Abstract

With this work we focus on the structure and dynamics of aqueous solutions of extracellular polymeric matrix (EPS-matrix) components of the gram-positive bacterium *Bacillus subtilis* subs. subtilis strain NCIB 3610. In a sucrose-rich liquid medium this organism forms a complex biofilm with the polysaccharide levan as its most abundant component. The challenge of this dissertation was microbiological as well as physico-chemical. Namely, it was first necessary to grow a sufficient amount of biofilm, then to isolate EPS-matrix, and eventually to purify a sufficient amount of individual EPS-matrix components (levan, nucleic acids). Aiming to recognize the individual contributions of the components to the structural developments in the formation of the biofilm the dynamic rheology, static (SLS) and dynamic light scattering (DLS), and small-angle X-ray scattering (SAXS) techniques were applied. SAXS data were evaluated utilizing two approaches; the classical Ornstein-Zernike model with an additional Debye-Bueche term and also the so called »string-of-beads« model.

In the first part of our research structural and dynamic properties of the major EPS-matrix component (~90 %), polysaccharide levan were investigated. The results for solutions of *B. subtilis* levan were compared to the ones of the commercially available levans of gram-negative bacteria *Zymomonas mobilis* and *Erwinia herbicola*. It is known that levans of different bacteria differ in their degree of polymerization, as well as in the number of their branching points. The rheological results interestingly showed viscosity independent of the shear rate of aqueous solutions of levan at low concentrations but at the same time surprisingly pointed out the elastic character of these solutions on the macroscopic level. The latter result was supported by the DLS results showing strong nondiffusive relaxation processes on the microscopic level that could be explained by the strong intramolecular interactions of levan at such low concentrations. The relaxation times of the dynamic response of these samples on the microscopic scale were in accordance with the sample viscosities. The strong intramolecular interactions manifest themselves also in the presence of the large levan particles in these samples that were confirmed by the microscopy results. SLS results showed that the size of the levan particles increases in the following direction: *Bacillus subtilis* < *Zymomonas mobilis* < *Erwinia herbicola*. 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. Slightly more intensive hydration was observed only in the case of *B. subtilis* levan samples that were found to consist of the most soluble levan. To

complement the macroscopic rheological and other results the SAXS experiments were performed. They revealed the structural details of these systems on the nanoscale via the resulting correlation lengths of the polymer molecules. This study therefore demonstrates that especially solutions of *B. subtilis* levan have extremely low viscosities and do not form gels. This is an interesting result, because the levan is found as the most abundant component of the bacterial biofilm being a compact gel. This suggests that the auxiliary structural biofilm components (nucleic acids and proteins) play an important role in the formation of the biofilm.

In the second part of our research the auxiliary components of the biofilm in terms of its gel-like structure (DNA and model protein collagen) were added to the aqueous solution of levan. These mixtures were prepared in the same concentration ratios as were found in the native *B. subtilis* biofilm. To study the effect of simple electrolytes on such samples similar mixtures were prepared also in the solutions of simple electrolytes of the SYM growth medium (SYM electrolytes). The corresponding rheological measurements indicate that already a very low percentage of additives (DNA, collagen) can strongly change the rheological behaviour of the levan solutions. We found that the levan acts like some kind of a »filling agent« in these systems primarily increasing the sample's viscosity, whereas the DNA and collagen cause a significant increase of the system's elasticity, with the latter increasing also the system's rigidity. In this way we showed that the auxiliary structural biofilm components are essential for the biofilm formation. Also these findings were complemented by SAXS results that revealed some significant differences in the structure of the studied polymer mixtures at the molecular level.

In the first chapter of this PhD thesis the general characteristics of biofilm and its components are presented. The second chapter is dedicated to the basics of the methods and physico-chemical models used in this research. In the third chapter the procedures of bacterial cultivation, the isolation of individual polymer components from *B. subtilis* biofilm and the experimental conditions used in this research were described. The fourth chapter contains results and discussion, while the fifth chapter contains conclusions. Finally, in the sixth chapter literature is collected.

Key words: Levan, *Bacillus subtilis*, EPS-matrix, biofilm, SAXS, SLS, DLS, rheology, polymer branching, polymer hydration, string-of-beads model.