Removal of hydrogen sulphide by immobilized *Thiobacillus thioparus* in a biofilter packed with polyurethane foam

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ABSTRACT. In this work, a laboratory scale biofilter was design for removal of hydrogen sulphide packed with *Thiobacillus thioparus* immobilized in polyurethane foam particles (the initial immobilized biomass was 1.6×10^{11} cel g^{-1} of solid support). Three empty bed residence times (EBRT) were studied (100, 70 and 30 second) and the initial H_2S concentration, ranging from 25 to 750 ppmv, in the inlet gas stream were varied from low to high values in order to determine maximum biofilter efficiency. In these conditions, the biofilter reached efficiency >95% for 300, 150 and 100 ppmv of H_2S concentration at 100, 70 and 30 second (EBRT) respectively, but when increasing the inlet concentration of the hydrogen sulphide, the removal efficiencies of H_2S decreased.

1 INTRODUCTION

Recently, people in many countries have encountered numerous air pollution problems, especially those associated with bad odour. It is well known that more than three hundred substances can cause this problem, but hydrogen sulphide is the ones that often exist in our surroundings. Large amounts of H_2S is generated and released from industrial processes, such as wastewater treatment, petrochemical refining, etc. Excess of these substances have to be removed for the sake of safety and health and also for the reduction of environmental impacts, so a growing awareness and concern for air quality is driving a search for economical and efficient abatement technology to reduce the impact of the industry in the environment.

Usually, the concentrations of these odorous compounds in the gas emissions are so dilute, traditional air pollution control technologies such as incineration, adsorption or chemical scrubbing tend to be costly and are associated with their own pollution problems. So, based on the cost for the equipment and operation, biological treatment is believed to be the most economical option for treating ammonia and hydrogen sulphide. Ammonia is colourless, but irritant and smelly, while hydrogen sulphide is corrosive, extremely toxic, and also smelly.

For removal of hydrogen sulphide the packing material media used in conventional biofilter beds consist mostly of peat and compost (Cho *et al*., 1992; Park *et al*., 1993;

Hartikainen *et al*., 2001; Oyarzún *et al*., 2003), but a wide variety of other materials has been used, ca-alginate (Chung *et al*., 1996; Chung *et al*., 1998; Park *et al*., 2002), polypropylene Pall rings (Cox *et al*., 2001; Wu *et al*., 2001), porous lava (Cho *et al*., 2000), wood bark (Van *et al*., 1986), and polyurethane foam (Gabriel *et al*., 2004). Literature contains few accounts of the use of polyurethane foam for removal of malodorous gases with biofilters.

Many microorganisms has been used for the removal of hydrogen sulphide, principally bacteria. The most active biomass in a biofilter are the heterotrophic and chemoorganotrophic groups. *Thiobacillus* is most prevalent species involved in H_2S removal. Literature have reported the use of different *Thiobacillus* species (*thioparus, thiobacilli, denitricans, thiooxidans, ferroxidans*) and other microorganisms (*chlorobiaciae, xanthomonas*) (Jensen *et al*., 1995) converting the H_2S into reduced sulphur compounds.

In this work, the removal of hydrogen suphide with *Thiobacillus thioparus* immobilized in polyurethane foam particles was studied. From experimental data obtained, it is possible to determine H_2S removal kinetics at various loading rates.

2 MATERIALS AND METHODS

2.1 Organism cultivation and medium preparation

The original pure-culture strain of autotrophic *Thiobacillus thioparus* ATCC 23645 was obtained from the American Type Culture Collection. *T. thioparus* is motile, gram-negative and short-rod. This stock culture was grown using a rotary shaker at optimal temperature (30ºC) and pH 7. The mineral medium was the ATCC Medium (#290 Broth: S6 Medium of Thiobacilli). The S6 medium (in grams of litre), including Na_2HPO_4 1.2 g, KH_2PO_4 1.8 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, $(NH_4)_2SO_4$ 0.1 g, $CaCl_2$ 0.03 g, $FeCl_3$ 0.02 g, $MnSO_4$ 0.02 g, $Na_2S_2O_3$ 10g.

The strain was maintained on a thiosulphate-agar slant (1.5% w/v of agar) and transferred to fresh slants every two months.

2.2 Packing material

Polyurethane foam (PUF) cubes of 1 cm^3 were used as filter packed bed. This packing material has a surface area of approximately 600 m^2/m^3 and a density of 20 kg/m^3 (Devinny *et al*., 1999). It is an inert material with low density, large porosity (near 96%), good scaling-up possibilities and very low commercial cost (McNevina *et al*., 2000; Moe *et al*., 2000). Low density provides advantage in construction and minimizes problems of compaction of packing material. High porosity permits uniform gas flow distribution needed for maximum contact between the gas stream and biofilm biomass. The total weigh of PUF in the biofilter was 10 grams.

