The determination of mechanisms affecting the formation of microbial communities is crucial for their use in environmental biotechnology. Current knowledge about the formation of biofilms and aerobic granules concerns primarily the technological conditions that must be met in order to effective granulation and formation of biofilm. The key role in the formation of technical biocenoses in wastewater treatment systems is played by extracellular polymers (EPS) secreted by microbial cells. The main components of EPS involved in the formation of granules and biofilms are polysaccharides, proteins and eDNA. Data on the function of bacterial species in the formation of biocenosis are very general. The aim of the project is to determine the effect of the type of treated wastewater on the detailed characteristics of polysaccharides and proteins during aerobic granulation and biofilm formation. Biosynthesis of EPS varies depending on the bacterial strain and depends on the genes and enzymes involved in the production and secretion of EPS. The main polysaccharides involved in biofilm formation are alginate, Pel and Psl, but studies of these polysaccharides concern the formation of biofilm by one species of bacteria. The study will determine the expression of genes responsible for the synthesis of these polysaccharides to assess the role of Pel and Psl in the formation of microbial communities under wastewater treatment conditions. Metatranscriptomics will also be carried out for a detailed understanding of the ecology of microorganisms during the formation of biofilms and aerobic granules. During the granulation and formation of biofilms, bacteria communicate with each other using chemical molecules called autoinducers. Autoinducers are used to regulate the expression of genes associated with the formation of communities. Due to the important role of intercellular communication in creating biocenosis, the studies will determine the presence of autoinducers during granulation and biofilm formation.

The project is divided into two stages depending on the type of wastewater used for formation of biocenosis. In the first stage, synthetic and real municipal wastewater will be introduced into the reactors during the formation of biofilms and aerobic granules. In the second stage, two types of wastewater with a high content of organic compounds (dairy wastewater and wastewater from local juice factory) will be used for granulation and biofilm formation. Two reactors will be used in each stage: one in which conditions favor formation of aerobic granules and the second with filling on which biofilms will be formed. In each reactor, biomass samples will be collected during the formation of biocenosis. EPS fractions (soluble, loosely bound and strongly bound) will be isolated from the collected samples. Detailed analysis of proteins, carbohydrates and e-DNA will be performed in each fraction of EPS (using standard Anthrone and Lowry methods, cuvette tests for monosaccharides, disaccharides and trisaccharides, FTIR and high-performance liquid chromatography (HPLC). eDNA will be used to determine the qualitative and quantitative composition of microorganisms in EPS fractions during the formation of biocenosis.

The research is aimed at determining the impact of wastewater composition on microbial communities in EPS fractions, on the composition and amount of EPS, intercellular signaling and expression of polysaccharide genes involved in the formation of granules and biofilms. Increased knowledge about the formation of microorganism communities and interspecies interactions gives the potential for further development and improvement of processes involving biofilms and aerobic granules.