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Operation of the Nitrifying Pilot reactor

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1.- Introduction

The third compartment of the MELISSA loop has been conceived as a packed bed reactor with *Nitrosomonas - Nitrobacter* cells immobilised on a solid support (Forler, 1994). Due to the very low maximum specific growth rate of both species (Hunik et al., 1993) a wash-out of the microorganisms could be a main drawback in continuous cultures. It can be overcome by biomass retention as applied in biofilm reactors. In addition, the lack of interest in generating cells that can not be used as food did that the nitrifying process was conceived as an immobilized cells bioreactor. The support material was selected in previous studies (Zeghal, 1992) and consists of polystyrene beads.

The previous work carried out in the MELISSA Pilot Plant with the third bioreactor compartment has been focused on its physical characterisation and definition of the control system (TN 25.310, TN 25.330). This includes the determination of phase hold-up, residence time distribution experiments in order to know the liquid phase flux model in the reactor, K_La determination at different operating conditions. The characterization of the instrumentation of the reactor, and the corresponding control loops, under static and dynamic conditions has been also performed. The already implemented control loops (temperature, ammonium concentration, liquid level, pH, dissolved oxygen and pressure), and the on-line monitoring of the nitrate concentration may permit to maintain the operation conditions in the pilot reactor.

Also, the communication between this compartment (III Compartment of the MELISSA loop) and the system station and the GPS was realized and documented in TN 25.320, with the configuration of the corresponding groups, Nitrifying, Nitrifying II, and nitr.gps.

Finally, the pilot reactor was modified with respect to its first design, as reported in TN 37.410, by the replacement of the bottom and top sections previously made on teflon material with stainless steel.

With all these steps, the pilot plant was prepared for its continuous operation. A critical step for this sterilisation, that needs to be done very carefully, as the BIOSTYR beads are not sterilisable by heat. This sterilisation procedure was studied as reported in TN 37.510 for the bench columns, and is summarised in the next point.

After sterilisation, the reactor is inoculated and operated at a low flow-rate to allow cell attachment. Next, continuous operation follows, with different experimental conditions studied. After fixing a given set of conditions of operation, the evolution of the reactor performance is monitored until reaching a steady-state. Once this steady state is characterised, a new set of conditions is fixed. In the present technical note, the results obtained in the operation of the pilot nitrifying reactor at different conditions during a period of five months is reported.

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

In order to maintain the sterility of the cultures of the pilot reactor, a sterilisation operation scheme was proposed by Forler (1994). The main problem of the procedure is the sterilisation of the polystyrene beads, due to the fact that this support does not resist the high temperature and pressure of the most conventional steam sterilisation processes (120 °C, 2,2 atm). Therefore, a sterilisation procedure based on acid/base treatment had been designed (Forler, 1994).

After several problems of cell contamination, this sterilisation process was slightly modified, in order to obtain a greater degree of sterility. The modifications performed were similar to the described for the nitrifying bench columns (TN 37.510). The two main steps of the sterilisation process are:

- Sterilisation of the reactor (empty of beads) with flowing steam (without pressure) during 2 hours. During this period of time the pressure is maintained at 1.1 atm and the temperature over 100 °C.

- Once the vessel and the gas loop are sterile, the next step is the filling of the bed with the beads support (the polystyrene beads are preliminary treated with acid/base solution and washed with water). Then, after the reactor is closed again, the chemical sterilisation process starts, following the next cycles:

    - reactor filling with absolute ethanol for 4-6 hours ;
    - washing phase with sterile distilled water ;
    - reactor filling with NaOCl (1-1.4 % available Cl_2) for 4-6 hours ;
    - washing phase with sterile distilled water ;
    - reactor filling with HCl (pH=2) for 4-6 hours ;
    - washing phase with sterile distilled water ;
    - reactor filling with NaOH (pH=12) for 4-6 hours ;
    - washing phase with sterile distilled water .

The co-culture (*Nitrosomonas europaea* and *Nitrobacter winogradskyi*) obtained from the operation of *Biostat B* fermenter (as described in TN 37.410) have been used to inoculate the pilot reactor. The innoculum volume was approximately 700 ml and the cell density estimated as 50 mg/L.

