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Oxidation of volatile reduced sulphur compounds in biotrickling filter inoculated with *Thiobacillus thioparus*

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Abstract Reduced volatile sulphur compounds generate an impact on the environment, because of the bad smell and its low odour threshold. Compared with the existing physicochemical technologies for their elimination, biotrickling filters are an economically and environmentally sustainable alternative. Usually mixed cultures of microorganisms are used for inoculating biotrickling filters, in this case a pure culture of *Thiobacillus thioparus* is used for generating a biofilm, allowing to measure its capacity for the oxidation of four volatile reduced sulphur compounds: hydrogen sulphide, dimethyl sulphide, methyl mercaptan and dimethyl disulphide, using a residence time of 0.033 hrs. The viable cells of the biofilm were quantified by epifluorescence microscopy, staining the cells with ethidium bromide and acridine orange, polymerase chain reaction analysis in real time was used for testing the predominance of *T. thiopharus* in the biofilm. The microorganism was able to adhere and grow on the surface of rings made of polyethylene, with a viable population of $7 \cdot 10^7$ cell·ring^{-1}, a 74% of total cells. The real time PCR showed a persistence of the population of *T. thioparus* for more than 300 days of operation, without being displaced by other microbial species. The maximum elimination capacities for each compound were 34.4; 21.8; 30.8 and 25.6 gS·m^{-3}·h^{-1} for H_2S, dimethylsulphide, dimethyldisulphide and methyl mercaptan, respectively. We conclude that it is possible to implement a biotrickling filter with the bacteria *T. thioparus*, which can oxidize volatile reduced sulphur compounds efficiently.

Keywords: biofiltration, odour removal, volatile sulphur reduced compounds

INTRODUCTION

Emissions of gaseous pollutants generated by industries such as oil refining, rendering plants, paper mills (Kraft process) or waste water treatment plants, contain volatile reduced sulphur compounds, also known as VRSC, among these, hydrogen sulphide (H_2S), methyl mercaptan (MM), dimethylsulphide (DMS) and dimethyldisulphide (DMDS) are present in variable composition (Ruokojärvi et al. 2001). These compounds have an unpleasant smell and can be perceived at great distances even when they are at very low concentration because of its low odour threshold, it reduces the quality of life of the people living in surrounding areas and creates conflict between the population and the emitting source; industry, water treatment plants (Burguess et al. 2001). To solve this problem, physicochemical technologies, such as condensation, adsorption or incineration can be used, however these technologies are too expensive when dealing with large volumes (flows) of gas, and usually generate an additional problem in the disposition of the exhausted reagents (Chan, 2006). In this context, the biological treatment of these emissions, known as biofiltration, have comparative advantages such as low investment, low operation and maintenance costs, and above all these are environmentally sustainable (McNevin and Barford, 2000).

Biofiltration use the capability of certain microorganisms that grow and form biofilms on a solid matrix (organic or inorganic) called support, and use the pollutants as an energy and or carbon source (Kennes and Veiga, 2001). When pollutants treated are extremely corrosive or generate extreme conditions, such as a drastic decrease in pH, like in the case of reduced sulphur compounds, the organic media have a short useful life, making the process impractical, so in these cases the support used is inorganic and nutrients are supplied through out a solution that is recirculated, allowing the

