

Changes in the Nutrients from a Dairy Wastewater in Batch-Reactor at High pH and Organic Load

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Dairy effluents comprise mainly milk, alkaline washing wastes, and water. In accordance with the characteristics of this type of industrial wastewater, a biological treatment was applied in a batch reactor. This work is centred on the use as fertilizers of nutrients from dairy wastewater, and studies the effect that the time of an initial anoxic stage has on mineralization of the organic matter of a dairy wastewater. The experiment was carried out using milk and water mixtures from the secondary sedimentation tank of a wastewater treatment plant (WWTP) that were inoculated with micro-organisms found naturally in wastewater (adapted to 30 °C) and in activated sludge systems. The operational conditions were 4000 mg L⁻¹ COD, alkaline pH of 11.5, and temperature of 30 °C.

Six experiments were performed, varying only the period without initial aeration. The results are discussed in two stages:

1 *Degradation of the organic matter.* The organic load is reduced and mineralized, liberating ammonium and phosphate. This stage lasts approximately 10 days. It is affected by the period without initial aeration; highest degradation is achieved using periods of 48 hour without initial aeration, ensuring a greater microbial acclimation and hydrolysis of the organic matter. In contrast, NH₄⁺ liberation is independent of whether aeration is applied from the start or after 6 or 48 hours. In no case was it advantageous to use a period of 10 hours without initial aeration.

2 *Nitrification.* There is hardly any organic matter, and the ammonium is nitrified by nitrifying bacteria. Nitrification is not affected by the conditions of a prior stage without initial aeration, but it is affected by light, pH, and the concentration of phosphorus. Highest nitrate concentrations are obtained when the concentration of phosphorus in the medium is highest and pH is lowest. pH affects both the solubilization of calcium phosphate precipitated and the phosphate-phosphate monoacid balance.

Keywords:

Anoxic/aerobic system, aerobic biological treatment, batch reactor, bio-mineralization, dairy industry, nitrification.

Introduction

Dairy effluents have an alkaline pH value (6.6–12.2) and are rich in organic matter, 4000 mg L⁻¹ COD (Gonzalez et al, 1982).¹ This type of industrial wastewater contains milk and, therefore, ammonium (from the amino acids) and phosphate (from the caseins). Consequently, these components are a factor in eutrophication of natural resources. Some scientists (Blankenship, 1993)² believe that the presence of nitrogen could increase the reduction of phosphorus in fresh water. Phosphorus encourages the growth of nitrogen-consuming algae. When the phosphorus is removed, algal growth does not take place and the residual nitrogen remains in the water. Nevertheless, this nutrient would reach the sea, where it is available for other algae, and the eutrophication would take place there. It has been found (Neethling, 1997)³ that

wastewater treatment plants are responsible for eutrophication of natural resources. The ammonium content of effluents from wastewater treatment plants will therefore have to be reduced in the next few years. A solution for the problems arising from the two types of wastewater could be sought by testing a mix of them.

This work is aimed at taking advantage of these nutrients in the wastewater by isolating them and using them as fertilizers. Several experiments have been carried out in batch reactor, studying the purification of this type of industrial wastewater by active sludge treatment with an anoxic/aerobic stage (varying the duration of the anoxic stage), at alkaline pH 11.5, and at a temperature of 30 °C. The ammonium and the phosphate present has been monitored to establish the optimal duration of the anoxic stage for ammonification and nitrification of

the organic matter. At the same time, a good purification is achieved. The advantage of working at this pH is that it provides good yields in reduction of the organic matter without the use of acids for neutralization. Municipal wastewater from the secondary sedimentation tank of a wastewater treatment plant was used to inoculate the dairy effluents. Nitrification was accomplished by enriching the medium with other types of micro-organism.

