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Biofilm mechanics in an extreme acidic environment: Microbiological significance

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We have performed rheological experiments of the different samples listed in Table 1. From the dynamic amplitude strain sweeps, the curves of shear amplitude strain, γ, versus stress, σ, obtained in at least three replicates are shown in Fig. S1A. The average results from these raw data are depicted in Fig. S1B.

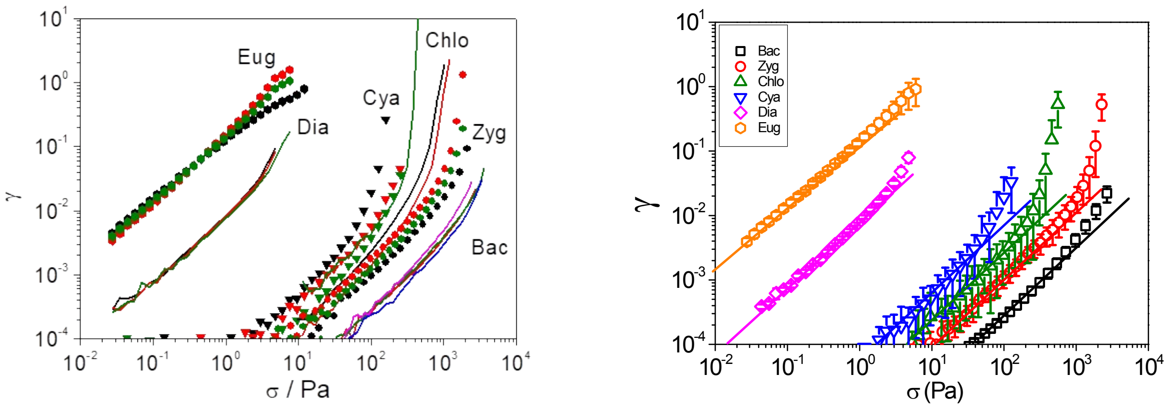

Fig. S1. Left. Raw data of strain amplitude measurements (3 to 5 tests) of the samples under study. Right. Averaged results of the raw data.

Application of Burger's model

The Burgers model contains 4 mechanical elements disposed as shown in Fig. S3. In this model, Maxwell and Kelvin models are connected in series, and it has been usually applied to biofilms [see reference Towler et al. 2003], mainly to creep and recovery experiments.

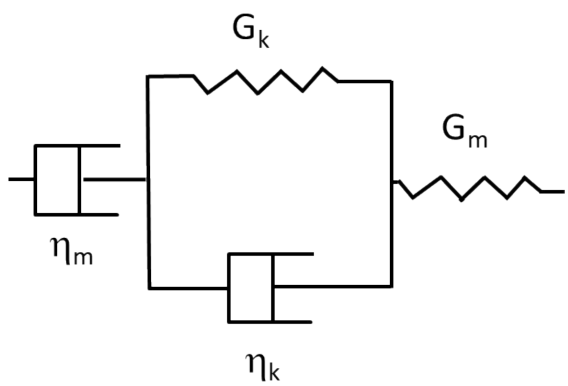

Fig. S2. Representation of the Burgers model.

This model combines two elastic and two viscous mechanical elements which can be represented as springs and dashpots, respectively (Fig. S2). The specific combination of Burgers model leads to a constitutive equation of constant coefficients that establishes the relation between the stress and the strain as a function of time:

$$\sigma + \left(\frac{\eta_m}{G_m} + \frac{\eta_m}{G_k} + \frac{\eta_k}{G_k}\right)\frac{\partial \sigma}{\partial \sigma} + \left(\frac{\eta_m \eta_k}{G_m G_k}\right)\frac{\partial^2 \sigma}{\partial t^2} = \eta_m \frac{\partial \gamma}{\partial t} + \left(\frac{\eta_m \eta_k}{G_k}\right)\frac{\partial^2 \gamma}{\partial t^2} \qquad \text{(S1)}$$

