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Hydrogen Sulfide Removal from Air by *Acidithiobacillus thiooxidans* in a Trickle Bed Reactor

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ABSTRACT. A strain of *Acidithiobacillus thiooxidans* immobilized in polyurethane foam was utilized for H_2S removal in a bench-scale trickle-bed reactor, testing the limits of acidity and SO_4^{2-} accumulation. The use of this acidophilic strain resulted in remarkable stability in the performance of the system. The reactor maintained a >98–99 % H_2S removal efficiency for c of up to 66 ppmv and empty bed residence time ≤12–15 s. Removal of >98 % H_2S was achieved under steady-state conditions, over the pH range of 0.44–7.30. Despite the accumulation of acidity and SO_4^{2-} (up to 97 g/L), the system operated without inhibition.

Abbreviations

c_{in}	inlet H_2S concentration, g S/m^3	ppmv	parts per million by volume
c_{out}	outlet H_2S concentration, g S/m^3	PUF	polyurethane foam
EC	elimination capacity, g S m^{-3} h^{-1}	Q	air flow rate, m^3/h
EBRT	empty-bed residence time, min and/or s	R	removal efficiency, %
L	load, g S m^{-3} h^{-1}	V_b	bed volume, m^3

Based on the cost analysis, biological treatment appears to be favorable for the H_2S removal from diluted streams (Wani *et al.* 1999). However, the *practical* use of H_2S biofiltration has been hindered by high standards for its removal. Since this common air pollutant retains toxicity and odor even at low concentration (Jin *et al.* 2005; Lee *et al.* 2006), guaranteed >97–99 % removal is required. This challenge is enhanced by the stoichiometric accumulation of a product, H_2SO_4. Both high $[H^+]$ and $[SO_4^{2-}]$ are known to be toxic to bacteria (Tanji *et al.* 1989; Ruokojärvi *et al.* 2001; Jin *et al.* 2005; Sercu *et al.* 2005; Chen *et al.* 2006).

Various microorganisms have been used for H_2S removal. Neutrophilic bacteria, such as *Thiobacillus thioparus* (Tanji *et al.* 1989; Cho *et al.* 1992; Chung *et al.* 1996), have been used in most cases, as well as heterophiles, *e.g.*, *Xanthomonas* sp. (Cho *et al.* 1992), *Pseudomonas* sp. and *Arthrobacter* sp. (Chung *et al.* 1996). Efficient H_2S removal was obtained while using any of these bacteria as long as the mineral medium was frequently replaced (*i.e.* in biotrickling reactors) to prevent $[H^+]$ and $[SO_4^{2-}]$ accumulation (Tanji *et al.* 1989; Ruokojärvi *et al.* 2001; Sercu *et al.* 2005).

However, frequent medium change leads to higher costs. Thus, even though the rate-limiting step under optimum conditions does not involve biochemical reactions, the problem of long-term stable bioreactor performance demands a microbiological solution. The use of acidophilic bacteria, *e.g.*, *Acidithiobacillus thiooxidans* or *T. thiooxidans*, was explored in earlier papers (Shinabe *et al.* 1995; Sercu *et al.* 2005; Lee *et al.* 2006; Aroca *et al.* 2007). This approach may meet the challenge of accumulation of toxic products, particularly since these bacteria exhibit tolerance to high levels of both $[H^+]$ and $[SO_4^{2-}]$ (Lee *et al.* 2005).

Biotrickling filters with acidophiles were successfully operated without pH control (Sercu *et al.* 2005; Aroca *et al.* 2007). However, the quantitative limits of tolerance affecting the H_2S removal have not been defined. This paper addresses this issue by subjecting a biotrickling reactor to a wide range of $[H^+]$ and $[SO_4^{2-}]$ while monitoring the H_2S removal.

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MATERIALS AND METHODS

Microorganism and medium. A strain of *Acidithiobacillus thiooxidans* DSM 11478, obtained from Minas Gerais (Brazil), was cultivated at its optimum growth temperature (30 °C) and pH 2.5 in 9K medium (Silverman and Lundgren 1959) of the following composition (g/L): $(NH_4)_2SO_4$ 3, $MgSO_4$ 0.5, K_2HPO_4 0.5, $Ca(NO_3)_2$ 0.5, KCl 0.1, supplemented with powdered sulfur (10 g/L) as the sole energy source.

