Macrothink Institute™

Journal of Applied Biotechnology
ISSN 2327-0640
2018, Vol. 6, No. 1

An Optical Microbial Biosensor Based on Whole Cell of *Acidithiobacillus thiooxidans* for Hydrogen Sulfide Determination

Odemar Cardoso Silva (Corresponding author)

Dept. of Chemistry, Federal Center for Technological Education of Rio de Janeiro

Av. Maracanã, 229, Rio de Janeiro, P. O. Box 20271-110, Brasil

Tel: +55-21-2566-3166 E-mail: odemar.silva@cefet-rj.br

Andréa Medeiros Salgado

Dept. of Biochemical Engineering, Federal University of Rio de Janeiro

Av. Athos da Silveira Ramos, 149, Rio de Janeiro, P. O. Box 21941-909, Brasil

Tel: +55-21-3938-7648 E-mail: andrea@eq.ufrj.br

Francisca Pessoa de França

Dept. of Biochemical Engineering, Federal University of Rio de Janeiro

Av. Athos da Silveira Ramos, 149, Rio de Janeiro, P. O. Box 21941-909, Brasil

Tel: +55-21-3938-7648 E-mail: fpfranca@eq.ufrj.br

Received: January 8, 2018 Accepted: February 12, 2018 Published: February 14, 2018

Doi: 10.5296/jab.v6i1.12445 URL: http://doi.org/10.5296/jab.v6i1.12445

Abstract

Due to its corrosive and highly toxic character, the generation of hydrogen sulfide is a serious problem for the environment, human health, and the industry. This paper reported a new and simple methodology for aqueous hydrogen sulfide determination through the development of an optical microbial biosensor. The principle of detection was based on the aerobic and chemolithotrophic metabolism of *Acidithiobacillus thiooxidans* bacterial cells. Under low

oxygen concentration and acidic conditions *A. thiooxidans* can rapidly oxidize H_2S to elemental sulfur. The biochemical formation of elemental sulfur can be spectrophotometrically detected and the increase in absorbance at 620 nm exhibited a linear relationship to an H_2S concentration up to 100 $mg.L^{-1}$. The parameters concerning the analytical performance of the biosensor such as cell harvesting time and pH influence were measured and optimized through the optical absorption value. The biosensor was selective to H_2S with no important disturbance by tested species except thiosulfate ion (11.5% error). Biosensor response expressed good repeatability (RSD = 4.46 %) and reproducibility (RSD = 4.66 %). The low cost of cell cultivation and the absence of the immobilization step make feasible the optic biosensor application.

Keywords: *Acidithiobacillus thiooxidans*, biosensing system, hydrogen sulfide, spectrophotometry

1. Introduction

Hydrogen sulfide is a flammable gas with a characteristic odor of rotten egg in low concentrations and of a highly toxic and corrosive character. Every year, millions of tons of H_2S is produced around the world from oil-refinery plants or natural-gas extraction, which is predicted to increase in the future (Chaudhari et al., 2011). Its anthropogenic sources include the paper industry, coal mines, sewage treatment and oil and gas desulfurization (Ghadiri et al., 2013). At low concentrations, it can cause problems to human health, such as headache, eye damage and breathing difficulties, while exposure to 600 mg. L^{-1} H_2S can lead to death in less than 15 minutes (Redondo et al., 2008). It's toxic and corrosive characteristics stimulated the development of several techniques for the removal and quantification of H_2S by amperometric (Dilgin et al., 2012), potentiometric (Fagnani et al., 2012), spectrophotometric (Ghadiri et al., 2013), voltammetric (Li et al., 2017) and interferometric methods (Kumeria et al., 2011). Among the techniques for H_2S elimination, biochemical processes such as biofiltration have received special attention due to their lower cost, low energy consumption and little generation of waste to be disposed of (Charnnok, 2012).

Sulfur oxidizing bacteria (SOB) have been used in H_2S biofiltration systems, especially the genus *Acidithiobacillus*, efficient in aerobic media over a broad pH range, whose acidity varies from neutrality to pH equal to 2 (Chaiprapat et al., 2011). Biofilters applying bacteria of the species *Acidithiobacillus thiooxidans* achieved more than 97% efficiency in the removal of H_2S in concentration up to 10000 ppmv (Aita et al., 2016). The biochemical process involved is based on its ability to use reduced or partially oxidized sulfur species as electrons source on its metabolic process. It generates sulfuric acid as final oxidation product, promoting a reduction in the pH of the medium and an increase in the sulfate concentration, through the following reactions (Montebello et al., 2010; Jensen et al., 2011; Ginkel et al., 2010; Yousefi et al., 2013).

$$H_2S + 0.5\ O_2 \rightarrow S + H_2O \quad (1)$$

$$S + 1.5\ O_2 + H_2O \rightarrow H_2SO_4 \quad (2)$$

$$H_2S + O_2 \rightarrow 0.5\ S_2O_3^{=} + 0.5\ H_2O + H^{+} \quad (3)$$

$S_2O_3^{=} + \quad 2\ O_2 \quad + H_2O \quad \rightarrow 2\ H^+ + 2\ SO_4^{=}$ (4)

$H_2S + 2\ O_2 \rightarrow H_2SO_4$ (5)

As can be seen from equations 1 to 5, there is a stoichiometric dependence between the amount of available oxygen and the formed main product (Montebello et al., 2010). Under conditions of sufficient oxygenation, there is a formation of H_2SO_4 as the final product, with consequent increase of the medium acidity. The Henry constants for H_2S and O_2 are 483 atm and 41100 atm, respectively. These values indicate that the solubility of oxygen in water is 85 times lower than H_2S (Aita et al., 2016). In the way that there must be a high flow rate of O_2 to compensate its low solubility. When oxygen supply for bioreaction is limited, H_2S oxidation is incomplete and elemental sulfur formation prevails, being observed as yellowish particles. (Chaiprapat et al., 2011). The pH also exerts influence on the formed main product. The decrease in pH increases the ionic strength of the medium and reduces the gases solubility. The oxygen is released into the gas phase, being less available in the liquid film for biochemical reaction, favoring the formation of elemental sulfur (Chaiprapat et al., 2011).

