Evaluating biofilm prevention strategies with agent-based modelling

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1 Introduction

Bacterial biofilms form when free-floating planktonic microorganisms attach to wet surfaces in clusters, creating a microbial assemblage of cells. Such structures allow for the transfer of nutrients via an extracellular polymeric substance (EPS) secreted by participating microbes [6]. The resulting EPS matrix acts as a 'glue' which holds cells together granting the biofilm structural resistance to shear stress, and protection against other unfavourable environmental factors such as (lack of) water, extreme pH levels, and substances toxic to microorganisms [8].

Although common examples of biofilm formation include the relatively harmless dental plaque on teeth and algae on submerged rock structures, unintended biofilm development can also occur on medical indwelling devices such as catheters or artificial organs and implants [8]. Biofilm growth on such devices can lead to bacterial biofilm infections, implying a large range of health complications and increased healthcare costs for patients [21].

While various strategies for preventing biofilm formation exist, biofilms' resistance to antibiotics and environmental protection from the EPS matrix means there is still no general approach to thwarting biofilm growth. That being said, relatively new research has paved the way for novel anti-biofilm technologies which aim to combat biofilm formation [4]. In this report, we propose and make use of an agent-based model (ABM) to assess and evaluate the effectiveness of select methods for biofilm treatment, ultimately aiming to provide an answer to the question: what are some effective interventions for reducing biofilm formation?

1.1 Literature review

The majority of early biofilm formation models were mathematical systems which solved partial or ordinary differential equations representing dynamical biofilm growth [15]. Following these, cellular automaton models were developed to explore the diffusive growth of biofilms, but the models were restricted to discrete space and time [2]. The introduction of *BacSim* in 1998 by Kreft et al. led to a paradigm shift in biofilm modelling —the seminal paper developed a state-of-the-art individual-based model for microbial colonies [10].

BacSim simulated colony growth and assemblages indirectly —that is, colony growth (and biofilm formation) was to be observed as an emergent property of simulation without explicit modelling. Since BacSim, the increased popularity of cell-based simulation has led to the development of more complex models that focus on extending cell representation, such as *iDynoMiCS* [11] and others [1].

In the current landscape of biofilm ABMs, most models tend to focus on simulating the emergent property of biofilm growth and do not explore implementations for biofilm treatments. Therefore, our report aims to explore implementations of strategies for biofilm prevention and formation.

2 Model design

The model described in this report was built with the modelling software NetLogo [19]. For clarity,

transparency, and to justify how our model is built to serve our research question, we follow the Overview, Design concepts, and Details (ODD) format proposed by Grimm et al. [7] to describe our model.

Purpose

The purpose of our model is to explore different strategies for reducing, limiting, and preventing biofilm growth. By simulating the dynamic formation and growth of biofilms alongside various treatments, we aim to use the model to answer the aforementioned question: what are some effective interventions for reducing biofilm formation?

Entities, state variables, and scales

Our model has two entities: microbes and environmental land (known in NetLogo as 'patches'.) Our model environment is described by an enclosed two-dimensional 300×200 grid of patches, where each square is characterised by either belonging to the substratum; the (assumed) aquatic surrounding environment; or is an EPS square forming a larger EPS matrix. Microbes (agents) move in continuous time with a cell-specific speed, and can be free-floating, exhibiting approximate Brownian motion; attached to the substratum; or submerged in EPS. In our model, one time-step is equivalent to roughly four minutes. See Appendix A.1 for the relevant derivation.

Process overview and scheduling

Figure 1 displays the general process flow of our model. Agents who are free-floating or submerged in EPS exhibit approximate Brownian motion, and may move uninterrupted in one direction or collide with the model walls, substratum, or other agents. Global updating of the model state is done in varying time-step increments —i.e., the model exhibits continuous time. All cells periodically emit and receive quorum-sensing molecules (QSMs) or *autoinducers* which trigger EPS secretion of attached molecules.

Free-floating cells that collide with the surface have a chance to attach to the substratum and stop moving, potentially signaling the beginning of the biofilm formation process. Cells that are temporarily attached to the surface may detach or permanently (irreversibly) attach with a probability. Irreversibly-attached agents have a much lower (but still nonzero) probability to detach over time. Once agents are irreversibly attached to the substratum, if the cell receives an autoinducer that binds to the cell's receptor, EPS secretion is genetically triggered, and the cell releases a radius of EPS.

Upon entering and leaving an EPS-submerged area, microbe speed is dramatically reduced and restored, respectively. Within an EPS matrix, cells have a greatly increased chance of irreversible attachment, and either randomly explore (and remain inside) the matrix, or, more often, head towards the substratum to simulate shoving: competition for nutrients and space.

Design concepts

Emergence. The structural make-up and formation of biofilms in our model is a non-deterministic process that arises solely from modelling individual cells, which exhibit behaviour determined by simulated competition for substratum nutrients and the reception of autoinducers. This emergence of diffusive growth is the basic principle explored in our model.

Observation. We make use of the following metrics within the model to evaluate biofilm formation: the mean shortest distance to every other microbe; the proportion of agents who are free-floating; and the proportion of EPS patches.

