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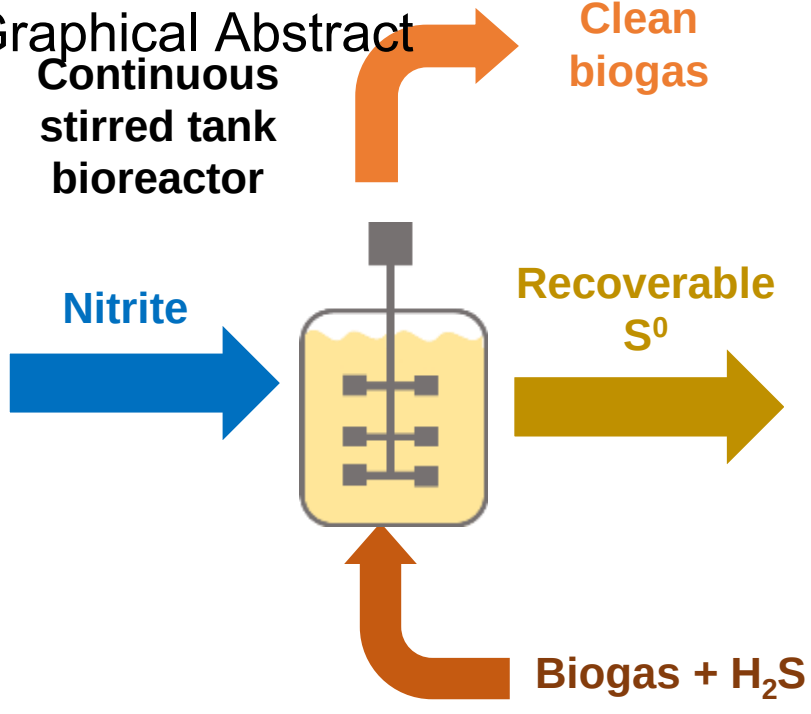
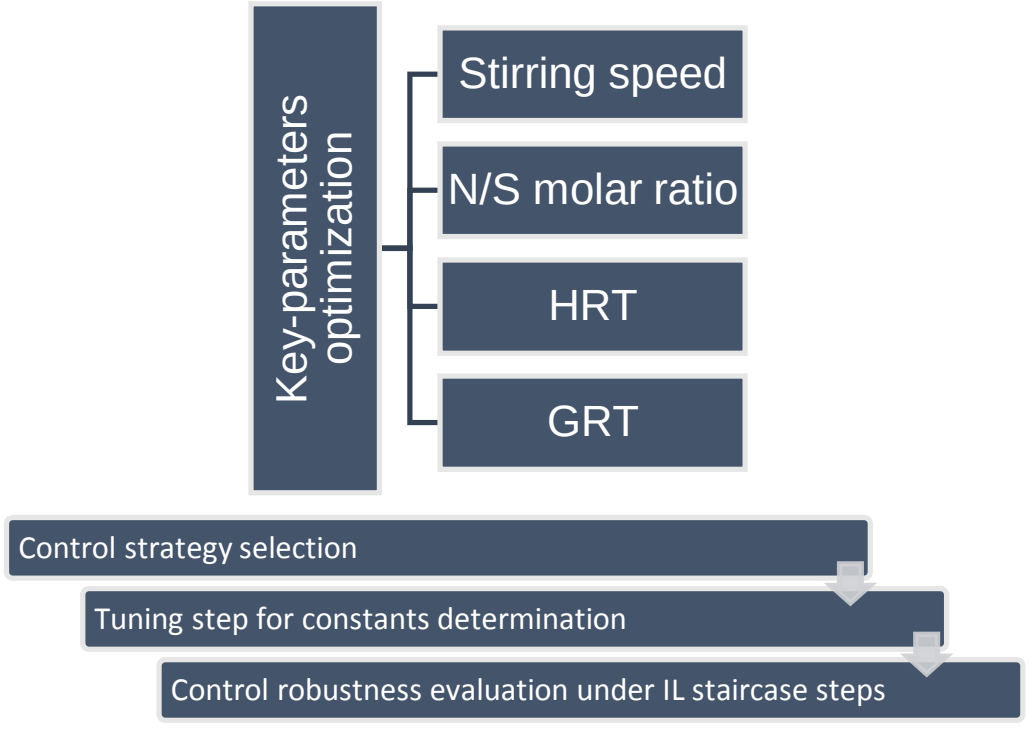
Graphical Abstract





- Nitrite-reducing sulfide-oxidizing bacteria is sensitive to shear stress forces.
- N/S molar ratios below 1.1 mol mol^{-1} compromises the H_2S RE.
- The highest dilution times biomass product was found at an HRT of 42 h.
- An EC of 166.0 ± 7.2 gS-H_2S m^{-3} h^{-1} (RE = 71.7%) was obtained under a GRT of 41s.
- Proportional – Integral control worked successfully under stepwise variable IL.

Anoxic biogas biodesulfurization promoting elemental sulfur production in a Continuous Stirred Tank Bioreactor

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Keywords: biogas; elemental sulfur; CSTBR; hydrogen sulfide; autotrophic denitrification; feedback control

Abstract

Biological desulfurization of biogas has been extensively studied using biotrickling filters (BTFs). However, the accumulation of elemental sulfur (S^0) on the packing material limits the use of this technology. To overcome this issue, the use of a continuous stirred tank bioreactor (CSTBR) under anoxic conditions for biogas desulfurization and S^0 production is proposed in the present study. The effect of the main parameters (stirring speed, N/S molar ratio, hydraulic residence time (HRT) and gas residence time (GRT)) on the bioreactor performance was studied. Under an inlet load (IL) of 100 g S-H_2S m^{-3} h^{-1} and a GRT of 119 s, the CSTBR optimal operating conditions were 60 rpm, N/S molar ratio of 1.1 and a HRT of 42 h, in which a removal efficiency (RE) and S^0 production of 98.6 ± 0.4% and 88% were obtained, respectively. Under a GRT of 41s and an IL of 232 g S-H_2S m^{-3} h^{-1} the maximum elimination capacity (EC) of 166.0 ± 7.2 g S-H_2S m^{-3} h^{-1} (RE = 71.7 ± 3.1%) was obtained. A proportional-integral feedback control strategy was successfully applied to the bioreactor operated under a stepped variable IL.

Keywords: biogas; elemental sulfur; CSTBR; hydrogen sulfide; autotrophic denitrification

1.- Introduction.

In a world with an ever-growing energy demand, the search for alternatives to the use of fossil fuels has received much attention over the last two decades. The promotion of renewable energy sources has been considered as an environmentally friendly and sustainable solution to meet the needs of tomorrow's society [1]. Among other renewable energy sources such as wind or solar energies, biogas has gained interest in terms of its regular and predictable production flow rates [2].

After proper treatment, biogas can be used in electricity and heat generation using a combined heat and power engine or as fuel in a solid oxide fuel cell [3]. Moreover, if the methane (CH_4) concentration is increased, the upgraded biogas can be used as fuel for vehicles or injected into gas grids [4]. Biogas, which is originated from the anaerobic digestion of organic matter, is mainly composed of CH_4, in a range of 55-75 vol%, and carbon dioxide (CO_2), in the range of 30-45 vol%. The final composition of the biogas is mainly dependent on the organic matter source and the digestion system. Apart from the major components, biogas is composed of other trace pollutants. Among these, hydrogen sulfide (H_2S), whose concentration in biogas can reach significant values ranging from 0.5 to 2 vol%, is widely considered to be the most toxic and corrosive and its mere presence can severely limit biogas usage [5]. For example, it has to be removed from biogas because, during combustion, the presence of H_2S can lead to harmful by-products such as sulfur oxides (SO_x) and cause corrosion damage to equipment. Hence, biogas has high energy content but must be desulfurized for its valorization.

H_2S can be removed from biogas by physico-chemical techniques (adsorption, absorption, membrane separation, etc.) [6] or by biological techniques where microorganisms degrade the pollutants. Nowadays, biological processes are considered to be more environmentally friendly and cost-efficient [7]. So, their use has gained

interest over the last two decades. Traditionally, biological desulfurization has been carried out by headspace micro aeration of digesters [8] and through the use of biotrickling filters (BTFs) under aerobic or anoxic conditions using oxygen (O_2) or nitrate/nitrite (NO_3^-/NO_2^-) as electron acceptors, respectively [9–14]. Despite the BTF being shown to achieve high elimination capacities (ECs), robustness and cost-effectiveness, a common drawback of its use in biological desulfurization is the accumulation of elemental sulfur on the packing material. This sulfur aggregation, provoked by working with low concentrations of the electron acceptor, results in pipe clogging, which leads to higher pressure drops and system flooding, causing operation shutdowns and high maintenance costs [15,16]. Sulfur production can be reduced by a higher electron acceptor feeding rate favoring complete oxidation of sulfide to sulfate. However, the production of sulfate as the main oxidation product of the anoxic desulfurization is undesirable because firstly, its production entails a high operational cost if commercial nitrate/nitrite is used , and secondly, it can be reduced again to sulfide under anaerobic conditions [17]. Nevertheless, the operational costs can be greatly reduced if the nitrate/nitrite is produced by nitrification of ammonium-rich wastewater [7]. The partial oxidation of sulfide to sulfur is advantageous because it can be recovered from the effluent by settling and used as an electron donor for autotrophic denitrification [18,19] and as a renewable feedstock for the fertilizer and chemical industries [20].

A possible solution to overcome the main shortcomings of BTF usage for sulfur production would be the use of suspended biomass bioreactors (SBB). Sulfur could be recovered from these bioreactors because of the absence of support to which it would otherwise become adhered. Some previous work on sulfide partial oxidation to sulfur in SBBs has been carried out using oxygen as electron acceptor [21–23]. However, despite the application of continuous SBBs to biological aerobic desulfurization of biogas being

widely investigated, scarce research has been conducted on the performance of the SBB under anoxic conditions for biogas desulfurization. Anoxic desulfurization has some advantages over the aerobic process such as the absence of biogas dilution, no oxygen mass transfer limitation, reduction in explosion risks and a better N/S molar ratio manipulation [11,24]. NO_2^- has been successfully used in BTFs [9] without reduction of the H_2S removal efficiency (RE) (94.74 ± 0.01%). Bearing this in mind, the use of NO_2^- is proposed in the present work as electron acceptor.

The main disadvantages of anoxic biodesulfurization versus the aerobic type are the higher operation costs if the source of NO_3^-/NO_2^- is a chemical reactant and the associated storage risks. These problems could be solved by using an effluent from a nitrification process. In this way, two toxic pollutants such as NH_4^+ and H_2S can be transformed into harmless oxidation products such as N_2 and elemental sulfur. The production of NO_2^- by partial nitrification over NO_3^- has several advantages such as lower operation costs (less aeration is required) and its employment has been demonstrated to allow a faster growth rate, and biological reaction, in the case of partial oxidation of sulfide to sulfur [25]. Whether chemically or biologically produced, the nitrite feeding has to be optimized in order not to create extra costs and a sulfate- and nitrite-rich purge. In order to accomplish this task, several feedback control strategies (e.g. on-off, proportional (P), proportional-integral (PI) and proportional-integral-derivative (PID)) have been successfully applied to anoxic desulfurization systems [26,27]. Among all these control strategies, PI control mode stands as the most suitable for implementation in an anoxic biodesulfurization process because results obtained by other researchers showed that its application led to less oscillation in the nitrite feeding [27]. However, to the best of our knowledge, the application of control strategies to the anoxic biodesulfurization in SBBs has not been reported.

Low biomass concentration, high shear stress forces, or lower mass transfer rates are some of the characteristic features of CSTBRs that widely differ from BTFs and which have not been widely studied. Taking into consideration the aforementioned matters, the study of the anoxic desulfurization process in a CSTBR becomes necessary to gain knowledge about the effect of different operational parameters on the overall performance. Therefore, the work reported here was aimed at studying the effect of the governing parameters in the bioprocess, such as stirring speed, dilution rate (D), N/S molar ratio and gas residence time (GRT) in order to optimize the bioprocess performance and enhance the sulfur production.

