Biogas upgrading from vinasse digesters: a comparison between an anoxic biotrickling filter and an algal-bacterial photobioreactor

Short title: Biogas upgrading in anoxic biofilters and algal-bacterial photobioreactors

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Abstract

BACKGROUND

The performance of an anoxic biotrickling filter (BTF) and an algal-bacterial photobioreactor (PBR) for the upgrading of real biogas was comparatively evaluated.

RESULTS

A H_2S removal efficiency of ~100% was consistently recorded in both systems, with elimination capacities of up to 1200 g S-H_2S m^{-3} h^{-1} at low empty bed residence times (EBRTs ranging from 30 to 146 s). Both bioreactors demonstrated a high robustness towards fluctuations in biogas composition and flowrate, maintaining nearly complete desulfurization despite the variations in sulfur load and EBRT. The BTF also showed an immediate recovery from a 15 days operational shut-down, and the ability to utilize the nutrients from nitrate-supplemented digestate during biogas desulfurization. In addition, the PBR supported an average CO_2 removal of 23.0 ± 11.8 % at EBRT of 37-146 s and a carbon fixation rate of 285 mg CO_2 L^{-1} d^{-1}.

CONCLUSIONS

The potential of anoxic biotrickling filters and algal-bacterial photobioreactors as efficient and robust technologies for the desulfurization of real biogas was demonstrated. The CO_2 fixation capacity of microalgae contributed to an enhanced biogas purification.

Keywords: algal-bacterial symbiosis, anoxic biofiltration, biogas upgrading, biotrickling filter, photobioreactor

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Introduction

The development of renewable energy sources has become a priority worldwide as a result of the steady rise in oil prices, the increasing dependence on fossil fuels, the gradual depletion of non-renewable energy sources and the increasing concern on global warming. Biogas from the anaerobic digestion of organic substrates constitutes a valuable bioenergy source that indeed contributes to the reduction of greenhouse gas (GHG) emissions. [1-2] Biogas production has steadily increased over the last years in the EU, with a total production of 13.4 Mtoe in 2013, which represents an increase of 10.2% compared with the production in 2012. [3]

The type and content of organic substrate digested stoichiometrically determine the yield and composition of biogas, which usually contains CH_4 (50 to 75%), CO_2 (25 to 50%), H_2S (0 to 2%) and other gas pollutants such as siloxanes or NH_3 at trace level concentrations. [1] The presence of CO_2 and H_2S in biogas hinders its direct use as a substitute of natural gas, biogas upgrading being essential in order to meet the required quality specifications for injection into natural grad grids or use as autogas. The high concentration of CO_2 increases biogas transportation costs and contributes to GHG emissions, besides reducing the specific biogas energy content. Likewise, a reduction in H_2S content is crucial due to its odorous and toxic nature, and to the need to prevent corrosion and mechanical wear in biogas combustion systems. Indeed, H_2S purification from biogas is sufficient for its direct combustion in the plant.

In this context, physicochemical technologies such as membrane separation, adsorption or scrubbing are capable of efficiently removing CO_2 and H_2S from biogas, but at the expenses of prohibitive operating costs and high environmental impacts. On the other hand, biological biogas purification methods are capable of removing either CO_2 (conventional microalgae photobioreactors) or H_2S (aerobic or denitrifying biotrickling

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filtration) at significantly lower operating costs in a more environmentally friend way. [2] Aerobic H_2S biotrickling filtration requires an external oxygen supply, which must be carefully controlled in order to avoid explosion risks and reduce the dilution of methane concentration in the purified biogas.[4] Autotrophic denitrification constitutes an alternative that overcomes these two operational problems based on sulfide oxidation via dissimilatory nitrate reduction (Eq. 1). In this technology, no mass transfer limitation of the electron acceptor occurs since nitrate is already dissolved in the tricking liquid media. [2,5]

$$15NO_3^- + 12H_2S \rightarrow 9H_2O + 6S^0 + 6SO_4^{2-} + 5N_2 + 2OH^- + 4H^+ \quad \text{Eq. 1}$$

Recent economic evaluations have demonstrated that the upgrading cost of one cubic meter of biogas amounts to 0.024 and 0.30 € m^{-3} for $FeCl_3$ and chemical scrubbing, respectively, decreasing to 0.016 € m^{-3} when anoxic biofiltration is applied. [5,6] However, this technology is only feasible as a pre-treatment for H_2S removal from biogas.

In this context, the simultaneous removal of CO_2 and H_2S by algal-bacterial symbiosis in photobioreactors represents a low-cost and environmentally-friendly alternative for an integral biogas upgrading. This technology is based on the fixation of CO_2 from biogas by microalgae using solar energy via photosynthesis, with the concomitant production of O_2. Meanwhile, sulfur oxidizing bacteria will use this photosynthetically produced oxygen to oxidize the biogas H_2S to sulfate according to Eq. 2. [7,8]This would avoid the operational problems of clogging often found in desulfurizing packed bed-biofilters due to elemental sulfur accumulation (Eq. 3).

$$HS^- + 2\,O_2 \rightarrow SO_4^{2-} + H^+ \quad \text{Eq. 2}$$

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$$HS^- + \tfrac{1}{2}\,O_2 \rightarrow S_0 + OH^-$$ Eq. 3

In brief, algal-bacterial symbiosis and autotrophic denitrification have emerged in the past years as promising platforms for biogas upgrading, but to the best of our knowledge there are no studies comparing their performance during the upgrading of real biogas emissions. This study aimed at comparatively evaluating the feasibility of an algal-bacterial column photobioreactor and an anoxic biotrickling filter for the upgrading of real biogas from two pilot scale Upflow Anaerobic Sludge Blanket (UASB) reactors treating vinasse.

