RHEOLOGICAL PROPERTIES OF BIOFILMS: STEADY AND TRANSIENT SHEAR FLOW MODELING

L. Prades[1] , Y. Bautista[2], J. Climent[3], V. Sanz[2], S. Chiva[3], A. D. Dorado[1] and X. Gamisans[1*]

[1] Department of Mining, Industrial and ICT Engineering, Universitat Politècnica de Catalunya, Spain
[2] Instituto Universitario de Tecnología Cerámica, Universitat Jaume I, Spain
[3] Department of Mechanical Engineering and Construction, Universitat Jaume I, Spain
*email: xavier.gamisans@upc.edu, phone: +34938777234

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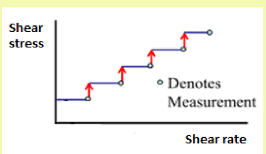
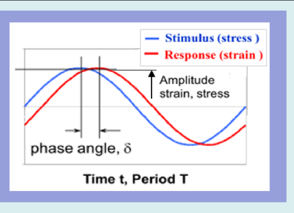
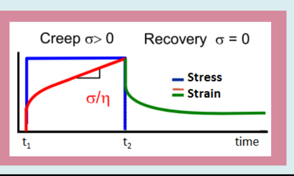
INTRODUCTION

Biofilms are considered as complex microbial structures containing mainly microorganisms, nucleic acids, proteins and extracellular polymeric substances (EPS). **The shear stress caused by the fluid flow over fixed biofilms is a factor of paramount importance which influences** their development, mass transfer and detachment and, hence, affecting **the bioreactor operation**. **The aim of this study was to investigate extensively the rheological properties of heterotrophic biofilms** present in bioreactors, by performing tests and models development. The flow effect characterization on biofilms was performed under steady shear, oscillatory and transient measurements. Suspended biomass (SB) samples were also analyzed to complete the study, comparing their rheological behavior with that obtained from the biofilms.

EXPERIMENTAL SET-UP

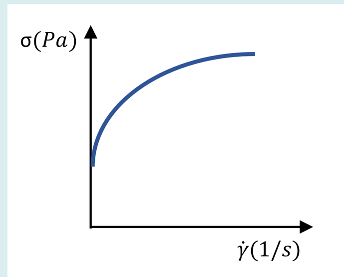
Samples of **various concentrations** from aerobic **heterotrophic biofilms** of a flat plate bioreactor and **suspended biomasses** from the same heterotrophic inoculum were analyzed.

Rheological tests were performed in a **BOHLIN CVO 120 HR rheometer** using a cone–plate geometry and a solvent trap to avoid evaporation. The experimental essays were carried out in **three different shear modes**:

Shear flow		Method	Input	Information
Steady		Shear stress ramp	Increasing shear stress	Yieldpoint
Oscillatory		Amplitude sweep	Stepwise increasing stress (10 to 200 Pa, ω = 1Hz)	Network stability
		Frequency sweep	Stepwise increasing frequency (0.01 to 10 Hz, 1% strain)	Time dependence
Transient		Creep test	Constant shear stress (12 and 40 Pa during 180 s)	Deformation

MODELS DEVELOPMENT

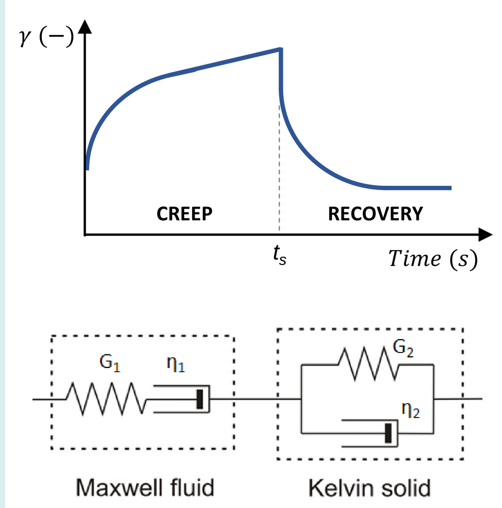
Steady shear flow model: The **Herschel-Bulkley model** (HBM) was adopted to characterize the behavior of biofilm and suspended biomass samples. The shear stress (σ) and the viscosity (η) are described as (Mezger, 2006):



$$\sigma = \sigma_y + K \cdot \dot{\gamma}^n \qquad \eta = \sigma / \dot{\gamma}$$

where σ_y is the yield stress (Pa), K is the fluid consistency index (Pa s), n is the flow behavior index (-) and $\dot{\gamma}$ is the shear rate (s^{-1}).

