Supplemental information for

Chelate chemistry governs ion-specific stiffening of *Bacillus subtilis* B-1 and *Azotobacter vinelandii* biofilms

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1. Purity of purified γ-PGA

As in a previous study from our group [1], we purified γ-PGA from liquid cultures of *B. subtilis* B-1 bacteria following a purification procedure established for different bacteria of the genus *Bacillus*, such as *B. subtilis natto* [2].

As described there, the identity of γ-PGA can be verified spectroscopically by comparing it to commercial PGA (obtained from Sigma Aldrich, St. Louis, USA) as shown below:

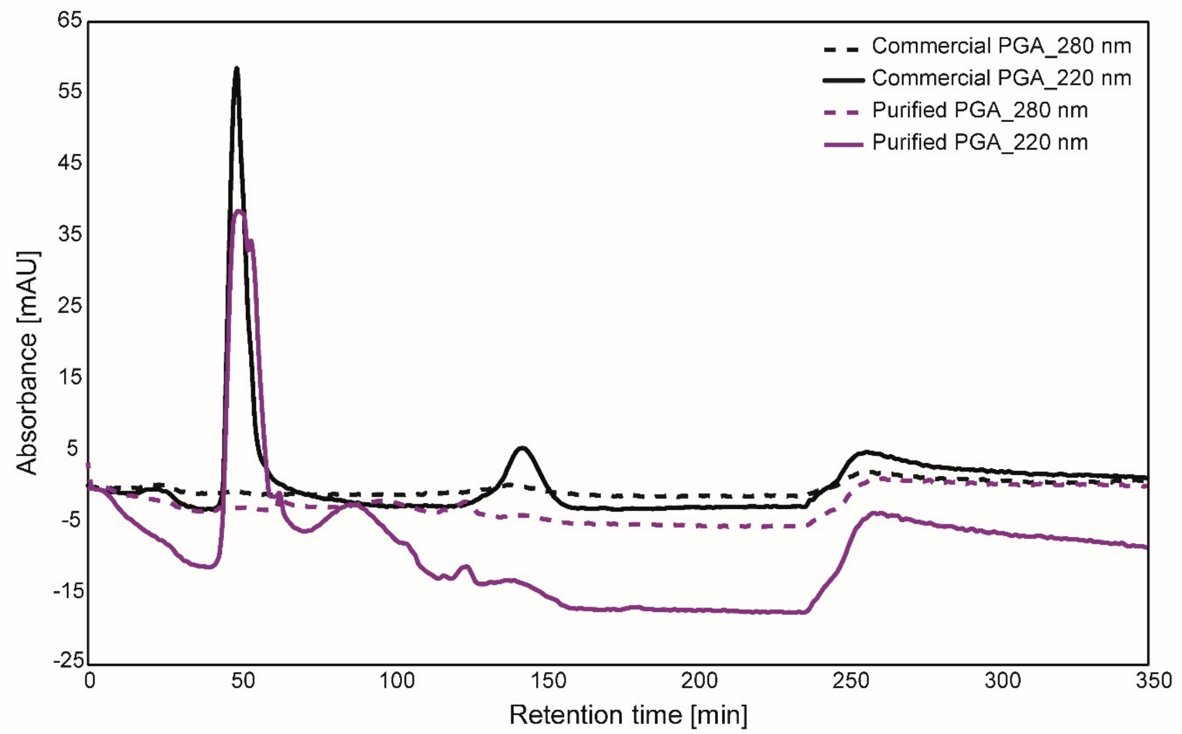

Figure S1: Gel filtration chromatograms of commercial and in-lab purified PGA. The same amount of a commercial or purified PGA solution (concentration 0.05% (w/v)) was run through a Sepharose 6FF XK50/100 column, and the absorbance at 220 nm and 280 nm was compared. Both PGA variants give a strong peak at 220 nm and at the same retention time. Moreover, neither sample exhibits significant absorption at 280 nm, which shows that the purity of both samples is comparable.