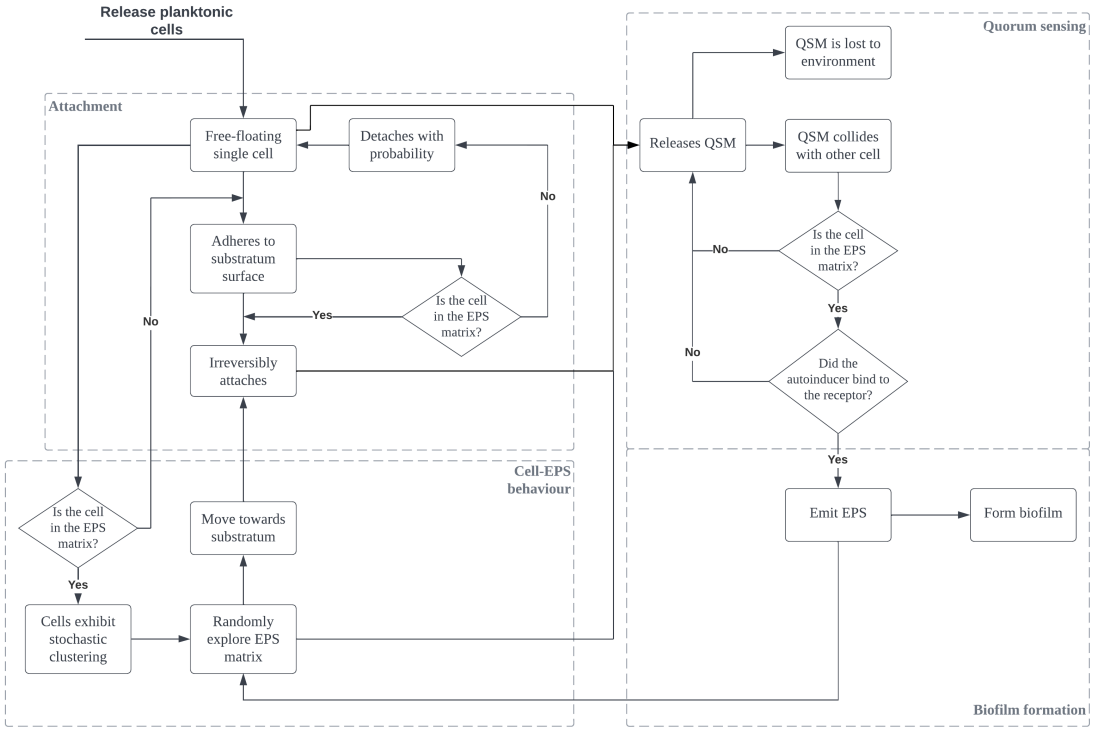

Figure 1: Cell behaviour, flow, and intentions in the NetLogo model.

Interaction. Agents exhibit direct interaction when they collide with one another, prompting a re-calculation of their trajectories and speeds. Indirect action with other agents occurs when cells enter an EPS matrix built by one or more preceding agents. Microbes also exhibit EPS secretion due to the absorption and binding of QSMs emitted by at least one other microbe — this is another example of indirect interaction.

Stochasticity. Our model heavily features randomness to replicate the complex interactions between microbes and their real environments. Agents begin with random speeds and headings, and the majority of behaviours are functions of probabilities: reversible and irreversible attachment occur with a certain probability for each agent, along with QSM emission and EPS secretion.

Initialisation

In our model, agents begin with random headings and speeds. Patches below a set height are coloured to indicate they are part of the substratum; in our model this height is fixed at an arbitrary value, $y = -75$, as varying the model space is unrelated to our research topic.

Input data

Our model solely relies on parameters which are user input. See Appendix B.1 Table 1 for default parameter values.

Submodels

Free-floating agents and Brownian motion. Our model implements and extends the GasLab Circular Particles model [20] to simulate Brownian motion and momentum-conserving collisions: agents who are free-floating or submerged in EPS continuously have their trajectories evaluated, and the model is updated in time-steps (or *ticks* in NetLogo) t such that $0 < t \leq 1$, such that $t < 1$ if a collision will take place in less than one global tick, and $t = 1$ otherwise.

Reversible and irreversible attachment. Each time

a microbe collides with the substratum, it has a user-defined probability to *reversibly attach* to the surface. This is the beginning of the biofilm lifecycle [6], and occurs because of physical phenomena, such as van der Waals forces. This temporary attachment can either lead to detachment or *irreversible attachment*.

As our model makes use of continuous time, we evaluate the decision to detach or irreversibly attach a reversibly-attached microbe every 15 time-steps. Cells may detach from the wall at the same speed upon their collision. Irreversible attachment simulates the more permanent adhesion of a microbe to a substrate, the second main component of biofilm formation. Irreversibly-attached cells may secrete EPS upon receiving QSMs from other cells, and may only detach if they have not secreted EPS. Their detachment is evaluated every 100 time-steps.

Quorum sensing and EPS secretion. When cell density reaches a sufficiently high threshold, microbes emit EPS to form a matrix which acts as the basis of biofilms. In microbiology, this is known as quorum sensing (QS) [18]. Cells do not observe their environment directly, however. Instead, they communicate with one another via the production, release, and detection of QSMs or autoinducers. Changes in the concentration of autoinducers allow cells to alter patterns of gene expression, triggering, for example, EPS secretion [14].

In our model, quorum sensing is recreated by the following process: every 15 time-steps, cells may emit a QSM (autoinducer) that travels at a high speed for a short period of time before being 'lost' to the environment. Every 2 time-steps, cells are able to detect and absorb surrounding QSMs within a radius or via direct collision. To simulate concentration detection, each absorption of a QSM by a cell may cause the microbe to emit EPS within a radius. In the case that a cell absorbs a QSM and is already in an EPS matrix, it is guaranteed to secrete EPS.

Microbe behaviour in EPS matrix. Cells within EPS matrices will randomly explore the matrix 25% of the time, and will otherwise face toward the substratum, attempting to reach and attach to the surface. This latter behaviour aims to simulate competition for nutrients from the substrate, and competition for space (shoving).