To decrease the risk of microbial contamination, the continuous running of the culture was immediately initiated after inoculation using a low dilution rate and being increased progressively. This "step by step" procedure was suitable to increase the ammonium load to be treated in the compartment while allowing satisfactory cell attachment onto the beads.

It is important to bear in mind that the system is intrinsically in a pseudo-stationary state relating to the cell population. When increasing the ammonium load, enough time to the cells is necessary to be accustomed to the new operations conditions, and so, to change the size, shape and thickness of the biofilm.

This period may be variable in functions of operation conditions, nature of the biofilm, type of cell attachment, shear stress and so on, but it is clearly demonstrated by the transient phenomena involved when, for instance, the load is varied. Direct consequences of this fact can be appreciated if analyses of nitrogen compounds are made. Mass balances can not be accomplished for the liquid phase, and undesirable levels of reaction intermediates, such as nitrite, may appear.

3.- Evolution of the reactor operation: experimental results

Due to the fact that the start-up of the culture was performed working in continuous operation, the phase of cell attachment on the surface of the solid particles occurred during this operational mode. While the dilution rate was lower than the referenced μ_{max} , this operation allows cell attachment without any risk of washing-out phenomena in the reactor.

The operating conditions of the pilot reactor are specified in **table 1**.

VARIABLE	VALUE	RANGE
Temperature	29 °C	±0.5
pH	8.1	±0.2
Recirculation	10 mL/min	±1
Stirring	400 r.p.m.	-
Dissolved oxygen	80 %	±3 %
Air flow-rate	3 L/min	±0.2
Light conditions	darkness	-

Table 1.- Culture conditions of the pilot reactor for its start-up phase.

The normal action of the pH control is to add base (sodium carbonate solution, with a concentration of 300 g/L) to compensate for the acidification of the medium due to the bacterial degradation. To maintain the pH in an appropriate range, the frequency and the duration of the additions could be variable. In these experiments, the changes in these parameters were performed manually, but considering the information extracted from the previous experiments. In future applications, and as an improvement in the system but not strictly necessary, it could be possible to configure an adaptive variation of the frequency and the duration of the addition depending on the ammonium input load. This control could act using either a prefixed or programmed relation between the key variables.

The dissolved oxygen control at the beginning of the reactor continuous operation was done using as measured variable an arithmetical mean of the measurements (readings) of the two probes, situated in the top and bottom sections of the reactor. However, after some time of operation, the behaviour of the oxygen bottom probe changed drastically, probably indicating that a biofilm of cells had been attached onto the membrane surface. Although the measurement of the probe could not correspond to the real value in the reactor, considering the oxygen depletion from the cells located at the membrane, the arithmetic mean or whatever weighted measure seemed not to be suitable to perform the control of the dissolved oxygen in the liquid phase. Due to this fact, the dissolved oxygen control was changed, using directly as measured variable the dissolved oxygen in the top section of the pilot reactor.

The evolution of the nitrate, nitrite and ammonium concentrations during the operations of the reactor was determined (**table 2**) from sample taken at its exit flow, using the analytical methods described in Appendix of TN 37.510. 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.

Once some of the cells were attached onto the beads and consequently the biofilm formation had started his development, the ammonium input load was progressively increased, by augmentation of input liquid flow-rate, until to reach a residence time of 5 h (18.4 L/day) (**figures 1 and 2**). To corroborate the satisfactory biofilm proliferation and bioreactor stability between stages it was observed that the ammonium conversion was quite complete during several residence times (97 - 99 %) (externally, the beads had been changing their colour, thus showing that cell attachment was occurring).

The last test concerning ammonium input load changes was to increase the input medium concentration from 0.3 to 0.6 g/L $N\text{-}NH_4^+$ (**figure 3**).