Materials and methods

Artificial wastewater and inoculum

Dairy wastewaters comprise mainly milk (responsible for their oxygen demand), as well as remains of cleaning agents (responsible for their high alkalinity). The experiment was carried out using milk and water mixtures from the secondary sedimentation tank of a wastewater treatment plant (WWTP), to avoid thick solids and to obtain a municipal wastewater rich in micro-organisms. The substrate for the micro-organisms was milk/water mix, adjusted to 4000 mg L⁻¹ COD because the dairy effluent has an organic matter value equivalent. The medium was inoculated with a culture prepared from dairy effluents, incubated at 30 °C for five days at work conditions. The initial pH value was 11.5, chosen because dairy effluents are alkaline due to the presence of washing products (Gonzalez et al, 1982),¹ and the maximum rate of deamination of the organic matter by alkaline hydrolysis of proteins takes place at this pH (Guillen-Jimenez et al, 2000),⁴ following nitrification. The operational temperature was maintained at 30 °C, optimal for nitrification of dairy effluents (Alvarez-Mateos, 1998).⁵ The same operational conditions were used for the six experimental series, differing only in the duration of the anoxic stage (from 0 to 48 hours without aeration).

Control of variables

Changes in organic content of the samples were followed via the chemical oxygen demand (COD) at each moment, and variations caused by reactions in the medium were monitored via pH. Mineralization of the organic matter was monitored by analysis of ammonium, nitrites, and nitrates. All analytical methods followed the procedures outlined in Standard Methods (APHA, 1980 and 1998)⁶; analyses were performed for pH (pH-meter, 423), ammonium-N (Nesslerization, 417 A and 417 B), nitrite-N (spectrophotometry, 419), nitrate-N (spectrophotometry, 418), COD (dichromate reflux method, 508), and phosphate (ascorbic acid method). The results are given for N-NH₄⁺, N-NO₂⁻

and N-NO₃⁻ as mg L⁻¹ N, PO₄³⁻ as mg L⁻¹ P and mg L⁻¹ O for COD and pH as pH units, dissolved oxygen (DO, membrane electrode method, 4500-G) and Total Solid (TS, Total, fixed and volatile solids in solid and semisolid samples 2540 G).

Equipment

The assembly, Figure 1, consisted of six borosilicate glass reactors thermostated of 12-litre (arranged in two columns, each of three rows) fitted to a metal support anchored to the floor. The reactors were thermostated and stirred by air injection. Air was supplied through a membrane pump fitted with a filter to prevent entry of particles from the environment. Each reactor was installed with such pump. The reactor had three outlets at the bottom – an aeration mechanism was placed in each of the two side outlets and the samples were collected through the central one. The cover of the reactor was provided with an opening, filled with water-repellent cotton wool, to permit the escape of gases produced by the metabolic reactions taking place in the medium. Three of the four walls of the room containing the reactors were covered with black plastic to prevent the inhibition of nitrifying micro-organisms by light. Experiments were monitored by daily sampling.

Results and discussion

The results are discussed in two stages:

1 *Degradation of the organic matter*, when the organic load is reduced and mineralized, liberating ammonium and phosphate. This stage lasts approximately 10 days.

2 *Nitrification*, when there is hardly any organic matter and the ammonium is nitrified by nitrifying bacteria.

1st stage: degradation of organic matter

Table 1 shows the conditions at the start of each experiment and at the start of aeration.

It can be seen that as the time without aeration increases, the mass concentrations of dissolved oxygen (DO) in the medium decrease, while the levels of pH, COD and total solids (TS) remain practically unaltered. This is because in the hydrolytic stage the largest molecules are cleaved and reduced to progressively smaller ones, facilitating their assimilation, but without appreciable consumption. Thus, the COD values are similar (Barker and Dold, 1997)⁷. The only source for the appearance of NH₄⁺ in the medium is degradation of the organic matter to soluble forms followed by ammonification. Such reactions take place thanks to the activity of

