Microaerobic digestion of sewage sludge on an industrial-pilot scale: The efficiency of biogas desulphurisation under different configurations and the impact of O2 on the microbial communities

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Abstract

Biogas produced in an industrial-pilot scale sewage sludge reactor (5 m3) was desulphurised by imposing microaerobic conditions. The H2S concentration removal efficiency was evaluated under various configurations: different mixing methods and O2 injection points. Biogas was entirely desulphurised under all the configurations set, while the O2 demand of the digester decreased over time. Although the H2S removal seemed to occur in the headspace, S0 (which was found to be the main oxidation product) was scarcely deposited there in the headspace. O2 did not have a significant impact on the digestion performance; the VS removal remained around 47%. Conversely, DGGE revealed that the higher O2 transfer rate to the sludge maintained by biogas recirculation increased the microbial richness and evenness, and caused an important shift in the structure of the bacterial and the archaeal communities in the long term. All the archaeal genera identified (Methanosaeta, Methanospirillum and Methanoculleus) were present under both anaerobic and microaerobic conditions.

1. Introduction

Anaerobic digestion (AD) is one of the oldest and most widely used processes for wastewater sludge stabilisation. It successfully reduces the quantity of solids, destroys pathogenic organisms, minimises odour problems, and produces an agricultural fertiliser, the digestate, and a renewable and versatile energy source, the biogas (Ward et al., 2008). Thus, AD optimises the costs of wastewater treatment plants (WWTPs) (Appels et al., 2008).

The biogas produced during AD is commonly used for heat and electricity production (Rasi et al., 2007) in relatively small and easy-to-manage industrial units (Antoni et al., 2007). A well-operated anaerobic reactor produces more energy (in the form of biogas) than that required on site, and the excess is usually sold to a local utility for domestic use (Turovskiy and Mathai, 2006). Alternatively, it can be fed to the gas grid, or used as a fuel in combustion engines, cars, or fuel cells (Appels et al., 2008).

Biogas from sewage digesters usually contains around 55–65% of CH4, 35–45% of CO2, and 1% of N2. Nevertheless, it typically also presents traces of H2S and other S-containing compounds (Rasi et al., 2007). Besides its bad smell, H2S is highly non-desirable in energy-recovery processes because it converts into SO2 and H2SO4, which are highly corrosive, unhealthy and environmentally
hazardous compounds (Abatzoglou and Boivin, 2009). Besides, H2S itself is reactive with most metals (Appels et al., 2008); it attacks iron, copper, cement, etc (Noyola et al., 2006). As a result, the biogas quality in the different technologies for energy recovery is assessed as H2S content.

The biogas generated during AD of sewage sludge can present H2S concentrations above the technical limits recommended by the facility manufacturers; it can contain up to 10,000 ppmv. Nonetheless, the usual H2S concentration in the biogas produced in WWTPs is about 1,000 ppmv. Therefore, with traditional boilers and internal combustion engines, in most cases the biogas can be used without any treatment, since the recommendations are that hydrogen sulphide content should not be more than 1,000 ppmv. Conversely, when the biogas is not used on site, the exhaustiveness required in the desulphurisation is much higher. The strictest limits of H2S are set for fuel cells, where its concentration in the biogas must not exceed 0.1 ppmv (Rasi et al., 2011).

The removal of H2S from biogas can be carried out by biological and physico-chemical treatments. Besides being ecologically cleaner, the biological desulphurisation techniques (the most notable of which are biofilters, biorickling filters and biosscrubbers) are inexpensive in terms of capital outlay and operating costs (Burgess et al., 2001). Nevertheless, physico-chemical processes are the most common methods for biogas desulphurisation. Among them, the dosing of iron chloride to the digester is one of the most widespread solutions (Persson and Wellinger, 2006), especially for sewage sludge digesters, where H2S concentrations of below 150 ppmv can be achieved (Deublein and Steinhauser, 2008). However, it implies high costs due to large chemical consumption and iron sludge production (Abatzoglou and Boivin, 2009). The rest of the popular physico-chemical methods are applied in the gas stream and in the upgrading process, such as adsorption on activated carbon (Krischan et al., 2012) and water scrubbing (Appels et al., 2008), respectively.