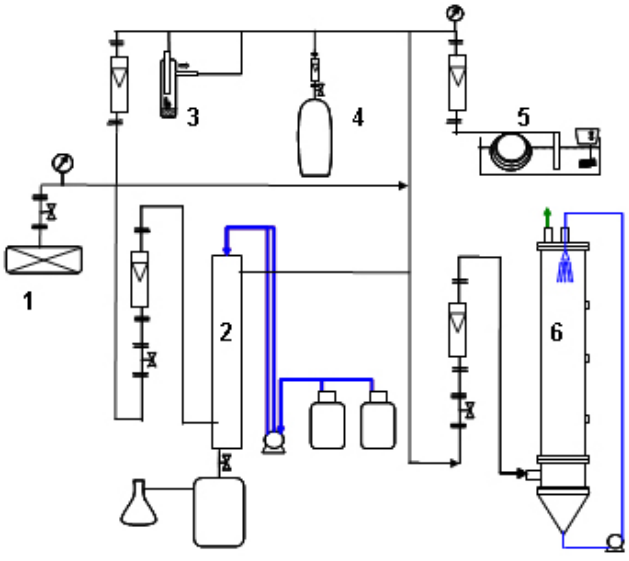

Fig. 1 Experimental system for the oxidation of sulphur compounds in biotrickling filter. (1) Compressor; (2) H_2S Generator; (3) Generator DMDS; (4) Cylinder MM; (5) DMDS Generator; (6) Biotrickling filter.

control of the pH, this type of configuration is called biotrickling filter (Gabriel and Deshusses, 2003).

An example of VRSC gases treatment by biotrickling filter, is the work of Ruokojärvi et al. (2001) who reached elimination capacities of 48, 37 and 3 $gS \cdot m^{-3} \cdot h^{-1}$ for H_2S, DMS and MM, respectively, using a biotrickling filter inoculated with a microbial consortium. In another example, Hartikainen et al. (2002) reached 6, 7 and 4 $gS \cdot m^{-3} \cdot h^{-1}$ for H_2S, DMS and MM, respectively using a non inoculated fibrous peat as support. However, it has been reported that biofilters inoculated with pure cultures, mainly bacteria from the genera *Thiobacillus*, *Acidithiobacillus* and *Hyphomicrobium*, have a lower start-up time and a more stable operation over the time depending on the biofilm generation conditions (Sercu et al. 2005; Aroca et al. 2007).

The objective of this work is the quantification of the elimination capacity of four volatile reduced sulphur compounds: H_2S, DMS, MM and DMDS, treated separately in a biotrickling filter inoculated with a pure culture of *Thiobacillus thioparus* ATCC 23645 at neutral pH conditions.

MATERIALS AND METHODS

Inoculation of the support and biofilm formation

Thiobacillus thioparus ATCC 23645 was used to inoculate rings of polyethylene used as support. They were inoculated with 1 L of a culture of the bacterium grown in thiosulphate liquid medium ATCC 290, with a composition in $g \cdot L^{-1}$ of: Na2HPO4·7H2O, 2.27; KH_2PO_4, 1.8; $MgCl_2 \cdot 7H_2O$, 0.1; $(NH_4)_2SO_4$, 1.98; MnCl2·H2O, 0.023; $CaCl_2$, 0.03; $FeCl_3 \cdot 6H_2O$, 0.033; Na_2CO_3, 1; $Na_2S_2O_3 \cdot 5H_2O$, 15.69; and the pH adjusted in 6.8. The cell concentration of the culture to inoculate the support was $1.5 \cdot 10^{10}$ $cell \cdot L^{-1}$. The cell suspension was recirculated for two days through out the packed column filled with the support to allow adsorption of the microorganisms.

Biotrickling filter

A biotrickling filter was set up with a column of polyvinilclorure (PVC) 40 cm height and 6.5 cm in diameter with (useful volume of 1.3 L), it was filled with rings of polyethylene 1.02 $kg \cdot L^{-1}$; 2.3 $m^2 \cdot L^{-1}$; 77% free volume. The column has a sampling port at the bottom and one at the top of the reactor, for the inlet and exit of the gas flow respectively, and three equidistant sampling ports through the packed support.

Biofiltration

After development of the biofilm by *T. thioparus*, liquid culture medium ATCC 290 without thiosulphate was circulated through out the packed column by spraying it to the top of the column. Every two days 0.5 L of the circulated solution was replaced by fresh solution to maintain a pH of 6.8 and a sulphate concentration under 10 $g \cdot L^{-1}$.

