S. aureus* colony is then surrounded by *P. aeruginosa* bacteria, which start to penetrate it and break it apart, whilst mixing with the *S. aureus* bacteria [2]. Since the *P. aeruginosa* bacteria currently start randomly distributed in the simulation, they naturally surround the *S. aureus* colony as they approach it from all directions. However, by adjusting the starting positions of the *P. aeruginosa* bacteria in the simulation so that they all start close to one another, we can verify whether the simulated *P. aeruginosa* bacteria also surround the *S. aureus* colony, or whether they all remain on one side of the colony. Since the live *P. aeruginosa* bacteria likely end up surrounding the colony because they break up the gradient of chemo-attractants as they move towards the colony, this would also provide further insight into whether the production and degradation of the chemo-attractant in the model and its implementation require adjustments.

After carrying out these first two tests, we intended to implement some changes to the model and its implementation. One change that we thought was particularly promising, was adding a force to the model that corresponds to the fact that *S. aureus* is capable of clinging to the substrate on which it lives [21], which may mean that a certain amount of force would be required to overcome *S. aureus*'s adhesion to its substrate and bring it into motion. Modelling such a force would entail adding a threshold force that is required to get *S. aureus* to move, and this force would correspond with the force required to get *S. aureus* to detach from its substrate. We wanted to investigate whether it would be more realistic to implement this force as a friction force (in which case it would remain relevant even after the *S. aureus* bacteria had been brought into motion), or as a one-time

threshold that needed to be passed for the movement of *S. aureus* to start, but which would no longer be relevant afterwards. Since it was difficult to determine a realistic magnitude for this force purely on the basis of existing literature (this force seems to depend on multiple factors, including the nature of the substrate), some simulations would probably have been required to gauge what would seem like a reasonable magnitude for the purpose of this model.

Another aspect of the model which we initially thought might benefit from a change, involves the adhesion force between simulated *P. aeruginosa* bacteria. The reason for this consideration, was that on the basis of the explanation of this adhesion force in Analikwu's internship report [6], it remained somewhat unclear to us how, precisely, the expression that was used for this force had come to be. However, after reading more about the type of potential on which this force was based (the Lennard-Jones potential or, more generally, Mie potentials), it became clearer how the expression for this force may have been determined. More about this in the relevant part of the Model section of this report.

Thus far, we have only considered goals involving testing the original model and its implementation against experimental data, making changes to the model and its implementation, and subsequently testing these changes against the experimental data to gauge whether these changes actually led to improvements. However, once we would have finished this stage of the project, we also intended to use the version of the model and its implementation that we would have obtained by then in order to investigate the short-term interactions between *S. aureus* and *P. aeruginosa*. Some aspects of these interactions that seemed of particular interest to us, were how long it would generally take *P. aeruginosa* to dismantle a *S. aureus* colony, and how changing certain parameters in the simulations would affect the outcome (for instance, changing the propulsion force of *P. aeruginosa*, or the amount of chemo-attractants emitted by *S. aureus*).

2 Literature Study

2.1 The Nature of the Short-Term Interactions

The model that was used in this project is based on the experimental findings of Limoli et al. [2, 6]. The setting that was studied by Limoli et al. [2] and which Analikwu's model [6] was designed to simulate, is the following. *P. aeruginosa* exists initially as a swarm (or, as it is also referred to in [2], in a raft) as it moves and replicates. Once *P. aeruginosa* detects *S. aureus*, which exists in a stationary colony, it switches from collective motion to individual motion [2]. What this means, is that individual *P. aeruginosa* bacteria break apart from the swarm to which they initially belonged, and start to make their own way towards the *S. aureus* colony by means of chemotaxis. The *S. aureus* colony is surrounded by individually moving *P. aeruginosa* bacteria, which ultimately penetrate it, break it apart, and mix with the *S. aureus* bacteria that used to make up the colony [2]. Why *P. aeruginosa* displays this type of behaviour is not completely clear [2]. It may be that *S. aureus* produces substances that are beneficial to *P. aeruginosa* and which *P. aeruginosa* therefore seeks to claim for itself by being in as close a proximity to *S. aureus* as possible. It may also be that *P. aeruginosa* perceives *S. aureus* as competition, which it may seek to reduce or eliminate by breaking apart the colony and killing *S. aureus* through direct cell to cell contact and secreted antimicrobials [2].

What is much clearer than the exact motives of the behaviour that is displayed by *P. aeruginosa*, are the results of this behaviour. As is observed by Limoli et al. [2], the behaviour of *P. aeruginosa* appears to inhibit the growth of the *S. aureus* colony. This, at least, would seem like a negative outcome for *S. aureus*. However, there are also other results of *P. aeruginosa*'s behaviour, which may in fact be beneficial to *S. aureus*. For when *P. aeruginosa* and *S. aureus* become mixed, both of the species of bacteria become more difficult to treat with antibiotics than they are when isolated from one another [5]. This, in turn, leads to worse treatment prospects for patients suffering from infections in which both *P. aeruginosa* and *S. aureus* are involved than for patients suffering from infections in which only one of these two species of bacteria is involved. As such, as is also argued by Limoli et al. [2], inhibiting these short-term interactions from occurring would be beneficial, as it would prevent a situation in which *S. aureus* and *P. aeruginosa* are more resilient to treatment using antibiotics from occurring.

So how, precisely, does *P. aeruginosa* sense and approach the *S. aureus* colony? To answer this question, we need to understand how *P. aeruginosa* moves across the substrate on which the interactions occur and how it detects *S. aureus*. We shall delve into these topics in the next subsection.

2.2 A Brief Overview of *P. aeruginosa* Motility

A bacterium that is capable of propelling itself through its environment is called motile, whereas bacteria that lack this capability are referred to as non-motile [8]. The ways in which a motile bacterium may move through its environment depend both on the species of bacterium at hand and on the type of environment that the bacterium may find itself in [8]. Different forms of bacterial motility can furthermore require the use of different organelles, the two most important types of which are flagella and pili [9, 10].

A pilus is hair-like structure on a bacterium's exterior that is often used for adhering to substrates

or other objects, including fellow bacteria [10]. A bacterial flagellum, on the other hand, can be somewhat thought of as a propeller. It is composed of a thin, helical appendage called a flagellar filament, which protrudes from the cell, a rotary motor that can turn the filament clockwise or counterclockwise, and the components that connect them [8]. A famous example of flagellum-mediated swimming is provided by the model-bacterium *Escherichia coli*, which can alternate between swimming in a straight line and tumbling erratically by rotating its flagella counterclockwise or clockwise, respectively [11]. Since the interactions between *P. aeruginosa* and *S. aureus* that this project focuses on do not occur in an environment that is sufficiently liquid for swimming motility to occur [2], however, this form of bacterial motility is of little relevance to the topic at hand.