As an alternative to internally and inexpensively desulphurise biogas, air/O2 can be injected into the anaerobic reactor; thus, there is no hazardous reagent usage and chemical sludge production and H2S levels of less than 50 ppmv can be achieved (Díaz et al., 2010b). The basis of this method lies in the presence of sulphide-oxidising bacteria (SOB) in the feedstock (Weiland, 2010), which proliferate in the reactor, which and convert dissolved sulphide into SO4^2- and SO3^2-. (van der Zee et al., 2007). The ratio of SO4^2- /S0 depends on the dissolved O2 concentration; limited O2 availability and high sulphide loads favour S0 production (Fortuny et al., 2008). Moreover, under such conditions, SOB can effectively compete with the chemical sulphide oxidation (Robertson and Kuenen, 2006). In comparison to the other biological processes, microaeration/microoxygenation of “anaerobic” digesters has a smaller footprint, and entails lower capital outlay and only a minor modification of the process flowchart.

The feasibility of sulphide oxidation during digestion under microaerobic conditions has been widely demonstrated; the process successfully competes for O2 with other oxidative processes, and is faster than re-reduction of the oxidised sulphur species (van der Zee et al., 2007). Moreover, the presence of O2 has been proved not to have a negative effect on either the organic matter removal, the biogas or the methane productivity (Fdz-Polanco et al., 2009; Díaz et al., 2010a,b). In fact, the full-scale experiments carried out by Jenicek et al. (2008, 2010) revealed that, besides efficiently desulphurising biogas, an intimate contact between the O2 and the liquid phase can improve organic matter degradation, dig- estate quality and productivity of both biogas and CH4 during municipal sludge digestion. This can arise from higher hydrolysis rates (Botheju et al., 2010; Lim and Wang, 2013), alleviation of sulphide toxicity (Jenicek et al., 2010; Zhou et al., 2007), and shifts in microbial activity and populations (Jenicek et al., 2011; Rodríguez et al., 2012; Tang et al., 2004; Zhou et al., 2007). Specifically, Zhou et al. (2007) observed that the rod-shaped methanogens almost disappeared and were replaced by cocci-shaped ones after imposing microaerobic conditions. Tang et al. (2004) reported a dramatic increase in the population size of Methanothrix due to microaeration. In the meantime, they found a significant decrease in the population of Methanosarcina, which suggested that Methanothrix sp. out-competed Methanosarcina sp. due to a higher tolerance for O2.

The most profitable oxidative reactant for imposing microaerobic conditions during digestion is O2 since air implies further biogas dilution (Díaz et al., 2010a; Jenicek et al., 2010). On the other hand, Díaz et al. (2010b) demonstrated that the most economical configuration for desulphurising biogas during the pilot-scale digestion of sewage sludge consisted of O2 supply to the headspace and liquid recirculation, which is consistent with the fact that the H2S removal takes place in the gas space of the reactor (Ramos et al., 2012). However, further research is required in order to corroborate these findings on a larger scale. Hence, the main aims of this research were:

- To investigate the efficiency of the process of biogas desulphurisation in an industrial-pilot scale digester under different configurations (mixing method and concentrated O2 dosing point).
- To study the impact of micro-oxygenation on the microbial communities.

2. Methods

2.1. Industrial-pilot scale reactor

The research was carried out in a continuous stirred tank reactor with 5 m3 (7 m3 of total volume) located at the WWTP of Villalonquijar (Burgos, Spain). Temperature (35 °C) was maintained by a water heat exchanger and monitored by probes (Fig. 1). Mixed sludge produced in the aforementioned municipal WWTP was continuously pumped to the digester by a screw pump. It presented a highly variable organic load, with a VS concentration ranging between 24.7 and 44.9 g/L. As a result, its rheology was highly changeable, which in turn led to fluctuations in the feeding flow rate. Hydraulic retention time (HRT) was approximately 20 d, and the organic loading rate ranged between 2.2 and 1.5 kg VS/m3/d. The reactor’s content was recirculated by a peristaltic pump at a rate of approximately 25 m3/d. Depending on the operational period, a compressor provided 21 m3/d of biogas recirculation (Fig. 1). Microaerobic conditions were implemented from the period P2 to P8 using an O2 concentrator (Table 1), which achieved a purity of 92–98%. O2 was introduced intermittently (approximately every 10 min). Fig. 1 shows the O2 dosing point in each operational period.

