Agent-based modelling of biofilm formation and inhibition in *Escherichia coli*

Anusha Modwal* and Shrisha Rao

International Institute of Information Technology, Bengaluru 560 100, India

Biofilm formation by bacteria such as *Escherichia coli* is a serious challenge faced in the treatment of infections. Biofilms provide a protected environment for the pathogens, where they may persist despite environmental adversities and treatments causing chronic infections. Furanones, both naturally occurring and synthetic, have been found to inhibit biofilm formation. An agent-based model of the behaviour of *E. coli* with regard to formation and inhibition of biofilms, is described here. Analytical tools used in this article allow us to find the optimal range of inhibitor concentration for Gram-negative bacteria. This is made possible by appropriate mathematical analysis, reducing the need for laborious experimental verification. The results are seen to be consistent with published experimental data on biofilm thickness of *E. coli* when acted upon by furanones. Our model permits estimation of concentration of the inhibitors needed to properly curb biofilms. This in turn has therapeutic implications, in that it may help formulate strategies to prevent the formation and growth of biofilms, especially in the context of devices placed inside the body, like catheters and implants.

Keywords: Agent-based modelling, biofilm, *Escherichia coli*, furanones.

BIOFILM is a protective layer formed by bacteria. When a significant number of bacteria are present, chemical signals referred to as quorum sensing molecules (QSMs) are released by the bacteria, which act on other bacterial cells to initiate the process of quorum sensing, by which the cells become upregulated and trigger activity and release of more QSMs. Under suitable conditions, a cluster of bacteria may adhere to a surface and secrete an extracellular polymer matrix called a biofilm, which covers and protects the entire cluster[1,2].

In a cluster of bacterial cells, quorum sensing is the mechanism by which majority of the bacteria decide to initiate the process of biofilm formation. It is an intercellular signalling mechanism involving receiving and producing of bacterial signalling molecules by which bacteria can communicate and coordinate gene expression. Quorum sensing can initiate regulatory pathways and affect gene expression in multiple bacteria within the biofilm[1]. In addition, the upregulated cells produce extracellular polysaccharide (EPS) material that forms a matrix, which in turn provides a protected environment for the bacteria. Bacteria in a cluster protected by the biofilm are also called persister cells – they remain protected and can survive antibiotics, attacks by white blood cells, and other hostile conditions. Such protected bacteria may be repeatedly released from under the biofilm in planktonic form, to cause recurrent or persistent infections in the host. Biofilm formation requires intercellular communication within the cluster of cells for coordinated behaviour. This communication is affected by QSMs such as AHL (acyl homoserine lactone), AI-1 (autoinducer-1) and AI-2 (autoinducer-2), which are key to the formation and sustenance of biofilms.

Bacteria live in communities and form biofilms in order to protect themselves from environmental attacks. Biofilm formation is a protective mechanism of bacteria which decreases their susceptiblity to antibiotics and antiseptics[3,4]. Infections can become untreatable and chronic due to repeated release of planktonic forms of bacteria from biofilms, where they remain protected from antibiotics and immune system of the host[5]. Biofilm infections, in relation to catheters, implants, and other medical devices are a cause of serious concern, as they may result in persistent infections, prolonged illness, high treatment costs, and at times the need for surgical removals and change of devices.

Agent-based modelling[6] explores the individual and collective behaviours of agents, typically in natural systems which obey certain rules. One of the earliest agent-based models described how agents interact in a system, and the expected outcome[7]. It was later established that agent-based modelling helps us understand the systems better, which in turn allows us to design a better approach considering the behavioural patterns of the defined system and its individual agents[8,9]. Agent-based models are applied in many fields, such as studying and simulating crowd behaviour in emergencies[10], computer networking[11], prediction and analyses of air-cargo demand[12], business analyses[13,14], auction theory[15], social science[16], and cloud computing[17].

Agent-based modelling has also been used extensively in the biological sciences; for instance, in bioinformatics[18], and in biomedical applications such as inflammation and the human immune system[19], and to study brain

*For correspondence. (e-mail: anusha.modwal@iiitb.org)

tumours[20]. Agent-based or individual-based models are also used for bacterial systems[21], to help understand emergent properties of bacteria at the population level given their individual behaviours. The study of bacterial coordination, communication and cooperation as required for biofilm formation and growth, can mitigate the cumbersome and expensive process of testing every hypothesis experimentally. Agent-based models demonstrate the relationships between microscopic properties of the agents and macroscopic behaviours of the community[22].

