School of Physics and Astronomy

Modelling the growth of microbial populations in heterogeneous antimicrobial concentrations First Year Report and Literature Review

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Abstract

Spatial gradients of antimicrobial chemicals are ubiquitous in both modern medicine and industry, from bacterial biofilms caused by infection and the application of antibiotics, to marine biofouling and the leaching of antimicrobials from specially designed shipping hull coatings. This project aims to use stochastic computer modelling techniques to investigate how the presence of antimicrobial gradients can affect the growth and proliferation of microbial populations in order to better understand how to minimise the formation and persistence of microbial biofilms.

Signature: Date:

Supervisor: Dr. Rosalind Allen

Contents

1 Background

Since their discovery in the early 1900s [1], antibiotics have shaped modern medicine, and indeed modern society as a whole. Yet despite their prevalence, with over 260,000,000 courses of antibiotics prescribed in the USA alone each year [2], very little is still known about the actual underlying pharmacodynamics, i.e., how these chemicals actually regulate the growth and death of bacteria. This lack of understanding is becoming increasingly significant with the rising emergence of antibiotic resistance. There were over 58,000 deaths in newborns under the age of a year in India in 2013 due to drug-resistant strains of bacteria [3], with experts predicting that the number of deaths from antibiotic-resistant bacteria will number in the millions by 2050 [4].

Part of the work involved in this PhD aims to shed further light on how the application of antibiotics causes bacterial populations to evolve and proliferate over time. In particular, in the cases of when the applied antibiotic concentration has the form of a gradient. A considerable portion of antibiotic research has been performed under laboratory conditions with constant, uniform antibiotic concentration [5], however it has now become clear that the effects of spatial heterogeneity may be a major factor in the emergence of resistance and the efficacy of drugs [6]. While these concentration gradients can simply arise due to scenarios such as diffusion throughout body tissue [7], the scenario which is most pertinent to this project is that of antibiotic concentration gradients in biofilms.

Biofilms arise when microbial organisms adhere irreversibly to a surface and then begin to secrete various polymers, which further aid in surface and inter-microbial attachment [8], so that the microbial population create a "slimey" surface. These structures are particularly problematic as bacteria found in biofilms are innately more resistant to applied antimicrobials and it is also difficult to achieve sufficient drug penetration throughout the biofilm to adequately curtail microbial growth, which leads to an increase in the persistence of infections [9].

The ability to inhibit and even prevent biofilm growth and formation not only has a multitude of medical applications, but has industrial applications as well. In the shipping industry it is estimated that around 10%, up to even 45%, of all fuel consumption of large shipping vessels arises from overcoming the hydrodynamic drag caused by algal and other microbial biofilm formation on ship hulls below the water level [10]. This not only has economic influences, but also major environmental implications. When compared to other nations, the shipping industry is the 7th largest producer of CO_2 on the planet [11]. Therefore, research into how these marine biofilms form and develop is incredibly important. To this end, this PhD also involves collaboration with AkzoNobel, an industrial paint company, which will entail modelling the formation of biofilms on the exterior of ship hulls in the presence of antimicrobial chemical gradients.

Recently, several physical methods of reducing marine biofouling have been developed, which range from physical coatings that inhibit microbial attachment due to their topography [12] to usage of ultraviolet radiation [13]. However, the most widespread technique is that of anti-microbial paints which are applied to the boat hulls and then leach various antimicrobial compounds over time which inhibit biofouling [14]. It is this latter method which is of relevant interest to this project, as it has analogies with the application of antibiotics to bacterial biofilms.

This project will involve creating computer simulations of a range of scenarios where

microbial populations experience gradients of antimicrobial chemicals, and will investigate how these populations develop over time. Work so far has included differing types of growth-rate dependent antibiotics and populations with heterogeneous species and resistance distributions.

2 Review of Background Bibliography

2.1 Antibiotic gradients

The issue of antibiotic resistance is one of the key issues plaguing modern science as of today. As such, the field commands a large volume of dedicated research utilising a wide range of methods. Ranging from experimental to theoretical techniques including both modelling and more in-depth simulation [15, 16, 17]. Current research is investigating a wide variety of factors which contribute to the development of resistances. From mutational path lengths [18] to the synergistic effects of various antibiotics [19]. However, the majority of these studies, including all studies referenced so far in this section, are performed with constant and uniform concentrations of antibiotics.

While this situation tends to be more convenient for idealised in vivo experiments, many real-world in vivo scenarios do not have these conditions. Many naturally occurring structures which the drugs are intended to target, tend to not allow the drugs to fully permeate throughout the region, creating gradients where the drug concentration can vary noticeably over space. These gradients can arise in a variety of situations, from tissue [20] to bacterial biofilms [21].

It is only in recent years that the effects of these spatial heterogeneities have been considered a serious influence on the evolution of resistance. In fact, models have been constructed which predict that antimicrobial gradients can actually accelerate the evolution of resistance [22]. These models were inspired by an experiment conducted by Zhang et al. [6]. In which they constructed a microfluidic device involving an array of several interconnected microhabitats with an antibiotic gradient of ciprofloxacin. This gradient ranged from no discernible antibiotic concentration at the top of the array to a concentration of 10μg/ml at the bottom. This concentration is around 200 times the minimum inhibitory concentration (MIC, i.e., the minimum necessary concentration of an antibiotic required to prevent observable growth of bacteria) of ciprofloxacin [23].

The array was then inoculated in the central microhabitats with around 10^6 wild type *E. coli*. Chemotaxis due to nutrient consumption then drove the bacteria towards the perimeter microhabitats. Once resistant mutants had fixed, they then spread and propagated throughout the array, as shown in Figure 1.

To confirm that it was indeed the gradient which allowed for this enhanced development of resistance, Zhang et al. conducted a range of further experiments. Firstly they eliminated the gradient by including ciprofloxacin at both ends of the array. This uniform antibiotic concentration resulted in no growth from the inoculated wild-type *E. coli*, as can be seen in Figure 2.

