Microaerobic desulphurisation unit: a new biological system for the removal of H_2S from biogas

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Abstract

A new biotechnology for the removal of H_2S from biogas was devised. The desulphurisation conditions present in microaerobic digesters were reproduced inside an external chamber called a microaerobic desulphurisation unit (MDU). A 10L-unit was inoculated with 1L of digested sludge in order to treat the biogas produced in a pilot digester. During the 128d of research under such conditions, the average removal efficiency was 94%. The MDU proved to be robust against fluctuations in biogas residence time (57-107min), inlet H_2S concentration (0.17-0.39%v/v), O_2/H_2Ssupplied ratio (17.3-1.4v/v), and temperature (20-35ºC). Microbiological analysis confirmed the presence of at least three genera of sulphide-oxidising bacteria. Approximately 60% of all the H_2S oxidised was recovered from the bottom of the system in the form of large solid S0 sheets with 98%w/w of purity. Therefore, this system could become a cost-effective alternative to the conventional biotechniques for biogas desulphurisation.

Keywords

Biogas desulphurization; hydrogen sulfide; elemental sulfur; microaerobic; oxygen

1. Introduction

In the current energy context, the possibility of generating heat or electricity generation from biogas are attractive reasons for the application of anaerobic digestion to treat organic wastes (Pölsch et al., 2010). Biogas can also be used as vehicle fuel and for chemicals production (Apples et al. 2008). Whatever the application, its quality is crucial in terms of both CH_4 content (which determines its calorific value) and purity.

H_2S is typically the main pollutant in biogas derived from sludge digesters in municipal wastewater treatment plants, with concentrations ranging from 1.0 to 0.1%v/v (10,000-1,000ppmv) (Rasi et al., 2011). It is produced by the anaerobic degradation of S-containing compounds (mainly proteins) and the reduction of anionic species (particularly SO_4^{2-}) contained in the feedstock of the digester (Stams et al., 2003). H_2S has a great influence when the different uses of biogas are considered, due to the corrosion problems that it causes in the installations for energy recovery. Therefore, manufacturers of biogas facilities establish limit concentrations; e.g., the biogas sulphide content should not be more than 1,000 and 0.1ppmv, respectively, in internal combustion engines and molten carbonate fuel cells (Rasi et al., 2011). Likewise, in combined heat and power plants, which are mainly implemented for the utilisation of biogas, levels below 250ppmv are required (Weiland, 2010). Nonetheless, H_2S removal is also required for health and safety reasons. State laws and regulations have been issued in Europe to minimise its presence in all parts of the biogas plants, including in bioreactors, gasholders, ignition and storage tanks, etc (Deublein and Steinhauser, 2008).

Most of the commercial technologies for H_2S removal are based on physicochemical processes; namely, absorption and adsorption. In order to overcome the chemical, energy and disposal costs of these desulphurisation methods, biologically-based removal processes were developed. Biological methods are the cost-effective and environmentally friendly solution to these techniques, since they can proceed at lower temperatures and pressures, and with limited or no reagent consumption (Syed et al., 2006).

Biogas can be biologically desulphurised in additional units, represented mainly by biofilters, biotrickling filters, and bioscrubbers, or directly into the anaerobic reactor, that is, by applying microaerobic conditions during digestion. All these processes are based on the S cycle, and more specifically, in H_2S oxidation. In the aforementioned extra units, H_2S is solubilised in a humid packed bed where aerobic species of sulphide-oxidising bacteria (SOB) are immobilised and grown as a biofilm in the presence of O_2 (Noyola et al., 2006). By contrast, these microorganisms are naturally present inside reactors; many organic wastes treated by AD have SOB (Weiland et al., 2010).

Elemental sulphur (S^0) (Eq. (1)) and SO_4^{2-} (Eq. (3)) are the thermodynamically stable by-products from biological H_2S oxidation, which, it has been proposed, proceeds through several intermediates. Duan et al. (2005) suggested the following pathway of chemoautotrophs: $H_2S \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow S_3O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$. The main reactions carried out by SOB are shown below (Tang et al., 2009). At this point, it should be noted that H_2S oxidation in biological systems occurs concurrently with chemical reactions (van der Zee et al., 2007), where $S_2O_3^{2-}$ is the main by-product

(Janssen et al., 1995).

$H_2S + 0.5O_2 \rightarrow S^0 + H_2O$; ΔG^0=-209.4kJ/reaction (1)

$S^0 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$; ΔG^0=-587.41kJ/reaction (2)

$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$; ΔG^0=-798.2kJ/reaction (3)

$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+$; ΔG^0=-818.3kJ/reaction (4)

Considerable efforts are still required concerning the packed-media based biotechnologies; though effective, they have strict requirements in terms of both monitoring and maintenance due to the bacteria's high sensitivity to fluctuations in operational conditions (Burgess et al., 2001), which translates into costs. Thiopaq® (Paques) and Biopuric® (Biothane Corporation) are indeed the only two industrial biotechnologies that have been specifically developed for the H_2S removal from biogas. Both of them combine a chemical scrubber and a bioreactor (Fortuny et al., 2008). Additionally, the Thiopaq® process includes a settler to separate the formed solid S^0 from the liquid phase (http://www.paques.nl/). Besides as fertiliser, S^0 can be applied in bioleaching and processes, and is suitable for producing H_2SO_4 (Kleinjan, 2005).
In microaerobic reactors, the sulphide-oxidising population specifically develop in the headspace, and as a result, the biogas desulphurisation and the consequent S^0 deposition both take place in this area (Díaz et al., 2010; Kobayashi et al., 2012). Therefore, periodic cleaning is required in order to prevent clogging problems and ensure stable H_2S removal efficiency, which in turn implies extra costs (Díaz, 2011).