3 Methods

In total, we examine four interventions for biofilm formation: two known (or *classical*) approaches [8], and two relatively novel treatments [4]. We conduct sensitivity analysis to observe how attachment and quorum sensing probability parameters impact our model output. Finally, we briefly perform robustness analysis to examine how the quorum sensing submodel affects our results.

3.1 Experiments

For experiments that did not involve chemical doses, simulations were run to a maximum of 3,000 time-steps. Otherwise, the time of supplying the dose was varied in 500 time-step increments, and simulations were halted 1,000 time-steps after administration. For further explanation of our methodology, see B.1 and Table 1 of Appendix B. Demonstrative images are given when applicable for each treatment.

Experiment I. Antibiotics

Antibiotics are a common treatment prescribed to patients suffering from biofilm growth on indwelling devices. For this experiment, we release antibiotic cells that seek and kill bacterial cells with an 80% efficacy. To simulate the high resistance of bacterial biofilms to antibiotics [8], we implement a cumulative global resistance to antibiotics incremented when antibiotics fail to kill a microbe.

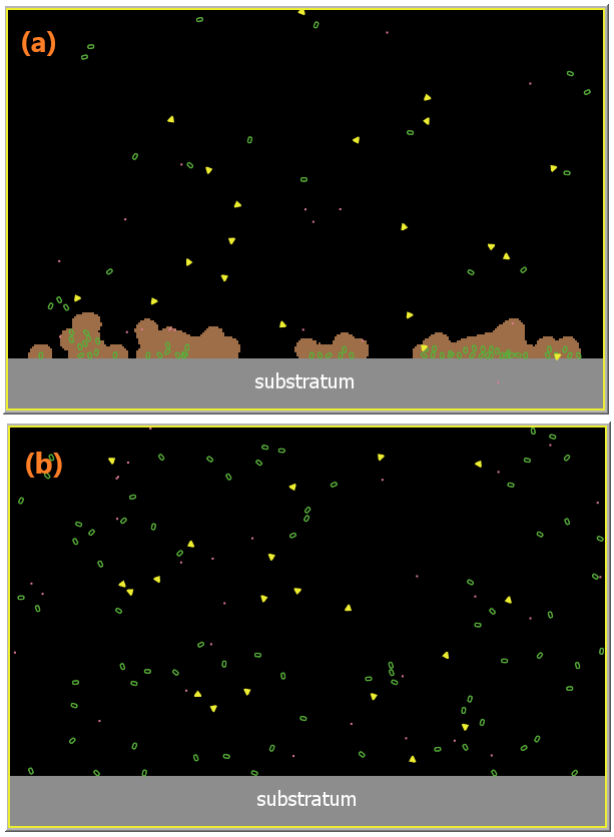

Figure 2: **a.** Antibiotics (yellow triangular cells) administered after biofilm maturation. **b.** Antibiotics administered prior to biofilm formation. Images were captured at similar time-steps.

Experiment II. QS inhibitors

To limit the growth, spread, and development of biofilms, targeting the quorum sensing process was popularised as a biofilm infection intervention at least 18 years ago [22]. By introducing inhibitors that limit QS processes and thus EPS emission, QS-targeting particles have been shown to decrease biofilm formation [22]. To simulate the affect of these QS-inhibiting cells, we variably decrease the QSM absorption (EPS emission) chance.

Experiment III. Matrix-targeting enzymes

Rather than focusing on microbes, a relatively novel technique is to introduce enzymes into patients that can target the EPS matrix, disrupting biofilms by weakening structures, ultimately leading to a dispersion of microbes. In our model, we simulate adversarial agents with limited lifespans that seek and destroy EPS patches.

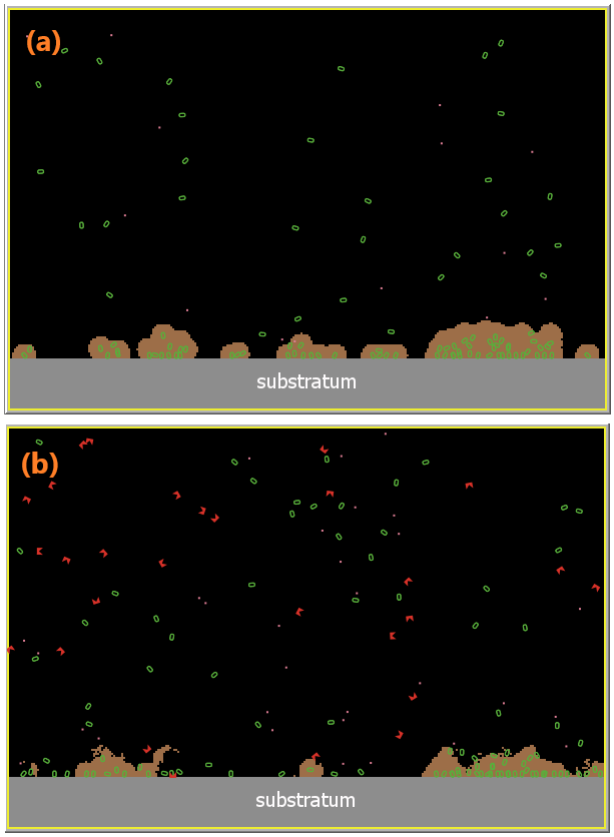

Figure 3: **a.** Mature biofilm formation prior to treatment. **b.** Deterioration of EPS matrix and dispersion of microbes after matrix-targeting enzymes (red shapes) administered to system.

