

On the calculation of the elastic modulus of a biofilm streamer

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Abstract

Biofilm mechanical properties are essential in quantifying the rate of microbial detachment, a key process in determining the function and structure of biofilm systems. Although properties such as biofilm elastic moduli, yield stress and cohesive strength have been studied before, a wide range of values for the biofilm Young's modulus that differ by several orders of magnitude are reported in the literature. In this article, we use experimental data reported in Stoodley *et al.* [Biotech Bioeng, 65(1):83-92] and present a methodology for the calculation of Young's modulus, which partially explains the large difference between the values reported in the literature.

Keywords: biofilms, biofilm mechanical properties, biofilm elastic modulus, biofilm detachment

Introduction

Biofilm accumulation depends on its mechanical strength or its ability to sustain forces applied on it by the surrounding fluid. Such forces lead to its scouring or sloughing off the surface where it is growing on, making microbial detachment a key process in determining the function and structure of both natural and engineered biofilm systems. Biofilms are known to be viscoelastic materials with an elastic relaxation time on the order of a few minutes (Klapper *et al.*, 2002; Vinogradov *et al.*, 2004; Shaw *et al.*, 2004). The elastic properties of biofilms play an important role when short time response is of interest. A series of properties have been studied and connected to biofilm detachment, such as cohesive strength of microbial biofilms (Ohashi and Harada, 1994, 1996; Poppele and Hozalski, 2003; Ahimou *et al.*, 2007), elastic moduli (Stoodley *et al.*, 1999, 2001), Young's modulus and yield stress (Körstgens *et al.*, 2001), mechanical failure (Cense *et al.*, 2006), as well as biofilm viscoelastic properties (Vinogradov *et al.*, 2004; Shaw *et al.*, 2004; Klapper *et al.*, 2002). Overall, very few experimental studies have been conducted for the determination of the material properties of intact biofilms, mostly due to the technical difficulties associated with such testing. Due to the scarcity of experimental data, any reported mechanical properties are very valuable and are being used extensively by modeling studies, which need reliable experimental data in order to produce meaningful results. Specifically for the Young's modulus (E) of a biofilm, a measure of compliance or stiffness of a material, there is a wide range of reported values in the literature, different by over one order of magnitude. Since biofilms are greatly heterogeneous and biofilms grown under different conditions may exhibit different mechanical properties, some variability in the reported values is to be expected; however, such great differences indicate that there may be an error in the way this property is calculated from the interpretation of the experimental measurements. In particular, the values reported by Stoodley *et al.* (1999) for the apparent Young's modulus

(E_{app}) are one order of magnitude smaller than those reported by Körstgens *et al.* (2001).

We present a new rigorous methodology for the determination of E_{app} from the reported experimental results in Stoodley *et al.* (1999); our results show that proper interpretation of the experimental measurements of Stoodley *et al.* (1999) results in values of the Young's modulus which are consistent with what is reported usually in the literature.

Materials and Methods

As discussed in detail in the following, it appears that there is some confusion on the proper definitions of the Young's modulus (E) and the elastic shear modulus (G), as they are reported in the literature relevant to the biofilm mechanical properties. In order to set the stage for the discussion that follows, we present first a short primer on the appropriate definition of the aforementioned elastic moduli.

Forces that are applied in the direction of the axis of a bar cause a change of its length. If the forces are directed "into" the material, the bar is in compression and shortens by an amount $\Delta\ell$. Similarly, if the axial forces are directed "out" of the material, the bar is in tension and increases its length by $\Delta\ell$. The axial force per unit cross-sectional area defines the axial (normal) stress σ . Let ℓ be the original length of the bar. If the bar is uniform and the stress is also uniform along the bar, the ratio $\Delta\ell/\ell$ defines the nominal axial strain ε of the bar¹. In such a case, the Young's modulus E is defined as initial the slope of the uniaxial stress-strain curve, i.e., as the ratio of the axial (normal) stress σ to the corresponding axial (normal) strain ε in the initial linear part of the σ - ε curve, i.e.,

$$E = \frac{\sigma}{\varepsilon}. \quad (1)$$

¹ This definition of strain is appropriate provided that the ratio $\Delta\ell/\ell$ is much smaller than unity ("infinitesimal strain"); more sophisticated definitions are required in the case of "finite strains" (e.g., Malvern (1969)).

