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RESEARCH ARTICLE

Comparison on the removal of hydrogen sulfide in biotrickling filters inoculated with *Thiobacillus thioparus* and *Acidithiobacillus thiooxidans*

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Abbreviations: PVC: polyvinilclorure
PE: polyethylene
TRS: Total Reduced Sulfur
TZ: tezontle

Emissions of hydrogen sulfide (H_2S) by industrial activities is frequent cause of corrosion and unpleasant odours. Treatment of gaseous emissions contaminated with H_2S by biotrickling filters inoculated with single cultures of sulfur oxidizer bacteria exhibit several advantages over physicochemical methods, such as shorter adaptation times and higher removal ability. Biofilms of *Thiobacillus thioparus* and *Acidithiobacillus thiooxidans* have proved to exhibit high removal capacities, yet no comparative studies between them have been reported. This article reports the efficiency of biotrickling filters inoculated with *T. thioparus* and *A.*

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***thiooxidans* under similar conditions excepting the pH, that was the optimal for the bacterial growth, for the removal of H_2S. The support was selected by determining the respirometric coefficients of the biomass. The maximum removal capacity of the biofilter inoculated with *T. thioparus*, operating within the range of pH (5.5-7.0) was 14 gS m^{-3} h^{-1}, lower the value obtained for the biotrickling filter inoculated with *A. thiooxidans*; 370 gS m^{-3} h^{-1}. Therefore, it is concluded that acid biotrickling filter inoculated with *A. thiooxidans* constitute the best strategy to remove H_2S, with the advantage that the system not require an exhaustive pH control of the liquid media.**

Hydrogen sulfide (H_2S) is being emitted by many industrial activities such as petroleum refining, natural gas and petrochemical plants, craft pulp manufacturing, viscose rayon manufacturing, food processing, tanneries, aerobic and anaerobic wastewater treatments. H_2S is a colourless, flammable and highly toxic gas, and heavier than air. Its value of Henry's Law constant for the air-water-H_2S system at 25ºC is 0,41 (H_2S concentration in air/H_2S concentration in water). Usually H_2S is found in mixture with other organic sulfur compounds such as methanethiol, dimethylsulfide and dimethyldisulfide. This mixture is known as the Total Reduced Sulfur Compounds (TRS) (Ruokojarvi et al. 2000).

The physicochemical methods used at present for the treatment of gaseous emissions containing H_2S and reduced sulfur compounds, such as adsorption, absorption, incineration, have relatively high energy requirements and/or high chemical and disposal cost. Several biological processes used to treat gaseous emissions contaminated with TRS, and specifically with H_2S, have been reported (Jensen and Webb, 1995; Smet et al. 1998).

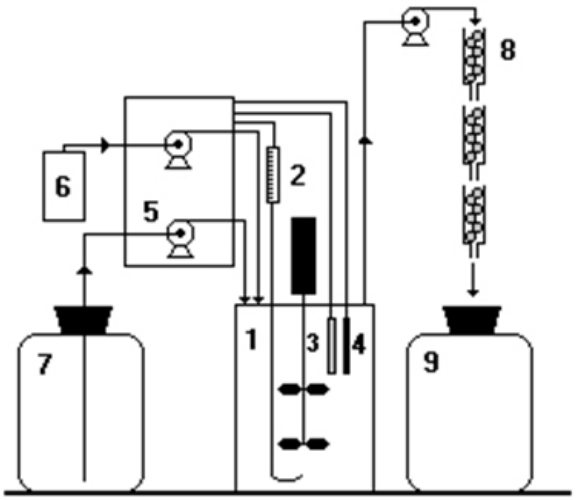

Figure 1. Diagram of the system for generating a biofilm of *Thiobacillus thioparus*. (1) Bioreactor; (2) Flowmeter; (3) Sensor of pH; (4) Heater; (5) Peristaltic pump; (6) NaOH; (7) Liquid culture medium; (8) Glass columns packed with the carriers; (9) Effluent accumulation.