According to the above observations, a potentially ideal external process for the removal of H_2S from biogas would integrate the simplicity of the desulphurisation process in microaerobic digesters. On this basis, a new biological technology was devised. The objective of this research was to evaluate the feasibility of this system, and to carry out a preliminary assessment of some of the variables that could affect its performance.

2. Materials and methods

2.1. Experimental set-up and performance monitoring

In this research, a variable flow rate of biogas with a changeable H_2S content from a pilot-scale anaerobic digester of sewage sludge was treated (Table 1). As illustrated in Fig. 1, it was mixed with pure O_2, the dose being regulated by a mass flow controller (Bronkhorst EL-FLOW Select). Prior to entering a thermostated chamber, the untreated biogas was analysed by a VARIAN MicroGC in terms of CH_4, CO_2, N_2, O_2, H_2 and H_2S according to Díaz et al. (2010). Subsequently, it passed through a 10L-system called a microaerobic desulphurisation unit (MDU), where it was desulphurised in the presence of 1L of inoculum taken from the anaerobic digester used as the biogas source. Thus, considering the amount of biogas yet to be treated, a biogas residence time (BRT) of approximately 60min would be maintained. This period has been proved to be sufficient for an efficient hydrogen sulphide removal in microaerobic reactors (Ramos et al., 2012). Then, the biogas composition was determined again. Finally, the biogas flow rate leaving the system was measured volumetrically by water displacement. Treatment performance was assessed by calculating mass loading rate (MLR), elimination capacity (EC) and removal efficiency (RE).

In order to increase the support area for SOB, wires, plastic tubing and paper strips (i.e., objects with low specific surface area) were tied to a small metal grid hanging from the top of the MDU (Fig. 1). It should be noted that they were partially submerged in the liquid phase.

2.2. Chemical analysis

The inoculum was characterised in terms of total and volatile solids (TS and VS, respectively) according to APHA (1998). Additionally, C and S concentrations in the inoculum and S^0-rich biomass accumulated inside the MDU were determined by a LECO CS-225.

2.3. Microbiological analysis

In order to identify the bacteria present in the MDU, an inoculum sample was drawn and frozen immediately after the system shutdown. DNA extraction, polymerase chain reaction (PCR) amplification and denaturing gradient gel electrophoresis (DGGE) analysis were carried out according to Lebrero et al. (2012). The universal bacterial primers 968-F-GC and 1401- R were used for the PCR amplification procedure (Sigma-Aldrich, St. Louis, MO, USA).

The DGGE profile was processed using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). After image normalisation, bands were defined by the program search algorithm. Subsequently, the Shannon-Wiener diversity index (H) was determined according to the expression: $H= -\Sigma[P_i\ ln(P_i)]$, where P_i is the importance probability of the bands in a lane, and is calculated as n_i/n, where n_i is the

height of an individual peak, and *n* the sum of all peak heights in the densitometric curves of the DGGE profile. Therefore, this index reflects both the sample richness (relative number of DGGE bands) and evenness (relative intensity of every band). According to McDonald (2003), it ranges from 1.5 to 3.5 (low and high species evenness and richness, respectively).

Based on a visual analysis of the gel, some DGGE bands were excised according to Lebrero et al. (2011). The closest matches to each sequenced band within a confidence level of 50%, and its respective taxonomic position were obtained by the Blast search tool at the National Centre for Biotechnology Information (McGinnis and Madden, 2004), and the Ribosomal Database Project (RDP) classifier tool (Wang et al. 2007), respectively. All the nucleotide sequences were deposited in the GenBank database under accession numbers KC306914 to KC306931.

3. Results and discussion

3.1. Feasibility of the system

An abiotic experiment was performed initially (period A in Table 1). It was contemplated that some SOB could reach the MDU carried along by the biogas. Therefore, the objective of that stage was to evaluate H_2S removal by bacteria reaching the system in this way. In order to provide sufficient moisture conditions, 1L of water was introduced into the MDU. It must be noted that a significant H_2S removal by adsorption and/or absorption during the stage A was ruled out by passing biogas through the system during the preceding 3 days.

RE ranged between 23 and 31% during the first 2 days of the period A (Fig. 2b). However, O_2 consumption was negligible (or even zero), which pointed to metal sulphides formation on the grid surface (Fig. 2a). Furthermore, RE was seen to decline thereafter, and although a higher O_2 flow was supplied to increase the O_2 availability, RE continued decreasing, suggesting saturation of the metal surface.

