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MELISSA

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Operation of the bench nitrifying reactors

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Draft	0	1/10/97	Original Version
1	1	18/12/97	Final Version
Draft,V.2	0	15/11/98	Expanded Version
2	1	12/02/99	

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1.- Introduction

The compartment III of the MELISSA loop consists of a packed-bed reactor with immobilised cells (*Nitrosomonas europaea* and *Nitrobacter winogradskyi*).

The low growth of the bacteria that carry out the biological nitrification process, as it has been discussed in previous technical notes of this compartment, limits the number of experiments to be conducted about the nitrification process.

In order to obtain more information about the nitrifying process it was decided to design and construct three bench columns, additionally to the pilot plant packed-bed reactor. With these bioreactors it will be possible to operate simultaneously the pilot reactor and the bench columns using different operating conditions. The preliminary design of these columns was proposed in TN 25.6. The general aim of this design is to reproduce the same configuration that has the pilot reactor taking into account the limitations associated with scale.

The main difficulty to attain the continuous operation of the bench nitrifying reactors is to reach enough cells to inoculate the bench columns (as it was already discussed in the TN 37.410), and also to carry out the process of support sterilisation, as Biostyr (polystyrene beads) is not directly steam sterilisable.

2.- Set-up of the bench columns.

The detailed design of the different parts conforming bench columns is described in **figures 1** to **4**.

The final configuration of these reactors is presented in **figure 5**, in this figure the general scheme of all the instrumentation and controls that have been implemented in the bench columns is given. The references given in figure 5 are described in **table 1**.

In each of the bench columns there have been implemented pH measurement and control, and temperature control (a thermostatic bath feeds the column external jacket allowing to maintain its temperature). The dissolved oxygen measure is available in one of the columns. The cell support is the same used in the pilot reactor : polystyrene beads (Biostyr) of 4.1 mm of mean diameter.

A more detailed description of the pH control is presented in **figure 6.** In that scheme the control element and the signal transmission process from the pH probe to the controller (pHrocon-18, Crison) are shown. The controller actuates when the $pH_{measured} > pH_{set\ point}$, and the actuator element is the solenoid valve that allows CO_2 entry to the column (through the gas sparger).

The general trend of pH variation in the system are that the pH of the culture decreases with cell metabolism, but the aeration flow produces a certain stripping of the dissolved CO_2 from the liquid phase to the gas phase. In consequence, when the effect of the stripping of CO_2 is higher than the variation of pH by cell growth, the pH of the culture would increase (this fact happen usually in batch operation when microbial activity is relatively low).

The base used to increase the pH of the culture is a Na_2CO_3 solution (30 g/L), and the additions are manual, as this action is required only occasionally during the set-up phase (in batch operation).

The control law used in the bench columns is that supplied by a Crison Automatic Proportional Controller. This control consists mainly in the definition of two areas : proportional band and the dead band. The actions of the control are only made within the proportional band. The duration and the frequency of the actions is variable, according to the difference $pH_{measured}$ - $pH_{set\ point}$. The frequency of the action can be modified by the user, and the duration of the addition can be changed by variation of the proportional band. After studying different possibilities by means of an experimental procedure the parameter values selected have been : cycle = 8 s, and proportional band = 7 pH units and dead band = 0.1 pH units ; that assures a good behaviour of the control system in terms of response time and precision, preventing that overshoots and off-sets appear.

The set-up of bench columns in the MELISSA Pilot Plant Laboratory is presented in **figure 7**.

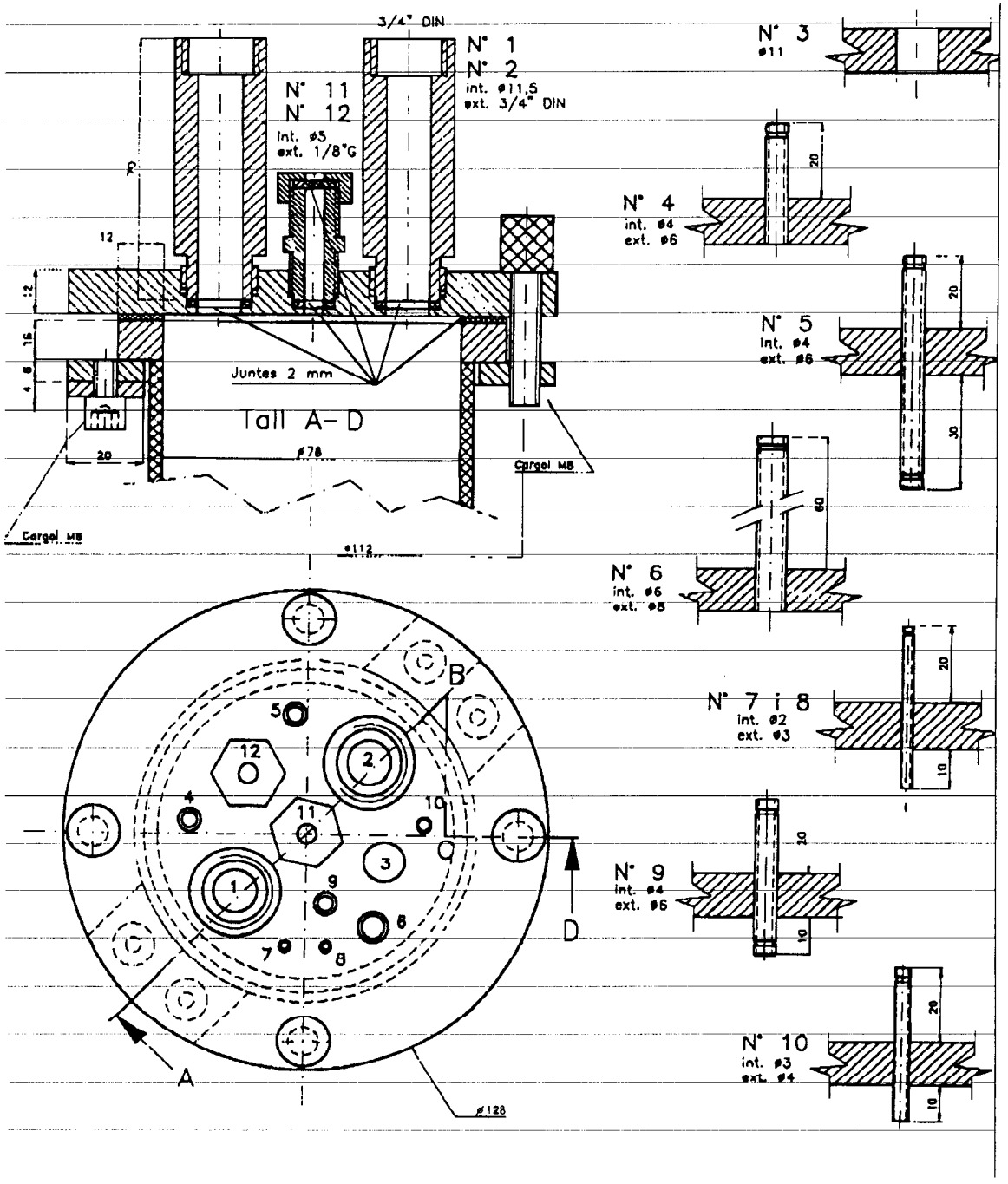

Figure 1.- Detailed design of the lid (stainless steal AISI-316) of the bench columns.

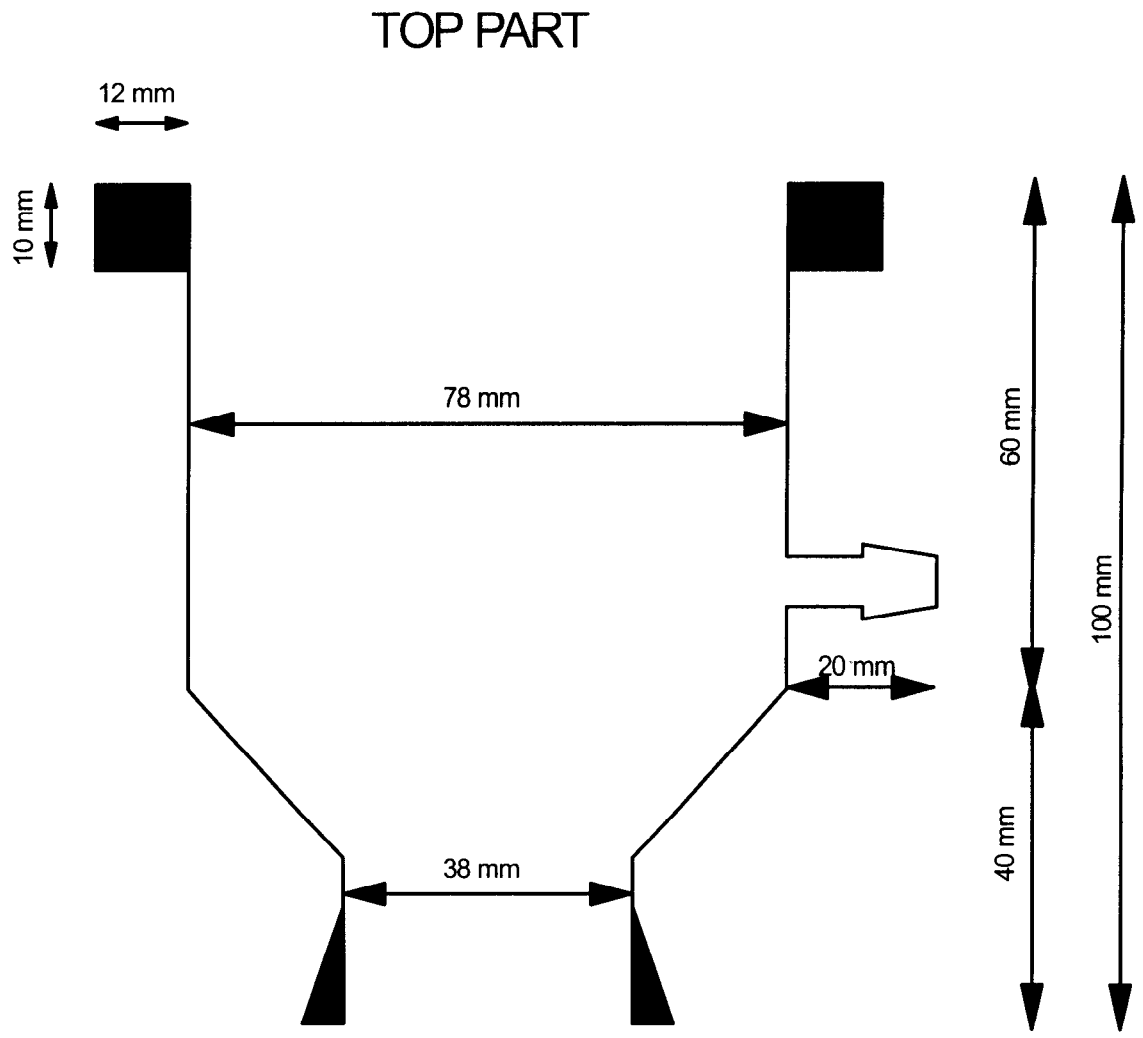

Figure 2.- Design of the top part (glass) of the bench columns.

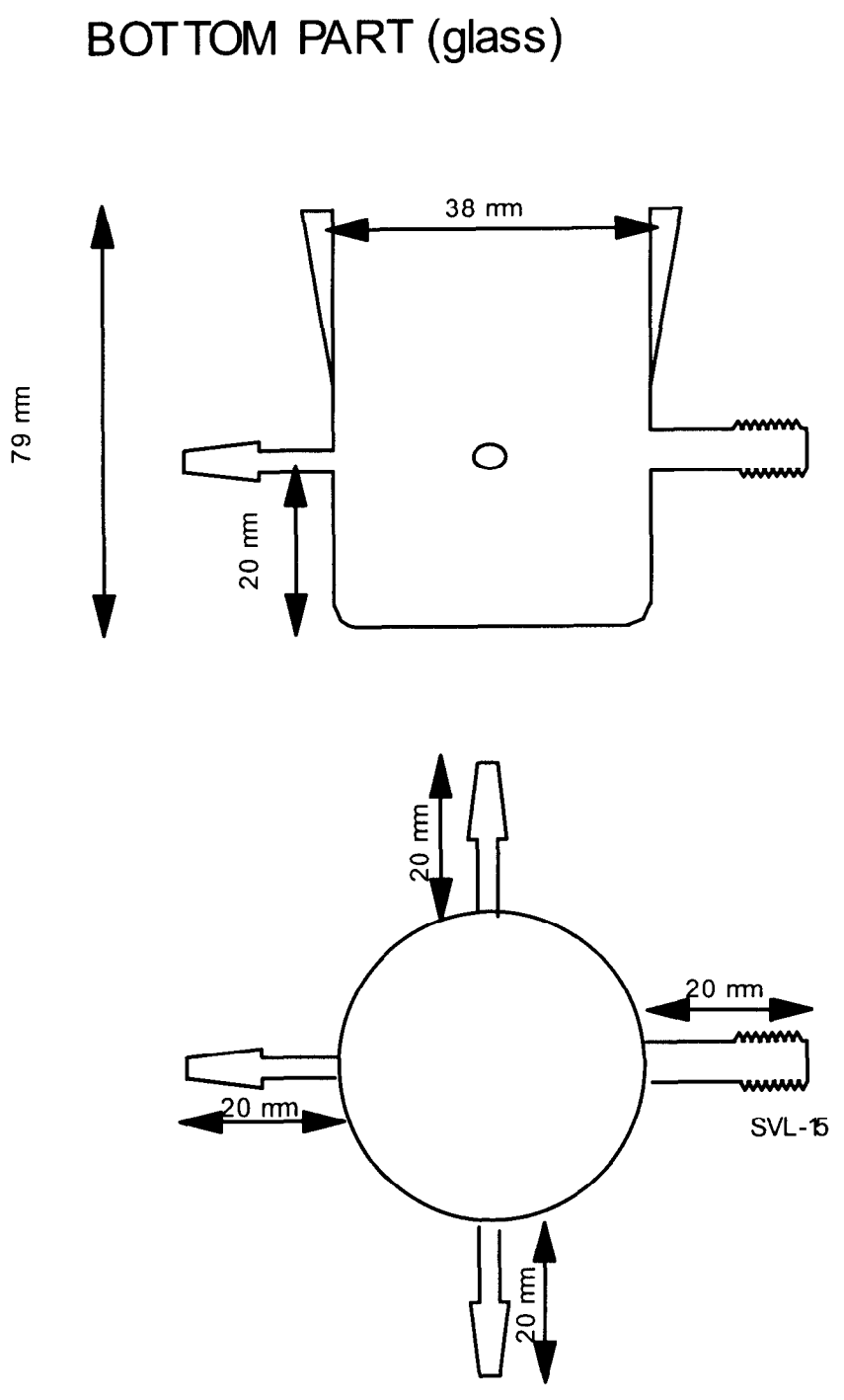

Figure 3.- Design of the bottom part (glass) of the bench columns.

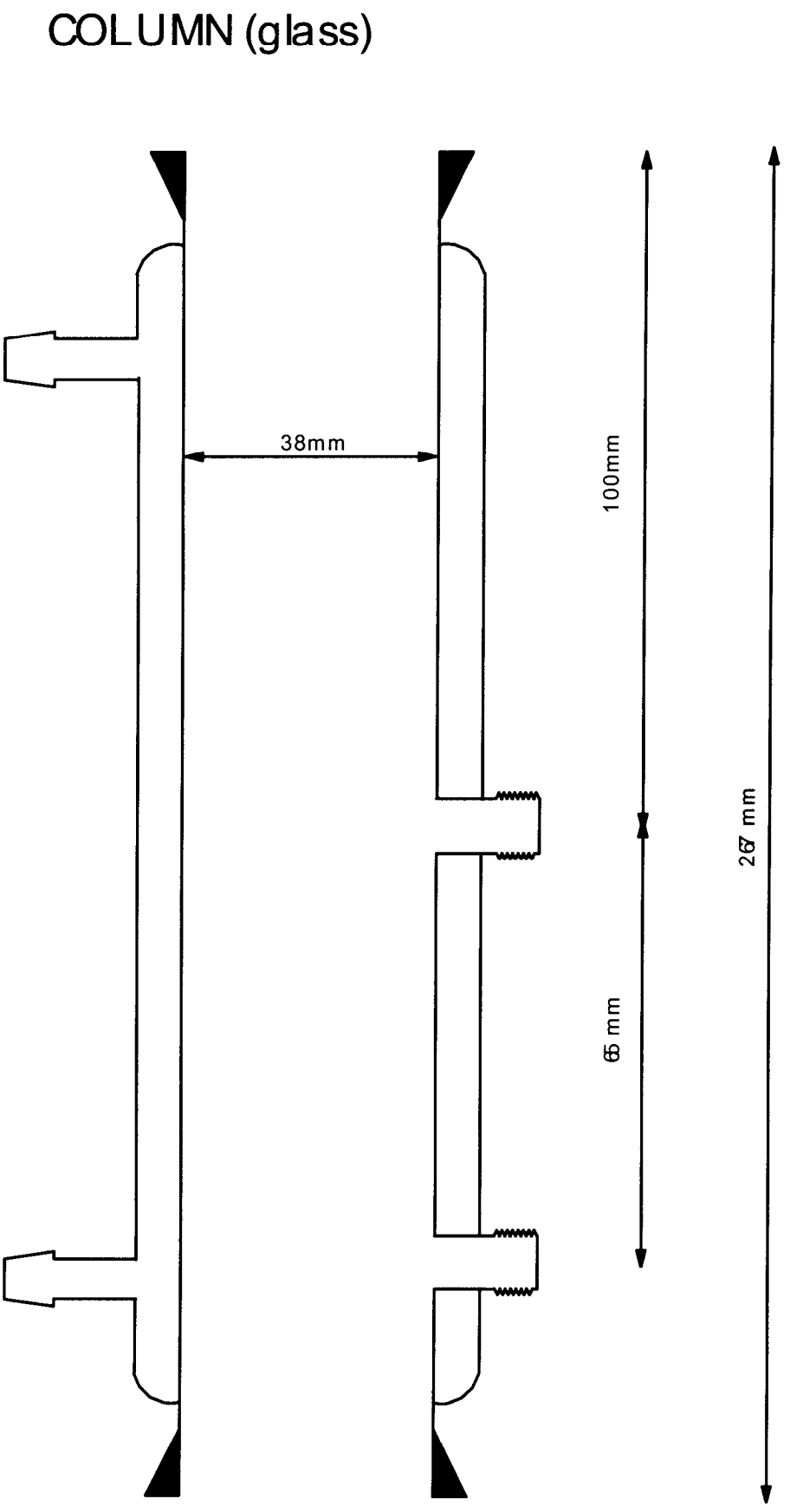

Figure 4.- Design of the central part (glass) of the bench columns.

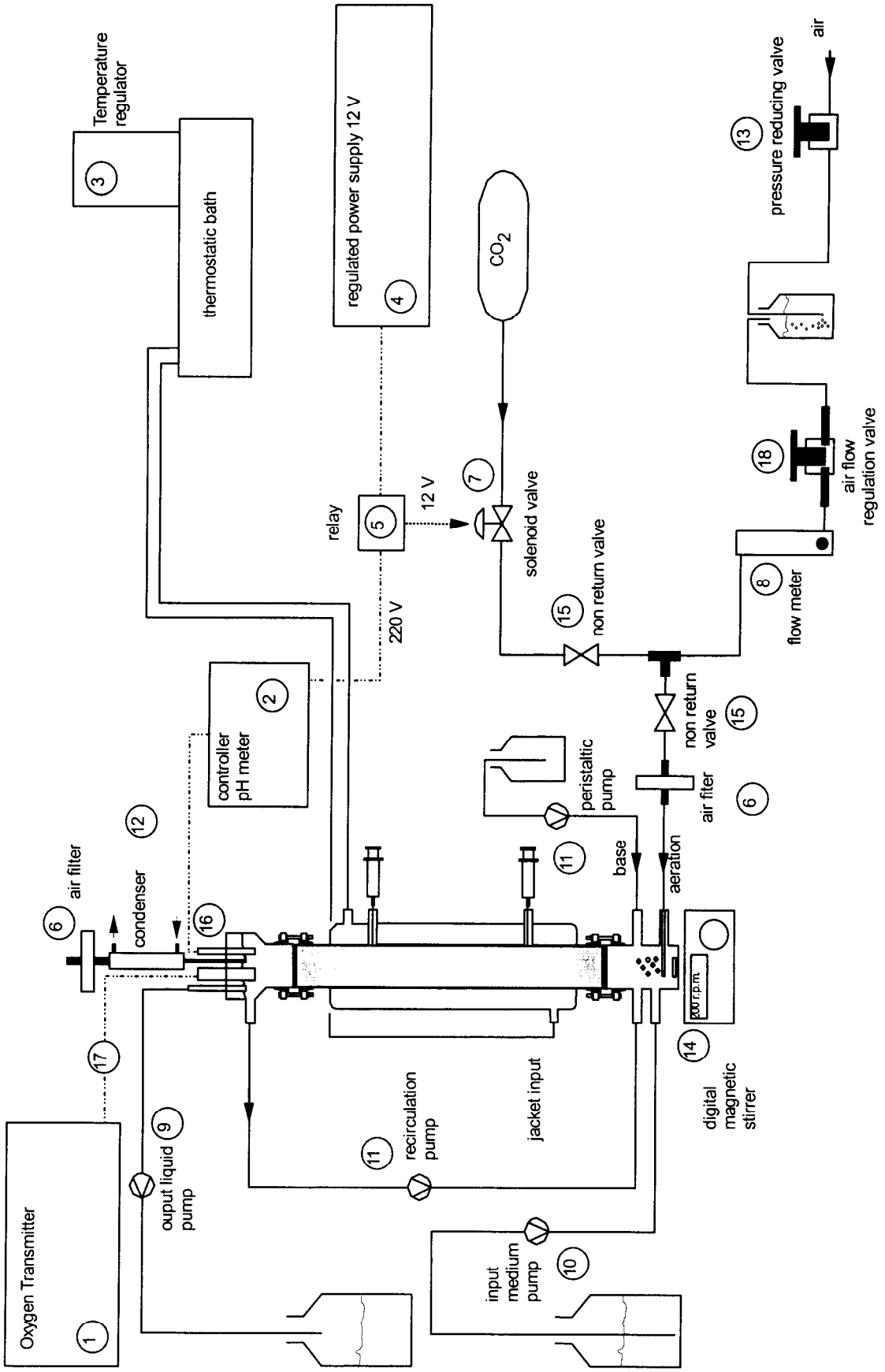

Figure 5.- Final operating configuration of the bench columns.

REF.	MATERIAL	MODEL/TRADEMARK
1	O_2 Transmitter	4500 (Mettler-Toledo)
2	pH measurer/controller (2 channels)	pHROCON 18 (Crison)
3	Thermostatic bath	DT-1 (Heto Lab Equipment)
4	Regulated power supply	U1413 (P.Fontaine Électronique)
5	Relay	220 V (Matsushita)
6	Air filter	0.22 μm (Millipore)
7	Plastic micro-solenoid valve (12V, 2.5 W)	EN 3 (Sirai)
8	Flow meter	FM 083-03 (Aalborg Instruments)
9	Peristaltic pump (4 channels)	ISM-850 (Ismatec)
10	Peristaltic pump (4 channels)	ISM-828 (Ismatec)
11	Peristaltic pump (2 channels)	T1A (Ismatec)
12	Glass condenser	Alco
13	Pressure reducing valve (with manometer)	Festo
14	Magnetic stirrer	AGIMATIC REV-S (Selecta)
15	Non return valve	H-QS-6 (Festo)
16	pH electrode	12/120 InPro 3000 (Ingold)
17	O_2 probe	12/120 T-TYPE (Ingold)
18	Air flow regulation valve	Whitey
--	Gas loop: rigid tube	4x6 (Mazzernew)

Table 1.- Description of the used materials according to references in the figure 5.

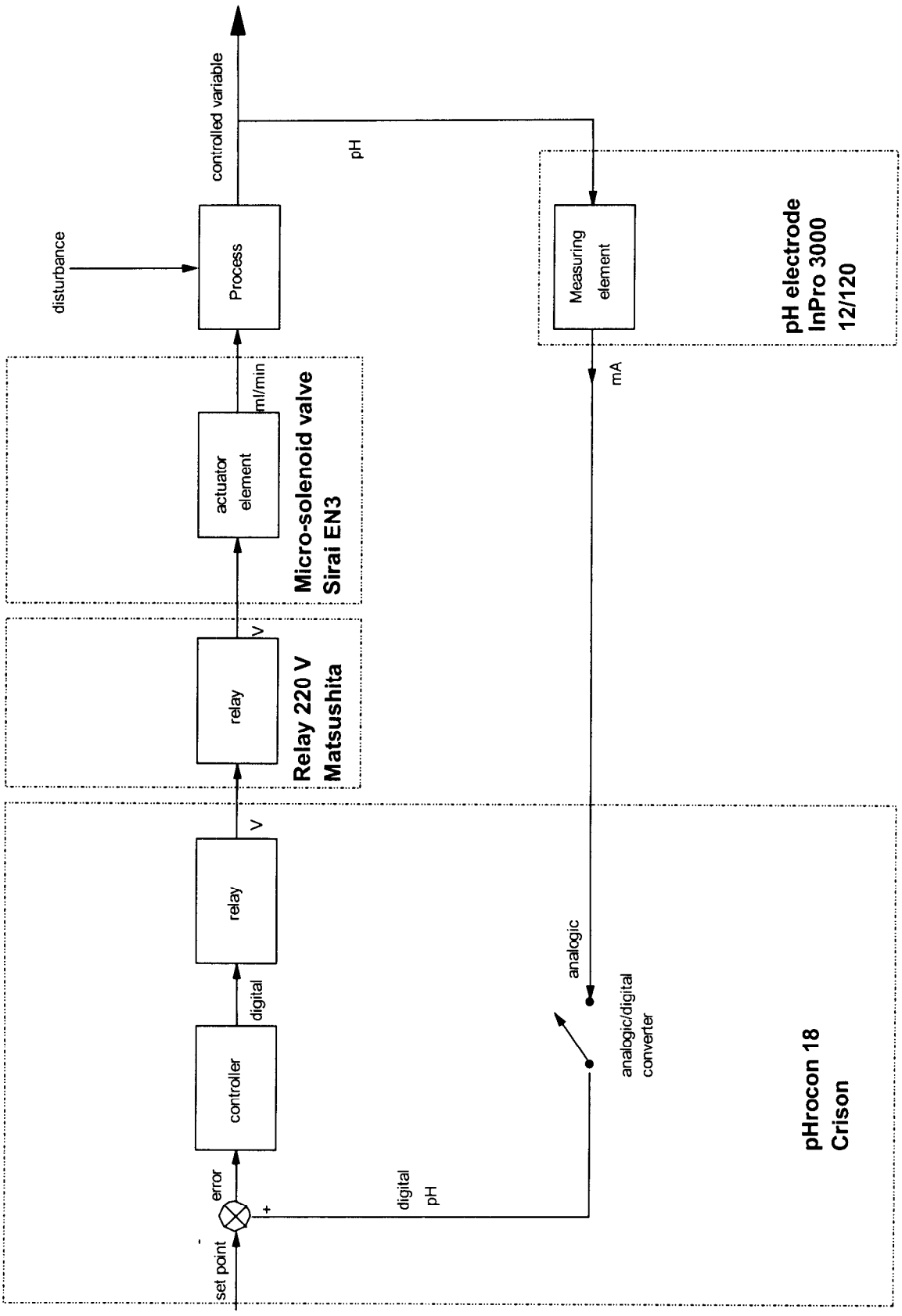

Figure 6.- Detailed scheme of the pH control loop.

