Appl Biochem Biotechnol (2012) 168:1643–1654
DOI 10.1007/s12010-012-9885-3

A Two-Stage Aerobic/Anaerobic Denitrifying Horizontal Bioreactor Designed for Treating Ammonium and H_2S Simultaneously

F. A. Chinalia · L. H. P. Garbossa · J. A. Rodriguez · K. R. Lapa · E. Foresti

Received: 27 February 2012 / Accepted: 28 August 2012 /
Published online: 7 September 2012

Abstract A two-stage bioreactor was operated for a period of 140 days in order to develop a post-treatment process based on anaerobic bioxidation of sulfite. This process was designed for simultaneously treating the effluent and biogas of a full-scale UASB reactor, containing significant concentrations of NH_4 and H_2S, respectively. The system comprised of two horizontal-flow bed-packed reactors operated with different oxygen concentrations. Ammonium present in the effluent was transformed into nitrates in the first aerobic stage. The second anaerobic stage combined the treatment of nitrates in the liquor with the hydrogen sulfide present in the UASB-reactor biogas. Nitrates were consumed with a significant production of sulfate, resulting in a nitrate removal rate of 0.43 $kgN m^3 day^{-1}$ and ≥92 % efficiency. Such a removal rate is comparable to those achieved by heterotrophic denitrifying systems. Polymeric forms of sulfur were not detected (elementary sulfur); sulfate was the main product of the sulfide-based denitrifying process. S-sulfate was produced at a rate of about 0.35 $kg m^3 day^{-1}$. Sulfur inputs as $S–H_2S$ were estimated at about 0.75 $kg m^3 day^{-1}$ and Chemical Oxygen Demand (COD) removal rates did not vary significantly during the process. DGGE profiling and 16S rRNA identified *Halothiobacillus*-like species as the key microorganism supporting this process; such a strain has not yet been previously associated with such bioengineered systems.

Keywords Biogas treatment · Sulfide anaerobic oxidation · Denitrification · Lithotrophic denitrification

F. A. Chinalia (✉)
Instituto de Ciencias e da Saude, Universidade Federal da Bahia, av Reitor Miguel Calmon s/n, 40110-100 Salvador, Brazil
e-mail: chinalia@hotmail.com

L. H. P. Garbossa · K. R. Lapa · E. Foresti
Departmento de Hidráulica e Saneamento, Escola de Engenharia e São Carlos, Universidade de São Paulo, Av do trabalhador são carlense, 400, 13560-970 São Carlos, SP, Brazil

J. A. Rodriguez
Escuela de ingeniería de recursos naturales y del ambiente, Universidad del Valle, Ciudad Universitaria Meléndez, Calle 13 No. 100-00, 25360 Cali, Colombia

Introduction

Modern advances in post-treatment technology have considerably improved treatment efficiency and quality of effluents and gas emissions from wastewater treatment plants (WWTP). Nevertheless, there are still improvements to be made in respect to treating nitrogenated compounds and sulfide gaseous emissions produced at WWTP. Several aerobic and anaerobic post-treatment processes have been tested for this purpose [1–3], but the heterotrophic denitrifying process is often praised as the most cost-effective in the case of nitrate removal [4]. This bioprocess, however, relies heavily on the use of additional carbon sources and it does not address problems with sulfite gaseous emissions [3]. Anaerobic bioxidation of sulfite is considered a more innovative post-treatment approach for it combines the removal of nitrates with the detoxification of sulfite [3]. Denitrifying sulfur bacteria are capable of autotrophically using sulfite instead of organic compounds as an energy source [5, 6] and, consequently, such metabolism can significantly reduce operating costs.

Specially designed systems have been recently developed for promoting the anaerobic bioxidation of sulfite [1, 7, 8]. In these trials, the preparation of a pre-enriched inoculum seemed to have been a prerequisite for favoring high performances [3, 9–12]. Limited information is currently available in the literature describing the start-up of a bioengineered system based on the anaerobic bioxidation of sulfide without specific pre-enriched inoculation. Furthermore, it is also rare to find scientific reports of sulfide anaerobic bioxidation experiments which were carried out using the effluent and gas emissions produced by a full-scale anaerobic digester without previous adjustments. It was the goal of this work to address these issues.

There are several stoichiometric reactions proposed for the anaerobic bioxidation of sulfite [1]. The metabolic reaction described by Eq. 1 was adopted as the model for comparing the results reported in this research once elementary sulfur and nitrite were not detected during the phase of anaerobic bioxidation of sulfite. Baspinar et al. [1] discuss alternative stoichiometric reactions showing that, depending on the compounds involved in

