PAPER

Shape of the growing front of biofilms

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Abstract

The spatial organization of bacteria in dense biofilms is key to their collective behaviour, and understanding it will be important for medical and technological applications. Here we study the morphology of a compact biofilm that undergoes unidirectional growth, and determine the condition for the stability of the growing interface as a function of the nutrient concentration and mechanical tension. Our study suggests that transient behaviour may play an important role in shaping the structure of a biofilm.

1. Introduction

The stability of a uniform front to small disturbances is a framework for understanding pattern formation in many physical and biological systems \cite{1}, with a well-known example in material science being the fingering pattern formed due to supercooling of an alloy, as first characterized by Mullins and Sekerka \cite{2}. In nonequilibrium physics, front instabilities are prevalent \cite{3–5} such as classic examples in Hele-Shaw cells \cite{6–9} and crystal growth \cite{10}. In contrast, the self-organization and collective behaviour of living and synthetic active systems have been intensely studied in recent years \cite{11–16}, with an area of specific focus being spatial patterns generated by microbial systems \cite{17–25}. One particular example in the biological sciences that is receiving much recent attention concerns the growth and spatial structure of biofilms, which are densely packed bacterial communities \cite{26–30}.

Bacteria have been experimentally observed to form different patterns in the form of growing colonies when cultured on agar plates at different levels of nutrient concentration \cite{19, 31–34}. Specifically, the surface of growing colonies form circular (or flat) patterns when the nutrient concentration is high, while the patterns are fractal (or rough) when the nutrient concentration is low. The pattern formation driven by nutrient availability has been theoretically studied \cite{35–37} using various models such as the Fisher–Kolmogorov equation \cite{38, 39}, which combines bacterial diffusion, bacterial growth and nutrient diffusion, all in the dilute limit. Recent studies have also highlighted the importance of the mechanical interactions between the cells \cite{40, 41}.

Given the wide diversity of microbial systems and their impact on both medical and natural systems, it is important to provide quantitative guidelines for instabilities that may influence the three-dimensional structure of growing biofilms. In this paper we present steps in this direction by analysing the influence of two measurable quantities, namely the nutrient concentration and the effective surface tension that results from active mechanical interactions between bacteria \cite{42}, on the instability of a planar growing front of bacteria.

The shape instabilities introduced by nutrient factors have been recently studied using numerical simulations \cite{19, 35, 40, 43, 44}, which capture the main features of patterning in the experiments. In this paper, we study the stability of the growing front of biofilms in a unidirectional planar growth using a perturbative analysis. We delineate various growth and patterning behaviours as a function of two key control parameters. With the mathematical criterion for the stability analysis, our study illustrates when and how the growing front of biofilms becomes unstable and thus provides insight for understanding microbial growth.
2. Description of the system

Consider the growth of a biofilm made of a single bacterial species. The scenario for culturing the system is depicted in figure 1(a), where nutrient is supplied from the top of the domain. Denote the nutrient concentration as \( c(x, y, z, t) \), where \( x, y, z \) are the spatial coordinates and \( t \) the time coordinate. The density of bacteria is \( \rho \sim 1/b^3 \), with \( b \) the characteristic length of a single bacterium. Within the biofilm, nutrient is consumed at a rate \( k(c) \) by each cell, where \( k(c) = k_c \frac{c}{c + K_m} \) is a Michaelis–Menten form. This is a nonlinear form that describes crossover from a reaction-limited regime where the nutrient is abundant to a diffusion-limited regime where nutrient is scarce. While at the top layer of the growing biofilm, any of these regimes could be dominant, depletion of nutrient by every layer necessitates that at some depth there will be a crossover to the diffusion-limited regime. For the convenience of analysis, we apply \( k_c \approx k_0c \) everywhere as an approximation. Because a diffusion process is involved, the nutrient concentration satisfies the equation:

\[
\partial_t c - D \nabla^2 c + \rho k(c) \theta(-z + L_H(x, t)) = 0,
\]

where \( D \) denotes the diffusion coefficient, \( \theta(z) \) is the Heaviside step function, and \( L_H(x, t) \) is the biofilm surface at position \( x = (x, y) \) and time \( t \) (figure 1(a)).

Denote the velocity of the growing front as \( V(x, t) \), then

\[
L_H(x, t) = \int_0^t V(x, t') \, dt' + L_H(x, 0).
\]

Supposing that \( N_u \) nutrient molecules are consumed on average to make a single bacterium, we can calculate the velocity as
where $H$ is the depth of the active growing region within the biofilm (figure 1(a)). The nutrient concentration and flux should be continuous at the biofilm surface (note that we have chosen the diffusion coefficients equal for simplicity). Nutrient diffusion is subject to boundary conditions

\[
\begin{align*}
\frac{\partial}{\partial z} c_{z=L^+} &= \frac{\partial}{\partial z} c_{z=H}, \\
\frac{\partial}{\partial z} c_{z=L^-} &= \frac{\partial}{\partial z} c_{z=H^-},
\end{align*}
\]

(4)

where $L^+$ denotes the boundary just above the biofilm surface, $L^-$ the boundary below the surface, and $\perp$ the direction perpendicular to the surface. In the initial state, the nutrient concentration is homogenous within the culturing system, i.e. $c_{z=0} = c_{\infty}$ (figure 1(a)).