Experiment IV. Bactericidal/Bacteriostatic Coatings

Anti-biofilm coatings directly alter the substratum: before the embedding of devices, chemically distinct coatings can be applied to surfaces which aim to limit attachment of microbes thereby preventing biofilm development. We implement this in two distinct ways: (1) by directly decreasing the reversible attachment probability; (2) by creating a layer on the exposed substratum that decreases attachment probabilities, incrementally wearing off over time and with microbe collisions.

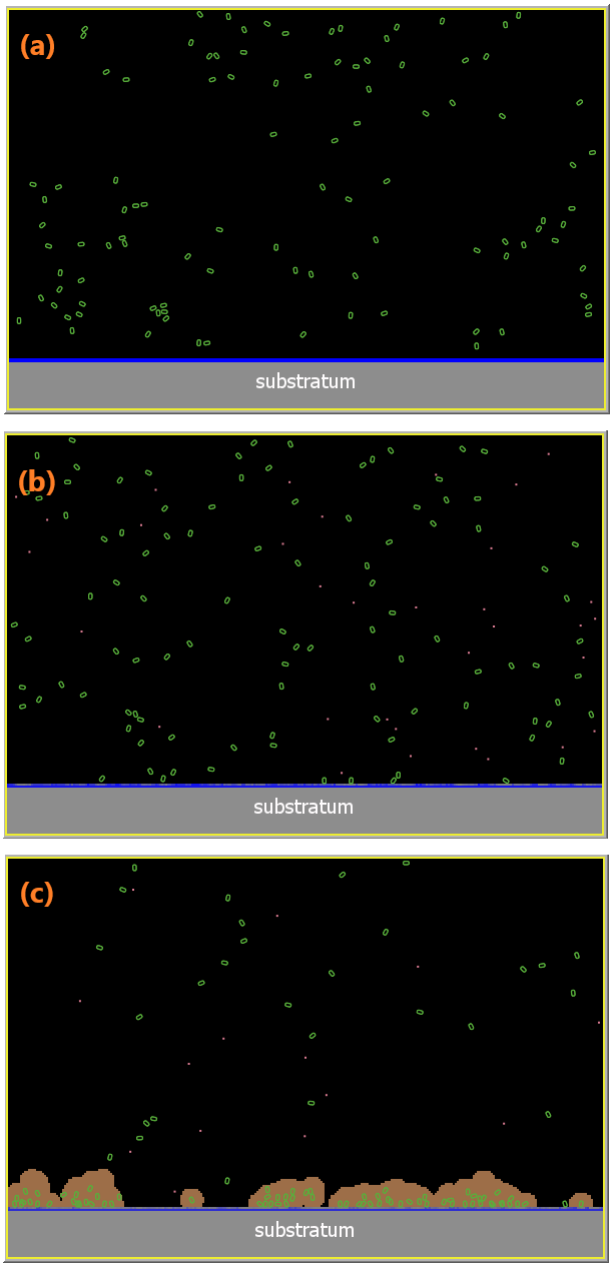

Figure 4: Strategy (2). **a.** Initial state of coating. **b.** Deterioration of coating owing to time and collisions. **c.** Biofilms forming after deterioration of coating.

3.2 Sensitivity analysis

To examine model sensitivity to user-defined variables, we examine the following sets of parameters, terminating simulations at 2,000 time-steps.[1] Parameter values were generated by Sobol sequences to create 64 unique parameter-value pairs.

Attachment probabilities. We vary the reversible and irreversible attachment probabilities for microbes.

Quorum-sensing procedures. We vary the chance for particles to emit QSMs and the propensity for autoinducers to bind to cell receptors.

3.3 Robustness analysis

We perform robustness analysis by comparing biofilm formation times[2] with a simpler model under identical parameters. The simpler model omits the QS submodel outlined in Figure 1. Instead, microbes may secrete EPS if they a sufficient number of neighbouring microbes (see Appendix C for full implementation details).

4 Results

4.1 Sensitivity and robustness analysis

Figure 5 displays results for sensitivity analysis and Figure 6 displays model comparisons for robustness analysis.

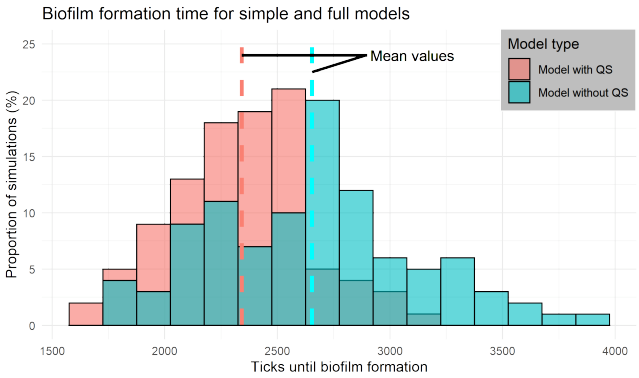

Figure 6: Time-steps needed to reach mature biofilm growth. Dashed lines represent mean time-step values.

4.2 Experiments

Figures 7 to 13 display results for the outlined experiments. Figure 12 compares the effect of antibiotics and matrix-targeting enzymes.

[1] By observation and analysis, it was discovered that most biofilms had visually formed under Table 1 parameter values by 1,500 time-steps.

[2] By experimentation, we judged the majority of biofilms to be 'mature' (sufficiently large) when the mean distance to each microbe's closest neighbour was ≈ 7. Thus, simulations were halted when this condition was satisfied.