In this work, an agent-based model is used to understand and mathematically evaluate the behaviour and interactions of *Escherichia coli* bacteria, with each biofilm-forming cluster (which may initially consist of a single bacterium, but may reach a much larger maximum size) considered an individual agent. This model helps us grasp the behaviour of bacteria considered individually, as well as their collective (emergent) behaviour in nature, with particular reference to biofilm formation. It can thus help us gain insights into the manner in which bacteria form biofilms, and how these may be inhibited to prevent and treat infectious diseases. Though our focus is on *E. coli* as a widely studied bacterium with a well-known connection to an infectious disease, our analytical tools may be used, *mutatis mutandis* for other infectious pathogens as well.

Later in this article, we use the term 'cell' to refer to an individual bacterium, to distinguish it from an agent or a cluster of bacteria.

The agent-based modelling approach given here to define bacterial behaviour makes it easier to predict results which can then be put to experimental evaluation. The modelling approach thus narrows down the range of necessary rigorous experimental work to a specific set of results which needs to be evaluated, avoiding the need to experimentally check all possible conditions. The model of the behaviour of bacteria also helps us to understand better how a biofilm is formed, what factors promote its growth, and what would curb it.

Using agent-based modelling, we define an agent as having a specific set of behavioural rules. This modelling is done in a system called a biofilm reactor, which is filled with substrate and has a surface on which the bacteria grow and form biofilm.

Chemicals known as inhibitors stop biofilm formation by competing with QSMs, thus inhibiting the upregulation of bacterial cells; this in turn lowers QSM production because of the decreased number of upregulated cells which start the process of biofilm formation in the first place[1,23,24]. Hence, the system is studied with and without inhibitors in varying concentrations, to observe the impact on biofilm formation and growth. (Furanones are considered as the inhibitor in our evaluations.) Our model incorporates the role of inhibitors and explores the system behaviour in the presence of inhibitors. The model allows us to predict when and how much inhibitor to introduce in the system, in order to impair the very mechanism of biofilm formation and growth.

There has been much study on the effect of various chemicals on biofilms, in order to address the problem. The behaviour of *E. coli*, with reference to its biofilm formation and growth, has been modelled in various conditions by different researchers – such as static conditions, slow-flow conditions[25], fast-flow conditions and in the presence or absence of inhibitors. Fozard *et al.*[26] have modelled the effect of inhibitors on biofilms keeping the concentration of inhibitors constant and studying the effect of varying their time of addition. They found that early addition of inhibitor resulted in greater inhibition of biofilm compared to later addition[22].

Furanones are halogenated compounds found in nature in red algae; they can also be synthesized chemically[27]. They have been found to inhibit bioflm formation and growth by inhibiting auto-inducers such as AHL, which is the main quorum sensing pathway in Gram-negative bacteria[1,23,24]. Inhibition of auto-inducers results in interference with quorum sensing and thus inhibits biofilm formation. Prior experimental work[28,29] on the effect of furanones on biofilms has evaluated their effect on the viability of bacteria. The study suggests that increased concentrations of furanones results in greater inhibition of biofilm formation and a decrease in the number of live cells, which is attributed to the collapse of the EPS matrix, rendering the cells in the cluster exposed and vulnerable to attacks – the furanones are not found to be directly toxic to the bacteria.

The rest of this article is organized as follows. The next section presents a description of the agent-based model – what an agent is and how it behaves in the system. A mathematical derivation of the inverse proportionality between upregulation and inhibitor concentration is given, as is a detailed flowchart covering different aspects of biofilm formation and inhibition under various conditions. The results of the study present various graphs depicting analysis using our model, and comparison with the existing experimental data. This is followed by the conclusion.

Model description

The biofilm reactor depicted in Figure 1 consists of two compartments: a biofilm compartment and a bulk liquid compartment. The three-dimensional biofilm compartment is made up of many voxels, each being a unit volume of the biofilm compartment. The bulk liquid compartment has a mixture of three diffusible substances: (i) a substrate, which is consumed by bacteria for their sustenance and growth, (ii) QSMs, and (iii) a quorum sensing inhibitor (QSI). The biofilm compartment is in contact with the bulk liquid compartment so that the two can exchange solutes by diffusion. The biofilm compartment is a

rectangular cuboid, with x and y boundaries which are periodic, and a planar support at the base so that the biofilm can be formed there. In addition to the substrate, QSM and QSI, the biofilm compartment also contains biomass and EPS as described elsewhere[26].