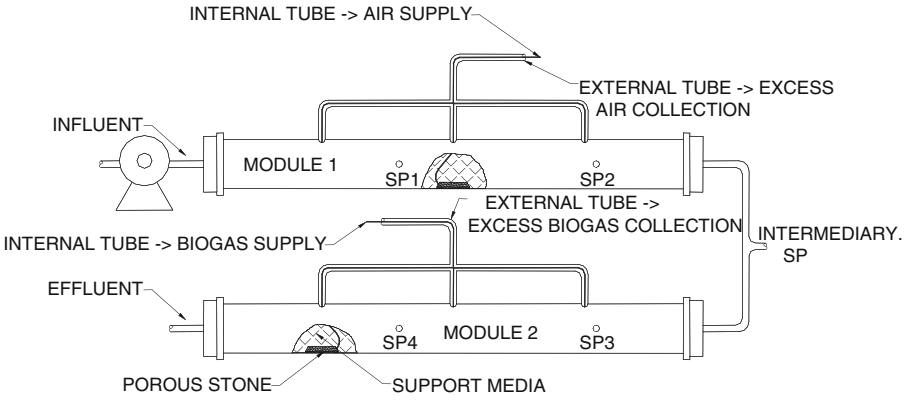

Fig. 1 Schematic diagram of the two-stage horizontal bed-packed reactor. The first stage (*module 1*) was designed to provide the conditions for a nitrifying environment by supplying atmospheric air. The second stage (*module 2*) was designed to promote sulfur-dependent chemolitotrofic denitrification using biogas inputs from a full-scale UASB reactor. Modules were interconnected at all times and atmospheric air and biogas were introduced and controlled separately. Sampling ports were strategically positioned to allow chemical and biological analysis. Two sampling ports in module 1 (SP1 and SP2) and two sampling ports in module 2 (SP3 and SP4) were positioned according to the course of the influent. An intermediary sampling port was also used for chemical monitoring

the reaction, S/N molecular ratios may vary from 0.44 to 2.89. The model described by Eq. 1 assumes an S/N ratio of 0.62, which is close to the sulfur to nitrogen concentration ratios observed in this experiment.

$$5HS^- + 8NO_3{}^- + 3H^+ \rightarrow 5SO_4{}^{2-} + 4N_2 + 4H_2O \quad (1)$$

In this research, the anaerobic bioxidation of sulfite was achieved in a two-stage system capable of combining the biogas and effluent of the same full-scale up-flow anaerobic sludge blanket reactor (UASB reactor). The bioreactor was being used for treating domestic wastewater. In the first stage, ammonium was aerobically transformed into nitrates, and in the second anaerobic stage, sulfite was used for removing nitrates (Eq. 1). Thus, the two-stage system comprised of two horizontal-flow anaerobic immobilized sludge bed-packed reactors arranged in sequence. This design is known for enhancing liquid–gas contact [13] and for favoring denitrifying processes [14]. Therefore, the aim of this work was to estimate the potential of such a two-stage system for combining nitrogenated compound and sulfide removal in a post-treatment approach without pre-enriched inoculation and the addition of organic supplementation.

Material and Method

Reactor Design, Operating Variables, and Analytic Methodology

PVC tubes with dimensions of 1.5 m of length and 0.15 m of diameter were used to build the two-stage system as shown in Fig. 1. This design is an adaptation of the horizontal reactor described by Zaiat et al. [13]. Bioreactors were filled with polyurethane foam cubes with sides of 10 mm which were inoculated as described previously [13]. For the inoculation procedure, active biomass was collected from a full-scale UASB reactor and a nitrifying reactor in the proportion of 1:3, respectively. The microbial biomass was not pre-adapted specifically to anaerobic oxidation of sulfide, but only with ammonium oxidizing bacteria. Effluent and biogas were obtained from a UASB reactor situated at the University of São Paulo, São Carlos Campus, Brazil (EESC/USP). The anaerobic digester is used to treat a food waste influent with a chemical oxygen demand loading ratio of about 3.4 $kgCOD\,m^3\,day^{-1}$. The anaerobic process yields a biogas with an average CH_4, CO_2, and H_2S ratios of 57:41:2 %, respectively.

During the operation of the two-stage system, atmospheric air (oxygen) was injected in the first module and UASB-reactor biogas was injected in the second module. The system was carefully sealed and monitored to prevent gas leaks or exchanges. Gas injection was achieved using three 10 cm diameter porous stones uniformly arranged in each module (Fig. 1). UASB-reactor effluent was continuously feed into the two-stage system with an average inflow of 0.43 $m^3\,day^{-1}$, which corresponded to a total HRT of 11.8 h. The effluent showed a COD, TSS, and VSS of 144 (±32), 23 (±12), and 19 (±10) $mg\,l^{-1}$, respectively. Concentrations of nitrogenated compounds are shown in Table 1. Prior to feeding into the two-stage system, UASB-reactor effluent was collected and stored in a 300-l tank and the pH was adjusted with bicarbonate (~7).

The operation of the two-stage horizontal reactor was divided in three phases. In the first phase (0–14 days), neither atmospheric air nor biogas was injected into the system. This phase was therefore used as a control for the experiment. In the second phase (15–84 days), only the first stage received the injection of atmospheric air. In the third phase (85–140 days), biogas was supplied in the second module in parallel with the oxygen being injected in module 1. This approach was adopted to favor a clear comparison between the processes of ammonium oxidation and hydrogen sulfide oxidation in phases 2 and 3, respectively.

Table 1 Chemical characteristics of the influents fed in the two-stage bioreactor during the period of biogas treatment

Sampling ports	COD (mgl^{-1})	Organic N (mgl^{-1})	$N–NH_4^+$ (mgl^{-1})	$N–NO_3^-$ (mgl^{-1})	Alkalinity (mgl^{-1})
Influent	142 (±33)[a]	3.2 (±1.14)	36.7 (±7.48)	BDL	354 (±67)
Intermediary	61 (±12)	1.5 (±0.82)	15.6 (±2.52)	14.2 (±4.8)	178 (±6)
Effluent	68 (±9.2)	[c]	12.7 (±6.3)	BDL[b]	67 (±7.1)

Details of sampling ports are shown in Fig. 1

[a] Standard deviation, $n=3$

[b] Below detection limits (<0.01 mgl^{-1})

[c] Not measured

The air supplied in the first module was provided by a pump with a continuous flow of 2.8 $m^3 day^{-1}$, approximately. During the experiment, oxygen concentrations within module 1 were of about 2.5(±1.2) mgl^{-1}. Dissolved oxygen was not detected in module 2 at any time. In a similar procedure, biogas was injected with a continuous flow of 1.8 $m^3 day^{-1}$ resulting in an $S–H_2S$ loading rate of about 0.75(±0.05) $kg m^3 day^{-1}$.

Samples were analyzed for COD, total nitrogen, ammonium, nitrate, sulfate, sulfide, and pH. The analyses were carried out according to the Standard Methods for the Examination of Water and Wastewater [15]. Ammonium concentrations were determined by distillation (method 4500-NH3 B) and titration (method 4500-NH3 C). Nitrite and nitrate were determined by flow injection analysis (FIA) as described in the method 4500-NO3 I. Alkalinity was determined according to Ripley et al. [16].

DNA Extraction and Molecular Analysis

At the end of the trial, ten polyurethane cubes were collected from the sampling-ports SP3 and SP4 (Fig. 1) and used for the molecular analysis. In order to detach immobilized microbial biomass, polyurethane cubes were macerated with a pistil/mortar and phosphate buffer (pH 7.0) until they reached a clean look. The suspension containing the detached microbial biomass was centrifuged and 0.5 g of the pellet transferred to a 15-ml centrifuge tube for the DNA extraction. Glass beads (0.5 g), phenol (1 ml), chloroform (1 ml), and phosphate buffer (pH 8.0) were added to the tube and vortexed for 1 min as described in the approach described by Daniel et al. [17]. PCR and DGGE profiling were carried out according to Muyzer et al. [18]. DNA bands were extracted from the DGGE gel and eluted in DNA/RNA free water for 12 h. PCR products were cloned into a TA-cloning vector according to the manufacturer' instructions (Invitrogen-UK). Clones were sequenced using ABI 377 DNA Sequencer-Perkin-Elmer as described by Gusmão et al. [14]. A phylogenetic tree was constructed using bacterial 16S DNA sequences downloaded from the Ribosomal Database Project [19] using PULP and Kimura 2-parameter. The DNA sequences were submitted to the NCBI Genebank under the accession numbers EU092238 to EU092241, respectively.

Results

The results characterizing the three distinct operating phases are shown in Fig. 2. Without aeration in the first phase, ammonium concentrations did not vary significantly during

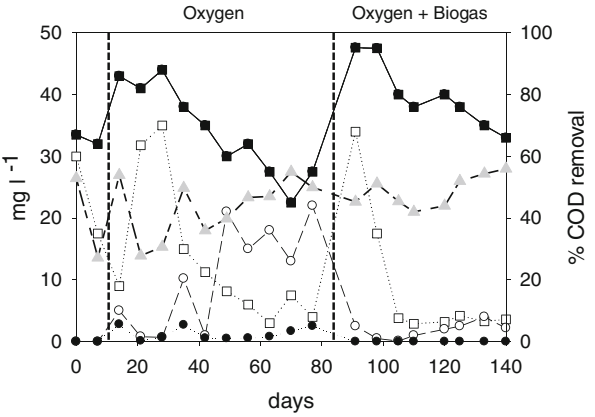

Fig. 2 Variations of total COD removal efficiency (*gray triangle*), N-ammonium in the influent (*filled square*) and effluent (*square*). N-nitrite (*filled circle*) and N-nitrate (*circle*) concentrations obtained at sampling port SP4 module 2; see Fig. 1. This figure summarizes three operating phases as described in the "Material and Method" section. In phase 2, oxygen was introduced in module 1, and in phase 3, biogas was simultaneously feed into module 2

14 days. In the second phase, however, oxygen was supplied and significant amounts of nitrate were detected within module 1. Thus, the success of this approach can be assessed by comparing N–NH_4 removal in phase 1 and 2, respectively. Dissolved oxygen concentrations within module 1 were kept constant from the start of phase 2 onwards ($2\pm1.2\ mgl^{-1}$). In phase 3, biogas was supplemented into the bioreactor's second stage (module 2). The goal of the third phase (84–140 days) was to promote anaerobic bioxidation of sulfite within module 2. Biogas containing sulfite was supplemented at rate of $1.8\ m^3 day^{-1}$. The success of this approach was assessed by comparing the production of SO_4 and N–NO_3 removal rates in phase 2 and 3, respectively. Sulfite was not detected in the biogas after passing through the reactor's module 2, and increases in sulfate concentrations are shown in Fig. 3.

