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Operation of the packed-bed pilot scale bioreactor

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1. Introduction

The preliminary work carried out in the MELISSA Pilot Plant with the third compartment bioreactor has been focused on its physical characterisation and definition of the control system (TN 25.310, TN 25.330).

The nitrifying pilot reactor has been operated throughout a first period of approximately one year. During this period of time a set of experiments was carried-out, and both transient and steady states were studied in different situations (TN 37.42.): different conditions of ammonium input load with steps in both influent ammonium concentration and flow-rate were investigated, providing a full set of results about the stability and high conversion of the influent to nitrate. Nevertheless, the operation of the third compartment of the MELISSA Pilot Plant had to be stopped due to the clogging of the packed bed, after one year of operation. This fact obliged to stop the continuous experiments, and introduce a number of changes in the hardware of this reactor, that would ensure a proper operation for even longer periods, without bed compactation or clogging. This upgrade of compartment III pilot reactor is reported in TN 47.2., and has been successfully applied. Indeed, a new operation period of one year has been completed, and the results are presented in this technical note. It is remarkable that no compactation or clogging limitations have appeared in this period, as a result of the upgrade of the hardware to prevent this problems, and the continuous operation of the reactor is kept beyond one year, to complete other experiments such as the connection of compartment III and IV at pilot scale.

In this present technical note, the results of this second year of continuous operation after the reactor upgrade are reported. They include the start-up of the reactor and the study of the effect of oxygen concentration and temperature on the evolution and

steady state of the concentration of ammonium, nitrite and nitrate in the effluent of this compartment.

2.- Start-up procedure for the nitrifying pilot reactor

To start-up the operation of the reactor, the procedure previously desecribed (TN 37.420.) was followed, particularly, with respect to the sterilisation protocol. The reactor was inoculated with a co-culture of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* obtained from the operation of the *Biostat B* fermenter (as described in TN 37.410.), the volume used was 700 mL.

The strategy used to carry out the start-up of this bioreactor has been to operate in continuous mode just after the inoculation. In that way, the risk of cellular contamination is lower and the attachment phase is stimulated (van Loosdrecht *et al.*, 1993).

Specifically, the residence time used initially was 80 h, and after 30 days it was decreased to 56 h. In table 1 a comparison between the conditions used in the start-up of the reactor in the first and second period of operation are detailed. In the present, the time required to complete the start-up and the initial development of the biofilm is lower than in the previous period of operation. This is a relevant fact, since due to the low growth rate of these bacteria, start-up time is an operational condition that should be reduced.

The evolution of the ammonium, nitrite and nitrate concentrations during this period of operation are detailed in figure 1.

Table 1.- Duration and residence time in the start-up period of the nitrifying pilot reactor.

	Approximate interval of start-up (days)	Residence time employed (h)
First period of operation	70	35
Current period of operation	45	80/56