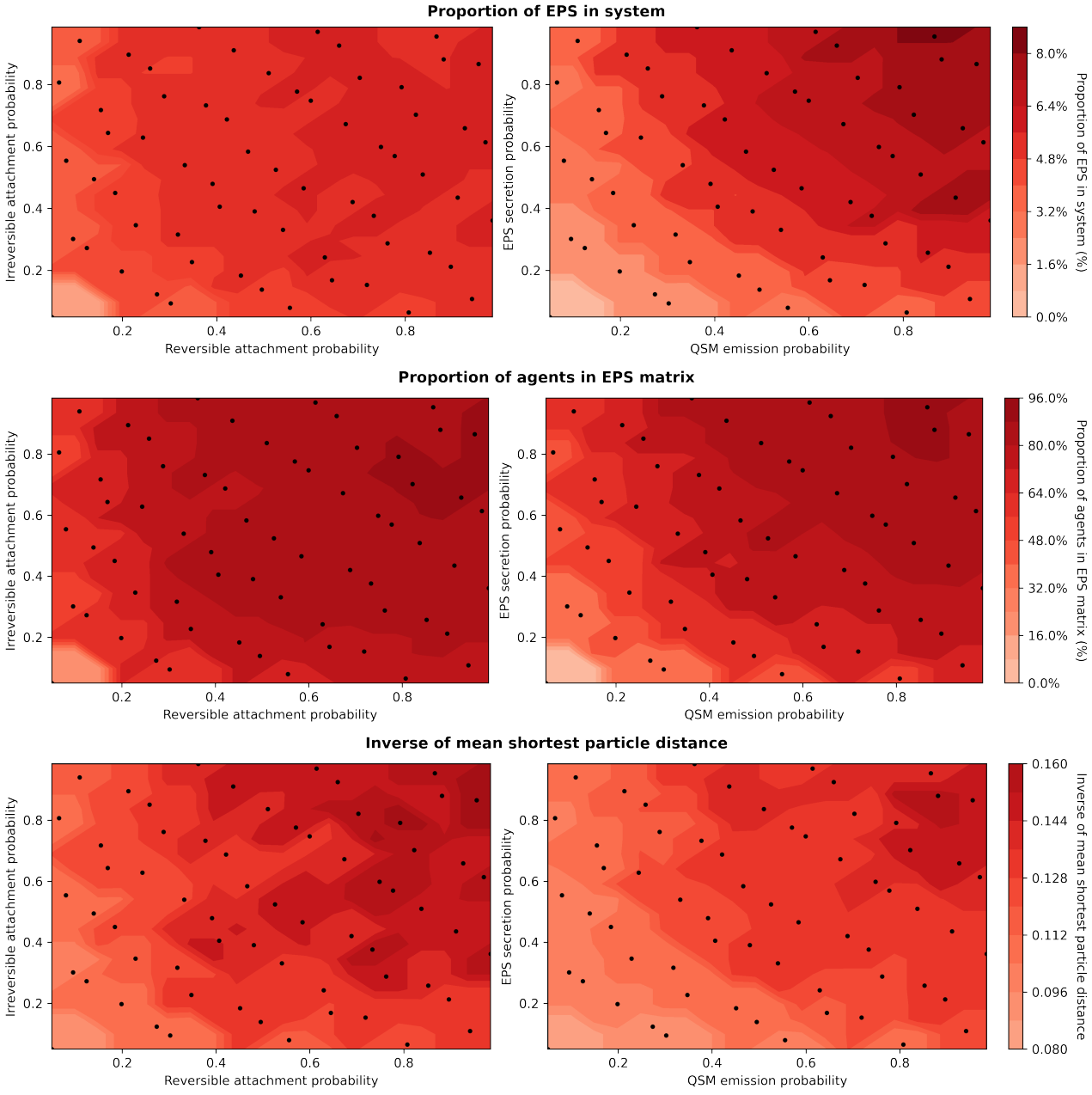

Figure 5: Resulting sensitivity of model after varying probabilities for attachment and QS submodels. Sensitivity of model output using proportion of EPS patches as metric (top row), number of microbes submerged in EPS upon simulation end (middle row), and inverse mean of shortest distance to each microbe's closest neighbour (bottom row).

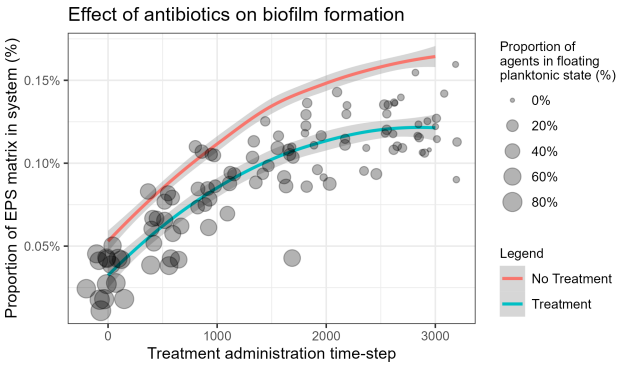

Figure 7: Experiment I. Metrics for antibiotics 1000 time-steps after administration. Lines are mean values.

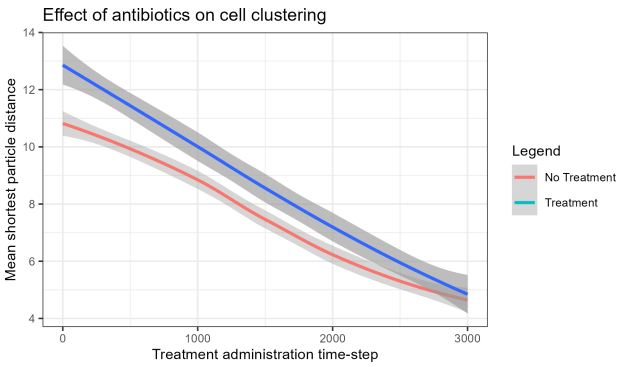

Figure 8: Experiment I. Average cell separation 1000 time-steps after administering antibiotics.