A comparison between phases showed that ammonium concentrations significantly decreased in phase 2 and 3, respectively. Nitrite was only detected in phase 2 and nitrate concentrations showed a reduction of approximately 82 % during phase 3 (Fig. 2). An overall profiling of nitrogenated compounds was carried out at the last day of the second and third phase, respectively (Fig. 4). The results show a significant correlation between the decrease of ammonium, with an increase of nitrite and nitrate concentrations in phase 2. In the third phase, however, nitrite was no longer detected and nitrate concentrations (N–NO_3^-) decreased 7.7 times in correlation with sulfate production (Fig. 3).

Nitrogen mass balance shown in Fig. 4 suggests that about 40 % of the initial nitrogen present as ammonium (N–NH_4) was transformed into nitrogen oxides within module 1, and over 92 % of the available nitrate was consumed in module 2 during phase 3. Calculations based on the hydraulic retention time (HRT), and the differences between N-nitrate concentrations in module 2 influent and effluent (Table 1), indicated a nitrogen removal rate of $0.43\ kgN m^3 day^{-1}$. COD concentrations did not vary significantly, but a significant reduction in alkalinity (CO_2 consumption) was observed in phase 3 (Table 1). In addition, COD removal ratios for the two-stage system were always lower than 60 % (Fig. 2) suggesting very low heterotrophic activity.

The stoichiometric model shown in Eq. 1 suggests that for sustaining an N-removal rate of $0.43\ kgm^3 day^{-1}$, a corresponding S–H_2S consumption of $0.61\ kgm^3 day^{-1}$ is necessary.

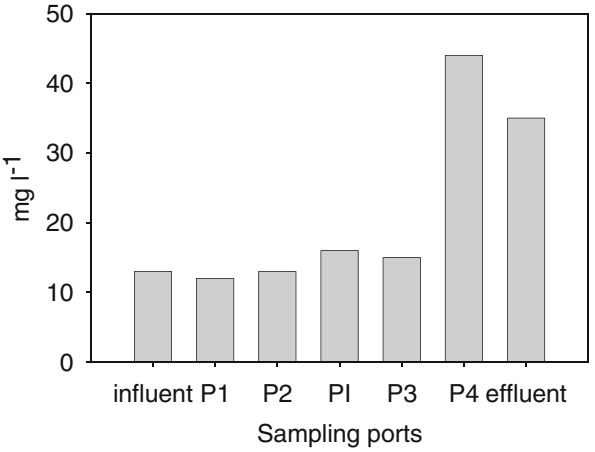

Fig. 3 $S–SO_4$ profiling of the two-stage horizontal reactor. Samples were obtained in the last day of phase 3 (Fig. 2a), which is coincident with nitrogen balance shown in Fig. 4b

The amount of sulfur injected into the system was estimated as 0.75 $gS–H_2S l^{-1} day^{-1}$. Assuming that the difference in sulfate concentrations between influent and effluent was in average around 25 $mg l^{-1}$ (Fig. 3) at an HRT of 11.8 h, calculations suggest an $S–SO_4$ production ratio of about 0.35 $kg m^3 day^{-1}$ in phase 3. It is known that some bacterial strains may produce elemental sulfur instead of sulfate, but X-ray absorption spectroscopy of the microbial biomass did not show the presence of polymeric sulfur in the biofilm at any phase.

DGGE profiling characterizing the biofilm suggests a microbial consortium composed of at least four different species in module 2 (Fig. 5). Phylogenetic analysis of DGGE band LH03 showed that such fragment has a high similarity to *Halothiobacillus*-like species and band LH02 grouped with *Flavobacterium*-like organisms (Fig. 5). Bands LH01 and LH04 were closely related to a new branch of the phylum Chloroflexi (16S rRNA fragments received the accession numbers EU092238-41, respectively).

Discussion

In this work, H_2S from biogas and nitrates resulting from the aeration of UASB-reactor effluent were simultaneously removed through the process of anaerobic bioxidation. This process has been identified as an ideal WWTP post-treatment approach when compared to the heterotrophic alternative for it does not rely on the supplementation of carbon sources [3, 4]. There are different routes for achieving anaerobic bioxidation of sulfite. For instance, it may occur during anaerobic photosynthesis, but this process is strongly affected by the availability of light and it has a strong negative effect on COD removal rates [20]. On the other hand, if the oxidant agent is nitrates, the lithotrophic reaction is independent of light and it produces less microbial biomass (COD). It was observed in this research that more than 40 % of the ammonium present in the UASB-reactor effluent was aerobically converted into nitrogen oxides in the first module of a two-stage bioreactor (Fig. 4) and the compound was then anaerobically consumed with the production of $S–SO_4$ in the second module (Fig. 3). This process occurred only when biogas containing hydrogen sulfide was introduced into the system at a rate of 0.75 $kgS–H_2S m^3 day^{-1}$ (Fig. 4). COD concentrations did not vary significantly (≤60 %) and dissolved oxygen was never detected in module 2.