Transient shear flow model: The **four elements Burger approach** was selected to model viscoelastic Behavior (Towler et al., 2003). It is made up of a Kelvin-Voigt solid (spring G_1 and dashpot η_1) and a Maxwell liquid (spring G_2 and dashpot η_2) linked in series to each other. It is solved for the creep and recovery shear strain as follows:



$$\gamma(t) = \sigma \cdot \left[\frac{1}{G_1} + \frac{t}{\eta_1} + \frac{1}{G_2} \cdot \left(1 - e^{-G_2 t/\eta_2}\right)\right]$$

$$\gamma(t) = \sigma \cdot \left[\frac{t_s}{\eta_1} + \frac{1}{G_2} \cdot \left(e^{-G_2 t_s/\eta_2} - 1\right) \cdot e^{-G_2 t/\eta_2}\right]$$

where Υ(t) is the shear strain (-), t is time (s), σ is the applied shear stress (Pa), G_1 and η_1 are the Kelvin-Voigt elements, G_2 and η_2 are the Maxwell element and t_s is the time (s) at the end of the creep period.

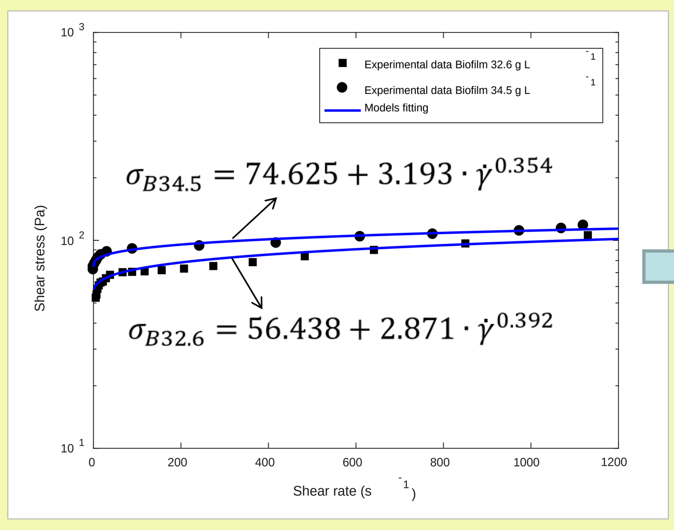
RESULTS AND DISCUSSION

Firstly, in the steady shear flow measurements, the deformation under a shear stressing flow was measured, recording the shear rate to obtain the basic flow behavior of the biofilm and suspended biomass (SB) samples, and characterizing the viscous and viscoplastic properties in detail. Secondly, dynamic strain-sweep measurements were performed to determine the linear viscoelastic regimen (LVR) and to examine the viscoelastic behavior in large amplitude oscillatory shear and, dynamic frequency-sweep measurements were conducted in order to interpret the relationship between the linear viscoelastic behavior and the microstructure of biological samples. Finally, the time-dependent nature of the samples in the linear region was proved performing the creep and recovery tests at various shear stresses.

RHEOLOGICAL CHARACTERIZATION IN THE STEADY SHEAR FLOW

Two **biofilm samples at different concentrations (X) were analyzed**, exhibiting both similar behavior:

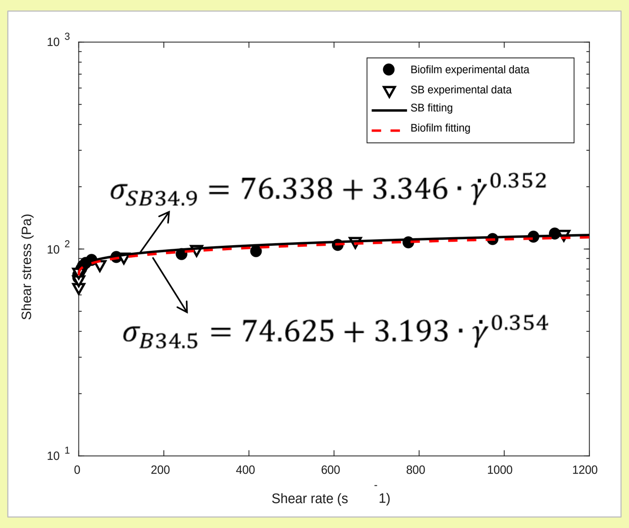
- **Being shear-thinning** fluids with **yield stress**, (**characteristic of gel-like structures**).
- The estimated HBM parameters revealed an **influence of the biofilm concentration in the rheological behavior.**



If ↑X then ↑σ_y ↑K ↓n

A **comparison** of the **rheological properties between biofilms and SB** was performed, since both biological samples are of analogous nature.