By means of these consecutive steps, the ammonium load to the pilot nitrifying column was increased 20 times with respect to its initial values, reaching levels comparable to these processes running with non-axenic systems (Garrido, 1996), always with high percentage of ammonium degradation and very low levels of nitrite

accumulation. It should also be emphasised that reactor stability is an important aspect to demonstrate in the operation of this reactor, each steady-state operation was maintained during a reasonably long period, from 25-40 residence-times.

In figure 1, it can be observed the long time required for the start-up of the system, allowing finally to consume all the ammonium load to the reactor without any nitrite accumulation. For this first time that the pilot reactor was operated this period was 90 days. After this period, the reactor operation was very stable, after 25 residence times (36 days) at steady state the liquid residence time was decreased from 35 to 20 h, and non major change in the concentration profiles were observed, thus, the reactor also operated very satisfactorily. This operation was maintained for 35 residence times (29 days).

The change at the residence time number 65 (from θ = 35 h to θ = 20 h) is not abrupt enough to be detectable (to have effect) at the level of cell metabolism behaviour. That is to say that, in spite of the change, the cells are able to adapt quickly to the new load, in such a way that the total conversion is maintained, in a global level.

In figure 2, the concentration profiles corresponding to the evolution of the reactor operation when the residence time is decreased from 10 to 5 h (the input medium flow-rate change from 9.2 L/day to 18.4 L/day) are showed. It can be observed that in this occasion, the reactor was under unsteady operation for a certain period, with non-complete metabolization of ammonium, and the accumulation of nitrite up to 6 ppm. After 10 residence times steady-state operation, with complete ammonium removal was obtained, and maintained for 30 residence times (6 days). It is interesting to point out that the same conditions of load where also investigated in the bench nitrifying reactors (as reported in TN 43.41). In this case, the operation of the reactor after the change in the residence time never reached complete ammonium conversion (as shown **figure 4**) probably showing the important and limiting role of oxygen in the conversion. Indeed, in the pilot reactor the oxygen level is controlled by enrichment of pure oxygen in the gas phase, while in the bench columns only a constant air flow rate is maintained. The results obtained comparing both experiments are presented in **table 3**.

Time (days)	Number of residence times	g N-NH_4^+ / L	g N-NO_2^- / L	g N-NO_3^- / L	g total N / L	N-balance%
0	0	0.318	0.0	0.056	0.374	--
2	1.37	0.150	0.001	0.148	0.299	100
15	10.29	0.298	0.0	0.002	0.300	100
20	13.71	0.298	0.0	0.005	0.303	101
32	21.94	0.273	0.0	0.003	0.276	92
43	29.49	0.021	0.079	0.193	0.293	98
50	34.29	0.003	0.034	0.283	0.320	107
56	38.40	0.003	0.0	0.297	0.300	100
63	43.20	0.002	0.001	0.301	0.304	101
77	52.92	0.002	0.001	0.304	0.307	102
83	58.37	0.004	0.002	0.300	0.306	102
86	61.97	0.003	0.002	0.309	0.313	104
89	**65.12**	**0.002**	**0.001**	**0.309**	**0.312**	**104**
92	68.43	0.002	0.001	0.301	0.304	101
96	73.23	0.001	0.001	0.298	0.300	100
103	81.63	0.002	0.001	0.298	0.301	100
111	91.23	0.001	0.001	0.302	0.304	101
115	**96.43**	**0.002**	**0.001**	**0.308**	**0.311**	**104**
127	120.43	0.001	0.002	0.299	0.302	101
128	122.03	0.001	0.002	0.290	0.293	98
132	141.23	0.001	0.001	0.293	0.295	98
135	148.43	0.001	0.0	0.296	0.297	99
137	**153.23**	**0.001**	**0.0**	**0.279**	**0.280**	**93**
137	154.63	0.050	0.006	0.220	0.275	92
138	157.38	0.057	0.006	0.207	0.270	90
138	158.93	0.036	0.005	0.237	0.278	93
139	162.56	0.009	0.003	0.274	0.286	95
140	167.56	0.004	0.004	0.266	0.274	91
143	181.96	0.002	0.001	0.286	0.289	96
144	188.01	0.001	0.001	0.282	0.284	95
145	191.56	0.001	0.001	0.271	0.273	91
146	196.36	0.002	0.001	0.267	0.270	90
157	249.16	0.001	0.001	0.301	0.303	101
159	**258.76**	**0.001**	**0.001**	**0.307**	**0.308**	**103**
159	259.86	0.001	0.001	0.455	0.457	76
160	263.46	0.002	0.001	0.560	0.563	94
163	277.86	0.001	0.001	0.566	0.568	95
164	280.26	0.0	0.001	0.586	0.587	98
165	286.06	0.0	0.001	0.580	0.581	97
166	290.86	0.001	0.002	0.576	0.579	97