Biological treatments using biotrickling filters have been proposed as convenient alternatives for treating air streams containing low concentration of contaminants (Deshusses, 1997; Kennes and Thalasso, 1998; Gabriel and Deshusses, 2003). These are packed columns where a bacterial biofilm is formed on the surface of an inert packing material. The contaminated air stream is concurrently or counter-currently contacted with a liquid phase that provides nutrients and conditions to keep the viability and activity of the biofilm. The gas is absorbed in the liquid phase and biologically oxidized, thus converting H_2S compounds into oxidized sulfur compounds such as sulfur and sulfate, chemicals that will go out of the bioreactor in the liquid phase (Alonso et al. 1997).

Although it is possible to establish a complex microbial population with the ability to oxidize H_2S, *i.e.*: by inoculating the biofilter with biological complex inoculums such as active sludge, a dominating microbial population exhibiting the highest degradation activity develops after a period of adaptation (Hirai et al. 1990; Cho et al. 1992). However in some cases, the efficiency of these systems is limited and generally its removing capacity is not constant (Wani et al. 1997). The use of biofilms generated with single bacterial cultures has been proposed for improving the efficiency and removal capacities of the biotrickling filters. Chemoheterotrophic microorganisms such as *Pseudomonas acidovorans* DMR-11 and *Pseudomonas putida* show degrading activity of H_2S and organic sulfur compounds. Bacteria from the genus *Acidithiobacillus*, such as *A. thiooxidans* that use H_2S as energy source, seem to be appropriate because of their low nutritional requirements (Cho et al. 2000; Sercu et al. 2005). The inoculation of biotrickling systems with single cultures leads to the shortening and even the absence of bacterial lag phase, as well as to the increase in the efficiency for removing H_2S and other organosulfur compounds during a stable operation. *T. thioparus* strains CH11 (Chung et al. 1997) and *T. thioparus* DW44 (Cho et al. 1992) shown high efficiency for removing H_2S, being also able to degrade methanethiol, dimethylsulfide and dimethyldisulfide, and to use carbon disulfide as energy source (Smet et al. 1998). Other bacterial species that have been evaluated for the removal of sulfur reduced compounds are *T. denitrificans* (Sublette and Sylvester, 1987), *A. ferrooxidans* (Pagella and De Faveri, 2000), *T. novellus* (Cha et al. 1999), and *A. thioxidans* (Oprime et al. 2001). There is no comparison in the literature about the performance of biotrickling filters inoculated with different microorganism for the bioxidation of H_2S.

Various materials have been used to support biofilms of *Thiobacillus*, among them polypropylene rings (Tanji et al. 1989), polystyrene foam, diatomaceous earth, ceramics, polystyrene mixed with active carbon, pellets of synthetic materials and perlite (Cox et al. 1997). In general, porous and non hydrophobic surfaces with high specific surface seem to facilitate or promote colonization by microorganisms and the subsequent formation of biofilms

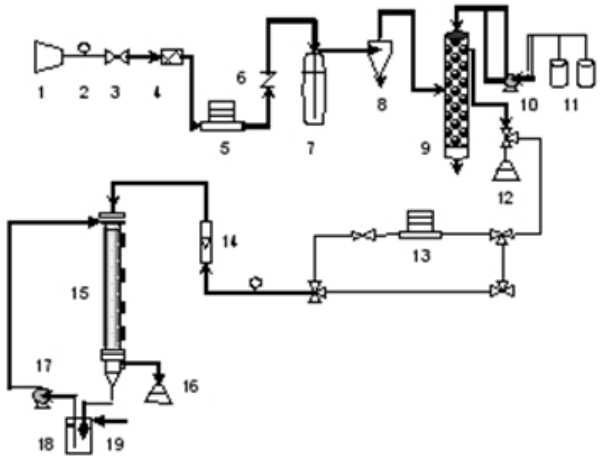

Figure 2. Laboratory-scale experimental biotrickling filter system. (1) Air compressor; (2) Pressure gauge; (3) Needle Valve; (4) Air filter; (5) Mass flow controller; (6) Check valve; (7) Humidification; (8) Mist removal chamber; (9) H_2S generator; (10) Peristaltic pump; (11) Solutions of Na_2S and HCl; (12) Lead acetate solution; (13) Mass flow controller; (14) Flowmeter; (15) Biotrickling filter; (16) Air outlet; (17) Recycling pump; (18) Solution recycle; (19) Solution make-up.

