Modeling Social Interactions of Yeast Biofilms with a Stochastic Spatial Simulation

Aparajita Sur

Follow this and additional works at: https://scholarworks.wm.edu/honorstheses

Part of the Applied Mathematics Commons, and the Microbiology Commons

Recommended Citation
https://scholarworks.wm.edu/honorstheses/1212

This Honors Thesis is brought to you for free and open access by the Theses, Dissertations, & Master Projects at W&M ScholarWorks. It has been accepted for inclusion in Undergraduate Honors Theses by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.
Modeling Social Interactions of Yeast Biofilms with a Stochastic Spatial Simulation

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Mathematics from The College of William and Mary

by

Aparajita Sur

Accepted for honors
(Honors)

[Signatures]

Dr. Leah Shaw

Dr. Helen Murphy

Dr. Lawrence Leemis

Williamsburg, VA
May 1st, 2018
Modeling Social Interactions of Yeast Biofilms with a Stochastic Spatial Simulation

Aparajita Sur

Department of Mathematics,
College of William and Mary,
Williamsburg, VA 23187-8795, USA

Email: asur@email.wm.edu
Acknowledgements

I would like to express deep gratitude to my advisors Dr. Leah B. Shaw and Dr. Helen Murphy for their support, guidance, and commitment to this thesis. Graduate students Adrienna Bingham and Sofya Zaytseva have been very helpful as mentors of this project. I also wish to thank the additional member of my committee, Dr. Lawrence Leemis. Finally, I wish to thank the Honors Fellowship and the Charles Center for supporting my research.
Abstract

Biofilms are microbial communities that are anchored to a surface and enmeshed in a protective extracellular matrix, shielding the microorganisms from antibiotics and other environmental hazards. As such, eradication of biofilms in medical and industrial settings can be challenging. These communities require individuals to cooperate and produce goods that will be used by all members, and thus are susceptible to cheaters who do not produce public goods, yet benefit from them. However, some cooperators can exhibit kin recognition, in which case they cooperate exclusively with themselves and not with another cell type such as a cheater. In which conditions does a cheater strain dominate cooperators exhibiting kin recognition? We use a stochastic spatial simulation to simulate the inoculation and growth of a yeast biofilm and to model social interactions between strains such as cooperation, competition, cheating and kin recognition. We vary social interaction parameters and define quantitative metrics to measure spatial segregation and cell distribution throughout and at the outside surfaces of a biofilm. These metrics help explain how social interactions affect a biofilm spatially. Understanding the spatial effect of social interactions on a biofilm can eventually help determine the optimum conditions for designing an engineered cheater strain to disrupt cooperative yeast biofilms or yeast infections.
# Contents

1 Introduction 1
   1.1 Biofilms 1
   1.2 Social Interactions in a Biofilm 2
   1.2.1 Spatial Positioning Affects Community Function 3
   1.2.2 The Effects of Social Interactions and Initial Cell Densities on Spatial Positioning 4
   1.3 Motivation 5
   1.4 Yeast Biofilm Lab Experiment 6
   1.5 Outline 7

2 Simulation Methods 8
   2.1 Simulating the Biofilm Structure and Inoculation of Cells 9
   2.2 Simulating Biofilm Growth 10
      2.2.1 Random Sequential Update and Stochastic Cell Growth 11
      2.2.2 Fitness Model to Determine if a Cell Grows 11
      2.2.3 Determining Where a Cell Will Grow 13
   2.3 Simulating Different Social Dynamics Exhibited in Biofilms 14
      2.3.1 Baseline Competition (No Cell-Cell Social Interactions) 16
      2.3.2 Simple Cheating 17
      2.3.3 Kin Recognition 17
   2.4 Differences Between Our Simulation and Prior Literature 18
      2.4.1 Different Biology Simulated 18
      2.4.2 Different Growth Geometry 18
      2.4.3 Different Placement for a Dividing Cell 19

3 Quantitatively Measuring Social Dynamics and Spatial Effects 20
   3.1 Final Proportions of Cell Types in the Biofilm 20
   3.2 Proportion of Cell Types Reaching the Outside Surface of a Biofilm 21
   3.3 Spatial Segregation 22
      3.3.1 Large Scale Spatial Segregation: Cluster Lengths 23
      3.3.2 Small Scale Spatial Segregation: Spatial Assortment 24

4 Results and Discussion 25
   4.1 Proportion of Cells in the Biofilm and on the Surface of the Biofilm 26
4.2 Spatial Segregation ......................................................... 29
  4.2.1 Large Scale Segregation ............................................. 29
  4.2.2 Small Scale Segregation .............................................. 32

5 Conclusions and Future Work ......................................... 35
Chapter 1

Introduction

1.1 Biofilms

In nature, many microorganisms exist within biofilms, rather than as solitary and free-living species. Biofilms are complex communities that are attached to a surface and encased in an extracellular matrix. These extracellular matrices provide protection from environmental stresses, antibiotic or antifungal treatments and other external threats. As a result, biofilms can cause resilient infections and are often difficult to eradicate [6].

Biofilms can be found in aquatic and terrestrial systems, in our bodies, all throughout our homes, and even on medical devices such as catheters [4]. The prevalence of biofilms and their resistance to external threats make them a great concern in medical and industrial settings. In this thesis, we study yeast biofilms because yeast or fungal biofilms in particular can cause disease in humans through yeast infections, oral thrush, and rashes. Additionally, yeast infections can be fatal in healthy patients with implanted medical devices, or in immunocompromised patients. For patients with implanted catheters, yeast biofilms are largely resistant to current antifungal drugs; thus, high antifungal doses together with removal of the medical device are generally required to treat infections. However, the removal of these medical devices is costly and, in some cases, dangerous,
and administration of high doses of antifungal agents can cause complications, such as kidney and liver damage. Consequently, these treatments are often not possible, as many critically ill patients are unable to tolerate them [4]. Thus the question arises: how do we disrupt these yeast biofilms effectively when they pose public health risks and are fiercely resistant to antifungal drugs? What other methods besides medical treatments can be used to disrupt a yeast biofilm and subsequently a yeast infection? Can an understanding of the social interactions within a biofilm benefit us in this regard?

1.2 Social Interactions in a Biofilm

Biofilms consist of cooperating individuals that secrete products or public goods that can be used by all the members in the community. Consequently, cheaters or individuals who do not expend energy producing the public goods but take advantage of them, can exploit the cooperating individuals. Non-cooperators also do not expend energy producing the public goods, but unlike cheaters, they do not take advantage of the public goods. Additionally, unless there is an excess of shared resources, competition always occurs within biofilms, particularly for limited space, nutrients and public goods. Cooperation, non-cooperation, cheating and competition are some of the different types of social interactions that can occur in a biofilm because often there are two or more strains interacting with one another [6].