Pressure was monitored by a sensor. Biogas production was quantified by a thermal mass flow meter, and its composition was determined by gas chromatography (GC) (CP-4900 Micro-GC) according to Díaz et al. (2010b) from the beginning of the study to the 87th day. From the 117th day, it was measured by a gas analyser (GA-3000) whose precision in determining CH4, O2 and H2S was ±3%/v, ±1%/v, and ±5% of the result or 500 ppm (the largest value), respectively. SCADA software was used for monitoring; data (pressure, temperature, biogas production and recirculation rate) were displayed and stored in real-time in a computer.

2.2. Experimental procedure

The reactor was inoculated with sludge from a full-scale anaerobic reactor located at the aforementioned WWTP. It was started-
Table 1
Sequence of variations applied to the digester.

<table>
<thead>
<tr>
<th>Period</th>
<th>Conditions</th>
<th>Recirculation</th>
<th>O$_2$ dosing point</th>
<th>O$_2$ flow rate (L/m$^3$/d)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Anaerobic</td>
<td>Sludge</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P2</td>
<td>Microaerobic</td>
<td>Sludge</td>
<td>Headspace</td>
<td>12–34 (increasing)</td>
</tr>
<tr>
<td>P3</td>
<td>Microaerobic</td>
<td>Sludge and biogas</td>
<td>Headspace</td>
<td>34–14 (decreasing)</td>
</tr>
<tr>
<td>P4</td>
<td>Microaerobic</td>
<td>Sludge and biogas</td>
<td>Biogas recirculation</td>
<td>14</td>
</tr>
<tr>
<td>P5</td>
<td>Anaerobic</td>
<td>Sludge and biogas</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P6</td>
<td>Microaerobic</td>
<td>Sludge and biogas</td>
<td>Biogas recirculation</td>
<td>14</td>
</tr>
<tr>
<td>P7</td>
<td>Microaerobic</td>
<td>Sludge</td>
<td>Liquid phase</td>
<td>14–5 (decreasing)</td>
</tr>
<tr>
<td>P8</td>
<td>Microaerobic</td>
<td>Sludge</td>
<td>Headspace</td>
<td>5</td>
</tr>
</tbody>
</table>

$^a$ Expressed as L of O$_2$ per m$^3$ of reactor and per day.

Fig. 1. Digester diagram. Operational periods (P1–P8) are indicated in brackets.

Fig. 2. Concentration of H$_2$S (△) and O$_2$ (○) (a), and CH$_4$ (□) and CO$_2$ (■) (b). Anaerobic periods are represented in white.
up under anaerobic conditions, using sludge recirculation as the mixing method. HRT was set at about 24 d until the 50th day; thereafter, it was around 20 d. At day 135 (day 0 in Fig. 2), it was considered that a stationary state had been achieved.

The study was divided into eight operational periods (Table 1): two anaerobic (P1 and P5) and six microaerobic (P2, P3, P4, P6, P7 and P8). In P1, P2, P7 and P8, the reactor’s content was mixed by sludge recirculation (Fig. 1). In the rest of the experimental periods, the digester operated with both biogas and sludge recirculation.

