Quantification of Methanogens during the Start-up of Biogas Reactors using DNA microarrays and Real-time PCR

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Objectives

- To test if the establishment of a stable methanogenic community can be achieved in the start-up of a biogas reactor filling it directly with the substrate for anaerobic digestion (cattle manure)
- To test if the methanogenic community establishment is accelerated by using anaerobic sludge from an operational biogas plant as seeding material

Materials and Methods

Two different start-up strategies were examined in continuously stirred tank reactors (CSTR, 75 L):

Reactor MAN	Reactor SEED
• 100 % cattle manure	• 20 % Anaerobic sludge • 80 % water • fed daily with cattle manure

- 4 wk operation at 37 °C, sampling every 3.5 d
- Extraction of total DNA
- Microarray ANAEROCHIP: PCR amplification of 16S rRNA gene using universal archaeal primers 109F and 934 R
- Real-Time PCR: primers targeting specific genera of methanogens

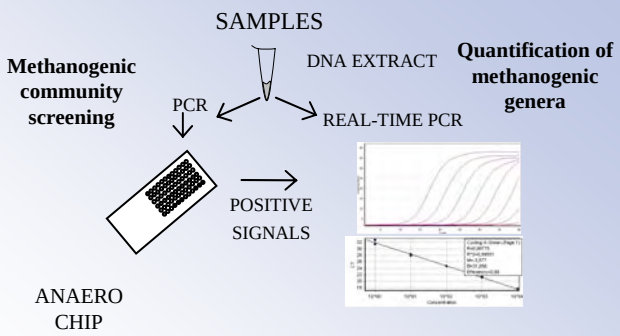

Fig. 1: Strategy of fingerprinting the methanogenic community

Results

Six methanogenic genera were found to be present in significant numbers combining both techniques: *Methanosarcina*, *Methanosaeta*, *Methanocorpusculum*, *Methanobrevibacter*, *Methanosphaera*, *Methanobacterium*

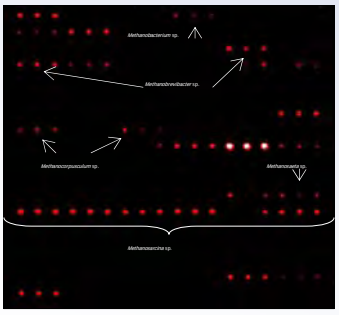

Fig. 2: Hybridisation of Cy5-labelled cattle manure 16S rRNA gene products

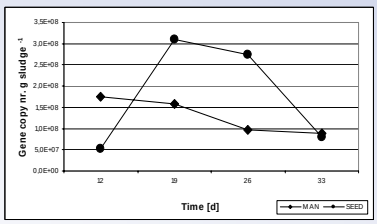

Fig. 3: Evolution of *Methanosarcina* sp. gene copy numbers during the experiment detected by real-time PCR

	cattle manure		anaerobic sludge (seed)	
	copies g sludge^{-1}	% of total methanogens	copies g sludge^{-1}	% of total methanogens
Methanosarcina sp.	$2.3\ 10^{7}$	82.85	$6.9\ 10^{7}$	99.36
Methanocorpusculum sp.	$2.7\ 10^{6}$	9.74	$4.6\ 10^{2}$	0.00
Methanobrevibacter sp.	$1.6\ 10^{6}$	5.73	$3.2\ 10^{5}$	0.46
Methanosaeta sp.	$3.1\ 10^{5}$	1.09	$2.8\ 10^{4}$	0.04
Methanosphaera sp.	$1.6\ 10^{5}$	0.58	$8.1\ 10^{3}$	0.01
Methanobacterium sp.	$2.8\ 10^{3}$	0.01	$9.1\ 10^{4}$	0.13
Total methanogens	**$2.8\ 10^{7}$**		**$6.9\ 10^{7}$**	

Tab. 1: Gene copy numbers of methanogens in the initial materials (cattle manure, anaerobic sludge) detected by real-time PCR

The acetrotrophic methanogens, foremost *Methanosarcina* dominated both reactors for the duration of the experiment. Its biomass increased in reactor SEED peaking at day 19 and progressively decreased reaching similar levels in both reactors at day 33. The abundance of the hydrogenotrophic methanogens decreased in both reactors with time (data not shown.)

Conclusion

Inoculation of biogas reactors treating cattle manure with anaerobic reactor sludge is not necessary, because cattle manure contains a diverse and abundant methanogenic community that should ensure a successful start-up.