Specifically, social interactions are the ways an individual or cell can positively, neutrally or negatively affect the reproductive output or fitness of other individuals or cells growing in the same neighborhood within the biofilm [5]. A neighborhood is the space in a biofilm within which individuals grow and interact with one another. Since cooperative cells produce the public good, they positively affect the reproductive output of other cells, such as other cooperators and cheaters. Cheaters have direct neutral effects on other cells (asides from affecting other cells through competition). Although not investigated in this thesis, biofilms can also have antagonistic social interactions in which a cell, such as a
toxin-inducing cheater, negatively affects the fitness or reproductive output of another cell [6]. These social interactions can affect the spatial positioning and structure of the biofilm.

1.2.1 Spatial Positioning Affects Community Function

Social interactions such as cooperation and competition influence the relative spatial positioning of members within the biofilm, which can subsequently affect the functioning of a community. For example, in microbial biofilms, certain bacterial species that create public goods and compete for resources can only grow if there is an intermediate distance between each of the species [5]. Additionally, the spatial positioning of members at the edges of a biofilm is particularly important. Since biofilms are communities attached to a surface, cells compactly grow on top of one another, causing the cells at the edges to have the best access to limiting nutrients, space and oxygen [3]. Recent microbial experimental studies have highlighted the importance of the edges or the “expanding frontiers” that can drive population differentiation by random genetic drift [8]. It has also been found in a biofilm with two cooperating strains that the spatial structure can promote enhance the mutual relationship between the strains [6].

Additionally, in biofilms with multiple bacterial or yeast strains that are not all cooperative, the spatial positioning of the cells play a key role in determining which strain will dominate the other(s). Domination can include occupying the most space in the biofilm, “trapping” another strain by surrounding it from above and from the edges, primarily residing in the outside edges of the biofilm with the best access to nutrients and out-performing the other strain(s) over time by colonizing new areas [8]. Prior literature on bacterial range expansion also determined that spatial positioning can determine whether strains will co-exist [6, 8, 9]. Thus, it is vital to understand how social interactions affect spatial positioning in multispecies biofilms.
1.2.2 The Effects of Social Interactions and Initial Cell Densities on Spatial Positioning

In 2012, Momeni et al. systematically investigated how different types of social interactions in biofilms with two cell types can lead to distinct biofilm patterning, by employing a mathematical model [5]. They specifically investigated “baseline” competition (when the only social interaction is competition), and scenarios in which each cell type cooperates with the another, both cell types negatively affect the other and in which one cell type cooperates while the other cell type negatively or neutrally affects the other. The authors found that in simulations as well as lab experiments with yeast and microbial biofilms, biofilms with baseline competition led to cell types forming columns that are spatially segregated from each other. In biofilms that exhibit baseline competition and in which one cell type is cooperative and the other cell type neutrally affects the fitness of the cooperative strain, frequently one of the cell types would grow on top of and cover the other population spatially. When both cell types exhibit cooperation, the cell populations were intermixed and the two cell types grew on top of each other. The authors defined intermixing as alternating changes in cell types along the height of a biofilm [5].

In addition to social interactions, the initial inoculation of cells can affect spatial pattern formations such as intermixing or segregation. Although Momeni et al. found that the level of intermixing in cooperative communities was insensitive to initial cell type ratios (the ratio of a cell type to another cell type at the time of inoculation), they found that overall high cell densities can promote intermixing, even in the absence of cooperation, because high cell densities put different cell types near one another [5].

Gestal et al. found similar results when they examined how initial cell densities impact spatial pattern formation [2]. They show experimentally and through a mathematical model that at low initial density of cells, cell types segregate in space whereas at high initial cell densities, intermixing occurs. The authors measured spatial pattern formation by calculating the average frequency of a cell type surrounding itself minus the average
frequency of a cell type surrounding the other species. From both studies, it is evident that the overall cell density inoculated at the onset of biofilm growth affects spatial formation. Other studies examining bacterial range expansion found that the inoculation of cells as well as the relative strain growth rates had strong effects on spatial positioning and the coexistence of strains [8].

Aside from Momeni et al.’s work [5], to our knowledge, all bacterial range expansion literature and studies on microbial and yeast biofilms use two-dimensional mathematical models to computationally represent experiments. In lab experiments, one cannot tune parameters easily. Thus, due to the lack of full control and efficiency to systematically investigate the spatial effects of social interactions, mathematical models that more closely correspond to biofilm lab experiments should be used. Since biofilms are three-dimensional, mathematical models should simulate biofilm growth three-dimensionally to fully understand the impact of social interactions on spatial structure.

1.3 Motivation

Our main motivation is to expand upon prior literature by investigating the spatial effects of social interactions with a three-dimensional simulation that more closely represents biofilm lab experiments. Although Momeni et al. examined how social interactions and initial cell densities affect a biofilm spatially three-dimensionally, there are facets of their mathematical model that do not accurately represent biofilms growing in lab experiments.

For example, Momeni et al. defines social interactions as the effect of a cell type upon another cell type, and not upon itself [5]. This definition does not model the behavior of cooperative cells in the lab experiments. In fact, some cooperative yeast cells can cooperate exclusively with themselves and not with another cell type. This behavior is a social interaction known as “kin recognition” [7]. Consequentially, it is necessarily to model how a cell type affects itself as well as other cell types; Momeni et al.’s simulation does not have the ability to do so.
Additionally, Momeni et al. found that initial cell type ratios do not significantly affect spatial patterns in cooperative communities. In this thesis, we will test if initial cell type ratios affect spatial patterns in communities with other social interactions such as baseline competition, cheating and kin recognition. Furthermore, Momeni et al. did not examine the behavior at the edges of a biofilm [5]. Determining which strains populate the edges can determine which strain is dominant in the biofilm. Thus, investigating the edges of a biofilm is another focus of this thesis.

Aside from understanding the spatial effects of social interactions, an eventual goal is to determine a strategy and the optimal conditions to disrupt a yeast biofilm. For treating bacterial disease, Brown et al. proposed a “Trojan horse” strategy to introduce engineered microbial cheats to disrupt biofilms [1]. Specifically, they proposed the following strategy: (1) introduce a “Trojan horse” strain into a biofilm; (2) induce it to excrete its toxin while protecting itself with an anti-toxin; and (3) when it has killed the majority of the cooperator strains, change the conditions to stop production of the anti-toxin, and thus kill itself. Their proposed strategy was not modeled in a fully spatial system. A better understanding of social interactions can aid us in implementing this novel evolutionary strategy to disrupt yeast biofilms in the future.

