Removal of ammonia by immobilized Nitrosomonas europaea in a biotrickling filter packed with polyurethane foam

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ABSTRACT

A chemolithoautrotrophic microorganism *Nitrosomonas europaea* has been utilized to remove gaseous ammonia in a biotrickling filter packed with polyurethane foam. The optimal pH for removing was 7.5 and the biological removal efficiency was zero at pH 6.5. Empty bed residence time of 150, 100, 50, 25, 20, 11 and 5 seconds were tested; the removal efficiency was of 100% in all range for a constant load of 8 $gN.m^{-3}.h^{-1}$. The critical elimination capacity was 270 $gN.m^{-3}.h^{-1}$ while the nitrite concentration was below of 100 mM (EBRT of 11 second, pH 7.5-7.6). Therefore, these results demonstrate that is possible to reach a high removal of ammonia using polyurethane foam, as solid support for *Nitrosomonas europaea,* and a biotrickling filter system.

1 INTRODUCTION

Ammonia (NH_3) is a colourless air pollutant with a strong and irritating odour. Breathing levels about 100 ppmv ammonia in air, noticeable irritation of eyes and nasal passages after few minutes exposure (Carson and Mumford, 2002). Ammonia is released into the atmosphere from various sources, such as sludge and wastewater treatment plants, composting plants, livestock farms, wastewater treatment plants (Chung *et al*., 1996; Smet *et al*., 2000; Busca and Pistarino, 2003).

Ammonia emissions control is essential to protect the environmental impact and the public health (Erisman *et al.,* 2003). The technologies involved in the treatment of waste gases containing ammonia are based on physical and chemical process, these technologies include incineration, condensation, absorption and adsorption (Busca

and Pistarino 2003). However, biological treatments have become an effective and inexpensive alternative to physico-chemical process (Devinny *et al.,* 1999).

Up to the present few studies on biofiltration using biotrickling filter system for treatment ammonia emissions are available (Kanagawa *et al.,* 2004; Melse and Mol 2004; Sakuma *et al.,* 2004; Chou and Wang, 2007). The objective of this work was to study the feasibility of a biotrickling filter packed with polyurethane foam particles inoculated with *Nitrosomonas europaea*. The following operations variables were tested: rate of the recirculation liquid, nitrite concentration in the recirculation liquid, pH, EBRT, ammonia load and pressure drop.

2 MATERIALS AND METHODS

2.1 Organism cultivation and medium preparation

The original pure-culture strain of autotrophic *Nitrosomonas europaea* ATCC 19718 was obtained from the American Type Culture Collection. *Nitrosomonas europaea* is a chemolithoautrotrophic soil bacterium which obtains all its energy and reducing power from the oxidation of NH_3 to NO_2^- (Prosser 1989; Stein and Arp 1998). This stock culture was grown using a rotary shaker at optimal temperature (30ºC) and pH 8.0 in the dark. The mineral medium was the ATCC Medium #2265: Solution 1: 4.95 g of $(NH_4)_2SO_4$ (for 50 mM NH_4^+), 0.2 g of KH_2PO_4, 0.27 g of $MgSO_4 \cdot 7H_2O$, 0.04 g of $CaCl_2$, 0.5 ml of $FeSO_4$ (30 mM in 50 mM EDTA at pH 7.0), 0.2 mg of $CuSO_4 \cdot 5H_2O$ in 1.2 litre of distilled water. Solution 2: 8.2 g of KH_2PO_4, 0.7 of NaH_2PO_4 in 0.3 litre of distilled water (pH 8.0 with NaOH 10N). Solution 3 (buffer): 0.6 g of Na_2CO_3 in 12 ml of distilled water. The three solutions were sterilized at 121ºC during 20 min and mixing at room temperature.

2.2 Packing material

Polyurethane foam as used in this study as carrier. This 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 (McNevin and Barford 2000; Moe and Irvine 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.