Table 2.- Ammonium, nitrite and nitrate concentrations in the start-up of the pilot reactor. The changes in the reactor input load are indicated in the table using marked cells.

The change at residence time 96 (from θ = 20 h to θ = 12 h) has not been characterised in a complete form, because in the first samples after the change, the reactor had a good and quick response, degrading the NH_4^+. Thus to progress in these long experiments, it was decided to increase (next step) the load in the reactor (θ = 10 h).

The oxidation of ammonium 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 "wash-out", or other operational problems in the biological reactor.

Nitrosomonas

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

Nitrobacter

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

Overall process:

$$NH_4^+ + 1.86\ O_2 + 1.98\ HCO_3^- \text{ --->} 0.020\ C_5H_7NO_2 + 0.98\ NO_3^- + 1.88\ H_2CO_3 + 1.04\ H_2O$$

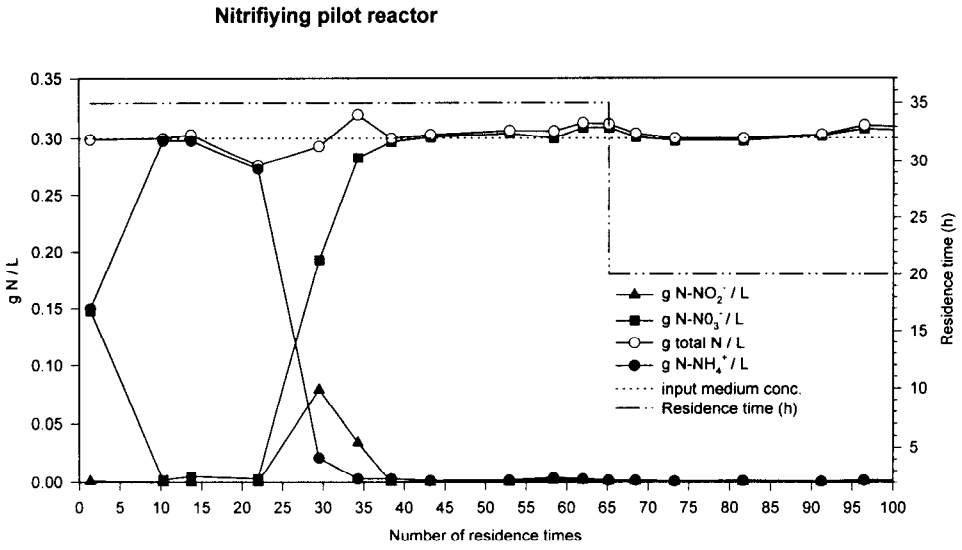

Figure 1.- Evolution of ammonium, nitrite and nitrate concentrations in the pilot reactor.