Our model defines the agent with a specific set of behavioural rules, such as state, thickness, etc. which allow us to clearly understand the relationship between upregulation and QSM concentration as opposed to the existing model[22], which does not account for the thickness of biofilm, in particular, a crucial parameter to be considered when modelling biofilm formation and growth. Our model allows a nonlinear relationship between thickness of biofilm and the number of upregulated cells in an agent. This allows us to correlate with the experimental data of biofilm thickness, and analyse how is it affected by the concentration of inhibitor, further making it possible to find the optimum concentration of inhibitor for maximal inhibition. In order to substantiate our work, we take the combination of *E. coli* as the bacteria and furanones as the inhibitor.

The original model of biofilm[24] considers multidimensional spaces, multiple biomass, inert biomass, etc. but does not incorporate EPS production, quorum-sensing, inhibitors, etc. Later work[26] proposed a simpler voxel-based model which included these. Based on this voxel model, our model defines an agent as the bacterial cluster which forms a biofilm, and is governed by a set of behavioural rules. This model makes it easier to predict results, as we have narrowed down our computational domain to a voxel and have constrained the scope of the same.

Our model, also being a voxel-based model, aims at establishing a clear understanding of how and when a biofilm is formed. We define an agent and its behaviours, and study the formation and growth of biofilm in our computational domain, which is a voxel. So, we consider a voxel to be of a size sufficient to accommodate an agent of maximum possible size. A collection of such voxels comprises of the biofilm reactor. Thus, the dimensions of the biofilm reactor turn out to be an integral multiple of the size of a voxel.

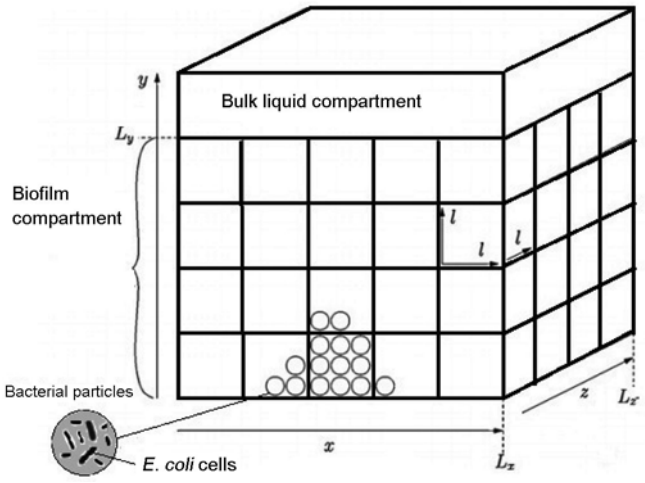

Figure 1. Biofilm reactor.

A voxel may contain a variable number of cells, and EPS is released in proportion to this. The agent consumes substrate and grows in size till it attains a maximum size, after which it divides and produces new agents. The agent forms the biomass and the EPS combining together to form a biofilm. After the biofilm-covered agent attains a certain size, disruption of the biofilm surface occurs, and planktonic bacteria are discharged from the agent into the host.

Bacterial cells at any point of time are either in an upregulated state (as a result of quorum sensing) or a downregulated state. In the upregulated state, genetic expression occurs, EPS is secreted by the cells, and more QSMs are produced, which further converts more cells into upregulated form. The upregulated cells also change to downregulated state continually[26].

The state of the system changes during the simulations for each of the components of the model the state changes are seen from $T = t$ to $T = t + \Delta t$, where Δt is a global timestamp for the model, and t is an integer index.

Biofilm formation

Biomass generation: The cells in an agent consume substrate proportional to the mass of the agent and concentration of the substrate in the voxel. Substrate uptake v_j (refs 30, 31) is given by

$$v_j = V_{\max} \frac{a_q}{K_s + a_q} M_j(t),$$

where K_s is the half-saturation constant and $V_{\max}$ the maximum substrate uptake rate (see Box 1 for notation). The time evolution using the forward Euler method[26] is given by

$$M_j(t+1) = M_j(t) + \Delta t Y_{\max}(v_j(t) - mM_j(t)),$$

where $M_j(t)$ denotes the mass of the agent with index j at $T = t$.