(Chitwood and Devinny, 2001). Since these materials offer different characteristics for bacterial growth and for the operating conditions, it is important to develop analytical techniques to evaluate and compare their behaviour.

This article reports the performance in the bioxidation of H_2S using biotrickling filters inoculated with *T. thioparus* operated at neutral pH conditions, and a biotrickling filter inoculated with *A. thiooxidans* in an acid environment. The selection of the supporting material was made by using respirometry as an index of the sulfur oxidizing capacity of the biofilm formed on three materials: (i) volcanic stones (tezontle (TZ)); (ii) polypropylene rings and (iii) polyvinilclorure (PVC).

MATERIALS AND METHODS

Microorganisms

Thiobacillus thioparus (ATCC 23645) and *Acidithiobacillus thiooxidans* (ATCC 19377) were used to inoculate the solid supports and to initiate the generation of the biofilms in the biotrickling filters. The liquid medium used to culture *T. thioparus*, was a variation of the Thiosulfate medium (ATCC 290), the composition in g L^{-1} was: $Na_2HPO_4*7H_2O$ 2.27; KH_2PO_4 1.8; $MgCl_2*7H_2O$ 0.1; $(NH_4)_2SO_4$ 1.98; $MnCl_2*H_2O$ 0.023; $CaCl_2$ 0.03; $FeCl_3*6H_2O$ 0.033; Na_2CO_3 1; $Na_2S_2O_3*5H_2O$ 15.69 and pH 6.8. The composition of the liquid culture medium used to growth *A. thiooxidans* was (g L^{-1}): KCl 0.1; K_2HPO_4 0.5; $Ca(NO_3)_2$ 0.01; $MgSO_4*7H_20$ 0.5; Sº 10 and pH 2 - 4.

Selection of the support

A continuous culture of *T. thioparus* was set up in a bioreactor, using a volume of 1 L and a dilution rate (D) of 0.03 h^{-1}, 1 vvm of aeration, and controlling the pH at 6.0 and the temperature at 30ºC. The formation of the biofilm on the solid supports tested was conducted by feeding the outlet stream of the continuous culture from the top of three glass columns connected in series, each one packed with one of the following supporting materials: polyethylene rings, pieces of PVC, and porous volcanic stones (TZ). A diagram of this system is shown in Figure 1.

The activity of the biofilm formed on the supporting materials was evaluated by respirometry measuring the ability of the biomass to oxidize thiosulfate using an oxymeter (Yellow Spring Instrument, USA), that was calibrated with distilled water saturated with oxygen. The biomass was released from the supporting material using ultrasound in a solution 0.85% p/v of NaCl. The suspended biomass was washed, centrifuged and resuspended three times to remove the remaining thiosulfate in the solution. The last pellet was suspended in a fresh medium without thiosulfate to obtain a final concentration of 10 g L^{-1} of protein content. 1 mL of cell suspension was used for the respirometry determinations. The rates of oxygen consumption were calculated taking into account the endogenous respiration and chemical oxidation measuring the oxygen consumption without cell suspension. Observations of the biofilm were made by scanning electron microphotography (Electronic microscope JOEL, model 5410).

Biofiltration experiments

Two biotrickling filter were set up using acrylic columns of 50 mm of inner diameter and 600 mm height. The column was filled with polyethylene rings (Kaldness MilijOteknology AS, Norway), the density of the support is 280 kg m^{-3}, specific surface 340 m^2 m^{-3} and it has a 73% of free volume.

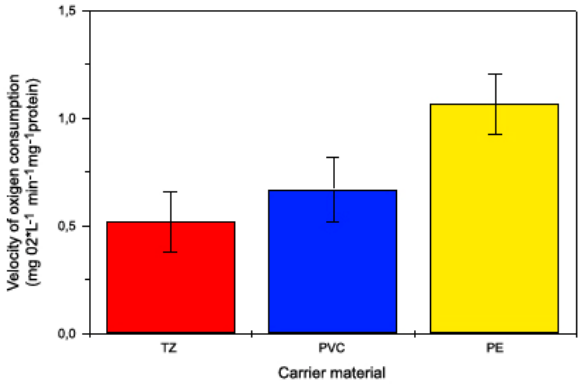

Figure 3. Oxidation rate of thiosulfate (10 g/L) by the biomass detached from TZ, PVC and Polyethilene rings (PE), at 30ºC and pH 6,0.

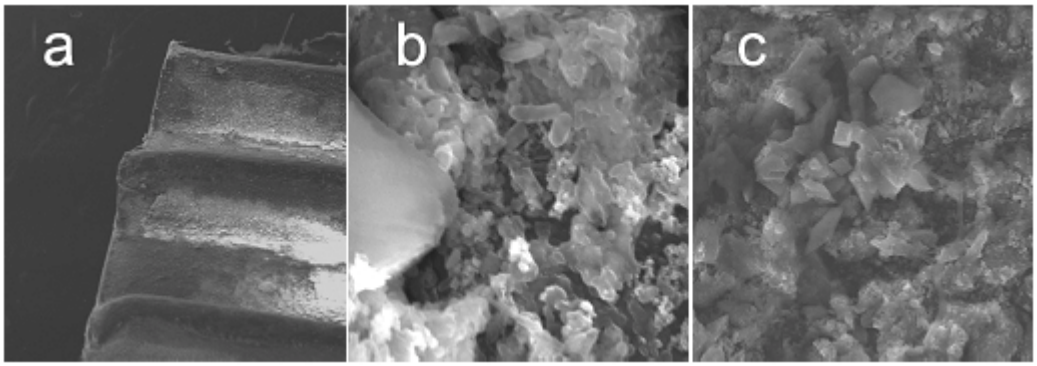

Figure 4. Scanning electron microphotograph of biofilm generated on polyethylene rings by *T. thioparus*.

The experimental setup is described in Figure 2. Teflon was used for all fittings, connections and tubing. The control of the flow was made by using needle valves and a flow mass controller (Aalborg Model GFC17, USA). The air was supplied by a compressor (Montecarlo, Model 24I ABAC, Spain), and the air was filtered and humidified by bubbling in an absorption column. H_2S was continuously generated in a column by the reaction between solutions of Na_2S and HCl. H_2S was transported to the biotrickling filter by an humidified air flow.

The biotrickling filter packed with polyethylene rings was inoculated with a continuous culture of the corresponding bacteria under similar conditions, excepting the pH, that was controlled in the range of optimal for growth in each case; 5,5-7,0 for *T. thioparus*, and 1,8-2,5 for *A. thiooxidans*. After 30 days the biofilms were clearly observed on the surface of the polyethylene rings. When the feed of H_2S started the liquid recirculated through out the column was the culture medium without the energy source.

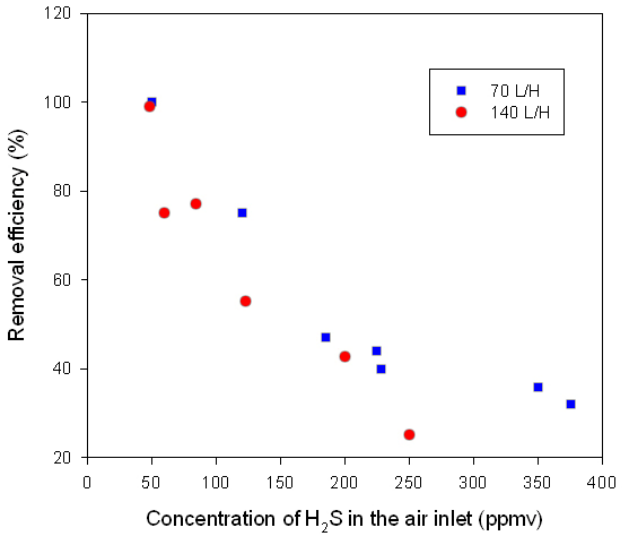

Figure 5. Removal efficiencies of H_2S in a biotrickling filter using a biofilm of *T. thioparus* (pH 5,5-7,0).

Analytical methods

H_2S was determined by a gas chromatograph (Perkin Elmer, USA) equipped with a detector for thermal conductivity, using a column packed with Super Q 80/100 (Alltech, USA). Helium was used as the carrier gas at a flow rate of 40 ml/min and the temperatures used in the injector, oven, and detector were 70ºC, 80ºC and 90ºC, respectively.

The biomass growing over the solid supports was suspended in liquid using ultrasound. In liquid culture the suspended biomass was determined by turbidimetry or by their total protein content. In both cases, the culture was previously filtered to remove colloidal sulfur. Protein content was determined to the suspension by using the method of Lowry (Lowry et al. 1951).

RESULTS AND DISCUSSION

The levels of oxygen consumption indicated that the oxidation of thiosulfate was higher with biomass suspended from biofilms formed by *T. thioparus* growing over polyethylene rings (Figure 3). Volcanic rocks (TZ) always exhibited the highest biomass production, a fact that probably due to the irregular surface and its highly porous structure, but the accumulation of biomass and the elementary sulfur generated as a product of the biological oxidation of thiosulfate obstructed the column and it causes the canalization of the flow through it. These conditions might have generated areas in the supporting material lacking in oxygen and nutrients, a fact that might explain the low activity showed, as a consequence of diffusional restrictions (Cox et al. 1997).

The higher rates of thiosulfate oxidation showed by cells detached from polyethylene rings may be attributed to the adsorbing properties of the surface of the material that leads to the development of a homogeneous biofilm, as shown by electron microscopy (Figure 4). This fact might result in a higher availability of oxygen and nutrients for the immobilized cells thus maintaining them metabolically active.

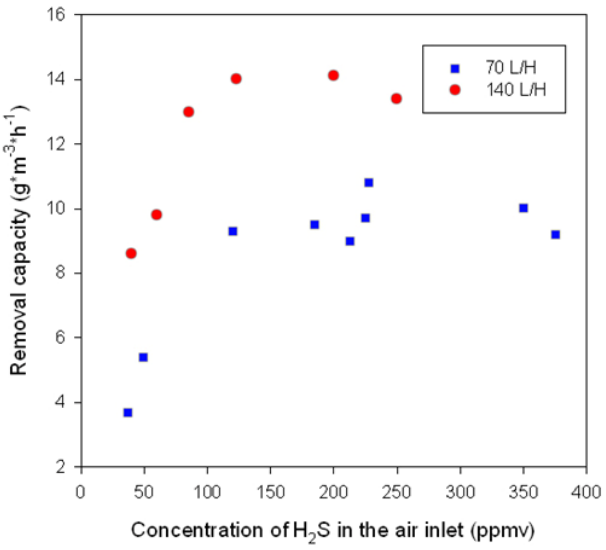

Figure 6. Removal capacity in a biotrickling filter using a biofilm of *T. thioparus* (pH 5,5-7,0).

Figure 4 shows a microscopy photograph of the biofilm formed by *T. thioparus*. In Figure 4a considerable development of bacteria associated to the rings is observed, whereas in Figure 4b and Figure 4c abundant elementary sulfur generated on the surface of the polyethylene ring was observed. This material was produced by oxidation of thiosulfate, producing crystals with their typical octahedral structure. The analysis of the secondary emission revealed that the elementary sulfur was present in an significant proportion (34,72% p/p). Similar results were obtained for *A. thiooxidans*.

Figure 5 and Figure 6 show the results obtained in the removal of H_2S when using the biotrickling filter with a biofilm formed by *T. thioparus*, operated at a range of pH between 5,5 and 7,0 as to provide the optimal conditions for growth. The maximal removal capacity attained in this bioreactor was 14 gS m^{-3} h^{-1} at 30 gS m^{-3} h^{-1} of inlet load, 47% of removal efficiency at a residence time of 26 sec.

Better results were obtained in the biotrickling filter inoculated with *A. thiooxidans*, and operated without pH control at high inlet concentrations of H_2S. The results are shown in Figure 7 and Figure 8.

Removal efficiency of 100% were achieved for higher inlet concentration of H_2S (4600 and 982 ppmv) for 120 sec and 45 sec of residence time, respectively, therefore, better removal capacities were obtained as compared with *T. thioparus*. Also,complete oxidation of H_2S (100%) was achieved with an inlet load of 240 gS m^{-3} h^{-1} of inlet load. The highest removal capacity was 370 gS m^{-3} h^{-1} at 45 sec of residence time, and 405 gS m^{-3} h^{-1} of inlet load (91% of efficiency), which represents eliminations capacities of a similar level that previously reported by Cho et al. (2000), who obtained the best results using biotrickling filter packed with porous lava inoculated with *A. thiooxidans* (428 gS m^{-3} h^{-1}).