2. Materials and Methods

2.1. Experimental Setup Description

The experiments were carried out in a CSTBR (Applikon Biotechnology BV, The Netherlands) with a working volume of 5.5 L (Fig. 1). The temperature was controlled by a thermostat (RM6 Lauda, Germany) at 30ºC. The bioreactor was fed with biogas substitute (mixture of H_2S and N_2) controlled by two mass-flow controllers (F-201C, Bronkhorst High-Tech B.V., The Netherlands). Oxidation-reduction potential (ORP) and pH were measured with a multiparametric analyzer (Crison Multimeter 44, Hach Lange S.L.U, Spain). The pH was set to 7.8 and controlled by the addition of H_3PO_4 (2N) and NaOH (2N). The system was monitored and controlled using LabVIEW™ (Version 2015, National Instruments™, USA).

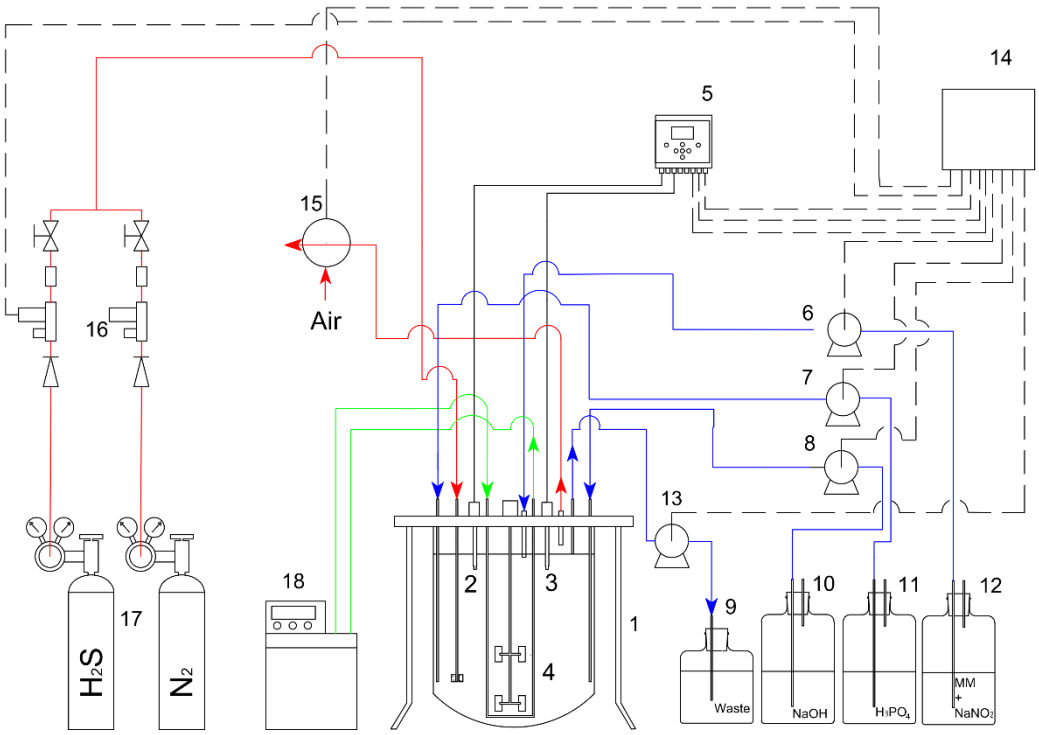


Fig. 1 Experimental setup. (1) CSTBR, (2) ORP probe, (3) pH probe (4) Stirrer, (5) Multimeter 44, (6) Analog peristaltic pump, (7) H_3PO_4 peristaltic pump, (8) NaOH peristaltic pump, (9) Waste container, (10) NaOH container, (11) H_3PO_4 container, (12) Mineral medium + $NaNO_2$ container, (13) Discharge peristaltic pump, (14) PC and control system, (15) H_2S sensor, (16) Mass flow controllers, (17) Gas cylinders, (18) Thermostatic bath.

2.2. Mineral medium composition

The mineral medium was adapted from ATCC-1255 *Thiomicrospira denitrificans* medium [24] by enriching with $NaNO_2$ (1.74 – 3.12 g $N\text{-}NO_2^-$ L^{-1}) and $NaHCO_3$ 1.89 (g L^{-1}) as carbon source.

2.3. Inoculum preparation

The bioreactor was inoculated with biomass from an anoxic BTF fed with nitrite used in previous works [27]. To desorb the microorganisms from the packing material, a selection of Pall rings was extracted from the top of the packed bed of the BTF. These were submerged in 200 mL of mineral medium and sonicated using an ultrasonic bath

(Ultrasons-H, Selecta, Spain) at 40 kHz for 15 min. The mineral medium with suspended microorganisms was subsequently inoculated to the CSTBR.

2.4. Reactor operation

Throughout the long-term operation of the CSTBR (more than 160 days), a series of six experiments was conducted. The main experimental conditions are listed in Table 1. All the experiments were performed in continuous operation mode except Exp.1 which was accomplished in batch mode.

Initially, a start-up period of 60 days was required to acclimatize the bacterial consortium and find the proper conditions to grow it. Once the steady-state conditions were reached, the experiments were conducted.

In Exp 1. the effect of stirring speed (under a constant IL of 70 g S-H_2S m^{-3} h^{-1}) on H_2S RE was studied. For nitrite feeding regulation, the automated feedback control mode proposed by Almenglo et al. [15] was applied. Herewith, a concentrated nitrite solution (400 g $NaNO_2$ L^{-1}) was discontinuously fed into the bioreactor using ORP measurement as a control variable (Fig. S2, control loop 5). So, when nitrite concentration decreased in the medium, H_2S started to accumulate in the broth leading to a sharp decrease in ORP. Once the ORP value reached the set-point (-400 mV), 6 mL of the concentrated nitrite solution was automatically supplied to the bioreactor. This addition, which caused a nitrite concentration increase in the bioreactor up to 90 mg N-NO_2 L^{-1}, led to an ORP increase to normal working values (from -360 to -380 mV). Every 24 h the stirring speed was stepwise increased by 50 rpm from 60 to 210 rpm while the H_2S_{out} and ORP behavior were monitored.

The effect of the N/S molar ratio on H_2S RE was studied in Exp. 2 at constant IL (100 g S-H_2S m^{-3} h^{-1}). The N/S molar ratio is the ratio between the amounts in moles of nitrogen

in the form of nitrite present in the liquid feed and sulfur in the form of sulfide present in the biogas substitute. The outlet H_2S concentration was monitored while N/S molar ratio values were changed every 2 h. Different N/S molar ratios were tested by modifying the inlet flow of nitrite, which resulted in a HRT variation considered to be negligible due to the short duration of the experiment.

Exp. 3 consisted of an optimization of the hydraulic retention time (HRT). Different HRT values ranging from 55 to 30 h were studied under constant IL (100 g S-H_2S $m^{-3}h^{-1}$) and N/S molar ratio (1.1). The experiment lasted for 40 days, and was aimed at obtaining consistent data at the steady-state. Conditions were maintained until the bioreactor was considered to be at steady state, i.e. when changes in biomass concentration were close to zero over a minimum of 3 times the HRT. Punctual measurements were performed to monitor biomass concentration and sulfur production for ~5 days after the steady-state condition was reached.

The influence of GRT was investigated in Exp. 4 for 48 h. Initially, under the previously optimized experimental conditions of N/S molar ratio (1.1) and HRT (42 h), the inlet gas flow was progressively increased every 2 h to test GRTs from 119 to 40 s keeping the IL constant at 100 g S-H_2S m^{-3} h^{-1} (Exp. 4.1). In this way, the $[H_2S]_{in}$ decreased along the duration of the experiment from 2500 to 853 ppm_v. In the second part of the experiment, $[H_2S]_{in}$ was kept constant at 2000 ppm_v while the GRT was lowered in the same way as in Exp 4.1. Thus, the IL was increased from 79 to 232 g S-H_2S m^{-3} h^{-1}.

Finally, a PI feedback control was implemented in the system. Previous studies with anoxic BTFs [26,27] demonstrated that the use of a PI control led to better stability of the controlled variable compared with a PID strategy. In PI control the error between the set-point and the controlled variable was used to calculate the proportional and integral terms. By the calculus of these terms, the action to be applied to the manipulated variable, in this

case, the flow of the analog pump that feeds nitrite (Fig. S3, control loop 2), could be determined. The method used to estimate the controller parameters as proportional gain (k_p) and the integral time (τ_i) was the Ziegler-Nichols rule [28] based on step response. Hence, the inlet H_2S concentration was increased by 20% from 2200 to 2640 ppm_v (IL from 87 to 104 g S-H_2S m^{-3} h^{-1}). The graphical information obtained during this step-response test in open-loop was used to determine the gain (K), delay time (L), and constant time (T). K corresponds with the response increase while L and T were obtained using the maximum slope tangent of the response curve. The k_p and τ_i constants were determined using the mathematical equations Eq. 1 and Eq. 2 reported by Hägglund and Åström [29]. The integral (k_i) gain was subsequently determined using Eq. 3 [28].

$$k_p = \frac{0.9\,T}{KL} \quad \text{Eq. 1}$$

$$\tau_i = 3L \quad \text{Eq. 2}$$

$$k_i = \frac{k_p}{\tau_i} \quad \text{Eq. 3}$$

Under this PI control, a profile of stepped variations in the IL (139.2 to 73.2 g S-H_2S m^{-3} h^{-1}) was applied to test the viability of the proposed control over an 8 day period.

220

221

222 **Table 1.** Operational conditions in the different experiments carried out.

Exp.	IL ($gS\text{-}H_2S\ m^{-3}\ h^{-1}$)	HRT(h)	GRT(s)	N/S molar ratio ($mol{:}mol^{-1}$)	Stirring speed (rpm)	$[H_2S]_{in}$ (ppm_v)	Studied Variable	Days
1	70	-	139	Variable	60 110 160 210	1800	Stirring speed	7
2	100	66 47 36 30	119	1.99 1.6 1.33 1.06 0.78	60	2500	N/S molar ratio	1
3	100	55 48 42 36 30	119	1.1	60	2500	HRT	40
4.1	100	42	119 104 89 73 57 41	1.1	60	2500 2171 1841 1512 1182 853	GRT (constant IL)	1
4.2	79 90 106 129 165 232	59 51 43 36 28 20	119 104 89 73 57 41	1.1	60	2000	GRT (constant $[H_2S]_{in}$)	1
5	139-73	40-21.5	119	1.6-0.9	60	3500-1850	Stepwise changes in IL	3

223

224 ## 2.5. Analytical methods

225 A biogas substitute (H_2S +N_2) was used to feed the system. The concentration of H_2S in
226 the inlet gas stream (>1000 ppm_v) was measured using a gas chromatograph (450-GC,
227 Bruker, Spain) with a thermal conductivity detector (TCD) and Poraplot Q plot FS 25 m
228 x 0.53 mm column. The oven temperature was set at 33 ºC (2 min) and then increased
229 33–80 ºC (10 ºC min^{-1}), while the temperatures of the injector and detector were set at
230 150 ºC. Samples with H_2S concentrations below 100 ppm_v were measured with a gas
231 chromatograph (450-GC, Bruker, Spain) equipped with a pulsed flame photometric
232 detector (PFPD) and Wcot Fused Silica 30m x 0.32 mm capillary column, under the

following experimental conditions; oven temperature: 35 ºC (1.5 min), 33–80ºC (15 ºC min^{-1}), injector temperature: 250 ºC and detector temperature: 200 ºC.

The CSTBR outlet gas stream was monitored using an electrochemical H_2S sensor (SureCell, Euro-Gas Management Services, UK). Due to the electrochemical H_2S sensor having a limited detection range of 0–200 ppm_v, the outlet gas stream was diluted with air before passing through the sensor.