2.3 Immobilization method

A PVC column (63 mm of diameter) was used to build-up the biotrickling filter with a working volume of 1.0 L. This column was packed with 10 grams of polyurethane foam cubes. A culture of *Nitrosomonas europaea* was continuously

recirculated over the packed bed using a centrifugal pump (EHEIM 1046) at a constant volumetric flow rate of 26.7 $L.h^{-1}$. The temperature was controller at 30ºC (Heildoph EKT 3001), and the culture was mixing at 200 rpm (Agimatic-N, Selecta) in the dark. When the pH decreased below 6.0 the total medium was drained and replaced with 1 L 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. As biotrickling filter it was used the same column that has been used for the immobilization. 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 μm). Air was humidified using fine bubble diffusion. Flow rates were controlled with mass flow controller (Bronkhorst, Model F-201C). For generation of high loads it was used an ammonia generator column of PVC (63 mm of diameter, packed with glass beads of 5 mm, 25 mm height). A solution whose composition was similar to the liquid culture medium without $(NH_4)_2SO_4$, the energy source, was added to supplement nutrients. The pH of medium was controller at 6.5-6.6 with addition of $NaHCO_3$ and controller (CRISON PH28). The temperature of experiment was maintained at 30ºC.

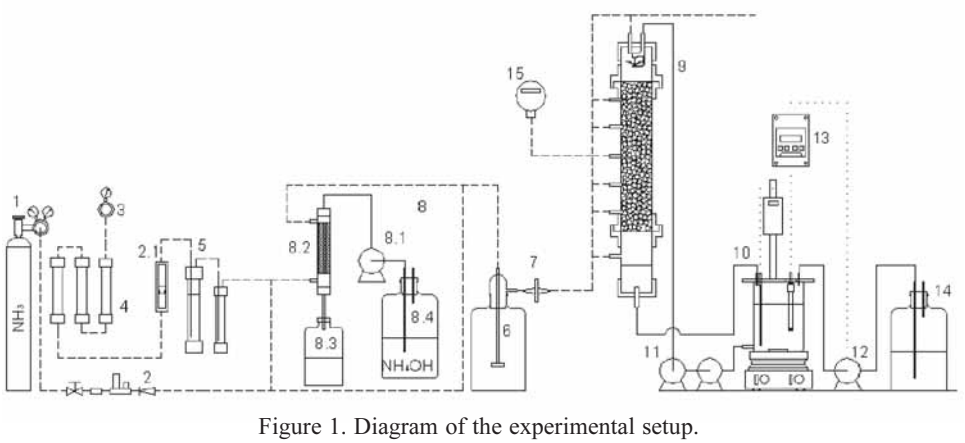

Figure 1. Diagram of the experimental setup.

1. Ammonia gas cylinder (NH_3/sintetic air); 2. Mass flow controller; 2.1 Rotameter 3. Pressure air regulator; 4. Air prefilter; 5. Humidification system; 6. Expansion deposit; 7. Air filter; 8. Ammonia generation system; 8.1 Peristaltic pump; 8.2. PVC column with glass beads; 8.3. Discharge deposit; 8.4. NH_4OH deposit; 9. Biotrickling filer; 10. Recirculation tank; 11. Recirculation pumps; 12. pH control pump; 13. pH controller; 14. $NaHCO_3$ deposit; 15. NH_3 sensor.

2.3 Analytical techniques

2.3.1 Gas

Ammonia was analysed using an electrochemical sensor of Crowcon (Model GASFLAG, TXGARD-IS).

2.3.2 Substrate and product concentration

Ammonia in the recirculation liquid was measure using a Nessler Method and nitrite was measure using a colorimetric method (Clescerl *et al.*, 1989).

2.3.3 Immobilized biomass

Immobilized biomass concentration was measured by counting of total biomass in a Neubauer chamber. A unit of carrier was removed from the reactor and squeezed lightly in order to remove the interstitial liquid. Then, it was submerged in an erlenmeyer flask containing 25 ml of sodium phosphate buffer solution (pH 7.0). In a second step, the flask was 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 was 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 (Gómez *et al.*, 2000; de Ory *et al.*, 2004). 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 the carrier, 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 $3.29 \pm 0.52 \cdot 10^{10}$ cel g^{-1} of solid support in 10 cycles. The duration of the experiment was of 310 hours. The Figure 2 shows the total immobilized biomass in each cycle. The immobilized biomass was very inhomogeneous. In the fourth cycle the biotrickling filter was inundate to homogenize the system and to improve the immobilization. The inundation was realized during one hour before the reposition of recirculation liquid.