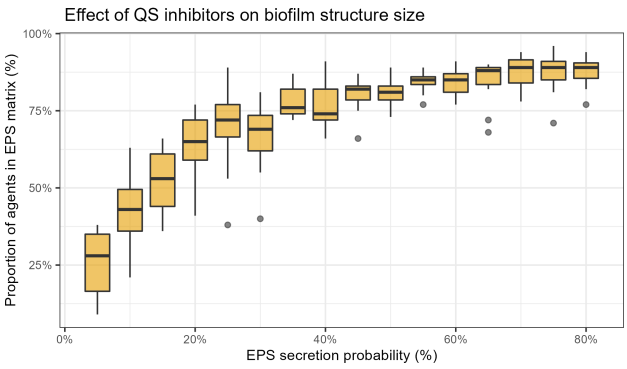

Figure 9: Experiment II. Boxplots for QS inhibitors affecting biofilm sizes.

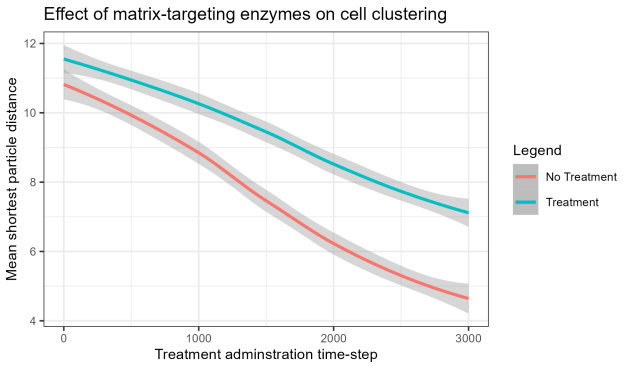

Figure 10: Experiment III. Average cell separation 1000 time-steps after administering matix-targeting enzymes.

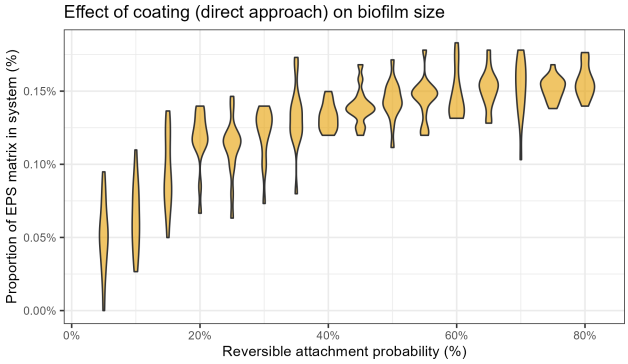

Figure 11: Experiment IV (a). Direct approach for coating affecting biofilm sizes.

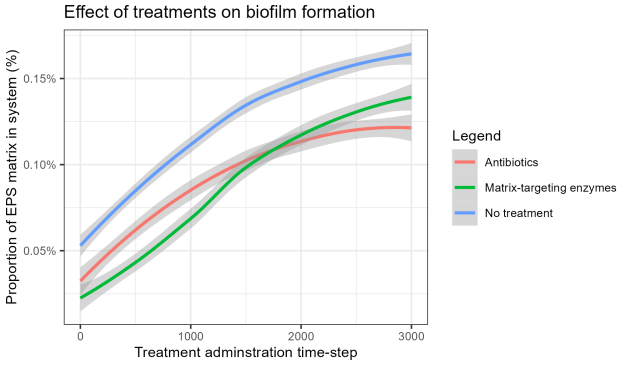

Figure 12: Experiment I & III. Comparing impacts of antibiotics and matrix-targeting enzymes.