It should be emphasized though that, if the stress and strain distributions are not uniform along the bar, the strain must be defined properly at every point along the bar, as described in the following, and equation (1) is valid locally.

On the other hand, forces that are applied tangentially on the surface of a rectangular block of material create shear stresses and strains. The shear stress τ is defined as ratio of the tangential force divided by the area over which the force is applied. The infinitesimal shear strain γ is defined as the (small) change (in radians) of the original right angles of the sides of the rectangular block. If the block is uniform, the shear modulus G is defined by the ratio of the shear stress τ to the shear strain γ :

$$G = \frac{\tau}{\gamma}. \quad (2)$$

Equations (1) and (2) make it clear that normal stresses and strains are required for the definition of E , whereas shear stresses and strain enter the definition G .

There are various cases in the literature in which the elastic moduli E and G are estimated incorrectly from experimental data, either because the wrong definition of E and ε are used and the axial stress and strain fields are non-uniform (Stoodley *et al.* (1999)) or G is calculated as the ratio of shear stress to axial (as opposed to shear) strain (Stoodley *et al.* (2001), Klapper *et al.* (2002)). It should be noted also that the correct formulae for the determination of G from experimental data has been used by Stoodley *et al.* (1999) and Rupp *et al.* (2005).

Next, we present a rigorous methodology for the calculation of E from the data of Stoodley *et al.* (1999), who carried out carefully designed experiments on biofilms *in situ* and studied the deformations caused by changes in the hydrodynamic shear stress (τ_w). They presented images of side views of a biofilm streamer growing on a flow cell under known hydrodynamic shear stresses and measured the change in the length of the streamer, as τ_w

was varied. A schematic representation of the geometry used for the measurements is shown in Fig. 1, where the fluid flow is from right to left, and the streamer DB is attached firmly to the wall at its downstream “tail”. Stoodley *et al.* (1999) used paired fluorescent particles at various locations along the streamer and measured the change in their distance when the shear stress τ_w is varied. Two such pairs of points are $\beta - \delta$ and $\zeta - \kappa$ as shown in Fig. 1. Based on such measurements they plotted τ_w versus $\Delta\ell/\ell$, where ℓ is the original distance between the two points and $\Delta\ell$ the change in ℓ due to τ_w . The apparent modulus (E_{app}) was then identified by initial the slope of the $\tau_w - \frac{\Delta\ell}{\ell}$ curve; i.e., E_{app} was defined as

$$\boxed{E_{app} = \frac{\tau_w}{\Delta\ell/\ell}} \quad (3)$$

There two major problems with this evaluation of E_{app} : i) it involves the ratio of the shear stress τ_w to an axial (as opposed to shear) strain-like quantity $\Delta\ell/\ell$, and ii) the ratio $\Delta\ell/\ell$ is not a proper definition of the axial strain, since the axial stress field is not uniform along the streamer. As discussed in the following, the proper definition of the apparent Young’s modulus requires the calculation of the normal stress (σ) and strain (ε) along the streamer, so that E_{app} can be evaluated as the ratio σ/ε .

Assuming that the cross-section of the streamer is approximately uniform between points B and D of Figure 1, the average axial stress σ on any cross section in that region can be calculated by considering the equilibrium of the part shown in Figure 2:

$$\sigma A = \tau_w C(L-x) \Rightarrow \sigma(x) = \frac{\tau_w C}{A}(L-x), \quad (4)$$

where L is the length of part BD as shown in Fig.1, A is the cross-sectional area, C the perimeter of the cross section and the x -axis is directed from right to left, as shown in Fig. 1. In equation (4), the normal stress is non-uniform along the streamer but varies with

position, i.e., $\sigma = \sigma(x)$. Also, the hydrodynamic shear stress (τ_w) is multiplied by the side area of the streamer on which it is applied, in order to calculate the total axial force applied on the streamer. In deriving equation (4), we assume that the shear stress τ_w is distributed uniformly on the lateral surface of the streamer. However, in reality, the local stress distribution is three dimensional and σ is to be interpreted as the average axial stress on the cross-section of the streamer (Figure 2). In fact, it is expected that the shear stress takes higher values on the surface of the streamer that faces the interior of the flow cell and smaller values on the part that faces the wall (Figure 1). In order to account for such a non-uniform distribution of τ_w on the lateral surface one may replace C by αC where α takes values in the region $1/2 < \alpha < 1$.