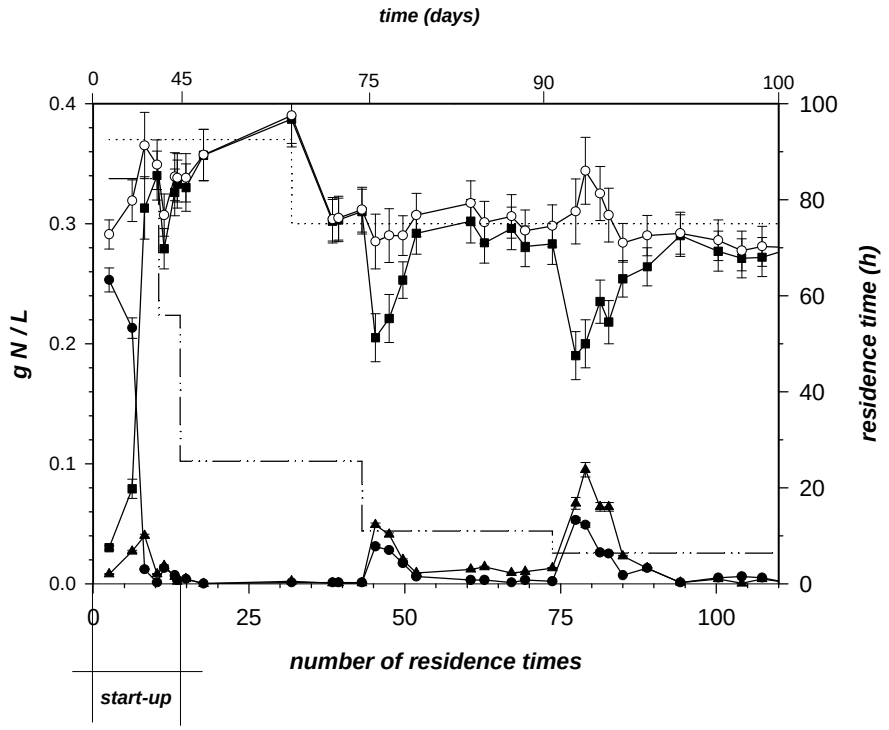

Figure 1.- Start-up of the pilot reactor. Evolution of the ammonium (●), nitrite (▲), nitrate (■), and total nitrogen ('❍', sum of the ammonium, nitrite and nitrate concentrations). The dashed line is the residence time. The dotted line is the ammonium input medium concentration.

The experimental conditions used during this period were: (30±1) ºC, pH of (8.1±0.1), 400 r.p.m. with magnetic stirring in the bottom section. The recirculation flow-rate was maintained 6 times higher than the input flow-rate.

As in the case of the first period of operation (described in TN 37.420.) once the initial development of the nitrifying biofilm had been attained, the ammonium input load was increased using lower residence times trying to determine the maximal load treated with high conversion by the pilot reactor.

3.- Maximal ammonium load

The steady state achieved during the operation of the reactor in which the load was increased to attain the maximal ammonium input load treated with high conversion by the pilot reactor are detailed in table 2. The values of load that appear in table 2 are calculated per unit of total reactor volume, in order to compare with values obtained from bibliography for this kind of systems in wastewater treatment.

Table 2.- Steady states in nitrifying pilot reactor in different conditions of ammonium input load.

Input concentration (g N-NH_4^+ / L)	Input ammonium load (kg N · m^{-3} · day^{-1})	Output ammonium load (kg N · m^{-3} · day^{-1})	Output nitrite load (kg N · m^{-3} · day^{-1})	Ammonium removed load (%)	Conversion to nitrate (%)
0.3	0.13	$2.3 \cdot 10^{-4}$	$3.0 \cdot 10^{-4}$	99.8	99.6
0.3	0.53	$8.8 \cdot 10^{-4}$	$2.7 \cdot 10^{-3}$	99.8	99.3
0.3	1.29	$1.2 \cdot 10^{-2}$	$4.8 \cdot 10^{-2}$	99.0	95.4
0.6	0.93	$7.9 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$	99.9	99.8
0.6	1.35	$3.4 \cdot 10^{-3}$	$3.4 \cdot 10^{-3}$	99.8	99.5
1.1	1.18	$1.3 \cdot 10^{-3}$	$1.9 \cdot 10^{-3}$	99.9	99.7

The maximal load attained in the pilot reactor is 1.35 kg N-NH_4^+ · m^{-3} · day^{-1}. The volumetric nitrification load in floating beds from bibliography is in the range 0.5 – 1.5 kg N-NH_4^+ · m^{-3} · day^{-1} (Martins dos Santos *et al.*, 1998) in systems with specific area between 200 and 700 $m^2 \cdot m^{-3}$. The specific area of the pilot reactor has been estimated in 705 $m^2 \cdot m^{-3}$. In this type of bioreactors the nitrification capacity is limited precisely by the specific area (Campos 2000, Martins dos Santos *et al.*, 1998). The comparison between reactors is difficult due to the following causes: the operating conditions used and the nitrification capacities of the reactors are in general referred to the reactor volume, therefore without taking into account the percentage of the total volume occupied by the bed. Due to these reasons, the values of nitrifying capacities have a direct relationship not only with the operating conditions used (pH and temperature among others), but also with the specific area. Taking into account the specific area in each case (that is, experimental values and values from the literature), it is possible to calculate the value of capacity in kg N-NH_4^+ · m^{-2} · day^{-1}:

$1.9 \cdot 10^{-3}$ kg N-$NH_4^+ \cdot m^{-2} \cdot day^{-1}$ (experimental for the pilot reactor), and

$1.7 \cdot 10^{-3}$ kg N-$NH_4^+ \cdot m^{-2} \cdot day^{-1}$ (mean referenced for floating beds).

Therefore, the maximal ammonium load treated is high and agrees with the maximal values reported in the bibliography for these type of reactors.

