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Operation of the bench nitrifying reactors

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1.- Introduction

The compartment III of the MELISSA loop (nitrifying compartment) consists of a packed-bed reactor with cells of two bacteria strains (*Nitrosomonas europaea* and *Nitrobacter winogradskyi*) immobilised on polystyrene beads (Biostyr), with the objective to transform the ammonium ions present in the exit stream from compartment II into nitrate, a nitrogen source more suitable for the cells cultured in compartment IV (*Spirulina platensis*).

As discussed previously (TN 25.6), it was decided to set-up three bench scale reactors, in addition to the pilot scale packed reactor, in order to generate more results to fully characterise this system. The detailed design, sterilisation procedure and start-up of these columns was presented already in TN 37.510.

Two of these columns have been in continuous operation for a period of six months, the third one not being operated yet to allow to carry out experiments of physical characterisation. In these two columns different tests related to the overall performance of the nitrifying system have been conducted.

A number of environmental factors influence the nitrification, among others, the substrate concentration, temperature, oxygen, pH and toxic substances. The main objectives for this workpackage were to investigate the effects of ammonium load in terms of concentration and flow-rate on the ammonium conversion. In order to study and describe quantitatively these effects, the NH_4^+, NO_2^- and NO_3^- were measured periodically. Among other variables studied, the aeration flow-rate was also studied allowing to obtain different steady states when increasing or decreasing the aeration. The results obtained during this period are discussed in the present report.

2.- Phases and operational conditions of the experiments.

In both columns, the operation was carried out in two phases. In the first phase, after sterilisation and placement of the beads (according to the protocol discussed in TN 37.510), the columns were inoculated with 150 mL of a co-culture of *Nitrosomonas europaea* and *Nitrobacter winogradskyi*, and operated batchwise, with complete recirculation of the liquid medium (8 ml/min). This phase was necessary to allow cell attachment onto the surface of the beads, by formation of biofilms.

The concentrations of NH_4^+, NO_2^-, NO_3^- were measured during this period (the analysis methods used for these determinations are described in the appendix at the end of the present technical note), that required about 100 days. After that, continuous operation was started up. Different flow-rates of increasing magnitude were used until the outlet liquid stream from the columns appeared without an important amount of free cells. At that time it was considered that the biofilm formation had been achieved, as the ammonium conversion was quite complete (95 - 99 %) (externally, the beads had changed their colour, thus showing that cell attachment had occurred).

Since that point, the operation conditions of the columns were fixed, and the concentration changes followed until a steady-state was achieved. The two columns have been operated at different conditions, presented in **table 1**. The variables that were studied (and will be followed in the next phase of the operation of these columns) are : feed flow-rate (thus, dilution rate), feed ammonia concentration and aeration flow-rate.

Other variables that will also be studied include recirculation rate (thus, internal mixing) and dissolved oxygen concentration. In all cases, the physical conditions used in both reactors are given in **table 2**.

Column	Input medium concentration (g N-NH_4^+ / L)	Dilution rate (h^{-1})	Aeration flow-rate (mL / min)	Ammonium load (g / (L·h))
1	0.300	0.075	9	0.026
2	0.300	0.075	15	0.026
1	0.400	0.075	9	0.030
2	0.300	0.100	40	0.030

Table 1.- Operation conditions of the bench columns.

VARIABLE	VALUE
Temperature	30 °C
pH	8.1
Recirculation flow-rate	8 ml/min
Stirring	300 r.p.m.
Light conditions	darkness

Table 2.- Culture conditions of the bench columns.

3.- Experimental results

The evolution of the concentrations of NH_4^+, NO_2^-, NO_3^-, at the different conditions of dilution rate, aeration flow-rate, and input medium concentration according to table 1, are given in **figure 1**, **table 3** for column 1 and **figure 2** and **table 4** for column 2.

In table 3 and 4 the experimental concentration of the measured compounds are given with respect to the day of operation of the reactor, while in figure 1 and 2 the results are given with respect to the number of residence times in the continuous operation of the reactor.

In both figures, θ_1 and θ_2 correspond to the transition in the reactors operation from batch to continuous, as it was mentioned before the residence time was gradually increased until a clean effluent free of cells was obtained from the columns. θ_3 in figure 1 and θ_3 , θ_4 in figure 2 are the final residence times at which continuous operation has been

kept for a reasonable long operational period to characterise the operation of the column and its stability.

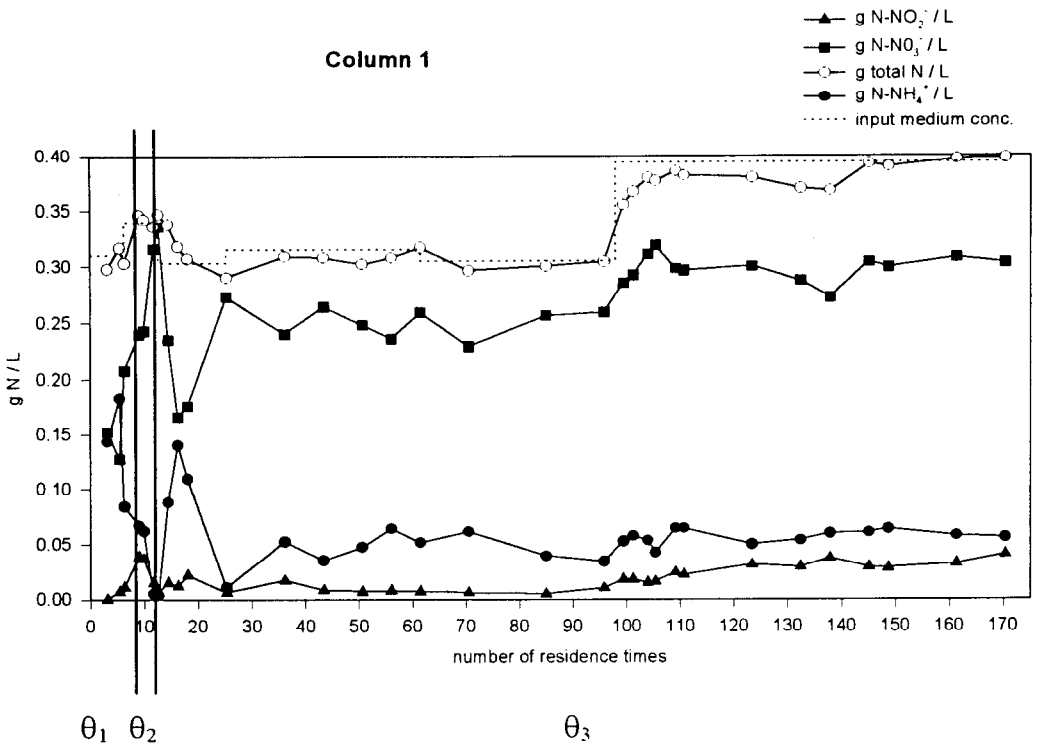

Figure 1.- Evolution of the ammonium, nitrite and nitrate concentrations in the column 1.The different conditions of every steady state are detailed in the table 1. $\theta_1 = 53$ h ;$\theta_2 = 27$ h ;$\theta_3 = 12$ h.

It can be observed that a certain degree of fluctuation in the measured concentration is taking place during the continuous operation of the reactor at fixed conditions.

These variations are due basically to two reasons. First, a certain variation in the liquid and aeration flow-rates in the columns, mainly due to changes in their pressure drop, is almost unavoidable. Second, the biofilm around the solid support particles never reaches a complete stationary state, but a pseudo-stationary state : some cells will detached from the support, as some new cell will grow and attached to it. A physical evidence of this process is given in **figure 3**, were a microscopic view of fractions of detached biofilm is shown.

Time (days)	g $N\text{-}NH_4^+$ / L	g $N\text{-}NO_2^-$ / L	g $N\text{-}NO_3^-$ / L	g total N / L *
0	0.143	0.000	0.152	0.298
5	0.183	0.007	0.127	0.317
7	0.084	0.011	0.208	0.303
10	0.067	0.039	0.240	0.346
11	0.062	0.037	0.243	0.342
13	0.005	0.015	0.316	0.336
17	0.004	0.007	0.336	0.347
19	0.088	0.015	0.235	0.338
20	0.140	0.012	0.166	0.318
21	0.109	0.022	0.176	0.307
24	0.011	0.006	0.273	0.290
30	0.052	0.017	0.240	0.309
34	0.035	0.009	0.264	0.308
38	0.047	0.007	0.248	0.302
41	0.064	0.008	0.236	0.308
47	0.051	0.007	0.259	0.317
52	0.061	0.006	0.229	0.296
60	0.039	0.005	0.256	0.300
66	0.034	0.010	0.259	0.304
68	0.052	0.018	0.285	0.355
69	0.057	0.018	0.292	0.367
70	0.053	0.015	0.311	0.380
71	0.042	0.016	0.319	0.377
73	0.064	0.024	0.298	0.386
74	0.064	0.022	0.296	0.382
81	0.049	0.031	0.300	0.380
86	0.053	0.029	0.287	0.370
89	0.059	0.037	0.272	0.368
93	0.060	0.029	0.304	0.393
95	0.063	0.028	0.299	0.390
102	0.057	0.032	0.308	0.397
107	0.055	0.040	0.303	0.398

Table 3.- Evolution of the ammonium, nitrite and nitrate concentrations in the column 1. *g total N/L = g $N\text{-}NH_4^+$/L + g $N\text{-}NO_2^-$/L + g $N\text{-}NO_3^-$/L.

In general, the nitrogen mass balance is accomplished taking into account the precision of the analytical methods used in the determination of ammonium, nitrite and nitrate.