Samples were taken from the outlet liquid stream. Sulfate and nitrite were measured by a turbidimetric method (4500-sulfate E) and a colorimetric method (4500-nitrite B), respectively, according to the Standard Methods [30] using a Spectroquant® Pharo 300 spectrophotometer (Merck, Germany). Sulfur production was determined by making a mass balance by subtraction [24]. Biomass concentration was determined by total Kjeldahl nitrogen (TKN) using a Kjletec8200 Unit (Foss, Sweden). To perform this analysis, 100 mL of the medium present in the CSTBR was centrifuged (13,100 x g, 6 min) and resuspended in distilled water twice to wash out the residual ammonium present in the medium. Next, the pellet was transferred to a digestion tube in which 7 g of K_2SO_4 and 0.8 g of $CuSO_4 \cdot 5H_2O$ were added and dissolved into 12 mL of 95% (w/w) H_2SO_4. Then, the sample was digested at 420 ºC for 60 min. After digestion, the samples were cooled to room temperature. Finally, the ammonium present in the digested samples was transferred to a receiver solution (H_3BO_3 4% (w/v)) by distillation (destilator Kjeltec™ 8200, Foss Iberia, S.A, Spain), using 80 mL of distilled water and 40% (w/w) NaOH. The final amount of ammonium present in the sample was determined by acidometric titration of the receiver solution with a standardized HCl solution (between 0.1 N and 0.2 N).

3.- Results and discussion

3.1 Effect of the stirring speed on the RE

In a CSTBR the stirring speed has a large influence on the mass transfer of compounds from the gas phase to the liquid phase [31]. Using the experimental set-up described above, when the stirring speed was increased while keeping the GRT constant (139 s), the volumetric oxygen transfer coefficient (k_La) increased from 7.56 h^{-1} at 60 rpm to a maximum of 14.94 h^{-1} at 210 rpm. The same test was performed using a realistic concentration of elemental sulfur and no significant influence on the mass transfer from gas to liquid phase was found (Fig. S1). Therefore, an improvement in the H_2S RE could be expected by increasing the stirring speed.

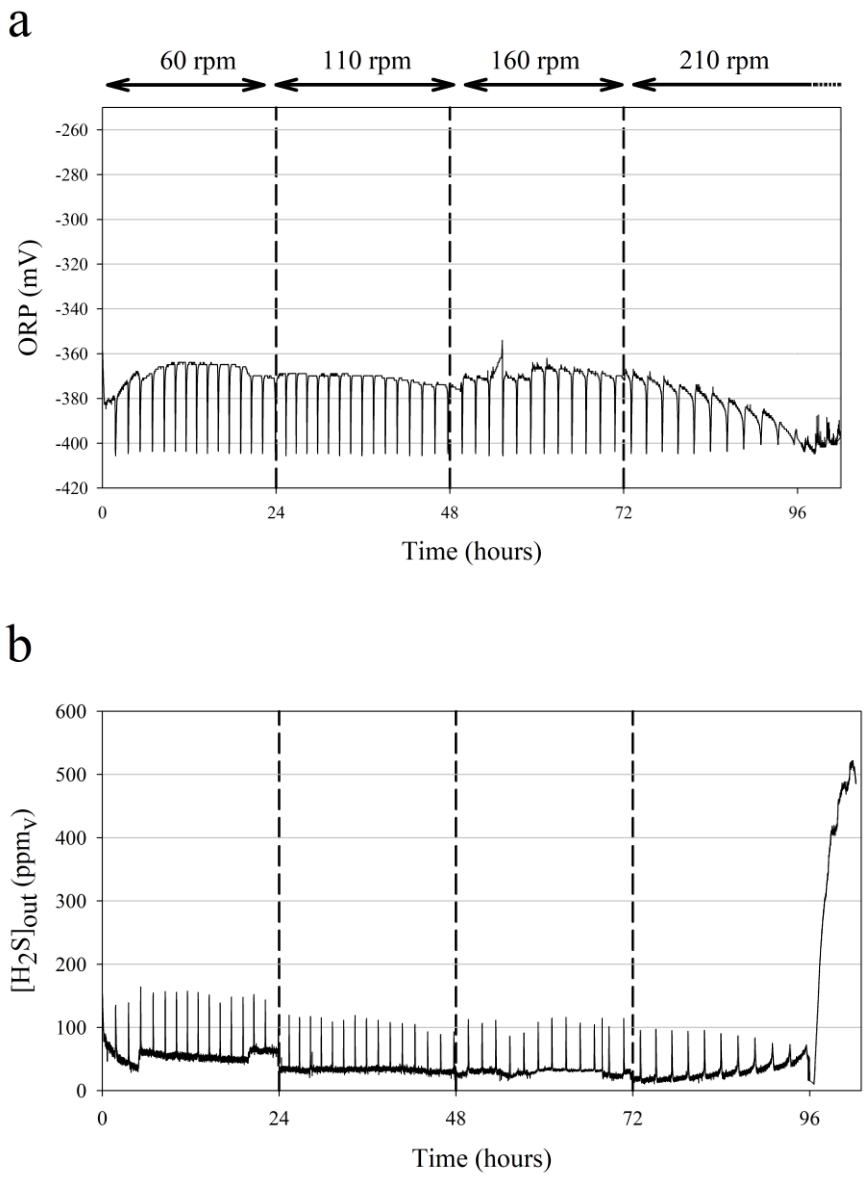


Fig. 2 - Profiles during Exp. 1 of: (a) ORP values; (b) H_2S concentration present in the outlet.

After inoculation, the CSTBR was operated for more than 60 days in order to obtain an acclimatized biomass and reach a pseudo-steady state to start Exp. 1 (data not shown). The effect of the stirring speed on the H_2S_{out} and ORP profiles is represented in Fig. 2. These profiles are characteristic of the feedback control [9,15] operation mode previously described in Section 2.4. Once nitrite was depleted, H_2S_{out} increased from 32.6 ± 11.9 ppm_v to 123.7 ± 32.6 ppm_v and H_2S started to accumulate in the medium leading to a rapid ORP decrease. When the ORP reached the set-point (-400 mV), the supplied nitrite was immediately used by the bacterial consortium to degrade H_2S, restoring the H_2S_{out} and ORP to normal working values. Fig. 2a shows how the ORP profiles were quite similar when low stirring speeds (60-160 rpm) were applied, showing an average of -371.5 ± 0.8 mV. In contrast, when the bioreactor was operated at 210 rpm, ORP working values started to decrease (385.8 ± 12.0 mV), indicating sulfide accumulation in the medium and leading to a working operation shutdown. Similar behavior was identified when H_2S_{out} was analyzed (Fig. 2b). It remained approximately constant during the first three stages (41.3 ± 16.9 ppm_v). When the CSTBR was operated at 60, 110 and 160 rpm, the average H_2S_{out} ranged from 31.5 ± 11.2 ppm_v to 58.0 ± 11.9 ppm_v. However, when the stirring speed was increased to 210 rpm, the H_2S_{out} started to increase until a large peak of H_2S up to 522.2 ppm_v was found in the outlet. At the end of the experiment, aiming to gain insights into the ability of the biomass to recover from potential damage, the CSTBR stirring speed was lowered to 110 rpm and a lower IL (57 g S-H_2S m^{-3} h^{-1}) was applied to the bioreactor. Despite these "soft" conditions being maintained for three days, the biomass was not able to recover forcing a re-inoculation of the bioreactor.

All these data indicate that the biomass present in the CSTBR responsible for the sulfide oxidation is shear stress sensitive. The shear forces are mainly due to fluid-mechanical

stress induced directly by the stirring devices or by gas bubbles bursting [32]. An acceptable estimation of the shear stress (τ_{Avg}) and rate (γ_{Avg}) coefficients for Newtonian fluids could be made using their relation (Eq. 4) with viscosity (μ) and the Metzner and Otto equation (Eq. 5), which has been demonstrated for Newtonian-fluids in laminar flow, transitional flows, and a portion of the turbulent regime. K is the Metzner – Otto constant and depends on the impeller.

$$\tau_{Avg} = \mu \cdot \gamma_{Avg} \quad \text{Eq. 4}$$

$$\gamma_{Avg} = K \cdot N \quad \text{Eq. 5}$$

The γ_{Avg} near the Rushton impeller installed in the bioreactor ranged from 12 to 42 s^{-1} at 60 and 210 rpm, respectively. The γ_{Avg} forces calculated are in line with those reported in stirred vessels by Merchuk [33] .These γ_{Avg} corresponded to τ_{Avg} which ranged from 0.012 to 0.042 Pa. Many anaerobic bacteria are shear sensitive [34], but damage usually occurs at higher agitation rates than those used in this study [35,36]. Therefore, the present bacterial consortium seems to be especially sensitive to these forces. Possible solutions to avoid causing damage to the bacterial consortium could be a reduction of the stirring speed, which would in turn decrease the energy requirements of the bioreactor, or the use of stirrers designed to minimize shear forces.

3.2 Effect of N/S molar ratio on RE

Determination of the optimal N/S molar ratio is essential because of its great influence on the product selectivity and H_2S RE. Since elemental sulfur is the desired oxidation product and most authors report that low N/S molar ratios favor the production of elemental sulfur over sulfate [37–39], Exp. 2 was aimed at finding the lowest N/S molar ratio without a decrease in the H_2S RE. At a constant IL of 100 g S-H_2S m^{-3} h^{-1}, H_2S_{out}

concentration values were monitored while the N/S molar ratios were progressively lowered.

Average H_2S RE values versus the different N/S molar ratios are shown in Fig. 3. N/S molar ratio determines the sulfur selectivity [24,40,41]. Low N/S molar ratio values favor elemental sulfur production (Eqs. 6 and 7) over sulfate (Eqs. 8 and 9) [37].

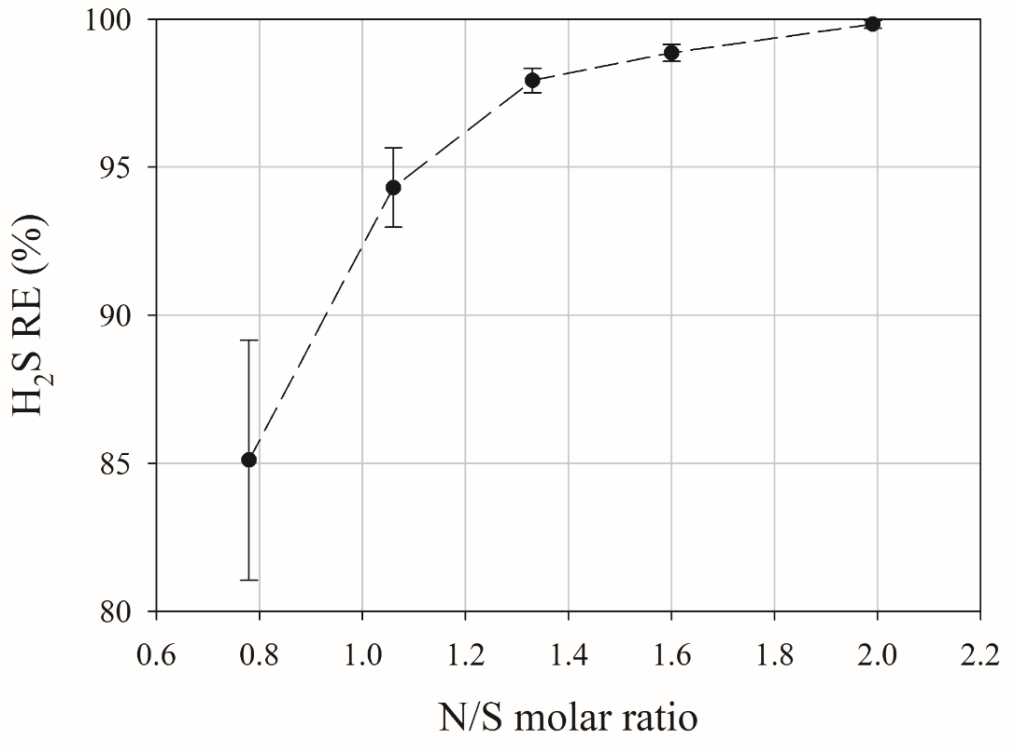
$$5\,HS^- + 2\,NO_3^- + 7\,H^+ \rightarrow 5\,S^0 + N_2 + 6\,H_2O \qquad (\Delta G° = -1264\ \text{kJ mol}^{-1}) \qquad \text{(Eq. 6)}$$

$$S^{2-} + 0.67\,NO_2^- + 2.67\,H^+ \rightarrow S^0 + 0.33\,N_2 + 1.33\,H_2O \quad (\Delta G° = -240.3\ \text{kJ mol}^{-1}) \qquad \text{(Eq. 7)}$$

$$5\,HS^- + 8\,NO_3^- + 3\,H^+ \rightarrow 5\,SO_4^{2-} + 4\,N_2 + 4\,H_2O \qquad (\Delta G° = -3848\ \text{kJ mol}^{-1}) \qquad \text{(Eq. 8)}$$

$$S^{2-} + 2.67\,NO_2^- + 2.67\,H^+ \rightarrow SO_4^{2-} + 1.33\,N_2 + 1.33\,H_2O \quad (\Delta G° = -920.3\ \text{kJ mol}^{-1}) \qquad \text{(Eq. 9)}$$

These experiments revealed that N/S molar ratios did indeed affect the H_2S RE in contrast to the behavior observed in BTFs. In BTFs, the N/S molar ratio can be reduced below the theoretical needs, which are 0.4 and 0.6 mol mol^{-1} for nitrate and nitrite, respectively, without a significant decrease in the H_2S RE [9,41].



