# RHEOLOGY OF BIOFILMS

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## Abstract

The paper describes an experimental study concerning the mechanical properties of bacterial biofilms formed from the early dental plaque colonizer Streptoccocus mutans and pond water biofilms. Experiments reported in this paper demonstrate that both types of biofilms exhibit mechanical behavior similar to that of rheological fluids. The time-dependent properties of both biofilms have been modeled using the principles of viscoelasticity theory. The Burger model has been found to accurately represent the response of both biofilms for the duration of the experiments. On this basis, the creep compliances of both biofilms have been characterized, and the respective relaxation functions have been determined analytically.

## Introduction

Microbial biofilms are the populations of micro-organisms that concentrate at wetted solid surfaces in both industrial and natural environments. Biofilm cells are typically surrounded by an extracellular polymeric slime (EPS) matrix consisting of cell clusters (aggregates of cells) surrounded by interstitial water channels. The propensity in multicellular biofilm communities to attach to surfaces is widespread. It represents an integral component of the microbial life cycle in these organisms. Typically, biofilms tend to grow in aqueous environments under a wide range of environmental conditions. Biofilm formation presents serious problems in industry causing, for example, product contamination and corrosion. In the medical field, biofilms can cause infection of indwelling devices such as catheters. Dental plaque biofilms lead to cavities and gingivitis.

At present, there is a growing interest in the mechanical properties of biofilms that appear to exhibit the behavior of rheological fluids. The understanding of these properties provides the necessary foundation for effective biofilm control in industrial and medical environments. Stoodley *et al.* (1999 a, b). In particular, important applications of biofilm rheology concern detachment processes and frictional energy losses in transport pipelines, Picaloglou et al. (1980). Similarly, of critical importance is the problem of biofilm detachment in food production facilities and drinking water systems, which may result in the potential transmission of pathogens via contaminated food, Zottola & Sasahara (1994), drinking water, Piriou et al. (1997), or aerosols, Walker et al. (1995).

To date, a very limited number of studies have been conducted regarding the mechanical properties of biofilms. Yet, these properties play a decisive role in various biofilm processes and behaviors. The studies of biofilm rheology reported in the literature usually involve removing the biofilm and then testing the suspension, Ohashi & Harada (1994). Although this approach

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can supply interesting rheological data it does not provide direct information regarding the *in situ* behavior of intact biofilms.

This paper describes an experimental study that helps to shed light on the rheology of nearly undisturbed biofilms in their natural environments. Two types of biofilms have been investigated, dental plaque colonizer Streptoccocus mutans and mixed culture pond water biofilms. The rheological properties of both biofilms are characterized using a viscoelastic constitutive model.

## Experimental

In this study, Streptoccocus mutans were grown in a modified Rotating Disk Reactor (RDR). The procedure of growing biofilms in this system complies with a registered ASTM standard method, E-2196-02. The RDR was modified to accept 25 mm diameter anodized flat rheometer disks that had been coated with hydroxyapatite (Clarkson Chromatography Products Inc. Williamsport, PA). The biofilms were grown on BHI medium with 2% sucrose under a $CO_2$ headspace for 3 days.

After the growth period, disks were removed individually and tested using a TA Instruments rheometer AR 1000 Series. In the rheometer, biofilms samples were sandwiched between two parallel disks and subjected to a normal force of 0.1 N. This step has been intended to ensure continuous contact and uniformity of biofilm samples. Following a waiting period of two to five minutes in order to stabilize the samples within the rheometer, biofilms were subjected to constant shear creep tests. A total of twelve samples of Streptoccocus mutans have been tested over a range of stresses from 0.05 Pa to 55 Pa. A similar experimental procedure was used in the case of pond water biofilms. In the latter case, a total of 10 biofilms were tested using either 2 or 3 plates from each of 4 independent experiments. The pond water biofilm study has been described in detail in a pending publication by Towler et al. (2003).

As a result of both experimental programs, it has been observed that both types of biofilms have demonstrated the time-dependent response similar to that of rheological fluids. The experimental responses of Streptoccocus mutans and pond water biofilms are depicted in Fig. 1

