

## Removal of hydrogen sulfide and ammonia from gas mixtures by co-immobilized cells using a new configuration of two biotrickling filters

M. Ramirez, J. M. Gómez, G. Aroca and D. Cantero

### ABSTRACT

The simultaneous removal of H<sub>2</sub>S and NH<sub>3</sub> was investigated using two biotrickling filters packed with polyurethane foam cubes. One biotrickling filter was inoculated with *Thiobacillus thioparus* ATCC 23645 for the removal of H<sub>2</sub>S (BTT) and the other filter with *Nitrosomonas europaea* ATCC 19718 for the removal of NH<sub>3</sub> (BNE). Three different configurations were studied by modification of the gas line and recirculation medium line. The best results were obtained with the BNE biotrickling filter after the co-immobilization of the two bacteria. A removal efficiency of 100% for 230 ppmv of NH<sub>3</sub> and 129 ppmv of H<sub>2</sub>S was reached at an EBRT of 60 seconds. The results obtained show that it is possible to co-immobilize both microorganisms using the same recirculation medium and remove successfully H<sub>2</sub>S and NH<sub>3</sub> from a gas mixture.

**Key words** | ammonia, biotrickling filter, hydrogen sulfide, *Nitrosomonas europaea*, *Thiobacillus thioparus*

M. Ramirez (corresponding author)

J. M. Gómez

D. Cantero

Department of Chemical Engineering,  
Food Technology and Environmental Technologies,  
Faculty of Sciences,  
University of Cádiz,  
Puerto Real (Cádiz) 11510,  
Spain  
E-mail: martin.ramirez@uca.es

G. Aroca

School of Biochemical Engineering,  
P. Catholic University of Valparaíso,  
Av. Brasil No. 2147,  
Valparaíso,  
Chile

### INTRODUCTION

H<sub>2</sub>S and NH<sub>3</sub> are highly toxic, colorless, and irritating malodorous gases. Considerable concentrations of H<sub>2</sub>S and NH<sub>3</sub> are released from industrial processes such as petrochemical plants, food preparation, wastewater treatment plants, landfills for waste disposal, live-stock farming, and the paper industry. Conventional removal processes such as active carbon adsorption, ozone oxidation, incineration and air-stripping involve physicochemical principles that use physical treatment or chemical oxidation. These traditional techniques are less effective and more expensive for the treatment of diluted waste gas streams (Leson & Winer 1991). Typically, waste gas biotreatment consists of two steps: firstly, the pollutant is removed from the air stream by transfer to the liquid film, followed by adsorption onto a solid support; secondly, the pollutant is degraded by microorganisms living in the liquid phase or on the packing material. There are a few studies concerning the biological

treatment of both NH<sub>3</sub> and H<sub>2</sub>S in a single air stream (Chung *et al.* 2000; Chung *et al.* 2001; Park *et al.* 2001; Kim *et al.* 2002; Lee *et al.* 2002; Malhautier *et al.* 2003; Chen *et al.* 2004; Chung *et al.* 2004a, 2004b; Jones *et al.* 2004; Chung *et al.* 2005; Park & Chung 2006; Chung *et al.* 2007). Nevertheless, there are also studies that describe indirect ammonia removal through the chemical reaction between ammonia and sulfate (a product of H<sub>2</sub>S oxidation) (Tanji *et al.* 1989; Cho *et al.* 1992; Park *et al.* 1993; Kim *et al.* 2003; Lee *et al.* 2005). The main microorganisms used for NH<sub>3</sub> and H<sub>2</sub>S removal are *Thiobacillus thioparus*, *Nitrosomonas europaea*, *Arthrobacter oxydans*, *Pseudomonas putida* and active sludges.

In the work described here, the removal of two compounds (NH<sub>3</sub> and H<sub>2</sub>S) from gaseous effluent is considered. The main objective of this study was to determine the appropriate configuration to remove both compounds.

