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The Structure of the Bacterial and Archaeal Community in a Labscale Hybrid Bio-Methane Reactor as Revealed by Denaturing Gradient Gel Electrophoresis and 16S rDNA Sequencing Analysis

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Aim of the study: Anthropogenic activities produce huge amaounts of CO_2 all over the world. Biogas draws significant attention due to the fact it is sustainable, clean, environmentally friendly and highly efficient and can be produced for wide range of organic waste material. In this study, it was aimed to identify the shifts and dynamics in archeal and bacterial profiles in a hybrid anaerobic bio-methane reactor fed with only H_2 and CO_2 gasses by 16s rDNA-based methods.

Material and Methods: Different H_2/CO_2 rates were tested during the study No additional organic material was fed into the bioreactor. The dynamics of microbial communities were quantified by real-time PCR analysis. All quantitative analysis were carried out with LightCycler 1.5 and reaction mixture was prepared using LightCycler TaqMan Master kit (Roche Diagnostics, Mannheim, Germany). Also, denaturing gradient gel electrophoresis (DGGE) method was used for determining microbial profile changes under different H_2/CO_2 ratios. DGGE of the PCR products was performed by DCode Universal Mutation System (Bio-Rad, USA) on 8% polyacrylamide gels with different denaturing gradients (35-70% for ARC,MBT,MSL and MMB, 40-60% for BAC samples) of urea-formamide (100% correspondent to 7M urea and 40% [v/v] formamide). The electrophoresis conditions were 135 V in 1xTAE buffer at 60°C for 8 h. Separated DNA bands were excised, re-amplified and analyzed using Sequencher 5.4.5 Sequence Analysis Software (Gene Codes, US). Bacterial V3-V5 variable region specific and archeal group-specific (*Methanobacteriales, Methanomicrobiales, Methanosarcinales* and total Archaea) primer and probe sets were used.

Results: The group-specific primer and probe sets targeting methanogenic archea showed that *Methanobacteriales* members were more intense, followed by *Methanosarcinales* members and *Methanomicrobiales* members had lower numbers. The number of archaea is generally approximately 1 log (10 times) more than the number of bacteria. DGGE results indicated that *Methanosaeta concilii, Methanoculleus* sp., *Methanosphaerula palustris*, *Methanofollis formosanus, Methanolinea* sp. and *Methanobacterium palustre* were the most dominant methanogens depending on different H_2/CO_2 ratios. DGGE profiles suggested both hydrogenotrophic and acetoclastic species were responsible of producing methane. Syntrophic bacteria and acetoclastic methanogens were thought to be survived by organic materials provided by dead cells. To the best of our knowledge, this is the first microbial profile detection study in the hybrid bioreactor system operated with only pure hydrogen and carbondioxide gas supply.

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