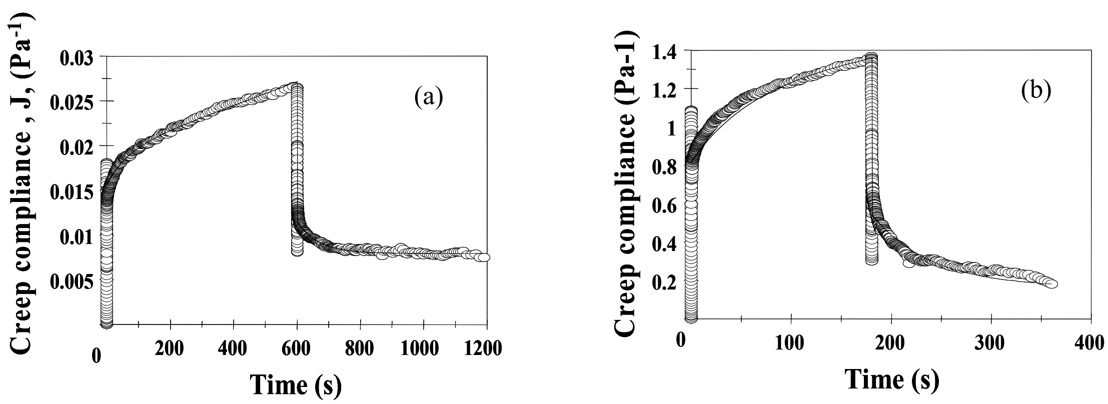


Figure 1. Creep and recovery
(a) Streptoccocus mutans; (b) Pond water biofilms.

The creep compliances of both biofilms have been obtained under shear stress conditions such that $\tau = 1$ Pa in the case of Streptoccocus mutans, and $\tau = 0.1$ Pa in the case of pond water biofilms. Further, it has been determined that both, Streptoccocus mutans and pond water biofilms, have demonstrated linearly viscoelastic behavior within the range of stresses (3.5 – 5) Pa and (0.1 – 0.5) Pa, respectively. The elastic response of the biofilms at the time of the load application, t = 0, has been defined in terms of the instantaneous shear modulus G. The values of G have been obtained such that $G_{sm} = 63.9$ Pa, and $G_{fw} = 1.2$ Pa for Streptoccocus mutans and pond water biofilms, respectively.

## Viscoelastic Response of Biofilms

Within the framework of viscoelaticity theory a distinction is made between viscoelastic solids and viscoelastic fluids. A viscoelastic solid subjected to a fixed deformation usually responds with a corresponding component of stress, which remains nonzero as long as the deformation is maintained. In contrast, a viscoelastic or rheological fluid under similar conditions produces a stress state that tends to decay to zero. In other words, a viscoelastic fluid exhibits an unlimited number of underformed configurations, whereas a viscoelastic solid may have only a single equilibrium configuration, Lakes (1998) and Christensen (1971).

A distinct class of biological materials defined as bioviscoelastic fluids comprises such materials as saliva, mucus, sputum and synovial fluids. The most prevalent viscoelastic biofluid is protoplasm, the contents of all living cells, Fung (1993). It is apparent that biofilms exhibit a similar type of behavior that can be described in terms of viscoelasticity theory.

In this study, the viscoelastic behavior of Streptoccocus mutans and pond water biofilms has been represented by the Burger model composed of the Maxwell and Kelvin elements, as shown in Fig. 2. The parameters of the Burger model for both biofilms are summarized in Table 1.

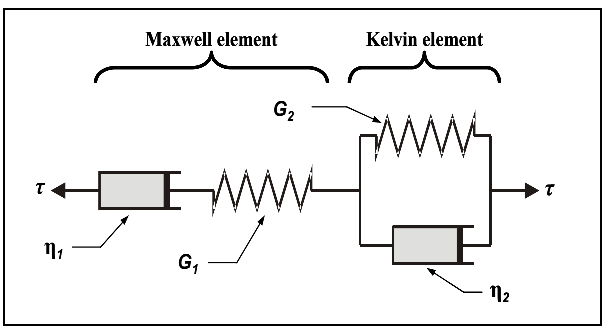


Figure 2. Burger model.

TABLE 1. Burger model characteristics of biofilms.

| Burger model parameters | Streptoccocus mutans | Pond water biofilm |
|---|---|---|
| $G_1$ | 63.9 Pa | 1.225 Pa |
| $G_2$ | 283 Pa | 2.526 Pa |
| $\eta_1$ | 75534 Pa*s | 1100 Pa*s |
| $\eta_2$ | 19028 Pa*s | 149 Pa*s |

The agreement between the experimental data and the viscoelastic response of biofilms predicted by the Burger model is illustrated in Fig. 3. The respective relaxation functions for both biofilms have been derived analytically, as illustrated in Fig. 4.