3. Growth of a flat front

Let us assume that the front grows in a steady state. To facilitate analysis, we approximate the system as semi-infinite in the $z$-direction, and then we transform to a moving reference frame, $x' y' z' t'$, with $(x', y', z', t') = (x, y, z - L_t, t)$ and $c'(x', y', z', t') = c(x, y, z, t)$. In this case, the nutrient concentration satisfies the equation

\[
\partial_t c' - V \partial_z c' - D \nabla^2 c' + \rho k(c') \theta(-z') = 0.
\]

(5)

For a uniform stationary moving front, the one-dimensional description is (here $k(c) \approx k_0 c$)

\[
-V \frac{dc'}{dz'} - D \frac{d^2 c'}{dz'^2} + \rho k_0 c' \theta(-z') = 0.
\]

(6)

Combined with the boundary conditions, the solution can be determined (see appendix A for details):

\[
c'(z') = \begin{cases}
C_{\infty} (1 + B e^{-\nu z'/H}) & (z' \geq 0) \\
C_{\infty} (1 + B) e^{\nu z'} & (z' < 0) \\
C_{\infty} & (z' = 0) \\
0 & (z' < 0)
\end{cases}
\]

(7)

where the coefficients are

\[
C_{\infty} = \frac{N_0 \rho}{1 - \exp(-\sqrt{\zeta})}, \\
B = -\frac{1}{\zeta} \ln^2(1 - N_0 \rho/C_{\infty}), \\
\lambda_1 = -\frac{1}{H} \ln(1 - N_0 \rho/C_{\infty}), \\
\zeta = H^2 \rho k_0 / D.
\]

(8a, 8b, 8c, 8d)

In addition, the front velocity has a solution of the form:

\[
V = \begin{cases}
\frac{B}{H} \left[ \ln \left( 1 - \frac{N_0 \rho}{C_{\infty}} \right) - \frac{\zeta}{\ln \left( 1 - \frac{N_0 \rho}{C_{\infty}} \right)} \right] & (C_{\infty} \geq C_{\infty}^c) \\
0 & (C_{\infty} \leq C_{\infty}^c)
\end{cases}
\]

(9)

The velocity in steady state is zero when the nutrient concentration $C_{\infty} < C_{\infty}^c$, while $V$ grows monotonically with $C_{\infty}$ when above the threshold $C_{\infty}^c$. Equation (9) is plotted in figure 2 for three different values of $\zeta$.

4. Growth rate for deformation modes

When $C_{\infty} > C_{\infty}^c$, and thus $V(t \to \infty) > 0$, consider the case that a growing front is slightly perturbed from the flat geometry, with the deformation described by a height profile function $h(x, t)$. For convenience, we define $C^2$ as $c'$ above (+) or below (−) the biofilm surface. Then, for a small perturbation of $h(x, t)$ the boundary conditions at the biofilm surface can be approximated as
We can construct a general solution for equation (5) of the form

\[
\begin{align*}
C^+|_{z'=h(x,t)} &= C^-|_{z'=h(x,t)}, \\
\partial_z C^+|_{z'=h(x,t)} &= \partial_z C^-|_{z'=h(x,t)}.
\end{align*}
\]

We can construct a general solution for equation (5) of the form

\[
\begin{align*}
C^+ &= C_0^+ + \int q A_+(q, t) e^{i q \cdot x} e^{-\alpha_+(q) z}, \\
C^- &= C_0^- + \int q A_-(q, t) e^{i q \cdot x} e^{\alpha_-(q) z},
\end{align*}
\]

where \(C_0^+ \equiv C_\infty(1 + B e^{-V_2/D}), C_0^- \equiv C_\infty(1 + B e^{k_2}),\) and \(\int_q \equiv \int \frac{dq}{(2\pi)^2}.\) By substituting equation (11) into equation (5), we find \(A_\pm(q, t)\) and \(\alpha_\pm(q)\) satisfy the following equations:

\[
\begin{align*}
\partial_t A_+ + (V \alpha_+ - D \alpha_+^2 + D q^2) A_+ &= 0, \\
\partial_t A_- - (V \alpha_- + D \alpha_-^2 - D q^2 - \rho_0) A_- &= 0.
\end{align*}
\]

Combined with equation (10), one finds that \(A_\pm(q, t)\) is of order \(h.\) Approximating these equations to the first order of \(h,\) we get the Fourier coefficients as

\[
A_+ = A_- = -\frac{C_\infty(1 + B) \rho_0 / D}{\alpha_+ + \alpha_-} h(q, t).
\]