Thus, in this project we explore how baseline competition, cooperation, kin recognition and non-cooperation affect the spatial structure of a biofilms, in a computational stochastic simulation that represents biofilm growth in a lab experiment. We are especially interested in kin recognition as this social interaction has not been modeled with a three-dimensional simulation in previous literature.

1.4 Yeast Biofilm Lab Experiment

To grow a yeast biofilm in the biology lab, yeast cells (typically two cell types) are inoculated in a droplet of growth media in the center of a plate. The droplet is heavily diluted. Yeast cells grow to the sides when there is available space and grow upward
when confined. A cell will grow based on the competition for resources present in their neighborhood, the social interactions of the cells around them in their local neighborhood and a degree of randomness. No cell will always grow and it is unlikely that replicate lab experiments will produce an identical yeast biofilm.

Consequently, we will computationally simulate yeast biofilm growth and inoculation stochastically. The specific methods for inoculation and growth will be discussed in the following chapter.

Over time, a three-dimensional biofilm will grow. Environmental conditions can be manipulated to control the degree of cooperation for a cooperative strain, and strains can be engineered to neutrally or negatively affect other strains. Cells are often inoculated in varying initial densities to examine the differences in resulting spatial structure.

### 1.5 Outline

Chapter 1 summarizes prior literature on social interactions within biofilms and the motivation of this thesis. Chapter 2 describes the simulation methods to computationally represent a yeast biofilm. Chapter 3 presents an overview of our quantitative measures when analyzing the simulation biofilms. Chapter 4 discusses the results. Chapter 5 states our conclusions and future work.
Chapter 2

Simulation Methods

The previous chapter presented an overview of biofilms and prior literature that examined how social interactions spatially affect biofilms. In the lab experiments described in Section 1.4, two interacting yeast strains (population 1 and population 2) grow together in the biofilm. We discussed how social interactions between interacting strains can impact the spatial positioning of cells, which in turn can be critical for the proper functioning of a yeast community. Spatial positioning of cells can also indicate which strain is dominating within the biofilm (Section 1.2.1).

This chapter presents how we computationally represent aspects of a yeast biofilm lab experiment such as cell inoculation, growth and possible types of social cell-cell interactions. Our simulation methods were adapted from Momeni et al’s 3D simulation of biofilms [5]; however, there are notable differences (Section 1.3) that we implement to model lab experiments more accurately.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_c$</td>
<td>Maximum biofilm length and width</td>
<td>50</td>
</tr>
<tr>
<td>$N_z$</td>
<td>Maximum biofilm height</td>
<td>100</td>
</tr>
<tr>
<td>$d$</td>
<td>Droplet length</td>
<td>15</td>
</tr>
<tr>
<td>$n$</td>
<td>Maximum number of simulated cells grown</td>
<td>50000</td>
</tr>
<tr>
<td>$f$</td>
<td>Fraction of droplet filled with cells</td>
<td>0.05</td>
</tr>
<tr>
<td>$f_1$</td>
<td>Fraction of type 1 inoculated cells</td>
<td>0.3, 0.5, 0.7</td>
</tr>
<tr>
<td>$f_2$</td>
<td>Fraction of type 2 inoculated cells</td>
<td>0.3, 0.5, 0.7</td>
</tr>
<tr>
<td>$\Delta t$</td>
<td>Time step</td>
<td>0.1</td>
</tr>
<tr>
<td>$r_1$</td>
<td>Population 1 growth rate</td>
<td>Calculated in simulation</td>
</tr>
<tr>
<td>$r_2$</td>
<td>Population 2 growth rate</td>
<td>Calculated in simulation</td>
</tr>
<tr>
<td>$r_{10}$</td>
<td>Baseline growth rate for cell type 1</td>
<td>0.05</td>
</tr>
<tr>
<td>$r_{20}$</td>
<td>Baseline growth rate for cell type 2</td>
<td>0.05, 0.075, 0.1</td>
</tr>
<tr>
<td>$r_{11}$</td>
<td>Social effect of cell type 1 on itself</td>
<td>0, 0.1, 0.3, 0.5, 0.7</td>
</tr>
<tr>
<td>$r_{21}$</td>
<td>Social effect of cell type 1 on cell type 2</td>
<td>0, 0.1, 0.3, 0.5, 0.7</td>
</tr>
<tr>
<td>$r_{22}$</td>
<td>Social effect of cell type 2 on itself</td>
<td>0</td>
</tr>
<tr>
<td>$r_{12}$</td>
<td>Social effect of cell type 2 on cell type 1</td>
<td>0</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Competition effect</td>
<td>1</td>
</tr>
<tr>
<td>$\phi_1$</td>
<td>Fraction of cell type 1 in cell neighborhood</td>
<td>Calculated in simulation</td>
</tr>
<tr>
<td>$\phi_2$</td>
<td>Fraction of cell type 2 in cell neighborhood</td>
<td>Calculated in simulation</td>
</tr>
<tr>
<td>$R_i$</td>
<td>Interaction radius</td>
<td>3</td>
</tr>
<tr>
<td>$R_d$</td>
<td>Displacement radius</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2.1: Parameters to simulate biofilm inoculation and cell growth. Section 2.1 and 2.2 describe how the parameters are used.

### 2.1 Simulating the Biofilm Structure and Inoculation of Cells

Since experimental biofilms are three-dimensional structures, our model simulates the inoculation and growth of yeast cells in a three-dimensional (3D) cubic array of an $(x,y)$ domain and a height $z$. Each array element represents one cell. The maximum $x,y$ width and length and the maximum biofilm height $z$ are set equal to pre-specified parameters $N_c$ and $N_z$, respectively. In all of our simulations, the biofilm was represented by a three-dimensional simulation grid containing up to $50 \times 50 \times 100$ cells.

To represent the inoculation of yeast cells in the biology lab, we consider the bottom
(x,y) surface of the 3D cell array as the plate, and allocate the cells in a centered square droplet. As described in Section 1.4, the droplet of cells inoculated at the start of the lab experiment is heavily diluted with growth media and only a small fraction of the droplet is yeast cells. We calculate the droplet area as the area of the square with a length \( d \). Therefore, cells are randomly distributed within a fraction \( f \) of the square droplet area in the simulation (Figure 2.1). We set \( f = 0.05 \) in all simulations (Table 2.1). In a biofilm with two strains 1 and 2, we can set the fraction of inoculated cells that are of type 1 (\( f_1 \)) and type 2 (\( f_2 \)) to understand how initial population ratios impact biofilm growth and the spatial positioning of cells.