Different loads of each compound were fed to the biotrickling filter separately, measuring the elimination capacity for each compound. All the experiments were

done at a retention time of 120 s^{-1}. The elimination capacity (EC) of sulphur and sulphur load (Ls) were calculated according to:

$$EC = \frac{(C_{in} - C_{out}) \cdot Q}{V} \quad [gS \cdot m^{-3} \cdot h^{-1}] \qquad \textbf{[Equation 1]}$$

$$L_s = \frac{C_{in} \cdot V}{Q} \quad [gS \cdot m^{-3} \cdot h^{-1}] \qquad \textbf{[Equation 2]}$$

Where C_{in} and C_{out} are the inlet and outlet sulphur concentrations in $gS \cdot m^{-3}$, respectively, Q is the volumetric flow of gas fed into $m^3 \cdot h^{-1}$ and V is the volume of support packed in m^3. A diagram of the experimental system is presented in Figure 1.

Determination of viable cells in biofilm

The number and viability of cells in the biofilm was monitored by direct using epifluorescence microscopy (model Eclipse, Nikon, Japan). The biomass attached to the rings of polyethylene was released using ultrasound 43 kHz for 5 min and suspended in 10 mL of sterile medium ATCC 290 without thiosulphate. To stain the samples were treated with ethidium bromide and acridine orange, which gave a characteristic green colour to viable cells and orange colour to the non-viable cells, the samples were observed with the epifluorescence microscope using a filter light B-2A (EX: 460 EM: 540).

To test the stability of the biofilm and the predominant presence of *T. thioparus* in the biotrickling filter, 10 rings were taken from the column and sonicated 5 min at 43 kHz for getting the cells from the biofilm in a sterile liquid culture medium ATCC 290 without thiosulphate. Analysis of 16s rDNA was carried out by amplifying (using nested PCR) an initial sequence of 566 bp (primers 341f and 907r), and then a smaller fragment of 193 bp (primers 534r and 341f) in a gradient thermocycler (Master Cycler, Eppendorf, USA). Both reactions were performed with a "touch-down" program to increase the affinity of DNA primers. Verification of the melting temperatures of the fragments amplified by PCR was performed.

Table 1. Maximum elimination capacities (EC_{max}) for H_2S, MM, DMDS and DMS obtained by non-linear regression in biofilter inoculated with *T. thioparus* at 120 (s) of residence time.

Gas	Ls ($gS \cdot m^{-3} \cdot h^{-1}$)	EC_{max} ($gS \cdot m^{-3} \cdot h^{-1}$)	R
H_2S	38.6	34.4	0.996
MM	42.1	25.6	0.997
DMDS	28.8	30.8	0.987
DMS	47.7	21.8	0.995

Generation and determination of H_2S, DMS, MM and DMDS

The H_2S was generated by reaction between sodium sulphide solutions (Na_2S, 1-3%) and hydrochloric acid (HCl, 0.5 N) using a device specially designed for this (Oyarzún et al. 2003). The MM stream was generated by diluting known volumes of 99% MM in the inlet flow of air. In the case of DMS, the gas flow was generated using a capillary diffusion system described by Smet et al. (1996). To generate a gas stream of DMDS (boiling point: 119ºC) a generation system by forced convection was implemented a stream of air impact vertically on the surface of liquid DMDS in a tube of 1 cm in diameter fitted with an outlet for the gas flow near the top of the tube. Figure 1 shows a diagram of the experimental set up.

The concentration of the sulphur compound was determined by gas chromatography (Perkin Elmer Clarus 500, USA), using a column-packed Supelpack S (Supelco) and a flame photometric detector (FPD), the operating conditions of the chromatograph were: injector temperature: 60ºC, initial oven temperature 60ºC for 1 min to 200ºC at a rate of 30ºC-min^{-1}, detector temperature: 400ºC; using Helium as carrier gas at a flow rate of 30 cm^3-min^{-1}.