4.- Oxygen supply

In the pilot reactor the aeration flow-rate is maintained constant (3 L/min) during all the operation, and dissolved oxygen set point is achieved adding to the gas loop pure

oxygen or nitrogen using the mass flow controllers. In that way, it is possible to enrich the composition in oxygen or nitrogen to manipulate the level of dissolved oxygen required in all the possible situations.

To study the effect of the dissolved oxygen in the nitrification process a set of experiments have been carried out. Using a high ammonium input load (1.18 kg $N\text{-}NH_4^+ \cdot m^{-3} \cdot day^{-1}$), the dissolved oxygen level in the bioreactor was decreased to know the effect in the ammonium and nitrite degradation by *Nitrosomonas europaea* and *Nitrobacter winogradskyi* respectively.

The changes in the dissolved oxygen concentration were made by decreasing the dissolved oxygen set point in a step from 80 % to 40 %, and also to 20 %. After this step, the dissolved oxygen concentration was set again to 40 % and after this, returned to the initial 80 %. The evolution of the ammonium, nitrite and nitrate concentrations were determined, and are shown in figures 2-5, and also in the table 3.

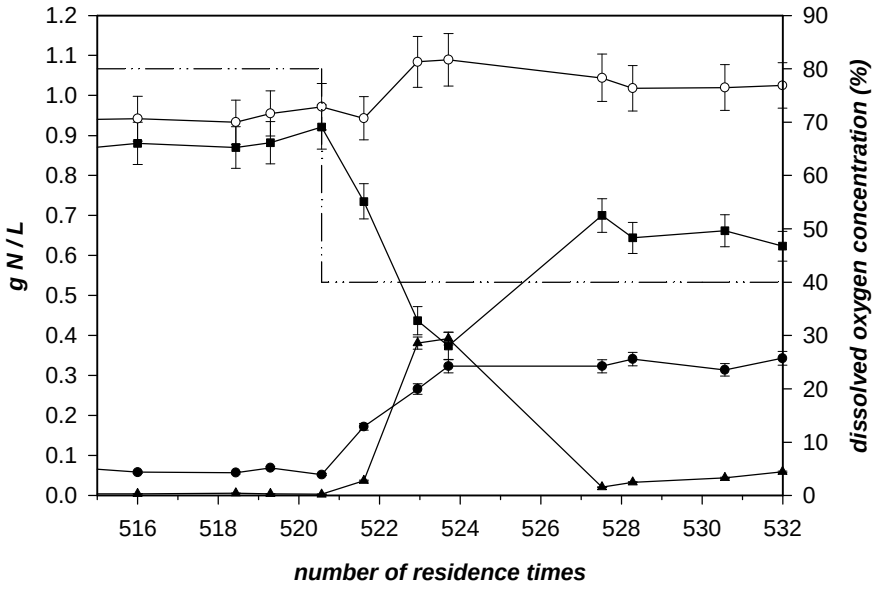

Figure 2.- Step perturbation of the dissolved oxygen from 80 to 40 %. Evolution of the concentration of ammonium (●), nitrite (▲), nitrate (■), and total nitrogen ('○', sum of ammonium, nitrite and nitrate concentrations). The dashed line is the dissolved oxygen concentration of the set point. The residence time employed is 10 h. The ammonium input concentration in all the experiments was 1.1 g N/L.