2. Exemplary large amplitude oscillatory shear (LAOS) measurements

To determine the linear response range of both, soft (= treated with water) and stiff (= metal ion enriched) biofilm samples, LAOS measurements are conducted as shown below. The excellent reproducibility of those measurements also shows that surface slippage events do not occur with the slimy, sticky biofilm material.

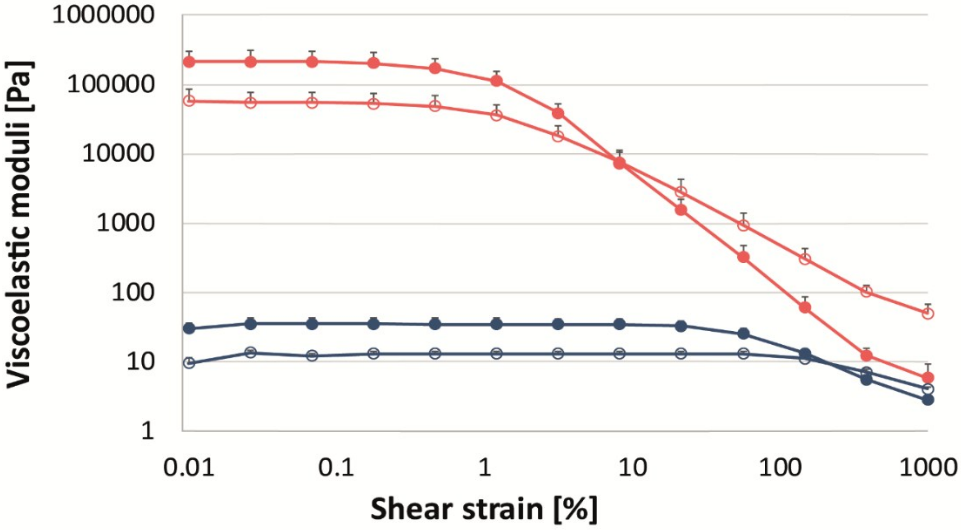

Figure S2: Viscoelastic behavior of *B. subtilis* B-1 biofilms at increasing oscillatory shear strain (LAOS measurements). Viscoelastic moduli are shown in dependence of the shear strain, and biofilm samples treated with water (blue symbols) and $CuCl_2$ (pink, final concentration in the samples was 250 mM), respectively, are compared. Full symbols denote the storage modulus, *G*', and open symbols denote the loss modulus, *G*''. The values shown represent the mean obtained from three independent measurements, and the error bars (which are sometimes smaller than the symbol size) represent the standard deviation.