In P2, O₂ was supplied to the gas space, just above the liquid interface, where the H₂S removal from biogas reportedly occurs (Abatzoglou and Boivin, 2009; Weiland, 2010), and the flow rate was gradually raised from approximately 12 to 34 L/m³/d, as shown in Table 1. During P3, the micro-oxygenation level was gradually lowered to 14 L/m³/d. Micro-oxygenation was interrupted in order to evaluate the H₂S production during P5. In P6, the configuration and the O₂ flow rate set in P4 were restored. During P7, the biogas recirculation was stopped, and O₂ was injected into the liquid phase. The O₂ dosing point was changed; it was injected into the biogas recirculation at a constant flow rate (14 L/m³/d). Micro-oxygenation was interrupted in order to evaluate the H₂S production during P5. In P6, the configuration and the O₂ flow rate set in P4 were restored. During P7, the biogas recirculation was stopped, and O₂ was injected into the liquid phase, just above the midpoint of the digester. As shown in Table 1, the O₂ flow rate was gradually lowered from 14 to 5 L/m³/d. In the last experimental period (P8), the configuration applied during P2 was restored, while the micro-oxygenation level remained constant (5 L/m³/d).

2.3. Chemical analysis

The feed and the digested sludge were analysed in terms of total and soluble chemical oxygen demand (TCOD and SCOD), total and volatile solids (TS and VS), carbon (C) and sulphur (S), total kjeldahl nitrogen (TKN), N–NH₄⁺, NO₂⁻, NO₃⁻, PO₄³⁻–, sulphide, SO₂⁻ and S₂O₃²⁻. Specifically, NO₂⁻, NO₃⁻, PO₄³⁻ were analysed by UV–visible spectrophotometry (APHA, 1998). SO₂⁻ and S₂O₃²⁻ concentrations were determined by ion chromatography (APHA, 1998) and high-performance liquid chromatography (van der Zee et al., 2007), respectively. Dissolved sulphide was analysed by potentiometric titration (APHA, 1998), and elemental analysis (C and S) were carried out by IR spectroscopy (LECO CS-225). The pH of the reactor was monitored by a pH-metre with a temperature probe.

2.4. Microbial analysis

In order to evaluate how the presence of O₂ affected the microbial community, four biomass samples (S1–S4) were collected and stored immediately at −20 ºC. Samples S1–S4 were taken in P1 (18rd day), P2 (67rd day), P3 (109th day) and P4 (165th day), respectively.

The V6–V8 and the V2–V3 regions of the bacterial and the archaeal 16S rRNA genes were amplified using polymerase chain reaction (PCR) using the universal bacterial primers 515-GC-R and A 109(T)-F and 1401-R, and A 109(T)-F and 515-GC-R (Sigma–Aldrich, St. Louis, MO, USA), respectively (Rodríguez et al., 2012). The PCR mixture contained 1 μL of each primer (10 ng μL⁻¹ each primer), 25 μL of BIOMIX ready-to-use 2× reaction mix (Bioline, Ecogen), PCR reaction buffer and deoxynucleotide triphosphates, 2 μL of the extracted DNA, and Milli-Q water up to a final volume of 50 μL. PCR was performed in a iCycler Thermal Cycler (Bio Rad Laboratories, Inc.) applying the thermo-cycling program described by Rodríguez et al. (2012). DGGE analysis of the bacterial and archaeal amplicons were performed according to Rodríguez et al. (2012). DGGE profiles were compared using the GelCompar ITMM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). The Shannon–Wiener diversity index (H) of every sample and the pair-wise similarity coefficient were both calculated according to Lebrero et al. (2013). Similarity dendrograms were constructed by using UPGMA clustering with error resampling (500 resampling experiments).

Individual bands were excised from the archaeal DGGE gel. Both DNA extraction and purification of PCR products were carried out according to Rodríguez et al. (2012). The taxonomic position of the sequences was obtained using the RDP classifier tool (80% confidence level) (Wang et al., 2007). Moreover, the sequences were compared with those included in GenBank by the BLAST search tool at the National Centre for Biotechnology Information (NCBI) website (McGinnis and Madden, 2004). Along with BLAST, DECIPHER was used as the chimera checking tool (Wright et al., 2012). Finally, the sequences were deposited in GenBank under accession numbers KJ402278–KJ402293.