Materials and Methods

Microorganisms and culture conditions

Anaerobic sludge from a UASB reactor located in a poultry slaughterhouse (Dacar, Tietê/SP, Brazil) was employed as the inoculum in the anoxic biotrickling filter (BTF). The anaerobic sludge (200 mL) was acclimated for 45 days to the anoxic biodegradation of sulfide in two bottles incubated at 35ºC under continuous agitation and periodically supplemented with Na_2S (to a final concentration of 20 mg S^{-2} L^{-1}) and KNO_3 (to a final concentration of 34 mg N-NO_3^- L^{-1}, ratio N/S = 1.7) under an argon headspace. The Mineral salt medium (MSM) used for sludge acclimation and BTF operation was composed of (mg L^{-1}): $NaHCO_3$ (2000); KNO_3 (144); KH_2PO_4 (36); NH_4Cl (16); $MgCl_2 \cdot 6H_2O$ (28) and $CaCl_2 \cdot 2H_2O$ (18). Trace elements were supplied by adding 2 mL L^{-1} of a stock solution containing (mg L^{-1}): EDTA (500); $ZnSO_4 \cdot 7H_2O$ (40); $CaCl_2 \cdot 2H_2O$ (70); $MnCl_2$ (30); $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (10); $CuSO_4 \cdot H_2O$ (20) and $CoCl_2 \cdot 6H_2O$ (20).

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The photobioreactor (PBR) was inoculated with a mixture of aerobic sludge from an activated sludge process (Volkswagen, São Carlos/SP, Brazil) and *Chlorella sp*. (BIOTACE, Escola de Engenharia de São Carlos, São Paulo University). The aerobic activated sludge (100 mL) was acclimated for 26 days to the aerobic biodegradation of sulfide by periodical addition of Na_2S to the cultivation broth (to a final concentration of 20 mg S^{-2} L^{-1}) and renewal of the air headspace in order to ensure aerobic conditions. *Chlorella sp*. was cultivated in 4 flasks of 200 mL for 9 days with atmospheric CO_2 supply. The MSM used for aerobic sludge acclimation, Chlorella sp. cultivation and PBR operation was composed of (mg L^{-1}): $NaNO_3$ (85.0); $CaCl_2 \cdot 2H_2O$ (36.8); $MgSO_4 \cdot 7H_2O$ (37.0); $NaHCO_3$ (12.6); $Na_2SiO_3 \cdot 9H_2O$ (28.4) and K_2HPO_4 (8.7); 1 mL L^{-1} of a trace elements solution and 1 mL L^{-1} of a vitamins solution. The trace elements stock solution was composed of (mg L^{-1}): $Na_2EDTA \cdot 2H_2O$ (4360); $FeCl_2 \cdot 6H_2O$ (3150); $CuSO_4 \cdot 5H_2O$ (10); $ZnSO_4 \cdot 7H_2O$ (22); $CoCl_2 \cdot 6H_2O$ (10); $MnCl_4 \cdot 4H_2O$ (180); $Na_2MoO_4 \cdot 2H_2O$ (6) and H_3BO_3 (1000); while the vitamins stock solution consisted of (mg L^{-1}): thiamine HCl (vitamin B1) (100); biotin (vitamin H) (0.5) and cyanocobalamin (vitamin B12) (0.5).

Experimental set-up

The biogas fed to both the BTF and the PBR was obtained from two pilot-scale UASB digesters anaerobically treating vinasse. The PVC digesters had a working volume of 63 L (height = 2 m, diameter = 20 cm), a total volume of 126 L (4 m total height) and were equipped with gas-solid separators according to Cavalcanti et al. [9] The digesters were agitated via liquid recirculation to prevent biomass compaction. The vinasse was collected in a sugarcane biorefinery and diluted to a final concentration of ~ 20 g COD

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L^{-1}. The UASB reactors were operated at different organic loads and recirculation rates, resulting in varying biogas production rates and compositions.

The biotrickling filter consisted of a cylindrical PVC column with a total working volume of 2.5 L (height = 0.5 m, inner diameter = 0.08 m) (Figure 1A). The BTF was packed with 2 L of 1 cm^3 cubes of polyurethane foam inserted into plastic curls in order to provide the packing material with a higher structural stability. The BTF was operated at ambient temperature in a countercurrent flow configuration: the biogas from UASB 1 was fed from the bottom of the column, while MSM was continuously irrigated from the upper part of the bed. The trickling solution was conducted to a 1.75 L external tank (height = 0.25 m, inner diameter = 0.09 m) operated under continuous agitation and recycled at ~2 m h^{-1} (diaphragm metering pump, Prominent, Germany).

The photobioreactor consisted of a transparent PVC column (total height = 0.9 m, inner diameter = 0.09 m) filled with 2 L of MSM and illuminated at ~230 µmol m^{-2} s^{-1} by 6 fluorescent lamps arranged in a circular configuration (Figure 1B). The algal-bacterial cultivation broth was continuously recycled from the top to the bottom of the PBR at 350 mL min^{-1} (3.3 m h^{-1}) in order to prevent biomass sedimentation. The biogas from UASB 2 was fed via a ceramic sparger from the bottom of the PBR, which was operated at ambient temperature.

Bioreactors operation

The acclimated anaerobic sludge (200 ml with a total suspended solid (TSS) and volatile suspended solid (VSS) concentration of 3.9 ± 0.1 and 1.7 ± 0.0 g L^{-1}, respectively) was mixed with the BTF packing material. After inoculation, the BTF was operated for 73 days with synthetic MSM. From day 74 onward, the effluent from the

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anaerobic digester previously aerated for 24 hours and supplemented with NO_3^- (by means of $NaNO_3$ addition to an initial concentration of 3 g NO_3^- L^{-1}) was used as a nutrient and electron donor source (simulating a partially nitrified digestate) in order to integrate biogas upgrading and wastewater treatment. For the first 47days, 200 mL of MSM were daily exchanged, increasing the MSM (or digestate) exchange rate to 250 mL day^{-1} from day 48 onward in order to prevent sulfate build-up. The digestate was characterized by a COD concentration of 1.82 ± 0.5 g O_2 L^{-1}, a sulfate concentration of 139.6 ± 38.5 mg L^{-1}, an alkalinity of 3553 ± 494 mg L^{-1} and a pH = 8.1 ± 0.2. The pH of the tricking solution was manually adjusted to ~ 7 by addition of NaOH (2 M) for an optimum growth of the denitrifying community. A shutdown period due to digester operational failure occurred from days 28 to 46. During the experimentation period, the biogas empty bed residence times (EBRTs) ranged from 30 to 100 s as a result of the variation on the biogas productivity of UASB 1.

The photobioreactor was inoculated with a mixture of acclimated aerobic sludge (200 ml with a TSS of 0.6 g L^{-1} and a VSS concentration of 0.3 g L^{-1}, respectively) and *Chlorella sp*. (800 mL with a VSS concentration of 0.2 g L^{-1}). The PBR was operated for 62 days at EBRTs ranging from 37 up to 146 s as a result of the variation on the biogas productivity of UASB 2. The pH was manually adjusted to ~ 7 by NaOH (2 M) addition. Cultivation medium (250 mL) was daily replaced with fresh MSM in order to provide nutrients for microalgal growth and prevent sulfate accumulation.

The inlet and outlet biogas composition (CH_4, CO_2, N_2 and H_2S) from both bioreactors was daily analyzed in a GC-TCD. Liquid samples were also periodically withdrawn from both bioreactors for the determination of sulfide, sulfate, thiosulfate, nitrite and nitrate concentrations. The optical density from the liquid medium of the PBR was also analyzed. Finally, biomass samples from the inoculum, the digestate and the BTF

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packed bed at three different heights at the end of the operation were collected and stored at −20 °C in order to evaluate the evolution of the bacterial communities.

Analytical procedures

CH_4, CO_2, N_2 and H_2S gas concentrations were analyzed in a Shimadzu GC-2014 coupled with a thermal conductivity detector and equipped with a HP-PLOT/Q (30 m × 0.54 mm × 40 µm) column. The injector and detector temperatures were set at 160 and 170 ºC, respectively. The oven temperature was initially maintained at 35 ºC for 2 min, and increased at 60 ºC min^{-1} up to 170 ºC. Hydrogen was used as the carrier gas at 24 mL min^{-1}. Sulfide concentration in the liquid phase was determined by the colorimetric methylene blue method, while sulfate, thiosulfate, nitrite and nitrate concentrations were analyzed by HPLC-IC according to Standard Methods for the Examination of Water and Wastewater. [10] The culture absorbance was analyzed by spectrophotometry (Hach DR/4000V, US).

The polyacrylamide DGGE gels for the characterization of the bacterial communities were made with denaturing gradient ranging from 45 to 65% (where 100% denaturant contains 7 M urea and 40% (v/v) deionized formamide). The electrophoresis was run for 16 h at 60 ºC and 75 V. The gel was stained with ethidium bromide and imaging was performed using an Eagle Eye TM III (Stratagene) at a UV wavelength of 254 nm. Gel images were analyzed using Bionumerics Software Vers.2.5 (Applied Maths, Belgium). The similarity coefficients of the DGGE profiles were calculated using the Pearson correlation coefficients (densitometric curve-based) and clustered using UPGMA (Unweighted Pair Group Method with Arithmetic).

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Results and Discussion

Anoxic biotrickling filter

The composition of the raw biogas fed to the anoxic BTF varied from 47 to 74% v/v for CH_4, from 16 to 48% v/v for CO_2 and from 0.4 to 3.7% v/v for H_2S, depending on the performance of UASB 1 (Figure 2A). From day 6 to 28, no H_2S was detected in the upgraded biogas regardless of the fluctuations in the EBRT, which ranged from 30 up to 100 s (Figure 2A). This removal efficiency (RE) > 99% was immediately restored after the resumption of biogas feeding following the 15-days shutdown period. Moreover, the substitution of the recycling synthetic MSM by NO_3^--supplemented effluent from the anaerobic digester did not result in a deterioration of the BTF performance, a complete H_2S removal being maintained until the end of the operating period (Figure 2B). The desulfurization performance was not affected by the sulfur inlet load (IL), which varied from 187 to 1260 g S m^{-3} h^{-1} (Figure 3B). This demonstrated the robustness of the anoxic BTF under real operating conditions (with inherent variations in the composition and flow rate of the biogas). In this sense, both a sustained elimination capacity (EC) of 677 ± 236 g S-H_2S m^{-3} h^{-1} during steady state operation and a maximum EC of 1260 g S-H_2S m^{-3} h^{-1} were found at a 100 % removal efficiency, even at the lowest EBRT of 30 s, while previous studies observed a critical EBRTs of 90 s for a complete anoxic degradation of H_2S in BTFs. [2, 11] On the contrary, no significant reductions in CH_4 or CO_2 concentrations were recorded despite the high H_2S RE achieved, with average decreases in the outlet biogas concentrations of 1.7 ± 2.7 % for CH_4, and 4.0 ± 5.2 % for CO_2 (Figure 2B). This absence of methane removal constitutes one of the main advantages of anoxic over aerobic desulfurization, where dilution of the upgraded

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biogas as a result of O_2 and N_2 (when using air as a source of O_2) addition is unavoidable. [2]

In order to ensure that H_2S was not only removed by physical absorption into the trickling liquid but also biologically oxidized, sulfide and sulfate concentrations in the liquid phase were periodically analyzed. Accumulation of S^{2-} was only detected immediately following the start-up and resumption of the BTF (Figure 3A). Hence, S^{2-} concentration reached 7.5 mg S^{-2} L^{-1} by day 9 and averaged 0.49 ± 0.28 mg S^{-2} L^{-1} from days 11 to 28. Likewise, S^{2-} concentration initially increased for two days following biogas resumption up to a maximum concentration of 11.5 mg S^{-2} L^{-1} by day 47. However, from day 50 onward, stable concentrations of S^{2-} of 0.31 ± 0.30 mg S^{-2} L^{-1} in the recycling medium were recorded despite supplementing the BTF with digester effluent. Similarly, thiosulfate was not detected in the liquid phase during the entire experimentation period. Therefore, the biological degradation of the H_2S transferred from the biogas was confirmed as sulfide was mainly oxidized to either sulfate or elemental sulfur according to Eq. 1. In this sense, sulfate initially accumulated during the first 28 days of operation reaching a maximum concentration of ~1.0 g S-SO_4^{2-} L^{-1} by day 23 (Figure 3A). The MSM renewal rate was increased from 200 mL day^{-1} to 250 mL day^{-1} from day 46 onward in order to decrease the SO_4^{2-} concentration in the recycling media. This operational change resulted in maximum sulfate concentrations of 0.66 g SO_4^{2-} L^{-1} by day 55 and in a gradual decrease to stable values of 0.37 ± 0.03 g SO_4^{2-} L^{-1} from days 62 to 73. The concentrations here recorded remained under the inhibitory values reported in literature (e.g. critical S-SO_4^{2-} concentration of 11 g L^{-1} at high sulfur ILs). [5]

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A partial oxidation of S^{2-} to S^0 has been widely reported in anoxic BTFs, the ratio between the nitrate supplied (mol N-NO_3^-) and the sulfide removed (mol S-H_2S) determining the S-SO_4^{2-}/S^0 ratio obtained. [11, 12] A sulfur mass balance was performed by subtraction in order to estimate the amount of sulfur produced in the reactor. [13] In our particular study, the molar N/S ratio fluctuated along the BTF operation from 0.4 to 1.3 mol mol^{-1} as a result of the fluctuating H_2S ILs. These values were below the minimum N/S ratio of 1.6 mol mol^{-1} required for complete H_2S oxidation to sulfate. [14] This was confirmed by the S-SO_4^{2-}/S^0 ratios estimated from a sulfur mass balance, which fluctuated from 5% to 84%. Several authors have reported the feasibility to control the oxidation products by modifying the N/S ratio during operation at a constant IL. [15] However, no clear correlation between the N/S molar ratio and the sulfate selectivity was observed in our particular system, despite the higher N/S molar ratios entailed higher S-SO_4^{2-}/S^0 values (e.g. 1.1 mol mol^{-1} resulted in 84% SO_4^{-2} conversion). These results could be attributed to the rapidly changing conditions in the BTF and the unstable nitrate and H_2S concentrations. Thus, a precise control of the sulfide oxidation by programmed NO_3^- feeding might not be feasible under real operating conditions, as previously stated by Fernández et al., [5] due to the rapidly changing H_2S loads.

Both the supply and concentration of nitrate must be carefully controlled as it acts as the electron acceptor for sulfide-oxidizing bacteria. Nitrate initially accumulated in the liquid media reaching a concentration of ~415 mg N-NO_3^- L^{-1} by day 26 (Figure 3A). In order to avoid nitrate build-up in the liquid phase, the concentration of nitrate in the fresh MSM was decreased after reactor resumption, resulting into roughly stable nitrate concentrations of 375 ± 43 mg N-NO_3^- L^{-1} from day 61 onward. According to previous studies, a minimum nitrate concentration of 20 mg N-NO_3^- L^{-1} is required to maintain maximum H_2S removals at an IL = 4.9 g S m^{-3} h^{-1}, [15] thus no nitrate limitation was

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likely to occur during the experimental period. The recorded nitrate reduction rates, which ranged from 0.01 to 0.1 g $N\text{-}NO_3^-$ L^{-1} d^{-1}, fluctuated concomitantly with the H_2S load (Figure 3B). These reduction rates were in accordance with previous denitrification data in anoxic BTFs, although they were highly dependent on the nitrate concentration and the H_2S IL. [5] The rapid reduction of nitrate compared with that of nitrite resulted in the accumulation of this latter intermediate product in the liquid media. Thus, $N\text{-}NO_2^-$ concentration gradually increased to stable values of 96 ± 15 and 183 ± 41 mg $N\text{-}NO_2^-$ L^{-1} following the start-up and resumption of BTF operation, no inhibitory effect being observed due to its accumulation. Previous studies operated at nitrite concentrations of up to 300 mg $N\text{-}NO_2^-$ L^{-1} reported no significant inhibition of the performance of the anoxic biofiltration process. [5, 14]

A typical liquid recycling velocity (U_L) of 2 m h^{-1} was set in the BTF. Despite prior studies have reported clear effects of the U_L on the H_2S mass transfer from the gas to the liquid phase (and therefore on H_2S removal), the ILs prevailing during the complete experimental period never resulted in mass transfer limitations. In this sense, minimum trickling velocities of 4.6 and 15 m h^{-1} are recommended when working at IL > 93 and 201 g S m^{-3} h^{-1}, respectively. [2, 5] Moreover, previous studies addressing the influence of U_L and EBRT on the volumetric mass transfer coefficient (k_La) of methane in a BTF demonstrated a low sensitivity of this parameter towards variations in the liquid recycling velocity during operation at high EBRTs. [16]

Finally, the microbiological analysis showed an acclimation of the microbial communities along the experimental period together with a decrease in bacterial diversity (Figure 4), which could be confirmed by the low similarity between both the inoculum and vinasse samples and the communities developed in the BTF. However,

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similar populations (>86% similarity) were established throughout the packed bed as shown by the DGGE profile of R1, R2 and R3.

Photobioreactor

Biogas from UASB 2 exhibited an average composition of 73 ± 4.9 % for CH_4, 21.5 ± 5.6 % for CO_2, 5 ± 2.5 % for N_2 and 0.5 ± 0.2 % for H_2S. On the other hand, the biogas flow rate ranged from 0.8 to 3.3 L h^{-1}, resulting in EBRTs from 37 up to 146 s (Figure 5A). Complete H_2S removal was recorded in the PBR regardless of the EBRT and the IL, achieving maximum ECs of 1052 g S-H_2S m^{-3} h^{-1} (Figure 5B). This sustained H_2S abatement despite the variations in biogas composition and flowrate confirmed the high desulfurization robustness of the FBR. However, a varying CO_2 removal was recorded during the complete experimentation period. A CO_2 fixation rate of 285 mg-CO_2 L^{-1} d^{-1} (assuming 0.5 g C per gram of biomass) was achieved based on the maximum biomass productivity recorded during the first 25 days of experimentation. The carbon mass balance showed that 100% of the C-CO_2 removed in the PBR was recovered as biomass. It was thus hypothesized that the low CO_2 mass transfer recorded, likely mediated by the low pH and to the low EBRT tested, might have limited microalgal growth and hence CO_2 removal from biogas. In this sense, the C-fixation rate resulted in removal efficiencies in the PBR ranging from 4.2 to 57.4 %, with an average RE of 23.0 ± 11.8 % (Figure 5B). In order to elucidate the limiting step during biogas in the PBR, an experiment was performed by day 62 which consisted of increasing the pH of the cultivation medium to 8.1 by NaOH addition and measuring the corresponding CO_2 concentration in the gas phase (data not shown). The results showed that the enhanced CO_2 concentration gradient between the bulk gas phase and the aqueous phase at a higher pH supported CO_2 removals of 62%. Thus, a better biogas upgrading

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performance in the PBR would be expected at higher operational pHs as a result of the enhanced CO_2 mass transfer and the herein confirmed subsequent CO_2 sequestration by microalgae. [17] At this point it should be highlighted that the pH in the PBR was maintained at 7 to allow for a fair performance comparison with the anoxic BTF. Finally, a negligible average methane removal of 5.7 ± 4.6 % was recorded in the PBR (Figure 5B). Moreover, no inhibition of the microalgal-bacterial activity at methane concentrations of up to 80 % was observed, as recently demonstrated by Wand and co-workers [18]. The maximum growth rate estimated (155 g L^{-1} d^{-1}) was comparable with previous reported values for *Chlorella* sp. MB-9 cultures (276 g L^{-1} d^{-1}) supplemented with artificial biogas with a composition of 80% of CH_4 and 20% of CO_2. [19]

The initial increase in sulfate concentration in the cultivation medium up to ~400 mg S-SO_4^{-2} L^{-1} by day 24 and its subsequent stabilization at 409 ± 67 mg S-SO_4^{-2} L^{-1} showed that H_2S was not only transferred from the biogas to the liquid phase, but also biologically oxidized according to Equation 2 (Figure 6). This result was further confirmed by the low S^{2-} concentrations recorded, which remained constant throughout the experimental period at 1.0 ±0.4 mg S^{-2} L^{-1} (Figure 6). In this context, the measurement of the dissolved O_2 concentration showed a roughly stable value of ~ 2.3 mg L^{-1} as a result of the balance between the photosynthetically O_2 produced and the O_2 consumed by bacteria for H_2S oxidation. This guaranteed the almost complete oxidation to sulfate of the H_2S transferred from the biogas.

Whereas several authors have previously investigated the CO_2 fixation capacity of *Chlorella* sp., [17-19] the number of studies devoted to elucidate the potential of this microalga for biogas upgrading is still scarce. In this context, Mann et al. [20] successfully eliminated up to 97% of CO_2 and 100% of H_2S from a synthetic biogas (41 % CO_2 and 438 ppm_v H_2S) in a batch biogas upgrading experiment (gas residence time = duration

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of the experiment) conducted in tubular photobioreactor. Nevertheless, the upgraded biogas was contaminated with a high content of oxygen with the subsequent decrease in methane content. Following this study, Bahr et al. and Serejo et al. [7, 21] demonstrated the potential of an alkaliphilic microalgal-bacterial consortium for the simultaneous removal of H_2S and CO_2 from biogas coupled with nutrient removal from centrates and anaerobically digested vinasse, respectively. The pilot high-rate algal ponds interconnected to external CO_2–H_2S absorption columns used in those studies supported removals of 100 % from H_2S and 40-80 % for CO_2. However, these high CO_2 removals were obtained at the expenses of an additional gas-liquid absorption stage operated at significantly higher EBRTs (from 16 min up to 8.3 hours) than those tested in our study (37 - 146 s). Moreover, the composition of the synthetic biogas used by Bahr et al. and Serejo et al. [7, 21] was constant and H_2S concentration was <5000 ppm_v, far below the values reached by the UASB biogas treated in our system. Finally, it is important to remark that, despite the CO_2 removal efficiencies here obtained were moderate, they were comparable to those achieved under indoors microalgae cultivation, and higher fixation rates, and therefore enhanced CO_2 removals from biogas, would be expected if the PBR was operated outdoors.

In brief, the desulfurization performance achieved in both BTF and PBR was similar, although slightly lower S^{2-} concentrations were recorded in the liquid medium of the BTF. Both systems exhibited a high robustness towards the variations in biogas flowrate and composition typically found in real biogas from anaerobic digesters. Moreover, the desulfurization capacity of the BTF was immediately recovered after a 15-days biogas shutdown. Despite CO_2 removal in the PBR was limited by the mass transport of this biogas pollutant to the aqueous phase, higher removals were recorded compared with

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those of the BTF. This study confirmed the potential of the symbiosis between microalgae and bacteria as a technological platform for the simultaneous removal of CO_2 and H_2S from biogas.

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Figure captions

Figure 1. Schematic diagram of the biotrickling filter (A) and the photobioreactor (B) for biogas upgrading.

Figure 2. Time course of (A) the inlet (■) and outlet (□) H_2S concentrations, and EBRT (×) of the biogas upgraded in the BTF; and (B) CH_4 (♦), CO_2 (◊) and H_2S (□) removal efficiencies in the BTF. The grey area represents the shutdown period and the vertical dashed line the beginning of digestate supplementation.

Figure 3. Time course of (A) sulfate (□), nitrate (●), nitrite (○) and sulfur (×) concentrations in the BTF recycling media; and (B) Sulfur inlet load (◆) and nitrate consumption rate (◇). The grey area represents the shutdown period and the vertical dashed line the beginning of digestate supplementation.

Figure 4. Bacterial similarity dendogram (UPGMA clustering) and DGGE profile of the bacterial communities present in the inoculum, digestate and the samples from the carrier at the top (R1), medium (R2) and bottom (R3) of the BTF.

Figure 5. Time course of (A) the inlet (■) and outlet (□) H_2S concentrations, and EBRT (×) of the biogas upgraded in the FBR; and (B) CH_4 (♦), CO_2 (◊) and H_2S (○) removal efficiencies in the FBR.

Figure 6. Time course of sulfate (□) and sulfur (×) concentration in the PBR cultivation broth, and sulfur inlet load (◆).

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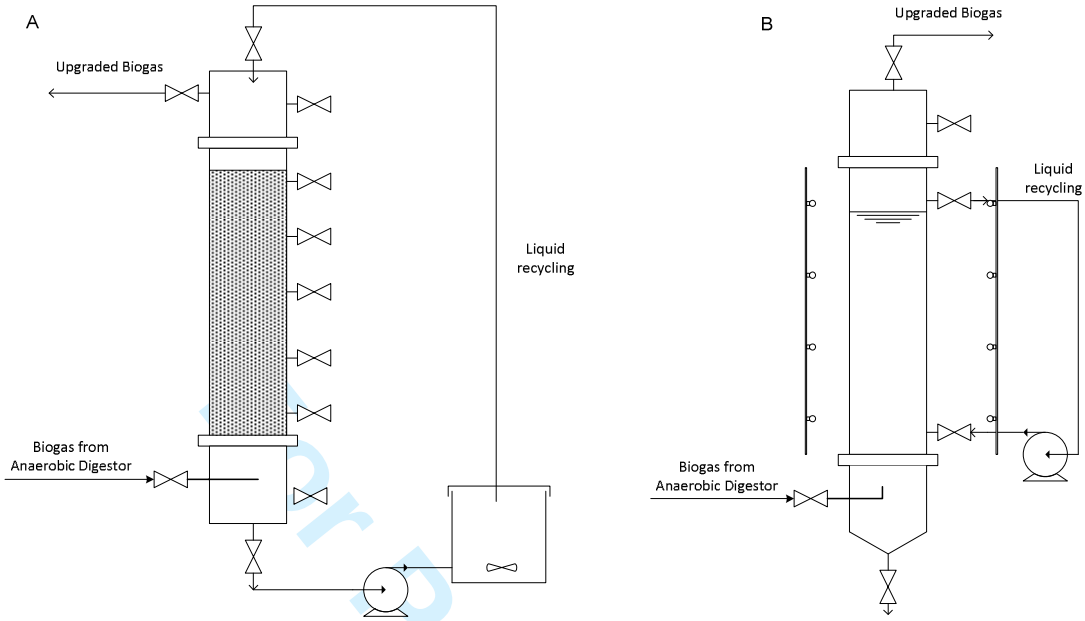
Figure 1.