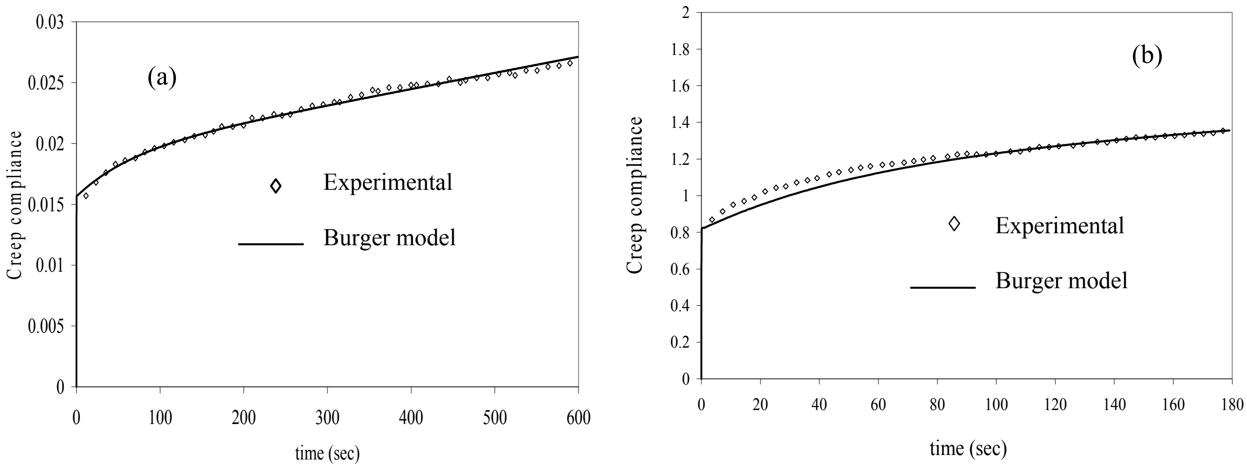


Figure 3. Creep compliances
(a) Streptoccocus mutans; (b) Pond water biofilms.

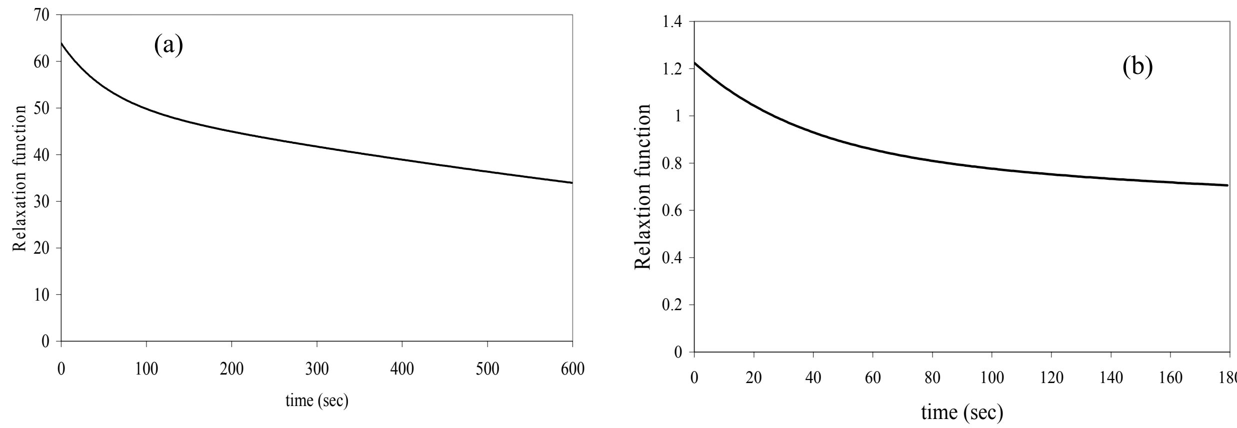


Figure 4. Relaxation functions
(a) Streptoccocus mutans; (b) Pond water biofilms.

## Conclusions

The study presented in this paper concerns the mechanical properties of two biofilms, Streptoccocus mutans and pond water biofilms. Based on rheological measurements it has been observed that, within certain limits, both biofilms have exhibited linearly viscoelastic behavior. The Burger model has been used to describe the creep compliances of the biofilms, showing good agreement with the obtained test data for the duration of the experiments. The respective relaxation functions have been determined analytically.

It is of interest that, in general, biomaterials appear to exhibit remarkable resemblance with the response of synthetic polymers, e.g. Janmey et al. (1990). It is evident that qualitative characterization of the mechanical properties of biofilms is critical in terms of enhancing the understanding of their biological functions, survival mechanisms, and surface attachment processes. On this basis, innovative strategies for mechanical removal of problematic pathogen containing biofilms can be developed.

## Acknowledgements

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## Appendix 1. Notation

G - instantaneous shear modulus

$\tau$ - shear stress

$G_1$, $G_2$, $\eta_1$ and $\eta_2$ - Burger model parameters