3. Results and discussion

3.1. Biogas quality

3.1.1. H₂S and O₂

The average sulphide content of the biogas produced in P1 (under anaerobic conditions) was 0.49%v/v (Table 2). Microaerobic conditions were applied on the 29th day (period P2) by injecting around 12 LO₂/m³/d into the gas space, just above the liquid interface (by a single point) (Table 1). Under such a configuration, presumably the O₂ transfer rate to the sludge was significantly limited. Moreover, it is possible that O₂ could only be transferred to a limited area in the headspace (around the injection point). Within 24 h, the H₂S concentration decreased to 0.35%v/v (Fig. 2a). It indeed remained at around that value until the 54th day, despite the gradual increase in the O₂ supply. Thereafter, at micro-oxygenation levels above 29 L/m³/d, the average H₂S and O₂ concentration in biogas was 0.02 and 4.3%v/v, respectively. It is worth noting that at even the highest micro-oxygenation level, the biogas N₂ content did not exceed 0.1%v/v.

On the 74th day (period P3), the biogas recirculation was started, while the O₂ supply was maintained at approximately 34 L/m³/d (Table 1). As expected, the biogas O₂ content dropped significantly (to approximately 1.2%v/v), which was attributed to improved O₂ transfer due to higher contact between the gas and the liquid phase. The H₂S concentration in biogas also decreased, and stabilised at around zero. Hence, from the 77th to the 87th day, the O₂ flow rate was gradually reduced from approximately 31 to 19 LO₂/m³/d. This hardly affected the H₂S and the O₂ concentration in the biogas. Regarding the O₂ content in the biogas, it presumably remained constant due to lesser O₂ consumption in unidentified processes, which could in turn result from the lower gradient of O₂ in the liquid phase.

In contrast to Díaz et al. (2010b), who reported a decrease in dissolved sulphide in the digester from approximately 130 mg/L to less than 40 mg/L due to the implementation of biogas recirculation, the digestate sulphide content in P3 did not decrease.

<table>
<thead>
<tr>
<th>Period</th>
<th>O₂ (%v/v)</th>
<th>H₂S (%v/v)</th>
<th>CH₄ (%v/v)</th>
<th>CH₄/O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>0.0</td>
<td>0.49</td>
<td>63.5</td>
<td>1.8</td>
</tr>
<tr>
<td>P2</td>
<td>1.7</td>
<td>0.21</td>
<td>62.4</td>
<td>1.8</td>
</tr>
<tr>
<td>P3</td>
<td>0.9</td>
<td>0.01</td>
<td>62.0</td>
<td>1.7</td>
</tr>
<tr>
<td>P4</td>
<td>0.1</td>
<td>0.02</td>
<td>59.1</td>
<td>1.5</td>
</tr>
<tr>
<td>P5</td>
<td>0.0</td>
<td>0.25</td>
<td>58.8</td>
<td>1.5</td>
</tr>
<tr>
<td>P6</td>
<td>0.1</td>
<td>0.01</td>
<td>59.4</td>
<td>1.5</td>
</tr>
<tr>
<td>P7</td>
<td>0.0</td>
<td>0.00</td>
<td>60.1</td>
<td>1.5</td>
</tr>
<tr>
<td>P8</td>
<td>0.0</td>
<td>0.00</td>
<td>59.2</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Table 2

Biogas quality.
significantly in relation to the previous operational periods. The feeding sulphide content was around 26 mg/L during the whole study, while the sulphide concentration in the effluent in P1, P2 and P3 was 58, 63 and 50 mg/L, respectively.

During P4, the O2 was introduced in the biogas recirculation instead of in the gas space (Table 1). As expected, the H2S and the O2 concentration in the biogas remained almost constant (around zero). Regarding the O2 concentration, it must be noted that the difference between the first and the last 12 days of P3 was due to the change in the measuring device (Fig. 2a).

In P5, the micro-oxygenation was stopped in order to evaluate the H2S concentration under anaerobic conditions (Table 1). The biogas sulphide content remained around 0.25%v/v (Table 2). The considerable decrease in the H2S concentration in relation to P1 was related to the sludge variability. At this point, it must be noted that, besides a variable organic load, the feeding presented a highly variable concentration of S. Specifically, the C and S content of the sewage sludge was in the range of 29–37 and 0.5–2.0%w/w, respectively.

