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Technical Note

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

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ABSTRACT

A biotrickling filter with *Nitrosomonas europaea* immobilized on polyurethane foam is proposed for treating ammonia contaminated air. The effect of the surface velocity of the recirculation medium, nitrite concentration, pH, empty bed residence time (EBRT) and ammonia inlet load on the NH_3 removal process was investigated. The total amount of biomass immobilized on the carrier was $3.29 \pm 0.52 \times 10^{10}$ cells g^{-1} dry carrier. The maximum elimination capacity of the biotrickling filter was 270 g N m^{-3} h^{-1} at pH 7.5, an EBRT of 11 s, and nitrite concentrations below 100 mM. These results show that system studied can be considered as a viable alternative for the treatment of gaseous emissions containing high concentrations of ammonia.

1. Introduction

Ammonia is a colourless, toxic, reactive and corrosive gas with an unpleasant and pungent smell (Busca and Pistarino, 2003). Considerable volumes of ammonia are released by process industries such as petroleum refining, metal manufacturing, food and textile production, and in waste treatment and the composting of plants (Busca and Pistarino, 2003). In livestock farming, emissions of ammonia range from 10 to 60 ppmv (Chung et al., 1996). In plants for compost production from solid wastes, concentrations of up to 394 ppmv (at 30 °C) have been measured (Smet et al., 2000), while in sludge composting plants concentrations of up to 1023 ppmv (at 30 °C) can be found (Haug, 1993).

Exposure to ammonia principally causes irritation to the mucous membranes, generating a burning sensation in the eyes, nose and throat; this sensation may be caused at very low concentrations from 50 to 100 ppmv (Busca and Pistarino, 2003). At concentrations of 400 ppmv the irritation is immediate, at 1500 ppmv ammonia causes coughing, and at 2500 ppmv it is life-threatening (Helmers et al., 1971; Ferguson et al., 1977).

Most of the studies published on the biofiltration of ammonia concern the use of biofilters with organic supports, while very few studies have been conducted with biotrickling filters (Kanagawa et al., 2004; Melse and Mol, 2004; Sakuma et al., 2004; Chou and Wang, 2007). Compost and peat are predominant among the biofilters described and these are usually mixed with inorganic compounds like activated carbon, perlite (Chen et al., 2005) and even shredded high density plastics (Taghipour et al., 2006) in an effort to improve pH control or as a bulking agent. The main advantages of biotrickling filters include the easy removal of reaction products by washing-out, easy control of the biological process and good adaptation capacity of the active biomass (Kennes and Thalasso, 1998).

Immobilized *Nitrosomonas europaea* (ATCC 19718) biofilters have been successfully applied to the removal of NH_3 alone (Chung and Huang, 1998) and for the treatment of mixtures of H_2S and NH_3 with a two-stage biofilter (Chung et al., 2007).

The objective of the work described here was to study the feasibility of treating air contaminated with ammonia using a biotrickling filter packed with cubes of polyurethane foam inoculated with *N. europaea*. This process was used to conduct a quantitative investigation of a biofilter system for the removal of NH_3.

2. Materials and methods

2.1. Microorganism and cultivation medium

A pure culture of *N. europaea* (ATCC 19718) was obtained from the American Type Culture Collection. The composition of the ATCC #2265 mineral medium was as follows: 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 L of distilled water; Solution 2: 8.2 g of KH_2PO_4, 0.7 g of NaH_2PO_4 in 0.3 L of distilled water (pH 8.0 with NaOH 10 N). Solution 3 (buffer): 0.6 g of Na_2CO_3

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in 12 mL of distilled water. The three solutions were sterilised at 121 °C for 20 min and mixed at room temperature.

2.2. Characteristics of the carrier material

Polyurethane foam cubes of 1 cm^3 were used as the carrier. Polyurethane foam is an inert material with good scale-up possibilities and a very low commercial cost. The principal relevant properties of this material are density (20 kg m^{-3}) and porosity (96%).

