Microbiological aspects of methane production during pig manure storage

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Introduction

The natural degradation of livestock wastes during their storage leads to the release of CH_4 to the atmosphere due to anaerobic decomposition of organic matter. These emissions originate mainly from swine (38%), dairy (21%) and poultry (9%) livestock wastes. However, the amount of methane emitted varies greatly according to the animal type, its diet or the related manure management practices (Martinez et al., 2000, 2003). With the goal of understanding the factors influencing methane emissions, it is important to analyse the microbiological aspects of manure anaerobic digestion.

In this work the bacterial and archaeal communities of manures with different methane production behaviour were analyzed at the beginning and at the end of laboratory scale incubations using a fingerprinting technique that targets microbial 16S ribosomal genes (Amann et al., 1995). This technique allows the detection of dominant microbial populations whatever their cultural ability.

Materials and methods

Manure incubations and methane production

Thirteen slurries covering various animal types (pig, bovine and duck), age classes, feed regimes and manure management systems, were studied (Vedrenne et al., 2007). The incubations were started with either (a) raw manure, (b) manure diluted with water or (c) manure diluted and inoculated with 10% amount of methanogenic anaerobic digester sludge. Storage of the slurries was simulated in 250-1000 mL glass bottles during 120-150 days at 30°C. During the simulated storage, biogas production was monitored by pressure measurement. Gas samples were collected and analysed for CH4. At the beginning and at the end of the storage experiments, slurries samples were taken for physico-chemical, microbiological and organic matter analysis.

Bacterial and archaeal communities fingerprinting and identification

Evolution of manure microbial communities during incubations was analysed by 16S rDNA targeted fingerprinting methods as described in Peu et al. (2006). Briefly, manure samples were collected at the beginning and at the end of the incubations and immediately frozen at -20°C. Total genomic DNA was extracted from about 0.7 ml manure using the QIAAMP DNA stool kit (Qiagen) according to supplier recommendations.

The V3 region of the microbial 16S rRNA genes was amplified by PCR using bacterial and archaeal primers and the *Pfu* Turbo DNA polymerase (Stratagene). PCR reactions were performed in 25 µl volumes in a MiniMJ thermocycler (Biorad). Twenty five cycles reactions were used for bacterial genes amplification while archaeal genes amplification required a semi-nested PCR of 2 times 30 cycles. The PCR products were then denaturated and separated by CE-SSCP electrophoresis using a 5.58% CAP – 10% glycerol polymer and an ABI 310 genetic analyser (Applied Biosystems). The fingerprints obtained were aligned and compared by using the GeneMapper software (Applied Biosystems).

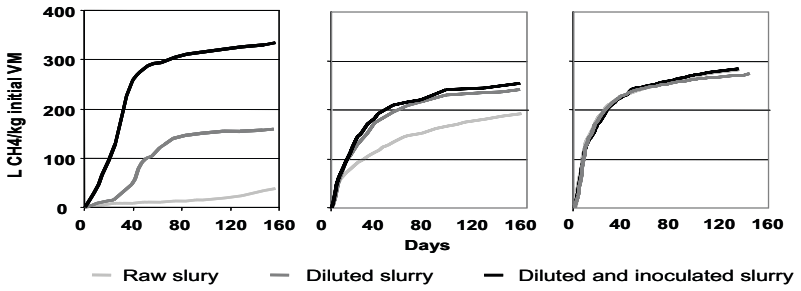
For the identification of archaeal populations, the archaeal 16S rDNAs were amplified again using the Red*Taq* polymerase (Sigma) and cloned into *E.coli* using a TOPO-TA vector cloning kit (Invitrogen) according to supplier recommendations. Recombinant clones were screened by CE-SSCP and sequenced at the Ouest-Genopole facility (Roscoff, France). The cloned 16S rDNA were finally identified by sequence comparison with their closest relative available in the Genbank database using the BLAST software from NCBI.

Results and discussion

The analysis of the microbial communities of five fresh manures before incubation (3 pigs, one cattle and one duck) showed a high diversity of bacteria with 15 to 20 dominant populations. The *Archaea*, on the contrary, presented a limited range of up to 10 species, with always one or two populations that strongly dominated the community. For swine manure, one of these populations was always identified as the hydrogenotrophic methanogen *Methanobrevibacter*. This species is naturally present in the digestive tract of animals and produces methane from H_2 and CO_2 (Garcia et al., 2000). Other methanogens identified belonged to the hydrogenotrophic group of the *Methanobacterium* or the acetotrophic group of the *Methanosaeta*. Manures from duck and cattle exhibited different populations from those of swine manure but could not be identified. The predominance of hydrogenotrophic *Archaea* in fresh manure confirms previous observations and suggests a slow adaptation of the animal digestive microbial community to its new environment (Whitehead and Cotta, 1999; Snell et al. 2005).