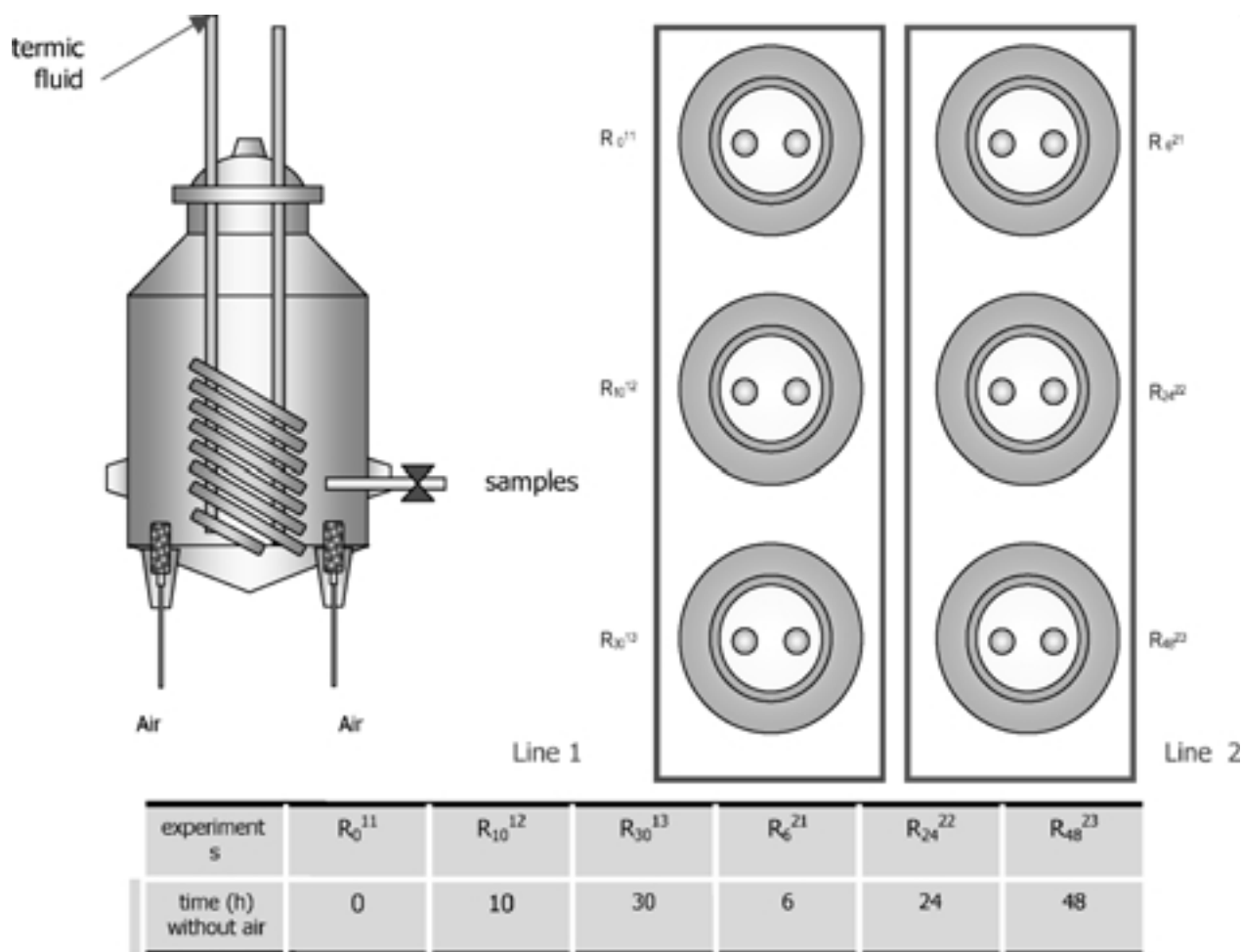


Fig. 1 – Scheme of the assembly for the wastewater treatment

Table 1 – Working conditions

Time t/h			Initial conditions				Conditions at the start of aeration				
Assay	Time t/h without air	Temperature $T/^\circ\text{C}$	pH	COD $\gamma/\text{mg L}^{-1}$	NH_4^+ $\gamma/\text{mg L}^{-1}$	TS $\gamma/\text{mg L}^{-1}$	DO $\gamma/\text{mg L}^{-1}$	pH	COD _i $\gamma/\text{mg L}^{-1}$	NH_4^+ $\gamma/\text{mg L}^{-1}$ N	TS $\gamma/\text{mg L}^{-1}$
R_0^{11}	0						5.9	11.79	3847	13.4	2915
R_6^{21}	6						5.8	11.68	3755	11.7	2910
R_{10}^{12}	10						5.3	11.68	3847	7.4	–
R_{24}^{22}	24	30.0	11.79	3847	13.4	2915	4.8	11.59	3847	7.4	3000
R_{30}^{13}	30						3.9	11.63	3877	7.7	3070
R_{48}^{23}	48						2.8	11.53	3633	7.6	2880

NO_2^- , NO_3^- and PO_4^{3-} could not be determined because the samples were initially turbid

heterotrophic organisms, independently of the available oxygen concentration. The decrease in NH_4^+ concentration when the supply of air is delayed is associated to losses as NH_3 (gas), given the conditions of high pH and temperature (Ferrara and Dimino, 1985⁸; Lin and Wu, 1996⁹) in the medium.