The corresponding axial strain $\varepsilon(x)$ is found from $\sigma(x)$ through the Young's modulus

E :

$$\varepsilon(x) = \frac{\sigma(x)}{E} = \frac{\tau_w C}{E A} (L - x). \quad (5)$$

Equation (5) makes it clear that the strain ε is non-uniform along the streamer.

Let $u(x)$ be the displacement (directed to the left) of the cross section at position x .

The derivative $\frac{du(x)}{dx}$ equals, by definition, the axial strain $\varepsilon(x)$, (e.g., Malvern (1969)).

Therefore, in view of (5),

$$\frac{du(x)}{dx} = \varepsilon(x) = \frac{\sigma(x)}{E} = \frac{\tau_w C}{E A} (L - x). \quad (6)$$

Integrating equation (6), we find

$$u(x) = \frac{\tau_w C}{A E} \left(L x - \frac{x^2}{2} \right) + u_0, \quad (7)$$

where u_0 is the axial displacement of the cross section at $x=0$. The elongation $\Delta l_{\zeta\kappa}$ of the part of the streamer between points ζ and κ equals the difference $u(x_\zeta) - u(x_\kappa)$ and, in view of (7), takes the value

$$\Delta l_{\zeta\kappa} = \frac{\tau_w C}{AE} \left[L(x_\kappa - x_\zeta) - \frac{x_\kappa^2 - x_\zeta^2}{2} \right] = \frac{\tau_w C l_{\zeta\kappa}}{AE} (L - x_m), \quad (8)$$

where $l_{\zeta\kappa} = x_\kappa - x_\zeta$ is the length of the part of the streamer between points ζ and κ , and $x_m = \frac{x_\kappa + x_\zeta}{2}$ the x -coordinate of the middle point between ζ and κ (Fig. 1). Solving

equation (7) for the Young's modulus (E), we obtain:

$$E = \lambda \frac{\tau_w}{\Delta l_{\zeta\kappa} / l_{\zeta\kappa}}, \quad \text{where} \quad \lambda = \frac{C(L - x_m)}{A}. \quad (9)$$

Note that the above equation for E differs from formula (3) used by Stoodley *et al.* (1999) by the factor $\lambda = \frac{C(L - x_m)}{A}$. For a circular cross section of radius R , $C = 2\pi R$ and $A = \pi R^2$,

so that

$$\lambda = \frac{2(L - x_m)}{R}. \quad (10)$$

From Figures 3 and 4 of Stoodley *et al.* (1999), we conclude that λ is of order 10, meaning that the correct value of E should be one order of magnitude higher than that reported in Stoodley *et al.* (1999).

The presentation in this article assumes that the biofilm behaves as a circular bar, which is not always the case, since biofilms are natural systems and can take various shapes. For a more exact calculation of the Young's modulus and if the profile of the streamer is relatively well defined, we can use a more accurate calculation, taking into account the variable biofilm geometry in the x -direction. In that case, for a "slice" of the streamer with length dx , we have the stresses as shown in Figure 3. We follow the same logic as before

with the only difference that the cross-sectional area A and the side surface area C vary along the streamer, i.e., $A = A(x)$ and $C = C(x)$. Calculations take the following form:

$$A(x)d\sigma = -C(x)dx\tau_w \Rightarrow \frac{d\sigma(x)}{dx} = -\frac{C(x)}{A(x)}\tau_w \quad (11)$$

Integration of equation (11) yields

$$\sigma(x) = \tau_w \int_x^L \frac{C(x')}{A(x')} dx', \quad (12)$$

where it was taken into account that the axial stress σ vanishes at the free end, where $x = L$.