The kinetics of methane production varied widely from one incubation to another and could be classified in 3 groups (Figure 1): i) manures with poor methanogenic kinetics that required dilution (and / or inoculation) to reach a significant methane production; ii) manures with intermediate methanogenic kinetics where dilution (and / or inoculation) improved substantially methane production and iii) manures with high methanogenic kinetics where dilution (and / or inoculation) had little impact on biogas production.

Figure 1. The three different behaviours of methane production observed during manure incubations



Analysis of the manure microbial communities present at the beginning and at the end of the incubations revealed different methanogenic archaeal populations.

Whatever the conditions were, the bacterial communities of the 5 manures analysed evolved during incubation without any obvious correlation between the dominant populations observed and the capabilities of methane production.

In contrast, a change in the dominant methanogenic *Archaea* was observed each time that a good methane production was recorded during incubation. This change happened spontaneously for raw manures starting with a high methanogenic potential (Figure 2), or after dilution and/or inoculation for manures starting with a low methanogenic potential

(not shown). In all cases, the proportion of *Methanobrevibacter*, usually dominant in the community at the beginning of incubation, had diminished to give way to other methanogenic populations. These populations were affiliated to *Methanoculleus* (a hydrogenotrophic methanogen regularly encountered in mesophilic anaerobic digesters), or *Methanosaeta* (an obligate acetotrophic methanogen frequently encountered in anaerobic digesters treating effluent with high acetate concentration).

Figure 2. Evolution of the dominant archaeal populations of pig manure with good starting methane production during incubation of raw, diluted or diluted and inoculated manure

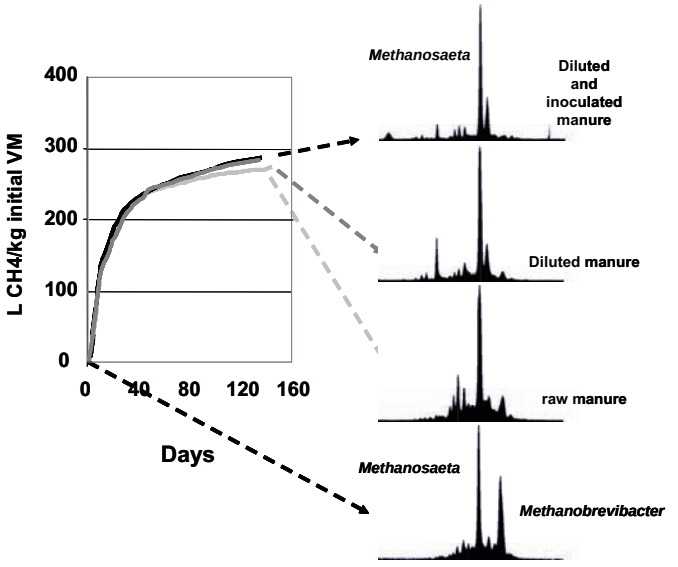
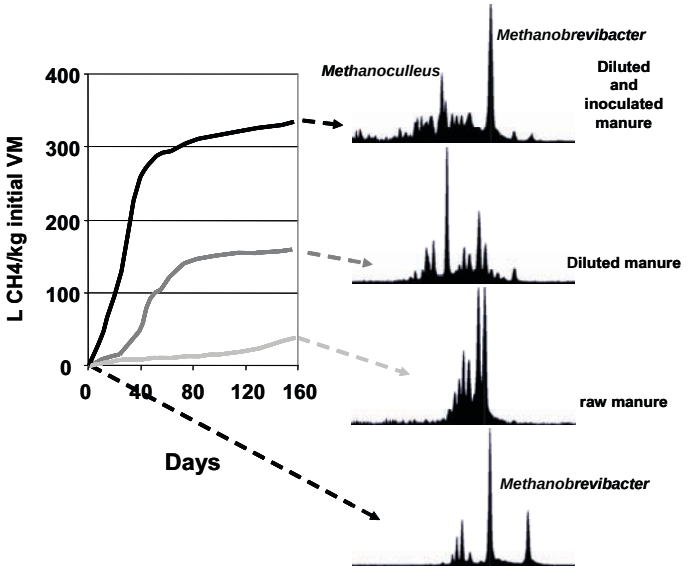


Figure 3. Evolution of the dominant archaeal populations of pig manure with poor starting methane production during incubation of raw, diluted or diluted and inoculated manure



Finally, the analysis of the inoculation experiments showed that every positive effect observed on methane production correlated with the installation of the inoculated *Archaea* during incubation.

As a conclusion, the variability of methane production observed between different manures results partly from the microbial community composition in the manure. Dilution and /or inoculation of manure can induce a shift of the dominant faecal methanogenic species present in the raw manure towards species commonly found in industrial (biogas) anaerobic digesters, thus having a beneficial impact on methane production

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