As deformation is involved, there are two sources of contributions to the local front velocity \(v(x, t),\)

\[
v(x, t) = v_f(x, t) + v_b(x, t),
\]

where \(v_f(x, t)\) represents the biofilm growth caused by nutrient flux,

\[
v_f(x, t) = \frac{1}{N_{d,n}} \int_{h(x,t)-h}^{h(x,t)} dz'(V \partial_z C^- + D \partial_z^2 C^-),
\]

while \(v_b(x, t)\) is a surface related contribution (a new source) that resists deformation in the growing front, as might be expected owing to cell–cell adhesion or the influence of type IV pili. For simplicity, we use the following generic form

\[
v_b(x, t) = \nu \nabla^2 h(x, t),
\]

where \(\nu\) is the effective surface tension coefficient (see appendix D for details). We note that the stresses that determine the dynamics of a bacterial biofilm in the presence of extracellular matrix, such as exopolysaccharides (EPS), and surface attachment will require a more complex treatment than a simple surface tension description can provide. However, our focus here is on the growing front of a biofilm, which we can reasonably assume will not be influenced by the EPS production and surface attachment. Hence, a simple scalar description of the surface stress coming from a surface tension term should provide a sufficiently accurate starting point in our preliminary study. Furthermore, while at the length scale of single bacteria the granularity of the medium will be important and the stresses will be affected by the anisotropies of the underlying structure, such effects will be sub-dominant as we coarse-grain to the level where the interface can be described by a smooth function, which is the low \(q\) limit. Therefore, our work is consistent with this approximation since we are focused on the large length scale behaviour of the surface deformation modes.
Developing an approximation to the first order of $h$ in equation (14) yields

$$v_f \approx V [1 + \lambda h(x, t)] + \frac{V}{N_a \rho} \int q A_- e^{i q \cdot (1 - e^{-\alpha_H})}$$

$$+ \frac{D}{N_a \rho} \int q A_- e^{i q \cdot (1 - e^{-\alpha_H})} \alpha_-.$$  \hfill (17)

Meanwhile $L_{1f}(x, t) = L(T) + h(x, t)$, where $L(T)$ denotes the average over the $x$ and $y$ axes in $L_{1f}(x, t)$. By applying $\partial_\alpha$ on both sides:

$$v(x, t) = V + \partial_\alpha h(x, t).$$  \hfill (18)

Combined with equations (14)–(17), in the Fourier space, we find that

$$\partial_\alpha h(q, t) = [\lambda(q) - \nu q^2] h(q, t),$$  \hfill (19)

where

$$\lambda(q) = V \lambda_0 - \frac{V \lambda_0 (V + D \alpha_+) (1 - e^{-\alpha_H})}{D (\alpha_+ + \alpha_-) (1 - e^{-\lambda H})}.$$  \hfill (20)

Combining equation (19) with equations (12) and (13), we find that the unidentifiable functions $\alpha_\pm(q)$ are subject to the following restrictions:

$$\lambda - \nu q^2 = D \alpha_+^2 - V \alpha_+ - D q^2,$$  \hfill (21a)

$$\lambda - \nu q^2 = D \alpha_-^2 - V \alpha_- - D q^2 - p_k q,$$  \hfill (21b)

Equations (20) and (21) are in a closed form, from which we can obtain $\alpha_\pm(q)$. Furthermore, from equation (19) it is clear that the stability of mode $q$ in the growing front is determined by the sign of $\lambda(q) - \nu q^2$: when $\lambda(q) - \nu q^2 > 0$, the deformation mode increases with time, and thus leads to an instability. Consequently, a growing front is stable only under the condition that there is no unstable mode, i.e. $\lambda(q) - \nu q^2 \leq 0$ for all $q$.

The dependence of instability on $q$ and $C_\infty$ is shown in figure 3 with different values of $\zeta$ and $\nu/D$, we find that in stable regions (figure 3), $\lambda - \nu q^2$ peaks at $q = 0$. Thus, we can obtain the stability behaviour (and thus the shape) of the growing front via the analysis of small $q$ regimes ($q \to 0$).

Since $\alpha_\pm(q = 0) = V/D$, $\alpha_- (q = 0) = \lambda_0$ and $\lambda(q = 0) = 0$, using perturbation analysis, we find the following asymptotic behaviour when $q \to 0$:

$$\lambda(q) - \nu q^2 \approx \left[ \frac{1 - \nu/D}{1/f(C_\infty)} - 1 \right] D q^2,$$  \hfill (22)

where

$$f(C_\infty) \equiv \ln^2 \left( 1 - \frac{N_a \rho}{C_\infty} \right) \zeta^{-1}$$

$$- \left( C_\infty \frac{N_a \rho}{C_\infty} - 1 \right) \ln \left( 1 - \frac{N_a \rho}{C_\infty} \right) \frac{1 - \ln^2 \left( 1 - \frac{N_a \rho}{C_\infty} \right) \zeta^{-1}}{1 + \ln^2 \left( 1 - \frac{N_a \rho}{C_\infty} \right) \zeta^{-1}}.$$  \hfill (23)

When $C_\infty \geq C^\ast_c$, $f(C_\infty)$ is a monotonically decreasing function of $C_\infty$, with $f(C_\infty = C^\ast_c) = 1$ and $f(C_\infty = \infty) = -1$. So if $\nu/D < 1$, for equation $f(C_\infty) = \nu/D$, there is only a single root, and we denote it $C^\ast_c$ (i.e. $f(C^\ast_c) = \nu/D$). Then, one finds that the biofilm surface is stable for $C_\infty \geq C^\ast_c$ (Region I, figure 1(b)), while it is unstable for $C^\ast_c < C_\infty < C^p_c$ (Region II, figure 1(b)). On the other hand, if $\nu/D > 1$, the growing front is always stable because $\lambda(q) - \nu q^2 < 0$ for all $q$ (Region IV, figure 1(b)).