The displacement $u(x)$ is calculated as follows:

$$\frac{du(x)}{dx} = \varepsilon(x) = \frac{\sigma(x)}{E} \Rightarrow u(x) = u_0 + \frac{1}{E} \int_0^x \sigma(x') dx'.$$

Finally,

$$\Delta\ell_{\zeta\kappa} = u(x_\kappa) - u(x_\zeta) = \frac{1}{E} \int_{x_\zeta}^{x_\kappa} \sigma(x) dx \Rightarrow E = \frac{1}{\Delta\ell_{\zeta\kappa}} \int_{x_\zeta}^{x_\kappa} \sigma(x) dx$$

or, in view of (12),

$$E = \frac{1}{\Delta\ell_{\zeta\kappa}} \int_{x=x_\zeta}^{x_\kappa} \left[\tau_w \int_{x'=x}^L \frac{C(x')}{A(x')} dx' \right] dx. \quad (13)$$

In such a case, the calculation of E requires, in general, numerical integration.

Results and Discussion

The Young's modulus values for biofilm reported in the literature vary greatly in size. Stoodley *et al.* (1999) report E_{app} values that range between 17 and 240 (N/m², or Pa), while Körstgens *et al.* (2001) experimentally determine E_{app} values of 6500 ± 500 Pa. The calculation of E proposed in this article can cover some of that difference. According to equation (8) and if we approximate the biofilm streamer with a circular bar—equation (9), the

error in the calculation of E will be related to the factor λ . For a factor $\lambda \approx 10$ —which is roughly the case for the biofilm streamer presented in Stoodley *et al.* (1999)—there is one order of magnitude error, and this error will be magnified the longer the bar is. This new proposed calculation consolidates the difference between the values reported by Stoodley *et al.* (1999) and Körstgens *et al.* (2001), bringing them at least in the same order of magnitude for the higher values of Stoodley *et al.* (1999) and the lower values of Körstgens *et al.* (2001).

The large variability in biofilm moduli has been dealt with in the literature by other researchers. Using spinning disk rheometry, Shaw *et al.* (2004) and Vinogradov *et al.* (2004) found that, for biofilms formed by the dental pathogen *S. mutans*, the shear modulus ranged over three orders of magnitude. Cense *et al.* (2006) used a micro-indenter and found a variation of a factor of eight in the magnitude of the storage modulus of the same organism. Ahimou *et al.* (2007) also discuss the variability in the reported literature. Naturally, there are other reasons that could justify the large differences in biofilm moduli values reported in the literature than the discrepancies we show in this paper. We mention two here:

First, the measurements of Stoodley *et al.* (1999) refer to a tensile state of stress in the streamer, whereas the data of Körstgens *et al.* (2001) are obtained from uniaxial compression of a “bulk” specimen. The difference in stress state, i.e. tension vs. compression, can be partly responsible for the observed difference in Young’s modulus. In fact, Lapidou and Aravas (2007) have shown recently that compressive stresses lead to increased values of the apparent Young’s modulus in biofilms. They show that, when a heterogeneous voided medium, like the biofilm, is loaded in compression, the resulting gradual void closure leads to “stiffening”, i.e., to increased values of the slope of the $\sigma-\varepsilon$ curve as the deformation increases. Once complete void closure is achieved, the material reaches its maximum stiffness. Therefore, it is expected to observe higher Young’s modulus in biofilms under compression (as in Körstgens *et al.* (2001)).

Second, it should be emphasized that the Young's modulus data compared in this study (Stoodley *et al.* (1999) vs. Körstgens *et al.* (2001)) differ in ways other than the state of stress: the former is a biofilm streamer, whereas the latter is bulk biofilm. It is possible that the mechanical properties of the streamer are different from those of the bulk material. However, there is such a limited amount of experimental data on biofilm mechanical properties that any improvement is worthwhile.