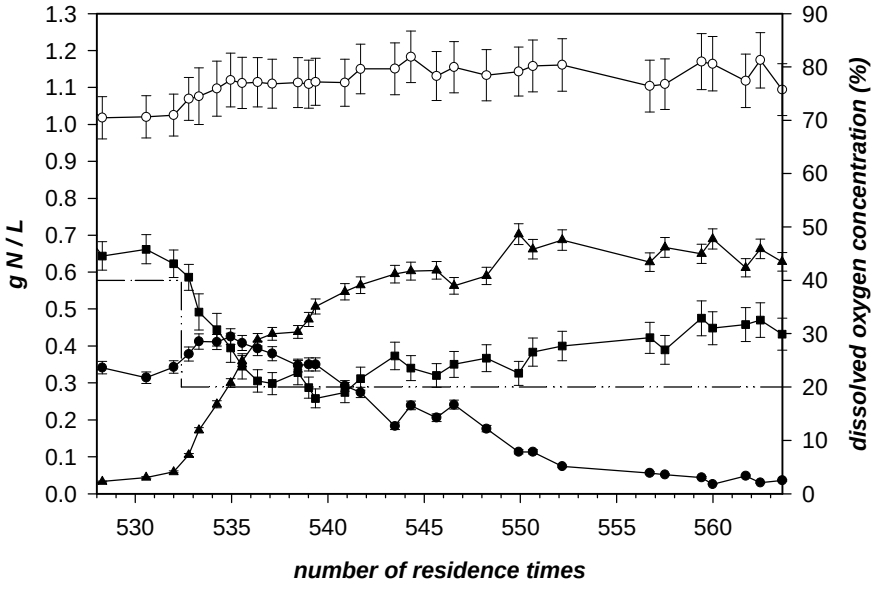

Figure 3.- Step perturbation of the dissolved oxygen from 40 to 20 %. Evolution of the concentration of ammonium (●), nitrite (▲), nitrate (■), and total nitrogen ('ↄ', sum of ammonium, nitrite and nitrate concentrations). The dashed line is the dissolved oxygen concentration of the set point. The residence time employed is 10 h. The ammonium input concentration in all the experiments was 1.1 g N/L.

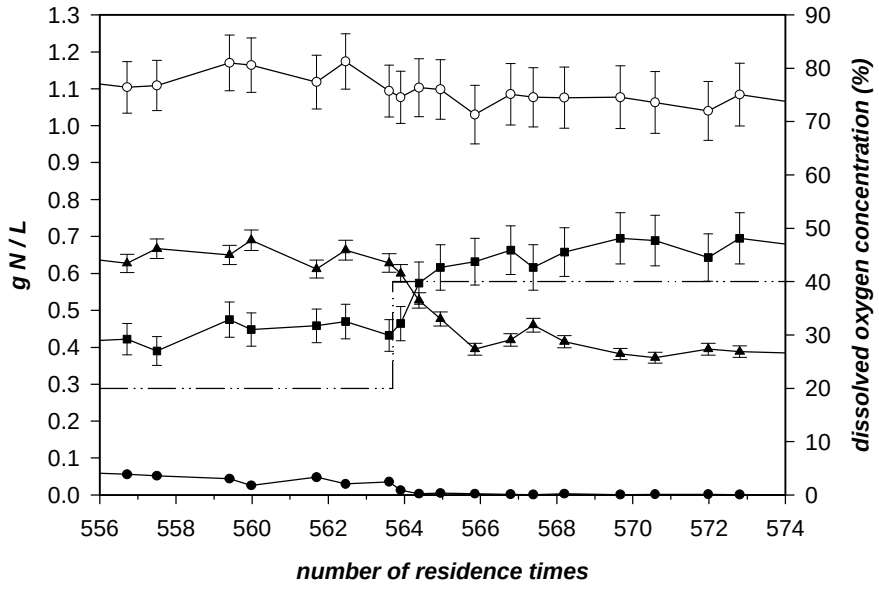

Figure 4.- Step perturbation of the dissolved oxygen from 20 to 40 %. Evolution of the concentration of ammonium (●), nitrite (▲), nitrate (■), and total nitrogen ('ↄ', sum of ammonium, nitrite and nitrate concentrations). The dashed line is the dissolved oxygen concentration of the set point. The residence time employed is 10 h. The ammonium input concentration in all the experiments was 1.1 g N/L.