These characteristics of *A. thiooxidans* strains, allied to its obligate chemolithotrophic character, which confer them analytical selectivity, potentiate its application in the development of biosensors for H_2S quantification (Mirzaei et al., 2014). In the recent years microbial biosensors have been developed for the detection of H_2S based on the variation of oxygen consumption by *T. thioparus* (Mirzaei et al., 2014; Vosoughi et al., 2015), *A. thiooxidans* e *A. ferrooxidans* (Janfada et al., 2015); potentiometric variation using immobilized *A. thiooxidans* cells and an enzymatic spectrophotometric biosensor based on the inhibition of the enzyme peroxidase by hydrogen sulfide (Ghadiri et al., 2013).

Nevertheless, to the best of our knowledge, there are no studies involving the determination of H_2S by spectrophotometric method applying cellular biosensors. The aim of this work is the presentation of a simple method of H_2S determination through a microbial optical biosensor using *A. thiooxidans* cells as a biological recognition element.

2. Materials and Methods

2.1 Cell Culture and Media

A. thiooxidans FG-01 bacterial cells were obtained from Center for Mineral Technology (CETEM), Rio de Janeiro, and utilized in all experiments. The microorganism was grown in ATCC® 8085™ liquid medium, containing (g. L^{-1}): $(NH_4)_2SO_4$, 0.2; KH_2PO_4, 3.0; $MgSO_4$, 0.5; $CaCl_2$, 0.25; $FeSO_4$, 0.005; sulfur, 1.0. The cultivation was carried out in 250 mL Erlenmeyer flasks containing 100 mL of ATCC® medium with 20% inoculum. The pH was adjusted to 3 with NaOH 1 mol.L^{-1} solutions and were further incubated on a rotary shaker at 30 ºC and 150 rpm.

2.2 Growth Curve and Dry Cell Weight

The growth curve was determined by turbidimetry using a spectrophotometer (Q798DRM – QUIMIS) at wavelength of 620 nm (Liu et al., 2008a). The unconsumed sulfur was removed by repeated centrifugation at 25 × g for 5 min and 10 mL of cell culture was withdrawn and

filtered through a 0.2 μm filter membrane for dry cell weight (DCW) determination. Optical density value (OD_{620}) was daily measured until the stationary growth phase was reached. The concentration of total biomass was estimated by cell counting in a Neubauer chamber with a depth of 0.1 mm and an area of 0.0025 mm^2. An optical microscope (Olympus CX-31) with 1000 × magnification was used for this purpose (Bajestani et al., 2014).

2.3 Sulfide Measurements Procedure

Hydrogen sulfide solutions at concentrations between 10 $mg.L^{-1}$ and 100 $mg.L^{-1}$ were obtained from the dissolution of an appropriate mass of sodium sulfide ($Na_2S.9H_2O$) in distilled water (Jensen et al., 2011). The solubility of H_2S in water at 25 °C is approximately 3000 $mg.L^{-1}$ (Zirrahi et al., 2012). Sodium sulfide undergoes rapid dissolution, with hydrolysis of the sulfide ion and ionization of the formed H_2S. The species $S^{=}$, HS^{-} and H_2S are generated on chemical equilibrium although below pH 3 almost all of sulfide is present as the unionized form (Rabbani et al., 2015). The dissolved H_2S concentration was determined by iodometric titration using standardized sodium thiosulfate solution as titrant and starch indicator. Depending on the estimated concentration of H_2S a certain volume of sample (H_2S solution) was added to triiodide solution upon the same conditions used for blank determination and the following equation was used to calculate the H_2S concentration, adapted from Vosoughi et al. (2015).

$$H_2S = \frac{M \,.\, (Va - Vb) \,.\, 34 \,.\, 1000}{2 \,.\, Vc} \qquad (6)$$

In Equation (6) M is the amount-of-substance concentration of sodium thiosulfate, Va and Vb are the titrant volume utilized in the blank and sample determination, respectively and Vc is the H_2S solution volume added to the triiodide solution.

2.4 Determination of Sulfide Using the Developed Biosensor

Cell culture system was centrifuged twice at 25 × g for 5 min for removal of the unconsumed elemental sulfur. Resulting cell suspension was centrifuged at 5400 × g for 20 min and cell pellet formed was ressuspended with 0.85 % NaCl solution and the acidity was adjusted with NaOH 1 mol/L or HCl 10 % until reaching the expected optical density and pH.

In all experiments for determination of best harvesting time and evaluation of pH and sulfide concentration influence on biosensor response equal volume of H_2S solution and cell suspension (1.7 mL) was directly mixed in a 1.0 cm path length quartz cuvette. The elemental sulfur formed in the reaction chamber was analyzed by spectrophotometry measuring the absorbance at 620 nm. On the biosensor selectivity experiments 1.0 mL of sulfide solution (50 mg/L) was directly mixed on a quartz cuvette containing 1.7 mL of cell dispersion (OD_{620}=2.0) and 1 mL of each possible interference species tested. The same procedure was used to determine the repeatability and reproducibility of biosensor response, replacing the interference specie for equal volume of distilled water. In all experiments, the spectrophotometric reading was set to zero after mixing to discount the absorption reduction due to cell dispersion dilution. The subsequent increase in absorption at 620 nm was

accompanied along time. The response time was defined as the time interval required for the sensor response to stabilize.

3. Results and Discussion

3.1 Detection Principle

Acidithiobacillus thiooxidans species are aerobic and chemolithotrophic microorganisms. They use CO_2 as carbon source for biomass production and H_2S as electron donor in their process of obtaining energy. Studies involving its application in bioleaching or biofiltration processes usually seek conditions that overcome the oxygen limitation and favor the formation of sulfuric acid as the final product of sulfide oxidation (Rodriguez et al., 2014). In this work, however, we intend to reach system conditions that maximize the natural formation of elemental sulfur, a product of the first stage of H_2S oxidation, favored by the limitation of dissolved oxygen available for bioreaction. The produced elemental sulfur is insoluble in water. It appears in the colloidal form, modifies the absorbance of the reaction medium and its formation can be observed by spectrophotometric method. In addition to H_2S, the main chemical species present at the moment of the reaction mixture are the Na^+ and Cl^- ions. Along the bioreaction, other inorganic sulfur species can be produced beyond elemental sulfur, possibly interfering on the biosensor response. However, as can be observed in Figure 1, regions of absorbance response from 300 nm are allowed, since from this wavelength the absorbance of H_2S, NaCl and the ions $S_2O_3^{=}$ and $SO_4^{=}$, possible reaction products, are despicable. The biosensor response was accompanied at 620 nm, the same wavelength selected for cell optic density measurements. After blanking the spectrophotometric reading to discount the cell absorption, any detected increase in absorption at 620 nm is straight related to elemental sulfur production and can be associated to the initial concentration of H_2S available for bioreaction.

