Biogas is a promising renewable energy source due to its methane content. Methane itself seems to have high energy and heating potential, its heating value was estimated as higher (55.5 MJ/kg) (Fountaoulakis et al. 2009). Another advantage of biogas production is the possibility of the use of many different organic wastes as a substrate in the process (Amon et al. 2007; Li et al. 2011). Biogas is produced in the anaerobic digestion process that consists of 4 stages: hydrolysis, acidogenesis, aceticogenesis and methanogenesis (fig. 1 (Li et al. 2011). Each stage is conducted by specific microbial community able to interact with each other in a syntrophic manner (Weiland 2010; Worn et al. 2010). The last step can be carried out by hydrogenotrophic (CH4 production from H2 and CO2) or acetotrophic (CH4 production from acetate) methanogens (Garcia et al. 2000). It has been already proved that there is a syntrophy between some particular methanogens and some groups of bacteria (Stams et al. 1992). The project assumption is that there might be a syntrophic relationship (based on H2 partial pressure) between some fermentative hydrogen-producing bacteria and hydrogenotrophic methanogens. In addition, hydrogenotrophic methanogens were proposed by Zgarnen et al. (2011) to give lower methane yields compared to acetoclastic methanogens. Thus, gene-monitoring specific for the particular hydrogen-producing bacteria (being in correlation with hydrogenotrophic Archaea) will bring the information about the methane yield (low specific gene abundance – high methane yield). The project will bring a robust molecular biology technique of monitoring the methane production in anaerobic digesters.

**Aims**

The study on the possible shifts in microbial community as a result of various reactor performance – especially in terms of feedstock

The investigation of possible syntrophy between candidate division hydrogen-producing bacteria and hydrogenotrophic methanogens and its influence on methane yields by using metagenomic tools

The development of robust technique (targeting the biomarker – gene of candidate division bacteria) to monitor methane yields in anaerobic digesters

**Methods**

Collection of a sample

- High molecule-yielded two stage anaerobic digester (AFBI, Hillsidebrook; Biological Incubator digester)

DNA extraction – Power DNA isolation kit (MoBio Sciences)

Amplification purification – GenoBAC Gel Extraction kit (Thermo Science)

PCR with Mi primer and mcrA-rev primer

Cloning the plasmid with an insert into a 2-clusten competent cells

Sanger sequencing of plasmids

PCR with Mi primer and some sets of primers

454-pyrosequencing bacterial and archaeal 16S investigation and processing method in QCsME

**Results**

454-pyrosequencing of the sample revealed that the most abundant group of bacteria was Firmicutes (especially order Clostridiales). The most dominant Archaea were Euryarchaeota (especially order Methanosarcinales). More details on fig. 2 and fig. 3.

**Conclusions and future plans**

The pyrosequencing results indicate that the most abundant Archaea in the AFBI digester is Methanosarcina which is classified as acetoclastic methanogen group. The predominance of such methanogens with simultaneous absence of particular candidate division bacteria in the digester characterized by high methane production is in agreement with our hypothesis. In contrast, hydrogenotrophic methanogens predominance with the presence of particular candidate division hydrogen-producing bacteria would be connected with non-optimal digester performance. However, the hypothesis still needs confirmation by investigating samples (by 454-pyrosequencing and qPCR) from other full- and lab-scale (under construction) reactors.

References:


