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Technical, Economical, and Microbiological Aspects of the Microaerobic Process on H_2S Removal for Low Sulfate Concentration Wastewaters

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Abstract We studied the feasibility of the microaerobic process, in comparison with the traditional chemical absorption process (NaOH), on H_2S removal in order to improve the biogas quality. The experiment consisted of two systems: R1, biogas from an anaerobic reactor was washed in a NaOH solution, and R2, headspace microaeration with atmospheric air in a former anaerobic reactor. The microaeration used for low sulfate concentration wastewater did not affect the anaerobic digestion, but even increased system stability. Methane production in the R2 was 14 % lower compared to R1, due to biogas dilution by the atmospheric air used. The presence of oxygen in the biogas reveals that not all the oxygen was consumed for sulfide oxidation in the liquid phase indicating mass transfer limitations. The reactor was able to rapidly recover its capacity on H_2S removal after an operational failure. Bacterial and archaeal richness shifted due to changes in operational parameters, which match with the system functioning. Finally, the microaerobic system seems to be more advantageous for both technical and economical reasons, in which the payback of microaerobic process for H_2S removal was 4.7 months.

Keywords Anaerobic · Biogas desulfurization · DGGE · Hydrogen sulfide · Microaerobic process

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Introduction

Anaerobic digestion is presented as an attractive technology in wastewater treatment, which involves the biological degradation and stabilization of organic matter and transformation into biogas. The biogas quality is very important when its potential for energy use is considered.

The biogas contains considerable amounts of pollutants, which are produced during anaerobic digestion. Among these pollutants, hydrogen sulfide deserves major attention because it is highly toxic and can cause problems in the nervous system, eyes, and respiratory tract [1]. In addition to public health problems, hydrogen sulfide can cause an inhibitory effect in several microorganisms, including methanogens, which will result in a decrease in the methane production and, consequently, decrease the energy potential production. Moreover, hydrogen sulfide is very corrosive, which reduces the lifetime of pipework and other installations needed for biogas utilization [2–5]. Therefore, the sulfide content in the biogas is important to be controlled to prevent damage and fulfill the quality standards for biogas use [6].

In order to prevent a high concentration of hydrogen sulfide in anaerobic reactors, some investigations have been performed introducing a limited amount of oxygen/air to anaerobic bioreactors as a strategy to lower the levels of sulfide. This technique involves the oxidation of sulfide by sulfur-oxidizing bacteria (SOB) under limiting oxygen conditions [7]. Several studies have shown that microaeration of wastewater or solid wastes containing high sulfate concentration is a promising technology, which promotes the oxidation of the generated sulfide in elemental sulfur [8–12].

It was proved in a mixed culture that even strict anaerobes are not inhibited by oxygen, assuming that facultative microorganisms are able to consume this oxygen quickly and completely [8]. This technology brings many benefits, mainly related to the excellent efficiency of treatment, no necessity of a specific unity and a subsequent reduction in energy consumption [13]. Currently, microaerobic systems have been used for efficient removal of hydrogen sulfide from biogas, mainly in anaerobic digesters treating both domestic and agroindustrial wastes [2, 4, 9]. Therefore, low concentrations of dissolved oxygen play an important role on sulfide oxidation to elemental sulfur according to the following equation (Eq. 1) [14]:

$$2HS^- + O_2 \rightarrow 2S^0 + 2OH^- \quad (1)$$

However, literature lacks an investigation of microaerophilic treatment for low sulfate concentration wastewaters (e.g., sewage), especially analyzing all technical, economical, and microbiological aspects. Therefore, this study evaluated the feasibility of the microaerobic process, in comparison with the traditional chemical absorption process (NaOH), for H_2S removal in order to improve the biogas quality and minimize odor generation in anaerobic reactors. Moreover, the impact of microaeration over the anaerobic system stability was evaluated. The microorganisms change in both systems was analyzed by using molecular biology tools such as DGGE. Finally, a technical and an economical analysis were performed after scale-up of the systems.

Material and Methods

Sludge Source

The reactors were inoculated with sludge provided by the Brazilian Agricultural Research Corporation (EMBRAPA) from an anaerobic reactor used in the treatment of banana

pseudostem. The sludge had a concentration of 30 and 13 g TVS L^{-1}. The sludge choice was just based in the availability and good methanogenic activity.

Continuous Flow Reactors

The experiment was conducted in two lab-scale continuous-flow anaerobic reactors (R1 and R2) (Fig. 1). The total volume of each reactor was 3.5 L (working volume of 2.8 L).

The reactors were made of acrylic (polymethyl methacrylate) and operated at a hydraulic retention time (HRT) of approximately 11 h. The organic loading rate (OLR) of about 2.0 kg COD m^{-3} day^{-1} was applied.

The experiment consisted of two different systems: (1) biogas from an anaerobic reactor was washed in a NaOH solution to remove H_2S (R1) and (2) headspace microaeration with atmospheric air in a former anaerobic reactor (R2) to convert the H_2S present in the liquid phase into elemental sulfur (Table 1).

The reactors feeding was performed by using a peristaltic pump (Watson Marlon, Model 323U / D), with an average flow rate of 6 L day^{-1}. The reactors were subjected to recirculation with metering pumps (ProMinent, model Concept Plus CNPA 1000 NPB2 00A01) at a flow rate of approximately 1 L h^{-1}, which provided an upflow velocity of 0.5 m h^{-1}. Reactors were equipped with electrodes for online redox potential (ORP) measurements.

Biogas production was calculated by counting the number of pulses generated in a water displacement device (25 mL per pulse).

Operational Conditions

The feeding consisted of synthetic wastewater with ethanol as the electron donor (COD = 1 g L^{-1}) (99.8 % purity, Dynamics, Brazil). The composition of basal medium was according to Firmino et al. [15]. To increase the sulfide concentration in the biogas, sodium sulfate (400 mg L^{-1}) was added to the basal medium. The influent was placed under refrigeration, with a temperature of approximately 4 °C. To keep pH around 7.0, the

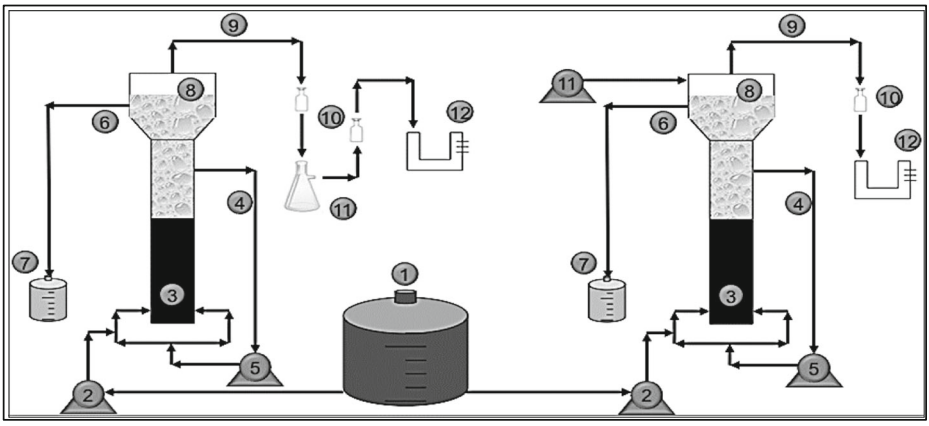

Fig. 1 Schematic of the experimental setup. R1: anaerobic system with caustic washing. R2: microaerobic system. *1* Affluent tank; *2*, peristaltic pump; *3*, reactor; *4*, recirculation line; *5*, recirculation pump; *6*, effluent line; *7*, effluent tank; *8*, headspace of the reactor; *9*, biogas line; *10*, biogas sampler; *11* (R1), caustic washing system; *11* (R2), microaeration system; *12*, biogas meter

Table 1 Operational condition during the experimental periods

Phases	R1		R2	
	Anaerobic	Anaerobic with caustic washing	Anaerobic	Microaerobic
Operation time (days)	74	41	33	87
HRT (h)	10.4	10.8	10.6	11.0
Inflow (L day^{-1})	6.56	6.54	5.98	6.17
Total COD (mg L^{-1})	910	940	930	960
Total sulfate (mg L^{-1})	137	141	136	143
DQO/sulfate	6.6	6.7	6.8	6.7
Airflow (mL min^{-1})	–	–	–	0.2

wastewater was buffered with sodium bicarbonate ($NaHCO_3$) in the proportion of 1 g $NaHCO_3$ to each 1 g COD ethanol. Operational conditions during the experimental periods are shown in Table 1.

The R2 system was preliminarily operated under anaerobic conditions until its stabilization, when air supply started in the operation day 33 onward. The biogas produced in R1 system was treated by caustic washing with 10 M NaOH, in which the efficiencies obtained were compared to those obtained in system R2 with microaerobic treatment. The microaeration strategy applied was the addition of atmospheric air directly into the reactor headspace by using a peristaltic pump (Minipuls 3, Gilson, USA) in a flow rate of 0.2 mL min^{-1}. Such a flow represents 5.2 dm^3 of O_2 per dm^3 of reactor.

Tygon™ tubing was used as the conduction material for the biogas as the usual silicon tubes are oxygen permeable.

Monitoring and Experimental Analysis

COD, sulfate, and dissolved sulfide were determined according to the Standard Methods for the Examination of Water and Wastewater [16]. Biogas characterization (Table 2) was performed by gas chromatography with thermal conductivity detector (GC-17A, Shimadzu Corporation, Japan) by assessing the content of air (O_2 + N_2), CO_2, and CH_4, according to Firmino et al. [17].