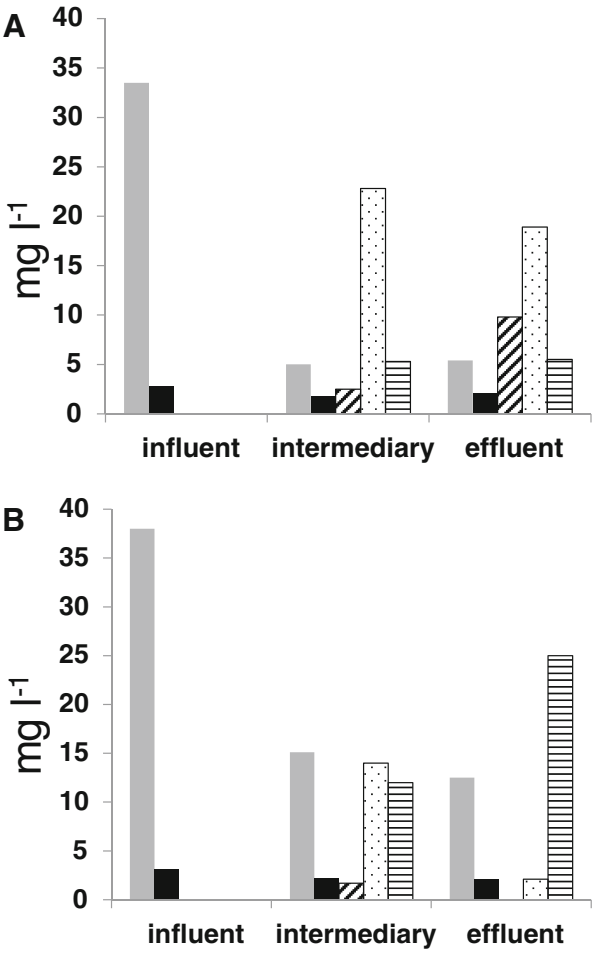

Fig. 4 Nitrogenated compounds mass balance. N-ammonium (*gray*), N-organic (*black*), N-nitrite (*slanted line*), and N-nitrate (*dotted*) concentrations profiles obtained in the last day of phase 2 (**a**) and phase 3 (**b**) of the reactor's 140 days trial. Potential concentrations of produced N_2 were also predicted and indicated in the graphic (*horizontal line*)

Therefore, these results are a significant indication of nitrogen removal coupled to anaerobic sulfite bioxidation. In this research, the nitrogen removal rate was 0.43 $kgNm^3 day^{-1}$, which is comparable to that obtained in heterotrophic systems, which are reported as ranging from 0.17 to 2.4 $gNl^{-1} day^{-1}$ [5].

The S/N ratio as seen in Eq. 1 was used for comparing the kinetics of sulfite, sulfate, and nitrogenated compounds. Thus, variations in the concentration of nitrogenated compounds and sulfate suggested a sulfur removal rate of 0.61 $kgS–H_2Sm^3 day^{-1}$, which is comparable to those obtained in similar systems [8]. An et al. [2], for example, observed that sulfate conversion rates are favored at sulfite to nitrate ratios of 0.62 and the results of this trial endorse such observation. Alternatively, Baspinar et al. [1] describe a lithotrophic process in which more than 95 % H_2S is removed when a continuous bioscrubber is operated within an $S–H_2S$ loading rate of between 2 to 4 $kgm^3 day^{-1}$. These are values considerably higher than the ones shown in this research. The former authors, however, worked with substantially