Subsequently, (period B1 in Table 1), the MDU was inoculated with digestate from the well-functioning bioreactor used as the biogas source, which operated under anaerobic and microaerobic conditions intermittently over the months preceding this study. The TS and VS content of the inoculum is shown in Table 2.

In B1, a significant linear correlation was observed between MLR and EC (Fig. 3a). As a result, REs higher than 94% were achieved. Moreover, the inlet and outlet O_2 concentrations in the biogas suggested H_2S oxidation (Fig. 2a and 2b), which was confirmed in the last period of the research (period B8 in Table 1). Once the micro-oxygenation had stopped, the biogas sulphide content leaving the MDU increased rapidly. ~~In fact, the outlet concentrations of H_2S during B8 were even slightly higher than the inlet ones, which indicated sulphate-reducing activity.~~ ~~Nevertheless, the microbial analysis performed after the system shutdown did not revealed the presence of sulphate-reducing bacteria in the inoculum.~~ In B8, REs of 4-8% were recorded, and O_2 consumption was almost zero. Taking into account that the H_2S concentration in the inlet biogas stream could fluctuate frequently due to its origin, 8% was not considered to be a significant removal.

The desulphurisation process in the MDU was considered to have occurred due to both chemical and biological reactions; some substances present in the inoculum could certainly have catalysed the chemical mechanisms (Kleinjan, 2005).

3.2. Effect of operational conditions

3.2.1. Biogas injection point

Once the feasibility of the MDU was confirmed, the effect of the biogas inlet point on its performance was studied. In B2 and B3, the biogas ceased to be bubbled into the sludge, and was injected at the middle and at the top of the gas space, respectively (Table 1). A substantial reduction in RE was expected in relation to B1; besides a lower transfer of O_2, it was anticipated that a substantial flow rate of untreated biogas left the MDU directly (especially in B3). However, RE remained fairly stable until the 31st day (Fig. 2b). Moreover, similar ECs were reached at almost equal MLRs (Fig. 3a). In this regard, it must be noted that as in B1, both parameters increased and decreased simultaneously. Therefore, the overall MDU performance was concluded to be independent of the biogas injection point.

3.2.2. Residence time and H_2S content of biogas

The average RE from B1 to B3 was 96%. Such successful performance was achieved under variable BRT, which specifically ranged from 59 to 97min (Fig. 2b). Furthermore, the biogas H_2S content during B1, B2 and B3 oscillated between 0.22 and 0.35%v/v (Fig. 2a). Hence, the MDU proved its robustness towards changes in both the flow rate of biogas yet to be treated and the inlet concentration of H_2S. Additionally, it was deduced that O_2 availability was not a factor limiting the process.

3.2.3. Inlet O_2/H_2S ratio

From B1 to B3, it was observed that the $O_2/H_2S_{consumed}$ ratio (v/v) increased and decreased concurrently with the $O_2/H_2S_{supplied}$ ratio (v/v) (Fig. 3b). It was at least partially attributed to variations in the $SO_4^{2-}/S^0_{produced}$ ratio. Fortuny et al. (2008) showed that even at an $O_2/H_2S_{supplied}$ ratio of 1.6, 3-4% of the H_2S removed in a laboratory-scale biotrickling filter was oxidised to SO_4^{2-}, and its production increased up to 70% when that ratio was raised to 5.3; obviously, the rest of the H_2S was converted into S^0. This agrees with the relatively high $O_2/H_2S_{consumed}$ ratios maintained in this study even at the lowest $O_2/H_2S_{supplied}$ ratios. Nonetheless, it must be considered that reactions such as those showed in Eq. (2) and (4) could also take place. It is worth noting the ruling out of the possibility that some species of SOB might become dominant according to the $O_2/H_2S_{supplied}$ ratio; SOB have been proved to be capable of switching from SO_4^{2-} to S^0 formation really fast, and vice versa (Janssen et al., 1995). As a result, the system performance was found to withstand variations in $O_2/H_2S_{supplied}$ ratio.

In order to determine the minimum $O_2/H_2S_{supplied}$ ratio needed in order to achieve high REs, the O_2 supply was gradually lowered during the period B7 (Table 1). The decline in this variable is reflected in Fig. 2a. As the $O_2/H_2S_{supplied}$ ratio decreased, the efficiency of O_2 utilisation inside the MDU increased; hence the relatively high relationship between the $O_2/H_2S_{supplied}$ ratio and the $O_2/H_2S_{consumed}$ ratio in relation to the preceding experimental periods. Specifically, the O_2 percentage leaving the system decreased by approximately 23%; in the preceding periods, only 30-40% of the O_2 supplied was consumed inside the MDU. As a result, 95% of the H_2S reaching the system in B7 was removed (Fig. 2b). The decreases in RE were attributed to insufficient O_2 availability;

specifically, at $O_2/H_2S_{supplied}$ ratios below 2.1, RE ranged from 73 to 97%.

A significant change in the relationship between the $O_2/H_2S_{supplied}$ ratio and the $O_2/H_2S_{consumed}$ ratio was observed when the $O_2/H_2S_{supplied}$ ratio decreased below approximately 4.0 (Fig. 3b). According to Janssen et al., (1995), it could be related, at least in part, to variations in the relative contribution of the mechanisms of H_2S oxidation. They pointed out that limitations in biological activity can take place at low $H_2S/O_{2supplied}$ ratios, and as a result, rates of chemical H_2S oxidation can rise.

3.2.4. Temperature

Temperature was maintained at 29ºC from the 35th to 40th day (period B4 in Table 1). RE decreased in relation to the last days of the period B3 (86 against 91%), when the system operated at 34ºC (Fig. 2b). In fact, it started to increase immediately after restoring the temperature to 34ºC (period B5 in Table 4), and continued rising during the following 2 days, when a RE of 96% was achieved. Nevertheless, values of up to 86% were reached shortly afterwards (44th day). In fact, taking into account the profile of this parameter during B5, the deterioration in the system performance during B4 could not be unequivocally linked with the reduction in temperature.

It is worth noting that a lower correlation between the $O_2/H_2S_{supplied}$ ratio and the $O_2/H_2S_{consumed}$ ratio was observed during B4 and a part of B5 (until the 51st day). Additionally, the relationship between these variables decreased significantly once the temperature was restored to 34ºC. The average O_2 percentage consumed inside the system up until the 45th day was only 17%; it re-normalised thereafter. Therefore,

temperature did seem to influence the SOB population.

In order to further assess the influence of temperature on the process performance, the MDU was subject to continuous temperature fluctuations from the 70th day onwards (period B6 in Table 1). As shown in Fig. 2b, although the average temperature was significantly lower during the first 11 days of the B6 period (25 ºC) than in the B4 period (29ºC), even reaching 20ºC (it specifically oscillated between 20 and 29ºC), the average RE was slightly higher in B6 (88 against 86%). Hence, further research about the temperature effect on the MDU performance is needed.

The average RE in B6, with temperatures ranging from 20 to 35ºC, was 91% (Fig. 3c). It must be noted that the lowest REs (≈75%) coincided with the largest temperature drops, and above 32ºC, this parameter remained over 95%. Therefore, the MDU demonstrated considerable robustness towards fluctuations. Moreover, it is worth mentioning that the correlation between the $O_2/H_2S_{consumed}$ ratio and the $O_2/H_2S_{supplied}$ ratio was fairly changeable in B6 (Fig. 3b), which corroborated the evidence that temperature did affect the process.

3.3. S^0 deposition, recovery and characterisation

Since the walls of the MDU were translucent, S^0 accumulation in the walls could be observed. Immediately after the start-up, S^0 started to accumulate in the area nearest the liquid media; it specifically covered the sludge deposited on the MDU walls during the inoculation process and due to sludge splashes resulting from the biogas bubbling (Fig. 4a). Obviously, SOB grew there due to humidity and the availability of nutrients. In

fact, when the system was opened on the 14th day, S^0 was also observed on both the sludge surface (Fig. 4b) and the additional supports (namely, in the area nearest the liquid media).

S^0 continued accumulating on the walls during B2 (Fig. 4c). Nevertheless, the deposition rate slowed thereafter; as reflected in Fig. 4f, minor differences were found between the system state on the 19th day and the 138th day. This was attributed to the depletion of both humidity and nutrients. Sludge reached those areas only occasionally, due to the slight movements performed for the visual examination of the MDU, which also caused sporadic contacts between the walls and the additional supports; hence the presence of S^0 in the areas furthest from the liquid phase (Fig. 4f). Along with the previous observations, it suggested that most of this by-product was accumulating on the gas-liquid interface, where growth conditions were more favourable. That was indeed confirmed later (Fig. 4d and 4e). Note that the degree of S^0 accumulation on the extra supports hardly changed in relation to the 14th day (Fig. 4g).

S^0 particles started to consolidate into thin sheets before the 60th day (Fig. 4e); many of them were found immersed in the sludge on the last day of the research (Fig. 4h). They appeared to form on the liquid surface and settle at a later time. Hence the morphological differences observed between its two sides; one side was smooth and the other was rough (Fig. 4i). With a magnifying glass, it could be shown that S^0 sheets were formed from multiple layers (Fig. 4j).

The largest possible amount of S^0 sheets was collected, cleaned and dried immediately after the system shutdown. Elemental analysis revealed a content of S and C of 98%w/w and 1% w/w, respectively (Table 2). Therefore, assuming that all the H_2S removed during the study was partially oxidised to S^0, the recovered sheets accounted for 60%. The amount of S^0-rich biomass collected from both the walls and the additional supports was negligible. Nonetheless, it must be highlighted that due to their brittleness, many fragments of different sizes were resealed from them into the inoculum. As a result, the inoculum had a heterogeneous content of these S^0 structures, which explained the extremely high S content of the sample analysed. It must be noted that 0.8L of inoculum was measured at the end of the research. This reduction in the sludge volume was consistent with the substantial increase in the concentration of both TS and VS. Moreover, it should be indicated that the C concentration in the inoculum decreased considerably, which suggested that the inoculum was further degraded inside the MDU. Therefore, the MDU exhibited a high capability to oxidise H_2S to easily recoverable S^0; large aggregates with good settling properties were formed.

3.4. Characterisation and diversity of the bacterial community

From the DGGE gel, 17 bands belonging to four different phyla were sequenced (Table 2): *Proteobacteria* (6 bands), *Firmicutes* (7 bands), *Actinobacteria* (1 band) and *Verrucomicrobia* (1 band), while two bands remained unclassified. In general, the results given by the RDP classifier tool were consistent with the results obtained from the Blast search tool (Table 3).

Proteobacteria was the predominant phylum of SOB inside the MDU, with the genera *Acinetobacter, Arcobacter* and *Sulfuricurvum* as representatives. A strain of *Acinetobacter* sp. (DGGE bands 4 and 5) was partially responsible for H_2S removal in the bioscrubber system developed by Potivichayanon et al. (2006) in the 28-33ºC temperature range. Members of the genus *Arcobacter* (DGGE bands 2 and 3) were found in the headspace of microaerobic digesters treating dairy cow manure (Kobayashi et al., 2012) and sewage sludge (Díaz et al., 2010) at 35ºC. Finally, a bacterium belonging to the genus affiliated to the DGGE band 4, *Sulfuricurvum kujiense*, was described by Kodama and Watanabe (2004) as a chemolithoautotrophic SOB capable of oxidising H_2S, S^0 and $S_2O_3^{2-}$ under microaerobic conditions in the 10-35ºC and 6-8 temperature and pH range, respectively.

From the *Firmicutes* phylum, the Blast search tool assigned the DGGE band 13 to the genus *Alicyclobacillus* with an identity of100%. Species belonging to this genus were reported in the study of Díaz et al. (2010).

Despite the stringent growing conditions inside the MDU, the Shannon-Wiener diversity index was 3.5, which revealed the high bacterial community diversity of the inoculum.

3.5. Competitive advantages of the MDU

Although further research is being carried out in order to optimise operational conditions and thus evaluate the maximum removal capacity of the MDU, a preliminary comparative assessment in relation to the current biotechnologies for biogas

desulphurisation can be performed based on the results of this study.

In contrast to other bioprocesses involving a chemically-based previous step (such as a scrubber), the MDU is a single-stage process, which could translate into lower space requirements and capital investment. It must be taken into account that additional equipment such as pumps, storage tanks or settlers are not needed. With regard to this, it should be emphasised that the S^0 formed during this research settled by itself inside the MDU.

Due to the extremely simple configuration of the MDU, its construction would be easier than that of other types of bioreactors. Furthermore, it is worth noting that, whereas the biomass growth could increase the treatment capacity of the MDU over time, this is a common problem in packed media-based bioreactors (clogging) (Kennes et al., 2009). Therefore, the MDU would largely reduce the frequency of supervision tasks and shutdowns.

The MDU proved to be at least in the medium-term self-sustaining and self-regulating. Conversely, other bioprocess require a strict control of the operational conditions, such as pH, moisture content or nutrient balance, which in turn entails the need for specific instrumentation and frequent monitoring tasks. Hence, besides the chemicals required in the previous chemical process, they require nutrients solutions. Therefore, liquid effluents are constantly generated. Overall, the MDU could largely reduce the capital cost, as well as the outlays for operation and maintenance.

4. Conclusions

A novel ~~cost-effective~~ alternative to the conventional biotechniques for biogas desulphurisation was developed. 1L of digested sludge was proved to be an efficient reaction media for H_2S removal in a 10L-system during 128d. Although the MDU was subjected to several fluctuations in BRT, concentration of H_2S, $O_2/H_2S_{supplied}$ ratio, and temperature, and neither nutrients nor water were added, the average RE was 94%. The system presented a really high bacterial diversity, including at least three genera of SOB. S^0 was the major by-product; it accumulated in the form of extremely pure multilayered sheets, which settled to the bottom of the system.

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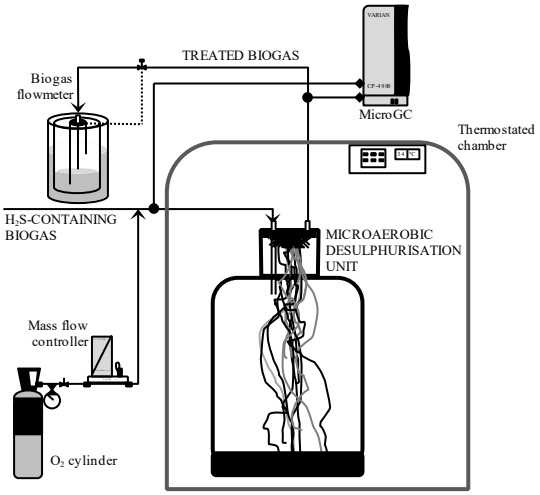

Fig. 1. MDU diagram.

Table 1. Sequence of variations applied and desulphurisation performance.

Period	Reaction media	Biogas injection point	Temperature (ºC)	BRT (min)	Inlet H_2S (%v/v)	Outlet H_2S (%v/v)
A	Water	Liquid phase	34	68	0.30	0.26
B1	Digestate	Liquid phase	34	68	0.33	0.01
B2	Digestate	Gas space (middle)	34	61	0.29	0.01
B3	Digestate	Gas space (top)	34	74	0.28	0.01
B4	Digestate	Gas space (top)	29	88	0.37	0.05
B5	Digestate	Gas space (top)	34	72	0.32	0.02
B6	Digestate	Gas space (top)	20-35	80	0.29	0.02
B7	Digestate	Gas space (top)	34	84	0.30	0.02
B8	Digestate	Gas space (top)	34	100	0.28	0.27

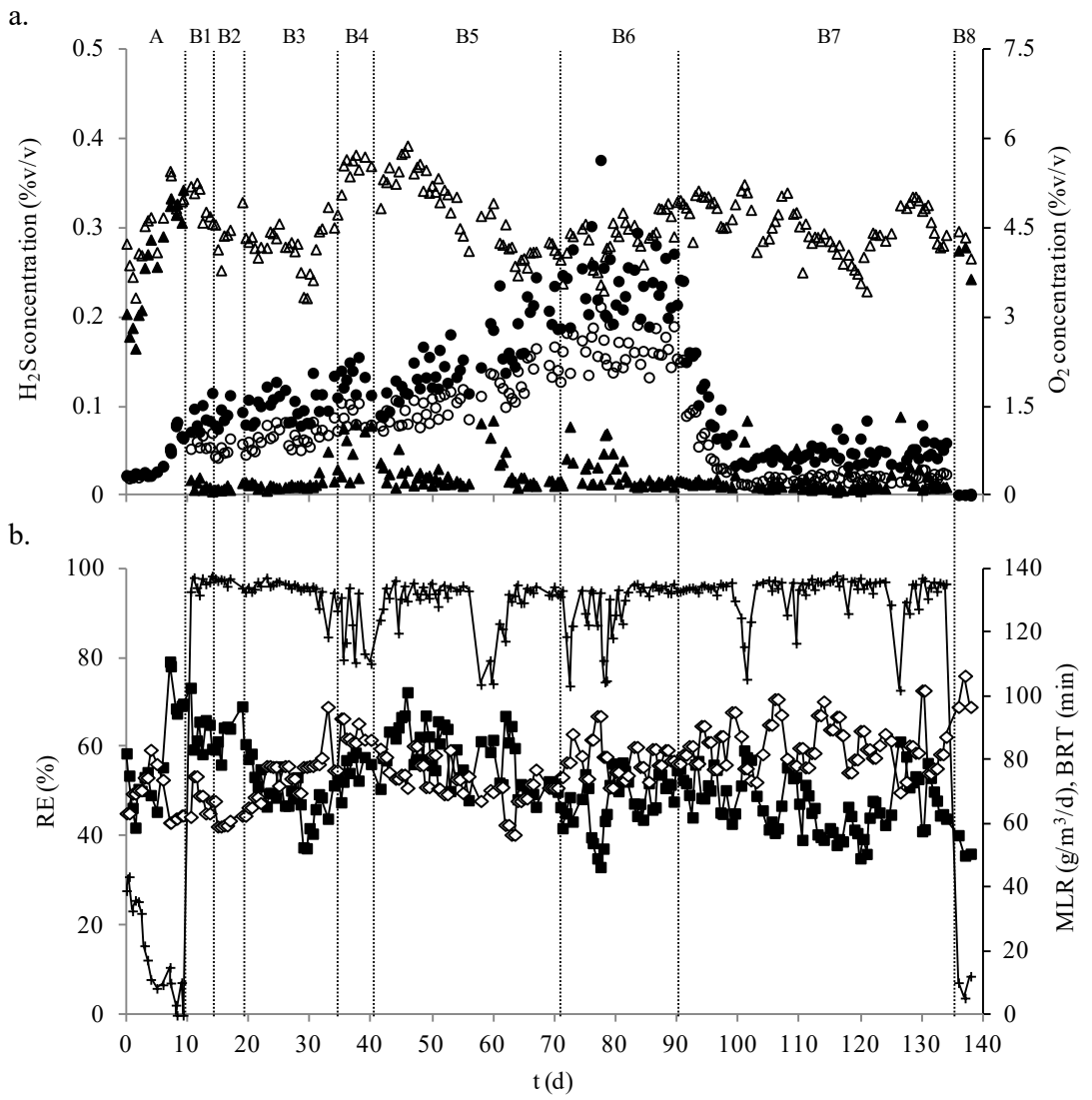

Fig. 2. (a) Input H_2S (Δ) and O_2 (●) concentrations, and output H_2S (▲) and O_2 (o) concentrations, and (b) RE (+), MLR (■) and BRT (◊) during the research.

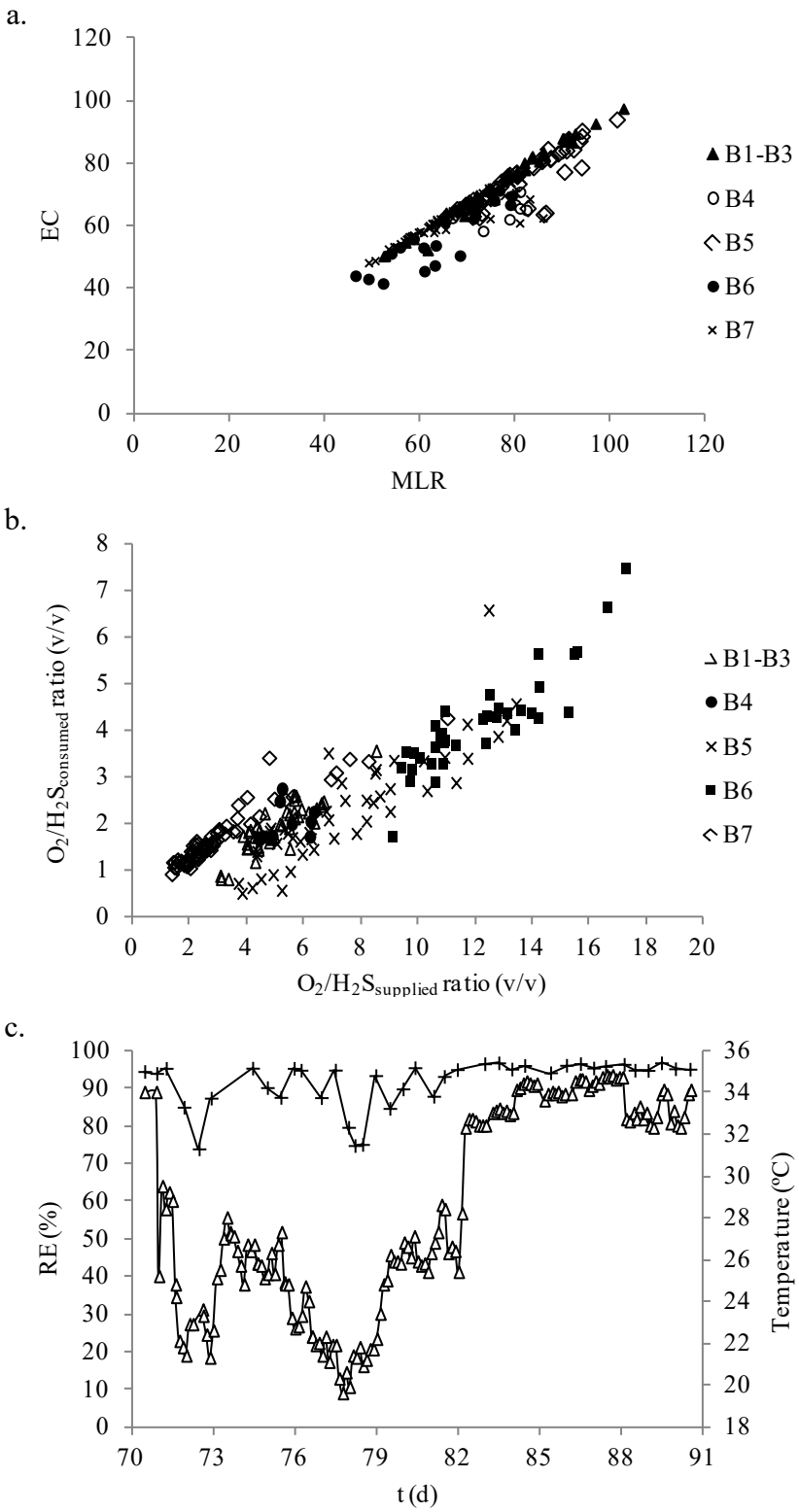

Fig. 3. (a) MLR against EC, and (b) M against R in the different experimental periods. (c) RE (+) and temperature (Δ) in B6.