Both values obtained in biotrickling filters inoculated with *A. thiooxidans* are considerably higher than the capacities reported in biofiltration systems packed with natural carriers (Cho et al. 1992; Yang and Allen, 1994; Wani et al. 1999; Elias et. al. 2002; Oyarzún et al. 2003), possibly due to the possibility to drain sulfur and sulfate.

CONCLUDING REMARKS

The draining of cellular suspension appears to be a simple and efficient system for the generation of biofilms, Volcanic stones showed favourable characteristics for microbial colonization, although the excessive accumulation of biomass constitutes a disadvantage in the use of biotrickling filters, since obstruction of the reactor bed and canalization problems could be generated with this support.

The determination of the biooxidation capacity of the biomass released from the biofilm offer a suitable criterion of carrier selection for biotrickling filters, since they constitute an approach for the metabolic state of the cells within the biofilm. Polyethylene rings exhibit the most suitable properties as supporting material in a biotrickling filter, as shown by the higher rates of thiosulfate oxidation and homogeneous cellular colonization of the carriers.

The removal capacity of the biotrickling filter inoculated with *T. thioparus* operating in the range of pH between pH 5.5-7.0, the range allowing the maximum reported growth rate, is lower than the removal capacity of acidophilic *A. thiooxidans* showing that *A. thiooxidans* is the most suitable microorganism for the biooxidation of H_2S in biotrickling filters.

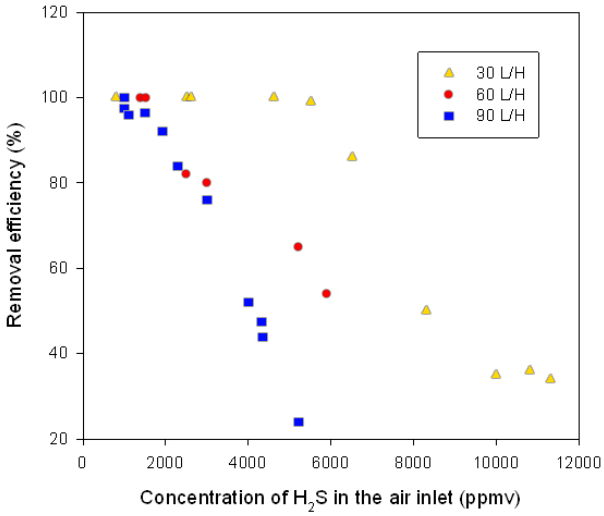

Figure 7. Removal efficiencies in a biotrickling filter inoculated with *A. thiooxidans*.

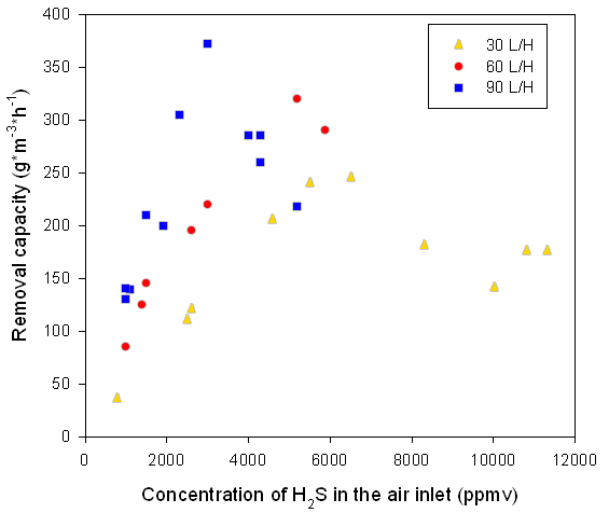

Figure 8. Removal capacity of a biotrickling filter inoculated with *A. thiooxidans*.

An important advantage of the acid biotrickling filter is that it does not require an exhaustive pH control of the liquid media as it occurs in the biotrickling filter inoculated with *T. thioparus*, in which any pH variation produces a drastic change on the H_2S biooxidation efficiency.

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