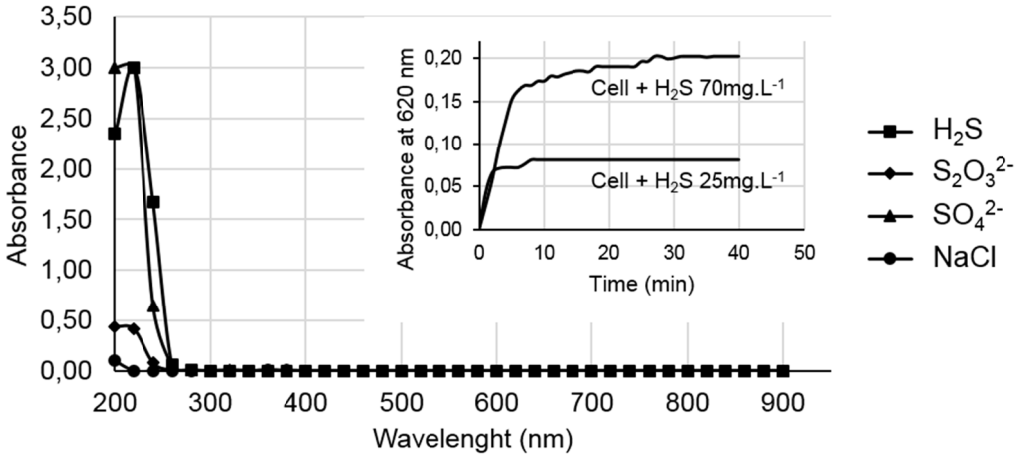

Figure 1. Absorption spectra of possible interference species on biosensor response. Inset shows the increase in absorption at 620 nm for systems containing hydrogen sulfide sample solutions and *Acidithiobacillus thiooxidans dispersions* (OD_{620}=2.0; pH=1).

3.2 Growth Curve and Best Harvesting Time

The growth of *A. thiooxidans* is slow, taking about 10 days to start the stationary phase (Nguyen et al., 2016). The elemental sulfur employed as energy source for cell growth is hydrophobic and remain floating over the growth medium limiting its disponibility for bacterial consumption. The growth rate increase as a consequence of cell adhesion and production of extracellular polymeric substances (EPS) on the sulfur surface. The EPS acts as surfactants and the negatively charged cell membrane of adhered bacteria enhance the hydrophilic behavior of colonized sulfur particles becoming this way more disperse and available for cell consumption (Sharihari et al., 1993). Figure 2 shows the growth curve for *A. thiooxidans* and the effect of cell age on the biosensor response.

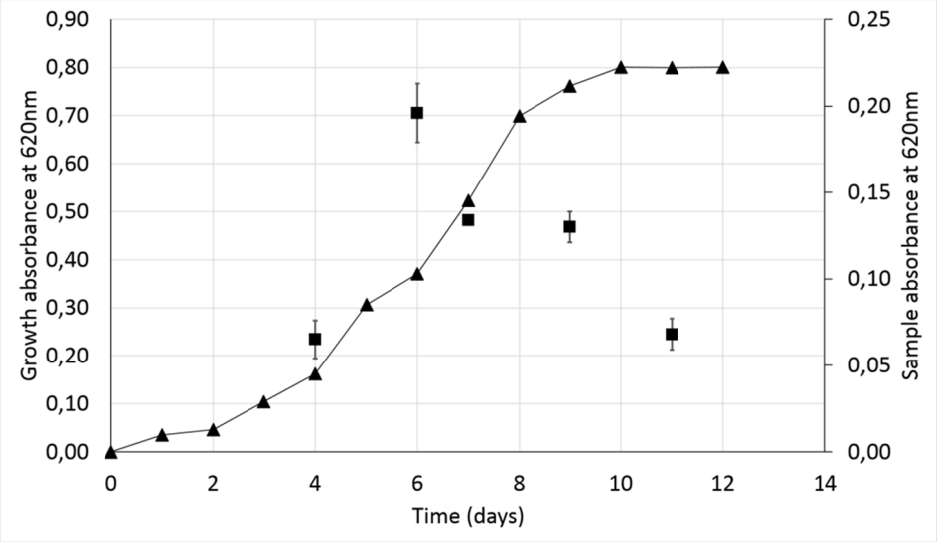

Figure 2. Growth curve and effect of cultivation age on the biosensor response. Symbols: triangles, growth curve; squares absorbance at steady state for H_2S solution analysis, and error bars represent the corresponding standard deviations

Cells were collected at different points of logarithmic and stationary phase and applied at pH 2 for 50 $mg.L^{-1}$ H_2S solutions analysis. Cell growth reached the log phase at about 4 days. During this phase, the biosensor response increased since the metabolic rate and nutrients consumption reaches the maximum speed (Janfada et al., 2015). The highest biosensor response was observed using 6 days old cell cultures. Based on the highest biosensor response, the optimum harvesting time was defined as 6 days old cell cultures and used throughout this work.

3.3 Effect of pH on H_2S Consumption

In water H_2S is a weak acid, which at pH between 7 and 12.5 exists predominantly as hydrogen sulfide anion (HS^-). The extremely low second dissociation constant of H_2S (pk_2=14.15) is responsible for the fact that the S^{-2} ion can only be a minority species in water, while under mesoacidophilic conditions (about 20 ºC-40 ºC and pH below 3) H_2S prevails in its non-ionized form (Lawrence et al., 2007; Zhang et al., 2008).

On its metabolism hydrogen sulfide is transported into the periplasmic space and converted to hydrogen persulfide (H_2S_2) by sulfide:quinone oxidoreductase (SQR) which is located in the periplasmic surface of the cytoplasmic membrane (Yin et al., 2014). After a sequence of disproportionation reactions from disulfide and trisulfide anions long chain polysulfides ($H\text{-}S_n^-$) are formed (Griesbeck et al., 2002). However, polysulfides are not stable in acidic periplasmic condictions and decompose to form octasulfane rings system (S_8) and sulfide (Rohwerder & Sand, 2003).