The experiments lasted 90 days, and were aimed at studying nitrification. In each assay, the start of aeration was taken as day 0, in order to estimate and compare the effect of the initial stage without air. The changes in COD, pH, DO and ammonium (Figure 2) are similar in all the assays. DO increases from a minimal value (due to degradation

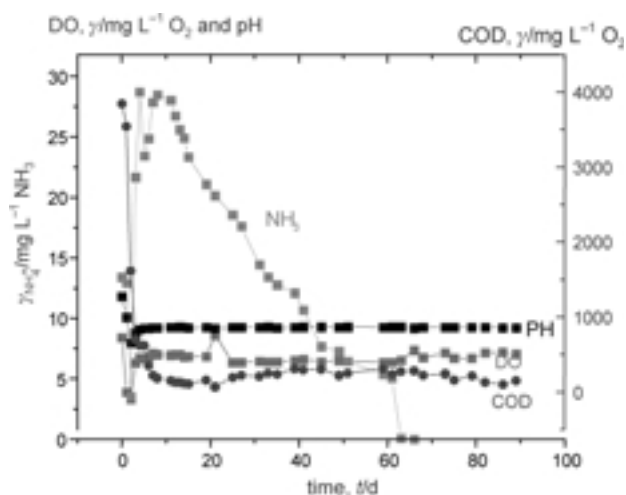


Fig. 2 – Evolution of ammonium, COD, pH, and DO in the experiment with initial aeration

of organic matter and its microbial consumption) and stabilizes from the fifth day at around 7 mg L⁻¹ O in all the assays. This guarantees perfectly aerobic conditions. COD decreases sharply at the start of aeration, and then falls exponentially, with 96 and 92 % of total COD degradation being reached after 10 days. During the stage without aeration, the pH of the medium hardly changes (Table I). However, when aeration begins, pH decreases sharply, and reaches values of around 7.5 units (Figure 2) before stabilizing at above 9. This decrease is associated to the appearance of organic acids in the medium, coming from degradation of the organic matter. The time of the minima varies, depending on when air is introduced. Consequently, it can be stated that during the stage without aeration, conditions of both COD and pH are highly unfavourable for the micro-organisms, which are hardly active. However, the supply of air stimulates their activity. Ammonium behaviour is also seen as various stages:

I. Assimilation: the initial free ammonia remains either practically constant, or decreases slightly, during the time when aeration is not applied, being lost as ammonia as a result of the high pH values of the effluent. However, from the moment when air is introduced, NH_4^+ is assimilated by the micro-organisms. Consumption continues until a minimum of air supply is reached after 24 or 48 hours. This minimum, as mentioned before, coincides with the lowest pH values.

II. Ammonification of organic matter, permitting the release of ammonium: after the minimum point, the protein organic matter begins to be degraded, and NH_4^+ is liberated. This liberation reaches its peak between 3 and 5 days. Depending on the time of the initial anoxic stage, some differ-

ences are observed in the deamination of nitrogen compounds (1st step). Heterotrophic micro-organisms, responsible for this process, have to adapt to the medium, developing systems to buffer pH, which is, under these conditions, the operational parameter unfavourable for their growth. In the first step micro-organisms are affected. The first step is the best for this type of micro-organism, whereas an alkaline hydrolysis of organic matter, favouring the release of ammonium, takes place in the other two (Guillen-Jimenez, et al, 2 000).⁴

III. A new stage of consumption: there is a further decrease in concentration; now microbial nitrification reactions are taking part preferentially.

The rates of deamination of ammonium depend on the initial conditions – that is, on the length of the stage without air supply. The rates increase as the period without initial aeration lengthens (except when the period is 10 hours). In contrast, the rate of assimilation, as that of increase in pH, is practically constant in this stage. There is a slight increase with delay in aeration, values being slightly higher in the experiment of 6 hours without initial aeration. Again, R_{10} ¹² presents the lowest values.

In all the experiments, the system is activated by introduction of the air. In the prior stage without aeration, the micro-organisms try to adapt to the highly unfavourable conditions of the medium, at the same time as there could be loss of NH_4^+ as NH_3 (gas).