P. aeruginosa is a rod-shaped, flagellated bacterium that can also produce pili at its poles [2, 12]. In the moist environment in which the interactions between *P. aeruginosa* and *S. aureus* take place, the two main modes of motility displayed by *P. aeruginosa* are swarming and twitching [2]. Swarming is the type of motility that *P. aeruginosa* displays before it detects the *S. aureus* colony. Like swimming, swarming is a flagellum-driven form of bacterial locomotion [8]. However, unlike swimming, which occurs in liquid environments, swarming occurs on solid substrates that are covered in a thin film of fluid (hence, moist) [8]. Furthermore, whereas swimming is typically an individual form of motility, swarming is a collective form of locomotion, in which individual bacteria travel closely together and in the same direction [7]. Oftentimes, bacterial swarming involves the production of additional flagella on each individual bacterium (a process known as hyperflagellation) and the elongation of the individual cells [7, 8]. This cell elongation may contribute to the lengthwise alignment that is necessary for all the involved cells to move in the same direction, and hyperflagellation may help to overcome the greater resistance that the bacteria experience when moving in a heavily crowded swarm [8]. However, these cellular changes are not fully understood, and furthermore, they are not observed in all species of swarming bacteria [8].

Whereas swarming relies on the cell's flagella, twitching, the form of locomotion to which *P. aeruginosa* switches after detecting the *S. aureus* colony, is mediated by the cell's pili [12]. The specific kind of pilus that is involved in bacterial twitching motility, is known as the type IV pilus, also denoted as T4P [12]. The ends of type IV pili can adhere to many different kinds of substrates, including other bacteria [2, 12]. They can be extended and retracted, and it is by this means that they can produce a twitching motion [8]. This works as follows. When a pilus is first attached to a solid substrate and then starts to retract, this action generates a pulling force in the direction of the point where the pilus is attached to the substrate [8]. By alternating between extending, attaching and retracting pili, cells can then generate a twitching motion in a certain direction [8]. In *P. aeruginosa* and other rod-shaped bacteria, pili are located at the poles of the cell [12]. Tension can be generated by pili that try to simultaneously pull the cell in opposite directions. If the pili that were attached to the substrate on one side of the cell are suddenly released while such tension exists, the cell can be rapidly propelled in the opposite direction in a slingshot-like motion [8].

After breaking apart from the swarm, *P. aeruginosa* uses chemotaxis to determine the direction in which it moves and thus, to make its way towards the *S. aureus* colony [2]. In other words, *P. aeruginosa* can sense the gradient of chemo-attractants that are secreted by *S. aureus*, and uses this to determine the direction in which it moves. Whereas flagellum-driven chemotaxis for swimming motility has been studied in great detail and is generally well understood, less is known about chemotaxis-directed twitching motility [13]. Let us look, however, at two important concepts that are known and which concern the way in which *P. aeruginosa* uses chemotaxis to navigate chemical gradients on moist, solid surfaces.

Firstly, *P. aeruginosa* uses a different transduction system while moving by means of twitching on a moist, solid substrate than it does while swimming through a liquid environment [13]. A study by Oliveira et al. [13] of single-cell twitching chemotaxis in biofilms found that even when the transduction system that is involved in swimming chemotaxis is knocked out in *P. aeruginosa*, the bacterium could still carry out twitching chemotaxis on moist, solid surfaces. Furthermore, the Chp system, which was previously only known to be associated with the biosynthesis of type IV pili, was also found to be involved in the transduction of chemotactic stimuli in twitching *P. aeruginosa* bacteria in that same study [13].

Secondly, *P. aeruginosa* employs what is described as a pessimistic chemotactic strategy while using twitching motility to move across a moist surface [13]. While moving by means of twitching, *P. aeruginosa* alters the frequency with which it changes direction based on whether it senses that it is moving away from the source of the chemo-attractant, as opposed to towards it [13]. Oliveira et al. found that *P. aeruginosa* cells almost double their reversal rate when they detect that they are moving away from the source of the chemo-attractant [13]. Thus, *P. aeruginosa* is more likely to change direction while moving away from the source of the chemo-attractant, which in turn helps the bacterium to ultimately move towards the source. Oliveira et al. [13] call this form of chemotaxis "pessimistic" chemotaxis. This is likely done to emphasise the difference between this form of chemotaxis and the well-studied form of chemotaxis that is employed by swimming *Escherichia coli* bacteria. To fully appreciate the difference in chemotaxis techniques, it is helpful to understand how *E. coli* employs chemotaxis too. While swimming through a liquid environment, *E. coli* alternates between rotating its flagella counterclockwise and clockwise [11]. During counterclockwise rotation of its flagella, *E. coli* moves forwards, and during clockwise rotation of its flagella, *E. coli* tumbles erratically [11]. By alternating between counterclockwise and clockwise rotation of its flagella, *E. coli* achieves periods of swimming in a certain direction, followed by a period of tumbling during which a new swimming direction is randomly picked, followed by a period of swimming in this new direction, etc. [11]. If *E. coli* senses that it is swimming in the direction of a positive gradient of a chemo-attractant, it will tumble less frequently than if it does not detect a positive gradient of chemo-attractant along its current direction [11, 13]. Oliveira et al. [13], call this type of chemotaxis "optimistic" chemotaxis, as it works in a similar yet opposite fashion to "pessimistic" chemotaxis.

It is interesting to note that it recently was discovered that *S. aureus*, which was previously thought to be altogether non-motile, can in fact move [14]. In an article on this subject, Pollitt et al. [14] describe two distinct forms of locomotion for *S. aureus*. The first form of motility that Pollitt et al. [14] describe is called spreading, which is a type of sliding motility. The second form of motility involves comet formation, which is more similar to gliding motility [14]. Since sliding motility does not tend to require active propulsion, it is generally regarded as a passive form of motility [8]. Gliding motility, on the other hand, does require active propulsion and is therefore considered to be an active form of motility [8]. As such, it is primarily because of the locomotion by means of comet formation that *S. aureus* may be seen as a motile bacterium species [14]. However, since neither of these two forms of movement occurs perceptibly in the experiments carried out by Limoli et al. [2] it is not necessary to include them in the model.

2.3 *S. aureus* Colonies

As was already mentioned in the Introduction, *S. aureus* is a well-known and relatively commonly occurring pathogen that is involved in several potentially life-threatening conditions [15]. We also

saw that while *S. aureus* can move on its own accord, it is not a particularly motile bacterium [2, 14], and that in the interactions that are the topic of this project, a colony of this species of bacterium is surrounded and broken apart by *P. aeruginosa*[2]. But how do such colonies of *S. aureus* come to be, and what roles can they play in pathogenesis? We shall address these questions in this subsection.