EPS production: Bacteria produce EPS at a much higher rate in the upregulated state than the downregulated state. The amount of EPS generated at each time step in each voxel is given by

$$E(t+1) = E(t) + \sum_{j \in B_j} (Z_{E,d} d_j(t) + Z_{E,u} u_j(t)),$$

where $Z_{E,d}$ and $Z_{E,u}$ are the constant rates at which EPS is produced by downregulated and upregulated cells respectively, B_j the agent contained in voxel j and m is the

apparent maintenance rate of the cells (per unit mass, and in terms of the substrate consumed) at zero growth rate. When E exceeds a certain value, so that the volume of EPS is the same as that of the biomass before division, a new EPS particle is generated. The EPS particle does not grow or change after it is generated.

Quorum sensing: This involves upregulation of cells and production of QSMs. The cells switch randomly between upregulated and downregulated states, depending upon the concentrations of QSMs and QSI in a voxel. In the presence of inhibitor, the transition rate from downregulated to upregulated state[26] is taken to be

$$Q^+ = \frac{\alpha a_q}{1+\gamma(a_q + a'_q)}, \quad (1)$$

where $a_{q,e}$ is the concentration of quorum sensing molecules in voxel e; $a_{q',e}$ the concentration of inhibitor in voxel e and γ is a constant denoting the relative ease with which the inhibitor combines with the receptor. The transition rate from upregulated to downregulated states[26] is taken to be

$$Q^- = \frac{\beta(a'_q + 1)}{1+\gamma(a_q + a'_q)}. \quad (2)$$

Box 1. Notation

Symbol	Meaning
K_s	Half-saturation constant
M_j	Mass of the B_jth agent
x_j	Thickness of the B_jth agent
V_{max}	Maximum substrate uptake rate
a_q	Concentration of quorum sensing molecules in a voxel
$a_{q'}$	Concentration of inhibitor in a voxel
m	Apparent maintenance rate of the cells
n_j	Number of cells in the agent
u_j	Number of upregulated cells in the agent
d_j	Number of downregulated cells in the agent
v_j	jth agent, substrate uptake
α	Number of cells upregulated in unit time
β	Number of cells downregulated in unit time
B_j	jth agent containing one or more cells
k	Total number of cells in the agent; varies in each agent
$Z_{E,d}$	Rate at which extracellular polysaccharide (EPS) is produced by downregulated cells
$Z_{E,u}$	Rate at which EPS is produced by upregulated cells
Q^+	Conversion rate from down to up, in the presence of inhibitor
Q^-	Conversion rate from up to down, in the presence of inhibitor
W^+	Conversion rate from down to up, in the absence of inhibitor
W^-	Conversion rate from up to down, in the absence of inhibitor
γ	Relative ease with which inhibitor combines with receptor
Y_{max}	Yield (efficiency at which substrate is converted into biomass)

Agent

B_j denotes the jth agent which can have a single cell or a cluster of cells depending on the time of observation. The attributes of the agent are as follows:

The number of *E. coli* cells in the agent, denoted as n_j. These can be calculated as

$$n_j = \frac{M_j}{0.95},$$

where M_j is the mass of the agent and 0.95 pg is the average mass of *E. coli*. The number of upregulated cells in the agent, denoted as u_j. The number of downregulated cells in the agent, denoted as d_j. The thickness of the agent, denoted as x_j.

The behavioural features that govern an agent are:

State: Among the upregulated and downregulated cells in the agent, whichever has the majority denotes the state of that agent.

Consumption: On consumption of substrate, the agent grows in size, thus producing more QSMs and more EPS.

Size: The maximum size an agent can reach is defined by M_{max}; if it exceeds this size, it starts releasing planktonic cells.

QSM concentration: This is directly related to the number of QSMs and the number of upregulated and downregulated cells and how they change between these states.

