Bioresource Technology 101 (2010) 7252–7257

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Selective microbial aerosolization in biogas demonstrated by quantitative PCR

Marina Moletta-Denat *, Valérie Bru-Adan, Jean-Philippe Delgenes, Jérôme Hamelin, Nathalie Wéry, Jean-Jacques Godon

*INRA, UR50, Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, Narbonne F-11100, France*

ARTICLE INFO

*Article history:*
Received 9 November 2009
Received in revised form 3 April 2010
Accepted 13 April 2010
Available online 21 May 2010

*Keywords:*
Biogas
Microbial aerosols
Aerosolization
Microbial diversity
Quantitative real-time PCR

ABSTRACT

Aerosolization of Bacteria, Archaea, Synergistes, *Staphylococcus* spp. and *Propionibacterium acnes* was investigated *in situ* with quantitative real-time PCR of DNA isolated from sludge and biogases of anaerobic digesters. The data revealed that in biogas, *Staphylococcus* spp. and *P. acnes* were, respectively, aerosolized 30 and 220 times more and Archaea and Synergistes, respectively, 8 and 20 times less aerosolized than Bacteria. This is the first demonstration of selective microbial aerosolization for anaerobic digestors microorganisms. This study illustrates the fact that some microbial groups, such as opportunistic pathogens, are more susceptible to be aerosolized, since they use air as a dissemination vector, and that this has to be taken in account when up-grading biogas into natural gas networks.

© 2010 Published by Elsevier Ltd.

## 1. Introduction

Air is certainly the best vector for the dissemination for microorganisms between all ecosystems, and with a mean of 14 $m^3$ of air inspired per day and per person, an important source of microbial contact. One cubic meter of environmental air conveys around $10^6$ microorganisms (Peccia and Hernandez, 2006), which have entered the air by means of aerosolization and contribute to the scattered biogeography of these organisms. Aerosolization indicates the transfer (or "takeoff") of a microorganism from a solid or liquid phase to a gas phase. This transfer is primarily related to physical factors that create movement (wind, mechanical agitation, wave movement, etc.) (Blanchard and Syzdek, 1970). Although different aerosolization behaviors leading to the enrichment of certain microbial groups have been demonstrated by culture (Blanchard and Syzdek, 1970; Hejkal et al., 1980), *in situ* observation in the environment is rare (Hamilton and Lenton, 1998; Morris et al., 2004). A recent qualitative description of airborne microbial diversity, using molecular tools (molecular inventories based on 16S rDNA), identified preferentially aerosolized species in air (Angenent et al., 2005; Moletta et al., 2007; Tringe et al., 2008) and in biogases (Moletta et al., 2007).

The study of biogas is particularly relevant to comparing sources and aerosols (Vinneras et al., 2006), because of its *in situ* formation in a closed system. Moreover, the possible widespread injection of biogas in natural gas networks enhances the relevance of this aerosolization model. Indeed, a new valorization of the produced biogas is its use in replacement of fossil fuels (Weiland, 2010). During a previous study, the microbial communities of five biogases were described (Moletta et al., 2007) by providing qualitative data (16S rDNA sequences). The results revealed the high abundance of bacteria, or groups of bacteria, considered to be rare in such a source environment, i.e. an anaerobic digester. Around 86% of the species aerosolized in biogas did not result from the dominant diversity of the anaerobic digester, and the predominant aerosolized species were mainly those found in other ecological niches (water, air and soil) (Moletta et al., 2007). However, this molecular inventory was mostly qualitative and it was not possible to make any relevant comparisons.

During the present study, a quantitative comparison was made between some the microbial communities of a full-scale anaerobic digester and microbial aerosols in biogas. The microorganisms were quantified at several taxonomic levels, ranging from the species to the division.

## 2. Methods

### 2.1. Samples and total DNA extraction

Sampling sites were selected based on three criteria: the type of waste being treated, the type of process used to ensure anaerobic

* Corresponding author. Present Address: Université Paris-Est, Centre Scientifique et Technique du Bâtiment (CSTB), Département Energie Santé Environnement, Division Santé, Laboratoire de Recherche et d'Innovation pour l'Hygiène des Bâtiments, 84, av. Jean Jaurès – Champs-sur-Marne, 77447 Marne-la-Vallée, France. Tel.: +33 (0)1 64 68 87 77; fax: +33 (0)1 64 68 88 23.
*E-mail address:* marina.moletta@cstb.fr (M. Moletta-Denat).

0960-8524/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.biortech.2010.04.035

digestion and the operating temperature. Five different sites were chosen according to these criteria. A total of five digesters were investigated (DM, DT, DE, DI, LD) (Moletta et al., 2007). Two samples of biogas were collected at two of the sites, referred to as DE1 and DE2 and DM1 and DM2. Background air was also sampled at the DI site. The sampling system for DNA extraction consisted of a vacuum pump (which pumped the biogas through a 47 mm, 0.2 μM filter (polyethersulfone or black polycarbonate). The filter support was linked to a 300 ml frozen vial to collect water transported by the biogas. The system was joined up to the biogas network by a ¾ in. valve.

Two cubic meters of biogas were sampled with a 15 l/min vacuum pump. The collected water in the biogas (about 5 ml/$m^3$) was added to the filter by refiltration at the end of the filtration period. The filter was cut with sterile scissors and each half filter was placed in a 2 ml sterile tube and frozen at −80 °C for transport with dry ice. Negative extraction control, performed for all extraction series, consisted of passing sterile water through the sterile filter in which all the DNA extraction steps were performed.