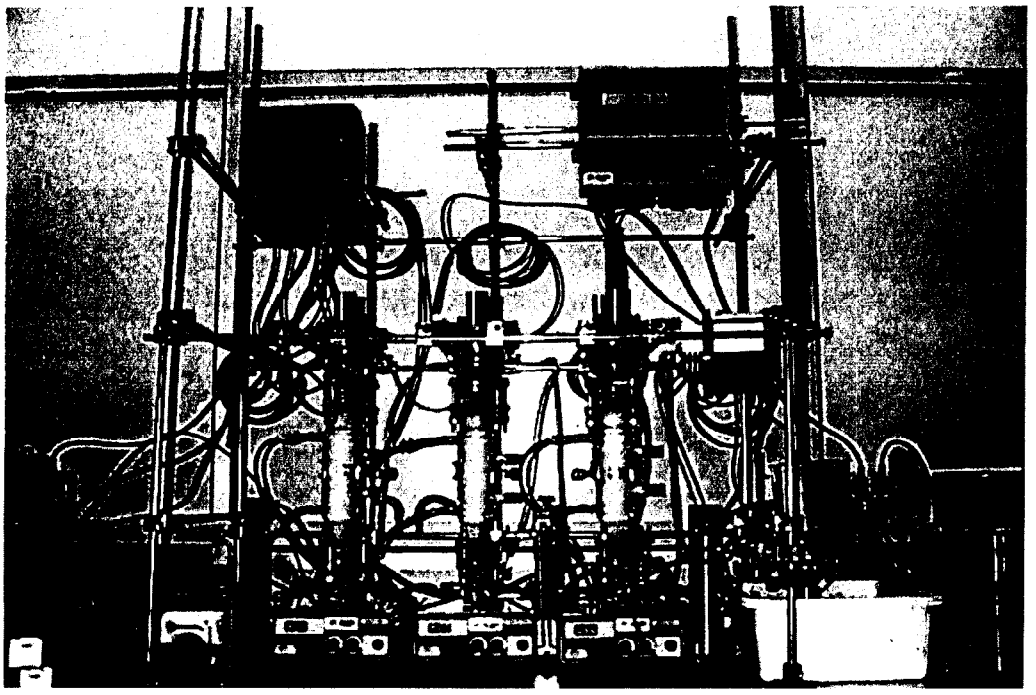

Figure 7.- Final set-up of the bench columns.

3.- Start-up procedure

The complete sterilisation process consists of two steps :

- Sterilisation of the reactor without the support : the reactor is sterilised in the autoclave (121 °C and 2.2 atm, during 20 minutes).

- Sterilisation of the support (Forler, 1994): once the support has been washed, it is introduced in the bench column ; then, the acid/base sterilisation process starts:

    - reactor filling with acid (pH = 2.0), during about 30 minutes ;
    - washing phase (sterile distilled water until pH = 6-7) ;
    - reactor filling with base (pH = 12), during about 30 minutes ;
    - washing phase (sterile distilled water until pH = 6- 7).

All the liquid solutions used in the sterilisation process were introduced in the column through a 0.22 μm filter.

Two bench columns were inoculated following this sterilisation procedure, and after two weeks of culture a microbial contamination in the two cultures was detected.

From the protocols used regularly in the plant cell cultures (Dixon, 1985), a new sterilisation procedure has been designed :

- support immersion in absolute ethanol for 20 minutes ;
- washing phase in sterile distilled water ;
- support immersion in NaOCl (1-1.4 % available Cl_2) for 20 minutes ;
- washing phase in sterile distilled water ;
- support immersion in HCl (pH = 2.0) for 1 hour ;
- washing phase in sterile distilled water ;
- support immersion in NaOH (pH = 12.0) for 1 hour ;
- washing phase in sterile distilled water.

This procedure has been carried out twice : first before to introduce the beads in the bench columns, and second, after the support was inside the columns, using filters of 0.22 μm. In this second sterilisation process, the time of immersion of the support in the different sterilising agents increases to 3 hours.

The co-culture (*Nitrosomonas europaea* and *Nitrobacter winogradskyi*) obtained from the operation of the fermenter Biostat B (as described in the TN 37.410) have been used to inoculate two of the bench columns. The inoculum volume was approximately 150 ml for every column.

A first approach to the liquid volume of the bench column has been determined : 510 ml. The value of the gas hold-up has been neglected, and a more accurate determination of the liquid volume of the bench column will be carried out during its final physical characterisation.

4.- Evolution of the culture operation

The first step in the operation of the nitrifying columns is to obtain satisfactory cell attachment onto the surface of solid particles. To achieve this objective, the columns were operated batchwise with medium recirculation, and the evolution of the nitrate and ammonium concentration was measured.

The operating conditions of this cell attachment period are specified in **table 2**.

VARIABLE	VALUE
Temperature	30 °C
pH	7.8-8.2 (controlled only by CO_2 addition)
Recirculation	3-8 ml/min
Stirring	300 r.p.m.
Light conditions	darkness

Table 2.- Culture conditions in the biofilm formation phase in the bench columns.

The evolution of the nitrate and ammonium concentrations was analysed during this phase (**table 3, figure 8**). The analytical methods used to do the analysis are described in the **Appendix.** Periodic microscopic observations were made along the process and microbial contamination was not detected.

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	0.125	n.d.	0.202	0.327
9	0.107	n.d.	0.235	0.342
24	0.080	n.d.	0.265	0.345
41	0.055	n.d.	0.280	0.335
57	0.028	n.d.	0.295	0.323
81	0.001	n.d.	0.296	0.297
104	0.143	0.0003	0.152	0.295
109	0.183	0.007	0.127	0.317

Table 3a.- Evolution of the ammonium, nitrite and nitrate concentrations in the column 1. * g total N/L = g $N\text{-}NH_4^+$ / L + g $N\text{-}NO_3^-$ / L + g $N\text{-}NO_2^-$ / L. (n.d. = not determined).

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	0.160	n.d.	0.151	0.311
9	0.113	n.d.	0.190	0.303
24	0.076	n.d.	0.234	0.310
41	0.046	n.d.	0.271	0.317
57	0.031	n.d.	0.281	0.312
81	0.002	n.d.	0.297	0.299
104	0.165	0.0002	0.156	0.321
107	0.163	0.0002	0.147	0.310
109	0.042	0.102	0.174	0.318

Table 3b.- Evolution of the ammonium, nitrite and nitrate concentrations in the column 2. * g total N/L = g $N\text{-}NH_4^+$ / L + g $N\text{-}NO_3^-$ / L + g $N\text{-}NO_2^-$ / L. (n.d. = not determined).

After more than 2 months of batch culture, the degradation of the initial ammonium was completed . Then, an addition of ammonium was done and after 20 days the continuous operation started up. The flow-rate used was 0.16 ml/min, the recirculation ratio 1:36 (5.8 ml/min) and the concentration of the input medium was 0.3 g $N\text{-}NH_4^+$ / L.

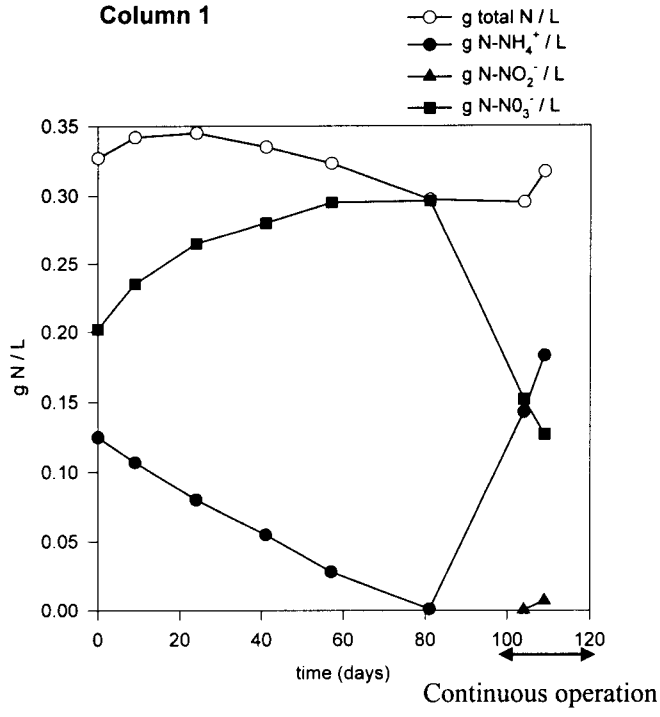

Figure 8a.- Evolution of the ammonium, nitrite and nitrate concentrations in the column 1 during bacth phase and continuous start-up. (g total N/L = g $N\text{-}NH_4^+$/L + g $N\text{-}NO_2^-$/L + g $N\text{-}NO_3^-$/L).