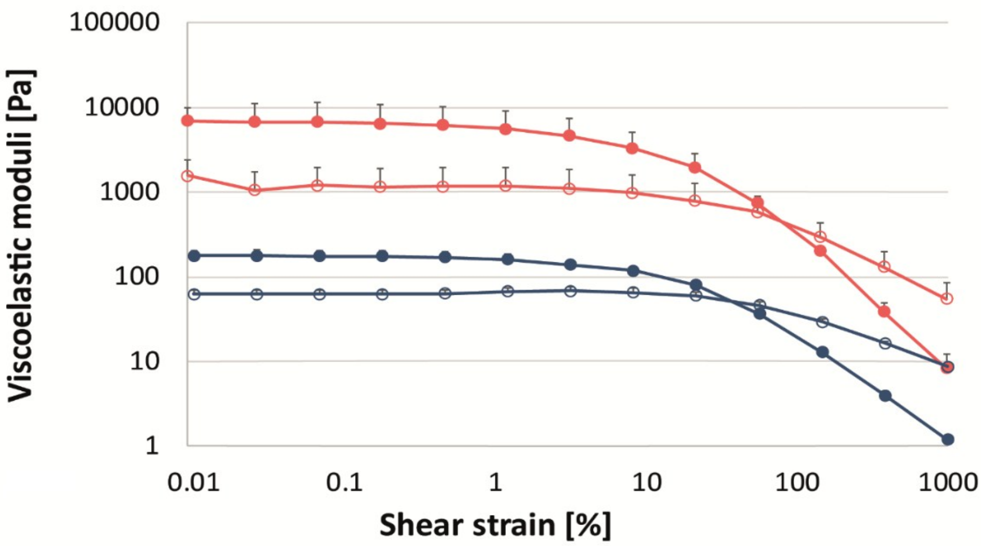

Figure S3: Viscoelastic behavior of *A. vinelandii* biofilms at increasing oscillatory shear strain (LAOS measurements). Viscoelastic moduli are shown in dependence of the shear strain, and biofilm samples treated with water (blue symbols) and $CuCl_2$ (pink, final concentration in the samples was 250 mM), respectively, are compared. Full symbols denote the storage modulus, *G*', and open symbols denote the loss modulus, *G*''. The values shown represent the mean obtained from three independent measurements, and the error bars (which are sometimes smaller than the symbol size) represent the standard deviation.

3. Exemplary frequency spectra

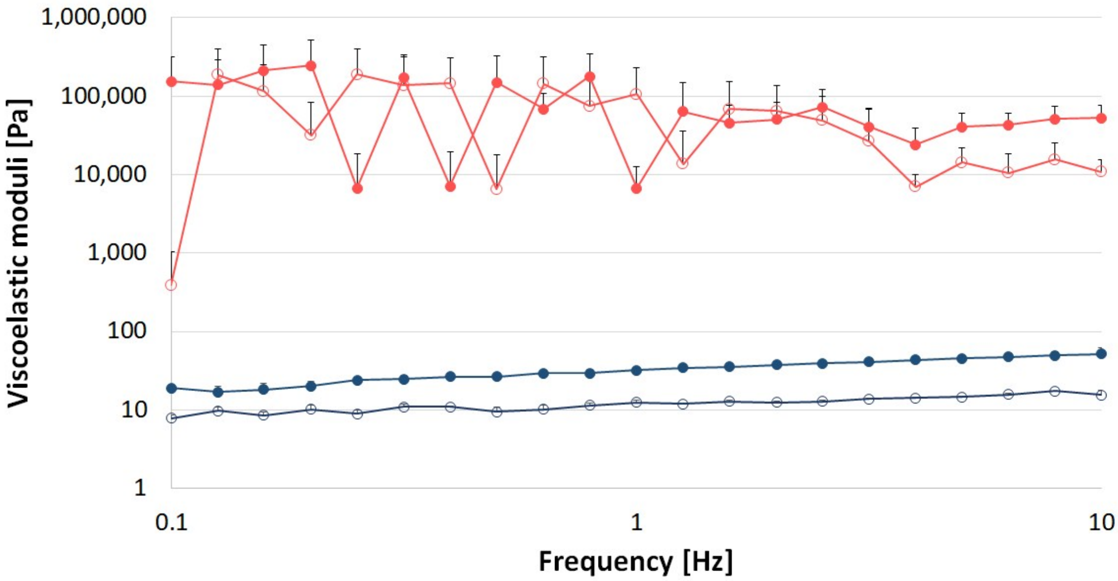

Figure S4: Viscoelastic response of *B. subtilis* B-1 biofilm with and without exposure to $CuCl_2$. Frequency spectra are shown for biofilm samples treated with water (blue symbols) and $CuCl_2$ (pink), respectively. In the second case, the final concentration of Cu^{2+} ions in the biofilm was set to 250 mM (using a $CuCl_2$ stock solution of 1 M which had a pH of 0.9). Full symbols denote the storage modulus, *G*', and open symbols denote the loss modulus, *G*''. The values shown represent the mean obtained from three independent measurements and the error bars (which are sometimes smaller than the symbol size) represent the standard deviation.