Extracellular and periplasmic pH is normally around 2 in acidophilic bacteria and the cytosolic pH is close to neutrality (Rohwerder & Sand, 2003). This creates a steep pH gradient and H^+ generated by the oxidation of sulfur compounds are released outside the cell, and cytochrome oxidase consumes H^+ within the cell to maintain the internal pH close to neutrality, involving soluble oxidative enzymes and membrane bound respiratory chains (Ghosh & Dam, 2009). Since alterations in external pH impact cell metabolism and consequently the hydrogen sulfide consumption we investigated the effect of pH on the absorbance at 620 nm for 30 $mg.L^{-1}$ hydrogen sulfide solutions maintaining cell concentration at 2.22 g/L (1.0 OD_{620}=1.11 g/L DCW=9 × 10^8 cells/mL) (Figure 3). The best biosensor responses are in the range of pH between 1 and 2. These results are in agreement to those found by Duan et al. (2006), achieving a H_2S biofilter eliminatory efficiency of 94-99% in this pH range. The 620 nm absorption showed a maximum value at pH 2 and markedly decreased at pH 0.5. Feng et al. (2015) described this acidity level as not deleterious to cell even though cell activity is slowed at pH below 0.9. Biosensor response also dropped significantly at pH 4 since this acidity level is out of the optimum pH range for *Acidithiobacillus thiooxidans* metabolism (Lors et al., 2009).

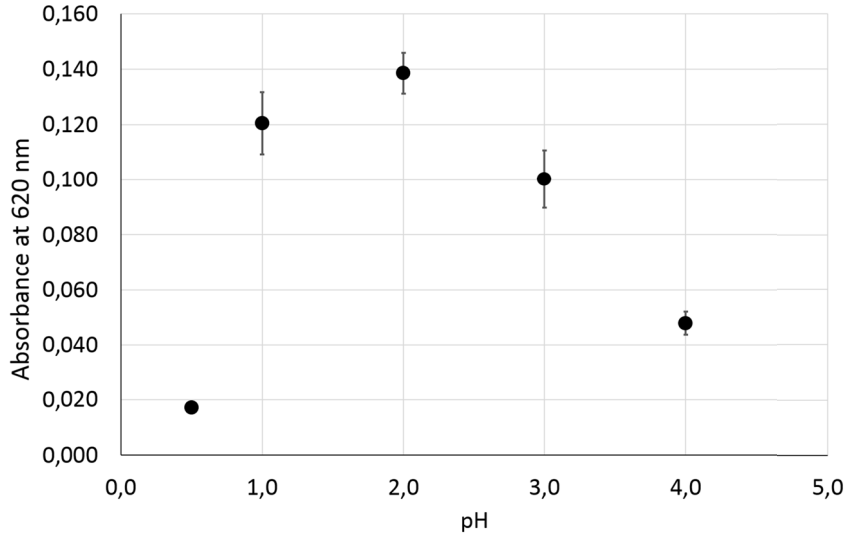

Figure 3. Influence of the pH on the biosensor response. Error bars represent the corresponding standard deviations

3.4 Influence of H_2S concentration on Biosensor Response

The influence of hydrogen sulfide concentration on the biosensor behavior was characterized by the analysis of solutions in the range of 10 mg. L^{-1} and 100 mg.L^{-1} (Figure 4). Hydrogen sulfide is a reactive molecule and a powerful reducing agent (Zhao et al., 2014). It can be chemically oxidized by air oxygen and its oxidation can be catalyzed by transition ions (Hughes et al., 2009). In order to verify the influence of hydrogen sulfide side reactions on the biosensor response two kinds of control assays were performed. The first test consisted in observing the biosensor response in the presence of *A. thiooxidans* cells (OD_{620}=2.0) but in the absence of hydrogen sulfide. In the second, the biosensor response to an H_2S solution at 30 mg. L^{-1} was monitored in the absence of cells, but in the presence of 1000 µmol. L^{-1} of the ionic species cited in Table 1 and Table 2. In both tests the increase in absorbance at 620 nm was negligible.

The oxidation rate of H_2S in alkaline medium is considerable, but the chemical oxidation by oxygen in the pH solution below 6 is very slow (Nielsen et al., 2006). The result of the first control test consisted of the blank on the calibration curve (Figure 6). According to Luther et al. (2011) the catalytic effect of transition metals in low concentration is more pronounced for Fe^{+2} and Mn^{+2} ions. However, elemental sulfur is not formed as a product of the chemical catalyzed oxidation of H_2S in acidic medium even in the presence of these metals. The major oxidation products under these conditions are sulfate and thiosulfate ions (Zhang & Millero, 1993). These species show a sharp increase in absorbance down to 250 nm wavelength region having a peak maxima at 218 nm assigned to the $S_2O_3^{=}$ ion (Khan, 2012). The production of these species cannot be detected in the second control test since its absorption at the 620 nm is neglectable and thiosulfate spectrum is overlapped by maximum absorption of H_2S at 230 nm (Hughes et al., 2009). Biological oxidation of H_2S takes a few seconds (Lee et al., 2006) and the increase in absorbance at 620 nm is observed immediately after adjusting the reading to zero. For concentrations below 15 mg. L^{-1} a small and rapid increase in biosensor response followed by a sharp decrease without a steady state occurs.

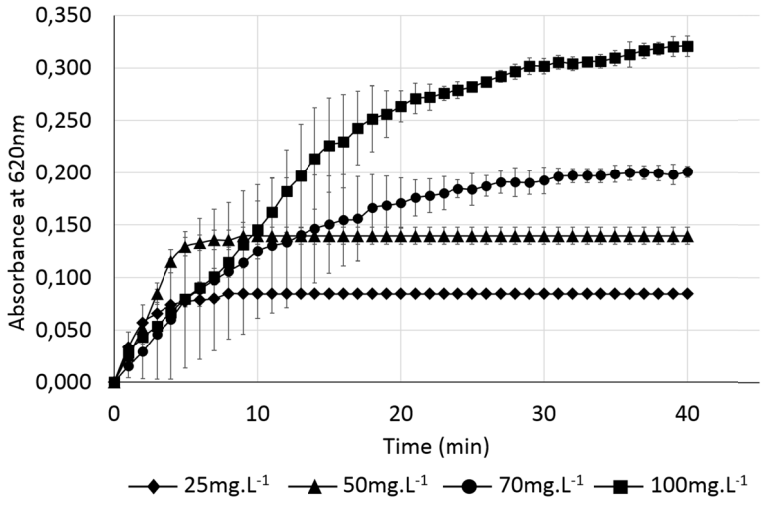

Figure 4. Biosensor response at different H_2S concentrations

In this way, the H_2S concentration of 15 mg.L^{-1} (0.44 mmol/L) was defined as the detection limit. At concentrations above 15 mg. L^{-1}, a linear increase in response was observed until a constant absorbance value was reached. The response time of biosensor was calculated based on the saturation of reaction (Ramesh et al., 2015), which was obtained after 300 s for concentrations up to 50 mg.L^{-1} (1.47 mmol/L). About 30 min were required to reach a steady state for 100 mg. L^{-1} H_2S solutions.