H_2S, NH_3, and O_2 concentrations were determined by using a portable gas meter (Dräger X-am® 5600, Drägerwerk AG & Co. KGaA, Germany).

Microbial Community Analysis

To evaluate the structure (diversity, evenness, and richness) of the microbial communities in the reactor R2, biomass samples were collected, consisting of seed; at the end of the anaerobic stage and at the end of the microaerobic stage. The samples were immediately frozen at −20 °C until DNA extraction was performed. Genomic DNA was extracted using the Fast DNA Spin Kit for Soil (MP Biomedicals, USA) according to the manufacturer protocol. DNA concentration was measured by using the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

Table 2 Chromatographic conditions of GC/TDC used in the experiments

CG-TCD	
Injection mode	Splitless
Sample loop (mL)	1
Injector (°C)	40
Carrier gas	He
Flow (mL min^{-1})	0,7
Oven (°C)	50
TCD (°C)	200
Run time (min)	5

Source: Firmino et al. [17]

TCD thermal conductivity detector

The 16S rRNA gene hypervariable regions V6–V8 of bacteria and V2–V3 of archaea were amplified by polymerase chain reaction (PCR) using the universal bacterial primers 1401-R and 968-F, and the archaeal primers A 109(T)-F and 515-R (IDT, USA). Primers 968-F and 515-R included a 40 pb GC-clamp at 5′ end (5′-CGCCCGGGGCGCGCCCCGGGCGGG GCGGGGGCACGGGGGG-3′).

The PCR mixture (50 μL) contained 10 μL of reaction buffer (5×), 5 μL of $MgCl_2$ (25 mM), 0.25 μL of Taq polymerase (5 μL) (Promega, USA), 1 μL of deoxynucleotide triphosphates (10 mM), 1 μL of the extracted DNA, 1 μL of PCR primers (10 μM), and nuclease-free water (Promega, USA) up to a final volume of 50 μL. PCR was conducted in a T100 Thermal Cycler instrument (Bio Rad Laboratories, USA). The PCR thermal cycling program for bacterial amplification consisted of 2 min of predenaturation at 95 °C, 32 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 45 s, and elongation at 72 °C for 1 min, with a final 30 min elongation at 72 °C. Archaeal amplification consisted of 2 min of predenaturation at 95 °C, 35 cycles of denaturation at 95 °C for 40 s, annealing at 53 °C for 40 s, and elongation at 72 °C for 1 min, with a final 30-min elongation at 72 °C. PCR products were verified in 1.7 % agarose (*w*/*v*) gel electrophoresis, using the 1-kb DNA Ladder (Promega, USA) as a molecular weight marker. The gels were stained with SYBR Green I nucleic acid gel stain (Sigma-Aldrich, USA) for 40 min, and the result was analyzed in a Universal Hood II Transilluminator (Bio Rad Laboratories, USA).

The double gradient DGGE analysis were performed in a D-Code Universal Mutation Detection System (Bio Rad Laboratories, USA) using polyacrylamide gels with a urea/formamide denaturing gradient of 42–67 % for bacterial community analysis and 30–60 % for archaeal community analysis, superimposed with a porous gradient of acrylamide/bisacrylamide (6–10 %). Electrophoresis was performed in 0.5× TAE buffer at 60 °C and 85 V for 16 h for bacterial amplicons and 60 °C and 65 V for 18 h for archaeal amplicons. The gels were stained with SYBR Green I nucleic acid stain (Sigma-Aldrich, USA) for 40 min.

The gel images were processed by using the Bionumeric software (Applied Maths BVBA, Sint-Martens-Latem, Belgium) to score the band pattern. Similarity values of the compared profiles were calculated from densitometric curves by using the Pearson product-moment

correlation coefficient [18] and were subsequently used to construct dendograms by UPGMA clustering. The ecological parameters of microbial communities, i.e., the range-weighted richness (Rr), the evenness/functional organization (F0) [19], and Shannon-Wiener diversity index (H) were calculated as described in Lebrero et al. [20].

Preliminary Economic Evaluation of Microaeration Process in Relation to the Chemical Absorption Process

Taking into account the data generated in the operation of both systems, we scaled up for a population of about 7500 inhabitants, which result in an influent flow rate of 945 m^3 day^{-1}.

Both systems R1 and R2 were investigated taking into account the payback after 10 years of operation.

Regarding operating costs, prices of sodium hydroxide on the Internet (http://www.alibaba.com/showroom/sodium-hydroxide-price.html) were surveyed and had adopted a value of $100.00 a ton, while the price of electricity was considered of US$0.18 per kWh.

Statistical Analysis

The StatGraphics Centurion XVI computer program was used for the statistical analysis of the data, being applied the Mann–Whitney rank sum test, a nonparametric procedure, which does not require a specific data distribution to compare the performance of the reactors during the experiments. The results of the tests were evaluated according to the p value. If $p \leq 0.050$, the null hypothesis is rejected, i.e., the data groups are considered statistically different.

Results and Discussion

Effect of Microaeration on Anaerobic Digestion

Throughout the experiment, the reactors were fed with an average COD of approximately 925 mg L^{-1} (R1) and 945 mg L^{-1} (R2). For R1, an average COD removal of 90 ± 7 % was obtained analyzing all stages investigated, which produced an effluent COD of 102 mg L^{-1}. The R2 system showed similar performance, with COD removal efficiency of 89 ± 6 %. Although no statistically significant difference between R1 and R2 was found in terms of COD ($p = 0.81$), the coefficient of variation for R1 system (13.9 %) was slightly higher compared to the system R2 (11.1 %), showing higher stability in R2. Such a result showed that the application of oxygen did not cause damage to the reactor performance in terms of organic matter removal, as also found by van der Zee et al. [2].

Shen and Guiot [21] reported that methanogenic community in granular sludge presented a higher tolerance to oxygen than those present in flocculent sludge due to the structure of the granular bead. Facultative anaerobic bacteria are prevalent on the outer layers of the granule whereas methanogenic archaea, which are oxygen-sensitive, stay protected in the core.

Jenicek et al. [22] described that the microaerobic system, unlike the anaerobic system, probably promotes the degradation of compounds that would be recalcitrant to degradation under strictly anaerobic conditions. The improved biodegradability is probably caused by the complementing of reducing and oxidizing processes but also by the augmentation of microbial species diversity of microaerobic population in comparison with a strictly anaerobic one [23].

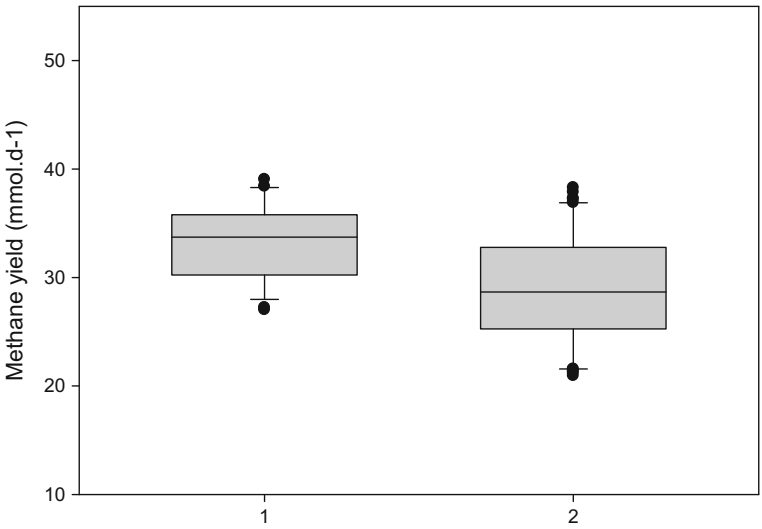

Fig. 2 Boxplots of the methane yield (mmol day^{-1}) of reactors: R1 (anaerobic with caustic washing) and R2 (microaerobic)

The daily production of methane (28 °C, 1 atm) in the systems R1 and R2 were 33.4 ± 3.29 and 28.6 ± 5.31 mmol day^{-1}, respectively (Fig. 2). As shown in Fig. 3, 13.8 % of the oxygen supplied to the system has been used for other unclear organic processes, which could be related to oxidation of organic matter. It is possible that these processes have interfered with the methane production, which was approximately 14 % lower in system R2 when compared to R1.

Therefore, as expected, that the microaerobic treatment promoted a dilution of the methane content, which was about 14 % lower in system R2. This dilution will be dependent on the amount of oxygen used, the sulfate removal in the bioreactor, the residence time of the oxygen in the liquid flow, among other factors. Nitrogen dilution is a disadvantage when using air instead of pure oxygen for microaeration. However, using

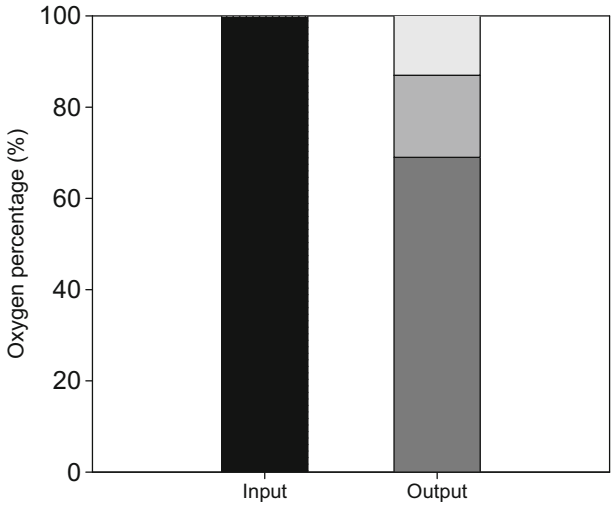

Fig. 3 Oxygen balance, consumption during the microaerobic phase (R2): O_2-added (■), O_2-S (■), O_2-others (□), and (□) O_2-biogas

pure oxygen might be dangerous since this gas and methane form an explosive gaseous mixture [24].

Diaz et al. [10] assessed the removal of hydrogen sulfide in a sludge digester with a working volume of 200 L, HRT of 20 days, and variable organic loading (COD_T max–min [80–37] g L^{-1}). These authors observed a reduction in methane concentration, which was 6 % lower in microaerobic conditions when compared to the anaerobic condition. Ramos et al. [6] state that the reduction in CH_4 concentration observed in microaerobic conditions occurs due to dilution of the biogas by air rather than aerobic oxidation of methanogenic substrate.

Sulfide Removal in Anaerobic and Microaerobic Systems

The system R1 showed 100 % efficiency in removing gaseous sulfide, as expected in a controlled chemical absorption process.

Figure 4 shows for the system R2 the concentration of hydrogen sulfide and oxygen in the biogas during the anaerobic (stage I) and microaerobic (stage II) conditions. In stage I, sulfide production in the biogas was 0.15 mmol day^{-1} (0,28 % *v*/*v*). After the introduction of atmospheric air (0.2 mL min^{-1}), the sulfide production decreased to 0.012 mmol day^{-1} (0,02 % *v*/*v*), corresponding to 93 % sulfide removal. Diaz et al. [10] found in pilot-scale experiments with 1.1 % of H_2S in biogas that the microaerobic treatment provided sulfide removal of 97 %, therefore very close to our results for low sulfate concentration experiments.

It can also be observed in Fig. 4 that the sulfide levels increased sharply in stage II, which was caused when air supply was interrupted by a power failure. An operational failure occurred on the 101st day, and the microaeration supply was stopped for 6 days.

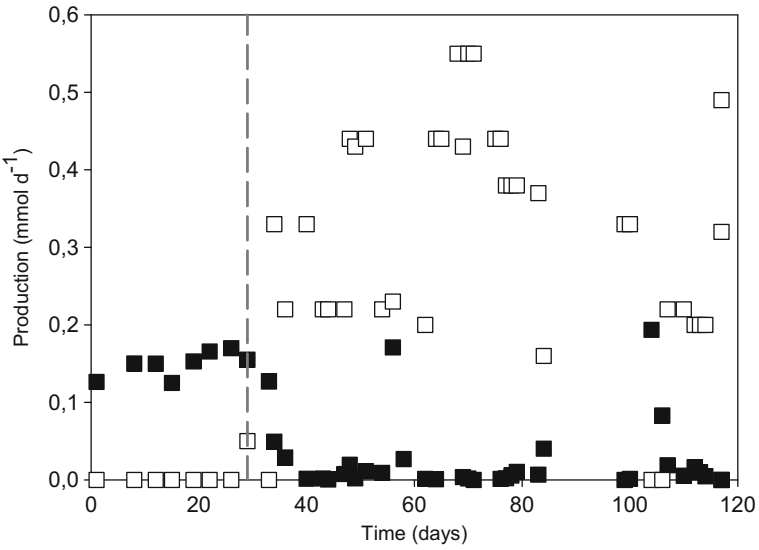

Fig. 4 R2, hydrogen sulfide (*filled squares*) and oxygen (*empty squares*) concentrations, under anaerobic and microaerobic conditions

The oxygen supply was fully restored at the 106th day and by the 107th day, the sulfide concentration reached 0.02 mmol day^{-1}.

According to van der Zee et al. [2], if microaeration stops and the oxygen is depleted, the sulfide concentration, produced from the reduction of its oxidized species, is higher than that obtained previously under anaerobic conditions. This condition was observed in the R2 system, which in anaerobic conditions reached a maximum of 0.17 mmol day^{-1} of sulfide whereas it reached 0.20 mmol day^{-1} during the days without microaeration.

Sulfur Mass Balance and Oxygen Utilization

Sulfur mass balance (Fig. 5) and oxygen utilization analyses were performed. The only sulfur source considered in the influent was sulfate, in which the sulfur sources for the effluent were sulfate, sulfide, and elemental sulfur. The other possible fractions of sulfur either precipitated or adsorbed in the sludge biomass reactor were not considered.