## METHODS

### Microorganisms and cultivation media

Pure cultures of *N. europaea* (ATCC 19718) and *T. thioparus* (ATCC 23645) were obtained from the American Type Culture Collection. The *N. europaea* was grown in ammonia medium (ATCC#2265) in the dark at 30°C and *T. thioparus* was grown in thiosulfate medium (ATCC 290:S6) at 30°C. The media were sterilized at 121°C for 20 minutes. The compositions of the media are shown in the Table 1.

### Characteristics of the carrier material

Small cubes of polyurethane foam (1 cm<sup>3</sup> in size) were used as the carrier. Polyurethane foam is an inert material with good scale-up possibilities and a very low commercial cost. The principal properties are density (20 kg m<sup>-3</sup>) and porosity (96%).

### Experimental setup

The experimental set up is illustrated in Figure 1. A PVC tube with an external diameter of 63 mm and a thickness of 1.5 mm was used to build each biotrickling filter with a working volume of 1 L. Two threaded PVC flanges were fitted to the two ends of the column; a silicon disc of 3 mm thickness, with perforations of 3 mm diameter, was placed on the lower flange to support the foam cubes. A diffuser

was fitted at the top of the column to spray the recirculation medium.

The air was supplied by an industrial compressor. Prior to entering the system, the air was passed through filters consisting of silica gel, activated carbon and glass wool (diameter: 32 mm, and height of filling: 30 mm). The flow rate of each stream was regulated by mass flow rate controllers (Bronkhorst F-201C). An expansion tank of 2.5 L capacity was used to homogenize the input stream, and a 0.45 μm filter was used at the end of the stream to sterilize the input stream to the biofilter (Millipore Filter SLG05010). The recirculation medium was recirculated by means of two centrifugal pumps (EHEIM 1046) connected in series for BNE and a single pump for BTT. The pH of the medium was controlled by the addition of NaHCO<sub>3</sub> using a pH controller (CRISON, PH28) for BNE and an ADI 1030 Biocontroller (Applikon) with an electrode and sleeve diaphragm (CRISON 5221). The temperature was maintained at 30°C by heating the recirculation tank using a temperature controller (Heidolph EKT 3001) and an agitator (Agitamic-N agitator, J.P. Selecta).

Three configurations were studied. In the first configuration (Figure 2a) biotrickling filters were arranged in series without co-immobilization (first BNE and second BTT). Each biotrickling filter had its own recirculation medium and the effluent gas passed first through BNE and then through BTT. In the second configuration (Figure 2b) the setup was modified by using the same recirculation medium flow. In the third configuration (Figure 2c) both filters initially used the same recirculation medium in order to allow co-immobilization and, subsequently, each filter had its own recirculation medium and gas feed mixture.

**Table 1** | Compositions of media for the cultivation of microorganisms

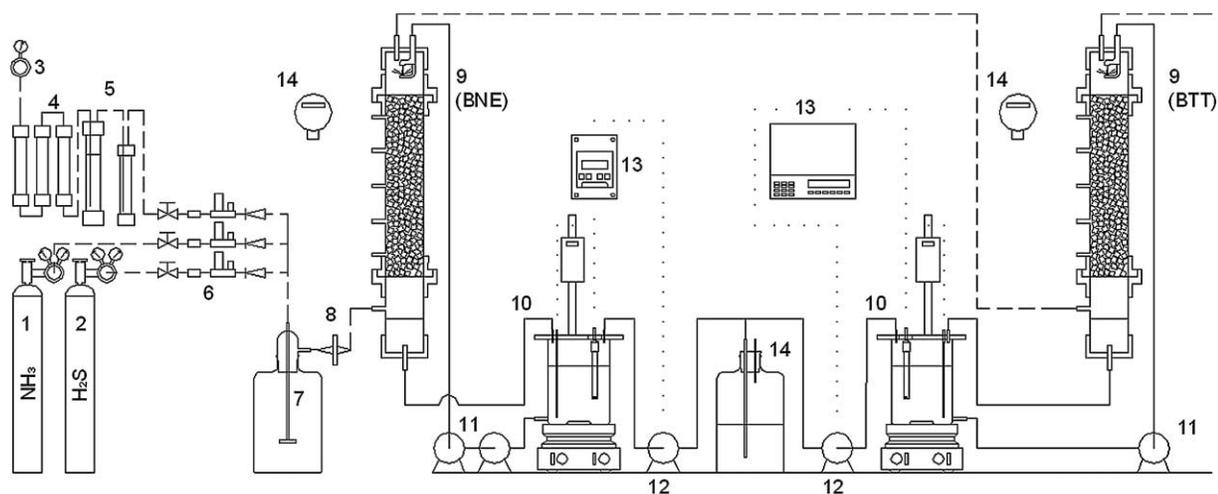
	ATCC#2265 <sup>1</sup>		ATCC 290:S6 <sup>2</sup> (g L <sup>-1</sup> )	
Solution 1	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	4.95 g	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	10 g
(in 1.2 L)	KH <sub>2</sub> PO <sub>4</sub>	0.2 g	Na <sub>2</sub> HPO <sub>4</sub>	1.2 g
	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.27 g	KH <sub>2</sub> PO <sub>4</sub>	1.4 g
	CaCl <sub>2</sub>	0.04 g	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.1 g
	FeSO <sub>4</sub> *	0.5 mL	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.1 g
	CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.2 mg	CaCl <sub>2</sub>	0.03 g
Solution 2**	KH <sub>2</sub> PO <sub>4</sub>	8.2 g	FeCl <sub>3</sub> ***	0.02 g
(in 0.3 L)	NaH <sub>2</sub> PO <sub>4</sub>	0.7 g	MnSO <sub>4</sub>	0.02 g
Solution 3	Na <sub>2</sub> CO <sub>3</sub>	0.6 g		
(in 12 mL)				