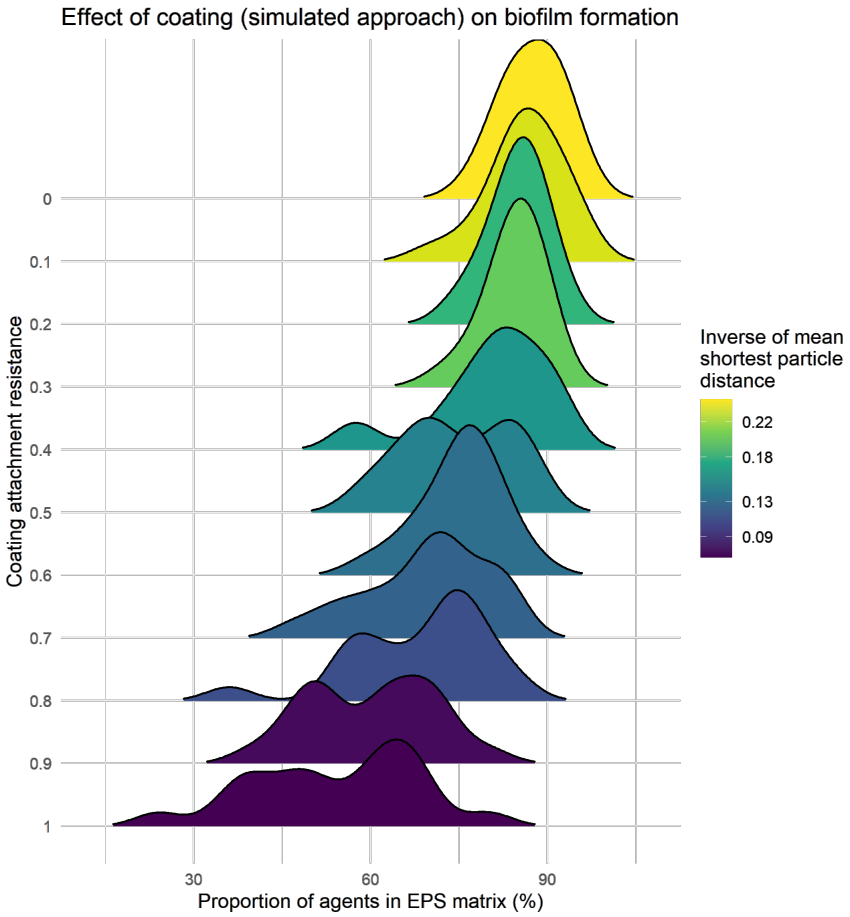

Figure 13: Experiment IV (b). Metrics for simulated approach of waning coatings.

5 Discussion

5.1 Model robustness

The model that omitted the QS submodel in favour of a simpler process exhibited a wider, flatter distribution of biofilm formation times and a mean formation time of 2654 time-steps in comparison to the full model's 2343 time-steps. The difference between mean formation times between the two models was statistically significant ($p < .001$), demonstrating the impact of including the QS submodel.

5.2 Sensitivity analysis

The top row of Figure 5 demonstrates that the model is less sensitive to changes in attachment probabilities: when quorum sensing is more likely, the model exhibits larger biofilm structures along a more gradual gradient, in comparison to the relatively constant amounts of EPS present when varying attachment chances. In other words, regardless of attachment probabilities, given enough time, microbes will eventually attach and form biofilms. In the other scenario, when QSM concentration is higher, agents who have recently joined an EPS matrix can immediately secrete EPS and grow the biofilm structure outwards, leading to an increased rate of biofilm growth and larger structures.

The second metric in Figure 5 highlights a key difference between varying attachment rates and QS likelihoods: although there is less EPS in the system when attachment chances are varied, agents join and form smaller biofilms faster than systems with relatively slow QS processes. Clustering of microbes also increases faster with attachment probabilities, as demonstrated in the third set of images —in the case of rapid QS processes, an overall higher propensity to secrete EPS

implies microbes will grow biofilm structures outwards, leading to larger structures, but with fewer participating microbes.

5.3 Experiments

The addition of microbe-targeting antibiotics systematically reduces the concentration of EPS in the system post-administration, as shown in Figure 7. Antibiotics are also able to lower microbe clustering in biofilms, but they appear to become less effective the later they are administered, as shown in Figure 8. This is in line with current research, which suggests antibiotic treatments for biofilm infections are only useful when they are early and aggressive [9] otherwise the dosage needed to counter mature biofilms and reach effective concentration levels is toxic [8].

Figure 9 demonstrates how QS inhibitors can reduce the proportion of agents in biofilm structures and lead to smaller biofilm formations over a constant time. However, significant reductions in biofilm formations occur mostly when the rate of EPS secretion is low ($< 30\%$) and biofilm formation is relatively stable until the inhibiting factor surpasses $\approx 65\%$. Achieving such high inhibiting factors is unrealistic, and difficult to match to real-world data [4].

Predictably, treating biofilm infections with matrix-targeting enzymes leads to an overall decrease in EPS after administration, and is more effective than antibiotics when biofilms are mature, as demonstrated in Figure 12. The higher effectiveness of enzymes reducing clustering in mature biofilms is also evident in Figure 10, conveying how the enzymes are able to disrupt a larger matrix and disperse microbes. While these *in silico* results are promising, the strategy is not a well-established technique, and its administration may lead to unintended chemical harm in humans [4].

The direct approach for the fourth strategy examined in this report is able to reduce EPS concentrations by $\approx 30\%$ as exemplified in Figure 11, but the implementation assumes a coating which never deteriorates. Correcting for this assumption, the simulated approach of varying coating efficacies is able to decrease cell clustering and biofilm formation, as shown in Figure 13. As the coating gets 'better,' microbes are less likely to attach to the substratum and form biofilms, and the distributions of agents in biofilms appear to become flatter.

This flattening can be explained by the formation process on a coating: with a more effective coating, biofilms are unlikely to uniformly develop on the substratum. However, if a small section of the coating wears off and becomes exposed, microbes can attach to the area and foster growth by secreting EPS, forming biofilms that are taller and more irregular, albeit smaller in area.

While coatings such as the ones simulated in the non-direct approach are promising *in silico* and *in vitro*, the most effective coatings to prevent attachment also appear to be the most disappointing *in vivo*: they are prone to wearing off quickly and causing harmful side effects such as cell death and the inactivation of important proteins [16].

In general, the effectiveness of the four investigated treatments varies. Antibiotics are a valid method of treatment, but as is the case with clinical results, treatments appear to only be impactful when administered early. While early detection of biofilm infections in patients is generally impossible, new research into rapid detection methods are works in progress [12]. QS inhibitors in our model achieved arguably the greatest success at limiting biofilm formation, however —as noted —the inhibiting factors that led to such success are unrealistic, and are incomparable to real-world data.

While this makes QS inhibitors somewhat less applicable than other treatments examined in this report, it has been shown that QS inhibitors can increase the susceptibility of bacterial biofilms to antibiotics [5], so a hybrid treatment could be investigated. In our model, matrix-targeting en-

zymes had a significant impact on biofilm growth, and were more effective than antibiotics when biofilms were mature. Despite these results, the effectiveness of matrix-targeting enzymes *in vivo* is still an ongoing area of research; the potential of the enzymes being limited by their harmful side effects [3].