328 **Fig. 3 -** Removal efficiencies at different N/S molar ratios (Exp. 2).

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330 Different N/S molar ratios of 1.99, 1.6, 1.33, 1.1, and 0.78 mol mol^{-1} were tested. The
331 H_2S RE depends on the mass transfer of the pollutant, which is related to the k_La and the
332 chemical characteristics of the H_2S, and the microbial activity. An increase in pH leads
333 to greater solubility of H_2S and, therefore, a greater mass transfer. When the highest N/S
334 molar ratio was applied (1.99), the H_2S RE was 99.8%. This high H_2S RE proved that
335 there were no limitations on the mass transfer of the pollutant contained in the biogas to
336 the liquid at these conditions. When the highest N/S molar ratio was applied (1.99), the
337 H_2S RE was 99.8%. This result highlights the low mass transfer limitation of the pollutant
338 from the biogas stream to the medium under these conditions. When N/S molar ratio was
339 lowered to 1.1 mol mol^{-1}, the H_2S RE was affected leading to a reduction to 94.2%.
340 Finally, the lowest N/S molar ratio used (0.78) led to an average H_2S RE drop to 84.9%
341 causing instability in the system.

342 This relationship between N/S molar ratios and H_2S RE was previously reported in the
343 literature in SBB for biogas desulfurization. Dolejs et al. [42] reported that N/S molar
344 ratio was decisive for the efficiency of a CSTBR removing H_2S using NO_3^- as the main
345 electron acceptor. In the same study, a sulfide RE drop from 96% to 55% was found when
346 the N/S molar ratio was reduced from 1.18 to 0.36. Another study performed by Li et al.
347 [40] found that, while a decrease in the N/S molar ratio did not affect the H_2S removal by
348 a BTF, the same low N/S molar ratio values diminished H_2S RE in a bubble column.
349 These differences were explained because of the vastly different mass transfer rates
350 between both types of bioreactors [43].

351 However, in this experiment we can assume that the low H_2S RE found at the lowest N/S
352 molar ratio tested cannot be explained by a mass transfer limitation because the ORP

353 decreased from -306 mV to -422 mV and the operational conditions (i.e. stirring speed
354 and GRT) remained constant during the whole experiment (data not shown). The ORP
355 decrease could be explained by a higher presence of HS^- ions in the medium, indicating
356 that mass transfer was not limited. A biomass concentration decrease could not be
357 responsible for the worse performance shown at the lowest N/S molar ratios tested. The
358 biomass concentration could be assumed to be constant due to the short duration of the
359 experiment. Therefore, the only reason that could explain the poor H_2S RE at N/S molar
360 ratios below 1.1 mol mol^{-1} is that the amount of nitrite supplied to the bacteria at these
361 N/S ratios was insufficient to carry out the biological desulfurization. According to the
362 stoichiometric equations of sulfide oxidation using nitrite (Eqs. 7 and 9), N/S molar ratios
363 of 0.67 and 2.67 mol mol^{-1} are required to achieve the partial oxidation of H_2S to
364 elemental sulfur and the complete oxidation to sulfate, respectively. Our data are in
365 complete agreement with those obtained by Mahmood et al. [44], who used the same N/S
366 molar ratio (1.1) to operate an anoxic desulfurization bioreactor using nitrite as electron
367 acceptor. In that study, sulfide REs over 95% were also obtained using that N/S molar
368 ratio and sulfate was again obtained as a minor product (~25%). The formation of sulfate,
369 as has been demonstrated in Exp. 3, is difficult to avoid and causes an increase in the
370 required N/S molar ratio of the bioprocess.

371 **3.3 Effect of HRT on process performance**

372 HRT or dilution rate (D) is an important parameter to optimize in most CSTBRs due to
373 its large influence on the bioreactor performance. The biomass productivity, i.e. dilution
374 rate times biomass concentration (DX), is a well-known parameter used to determine the
375 optimal HRT or dilution rate of the bioprocess. At the dilution rate in which the
376 production rate of biomass per unit reactor volume is considered to be maximum
377 (maximum DX product value), the H_2S EC is expected to be the highest. Therefore, this

test was aimed at studying and optimizing the effect of dilution rate on biomass concentration and productivity, H_2S RE, and sulfur production.

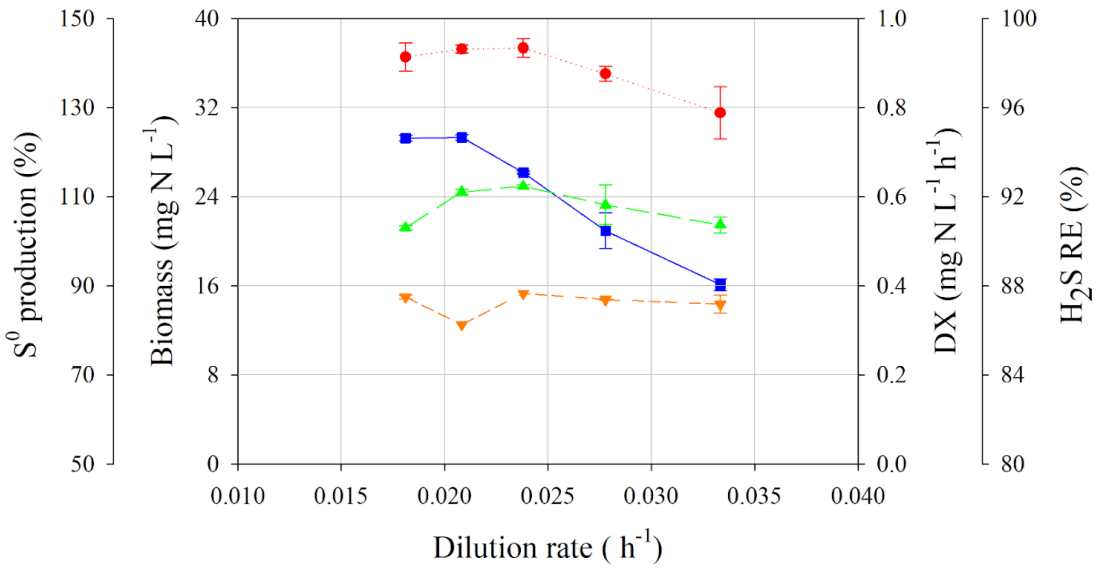

Fig. 4 – DX product (green triangle), biomass concentration (blue square), H_2S removal efficiency (red circle), and sulfur production (orange inverted triangle) versus dilution rate (Exp. 3).

In order to study the effect of dilution rate on biomass production and RE, Exp. 3 was carried out for 40 days. Initially, at the lowest D tested of 0.018 and 0.021 h^{-1} (HRTs of 55 and 48 h, respectively), the biomass hardly changed indicating that the maximum biomass concentration was reached (29.3 ± 0.4 mg N L^{-1}). An increase of dilution rate from 0.021 to 0.033 h^{-1} led to a proportional decrease in biomass concentration from 29.35 to 16.11 mg N L^{-1}. Hence, a DX curve could be made, and is presented in Fig. 4 as green triangles. This curve has a maximum DX value of 0.63 mg N L^{-1} h^{-1} at a dilution rate of 0.024 h^{-1}, which corresponds to an HRT of 42 h. From these data collected at pseudo-steady state, the biomass growth yield ($Y_{X/S^{2-}}$) was also calculated (Eq. S1). An average $Y_{X/S^{2-}}$ of 0.05 ± 0.003 g VSS (g S^{2-})$^{-1}$ was calculated assuming $C_5H_7O_2N$ as typical biomass composition [25]. Mora et al. [25] reported a higher $Y_{X/S^{2-}}$ of 0.328 ±

0.045 g VSS (g S^{2-})$^{-1}$ compared to the one obtained in the present work, which could probably be explained by the different compositions of the bacterial consortia.

H_2S REs were slightly affected under the different conditions tested during Exp. 3 (Fig. 4, red circles). The lowest average RE value (RE = 95.8 ± 1.2%) was found when the highest dilution rate was applied to the bioreactor (D = 0.033 h^{-1}; HRT = 30 h). On the other hand, the best performance (RE = 98.6 ± 0.4 %) was found when the highest DX product was obtained, at a dilution of 0.024 h^{-1} (HRT = 42 h). These data lend support to previous findings in the literature. Mahmood et al. [44] reported that the effect of HRTs ranging from 36 to 2.4 h had little impact on the H_2S RE, which ranged from 99.8 to 99.2%, of an airlift bioreactor using nitrite as the main electron acceptor. Also, Can-Dogan et al. [45] found that HRTs from 86.4 to 2h did not affect the sulfide RE, which always exceeded 92%.

Additionally, the influence of the HRT on sulfur production at the previously optimized N/S molar ratio in Exp. 2 (1.1 mol mol^{-1}) was studied. As in the case of H_2S RE, the sulfur production percentages were modestly influenced by HRT ranging from 81.3% to 88.3%. The highest sulfur production was found at a dilution rate of 0.024 h^{-1} (HRT = 42 h). These high values of sulfur production give an explanation to the milky yellow/white appearance of the bioreactor (Fig. S4) when low N/S molar ratios were applied. This characteristic appearance was previously linked, in the literature, with the accumulation of sulfur particles [46,47]. Despite sulfide removal by autotrophic denitrification having been carried out in SBBs, sulfur production data have not been widely reported. Most studies claim that sulfur accumulates in the bioreactor when working at low N/S molar ratios but scarce further data are given [42,47,48].

Mahmood et al. [44] reported that sulfur was obtained as the main oxidation product (around 66%) in a UASB reactor using nitrite as electron acceptor. Compared to aerobic

SBB, as happens in anoxic desulfurization, the limitation of the electron acceptor enhances the sulfur production. For example, Lohwacharin and Annachhatre [22] and Buisman et al. [21] reported that up to 90% of sulfide removed was converted to sulfur in an airlift bioreactor operating under oxygen-limited conditions. Also, a similar value of sulfur production (87.76%) was found under optimal operational conditions by Roosta et al. [49] using a model validated with an aerobic CSTBR. Lower values of sulfur production (65%) were obtained by Krishnakumar et al. [23] with an aerobic reverse fluidized loop reactor under high sulfide loads. Even though working at low N/S molar ratios is not feasible in BTFs because of the column clogging problems caused by sulfur accumulation, some studies are available. Cano et al. [41] obtained sulfur production up to 99% at N/S molar ratios as low as 0.34 using NO_3^- as electron acceptor. Brito et al. [9] obtained sulfur as the main oxidation product using a BTF fed with nitrite at N/S molar ratios ranging from 1.1 to 1.5. Also, Montebello et al. [50] obtained sulfur production rates ranging from 20 to approximately 60% using an aerobic BTF operating under acidic conditions.

Despite neither H_2S RE nor sulfur production differences among dilution rates being substantial, in the present study, taking into consideration that the best performance in terms of H_2S removal and sulfur production was obtained when the maximum DX product was found, subsequent experiments were conducted considering a D of 0.024 h^{-1} (HRT = 42 h) as optimum.

3.4 Effect of GRT

Among all parameters affecting bioreactor efficiency, the gas flow rate, which determines GRT, stands as one of the most important design parameters to optimize. Lower GRTs are strongly correlated with smaller bioreactors as well as lower costs of construction, maintenance, and operation. Decreasing the GRT results in an increase of the pollutant

loading rate to the CSTBR, and therefore the number of pollutants that can potentially be removed. So, in order to study the effect of the GRT on H_2S removal by the CSTBR, two different experiments were carried out (Exp 4.1 and 4.2).