2.3 Immobilization method

Three erlenmeyer flask of 1L were used. Each flask contains 500 ml of medium, 100 ml of inoculum and 3,5 g of PUF. The culture was incubate at 150 rpm and 30ºC. The evolution of substrate was followed and before it was exhausted, the medium was drained and replaced with 600 mL of fresh medium without inoculation.

Several consecutive batches were run on a “drawn and fill” basis until steady-state biomass levels had been achieved.

2.4 Experimental set-up

The experimental set up is shown in Figure 1. A PVC column (63 mm of diameter) was used to build-up the biofilter with a working volume of 0.8 L. The air supply used was compressed air available in the building. Pressure regulation and filtering were achieved by having four filters: silica gel, active carbon, wool glass and Millipore Filter SLG05010 (0.45 m). Air was humidified using fine bubble diffusion. Flow rates were controlled with Flow controller (Bronkhorst, Model F-201C).

A solution whose composition was similar to the liquid culture medium without thiosulphate, the energy source, was added to supplement nutrients (0.19 l/h flow rate). The pH of medium was controller at 7.0 with Bio controller ADI 1030 (Applikon) and the temperature of experiment was maintained at 30ºC.

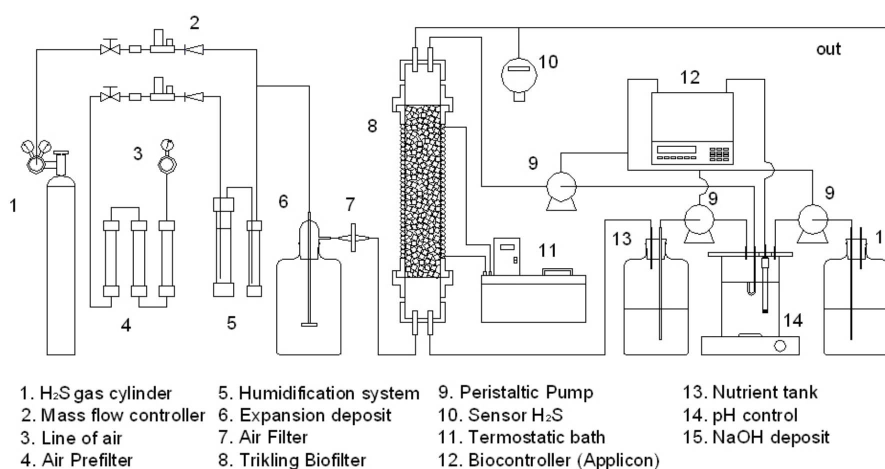

Figure 1. Diagram of the experimental setup

2.3 Analytical techniques

2.3.1 Gas

Hydrogen sulphide was analysed using a specific sensor of Crowcon (Model GASFLAG, TXGARD-IS)

2.3.2 Substrate and product concentration

Sulfate in the medium was measure using a modification of Classic Turbidimetric Method (Clesceri *et al.*, 1989) and thiosulphate was measure using an iodometric method (Rodier, 1998).

2.3.3 Immobilized biomass

Immobilized biomass concentration was measured by counting of total biomass in a Neubauer chamber. A unit of carrier is removed from the reactor and squeezed lightly in order to remove the interstitial liquid. Then, it is submerged in an erlenmeyer flask containing 25 ml of sodium phosphate buffer solution (pH 7.0). In a second step, the flask is placed in an ultrasonic bath at room temperature for 15 min. These conditions led to the total desorption of adhered cells. In the last stage, the Neubauer chamber re-count method for the submerged cells is carried out on the liquid phase. The carrier was subsequently removed from the flask and dried in an oven at 80 °C during 24 h. It was then possible to calculate the number of immobilized cells per milligram of carrier (Ory *et al.*, 2004; Gómez *et al.*, 2000). This technique has been previously validated by developing experiments concerned with cellular resistance to ultrasonic treatment and studying the desorption efficiency.

2.3.4 Electron microscopy

Scanning electron microscopy (SEM) was used for examination of immobilized bacterial in PUF, with a microscope FEI QUANTA 200 (Philips) of 2.5 nm of resolution. Fixation with glutaraldehyde (2.5%) at 4 °C for 1 h, cacodylate salt (0.1 M, pH 7.0) for 30 min, dehydration with acetone and drying, and metallization with gold.

3 RESULTS AND DISCUSSION

3.1 Immobilized biomass

The total immobilized biomass at the end of batches was of 1.6×10^{11} cel g^{-1} of solid support. The duration of the experiment was of 335 hours. In Figure 2 the different cycles of colonisation, with the consecutive charging and discharging of the medium, can be observed.