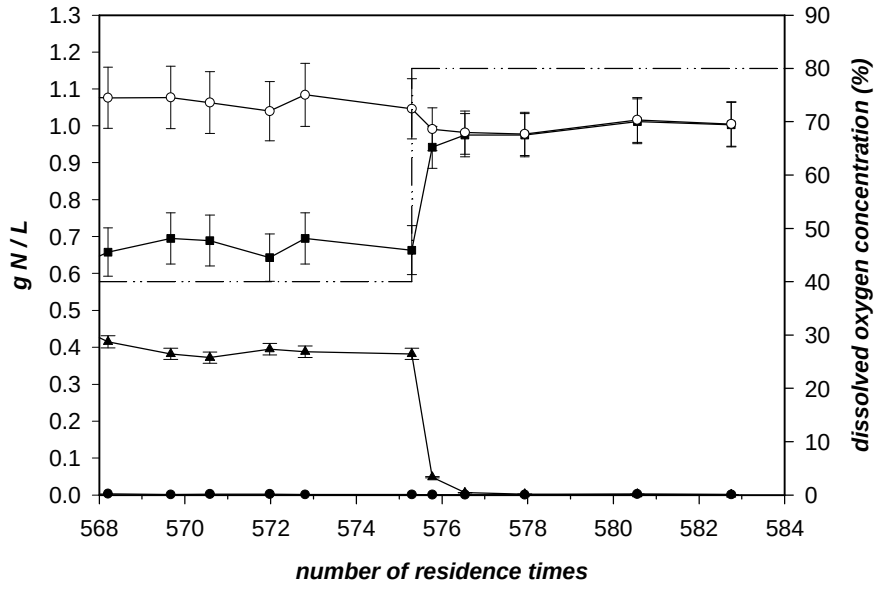

Figure 5.- Step perturbation of the dissolved oxygen from 40 to 80 %. Evolution of the concentration of ammonium (●), nitrite (▲), nitrate (■), and total nitrogen ('ↄ', sum of ammonium, nitrite and nitrate concentrations). The dashed line is the dissolved oxygen concentration of the set point. The residence time employed is 10 h. The ammonium input concentration in all the experiments was 1.1 g N/L.

Table 3.- Steady states in the different steps of dissolved oxygen concentration, the values of load are calculated per unit of total volume reactor. The ammonium input concentration in all the experiments was 1.1 g N/L, and the residence time used was 10 h.

Dissolved oxygen concentration (%)	Input ammonium load (kg N · m^{-3} · $día^{-1}$)	Output ammonium load (kg N · m^{-3} · $día^{-1}$)	Output nitrite load (kg N · m^{-3} · $día^{-1}$)	Ammonium removed load (%)	Conversion to nitrate (%)
40	1.18	$3.7 \cdot 10^{-1}$	$4.4 \cdot 10^{-2}$	68.5	64.8
20	1.18	$4.7 \cdot 10^{-2}$	$7.3 \cdot 10^{-1}$	96.0	34.3
40	1.18	$1.8 \cdot 10^{-3}$	$4.5 \cdot 10^{-1}$	99.8	61.6
80	1.18	$1.3 \cdot 10^{-3}$	$1.9 \cdot 10^{-3}$	99.9	99.7

Discussion

The partial nitrification to nitrite by manipulation of dissolved oxygen concentration has been described and studied by several authors. In the experiments carried out in the bibliography an increase in the nitrite concentration in the effluent is detected when a decrease of the dissolved oxygen is applied. Frequently the decrease in the dissolved oxygen concentration is an effect of a decrease in the superficial gas velocity (e.g. aeration flow-rate). This phenomenon has been studied in reactors with

immobilised biomass in different systems: moving bed (Nogueira *et al.*, 1998), gas-lift reactor (Garrido *et al.*, 1997), floating bed (Joo *et al.*, 2000).

During the experiments carried out in the present work, the partial nitrification to nitrite have been obtained by decreasing of the dissolved oxygen concentration, but in this case, manipulating the partial pressure of oxygen (because the gas flow-rate has been kept constant). Therefore, the decrease of the dissolved oxygen concentration has been achieved through variation of oxygen composition of the gas phase in the inlet flow to the bioreactor.

The partial nitrification to nitrite in free cells is produced because *Nitrosomonas europaea* has a higher oxygen affinity than *Nitrobacter winogradskyi* (Hendrikus *et al.*, 1993). Due to this fact, when competence is established in conditions in which the oxygen is limiting, an accumulation of nitrite (and not ammonium) is detected. In immobilised cells, nevertheless, the exact mechanism that produces the partial nitrification is unknown. It is still unclear if the partial nitrification is produced due to a spatial segregation of the two strains in the biofilm or equal than in free cells, the partial nitrification is due to different oxygen affinity of the two bacterial strains (Garrido *et al.* 1997).

In the experiments described by figures 2-5, it can be observed that the nitrite concentration in steady state increases when the dissolved oxygen concentration decreases. This happens in all the perturbations except in figure 2; in this experiment it can be observed an initial accumulation of both ammonium and nitrite in a transitory state. While in steady state, only the ammonium is maintained constant at a high value (0.330 g $N\text{-}NH_4^+$/L), and the nitrite is much more lower (0.039 g $N\text{-}NO_2^-$). This behaviour can not be attributed (at least totally) to inhibition of *Nitrosomonas* because the concentration of free ammonia do not attain (in the conditions of temperature and pH of the experiment) the values fixed by Anthonisen (1967). Therefore, in this case, the increase of ammonium in the effluent could be linked to the biofilm structure, concretely to the spatial distribution of the relative populations of *Nitrosomonas* and *Nitrobacter*.