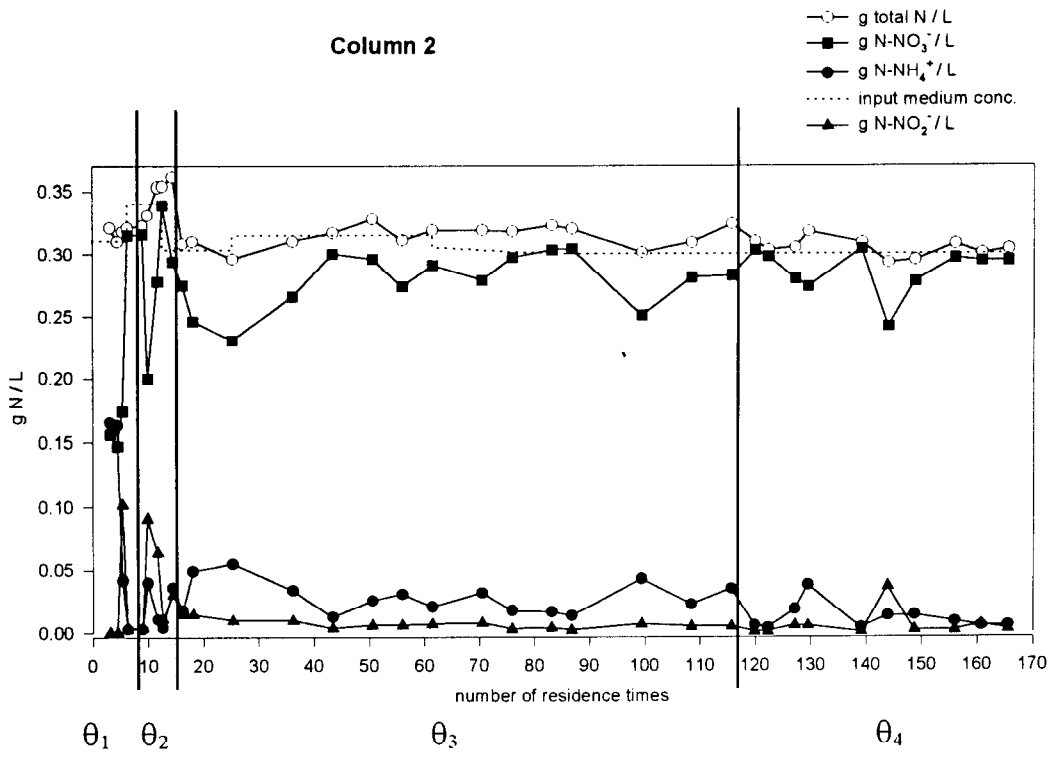

Figure 2.- Evolution of the ammonium, nitrite and nitrate concentrations in the column 2.The different conditions of every steady state are detailed in the table 1.
$\theta_1 = 53$ h ; $\theta_2 = 27$ h ; $\theta_3 = 12$ h ; $\theta_4 = 10$ h.

The nitrification process is performed by two autotrophic microorganisms. The process takes place in two steps as ammonium is oxidised to nitrite by *Nitrosomonas.* Subsequently nitrite is oxidised to nitrate by *Nitrobacter.*

The process for the ammonium oxidising bacteria (*Nitrosomonas*) is :

$$NH_4^+ + 3/2\ O_2 \text{ ----------> } NO_2^- + H_2O + 2\ H^+$$

The process for the nitrite oxidising bacteria (*Nitrobacter*) is :

$$NO_2^- + 1/2\ O_2 \text{ ----------> } NO_3^-$$

Time (days)	g $N\text{-}NH_4^+$ / L	g $N\text{-}NO_2^-$ / L	g $N\text{-}NO_3^-$ / L	g total N / L *
0	0.165	0.000	0.156	0.321
3	0.163	0.000	0.147	0.310
5	0.042	0.102	0.174	0.318
7	0.003	0.003	0.315	0.321
10	0.003	0.004	0.316	0.323
11	0.040	0.091	0.200	0.331
13	0.011	0.064	0.278	0.353
17	0.004	0.011	0.339	0.354
19	0.036	0.031	0.294	0.361
20	0.018	0.015	0.275	0.308
21	0.049	0.015	0.246	0.310
25	0.055	0.010	0.231	0.296
31	0.034	0.010	0.266	0.310
35	0.013	0.004	0.300	0.317
39	0.026	0.006	0.296	0.328
42	0.031	0.006	0.274	0.311
48	0.021	0.007	0.291	0.319
53	0.032	0.008	0.279	0.319
56	0.018	0.003	0.297	0.318
60	0.017	0.004	0.303	0.323
62	0.014	0.002	0.304	0.320
69	0.043	0.007	0.251	0.301
74	0.023	0.005	0.281	0.309
78	0.035	0.005	0.283	0.324
80	0.006	0.001	0.303	0.310
81	0.004	0.001	0.298	0.303
83	0.019	0.006	0.280	0.304
84	0.038	0.006	0.274	0.318
88	0.004	0.001	0.304	0.309
90	0.014	0.037	0.242	0.293
92	0.014	0.002	0.278	0.295
95	0.009	0.002	0.297	0.308
97	0.006	0.007	0.294	0.301
99	0.006	0.003	0.295	0.304

Table 4.- Evolution of the ammonium, nitrite and nitrate concentrations in the column 2. *g total N/L = g $N\text{-}NH_4^+$/L + g $N\text{-}NO_2^-$/L + g $N\text{-}NO_3^-$/L.

The nitrifying bacteria are a homogenous group of bacteria, characterised for example by a low growth rate, as stated in the start-up procedure. This is due to the low energy yield which is linked to the oxidation of ammonium and nitrite, respectively. The slow growth is a major problem, to conduct nitrification processes with free cell cultures, but not the

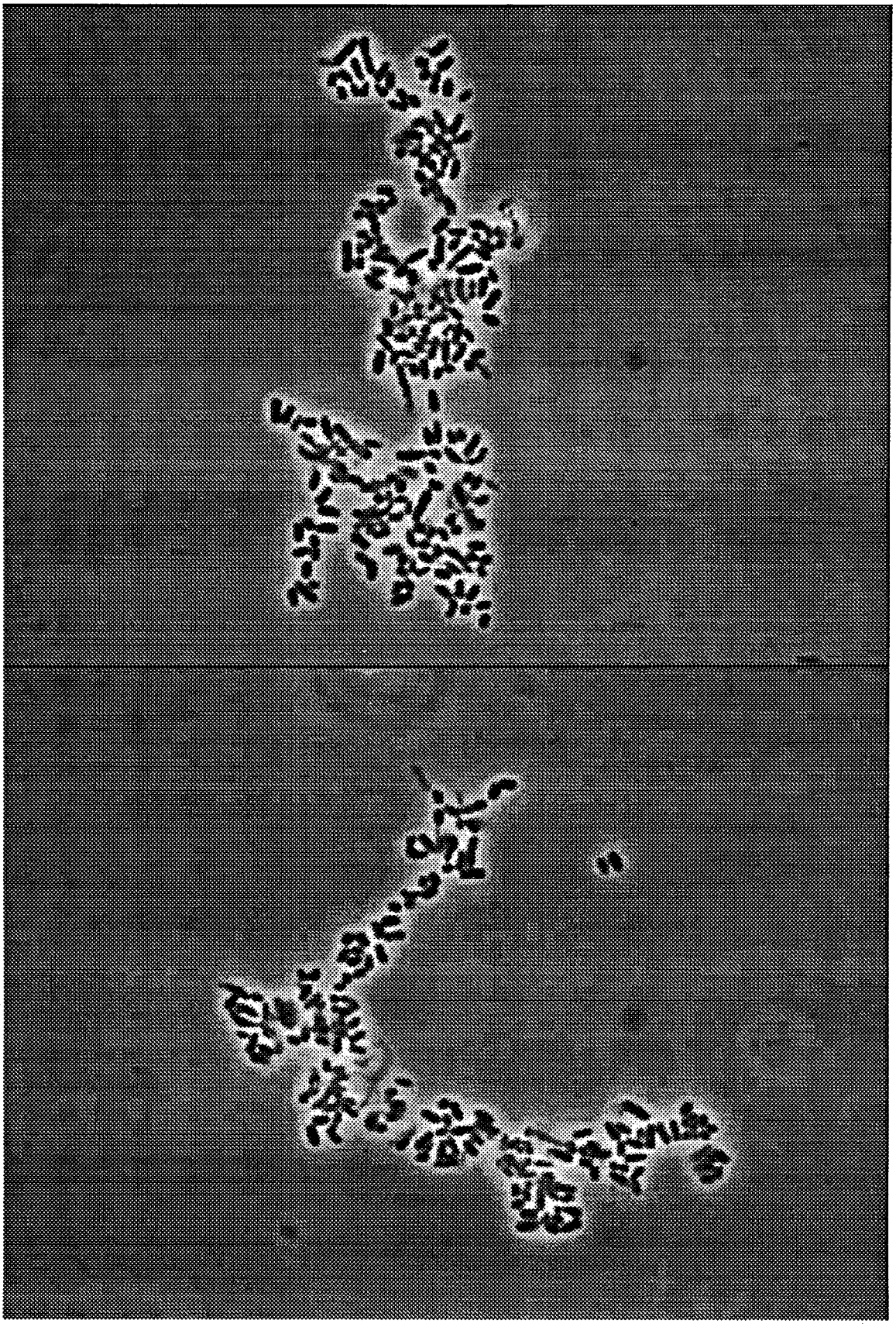

Figure 2.- Detachment of fragments of biofilm from the solid support of the bench columns.

only one. With fixed biomass cultures, problems such as substrate difussion (carbon, nitrogen and oxygen), toxic inhibition, or even substrate inhibition can be important bottlenecks once the biofilm proliferation have been reached.

It is important to note that in practice it is the oxidation of ammonium which is the rate limiting step in the overall process. This means that nitrite will only appear in large amounts when the considered process is non-stationary, for example because of varying loads, start-up and washout, or other operational problems in the biological reactor. In this sense, in figure 1 it can observed a certain increase in the NO_2^- concentration when changing the ammonium load that produces a non-stationary situation. Similarly, in the start-up procedure from batch culture to continuous culture an increase in NO_2^- is also present in the bioreaction system.

Moreover, the nitrification bacteria are more sensitive to low oxygen concentrations than the heterotrophic bacteria. The kinetics can be described using a Monod expression. Difussional limitation is an essential feature in packed-bed bioreactors, and hence the conditions in the system are determined by biofilm thickness, substrate load conditions, temperature, etc. In figure 1 can be observed that if the ammonium load is increased, but maintaining the same air flow-rate, that is, producing a decrease in dissolved oxygen, the consequence is a higher concentration of NO_2^- at the steady state conditions. Taking into account that most nitrifying bacteria are autotrophic and thus use carbon dioxide as the carbon source, this C-source should be reduced before the carbon can form part of the cell mass, and this reduction takes place through the oxidation of the nitrogen source of the organism concerned. Thus, considering the oxidation of the ammonia and nitrite and their contribution to growth, an overall equation of reaction for nitrification can be found as follows :

$$NH_4^+ + 1.86\ O_2 + 1.98\ HCO_3^- \rightarrow 0.020\ C_5H_7NO_2 + 0.98\ NO_3^- + 1.88\ H_2CO_3 + 1.04\ H_2O$$

This expression shows that when total conversion is obtained with enough dissolved oxygen present in the system, a nitrogen balance only based on dissolved nitrogen compounds can be closed at a maximum value of 98%. This value in addition

with the precision of the analyses must be the starting point to discuss any result concerning to the accomplishment of the nitrogen balance. It is necessary to take into account that the biofilm is a biological system in pseudo-stationary state, and so, certain growth of biomass is happening, specially when perturbations in the system are produced.

In figure 2 and table 4 NH_4^+, NO_2^-, NO_3^- concentration evolution at the different conditions of dilution rates, aeration flow-rate and input medium concentration for the column 2 are presented. The general behaviour is very similar to the observed for column 1. A high conversion on ammonium were observed not having high concentration of nitrite and with a high level of nitrogen balance accomplishment.

The air flow-rate applied in this second column were higher than in the first one. As a consequence, lower levels of nitrite are observed according to the concepts about nitrification processes described above. In column 1 the air flow-rate for the two different ammonium loads tested were 9 ml/min. Thus, an increase in the NO_2^- concentration was observed when increasing the ammonium load. On the contrary for the column 2, when the load was increased the air flow-rate was changed from 15 ml/min to 40 ml/min. Therefore, no significant variations on NO_2^- concentration appeared.

As a summary, in order to compare the overall performance of the two columns, and to dump the oscillations observed in the nitrogen concentrations, averaged values of NH_4^+, NO_2^-, NO_3^- concentrations at the different steady states reached in the experiments are presented in **table 5** and **figure 4**. Moreover, to obtain additional information from the degradation produced in the nitrification process, the conversion of ammonium have been plotted versus ammonium load and aeration flow rate (**figure 5**).

