The effect of substrate and operational parameters on the abundance of sulphate-reducing bacteria in industrial anaerobic biogas digesters

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HIGHLIGHTS

▶ Abundance of sulphate-reducing bacteria was determined by qPCR targeting dsrB in biogas digesters.
▶ Sulphate-reducing bacteria were tolerant to most operational strategies used in industrial biogas plants.
▶ High concentrations of ammonia and ammonium lead to decreased abundance of sulphate-reducing bacteria.

ARTICLE INFO

ABSTRACT

This study evaluated the effects of operational parameters and type of substrate on the abundance of sulphate-reducing bacteria in 25 industrial biogas digesters using qPCR targeting the functional dissimilatory sulphite reductase gene. The aim was to find clues for operational strategies minimizing the production of H_2S. The results showed that the operation, considering strategies evaluated, only had scarce effect on the abundance, varying between 10^5 and 10^7 gene copies per ml. However, high ammonia levels and increasing concentration of sulphate resulted in significantly lower and higher levels of sulphate-reducing bacteria, respectively.

1. Introduction

Biogas is produced through biological degradation of organic material in the absence of oxygen. The process occurs in natural environments, but is also exploited in commercial plants for waste treatment and for production of biogas. In commercial biogas plants, commonly used waste streams include sludge from wastewater treatment plants (WWTP), slaughterhouse waste, food waste, manure or other industrial waste streams, crops and crop residues.

The anaerobic degradation of organic material and production of biogas proceeds through four sequential steps and requires the activity of different microbiological groups (Angelidaki et al., 2011). The last step, methanogenesis, is performed by the activity of two main groups of methanogens, the hydrogenotrophic and acetoclastic methanogens, or by syntrophic acetate-oxidising bacteria (SAOB) operating in cooperation with hydrogenotrophic methanogens (Angelidaki et al., 2011; Westerholm et al., 2011).

The biogas produced in industrial biogas digesters mainly consists of methane and carbon dioxide, but also small amounts of other gases such as hydrogen sulphide. The later compound has corrosive properties causing damage on equipment and thus during industrial scale production the hydrogen sulphide has to be removed (Appels et al., 2008). In Sweden this is accomplished by precipitation of sulphides with ferric or ferrous iron inside the digester (Ek et al., 2011). Alternative methods are aeration of the gas to obtain elemental sulphur or biological treatment with for example *Thiobacillus* strains etc. (Ramírez et al., 2011; van der Zee et al., 2007). Regardless of the choice of technique, removing sulphides requires either expensive, extensive use of chemicals or large investments in new equipment. In addition to the problem related to gas usage, hydrogen sulphide may also cause inhibition to the microbial community by direct toxic effects or by precipitation of trace metals needed for enzymatic activity (Chen et al., 2008;

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Lopes et al., 2010a; van der Veen et al., 2007). The production of sulphides is influenced by different factors. Two important regulatory parameters being: (i) the amount of sulphur-containing amino acids in the incoming material, (ii) the level of sulphate in the incoming material (Dewil et al., 2008; Rabus et al., 2006).

In the presence of sulphate in a biogas process, SRB and methanogens compete for the same substrate, i.e. acetate and hydrogen/carbon dioxide. SRB typically win this competition owing to several interacting factors: (i) anaerobic respiration with sulphate as the final electron acceptor yields more energy for growth compared with carbon dioxide; (ii) SRB possess higher affinity for both hydrogen and acetate, enabling them to consume substrates below levels possible for use by methanogens (Rabus et al., 2006); and (iii) SRB generally have a higher specific growth rate than methanogens (Oude Elferink et al., 1994). Several previous studies have sought to decrease the activity of SRB and hydrogen sulphide production in biogas processes. These studies have mainly focused on changes in hydrogen sulphide production or levels of fermentation products. Parameters that have been investigated include COD:sulphate ratio in the substrate (Hirasawa et al., 2008; Lopes et al., 2010b), addition of different SRB inhibitors (Isa and Anderson, 2005; Nemati et al., 2001), pH (Visser et al., 1996; Chaiprapat et al., 2011) and temperature (Pender et al., 2004). Overall, these studies provide no conclusive solution for optimising the management of a biogas process towards lower sulphide levels in the biogas.

The overall aim of the present study was to obtain further information concerning SRB in biogas processes and by doing so find clues on how to decrease H_2S production. The specific objective was to determine SRB abundance in biogas digesters and to evaluate possible correlations to substrate composition and operational parameters. To our knowledge, no previous study has examined the effects of different management strategies on the level of SRB. In total, 25 large-scale biogas digesters in Sweden were analysed with quantitative PCR targeting the dissimilatory sulphite reductase gene (drsB). In addition, SRB abundance was investigated over time in one digester subjected to increasing sulphate concentrations in the incoming substrate.