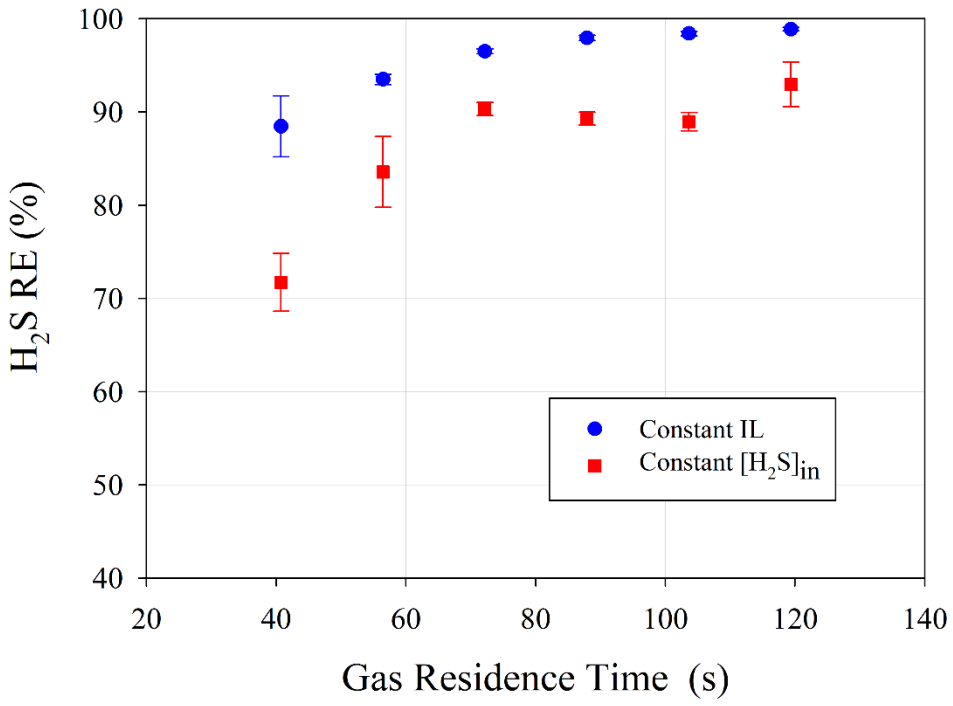

Fig. 5 – Removal Efficiency versus Gas Residence Time keeping constant: (1) Inlet Load (blue circles) and; (2) Inlet H_2S concentration (red squares).

Initially, when the IL was kept constant and GRT and $[H_2S]_{in}$ decreased in Exp. 4.1 (Fig. 5, blue circles), REs of 88.5 ± 3.3% and 93.4 ± 0.6% were obtained at the lowest GRTs tested (41 and 56 s, respectively). Nonetheless, once GRT was increased to 72 s, RE increased to 96.5 ± 0.3% revealing this GRT as a suitable choice to obtain REs above 95%. As expected, when GRT was further increased, REs in turn increased up to a maximum of 98.9 ± 0.2 at the highest GRT tested (119 s). Secondly, in Exp. 4.2 (Fig. 5, red squares) the same GRTs were tested but, this time, keeping the H_2S inlet concentration constant at 2,000 ppm_v, which in turn led to an IL increase from 90 to 232 g S-H_2S m^{-3} h^{-1}. This time, the differences between GRTs are more remarkable. At the highest IL (232

462 g S-H_2S m^{-3} h^{-1}) and the lowest GRT (41 s) the maximum EC in the present study was
463 found (EC = 166.0 ± 7.2 g S-H_2S m^{-3} h^{-1}; RE = 71.7 ± 3.1%). When the GRT was
464 increased, slight differences were found at GRTs ranging from 72 to 104 s where an
465 average RE of 89.5 ± 0.6% occurred. Finally, the maximum RE in Exp. 4.2 (93.0 ± 2.4%)
466 was obtained at the highest GRT of 119 s (IL = 79.0 g S-H_2S m^{-3} h^{-1}).

467 Comparison of these data with the literature is difficult because of the few studies about
468 the GRT effect in SBB for hydrogen sulfide removal. Most of the research on H_2S
469 removal in SBBs has been conducted using a sulfide salt solution as substrate so the
470 influence of GRT has not been widely studied. In this way, additional external absorption
471 units would be required for the operation of these bioreactors, involving extra capital and
472 operating costs. Amongst the authors who have used gas effluents with H_2S, Zytoon et al.
473 [51] fed an airlift bioreactor with a mixture of air and H_2S corresponding to a GRT of
474 1484 s. Also, a GRT of 300 s was applied by Li et al. [40] in a bubble column carrying
475 out anoxic desulfurization, achieving REs ranging from 66.2% to 99.6% under an IL
476 around 30 g S-H_2S m^{-3} h^{-1}.

477 In comparison with BTFs, Cano et al. [41] were able to achieve H_2S REs between 96-
478 98.5% in a BTF operating at empty bed residence times (EBRTs) ranging from 32 to 42
479 s and the same inlet H_2S concentration as used in this study (2,000 ppm_V). This better
480 performance shown by the BTF mentioned above could be explained in terms of its high
481 height/diameter ratio (9.8), which allowed it to obtain higher mass transfer rates.
482 Moreover, BTFs are capable of hosting large amounts of biomass fixed to the packing
483 material, which leads to higher substrate consumption rates in BTFs in comparison to
484 CSTBRs. However, the performance of other anoxic BTFs was affected at higher EBRTs
485 than those tested in the present study, probably due to the lower height/diameter ratios
486 compared to Cano et al. [41]. For example, Almenglo et al. [52] obtained a RE drop from

99% to 80% when the EBRT was decreased from 601 to 137 s. Also, the H_2S RE was greatly affected by EBRT in another anoxic BTF when EBRT was diminished from 121 s (RE = 98%) to 30 s (RE = 47%) [11].

The maximum EC obtained in the present study (EC = 166.0 ± 7.2 g S-H_2S m^{-3} h^{-1}; RE = 71.7 ± 3.1%) improves on the performance of most SBBs removing H_2S from biogas that have been reported in the literature. ECs around 25 g S-H_2S m^{-3} h^{-1} (RE = 99.6 ± 0.4%) were obtained in an anoxic bubble column under a GRT of 300 s [40]. A maximum EC of 113 g S-H_2S m^{-3} h^{-1} (RE ≈ 95%) was achieved by Zytoon et al. [51] in a pilot-scale airlift aerobic bioreactor at a GRT of 1484 s. Jiang et al. (2020) obtained a higher (EC 256 g S-H_2S m^{-3} h^{-1}) refluxing the outlet gas in an aerobic biological bubble column but with a much lower RE of 57%. Similar or better results were obtained in aerobic or anoxic bioreactors removing sulfide from wastewaters [23,44,46,54]. Also, the EC values obtained in the present study exceed the performance of several BTFs at comparable EBRTs [26,52,55,56]. Despite there being some BTFs that have higher ECs [33,48], the accumulation of elemental sulfur in the packing material would limit its application.

Therefore, it can be concluded that the H_2S RE values obtained from the studied CSTBR were better than those from other suspended biomass bioreactors and are in line with BTFs carrying out the anoxic desulfurization.

3.5 Use of PI control under stepped variation

In the last part of the study, the effect of inlet perturbation on H_2S RE under a PI control was studied. First of all, a suitable set of gain parameters had to be obtained in order to implement a convenient and robust control. Brito et al. [26] applied different feedback control strategies to an anoxic desulfurization system and concluded that a PI controller had a more stable behavior at different ILs than a PID controller. Therefore, in the present

work, a PI control was implemented. Due to its responsiveness, simplicity and adequacy, the tuning method used was the Ziegler-Nichols rule based on step response [27]. The final values for the different gain parameters were K_p= 0.104 and K_i = 0.0005.

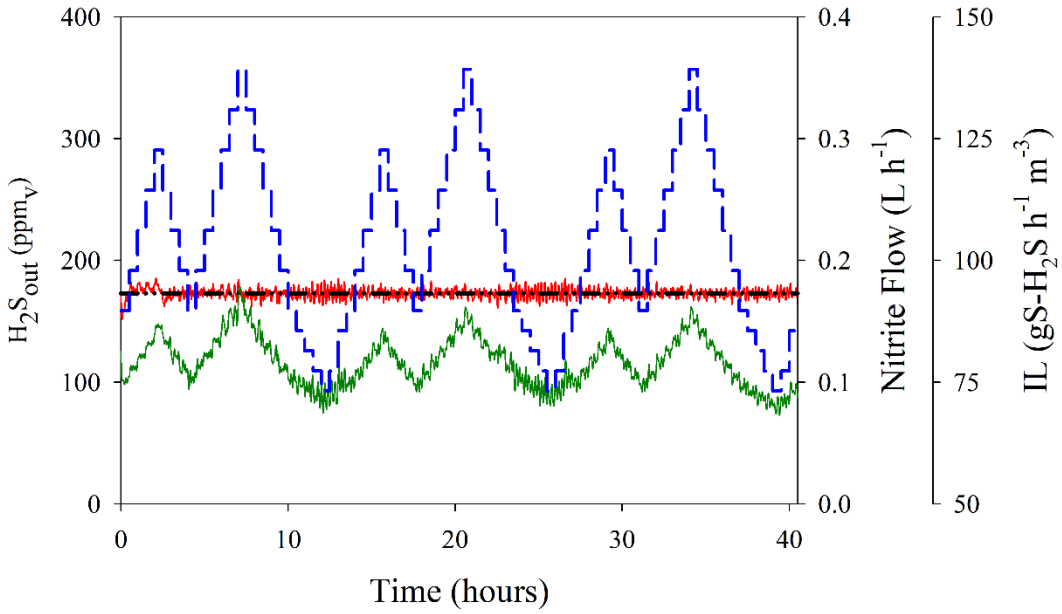

Fig. 6 – H_2S concentration present in the outlet (red) and set-point (black) under stepped variations in IL (blue). Nitrite inlet flow is represented in green.

Then, once the PI controller was implemented, the effect of a stepped inlet perturbation (Fig. 6, blue line) on the CSTBR under PI control was studied (Exp. 5). The results of this experiment are depicted in Fig. 6. The H_2S set-point selected was 173 ppm_v. This set-point was chosen because the outlet biogas from this bioreactor could be fed to an internal combustion engine, whose technical limit is 200 ppm_v [58]. The average H_2S outlet concentration was 173 ± 6.7 ppm_v. These results concur well with the data depicted in Fig. 6, in which it can be seen that the PI control successfully maintained the H_2S outlet concentration (Fig. 6, red line) near the set-point (Fig. 6, black line). The feedback control allowed us to adjust the outlet H_2S concentration by modifying the nitrite flow rate (Fig 6, green line) resulting in an average of 0.116 ± 0.021 L h^{-1}. Despite the N/S molar ratio varying throughout the experiment, these changes were not excessive and the N/S molar

ratio was kept almost constant to an average of 1.1 ± 0.1. This N/S molar ratio, applied by the automated feedback controller, ended up being the same as the previously optimized value in Exp 2. Herewith, the average RE of the whole experiment was 93.4 ± 0.3% which satisfactorily met the requirements of the system.

The application of feedback control strategies to a SBB for sulfide removal has not been reported previously. The studies carried out previously in anoxic BTFs used manual, programmed and continuously modulated control [15,26,27]. Manual feeding of nitrate is possible but not feasible in an industrial bioreactor due to the high operational costs and underperformance [26]. An automated method to supply nitrate was proposed by Almenglo et al. [15] by measuring ORP in the same way as Exp. 1. Even though this control strategy had already been applied in the pilot-scale, high concentrations of nitrate were found in the recirculation medium outlet stream in addition to not being able to prevent outlet peaks of H_2S in the outlet gas stream when nitrate was depleted. Different feedback strategies have also been applied to anoxic and aerobic BTFs for H_2S removal [26,27,39]. PID control using the H_2S outlet concentration as controlled variable was successfully applied to an anoxic BTF fed with nitrate to a set-point of 100 ppm_v. This control strategy maintained an average offset of ± 7 ppm_v under similar stepwise variations in IL. In another study, a PI control was successfully applied to an anoxic BTF for H_2S removal and tested under remarkably variable ILs ranging from 28 to 141 g S-H_2S m^{-3} h^{-1} [27]. Here, the PI control satisfactorily maintained the H_2S outlet concentration between 90 and 114 ppm_v using a set-point of 100 ppm_v.