In general ,the slight differences observed between the total nitrogen measured and the expected by the ammonium concentration supplied at the inlet stream can be explained by the analysis error in the determination of the NH_4^+, NO_2^- and NO_3^- concentrations. These errors can be estimated in the order of 5% -10%. However, there

are some cases in which perturbations on ammonium load, temperature, aeration, etc. affects in the overall nitrogen balance producing their unaccomplishment.

Steady states	Ammonium (g N-NH_4^+/L)	Nitrite (g N-NO_2^-/L)	Nitrate (g N-NO_3^-/L)	Total Nitrogen (g N /L)	Deviation Total Nitrogen (%)
Column 1 0.026 g/(L·h) 0.75 h^{-1} 9 ml air/min	0.044 (± 0.010)	0.008 (± 0.002)	0.251 (±0.010)	0.303 (±0.022)	+1.0
Column 1 0.030 g/(L·h) 0.75 h^{-1} 9 ml air/min	0.058 (± 0.003)	0.030 (± 0.004)	0.296 (± 0.007)	0.384 (± 0.014)	-4.0
Column 2 0.026 g/(L·h) 0.75 h^{-1} 15 ml air/min	0.025 (± 0.006)	0.005 (± 0.003)	0.287 (± 0.010)	0.317 (± 0.019)	+5.7
Column 2 0.030 g/(L·h) 0.100 h^{-1} 40 ml air/min	0.013 (± 0.007)	0.007 (± 0.007)	0.285 (± 0.010)	0.305 (± 0.024)	+1.7

Table 5.- Averaged values of ammonium, nitrite and nitrate concentrations in the different steady states described in table 1.

Another fact that may be responsible that the nitrogen balance (nitrogen soluble compounds) is not accomplished can be the possible denitrification developed in the system, generating gaseous nitrogen species.

By denitrification, microorganisms convert nitrate into atmospheric nitrogen. The process is anaerobic as nitrate is the oxidising agent. When nitrate is the oxidising agent, the process is called anoxic. It occurs where nitrate is present, provided that no oxygen (or not too much) is present at the same time. Most denitrifying

microorganisms are facultative and hence they use oxygen as an oxidising agent, provided it is present. Many of the commonest bacteria (including *Nitrosomonas*) have the ability to change their metabolism from using oxygen as the final electron acceptor, to using nitrate instead. The intermediate products by denitrification from nitrate are as follows :

$$NO_3^- \text{------}> \quad NO_2^- \text{----------}> \quad NO \text{----------}> N_2O \text{----------}> \quad N_2$$

Denitrification process I.

In addition, there have been presented evidence that N_2O was produced by *Nitrosomonas europaea* from unstable intermediates in ammonia oxidation and by nitrite reduction(denitrification). A kinetic analysis of ^{15}N and ^{14}N incorporation into N_2O produced from 99% $^{15}NO_2$ and 99% $^{14}NH_4^+$ demonstrated that N_2O production by nitrifiers was consistent with the hypothesis that denitrification was the sole mechanism of production under the conditions used (Poth and Focht, 1985, Poth, 1986). Given the certain chemolithotrophic nitrifiers are able to denitrifie (i.e., reduce nitrogen oxides to gaseous products), then the possibility exists that some nitrifiers are able to reduce nitrite completely to nitrogen gas the final product in the dinitrification process (Knowles, 1982 ,Payne.1981).

$$NO_2^- \text{----------}> \quad N_2O \text{----------}> \quad N_2$$ Denitrification process II

Studies of the kinetics of N_2O formation by *Nitrosomonas europaea* agrees with denitrification as the mechanism of N_2O production (Poth and Focht, 1985) and that it is nitrite reduction and not ammonia oxidation directly that account for the production of gaseous N species by nitrifiers. If nitrifiers do denitrify, then the reduction of NO_2- should be coupled to energy generation (electron transport). Nitrifiers can not be grown under anaerobic denitrifying conditions with either NH_4^+ (O_2 is required for the initial oxidation to NH_2OH) or NH_2OH, because of inhibitory effects which prevent even aerobic growth on this substrate. The definitive anaerobic, denitrifying growth of nitrifiers might await the advent of a suitable additional electron donor.

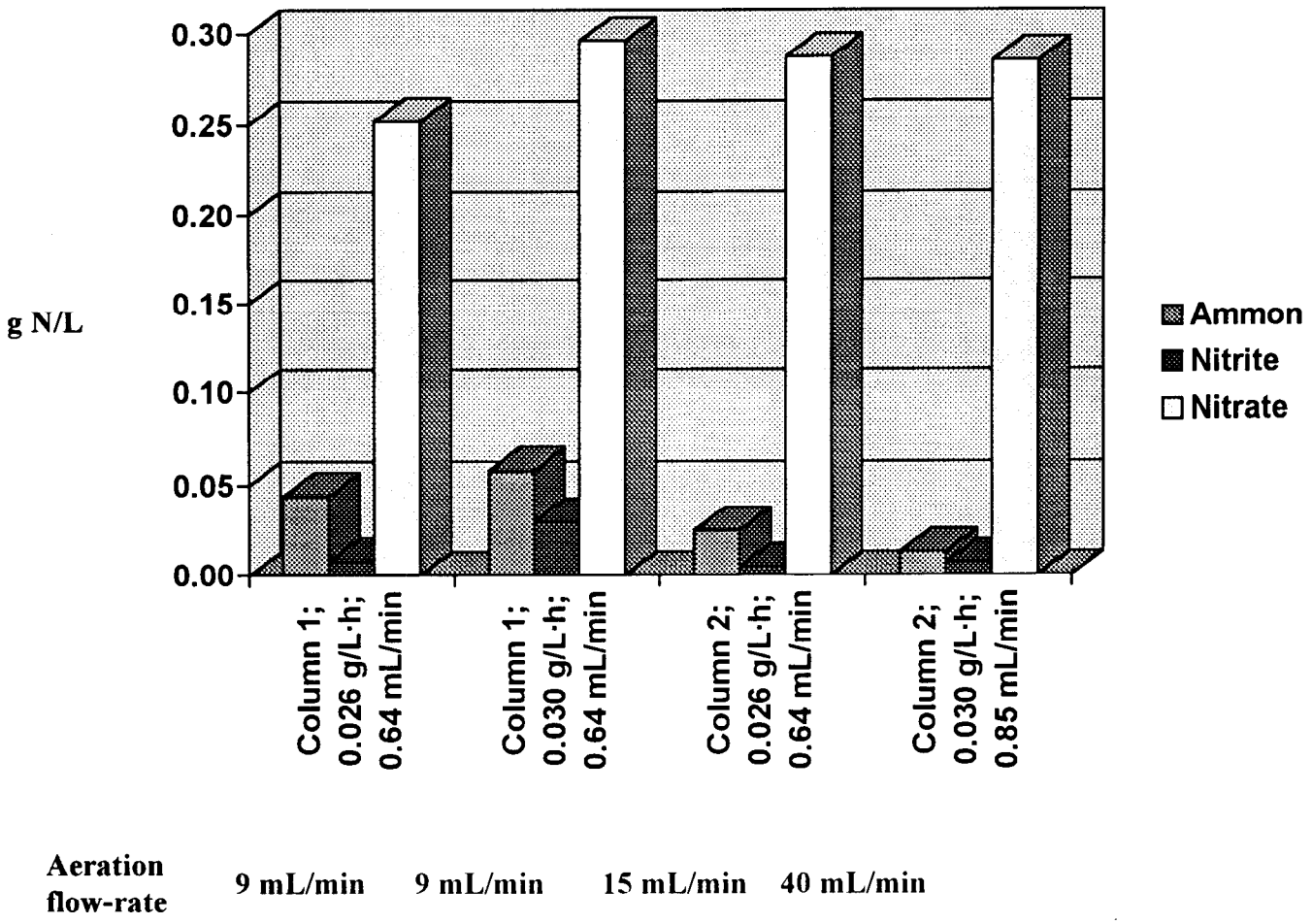

Figure 4.- Averaged values of ammonium, nitrite and nitrate concentrations at the different steady states described in Table 1.

In reference to the second step of nitrification, that is, nitrite conversion into nitrate, from the table 5 and figure 4, it can be stated that when increasing the ammonium load without enough dissolved oxygen, an increase of nitrite is obtained due to oxygen limitation as can be seen for column 1. If an increase of ammonium load is made jointly with the increase of aeration flow-rate the nitrite concentration does not change substantially.

From the results showed in figure 5 it can be concluded that the limiting substrate for *Nitrosomonas* can be ammonia or oxygen and for *Nitrobacter* nitrite or oxygen. It is clearly shown by the fact that the ammonium conversion for a fixed ammonium load depends on the aeration flow-rate, that is to say, it seems that the higher the aeration

flow-rate is, the higher the conversion attained. This fact has been widely supported by many authors in the open literature (Becari et al., 1992 ; Jayamohan et al., 1988 ; Hunik et al., 1993). However, there are some publications referring to oxygen toxicity at high concentration (Zeghal and Benyahina, 1993 ; Prosser, 1989). Thus, it might be very important to bear in mind this possible effect and their operational consequences. It must be taken into account the possibility to carry out further studies, if necessary, in order to investigate this phenomenon.

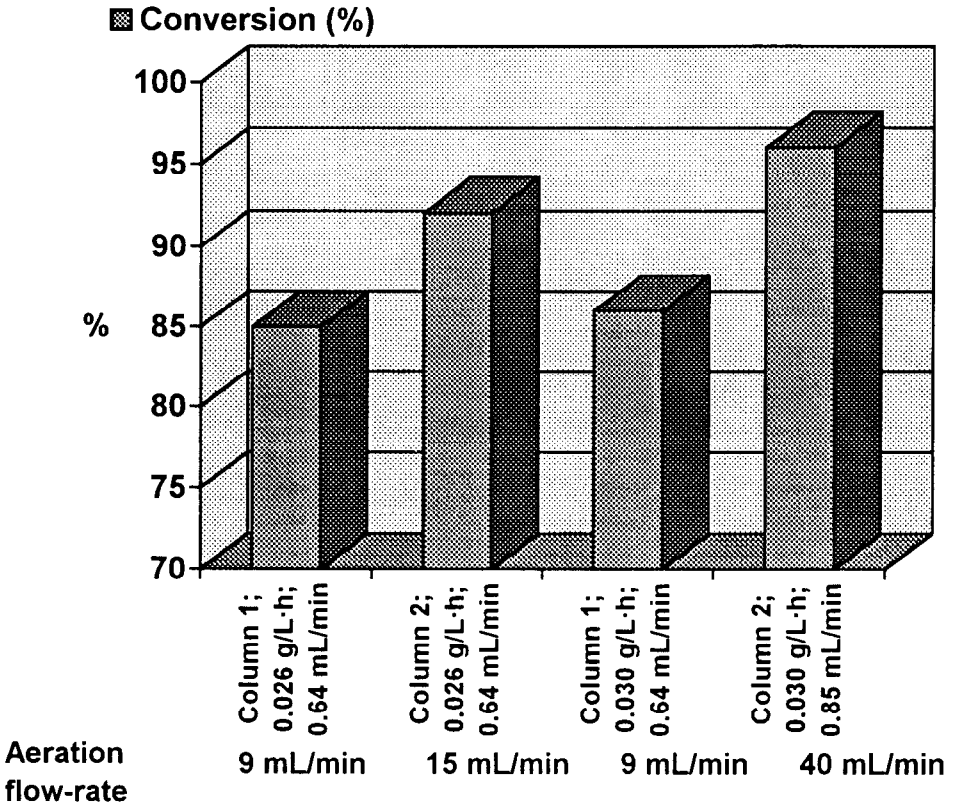

Figure 5.- Conversion (%) of ammonium at different ammonium loads and aeration flow-rates.

