

The biodiversity of microorganisms on Earth is almost unlimited. It is estimated that there are  $6 \cdot 10^{30}$  bacteria,  $1.3 \cdot 10^{28}$  archaea and  $3.1 \cdot 10^{29}$  eukaryote which made possible to develop microbial fermentation technologies and have been applied in environmental engineering and industrial or environmental biotechnology. Microorganisms are ubiquitous and play a major role in biochemical cycles that are fundamental to sustain life on Earth, but only around 150 types of microbiological processes have been applied in industrial scale for production of chemicals, antibiotics, vitamins, pharmaceuticals, sweeteners or biofuels. Hence, the metabolic potential of microbes still needs to be fully uncovered. Majority of the processes use pure cultures either adapted to the reactor environments or genetically engineered microorganisms using synthetic biology tools. Most of the biotechnological processes use pure culture fermentation as process parameters can be optimized for specific strains of microorganism. However, pure culture fermentation has a fundamental disadvantage – it requires sterile operating conditions and raw material of high quality and purity. One of the most common commercially applied processes that employs undefined mixed cultures is anaerobic digestion. This process plays a crucial role in waste management systems and produces renewable energy in the form of methane. Anaerobic digestion is entirely dependent on the complex syntrophic activity of microorganisms belonging to several functional groups. In general, this process can be divided into hydrolysis, acidogenesis, acetogenesis, and methanogenesis, where most organic matter (carbohydrates, lipids, proteins) except for lignin components, in the absence of oxygen, is degraded into methane and carbon dioxide. Conversion of wet organic waste into biogas is a well-established technology, whereas conversion of organic waste to high-value biochemicals is still only in the exploratory research phase. Volatile fatty acids such as acetic, propionic, butyric and valeric acids, which are key intermediate products in AD, can be considered as intermediate products for newly developed process creating carboxylic platform. The most abundant organic secondary resources which would be suitable for mixed culture fermentation include municipal solid waste, cattle and pig manure, fruit and vegetable waste, brewery spent grains, cheese whey, sewage sludge and other waste streams originating from agro-food industry. Crop field residues (such as wheat straw or maize stalks) as well as forestry field and processing waste are also of high interest but they require efficient pretreatment processing prior fermentation. In recent years, new function of the anaerobic mixed culture fermentation has been re-discovered i.e. carboxylate chain elongation via reverse  $\beta$ -oxidation.

The aim of the project is to understand the process of carboxylates chain elongation during the anaerobic mixed culture fermentation. In that process, volatile fatty acids are elongated into medium chain fatty acids via reverse  $\beta$ -oxidation pathway. In order the reaction to occur, adequate concentration of electron donor must be provided. So far, two compounds have been identified as the crucial ones i.e. ethanol and lactic acid, and henceforth the research focus has been mainly on the external delivering suitable concentration of one of them. The objective of the C-elong project is to understand how an electron donor (ethanol, lactic acid or other not identified yet) could be produced within the anaerobic mixed culture community and to reveal the key and hub microorganisms involved in the process. In the second step, those identified microorganisms will be bioaugmented to the anaerobic mixed culture fermentation in order to enhance the chain elongation process. The crucial point of the project is that the anaerobic system will not be bioaugmented with a microorganisms responsible for a final products but with the key or hub ones responsible for shaping and stabilizing reactor microbiome structure. This novel approach may lead to a development of a new method for shaping reactor microbiomes with unknown yet functions. The process performance will be monitored by advanced analytical and molecular techniques.

In recent years, the molecular biology tools became available to the environmental engineers. At first, they served only as tools to observe the composition of the mixed culture process, recently they have become popular to monitor the process performance. In order to find the most important microbes and the most crucial connections in the mixed culture systems, it is necessary to search for highly connected microorganisms which benefit from being specifically involved with high connectivity. This new approach will not only allow to monitor but also to control the biotechnological processes. In the C-elong process I aim at using those tools to understand the mixed microbial community structure and behaviour and shape that community to enhance chain elongation process via  $\beta$ -reverse oxidation. Development of such a method might open new opportunities for novel processes and it will have a tremendous impact on the role of biotechnological processes in the environmental engineering. In the long term (beyond the scope of the project), the outcome of the project will help to build up a method for designing the reactor microbiomes with yet unknown functions. In a longer perspective (15-20 years) it might help to create new branch of the environmental industry for treating waste streams and converting them into valuable biochemicals in a sustainable manner.