2.2.1. Growth kinetics and inoculum preparation

The propagation of *N. europaea* was carried out in a liquid culture (100 mL of ATCC #2265 medium) using a rotating shaker at 150 rpm and 30 °C (optimal temperature) in darkness. The cells were collected and concentrated by centrifugation at 15 000 rpm, 4 °C for 15 min. The pellet was resuspended in 5 mL of fresh culture medium. A volume of 100 mL of ATCC #2265 medium was inoculated with 2 mL of the resuspension of cells and used for growth kinetics and inoculation of the biotrickling filter.

2.3. Experimental configuration

The experimental set up is illustrated in Fig. 1. A PVC tube with an external diameter of 63 mm and a thickness of 1.5 mm was used to build the biotrickling filter and this had 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 into the column.

The air was supplied by an industrial compressor. Before entering the system, the air was passed through filters of silica gel, activated carbon and glass wool (diameter of 32 mm; packing height of 30 mm). The flow rates of each stream were regulated by mass flow rate controllers (Bronkhorst F-201C). A PVC desorption column packed with 5 mm glass spheres, with a packing height of 25 mm, was fed from the top of the column with a solution of NH_4OH to generate high ammonia loads. An expansion tank with a capacity of 2.5 L was used to homogenize the input stream, and a 0.45 μm filter was utilized at the end of the system to sterilise the input stream to the biofilter (Millipore Filter SLG05010). A solution of similar composition to the cultivation medium, but without the ammonium source, was recirculated by means of two centrifugal pumps (EHEIM 1046) connected in series. The pH of the medium was controlled (only in the ammonia gas removal study) by the addition of $NaHCO_3$ (2 M) using a pH controller (CRISON, PH28) and an electrode with a 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).

2.4. Method for the immobilization and adaptation of N. europea

The biotrickling filter was packed with 10 g of polyurethane foam in 1 cm^3 cubes and the initial volume of the packing was 1 L. A suspension of *N. europaea* cells was fed onto the top of the column at a constant flow rate of 26.7 L h^{-1} (surface velocity of 8.6 m h^{-1}). 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. In the first stage, 1 L of ATCC #2265 medium was inoculated at 10 vol% with a culture of *N. europaea* in the exponential growth phase. 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 (100% in subsequent cycles) and fresh medium was added. Successive cycles were performed until the maximum immobilized biomass was achieved.

In order to adapt the biofilm, the recirculation medium was replaced with a medium of identical formulation to ATCC #2265 but without the energy source (ammonium sulfate) and, at the same time, the feed of NH_3 in air was initiated. The flow rate of air employed was 120 L h^{-1} and the concentration of NH_3 was 50 ppmv.

2.5. Analytical techniques

A specific sensor from Crowcon (GASFLAG model, TXGARD-IS) was employed to analyse the ammonia in the gas phase. Direct nesslerization and colorimetric methods were employed to determine the concentrations of ammonia and nitrite, respectively, in the liquid medium (APHA, 2007).

The quantity of immobilized biomass in the cells per gram of dry carrier was determined by counting the number of bacteria on a unit of the carrier material and dividing the total quantity of biomass by the weight of the polyurethane foam (Gomez et al., 2000; de Ory et al., 2004). In the first stage, a unit of colonized carrier was removed from the reactor, dried with absorbent paper and submerged in an Erlenmeyer flask containing 25 mL of sterile ATCC #2265 medium. In the 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 final stage, the Neubauer chamber re-count method for submerged cells was