For the highest yields of organic matter degradation, periods without initial aeration of 48 hours should be used, ensuring a better microbial acclimation and a greater hydrolysis of the organic matter. In contrast, liberation of NH_4^+ is independent of whether aeration is carried out from the start or delayed for 6 or 48 hours. In no case was there advantage in working with a delay of 10 hours in aeration (F. Carta-Escobar et al, 1999)¹⁰.

2nd stage: nitrification

Figure 3 shows the evolution of the nitrogen and phosphorus compounds along the tests carried out without aeration during 6 hours (R_6 ²¹), 10 hours (R_{10} ¹²) and 24 hours (R_{24} ²²). Nitrifying bacteria are found in most aerobic environments where organic matter is mineralized. During the nitrification phase two reactions could be perceived: oxidation of ammonium to nitrite, and oxidation of nitrite to nitrate. These processes were simultaneous but at different rates. The factors affecting nitrification are (apart from pH and temperature) the concentrations of oxygen and phosphates. The concentration of dissolved oxygen is practically constant in all the assays, at around 6 mg L⁻¹ O₂, and as this amount is

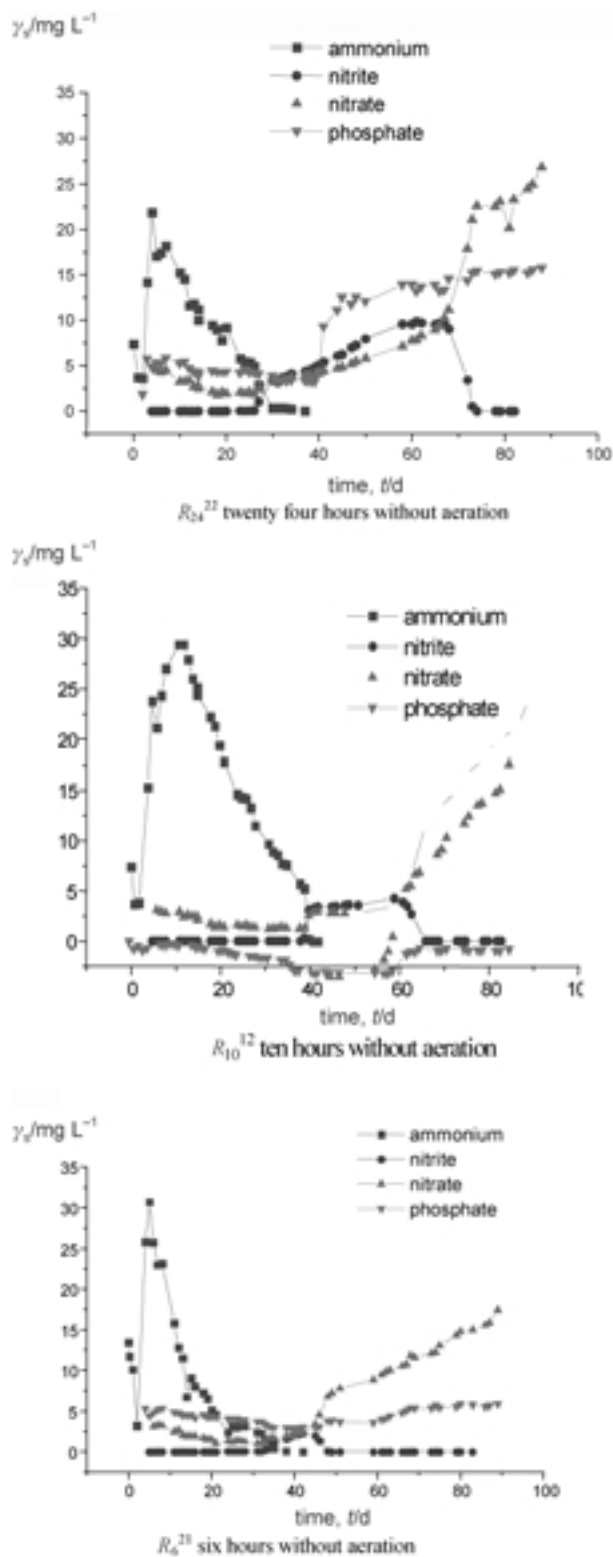


Fig. 3 – Evolution of ammonium, nitrite, nitrate and phosphate in experiments R_6^{21} , R_{10}^{12} and R_{24}^{22}

sufficient, oxygen is not a variable to be taken into account.