The external aspect of the two packed-bed bench columns is presented in **figures 6** and **7**. Regarding the appearance of the two columns, the characteristic colour of the biofilm developed by these cultures can be observed with an uniform distribution along the total height of the bed, this fact allowing to presume a good mixing in the liquid phase. Another additional observation is that the cells also attach onto the walls of the glass column, and on the internal surfaces of silicone tubing.

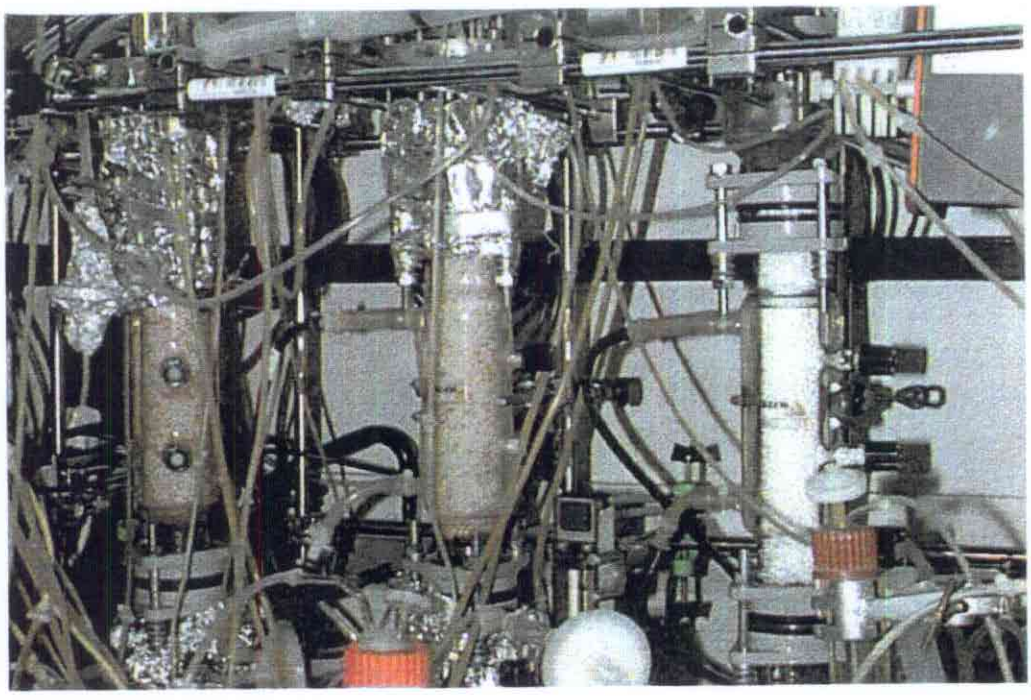

Figure 6.- General view of the three bench packed-bed reactors. The non-inocculated column is placed on the right of the figure.

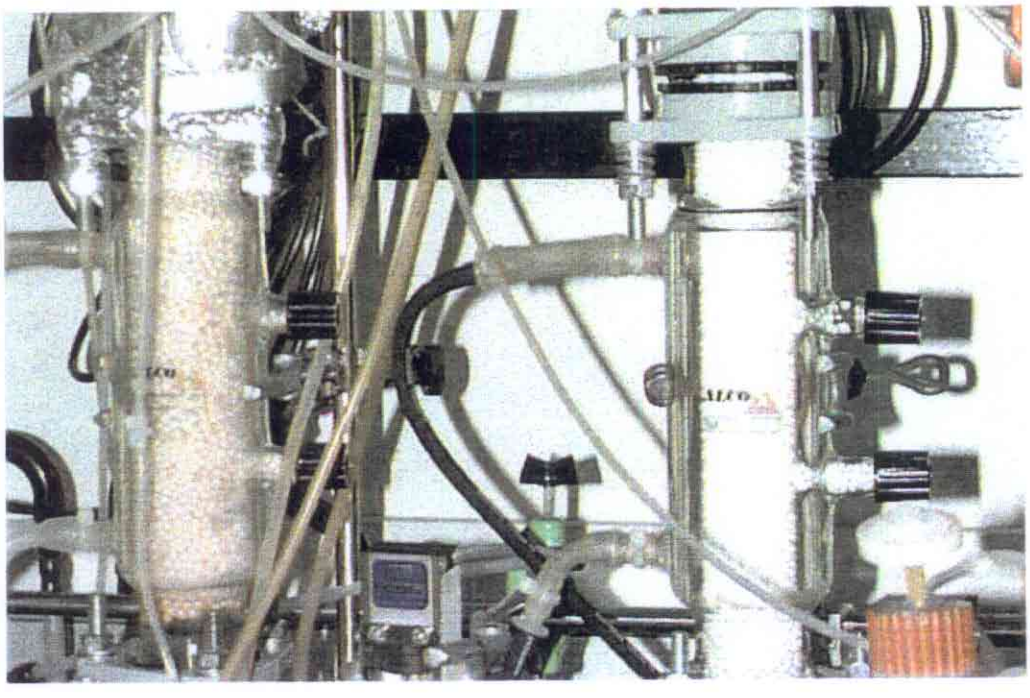

Figure 7.- Detailed comparison between a inoculated (left) and void (right) packed-bed column.

4.- Conclusions and future work.

The bench columns have been running during a period of about 8 months, since the inoculation. After more than three months of continuous operation the results obtained allow to affirm the bench packed-beds are able to carry out the nitrification process satisfactorily.