Biofilm and SB samples with very similar VSS concentration (34.5 and 34.9 g L^{-1} respectively) were contrasted:



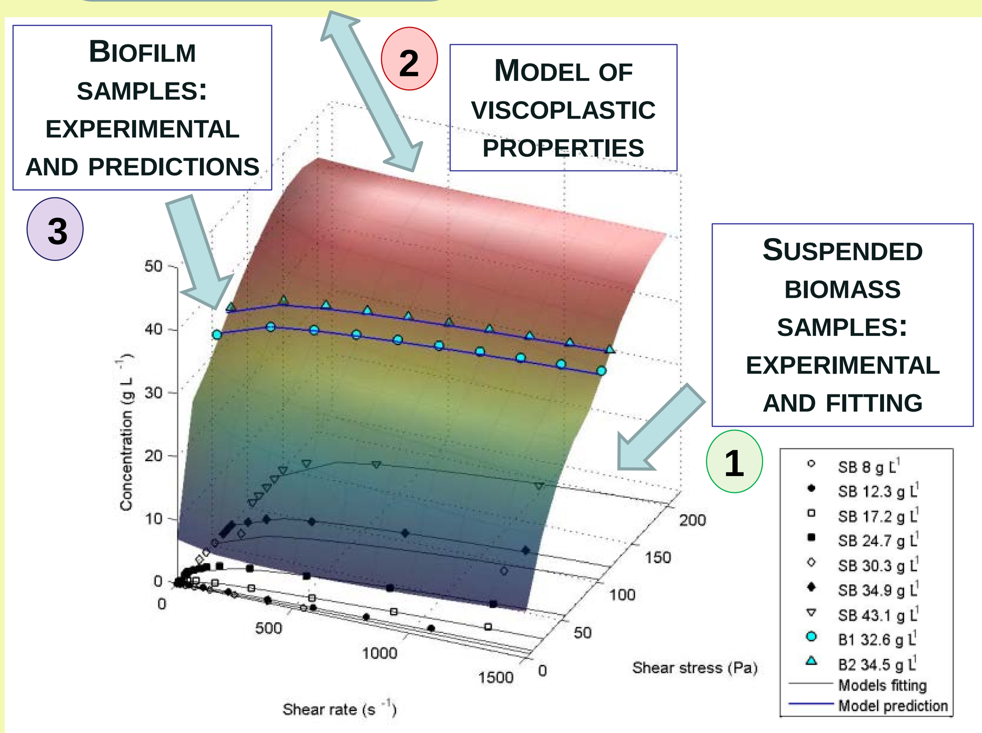
SB samples in a wide range of concentrations were also analyzed → to develop a complete characterization

Samples presented **analogous behavior**, since at higher shear stress, the **cellules were aligned into the flow direction** (Klapper et al., 2002) due to break down of aggregates.

Correlations to describe viscoplastic behavior of BIOFILMS in function of samples concentration (X):

$$\sigma_y = 9.3e^{-4} \cdot X^{3.17}$$
$$K = \mu_w \cdot (1 + 0.73 \cdot X^{2.38})$$
$$n = 0.09^X - 0.28 \cdot X^{0.94}$$

Defined based on η (X→0) = water viscosity (μ_w): *if* X → 0 *then* σ_y → 0, K → μ_w, n → 1, η_L → μ_w



1. **SB samples were characterized**, also showing a dependency with the X.
2. **A new set of HBM parameters were established** to describe accurately the rheological behavior of SB in function of X.
3. **The validation of the defined correlations** was achieved since **predicted flow curves for biofilms** showed the same behavior than experimental data.