Conversion of ammonium into nitrites by nitrifying organisms. The ammonia-oxidizing bacteria have been limited to strains of *Nitrosomonas*, al-

though similar results would be obtained with other genera (*Mossakowska*, 1997)¹¹. In this first stage of nitrification, the nitrites do not begin to appear until the ammonium has almost disappeared.

A kinetic study was performed (Figure 2) in the stage of ammonium disappearance, as researchers have quite often tried to fit their data to a zero-, first-, or second-order model, or sometimes a model with a fractional order. The general rate law forms the basis for such models. For disappearance of a compound this is

$$r = -\frac{d\gamma_s}{dt} = k \cdot \gamma_s^n$$

in which r is the rate of the reaction, γ_s the mass concentration of the reactant (ammonium concentration, $\text{mg L}^{-1} \text{N}_2$), k the rate constant, and n the order of the reaction. There are two methods to determine the dependence of r on reactant concentration: the differential method and the integral method (*Laidler*, 1987¹²; *Hill and Grieger-Block*, 1980¹³). For a first-order reaction and using the integral method, the equation integrated with respect to time is

$$\gamma_s = \gamma_{0s} + \gamma_{\text{NH}_4^+, \text{max}} \cdot e^{-\frac{(t-t_0)}{b}}$$

$\gamma_{\text{NH}_4^+, \text{max}}$ is the maximum mass concentration of ammonium when t is equal to t_0 , and equal to the height, which represents the maximum concentration of ammonium $\gamma_{\text{NH}_4^+, \text{max}}$, t_0 is the peak at time (t_{max}) required to give $\gamma_{\text{NH}_4^+, \text{max}}$, b is the width.

Table 2 shows the value of each constant in the kinetic equation, the value of the integral of the curve, the peak height, and the correlation coefficient (R^2). In the interpretation of this equation, the lower the value of b and t_0 , the lower will be the value of the integral (the area below the curve), and the higher will be the rate of this first stage in the nitrification.

In general, the values of R^2 fit sufficiently well to first-order kinetics (decreasing exponential). To reveal which experiment gives the highest rate, we divide t_0 by height to represent $\gamma_{\text{NH}_3, \text{max}}/t_{\text{max}}$. Figure

Table 2 – Kinetic constant values and statistics data

	$\gamma_{\text{NH}_4^+}$	t_0	R^2	b (width)	Height, h	Area, A
R_0^{11}	31.00	11	0.900	15.50	31.00	604.82
R_6^{21}	30.05	5	0.933	8.47	30.05	312.95
R_{10}^{12}	50.82	11	0.901	28.48	30.38	662.45
R_{24}^{22}	21.65	5	0.902	13.59	21.65	306.05
R_{30}^{13}	69.62	13	0.899	40.60	25.76	623.75
R_{48}^{23}	27.43	5	0.991	12.56	27.43	389.25

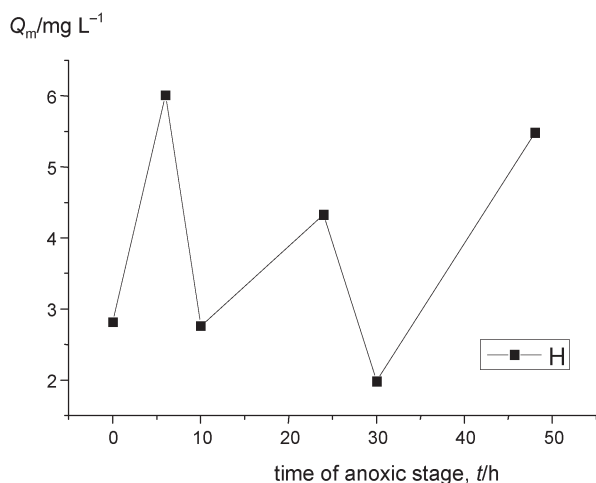


Fig. 4 – Maximum mass flow rate of ammonification at condition without aeration

4 plots this ratio against the number of hours without initial aeration. It can be seen that the experiment with the highest rate is that of 6 hours without aeration initial. Three of the six experiments have a high nitrification rate, which might be due to the effect of light, even though the room containing the reactors was 3/4 covered with black plastic: reactors R_0^{11} , R_{10}^{12} and R_{30}^{13} (line 1) were closest to the uncovered 1/4, and this row showed a slight inhibition caused by the effect of the light (Alleman, 1987¹⁴ and Holt et al, 1994¹⁵).