2. Methods

2.1. Samples and operational parameters

Representative samples were taken from 25 industrial biogas digesters at 17 different biogas plants in Sweden (Table 1). In total, six thermophilic digesters (H1, H2, K, M, N and P) and 19 mesophilic digesters (A1, A2, B1, B2, C, D, E, F, G, I1, I2, L1, L2, O1, O2, Q, R and S) were sampled, including two second stage digesters (D and F). At six of the biogas plants, two parallel digesters with slightly differing operational parameters were sampled. These digesters are denoted 1 and 2 accordingly for each plant (A1–A2, B1–B2, H1–H2, I1–I2, L1–L2 and O1–O2). The selected production plants included WWTP (L1, L2, O1, O2, P, Q and R) and co-digestion plants treating slaughterhouse waste or food waste as the main substrate (B1, B2, H1, H2, I1, I2, J, L1, L2, M, N and S). Digesters treating either brewery waste or crops (A1, A2, C, D, E and F) and digesters treating mainly manure (G and K) were also included. The samples taken from the digesters were frozen on-site (−20 °C) and sent to the laboratory for analysis. Information on substrate composition, iron addition and operational parameters for the different plants is presented in Table 1. Data on volatile fatty acids (VFA), pH, ammonium–nitrogen, hydrogen sulphide in raw gas (if available) were obtained from the different biogas plants. The fraction of ammonium–nitrogen that was present as ammonia was calculated according to Hansen et al. (1998) using

pH and temperature. The ammonium–nitrogen concentration was also adjusted according to this calculation to only show nitrogen in the form of ammonium.

In addition, consecutive sampling of one industrial plant (B) was performed during a period when the sulphate level in the substrate involuntarily increased on average by 870 mg/l. In total, eight samples were taken over a period of 70 days.

2.2. Sulphate analysis

Sulphate concentrations in typical substrates common for the biogas plants included in the study were analysed using a sulphate cell test manufactured by WTW PhotoLab Spektral, Weilheim, Germany. Samples were diluted to the desired concentration, centrifuged (20 min, 11,000g) and filtered (0.45 μm). Barium in excess was added to the samples and turbidity was measured using a WTW turbidity meter (WTW PhotoLab Spektral). Analysis of thin stillage was performed according to ISO 22743 using a Skalar San++ Continuous Flow Analyzer (Skalar, Breda, Netherlands).

The increased amount of sulphate added to digester B in the time study was calculated by analysis of sulphate concentration in the substrate (EN ISO 10304-1:2009, Eurofins Environment Sweden AB) and by multiplying this by the total amount of substrate added to the digester.

2.3. DNA extraction

Frozen samples were thawed and DNA was extracted from small aliquots (300 μl) with the FastDNA® SPIN kit for soil (MP Biomedicals, Solon, OH, USA), according to the protocol given by the manufacturer, with small adjustments to increase yield (SEWS-M washing was performed twice). For monitoring of DNA yield, Qubit® (Invitrogen, Carlsbad, CA, USA), fluorometric quantification was used.

2.4. Quantitative PCR

The functional gene dissimilatory sulphite reductase (dsrB) was amplified with primer pair DSRp2060F-GC (5′ CAA CAT CGT YCA YAC CCA GGG 3′) and DSR4R (5′ GTG TAG CAG TTA CCG CA 3′) according to Geets et al. (2006). For the analysis, the iQ™ SYBR® Green Supermix (Bio Rad, Hercules, CA, USA) was detected with a C1000™ Thermal Cycler, CFX96™ Real-Time System (Bio Rad). PCR was performed according to the protocol described by Dar et al. (2007), with adjusted initial touchdown protocol using 10 cycles instead of 20 for step-wise decrease of annealing temperature from 65 to 55 °C. PCR amplification was followed by a melt curve analysis. Each reaction was loaded with 10 μl Supermix, 1 μl forward primer (1 μM), 1 μl reverse primer (1 μM), 5 μl sterile water and 3 μl template DNA. The samples were analysed at different dilutions; 1:500, 1:100, 1:50 and 1:10 in order to find the optimal dilution and to ensure that no PCR-inhibiting substance affected the results. Most samples had optimal PCR performance at the 1:100 dilution. However, DNA samples from digesters D, O1 and O2 were diluted 1:50 and samples from digester E were diluted 1:500. The melt curve analysis of the real-time PCR showed a lower melt temperature for some samples (digesters C, D, E, G and J) of 83 °C, which was 7 °C lower than the standard curve, motivating sequence analysis of the targeted sequence (Uppsala Genome Center). The sequences obtained was aligned against known sequences with BLASTN and the result showed that the amplified sequence was of correct length and closely related to the dsrB sequence (data not shown).