System R1 presented a total input of 292 mg day^{-1} and output of 228 mg day^{-1} (Fig. 5). It was also observed that the H_2S fraction in the biogas was completely absorbed by the washing system. The amount of sulfur that left the system corresponded to 31 and 197 mg day^{-1} of sulfate and dissolved sulfide, respectively.

System R2 presented a total input of 275 mg day^{-1} and output of 166.25 mg day^{-1} (Fig. 5), in which the sulfur in the latter fraction corresponds to the forms of sulfate, dissolved sulfide, and H_2S in the gas phase. The amount of sulfur that left the system corresponded to 0.25 mg day^{-1} H_2S only. The gap of 108.75 mg L^{-1} (39.5 %) can be related to a higher assimilation by this microaerobic biomass, as well as the formation of thiosulfate (not analyzed) and elemental sulfur (not analyzed) during microaerophilic treatment. Therefore, the introduction of oxygen substantially altered the output of sulfur in the process.

Diaz et al. [5] stated that elemental sulfur was the main product of the oxidation of sulfide (31 %) when the microaeration was applied directly to the headspace, being considered the best dosing point. Under the same conditions, these authors observed 98 % removal of sulfide from the biogas.

The level of H_2S in the biogas substantially decreased (>0.001 %), indicating that moderate oxygenation can indeed be applied successfully as a strategy for the removal of sulfide from

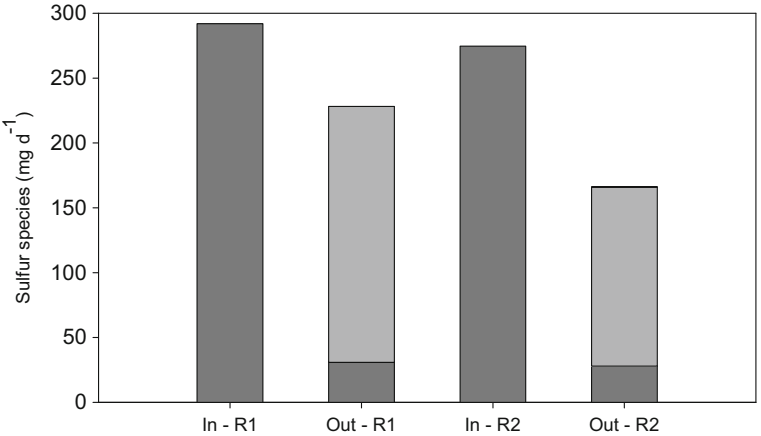

Fig. 5 Sulfur balance. Sulfur species: S-sulfate (▒), S-dissolved sulfide (▒), and (▒) S-H_2S gas

biogas in low sulfate concentration wastewater. Furthermore, it is clear that at least a fraction left the reactor as elemental sulfur since it was possible to identify this element in the headspace.

Comparing the sulfate input and output in both systems (Fig. 5), no statistically significant difference in terms of sulfate removal was found ($p > 0.20$), with an average efficiency of approximately 90 %. Therefore, SRB was not affected by the oxygen load in the system R2.

However, analyzing the sulfate removal in system R2 during stages I and II, there was a statistically significant difference ($p < 0.01$), in which the removals were decreased in the microaerobic stage. Likely, there was the formation of sulfate from the complete oxidation of sulfide when oxygen was available, as reported in Gutierrez et al. [25].

R1 and R2 systems showed no statistically significant difference in the concentration of the dissolved sulfide ($p = 0.71$), which is in agreement with Diaz et al. [10] who also investigated the use of anaerobic and microaerobic treatments for high sulfate concentration wastewaters.

As previously reported, 100 % sulfide removal was achieved in the caustic washing absorbed system who was used for biogas treatment. The microaerobic system produced biogas with low sulfide concentration (0.01 mmol day^{-1}). However, the formation of elemental sulfur in the reactor headspace was verified, as previously reported [2, 6, 10].

The oxygen injected into the microaerobic system was not fully consumed in the oxidation of sulfide, resulting in a 0.75 % (*v*/*v*) content in the biogas, indicating mass transfer limitations. Such a finding is in agreement with the results of 0.77 % (*v*/*v*) found by Diaz et al. [10]. Jenicek et al. [22], using air in the headspace of a sludge digester, found sulfide removals up to 99 and 1.0 % (*v*/*v*) oxygen in the biogas.

Molecular Biology

In order to evaluate the effect of the microaeration in the bacterial and archaeal communities of the R2 system, ecological parameters (richness, evenness, and Shannon diversity) were calculated by using the information from the DGGE patterns obtained. Dendrograms were designed by UPGMA clustering.

According to the classification of Marzorati et al. [19], the functional organization (Fo) of the bacterial community maintained medium values throughout the operation, although increased in anaerobic condition (45 %), decreasing after the introduction of oxygen (30 %) (Table 3). These medium range values indicate that the most fitting species were dominant, thus retaining a certain level of organization. According to Marzorati et al. [19], the elevated concentration of some species and the availability of many others suggest that the community can potentially deal with changing environmental conditions preserving its functionality.