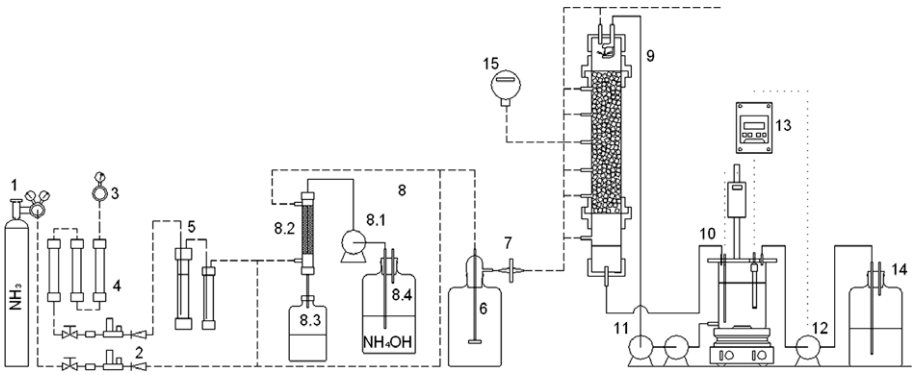

Fig. 1. Schematic diagram of the biotrickling filter. 1. Compressed gas cylinder (NH_3/synthetic air); 2. Mass flow controllers; 3. Air pressure regulator; 4. Air prefilters; 5. Humidifier and water trap; 6. Expansion tank; 7. Air filter; 8. System for generation by chemical desorption; 8.1 Peristaltic pump; 8.2. PVC column filled with glass spheres; 8.3. Discharge tank; 8.4. NH_4OH tank; 9. Biotrickling filter; 10. Recirculation tank; 11. Nutrient recirculation pumps; 12. Base addition pump; 13. Biocontroller; 14. $NaHCO_3$ tank; 15. NH_3 sensor.

carried out on the liquid phase. The carrier was subsequently removed from the flask and dried in an oven at 80 °C for 24 h. It was then possible to calculate the number of immobilized cells per gram of carrier. This technique has previously been validated by developing experiments concerned with cellular resistance to ultrasonic treatment and studying the desorption efficiency.

The colony forming units (CFU) were measured by the Spread Plate method on ATCC #2265-agar plates (2.0% w/v of agar) using serial 10-fold dilutions. A U tube with a scale in mm was filled with water and this was used to measure the pressure drop.

3. Results and discussion

3.1. Growth kinetics and biomass immobilized

N. europaea gain their energy by oxidizing ammonia to nitrite. As can be observed (Fig. 2), the ammonia concentration decreased from 50 to 27 mM and the nitrite concentration increased to 7.2 mM. The parameter that has the greatest influence on the metabolism of the bacteria is pH. The initial pH of the culture was 8.0 and once the exponential growth phase was reached the pH decreased in a linear manner to a constant value of 5.8 after 63 h. After this point the metabolism of the bacteria diminishes notably; the same result was reported by Chung and Huang (1998).

A maximum specific growth rate (μ) of 0.056 h^{-1} with a linear regression coefficient of 0.996 was obtained. The value obtained falls within the range reported by Prosser (1989) for continuous cultures of *N. europaea* (0.039–0.064 h^{-1}).

A total of 10 immobilization cycles were performed with a total duration of 310 h. The samples were taken in duplicate (A and B, Fig. 3) at the mid-point of the bed. The total quantity of biomass immobilized in each cycle is shown in Fig. 3 along with the corresponding substrate consumption rate. The carrier material presented a wide variation in the number of bacteria immobilized. As a consequence of this heterogeneity in the immobilization, when the 4th cycle was completed it was decided to flood the column for 1 h before replacing the medium. The objective of this flooding was to homogenize the system as a means of accelerating the immobilization process. This operation was repeated in the subsequent cycles.

The total quantity of biomass immobilized at the end of the cycles was 3.3 ± 0.5 × 10^{10} cells g^{-1} dry carrier (1.2 × 10^{9} CFU g^{-1} dry carrier).

In light of the results obtained, the substrate consumption rate could be taken as an indicator of the degree of immobilization and it could therefore be considered that, after five cycles (7–8 d), the maximum number of bacteria immobilized by this technique was reached.