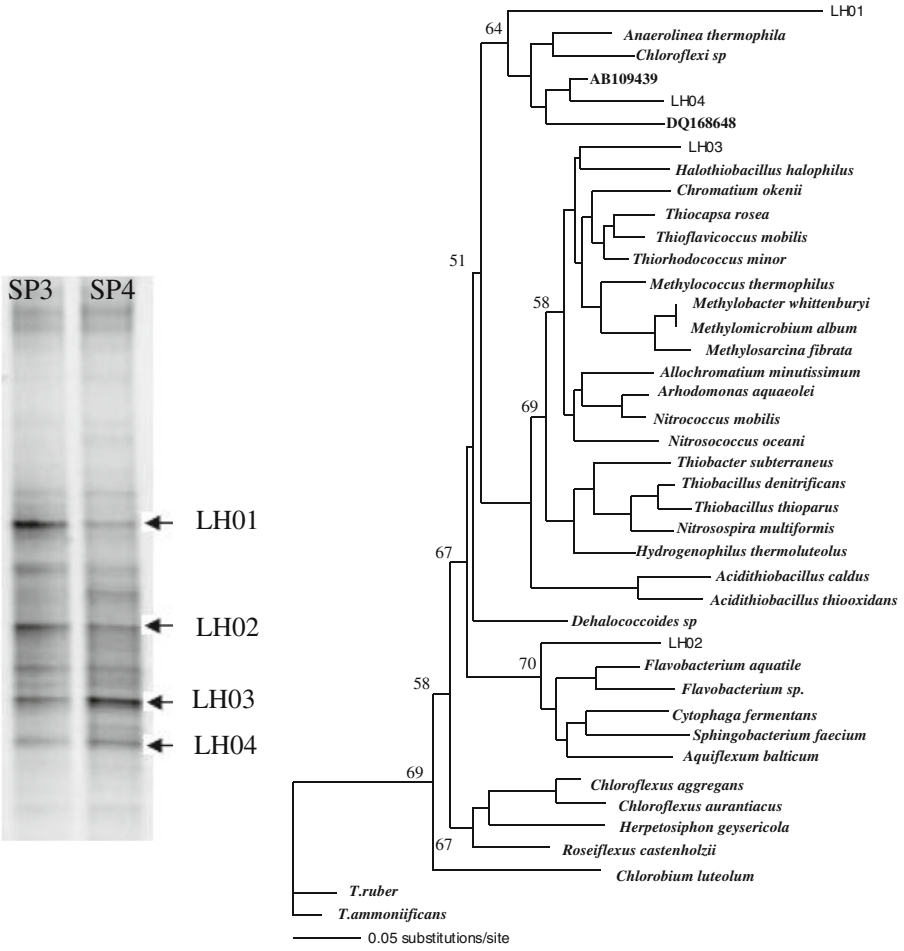

Fig. 5 DGGE profiling of the biofilm sampled in SP3 and SP4 sampling-ports (*left*), and the evolutionary distance tree based on comparative 16S rRNA analysis of the DNA sequences obtained with the bands LH01 to 04 and the sequences downloaded from the Ribosomal Data Project (RDP). Distance analysis was carried out using PAUP and Kimura 2-parameter and bootstrap values lower than 70 are shown in the tree

different conditions. The concentrations of nitrite and nitrate present in their effluent were considerably higher (46 and 79 $kgNm^3day^{-1}$, respectively) and sulfite supplementation was not directly provided from the anaerobic digester. In this research, apart from pH correction, effluent and biogas were used in nature throughout this experiment. Thus, the goal of this research was not to test the limits of sulfur and nitrogen removal by lithotrophic denitrification, but rather test the direct association of specific UASB-reactor by-products (effluent and biogas) as a means to promote such a biological process. In this regard, the results indicate significant success. Therefore, sulfur and nitrogen removal rates are significantly high considering the initial concentrations present in module 2, phase 3, respectively. Further tests should be carried out in order to ascertain the limits of sulfur and nitrogen removal rates when such a system is exposed to a different full-scale UASB reactor showing higher concentrations of these compounds. Rates of ammonium oxidation in stage 1 were possibly the limiting step in the whole two-system process (Table 1). This was an unexpected result

once a specific ammonium-oxidizing biofilm was used to inoculate module 1. Such a result reinforces the understanding that ammonium-oxidizing biofilm includes species with low growth rates and are very sensitive to potentially toxic substances [17].

DGGE profiling and DNA sequencing identified four main bacterial strains (Fig. 5). Among these four sequences, one matched a bacterial strain with metabolic potential for lithotrophic denitrification. The similarity of band LH03 to the *Halothiobacillus*-like species is supported by the bootstrap values shown in the phylogenetic tree (Fig. 5). Such a 16S rRNA sequence is distinct from the common strains belonging to the α-, β-, and δ-proteobacteria, such as *Chromatium* sp., *Thiobacter* sp., *Nitrospira* sp., *Acidithiobacillus* sp., *Thiobacillus* sp., *Methylobacter* sp., and others. According to some authors, sulfur-based lithotrophy is found scattered in distinct groups within the Proteobacteria [21]. Nonetheless, the 16S rRNA sequence found in this trial is similar to a particular strain that may unite the representatives of these groups into a different unique linage [22]. There is very little information on the phylogeny and physiology of *Halothiobacillus*-like bacteria. These organisms were commonly observed in environments containing high concentrations of salts [22], but the growth of halophyte strains was also observed in low salinity media [23, 24]. The LH03-band did not match any of the *Thiobacillus*-like bacteria commonly used in pre-enriched inoculum [3, 9–12]. It is possible that *Thiobacillus*-like species were not present in the original inocula. On the other hand, *Halothiobacillus*-like species may be more adaptable to bioengineered systems. It is known that, comparatively, they produce higher energy yields from the anaerobic oxidation of sulfide [23, 24]. Nevertheless, their accurate biochemical pathway and genetic control is still poorly understood [25]. Polyurethane matrices used as support material may have played a part in the selection of such *Halothiobacillus*-like species. They are known to enhance sulfide bioavailability in bioengineered systems [20]. The interaction between the physical substrate and the microorganism may be of pivotal importance for sustaining high rates of anaerobic oxidation of sulfur. This reaction is dependent on the activity of a sulfur-binding protein present in the bacterial membrane; which can be directly affected by close contact with sulfide-like compounds [26].

Bands LH01 and LH04 (Fig. 5) were associated with microbial strains of the Chloroflexi group. Fermenting strains in this group can efficiently grow in an environment containing very low amounts of organics, especially when in co-culture with hydrogenotrophic organisms [27]. Chloroflexi-like organisms are present in several habitats where anaerobic methane oxidation has been detected [28, 29]. In the present study, methane was the first largest constituent of the biogas feed in module 2, phase 3. Anaerobic oxidation of methane has been previously suggested in bioengineered systems under denitrifying and sulfate-reducing conditions but neither the organisms involved, nor the biochemical process are known [30–32]. In this work, however, it is more likely that the presence of sulfide may have enhanced the levels of toxicity within module 2 in phase 3. As a consequence, the autolysis of non-adapted microbial cells would have generated a yeast-like extract, which has been identified as an important source of nutrients capable of supporting the growth of such Chloroflexi-like organisms [33]. In addition, discharged microbial biomass is also constantly fed into module 2 from module 1 and such material is subject to the same toxic effect. Therefore, the eco-physiological function of these Chloroflexi-like organisms may contribute to sustain biofilm structure and biomass turnover in such bioengineered systems.