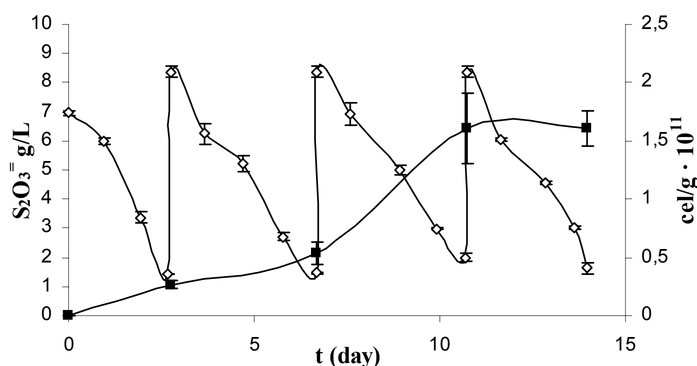

Figure 2. Evolution of thiosulfate and immobilized biomass as a function of time during repeated-batch cultivation.

Figure 3 are microphotographs sequence of colonised PUF for *T. thioparus* in various sizes. In the figure 3 (d) we can see the bacteria of *T. thioparus* immobilized on the surface of PUF. The previous microphotographs show the high amount of bacteria immobilized.

3.2 Removal of hydrogen sulphide

The biofilter operated in continuous mode during 118 days. Three EBRT (empty bed residence time) was studied (100,70 and 30 second) with H_2S concentrations, ranging from 25 to 750 ppmv, in order to determine removal capacity of the biofilter.

Several authors have studied biofilters to predict a mathematical models (Ottengraf, *et al*., 1983; Devinny *et al*., 1991; Shareefdeen *et al.,* 1993; Deshusses *et al*., 1995; Zarook *et al*., 1997). A simple model based on the Michaelis-Menten equation was using for Hirai *et al*. (1990), Cho *et al*. (1991), and Wani *et al*. (1999) assuming an ideal plug flow of gas without dispersion at steady state in the biofilter column. In steady state the biofilter can be modelled by the following equation:

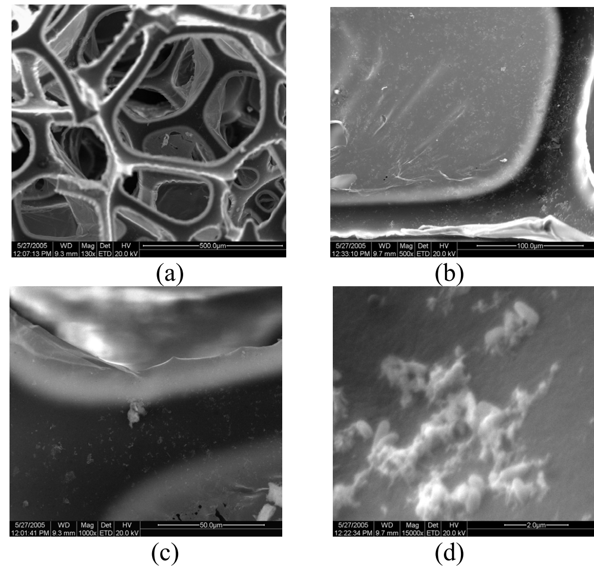

Figure 3. Scanning electron micrographs of biomass support particles removed during immobilization process.Magnification: (a) x130; (b) x500; (c) x1000; (d) x15000

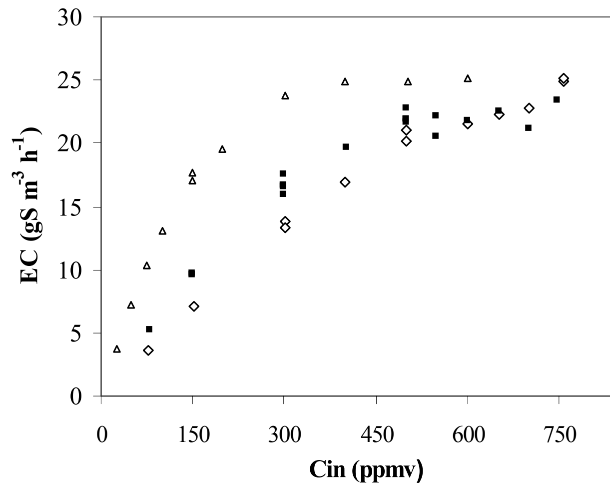

Figure 4. Evolution of elimination capacity versus inlet concentration at differents EBRT: (△) 30 s, (■) 70 s, (◇) 100 s

$$EC = \frac{V_{\max} C_{\ln}}{K_m + C_{\ln}} \quad (1)$$

Where, V_{max} is the maximum elimination rate (gS m^{-3} h^{-1}); k_m is the saturation constant (ppmv); C_{ln} is the log mean concentration: C_{ln}= $[C_o$-$C_e)/ln(C_o/C_e)]$ and EC is the elimination capacity (gS m^{-3} h^{-1}).