Both approaches to bactericidal and bacteriostatic coatings demonstrated a decrease of cell clustering and biofilm structure sizes when coatings were effective at limiting attachment. Although substratum coatings have promising model results and limited patient interaction, *in vivo* results show an underwhelming effectiveness owing mainly to cell death, oxidation damage, and other harmful side effects. That being said, it is not immediately clear if there is any current research comparing the potential side effects of each of these treatments against one another, so it is difficult to say how preferable coatings truly are to other interventions.

5.4 Model limitations

The above exploration into two classical and two novel interventions for treating biofilm formation serves to investigate the general effects of treatments on emergent biofilm formation. In our analysis, we highlight the effectiveness of each method in addition to its apparent disadvantages. However, we cannot directly comment on the biological accuracy of the impacts observed for each treatment explored in this report, as the model has various limitations.

Firstly, our model assumes a single-substrate, single-species biofilm, which is unrealistic [9]. The motion by which microbes move is modelled after gas particles, and the model lacks dynamic fluid simulation, which impacts growth patterns of EPS matrices [17]. We also do not model complex biological interactions between bacteria and the substratum, such as substrate uptake, nutrient transportation within biofilms, growth, replication, erosion of the substratum, and more [11].

Instead, our model attempts to encode these interactions directly via microbe behaviour, such as veering microbe headings toward the substratum to simulate nutrient-seeking behaviour. Lastly, we note that apart from visual assessment, we lack concrete metrics to determine whether biofilms have sufficiently 'formed' in simulations, and instead use the heuristics outlined in this report to judge formation. Our model could be extended upon by implementing at least some complex behaviour described above, exploring more accurate metrics for biofilm growth, and exploring mixed biofilm treatments (i.e., combining experiments).

6 Conclusion

In conclusion, research into treatments for mitigating the growth and formation of bacterial biofilms is ongoing, and the use of agent-based modelling approaches for simulating such treatments is an active yet relatively quiet field of research. In this report, we explored the implementation of four interventions for treating biofilm growth in our simple model, and evaluated the effectiveness of each approach, examining real-world implications of model results.

While we were not able to directly extrapolate our model results to real-world applications, we intend for the analysis of the investigated treatments to serve as benchmarks for more extensive models that may simulate similar treatments. In our discussion, we detailed limitations of our model and highlighted areas for development, with the intention that the intuition for our simple model may be extended to account for some of these limitations.

Finally, while no strategy for reducing biofilm formation was deemed to be the most effective, our evaluation concluded that all interventions —at least in our simple model —were effective to varying degrees with their respective drawbacks.

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Appendices

A Model

A.1 Processes

The equivalence of one time-step in our model is based on real-world data: first, we determined the average number of time-steps taken to form mature biofilms under parameters in 1. This was determined to be 2,343 time-steps. Next, we scaled real-world biofilm formation times to this number: biofilm formation is random, but usually begins within 2 days, and is mature by 4–8 days [13], and so we assume 6 days for growth.

Therefore:

$$\begin{aligned} 2343 \text{ ticks} &= 6 \text{ days} \\ 1 \text{ tick} &= 0.002561 \text{ days} \\ 1 \text{ tick} &= 3.688 \text{ minutes} \approx 4 \text{ minutes} \end{aligned}$$

B Experiments

B.1 Processes

For treatments that occurred at specific times (e.g., to release adversarial agents) simulations were run until the time-steps exceeded (time of administering dose + 1000). All time-specific treatments were compared to 'baseline' simulations run under Table 1 parameter values, terminating at times $\{1000, 1500, \ldots, 4000\}$. Non-time-specific experiments terminated at 3,000 time-steps.

Experiment I. 20 antibiotic agents were supplied to the system at different times to seek and kill microbes with an 80% probability. Microbes began with a 10% resistance to antibiotic attacks. If adversarial cells could not kill a microbe, all bacteria gained a 10% increased resistance to antibiotic attacks.

Experiment II. The chance for microbes to emit EPS when absorbing a QSM was varied from 5% to 80%, simulating varying concentrations of QS inhibitors.

Experiment III. 20 matrix-targeting enzyme agents were supplied to the system at different times to seek and destroy EPS matrix patches, dispersing submerged microbes. After destroying 100 patches, the adversarial agents would die.

Experiment IV. (1) The reversible attachment chances for microbes was varied from 5% to 80% to simulate coatings of various effectiveness; (2) the coating was applied to the substratum at the beginning of the simulation, and the dampening factor of the coating was varied from 0% to 100%.

B.2 Parameters

The following parameters were held constant during simulations unless stated otherwise.

Table 1: Parameter values for experiments.

Parameter	Value	Description
`initial-number-particles`	100	Number of initial planktonic, free-floating microbes
`rev-attach-chance`	70%	Chance for cells to reversibly attach to substratum
`irr-attach-chance`	5%	Chance for cells to irreversibly attach to substratum
`detach-chance`	20%	Chance for reversibly-attached cells to detach from substratum. Irreversibly-attached cells have a detach rate of ≈ 7 times less than this
`emit-qsm-chance`	15%	Chance for all microbes to emit QSMs at regular time intervals
`emit-eps-chance`	70%	Chance for irreversibly-attached cells to emit EPS

C Robustness analysis

To perform basic robustness analysis, our simpler model omitted the full model's quorum sensing submodel in favour of a radial-based approach. When microbes had at least two neighbours within a radius of four patches, the EPS secretion process was triggered (i.e., they were given a chance to secrete EPS).