Samples of the content of anaerobic digesters (sludge) were collected using the outlet valve of the anaerobic digester with respect to all biogas samples, except for LD and DM1 (for technical reasons). The principal characteristics of each digester are shown in Table 1.

DNA extractions were performed according to the methods described by Moletta et al. (2007). Anaerobic digester sludge samples (10 ml per sample) were extracted according to the method described by Godon et al. (1997).

### 2.2. Primers and probes

During this work, five different real-time PCR methods were used or developed, all based on the sequence of rRNA 16S. Microorganisms belong to different classifications: domains in Bacteria and Archaea, phylum for Synergistes, genus for *Staphylococcus* spp. and species for *Propionibacterium acnes* (Table 2). A previous study on microbial diversity in biogases and anaerobic digesters had identified them as being over-represented or under-represented in biogases when compared to the source, i.e. the anaerobic digester (Moletta et al., 2007). Indeed, Synergistes and Archaea are the only ones microbial groups found exclusively in anaerobe ecosystems and were under-represented in biogas. *P. acnes* were found in all biogas molecular inventories and *Staphylococcus* spp. was found in one biogas molecular inventorie but were found in all published air molecular inventories. The quantifications were performed on all seven biogas samples collected (Moletta et al., 2007).

All primers and the TaqMan® probe were synthesized by Sigma Proligo (Paris, France), while the MGB (Minor Groove Binding) probe was synthesized by Applied Biosystems.

The current specificity of all real-time amplification systems was tested *in silico* using Probematch software on the ARB database and NCBI Blast against all the 16S DNA sequences available in RDP and Genbank. Amplification reactions were carried out in triplicate for two consecutive 10-time serial DNA dilutions in a total volume of 25 μl, and were run and analyzed on an ABI Prism 7000 SDS version 1.01 (Applied Biosystems). All reaction mixtures contained DNA and the TaqMan® universal PCR Master Mix or Sybr Green I PCR Master Mix (Applied Biosystems). The cycle threshold (CT) was calculated as the cycle number at which the reaction became exponential. The cycle threshold of each sample was then compared to a standard curve and the result expressed as a numerical value of the number of target copies in the sample.

The total Bacteria real-time PCR system developed by Suzuki et al. (2000) was used according to the protocol described by Rousselon et al. (2004). The system Taqman MGB® system (W102, W105, W101) amplified a fragment of 123 bp (Table 2). The total Archaea real-time PCR system developed by Takai and Horikoshi (2000) amplified a fragment of 476 bp. Sequences of the primers (W178 and W179) and the Taqman® probe (W177) are listed in Table 2, together with the PCR reaction conditions described by the authors (Takai and Horikoshi 2000).

The *P. acnes* real-time PCR system was that described by Eishi et al. (2002) (Table 2). The Taqman® system was replaced by the Sybr Green I assay because of poor probe specificity. A total of 100 nM of each primer was added to amplify the 16S rDNA of *P. acnes*. Reactions occurred after 2 min at 50 °C and 10 min at 95 °C, followed by 40 thermocycles: 15 s at 95 °C and 1 min at 60 °C.

A new Taqman® real-time PCR system used for the quantification of Synergistes was applied using the primers described by

**Table 1**
Relative quantification of microbial groups in biogas and anaerobic digester samples.

| Sites | Description of sampling sites | | | | Archaea | | Bacteria | | *Staphylococcus* spp. | | *P. acnes* | | Synergistes | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Waste | Biogas production ($m^3$ $day^{-1}$) | Sludge | Temperature (°C) | Biogas (%) | Anaerobic digesters (%) | Biogas (%) | Anaerobic digesters (%) | Biogas (%) | Anaerobic digesters (%) | Biogas (%) | Anaerobic digesters (%) | Biogas (%) | Anaerobic digesters (%) |
| DI | Industrial lactoserum effluent | 4800 | Aqueous | 37 | 0.24[a] | 3.00 | 99.76 | 97.00 | 0.10 | 0.01 | 0.20 | 0.004 | 0.03 | 1.86 |
| DE1 | Liquid manure and garden refuse | 80–150 | Viscous | 35 | 0.53 | 0.59 | 99.47 | 99.41 | <DL | <DL | 11.21 | 0.001 | 2.98 | 1.82 |
| DE2 | Liquid manure and garden refuse | 80–150 | Viscous | 35 | 0.37 | 4.55 | 99.63 | 95.45 | <DL | <DL | 0.23 | 0.01 | 0.06 | 1.16 |
| DM1 | Domestic waste and garden refuse | 15,000 | Thick | 37 | 0.14 | 1.61 | 99.86 | 98.39 | 5.73 | >DL | <DL | 0.01 | 0.06 | 3.31 |
| DT | Domestic waste and garden refuse | 13,000 | Thick | 55 | 0.06 | 0.78 | 99.94 | 99.22 | <DL | <DL | <DL | <DL | 0.15 | 5.82 |
| LD | Domestic waste (landfill) | 20,000 | –[c] | – | <DL[b] | – | 100 | – | 0.94 | <DL | <DL | <DL | <DL | <DL |

[a] Percentage of Procarya.
[b] Assay results below the limit of detection.
[c] Not performed.

Download English Version:

https://daneshyari.com/en/article/682807

Download Persian Version:

https://daneshyari.com/article/682807

Daneshyari.com