Zhang et al. then performed the experiment in a 96 well plate with a gradient present, but with the microhabitats now disconnected from one another, with discrete antibiotic concentrations in each well, ranging from low to high concentrations as in the previous

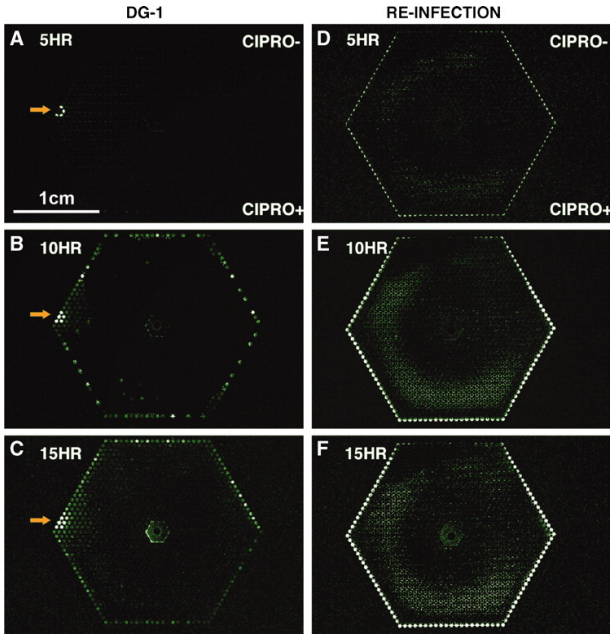


Figure 1: The proliferation of the bacterial population when exposed to an antibiotic gradient. The LHS shows the development of the population after the initial inoculation, and the RHS shows the development of an identical slide which was inoculated with the resistant mutants. Zhang et al., 2011

array. This also resulted in no resistance being developed, as the growth of the bacterial colonies simply decreased as the concentration of ciprofloxacin increased, thereby implying that bacterial motility across the gradients is what is key to the emergence of resistance.

As can be seen in Figure 3, even the most diluted populations developed resistance when exposed to the antibiotic gradient. These results heavily support the proposal that the source of emergent resistance is due to de novo mutations and not from preexisting mutants distributed amongst the wild-type. Zhang et al. offered the following explanation as to why antibiotic gradients allow for such prevalent opportunities for resistance to emerge.

> A spatially complex environment may lead to an enhanced rate of evolution for two reasons. First, if a stress gradient is imposed on a connected network of populations, and if a mutant acquires some resistance to the local stress, the relative fitness of the mutant is increased if it moves to join a population exposed to even higher stress. Second, because there are fewer individuals in the region of higher stress, the mutant can fix more quickly in the smaller population.
>
> (Zhang et al., 2011)

2.2 Modelling the effects of antibiotic gradients

As can be seen, there is experimental evidence supporting the notion that non-uniform drug distributions can accelerate the emergence of resistant organisms. To gain a better understanding of whether this is always the case and also how the mutants emerge and

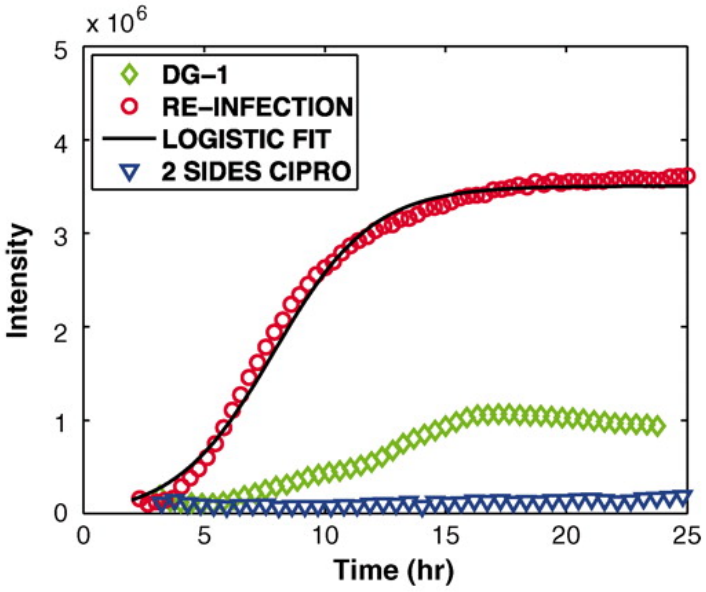


Figure 2: The summed growth over the entire array for various scenarios. The green diamonds are the initial experiment where the wild-type *E. coli* were placed in the antibiotic gradient. The red circles are the resistant mutants which were then used to re-inoculate an identically set up array. The blue triangles are the wild-type exposed to a uniform antibiotic concentration. Zhang et al., 2011

propagate, Greulich et al. (2012) [24] constructed a simple computational model which investigated how a bacterial population evolved along pathways in genotype space when exposed to both uniform and non-uniform antibiotic concentrations. It is this model which has also formed the basis for the other models constructed in this project, although the work conducted with these models has focused less on evolution and more on colonisation along the antimicrobial gradient.

The model consisted of L microhabitats interconnected in series with one another. Each microhabitat had a concentration of antibiotic c_i (where i is the index of the microhabitat), and a carrying capacity K. If the number of bacteria in the microhabitat was $\geq K$, then no bacterial growth could occur in this microhabitat until its population decreased below the carrying capacity.

Each bacterium had a numeric genotype m, which they could mutate between with a probability μ and had a maximum value of M. This genotype described the level of resistance the bacteria had to the antibiotic, with m_2 being more resistant than m_1 and so on. At each step in the simulation each bacteria could die or move to an adjacent microhabitat at constant rates d and b, or replicate at a rate given by

$$R_{rep} = \phi_m(c_i)(1 - \frac{N_i}{K}). \tag{1}$$

Where N_i is the total number of bacteria present in microhabitat i and $\phi_m(c_i)$ is the genotype and antibiotic dependent growth rate. This value decreases until the MIC for that particular genotype (β_m) is reached, after which that bacteria cannot replicate. The simulation is initialised by placing K bacteria of genotype $m = 1$ in the first microhabitat and then allowing them to proliferate throughout the system. To illustrate the effects of

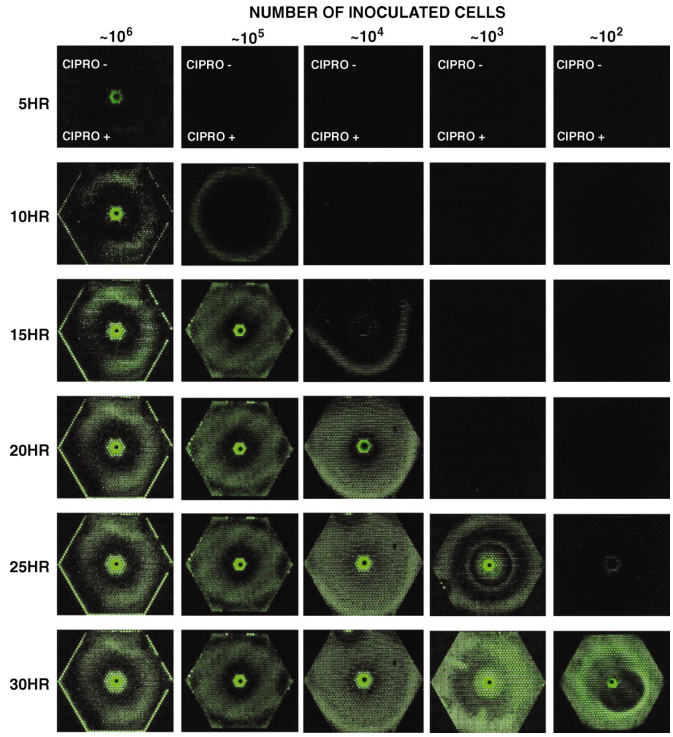


Figure 3: Bacterial growth over time in an antibiotic gradient up to 1 μg/ml of ciprofloxacin for various initial bacterial population sizes. The initial sizes range from the order of 10^6 to as low as 10^2. It can be seen that even at the lowest starting densities, resistance still emerges. Thus supporting the argument that resistance emerges due to de novo mutation and not preexisting mutants. Zhang et al., 2011