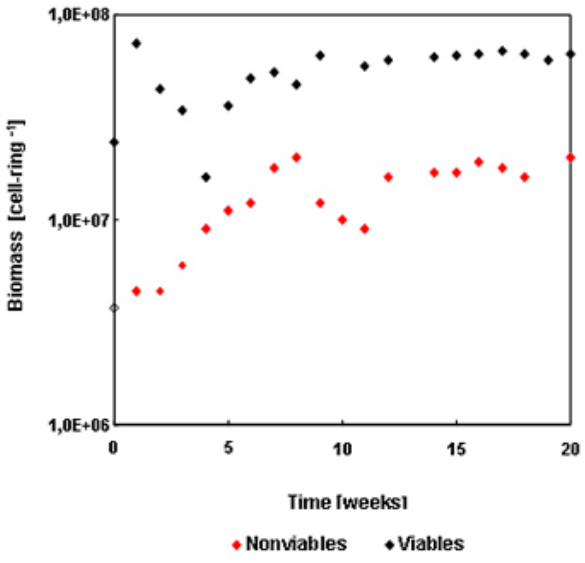

Fig. 2 Cell count in time of *T. thioparus* on biotrickling filter.

RESULTS

The bacterium, *T. thioparus*, was capable of forming a biofilm over PVC rings with VRSC oxidative capacity under conditions near pH 7. During the operation of the biotrickling filter a small variation in the number of viable cells in the biofilm was

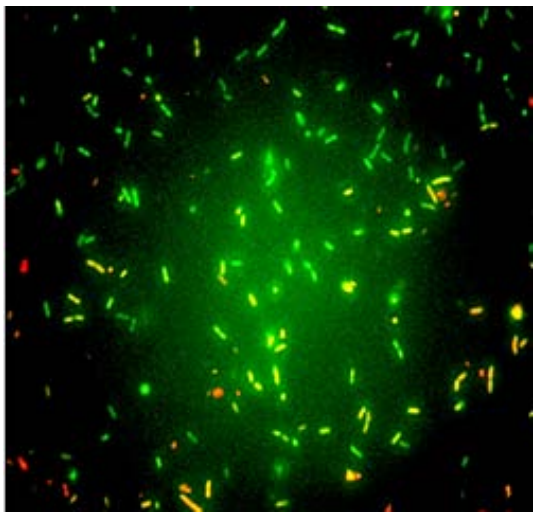

Fig. 3 Epifluorescence microscopy image of *T. thioparus* cells at 4 weeks of operation.

observed (Figure 2), the average cell concentration was $7.2 \cdot 10^7$ cells·ring^{-1} with a percentage of viable cells of 74%. Figure 3 shows an image of the viable and non viable cells view with epifluorescence microscopy at 4 week of operation, at that time the biofiltration experiments began.

According to the results of the PCR analysis, a peak at a temperature of 87.8ºC in the melting curve define the pure culture sample of *T. thioparus*, it is similar to the melting temperature of the samples of cells taken from biotrickling filter (88.3ºC), indicating that there is only one type of fragment amplified in the PCR reaction (Figure 4), therefore, the bacteria used as inoculum generates the biofilm and is the main specie present in the biofilm. This result allows to attribute the oxidation of sulphur compounds to the bacteria.

Table 2. Comparison of elimination capacities obtained from this work and from other authors.

EC H_2S ($gS \cdot m^{-3} \cdot h^{-1}$)	EC DMS ($gS \cdot m^{-3} \cdot h^{-1}$)	EC MM ($gS \cdot m^{-3} \cdot h^{-1}$)	EC DMDS ($gS \cdot m^{-3} \cdot h^{-1}$)	Reference
25	1	5	2	Cha et al. 1999
9.8	5.4	4.5	1.6	Cho et al. 1991
48	37	3	-	Hartikainen et al. 2002
6	7	4	-	Ruokojarvi et al. 2001
34.4	21.8	25.6	30.8	This work

*Different letters in the same column indicate significant differences, 5% level, Duncan's multiple range test.