The perturbative calculation near $q = 0$ gives us analytical insight into the condition of instability at the largest length scale across the biofilm. However, figures 3(a) and (b) show the instability persists up to a finite threshold in $q$, so it will be important to examine the fastest growing mode which corresponds to the maximum growth rate in $q$ space. Using a numerical solution of equations (20) and (21), we have calculated the overall growth rate as a function of $q$, as shown in figure 3(c), with the dependence of $q_{\text{max}}$ on the nutrient concentration shown in figure 3(d). The results show that the characteristic length scale of the growing pattern $2\pi/q_{\text{max}}$ exhibits sensitivity to the nutrient concentration in a narrow range, and disappears when the nutrient concentration is higher than the initial growth threshold by only 30%−50%.

5. Transient growth behaviour

When $C_\infty \leq C^\ast_c$, in the approximation that $L \approx \infty$, the growing front eventually stops (equation (9)), yet we can identify the shape of the front by analysing the transient behaviour before it stops. To study the transient
behaviour, we apply numerical studies on the growth process using finite difference methods. Difference equations are converted from equations (1)–(4), and in order to make variables and parameters dimensionless, we assume \( H/b \) to be a constant and use \( H \) as the unit length in the \( z \)-direction, while we define \( H/D^2t \) as the unit time interval. For convenience, define the following dimensionless variables:

\[
L_c/L, \quad H/H_c, \quad t/t^*, \quad V/V_h, \quad c/c_{\infty}, \quad \zeta, \quad \nu/\nu_h.
\]

In our calculations (figure 4), the time step for update is 0.002\( t \), and it makes little differences when we reduce the time steps.

We consider the case that a biofilm grows from a thin layer (e.g. \( L_H = 0.1H^* \)) towards the nutrient source. As is shown in figure 4(a), the growing front speeds up at first owing to incorporation of more layers of bacterial growth until \( H_H \approx H^* \) (denoted as Phase I). Then the translation speed decreases as the consumption of nutrient by the bacteria overwhelms the supply of diffused nutrient from the source (\( y = 0 \)) (denoted as Phase II). Finally, when \( L_H \) approaches \( L \), the front speed recovers since nutrient supply is increased in the region near the source (\( y = 0 \)) (denoted as Phase III).

To study the transient behaviour before the growing front stops, we ignore the growth process of Phase I by setting \( L_H^* = 0.1 \) (pink), \( \nu/D = 0.01 \) (plus yellow), and \( \nu/D = 0.01 \) (plus blue). (c) Functional dependence of \( \lambda - \nu q^2 \) on \( q \). \( \lambda - \nu q^2 \) peaks at \( q_{\max} \) for given parameters of \( \nu/D \), \( \zeta \) and \( C_{\infty} \). (d) In the stable regions (compared with (a) and (b)), \( q_{\max} = 0 \).

Figure 3. Stability of a growing biofilm front as a function of \( C_\infty \) and \( q \). (a) and (b) The unstable regions correspond to \( \nu/D = 0.1 \) (pink), \( \nu/D = 0.01 \) (plus yellow), and \( \nu/D = 0.001 \) (plus blue). (c) Functional dependence of \( \lambda - \nu q^2 \) on \( q \). \( \lambda - \nu q^2 \) peaks at \( q_{\max} \) for given parameters of \( \nu/D \), \( \zeta \) and \( C_{\infty} \). (d) In the stable regions (compared with (a) and (b)), \( q_{\max} = 0 \).
threshold, the moving front actually stops (see appendix A for details), and thus there is no Phase III when \( L \) is large.