the antibiotic gradient, the simulations were performed under two different conditions; with a uniform antibiotic concentration ($c_i = c$) and with an exponentially increasing antibiotic concentration

$$c_i = \exp(\alpha i) - 1. \tag{2}$$

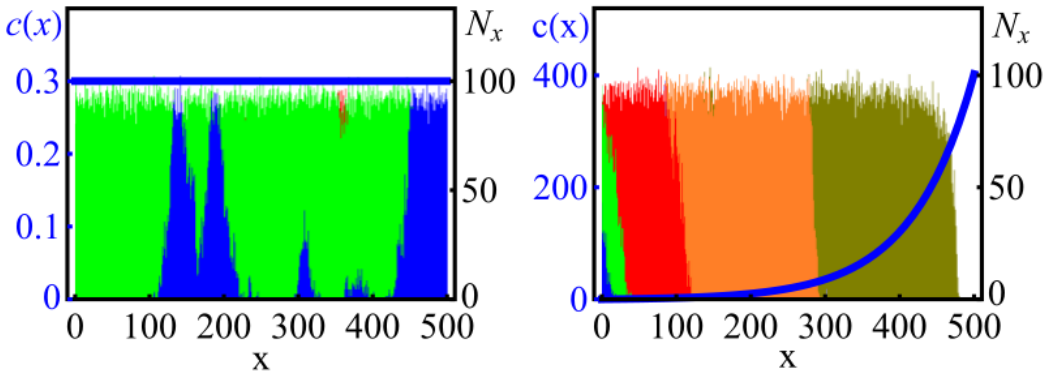


Figure 4: Distributions of bacterial genotypes throughout the series of interconnected microhabitats. The thick blue line is the concentration of antibiotic per microhabitat, and the colours represent the distributions of genotypes. $m = 1$ is blue, $m = 2$ green, with $m = 3, 4, 5$ shown by red, orange and olive respectively. For the uniform concentration, any mutations are sporadic and randomly distributed, however the exponential gradient shows clear emergence of resistance in a consistent manner. Greulich et al., 2012.

Once again, as shown in Figure 4, the dynamics of how evolution emerges varies greatly between the uniform and non-uniform drug distributions. For the uniform case, mutations arise sporadically and proliferate randomly, with the system as a whole generally evolving from one genotype to the other. However when exposed to the gradient, the genotypes tend to form "stationary fronts", with resistant mutants emerging at the tip of the colony, then quickly spreading to fill the remaining space until the MIC for the current advancing genotype is reached, at which point the process repeats itself. As Figure 4 illustrates, where the snapshots were taken after an equal amount of time had passed, the presence of the gradient causes the emergence of "population waves" of increasing resistance, which greatly reduces the time taken for resistance to emerge.

2.3 Growth rate dependency

It has long been known that the growth rate of bacteria affects their susceptibility, with slow growing or metabolically inactive bacteria having a much higher tolerance than their fast growing counterparts [25]. However Greulich et al., 2015 [26] proposed that this might not exclusively be the case, and instead that certain ribosome-targeting antibiotics are more effective against fast-growing or slow-growing cells depending on the mechanism which the antibiotic uses to bind to the target cell's ribosomes.

For example, the antibiotic tetracycline binds reversibly to the bacteria's ribosomes and is more effective when the bacteria are in a fast-growing state. In contrast, streptomycin binds irreversibly tot he bacterial ribosomes and is more suited for bacteria which are less metabolically active. It's been shown that the ribosome content of a cell correlates with the cell's growth rate [27], with fast-growing cells dedicating more of their resources to ribosome production than their slow-growing counterparts [28].

Although there are numerous factors which can affect a cell's growth rate, from oxygen limitation [29] to pH levels [30], the main environmental influence is that of nutrient availability, which was the variable Greulich et al. varied in order to alter the growth

rates of their bacteria. The behaviour of the nutrient-limited growth rate can be described by the Monod equation [31]

$$\mu(S) = \mu_{\max} \frac{S}{K' + S}. \tag{3}$$

Here $\mu(S)$ is the current growth rate of the bacteria in this environment, $\mu_{\max}$ is the maximum growth rate of the bacteria, S is a measure of the nutrient concentration present and K' is a constant which represents the nutrient concentration at which growth is half-maximal. As described this equation, the growth rate of bacteria decreases non-linearly as nutrient availability decreases.

2.4 Biofilms

Bacteria which aggregate onto a surface forming a biofilm have been shown to be much harder to eradicate than their free-flowing planktonic bacterial counterparts [32]. In fact, biofilm colonies can exhibit resistances to antibiotics 10-1000 times greater than their individual complements [33], making treatment plans an immensely more complicated affair. Whilst it is still relatively unclear as to what exactly affords biofilms these defences, experiments conducted in laboratory conditions heavily suggest that it is indeed the biofilm itself which possesses these qualities, rather than any external influence from the host or environment [34]. Biofilms can form on almost any surface where there is a consistent influx of bacteria, algae or other microorganisms, ranging from medical devices [35] to the exterior of ship hulls [36].

The formation of biofilms is a complex and little-understood process. Whilst it was originally thought that biofilms were formed by single organisms attaching to a surface, leading to the formation of micro-colonies and then 3D structures [37], there is also now evidence that pre-existing bacterial aggregates can also seed the formation of biofilms [38], the differing shape and composition of which in turn can influence the final established biofilm [39], making the developmental paths of biofilms increasingly hard to predict.

Regardless of how they come to be, biofilms have a range of features which distinguish them apart from a simple group of bacteria in close proximity to one another. One key trait that marks them apart is that of the inter-cellular matrix. When bacteria or algae aggregate into a biofilm, they secrete a variety of macromolecules such as exopolysaccharides, DNA and protein fragments [40], which allows the microorganisms involved to adhere to both surfaces and one another.