Therefore, taking into consideration the above, the CSTBR stands as a favorable alternative to BTFs to maintain a stable H_2S concentration in the outlet under variable ILs.

4.- Conclusion.

The use of a CSTBR to carry out the anoxic biogas desulfurization offers an alternative to the BTF. The optimal conditions to maximize elemental sulfur production without decreasing the H_2S RE at an IL of 100 g S-H_2S m^{-3} h^{-1} were: 60 rpm, HRT of 42 h, N/S molar ratio of 1.1 and GRT of 119 s, obtaining an H_2S RE of 98.6 ± 0.4 % and 88% of sulfur production. The maximum EC obtained was 166.0 ± 7.2 g S-H_2S m^{-3} h^{-1} (RE = 71.7 ± 3.1%) operating at a GRT of 41 s. The PI feedback control was able to keep the actual outlet concentration very stable and close to the set-point.

Acknowledgments

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Abstract

Biological desulfurization of biogas has been extensively studied using biotrickling filters (BTFs). However, the accumulation of elemental sulfur (S^0) on the packing material limits the use of this technology. To overcome this issue, the use of a continuous stirred tank bioreactor (CSTBR) under anoxic conditions for biogas desulfurization and S^0 production is proposed in the present study. The effect of the main parameters (stirring speed, N/S molar ratio, hydraulic residence time (HRT) and gas residence time (GRT)) on the bioreactor performance was studied. Under an inlet load (IL) of 100 g S-H_2S m^{-3} h^{-1} and a GRT of 119 s, the CSTBR optimal operating conditions were 60 rpm, N/S molar ratio of 1.1 and a HRT of 42 h, in which a removal efficiency (RE) and S^0 production of 98.6 ± 0.4% and 88% were obtained, respectively. Under a GRT of 41s and an IL of 232 g S-H_2S m^{-3} h^{-1} the maximum elimination capacity (EC) of 166.0 ± 7.2 g S-H_2S m^{-3} h^{-1} (RE = 71.7 ± 3.1%) was obtained. A proportional-integral feedback control strategy was successfully applied to the bioreactor operated under a stepped variable IL.

Credit Author Statement

José Joaquín González-Cortés: Investigation, Formal analysis, Writing- Original draft preparation. **Sandra Torres-Herrera**: Investigation. **Fernando Almenglo:** Conceptualization, Methodology, Supervision. **Martín Ramírez**: Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing- Reviewing and Editing. **Domingo Cantero**: Conceptualization, Project administration, Funding acquisition, Writing- Reviewing and Editing.

Novelty Statement

An efficient bioprocess (anoxic desulfurization from biogas) has been successfully optimized in a CSTBR for the first time, achieving similar elimination capacities than the widely-studied biotrickling filters. Additional novel features such as the SO-NR biomass sensitiveness to shear-stress forces or the dilution study are described.

Hydrogen sulfide (H_2S), a ubiquitous compound strongly toxic to the environment and the human-health, stands as the main hazardous material removed in the study. Its conversion to recoverable and re-usable elemental sulfur at the same time as nitrite (another toxic compound) is reduced to harmless N_2 is proposed in the present work.

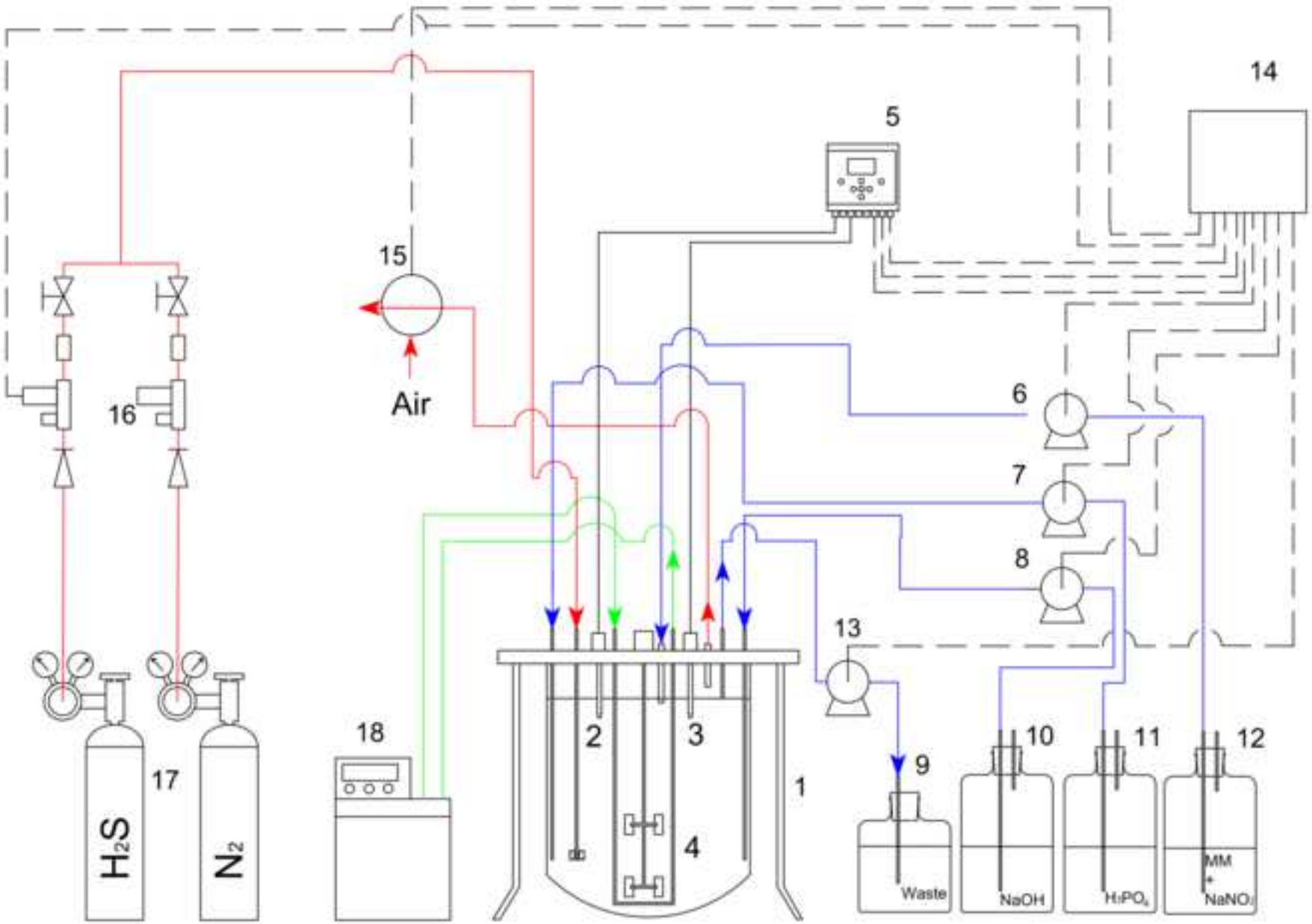
Table 1

Table 1. Operational conditions of the different experiments carried out.

Exp.	IL ($gS\text{-}H_2S\ m^{-3}\ h^{-1}$)	HRT(h)	GRT(s)	N/S molar ratio ($mol{:}mol^{-1}$)	Stirring speed (rpm)	$[H_2S]_{in}$ (ppm_v)	Studied Variable	Days
1	70	-	139	Variable	60 110 160 210	1800	Stirring speed	7
2	100	66 47 36 30	119	1.99 1.6 1.33 1.06 0.78	60	2500	N/S molar ratio	1
3	100	55 48 42 36 30	119	1.1	60	2500	HRT	40
4.1	100	42	119 104 89 73 57 41	1.1	60	2500 2171 1841 1512 1182 853	GRT (constant IL)	1
4.2	79 90 106 129 165 232	59 51 43 36 28 20	119 104 89 73 57 41	1.1	60	2000	GRT (constant $[H_2S]_{in}$)	1
5	139-73	40-21.5	119	1.6-0.9	60	3500-1850	Stepwise changes in IL	3

Figure 1

Click here to access/download;Figure;Figure 1.tiff



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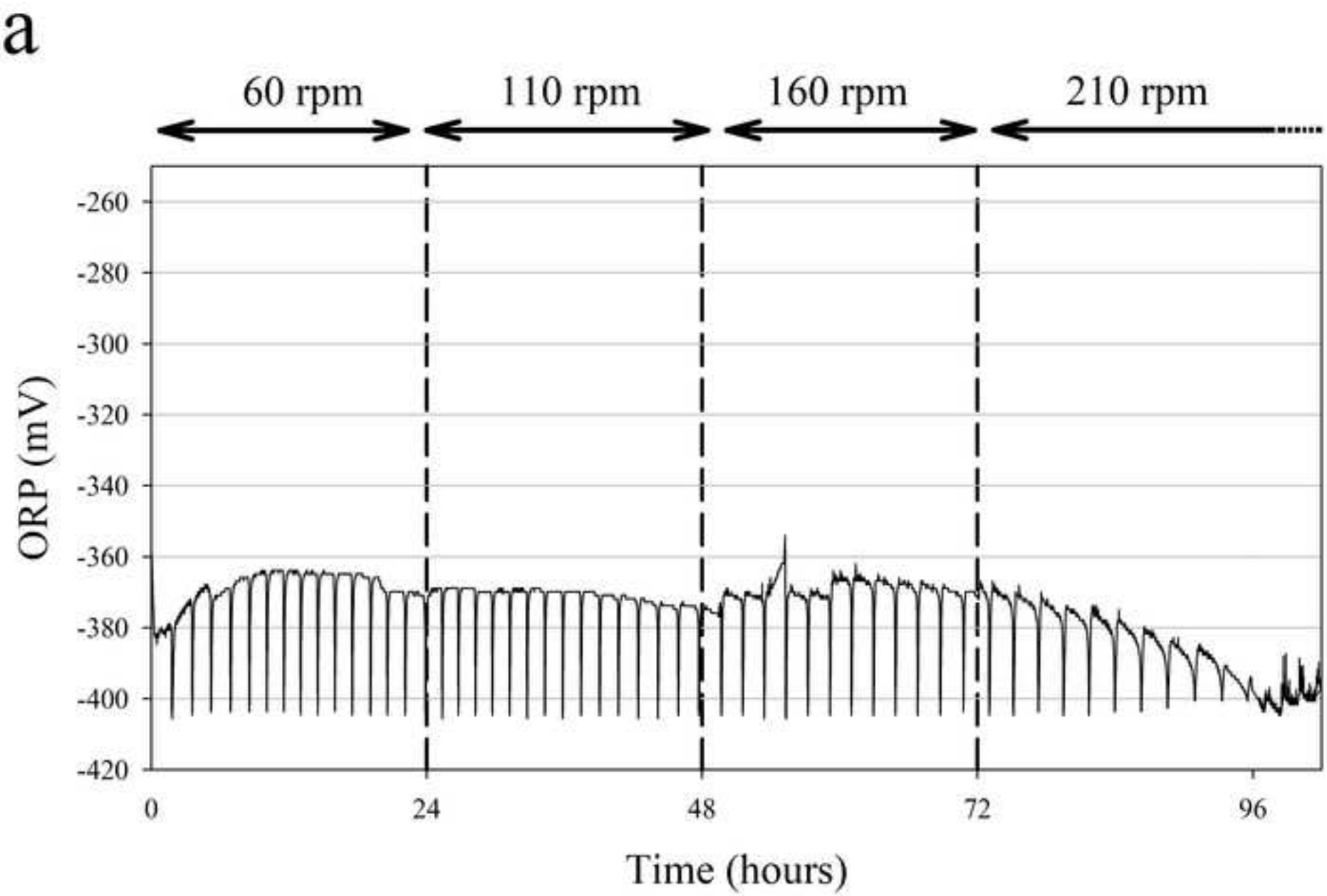