Since every cell going from downregulated to upregulated (or the reverse) state indicates that there is one more upregulated cell and one less downregulated cell (or the reverse), we can conclude that the total number of upregulated and downregulated cells in a voxel at any given time is some constant k. We also posit that the number of upregulated cells in an agent is related to the thickness x_j of the biofilm by

$$u_j = c_1 \cdot x_j^{c_2}, \quad (3)$$

where c_1 and c_2 are constants. Given suitable values for c_1 and c_2, there can be various types of correlation between the count of upregulated cells and biofilm thickness; specifically, the relationship becomes linear if c_2 is 1, but it is generally nonlinear.

The known mechanism of biofilm formation has several steps – each upregulated cell secretes QSMs that leads to the upregulation of more cells[3], which in turn leads to secretion of the EPS which contributes to the thickness of biofilm. In this way, the number of upregulated cells is related to the thickness of biofilm, but the relationship may not necessarily be linear.

As depicted in Figure 2, the agent, that is, the bacterial cell, on coming into contact with a suitable surface, adheres

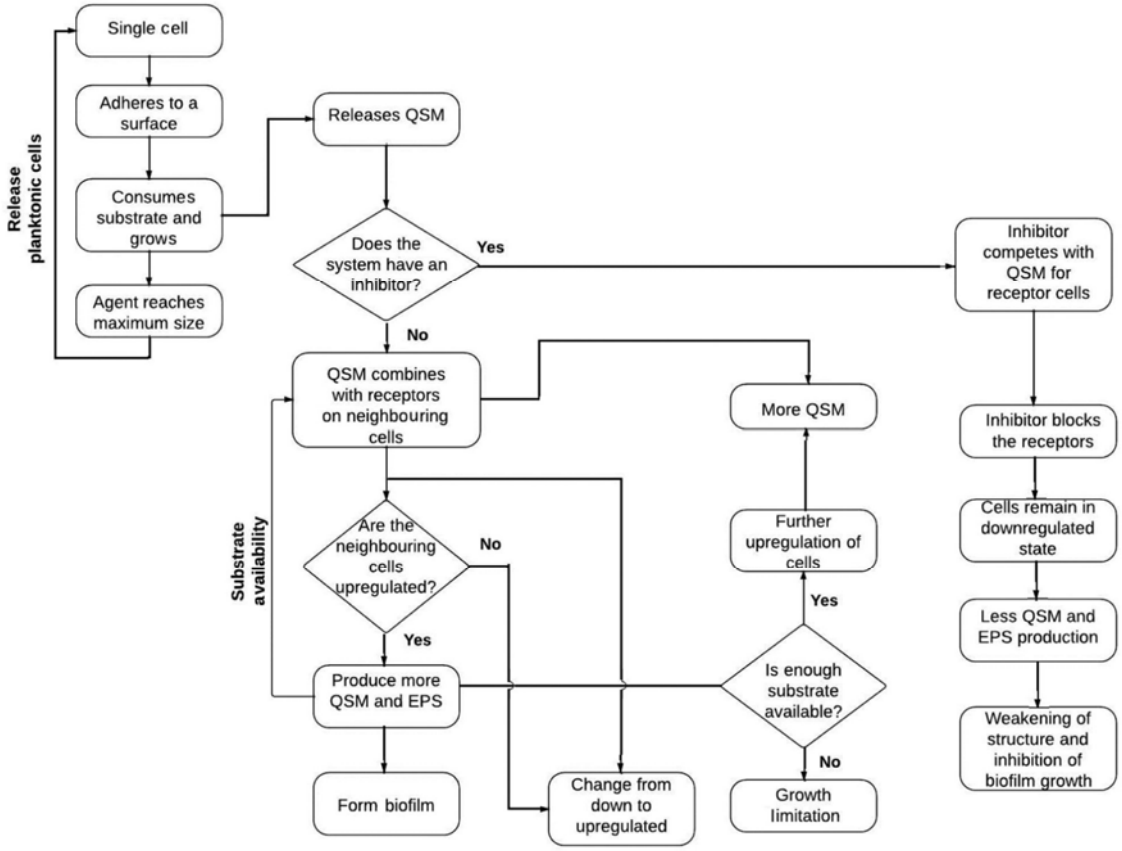

Figure 2. Agent-based model of biofilm formation under different conditions.

to it and releases QSMs. In the absence of inhibitors, these QSMs combine with the receptors on neighbouring cells, converting them from the downregulated to upregulated state. The agent in the upregulated state produces more QSMs and EPS, resulting in the formation and growth of biofilm. The cycle of upregulation and growth is limited by the availability of substrate in the bulk liquid compartment.