Conversion of nitrites into nitrates by nitrifying organisms (Nitrobacters). As can be seen in Figure 3, the nitrites begin to appear once the ammonium is consumed (the ammonium is consumed first). In the experiments with a little light, the nitrites do not begin to appear until day 30, while in the experiments exposed to greater light intensity, 40 days was required for nitrification.

The experiments where the nitrites begin to appear earliest are those in which the ammonium disappears earliest, confirming that in the later stages, the initial conditions have no effect, and the only effect is from the conditions of the medium: pH, DO, and con-

centration of phosphates. As the rate of oxidation of ammonium to nitrite (Mossakowska, 1997)¹¹ is much higher than that of oxidation of nitrite to nitrate, nitrites accumulate in the medium (Figure 3). Mossakowska states that the nitrite was accumulated during the nitrification process as long as there was ammonium present; after depletion of ammonium nitrogen, the concentration of nitrite began to diminish. This did not happen in any of our experiments.

The accumulated nitrite remained in the medium some 20 days, possibly due to the adverse conditions of pH, which stabilized at between 9.2 and 9.4 units, not optimal for nitrobacter growth (Groeneweg, 1994)¹⁶.

In such conditions, the concentration of NH_3 (g) – the main inhibitor of nitrifying bacteria – increases. In order to test this, the pH was lowered to 7.5 in assay R_{24}^{22} 40 days after the start of aeration. Table III shows the final values of the quantities COD_t , pH, NO_3^- and PO_4^{3-} . The concentrations of ammonium and nitrites at the end of the experiments were zero. The rates of nitrite disappearance ($-r_{\text{nitrites}}$) and nitrate formation (r_{nitrates}), were also calculated. As can be seen in the Table, the experiment with highest concentration in nitrates and phosphates is that in which pH was lowered (R_{24}^{22}). Figure 3 shows that the concentration of phosphates in the medium began to increase immediately as pH was lowered. This could be due to solubilization of calcium phosphate.

Under aerobic conditions, calcium phosphates are precipitated when pH values are around 8, so that lowering the pH solubilizes the phosphates (Zoltek, 1976;¹⁷ Arvin, 1979¹⁸ and Lan, 1983¹⁹) and their concentration increases. This makes the rate of nitrification (the step from nitrite to nitrate) higher than in the other experiments, because the nitrifying bacteria require phosphorus, among other nutrients. Phosphates are a controlling factor in the nitrification process (Shammas, 1986).²⁰ According to Mossakowska, the rate of nitrite disappearance should be equal to the rate of nitrate formation. However, it can be seen from Table 3 that this is

Table 3 – Concentrations at the end of the experiments and rates of nitrite consumption and nitrate formation

Assay	pH	COD_t $\gamma \text{ mg L}^{-1} \text{ O}$	NO_3^- $\gamma \text{ mg L}^{-1} \text{ N}$	PO_4^{3-} $\gamma \text{ mg L}^{-1} \text{ P}$	$-r_{\text{nitrites}}$	r_{nitrates}
R_0^{11}	9.2	99	23.48	6.03	-0.36	0.82
R_6^{21}	9.3	162	17.44	5.88	-0.65	1.12
R_{10}^{12}	9.2	130	23.87	5.46	-0.63	1.45
R_{24}^{22}	6.7	166	26.83	15.77	-1.55	1.77
R_{30}^{13}	9.3	236	14.32	4.62	-0.30	0.41
R_{48}^{23}	9.3	300	25.76	5.87	-0.29	0.79

true only in assay R_{24} ,²² in which pH was lowered to 7.5, stabilizing at the end of the experiment at 6.7, the optimum pH for the Nitrobacters (*Hofman and Less*, 1953)²¹. In the other experiments, the rate of nitrate appearance is approximately double to the rate of nitrite disappearance. This could be, because at these pH values (between 9.2 and 9.3) nitrogen exists in the medium in other inorganic forms (such as hyponitriles, dihydroxyammonium, nitroxyl, nitric oxides, nitrohydroxylamines) (*Nowak*, 1995)²², undetected by the analytical methods used. This can be confirmed from Figure 3, showing that in almost all the experiments, the nitrate concentration continues to increase even when there are no nitrites in the medium.

