# Biofilm Water Channel Network Model for Bacterial Communication

Yanahan Paramalingam, Hamidreza Arjmandi, and Adam Noel
*School of Engineering, University of Warwick, Coventry, United Kingdom*

***Abstract*—In nature, bacteria are often found in enclosed colonies called biofilms. A biofilm is comprised of the constituent bacteria and an extracellular polymeric substance (EPS), which acts as a protective layer that makes the biofilm difficult to eradicate. However, one way proposed to kill the bacteria in a biofilm is to disrupt the quorum sensing process. Within a biofilm, communication takes place primarily via diffusion using cylindrical channel networks called water channels and through the EPS. This abstract introduces a 2D model for anisotropic diffusion across a biofilm with water channels.**

***Index Terms*—Biofilm, Water Channels, Anisotropic Diffusion**

## I. INTRODUCTION

Over the past few decades, bacteria have been recognised as possessing complex structures that are able to adapt their behavioural reactions to environmental conditions, rather than being strictly unicellular organisms. Bacterial colonies have the capacity of multicellular organisation and cellular differentiation, which can lead to the formation of biofilms [1]. Biofilm is the name given to the aggregation of bacteria buried in or attached to the surface of an extracellular polymeric substance (EPS). Within a biofilm, substances such as extracellular DNA, amyloidogenic proteins, polysaccharides and proteins can be found that enable the biofilm to withstand harsh conditions. In healthcare, biofilm formation is a major concern due to it having multidrug resistance and having the ability to withstand other external stresses. This results in chronic bacterial infections worldwide [2]. A biofilm can consist of a wide range of bacterial colonies of different species.

Bacteria are able to chemically communicate cell-to-cell via extracellular signalling molecules called autoinducers. This process is called quorum sensing (QS) and relies on the production of and response to the autoinducers. Vicinal communities of bacteria have the ability to synchronously alter their behaviour in response to QS from changes in population density and species composition [3]. Spatial variation (i.e. heterogeneity in cell size and mass) is common in biofilms and affects their internal nutrient uptake. This is especially true in large biofilms, where the variation can deplete growth due to nutrient deprivation. Therefore, multiple transport mechanisms are required for nutrients to reach all parts of a biofilm.

The primary transport mechanism is diffusion. Diffusion via the bacteria EPS alone might be too slow for molecules to be readily distributed throughout the whole biofilm within a sufficient timescale. Fortunately, soluble signalling molecules, nutrients, and waste are also transported throughout the biofilm through "water channels", as seen in *Bacillus subtilis* [4] and *Escherichia coli*. Water channels provide less obstructed pathways, such that molecules can propagate much more efficiently [5].

In order to disrupt a mature biofilm, new strategies of prevention and treatment are required. Research in molecular communication can provide a greater understanding of the internal propagation of molecules, including autoinducers, potentially leading to strategies to improve the inhibition effectiveness of antimicrobial agents. Such improvements could increase our capability to disrupt and disperse the bacteria in biofilms. The presence of the water channel network suggests that conventional isotropic diffusion is inappropriate to model signal propagation within a biofilm. In this abstract, we propose the first anisotropic diffusion model for biofilm transport.

## II. GEOMETRY OF WATER CHANNELS

As discussed, the water channels found in biofilm are vital for the survival of bacteria embedded in the biofilm since it enables more effective molecule transport. The minimal dimensions considered for a water channel is a length of 1 $\mu$m and a width of 100 nm [6].

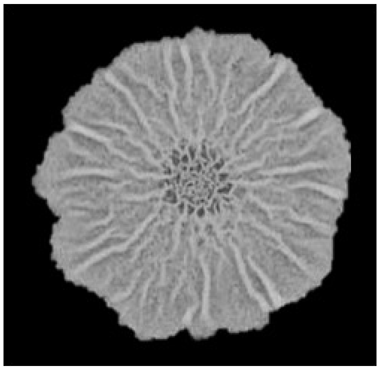

Figure 1. Biofilm growth of *B. subtilis* on the surface of an agar gel containing water and nutrients after 60 hours. The biofilm increases in height to hundreds of micrometres, spreads to reach a diameter of several centimetres, and forms macroscopic wrinkles. Image adapted from Wilking et al. 2013 [4].