In the presence of inhibitor, it competes with QSMs for receptor sites on the cell surface, resulting in decreased rate of upregulation of cells, decreased EPS and QSM production and hence, decreased growth of biofilm.

Mathematical evaluation

The following approach can be used to model the effect of furanones on biofilm formation in *E. coli*. In the process of biofilm formation, the rate of upregulation of cells in the absence of inhibitor would be

$$W^+ = \alpha \cdot a_q, \tag{4}$$

here γ is zero, as there is no inhibitor in the reactor.

Similarly, the rate of upregulation to downregulation is

$$W^- = \beta. \tag{5}$$

For a system without inhibitor, the total number of cells k in a voxel would be equal to the sum of the number of upregulated cells and the number of downregulated cells in that voxel, as previously noted.

The number of upregulated cells in a voxel = $W^+ \cdot k$. The number of downregulated cells in a voxel = $W^- \cdot k$, where

$$W^+ + W^- = 1.$$

Substituting values from eqs (4) and (5), we get

$$\alpha a_q + \beta = 1. \tag{6}$$

Similarly, we can find the number of upregulated and downregulated cells in a voxel for a system with inhibitor.

Adding eqs (1) and (2) we get

$$\frac{\alpha a_q + \beta(a'_q + 1)}{1 + \gamma(a_q + a'_q)} = 1. \tag{7}$$

Solving eq. (6) using eq. (7), we get

$$\alpha \cdot a_q = 1 - \beta. \tag{8}$$

Substituting from eq. (8) into eq. (7) we get

$$\frac{1-\beta+\beta\times(a'_{\mathrm{q}}+1)}{1+\gamma(a_q+a'_{\mathrm{q}})}=1,$$

$$1+\beta a'_{\mathrm{q}}=1+\gamma(a_{\mathrm{q}}+a'_{\mathrm{q}}). \quad (9)$$

If we consider a particular inhibitor as the base and rate the effects of others with respect to the said base, this permits us to assign the value of γ in eq. (9) as 1. We do this considering furanone as the base inhibitor. Thus, we have

$$1+\beta a'_{\mathrm{q}}=1+a_{\mathrm{q}}+a'_{\mathrm{q}}. \quad (10)$$

Simplifying eq. (10) and substituting from eq. (8) we get

$$-\alpha a_{\mathrm{q}}\times a'_{\mathrm{q}}=a_{\mathrm{q}}.$$

Finally, we get

$$a'_{\mathrm{q}}=\left(\frac{-1}{\alpha}\right). \quad (11)$$

Equation (11) establishes the inverse proportionality between concentration of inhibitor and rate of upregulation of cells.

Now, we compare the change in upregulated cells in the system without and with inhibitor. In eq. (12) below, we obtain the percentage of cells inhibited from being upregulated due to the effect of furanones. The number of cells already in the upregulated state is subtracted from the total number of cells k in the agent, to get the number of cells capable of being upregulated.

For the percentage decrease in the number of upregulated cells, we have

$$\frac{k\alpha a_{\mathrm{q}}-\dfrac{\alpha a_{\mathrm{q}}(k-k\alpha a_{\mathrm{q}})}{1+a'_{\mathrm{q}}+a_{\mathrm{q}}}}{k\alpha a_{\mathrm{q}}}\times 100. \quad (12)$$

Solving eq. (12) gives, for the percentage decrease in the number of upregulated cells, the following

$$\frac{a'_{\mathrm{q}}+100(1+\alpha)}{101+a_{\mathrm{q}}}\times 100. \quad (13)$$

Taking the concentration of QSMs as a constant, say 100 μg/ml, we find the change in upregulated cells at different concentrations of the inhibitor. Substituting the value of α from eq. (11) in eq. (13), for the percentage decrease in the number of upregulated cells, we have

$$\frac{a'_{\mathrm{q}}+100\left(1-\dfrac{1}{a'_{\mathrm{q}}}\right)}{101+a_{\mathrm{q}}}\times 100. \quad (14)$$

Results

Our model indicates an inverse relation between furanone concentration and rate of upregulation of cells. We consider furanone concentration to be variable, while holding the time of addition as a constant. Varying the concentration of furanones in the system impacts the rate of production of QSMs and the rate of upregulation of cells, which vary quantitatively in relation to the amount of furanone present in the system. This inhibition of upregulation of cells is quantified by calculating the percentage decrease in the rate of upregulation of cells. This is calculated in relation to the concentration of furanones and can be depicted graphically. We see a linear relationship at lower concentrations, but the graph reaches plateau at higher concentrations. This is because the receptors on the cells get exhausted and hence a further increase in concentration does not cause increased inhibition.