Range-weighted richness (Rr) parameter showed high values for the seed (50) and the anaerobic (84) samples, but a medium value for the sample collected under microaerobic conditions (25) (Table 3). According to Mc Donald [26], the Shannon diversity index (H) is used to calculate the diversity of species based on the evenness and richness, ranging from 1.5 (low species evenness and richness) to 3.5 (high species evenness and richness). The diversity analysis showed that the lowest value was observed in the microaerobic sample (2.46), while the inoculum (2.77) and the anaerobic sample (2.98) presented higher values (Table 3). Since the evenness presented a similar value under anaerobic

Table 3 Shannon diversity index (H), range-weighted richness (Rr), and functional organization (Fo) calculated from the DGGE patterns

Parameters	Innoculum	Anaerobic	Microaerobic
Bacteria			
Functional organization (Fo) (%)	31	45	30
Range-weighted richness (Rr)	50	84	25
Shannon diversity (H)	2.77	2.98	2.46
Archaea			
Functional organization (Fo) (%)	44	45	52
Range-weighted richness (Rr)	193	122	210
Shannon diversity (H)	3.18	2.62	2.74

Rr: low (< 10), medium (10–30), high (>30) [19]. Fo: low Fo/high evenness (25 %), intermediate Fo and evenness (30–70 %), high Fo/low evenness (70 %) [19]. H: 1.5 to 3.5 (low and high species evenness and richness, respectively) [26]

and microaerobic conditions, it can be assumed that microoxygenation mostly affected bacterial richness rather than community evenness, suggesting that some bacteria could be negatively affected by oxygen introduction. These shifts in community structure could be explained by competition between microbial populations, since those which are most fitted to the imposed substrate prevail, with dominance of the most adapted organisms Viana et al. [27].

According to Wittebolle et al. [28], the initial archaeal community (seed) showed conditions that favors an increased stability of the system, like high richness (193) and medium evenness (44). After adjustment under anaerobic conditions, it was observed that the archaeal community showed an increase in Fo value in the microaerobic stage (45 to 52 %). For the range-weighted richness (Rr) in anaerobic conditions, the difference is higher, from 193 (seed) to 122 (anaerobic) (Table 3). However, the archaeal community maintained a high richness in all stages, according to Marzorati et al. [19].

The decrease in Rr from the seed when compared to anaerobic condition could be related to the simplification of the feed, since the seed community was adapted to a more complex substrate (banana pseudostem) than ethanol. Thus, archaea in the seed was adapted to a major variety of compounds such as H_2, acetate, and other methyl compounds. After adjustment under anaerobic conditions, ethanol was the unique carbon and energy source. Therefore, only H_2 and acetate from syntrophic conversion of ethanol were available for archaea. Considering that usually sulfate-reducing bacteria (SRB) outcompetes archaea for H_2, archaea only had acetate as substrate for growth.

The higher Rr value of archaea observed in microaerobic conditions when compared to anaerobic conditions could be related to two main factors: (1) the structure of the granular sludge, which protects the oxygen-sensitive archaeal community in the core, whereas facultative anaerobic are prevalent on the outer layers, as reported by Shen and Guiot [21]; (2) the accessibility of methanogens to other substrates different than acetate (H_2 or even ethanol). This could be induced by changes in the anaerobic degradation process due to oxygen presence, once SRB could disproportionate or use sulfur intermediates (S^0, S_2O_3) generated from sulfide oxidation (sulfur intermediates: S^0, S_2O_3).

The comparison of the DGGE profiles of the archaeal community resulted in Pearson similarity coefficient (p value) of 59 % between seed and anaerobic (stage 1) and 48 %