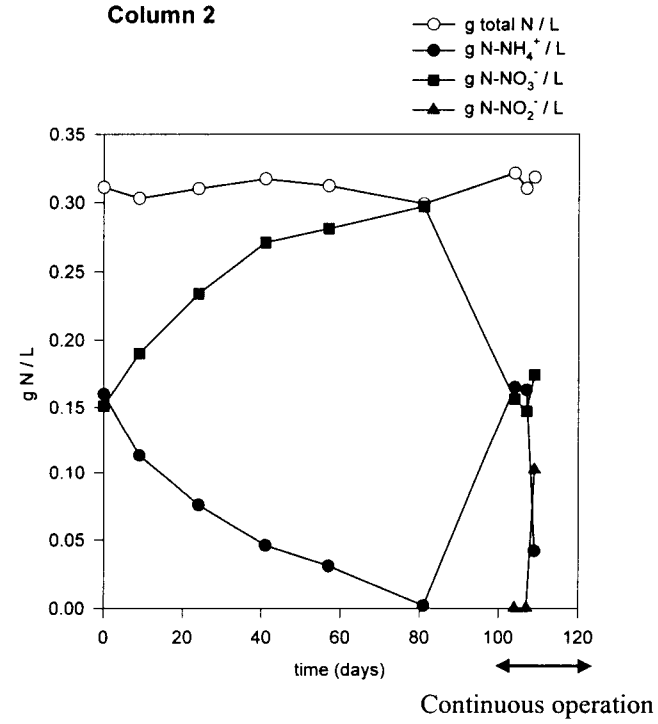

Figure 8b.- Evolution of the ammonium, nitrite and nitrate concentrations in the column 2 during bacth phase and continuous start-up. (g total N/L = g $N\text{-}NH_4^+$/L + g $N\text{-}NO_2^-$/L + g $N\text{-}NO_3^-$/L).

To determine the degree of cell attachment onto the beads, the input flow-rate of medium (0.16 ml/min, θ = 53 h) was increased progressively: first 0.32 ml/min (θ = 27h) and then 0.64 ml/min (θ = 13 h), until assure the effect of the free cells in the

ammonium degradation could be neglected. The last dilution rate used (0.08 h^{-1}) is higher than the referenced μ_{max} of the nitrifying bacteria (0.06 h^{-1} for *Nitrosomonas europaea* and 0.03 h^{-1} for *Nitrobacter winogradskyi*), and on the other hand, the concentration of free cells in the output flow is very low. The evolution of the ammonium, nitrite and nitrate concentrations when the input flow-rate of medium increased are presented in **figure 9**.

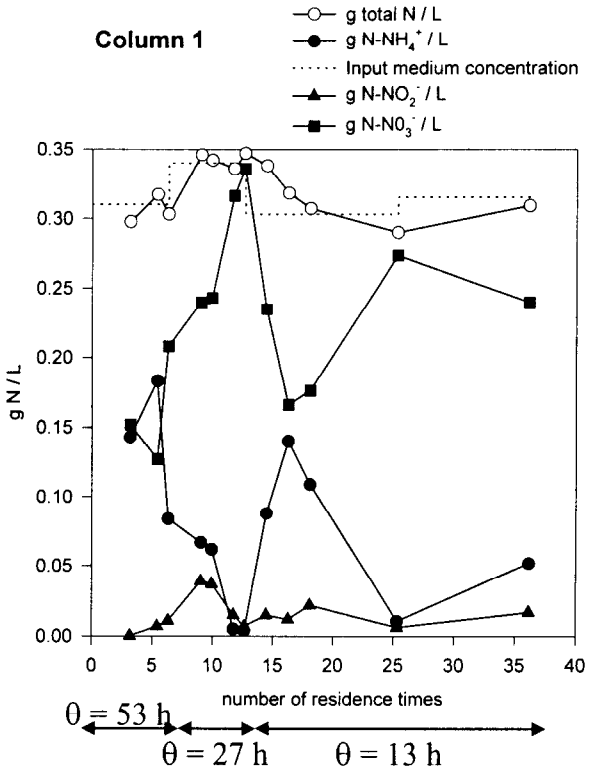

Table 9a.- Evolution of the ammonium, nitrite and nitrate concentrations in the output of the bench column number 1 during 35 days in continuous operation at different residence times. (g total N/L = g N-NH_4^+/L + g N-NO_2^-/L + g N-NO_3^-/L ; θ = residence time) .

For the continuous operation of the bench columns the automatic addition of base solution for pH control is necessary. The automatic additions of the Na_2CO_3 solution are performed in the top section of the bench columns (where the pH probe is placed). The controller and the control parameters are the same that in automatic acid additions (CO_2 gas), as previously described (page 5).

Regarding the high concentration of nitrite in the column 2, at number of residence times 5 and 10, a possible inhibition of *Nitrobacter winogradskyi* could have taken place. The partial nitrification to nitrite has already been studied by Garrido (1996), and one of the possible causes is a low level of dissolved oxygen concentration. For this reason, the air input flow to the column 2 was increased (at number of residence times 14).

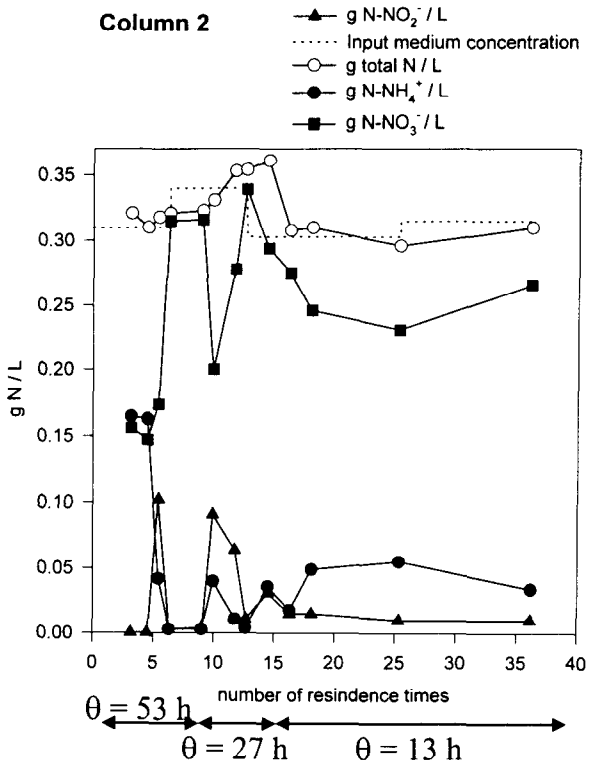

Table 9b.- Evolution of the ammonium, nitrite and nitrate concentrations in the output of the bench column number 2 during 35 days in continuous operation at different residence times. (g total N/L = g N-NH_4^+/L + g N-NO_2^-/L + g N-NO_3^-/L; θ = residence time).

5.- Physical characterisation of the bench columns

The study of the physical characteristics of the bench columns have been performed in order to obtain further information about the behaviour of these nitrifying bench packed bed reactors. Following the structure carried out in the pilot reactor (TN 25.330), the considered aspects included in this section are:

- Determination of the liquid, gas and solid volumes of the bench columns in the working conditions.
- Mixing degree of the liquid phase in the bench reactor, performing experiments of residence time distribution (RTD), using a dye as tracer (blue dextran).
- Mass transfer between different phases: oxygen transfer from gas to liquid. Experiments of determination of the coefficient K_La.

5.1.- Liquid, gas and solid volumes

The dimensions of the bench columns (having into account the thickness of the two grids and the four seals) and the calculated volumes of different sections of the bench columns are detailed in **table 4**.

TOP PART	Diameter (mm)	Height (mm)	Total volume (ml)	Total volume - solid volume (ml)
	38	22	24.95	24.95
	38-78	20	52.84	52.84
	78	30	143.35	143.35
MAIN PART				
	38	267	302.81	181.69
BOTTOM PART				
	38	79	89.60	89.60
(Grid and seals) x 2	38	8	9.07	9.07
TOTAL			622.62	**501.5**

Table 4.- Dimensions of the bench columns and theoretical liquid volumes.

Experimental measurements of the liquid, solid and gas volumes:

* Total volume: 650 ml

* Solid volume: 138 ml

* Gas volume (gas flow-rate = 15 ml/min): 30 ml
(gas flow-rate = 40 ml/min): 40 ml
(gas flow-rate = 500 ml/min): 47 ml

* Liquid volume (gas flow-rate = 15 ml/min): 512 - 30 = 482 ml
(gas flow-rate = 40 ml/min): 512 - 40 = 472 ml
(gas flow-rate = 500 ml/min): 512 - 47 = 465 ml

* Hold-up: (gas volume)·100/(gas volume + liquid volume)
(gas flow-rate = 15 ml/min): 6 %
(gas flow-rate = 40 ml/min): 8 %
(gas flow-rate = 500 ml/min): 9 %

5.2.- Liquid-phase mixing

In order to know the degree of mixing of the liquid phase in the bench reactors, and to compare pilot and bench columns, the conditions used to study the residence time distribution are detailed in **table 5**.