\* (30 mM in 50 mM EDTA at pH 7.0); \*\* pH adjusted to 8.0 with 10N NaOH; \*\*\* sterilized by filtration.

<sup>1</sup>Mixed at ambient temperature; <sup>2</sup> pH adjusted to 7.0 with 2N NaOH before sterilization.

### Method of immobilization and adaptation

Each biotrickling filter was packed with 10 g of polyurethane foam (1 cm<sup>3</sup> cubes) and the initial fill volume was 1 L. In the BNE a suspension of *N. europaea* cells was fed onto the top of the column at a constant flow rate of 26.7 L h<sup>-1</sup> (surface velocity of 8.57 m h<sup>-1</sup>). The volume of the recirculation medium was 1 L, the temperature was controlled at 30°C, and the medium was agitated at 200 rpm in the absence of light. As in the first stage, 1 L of ATCC#2265 medium was inoculated at 10% with a culture of



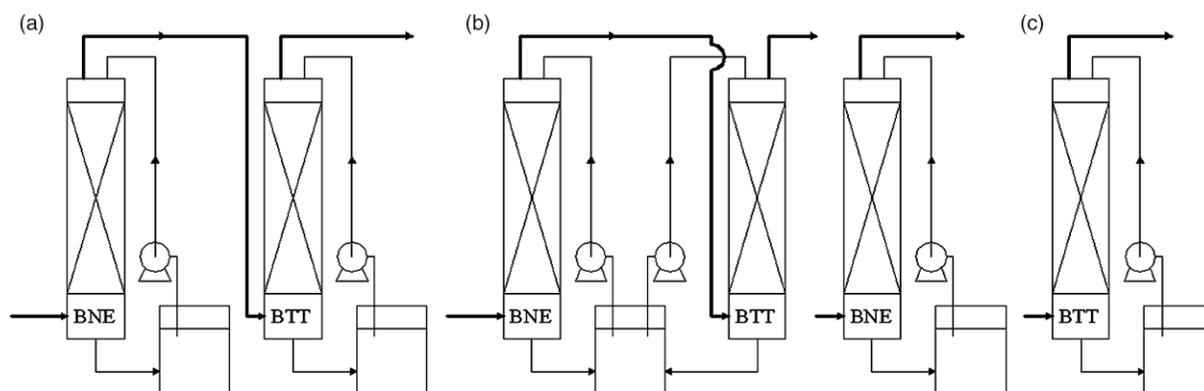
**Figure 1** | Schematic diagram. 1.  $\text{NH}_3$  gas cylinder (0.1% in air); 2.  $\text{H}_2\text{S}$  gas cylinder (0.5% in air); 3. Air manoreductor; 4. Air prefilters; 5. Humidifier and water trap; 6. Mass flow controller; 7. Expansion tank; 8. Air filter; 9. Biotrickling filter; 10. Recirculation tank; 11. Recirculation centrifugal pumps; 12. Base addition pumps; 13. pH controller; 14. Specific sensors.