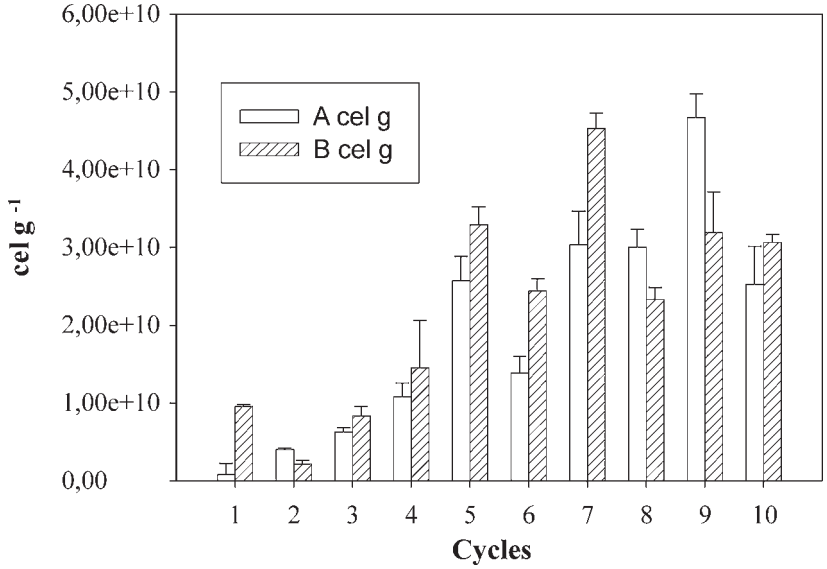

Figure 2. Immobilized biomass in each cycle.

In Figure 3, we can see the bacteria of *Nitrosomonas europaea* immobilized about surface of polyurethane foam.

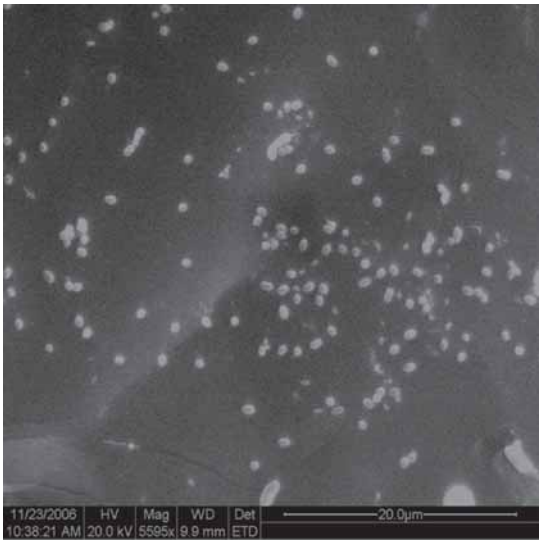

Figure 3. SEM of *Nitrosomonas europaea* immobilized.

3.2 Removal of ammonia

3.2.1 Effect of liquid recirculation velocity

The recirculation liquid in biotrickling filters use to have benefits on micronutrients distribution , absorb gaseous contaminants, remove metabolite, moisten the biofilm and control the thickness biofilm. Therefore the effect of liquid recirculation velocity on the ammonia removal efficiency was tested. The biotrickling filter was operated at 8.57, 3.55 and 1.59 $m.h^{-1}$. Results showed that the increase of liquid recirculation velocity didn't have a significant effect on the removal efficiency.

3.2.2 Effect of nitrite concentration

In the biological treatment of ammonia gas, ammonium and nitrite are produce and accumulate in the reactor. Therefore, the influence of ammonium and nitrite concentrations on *Nitrosomonas europaea* was examined. The EBRT was 30 second, the liquid recirculation velocity was 8.57 $m.h^{-1}$, the load was 6.76 $gN.m^{-3}.h^{-1}$ and the pH was controlled between 7.5-7.6. Removal efficiencies of 100% were obtained in this experiment, but the biological removal efficiency was not total. The biological removal efficiency was calculated using the following equation obtained from matter of balance:

$$(Rb) = \frac{(R)}{(E)} = \frac{Q\ (\overline{C}_0 - \overline{C}_S) - \dfrac{\Delta \overline{C}_L}{\Delta t}\ V_L}{\overline{C}_0\ Q}$$

where, Rb (%)= biological removal efficiency, Q ($m^3.h^{-1}$)= volumetric flow medium, $\overline{C}_0$ ($gN.m^{-3}$)= inlet ammonia concentration, $\overline{C}_S$ ($gN.m^{-3}$)= outlet ammonia concentration, t (h)= time, V_L (m^{-3})= volume of medium recirculation.

Because the biotrickling filter was inoculated with a pure culture, acclimation was unnecessary. These results show that the nitrite concentration must be kept below 100 mM $N(NO_2^-)$ by replacing the recirculation liquid with fresh medium. When the nitrite concentration exceeds 100 mM, the ammonia concentration in the recirculation liquid increased rapidly (Figure 4).

3.2.3 Effect of pH

The growth of *Nitrosomonas europaea* is optimal at pH of 7.5-8.0 (Hunik *et al.,* 1992). The effect of pH on ammonia removal efficiency was studied in the range from 6.5 to 8.2 (Figure 5). The rest of the parameters was fixed as follows: EBRT 30 second, the liquid recirculation velocity 8.57 $m.h^{-1}$, the load 6.76 $gN.m^{-3}.h^{-1}$ and the nitrite concentration smaller than 150 mM. When the pH decreased from 7.5 to 6.5, the biological removal efficiency decreased to zero.

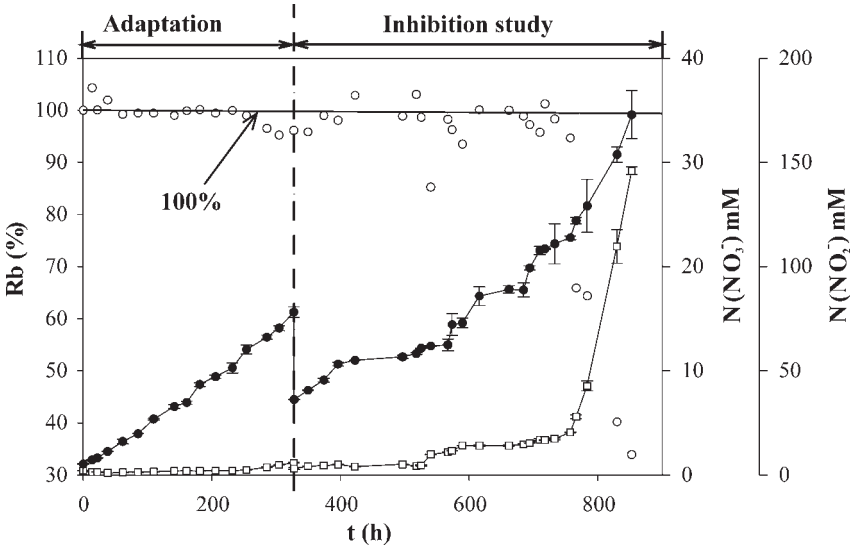

Figure 4. Effect of nitrite concentration. Ammonia concentration (□), nitrite concentration (○) and biological removal efficiency (●) versus time.

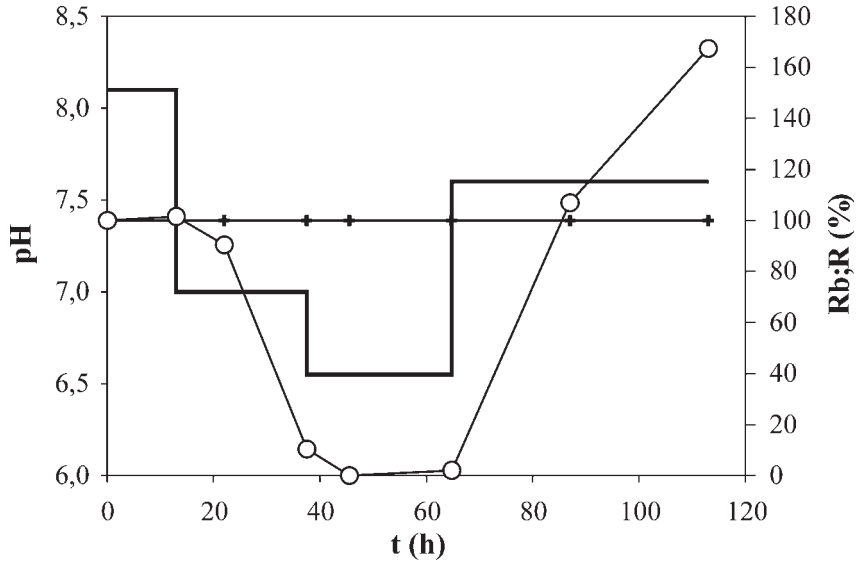

Figure 5. Effect of pH. pH (—). Removal efficiency (+) and biological removal efficiency (○) versus time.

Therefore the optimal pH was 7.5, the same value that obtained one for Chung and Huang (1998) using Ca-alginate beads with N*itrosomonas europaea*. This result showed that the pH control in the range is important for maintaining a high removal ratio of ammonia.