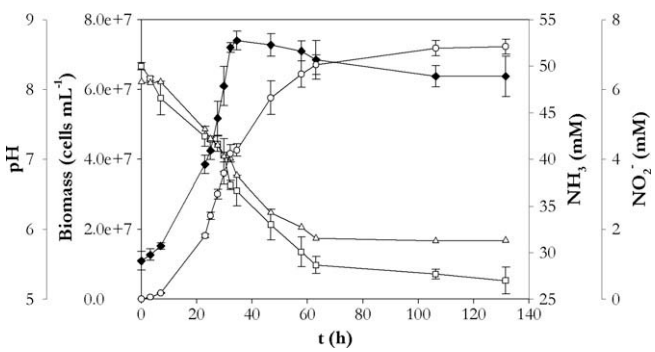

Fig. 2. Kinetic of *Nitrosomonas europaea*. pH (▲) and concentration of: biomass (♦), ammonia (□) and nitrite (●) versus time.

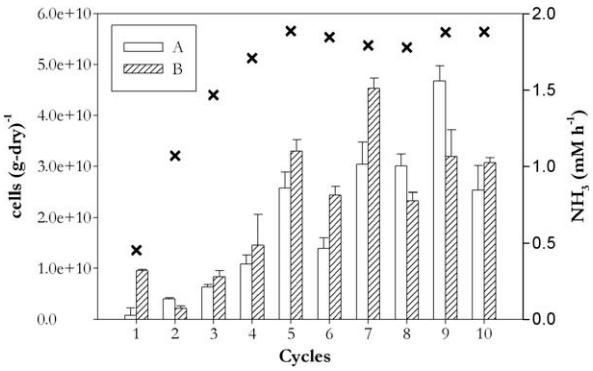

Fig. 3. Evolution of the immobilized biomass (A, B each sample) and rate of consumption of substrate in the process of immobilization (χ).

There are no antecedents concerning the use of polyurethane foam for the specific immobilization of *N. europaea*. However, Kim et al. (2002) employed polyurethane foam covered with a mixture of activated carbon and natural zeolite powder to immobilize ammonium-oxidizing populations originating from a sludge. The quantity of bacteria immobilized after 53 d of operation, as reported by Kim et al. (2002), was 1.6 × 10^{9} cells g^{-1}; this quantity is less than that obtained by the immobilization technique reported here. The immobilization of *N. europaea* has been performed by entrapment in materials such as calcium alginate (van Ginkel et al., 1983) and k-carrageenan (Wijffels and Tramper, 1989; Wijffels et al., 1994).

In biofiltration, the use of this bacterium in pure cultures (*N. europaea* ATCC 19718) for the removal of ammonia has been studied by Chung and Huang (1998), who performed an immobilization on calcium alginate beads starting from a concentration of 10^{5} CFU g^{-1} dry.

3.2. Effect of the surface velocity of the recirculation medium

The recirculation of the medium in a biotrickling filter enables the oxidation products to be removed easily. It allows the thickness and humidity of the biofilm to be controlled and facilitates the absorption of the contaminant of the gas. The biotrickling filter was operated with a constant surface velocity of the recirculation medium of 8.6, 3.5 and 1.6 m h^{-1}. The removal efficiency was constant at 100% for all three surface velocities. Chou and Wang (2007) studied the effect of the recirculation flow rate in a biotrickling filter packed with coke, at surface velocities between 0 and 2.9 m h^{-1}, and found insignificant differences in the ammonia removal results.

3.3. Effect of the concentration of nitrite

Once the adaptation phase had been completed and the recirculation medium was replaced with fresh medium, the next variable studied was the inhibitory effect of the nitrite concentration. According to previously published results, the two chemical species that inhibit the metabolism of *N. europaea* are ammonia and nitrous acid (Anthonisen et al., 1976; Sakuma et al., 2004; Baquerizo et al., 2005), although other factors – such as the increase in osmotic pressure due to high concentrations of salts – may also inhibit its activity (Hunik et al., 1992).