Conclusions

A rigorous methodology for the calculation of biofilm's Young's modulus from the experimental data of Stoodley *et al.* (1999) is reported. The improved calculations provide an explanation for the large differences in the reported values in the literature, bringing them closer in value. However, some variability in the experimental data is to be expected in view of the heterogeneity of biofilms and the difference in stress state (tension vs. compression).

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Figure 1. Schematic of flow cell with biofilm streamers formed along its walls and blow-up of streamer with tensions acting on it by the moving fluid.

Figure 2. Part of biofilm streamer in equilibrium, with tensions acting on it by the moving fluid.

Figure 3. Stresses on biofilm with variable geometry in the x direction.

Figure 1.

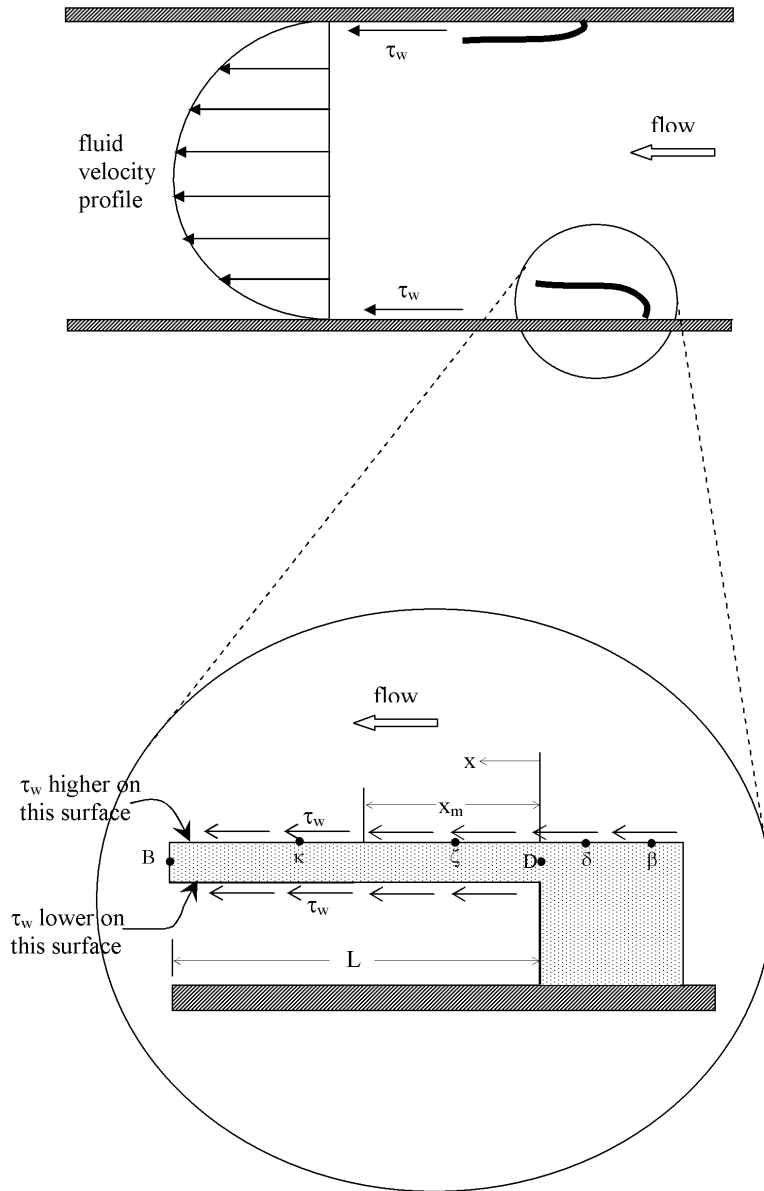


Figure 2.

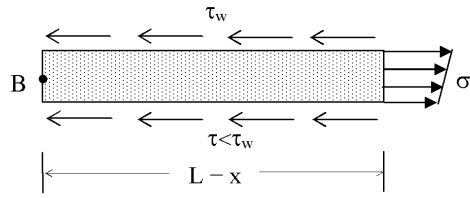


Figure 3.