This matrix forms just one of the biofilm's innate defence mechanisms. Although its composition is mainly water, perhaps up to 97% in some cases [41], the organic material present in it may act as a diffusion barrier to the applied antibiotic, preventing it from fully penetrating the biofilm. On average, the diffusion coefficient of an administered antibiotic in a biofilm matrix is roughly 40% that in pure water [42]. However it is not thought that this reduction in antibiotic mobility alone is enough to explain the levels of resistance which biofilms exhibit.

Another feature of biofilms which may explain their durability is that of the formation of microenvironments within their structure [43]. Factors such as oxygen limitation and poor nutrient diffusion throughout the biofilm matrix creates regions where the growth rate is substantially reduced compared to the outer sections. As mentioned previously,

the efficacy of many antibiotics are dependent on growth rate [44], this therefore causes many antibiotics to only be effective against specific sections of a biofilm, rather than the structure as a whole.

An example of which can be see in Figure 5, taken from an experiment by Pamp et al., 2008 [45]. Here they applied the antibiotic colistin to a biofilm of *P. aeruginosa* bacteria to show that it is bacterial metabolic activity which contributes to the tolerance of colistin. As shown in Figure 5, the metabolically active cells on the periphery of the biofilm are able to tolerate the application of the antibiotic. However the metabolically inactive cells towards the centre are unable to develop this tolerance, resulting in their death. These multitude of factors contributing to the persistence of biofilms make further research into how they develop over time essential. As despite their prevalence, surprisingly little is still known about their inner workings.

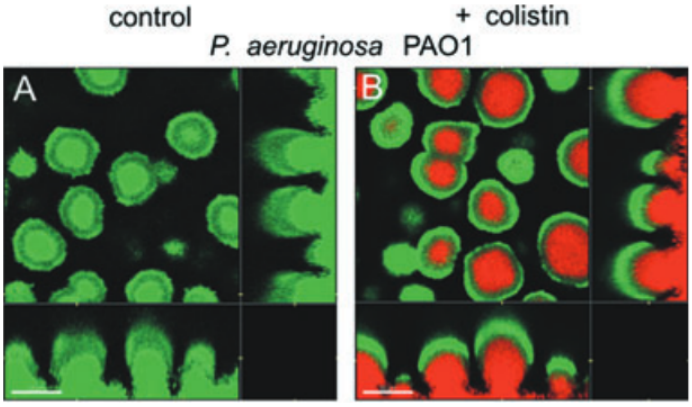


Figure 5: The effects of the antibiotic colistin on a biofilm consisting of *P. aeruginosa* bacteria. Colistin is an antibiotic which targets slow-growing, metabolically inactive cells, as can be seen, it is therefore more effective against the internal regions of the biofilm. Live cells are shown in green, dead cells in red.

2.5 Antimicrobial coatings

3 Progress to Date

3.1 Replication of Greulich et al.

The work undertaken so far in this PhD has comprised computer simulations investigating how microbial populations colonise along spatial concentration gradients of antimicrobial chemicals. The progress made up until now in this project can be organised into several sections. The initial few weeks of the project were spent replicating the results and techniques found in Greulich et al., 2012 [24]. Discussion was had on the subject of which algorithm would be optimal for updating the system over time.

Algorithms such as Gillespie [46] and τ-leaping [47] were proposed, but eventually a simple Monte-Carlo style selection process as detailed in the supplementary material of Greulich et al., was decided upon. The algorithm operated by selecting an individual cell at random and then summing together the migration, death and replication rates of the

cell ($R_{mig}, R_{dea}, R_{rep}$), as detailed in section 2.2. A random number r was then chosen between 0 and $R_{\max}$, where $R_{\max} \geq R_{mig} + R_{dea} + R_{rep}$. Depending on the value of r, the cell would then either migrate, die, replicate or do nothing. The time elapsed in the simulation was then increased by an increment $\Delta t = 1/(NR_{max})$.

This method was found to be faster than the standard Gillespie algorithm as the number of calculations required for each iteration is small, and the rate at which an event occurs (i.e., the likelihood of $r < R_{mig} + R_{dea} + R_{rep}$) is relatively high, and on average around 25%. The only downside when compared to Gillespie is that the time elapsed is discretised into increments of Δt rather than a continuous distribution. However the timescales that these simulations are performed for are much larger than Δt, so this is relatively inconsequential.

The purpose of this was mainly to establish useful methods for future projects, intended to increase fluency and familiarity in the techniques and background theory required for the modelling of biological systems. The results obtained from this body of work were rough, proof-of-concept illustrations, rather than the precise quantitative results obtained in the actual paper, intended more for comparative purposes to ensure the constructed model worked as intended.

3.2 Growth rate-dependant antibiotics

The majority of the time spent on this project has been on the modelling of growth rate-dependant antibiotics, based on the 2015 paper by Greulich et al. [26] as mentioned in section 2.3. The foundation of this model was heavily borrowed from the one created in the previous section, but with the key addition of nutrients, which were used to modify the growth rate in lieu of the carrying capacity factor from the previous model. Two simple functional forms for the MICs of fast-growing bacteria targeting antibiotics (FGBTA) and slow-growing bacteria targeting antibiotics (SGBTA) were constructed as follows: for the fast-growth targeting antibiotics;

$$\beta_{FGT} = 10 - 9\frac{\mu(S)}{\mu_{\max}} \tag{4}$$

and for the slow-growth targeting antibiotics;

$$\beta_{SGT} = 1 + 9\frac{\mu(S)}{\mu_{\max}}. \tag{5}$$

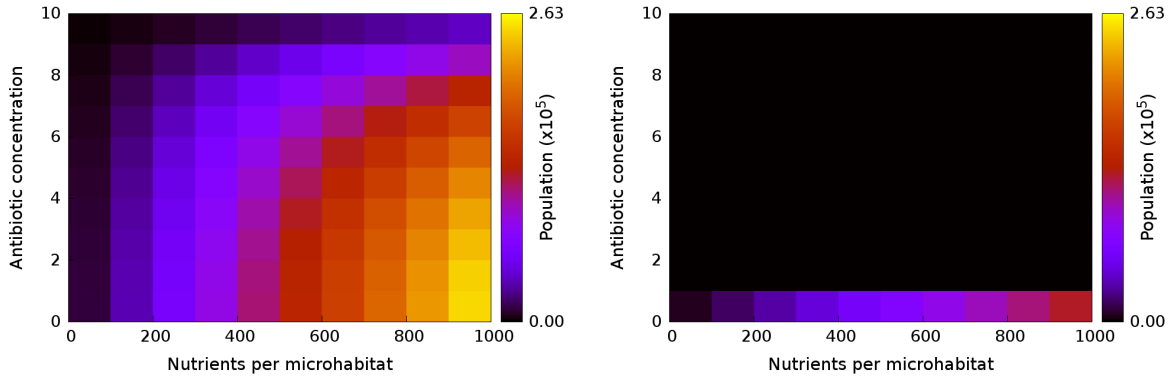
Here

$$\mu(S) = \frac{S}{K' + S} \tag{6}$$

where K' is a constant which represents the nutrient concentration at which bacterial growth is half-maximal, the value used here is for that of *E. coli* growing in glucose, and S is a measure of the nutrients present. Each replication by a bacteria consumes nutrients, altering the MIC in the microhabitat, which in turn alters the growth rate. Therefore the full expression for the growth rate experienced by the bacteria is given by