The curves for different concentrations of H_2S were obtained from the arithmetic mean of four runs. Error bars are representative of standard deviations. For concentrations between 50 and 100 mg. L^{-1}, a considerable variability in the rate of H_2S consumption was observed due to the heterogeneous response of living cells to different environmental conditions such as temperature, O_2 saturation level, and cellular adaptation to changes in pH and type of nutrient since they were adapted to elemental sulfur as a source of energy during cultivation. Nevertheless, the absorbance response for different concentrations achieve the steady state at a close value range. These results indicate that the steady state achieved is associated with the total consumption of H_2S and its conversion to elemental sulfur. To evaluate this hypothesis the absorption at 620 nm and pH was accompanied at a system with 25 mL of H_2S 50 mg. L^{-1} and 25 mL of bacterial dispersion (OD_{620}=2.0) for a longer period of 3.5 h. It was observed a sharp and linear increase in absorption until a maximum value, followed by a gradual reduction of the absorption value (Figure 5). The bacterial oxidation of hydrogen sulfide is easier than elemental sulfur oxidation since octasulfane ring must be first opened and activated prior to its transport through the cell membrane to periplasmic space (Yin et al., 2014). At the first 30 min, pH remained constant and slowly decrease accompanying the absorption reduction. These results indicate that in the first stage H_2S is converted to elemental sulfur and in a second stage elemental sulfur is oxidized to H_2SO_4 (Jensen et al., 2011).

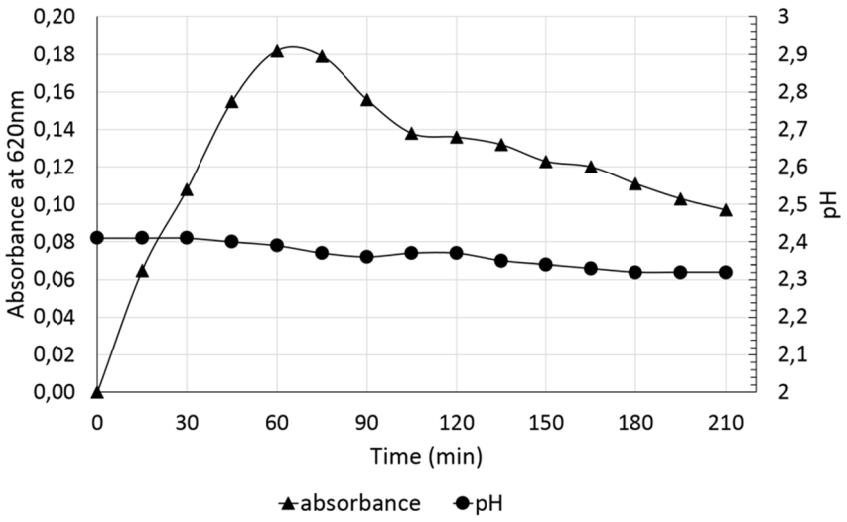

Figure 5. Monitoring of the absorbance at 620nm and pH profile for H_2S consumption by *A. thiooxidans* at a closed batch reactor

3.5 Calibration of the Absorption Response to H_2S

The absorption difference between the initial and the steady state value for the different H_2S concentrations presented in Figure 4 was used to plot a calibration curve. A linear relationship was observed between hydrogen sulfide concentration and absorbance at 620 nm from 15 to 100 mg. L^{-1} (Figure 6). The R-squared value (coefficient of determination) was 0.9964 (n = 7) in this concentration range.

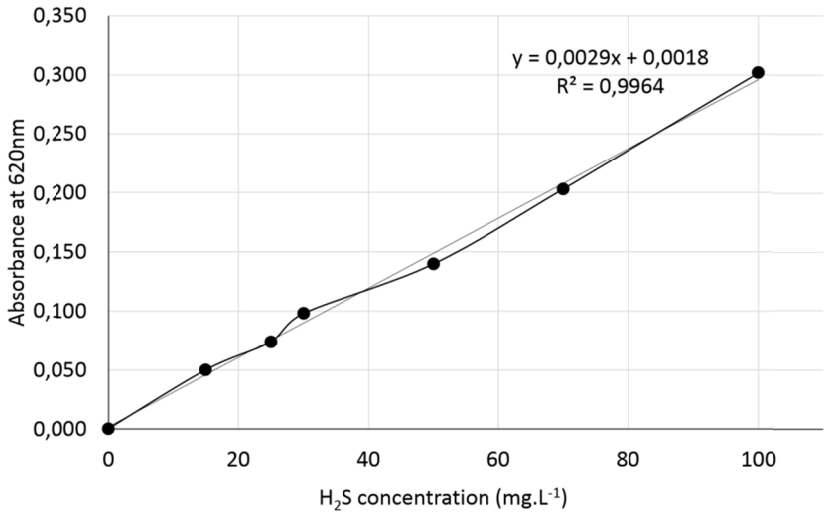

Figure 6. The biosensor calibration curve for H_2S determination applying *A. thiooxidans* dispersions at pH 1 and OD_{620} 2.0

3.6 Selectivity of the Biosensor

The test of potential interference of some common ionic species on the response of the sulfide biossensor was performed at pH 1 and in the presence of 1000 µmol/L of each possible interference species (Liu et al., 2008b). The steady-state absorbance value for each potential interference species was converted to the H_2S concentration in mg.L^{-1} according to the equation of the calibration curve shown in Figure 5. The influence of all possible interference ions on the biosensor response was determined by the relative error between the mean response in the absence of interference species (n = 3) and the mean H_2S concentration obtained in the presence of each species tested (n = 2). As can be seen in Table 1 a negligible interference on biosensor response was observed in the presence of studied cations (Rawal & Pudir, 2012). The highest interference of Cd^{2+} and Zn^{2+} is due to the low Kps and precipitation of their respective sulfides, since *A. thiooxidans* is not able attack insoluble sulfides (Son et al., 2007).