### 2.2 Simulating Biofilm Growth

Yeast biofilms in a lab experiment grow continuously throughout time. Although our simulation increments time discretely, we employ small time steps \( \Delta t \) to model continuous time.
After the simulation randomly distributes the two populations of cells on the bottom surface during the cell inoculation, the growth of cells is simulated until the biofilm has reached a specified number of cells $n$ (Table 2.1). After several trials, $n = 50000$ was chosen as the number of total cells to simulate to, as the amount represented a fairly “mature” biofilm. We define a biofilm as “mature” when the bottom surface is completely populated and cells grow upwards, making the simulated biofilm three-dimensional as the biofilm would be in lab experiments. In this thesis, we set $n = 50000$ throughout all our simulations so the simulations can be compared to one another.

### 2.2.1 Random Sequential Update and Stochastic Cell Growth

Since lab experiments will always have a degree of randomness, our model simulates cell growth stochastically by using a discrete time Monte Carlo simulation. Specifically, in each discrete time step $\Delta t$, (1) a random cell in the biofilm is chosen, (2) the cell’s growth rate $r$ is calculated with a fitness function (to be defined in Equation 2.2), (3) a random number between 0 and 1 is generated, and (4) if the generated random number is less than $r\Delta t$ (the probability of reproduction in a time step), the cell will divide. Within each time step, this random sequential update selection continues until the number of cells selected equals the number of cells present in the biofilm at the beginning of the time step.

### 2.2.2 Fitness Model to Determine if a Cell Grows

In a biofilm with two yeast strains or populations 1 and 2, the growth rate or fitness of a particular cell depends upon the cells in its cubic three-dimensional interaction neighborhood, which is defined by an interaction radius $R_i$ (Table 2.1) or the $R_i$-cell-width above, below and to the left, right, front, and back of the growing cell. In our simulations, $R_i = 3$ grid units which means the entire three-dimensional neighborhood consists
of $7^3 = 243$ cells.

Let $\phi_1$ and $\phi_2$ be the fraction of cell type 1 and 2 occupying the interaction neighborhood, respectively. To compute the fractions $\phi_1$, $\phi_2$, we calculate the number of cells of each cell type and divide the cell type counts by the volume of space within the 3D interaction neighborhood. After the fractions of each cell type are computed for the interaction neighborhood of the growing cell, they are passed on to the fitness model to calculate the growth rate $r$ to determine if a chosen cell will divide (Section 2.2.1). If a cell has a portion of its neighborhood extending beyond the “edges” or the “bottom layer” of our simulation, that portion of the neighborhood is not counted to compute the fractions. To make our algorithm efficient, we store the number of cells for each cell type to compute the fraction of a neighborhood for each cell that has already grown. If there are any changes to a cell site (such as when a cell is pushed to a different site), we flag all the cells within its neighborhood. Therefore, when a cell is flagged, its neighborhood has to be recalculated but if a cell is unflagged the algorithm does not have to check every site within the neighborhood to compute the fractions.

Regarding notation, an $r_{ij}$ social interaction is the effect of cell type $j$ on $i$ (in our simulations $i, j = 1$ or 2). Then the growth rate $r_1$ for a population 1 cell is calculated by the following fitness function:

$$r_1 = [r_{10} + r_{11}\phi_1 + r_{12}\phi_2][1 - \chi(\phi_1 + \phi_2)].$$

The fitness of a type 1 cell is influenced by the baseline fitness $r_{10}$ (growth rate of cell type 1 without any interactions (Section 1.2.2), the total fitness effect of cell type 1 on itself ($r_{11}\phi_1$), the total fitness effect of cell type 2 on the dividing type 1 cell ($r_{12}\phi_2$) and intra- and inter-population competition for shared resources ($1 - \chi(\phi_1 + \phi_2)$). If any of the $r_{ij}$ social interactions are positive (cooperation), then the fitness for a dividing cell may increase if the $\phi$ of a cooperating cell type is large, depending on values in the fitness function. The fitness may decrease as the neighborhood becomes more occupied because
of competition; if the neighborhood is completely occupied, the cell is prevented from growing in our simulation because we set $\chi = 1$

Similarly, the growth rate for cell type 2 is:

$$r_2 = [r_{20} + r_{22}\phi_2 + r_{21}\phi_1][1 - \chi(\phi_1 + \phi_2)].$$  \hspace{1cm} (2.2)

### 2.2.3 Determining Where a Cell Will Grow

Once the growth rate and random selection determines that a chosen cell will grow, the next process is to determine where the cell will grow. Neighborhoods determine where a cell will grow by searching for empty cell sites within the displacement radius ($R_d$ from Table 2.1) in the following order: immediate sites (horizontally adjacent and diagonally adjacent (Figure 2.2a), other sites in $(x,y)$ planar neighborhood (Figure 2.2b) and sites directly above the growing cell (Figure 2.2c). We set $R_d = 3$ for all our simulations, which means that for a growing cell the $(x,y)$ planar neighborhood the algorithm searches within is a 7 by 7 square.

To elaborate, to find empty sites, we first check the four sites that are horizontally adjacent to the growing cell. If more than one horizontally adjacent site is empty, one is randomly chosen. If no horizontally adjacent site is empty, then the four diagonally adjacent sites are checked and one is randomly chosen if more than one diagonal site is empty.

If none of the eight immediately adjacent sites are empty, every spot in the $(x,y)$ planar neighborhood is checked and the distance from each empty site to the growing cell is computed. The minimum distance is found and if there are multiple empty sites with the same minimum distance, one of those sites is randomly chosen. Afterwards, our simulation determines the optimum path to the chosen empty site and pushes the cells on the path to produce an adjacent empty space for a growing cell. We adapted this algorithm from Momeni et al.’s “tracepath” algorithm [5].
If no immediate sites or sites within the \((x, y)\) planar neighborhood of the growing cell are empty, a yeast cell will divide and place its daughter cell directly upwards. If there are already cells directly above the growing cell, the algorithm pushes them further upwards to create space to place a daughter cell directly above the growing cell.

**Tracepath algorithm**

If all the immediate sites of a growing cell are filled and there is an empty site elsewhere in the neighborhood, an optimum path to push cells must be determined in order to create an adjacent empty space for a growing cell to place its daughter. The “tracepath” algorithm first determines the general direction (east, west, north, south in the \(x, y\) plane) a chosen empty site is in comparison to the growing cell. The simulation then accordingly finds the three nearest neighbor sites (nearest site in the \(x\) direction, \(y\) direction and the diagonal). One of the three sites is chosen to pave the path, based on which one is most parallel to the line between the growing cell and the empty site. Specifically, the nearest neighbor site with the maximum cosine is chosen because the site’s angle \(\theta\) to the straight line between the growing cell and the empty site is closest to zero (Figure 2.3). While the empty site is not reached, the simulation continues finding the three nearest neighbor sites and choosing the optimum one for forging the path from the growing cell to the empty site. The angle \(\theta\) is always calculated from the site to the line \(AB\) (Figure 2.3). Once the path is determined, the simulation pushes all the cells up a spot on the path, so the growing cell can place its daughter in an adjacent site (Figure 2.2).