The empty bed residence time (EBRT) of the gas was 30 s, the inlet load was 6.76 g N m^{-3} h^{-1}, the surface velocity of the recirculation medium was 8.6 m h^{-1} and the pH was kept between 7.5 and 7.6. As can be observed in Fig. 4, the acclimation stage was non-existent, possibly because the biofilter was inoculated with a previously adapted pure culture. Since ammonia is a highly soluble

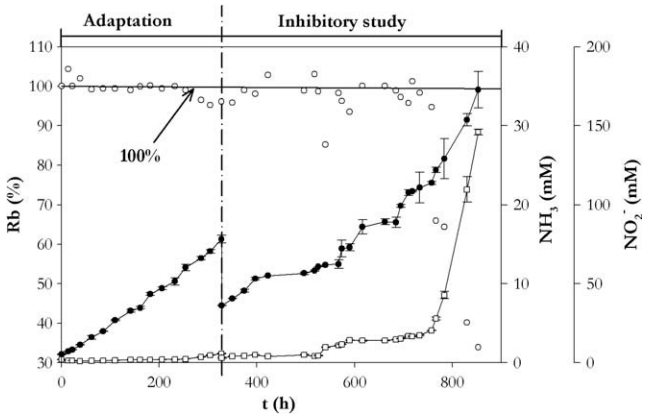

Fig. 4. Inhibitory study of the nitrite concentration. Concentration of: ammonia (□), nitrite (●) and biological removal efficiency (○) versus time.

compound, the elimination percentage due exclusively to the metabolism of the bacteria was calculated using the following equation, which was obtained from the ammonia mass balance of the system:

$$\text{Accumulation} = \text{In} - \text{Out} - \text{Reaction} \times (\text{Rate of } NH_3 \text{ removal by biodegradation}) \quad (1)$$

$$\text{Accumulation} = (A) = \frac{\Delta \bar{C}_L}{\Delta t} V_L \quad (2)$$

where $\bar{C}_L$ (g N m^{-3}) is the ammonia concentration in the recirculation medium and V_L (m^3) is the volume of the recirculation medium.

$$\text{In} - \text{Out} = (I) - (O) = Q(\bar{C}_0 - \bar{C}_S) \quad (3)$$

where Q (m^3 h^{-1}) is the gas flow rate, $\bar{C}_0$ (g N m^{-3}) is the input ammonia concentration and $\bar{C}_s$ (g N m^{-3}) is the output ammonia concentration.

Rearrangement and substitution into Eq. (1) gives the rate of NH_3 removal by degradation:

$$(R) = (I) - (O) - (A) = Q(\bar{C}_0 - \bar{C}_S) - \frac{\Delta \bar{C}_L}{\Delta t} V_L \quad (4)$$

The biological removal efficiency will be the fraction of the NH_3 removed by the biotrickling filter and this is expressed as a percentage:

$$(Rb) = \frac{(R)}{(E)} = \frac{Q(\bar{C}_0 - \bar{C}_S) - \frac{\Delta \bar{C}_L}{\Delta t} V_L}{\bar{C}_0 Q} \times 100 \quad (5)$$

The results show that the nitrite concentration must be kept below 100 mM by replacing the used recirculation medium with fresh medium. When the concentration of nitrite exceeded 100 mM, the concentration of ammonia in the recirculation medium increased rapidly and the percentage of biological elimination was reduced. In subsequent experiments concentrations of up to 300 mM of nitrite were reached (data not shown) and a progressive adaptation of the microorganism was observed. Control of the nitrite concentration is clearly an important parameter in the removal of ammonia by biofiltration.

3.4. Effect of the pH

The effect of pH on the removal of ammonia was studied in the range from 6.5 to 8.2 (Fig. 5). The inlet load was kept constant at

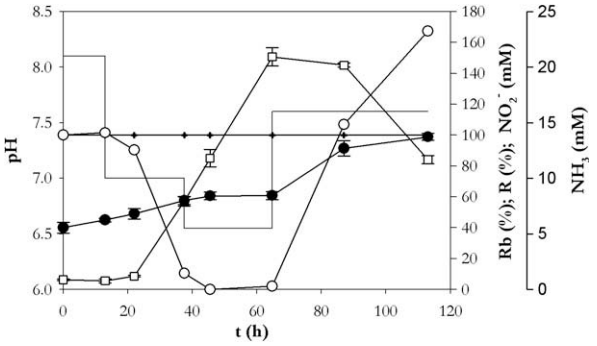

Fig. 5. Study of the effect of the pH. pH (—), removal efficiency (+), biological removal efficiency (○), ammonia concentration (□) and nitrite concentration (●) versus time.