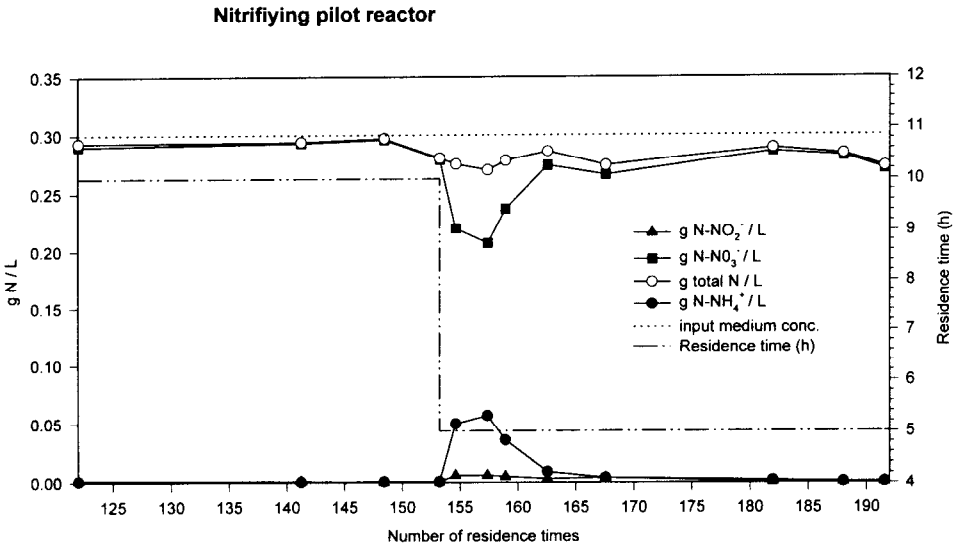

Figure 2.- Evolution of ammonium, nitrite and nitrate concentrations in the pilot reactor. Step in the dilution rate.

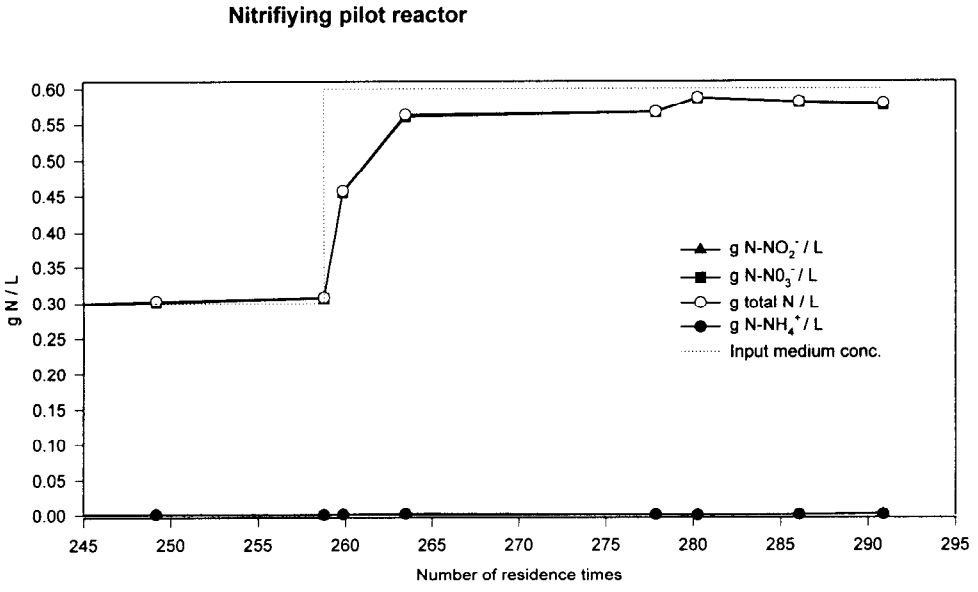

Figure 3.- Evolution of ammonium, nitrite and nitrate concentrations in the pilot reactor.