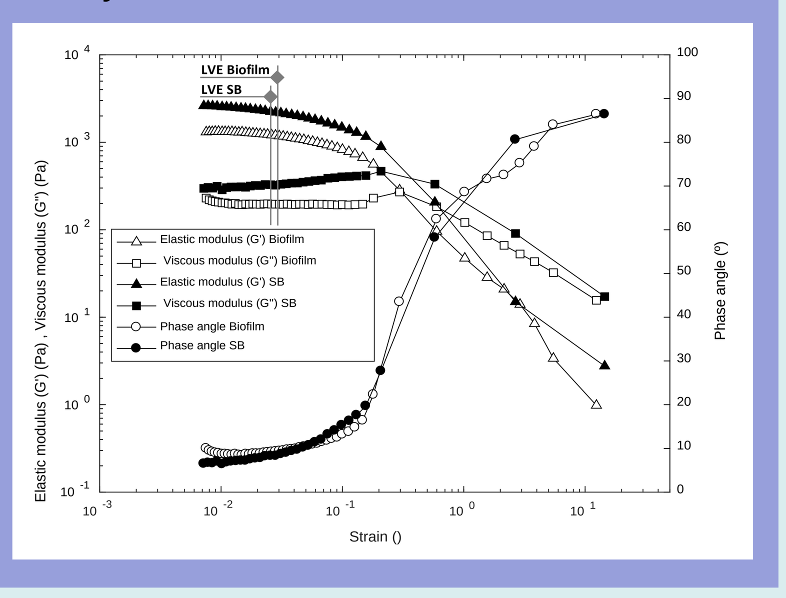
RHEOLOGICAL CHARACTERIZATION IN THE OSCILLATORY SHEAR FLOW

Biofilm and SB samples with very similar VSS concentration (34.5 and 34.9 g L^{-1} respectively) were contrasted.

The elastic behavior dominated the viscous one inside the LVR for both samples, **showing their gel character,** which agrees with other authors who studied mechanism and structure of biofilms (Wilking et al., 2011). Also, both samples had very close LVR limit values.

However, some **inequalities were detected in their structure and viscoelastic properties**:

- The **elastic (G') and** viscous (G'') modulus were greater for SB than biofilm sample, which indicated that the **interaction between the structure components of SB was stronger**.
- These differences may be related to the **higher EPS concentration of the biofilm** sample that might interfere in the direct interactions between microorganisms.



RHEOLOGICAL CHARACTERIZATION IN THE TRANSIENTSHEAR FLOW

Biofilm and SB samples with very similar VSS concentration (34.5 and 34.9 g L^{-1} respectively) were tested at different shear stresses inside the LVR.

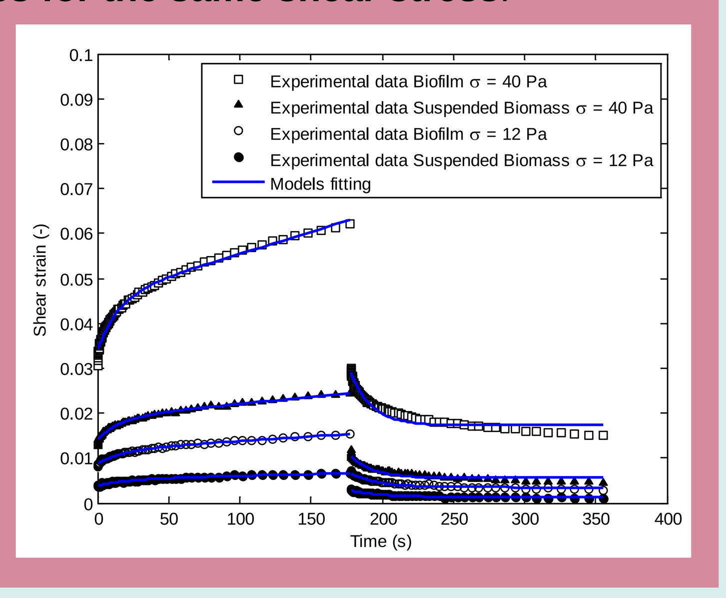
This **time-dependent strain response** undoubtedly pointed out that **both samples presented viscoelastic fluid behavior**, as reported in previous works of biofilms (Towler et al., 2003).

Biofilm and SB samples behavior showed **remarkable differences for the same shear stress**:

- **Biofilm exhibited higher strain** than SB for all tested shear stress. It is also observed in the **parameters** of Burger model:

Sample	G1 (Pa)	η1 (Pa s)	G2 (Pa)	η2 (Pa s)
Biofilm	1377	508261	3497	64044
SB	2992	1434600	8166	171549

- **Biofilm parameters** are **lower**, explaining why its **deformation was much greater** than for SB, which showed greater opposition to deformation, and indicating that **higher presence of EPS in biofilm** samples **affected** considerably their **internal structures and mechanical properties** (Wilking et al., 2011).



CONCLUSIONS

The **viscous and viscoelastic properties of biofilms and suspended biomasses were investigated via rheological analyses** under steady, oscillatory and transient shear flow. With this complete rheological characterization, **models for the description of the biofilm as a pseudo-plastic fluid as well as a viscoelastic material were developed**, allowing to define the biofilm as an independent fluid phase, which can be readily implemented coupled fluid dynamics codes. In addition, the findings suggest that the **suspended biomass could be used for the characterization of the biofilms viscosity and flow curves**, due to its feasibility to obtain the samples. For **strain modeling, biofilms samples** will be **needed** to accurate **reproduce their transient behavior**, since the important role of the EPS during the deformation was proven.

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