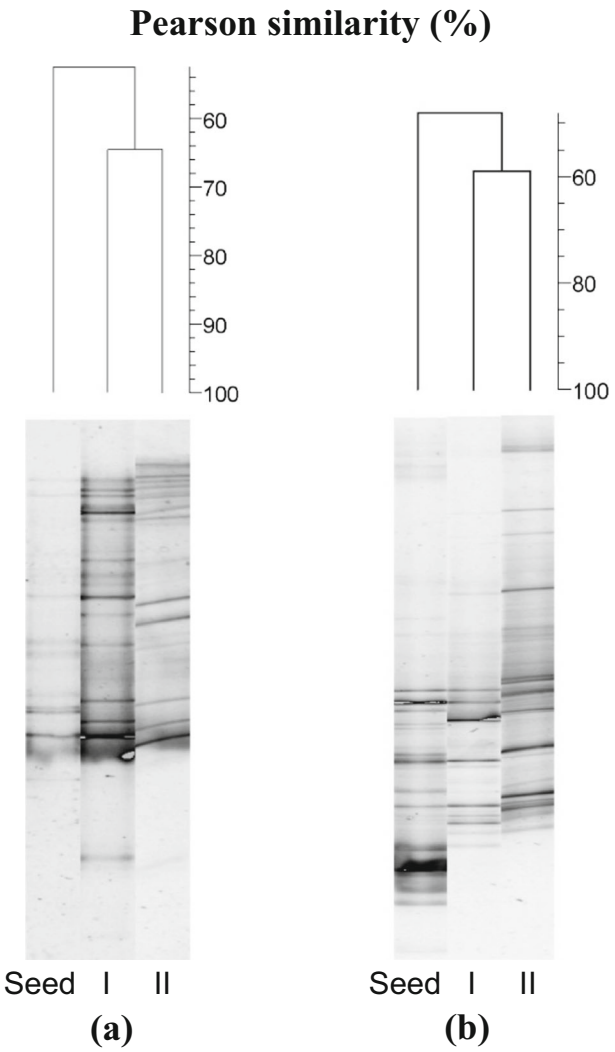

Fig. 6 Dendograms based on the 16S rRNA DGGE profiles of the bacterial (a) and archaeal (b) communities. The labels indicate the experimental period at which each sample was collected: anaerobic phase (I); microaerobic phase (II)

between microaerobic (stage 2) and the rest of samples, indicating the adaptation of the inoculum to the imposed conditions on the experimentation (ethanol as the sole carbon and energy source). Samples corresponding to anaerobic and microaerobic conditions showed a value of 53 %, suggesting a clear impact of microaeration on the archaeal communities. According to the obtained results of Rr and H, it was observed that the archaeal community was favored by the stress (introduction of oxygen) (Fig. 6).

The DGGE profiling in the bacterial community resulted a high *p* value (65 %) between seed and anaerobic stage (1) and 52 % compared to the microaerobic stage (2). The *p* value between stages 1 and 2 was approximately 57 % (Fig. 6). Therefore, the difference found could be explained by negative impact of microaeration in bacterial community.

The microaerobic system revealed a lower sulfate removal (93 %), although system stability was higher than in anaerobic conditions. The lower stability of the system when in anaerobic conditions could be explained by the microbial community adaptation to simplified feed and the competition for an exclusive substrate, both factors possibly capable of promoting changes in microbial structure. Despite strict anaerobic species did not support microaerobic conditions, syntrophic associations between bacterial and archaeal communities could be the reason of a major stability in the system. Changes observed in bacterial and archaeal richness match with the system functioning.

Preliminary Economic Evaluation of Microaeration Process in Relation to the Chemical Absorption Process

For system R1, according to experimental data obtained in the current work, it was necessary 38.2 g NaOH per g H_2S, which corresponds to 2283 g NaOH per m^3 of biogas. By scaling up this system for a population of 7500 inhabitants, it is estimated a biogas production of 324 m^3 day^{-1}. Therefore, the chemical absorption process would demand 270 t $year^{-1}$ NaOH, which is related to the fact that NaOH is not selective for sulfide, and compounds such as CO_2 can also be absorbed. This causes a decrease in the saturation time of the solution, demanding frequent replacements.

On the other hand, R2 would demand a 1.5-kW compressor with a flow rate of 170 L min^{-1} to provide enough oxygen for H_2S oxidation. It is necessary to install safety systems due to risk of explosion by the introduction of oxygen in an environment that contains methane.

Comparing the costs for deploying the technologies studied, it was observed that for the chemical absorption technology, the calculated cost was equivalent to the amount of reagent (NaOH), i.e., US$26,969.86. For the microaerobic system, there is no cost for reagent acquisition.

In relation to energy consumption, considering that the compressor used in system R2 would work 24 h a day, the operating cost would be US$3232.42.

Assessing the payback after 10 years of operation in both systems, we found that the microaerobic system has a payback of 4.7 months. At the end of a period of 10 years, the treatment plant would have saved approximately 84.6 % by using microaerobic system for removal of hydrogen sulfide in relation to chemical absorption process, which would result in a saving of US$228,102.90.

Conclusions

The microaeration used for low sulfate concentration wastewater did not affect the anaerobic digestion, but even increased system stability.

Presence of oxygen in the biogas (0.75 % *v*/*v*) reveals that not all the oxygen was consumed for sulfide oxidation in the liquid phase indicating mass transfer limitations.

Microaeration was an efficient strategy for H_2S removal (93 %) in the biogas, and the reactor was able to rapidly recover its capacity after an operational failure.

Bacterial and archaeal richness shifted due to changes in operational parameters, which match with the system functioning.

The payback of microaerobic process for H_2S removal was 4.7 months.

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