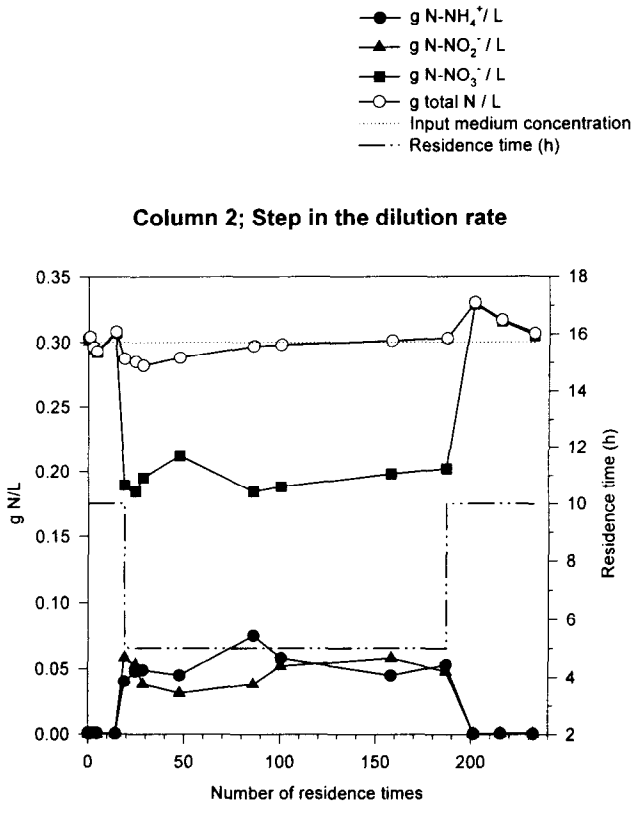

Figure 4.- Evolution of ammonium, nitrite and nitrate concentrations in one of the bench columns.

Reactor	Aeration	Residence Time (h)	Input conc. g/L $N\text{-}NH_4^+$	Removal efficiency (%)	Remained $N\text{-}NO_2^-$ (%)
Pilot column	Automatic control (pure oxygen) ; 0.8 vvm	10	300	99.7	0.3
		5	300	99.7	0.3
Bench column	Constant input flow-rate (air) ; 0.08 vvm	10	300	96	0.3
		5	300	67	15.7

Table 3.- Comparison between pilot and bench scale reactors during a step on ammonium load.

Stoichiometric equation (page 12 of TN 37.420), presented for the nitrification overall process is a theoretical equation. In fact, the biomass elemental composition is

only a bibliographic value, and of course, the stoichiometric coefficients are only a first approach.

In respect to the experimental value of conversion into nitrate, it would be interesting to note that having into account the experimental error of analyses of determination of ammonium, nitrite and nitrate concentrations, the conversion value can oscillate in the range 95-105%.

Table 4 is presented as a summary of results of different steady states reached in the pilot reactor at different ammonium input loads. The results presented in table 3 and 4 shows that nitrification with immobilized cells is mainly controlled by mass transfer of oxygen, in particular between gas and liquid phase. If enough oxygen is provided to the liquid phase nitrification conversion was always between 95-100% in both reactors for the range of ammonium load studied, showing that the proposed bioreaction system is a good alternative to be implemented as the Compartment III in the MELiSSA loop with high conversion and stability for long term operations.

Nevertheless, it is important to note that the sterilisation is a critical point for the success of the culture, mainly, due to the fact that the biomass support of the packed-bed is non sterilisable by heat, and this makes the sterilisation as a long and risky process with really high percentage of possibilities of suffering a cell contamination in the axenic co-culture. Other aspect that could be significant to outline is the slow start-up of this kind of co-cultures, this fact makes increase the importance of the consequences that an accident in the operation of the reactor can cause.

Residence time (h)	Air flow-rate (ml/min)	Input conc. g/L N-NH_4^+	Ammonium input load (kg/(m^3·h))	Ammonium output load (kg/(m^3·h))	Removal efficiency (%)
35	3000	0.300	0.0125	$3 \cdot 10^{-5}$	99.7
20	3000	0.300	0.015	$5 \cdot 10^{-5}$	99.7
10	3000	0.300	0.030	0.0001	99.7
5	3000	0.300	0.060	0.0002	99.7
5	3000	0.600	0.120	0.0002	99.7

Table 4.- Steady states reached in the pilot reactor. The removal efficiency varies slightly in every steady state, but the variation is produced in the second decimal, which is not shown in this table.

The goal of the experiments was to increase the ammonium load level to know what was the maximal value that the reactor could degrade. In this sense, the followed strategy during all the set of experiments was to increase, in a progressive way, the load, increasing the dilution rate (decreasing the residence time). For this reason experiments decreasing the ammonium load were not performed (on the other hand, these experiments do have been performed in the bench columns, obtaining the same results of conversion at the same conditions of dilution rate).

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