However, it is necessary to perform additional experiments in order to improve the knowledge of the system about its response on different changes of ammonia load, studying both transient and stationary states. It has been observed that the conversion attained is relatively high, but the new stationary states reached after changing the feeding flow rate or even ammonium concentration are sometimes very large in terms of number of residence times (10-20). This situation is probably due to phenomena involved with biofilm proliferation transients and/or the fact the biomass have to become accustomed to the new operating conditions.

On the other hand, the level of dissolved oxygen and the degree of mixing seems to be responsible of different stationary states and transient effects as in the case of ammonium load. This is an interesting aspect to be investigated in a future work in the bench columns. The proposal is to conduct additional experiments in order to study deeply the effect of mixing and dissolved oxygen on the general behaviour of the coculture in bench scale packed-bed reactors.

To maintain a constant aeration flow-rate during the experiments will become necessary to improve the installation of the gas loop, introducing flow mass controllers for every bioreactor, to have a better accuracy, precision and stability in these conditions.

Finally, effect of organic carbon sources on the evolution of nitrite, nitrate and ammonium concentrations may be studied for constant ammonium load and operating conditions (temperature, pH, dissolved oxygen, recirculation ratio, gas flow-rate, etc.)

5.- Appendix.

In this appendix the analysis methods used to determine the concentration of ammonium, nitrite and nitrate are described. The two principal methods used to analyse the concentration of the ions NH_4^+, NO_2^- and NO_3^- have been : capillary electrophoresis (Waters Capillary Ion Analyser) and colorimetric methods (LCK kits : 304 ammonium, 341 nitrite and 339 nitrate ; Dr. Lange).

The results obtained using these two methods have been compared and its precision is very similar. The fact of having two alternative measurements methods is judged important to assure the possibility to carry out the analysis during the long operation of the nitrifying bioreactors.

Description of the analysis methods

a.- Ammonium analysis (NH_4^+)

a.1.- Capillary electrophoresis (Waters Capillary Ion Analyser), 0.5-25 ppm N-NH_4^+:

Capillary electrophoresis works as follows :

- A sample is injected into the capillary, which is filled with electrolyte.
- Voltage is applied across the capillary, causing a differential migration of the charged sample components toward the respective electrode.
- Depending on the polarity of the power supply and the electrolyte composition, the separated analytes pass through the detector.

Capillary ion analysis analyses small molecular weight ions by directing analytes and osmotic flow toward the detector. Cation analysis require a positive power supply, with osmotic flow controlled by pH. Anion analysis requires a negative power supply and an osmotic flow modifier that is added to the electrolyte.

The analysis conditions to determine the ammonium concentration with this method are specified in **table A.1.**

Instrument	Capillary Ion Analyser
Electrolyte	UV-Cat 2, Tropolone, 18-crown-6 *
Capillary	75 μm x 60 cm fused silica
Power supply	Positive voltage
Applied voltage	20 KV
Current	12 μA
Injection mode	Hydrostatic, 10 cm height for 30 seconds
Detection	UV at 185 nm (Hg lamp)
Temperature	20 °C
Data System	Millenium 2010 Chromatography Manager
Sample Rate	20 points / second
Auto Purge	2.0 minutes

Table A.1.- Analytical conditions to determine the ammonium concentration, elimination the interference of the potassium cation. (* The composition of the electrolyte is 30 mg of Tropolone, 52 mg of 18-crown-6 and 60 mg of UV-Cat 2 per 100 ml of Milli-Q water).

a.2.- Colorimetric method (LCK kit 304, Dr. Lange, analysis range 0.015 - 2 ppm)

Principle : ammonium ions react at pH 12.6 with hypoclorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenol blue. The measure of absorbance is made with the spectrophotometer CADAS 100 (Dr. Lange), at a wavelength of 694 nm.

b.- Nitrate analysis (NO_3^-)

b.1.- Capillary electrophoresis (Waters Capillary Ion Analyser), 0.5-50 ppm $N\text{-}NO_3^-$:

In the case of nitrate analysis the analytical conditions of **table A.2.** were used. With the same analysis, determine the nitrite concentration is possible to obtain simultaneously to the nitrate concentration.

b.2.- Colorimetric method (LCK kit 339, Dr. Lange, analysis range 0.23 - 13.5 ppm)

Principle : nitrate ions solutions containing sulphuric and phosphoric acids react with 2,6-dimethylphenol to form 4-nitro-2,6-dimethyilphenol. The measure of absorbance is made with the spectrophotometer CADAS 100 (Dr. Lange), at a wavelength of 370 nm.

c.- Nitrite analysis (NO_2^-)

c.1.- Capillary electrophoresis (Waters Capillary Ion Analyser), 0.5-50 ppm $N\text{-}NO_2^-$:

In the case of nitrite analysis the analytical conditions are the same of the nitrate analysis because the analysis is simultaneous.

Instrument	Capillary Ion Analyser
Electrolyte	Ion select High Mobility Anion Electrolyte*
Capillary	75 µm x 60 cm fused silica
Power supply	Negative voltage
Applied voltage	15 KV

Current	14 μA
Injection mode	Hydrostatic, 10 cm height for 30 seconds
Detection	UV at 254 nm (Hg lamp)
Temperature	25 °C
Data System	Millenium 2010 Chromatography Manager
Sample Rate	20 points / second
Auto Purge	2.0 minutes

Table A.2.- Analytical conditions to determine the nitrate and nitrite concentrations. (* Patented by Waters).

c.2.- Colorimetric method (LCK kit 341, Dr. Lange, analysis range 0.015 - 0.6 ppm)

Principle : nitrites react with primary aromatic amines in acidic solution to form diazonium salts. These combine with aromatic compounds that contain an amino group or a hydroxyl group to form intensively coloured azo dyes. The measure of absorbance is made with the spectrophotometer CADAS 100 (Dr. Lange), at a wavelength of 524 nm.

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