When microaerobic conditions were restored with the same configuration as in P4 (period P6), the sulphide content of the biogas dropped to an average of 0.01%v/v, while the surplus of O2 hardly rose (Fig. 2a). It must be mentioned that the peaks of H2S concentration detected on the 223rd and 224th day could result from the increase in the biogas production recorded during those days. Once the biogas recirculation was stopped (period P7), the H2S concentration decreased negligibly. Regarding the biogas O2 content, it hardly changed, which could be related to the precision of the gas analyser; it was expected to rise due to lower O2 transfer to the sludge. In fact, from the 235th day, although the O2 supply was gradually lowered, the biogas composition remained almost constant in terms of both O2 surplus and H2S concentration. From the 250th day until the end of the study, the O2 flow rate was maintained at around 5 L/m3/d.

In P8, the H2S and O2 concentrations in the biogas were almost equal to those in P7 (Table 2), which highlighted that the success of the biogas desulphurisation was independent of the O2 transfer rate to the liquid phase. At this point, it should be mentioned that the objective of the configuration applied in P7 was to maintain a successful O2 transfer to all the surfaces of the headspace. As a result, it was concluded that the biogas desulphurisation took place predominantly in the gas space.

Despite applying the same configuration, the molar ratio of O2 supplied to H2S produced which was required to achieve similar H2S concentrations in the biogas in P2 was almost four times higher than in P8 (8 against 2 v/v, respectively) (Table 1). Maybe the population size of SOB in the area of the headspace with sufficient O2 availability during P2 (that around the O2 injection point) did not suffice to achieve efficient biogas desulphurisation. Presumably the SOB population in the gas space increased over time due to the accessibility of both H2S and O2.

3.1.2. CH4

The average CH4 content of the biogas produced in P1 was 63.5%v/v (Table 2). During P2, while the surplus of O2 in the biogas increased, the CH4 concentration declined gradually; it was specifically 62.4%v/v on average (Fig. 2b). It should be noted that this oscillated between 61.7 and 60.9%v/v from the 87th day. Subsequently, in P3, due to the implementation of biogas recirculation and the consequently higher O2 consumption in the reactor, the biogas CH4 content rose, remaining around 62.7%v/v until the 67th day. Consequently, the decrease in the CH4 concentration observed in P2 was attributed to dilution rather than aerobic oxidation of methanogenic substrate. In fact, the ratio of CH4 to CO2 was 1.8 in both P1 and P2 (Table 2). In P3, before the change in the measuring device of biogas composition, this variable was negligibly lower (1.7), which indicated that the additional O2 was scarcely employed to oxidise soluble organic matter by aerobic respiration; this finding was confirmed in the following operational periods. Nonetheless, it must be considered that slight changes in the biogas composition could arise from the variability of the feeding composition.

In P3, after the 117th day, due to the replacement of the measuring device, the CH4 and O2 concentrations were significantly lower, and the CH4/CO2 ratio remained around 1.5 (Fig. 2b). The average biogas CH4 content in P3 from the aforementioned day was specifically 60.0%v/v. In the rest of the operational periods, including P4, P5 (anaerobic), P6, P7 and P8, it was 59.1, 58.8, 59.4, 60.1 and 59.2%v/v (respectively) (Table 2). Regarding the CH4/CO2 ratio, it varied negligibly, which along with the biogas production (Table 3) suggested that O2 did not cause a significant impact on the reactor productivity, independently of its availability.

3.2. Digestion performance

Due to the continuous changes in the organic loading rate to the reactor arising from the variability of the feeding composition, the digestion performance (organic matter removal and biogas productivity) could not be estimated with the necessary precision in order to identify significant differences between the various operational periods, specifically, between P1, P2, P3 and P4, that is, the periods with a sufficiently long duration. With regard to this, it must be considered that the reactor was started-up and operated under the configuration set in P1 for 135 days.