Even though *S. aureus* can cause severe infections, many people are carriers of *S. aureus* without ever noticing it [15]. For this reason, a difference is often made between so-called colonisation, and actual infection by *S. aureus* (or by other species of bacteria). In this context, colonisation generally means that an organism merely carries *S. aureus* somewhere on their body. Unlike infection, which is directly related to disease, colonisation does not need to have any adverse consequences for the host, although this can ultimately change. In fact, as is described by Brown et al. [16], *S. aureus* colonisation is the greatest risk factor for infection, and may moreover modify the outcome of an eventual infection, as the initial period of colonisation may affect the reaction of the immune system to *S. aureus* during an actual infection. In this same study [16], the anterior nares (i.e., the nostrils) are named as the primary *S. aureus* reservoir in humans, with some areas outside of the nose that may also serve as colonisation sites including the skin, the throat and the gastrointestinal tract. An infection is generally only achieved by *S. aureus* if it manages to breach the epithelium (for instance, the skin) [16]. During infection, *S. aureus* may use several different strategies, one of which is the formation of a biofilm [16]. Since the *S. aureus* colonies that are involved in the short-term interactions that were studied in this project consist of a single layer of *S. aureus* bacteria that are grouped closely together and which adhere to each other [2], one may think of such a colony as a gateway to a biofilm of sorts.

3 Model

In this section, we shall discuss the model that was used to simulate the short-term interactions between *S. aureus* and *P. aeruginosa*. The model that is discussed in this section is the model that Analikwu described in his academic internship report [6]. As such, Analikwu's internship report [6] is an important reference for this entire section, which we mention here to avoid having to put this reference at the end of almost every sentence of the remainder of this section. The description below is in our own words, however, and whereas some parts of the model won't be discussed in as much detail as they were discussed in [6], other parts will be explained in a bit more detail than they were in Analikwu's internship report.

3.1 An Active Particle Model

The type of model that was employed by Analikwu [6] is known as an active-particle model. In an active particle model, the objects that we intend to study are represented by particles that have certain propulsion and interaction mechanics. Another important feature of Analiwu's model [6], is that it is made dimensionless. As a result, all parameters can be expressed in a small number of parameters. The results are thus more general, because they are no longer as bound to physical quantities, without losing their physical relevance. All the variables that occur in the model can be expressed in the fundamental quantities distance [L], time [T] and mass [M]. However, since mass is only used to calculate forces in this model, the quantity mass can be replaced with the derived quantity force $[\mathrm{F}] = [\mathrm{MLT}^{-2}]$ for convenience.

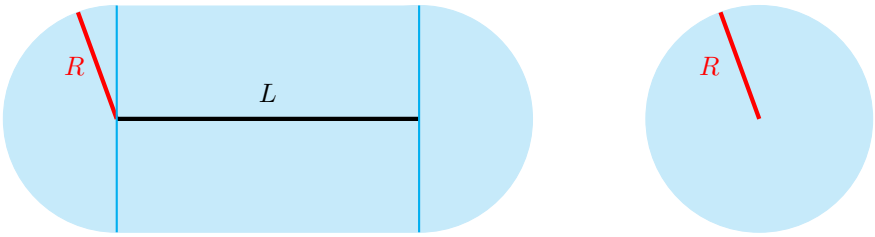

Figure 1: Two examples of a spherocylinder in two dimensions. The spherocylinder on the left has a spine length L that is greater than zero and is therefore a true spherocylinder, whereas the spherocylinder on the right has spine length zero and is therefore not a true spherocylinder. Note that both of the depicted spherocylinders have the same hemispherical radius R.

In the short-term interactions that the model is designed to simulate, the relevant particles are *P. aeruginosa*, *S. aureus* and the chemo-attractants that are produced by *S. aureus*. Within the model, the bacteria *P. aeruginosa* and *S. aureus* are represented by spherocylindrical particles. In three dimensions, a spherocylinder is a cylinder with a hemisphere attached to each end. Two examples of the two-dimensional equivalent of a spherocylinder, which is employed in the model, are shown in Figure 1. Observe that since the diameter of the hemispheres is equal to that of the cylinder, the geometry of a spherocylinder is smooth. Furthermore, the radius of the hemisphere (R) and the aspect-ratio of the spherocylinder (AR) are sufficient to completely determine the geometry of the particle. After all, since the aspect-ratio of a particle is the ratio of the length of the particle over its width, the length of a spherocylinder is equal to $L = 2R \cdot AR$. In the special case of a sphere, the length of the particle is equal to the diameter of the hemisphere, and hence, the aspect-ratio

is equal to 1 (since $L = 2R \implies 2R = 2R \cdot AR \implies AR = 1$). As such, we only speak of a "true" spherocylinder if the aspect-ratio is strictly larger than one. Hence, while both *S. aureus* and *P. aeruginosa* are represented by spherocylinders in the model, only the rod-shaped bacterium *P. aeruginosa* is represented by true spherocylinders, in contrast to the spherical bacterium *S. aureus*.

In two dimensions, a spherocylinder becomes a rectangle with a semicircle attached to each end, as is shown in Figure 1. Since the model is implemented for the two-dimensional case, the word spherocylinder will always refer to its two-dimensional counterpart from this point onward in this text, unless specified otherwise. Even though a spherocylinder is a symmetrical object, it is convenient to give it a set direction. This is done as is shown in Figure 2. First, note that the so-called spine of a spherocylinder is the line segment that connects the two points along the longest axis where the rectangle ends and the two semicircles start. We call the endpoints of the spherocylinder's spine A and B, and we let the vector that points from A to B be the direction vector of the particle.

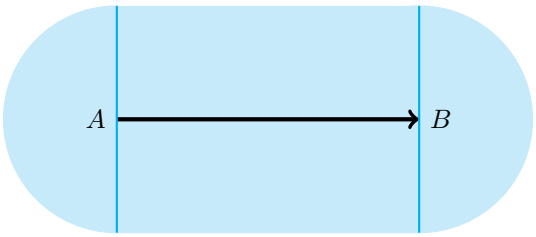

Figure 2: A true spherocylinder with an indicated direction. The line segment that connects the points A and B is the spine of the spherocylinder, and the vector that points from A to B serves as the direction vector of the spherocylinder.

The particles whose motions we are interested in modelling are bacteria. Since bacteria are microscopic particles, the Reynolds number, which is denoted by Re, is relevant for determining the type of fluid flow that the bacteria encounter. There are two typical regimes that one may encounter: if the Reynolds number is large ($Re >> 1$), then we have a turbulent flow. And if the Reynolds number is small ($Re << 1$), then we have a viscous flow. In turbulent flow, fluids mix easily and behave chaotically. In a viscous flow, fluids do not mix and behave much more predictably. Because of this, viscous flow is also referred to as laminar flow.