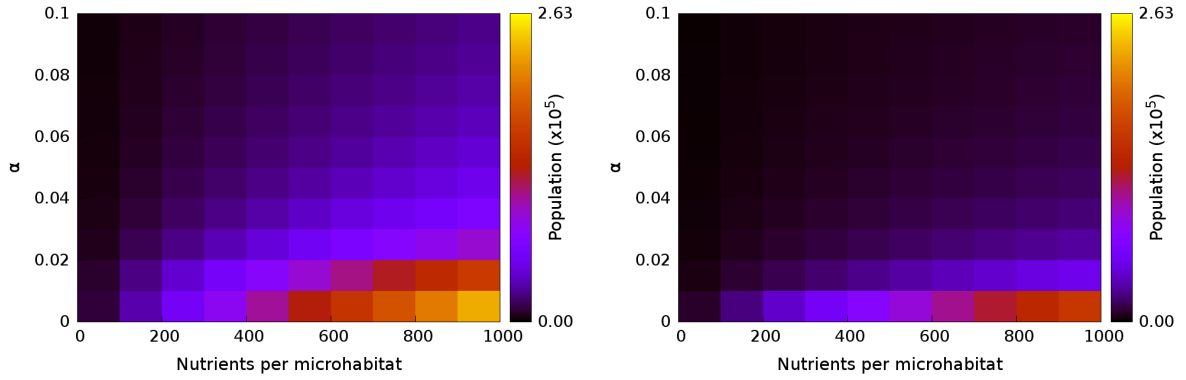
$$R_{rep} = \max\{0, 1 - (\frac{c}{\beta})^2\}\frac{S}{K' + S} \tag{7}$$

As $\mu_{\max}$ is a constant representing the maximum concentration of nutrients in each microhabitat, the ratio $\frac{\mu(S)}{\mu_{\max}}$ will decrease over time with each successful replication. Therefore for the bacteria exposed to the fast-growth targeting antibiotics, the tip of the advancing population will be more inhibited than the slow-growing bulk towards the rear of the colony, and vice versa for the slow-growth targeting antibiotics. Additionally, as a form of "control" group, a series of simulations were also performed utilising growth-independent bacteria targeting antibiotics (GIBTA) whose MIC didn't vary with S or c, but rather remained constant at all times.



(a) SGBTA (b) FGBTA

Figure 6: Overall sizes of bacterial populations after 1000 time units have passed, for a variety of uniform antibiotic and initial nutrient concentrations. From this it can be seen that the SGBTA are less effective at inhibiting overall bacterial growth, however the impact of a uniform antibiotic concentration has opposing effects for SGBTA and FGBTA. The presence of a gradient causes the SGBTA to be more effective inhibitor, while the reverse is true for the FGBTA.

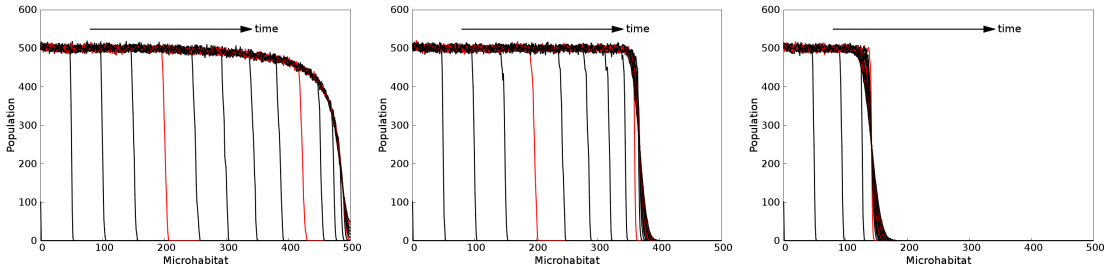


(a) SGBTA (b) FGBTA

Figure 7: Overall sizes of bacterial populations after 1000 time units have passed, for a variety of antibiotic gradients and initial nutrient concentrations. From this it can be seen that the SGBTA are less effective at inhibiting overall bacterial growth.

Some example results from these simulations are shown in Figures 6, 7 and 8. The first computational experiment performed entailed exposing the bacteria to a variety of nutrient and uniform antibiotic concentrations, as shown in Figure 6. This experiment, along with all the others, was initialised by setting

Some examples of the results that these experiments yielded are shown in Figures 7, 6 and 8. Figure 7 shows the total growth achieved by the bacteria for various initial nutrient concentrations and steepness of antibiotic gradients, whereas Figure 6 utilises uniform antibiotic concentrations. From these results two observations can be made. Firstly, if we compare Figures 7a to 7b and 6a to 6b, it can be seen that in both cases, the SGBTA are much less effective at inhibiting the growth of the population. However when the scenarios involving gradients are compared to those without, two differing features can be observed, which leads to the second observation; SGBTA are more effective inhibitors when they are applied in the form of a gradient, but the reverse is true for FGBTA, which are more effective in uniform concentrations.



(a) SGBTA (b) GIBTA (c) FGBTA

Figure 8: Spatial distributions of bacterial populations exposed to SGBTA, GIBTA and FGBTA respectively. The steepness of the antibiotic gradient has been chosen here such that the antibiotic concentration has a maximum of just over 10 in the final microhabitat. Once again, the SGBTA is inferior at inhibiting bacterial growth.

Following on from this, simulations were run to determine how gradients of these differing

antibiotic types affected the spatial distribution of the bacteria, an example of which is shown in Figure 8. Here once again we can see that the SGBTA allow for the bacteria to spread much further than the FGBTA in the same timeframe, with the GIBTA's efficacy being somewhere inbetween the two. Therefore showing that antibiotics which target the fast-growing tip of an advancing colony are more effective at impeding their progress.