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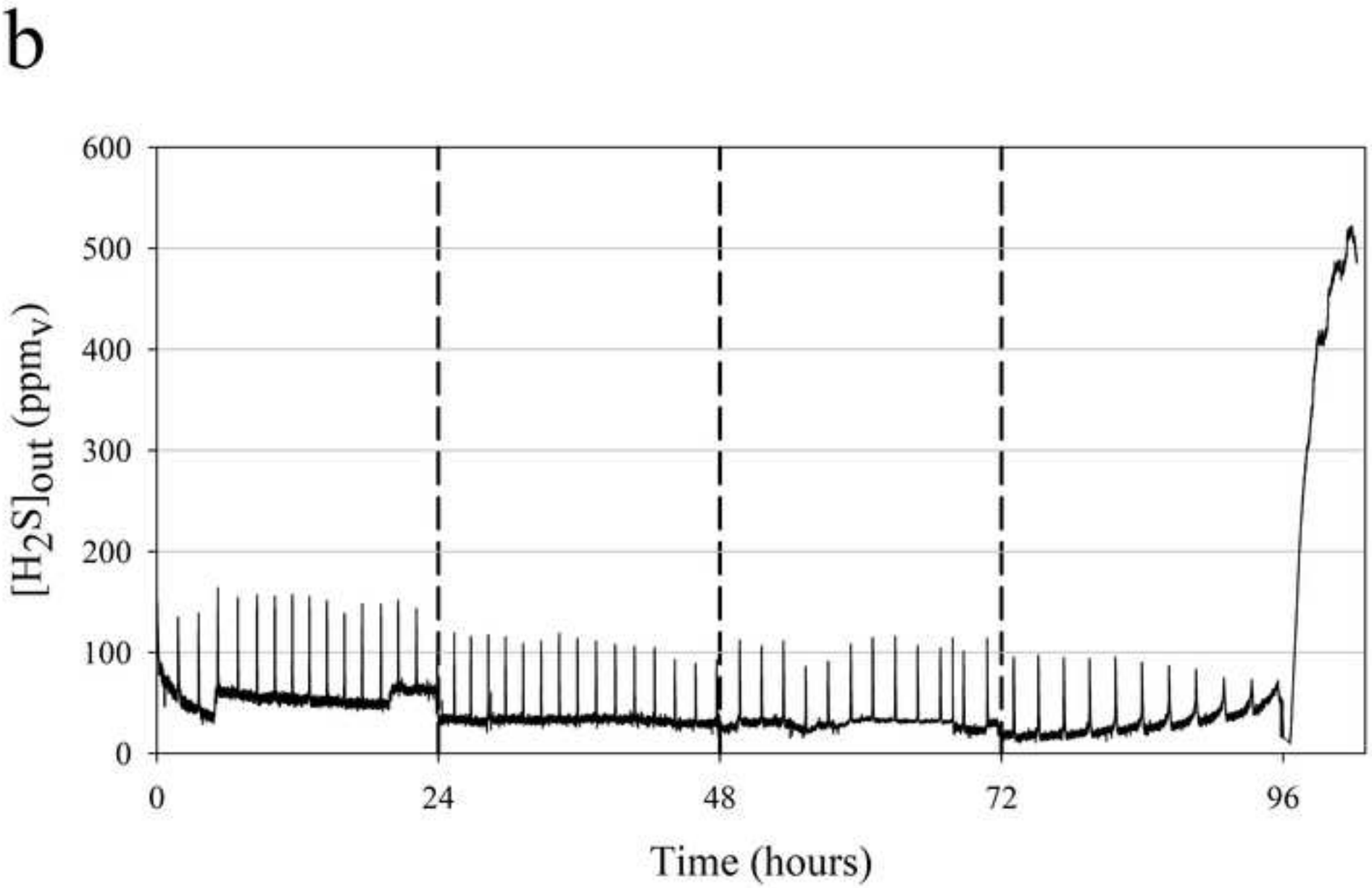

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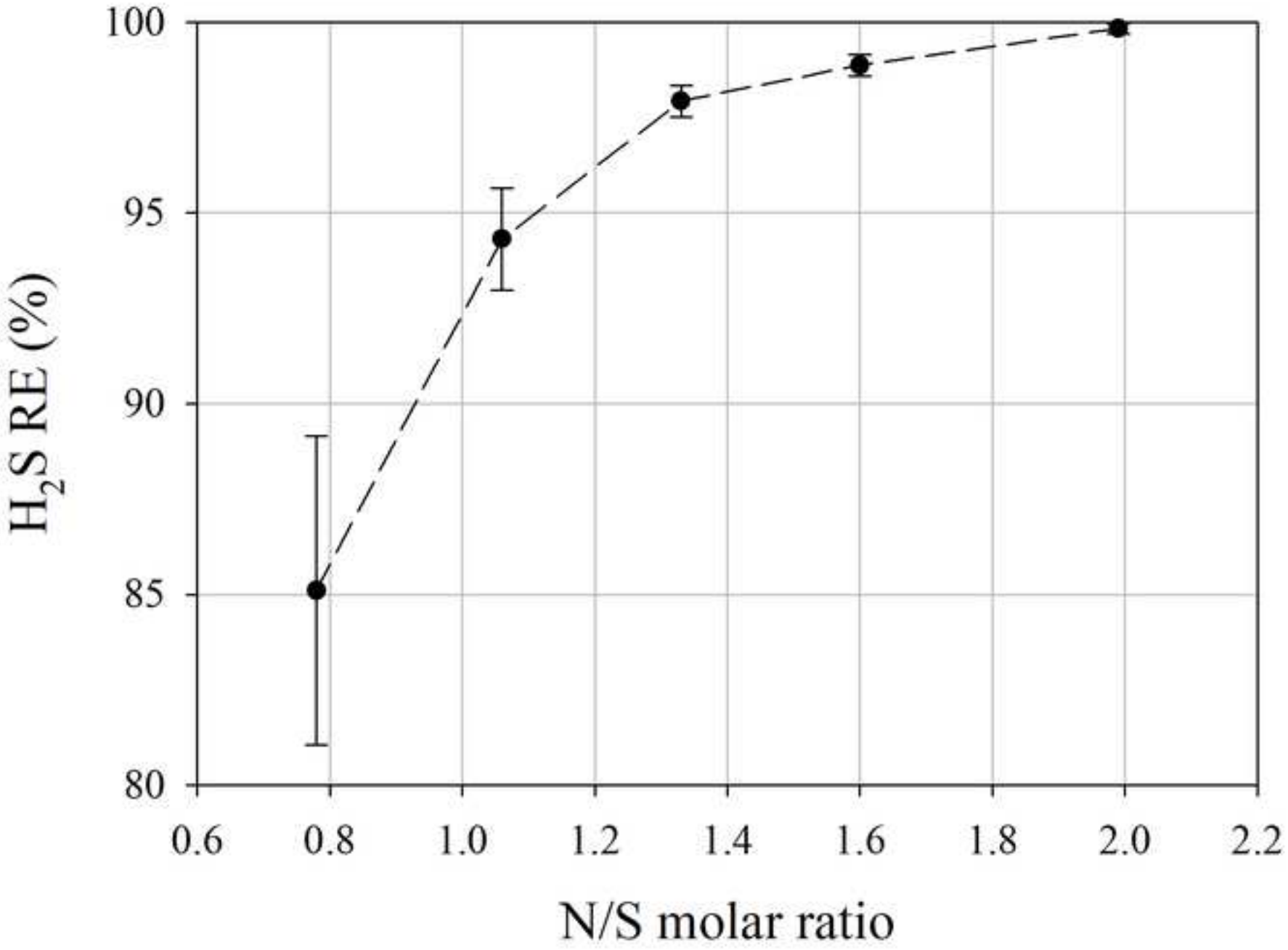

Figure 4

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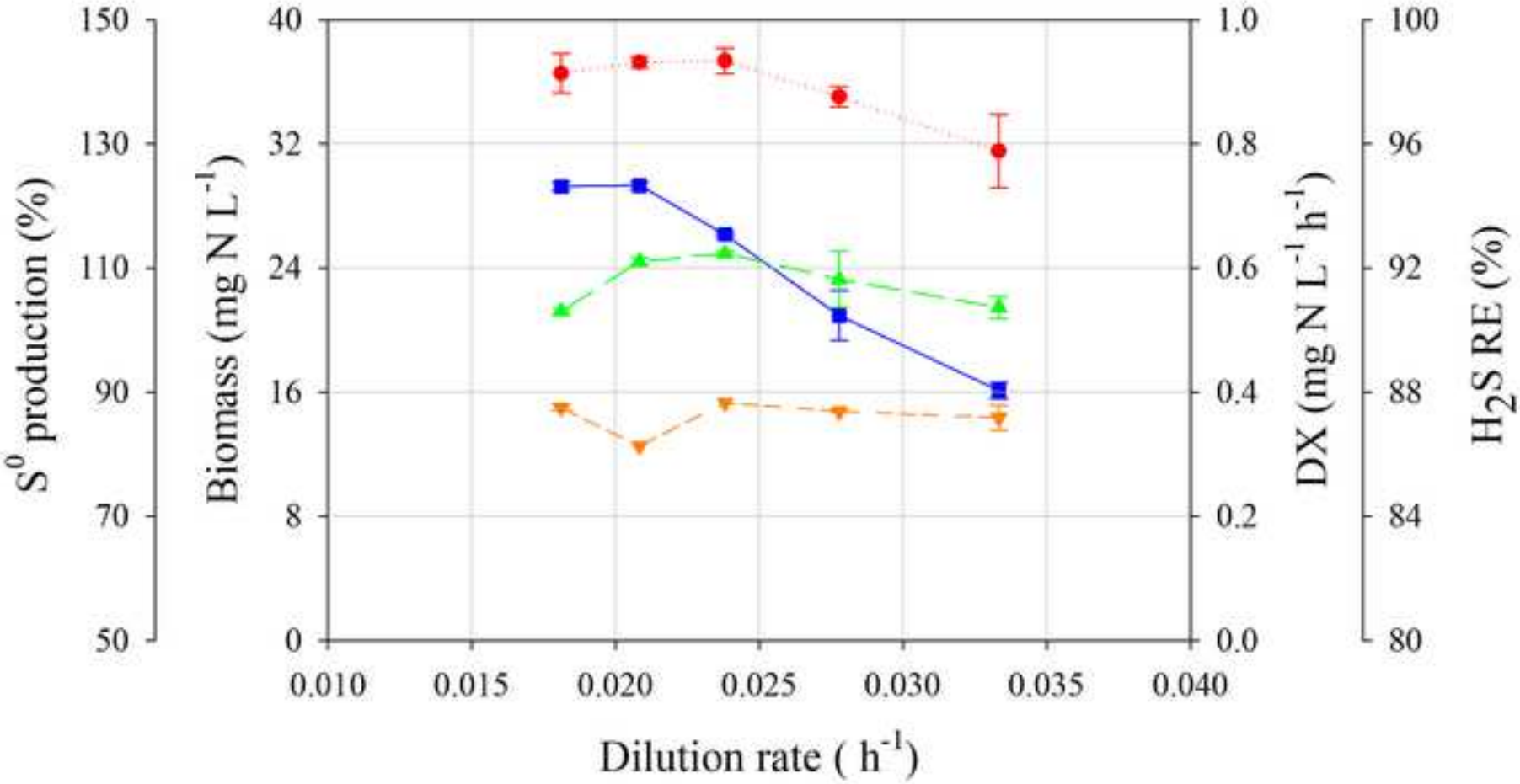