### 2.3 Simulating Different Social Dynamics Exhibited in Biofilms

To simulate different social dynamics or interactions in biofilms, we vary the baseline growth rates \((r_{10}, r_{20})\) and the cell-cell interaction effects \((r_{11}, r_{21}, r_{22}, r_{12})\) in the fitness
Figure 2.2: A budding cell in (a) divides into a horizontally adjacent empty site. A budding cell in (b) finds the optimum path and accordingly pushes the cells on the path to the empty site, to create space to place its daughter. A budding cell in (c) divides upwards. The blue square in (a) and (b) is the interaction neighborhood. In these figures, $R_d = 2$.

Figure 2.3: Finding the optimum path from a growing cell at site A to an empty site B. The optimum path is along the red sites and the yellow sites are the neighboring sites considered.
2.3.1 Baseline Competition (No Cell-Cell Social Interactions)

Competition for shared resources is always present in a biofilm. Thus, in all of our simulations we set $\chi = 1$ to represent full competition. A biofilm with two cell types co-existing without any cooperative or negative effects towards each other or towards themselves is a baseline competition biofilm, since there are no social cell-cell interactions beyond competition. The social interaction parameters $r_{11}, r_{21}, r_{22}, r_{12}$ (from Equation 2.2) are set to zero, leading to the following fitness functions to simulate a baseline competition biofilm:

\[
\begin{align*}
    r_1 &= r_{10}[1 - \chi(\phi_1 + \phi_2)]. \\
    r_2 &= r_{20}[1 - \chi(\phi_1 + \phi_2)].
\end{align*}
\]

In our simulations, the baseline growth rate $r_{10}$ is fixed at 0.05, and $r_{20}$ is varied from 0.05, 0.075 to 0.1.

The purpose of the baseline competition simulation is to isolate how baseline growth rates alone can impact the spatial positioning of populations. We also wanted to determine which relative baseline growth rate ratio ($\frac{r_{20}}{r_{10}}$) should be used for future simulations when a cheater or non-cooperator (Section 1.2.2) co-exist with a cooperator. If population 1 are the cooperators, and population 2 is a cheater or is a non-cooperator, $r_{20} > r_{10}$ because type 2 cells do not have to expend an energy cost to produce the public goods. Determining how the relative baseline growth rate ($\frac{r_{20}}{r_{10}}$) spatially affects a biofilm in these baseline competition simulations can help us choose a relative baseline growth rate for future simulations in which other social interactions are implemented.

We ultimately selected relative baseline growth rate $\frac{r_{20}}{r_{10}} = 2$ ($r_{10} = 0.05$, $r_{20} = 0.1$) because there is a qualitative discernible difference in the spatial positions of the two
populations (see Chapter 4 “Results and Discussion”).

2.3.2 Simple Cheating

A simple cheater is a cell type that takes advantage of the public goods produced by a cooperator, and has neutral effects on itself and the cooperator cell type. Since the simple cheater does not have to produce the public good, the cell type has a higher baseline growth rate than the cooperators. Let cell type 1 be the cooperators and cell type 2 be the simple cheaters. Then to simulate a biofilm with simple cheaters and cooperators, in Equation 2.2 we set $r_{20} > r_{10}$, $r_{11}, r_{21} > 0$, and $r_{22} = r_{12} = 0$.

Varying the cooperator’s degree of cooperation ($r_{11}$ and $r_{21}$) allows us to understand how the degree of cooperation spatially affects a biofilm when simple cheaters are present. We set $r_{11} = r_{21} = 0.1, 0.3, 0.5, 0.7$ and $r_{10} = 0.05, r_{20} = 0.1$.

Another key motivation for the simple cheater simulations is to determine the conditions in which cooperators emerge as the dominant population and in which conditions the simple cheaters have an advantage. Although the WM biology lab does not have experiments with simple cheaters, simple cheating can occur in microbial biofilms [6]. Understanding how cooperators behave amidst simple cheaters can aid us in designing a strategy to prevent microbial infections.

2.3.3 Kin Recognition

Kin recognition is a scenario in which the cooperators recognize and only cooperate with their own cell type or kin [7]. Therefore, non-cooperators (cells that do not produce public goods) will not benefit from the cooperators. However, since the non-cooperators do not produce the public good, their advantage is having a higher baseline growth rate.

Let cell type 1 be the cooperators with kin recognition and cell type 2 be the non-cooperators. Then to simulate a biofilm with non-cooperators and cooperators with kin recognition, we set $r_{20} > r_{10}$, $r_{11} > 0$, and $r_{21} = r_{22} = r_{12} = 0$ from Equation 2.2.
In all of our kin recognition simulations, we set $r_{10} = 0.05$, $r_{20} = 0.1$, and $r_{11} = 0.1, 0.3, 0.5, 0.7$. Kin recognition simulations are of particular interest because the cooperative yeast strains in the lab experiments solely exhibit kin recognition. Additionally, to our knowledge, no prior literature has developed a 3D model to study kin recognition behavior.

2.4 Differences Between Our Simulation and Prior Literature

As mentioned previously, this simulation is adapted from a past paper [5] that also modeled 3D biofilm growth and varied competition and cooperation parameters. To our knowledge, our simulation and the simulation in [5] are the only 3D models of biofilm growth in literature. However, there are some notable differences between the two models.

2.4.1 Different Biology Simulated

Our fitness model incorporates the effect of a cell type on itself. In lab experiments, cooperating cell types affect other cell types as well as themselves; therefore, it is crucial to implement a cell’s effect on itself to accurately represent the biofilms growing in a biology lab. Additionally, we simulate kin recognition, which is a behavior yeast cells can exhibit in biofilms.

2.4.2 Different Growth Geometry

Momeni et al. used periodic boundary conditions to structure their computational biofilm [5]. Instead, our biofilm is visualized as a cube rather than a torus. If a cell is at the edge, and the only empty spaces for a cell to grow are beyond the edge, the cell does not grow horizontally. If any neighborhood extends beyond an edge, the spaces beyond
an edge are not considered as part of the neighborhood. If a cell reaches the top of our simulated biofilm, the simulation stops. However, we set $N_z$ (Table 2.1) to be large so the cells always have ample space to grow up.

Furthermore, we allocate cells in the center of the bottom layer as a “droplet” instead of randomly allocating throughout the whole domain as Momeni et al. do [5]. Allocating cells in the center of the bottom layer serves as a more realistic representation of the allocation of cells in a biofilm experiment. Additionally, distinguishing the inoculated cells at the center from the edge allows us to study the effects of social interactions on the proportions of strains at the edges.