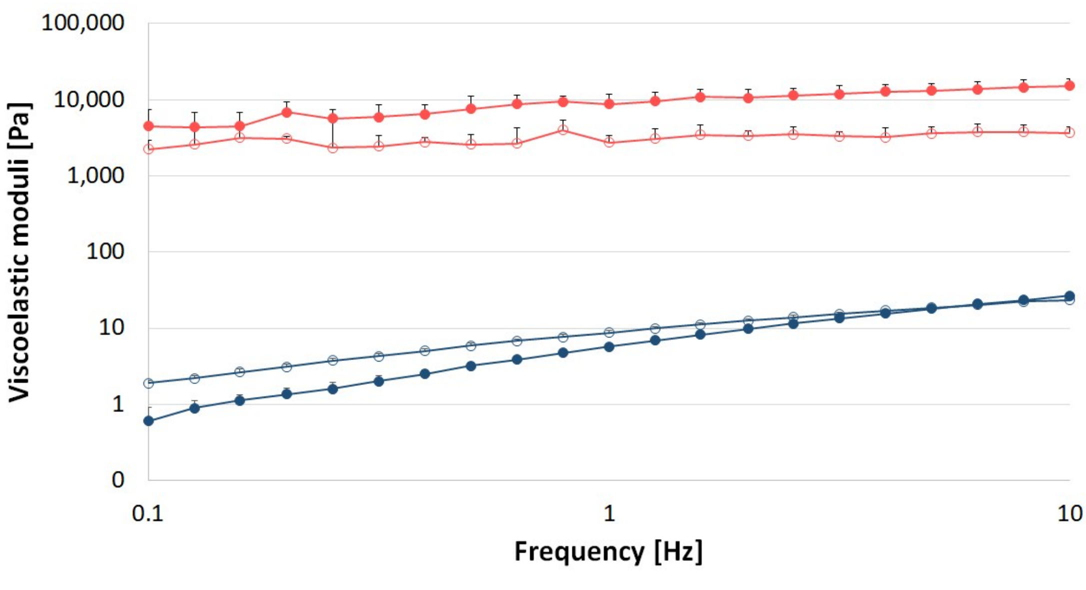

Figure S5: Viscoelastic response of a 1.5 % (w/v) γ-PGA solution with and without exposure to $CuCl_2$. Frequency spectra are shown for γ-PGA solutions treated with water (blue symbols) and $CuCl_2$ (pink), respectively. In the second case, the final concentration of Cu^{2+} ions in the biofilm was set to 250 mM (using a $CuCl_2$ stock solution of 1 M which had a pH of 0.9). Full symbols denote the storage modulus, *G*', and open symbols denote the loss modulus, *G*''. The values shown represent the mean obtained from three independent measurements and the error bars (which are sometimes smaller than the symbol size) represent the standard deviation.