Number of experiment	Air flow-rate (mL/min)	Liquid flow-rate (mL/min)	Stirring (r.p.m.)	Recirculation flow-rate (mL/min)
1	40	0.83	300	4.5
2	500	0.83	300	4.5
3*	40	0.83	300	4.5
4*	40	0.83	300	0

Table 5.- Conditions of residence time distribution in the experiments. * These experiments were performed injecting the tracer in a different way.

The samples obtained to determine the evolution of the absorbance were withdrawn from the centre of the section between the main body of the column and the top section (see **figure 12**).

Experiment 1

The results obtained in this experiment are presented in **figure 10a**. Although the RTD curve is similar to a well-mixed tank, the differences are slightly more significant that in the case of the pilot reactor. Two different behaviours can be appreciated in the RTD curve: a slight contribution of plug flow, and a high contribution of well-mixed tank. The equivalent RTD curve to a well-mixed tank is presented in **figure 10b** to observe the differences between the experimental RTD and the theoretical mixed tank.

The experimental residence time obtained is $\theta = 526$ min (regression coefficient 0.999), while the expected residence time for the liquid flow-rate used is 566 min.

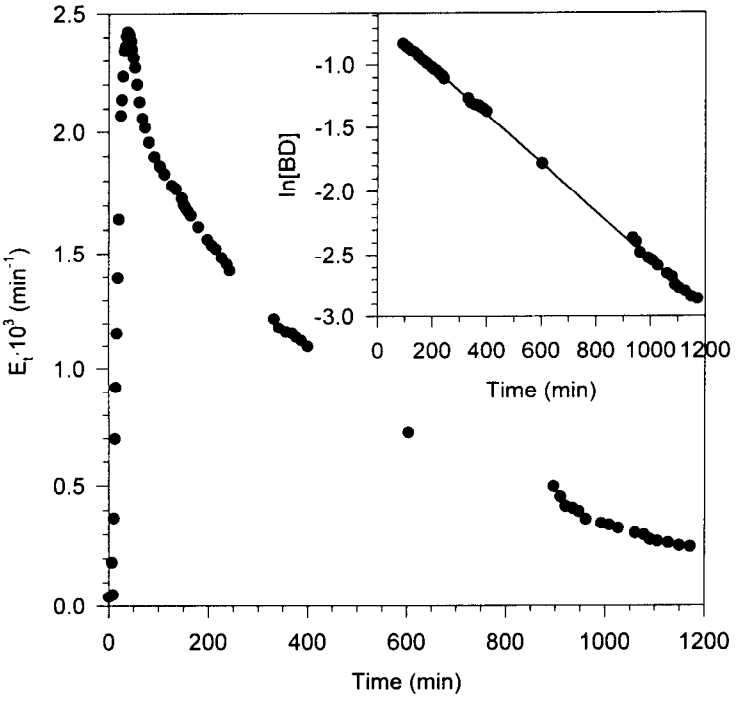

Figure 10a.- Experimental residence time distribution. Determination of the residence time value from the theoretical equation of an ideal mixed tank. Aeration flow-rate: 40 mL/min.

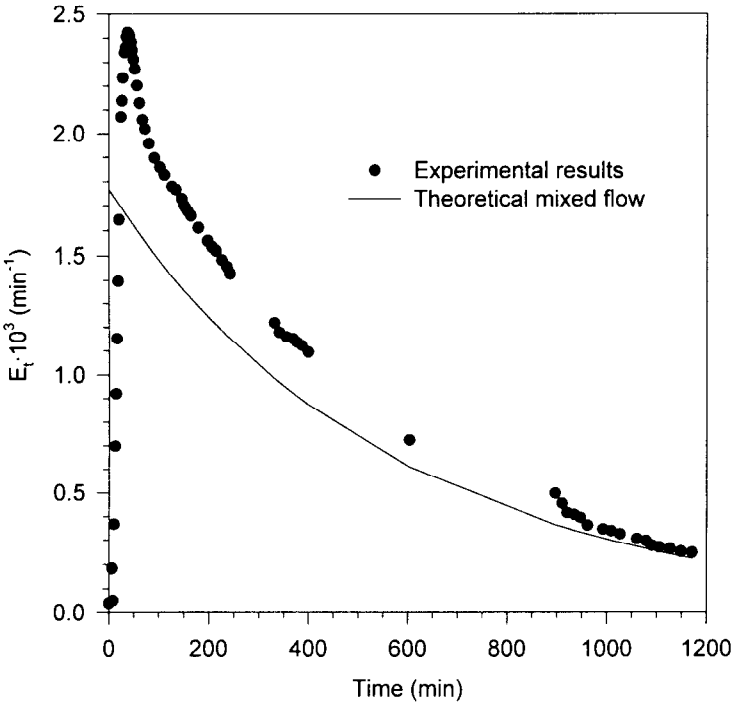

Figure 10b.- Experimental residence time distribution compared with the residence time distribution if the bench column had a behaviour of ideal mixed tank.

Experiment 2

In this experiment all the last conditions were kept constant except the aeration flow-rate, which increased to 500 mL/min. When the aeration flow-rate is increased the degree of mixing increase too. This experiment was carried out with the aim to have a relation reactor volume/aeration similar to the nitrifying pilot reactor. As the pilot reactor has a degree of mixing very close to a perfectly mixed tank (TN 25.330), working in similar conditions, the bench columns should have similar residence time distributions.

The results obtained in this experiment are presented in **figure 11**. The general behaviour is close to a well-mixed tank than in the first experiment. The experimental residence time obtained is $\theta = 526$ min (the residence time waited for a liquid flow-rate is 560 min).

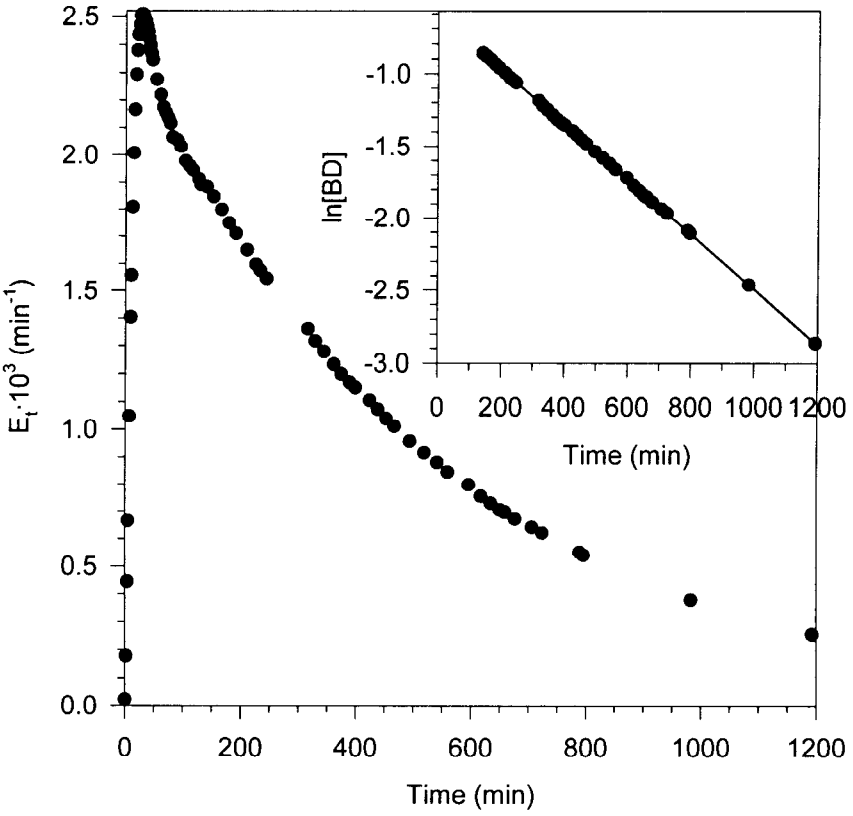

Figure 11a.- Experimental residence time distribution. Determination of the residence time value from the theoretical equation of an ideal mixed tank. Aeration flow-rate: 500 mL/min.

Experiment 3

In this experiment the tracer was injected in a different way. Using a needle placed between the bottom and the main body of the bench column, the dye was injected in the centre of the section (see **figure 12**). The conditions of this experiment were the same that in the first (aeration flow- rate of 40 mL/min).