It is of importance to find the optimum concentration of furanones required for effective inhibition of biofilms, which can easily be predicted by finding the region in the graph where it reaches a plateau. It is known from earlier studies[28,29] that the early introduction of any inhibitor into the system helps achieve more effective inhibition. Taking into consideration these findings, vulnerable surfaces where bacterial biofilm is liable to form may either be coated or impregnated with furanones in order to inhibit biofilm formation and prevent biofilm-related infections, which in turn would tend to ameliorate prolonged morbidity.

The rate of production of EPS is also dependent upon the percentage of upregulated cells, as these produce EPS at a much higher rate than downregulated cells. The structure of biofilm is formed by EPS, and hence its production affects the thickness and strength of the biofilm. In consideration of this, our model permits a nonlinear relation between the number of upregulated cells in an agent and the thickness of the biofilm of that particular agent. This allows us to correlate the decrease in the number of upregulated cells to the thickness of the biofilm. Further, we validate this assumption with previously available experimental data.

The effect of addition of quorum sensing inhibitors on the rate of upregulation of cells, and hence on the growth of biofilm, has been established previously. Using the results obtained mathematically, a graph between the percentage decrease in upregulation of cells and concentration of furanones in the bulk liquid compartment of the reactor can be plotted, as shown in Figure 3.

Figure 3 shows that the relation is asymptotic, first appearing linear but then tapering-off, indicating that the percentage decrease in the number of upregulated cells increases in proportion to the concentration of furanone up to a limit, beyond which the graph reaches plateau, showing that after such a limit, further increase in the concentration of furanones does not cause as much

inhibition. This is a logical outcome, considering that once most of the receptors on the cells for which furanones compete are saturated, further increase in concentration of furanones would have no impact on the rates of upregulation of cells.

This analysis helps in estimating the concentration which would be maximally effective in inhibiting biofilms. This can be subjected to experimental evaluation.

The upregulated cells produce QSMs and EPS in higher quantities; hence any decrease in percentage of upregulated cells would result in a decline in EPS production which forms the framework of the biofilm; it would also cause a decline in QSMs, which would in turn cause fewer cells to become upregulated, and finally result in the retardation or disruption of the growth of biofilm. Also, the size and thickness of the biofilm would decrease in the presence of furanone as a function of increasing concentration of the same.

Figure 4 shows a nonlinear relationship between percentage decrease in thickness of biofilm and concentration of furanone, as depicted in our model. The findings are consistent with published experimental observations[28], which indicate that thickness of the biofilm decreases by 37.7% at furanone concentration of 30 μg ml^{-1}, 42% at 50 μg ml^{-1} and 55% at 60 μg ml^{-1}. The decrease in size of the biofilm correlates with the increasing concentration, exactly as predicted by our mathematical model.

The experimental findings were compared with our mathematically calculated percentage decreases of upregulated cells at each concentration with respect to the percentage decreases in thickness of biofilm and a nonlinear pattern was observed. As the percentage of upregulated cells decreases, the thickness of the biofilm decreases more rapidly after a certain stage.

These findings substantiate the results eq. (3) that the relation between biofilm thickness and decrease in upregulated cells is nonlinear. Combining the findings depicted in Figures 3 and 4, we can estimate the concentration of furanone at which there is maximal percentage decline in the number of upregulated cells in correlation with accelerated decrease in thickness of the biofilm (Figure 5).

As has been shown by Ren *et al.*[28], early addition of inhibitor is more effective. Taking into account this observation as well, we can take suitable measures to apply furanones or other inhibitors early, where needed, in order to tackle the problem of biofilms. The range of concentration of inhibitors needed can be predicted through our analysis and used to achieve the most effective possible inhibition of biofilms.