### 2.4.3 Different Placement for a Dividing Cell

The following differences are minor. When checking for empty sites in the eight adjacent sites directly around the cell chosen to grow, our algorithm gives preference to the horizontal adjacent sites over the diagonal adjacent sites. Momeni et al. treats horizontal adjacent and diagonals as equal, although the growing cell is closer to the horizontal adjacent sites than the diagonals [5].

Additionally, when tracing the path to an empty site, our algorithm uses the cosine angle to choose the nearest neighbor site to include on the optimum path. Momeni et al. used the area of a parallelogram. Essentially, Momeni et al. paper regarded both the distance and angle while we focused on just the angle or how parallel a neighbor site is to the line between the growing cell and the empty site [5].
Chapter 3

Quantitatively Measuring Social Dynamics and Spatial Effects

This chapter describes the quantitative metrics for analyzing spatial effects and population proportions in biofilms. Particularly, we are interested in the final proportions of cell types, which cell types reach the outside surface of a biofilm (the top surface and the edges), and spatial segregation within a biofilm.

3.1 Final Proportions of Cell Types in the Biofilm

A key goal of this thesis is to computationally represent the behavior of biofilms in lab experiments. Therefore, since the biology lab measures the proportion of a cell type at the start and end of the experiment, we also calculate the proportion of a cell type at the start and end of the simulation. Additionally, we measure the relative change in abundance of each cell type.

The final proportion of a cell type is defined as the total number of cells of that cell type in the biofilm divided by the total number of cells present in the biofilm ($\frac{n_1}{n_1 + n_2}$ or $\frac{n_2}{n_1 + n_2}$). The relative change in abundance for cell type 1 is calculated by dividing the final
3.2 Proportion of Cell Types Reaching the Outside Surface of a Biofilm

Since the edges of the biofilm contain the nutrients (Section 1.3), it is important to calculate the proportion of cells reaching the edges for each cell type. We define the edges of the biofilm as the grid sites at the edges of the bottom layer (Figure 3.1). For cell type 1, if $n_{e1}$ is the number of type 1 cells at the biofilm edge, then the edge proportion for cell type 1 is \( \frac{n_{e1}}{n_{e1} + n_{e2}} \).

Additionally, we also calculate the proportion of cells that reach the top surface of the biofilm for each cell type, to understand if one cell type is surrounding the other cell type from above. The top of the biofilm is composed of the cells that are at the top of each column in the biofilm (Figure 3.2). For cell type 1, if $n_{t1}$ is the number of type 1 cells at the top of the biofilm, then the top proportion for cell type 1 is \( \frac{n_{t1}}{n_{t1} + n_{t2}} \).
3.3 Spatial Segregation

Quantitatively representing whether a biofilm is intermixed, well mixed or segregated is vital. We define intermixed as cells that are alternating in space or grow on top of one another. In segregated biofilms, it is common that a cell grows amongst cells of its own kind and is separated from the other cell type. Well mixed is a biofilm that is neither intermixed or segregated. Since we run numerous trials per simulation, summarizing the spatial segregation for all the trials must be done quantitatively to compare different types of simulations.

Determining a metric for spatial segregation is challenging, because differences in spatial segregation between distinct simulations can be more influenced by the metric definition rather than the spatial positioning of the cells. How does one define spatial segregation? As mentioned in Chapter 1, we adapted a small scale spatial segregation measure from Gestal et al. [2]. Additionally, we define a large scale spatial segregation measure.
Figure 3.3: Cluster lengths for a cooperator computed from one vertical slice in the baseline competition simulation with $r_{10} = 0.05$ and $r_{20} = 0.1$.

### 3.3.1 Large Scale Spatial Segregation: Cluster Lengths

We quantify large scale spatial distribution by calculating how the cell types are segregated in clusters. Are they intermixed with miniscule clusters? Are they segregated in large clusters or small clusters? We define a cluster as a row of cells of one type. Cluster lengths are calculated by choosing four random vertical slices or cross sections parallel to the $(x,z)$ plane in the biofilm, and counting the cluster lengths of each cell type in the lowest and middle height levels within the vertical slice (Figure 3.3). The height of the vertical slices may differ from one another. Thus, the middle of the vertical slice is calculated for each particular cross section. We compute the average cluster length and a histogram of cluster lengths for a cell type.
3.3.2 Small Scale Spatial Segregation: Spatial Assortment

Adapted from Gestal et al. [2], spatial assortment is defined by the average proportion of type 1 cells that surround a type 1 cell ($F_{11}$) minus the average proportion of type 1 cells that surround a type 2 cell ($F_{21}$). To elaborate, for each cell in the biofilm, the number of cells of each type within a three-dimensional cube of an assortment radius ($R_a$), is computed. The proportion of type 1 cells to total cells (not including the surrounded cell) in the $R_a$ defined cubic neighborhood ($\frac{n_1}{n_1+n_2}$)is calculated and the average for all type 1 cells is calculated to obtain $F_{11}$. A difference in our metric compared to Gestal et al.’s [2] is they only examine the 2D top surface of the biofilm while we calculate spatial biofilm for the entire 3D biofilm.

The degree of spatial assortment is a value between -1 and +1. When the degree of assortment is 1, cell type 1 and 2 cells are completely segregated in space. When the degree of assortment is 0, cell type 1 and 2 cells are well mixed in space. When the degree of assortment is negative, cell type 1 and 2 cells are intermixed.

The radius $R_a$ used to calculate the level of assortment is a parameter we specify. However, the size of $R_a$ affects the degree of assortment measured. In other words, the degree of assortment will be computed as high when $R_a$ is very small because the cells directly next to a chosen cell will likely be its offspring. Conversely, when $R_a$ is large, most cells in the biofilm are included in the assortment measurement so the degree of assortment will be low. The size of $R_a$ used to calculate assortment should correspond to or not be larger than the interaction radius $R_i$ (Table 2.1) because then we are measuring segregation on the same scale that the cells interact with one another. However, we typically use an interaction radius $R_i = 3$ so if $R_a = 3$, $242 ((7)^2 - 1)$ cells would be included in the three-dimensional cube surrounding the chosen cell, which may be too large to measure spatial assortment. In Chapter 4, we will compare the spatial assortment measure for $R_a = 1, 2, 3$ and choose an $R_a$ value.
Chapter 4

Results and Discussion

In order to understand how social interactions spatially affect a biofilm and impact the proportion of cell types, we analyzed our simulations with the quantitative measures described in Chapter 3.