Table 1. Effect of cations on sulfide determination

Interference cations	Sulfide detected (μmol.L^{-1})	Error (%)
Standard solution	34,07	-
Na^+	34,24	0,5
K^+	34,41	1,0
NH_4^+	35,05	2,9
Fe^{2+}	33,90	-0,5
Cd^{2+}	32,43	-4,8
Mn^{2+}	33,03	-3,1
Zn^{2+}	32,40	-4,9

Among the analyzed anions, only the thiosulfate ion significantly interfered in the biosensor response (Table 2). Strains of *A. thiooxidans* species utilize H_2S and $S_2O_3^=$ as energy source and the main product of the first stage of thiosulfate oxidation is also elemental sulfur (Yousefi et al., 2013). The consumption of both species present in this test caused an increase in the absorbance in relation to the absence of thiosulphate. No increase in absorbance was observed in the control test performed in the absence of cells and in the presence of 1000 μmol.L^{-1} of each interference species. This result indicates that the increases in absorbance observed in these experiments are not related to the reaction between H_2S and the tested chemical species but to the consumption of H_2S by the cells and the consequent formation of elemental sulfur.

Table 2. Effect of anion on sulfide determination.

Interference anions	Sulfide detected (μmol.L^{-1})	Error (%)
Cl^-	34,24	0,5
I^-	34,41	1,0
$S_2O_3^{2-}$	38,00	11,5
SO_4^{2-}	33,90	-0,5
NO_3^-	34,60	1,6
HPO_4^{2-}	33,90	-0,5

3.7 Repeatability and Reproducibility

An adaptation period of the cells to the new acidic condition was observed when the experiments were started immediately after cell resuspension in 0.85% NaCl and pH adjustment. The absorbance at steady state varies gradually for about half an hour until it

reaches a plateau. As earlier described, changing in medium acidity modifies the intracellular pH gradient by activating a mechanism of response to external pH perturbations. Mykytczuk et al. (2010) studied the effect of external pH perturbations on *Acidithiobacillus ferrooxidans* and inferred that a shift in the pH by 3.5 to 1.5 induce a reversible pH stress response increasing the synthesis of several lipids to decrease the membrane fluidity and maintain the internal pH homeostasis.

Considering 15 successive experiments the relative standard deviation (R.S.D) was 7.34% while considering only the last 12 experiments, performed after half an hour of adaptation, the R.S.D was found to be 4.46 %. Reproducibility was assessed from the arithmetic mean of five daily sequential measurements carried out over five days along a month and using different cultures. The experiments were performed under the same system conditions applied for repeatability and the RSD was 4.66 %.

4. Conclusions

This study reported the development and feasibility of a simple and new microbial biosensor for aqueous hydrogen sulfide. The age of culture and the pH of the cell suspension showed an important effect on the metabolism of A. thiooxidans and consequently on the biosensor response. These parameters were optimized and the biosensor performance under these conditions was characterized (Table 3).

Table 3. Figures of merit of the biosensor under optimum conditions.

Figures	Response
Harvesting time (day)	6
pH	1-2
Cell concentration ($g.L^{-1}$)	2,2
Equation for the calibration curve[(a)]	$A_{620} = 0{,}0029\ CH_2S + 0{,}0018$
Correlation coefficient	0,9964
Linear range ($mg.L^{-1}$)	15-100
Response time (min)	5

[(a)] A_{620} is response at 620 nm and CH_2S is H_2S concentration ($mg.L^{-1}$); [(b)] Steady-state absorbance for H_2S concentrations up to 50 mg. L^{-1}.

Good selectivity, repeatability and reproducibility were obtained (RSD<5 %) at pH 1. The biosensor showed good performance in hydrogen sulfide concentrations up to 100 $mg.L^{-1}$ and may be desirable for practical application in the petrochemical industry and in wastewater treatment plants.

Acknowledgments

The authors are grateful to the FAPERJ for providing financial support.

References

Aita, B. C., Mayer, F. D., Muratt, D. T., Brondani, M., Pujol, S. B., Denardi, L. B., Hoffmann, R., & Silveira, D. D. (2016). Biofiltration of H_2S-rich biogas using *Acidithiobacillus thiooxidans*. *Clean Technologies and Environmental Policy*, *18*, 689-703. https://doi.org/10.1007/s10098-015-1043-5

Mykytczuk, N. C. S., Trevors, J. T., Ferroni, G. D., & Leduc, L. G. (2010). Cytoplasmic membrane fluidity and fatty acid composition of Acidithiobacillus ferrooxidans in response to pH stress. *Extremophiles*, *14*, 427-441. https://doi.org/10.1007/s00792-010-0319-2

Bajestani, M. I., Mousavi, S. M., & Shojaosadati, S. A. (2014). Bioleaching of heavy metals from spent household batteries using *Acidithiobacillus ferrooxidans*: Statistical evaluation and optimization. *Separation and Purification Technology*, *132*, 309-316. https://doi.org/10.1016/j.seppur.2014.05.023

Chaiprapat, S., Mardthing, R., Kantachote, D., & Karnchanawong, S. (2011). Removal of hydrogen sulfide by complete aerobic oxidation in acidic biofiltration. *Process Biochemistry*, *46*, 344-352. https://doi.org/10.1016/j.procbio.2010.09.007

Charnnok, B., Suksaroj, T., Boonswang, P., & Chaiprapat, S. (2013). Oxidation of hydrogen sulfide in biogas using dissolved oxygen in the extreme acidic biofiltration operation. *Bioresource Technology*, *131*, 492-499. https://doi.org/10.1016/j.biortech.2012.12.114

