Methane and carbon dioxide are the main final products of the decomposition of biomass under anaerobic conditions in environments where the concentration of other electron acceptors, such as nitrate, sulfate, Fe(III) and Mn(IV), is low. It is a complex process that results from the interaction of many groups of microorganisms requiring four major steps. The first one is hydrolysis of complex organic polymers (e.g. polysaccharides, lipids, proteins) to monomers (sugars, fatty acids, amino acids) by fermentative bacteria. The second step is acidogenesis that results in formation of hydrogen and carbon dioxide as well as non-gaseous fermentation products, i.e. low-molecular-weight organic acids and alcohols. These products are further oxidized to hydrogen, carbon dioxide and acetate. This step is called acetogenesis and involves mainly syntrophic degradation of non-gaseous fermentation products. The forth step is methanogenesis. The two last steps, acetogenesis and methanogenesis are closely related and involve syntrophic associations between hydrogen-producing acetogenic bacteria and hydrogenotrophic methanogens. These associations keep the hydrogen partial pressure low enough to allow acetogenesis to become thermodynamically favorable. Three groups of substrates for methane production and three types of methanogenic pathways are known: splitting of acetate (aceticlastic/acetotrophic methanogenesis); reduction of carbon dioxide with hydrogen or formate and rarely ethanol or secondary alcohols as electron donors (hydrogenotrophic methanogenesis); reduction of methyl groups of methylated compounds such as methanol, methylated amines or methylated sulphides (hydrogen-dependent and hydrogen independent methylotrophic methanogenesis).

The aim of the study is recognition and description of poorly-recognized/unknown mechanisms of acetogenesis, the third step of anaerobic digestion of organic matter to methane and carbon dioxide. Our understanding of the microbial ecology and physiology associated with anaerobic digestion is incomplete because it is restricted to culture-dependent techniques. In fact the majority of microorganisms involved in the process of anaerobic digestion have not been cultivated yet. It is noteworthy that acetogenic bacteria are not able to grow without their syntrophic partner and cannot be grown as a single pure culture. Thus the mechanisms of transformation of the main products of acidogenesis leading to formation of substrates for methanogenesis are poorly recognized. Using culture-independent techniques we want to trace and fully understand the metabolic pathways of acetate, lactate, butyrate and propionate transformation in the methane-yielding communities processing artificial media imitating non-gaseous products of different types of bacterial fermentations.

Another aim of the study is isolation, identification of new species of methanogenic archaeons, especially acetotrophs. So far only two genera of acetoclastic methanogens, Methanosarcina and Methanosaeta, have been recognized. Interestingly, it is thought that two-thirds of the methane generated in anaerobic digesters is produced by acetotrophs.

Research object will be continuous and stationary-phase cultures of methane-producing communities on the artificial media imitating acid effluents (non-gaseous products) from different types of microbial fermentations.

Studies on efficiency of methane production by the examined microbial communities will supply detailed data showing reactor performance (pH, redox potential, chemical oxygen demand, total rate of gas production, continuous gas analysis using mass spectrometer; analysis of effluents after fermentation processes using high performance liquid chromatography, gas chromatography).

Isotopic labelling of the media components allows tracing the metabolic pathways of acetate, lactate, butyrate and propionate in the continuous and stationary-phase cultures of methanogenic communities.

We will use high throughput DNA and RNA sequencing technologies to metagenomic and metatranscriptomic analyses of the methane-yielding communities. Metagenomic analysis involves isolation and sequencing of total DNA from the microbial communities to determine its composition (biodiversity). Metatranscriptomic analysis involves isolation and sequencing of mRNA from the microbial communities and provides a way to measure in situ gene expression.

A combination of metagenomics, metatranscriptomics, isotope labelling techniques and analysis of reactor performance enables to reconstruct and understand key metabolic pathways in methane-yielding microbial communities. Such an approach is in accordance with the current trends.

Identification of potentially new species of methanogenic Archaea will be based on the mcrA gene and 16SrRNA encoding gene sequences.

Process of anaerobic digestion of biomass was used by man to produce biogas as alternative energy source. Currently there is great interest in the development of new technologies aimed at producing energy from renewable sources, of which gaseous fuels production show great promise. Searching for syntrophic cooperation between microorganisms is crucial for attempts to optimize anaerobic digestion processes. The long term fundamental studies are usually the basis for applied research. Thus recognition of acetogenesis step on molecular level is very important. Furthermore the mechanisms of particular steps of anaerobic digestion are similar in natural environments as well as in anaerobic bioreactors aimed at gaseous fuels production. For this reason the results obtained in this project will be interested for environmental microbiology, biotechnology, geology and geochemistry.