These simulations were then performed using more "realistic" expressions for the MICs and replication rates of relevant bacteria, from the findings in Greulich et al., 2015, which continue to corroborate these findings. This component of the project has now deemed to have been completed and is currently in the process of being written up into a paper intended for publication.

3.3 Multispecies models

While many experimental investigations in laboratory conditions utilise biofilms containing only one species of bacteria, in nature the vast majority of biofilms are actually composed of a wide variety of species, which have differing physical properties and typically are in competition with one another [48]. Motivated by our discussion with AkzoNobel, where they highlighted the importance of understanding how these multispecies biofilms behave in order to better design the combinations of anti-microbials they include in their anti-fouling paints.

To this end, work has now begun on creating a model which contains a variety of species of bacteria, each with differing resistances to the applied antibiotic. This design is currently in its infancy, and is little more than a toy at the moment. However there are plans to incorporate real lab data supplied by AkzoNobel to allow the model to utilise growth curves and other relevant features of observed microorganisms from real-world environments, rather than the simple numeric approximations the model currently has in place. Additionally discussions have been had about how best to incorporate another external source of bacteria to the system, representing the influx of microorganisms which one would experience in the wild.

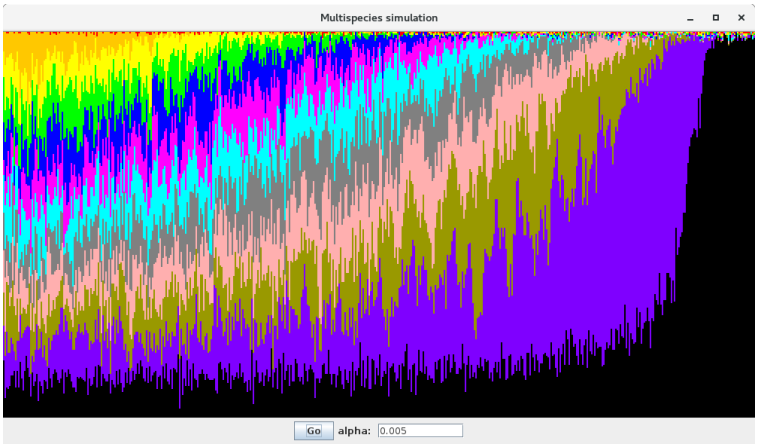


Figure 9: Snapshot of the GUI for the current multispecies model. The most resistant species are shown at the bottom of the picture, with the resistances decreasing towards the top. The system is exposed to an antibiotic gradient which is at its lowest on the left of the system, and increases exponentially towards the right.

4 Proposal

Over the next year, the first step will be to complete writing the paper on the growth-rate dependent antibiotic model. This should not take longer than a few weeks, as all the results have now been collated, so a write-up and commentary, along with a discussion section is all that remains. Along with some mainly stylistic editing.

Following on from this, work will continue on the multispecies model. The current version is a simplistic toy model, similar to the one in the Greulich paper [26]. Ideally our industrial partners AkzoNobel will provide us with some more realistic parameters for factors such as growth and death rates for a variety of microorganisms, and their susceptibility to various biocides. When incorporated into the current model, this should provide us with some more accurate, if still overly simplified, results for how a system with multiple species compete with each other over time. Additionally, the current models only consider freely moving, independent bacteria. But as seen in the previous literature, there is a vast difference between the behaviour of these planktonic bacteria and those which have aggregated to form a biofilm. As such, it seems that the next logical step is to incorporate the formation and subsequent property-altering qualities of biofilms. I estimate that at least, but no more than a few months will be spent working on this model.

While these additions should certainly yield some interesting results, there is only so far that these simplistic models can take us in providing a clear picture of the mechanics involved in how biofilms respond to the application of antibiotics. Therefore the plan at present is to move on from this 1D "lattice" model and begin work on a more intricate continuum model. This model would allow us to incorporate key features of biofilms and the environment which surrounds them, such as surface roughness and flow around the biofilm. These models should allow us to perform research on both the medical and industrial scenarios where biofilms form, by varying the side of the biofilm that the drug gradient arises from, and discerning whether these two situations create differing outcomes. I predict that this will consume the majority of the second year of my PhD.

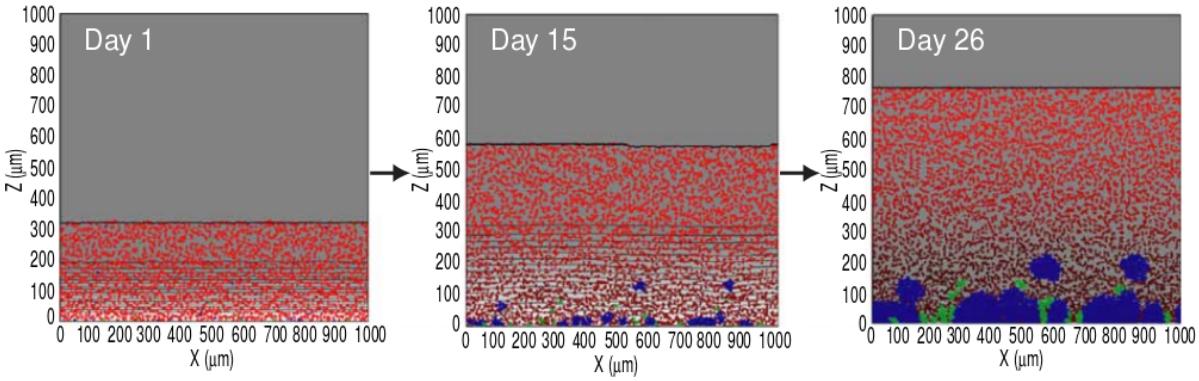


Figure 10: Screenshot taken from the paper by Matsumoto at al., 2007 [49] which contained several differing species of bacteria whose growth rates varied depending on the atmospheric composition of the biofilm. This is included for illustrative purposes as an idea of what the simulations constructed in the later stages of this PhD project might resemble.

In conjunction with AkzoNobel, some wet work will be undertaken at their laboratory in

Newcastle. This would entail cultivating biofilms on various surfaces akin to industrial ship hulls and exposing them to a variety of biocidal paints and then examining the composition of the established biofilm to compare the model with reality. There might also be opportunity to undertake part of the metagenomic analysis of these cultivated biofilms. This aspect of the project is intended as more of an interesting aside, rather than as a basis of the overall PhD project. It should only require a month or so of involvement, and will likely be performed alongside my other commitments.

5 Summary

Simple models investigating the effects of antibiotic gradients have been constructed which demonstrate the relation between gradient steepness and time taken to evolve resistance, the differing inhibition properties of growth-rate dependent antibiotics and how colonies involving multiple species with differing levels of resistance develop over time. An article is currently being written on the findings of the growth-rate dependent simulations.