This constitutive equation should apply for the different experiments (dynamic, creep and recovery) using the same set of coefficients. We have applied this model using the corresponding equations for each experiment:

$$\gamma = \frac{\sigma}{G_m} + \frac{\sigma}{\eta_m} + \left[1 - exp\left(-\frac{G_k}{\eta_k} t\right)\right] \tag{S2}$$

for the strain time evolution in creep experiments,

$$\gamma = \frac{\sigma}{\eta_m} t_c + \frac{\sigma}{G_k}\left[1 - exp\left(-\frac{G_k}{\eta_k} t_c\right)\right] exp\left(-\frac{G_k}{\eta_k} t\right), \tag{S3}$$

for the strain time evolution in recovery experiments, for $t > t_c$,

$$G' = \frac{J'}{J'^2 + J''^2} \tag{S4}$$

$$G'' = \frac{J''}{J'^2 + J''^2} \tag{S5}$$

$$J' = \frac{1}{G_m} + \frac{G_k}{G_k^2 + \omega^2 \eta_k^2} \tag{S6}$$

$$J'' = \frac{1}{\omega \eta_m} + \frac{\omega \eta_k}{G_k^2 + \omega^2 \eta_k^2} \tag{S7}$$

for dynamic experiments. A set of coefficients, G_m, G_k, η_m and η_k, from the creep experiment is obtained for each sample (see Table S1). The fit of Eq. S2 to creep data is observed in Fig. S3.

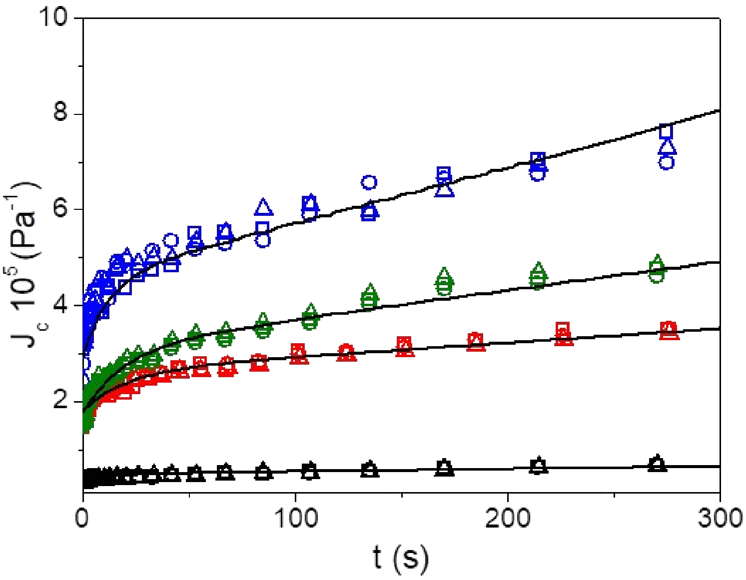

Fig. S3. Creep compliance, J_c, versus time of the samples studied at different shear stress values, σ. (○ 1000 Pa, ℘ 500 Pa, □ 250 Pa) CEM-Bac; (○ 600 Pa, ℘ 300 Pa, □ 150 Pa) CEM-Zyg; (○ 200 Pa, ℘ 150 Pa, □ 75 Pa) ANG-Chlo; (○ 60 Pa, ℘ 30 Pa, □ 15 Pa) CEM-Cya. Burgers model (Eq. S2) is represented by the solid lines. The experimental data are the same than in Fig. 6A of the main manuscript.

Table S1. Burgers parameters for the samples studied.

Sample	G_m (kPa)	η_m(kPa s)	λ_m(s)	G_k(kPa)	η_k(kPa s)	λ_k(s)
CEM-Bac	266.3	186,000	698	939.1	15,200	16.2
CEM-Zyg	54.0	44,720	828	121.5	1,690	13.9
ANG-Chlo	47.2	13,800	292	116.5	1.066	9.2
CEM-Cya	34.1	8,600	252	61.8	745.0	12.1

In the case of the Burgers model, two characteristic relaxation times, λ_m and λ_k can be defined ($\lambda_m=\eta_m/G_m$ and $\lambda_k=\eta_K/G_k$). We have obtained that the Burgers model, with two well defined relaxation events is not able to describe the complete set of dynamic experiments in these power-law materials, likely due to its simplicity. This is a clear indication of the broad relaxation spectrum related to the specific microstructure composing these power law materials.

For this reason, we decided to apply the fractional Maxwell model, which is capable to describe broad relaxation events in complex networks (Jaishankar & McKinley 2013).

