

Estimation of glucose concentration

Let us consider a channel of height h , width w and length L . We first consider the case of a biofilm attached to the channel bottom surface at $z=0$ with a thickness $e \ll h$, under continuous supply of a medium, and that consumes glucose.

A molecule of glucose entering the channel at height $z=z_0$ takes a mean time $t_{ADV}=x_0/\langle v \rangle$ to reach the distance x_0 , where $\langle v \rangle$ is the mean fluid velocity. The mean time taken to diffuse to the channel bottom at $z=0$ from a height z_0 is $t_{DIF}=z_0^2/D$, where D is the diffusion coefficient of glucose. These two times define a curve:

$$z^*(x)=(x D/\langle v \rangle)^{1/2} \quad (1)$$

below which the concentration of glucose is less than c_0 because of the consumption by the biofilm, and above which the concentration of glucose is c_0 . Here c_0 is the concentration of glucose in the culture medium.

The flow of glucose at $z=e \approx 0$ through a horizontal slice of area A is equal to C , the number of molecules of glucose consumed per second by the bacterial biofilm within the slice.

To estimate the concentration c_s of glucose just above the slice, let the current of glucose along the z axis be:

$$j_z(x)=-D (c_0-c_s)/z^*(x)=-C/A \quad (2)$$

By combining (1), (2) and $Q=\langle v \rangle w h$, where Q is the fluid flow rate, c_s can be estimated by:

$$c_s(x)=c_0 - C/A *(x h w/(Q D))^{1/2} \quad (3)$$

with $c_0=1.36 \times 10^{24}$ molecules/m³ (corresponding to 0.4% glucose), $D=5.7 \times 10^{-10}$ m²/s and $Q=1$ ml/h $=2.78 \times 10^{-10}$ m³/s.

C/A is the consumption of glucose by unit area. The experimental data (recovering biofilm cells and measuring the optical density, not shown) suggest that there are $\approx 10^9$ bacteria in a biofilm. By taking a maximum consumption rate $c_M=197$ molecules of glucose per second per cell (Natarajan, A. & Srienc, F. Dynamics of glucose uptake by single Escherichia coli cells. *Metab Eng* **1**, 320-333, (1999)), we estimate: $C/A= c_M/(hw)=6.6 \times 10^{15}$ molecules of glucose per m² per s.

The lowest concentration of glucose is found at the bottom of the channel near the outlet, at $(x,y,z)=(L,y,0)$, and is:

$$c_s(L)=c_0-C/A *(L h w/Q D)^{1/2} \quad (4)$$

It allows us to estimate c_s/c_0 to be 0.998 in the 1 mm height channel, and 0.999 in the 250 μ m height channel.

If we take into account the variation of velocity along the z axis, using a Poiseuille velocity field, it does not affect significantly the result (not shown).

In the 250 μ m height channel, the biofilm grows from the edges (see text). The estimation of c_s in that case can be obtained by considering two slices of biofilm growing at $z=0$ and $z=h$, in a channel where $(w,h,L)=(0.25$ mm, 1 mm, 30 mm). The function $z^*(x)$ is the same as (1) and gives $z^*(L)=0.124$ mm $< h/2$; thus, the glucose-depleted regions generated by both biofilm slices, where $c < c_0$, do not overlap. The lowest concentration of glucose is then calculated at $x=L$ using (3), which still gives (4).

In this estimate, the y -dependence of velocity has been neglected.

Experimental clues

The above theoretical evaluation was confirmed by experimental observations showing that no significant biofilm growth decrease occurred along the channel x axis (direction of the flow) — if the glucose were depleted, the biofilm would be progressively less dense toward the channel outlet. We measured light transmission I along the channel length x from the inlet to the outlet, for two 1 mm-height channels, in the presence of biofilm after 24h growth; Figure SII shows $\ln(I_0/I)$ vs x .

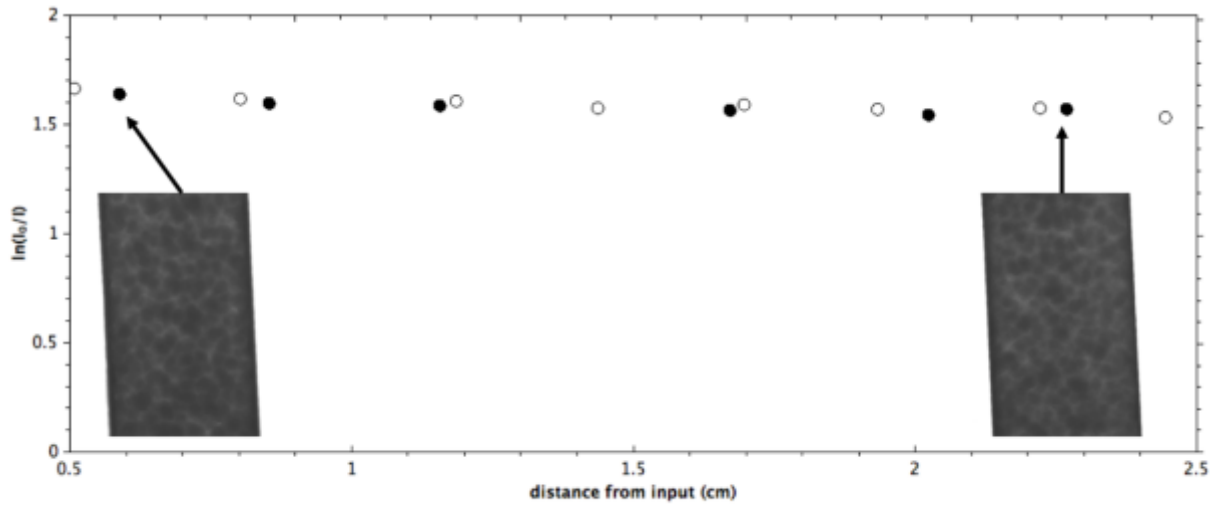


Figure SII: $\ln(I_0/I)$ vs x , distance from channel inlet in cm (the length of a channel is 3 cm; $x=0$ corresponds to the channel inlet). Filled and open circles: measurements in two different 1 mm height channels after 24 h of biofilm growth. Pictures: snapshots — field of view: 1 mm x 1.65 mm — of one channel taken with a 4x objective, near inlet (left) and near outlet (right), as indicated by the arrows; no significant differences are observed in biofilm microscopic absorbance.