The Reynolds number can be determined as the ratio between inertial forces (which result from changes in the velocity) over the viscous forces (which can be thought of as the force required to push an object through the medium that it is in). Hence, it can be calculated as follows:

$$Re = \frac{\text{intertial forces}}{\text{viscous forces}} = \frac{\rho v L}{\eta}. \tag{1}$$

Here, ρ is the density of the medium (for instance, a fluid), v is the velocity of the moving object, L is the relevant size of the object (which may, for instance, depend on whether a non-symmetrical object is moving forwards or to the side), and η is the viscosity of the medium. The Reynolds number for *P. aeruginosa* in water can be calculated as follows:

$$\rho = 10^3 \text{ kg m}^3,\ v = 0.1\ \mu\text{m s}^{-1},\ L = 065\ \mu\text{m},\ \eta = 10^{-3} \text{ s kg}^{-1} \text{ m}^{-1} \implies Re \approx 6.5 \cdot 10^{-8}, \tag{2}$$

where the values of v and L were respectively taken from and based on Limoli et al. [2] by Analikwu [6] (and the estimation of L was carried out again in this project, to see if a similar value would be

found). Since $Re << 1$, the viscous forces should be considerably greater than the inertial forces in this model. Because of this, we can essentially relate the velocity of a particle to the force that is acting on it, instead of calculating the acceleration first. Hence, the Stokes law can be used to describe the relation between the force that is acting on a particle, and the velocity that the particle will have:

$$F_{\text{drag}||} = -2v\gamma_{||},$$

where v is the velocity of the particle and $\gamma_{||}$ is the drag coefficient for the drag force when the particle is moving in a direction that is parallel to the long axis of its body. Similar relations hold for rotation and orthogonal motion, but the drag coefficients are different for such motions (for instance, the drag coefficients will typically be bigger for orthogonal motion than it is for parallel motion). The drag coefficients that Analikwu provided in his report [6] are the following:

$$\gamma_{||} = \frac{1}{3}\frac{\zeta L}{\ln(AR) - 0.207 + \frac{0.98}{AR} - \frac{0.133}{AR^2}}, \tag{3}$$

$$\gamma_{\perp} = \frac{2}{3}\frac{\zeta L}{\ln(AR) + 0.839 + \frac{0.185}{AR} + \frac{0.233}{AR^2}}, \tag{4}$$

$$\gamma_{\text{rot}} = \frac{1}{18}\frac{\zeta L^3}{\ln(AR) - 0.662 + \frac{0.917}{AR} - \frac{0.050}{AR^3}}. \tag{5}$$

The above values were based by Analikwu [6] on the reference [17]. Within the model, all forces that act on the active particles representing *P. aeruginosa* and *S. aureus* are placed on the two spine endpoints. This is sufficient because the particles are modelled as rigid bodies.

3.2 Relevant Interactions

Within the model, three types of interactions occur: particle-particle adhesion, particle-particle repulsion, and particle-boundary interactions, the former two of which we shall collectively refer to as particle-particle interactions. The idea behind the particle-particle interactions is that there are three different regimes that may occur and which determine the nature of these interactions. Firstly, if two particles are too far away from each other to interact, they should neither adhere to one another nor repulse each other. Secondly, if the two particles come within reach of one another, they start to adhere to one another. The biological reason for this is that certain protein structures on the cell walls of the bacteria allow them to cling to one another, which leads to an adhesion force keeping them together. Thirdly, if the two particles come too close to one another, they start to repulse each other. The biological reason for this is that if two bacteria are pressed into one another, their cell walls and membranes start to deform. This deformation leads to a force pushing the bacteria away from each other: a repulsion force. Finally, since the interactions between the particles and the simulated boundaries are of much less importance than the interactions between the particles themselves in this model, the simplest possible option is chosen for the particle-boundary interactions, namely a repulsion force that acts on the particles if they get too close to the boundary.

Now, let us consider the individual interactions in more detail, starting with particle-particle adhesion. Adhesion between bacteria of the same species or strain is known as auto-aggregation, whereas adhesion between bacteria of different species or strains is known as co-aggregation. Both processes may occur in tandem, and both can lead to biofilm aggregation [18]. However, in this model, only auto-aggregation between particles of the same species is taken into consideration [6]. The

main reason that was provided for this choice, is that during the short-term interactions between *S. aureus* and *P. aeruginosa*, *P. aeruginosa* does not seem to significantly stick to *S. aureus*. While auto-aggregation occurs by different underlying mechanics in both species of bacteria, they can both be simplified as a distance-based attraction force. In the model, it is assumed that both of these forces have similar orders of magnitudes. Whether this is actually the case is irrelevant, firstly because no direct comparison between the two forces is made, and secondly because the model was made dimensionless.

The function that the particle-particle adhesion force is modelled as, is similar to the Lennard-Jones potential, which models simple, intramolecular interactions and is often given in the following form:

$$V(r) = 4\varepsilon \left(\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6} \right),$$

where r represents the distance between the interacting particles, ε is the so-called depth of the potential well (the energy required for the two particles to move away from each other), and σ is the distance at which the particle-particle potential energy V vanishes [19]. It models an adhesion force that turns into a repulsion force when the two particles get too close to each other [19]. The reason why we spend a little bit of time on the Lennard-Jones potential, is because it helps to understand where the adhesion force that is used in the model for *P. aeruginosa* and *S. aureus* comes from, and moreover has a very similar type of behaviour. The exact expression that was used to model said interactions between *P. aeruginosa* and *S. aureus* is shown below.

$$F_{\text{adh}}(r) = \varepsilon \left(\left(\frac{\sigma}{r}\right)^{5} - \left(\frac{\sigma}{r}\right)^{9} \right), \text{ for } r \geq \sigma, \tag{6}$$

where r is the distance between the spines of the two particles, ε is used for scaling the force, and σ is the distance between the two spines at which the two particles begin to experience an adhesive force. That is, σ is equal to the sum of the radii of the two interacting particles (as the size of the protruding structures that play a role in cell-to-cell adhesion are assumed to be much smaller than the cells themselves and can therefore be neglected). If we recall that force and potential are related to one another as

$$F(x) = -\frac{\mathrm{d}}{\mathrm{d}x} V(x), \tag{7}$$

we see just how closely this force resembles the force corresponding to the Lennard-Jones potential, with the main difference being that one of the two exponents is 9 instead of 11. As is discussed in Analikwu's internship report [6], the exponents for this adhesion force were chosen based on observations that were made by Rachel Los, who at the time worked on a similar particle-particle adhesion system. Furthermore, it is noted that this adhesion force corresponds to a so-called Mie potential, which is a generalisation of the Lennard-Jones potential, and is any potential that is of the following form [20]:

$$V(r) = C\varepsilon \left(\left(\frac{\sigma}{r}\right)^{n} - \left(\frac{\sigma}{r}\right)^{m} \right). \tag{8}$$

While the Lennard-Jones potential and, more generally, the Mie potential is typically used to describe interactions on the atomic level, the same principle can also be applied to other particles (such as bacteria) that adhere to each other at mid-close distances, and repulse each other at close distances. However, in the case of bacteria, adhesion and repulsion come forth from distinct physical processes, and as such, they should also be modelled differently. This is why equation (6) is only used for

$r \geq \sigma$, i.e., for distances at which adhesion would actually take place. Furthermore, it is important to note that the protein structures that are responsible for adhesion may touch at more than one point. As a result, the total adhesion force from one bacterium on another should be computed as the sum of the adhesion forces acting on all the points where the protein structures may adhere to one another. If it is assumed that these structures are homogeneously distributed over the cell wall and that they are much smaller than the cell wall itself, then we can approximate this sum by the following integral:

$$\vec{F^i}_{\text{adh}} = \int_0^1 \left(F_{\text{adh}}(|\vec{x}_s - \vec{x}_i|) \frac{\vec{x}_s - \vec{x}_i}{|\vec{x}_s - \vec{x}_i|} \right) \mathrm{d}s. \tag{9}$$

Here, F_{adh} is the adhesion force given in equation (6), $\vec{x}_i$ is the position vector of the point i where the adhesion force is evaluated, and $\vec{x}_s$ is the position vector for a point along the other particle's spine, which has been parameterised on $[0, L]$, where L is the length of the other particle's spine.