The elimination capacity is EC=[(C_o-C_e) x Q/V x], where, Q is the air flow (m^3 h^{-1}), C_o, the concentration of H_2S at the inlet (ppmv); C_e, the concentration of H_2S a the outlet; V, is the volumen of the biofilter (m^3) and is a factor of conversion:
=[(M x 10^{-3})/{22,4 x (273+T)/273}], where M is pollutant molecular weight and T the operating temperature (ºC).

The linealization of the equation 1: $\frac{1}{EC} = \frac{K_m}{V_{max}} \frac{1}{C_{ln}} + \frac{1}{V_{max}}$ (2)

The kinetics parameters V_{max} and k_m were estimated of equation 2. Values for the Vmax and Km are summarized in the Table 1.

Table 1. Reaction rate parameters.

EBRT	K_m	V_{max}
100	101,76	29,98
70	56,31	24,67
30	44,56	26,60

Figure 5 and 6 shows experimental data obtained and values predicted by model. The application of this model allows obtaining elimination capacity for any value of EBRT and inlet concentration. The removal capacity is >95% in a wide range of concentrations for the different time residence studied.

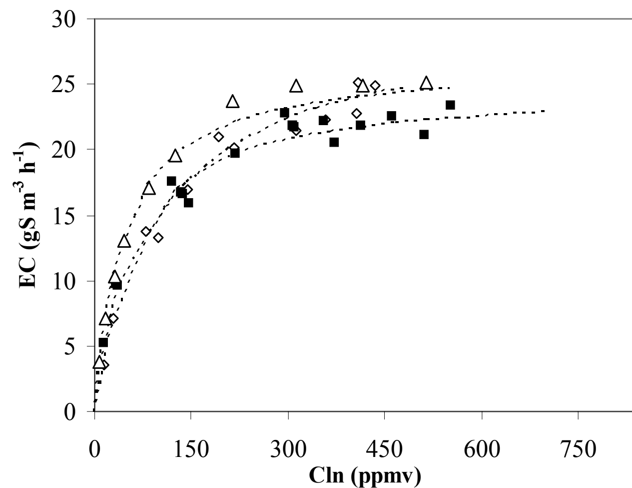

Figure 5. Evolution of elimination capacity versus the log mean concentration at differents EBRT: (△) 30 s, (■) 70 s, (◇) 100 s

The sulfate in the media of biofilter is a toxic compound. The principal problem is that sulfate is a product of the biological oxidations of hydrogen sulphide. Yang *et al*. (1994) found that sulphate concentrations of more than 24 mg-S (g dry compost) inhibited the performance of a hydrogen sulphide removing compost biofilter. In this work the elimination capacity decreased quickly when the concentration of sulfate was greater of 5 g/l, nevertheless the biofilter had a fast capacity of recovery (replace media, decrease load). In all cases, the sulfate concentration was kept at 3-4 g/l.

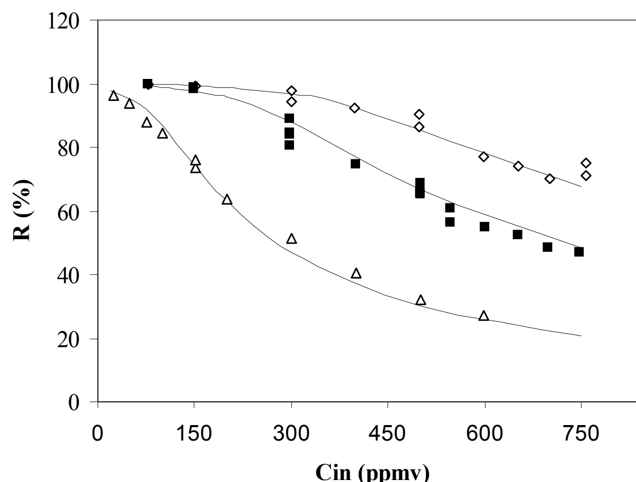

Figure 6. Evolution of removal capacity versus the inlet concentration at differents EBRT: (△) 30 s, (■) 70 s, (◇) 100 s

4 CONCLUSIONS

The maximum biomass immobilized in PUF was 1.6 10^{11} cel g^{-1} in four cycles. It was possible to reach a high removal of hydrogen sulphide using polyurethane foam as solid support for *Thiobacillus thioparus* immobilization. The removal capacity is >95% for the next concentration: 300, 150 and 100 ppmv for 100, 70 and 30 second (EBRT). The sulfate is a toxic compound and its concentration must be smaller of 5 g/l. It was possible to predict the performance of the biofilter using a simple model based on the Michaelis-Menten equation.

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