As described in Section 2.3, in the simple cheater and kin recognition simulations, a cooperator is growing with a cheater in the simple cheater simulation and a non-cooperator in the kin recognition simulation. Since cheaters and non-cooperators do not expend energy producing public goods, they have a higher baseline growth rate than the cooperators \((r_{20} > r_{10}\) from Table 2.1). To select the relative baseline growth rate of cheaters or non-cooperators to cooperators \((\frac{r_{20}}{r_{10}}\) from Section 2.3.1), we simulated biofilms exhibiting no social interactions except for baseline competition, where there are two cell types growing with different baseline growth rates. We chose to use a highest relative baseline growth rate \((\frac{r_{20}}{r_{10}} = 2)\) for all of our kin recognition and simple cheater simulations, since one can qualitatively discern a difference between the distribution of cheaters or non-cooperators and cooperators (data not shown).
4.1 Proportion of Cells in the Biofilm and on the Surface of the Biofilm

A key measurement in biofilm lab experiments, is the final proportion of a cell type at the end of the biofilm. Since we are interested in the impact that an introduced cheater or a non-cooperator has the population of cooperators, we present the results of the final, top and edge proportion of cooperators. All of the results are average proportions of 20 trials.

As expected, in both the kin recognition and simple cheater simulations, the proportion of cooperators increased as the degree of cooperation and initial fraction of cooperators increased (Figure 4.1). The total proportion of cooperators was higher overall for the kin recognition simulation than the simple cheater simulation. In fact, cooperators with kin recognition almost entirely populate the biofilm when they have a high degree of cooperation ($r_{11} = 0.5, 0.7$) whereas cooperators in the simple cheater simulation are slower to populate the biofilm because the cheaters can exploit the cooperators by taking advantage of the public goods without producing any. Since cooperators only benefit themselves when they exhibit kin recognition, they are at a lower risk of being taken advantage of by the non-cooperators, despite the non-cooperator’s faster baseline growth rate.

The proportion of cooperators at the top surface (Figure 4.1 c,d) closely mirrors the final proportion of cooperators (Figure 4.1 a,b). If cooperators are the overall advantageous population in the biofilm, it is expected that they are the cells that reach the top surface. Lab experiments use fluorescence to calculate the final proportions of each cell type in the biofilm. However, the fluorescence could be primarily capturing the top surface rather than the entire biofilm. Thus, it is reassuring that the proportion of cells at the top surface closely mirror the proportion of cells throughout the biofilm.

Cooperators with kin recognition reach the edges of the biofilm faster than the coop-
Figure 4.1: (a) Simple Cheater, (b) Kin Recognition (see Sections 2.3.2 and 2.3.3). The varying degrees of cooperation are $r_{11}, r_{21}$ for (a) and $r_{11}$ for (b), and varying initial ratios of the cooperator are $f_1$ from Table 2.1. From equation 2.2, $r_{10} = 0.05$, $r_{20} = 0.1$ for (a) and (b). For (a) $r_{21} = r_{11} = 0.1, 0.3, 0.5, 0.7$ and $r_{22} = r_{12} = 0$. For (b), $r_{11} = 0.1, 0.3, 0.5, 0.7$ and $r_{21} = r_{22} = r_{12} = 0$. The final and top proportions are shown (see Section 3.2).
operators in the simple cheater simulation (Figure 4.2). In both simulations, it is evident that the population of cooperators at the edges do not mirror the final population of cooperators in the biofilm (Figure 4.1). Cooperators grow to the outside edges of the biofilm at a slower rate than their growth throughout the entire biofilm. The initial ratio of cooperators and the degree of cooperation both impact the proportion of cooperators at the edges.

Figure 4.3 depicts the relative change in abundance of cooperators \( \frac{n_1}{n_1+n_2} \) from Section 3.1) from the start to the end of the simulations. In the kin recognition, when cooperators have a high degree of cooperation \( r_{11} = 0.5, 0.7 \), the relative change in abundance decreases when the initial fraction of cooperators increases, since the cooperators are already the dominant population. The relative change in abundance of cooperators from the onset of biofilm growth to the end of the simulation is higher in the kin recognition case compared to the simple cheater case because kin recognition provides an advantage to cooperators.
4.2 Spatial Segregation

4.2.1 Large Scale Segregation

To investigate large scale segregation, we examined the vertical cross sections of biofilms and calculated the cluster lengths of cooperators (see Section 3.3.1 and Figure 3.3). We first looked at baseline competition to determine how the relative baseline growth rate \( \frac{r_{20}}{r_{10}} \) was associated with the average cluster length (Figure 4.4). In the kin recognition and simple cheating simulations, \( \frac{r_{20}}{r_{10}} = 2 \), so we wanted to isolate how \( \frac{r_{20}}{r_{10}} = 2 \) affects cluster lengths to gain context when looking at the simulations with social interactions. All of the cluster lengths are averages of clusters found in 80 vertical cross sections (4 vertical slices for each of the 20 replications per simulation).

As \( \frac{r_{20}}{r_{10}} \) increased, the cluster lengths increased, only for the cell type with the larger growth rate \( r_{20} \). Intuitively, if population 2 grows at a faster rate, there will be more type 2 cells in the biofilm and they will occupy more space. Momeni et al. found that unequal-fitness competition communities formed segregated columns with the faster growing cell type dominating the top surface of the biofilm [5]. Our results are consistent with Momeni
Figure 4.4: Cluster lengths for baseline competition simulation. The parameters are \( f_1 = f_2 = 0.5, r_{11}, r_{21}, r_{22}, r_{12} = 0, r_{10} = 0.05 \) and \( r_{20} = 0.05, 0.075, 0.1 \) from Equation 2.1. 

et al.’s findings as the cluster lengths for the fastest growing cell type 2 formed clumps of 28 consecutive cells on average out of a maximum length of 50 cells in the biofilm (Figure 4.4). Additionally, the fastest growing population 2 \((r_{20} = 0.1)\) occupied 100% of the top surface in the biofilm.

Comparing kin recognition and simple cheating, the average cooperator clusters lengths for kin recognition are larger than the simple cheating simulations (Figure 4.5), indicating that kin recognition biofilms could be more segregated than biofilms with simple cheaters. Biologically, since cooperators with kin recognition only benefit themselves, non-cooperators do not benefit from being in close proximity to cooperators. Therefore, kin recognition biofilms will intuitively be more segregated than biofilms with simple cheaters.

Additionally, in both types of simulations, as the cooperation level \((r_{11}, r_{21} \text{ for (a) and } r_{11} \text{ for (b) from Equation 2.2})\) increased, the average cluster length increased. However, the cooperation level appears to have a stronger effect on average cluster length in the kin
Figure 4.5: (a) are the average cooperator cluster lengths for the simple cheating simulations and (b) are the average cluster lengths for kin recognition simulations. The parameters are the same as in Figure 4.1. Standard deviations are similar to those in Figure 4.4.