Next, let us consider the particle-particle repulsion. As was mentioned before, the mechanics that are responsible for adhesion and repulsion between bacteria are different from one another: the prior results from protein structures clinging to one another, the latter from the deformation of the cell walls and cell membranes. Since repulsion results from deformation, a simple way to model it is as a spring force, with spring constant K_{OV} (where OV stands for overlap). As when modelling the adhesion force between two particles, the closest distance between the spines of particles is used to calculate the total repulsion force. The reason for this is that the closest distance between the particles gives the greatest deformation, and hence also the greatest contribution to the repulsion force. As a result, the point where the distance between the particles is the smallest will also have the most significant contribution to the repulsion force.

Since the repulsion force is modelled as a spring force, Hooke's law (opposing force is compression times spring constant) can be used to calculate the exact force and direction due to this repulsion. Subsequently, as the two end points are the only two points in the particle to which force is applied in the model, the repulsion force acting on the particles is distributed over these two points depending on the relative distance to these points. This relative distance is given by s for endpoint A, and by $1 - s$ for endpoint B. As a result, the following repulsion forces are found:

$$\vec{F}_{\text{rep}} = K_{OV} \cdot (\vec{x}_1 - \vec{x}_2), \tag{10}$$

$$\vec{F}_{A,\text{rep}} = \vec{F}_{\text{rep}} \cdot s, \tag{11}$$

$$\vec{F}_{B,\text{rep}} = \vec{F}_{\text{rep}} \cdot (1 - s). \tag{12}$$

In the above equations, $\vec{F}_{\text{rep}}$ is the total repulsion force acting on the particle, as is given by Hooke's law. $\vec{x}_1$ represents the point of contact on one particle, and $\vec{x}_2$ represents the point of contact on the other particle. $\vec{F}_{A,\text{rep}}$ and $\vec{F}_{B,\text{rep}}$ are the repulsion forces acting on point A and on point B, respectively (note that their sum is equal to $\vec{F}_{\text{rep}}$, as it should). Finally, s represents the normalised distance between the point of contact along the spine and the spine end point A of particle 1 (so $s \in [0, 1]$). As such, if the closest point of contact is located at either of the two spine end points, then there won't be a repulsion force acting on the other end point. Finally, by Newton's third law, the repulsion force that is applied to particle 1 is also applied to particle 2, except in the opposite direction.

Now that we have covered both types of particle-particle interactions in the model, let us finish our discussion of the relevant interactions by considering the interactions between the particles

and the boundary. As we already touched upon at the start of this section, the particle-boundary interactions are far less important in this model than the particle-particle interactions. After all, the *S. aureus* colony is located near the center of the domain, and since we are primarily interested in the short-term interactions between *P. aeruginosa* and *S. aureus*, the most relevant interactions will typically not occur close to the boundary. As such, a simple form of interactions can be chosen for the interactions between the particles and the boundaries without any serious repercussions for the model. The type of interaction that was chosen for the particle-boundary interactions, is another repulsion term. The boundary is treated as a physical obstacle for the simulated bacteria, which means that if a simulated bacterium moves into the boundary, its cell wall and cell membrane will be deformed just as would be the case if it ran into another bacterium. As such, the spring constant that is chosen for the particle-boundary repulsion force is the same as the spring constant that was used for the particle-particle repulsion force, namely K_{OV}.

Whereas particles could collide into one another from any direction, the same does not hold for the boundary. Since the boundary is stationary, the only way for a particle to collide with it, is to move into it. Because the moving particles are (true) spherocylinders, this means that particles can only collide with the boundary with one end or with both (the latter only occurs if they happen to be aligned parallel to be boundary). It is impossible, however, for a particle to have the boundary collide with any other points on its body. This also makes it simpler to calculate the repulsion force: we calculate the distance from both end points to the boundary and subsequently use this distance to calculate the repulsion force that the particle experiences from the boundary. Thus, we find that the repulsion force from the boundary on a particle is given by

$$\vec{F}_{i,\text{bnd}} = -K_{OV} \cdot \vec{x}_i. \tag{13}$$

Here, i is either end point A or B of the particle and $\vec{F}_{i,\text{bnd}}$ is the repulsion force due to the interaction of point i with the boundary. $\vec{x}_i$ is the distance between the boundary and the closest point on the particle from end point i.

3.3 Bacterial Behaviour

In the previous subsection, we covered the interactions that may occur between the particles themselves and between the particles and the domain. In this subsection, we shall consider another important aspect of bacterial behaviour that comes into play in the model, namely chemotaxis.

As is discussed in more detail in the Literature Study section of this report, *P. aeruginosa* is a motile bacterium that, depending on the situation, can switch between different forms of movement. In the particular setting that this model intends to simulate, *P. aeruginosa* is initially moving as a swarm, until it detects a colony of *S. aureus* through the chemo-attractants that this colony produces [2]. At that point, *P. aeruginosa* switches to individual movement, with each *P. aeruginosa* bacterium individually using chemotaxis to make its way towards the *S. aureus* colony. That *P. aeruginosa* uses chemotaxis like this was one of the major findings from Limoli et al. [2].

Since chemotaxis plays a key role in the short-term interactions that occur between *P. aeruginosa* and *S. aureus*, it is little surprise that it is also a critical component in the model that is made to simulate these interactions. Chemotaxis is a complex process, however, and many of the finer details needn't be included in a model that seeks to simulate this type of behaviour. As such, some simplifications were made in the implementation of chemotaxis in the model.

The two simplifications that are made to the chemotaxis behaviour of *P. aeruginosa* involve the twitching motility of *P. aeruginosa* and the way in which *P. aeruginosa* senses the local concentration gradient of the chemo-attractant. Whereas *P. aeruginosa* uses its pili for the twitching motility that is part of its chemotaxis behaviour in real life, the spherocylinders that represent *P. aeruginosa* in the model do not possess pili. Instead, the spherocylinders propel themselves using a force that stems solely from chemotaxis, and the twitching fluctuations that are seen in live *P. aeruginosa* bacteria are omitted from the model. This first simplification is justified because the behaviour that the model is aimed at simulating (namely the surrounding and breaking apart of the *S. aureus* colony by *P. aeruginosa*) occurs at a much greater timescale than the individual twitches. Hence, even if they were to be included in the model, they would hardly be discernible. Furthermore, the exact source of the force does not matter for the simulated bacteria, and hence, the omission of the pili in favor of a force that stems purely from chemotaxis should not be problematic. The second simplification is more born from the fact that the way in which *P. aeruginosa* senses the local concentration gradient of chemo-attractants is not yet fully understood. And as such, it also cannot be modelled in complete detail. As with the twitching motility, the noise that accompanies *P. aeruginosa*'s chemotactile movement is ignored. Furthermore, it is assumed that the bacteria only make slow turns, instead of also turning rapidly.