Analyses. A *Crison* 5202 pH meter was used to monitor pH. The $[SO_4^{2-}]$ was measured using the classical turbidimetric method (Clesceri *et al.* 1989), H_2S being quantified using a specific Crowcon sensor (model Gasflag TXGARD-IS).

Immobilization. To monitor immobilization, the entire unit of packing material was submerged in an Erlenmeyer flask containing 25 mL of 9K medium and placed in an ultrasonic bath for ¼ h. This treatment resulted in a complete desorption of adhered cells while retaining their viability (de Ory *et al.* 2004). Suspended bacteria were counted in a Neubauer chamber (using an *Olympus* BH-2 optical microscope). Upon drying the packing for 1 d at 80 °C, the cell count per g of dry mass was obtained.

PUF cubes (1 cm^3 size, 7.8 g/L density) were used as packing material. Three Erlenmeyer flasks, each containing 500 mL medium, 6 g sulfur, 100 mL inoculum, and PUF (10 g total), were used for immobilization. The culture was incubated at 2.5 Hz and 30 °C. Once the pH reached 1.0, the liquid was replaced with 600 mL of fresh medium. This procedure was repeated until the biomass level stabilized at $(1.6 \pm 0.5) \times 10^{10}$ cells per g by the end of 4th "drown-and-fill" cycle (30 d). Then the bacteria-laden PUF was plaed into the trickling bioreactor.

Parameters were calculated as defined by Devinny *et al.* (1999):

$$\text{EBRT} = 60\ V_b/Q\ (\text{min}) \quad (1)$$

$$\text{R} = (c_{in} - c_{out})/c_{in} \times 100\ (\%) \quad (2)$$

$$\text{EC} = (c_{in} - c_{out}) \times Q/V_b\ (\text{g S m}^{-3}\ \text{h}^{-1}) \quad (3)$$

$$\text{L} = c_{in} \times Q/V_b = 3600\ c_{in}/\text{EBRT}\ (\text{g S m}^{-3}\ \text{h}^{-1}) \quad (4)$$

where g S is grams of sulfur upon recalculation from H_2S.

$$\text{EC} = \text{R} \times \text{L}/100\ (\%) \quad (5)$$

yields a line on an EC *vs.* L plot, with a slope of R/100. The critical value (R = 97–99 %) has been used for assessing the H_2S biofiltration efficiency (Shinabe *at al.* 1995; Jin *et al.* 2005).

Reactor: stages, sections, and subsets. For experimental setup *see* Fig. 1. A PVC column (∅ 63 mm) was used to build the trickling reactor with two *stages*, 0.21 m of bed height each (with a total packing voume of 1.278 L). In turn, each stage was equipped with 3 outlets at fixed bed heights of 0.06, 0.13, 0.21 (end of the 1st stage), 0.27, 0.34, and 0.41 m (the end of reactor), thus defining 6 reactor *sections*. The consecutive combined sections, starting from the inlet, comprised 6 reactor *subsets* with bed volumes (proportional to the

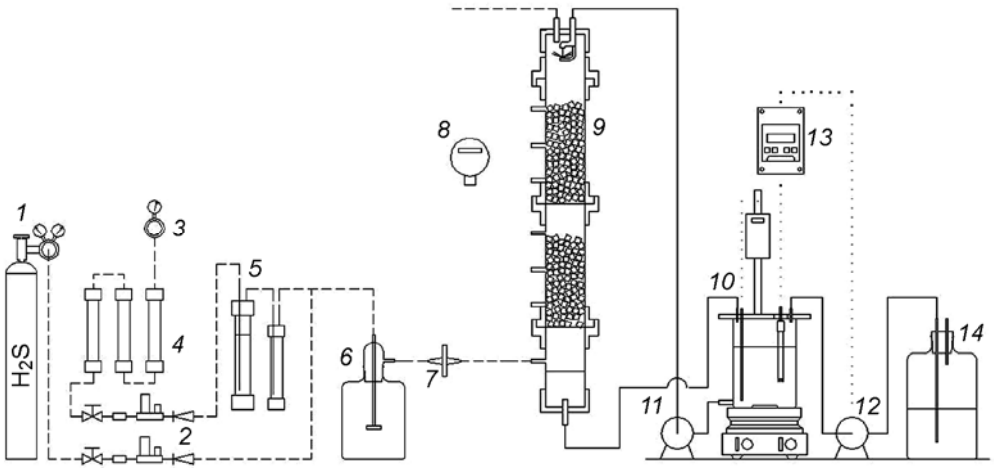

Fig. 1. Experimental setup;

1	H_2S or air cylinder	*6*	expansion deposit	*11*	recirculation pump
2	flow controller	*7*	air filter	*12*	pH control pump
3	pressure regulator	*8*	H_2S sensor	*13*	pH controller
4	prefilter	*9*	biotrickling reactor	*14*	NaOH deposit
5	humidification system	*10*	medium tank		

bed height) increasing toward the end of the reactor, *e.g.*, section *1* (1st subset), followed by combined sections *1–2* (2nd subset), *1–3* (3rd), *1–4*, *1–5*, and, finally, the whole reactor. EBRT values were calculated for each of the subsets according to Eq. (*1*). H_2S concentration was monitored at the inlet of the reactor and 6 outlets, thus allowing the calculation of R, EC, and L for each subset according to Eqs (*2*)–(*4*).

Setup. Compressed air was passed through 3 filters, filled with silica gel, active carbon, and glass wool, respectively; then it was sterilized using a *Millipore* filter SLG 05010 (0.45 μm; *not shown* in Fig. 1), and humidified by passing through a column filled with tap water. Air flow was set with a *Bronkhorst* model F-201C flow controller.

The medium was recirculated through the reactor (19 L/h). The pH (2) and temperature (30 °C) were controlled by using a pH controller (*Crison* PH28) and *Heidolph* EKT3001 thermostat, respectively. Fresh medium was added whenever [SO_4^{2-}] reached 30 g/L unless stated otherwise.

RESULTS AND DISCUSSION

The reactor was run for 580 h (24⅙ d) under an EBRT of 24 s and H_2S loads of 12–26 g S m^{-3} h^{-1} without any changes in performance (R > 98 %). The H_2S removal was not affected by medium replacement; *i.e.*, the suspended biomass did not affect the performance of the system.

Effect of H_2S c_{in} *and* EBRT. To ensure the correct assessment of the effects of [H^+] and [SO_4^{2-}] accumulation on H_2S removal, critical system performance parameters were determined. The H_2S load was set at 4 g S m^{-3} h^{-1} and then incrementally increased to 59 g S m^{-3} h^{-1} (by raising the c_{in} while keeping the whole EBRT constant of the reactor at 24 s). Any changes in the reactor operational parameters were made once in 2–3 h, with the H_2S measurements conducted at the end of this time period, to ensure the achievement of steady state (small transient effects subsided within 1 h). The pH was maintained at 2.0–2.1 and [SO_4^{2-}] was 23–30 g/L (Fig. 2A).

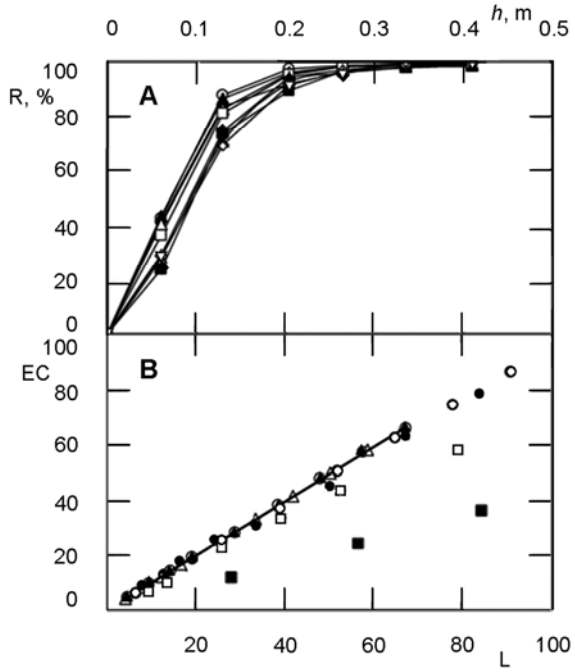

Fig. 2. Varying the load by H_2S c_{in};
A: Removal efficiency (R, %) along the bed height (*h*, m) at a constant EBRT of 24 s; loads (L, g S m^{-3} h^{-1}):
full circles – 4 *open circles* – 8
full triangles – 12 *open triangles* – 16
full squares – 25 *open squares* – 33
full rhombs – 42 *open rhombs* – 50
reverse triangles – 59.
B: Elimination capacity (EC, g S m^{-3} h^{-1}) of the reactor and its subsets *vs.* load (L, g S m^{-3} h^{-1}); EBRT (in s):
full squares – section *1* – 3.6
open squares – sections *1–2* – 8
full circles – sections *1–3* – 12
open circles – sections *1–4* – 16
full triangles – sections *1–5* – 20
open triangles – whole reactor – 24.
The line corresponds to R = 98 %.

R increased gradually for larger reactor subsets until it became >98 % for any H_2S load within the range used. This feature is underscored in Fig. 2B [*i.e.*, a EC *vs.* L plot; Eq. (*5*)]. Deviations from the R = 98 % line occur because smaller reactor subsets fail to meet this specification at a certain threshold load reflecting the maximum value of cell catabolic activity. Once it is exceeded, the system is overloaded and R drops. The shorter bed height of the subset [*i.e.*, smaller volume and lower EBRT; Eq. (*1*)], the greater this effect.

The effect of EBRT was followed at a constant c_{in} of 66 ppmv, EBRT was varied by adjusting the air flow rate [Eq. (*1*)] (Fig. 3). The reactor performance improved along the bed height and upon increasing EBRT, similarly to the previous experiment. R > 98 % was observed up to a load of 28 g S m^{-3} h^{-1} and EBRT of 13 s.

Effect of the constant load. The next experiment was focused on the combined effect of changing both the air flow rate and c_{in} while keeping the load constant at 13 g S m^{-3} h^{-1} (Fig. 4). This test was aimed at determining whether the EBRT or c_{in} makes a stronger impact on the performance of the reactor. While in the previous experiments EBRT variations caused proportional changes in H_2S load, now this side effect was compensated by proportional changes in the H_2S inflow [Eq. (*4*)].

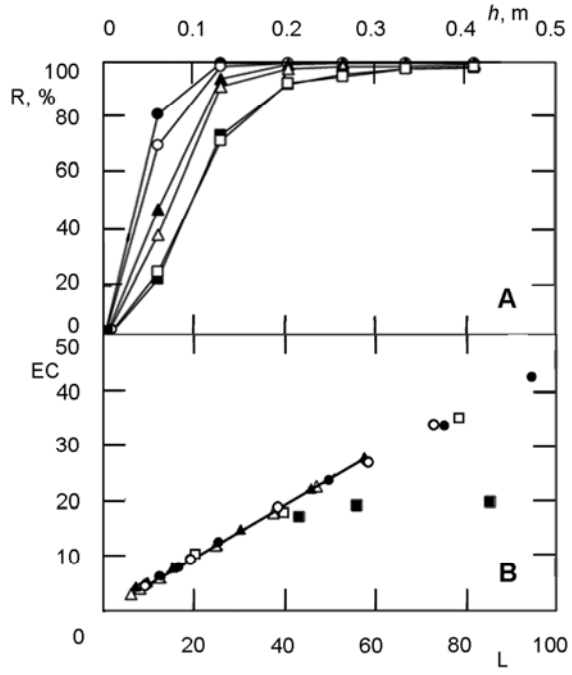

Fig. 3. Varying the load by air flow rate;
A: Removal efficiency (R, %) along the bed height (*h*, m) at a constant H_2S c_{in} of 66 ppmv:
EBRT (in s): *full circles* – 96; *open circles* – 73; *full triangles* – 49; *open triangles* – 24; *full squares* – 16; *open squares* – 13.
B: Elimination capacity (EC, g S m^{-3} h^{-1}) of the reactor and its subsets *vs.* load (L, g S m^{-3} h^{-1}):
full squares – section *1*
open squares – sections *1–2*
full circles – sections *1–3*
open circles – sections *1–4*
full triangles – sections *1–5*
open triangles – whole reactor.
The line corresponds to R = 98 %.