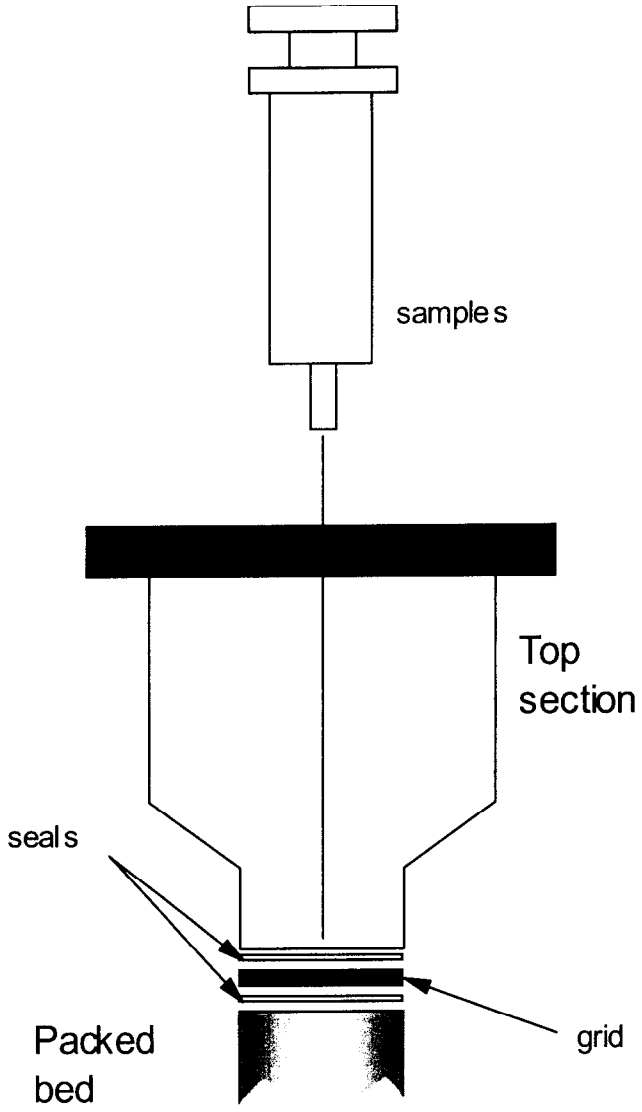

Figure 12a.- Detailed position of the sample extraction.

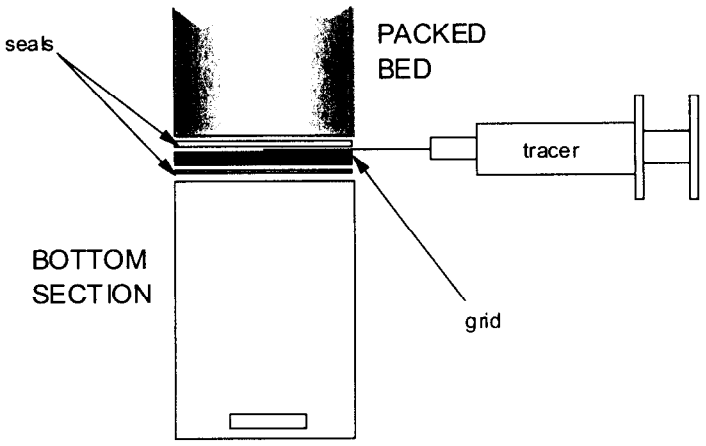

Figure 12b.- Detailed position of the tracer injection point.

The results obtained in this experiment are presented in **figure 13**. As it can be observed in this figure, the plug flow component is less accentuated than in the other experiments because the decrease of the tracer concentration, once the maximum is attained, do not have two different slopes, as the rest of residence time distribution curves have. On the other hand, the time between the initial point of the experience (tracer injection) and the maximum concentration detected is slightly higher than in the other experiments. This occurs because the tracer does not mix initially in the bottom section (by the effect of the aeration), and the effect of the recirculation makes possible the mix of the liquid volume of this section. The experimental residence time obtained is $\theta = 512$ min (the residence time waited for a liquid flow-rate is 566 min).

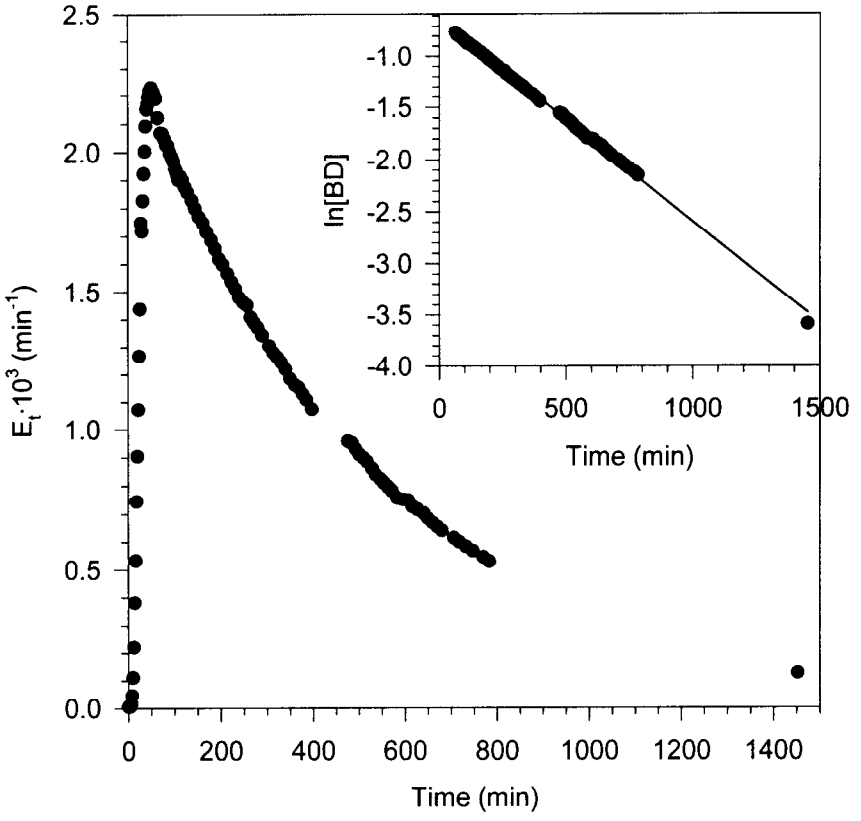

Figure 13.- Experimental residence time distribution. Determination of the residence time value from the theoretical equation of an ideal mixed tank. Aeration flow-rate: 40 mL/min.

Experiment 4

In this experiment the tracer was also injected in the way described in the last experiment (figure 12). The conditions of this experiment were the same that in the last experiment (aeration flow-rate of 40 mL/min), although without liquid recirculation.

The results obtained in this experiment are presented in **figure 14**. The residence time distribution curve has a strong component of plug flow, although the component of mixed tank is still visible. The mix of the liquid phase, in this case, is the effect of the

stirring (300 r.p.m.) and the aeration flow-rate. The experimental residence time obtained is $\theta = 348$ min (the residence time waited for a liquid flow-rate is 566 min), of course this value has an important deviation from a well-mixed tank, for the reasons explained previously.

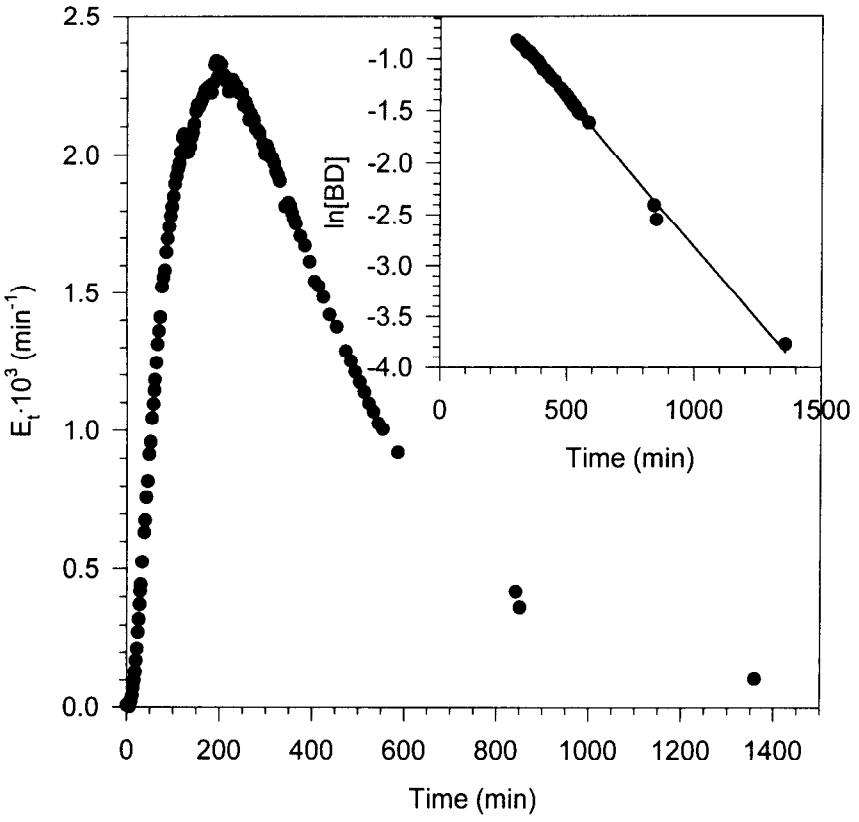

Figure 14.- Experimental residence time distribution. Determination of the residence time value from the theoretical equation of an ideal mixed tank. Aeration flow-rate: 40 mL/min; without recirculation of liquid.

After an analysis of all these results of residence time distribution, it is possible to affirm that the aeration, stirring and recirculation of the bench column make possible a reasonable mixed flow. However, a component of plug flow is detected, and this effect is higher, when the residence time of operation is decreased. On the other hand, comparing bench columns and pilot reactor is not possible in a direct way, due to the fact that the experiments of the residence time distribution of the two reactors are performed using different values of residence time. Anyway, the flow-pattern is clearly shown in the experiments and the similarity with the RTD for the pilot reactor is definitely confirmed.