3.4 Overdamped Motion

In subsection 3.1, we saw that the Reynolds number for *P. aeruginosa* in water is approximately equal to $6.5 \cdot 10^{-8}$. Since this is much smaller than 1, the viscous forces that *P. aeruginosa* experiences are far greater than the inertial forces that *P. aeruginosa* experiences in this setting. To see what this means precisely for the motion of the simulated particles, let us consider the following equation of motion:

$$m\frac{\mathrm{d}^2x}{\mathrm{d}t^2} = F - \gamma\frac{\mathrm{d}x}{\mathrm{d}t}. \tag{14}$$

Here, m is the mass of a particle, x is the location of a particle, F is the force that is applied to push the particle in a certain direction, and γ is the drag coefficient (and hence, $\gamma\frac{\mathrm{d}x}{\mathrm{d}t}$ is the drag force). Note that the right-hand side of the equation is the net force, written as the difference between the driving force and the drag force. Hence, this equation of motion corresponds with Newton's second law. If F is constant, then the terminal velocity of the particle is found as follows:

$$0 = F - \gamma\frac{\mathrm{d}x}{\mathrm{d}t} \implies \frac{\mathrm{d}x}{\mathrm{d}t} = F/\gamma. \tag{15}$$

Furthermore, using dimensional analysis, we can find that the time that it takes for a particle to reach this terminal velocity is proportional to m/γ. But since $Re << 1$, we also have that $m << \gamma$. And hence, the ratio m/γ is very small, which in turn means that it will take very little time for the particle to reach its terminal velocity. Since the terminal velocity is reached so quickly, the acceleration $\frac{\mathrm{d}^2x}{\mathrm{d}t^2}$ will be equal to zero for the vast majority of time. As such, we can simplify the equation of motion by setting the acceleration to zero. This yields the equation

$$F = \gamma\frac{\mathrm{d}x}{\mathrm{d}t}, \tag{16}$$

in which the acceleration is no longer taken into account and instead, the driving force acting on a particle is directly related to the velocity of the particle. This type of motion, which occurs when

the viscous forces greatly outweigh the inertial forces, is referred to as overdamped motion, and it is this type of motion that is used to simulate the movements of the active particles in Analikwu's [6] model.

Since we are using overdamped motion to describe the movements of the particles in the model, determining the new position of a moving particle is relatively straightforward. All that has to be done, is to identify all the forces that are acting on the particle, after which the particle's velocity can be immediately determined. This is then used to determine the particle's new position at the start of the next time step.

4 Simulation

The first goal of our simulations was to test the outcomes of Analikwu's model [6] and its implementation against the experimental data of Limoli et al. [2]. Doing so would have provided more insight into how accurate this model already was. Moreover, such a comparison may have served as an inspiration for further improvements to the model and its implementation and an indication of which possible changes were the most promising. Finally, it would have made for a good point of reference against which we could have gauged the accuracy of later versions of the model and its implementation.

With this goal in mind, we started our simulations with an unaltered version of Analikwu's implementation of his model. The implementation is in the programming language C++ and it consists of a main file that references numerous helper files. We ran the simulation on the hpc05 high-performance cluster of the Kavli Institute of Nanoscience Delft, as did Analikwu when he last used the code during his academic internship.

Unfortunately, running the code on the hpc05 cluster led to a segmentation fault. A segmentation fault is an error that typically occurs when a program either attempts to access a memory location that it is not allowed to access, or attempts to access a memory location in a way that is not allowed. For instance, a segmentation fault may occur when a program attempts to access the sixth entry of a list that only has five entries, or if it attempts to write to a read-only portion of memory. Whatever the cause of a segmentation fault, its effect is generally the immediate termination of the program. In the case of our simulations, this means that our simulations were aborted prematurely.

After repeatedly trying to run the same, unaltered code on the cluster, it became apparent that the program was always aborted after the same number of iterations. The code uses a pseudorandom number generator, and a seed was used to fix the sequence of numbers that it generated. Strikingly, changing the seed also changed the number of iterations that the simulation could go through before a segmentation fault was generated.

Another important remark concerning the segmentation fault, is that Analikwu did not experience any segmentation faults while running the code during his internship project. While Analikwu ran the code on the same cluster as the one that we used during our project, the cluster may have run on a different version of software during his project than it did during ours. Otherwise, it remains unclear what, precisely, caused the segmentation fault when the code was run during this project, and why it did happen before.

One of the first things that we intended to do after testing Analikwu's model and code against the experimental data from Limoli et al. [2], was changing the starting positions of *P. aeruginosa*and repeating the same test. For a notable difference between the current simulations and the experiments with live bacteria, is that in the simulation, *P. aeruginosa* starts out randomly distributed outside of the *S. aureus* colony, whereas in the experiments, *P. aeruginosa* starts grouped closely together in a so-called raft or swarm. This experimental setting appears to be more realistic, as it is believed that the short-term interactions between *P. aeruginosa* and *S. aureus* start when a traveling swarm of *P. aeruginosa* discovers an already established *S. aureus* colony, at which point the *P. aeruginosa* bacteria start to approach the *S. aureus* colony individually, and the *S. aureus* swarm breaks apart. As such, it was of interest to see if *P. aeruginosa* would also successfully surround the *S. aureus* colony if they started out grouped closely together, or if they would stick to one side of the colony. If this was not the case, then it may have been that in the simulations, the chemo-attractant is not

broken up quickly enough by *P. aeruginosa*, or that too much of it is being released by *S. aureus*.

Whereas completing our check of Analikwu's model and implementation against Limoli et al.'s [2] experimental data was impossible as long as the segmentation fault remained unsolved, it was possible to attempt to change the starting positions of *P. aeruginosa*. For while the segmentation fault prevented a full simulation from being carried out, it was possible to carry out a shorter simulation, such as one that lasted for one hundred iterations, instead of the original one thousand iterations. And while such a shorter simulation was not useful for properly testing the results of the simulation against those of live experiments, this was sufficient to check whether the starting positions of *P. aeruginosa* could be successfully altered, and perhaps also to see if the *P. aeruginosa* swarm would at least start to break apart as *P. aeruginosa* switched from group movement to individual movement.