3.2.4 Effect of load

The load on the biotrickling filter was gradually increased by increasing the gas concentration from 60 to 1600 ppmv. The effect of load on ammonia removal efficiency was studied in the range from 0.89 to 21.7 $gN.m^{-3}.h^{-1}$. The EBRT was maintained constant at 150 second. The pH was controlled between 7.5-7.6 and the liquid recirculation velocity was 8.57 $m.h^{-1}$. The ammonia gas was successfully treated in the whole range. Therefore, the biotrickling filter attained good operational efficiency (R=100% and Rbe≥100% for each load at stationary state). The nitrite concentration of the recirculation liquid was smaller than 150 mM.

3.2.5 Effect of empty bed residence time

The effects of EBRT of 150, 100, 50, 25, 20, 11 and 5 second on the ammonia removal efficiency were tested. The load was maintained constant at 8 $gN.m^{-3}.h^{-1}$ by increasing the gas concentration from 20 to 592 ppmv. The pH was controlled between 7.5-7.6 and the liquid recirculation velocity was 8.57 $m.h^{-1}$. In this study removal efficiency of 100% was reached, and the biological removal efficiency was higher than 100% (Figure 6). To avoid inhibition of ammonia removal owing to nitrite concentration, the liquid of recirculation was replaced for fresh medium at 143h of operation.

Liang *et al.* (2000) have also observed that the EBRT can be decreased further without decreasing the ammonia removal efficiency. Chung *et al.* (1997) obtained a decreased of 20% when the EBRT was decreased from 70 to 12 second working with a biofilter. In a biotrickling filter Chou and Wang (2007) studied the effect of EBRT from 236 to 30 seconds reached removal efficiency of 99 and 96% for EBRT of 59 and 30 seconds respectively.

To know the elimination limits, it was realized an experiment maintaining constant EBRT at 11 second and increasing the ammonia concentration from 134 to 1434 ppmv (loads from 24.7 to 270 $gN.m^{-3}.h^{-1}$). As shown in Figure 7, the biological removal efficiencies was higher than 100% when the nitrite concentration was smaller than 100 mM $N(NO_2^-)$. When the nitrite concentration was higher than 100 mM the biological removal efficiency decreased and the ammonia concentration increased rapidly. The maximum elimination capacity was 270 $gN.m^{-3}.h^{-1}$. Working with a biotrickling filter the maximum elimination capacity observed for other researchers were: 59.9 $gN.m^{-3}.h^{-1}$ (99.8%) (Kanagawa *et al.,* 2004), 33.83 $gN.m^{-3}.h^{-1}$ (90.0%) (Melse and Mol, 2004), 2.78 $gN.m^{-3}.h^{-1}$ (98.0%) (Sakuma *et al.,* 2004) and 10.16 $gN.m^{-3}.h^{-1}$ (94.2%) (Chou and Wang, 2007).

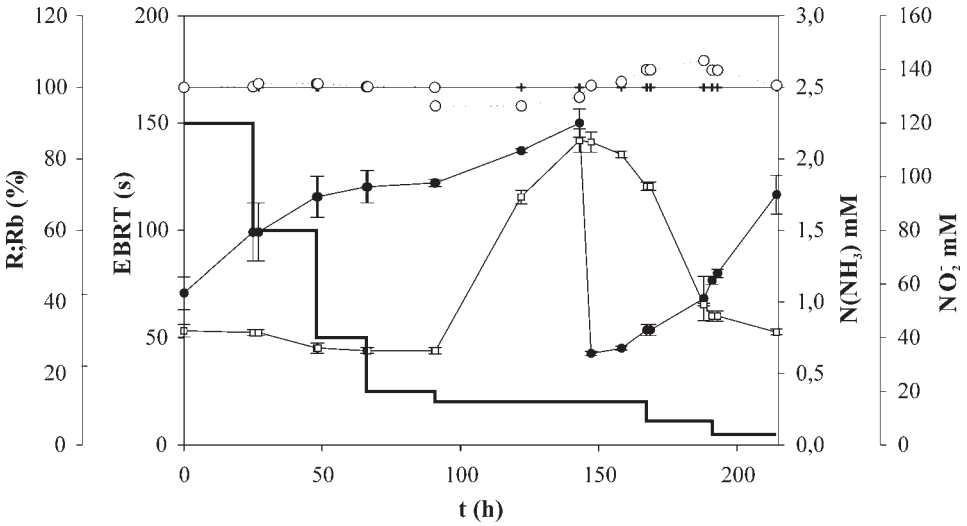

Figure 6. Effect of EBRT (—). Ammonia concentration (□), nitrite concentration (●), removal efficiency (+) and biological removal efficiency (○) versus time.