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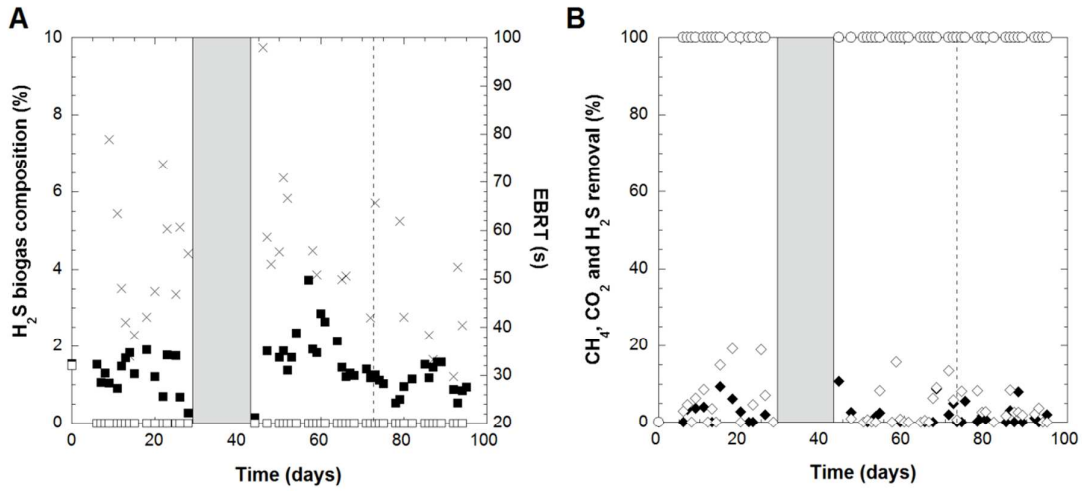

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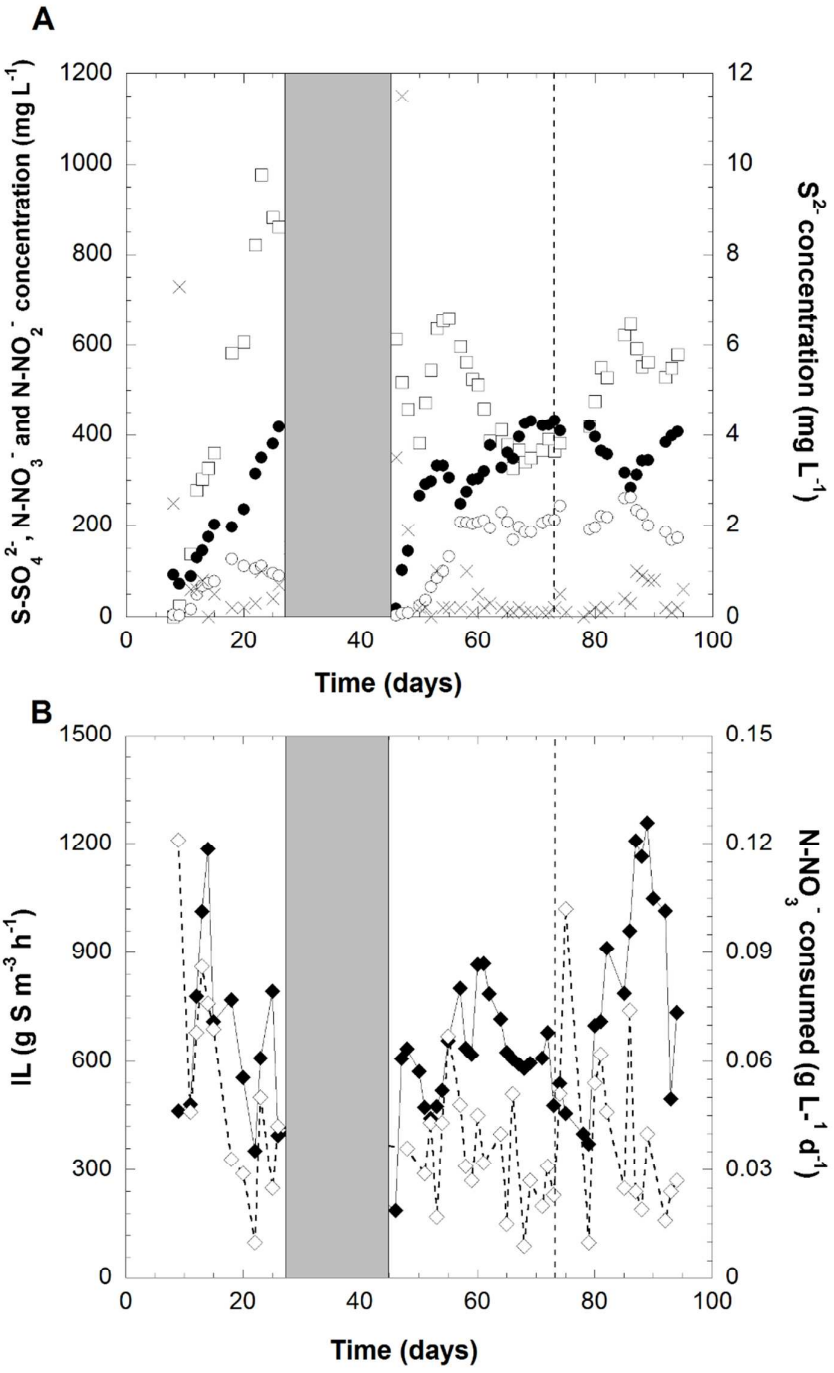