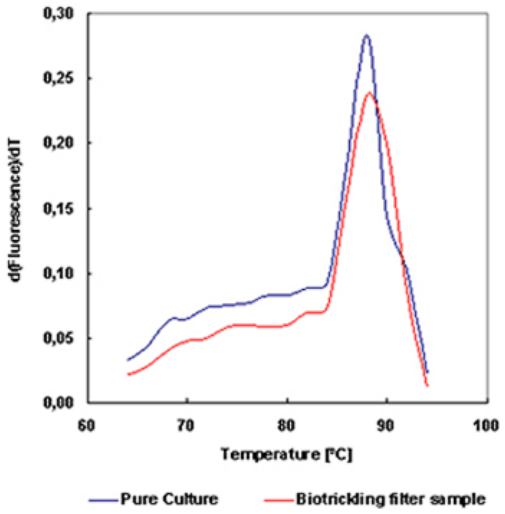

Fig. 4 Melting curves for pure culture and biotrickling filter inoculated with *T. thioparus*.

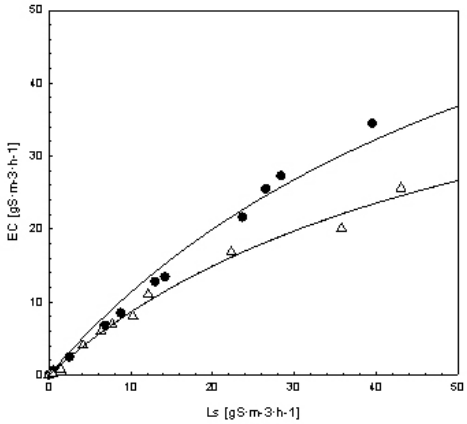

Fig. 5 Elimination capacity (EC) vs Load of sulphur (Ls) in biofilter inoculated with *T. thioparus* for H_2S (●) and MM (∆).

H_2S and MM, and DMDS and DMS respectively. Until 10 $gS \cdot m^{-3} \cdot h^{-1}$ of sulphur loads removal efficiencies greater than 95% were obtained for each compound, so this value correspond to the critical load for sulphur in the biotrickling filter used, regardless the compound treated.

In order to estimate the maximum elimination capacities of each compounds was used a non-linear regression, considering a expression saturation type. For the same residence time (120 sec) the higher elimination capacity was for H_2S and smaller for DMS. In Table 1 presents the values obtained by non-linear regression.

Concentrations along the column for each compound was also monitored, Figure 7 shows these profiles, all the gaseous compounds shows a similar behaviour with the highest removal in the first section of biotrickling filter.

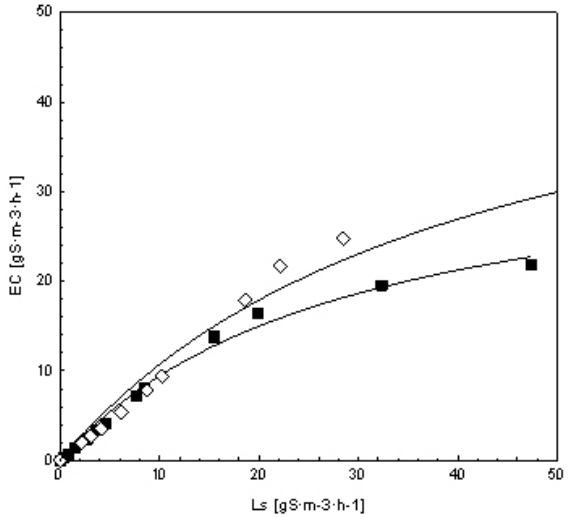

Fig. 6 Elimination capacity (EC) vs Load of sulphur (Ls) in biofilter inoculated with *T. thioparus* for DMDS (◊) and DMS (■).

DISCUSSION AND CONCLUSIONS

The bacteria *T. thioparus* remained the predominant species throughout the operation, which allows conferring in this case the oxidation of VRSC compounds to this particular microorganism.

