# STRUCTURAL INVESTIGATION OF AQUEOUS SOLUTIONS OF *Bacillus subtilis* BIOFILM COMPONENTS


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## MOTIVATION and PROSPECTS

***Bacillus subtilis*** (BS) biofilms are composed of sticky gel-like **EPS-matrix** (polysaccharide levan, proteins, nucleic acids and other biopolymers). In pure aqueous system non-ionic **levan** exhibits rather peculiar **non-gelling** behavior up to high concentrations (60 wt. %). However, BS biofilm forms a weak hydrogel only up to 8 wt. % of major polymeric component levan. Our goal was to explore these differences in behavior of BS levan from the structural point of view and to study the effect of minor biofilm components (nucleic acids, proteins, simple electrolytes) and bacterial cells on structure and dynamics of levan solutions.

The results obtained for BS levan were compared to the ones obtained for commercially available levans from ***Zymomonas mobilis*** (ZM) and ***Erwinia herbicola*** (EH). Levan samples and polymeric mixtures were investigated by rheological, small-angle X-ray scattering (SAXS), static and dynamic light scattering (SLS and DLS), density and sound velocity measurements, as well as by microscopy. One of the aims was also to compare the structural properties of native and **synthetic biofilm**. Determination of polymer branching after per-O-methylation analysis and basic chemical and spectrophotometric analysis of the purity of the original levan samples was also performed.

## EXPERIMENTAL

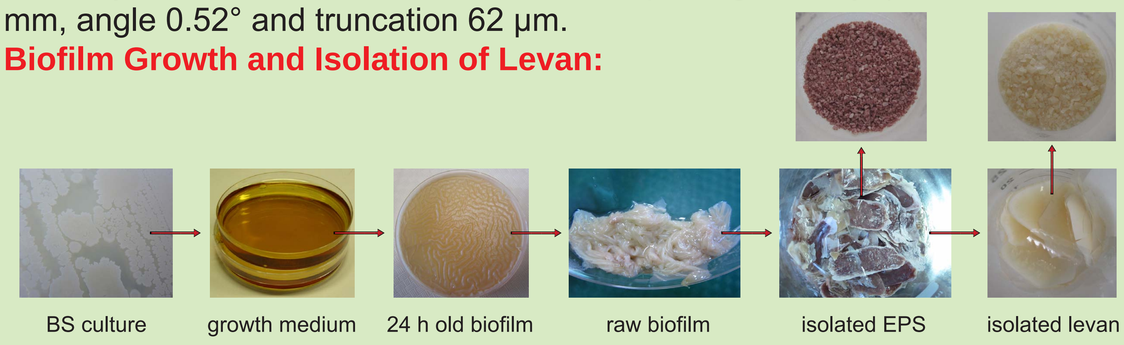
**Materials.** BS levan was isolated and purified directly from BS biofilm. DNA was isolated from BS cells. ZM and EH levans as well as protein collagen were purchased from Sigma Aldrich.

**SAXS Experiments** were performed on a modified Kratky camera with modern focusing multilayer optics at wavelength of $CuK_\alpha$ line.

**SLS and DLS Experiments** were performed on 3D-DLS Spectrometer in a 3D-cross-correlation mode at λ=632.8 nm.

**Dynamic Rheological Experiments** were performed on Anton Paar rheometers Physica MCR 302 and 301 with cone-plate geometry (with cone: r=60 mm, angle 0.52° and truncation 62 μm.

**Biofilm Growth and Isolation of Levan:**




## RESULTS and CONCLUSIONS

### BS, ZM, EH LEVANS

**Figure 1.** Viscosity curves - levans.

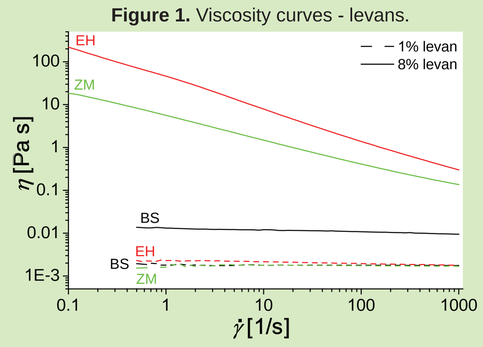
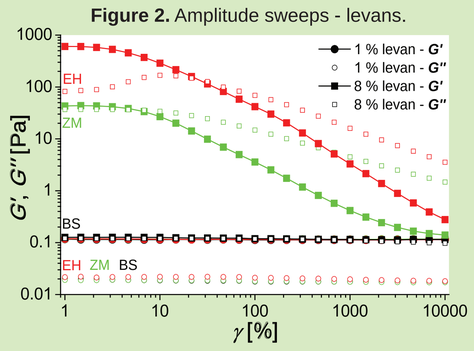