*N. europaea* in the exponential growth phase, grown in an orbital agitator incubator at 30°C, 150 rpm and in the absence of light. The culture was recirculated and the pH, ammonia concentration, nitrite concentration and biomass in suspension were monitored. When the pH decreased to below 6.0, a total of 90% of the recirculation medium was drawn off in the first cycle, and 100% in subsequent cycles, and fresh medium was added. Successive cycles were performed until the maximum immobilized biomass was achieved. In order to adapt biofilm, the recirculation medium was replaced with a medium with an identical formulation to ATCC#2265 but without the energy source (ammonium sulfate), and, at the same time, the feed of  $\text{NH}_3$  in air was initiated. The flow rate of air employed was 120 L  $\text{h}^{-1}$  and the  $\text{NH}_3$  concentration was 50 ppmv.

A similar procedure was used for BTT. The recirculation medium flow was 18.6 L  $\text{h}^{-1}$  (5.94 m  $\text{h}^{-1}$ ) and the medium was ATCC290:S6 without thiosulfate. The controlled parameter for immobilization was the thiosulfate concentration. When the thiosulfate concentration decreased to below 1 g  $\text{L}^{-1}$  the medium was changed. For adaptation the ATCC290:S6 medium without thiosulfate was used with a flow rate of air of 120 L  $\text{h}^{-1}$  and an  $\text{H}_2\text{S}$  concentration of 100 ppmv.

### Analytical techniques

Two specific sensors from Crowcon (GASFLAG model, TXGARD-IS) were employed to analyze  $\text{NH}_3$  and  $\text{H}_2\text{S}$  in the gas phase.



**Figure 2** | (a) First configuration: in series; (b) Second configuration: co-immobilization in series; (c) Third configuration: with independent recirculation after co-immobilization.

The concentration of ammonia in the recirculation medium was measured by direct Nesslerization, the thio-sulfate content was determined by titration using a standard potassium iodide-iodate method with a starch indicator, sulfate was determined by a turbidimetric method and nitrite by a standard colorimetric method (APHA/AWWA/WEF 1998).

The quantity of immobilized biomass was determined by counting the number of bacteria on a unit of the carrier material and the total quantity of biomass was divided by the weight of the polyurethane foam (Gomez *et al.* 2000; de Ory *et al.* 2004). To this end, a unit of the carrier material was taken and the retained liquid was removed; the sample was then gently dried with absorbent paper. The sample was immersed in 25 mL of sterile medium and submitted to ultrasound for 15 minutes in order to desorb the bacteria. The total quantity of bacteria was then counted using a Neubauer chamber. The precise quantity of carrier material used in the analysis was determined by drying the sample in an oven at 80°C for 24 hours. This technique was validated by studying the resistance of the cells to ultrasound and the desorption effectiveness of the cells of the carrier material.

A U tube with a scale in mm, filled with water, was used to measure the pressure drop.

## RESULTS AND DISCUSSION

### First configuration

According to previous reports the presence of H<sub>2</sub>S suppresses NH<sub>3</sub> removal from waste gases (Chung *et al.*

2000; Chung *et al.* 2001; Kim *et al.* 2002; Lee *et al.* 2002; Malhautier *et al.* 2003; Chung *et al.* 2005). Two-stage biofilters have been used to avoid this ammonia removal inhibition phenomenon. Chung *et al.* (2007) improved the NH<sub>3</sub> removal from mixtures containing H<sub>2</sub>S by working with a two-stage biofilter using granular active carbon as the carrier with the same microorganisms (the first inoculated with *T. thioparus* and the second with *N. europaea*). In our case we employed biotrickling filters with the first filter inoculated with *N. europaea* (BNE) and the second with *T. thioparus* (BTT). The filters were arranged in this order because if the first biotrickling were BTT, the ammonia would be absorbed in the recirculation medium.