6.10 g N m^{-3} h^{-1}, the input concentration was 90 ppmv of NH_3 and the EBRT of the gas was 30 s. The nitrite concentration was kept below 100 mM NO_2^- to prevent inhibition.

It can be observed that the calculated removal efficiency, based on the input and output concentrations in the biofilter, was maintained at 100%, but the biological removal efficiency was much lower for pH values between 7.0 and 6.5, falling to zero for a pH of 6.5. At pH 6.5 the removal of ammonia was exclusively due to the absorption of ammonia in water and formation the ammonium ion. The NH_4^+ can not be metabolized by *N. europaea* (Suzuki et al., 1974) and a low pH is therefore not recommended. The absence of metabolism of the bacteria was confirmed as the concentration of nitrite in the recirculation medium remained constant. Therefore, in light of the results obtained, it can be concluded that the optimum working pH is 7.5 and that control of this parameter is fundamental in the NH_3 removal process. This pH value is the same as that obtained by Chung and Huang (1998), who were working with the same bacteria immobilized on calcium alginate beads. This optimum working pH also coincides with the optimum value found for the species in submerged culture, which is in the range 7.5–8.0 (Hunik et al., 1992).

3.5. Effect of the load

The inlet load was increased by increasing the input concentration of ammonia from 60 to 1600 ppmv (0.9–21.7 g N m^{-3} h^{-1}). The EBRT of the gas was kept high at 150 s with the aim of minimising the mass transfer limitation and ensuring that the biological degradation was maximum. The pH was kept between 7.5 and 7.6, the surface velocity of the recirculation medium was 8.6 m h^{-1} and the nitrite concentration was kept below 150 mM. The biological removal efficiency was 100% for the entire range of concentrations once the stationary state had been reached, i.e. a constant concentration of ammonia for at least 12 h (Chen et al., 2004). The ammonia concentration in the outlet was therefore zero and the ammonia concentration in the recirculation medium did not increase. The maximum value of the elimination capacity for a biotrickling filter, which was obtained by Kanagawa et al. (2004), was 59.9 g N m^{-3} h^{-1} (removal efficiency of 99%).

3.6. Effect of the empty bed residence time

Studies were carried out in the range from 5 to 150 s for a constant inlet load of 8.0 g N m^{-3} h^{-1} (input concentrations from 20 to 592 ppmv of NH_3). The pH was controlled between 7.5 and 7.6 and the surface velocity of the recirculation medium was 8.6 m h^{-1}. A removal efficiency of 100% was obtained for all EBRT values assayed, once the stationary state had been reached. The percentage of biological removal efficiency was greater than 100%, meaning

that the immobilized biomass was able to eliminate not only the ammonia inlet load but also some of the ammonia already dissolved in the recirculation medium.

Liang et al. (2000) found a similar trend in that the decrease in the EBRT did not have a significant effect on the elimination of ammonia, although in studies carried out with biofilters the authors usually found a decrease in the elimination percentage when the EBRT was reduced (Chung et al., 1997); this decrease was explained as being due to mass transfer problems. In studies conducted with biotrickling filters, the problem of mass transfer has been minimised (Chou and Wang, 2007). Therefore the use of a biotrickling filter is much more appropriate for the treatment of gaseous effluents contaminated with ammonia, since the mass transfer from the gas to the liquid phase is improved and the EBRT of the gas does not have a significant effect.

With the aim of determining the ammonia removal limit, an experiment was performed at a constant EBRT of 11 s with successive increases in the ammonia concentration from 134 to 1434 ppmv (24.7–270 g N m^{-3} h^{-1}); this range is sufficiently wide to be able to treat effluents from composting plants (Haug, 1993; Smet et al., 2000) and fertiliser manufacturing plants, where concentrations of more than 1000 ppmv are encountered (Williams and Miller, 1992).