5.3.- Oxygen transfer from gas to liquid phase. Determination of the K_La coefficient.

The determination of the value of the overall oxygen transfer coefficient from the gas to the liquid phase is very important in process like the nitrification, which is an aerobic process. And moreover, having into account that the aeration is one of the key parameters in the biological degradation of the ammonium to nitrate.

The experimental procedure to determine the K_La coefficient is the same that described in TN 25.330. The simple method used can be briefly explained: first, the oxygen concentration in the liquid phase of the reactor is purged using nitrogen until the oxygen concentration in the liquid phase is reduced to a steady state value of approximately zero. Then, at steady fluid temperature a small air flow is introduced, producing an increase of dissolved oxygen concentration until a new steady state is reached.

After the oxygen probe characterisation (**figure 15**, response time 27.5 s), the response time of this probe is used to determine the K_La coefficient by curve fitting of the experimental data to the theoretical oxygen concentration time-profiles, using a non-linear procedure. The equation used is (Chisti, M.Y., 1989):

$$C_{med} = C^* + \frac{C^* - C_0}{1 - \tau K_L a}\left[\tau K_L a \exp\left(-\frac{t}{\tau}\right) - \exp(-K_L a t)\right]$$

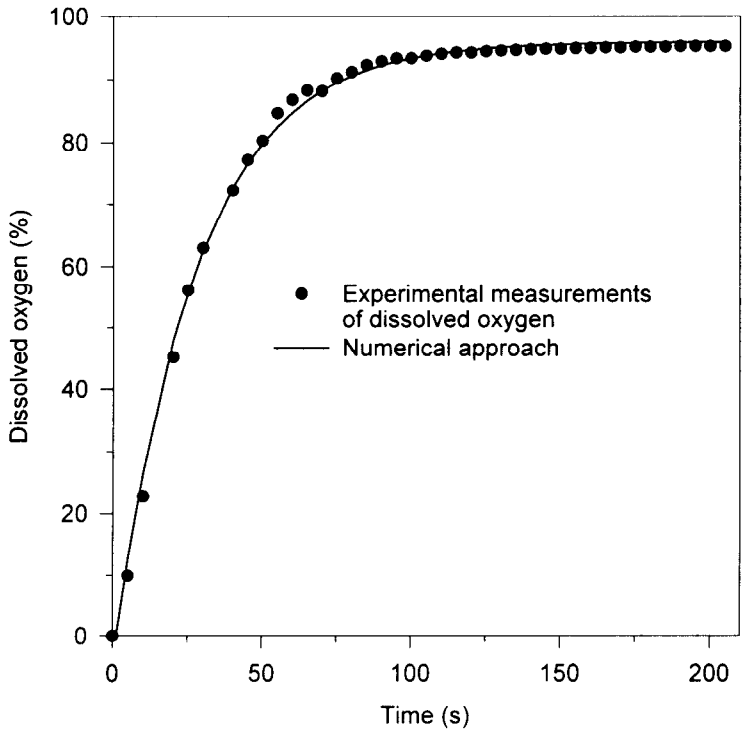

Figure 15.- Characterisation of the oxygen probe, determination of the response time.

In **table 7** the conditions used for the K_La determinations and the summary of the results of the coefficients determined are detailed. The temperature of all determinations is 28 °C.

Air Flow (mL/min)	Stirring (r.p.m.)	Rec.Flow (mL/min)	VVM (min^{-1})	K_La (s^{-1})
40	300	4.5	0.08	0.0013
40	300	9	0.08	0.0016
40	500	4.5	0.08	0.0014
100	300	4.5	0.2	0.0032
250	300	4.5	0.5	0.0072
500	300	4.5	1.0	0.012

Table 7.- Summary of results of K_La determinations.

An example of the K_La determination for given conditions is presented in **figure 16**, showing a good concordance between theoretical and experimental data.

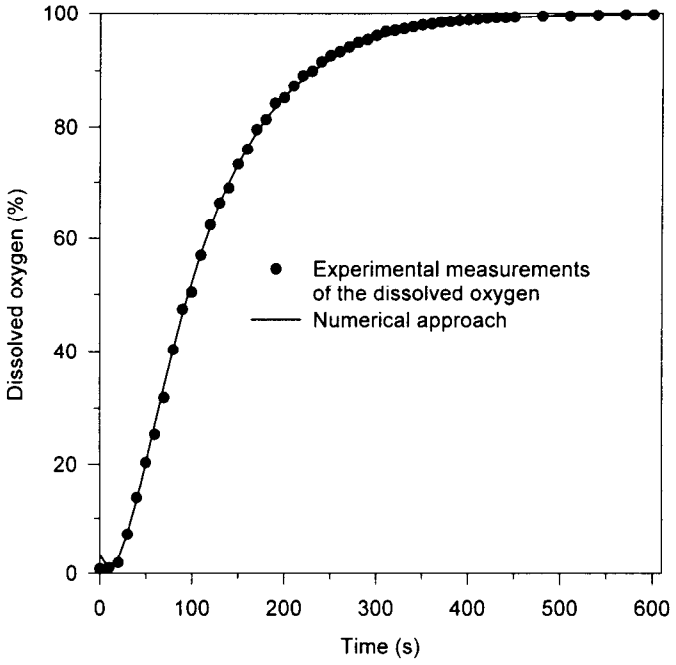

Figure 16.- Example of the K_La determination.

Regarding table 7, it is clear that the K_La value hardly increase when the stirring increase from 300 to 500 r.p.m. On the other hand, an increase in the recirculation flow-rate causes an increase in the K_La value. Nevertheless, the aeration flow-rate produces the more important effect in the K_La coefficient value.

From these values it is possible to fit a curve to describe the behaviour of the K_La when the aeration flow-rate is varying. This curve and the parameters that define his equation are detailed in **figure 17**.

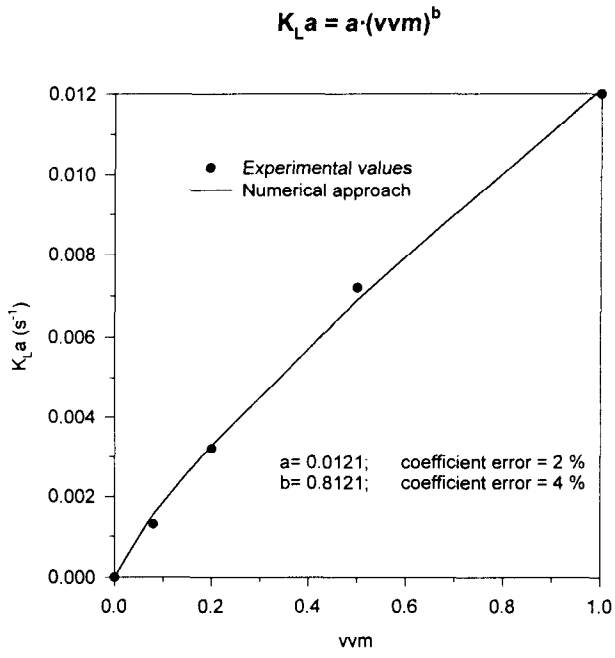

Figure 17.- Variation of the K_La versus reactor volume/aeration flow-rate (vvm).

Using the K_La values of the pilot reactor and all the results of the bench column, it is also possible to fit a curve to the evolution of the coefficient values with the aeration. So, the parameters of this equation and the graph of the curve fitted are presented in **figure 18**.

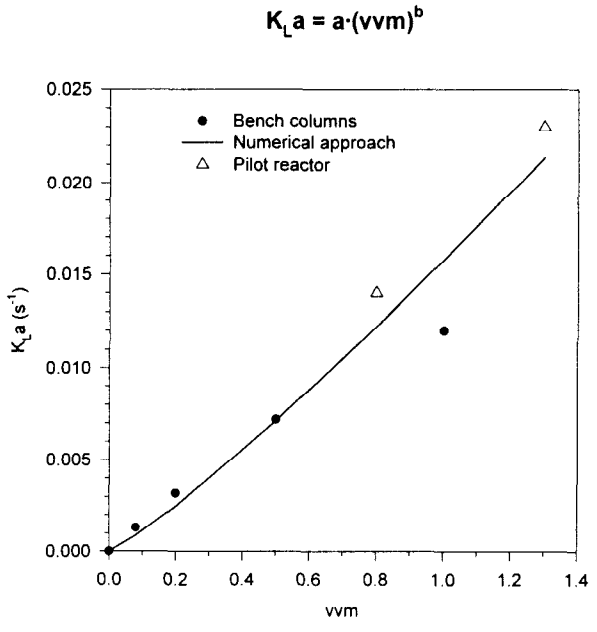

Figure 18.- Values of the K_La from the pilot reactor and from the bench column.

It can be concluded that the K_La in the bench column can be reach similar values that in the pilot reactor if aeration (vvm) is close to that used in the pilot reactor. Nevertheless, the K_La determinations are performed in the top of the bench column, where the superficial velocity of the gas phase has the lowest values along the column vertical axis, due to the important increase of the column section in the top part. For this reason it is possible that the real value of the mass transfer coefficient in the active zone of the reactor was higher than the determined experimentally.

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-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-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	Ionselect 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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