We next focus on the evolution process of Phase II. The stability of the growing front is determined by the local nutrient concentration around the biofilm surface. In the initial state, \( c(x, y, z) = C_\infty \) and \( L(t = 0) = 1.1 \), so that the local nutrient concentration around the growing front is much higher than that of the steady state (equation (7)). When \( \zeta \) is large, we find the velocity \( V \) decreases with time as \( V \sim t^{-0.55} \) especially if \( L \) is large (e.g. \( L^* = 250 \) in figure 4(c)). This result appears to be in good agreement with the experimental studies of biofilm growth reported in [45]. As an approximation, we use the time dependent velocity \( V(t) \) in this case as a quasi-steady state quantity to measure the local nutrient adequacy around the growing front. From equation (9) (and figure 2), one finds that there is a bijective mapping relation between \( V \) and \( C_\infty \) when \( V > 0 \), and thus we obtain \( V^p \equiv V(C_{\infty}^p) \) and \( V^c \equiv V(C_{\infty}^c) \), the mapping velocity of \( C_{\infty}^p \) and \( C_{\infty}^c \) in a quasi-steady state. As \( V(t) \) measures the local nutrient adequacy, so the growing front is stable when \( V(t) > V^p \), while it is unstable when \( V^p > V(t) > V^c \). If \( \nu/D > 1 \), the growing front is always stable (\( \forall q, \lambda(q) - \nu q^2 < 0 \)) before it stops, thus the biofilm surface is flat (Region V, figure 1(b)). However, if \( \nu/D < 1 \), \( V^p = V(C_{\infty}^p) > 0 \), since \( V(t \to \infty) = 0 \) and the velocity decreases with time in Phase II, then we can find times \( t^p > 0 \) satisfying \( V(t) < V^p \) when \( t > t^p \). Meanwhile \( V = V(C_{\infty}^c) = 0 \), thus when \( t > t^c \), \( V > V^c \). Consequently, the growing front is unstable before it stops, resulting in a rough surface (Region III, figure 1(b)).

6. Discussion

The growth and patterns formed by a biofilm are summarized in table 1. According to the mathematical model developed here, in theory, there are five distinct regions. If \( \nu/D > 1 \), the biofilm surface is always flat (Region IV and V), yet the growth is transient when \( C_\infty < C_{\infty}^c \) (Region V), while sustainable when \( C_\infty > C_{\infty}^c \) (Region IV). In reality, the value of \( \nu/D \) is usually significantly smaller than one (see appendix E for details), so these regions (Region IV and V) may be difficult to observe in experiments. If \( \nu/D < 1 \), the sustainable growth threshold is determined by the nutrient threshold \( C_{\infty}^p \), while the pattern formation is governed by the nutrient threshold \( C_{\infty}^c \).
(\(C_p^c > C_c^c\)). These two nutrient thresholds, obtained naturally from our analytical analysis, can illustrate the origin of thresholds for roughness and branching in the colony patterns of a recent simulation study [40]. Furthermore, the patterning in Regions I–III agree well with those of microbial colonies in the experimental studies [19,31–34]. To summarize, our analysis agrees qualitatively with experimental studies concerning patterning of microbial colonies and can illustrate puzzles that are not fully understood in the previous simulation studies. Our study will be relevant to a wide variety of practical questions such as the behaviour of multispecies biofilms, the impact of digestive enzymes that may free nutrients, and the influence of cooperation among cells or the presence of cheater cells on the evolution of a biofilm. Moreover, it is inherently related to the recent stability analysis that has been used to study the chemically driven growth and division of droplets [46] and may shed light on our understanding of division of proto-cells in early forms of life [46,47].

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**Appendix A. Nutrient threshold for growth**

Bacteria are living systems out of equilibrium. When the living environment is harsh, for instance, when nutrients are insufficient, some species of bacteria switch to a protective state named a spore, which is quasi inanimate with significantly lowered energy dissipation (so that the bacteria may survive for even hundreds of years in the harsh environment) [48–50]. There exists a minimum threshold of nutrient flux to initiate cell growth, and we can define it as \(\varepsilon\). To consider this effect, the formula of front velocity changes as:

\[
V = \frac{1}{N_\varepsilon} \int_{L_0-H}^{L_0+H} dz \left[ k_0 c(z) - \varepsilon \right] \theta \left( k_0 c(z) - \varepsilon \right),
\]

(A.1)

where we have applied \(L_0(t = 0) > H\). Due to the consumption by microbes, the nutrient is depleted very quickly when the concentration is below certain levels [51]. Therefore, we can consider \(\varepsilon\) to be small, and neglect it when there are enough nutrients to sustain bacterial growth. However, \(\varepsilon\) needs to be taken into consideration when the moving speed approaches to zero. For convenience, we can approximate (A.1) as

\[
V = \theta \left( \frac{1}{H} \int_{L_0-H}^{L_0+H} dz k_0 c(z) - \varepsilon \right) \frac{1}{N_\varepsilon} \int_{L_0-H}^{L_0+H} dz k_0 c(z).
\]

(A.2)

Using (A.2), we find that there is a threshold of velocity for biofilms growth: when \(V < \frac{H}{N_\varepsilon} \varepsilon\), \(V = 0\). Thus, when \(C_\infty < C_c^c\), as we find in figures 4(c) and (d), \(V_{\text{min}}\) depends inversely on the system size \(L\). When \(L\) is sufficiently large, so that \(V_{\text{min}} < \frac{H}{N_\varepsilon} \varepsilon\), then there is no Phase III in the growth process.