Conclusions

A stage without aeration at the start of the process does not affect the performance of the reactor, but it does affect the rate of deamination and assimilation of nitrogen by micro-organisms. It is not advantageous to work with a stage of 10 hours without initial aeration.

In the nitrification stage, conditions of the initial stage without aeration have no effect, but light, pH, and concentration of phosphorus do. The highest concentrations of nitrates are obtained when the concentration of phosphorus in the medium is highest.

pH affects, both, solubilization of calcium phosphate formed and the phosphate-phosphate monoacid balance.

In order for a rapid nitrification without accumulation of nitrites in the medium, there should be a good concentration of DO (to prevent the formation of nitrosamine and nitrous oxides that inhibit the process), a pH of between 7.5 and 8.9, and an adequate concentration of phosphates.

References

1. *González, J. A., García, A., and Romero, F.*, *Agroquímica, Tecnología de alimentos* **22** (4) (1982) 501.
2. *Blankenship, K.*, *Water Environment and Technology* **6** (1993) 19.
3. *Neethling, J. B., Pivetti, D., Giuliano, L., Bolding, D., Spani, C., Willey, B.*, *Water Environment and Technology*, October, 1997, pp. 34–40
4. *Guillen-Jimenez, E., Alvarez-Mateos, P., Romero-Guzmán, F. and Pereda-Marin, J.*, *Water Research* **34** (4) (2000) 1215.
5. *Alvarez-Mateos, P., Leon-Morillo, A. and Guillén-Jimenez, E.*, *Tecnología del Agua*, **174** (1998) 61.
6. APHA, AWWA, WPCP., *Standard Methods for examination of water and wastewater*. 15th Edition. American Public Health Assoc. Washington D.C., 1980 and 1998
7. *Barker, P. S. and Dold, P. L.*, *Water Environment Research* **69** (5) (1997) 985.
8. *Ferrara, R. A. and Dimino, M. A.*, *J. Water Pollut. Control Fed.* **57** (7) (1985) 763.
9. *Lin, S. H. and Wu, C. L.*, *Industrial & Engineering Chemistry Research* **35** (2) (1996), 553.
10. *Carta-Escobar, F., Alvarez-Mateos, P., Romero-Guzman, F. and Pereda-Marin, J.*, *Jornadas Internacionales de Ingeniería Ambiental (99-ICEE)*. 9 y 10 de Septiembre. Cartagena (Murcia), Spain, 1999.
11. *Mossakowska, A., Reinius, L. and Hultman, B.*, *Water Environment Research* **69** (6) (1997) 1128.
12. *Laidler, K. T.*, 3rd ed. Harper and Row, New York, 1987.
13. *Hill, C. G. and Grieger-Block, R. A.*, *Food Technology* **34** (2) (1980) 56.
14. *Alleman J. E., Keramida V. and Pantea-Kiser L.*, *Water Research* **21** (1987) 499.
15. *Holt, J. G., Krieg, N. R., Sneath, P. H., Staley, J. T. and Williams, S. T.*, Ed. Williams & Wilkins, Maryland, USA, 1994
16. *Groeneweg, J., Beate, S., Tappe, W.*, *Water Research* **28** (1994) 2561.
17. *Zoltek, J.*, *J. Water Pollut. Control fed.* **48** (1976) 179.
18. *Arvin, E.*, The influence of pH and calcium ions upon phosphorus transformations in biological wastewater treatment plants. In *Kinetics of Wastewater Treatment* (Edited by Jenkins S. H.). Pergamon Press. Oxford, 1979
19. *Lan, J., Benefield, L. and Randall, C.*, *Water Research* **17** (9) (1983), 1193.
20. *Shammas, N. Kh.*, *J. Water Poll. Control Fed.* **1** (1986) 52.
21. *Hofman, T. and Less, H.*, *Biochem. J.* **54** (1953) 579.
22. *Nowak, N., Svardal, K. and Schweighofer, P.*, *Water Science Technology* **31** (2) (1995) 115.