This is when we encountered a second problem with the code, namely that the starting positions of *P. aeruginosa* did not appear to be determined as we previously assumed. As was stated before, the code that was used for the simulations consists of a main file and numerous helper files. One of these files, init_particles.hpp, was believed by us to determine the starting positions of all *P. aeruginosa* and *S. aureus* bacteria in the simulation. As a first test, we attempted to change the part if this file that determined the starting positions of *P. aeruginosa* in such a way that *P. aeruginosa* could only start on one side of the colony. However, when the simulation was run with this altered starting condition, *P. aeruginosa* still seemed to be able to start anywhere outside of the colony. Several attempts to get *P. aeruginosa* to start on one side all proved unsuccessful, which led to the conclusion that perhaps, there were also other parts of the code that somehow influenced the starting positions of *P. aeruginosa*. Since the starting positions of *P. aeruginosa* could not be successfully changed, it was also impossible to verify whether the simulation could reproduce the behaviour of a *P. aeruginosa* swarm breaking up upon first detecting a *S. aureus* colony as the individual *P. aeruginosa* bacteria switch from collective movement to individual movement.

5 Results

Even though the full simulation could not be run as a result of the segmentation fault that, unfortunately, has remained unresolved, shorter simulations could be completed. In Figure 3, some snapshots are displayed of a simulation that ran through one hundred iterations, instead of the intended one thousand iterations. The setup that was used for this simulation is identical to the one that we presume was last used by Analikwu [6] (based on the code that we downloaded), except for two changes. Firstly, as we mentioned before, we reduced the number of iteration steps from one thousand to one hundred. Secondly, we changed the value of a Boolean that determines whether the diffusion data is saved during the simulation from false to true. The reason why this option was initially turned off seems to be that this helps to speed up the simulation, but we turned it on because we were also interested in the diffusion data. While this second change does not affect the outcome of the simulation (it only changes which kinds of data are saved), we still mention it here for the sake of completeness. Both of the two changes were made in the params.hpp file.

In Figure 3, we can see that *P. aeruginosa* starts distributed randomly outside of the *S. aureus* colony. Over the course of the simulation, *P. aeruginosa* follows the gradient of chemo-attractants which is secreted by *S. aureus* and which diffuses throughout the domain over time. The colony is surrounded and ultimately infiltrated by *P. aeruginosa*. However, because the simulation only lasts for such a limited duration, we cannot actually see the colony being fully dismantled, nor is an end state in which *P. aeruginosa* and *S. aureus* are mixed achieved.

Since we could not successfully adjust the starting positions of *P. aeruginosa* (such as by trying to make *P. aeruginosa* exclusively start on one side of the domain), the outcomes of simulations in which we only changed these starting conditions in the file init_particles.hpp are the same as those shown in Figure 3. In Appendix B, we have included the relevant part of the file init_particles.hpp, with both the original code and some of our attempts to change the starting positions of *P. aeruginosa*.

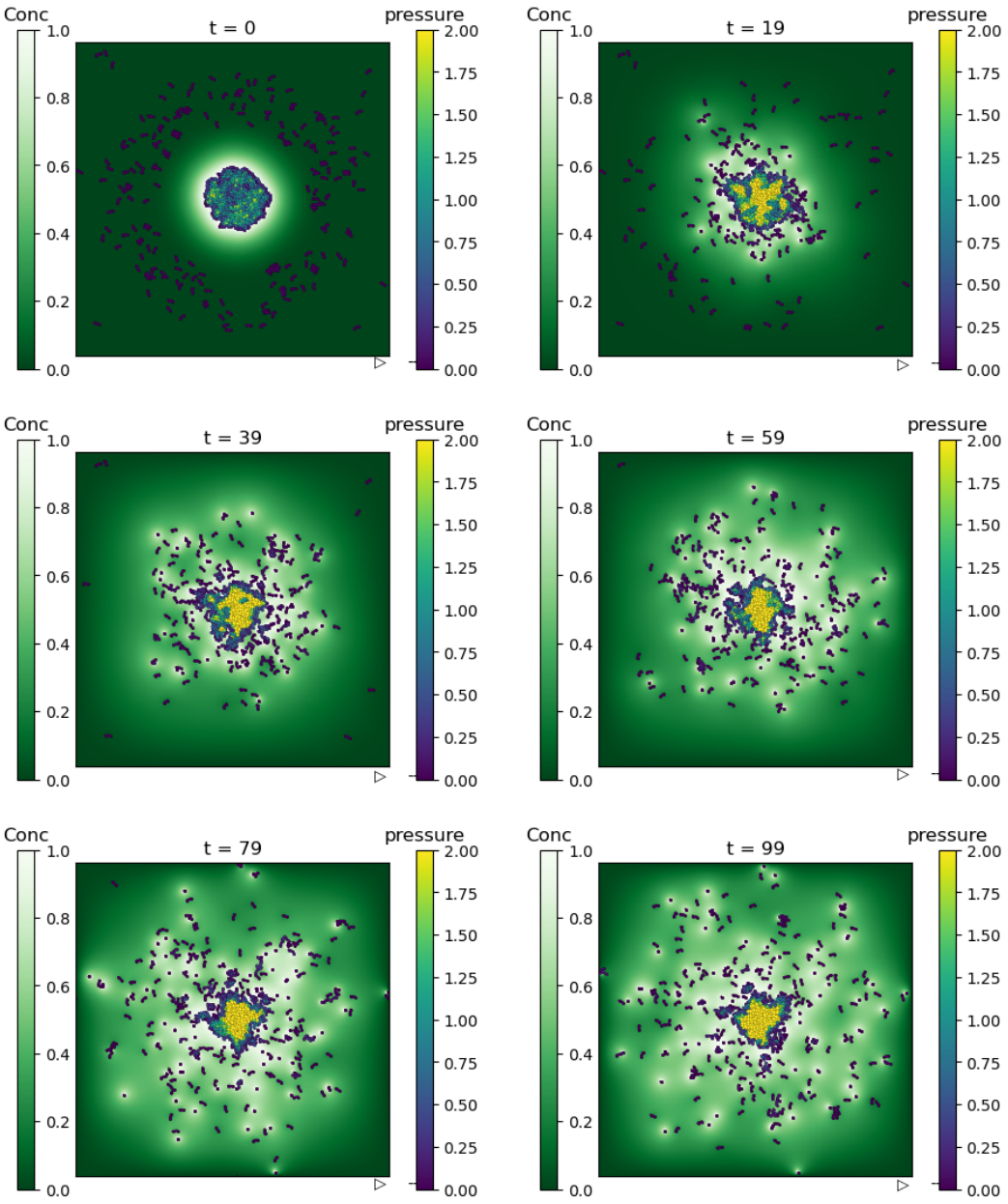

Figure 3: Snapshots of the simulation, after zero, nineteen, thirty nine, fifty nine, seventy nine and ninety nine iteration steps. *P. aeruginosa* starts randomly distributed outside of the *S. aureus* colony, follows the gradient of chemo-attractant towards the colony, and manages to somewhat penetrate the initial outer layer of the colony.

6 Conclusion and Discussion

In this project, we sought to use an existing model and its implementation to study short-term interactions between *P. aeruginosa* and *S. aureus*. The intention was to first test this model and its implementation against experimental data from Limoli et al. [2] and to subsequently implement some changes to the model, which would hopefully make it even more true to the experiments on which it is based. Finally, the thus obtained version of the model and its implementation would be used to study the short-term interactions that occur when *P. aeruginosa* first encounters a *S. aureus* colony.