**Figure 2.** Amplitude sweeps - levans.




The macroscopic rheological results showed Newtonian-like behavior of aqueous solutions of levan at low concentrations but at the same time pointed out the strongly elastic character of these solutions. This elastic character was supported by the DLS results showing strong nondiffusive relaxation processes which could be explained by the strong intramolecular interactions of levan at such low concentrations. The latter also manifest themselves in the presence of large levan particles that were confirmed by microscopy. The extent of branching and the hydration of the levan samples of different origin were also investigated, but they did not show any considerable mutual differences. Structural details on the nanoscale were obtained by SAXS.

**Figure 3.** Angle dependent DLS results - levans.

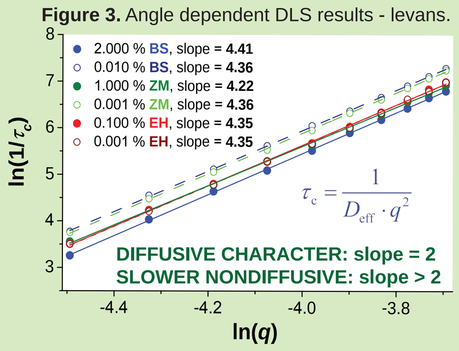



**Figure 4.** Hydration number - levans.

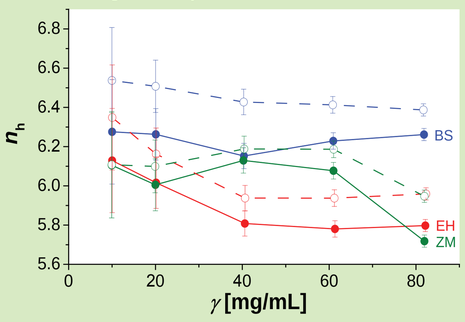



**Figure 5.** Microscopy images - levans.

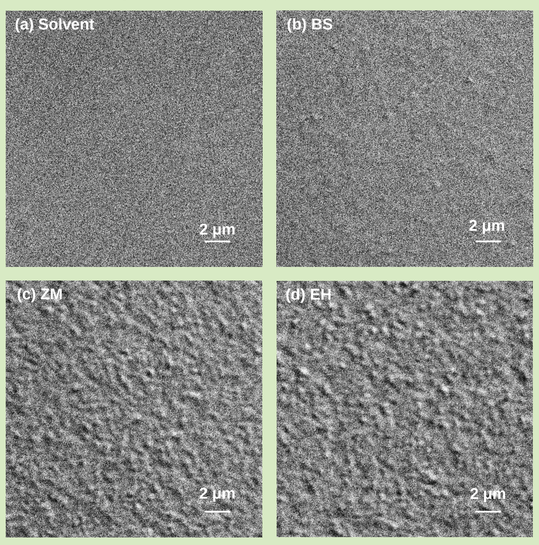
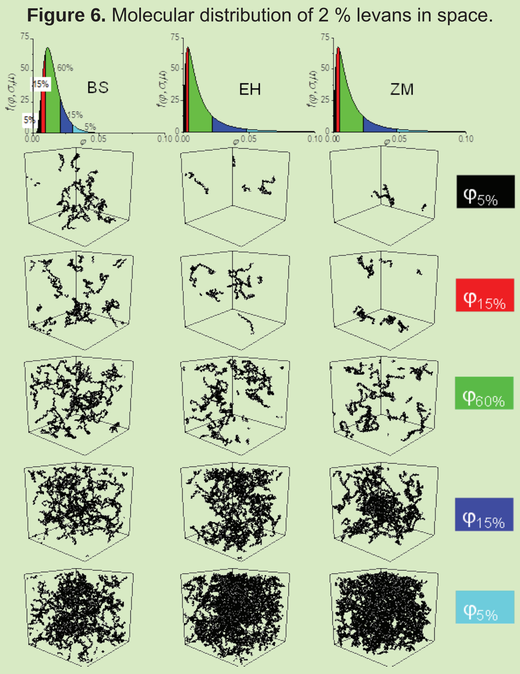