Chaudhari, N. S., Bhirud, A. P., Sonawane, R. S., Nikam, L. K., Warule, S. S., Raneb, V. H., & Kale, B. B. (2011). Ecofriendly hydrogen production from abundant hydrogen sulfide using solar light-driven hierarchical nanostructured $ZnIn_2S_4$ photocatalyst. *Green Chemistry*, *13*, 2500-2506. https://doi.org/10.1039/c1gc15515f

Dilgin, Y., Kizilkaya, B., Ertek, B., Eren, N., & Dilgin, D.G. (2012). Amperometric determination of sulfide based on its electrocatalytic oxidation at a pencil graphite electrode modified with quercetin. *Talanta*, *89*, 490-495. https://doi.org/10.1016/j.talanta.2011.12.074

Duan, H., Koe, L. C. C., Yan, R., & Chen, X. (2006). Biological treatment of H_2S using pellet activated carbon as a carrier of microorganisms in a biofilter. *Water Research*, *40*, 2629-2636. https://doi.org/10.1016/j.watres.2006.05.021

Fagnani, E., Guimarães, J. R., Grassi, M. T., & Fadini, P. S. (2012). Controle de qualidade na determinação potenciométrica de sulfetos volatilizáveis por acidificação (SVA) utilizando adições de padrão. *Química Nova na Escola*, *35*, 832-836. https://doi.org/10.1590/S0100-40422012000400033

Feng, S., Yang, H., & Wang, W. (2015). System-level understanding of the potential acid tolerance components of *Acidithiobacillus thiooxidans* ZJJN-3 under extreme acid stress. *Extremophiles*, *19*, 1029-1039. https://doi.org/10.1007/s00792-015-0780-z

Ghadiri, M., Kariminia, H., & Azad, R. R. (2013). Spectrophotometric determination of sulfide based on peroxidase inhibition by detection of purpurogallin formation. *Ecotoxicology and Environmental Safety*, *91*, 117-121. https://doi.org/10.1016/j.ecoenv.2013.01.015

Ghosh, W., & Dam, B. (2009). Biochemistry and molecular biology of lithotrophic sulfur-oxidation by taxonomically and ecologically diverse Bacteria and Archaea. FEMS Microbiology Reviews, 33, 999-1043. https://doi.org/10.1111/j.1574-6976.2009.00187.x

Ginkel, S. W. V., Hassan, S. H. A., & Oh, S. E. (2010). Detecting endocrine disrupting compounds in water using sulfur-oxidizing bacteria. *Chemosphere*, *81*, 294-297. https://doi.org/10.1016/j.chemosphere.2010.05.056

Griesbeck, C., Schutz, M., Schodl, T., Bathe, S., Nausch, L., Mederer, N., Vielreicher, M., & Hauska, G. (2002). Mechanism of sulfide-quinone reductase investigated using site-directed mutagenesis and sulfur analysis. *Biochemistry*, *41*, 11552-11565. https://doi.org/10.1021/bi026032b

Hughes, M. N., Centelles, M. N., & Moore, K. P. (2009). Making and working with hydrogen sulfide-The chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: A review. *Free Radical Biology & Medicine*, *47*, 1346-1353. https://doi.org/10.1016/j.freeradbiomed.2009.09.018

Janfada, B., Yazdian, F., Amoabediny, G., & Rahaie, M. (2015). Use of sulfur‐oxidizing bacteria as recognition elements in hydrogen sulfide biosensing system. *Biotechnology and Applied Biochemistry*, *62*, 349-356. https://doi.org/10.1002/bab.1282

Jensen, H. S., Lens, P. N. L., Nielsen, J. L., Bester, K., Nielsen, A. H., Jacobsen, T. H., & Vollertsen, J. (2011). Growth kinetics of hydrogen sulfide oxidizing bacteria in corroded concrete from sewers. *Journal of Hazardous Materials*, *189*, 685-691. https://doi.org/10.1016/j.jhazmat.2011.03.005

Khan, S. A. (2012). UV-ATR Spectroscopy study of the speciation in aqueous polysulfide electrolyte solutions. *International Journal of Electrochemical Science*, *7*, 561-568.

Kumeria, T., Parkinson, L., & Losic, D. (2011). A nanoporous interferometric micro-sensor for biomedical detection of volatile sulphur compounds. *Nanoscale Research Letters*, *6*, 634. https://doi.org/10.1186/1556-276X-6-634

Lawrence, J., Robinson, K. L., & Lawrence, N. (2007). Electrochemical determination of sulfide at various carbon substrates: A comparative study. *Analytical Sciences*, *23*, 673-676. https://doi.org/10.2116/analsci.23.673

Lee, E. Y., Lee, N. Y., Cho, K.S., & Ryu, H.W. (2006). Removal of hydrogen sulfide by sulfate-resistant *Acidithiobacillus thiooxidans* AZ11. *Journal of Bioscience and Bioengineering*, *101*, 309-314. https://doi.org/10.1263/jbb.101.309

Li, C., Zhang, D., Wang, J., Hu, P., & Zhaohua, J. (2017). Magnetic MoS2 on multiwalled carbon nanotubes for sulfide sensing. Analytica Chimica Acta, 975, 61-69. https://doi.org/10.1016/j.aca.2017.04.021

Liu, H. L., Y, F. C., Lin, H. Y., Huang, C. H., Fang, H. W., Tsai, W. B., & Cheng, Y. C. (2008a). Artificial neural network to predict the growth of the indigenous Acidithiobacillus thiooxidans. Chemical Engineering Journal, 137, 231-237. https://doi.org/10.1016/j.cej.2007.04.024

Liu, L., Zhinchun, C., Shaoming, Y., Jin, X., & Lin, X. (2008b). A novel inhibition biosensor constructed by layer-by-layer technique based on biospecific affinity for the determination of sulfide. Sensors and Actuators B, 129, 218-224. https://doi.org/10.1016/j.snb.2007.07.137

Lors, C., Chehade, M. H., & Damidot, D. (2009). pH variations during growth of *Acidithiobacillus thiooxidans* in buffered media designed for an assay to evaluate concrete biodeterioration. *International Biodeterioration & Biodegradation*, *63*, 880–883. https://doi.org/10.1016/j.ibiod.2009.06.012

Luther, G. W., Findlay, A. J., MacDonald, D. J., Owings, S. M., Hanson, T. E., Beinart, R. A., & Girguis, P. R. (2011). Thermodynamics and kinetics of sulfide oxidation by oxygen: A look at inorganically controlled reactions and biologically mediated processes in the environment. Frontiers in Microbiology, 62, 1-9. https://doi.org/10.3389/fmicb.2011.00062