During the first steady state (pseudo-steady) internal mass transfer in the biofilm restricts *Nitrosomomas* activity (due to its spatial distribution), despite its higher oxygen affinity. Nevertheless in these conditions *Nitrobacter* is inhibited by the free ammonia concentration (Anthonisen, 1976). If the nitrite concentration between residence times 528 to 532 (figure 2) is examined, it can be observed as this value has a little increase possibly due to inhibition. Therefore, the nitrite accumulation is determined not only by a different oxygen affinity of the strains, but also due to inhibition of *Nitrobacter* by free ammonia (the accumulation of ammonium is produced due to the spatial distribution in the biofilm, which determines that *Nitrosomomas* is limited by oxygen).

Therefore, in the concrete case of the biofilm formed in the bed studied, the partial nitrification to nitrite at low dissolved oxygen is produced due to two factors: the different oxygen affinity between the two strains, and on the other hand, the inhibition of *Nitrobacter* due to free ammonia concentration. Consequently, to attain in the studied conditions an effluent with a high nitrite content it has been necessary a previous stage in which *Nitrobacter* suffer inhibition by free ammonia due to the spatial distribution of the two strains in the biofilm.

On the other hand, in the experiment presented in figure 3 it can be observed a decrease of the ammonium concentration with time, during a very long transitory state (7.5 days). This behaviour may be caused due to two different situations: a phenomenon of adaptation of *Nitrosomonas*, influenced partially by *Nitrobacter* inhibition, or due to changes in the relative population and/or in the spatial distribution in the biofilm of the two strains. Up to now, it can cot be established neither which of the two situations is occurring, nor even whether a combined situation is proceeding.

After this situation, a new step to attain 40 % of dissolved oxygen was made (figure 4), and in this case, a “conventional” partial nitrification is observed, showing a hysteresis phenomenon if we compare with the results obtained in the same conditions during the first step (figure 2) are compared.

5.- Temperature effect

The effect of temperature on the nitrification process has been widely studied in wastewater treatment. The optimal temperature of the nitrifying process is in the range 28-30 ºC (Leenen *et al*., 1997; Polanco *et al*., 1994).

Although in the field of wastewater treatment the effect of temperature is a very important parameter, and its study is carried out in a wide range: 5 – 30 ºC, in the case of the MELISSA project, in which the bioreactors have an efficient temperature control, the range of temperatures studied has been narrowed.

While the reactor was operating at maximal ammonium load, perturbations in the temperature were carried out, determining the effect on ammonium, nitrite and nitrate concentrations in the effluent. The results obtained are shown in table 4 and figure 6.

Nitrosomonas europaea shows higher temperature sensitivity than *Nitrobacter winogradskyi* (Wijffels *et al.*, 1995), therefore, it can be observed a major ammonium accumulation (instead of a nitrite accumulation), when the temperature decreases.

On the other hand, Leenen *et al.* (1997), demonstrated that immobilised *Nitrobacter* cells have a reduced temperature sensitivity. If the obtained results are compared with the variation of the relative activity as a function of temperature in free cells (figure 7), it is observed a clear reduced temperature sensitivity of the immobilised cells. On the other hand, in figure 7 the results obtained in the pilot reactor are also compared with other references of immobilised cultures.

Table 4.- Decrease of the nitrification capacity as function of temperature. The relative activity has been determined from the total removed load at 30 ºC.

Temperature (ºC)	Output ammonium concentration (g N-NH_4^+/L)	Output nitrite concentration (g N-NO_2^-/L)	**Relative activity (%)**
30	0.019	0.002	**100**
28	0.032	0.001	**98**
24	0.039	0.002	**96**

