The objective of this project is to investigate the impact of bioaugmentation with the selected type strains on carboxylic fatty acid chain elongation to medium chain carboxylates and microbiome characterisation in mixed culture fermentation processes. Mixed culture fermentation is an anaerobic biological process based on a carboxylate platform, in which carbon and energy originating from different waste streams can be recovered by naturally occurring consortia of microorganism resulting in production of volatile fatty acids. The process can be then directed towards volatile fatty acid chain elongation to medium chain carboxylates. The final product is energetically richer than the substrate and consequently becomes suitable biofuels precursor. Due to the complexity of the processes and the interactions that occurs between particular microbial groups, the knowledge of mixed culture fermentation for the production of medium chain fatty acids is still insufficient, making it difficult to efficiently control the process and achieve maximum productivity. Current process management approaches are focused on control of operating conditions, such as pH, temperature, hydraulic retention time, substrate change, etc. As an alternative to control the process towards desired direction, bioaugmentation has the potential to substantially expand the field. Bioaugmentation is a microbial-based strategy that employs an introduction of a selected strain to the system for a process improvement.

In order to investigate the carboxylate chain elongation and the effect of bioaugmentation on the efficiency of the processes, in this project a series of the mixed culture fermentation with bioaugmentation will be carried out. Simple and complex equivalents of organic compounds that naturally occur in waste streams, such as glucose, short chain fatty acids, lactate and lactose, glycerol, starch, cellulose will serve as substrates. For inoculation the mixed culture will be separated from previously conducted process where caproic acid was produced. Selected type strains that show the metabolic potential necessary for chain elongation process will be used in the research. It should be noted that the successful application of bioaugmentation for any biological process is often limited by the lack of robust and simple methods for monitoring the survival and activity of introduced strains as well as the other subpopulations of microorganisms involved in the process. It is also important that most of the microorganisms that occur in mixed culture fermentation is unculturable using classical methods or the cultivation techniques are not known. Recent advancements in cultivation-independent investigations of the microbial communities now allow us to address these limitations. The fermentations and analysis will be performed using cutting-edge technologies with use of analytical and microbiological methods. Analytical methods will be performed at each stage of the research and it will enable to assess the impact of bioaugmentation on the emerging product spectrum and the fatty acid chain elongation. After the bioaugmentation the samples will be analyzed using advanced research tools, such as flow cytometry with microbial cell sorting or metagenomic sequencing. The combination of different methods will not only enable monitoring the introduced strain and assessing the impact of bioaugmentation on product formation. It will also let the multi-parameter assessment of the entire microbial population, the identification of key microbial communities involved in the process and assessment of interactions among the microbial community members.

Mixed cultures are broadly studied for biotechnological purposes application. However, the connection between microbiome structure and its functionality resulting from interactions between microbial community members remains still unexplained. Notwithstanding, little is known about the function of individual species within the mixed culture as well as the impact of microbial groups and strains on a particular reaction mechanisms of fatty acid chain elongation. The insufficient knowledge of mixed culture fermentation for the production of longer carboxylic fatty acids makes it difficult to efficiently control the process and hence, achieve maximum productivity. As the functioning and stability of the process largely depends on the structure and activity of the microbiome, there is a growing demand to develop a new microbial-based approach to achieve the goal. Bioaugmentation is an emerging technique in this field and the knowledge and understanding of its influence on mixed culture fermentation processes has not been deeply investigated so far. The project results will provide new knowledge in the discipline of environmental engineering on using bioaugmentation in mixed culture fermentation for longer fatty acid formation, which is of great importance from the standpoint of the resource recovery from waste.