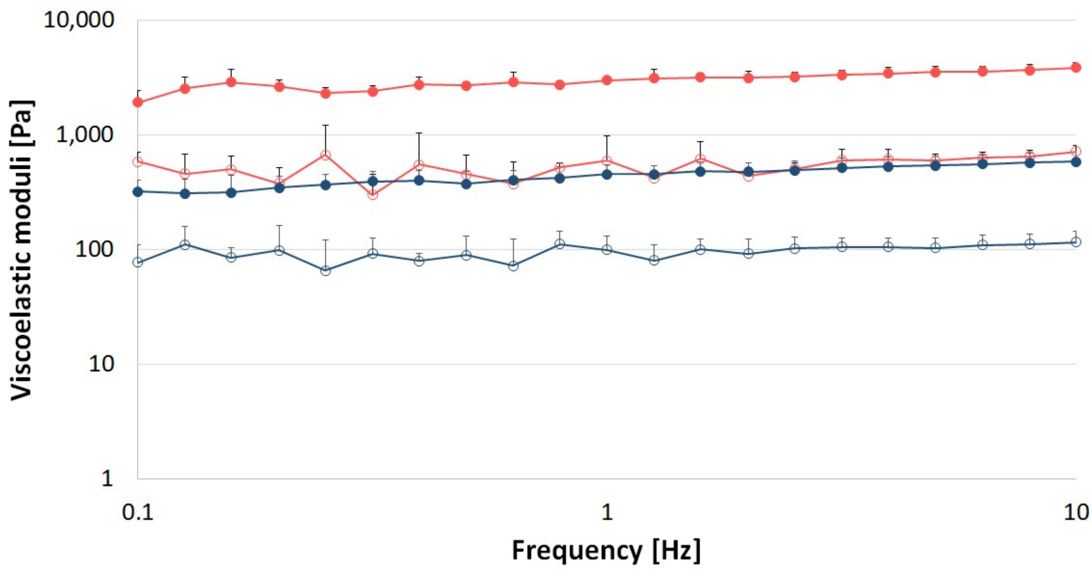

Figure S6: Viscoelastic response of an *A. vinelandii* biofilm with and without exposure to $CuCl_2$. Frequency spectra are shown for biofilm samples treated with water (blue symbols) and $CuCl_2$ (pink), respectively. In the second case, the final concentration of Cu^{2+} ions in the biofilm was set to 250 mM (using a $CuCl_2$ stock solution of 1 M which had a pH of 0.9). Full symbols denote the storage modulus, *G*', and open symbols denote the loss modulus, *G*''. The values shown represent the mean obtained from three independent measurements and the error bars (which are sometimes smaller than the symbol size) represent the standard deviation.

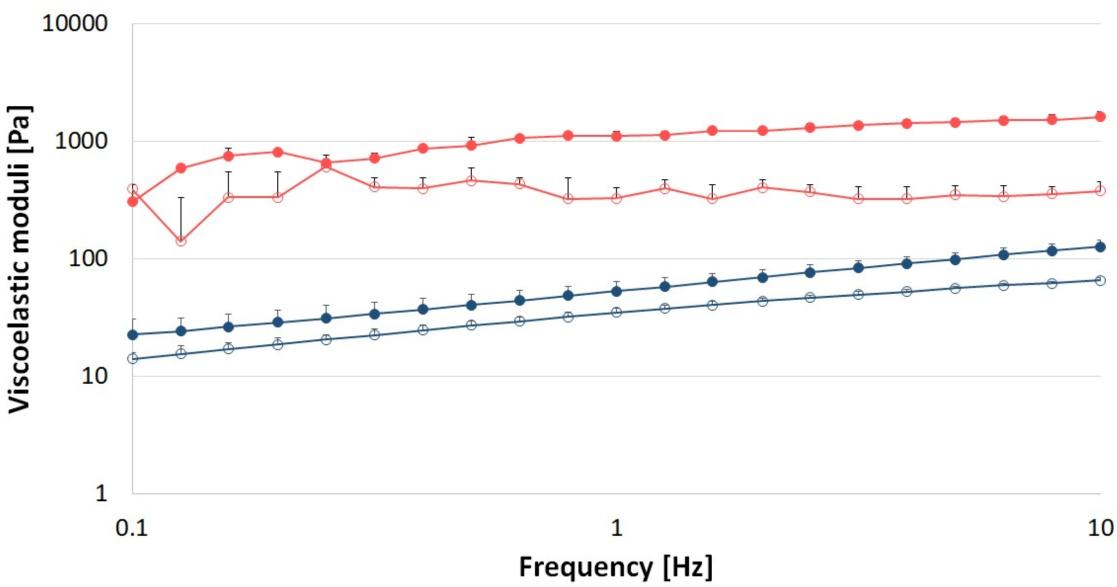

Figure S7: Viscoelastic response of 1.5 % (w/v) alginate solution with and without exposure to $CuCl_2$. Frequency spectra are shown for alginate solutions treated with water (blue symbols) and $CuCl_2$ (pink), respectively. In the second case, the final concentration of Cu^{2+} ions in the biofilm was set to 250 mM (using a $CuCl_2$ stock solution of 1 M which had a pH of 0.9). Full symbols denote the storage modulus, *G*', and open symbols denote the loss modulus, *G*''. The values shown represent the mean obtained from three independent measurements and the error bars (which are sometimes smaller than the symbol size) represent the standard deviation.