Few bacterial species can carry out S-dependent denitrification, but such metabolism is also a feasible process when using H_2 as an electron donor [34]. This is a metabolic process commonly carried out by *Flavobacterium*-like organisms and the phylogenetic analysis shown in Fig. 5 placed band LH02 into this group. Hydrogen gas was not assessed, but it may have been a constituent of the biogas feed into module 2, phase 3. *Flavobacterium*-like

organisms are also known to utilize sulfate as an electron acceptor. This explains some increase in COD removal rates at the end of the trial (Fig. 2) and the differences in sulfate concentrations between sampling port P4 and the effluent of module 2, phase 3 (Fig. 3). If sulfate was consumed in such a fashion, predictions of mass balance may be underestimated.

Conclusions

The two-stage system was capable of sustaining a stable process of nitrogen and sulfite removal based on a lithotrophic process with an efficiency of about 82 % and 99.9 %, respectively. The bioreactor was operated with an S–H_2S loading rate of about 0.75 $kgm^3 day^{-1}$ and sulfite was not detected in the biogas after the treatment. Nitrogen removal rates were comparable to heterotrophic systems showing values of 0.43 $kgNm^3 day^{-1}$. Sulfate concentrations in the bioreactor's effluent suggested an S–SO_4 discharging rate of about 0.35 $kgm^3 day^{-1}$.The goal of this research was not to test the limits of sulfur and nitrogen removal by lithotrophic denitrification, but rather test such an approach using the by-products of a specific UASB reactor (effluent and biogas). In this regard, the results indicate a significant success. Thus, this approach showed to be efficient in treating the by-products of a full-scale UASB reactor with a start-up period of less than 140 days. The main microorganism involved in this process was tentatively identified as a *Halothiobacillus*-like species, which has not yet been previously associated with post-treatment of liquid and gaseous effluents. The observed rates of denitrification were obtained without pre-enriched inoculation or bioaugmentation.

Acknowledgments The authors would like to acknowledge the Brazilian Research funding institutions CNPq (research grant 541044/2001-0), FAPESP (research grant 99/06152-8), and FAPESB (research grant 003/2011).

References

1. Baspinar, A. B., Turker, M., Hocalar, A., & Ozturk, I. (2011). Biogas desulphurization at technical scale by lithotrophic denitrification: integration of sulphide and nitrogen removal. *Process Biochemistry, 46*, 916–922.
2. An, S., Tang, K., & Nemati, M. (2010). Simultaneous biodesulphurization and denitrification using an oil reservoir microbial culture: effects of sulphide loading rate and sulphide to nitrate loading ratio. *Water Research, 44*, 1531–1541.
3. Manconi, I., Carucci, A., & Lens, P. (2007). Combined removal of sulfur compounds and nitrate by autotrophic denitrification in bioaugmented activated sludge system. *Biotechnology and Bioengineering, 98*, 551–560.
4. Ahn, Y.-H. (2006). Sustainable nitrogen elimination biotechnologies: a review. *Process Biochemistry, 41*, 1709–1721.
5. Boley, A., Müller, W. R., & Haider, G. (2000). Biodegradable polymers as solid substrate and biofilm carrier for denitrification in recirculated aquaculture systems. *Aquacultural Engineering, 22*, 75–85.
6. Robertson, L. A., & Kuenen, J. G. (1992). The colorless sulfur bacteria. In A. Balow, H. G. Trüper, M. Dworkin, W. Harder, & K. K. Schlefer (Eds.), *The procariotes* (pp. 385–413). New Yourk: Springer-Verlag.
7. Deng, L., Chen, H., Chen, Z., Liu, Y., Pu, X., & Song, L. (2009). Process of sumultaneous hydrogen sulfide removal from biogas and nitrogen removal from swine wastewater. *Bioresource Technology, 100*, 5600–5608.
8. Li, W., Zhao, Q. L., & Liu, H. (2009). Sulfide removal by simultaneous autotrophic and heterotrophic desulfurization-denitrification process. *Journal of Hazardous Materials, 162*, 848–853.
9. Chung, Y.-C., Huang, C., Pan, J. R., & Tseng, C.-P. (1998). Comparison of autotrophic and mixotrophic biofilters for H2S removal. *Journal of Environmental Engineering, 124*, 362–367.