Figs. 1 and 2, both from [4], are starting points for us to propose a suitable communication model that accounts for biofilm geometry. Fig. 1 is a scanning electron microscope

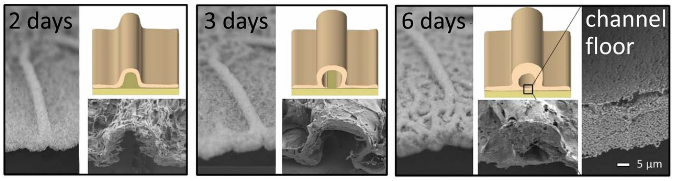

Figure 2. Structural evolution of the channels of *B. subtilis* biofilm. Photographs, SEM images, and illustrations depict the structural evolution of a channel over time. Image from [4].

(SEM) image of *B. subtilis* growth after 60 hours on an agar gel with nutrients and water. The wrinkly structure seen on the surface of the biofilm is formed by the water channels; they are connected in a spider-web like structure, which can result in transmission of autoinducers (signals) to different regions of the biofilm, hence be detected by different receivers.

To better inform the water channel geometry, Fig. 2 provides SEM images and illustrations of the structure of these water channels and how they evolve over time from 2-6 days from initial biofilm formation. Hence, it is reasonable for us to assume that water channels are cylindrical.

## III. DIFFUSION MODEL

In this section, we begin to construct a model to explain the propagation of autoinducers diffusing across a biofilm. For the bacteria to survive, it has to multiply and colonise a larger area. Our initial system model will focus on communication within a mature biofilm under laboratory conditions, such that the water channels are fixed within a 2D environment with a circular boundary. The reason for choosing a 2D circle as the biofilm shape is due to the relative thickness of the biofilm when grown in an agar plate, as seen in Fig.1.

As mentioned previously, autoinducers not only diffuse across the water channel, but can also diffuse across the rest of the EPS. Thus, a biofilm can be considered as a porous material, material where the diffusion is anisotropic, i.e., diffusion in different directions occurs at different rates. A good analogy representing the network of water channels within a mature biofilm is the London Tube map, where the tube routes are the water channels, the passengers are the autoinducers, and the destination is the receiver. Similar to Fig.1, in the centre there are multiple routes branching out into all directions and the further away you go from the centre, the fewer travel options are available. Travelling from Oxford Circus Station to Epping Station would be travelling from the centre to the edge of the map. The quickest route from Oxford Circus Station to Epping Station would be to take the Central line, but this journey can also be made by foot. However, it would take you several hours compared to roughly 45 minutes by tube.

To describe the anisotropic diffusion of the autoinducers within the biofilm, we employ the polar coordinate system where ($\rho$,$\theta$) denote radial and azimuthal coordinates, respectively. It is assumed that the autoinducer released in the environment will degrade, be consumed, or transform into another molecule with a certain lumped rate, $k_d\ s^{-1}$. Hence, we model this conversion as a first-order degradation reaction i.e.,

$$A \xrightarrow{k_\mathrm{d}} \phi. \tag{1}$$

The biofilm structure in Fig.1 shows that radial channels are formed within the biofilm. Considering the cylindrical structure for the individual water channels, these radial channels support the “VIP transmission” of autoinducers in this direction to and from the edges of the biofilm, leading to directed diffusion on radial axis. Thus, we model the effective diffusion coefficient in the radial direction as $D_\rho(\rho)$, which varies with $\rho$ and is invariant to $\theta$, due to the symmetry. Considering a lower density channel network map across the azimuthal direction, the diffusion in the azimuthal direction is expected to be slower than the radial direction. Then, we define the effective diffusion coefficient in the azimuthal direction as $D_\theta(\rho)$. To be variant in terms of $\rho$, we observe that the channel density varies in the radial direction and, such that there are fewer opportunities for azimuthal diffusion near the edges.

However, $D_\theta(\rho)$ is invariant in terms of $\theta$ due to azimuthal symmetry seen in Fig. 1. Thus, the diffusion of the autoinducer within the biofilm will be governed by the following partial differential equation with the boundary condition $\frac{\partial C}{\partial \phi} = 0$:

$$\begin{aligned}\frac{\partial C(\bar{r},t)}{\partial t} &= \nabla \cdot (D_\text{eff} \cdot \vec{\nabla}\ C(\bar{r},t)) \\ &\qquad - k_\mathrm{d} C(\bar{r},t) + S(\bar{r},t),\end{aligned} \tag{2}$$

where

$$D_\text{eff} = \begin{bmatrix} D_\rho & 0 \\ 0 & D_\theta \end{bmatrix}. \tag{3}$$

Solving (2) and (3) to find $C(\bar{r},t)$ will enable us to investigate the role of the water channel geometry to facilitate internal biofilm signal propagation.

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