The VS removal in P1, which was considered the baseline period, was 45% on average and, during P2, P3 and P4, this variable fluctuated around that value (Table 3). Likewise, neither the digestate quality deteriorated significantly nor the biogas production or its CH4 content decreased substantially during the different stages in relation to P1. Similarity to the CH4 concentration in the biogas, the TS and VS content of the digestate and the biogas production both fluctuated within a narrow range all through the research (31.6–32.8 g/L, 17.0–18.1 g/L, and 0.86–1.08 m3/m3/d, respectively). Moreover, these variables rose concurrently, which was consistent with the increasing VS content of the feeding (data not shown). These results are in turn consistent with those reported in literature, since even on an industrial scale, it has been broadly demonstrated that the presence of limited amounts of O2 in digesters does not negatively affect digestion performance (Jenicek et al., 2008, 2010).

3.3. Sulphide oxidation products

According to the SO42− and S2O32− analysis, H2S was mostly oxidised to SO42−. S2O32− was detected in negligible amounts in both
the sewage and the digested sludge. The average SO$_4^{2-}$ concentration in the feeding from P1 to P4 was between 141 (P4) to 238 mg/L (P1), while the effluent SO$_4^{2-}$ content remained between 24 (P1) and 29 mg/L (P2). According to the H$_2$S production and the operational conditions, the expected increase in the digestate S content during the microaerobic periods in relation to P1 was also negligible. Moreover, even if it had been significant, it would hardly have been detectable due to the aforementioned variable S concentration in the feeding. Nevertheless, it is worth noting that the effluent S and C content of the digested sludge oscillated continuously between 0.7 and 2.0%w/w, and between 22 and 28%w/w, respectively.

After approximately 415 days of operation (240 days under microaerobic conditions), the ceiling of the reactor was removed. A relatively thick deposit with 19%w/w of C and 58%w/w of S was observed at the area of the walls surrounding the liquid phase. Presumably it formed due to the slight, unavoidable fluctuations in the liquid level and even sludge splashes. The rest of the headspace was covered with a really thin, dry layer with 6 and 86%w/w of C and S, respectively. The high degree of dryness observed in most of the surfaces of the gas space after the operation shutdown suggested that moisture availability could limit the growth of SOB in the headspace. This could be the cause of the low desulphurisation performance and relatively high O$_2$ demand maintained at the early stage of the microaerobic operation (Fig. 2a).

Considering that the biogas desulphurisation appeared to occur predominantly in the headspace (see Section 3.1.1), and, as noted above, H$_2$S was mostly converted into S$^0$, larger amounts of this compound were expect to be found all over the headspace.

Presumably the S$^0$ formed there fell into the sludge, thereby leaving the reactor with the effluent. Since the main factor affecting the profitability of the implementation of microaerobic conditions in full-scale reactors is the required periodicity for cleaning the headspace (Díaz, 2011), this observation could be of utmost importance.

Fig. 4. Archaeal (a) and bacterial (b) similarity dendrogram (UPGMA clustering) and matrix with error resampling (500 resampling experiments).
Table 4

RDP classification of the archaeal DGGE bands sequenced with at least 80% of confidence level, and corresponding closest relatives in Genbank obtained by the BLAST search tool with their similarity percentages, and environments from which they were retrieved. Intensity < 35 = ×, 35 ≤ intensity < 80 = ■■, intensity ≥ 80 = ■■■.

<table>
<thead>
<tr>
<th>Taxonomic placement (confidence threshold ≥ 80%)</th>
<th>Band</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>Closest relatives (accession no. in Genbank)</th>
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Table 4 (continued)

<table>
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<th>S2</th>
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3.4. Microbial communities

3.4.1. Biodiversity

In order to evaluate the richness and evenness of species (diversity) in the reactor in P1 (sample S1), P2 (sample S2), P3 (sample S3), and P4 (sample S4) the Shannon–Wiener index (H) was calculated from both DGGE gels (Fig. 3a and b). This index typically ranges from 1.5 to 3.5 (McDonald, 2003). All the samples showed a moderate archaeal diversity, with H ranging from 2.4 to 2.8 (Fig. 3a). The diversity indices calculated from the bacterial DGGE gel were in the range of 2.6 to 3.1, which highlighted a moderately high bacterial richness and evenness (Fig. 3b).