Furthermore, we can derive a solution to the nutrient profile in the steady state (\(t \to \infty\)) when \(C_\infty < C_c^c\) (note that \(V(t \to \infty) = 0\)). Supposing that the growing front finally stops at \(L_0(t \to \infty) = L + L'\), and using coordinates \((x', y', z', t') = (x, y, z - L + L', t)\), we obtain the following equation as \(t' \to \infty\):

\[
-D \partial^2 z' c'(z') + \rho k_0 c(z') \theta(-z') = 0.
\]

(A.3)

For \(z' > 0\), \(C^+(z') = C_\infty(A_c + B_\lambda z')\); whereas \(z' < 0\), \(C^-(z') = C_\infty C_\xi \exp \left( z' \sqrt{\frac{\rho k_0}{D}} \right)\). The boundary conditions are:

| Region | Definition | Behaviour |
|--------|------------|-----------|
| I      | \(C^c > C_c^c, \nu / D \leq 1\) | \(V > 0\), Flat |
| II     | \(C^c > C_c^c, \nu / D < 1\) | \(V > 0\), Rough |
| III    | \(C^c > C_c^c, \nu / D > 1\) | \(V = 0\), Rough |
| IV     | \(C^c < C_c^c, \nu / D < 1\) | \(V > 0\), Flat |
| V      | \(C^c < C_c^c, \nu / D > 1\) | \(V = 0\), Flat |
\[
\begin{align*}
C'_{1}(z) & = C_{\infty}, \\
C'_{2}(z) & = 0, \quad z' = 0, \\
\partial_{z}C'_{2}(z) & = \partial_{z}C'_{2}(z), \quad z' = 0.
\end{align*}
\] (A.4)

In steady state \( V = 0 \), which means (see \( A.2 \))

\[
\frac{1}{H} \int_{-H}^{0} dz' k_{bc} c'(z') < \varepsilon. 
\] (A.5)

Combined with \( A.4 \), we obtain \( C_{1} = A_{c} = B_{c} = 1/(1 + \lambda_{0} L') \). When \( L \) is large, and \( \frac{L - L'}{L'} \ll 1 \), we can approximate \( L' \) as \( L \) and thus obtain the nutrient profile in equation \( (7) \).

When is \( L \) large enough to be approximated as \( \infty \)? The criterion lies in \( A.5 \), which requires a threshold value of the system size for the approximation of \( L \to \infty \). Specifically, if we take \( L_{0} \) as the threshold, then

\[
L_{0} = \frac{D_{c}}{H_{0} c_{c}} \left[ 1 - \exp \left( - \frac{H_{0} \sqrt{k_{bc}}}{V} \right) \right] = \frac{D}{\sqrt{\rho k_{c}}}
\]

\[
\approx \frac{D_{c}}{H_{0} c_{c}} \left[ 1 - \exp \left( - \frac{H_{0} \sqrt{k_{bc}}}{V} \right) \right].
\] (A.6)

where \( L_{0} \) exhibits an inverse dependence on \( \varepsilon \). When \( L \geq L_{0} \), we can approximate \( L \) as \( \infty \).

**Appendix B. Upper bound for the growth speed**

We assumed that the uptake rate of nutrient by bacteria takes the Michaelis–Menten form, i.e.

\[
k(c') = k' \frac{c'}{c' + K_{m}}.
\] (B.1)

with the maximum value of \( k(c) \) to be \( k' \). From equation \( (3) \), we find that there is an upper bound to the translation speed, i.e.

\[
V \leq \frac{k'H}{N_{a}}. 
\] (B.2)

Despite the existence of an upper bound to velocity, it is easy to find that the bijective mapping relation between \( C_{\infty} \) and \( V \) \((t \to \infty)\) still holds for \( C_{\infty} \geq C_{c}' \). Using the same analysis, we can get similar growth and patterning behaviour of a biofilm when we apply the correction that arises due to equation \( B.2 \).

**Appendix C. The depth of active growth region \( H \)**

The length scale \( H \) is determined by a combination of different factors, including nutrient depletion and other non-nutrient-related factors. The penetration depth of nutrients is given by \( 1/\lambda_{0} \), which depends on the front velocity \( V \) as follows (see equations \( (6) \) and \( (7) \)):

\[
\frac{1}{\lambda_{0}} = \sqrt{\frac{V}{2\rho k_{0}}} + \frac{D}{\rho k_{0}} + \frac{V}{2\rho k_{0}}.
\] (C.1)

When the local nutrient concentration near the biofilm front is low (and thus \( V \ll 2\sqrt{D\rho k_{0}} \)), \( 1/\lambda_{0} \approx 2\sqrt{D/\rho k_{0}} \); otherwise, \( 1/\lambda_{0} \gg \sqrt{D/\rho k_{0}} \) when \( V \gg 2\sqrt{D\rho k_{0}} \). The non-nutrient-related constraints are due to the mechanical interactions between bacteria in a densely packed biofilm, which can be exemplified by a nutrient independent length \( H_{0} \) and can be regarded as a constant. We thus have

\[
H = \min(H_{0}, 1/\lambda_{0});
\] (C.2)

as an approximation, we regard \( H \) as a constant in our analysis.