1. Elastic modulus of metal-ion treated biofilms as function of the ionic radius

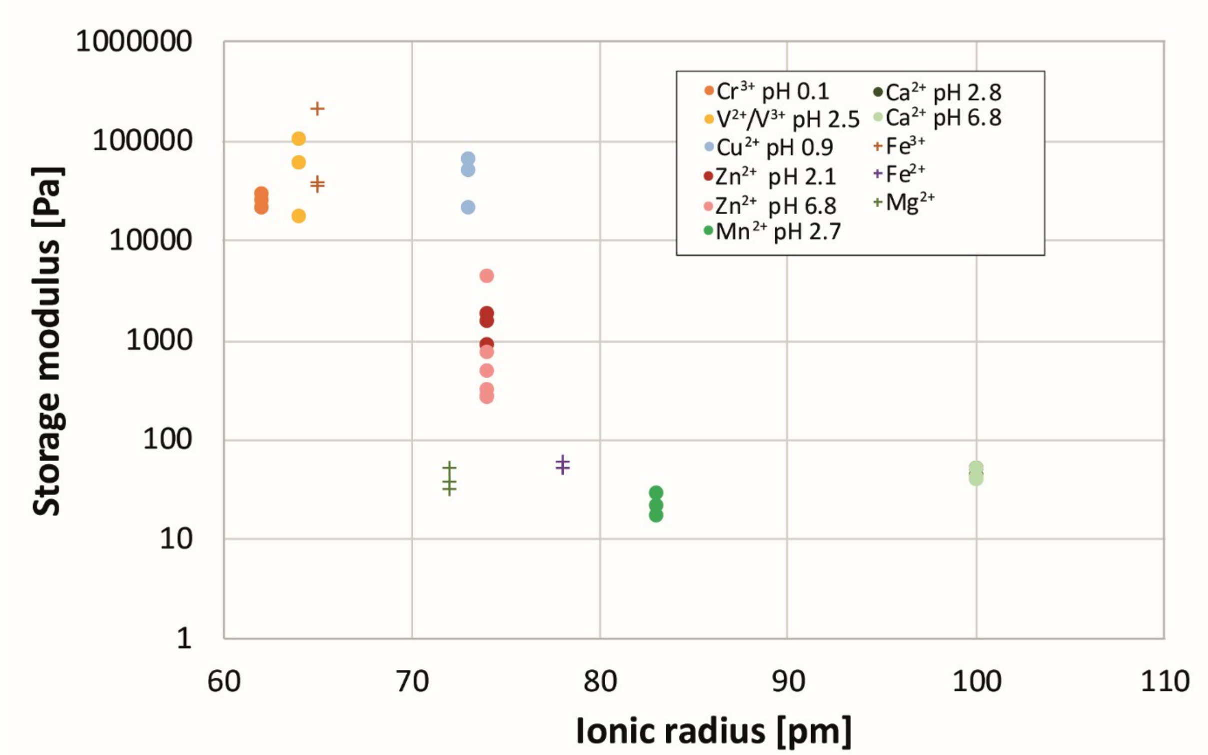

Figure S8: Plateau modulus of *B. subtilis* B-1 biofilms treated with different metal cations as a function of the ionic radius. Full circles denote the storage modulus, *G*', in the plateau regime as obtained in this study for individual samples. The final concentration of metal ions in the biofilms was 250 mM for all measurements conducted in this study (data depicted as circles). In addition, data points from a previous study by Grumbein *et al.* [3] is added to the graph (cross symbols). Samples from this earlier study had a final metal ion concentration of 50 mM. The ionic radius shown on the x-axis was chosen according to Riedel [4].