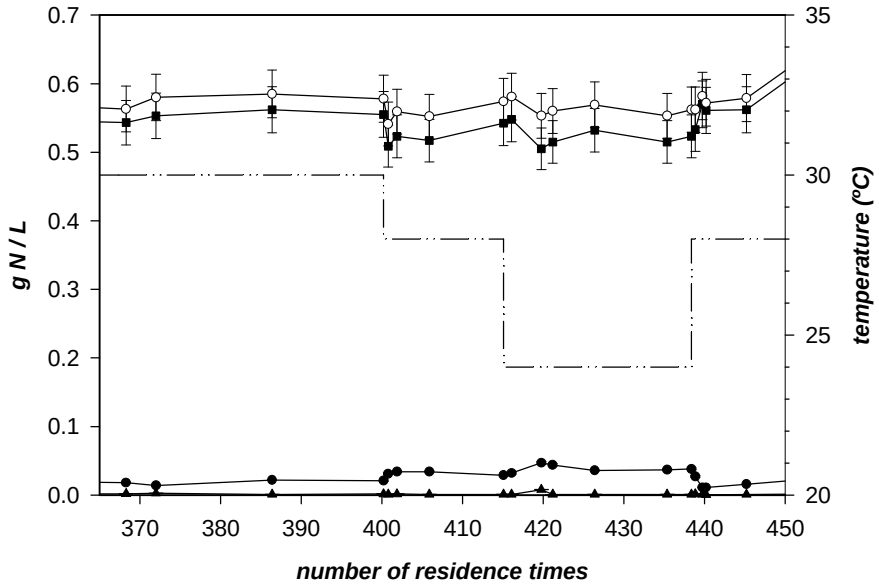

Figure 6.- Temperature step perturbations. Evolution of the concentration of ammonium (●), nitrite (▲), nitrate (■), and total nitrogen ('ↄ', sum of ammonium, nitrite and nitrate concentrations). The dashed line is the temperature of the reactor. The residence time employed is 5 h.

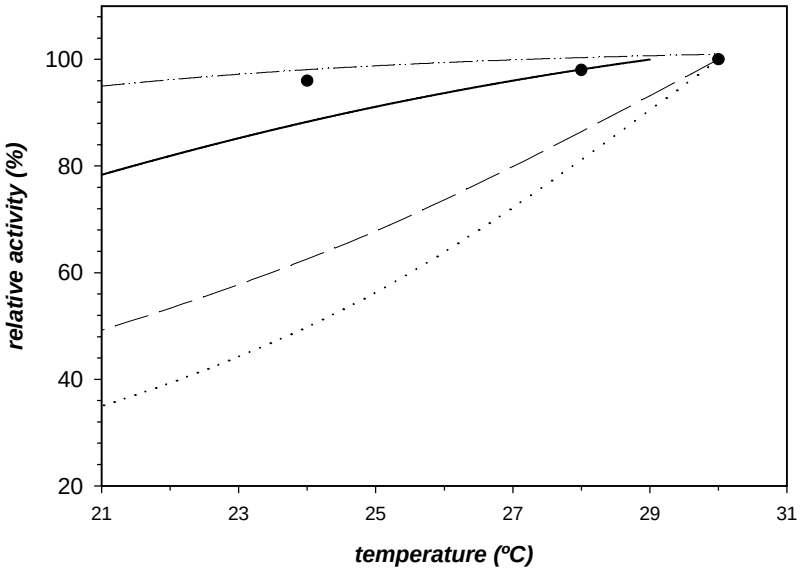

Figure 7.- Effect of temperature in the removed load (relative activity to 30 ºC). The simbols '●' are the experimental points. Solid line: temperature effect in an up-flow aerated biological filter with a support of clay particles (Polanco et al., 1994). Dashed and dotted line: temperature effect of *Nitrobacter agilis* immobilised as carrageenan beads (Leenen et al., 1997). Dashed line: temperature effect in a free cell culture of *Nitrobacter agilis* (Wijffels *et al.*, 1995). Dotted line: temperature effect of a free cell culture *Nitrosomonas europaea* (Wijffels et al., 1995).

6.- Conclusions

The experiments carried out with the pilot reactor investigating the effects of nitrification with reduced dissolved oxygen have revealed the importance of the internal structure of the biofilm. This fact acquires a major relevance in the case of the transitory states, in which the nitrite and ammonium accumulations are important. For this reason a simple modelization of the problem, which does not bear in mind the internal mass transfer limitations and the distribution and relative concentration of the two bacterial strains in the biofilm will not successfully predict these effects.

On the other hand, the effect of temperature in the removal efficiency has shown a clear reduced sensitivity of immobilised bacteria respect to free cells, what makes the compartment III of MELISSA more robust with respect to potential temperature fluctuations.

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