We thus propose that these observations can be used to advantage in tackling the problem of biofilm formation on catheters, devices and implants introduced into the human body, which may serve as surfaces for adhesion of bacteria followed by quorum sensing and biofilm formation. As furanones are more effective when present early in the process of biofilm formation[26], coating or impregnation of catheters and implants with inhibitors like furanones, in a manner that they are released into the system at an optimum concentration, would inhibit formation and growth of biofilms and thereby prevent the associated chronic illnesses.

Conclusion

We have proposed an agent-based modelling approach which defines bacterial behaviour vis-à-vis biofilm formation and inhibition; in particular, how bacteria react in the presence of QSM inhibitors that restrict biofilm formation, using *E. coli* for analysis. We define an agent with a specific set of behavioural features such as its state, growth,

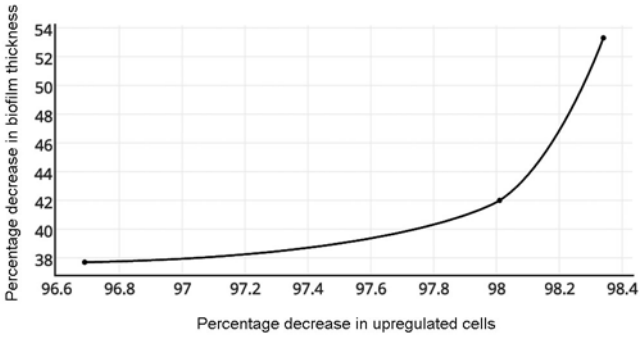

Figure 4. Graph based on mathematical evaluation and experimental data.

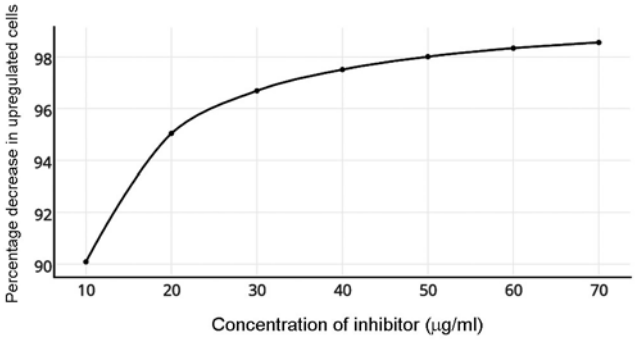

Figure 3. Graph based on mathematical evaluation.

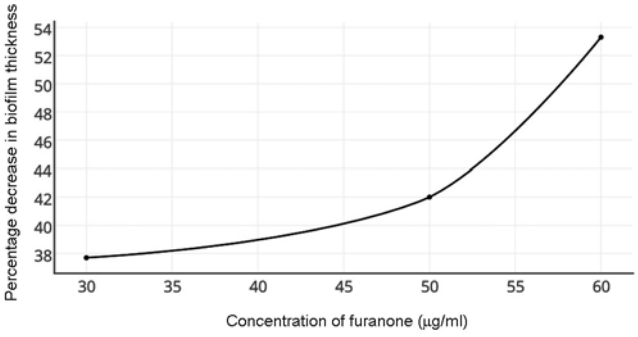

Figure 5. Graph based on experimental data.

size, concentration of QSMs, etc. Modelling these makes it easier to understand the phenomenon of biofilm formation, which can in turn allow us to find ways to control its growth. Thus, based on this model we have established an inverse relation between the concentration of inhibitor and rate of upregulation of cells. This relation is useful in finding the appropriate range of concentration for inhibitors which is appropriate for curbing biofilm growth. We have compared the results with published experimental data for validation, and find them to be consistent. We have demonstrated our concepts, model, analysis and results using *E. coli* and furanone inhibitor. This work can be applied to other Gram-negative bacteria as well and their corresponding inhibitors for similar analysis and results.

We suggest that furanones and other effective inhibitors of quorum sensing can be used to prevent bacterial disease and their recurrence, which would bring significant public health benefits. The presence of such inhibitors in the vicinity of bacteria in the early stages of biofilm formation can be achieved by incorporating them in devices placed in the human body, such as implants and catheters, which are known to serve as growth surfaces for infectious bacteria. Our model of such inhibition would be of use in planning countermeasures in an efficacious manner, in particular by helping estimate the concentration of the inhibitors needed to properly curb the menace of biofilm formation.

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