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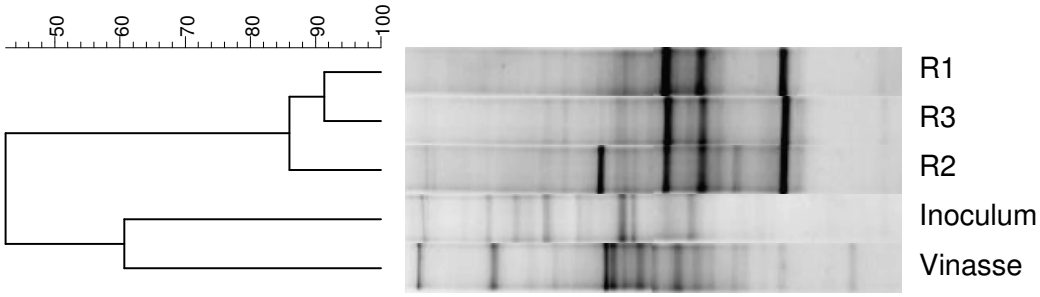

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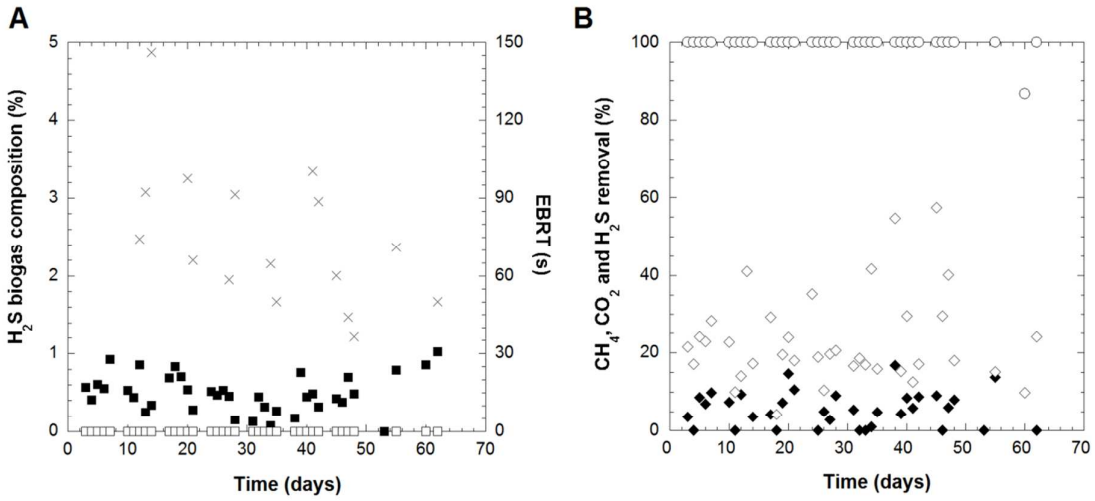

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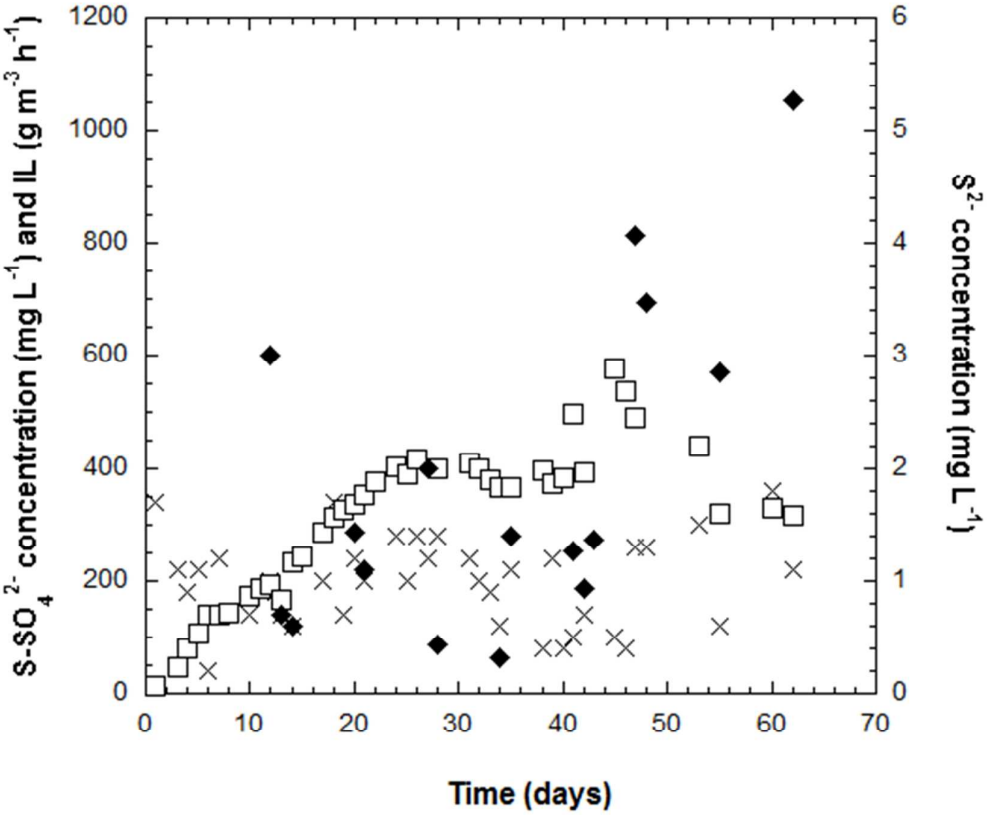

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