2. Time dependent biofilm stiffening induced by vanadium chloride

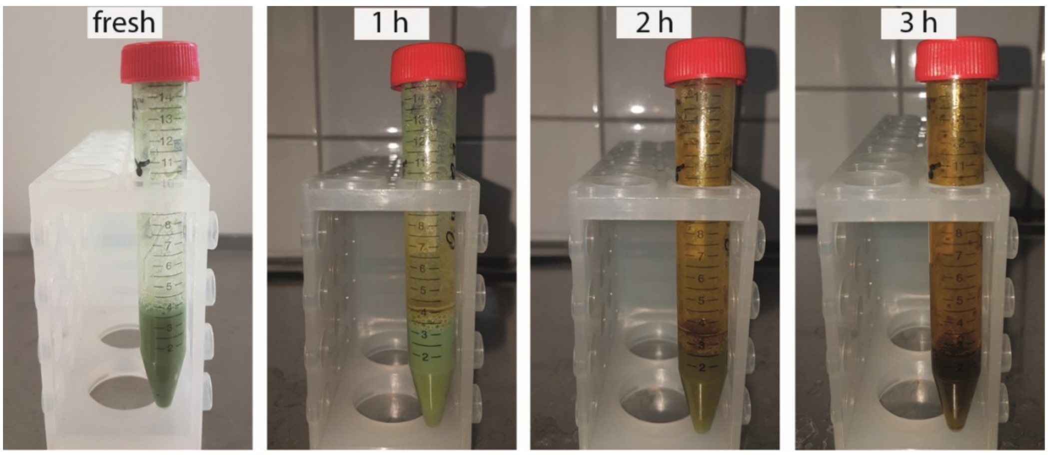

Figure S9: A vanadium chloride solution changes color as it oxidizes. A freshly prepared vanadium chloride solution (left) exhibits a mint-like, green color. Over time, this color changes into brown as the vanadium ions oxidize. The age of the solution is indicated above the respective image.

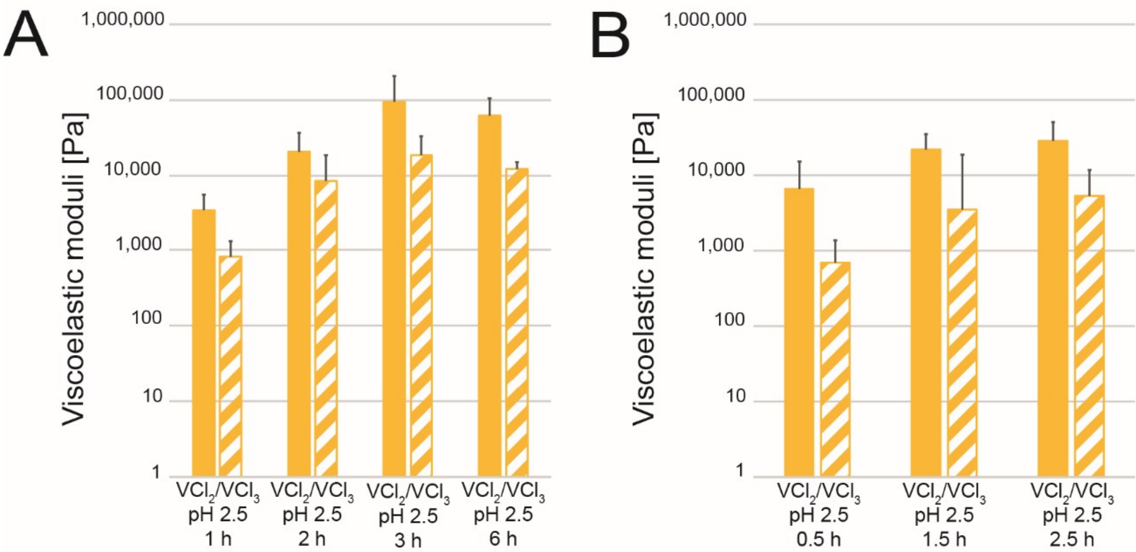

Figure S10: Viscoelastic moduli of *B. subtilis* B-1 biofilm (A) and γ-PGA solutions (B) treated with aging vanadium(II) chloride solutions. Full bars denote the storage modulus, *G*', and striped bares the loss modulus, *G*''. The samples were always treated for 5 minutes, only the age of the solution increased up to 6 hours. With increasing age of the vanadium(II) chloride solution, the ratio of V^{2+} and V^{3+} changes as V^{2+} oxidizes over time. The error bars represent the standard deviation as obtained from at least three independent samples.

Cited Literature

1. Hayta, E., Nur and O. Lieleg, *Biopolymer-enriched B. subtilis NCIB 3610 biofilms exhibit increased erosion resistance.* Biomater. Sci., 2019. **7**: p. 4675-4686.
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4. Riedel, E., *Anorganische chemie*. 2011, Berlin, New York: Walter de Gruyer.