Mirzaei, M., Amoabediny, G., Yazdian, F., Sheikpour, M., Ebrahimi, E., & Zadeh, B. E. H. (2014). An immobilized *Thiobacillus thioparus* biosensing system for monitoring sulfide hydrogen; optimized parameters in a bioreactor. *Process Biochemistry*, *49*, 380-385. https://doi.org/10.1016/j.procbio.2013.12.013

Montebello, A. M., Baeza, M., Lafuente, J., & Gabriel, D. (2010). Monitoring and performance of a desulphurizing biotrickling filter with an integrated continuous gás/liquid flow analyser. *Chemical Engineering Journal*, *165*, 500-507. https://doi.org/10.1016/j.cej.2010.09.053

Nguyen, T. A., Fu, C. C., & Juang, R. S. (2016). Biosorption and biodegradation of a sulfur dye in high-strength dyeing wastewater by Acidithiobacillus thiooxidans. *Journal of Environmental Management*, *182*, 265-271. https://doi.org/10.1016/j.jenvman.2016.07.083

Nielsen, A. H., Vollertsen, J., & Jacobsen, T. H. (2006). Kinetics and stoichiometry of aerobic sulfide oxidation in wastewater from sewers-effects of pH and temperature. *Water Environment Research*, *78*, 275-283. https://doi.org/10.2175/106143005X94367

Rabbani, K. A., Charles, W., Cord-Ruwisch, R., & Ho, G. (2015). Recovery of sulphur from contaminated air in wastewater treatment plants by biofiltration: a critical review. *Reviews in Environmental Science and Bio/Technology*, *14*, 523-534. https://doi.org/10.1007/s11157-015-9367-5

Ramesh, R., Puhazhendi, P., Kumar, J., Gowthaman, M. K., D'Souza, S. F., & Kamini, N. R. (2015). Potentiometric biosensor for determination of urea in milk using immobilized *Arthrobacter creatinolyticus* urease. *Materials Science and Engineering C*, *49*, 786-792. https://doi.org/10.1016/j.msec.2015.01.048

Rawal, R., & Pundir, C. S. (2012). Development of an amperometric sulfite biosensor based

on SOx/PBNPs/PPY modified ITO electrode. *International Journal of Biological Macromolecules*, *51*, 449-455. https://doi.org/10.1016/j.ijbiomac.2012.06.008

Redondo, R., Machado, V. C., Baeza, M., Lafuente, J., & Gabriel, D. (2008). On-line monitoring of gas-phase bioreactor for biogas treatment: hydrogen sulfide and sulfide analysis by automated flow systems. *Analytical and Bioanalytical Chemistry*, *391*, 789-798. https://doi.org/10.1007/s00216-008-1891-5

Rodriguez, G., Dorado, A. D., Fortuny, M., Gabriel, D., & Gamisans, X. (2014). Biotrickling filters for biogas sweetening: oxygen transfer improvement for a reliable operation. *Process Safety and Environmental Protection*, *92*, 261-268. https://doi.org/10.1016/j.psep.2013.02.002

Rohwerder, T., & Sand, W. (2003). The sulfane sulfur of persulfides is the actual substrate of the sulfur-oxidizing enzymes from *Acidithiobacillus* and *Acidiphilium* spp. *Microbiology*, *149*, 1699-1709. https://doi.org/10.1099/mic.0.26212-0

Sharihari, S. R., Bhavaraju, J. M., Modak, R., Kumar, R., & Ghandhi, K. S. (1993). Dissolution of sulphur particles by *Thiobacillus ferrooxidans*: substrate for unattached cells. *Biotechnology and Bioengineering*, *41*, 612-616. https://doi.org/10.1002/bit.260410603

Son, K. H., Lee, C. G., & Cho, N. (2007). Leaching of copper from furnace dust by pure and mixed culture of Thiobacillus ferrooxidans and Thiobacillus thiooxidans. *Bulletin of the Korean Chemical Society*, *28*, 1777-1780. https://doi.org/10.5012/bkcs.2007.28.10.1777

Vosoughi, A., Yazdian, F., Amoabediny, G., & Hakim, M. (2015). Investigating the effect of design parameters on the response time of a highly sensitive microbial hydrogen sulfide biosensor based on oxygen consumption. *Biosensors and Bioelectronics*, *70*, 106-114. https://doi.org/10.1016/j.bios.2015.03.025

Yin, H., Zhang, X., Li, X., He, Z., Liang, Y., Guo, X., Hu, Q., Xiao, Y., Cong, J., Ma, L., Niu, J., & Liu, X. (2014). Whole-genome sequencing reveals novel insights into sulfur oxidation in the extremophile *Acidithiobacillus thiooxidans*. *BMC Microbiology*, *14*, 179. https://doi.org/10.1186/1471-2180-14-179

Yousefi, A., Hejazi, P., & Allahverdi, A. (2013). Evaluation of effective strategies for cultivation of *Acidithiobacillus thiooxidans* as cement-degrading bacteria. *Iranian Journal of Chemical Engineering*, *10*, 55-66.

Zhang, J. Z., & Millero, F. J. (1993). The products from the oxidation of H_2S in seawater. *Geochimica et Cosmochimica Acta*, *57*, 1705-1718. https://doi.org/10.1016/0016-7037(93)90108-9

Zhang, L., Schryver, P., Gusseme, B., Muynck, W., Boon, N., & Verstraete, W. (2008). Chemical and biological technologies for hydrogen sulfide emission control in sewer systems: A review. *Water Research*, *42*, 1-12. https://doi.org/10.1016/j.watres.2007.07.013

Zhao, Y., Biggs, T. D., & Xian, M. (2014). Hydrogen sulfide (H_2S) releasing agents: chemistry and biological applications. *Chemical Communications*, *50*, 11788-11805.

https://doi.org/10.1039/C4CC00968A

Zirrahi, M., Azinb, R., Hassanzadeha, H., & Moshfeghian, M. (2012). Mutual solubility of CH_4, CO_2, H_2S, and their mixtures in brine under subsurface disposal conditions. *Fluid Phase Equilibria*, *324*, 80-93. https://doi.org/10.1016/j.fluid.2012.03.017

Copyright Disclaimer