**Appendix D. Effective surface tension coefficient \( \nu \)**

In equilibrium physics, surface tension can be defined and studied using energetic arguments, and then used in mechanical equilibration. Quite generically, it is possible to argue that the energetic cost of having an interface \( E \) should scale with the surface area \( A \), leading to the definition of an intensive quantity, the surface tension \( \sigma \), that relates the two extensive quantities as \( E = \sigma A \), in the language of thermodynamics. When studying mechanical stability of a droplet, i.e. an enclosed domain with a deformable surface, we find that the condition of stability
demands that the difference in the normal stress (or pressure) across the interface is balanced by the local mean curvature of the surface times the surface tension; a combination that is called the Laplace pressure. In the non-equilibrium case of a growing biofilm front, while we do not have access to the energetic definition of surface tension, we can use a generalization of the force balance argument to define a quantity that plays the same role as surface tension. Microscopically, we can use the Laplace pressure equilibration argument to define surface tension as the tendency to eliminate any curvature in the absence of normal stress difference. In the case of the biofilm, this mechanisms can be provided by the pulling of the pili: since they are attached to the neighbouring bacteria, any curvature in the profile necessitates an asymmetry in the distribution of pulling pili that will relax the bacterial configuration to a flat surface where there is minimal asymmetry. This crude generalization can be used to define an interfacial normal stress difference $\gamma$ that is proportional to the local curvature $\nabla^2 h$ (for a height profile $h$), as follows

$$\gamma = \sigma \nabla^2 h,$$

where the coefficient of proportionality plays the role of generalized surface tension.

By using a surface mobility coefficient $\mu_S$, we can relate the normal stress to the normal velocity of the interface as $v_n = \mu_S \gamma$, which yields

$$v_n(x, t) = \mu_S \sigma \nabla^2 h.$$  

A comparison with equation (16), the effective surface tension coefficient $\nu$ follows

$$\nu = \mu_S \sigma.$$

### Appendix E. Parameters estimation and discussions

Two parameters are most important in our model; one is $C_S$, the other is $\nu/D$. From equation (8a), $C_S$ is of order $N_R \rho$ for common carbon sources (e.g. glucose); $N_R$ is of the order of $10^9$ $\text{m}^{-1}$ $\text{s}^{-1}$ while $\rho$ is typically $b^{-3}$. Considering the spaces between bacteria in a biofilm, $\rho$ can be estimated as $10^{-1} - 1 \mu\text{m}^{-3}$, which yields values for $C_S$ of the order of $0.1 - 1 \text{ mol l}^{-1}$, which appears to be a high concentration. In most experiments using agar plates, nutrients can be supplied from the bottom part of the plate, resulting in a local nutrient concentration that has a steeper gradient than that of the steady state and thus could have a flat front propagating in the unsteady situation.

For $\nu/D$, we have $\nu = \mu_S \sigma$, and $\sigma$ can be estimated as $10^{-4} \text{ Nm}^{-1}$ $\text{m}^{-1}$ $\text{s}^{-1}$, while $\mu_S$ can estimated from Stokes’ law [56], to be of the order of $10^{-13} - 10^{-10} \text{ m}^2\text{s}^{-1}$. Meanwhile, $D$ is of the order of $7 \times 10^{-10} \text{ m}^2\text{s}^{-1}$ $\text{m}^{-1}$ $\text{s}^{-1}$. $\nu/D$ is of order $10^{-4} - 10^{-1} < 1$.