R > 98 % was observed for the whole reactor only as long as the EBRT exceeded its critical value of 12–14 s (Fig. 4). Thus, EBRT is a more important factor for the reactor performance than c_{in}. Apparently, the gas–liquid mass transfer is rate-limiting and requires minimum contact time.

For larger subsets of the reactor (sections *1–3* and beyond), the critical EBRT value was 12–15 s, *i.e.* statistically the same as for the whole reactor. In contrast, R was significantly lower for the 1st reactor section (*i.e.* its smallest subset) at any EBRT. Thus, this 1st subset of the reactor was deemed non-representative, due to its position facing the inlet flow. Combined sections *1–2* were short of R = 98 % at EBRT = 22 s for an effective load of 13 g S m^{-3} h^{-1} and 27 s for 39 g S m^{-3} h^{-1} (*not shown*). Thus, this subset is under moderate stress. Thus, if a reactor or its subsets experience a moderate stress, the critical EBRT may rise twice.

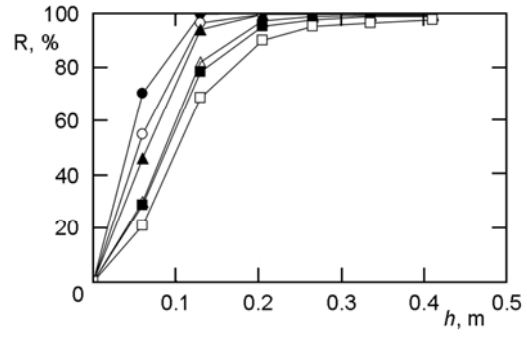

Fig. 4. Removal efficiency (R, %) along the bed height (*h*, m) at a constant load of 13 g S m^{-3} h^{-1};
EBRT (s) and c_{in} (ppmv):
full circles – 96; 259; *open circles* – 73; 197
full triangles – 49; 132; *open triangles* – 24; 66
full squares – 16; 44; *open squares* – 13; 35.

Setup for experiments on SO_4^{2-} *and acidity accumulation.* The key experiments here were conducted under the conditions of assured optimum performance (EBRT = 50 s, H_2S loads 4–13 g S m^{-3} h^{-1}). Low loads were also essential for preventing the SO_4^{2-} over-accumulation beyond 30 g/L (which corresponds to nearly 1 osmol/L total ion osmolarity, thus setting a reasonable physiological limit).

We set out to run the tests without replacing the medium, to let $[H^+]$ and $[SO_4^{2-}]$ accumulate naturally as a result of H_2S oxidation. Selected experiments were also conducted under a higher load of 26 g S m^{-3} h^{-1}, which would yield a lower R if the biocatalyst were under moderate stress (as shown *above*).

Effect of SO_4^{2-} *accumulation.* The influence of $[SO_4^{2-}]$ was examined under pH = 2 and EBRT = 50 s. During the 1st phase, the H_2S load was kept constant at 13 g S m^{-3} h^{-1}; then, after 13 d, it was increased to 26 g S m^{-3} h^{-1} (Fig. 5). Meanwhile, $[SO_4^{2-}]$ increased from 29 to 57 g/L as a result of H_2S biological oxidation (*i.e. biological* $[SO_4^{2-}]$). Throughout this experiment, R > 98 % persisted, despite a significant increase in $[SO_4^{2-}]$, indicating that $[SO_4^{2-}]$ accumulation did not affect the H_2S removal.

To further test this hypothesis, $[SO_4^{2-}]$ was increased, after 450 h of operation, to 97 g/L (through the addition of Na_2SO_4, *i.e. chemical* $[SO_4^{2-}]$). Note that this concentration was near 1 mol/L, hence the osmolarity approached 3 osmol/L. However, R > 98 % remained unaffected (*not shown*).