The maximum elimination capacities obtained for each compound are comparable to those reported in literature using an adapted mixed culture (Ruokojärvi et al. 2001),

reaching elimination capacities of 99% for each of the compounds with load of sulphur less than 10 $gS \cdot m^{-3} \cdot h^{-1}$. Table 2 shows a comparison between the maximum elimination capacity obtained using mixed cultures (Ruokojarvi et al. 2001; Hartikainen et al. 2002), *T. novelus* (Cha et al. 1999) and *T. thioparus* DW44 in a bifilter (Cho et al. 1991). In this work, better elimination capacities were obtained using a biotrickling filter.

The elimination capacity of a compound with respect to the other obtained in this study confirms what was observed previously (Cha et al. 1999) where it was observed the following order in elimination capacities: H_2S > MM > DMDS > DMS, who attributed this to the high specificity of *Thiobacillus novellus* SRM on the S-H bond of H_2S relative to C-S-H bond of the other sulphur compounds (MM, DMS and DMDS), which is consistent with the route for oxidation of sulphur compounds in *Thiobacillus* proposed by Smith and Kelly (1988), where the H_2S is the compound which less reactions required to be oxidized.

Figure 7 shows the concentration profiles obtained at different heights of the biofilter, these values are the mean of several determinations and showed a clear trend. The highest percentage of removal is achieved in the first 20 cm of biofilter, indicating that in the first contact between the biofilm and the gas flow is generated greater transfer of the pollutant by the concentration higher gradient, which is reflected in an increased microbial activity. This indicates that it could reduce the size of the biofilter by 50% while still achieving removal efficiencies over 90% for each VRSC. A similar behaviour

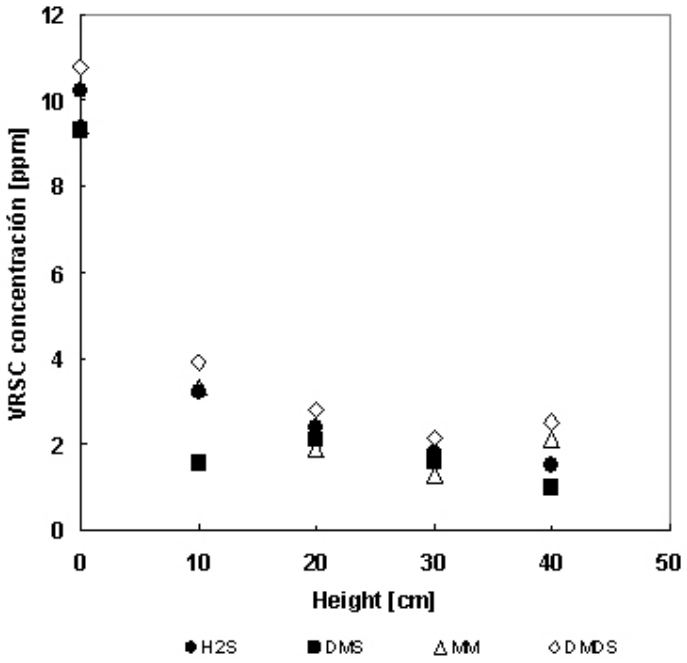

Fig. 7 VRSC concentration (ppm) vs. biofilter height (cm).

has been described in the work of Kennes et al. (2007) in the co-treatment of H_2S and methanol with a single-stage biotrickling filter under acidic conditions.

It must be stressed that the study made it possible here to verify the possibility of a biological technology for the treatment of volatile reduced sulphur compounds, in a simple, efficient and economical use of the bacteria *T. thioparus*; constitute the basis for upcoming works which are aimed at solving the problem that occurs when treating a mixture containing the four compounds mentioned here, as it is known (Ruokojärvi et al. 2001) that in this case, H_2S is preferentially degraded by the microbial population at the expense of other compounds, making a less effective system (Smet et al. 1998; Chan, 2006).

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