As is described in the Simulation section and in the Results section, problems were encountered while attempting to run the code that prevented most of the original goals of this project from being achieved. These problems are a segmentation fault that severely limited the number of iterations that the simulation could go through before being aborted and a strange problem concerning the way in which the possible initial positions of *P. aeruginosa* are determined. As long as these two problems remain unsolved, the current implementation of Analikwu's [6] model cannot be used to properly study the short-term interactions between *P. aeruginosa* and *S. aureus*. However, the model itself seems to be well-grounded, as our discussion of it in the Model section hopefully illustrates. The one part of the model that we initially had some doubts about was the expression for the adhesion force between simulated particles, but after learning more about Lennard-Jones and Mie potentials, this expression made much more sense to us.

Because of the above, our recommendation for a future project with the aim of studying the short-term interactions between *S. aureus* and *P. aeruginosa* would be the following. Since the model appears to be sound but the implementation has, at least for us, yielded some difficult problems, it may be worthwhile to consider making a new implementation based on this same model. The primary advantage of this option, as opposed to trying to solve the problems that were encountered with the current implementation of the model, is that with a brand new code and cautious documentation, if unexpected problems such as segmentation faults are to arise, finding their cause would be easier than with an already written code. While writing new code, the current implementation could still be preserved, and well-understood parts of it could serve as inspiration for this new implementation. This way, the work that was put into the current implementation will still remain relevant even if this implementation is ultimately replaced by a new one.

Interactions between *P. aeruginosa* and *S. aureus* remain a relevant subject of study as a better understanding of these interactions will likely lead to better prospects for preventing or treating harmful infections that involve these bacteria. Both simulations and experiments with live bacteria may contribute to such an understanding. The model that was described in Analikwu's internship report [6] and which was studied in this project may serve as a good starting point for simulating short-term *P. aeruginosa* and *S. aureus* interactions or, if adapted, possibly also for interactions between other species of bacteria.

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Appendix A

In this appendix, we include the planning that was made for this project when we first started it. While this planning could ultimately not be adhered to, it was still deemed worthwhile to put it in an appendix.

- Thought will need to be given to possible factors that might make worthwhile additions to the model. With a thorough understanding of the existing model, one can consider what things were omitted (e.g. the friction force) and ask oneself whether these omissions have a significant effect on the results that this model yields (approx. 40 hours).
- Once a list of possible factors of interest has been made, literature research will be needed to get a better idea of which factors are actually important enough to be added to the model. Obviously, there is no truly objective way of making such a decision, but it seems reasonable to take the magnitude of possibly missing parameters and the amount of time that they would add to iterations of the simulation into consideration. During this literature study, more factors that could be of interest may or may not come to light. If they do, they will be treated like those that were already found during the previous step (approx. 100 hours).
- After it has been decided which new components will be included in the model, it is time to start making actual adjustments to the model. This step can actually be divided into two smaller steps: the model needs to be revised on paper, and subsequently, these modifications will need to be added to the C++ implementation of the model. Before this second substep can be made, the author will also need to achieve a good understanding of the existing C++ implementation of the model (approx. 200 hours).
- Next, the simulation will have to be run on the cluster and the results will have to be examined. Most likely, modifications will have to be made to the C++ implementation and possibly also to the model itself. Maybe it will become apparent that some components that were added to the model weren't that relevant after all. Or maybe it will become evident that mistakes were made while modifying the model or its C++ implementation, which will obviously need to be corrected. In order to draw such conclusions, the results of the simulations that the updated version of the model will yield need to be compared to the results of the original version of the model, and experimental data that may be available (approx. 150 hours).
- Eventually, final decisions will be made concerning which extra components are or are not used in the final version of the model that will be used for this project. Once this decision has been made and once the model and its implementation have been successfully altered to reflect these alterations, the second and third goal of the project will have been fulfilled, and progress will begin to be made towards fulfilling the first goal as well. Before that can be done, however, yet another decision will have to be made. Namely: which facets of the hostile interactions between *P. aeruginosa* and *S. aureus* do we want to study in the most detail, and how can that best be done? Ideally, these decisions are made before we arrive at this point in the project, as they may also influence some of the decisions that are made earlier on (approx. 150 hours).
- The results of the simulation will be analysed (both qualitatively and quantitatively) to answer the questions that we shall have decided that we wish to answer concerning the interactions between *S. aureus* and *P. aeruginosa*. Again, these questions will have to be formulated

beforehand, but this hasn't been completely done at the moment of this writing (approx. 150 hours).

- A report will need to be written and, ultimately, a presentation will need to be given (approx. 100 hours).

Appendix B

In this appendix, we include the part of the init_particles.hpp file which we believe is used to determine the starting positions of the simulated *P. aeruginosa* bacteria. The comments containing the initials JvdP were made by us in order to clarify which parts of the original code are being replaced.

```
inline void set_start_coordinates_box(double& x0, double& y0, double& z0,
    const double& length, const int& id){
    // assigning initial coordinates so that particle types are put on
    //   different sides of the box
    // TODO:(klb) distance should be proportional to particle proportion,
    //not just half or 1/4?
    int numParticlesType1 = (int)(Parameters::params->NUM_PART *
        Parameters::params->PART_PROPOR);
    if (id < numParticlesType1) { // particle type 1
        double r = (Parameters::params->SPACE/3) * sqrt(fRand(0,1));
        // get random radius
        double theta = fRand(0,1) * 2 * M_PI;
        x0 = 0 + r * cos(theta); // centred around 0 in x
        y0 = 0 + r * sin(theta); // centred around 0 in y
    }
    else if (id >= numParticlesType1) { // particle type 2
        //if (id < numParticlesType1 + (Parameters::params->NUM_PART
        // - numParticlesType1) / 2) {
        // // taking only first half of type 2 particles for this side
        //    x0 = fRand(-(Parameters::params->SPACE - length / 2),
        // -3*(Parameters::params->SPACE - length / 2)/4);
        // // in left eighth of box
        //}
        //else if (id <= Parameters::params->NUM_PART) {
        // //taking second half of type 2 particles for other side
            //x0 = fRand(3*(Parameters::params->SPACE - length / 2)/4,
            // Parameters::params->SPACE - length / 2);
            // // in right eighth of box
        //    x0 = fRand(-(Parameters::params->SPACE - length / 2),
        // -3*(Parameters::params->SPACE - length / 2)/4);
        // // JvdP: this replaces lines 40 through 42; idea is to put all
        // the p.a. particles on one side
        //}
        x0 = fRand(-(Parameters::params->SPACE - length / 2), -3*
            (Parameters::params->SPACE - length / 2)/4);
        // in left eighth of box JvdP: this replaces lines 31 through 47
        y0 = fRand(-(Parameters::params->SPACE - length / 2),
            Parameters::params->SPACE - length / 2);
    }
    if (DIM == 3){
```

```
        z0 = fRand(-(Parameters::params->SPACE - length/2),
            Parameters::params->SPACE - length/2);
    }
}
```