10. Friedrich, U., Prior, K., Altendorf, K., & Lipski, A. (2002). High bacterial diversity of a waste gas-degrading community in a industrial biofilter as shown by a 16S rDNA clone library. *Environmental Microbiology, 4*, 721–734.
11. Oprime, M. E. A. G., Garcia, O., & Cardoso, A. A. (2001). Oxidation of H2S in acid solution by Thiobacillus ferrooxidans and Thiobacillus thiooxidans. *Process Biochemistry, 37*, 111–114.
12. Sublette, K. L., Kolhatkar, R., & Raterman, K. (1998). Technological aspects of the microbial treatment of sulfide-rich wastewater: a case study. *Biodegradation, 9*, 259–271.
13. Zaiat, M., Vieira, L. G. T., & Foresti, E. (1997). Spatial and temporal variations of monitoring performance parameters in horizontal-flow anaerobic immobilized sludge (HAIS) reactor treating synthetic substrate. *Water Research, 31*, 1760–1766.
14. Gusmao, V. R., Chinalia, F. A., Sakamoto, I. K., & Varesche, M. B. A. (2007). Performance of a reactor containing denitrifying immobilized biomass in removing ethanol and aromatic hydrocarbons (BTEX) in a short operating period. *Journal of Hazardous Materials, 139*, 301–309.
15. APHA, American Public Health Association. (1998). *Standard methods for the examination of water and wastewater* (19th ed.). Washington, DC: Water Pollution Control Federation.
16. Ripley, L. E., Boyle, W. C., & Converse, J. C. (1986). Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. *Journal Water Pollution Control Federation, 58*, 406–411.
17. Daniel, L. M., Pozzi, E., Foresti, E., & Chinalia, F. A. (2009). Removal of ammonium via simultaneous nitrification–denitrification nitrite-shortcut in a single packed-bed batch reactor. *Bioresource Technology, 100*, 1100–1107.
18. Muyzer, G., de Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology, 59*, 695–700.
19. Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., et al. (2007). The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. *Nucleic Acids Research, 35*, D169–D172.
20. Sarti, A., Pozzi, E., Chinalia, F. A., Zaiat, M., & Foresti, E. (2006). The performance of an anaerobic sequencing batch biofilm reactor treating domestic sewage colonized by anoxygenic phototrophic bacteria. *Chemosphere, 62*, 1437–1443.
21. Kelly, D. P., & Wood, A. P. (2000). Reclassification of some species of Thiobacillus to the newly designated genera Acidithiobacillus gen. nov., Halothiobacillus gen. nov. and Thermithiobacillus gen. nov. *International Journal System Evolution Microbiology, 50*, 511–516.
22. Sievert, A. M., Heidron, T., & Kuever, J. (2003). Halothiobacillus Kellyi sp. nov., a mesophilic, obligately chemolitroautotrophic, sulfur-oxidizing bacterium isolated from a shallow-water hydrotermal vent in the Aegean Sea, and emended description of the genus Halothiobacillus. *International Journal System Evolotion Microbiology, 50*, 1229–1237.
23. Oren, A. (1999). Bioenergetic aspects of halophilism. *Microbiol. Mol. Biol. R., 63*, 334–348.
24. Sorokin, D. Y., Tourova, T. P., & Muyzer, G. (2005). Oxidation of thiosulfate to tetrathionate by a haloarchaeon from hypersaline habitat. *Extremophiles, 9*, 501–504.
25. Friedrich, C. G., Bardischewsky, F., Rother, D., Quentmeier, A., & Fischer, J. (2005). Prokaryotic sulfur oxidation. *Current Opinion in Microbiology, 8*, 253–259.
26. Wood, A. P., & Kelly, D. P. (1991). Isolation and characterisation of Thiobacillus halophilus sp. nov., a sulphur-oxidizing autotrophic eubacterium from a Western Australian hypersaline lake. *Archives of Microbiology, 156*, 277–280.
27. Ohmura, N., Tsugita, K., Koizumi, J. I., & Saika, H. (1996). Sulfur-binding protein of flagella of Thiobacillus ferrooxidans. *Journal of Bacteriology, 178*, 5776–5780.
28. Sekiguchi, Y., Yamada, T., Hanada, S., Ohashi, A., Harada, H., & Kamagata, Y. (2003). Anaerolinea thermophila gen. nov., sp. nov. and Caldilinea aerophila gen. nov., sp. nov., novel filamentous thermophiles that represent a previous uncultured lineage of the domain Bacteria at the subphylum level. *International Journal System Evolution Microbiology, 53*, 1843–1851.
29. Inagaki, F., Suzuki, M., Takai, K., Oida, H., Sakamoto, T., Aoki, K., et al. (2003). Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Applications Environment Microbiology, 69*, 7224–7235.
30. Schwarz, J. I. K., Eckert, W., & Conrad, R. (2007). Community structure of Archaea and Bacteria in a profundal lake sediment Lake Kinneret (Israel). *Systematic and Applied Microbiology, 30*, 239–254.
31. Costa, C., Dijkema, C., Friedrich, M., Garcia-Encina, P., Fernandez-Polanco, F., & Stams, A. J. M. (2000). Denitrification with methane as electron donor in oxygen-limited bioreactors. *Applied Microbiology and Biotechnology, 53*, 754–762.
32. Islas-Lima, S., Thalasso, F., & Gómez-Hernandez, J. (2004). Evidence of anoxic methane oxidation coupled to denitrification. *Water Research, 38*, 13–16.

33. Sekiguchi, Y., Takahashi, H., Kamagata, Y., Ohashi, A., & Harada, H. (2001). In situ detection, isolation, and physiological properties of a thin filamentous microorganism abundant in methanogenic granular sludges: a novel isolate affiliated with a clone cluster, the green non-sulfur bacteria, Subdivision I. *Applied and Environmental Microbiology, 67*, 5740–5749.
34. Karanasios, K. A., Vasiliadou, I. A., Pavlou, S., & Vayenas, D. V. (2010). Hydrogenotrophic denitrification of potable water: a review. *Journal of Hazardous Materials, 180*, 20–37.