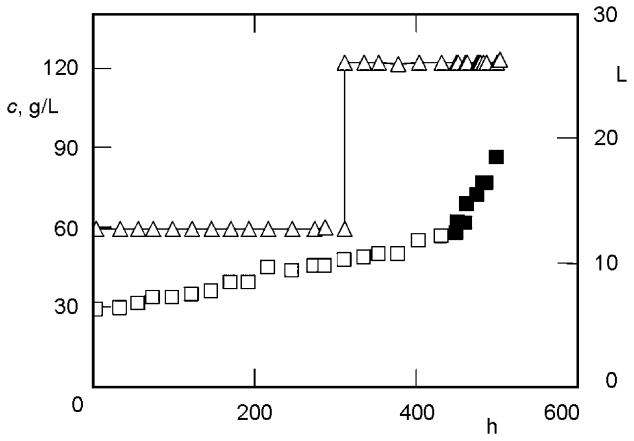

Fig. 5. Effect of SO_4^{2-} accumulation (*c*, g/L; *open squares* – biological, *full squares* – chemical) at two H_2S loads (L, g S m^{-3} h^{-1}; *triangles*); the constant R = 99 ± 1 is *not shown*.

In agreement with this result, Lee *et al.* (2005) reported that the accumulation of $(NH_4)_2SO_4$ up to 60 g/L had no effect on growth of *A. thiooxidans* TAS; however, the sulfur oxidation rate dropped to zero at $[SO_4^{2-}]$ of 80 g/L. Another acidophilic strain, *A. thiooxidans* AZ11, exhibited growth at 74 g/L SO_4^{2-} (Lee *et al.* 2006).

We proved that $[SO_4^{2-}]$ up to 97 g/L caused no effect on H_2S oxidation. Thus, under biofiltration conditions acidophiles are even more tolerant to SO_4^{2-}; perhaps, due to cell immobilization. In contrast, the use of neutrophilic strains necessitated medium change whenever $[SO_4^{2-}]$ exceeded 5–15 g/L (Tanji *et al.* 1989; Ruokojärvi *et al.* 2001; Sercu *et al.* 2005; Chen *et al.* 2006).

Effect of acidity. EBRT was kept at 50 s and $[SO_4^{2-}]$ was <30 g/L. In the 1st phase here, the controlled pH decreased from 7.3 to 1.0 with an H_2S load of 4 g S m^{-3} h^{-1}. Each pH value was maintained for >20 h to provide time for the development of inhibitory effects, if applicable. The 2nd phase started with the same load of 4 g S m^{-3} h^{-1} when the pH was gradually dropping below 1.0 (the medium was replaced at this point to remove the suspended biomass, which could artificially contribute to the H_2S removal). After 28 h, the load was increased to 8 g S m^{-3} h^{-1}, the maximum value to keep $[SO_4^{2-}]$ <30 g/L without frequent dilution.

Despite these pH variations, R > 98 % remained unchanged (Fig. 6). It corroborates the data of Shinabe *et al.* (1995), who observed that the H_2S removal by acidophiles (*T. thiooxidans*) was constant within a wide pH range (1–6), despite the growth optimum being at pH 2.5. Our work has further extended this pH operational range to even lower values beyond the observed physiological limits. These observations are consistent with mass transfer limitations of the process (underscored by a great influence of EBRT on R).

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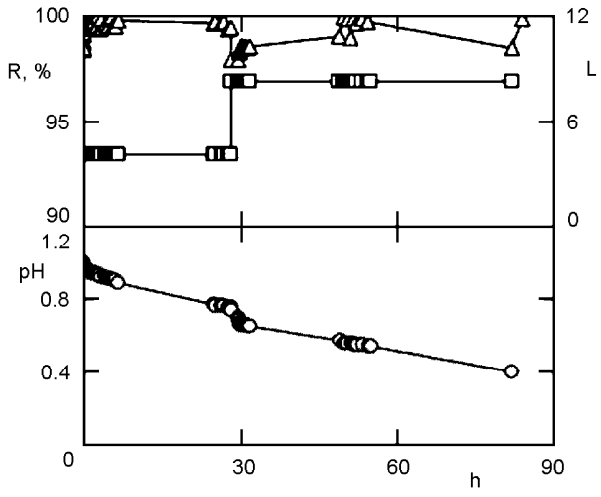

Fig. 6. Effect of pH on the H_2S removal efficiency (R, %; *triangles*), pH (*circles*) and H_2S load (L, g S m^{-3} h^{-1}; *squares*).

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