The aim for the next year or so is to finish writing the article regarding the work on the growth-dependent antibiotics, then to continue with the multispecies model and hopefully incorporate some realistic parameters contributed by AkzoNobel. Following on from this, work will begin on the proposed continuum model which could incorporate other factors such as surface texture and flow into the system. This will be accompanied by some wet work at the AkzoNobel laboratory at their Newcastle compound. This is mainly for interest and not intended to form a sizable component of the project. There has also been some discussion of my involvement in AkzoNobel's metagenomic analysis of organisms gathered from their laboratory to extract information about the growth rates and biocidal susceptibility from microorganisms taken from biofilms attached to industrial shipping vessels.

References

[1] K. Williams, "The introduction of chemotherapy using arsphenamine the first magic bullet," *Journal of the Royal Society of Medicine*, vol. 102, no. 8, pp. 343–348, 2009. PMID: 19679737.

[2] L. A. Hicks, M. G. Bartoces, R. M. Roberts, K. J. Suda, R. J. Hunkler, T. H. Taylor, Jr, and S. J. Schrag, "Us outpatient antibiotic prescribing variation according to geography, patient population, and provider specialty in 2011," *Clinical Infectious Diseases*, vol. 60, no. 9, pp. 1308–1316, 2015.

[3] R. Laxminarayan, A. Duse, C. Wattal, A. K. M. Zaidi, H. F. L. Wertheim, N. Sumpradit, E. Vlieghe, G. L. Hara, I. M. Gould, H. Goossens, C. Greko, A. D. So, M. Bigdeli, G. Tomson, W. Woodhouse, E. Ombaka, A. Q. Peralta, F. N. Qamar, F. Mir, S. Kariuki, Z. A. Bhutta, A. Coates, R. Bergstrom, G. D. Wright, E. D. Brown, and O. Cars, "Antibiotic resistance–the need for global solutions," *The Lancet Infectious Diseases*, vol. 13, no. 12, pp. 1057 – 1098, 2013.

[4] M. E. A. de Kraker, A. J. Stewardson, and S. Harbarth, "Will 10 million people die a year due to antimicrobial resistance by 2050?," *PLOS Medicine*, vol. 13, pp. 1–6, 11 2016.

[5] S. Grasso, G. Meinardi, I. De Carneri, and V. Tamassia, "New in vitro model to study the effect of antibiotic concentration and rate of elimination on antibacterial activity," *Antimicrobial Agents and Chemotherapy*, vol. 13, no. 4, pp. 570–576, 1978.

[6] Q. Zhang, G. Lambert, D. Liao, H. Kim, K. Robin, C.-k. Tung, N. Pourmand, and R. H. Austin, "Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments," *Science*, vol. 333, no. 6050, pp. 1764–1767, 2011.

[7] B. Fernando and N. MaraCristina, "Challenges: Selective compartments for resistant microorganisms in antibiotic gradients," *BioEssays*, vol. 19, no. 8, pp. 731–736.

[8] R. M. Donlan, "Biofilm formation: A clinically relevant microbiological process," *Clinical Infectious Diseases*, vol. 33, no. 8, pp. 1387–1392, 2001.

[9] J. W. Costerton, P. S. Stewart, and E. P. Greenberg, "Bacterial biofilms: A common cause of persistent infections," *Science*, vol. 284, no. 5418, pp. 1318–1322, 1999.

[10] M. W. Mittelman, *Bacterial Biofilms and Biofouling: Translational Research in Marine Biotechnology: Proceedings of the October 5-6, 1999, Workshop.* National Research Council (US) Board on Biology; National Research Council (US) Ocean Studies Board, 2000.

[11] E. Harrould-Kolieb and J. Savitz, *Shipping Solutions: Technological And Operational Methods Available To Reduce CO2.* Washington, D.C.: Oceana, 2010.

[12] C. M. Magin, S. P. Cooper, and A. B. Brennan, "Non-toxic antifouling strategies," *Materials Today*, vol. 13, no. 4, pp. 36 – 44, 2010.

[13] J. S. Patil, H. Kimoto, T. Kimoto, and T. Saino, "Ultraviolet radiation (uv-c): a potential tool for the control of biofouling on marine optical instruments," *Biofouling*, vol. 23, no. 4, pp. 215–230, 2007. PMID: 17653932.

[14] J. G. Burgess, K. G. Boyd, E. Armstrong, Z. Jiang, L. Yan, M. Berggren, U. May, T. Pisacane, ke Granmo, and D. R. Adams, "The development of a marine natural product-based antifouling paint," *Biofouling*, vol. 19, no. sup1, pp. 197–205, 2003. PMID: 14618721.

[15] R. Chait, A. Craney, and R. Kishony, "Antibiotic interactions that select against resistance," *Nature*, vol. 446, no. 668, 2007.

[16] Y. C. Wang and M. Lipsitch, "Upgrading antibiotic use within a class: Tradeoff between resistance and treatment success," *Proceedings of the National Academy of Sciences*, vol. 103, no. 25, pp. 9655–9660, 2006.

[17] J. P. Torella, R. Chait, and R. Kishony, "Optimal drug synergy in antimicrobial treatments," *PLOS Computational Biology*, vol. 6, pp. 1–9, 06 2010.

[18] R. L. Marvig, H. K. Johansen, S. Molin, and L. Jelsbak, "Genome analysis of a transmissible lineage of pseudomonas aeruginosa reveals pathoadaptive mutations and distinct evolutionary paths of hypermutators," *PLOS Genetics*, vol. 9, pp. 1–12, 09 2013.

[19] I. X. Liu, D. G. Durham, and R. M. E. Richards, "Baicalin synergy with lactam antibiotics against methicillinresistant staphylococcus aureus and other lactamresistant strains of s. aureus," *Journal of Pharmacy and Pharmacology*, vol. 52, no. 3, pp. 361–366.

[20] M. E. Bertazzoni, A. Benini, A. Muner, C. Bassi, H. Abbas, and P. Pederzoli, "Pefloxacin penetration into human necrotic pancreatic tissue," *J Antimicrob Chemother.*, vol. 38, pp. 237–43, Aug 1996.

[21] K. P. Kim, Y.-G. Kim, C.-H. Choi, H.-E. Kim, S.-H. Lee, W.-S. Chang, and C.-S. Lee, "In situ monitoring of antibiotic susceptibility of bacterial biofilms in a microfluidic device," *Lab Chip*, vol. 10, pp. 3296–3299, 2010.

[22] R. Hermsen and T. Hwa, "Sources and sinks: A stochastic model of evolution in heterogeneous environments," *Phys. Rev. Lett.*, vol. 105, p. 248104, Dec 2010.