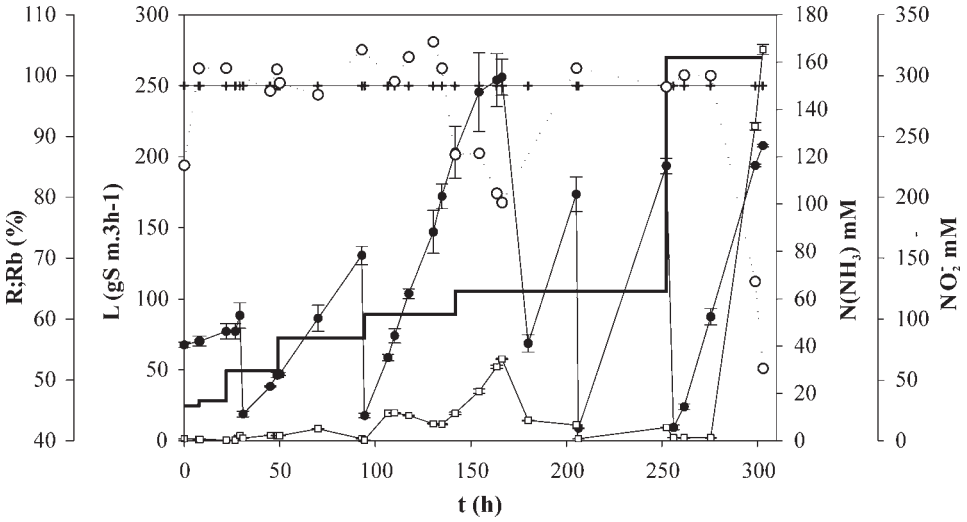

Figure 7. Effect of load. Ammonia concentration (□), nitrite concentration (●), removal efficiency (+) and biological removal efficiency (○) and Load (—) versus time. EBRT = 11 seconds.

3.2.6 STUDY OF PRESSURE DROP

The pressure drop in the biotrickling filter was measure before and after the immobilization. The pressure drop increased from 6.2 to 25.9 cm of water per meter of column when decreased de EBRT from 11 to 5 second after immobilization (Figure 8).

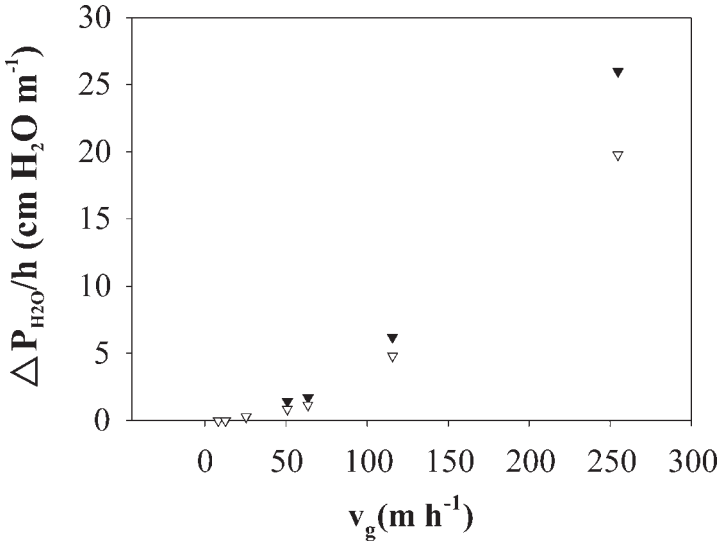

Figure 8. Pressure drop per meter of column versus superficial gas velocity.
▼ With biomass; ▽ without biomass.

4 CONCLUSIONS

The biotrickling filter can be considered as a good configuration for treatment waste gases contaminated with ammonia. This configuration allows a rapid absorption and biological oxidation of ammonia in the liquid. Ammonia gas was successfully treated at a load of 270 $gN.m^{-3}.h^{-1}$ when nitrite concentration was smaller than 100 mM $N(NO_2^-)$ at EBRT of 11 seconds. The more important parameters affecting the performance of this configuration are: pH (optimal 7.5) and nitrite concentration in the recirculation liquid (optimal <100mM).

5 ACKNOWLEDGEMENTS

Authors wish to express their sincere gratitude to Spanish Government for financial support of this work through Project PPQ2002-0217.

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