The inlet loading of H<sub>2</sub>S was increased from 2.89 to 15.82 gS m<sup>-3</sup> h<sup>-1</sup> (input concentration from 37.5 to 304.7 ppmv) and the loading of NH<sub>3</sub> was constant at 10 gN m<sup>-3</sup> h<sup>-1</sup> (296 ppmv). The EBRT was 60 seconds and the pH was controlled in the ranges 7.4–7.5 (BTT) and 7.5–7.6 (BNE).

On using this configuration the H<sub>2</sub>S accumulated in the BNE recirculation medium. The pH of the recirculation medium for BNE was controlled between 7.5–7.6 (pH > pKa<sub>1</sub>[H<sub>2</sub>S] = 7.02) and at this pH the concentration of H<sub>2</sub>S in BNE caused inhibition of *N. europaea* metabolism and led to an increase in the ammonia concentration in the recirculation medium.

The maximum concentration of total hydrogen sulfide in the recirculation medium was 231.4 mgS L<sup>-1</sup> (sum of the three species: H<sub>2</sub>S/HS<sup>-</sup>/S<sup>=</sup>). At 231.4 mgS L<sup>-1</sup> the ammonia concentration in the recirculation medium increased rapidly. This phenomenon has been observed by other authors (Beccari *et al.* 1980; Bentzen *et al.* 1995; Aesoy *et al.*

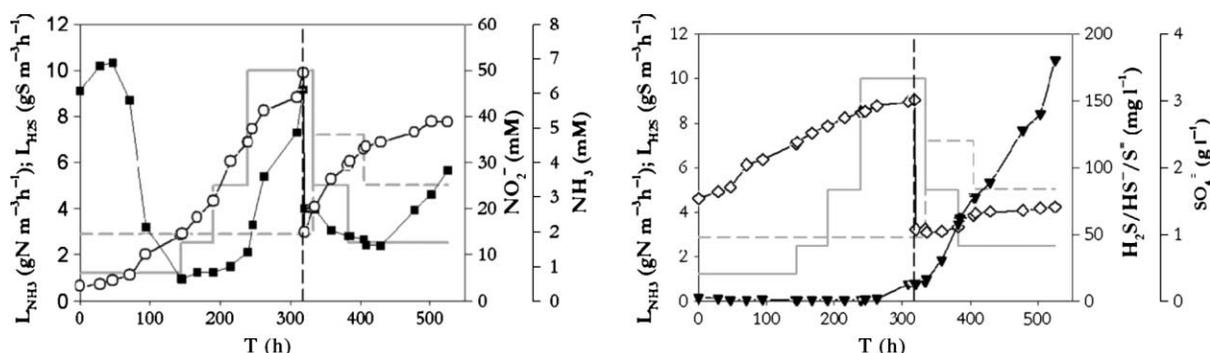
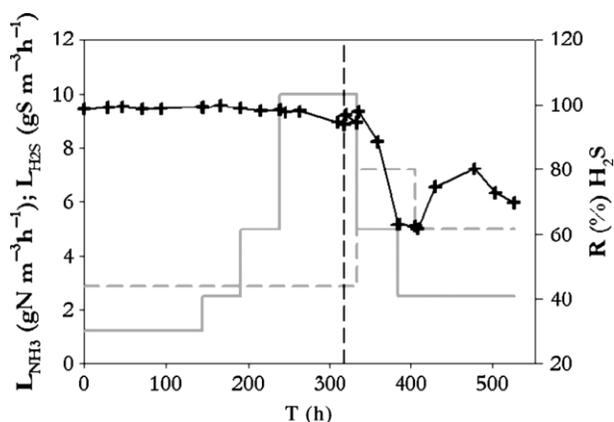


Figure 3 | Loadings: ammonia (—■) and hydrogen sulfide (---○). Concentrations: ammonia (■), nitrite (○), sulfur (▼) and sulfate (◇) versus time.