The archaeal diversity of the sample S3 (2.4) was negligibly lower than that of S2 and S1 (2.5), while S4 presented the highest H (2.8) (Fig. 3a). On the other hand, S1 and S4 exhibited the lowest and the highest bacterial diversity (2.6 and 3.1, respectively), while S2 and S3 presented the same H (2.9) (Fig. 3b). Therefore, the O2 availability in the sludge maintained during P2 sufficed to cause a significant increase in the bacterial diversity. Likewise, the archaeal and the bacterial richness and evenness both rose in P4. This indicated that the increase in the O2 transfer rate to the liquid phase arising from the implementation of biogas recirculation in P3 positively affected the microbial diversity in the long term (in period P4).

3.4.2. Similarity

The pair-wise similarity indices indicated a moderate-high correspondence between the microbial communities growing in the sludge at the different periods of the study (Fig. 4). The similarity coefficients between the archaeal communities fluctuated between 60 and 91% (Fig. 4a). Sample S1 and S2 presented the highest similarity, which suggested that the archaeal communities were only slightly affected by the injection of O2 into the headspace. This was consistent with the presumably low O2 transfer to the sludge maintained during P2. Likewise, a high similarity was also found between sample S3 and S4 (87%). By contrast, the similarity indices between the rest of the sample pairs were lower than 70%. Therefore, the implementation of biogas recirculation in P3 and the resulting increase in the O2 transfer rate to the sludge caused a significant impact on the structure of the archaeal communities.

The similarity coefficients between the bacterial communities fluctuated from 41 to 93% (Fig. 4b). The highest similarities were found between S1 and S2 (93%), S1 and S3 (91%), and S2 and S3 (90%). Conversely, S4 presented a similarity of between 41 and 53% with the rest of the samples. Hence, as in the archaeal communities, the bacterial communities were only slightly affected when O2 was injected into the gas space. The increase in the O2 transfer rate to the liquid phase during P3 also had a limited impact on them; however, in the long term (P4), it caused a significant change in the structure of the bacterial communities.

3.4.3. Archaeal DGGE dynamics

From the archaeal DGGE gel (Fig. 3a), sixteen bands were sequenced (Table 4). According to the RDP classifier (confidence threshold of at least 80%), all of them belonged to the Eur-yarchaeota phyla. Band 1 and 2 remained unclassified, and the rest were all ascribed to one class, Methanomicrobia, and two orders, Methanosarcinales (band 3–9) and Methanomicrobiales (band 10–16). According to the BLAST search tool, band 1 and 2 could belong to the Methanosarcinales order. The rest of the results obtained from this database were consistent with those given by the RDP classifier.

Most of the sequences were close to sequences of already-known methanogenic genera retrieved from reactors (Leclerc et al., 2004). The sequences affiliated to the Methanosarcinales order were assigned to the Methanosaeta genus (Table 4), which contains acetotrophic methanogens capable of utilising acetate. On the other hand, from all the DGGE bands ascribed to the Methanomicrobiales order, six (10–15) were assigned to Methanospirillum sp., and band 16 was assigned to Methanoculleus sp.. These two genera have been reported to consist of hydrogenotrophic methanogens (Robertson and Kuenen, 2006).

All the genera identified were present in the four samples. Moreover, according to the bands intensity, O2 increased the evenness of all these genera (Table 4). Therefore, in contrast to Tang et al. (2004), micro-oxygenation did not seem to cause a particular impact on any of the archaeal populations.

4. Conclusions

Biogas produced in an industrial-pilot reactor was efficiently desulphurised independently of the mixing method and the O2 dosing point. The O2 demand of the digester decreased with time. The H2S removal seemed to occur in the headspace; however, S0, which was found to be the main oxidation product, scarcely accumulated there in the headspace. O2 did not have a significant impact on digestion. In the long term, the relatively high transfer rate of O2 to the sludge maintained by the biogas recirculation...
caused a significant change in the structure and a considerable increase in the richness and evenness of the microbial communities.

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References