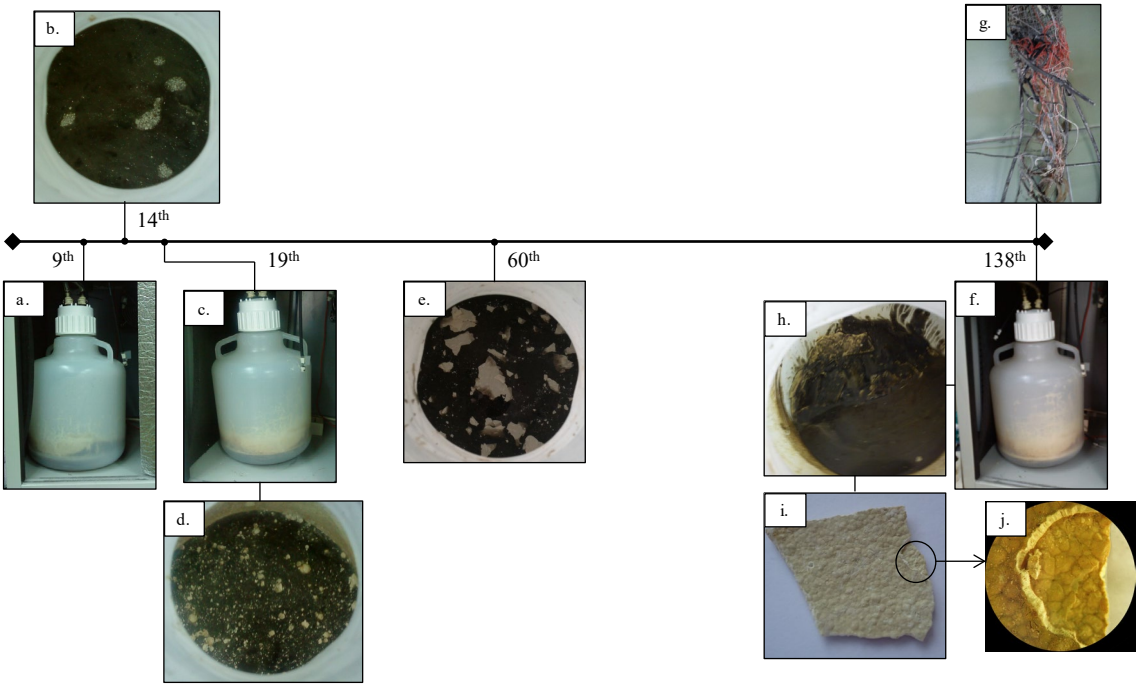

Fig. 4. MDU state with time.

Table 2. Elemental composition of the inoculum and S^0-rich biomass deposited in the MDU.

	Inoculum		**Walls**	**Sheets**
	After	Before		
C (%w/w)	30	13	6	1
S (%w/w)	1	50	90	98
TS (g/L)	24	41	-	-
VS (g/L)	15	27	-	-

Table 3. RDP classification of the DGGE bands sequenced with a 50% of confidence level, and corresponding matches according to the Blast search tool, with their similarity percentages, and environments from which they were retrieved.

Taxonomic placement	Band nº	Closest relatives (accession nº)	Similarity (%)	Source of origin
Phylum *Proteobacteria*	1	Uncultured bacterium (AB286499)	95	Activated sludge
Class *Epsilonproteobacteria*				
Order *Campylobacteraceae*				
Family *Campylobacteraceae*				
Genus *Arcobacter*	2	Uncultured *Arcobacter* sp. (HQ392829)	99	Headspace of a digester of sewage sludge under microaerobic conditions
	3	Uncultured *Arcobacter* sp. (HQ392829)	100	Headspace of a digester of sewage sludge under microaerobic conditions
Family *Helicobacteraceae*				
Genus *Sulfuricurvum*	4	Uncultured *Epsilonproteobacterium* (DQ295695)	99	Floating microbial mat in sulfidic groundwater (Movile Cave)
Class *Gammaproteobacteria*				
Order *Pseudomonadales*				
Family *Moracellaceae*				
Genus *Acinetobacter*	5	Uncultured *Acinetobacter* sp. (EU567041)	95	Oil field soil
	6	Uncultured *Acinetobacter* sp. (JN679106)	99	Membrane bioreactor
Phylum *Firmicutes*				
Class *Clostridia*				
Order *Clostridiales*	7	Uncultured bacterium (FJ978625)	96	Feces
Family *Peptostreptococcaceae*				
Genus *Clostridium XI*	8	Uncultured bacterium (JQ085717)	99	Anaerobic digester
Family *Syntrophomonadaceae*				
Genus *Thermohydrogenium*	9	Uncultured bacterium (HE681331)	96	Bioreactor
	10	Uncultured bacterium (GQ259594)	96	Bioreactor
Family *Clostridiales incertae sedis*				
Genus *Soehngenia*	11	Uncultured bacterium (AB114320)	90	Thermophilic anaerobic municipal solid waste digester
Family *Lachnospiraceae*	12	*Clostridium* sp. (GU247219)	93	Waste water of a pesticides firm
Class *Bacilli*				
Order *Bacillales*	13	Uncultured *Alicyclobacillus* sp. (HQ392831)	100	Headspace of a digester of sewage sludge under microaerobic conditions
Phylum *Actinobacteria*				
Class *Actinobacteria*				
Subclass *Actinobacteridae*				
Order *Actinomycetales*				
Suborder *Corynebacterineae*				
Family *Dietziaceae*				
Genus *Dietzia*	14	Uncultured *Dietzia* sp. (JN882177)	95	Crude oil samples
Phylum *Verrucomicrobia*				
Class *Optitutae*				
Order Optitutales				
Family Optitaceae				
Genus Alterococcus	15	Uncultured *Verrucomicrobia* (CU918353)	99	Mesophilic anaerobic digester which treats municipal wastewater sludge
Unclassified Bacteria	16	Uncultured bacterium (AB175392)	99	Mesophilic anaerobic BSA digester
	17	Uncultured *Firmicutes* (CU923016)	97	Mesophilic anaerobic digester which treats municipal wastewater sludge