**Figure 4** | Loadings: ammonia (—) and hydrogen sulfide (---). H<sub>2</sub>S removal efficiency (+) versus time.

1998). The inhibition of ammonia removal depends on several factors: biomass composition, grade of acclimation, H<sub>2</sub>S concentration and organic/inorganic matter (Æsoy et al. 1998). For pH < pKa<sub>1</sub>[H<sub>2</sub>S] the accumulation of H<sub>2</sub>S would be very low but the low pH caused inhibition of *N. europaea* metabolism (Hunik et al. 1992).

## Second configuration

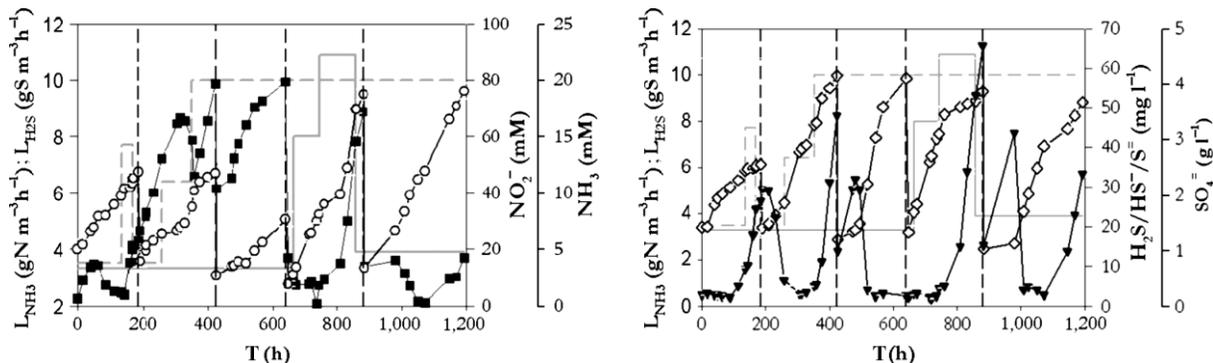
In an effort to solve the problems encountered with the first configuration, the microorganisms were co-immobilized to avoid the accumulation of H<sub>2</sub>S. The co-immobilization of the two microorganisms was carried out by removing one of the nutrient recirculation tan from the system, leaving only one tank with the same recirculation medium for both biofilters (50% of each medium: ATCC290:S6 and

ATCC#2265). This new configuration allowed the co-immobilization of *T. thioparus* and *N. europaea* by natural adsorption. This immobilization method has significant advantages in terms of gel entrapment: it is less sensitive to pH changes, has smaller diffusional limitations and higher stability in the presence of chelating compounds (Scott 1987).

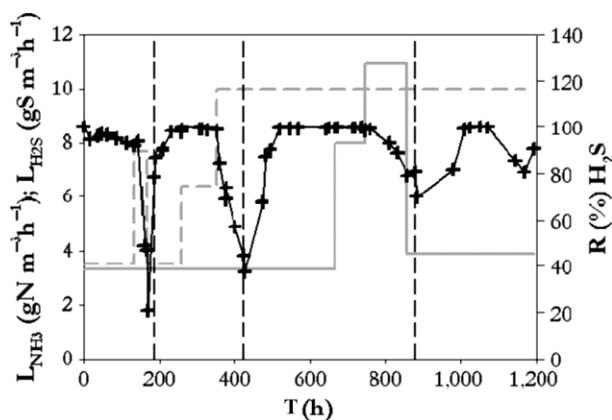
The inlet loading of H<sub>2</sub>S was increased from 2.89 to 7.19 gS m<sup>-3</sup> h<sup>-1</sup> (input concentration from 37.5 to 92.9 ppmv) and the loading of NH<sub>3</sub> from 1.24 to 10 gN m<sup>-3</sup> h<sup>-1</sup> (input concentration from 36.7 to 296 ppmv). The EBRT was 60 seconds and the pH was controlled in the range 7.5–7.6.