The maximum elimination capacity was 270 g N m^{-3} h^{-1} for nitrite concentrations below 100 mM (Fig. 6). The biological removal efficiency decreased when the nitrite concentration was greater than 100 mM. These values for the maximum elimination capacity are notably higher than those found in the bibliography for biofilters with the same characteristics: 59.9 g N m^{-3} h^{-1} (99%) (Kanagawa et al., 2004), 33.8 g N m^{-3} h^{-1} (90%) (Melse and Mol, 2004), 2.8 g N m^{-3} h^{-1} (98%) (Sakuma et al., 2004) and 10.2 g N m^{-3} h^{-1} (94%) (Chou and Wang, 2007).

3.7. Study of the pressure drop

The pressure drop in the biotrickling filter was measured before and after the immobilization. The pressure drop increased from 19.8 to 26.0 cm H_2O m^{-1} column for an EBRT of 5 s, and from 4.8 to 6.2 cm H_2O m^{-1} column for an EBRT of 11 s for the system without and with biomass, respectively.

As can be appreciated, the pressure drop per metre of column is increased slightly by having biomass present, according to the equation of Ergun (1952):

$$\frac{\Delta P}{hvg} = \alpha + \beta vg \tag{6}$$

where ΔP is the pressure drop along the bed length, h is the bed length, vg is the superficial gas velocity, and α and β the linear regression parameters.

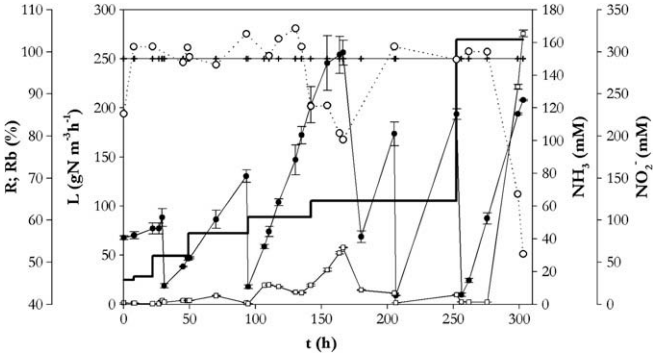

Fig. 6. Effect of the inlet load. Inlet load (—), ammonia concentration (□), nitrite concentration (●), removal efficiency (+) biological removal efficiency (○) versus time.

For each surface velocity there would be values of the constants alpha and beta from the equation, since the Ergun equation is only applicable to the movement of a fluid phase (Ergun, 1952). Of the two constants (alpha and beta) the beta value has the greatest influence on the increase in the load loss since it is the slope of the line. The values found for the beta constant were 0.033 and 0.025 Pa h^2 m^{-3} for the biotrickling system with and without biomass, respectively (surface velocity of the recirculation medium of 8.5 m h^{-1}). The values of the beta constant obtained are comparatively much lower than those obtained with other carrier materials: 1.64, 2.27, 4.17, 4.93 Pa h^2 m^{-3} for coconut husk, rice husk, maize stubble and bagasse, respectively (Ramirez et al., 2003).

4. Conclusions

The results obtained lead us to conclude that the parameters with the most influence on the functioning of the biofilter are the pH (optimum pH: 7.5) and the nitrite concentration in the recirculation medium (optimum: <100 mM). Careful control of these parameters allows 100% elimination to be achieved for loads of up to 270 g N m^{-3} h^{-1} with empty bed residence times in the order of 11 s. The system employed is therefore proposed as an effective alternative for treating highly concentrated effluents in a pilot plant. This system is capable of operating at low residence times for the gas, which would thus enable units of small size to be employed.

Polyurethane foam has been demonstrated to be a carrier material with a high capacity for the immobilization of *N. europaea* and it also offers a low resistance to the flow of gas, thus reducing the need for compression of the feed and the costs associated with this.

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