Figure 5

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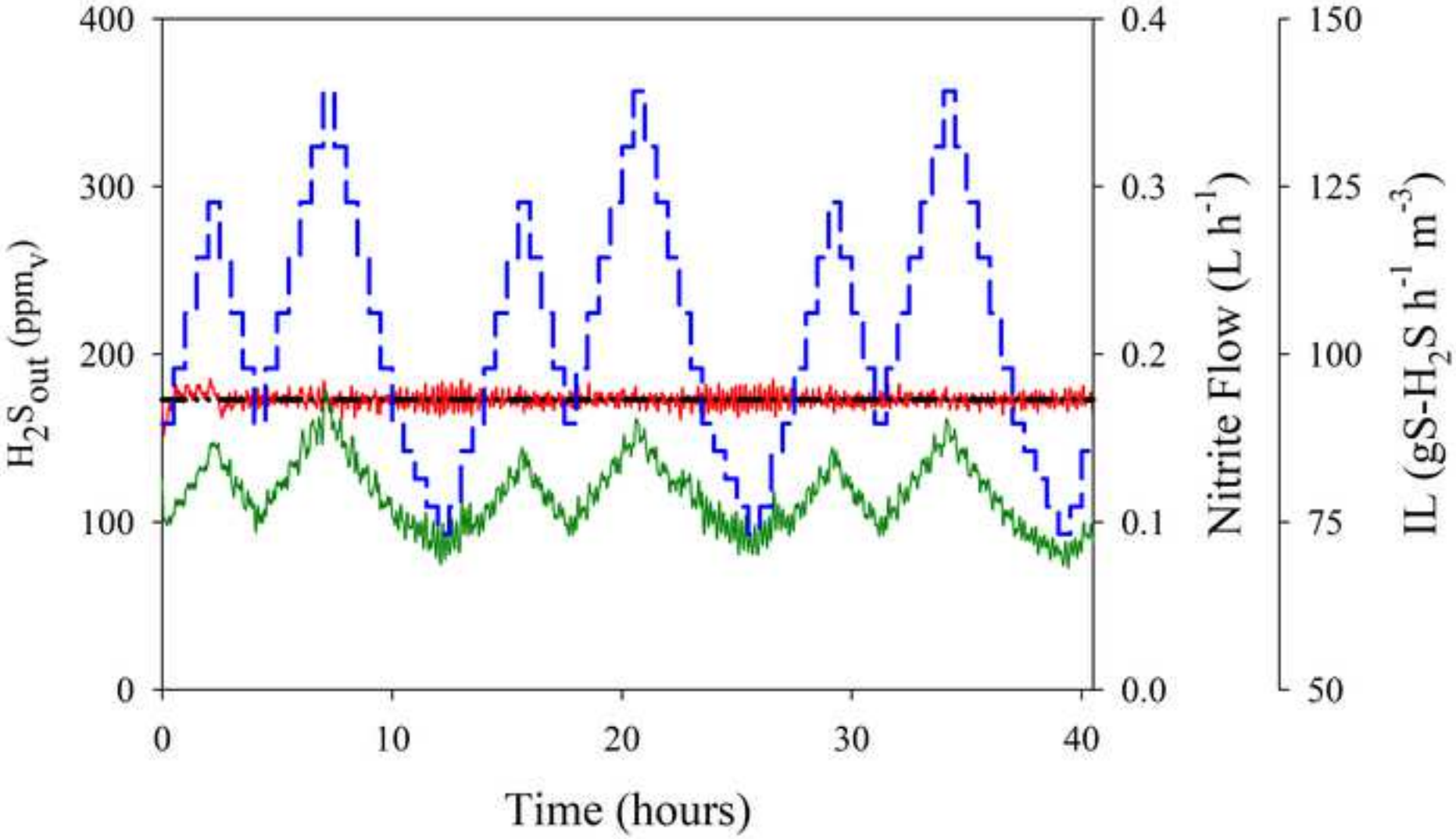

Figure 5.TIF

Anoxic biogas biodesulfurization promoting elemental sulfur production in a Continuous Stirred Tank Bioreactor

José Joaquín González-Cortés, Sandra Torres-Herrera, Fernando Almenglo, Martín Ramírez, Domingo Cantero.

SUPPLEMENTARY MATERIAL

Supplementary figures and tables.

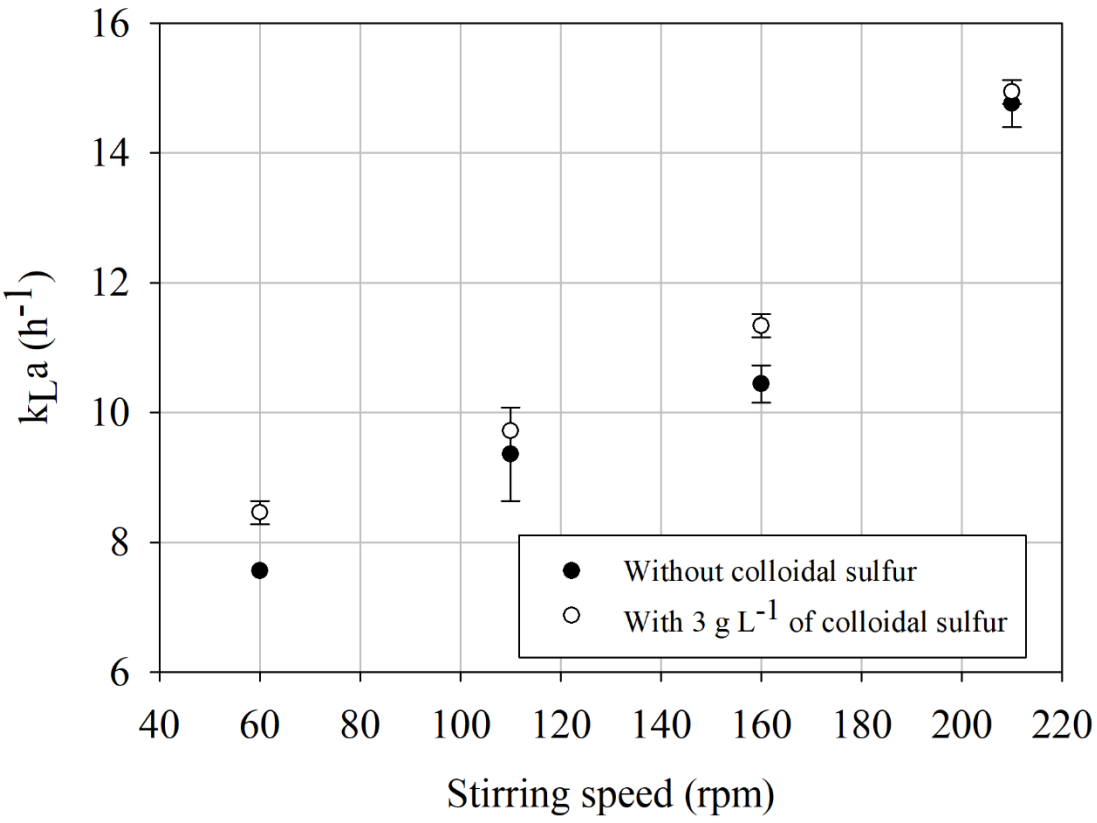

Fig. S1 – k_La determination at different stirring speeds under a GRT of 139 s.

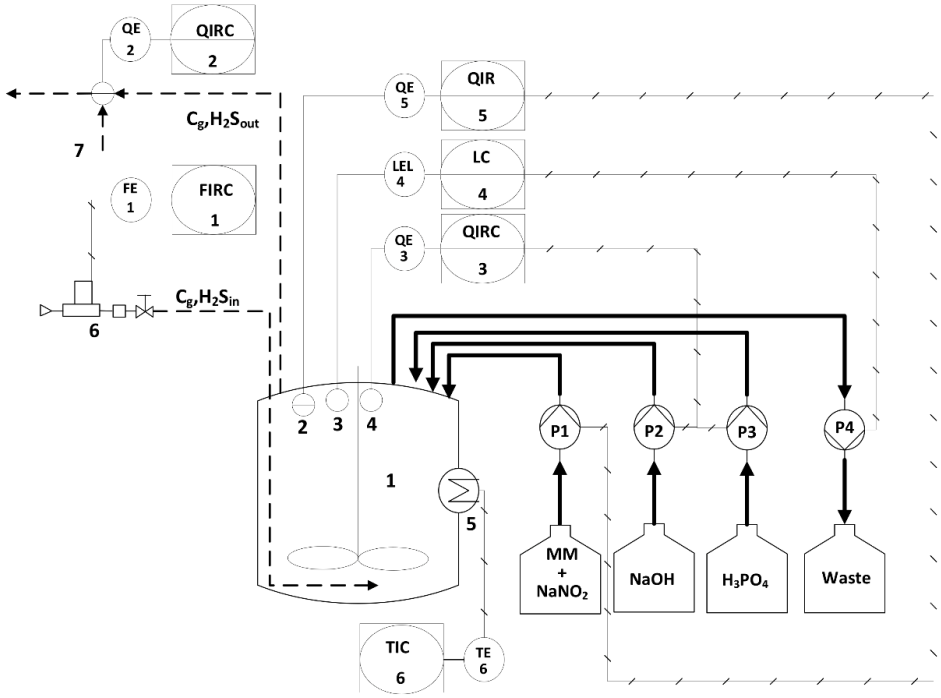

Fig. S2 – Schematic of the control system. 1. CSTBR; 2. ORP sensor; 3. Level sensor; 4. pH sensor; 5. Heat exchanger; 6. Mass flow controller. 7. H_2S sensor with a dilution system.
FIRC, flow rate indicator recording controller; QIRC, quantity indicator recording controller,
QIR, quantity indicator recording; LC, level control; TIC, temperature indicator controller. Control loops: 1 (gas flow rate), 2 (H_2S concentration), 3 (pH), 4 (level), 5 (nitrite flow rate) and 6 (temperature).

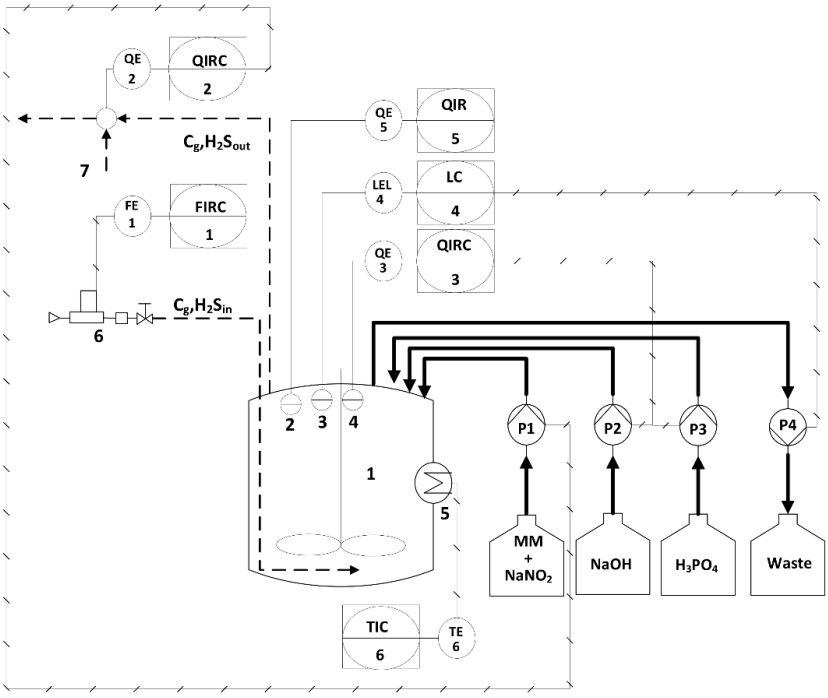

Fig. S3 – Schematic of the control system. 1. CSTBR; 2. ORP sensor; 3. Level sensor; 4. pH sensor; 5. Heat exchanger; 6. Mass flow controller. 7. H_2S sensor with a dilution system.
FIRC, flow rate indicator recording controller; QIRC, quantity indicator recording controller,
QIR, quantity indicator recording; LC, level control; TIC, temperature indicator controller. Control loops: 1 (gas flow rate), 2 (nitrite flow rate), 3 (pH), 4 (level), 5 (ORP) and 6 (temperature).

Fig. S4 – Picture of the stirred tank bioreactor accumulating elemental sulfur as the main oxidation product.

Biomass growth calculation

$$Y_{X/S^{2-}}\left(\frac{\text{g VSS}}{\text{g } S^{2-}}\right)=\frac{Dilution\,(h^{-1})\,x\,Biomass\,(g\,VSS\,m^{-3})\,x\,100}{IL\,(g\,S{-}H_2S\,m^{-3}\,h^{-1})\,x\,RE\,(\%)} \qquad \text{(Eq. S1)}$$