The evolution of the concentrations of oxidation products and substrates in the BTT and BNE recirculation medium can be seen in Figure 3. The recirculation medium was changed after 319 h of operation. The maximum product concentrations were 3.0 gL<sup>-1</sup> of sulfate and 49.5 mM of nitrite. The ammonia removal was not affected by the loading of H<sub>2</sub>S in the range studied, but the ammonia in the recirculation medium increased when the nitrite concentration exceeded 33–37 mM. Nitrite concentrations of around 33–37 mM caused inhibition of the *N. europaea* metabolism. The ammonia concentration at the outlet of BNE was zero. The removal efficiency of H<sub>2</sub>S was high after only a few hours of operation.

The evolution of the H<sub>2</sub>S removal efficiency (considering the concentration at the outlet of the second biofilter) can be seen in Figure 4 and this value decreases at high sulfur concentrations (H<sub>2</sub>S/HS<sup>-</sup>/S<sup>=</sup>) in the recirculation medium. The total hydrogen sulfide concentration



**Figure 5** | Loadings: ammonia (—) and hydrogen sulfide (---). Concentrations: ammonia (■), nitrite (○), sulfur (▼) and sulfate (◇) versus time.



**Figure 6** | Loadings: ammonia (—) and hydrogen sulfide (---). H<sub>2</sub>S removal efficiency (+) versus time.

(H<sub>2</sub>S/HS<sup>-</sup>/S<sup>=</sup>) increased in the second stage, possibly due to the presence of high nitrite concentrations (about 40 mM) depressing the sulfate concentrations. The best results were 100% and 98% removal efficiency for 296 ppmv of NH<sub>3</sub> and 36.7 ppmv H<sub>2</sub>S.

From the initial stages of operation a high hydrogen sulfide removal efficiency was achieved and this can be attributed to BTT alone, because sulfur oxidizing bacteria are not initially present in the BNE.

### Third configuration

Having completed the study with the two biofilters connected in series, the next step involved studying the behavior of the two biofilters separately. The BNE biotrickling filter gave the best results. In BNE a biofilm of *T. thioparus* formed on a biofilm of *N. europaea* (data for BTT not shown).

The inlet loading of H<sub>2</sub>S was increased from 3.5 to 10 gS m<sup>-3</sup> h<sup>-1</sup> (input concentration from 45 to 129 ppmv) and the loading of NH<sub>3</sub> from 3.3 to 10.9 gN m<sup>-3</sup> h<sup>-1</sup> (input concentration from 98 to 322 ppmv). The EBRT was 60 seconds and the pH was controlled in the range 7.5–7.6. The evolution of the concentration of oxidation products and substrates in the recirculation medium for BTT and BNE is shown in Figure 5. The recirculation medium was changed after 184.5, 423.8, 640.7 and 879.8 h of operation. The maximum product concentrations were 2.55, 4.15, 4.10, 3.86 and 3.67 g L<sup>-1</sup> for sulfate and 47.4,

46.8, 30.6, 74.9 and 76 mM for nitrite. During this experiment the total hydrogen sulfide concentration was below 70 mgS L<sup>-1</sup>.

The same trend was observed as described in previous sections in that an increase in the sulfur concentration was observed for nitrite concentrations of around 40 mM.

The ammonia concentration at the outlet of BNE was zero, but the hydrogen sulfide removal decreased as the sulfur concentrations increased as a result of the increase in the nitrite concentration (Figure 6).

The increase in the ammonia loading did not directly affect the H<sub>2</sub>S removal efficiency, but it did have an indirect effect by increasing the nitrite concentration. The BNE biotrickling filter had a removal efficiency of 100% for 230 ppmv of NH<sub>3</sub> and 129 ppmv of H<sub>2</sub>S at an EBRT of 60 seconds.

## CONCLUSIONS

The results of this study show that it is possible to co-immobilize the two microorganisms using the same recirculation medium and to remove H<sub>2</sub>S and NH<sub>3</sub> from a gas mixture successfully.

The main variable that affects the removal efficiency is the nitrite concentration. Control of the nitrite concentration is therefore very important in the system because at a nitrite concentration of around 40 mM the ammonia and sulfur compounds are absorbed in the recirculation medium. At this concentration the metabolisms of both microorganisms are inhibited.

BNE showed better results when the two bacteria were co-immobilized in the two biofilters and also in the third configuration, in which the bacteria were studied separately.

## ACKNOWLEDGEMENTS

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