Figure 4.5: (a) are the average cooperator cluster lengths for the simple cheating simulations and (b) are the average cluster lengths for kin recognition simulations. The parameters are the same as in Figure 4.1. Standard deviations are similar to those in Figure 4.4.

Challenges with the Cluster Length Metric

In a vertical cross-section, a cell type may have large clusters of length 30-40, with smaller clusters of length 1-5. Due to this cluster length metric, the standard deviations are large (Figure 4.4) and the distribution of cluster lengths often have wide spread (Figure 4.6a).

When we decided whether the mean or median cluster length should be calculated for the metric, we chose the mean because the frequency of smaller clusters will often be larger than the frequency of larger clusters (Figure 4.6b), often leading to a very small median that may not be representative of the cluster length distribution. In addition to skewed distributions, cluster length distributions can also be bimodal, in which a median measure would discount the large cluster lengths. However, mean cluster length also has its caveats - it will be affected by possible outliers and the naturally wide spread of the
Figure 4.6: Cluster lengths distributions for select baseline competition simulations. (a) is a cluster length distribution for cell type 2 when \( r_{20} = 0.1 \). The spread of the distribution spans from cluster lengths of 1 to 50. (b) demonstrates why the median may not be an ideal measure to represent a cluster length distribution. The cell type 1 cluster length distribution \( (r_{10} = 0.05, r_{20} = 0.1) \) has a median of 2.5 and a mean of 7; the median discounts the larger clusters.

Therefore, for future work, we might use the top 10th percentile of a cluster length distribution, rather than a mean or median, for comparisons between simulations.

### 4.2.2 Small Scale Segregation

As mentioned previously, we adapted our spatial assortment measure from Gestal et al. [2]. In all of our simulations, as we increased the assortment radius \( R_a \), the spatial assortment decreased (typical behavior shown in Figure 4.7) because there are more cells included in the cubic section of the biofilm surrounding the chosen cell. \( R_a = 1 \) leads to high spatial assortment because the metric primarily captures the offspring of the surrounded cell. For all simulations, we use \( R_a = 2 \) to calculate spatial assortment (see Section 3.3.2).

Similar to the large scale spatial segregation metric, the initial ratio of cooperators \( (f_1) \) does not appear to affect the level of assortment. Figure 4.8 shows the spatial as-
Figure 4.7: As the assortment radius $R_a$ increases, the spatial assortment decreases. The simulation shown has the following parameters: $r_{11} = 0.7$ and $r_{21} = r_{22} = r_{12} = 0$. This trend is generalizable to all simulations.

Figure 4.8: Spatial assortment for a simple cheater and kin recognition when $f_1 = 0.5$. The cooperation parameter $(r_{11}, r_{21})$ and baseline growth rates $(r_{10}, r_{20})$ have the same values for kin recognition and simple cheating as Figure 4.1. The assortment radius $(R_a)$ is 2.
Figure 4.9: Proportion of cooperators surrounding a cooperator ($F_{11}$) and proportion of cooperators surrounding a cheater ($F_{21}$) for (a) simple cheater and (b) kin recognition simulation when $f_1 = 0.5$. The cooperation parameter ($r_{11}$, $r_{21}$) and baseline growth rates ($r_{10}$, $r_{20}$) have the same values for kin recognition and simple cheating as Figure 4.1. The assortment radius ($R_a$) is 2.

Consistent with the results with the cluster lengths, kin recognition has a higher spatial assortment measure than simple cheating in all of the cooperation levels, indicating that kin recognition may lead to more segregated biofilms than biofilms with simple cheaters. A spatial assortment ($F_{11} - F_{21}$ from Section 3.3.2) above 0 indicates segregation, with 1 being complete segregation. As the cooperation level increases, the spatial assortment measure increases until the cooperation level is 0.7, because more cooperators are growing in the biofilm and surrounding each other ($F_{11}$) increases (Figure 4.9a). When the cooperation level is 0.7, more cooperators are present in the biofilm and the average proportion of cooperators surrounding a cheater ($F_{21}$) increases (Figure 4.9b), so the overall spatial assortment decreases. These findings are consistent with Momeni et al.’s work [5]. They observed that the initial partner ratio does not significantly affect the level of intermixing and all communities that do not have two populations cooperating with one another display segregation.
Chapter 5

Conclusions and Future Work

This project offers a foundation to understand how social interactions impact spatial structure in a biofilm, and our mathematical simulation is the first three-dimensional simulation to model kin recognition. It is evident that social interactions spatially affect a biofilm, including the outside edges and the top surface of a biofilm, cluster lengths and spatial assortment. Simulations when a population “entrap” the other population horizontally as well as vertically by dominating the edges and top surface can potentially help determine the optimal conditions to develop a successful cheater or eventually, a “Trojan horse” cheater. It is possible that a toxin-inducing cheater would most efficiently kill a population of cooperators if the cheaters can “trap” them.

Additionally, kin recognition spatially affects a biofilm differently than simple cheating does, indicating the importance of understanding the behavior of cooperators exhibiting kin recognition. The initial fraction of a cell type does not appear to affect spatial segregation, consistent with prior literature [5].

Regarding future work, in order to further our understanding of spatial segregation, further metrics should be designed and tested. The cluster length metric is sensitive to the number of cells in the biofilm. For example, if the number of cooperators in the biofilm increases, it is expected that the cooperator cluster lengths will increase as well. The
spatial assortment metric may also be affected by the number of cells in the biofilm; if there are more cooperators in a segregated biofilm, the metric may accentuate segregation because more cooperators would surround a cooperator. Therefore, the metrics should be normalized to the number of cells in the biofilm or redefined so they are not sensitive to the number of cells. Furthermore, it is unclear if a negative spatial assortment metric truly represents an intermixed biofilm from a lab experiment. Overall, spatial segregation is challenging to quantify and the next priority should be to develop better metrics.

Additionally, Momeni et al. [5] and Gestal et al. [2] found that a high initial cell density (\(f\) from Table 2.1) can lead to intermixing between populations and changes in spatial assortment. Further simulations can vary overall cell density to investigate how spatial structure is affected.

Another possible continuation of this project is to simulate a “Trojan horse” cheater [1]. The proposed “Trojan horse” strategy has not been tested in a fully spatial system. So how do “Trojan horse” cheaters spatially affect a biofilm? What are the optimum conditions in which a cheater can disrupt a biofilm, while coexisting with a cooperator that exhibits kin recognition? The trends from the simulations can be compared to the lab experiments to help identify the parameter ranges in the simulation that match the lab experiments. Once we attempt to accurately represent the strain conditions (degree of cooperation, competition, toxin range etc.) from the lab experiments in the simulation, we can attempt to engineer the optimum “Trojan horse” cheater. If successful, this novel evolutionary strategy could help eliminate microbial and yeast infections.
Bibliography