[23] C. Peloquin, T. Cumbo, D. Nix, M. Sands, and J. Schentag, "Evaluation of intravenous ciprofloxacin in patients with nosocomial lower respiratory tract infections: Impact of plasma concentrations, organism, minimum inhibitory concentration, and clinical condition on bacterial eradication," *Archives of Internal Medicine*, vol. 149, no. 10, pp. 2269–2273, 1989.

[24] P. Greulich, B. Waclaw, and R. J. Allen, "Mutational pathway determines whether drug gradients accelerate evolution of drug-resistant cells," *Phys. Rev. Lett.*, vol. 109, p. 088101, Aug 2012.

[25] R. M. Cozens, E. Tuomanen, W. Tosch, O. Zak, J. Suter, and A. Tomasz, "Evaluation of the bactericidal activity of beta-lactam antibiotics on slowly growing bacteria cultured in the chemostat.," *Antimicrobial Agents and Chemotherapy*, vol. 29, no. 5, pp. 797–802, 1986.

[26] P. Greulich, M. Scott, M. R. Evans, and R. J. Allen, "Growth-dependent bacterial susceptibility to ribosome-targeting antibiotics," *Molecular Systems Biology*, vol. 11, no. 3, p. 796, 2015.

[27] D. P. Bremer H, "Modulation of chemical composition and other parameters of the cell at different exponential growth rates," *EcoSal Plus*, 2008.

[28] M. Scott, C. W Gunderson, E. M Mateescu, Z. Zhang, and T. Hwa, "Interdependence of cell growth and gene expression: Origins and consequences," vol. 330, pp. 1099–102, 11 2010.

[29] H. Dalton and J. R. Postgate, "Effect of oxygen on growth of azotobacter chroococcum in batch and continuous cultures," *Microbiology*, vol. 54, no. 3, pp. 463–473, 1968.

[30] J. B. Russell and D. B. Dombrowski, "Effect of ph on the efficiency of growth by pure cultures of rumen bacteria in continuous culture.," *Applied and Environmental Microbiology*, vol. 39, no. 3, pp. 604–610, 1980.

[31] J. Monod, "The growth of bacterial cultures," *Annual Review of Microbiology*, vol. 3, no. 1, pp. 371–394, 1949.

[32] K. Lewis, "Riddle of biofilm resistance," *Antimicrobial Agents and Chemotherapy*, vol. 45, no. 4, pp. 999–1007, 2001.

[33] G. G. Anderson and G. A. O'Toole, *Innate and Induced Resistance Mechanisms of Bacterial Biofilms*, pp. 85–105. Berlin, Heidelberg: Springer Berlin Heidelberg, 2008.

[34] P. S. Stewart, "Mechanisms of antibiotic resistance in bacterial biofilms," *International Journal of Medical Microbiology*, vol. 292, no. 2, pp. 107 – 113, 2002.

[35] R. M. Donlan and J. W. Costerton, "Biofilms: Survival mechanisms of clinically relevant microorganisms," *Clinical Microbiology Reviews*, vol. 15, no. 2, pp. 167–193, 2002.

[36] L. Chambers, K. Stokes, F. Walsh, and R. Wood, "Modern approaches to marine antifouling coatings," *Surface and Coatings Technology*, vol. 201, no. 6, pp. 3642 – 3652, 2006.

[37] R. D. Monds and G. A. OToole, "The developmental model of microbial biofilms: ten years of a paradigm up for review," *Trends in Microbiology*, vol. 17, no. 2, pp. 73 – 87, 2009.

[38] L. Hall-Stoodley and P. Stoodley, "Biofilm formation and dispersal and the transmission of human pathogens," *Trends in Microbiology*, vol. 13, no. 1, pp. 7 – 10, 2005.

[39] J. B. Xavier, C. Picioreanu, and M. C. M. Van Loosdrecht, "A framework for multidimensional modelling of activity and structure of multispecies biofilms," *Environmental Microbiology*, vol. 7, no. 8, pp. 1085–1103, 2005.

[40] C. B. Whitchurch, T. Tolker-Nielsen, P. C. Ragas, and J. S. Mattick, "Extracellular dna required for bacterial biofilm formation," *Science*, vol. 295, no. 5559, pp. 1487–1487, 2002.

[41] X. Zhang, P. L. Bishop, and M. J. Kupferle, "Measurement of polysaccharides and proteins in biofilm extracellular polymers," *Water Science and Technology*, vol. 37, no. 4, pp. 345 – 348, 1998. Microorganisms in Activated Sludge and Biofilm Processes II.

[42] P. S. Stewart, "A review of experimental measurements of effective diffusive permeabilities and effective diffusion coefficients in biofilms," *Biotechnology and Bioengineering*, vol. 59, no. 3, pp. 261–272, 1998.

[43] K. S. L. Wimpenny, J. W. T., *Biochemical reactions and the establishment of gradients within biofilms. In: Microbial biofilms*, ch. 5, pp. 99–117. Cambridge University Press, 1995.

[44] T. R. Field, A. White, J. S. Elborn, and M. M. Tunney, "Effect of oxygen limitation on the in vitro antimicrobial susceptibility of clinical isolates of pseudomonas aeruginosa grown planktonically and as biofilms," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 24, p. 677, Oct 2005.

[45] S. J. Pamp, M. Gjermansen, H. K. Johansen, and T. TolkerNielsen, "Tolerance to the antimicrobial peptide colistin in pseudomonas aeruginosa biofilms is linked to metabolically active cells, and depends on the pmr and mexaboprm genes," *Molecular Microbiology*, vol. 68, no. 1, pp. 223–240, 2008.

[46] D. T. Gillespie, "A general method for numerically simulating the stochastic time evolution of coupled chemical reactions," *Journal of Computational Physics*, vol. 22, no. 4, pp. 403 – 434, 1976.

[47] D. T. Gillespie, "Approximate accelerated stochastic simulation of chemically reacting systems," *The Journal of Chemical Physics*, vol. 115, no. 4, pp. 1716–1733, 2001.

[48] S. Elias and E. Banin, "Multi-species biofilms: living with friendly neighbors," *FEMS Microbiology Reviews*, vol. 36, no. 5, pp. 990–1004, 2012.

[49] S. Matsumoto, A. Terada, Y. Aoi, S. Tsuneda, E. Alpkvist, C. Picioreanu, and M. van Loosdrecht, "Experimental and simulation analysis of community structure of nitrifying bacteria in a membrane-aerated biofilm," in *Water Science and Technology*, vol. 55 of *Water Science and Technology*, pp. 283–290, 2007.