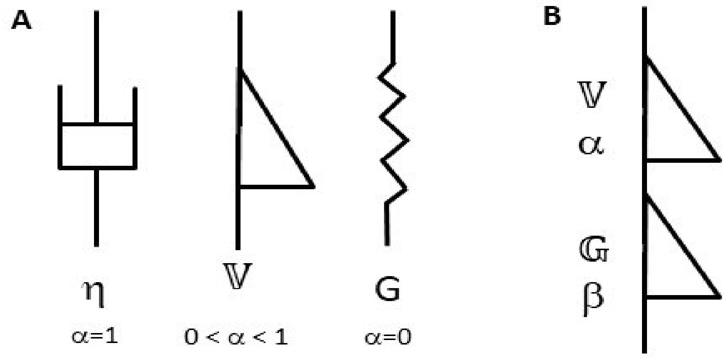

Fig. S4 A) is the representation of the spring-pot mechanical element described by the quasi-property, V, and the power-law exponent, α. The two extreme cases, viscous (α = 1) and elastic (α = 0), are also depicted. **B)** is the fractional Maxwell model mechanical analogue (two spring-pots disposed in parallel). This model is defined by four material parameters: two quasi-properties (V and G) and two power-law exponents (α and β). Both schemes adapted from Jaishankar & McKinley (2013).

In this approach a spring-pot unit is described, for which the constitutive equation is given by:

$$\sigma(t) = V\frac{d^{\alpha}\gamma(t)}{dt^{\alpha}} \tag{S8}$$

where V is a constant dependent on the material and d^{α}/dt^{α} is the fractional derivative operator. When α = 1, the material behaves like a dash-pot (viscous), and when α = 0, the material behaves like a spring (elastic). The spring-pot is then a viscoelastic element, and the "quasi-property" V characterizes the magnitude of the mechanical response of the material (Fig. S4A). We have considered the fractional Maxwell model (FFM), composed of two spring-pot elements in parallel as in Fig. S4B. In this case the model displays four parameters, V, α, G, and β, which should be obtained by curve fitting to the experimental results.

The corresponding equation for creep, recovery and dynamic experiments are as follows:

$$J(t) = \frac{\sigma \quad t^{\alpha}}{V\Gamma(1+\alpha)} + \frac{\sigma \quad t^{\beta}}{G\Gamma(1+\beta)} \tag{S9}$$

for the creep compliance response and

$$J(t) = \frac{\sigma\left[t^{\alpha} - (t - t_c)^{\alpha}\right]}{V\Gamma(1+\alpha)} + \frac{\sigma\left[t^{\beta} - (t - t_c)^{\beta}\right]}{G\Gamma(1+\beta)} \tag{S10}$$

for the recovery experiment, being t_c the creep time.

The characteristic time of the material is defined as:

$$\lambda_c = \left(\frac{V}{G}\right)^{1/(\alpha - \beta)} \tag{S11}$$

The corresponding equations for the storage and loss moduli in dynamic measurements are obtained as:

$$G'(\omega) = G_0 \frac{(\omega\lambda_c)^{\alpha} cos(\pi\alpha/2) + (\omega\lambda_c)^{2\alpha-\beta} cos(\pi\beta/2)}{(\omega\lambda_c)^{2\alpha-\beta} + 2(\omega\lambda_c)^{\alpha-\beta} cos[\pi(\alpha-\beta)/2] + 1} \quad \text{(S12)}$$

$$G''(\omega) = G_0 \frac{(\omega\lambda_c)^{\alpha} sin(\pi\alpha/2) + (\omega\lambda_c)^{2\alpha-\beta} sin(\pi\beta/2)}{(\omega\lambda_c)^{2\alpha-\beta} + 2(\omega\lambda_c)^{\alpha-\beta} cos[\pi(\alpha-\beta)/2] + 1} \quad \text{(S13)}$$

being G_0 the characteristic modulus of the material:

$$G_0 = V\lambda_c^{-\alpha} \quad \text{(S14)}$$

References

B. W. Towler, C. J. Rupp, A. B. Cunningham and P. Stoodley, *Biofouling*, 2003, **19**, 279-285.

A. Jaishankar and G. H. McKinley, *Proceedings of the Royal Society a-Mathematical Physical and Engineering Sciences*, 2013, **469**.