**Figure 6.** Molecular distribution of 2 % levans in space.




### SYNTHETIC BIOFILM BS

**Figure 7.** Viscosity curves - L, L+D, L+C, sEPS.

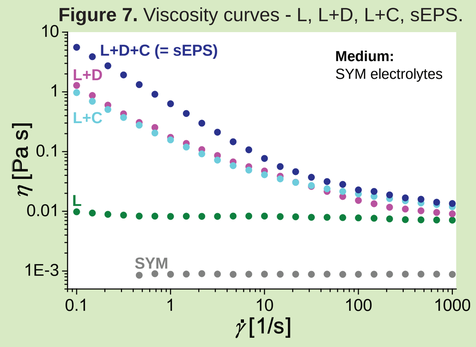



**Figure 8.** Viscosity curves - sEPS, sBF, nBF1, nBF2.

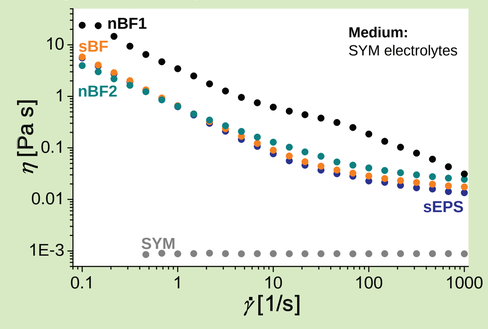



**Figure 9.** Amplitude sweeps - L, L+D, L+C, sEPS.

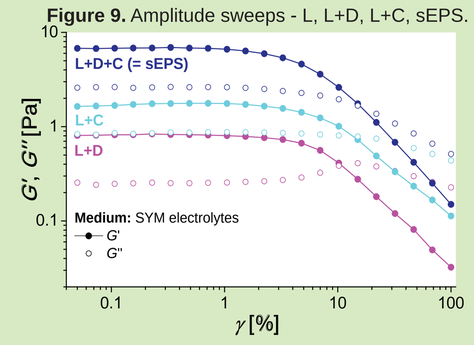



**Figure 10.** Amplitude sweeps - sEPS, sBF, nBF1, nBF2.

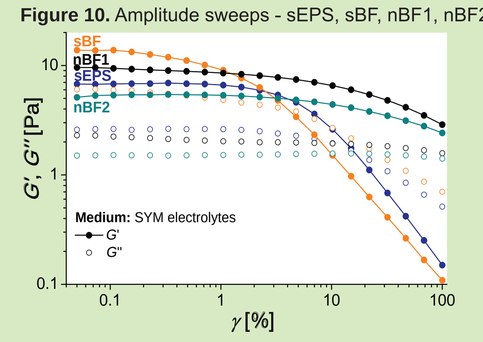



**Figure 11.** SAXS curves - L, L+D, L+C, sEPS.

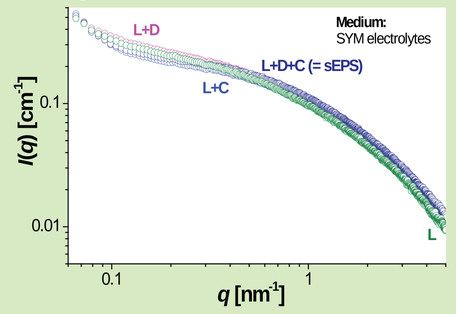
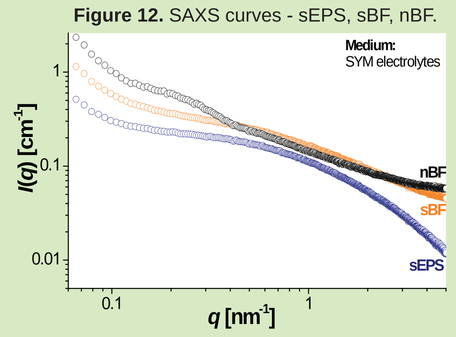



**Figure 12.** SAXS curves - sEPS, sBF, nBF.




The rheological results showed that the presence of DNA in levan solution caused significant increase of its viscosity and a strong pseudoplastic character, while the presence of collagen similarly caused a significant increase of viscosity and pseudoplastic behavior that was accompanied also by the notable increase in the rigidity of the system. These results therefore indicate that from the structural point of view levan in these samples has the role of a filling agent, long flexible DNA molecules obviously effectively interconnect the levan moieties in the system and introduce a strong pseudoplastic behavior, but less flexible pseudoplastic collagen fibrils significantly contribute to the structural rigidity of the system.

**Figure 12.** Microscopy images - L, L+D, L+C, sEPS.