Community shifts of methanogens during the start-up of a mesophilic biogas reactor treating cattle manure

Sabine Podmirseg*, Maria Gadermaier, Marta Goberna, Ingrid Franke-Whittle, Heribert Insam

University of Innsbruck, Institute of Microbiology, Technikerstr. 25d, 6020 Innsbruck, Austria
*sabine.podmirseg@uibk.ac.at

Objectives

1) To analyse the changes in methanogenic community during the start-up and first stable operating phase of a full-scale biogas plant and 2) to detect key organisms and their abundance.

Materials and Methods

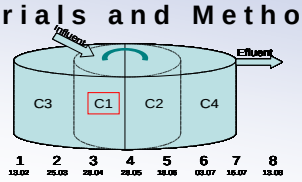

Figure 1: Scheme of the four-chamber biogas plant[1] including the eight sampling dates in 2008; C1-C4 refering to chamber 1-4; samples obtained from chamber 1;

Clone libraries were generated from the input material (cattle manure, S1) and from a sludge sample collected 4 months after the start-up (S5). All clones were screened with restriction digestion (HaeIII).
Real-time PCR was performed with specific methanogenic primers targeting the 16S rRNA gene in a Corbett Rotorgene 6000 cycler using SYBR Green I. Also chemical and physical parameters were analysed (Table 1).

Results: Clone libraries

Screening of the clone libraries revealed 38 ribotypes in the cattle-manure library and 18 in the sludge library, respectively. After sequencing and merging of phylogenetically close sequences (<3% difference), the final number of operational taxonomic units was reduced to 16 and 4, respectively. (Fig.3)

Results: Real-time PCR

The values obtained by real-time PCR ranged from 1.09 10^3 gene copies per mL^{-1} sample for *Methanosaeta* to 1.09 10^6 gene copies per mL^{-1} sample for *Methanobrevibacter.* There was a decrease over time of 37.7% in abundance of the total analysed methanogenic community. The real-time PCR data displayed the change from a community dominated by *Methanobrevibacter* (79.1%) and *Methanocorpusculum* (16.3%), two hydrogenotrophic methanogens, to a community comprised mostly of *Methanobrevibacter* (52.4%) and the metabolically versatile *Methanosarcina* (46.8%).

Table 1: Additional information to each sampling date;

sampling	[n]	T [°C]	pH	TS [%]	VS [%TS]	COD tot [mg/l]	NH4-N [mg/l]	CH4 [%]	CO2 [%]	O2 [%]
1	5	8.0	n.a.	3.7	67.8	39000	1372	n.a.	n.a.	n.a.
2	3	25.0	n.a.	4.7	72.4	55882	1368	12.0	17.2	6.7
3	3	33.6	7.39	3.6	65.3	36622	1238	63.0	36.6	0.3
4	3	39.3	7.37	4.8	69.5	46890	1383	44.6	34.0	3.9
5	5	37.0	7.47	4.9	69.1	59255	1346	41.0	32.2	3.8
6	3	n.a.	n.a.	4.1	64.6	47080	1176	34.8	25.1	3.6
7	3	n.a.	n.a.	3.9	69.0	48554	1035	29.1	21.0	6.8
8	3	36.1	7.50	4.4	69.6	49257	929	36.5	26.5	7.9

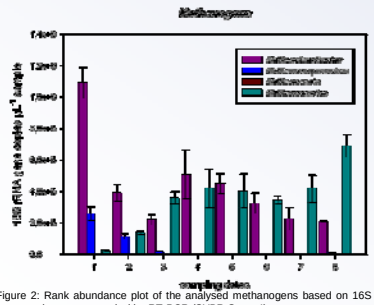

Figure 2: Rank abundance plot of the analysed methanogens based on 16S rRNA gene copy numbers; measured with qRT-PCR (SYBR Green I);

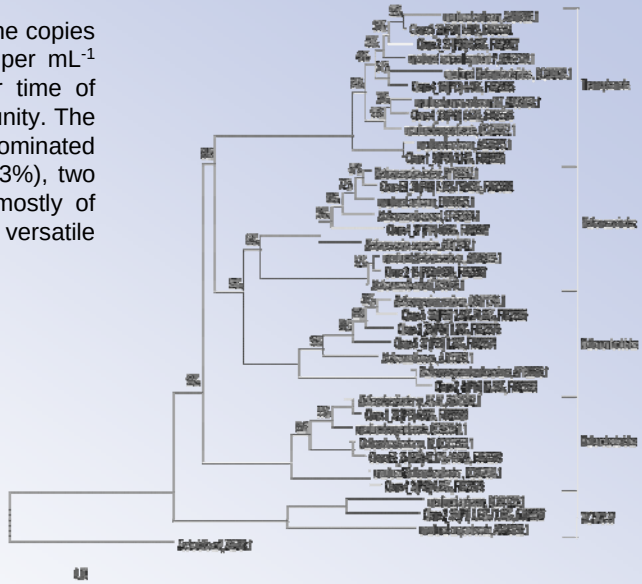

Figure 3: Phylogenetic distance tree of representative archaeal clones (16S rRNA gene sequences) and related sequences from the Greengenes database. Calculated with the neighbour joining method and *Escherichia coli* as outgroup. Bootstrap values (1000) shown at each node. Percentage values after brackets represent the abundance in the start-up (S1) and time point 5 (S5) clone libraries, respectively.

Conclusions: Besides a general decrease of archaeal diversity, the start-up process of this biogas plant led to the establishment of a specific methanogenic community with a dominance of only few genera.

References [1] Wett B., Schoen, M.; Phothilangka, P.; Wackerle, F.; Insam, H. (2007) Model based design of an agricultural biogas plant – application of Anaerobic Digestion Model No.1 for an improved 4 chamber scheme. Water Sci Technol 55, 21-28

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