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### References

[1] Cross M C and Hohenberg P C 1993 Rev. Mod. Phys. 65 851
[2] Mullins W W and Sekerka R 1964 J. Appl. Phys. 35 444–51
[3] Ben-Jacob E and Garik P 1990 Nature 345 233
[4] Witten T J and Sander L M 1981 Phys. Rev. Lett. 47 1400
[5] Bensimon D, Kadanoff L P, Liang S, Shraiman B I and Tang C 1986 Rev. Mod. Phys. 58 977
[6] Hele-Shaw H S 1999 Nature 384 34–6
[7] Saffman P G and Taylor G 1958 Proc. R. Soc. A 253 312–29
[8] Chuoke R, Van Meurs P and van der Poel C 1959 Trans. AIMF 216 188–94
[9] Ben-Jacob E, Godfrey R, Goldenfeld N D, Koplik J, Levine H, Mueller T and Sander L 1985 Phys. Rev. Lett. 55 1315
[10] Langer J S 1980 Rev. Mod. Phys. 52 1
[11] Persat A, Nadell C D, Kim M K, Ingreameu F, Siryporn A, Drescher K, Wingreen N S, Bassler B L and Stone H A 2015 Cell 161 988–97
[12] Ramaswamy S 2010 Annu. Rev. Condens. Matter Phys. 1 323–45
[13] Bielczyk W, Cavagna A, Giardina I, Mora T, Pohl O, Silvestri E, Viale M and Walczak A M 2013 Proc. Natl Acad. Sci. 111 7212–7
[14] Kim M K, Ingreameu F, Zhao A, Bassler B L and Stone H A 2016 Nat. Microbiol. 1 150005
[15] Hallatschek O and Nelson D R 2010 Evolution 64 193–206
[16] Saha S, Golestanian R and Ramaswamy S 2014 Phys. Rev. E 89 062316
[17] Costerton J W, Lewandowski Z, Caldwell D E, Korber D R and Lappin-Scott H M 1995 Annu. Rev. Microbiol. 49 711–45
[18] Nadell C D, Xavier J J and Foster K R 2009 FEMS Microbiol. Rev. 33 206–24
[19] Ben-Jacob E, Schochet O, Tenenbaum A, Cohen I, Czirók A and Vicsek T 1994 Nature 368 46
[20] Zhao K, Tseng B S, Beckerman B, Jin F, Gilbiansky M L, Harrison J J, Luijten E, Parsek M R and Wong G C 2013 Nature 497 388–91
[21] Gloag E S, Turnbull L, Huang A, Vallotton P, Wang H, Nolan L M, Miliilli L, Hunt C, Lu J and Osvath S R 2013 Proc. Natl Acad. Sci. 110 11541–6
[22] Cates M, Marenduzzo D, Pagonabarraga I and Tailleur J 2010 Proc. Natl Acad. Sci. 107 11715–20
[23] Drescher K, Dunkel J, Nadell C D, van Teefelen S, Gryna I, Wingreen N S, Stone H A and Basler B L 2016 Proc. Natl Acad. Sci. 113 E2066–72
[24] Gelisman A, Zhao K, Lee C K, Kranz W T, Wong G C and Golestanian R 2016 Phys. Rev. Lett. 117 178102
[25] Kranz W T, Gelisman A, Zhao K, Wong G C and Golestanian R 2016 Phys. Rev. Lett. 117 038101
[26] Costerton JW, Stewart PS and Greenberg EP 1999 Science 284 1318–22
[27] Hall-Stoodley L, Costerton JW and Stoodley P 2004 Nat. Rev. Microbiol. 2 95–108
[28] Stewart PS and Costerton JW 2001 Lancet 358 135–8
[29] Donlan RM and Costerton JW 2002 Clin. Microbiol. Rev. 15 167–93
[30] Costerton JW, Cheng K, Geesey GG, Ladd T I, Nickel JC, Dasgupta M and Marrie T J 1987 Annu. Rev. Microbiol. 41 435–64
[31] Fujikawa H and Matsushita M 1989 J. Phys. Soc. Japan 58 3875–8
[32] Wakita J, Itoh H, Matsuyama T and Matsushita M 1997 J. Phys. Soc. Japan 66 67–72
[33] Fujikawa H and Matsushita M 1991 J. Phys. Soc. Japan 60 88–94
[34] Matsushita M and Fujikawa H 1990 Physica A: Stat. Mech. Appl. 168 498–506
[35] Kawasaki K, Mochizuki A, Matsushita M, Umeda T and Shigesada N 1997 J. Theor. Biol. 188 177–85
[36] Kessler DA and Levine H 1998 Nature 394 556–8
[37] Matsushita M, Wakita J, Itoh H, Rafols I, Matsuyama T, Sakaguchi H and Mimura M 1998 Physica A: Stat. Mech. Appl. 249 517–24
[38] Ben-Jacob E, Cohen I and Levine H 2000 Adv. Phys. 49 393–554
[39] Murray JD 2003 Mathematical Biology II. Spatial Models and Biomedical Applications (New York: Springer)
[40] Farrell F, Hallatschek O, Marenduzzo D and Walawalkar B 2013 Phys. Rev. Lett. 111 168101
[41] Ghosh P, Mondal J, Ben-Jacob E and Levine H 2015 Proc. Natl Acad. Sci. 112 E2166–73
[42] Dewenter L, Volkmann T E and Maier B 2013 Integr. Biol. 7 1161–70
[43] Nadell CD, Foster KR and Xavier JB 2010 PLoS Comput. Biol. 6 e1000716
[44] Bonachela JA, Nadell CD, Xavier JB and Levin SA 2011 J. Stat. Phys. 144 303–15
[45] Dervaux J, Magniez JC and Libchaber A 2014 Interface Focus 4 20130051
[46] Zwicker D, Seyboldt R, Weber CA, Hyman AA and Julicher F 2017 Nat. Phys. 13 408–13
[47] Golestanian R 2017 Nat. Phys. 13 323–4
[48] Setlow P 2007 Trends Microbiol. 15 172–80
[49] Setlow P and Johnson EA 2013 Food Microbiology: Fundamentals and Frontiers (Washington, DC: ASM Press) pp 45–79
[50] Cano RJ and Borucki MK 1995 Science 268 1060
[51] Bren A, Hart Y, Dekel E, Koster D and Alon U 2013 BMC Syst. Biol. 7 27
[52] Phillips R and Milo R 2009 Proc. Natl Acad. Sci. USA 106 21465–71
[53] Neidhardt FC and Curtiss R 1999 Escherichia Coli and Salmonella: Cellular and Molecular Biology (Washington, DC: ASM Press)
[54] Oldewurtel ER, Kouzel N, Dewenter L, Henseler K and Maier B 2015 Elife 4 e10811
[55] Sabas B, Koch MD, Liu G, Stone HA and Shaevitz JW 2017 Proc. Natl Acad. Sci. 114 7266–71
[56] Ladle J 1978 Physical Chemistry with Biological Applications (New York: Benjamin–Cummings)