

## Influence of Inoculum and Initial pH on Dairy Effluent Biodegradation and Mineralization

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Dairy effluents are constituted mainly by milk, alkaline washing wastes, and water. In accordance too the characteristics of this type of industrial wastewater, a biological treatment was applied in a batch reactor. The process chosen was nitrification to reduce the nutrient content. In order to enhance ammonium oxidation, the medium was inoculated with municipal wastewater from a secondary clarification tank of the Ranilla treatment plant (Seville, Spain). Municipal wastewater of temperature at about 26 °C during the warmer season (September) showed higher concentrations of nitrite and nitrate. Different initial pH values (8.5, 10.5, 11.0, 11.5 and 12.0) were tested, and the organic matter content, expressed as Chemical Oxygen Demand (COD), was maintained constant at 1000 mg. The municipal wastewater was taken in two different seasons, winter (February) and summer (September), to know the effect of season on the nitrification. The results indicate a difference in the rate of the process but not in the behaviour. The reduction in COD is nearly the same in both cases, and the difference depends on the initial pH value rather than on the season of the inoculum.

### Key words:

Dairy industries, Nitrification, Biological treatment, Aerobic treatment, Batch reactor.

## Introduction

Dairy effluents have an alkaline pH value (6.6–12.2) and are rich in organic matter<sup>2</sup>. 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<sup>3</sup> believe that the presence of nitrogen could increase the reduction of phosphorus in fresh water. This latter element encourages the growth of algae which consume nitrogen. 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.

At the same time, it has been found<sup>4</sup> that wastewater treatment plants are responsible for eutrophication of natural resources. Consequently, the ammonium content of effluents from wastewater treatment plants will 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.

The aim of this paper is to study the reduction of ammonium content applying nitrification in dairy effluents in two different seasons, in winter and in summer. Municipal wastewater, from the secondary clarification tank of a wastewater treatment plant, was used to inoculate the dairy effluents. Nitrifica-

tion was accomplished by enriching the medium with other types of microorganism.

## Materials and methods

Dairy wastewaters contain mainly milk (responsible for their oxygen demand), as well as remain 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 microorganisms. Two municipal wastewater samplings were chosen, having the different weather characteristics of winter and summer, respectively. The field studies were performed in February and September 1996. The average values of the temperature parameter in these months were 12 and 26 °C, respectively. The substrate for the microorganisms was milk and water mixes, adjusted to 1000 mg l<sup>-1</sup> COD. The inoculum has a concentration of 500mgVSS/440mgCOD<sup>1</sup>. This inoculum was a mixture of 1:1 culture prepared with a dairy effluent incubated at 30 °C for five days, and a sludge from WWTP secondary clarifier.

The same operational conditions were applied for the two experimental series, differing only in the season, when the municipal wastewater inoculum was taken. Five initial pH values (8.5, 10.5, 11.0, 11.5 and 12.0), were studied. These val-

ues were chosen because the dairy effluents are alkaline due to the presence of washing products<sup>2</sup>.

The operational temperature was maintained at 30 °C, the optimal for nitrification of dairy effluents<sup>5</sup>. The experiments were carried out during 30 days, to obtain organic matter mineralization in aerobic conditions.

### Control of variables

The changes in organic content of the samples can be followed adequately through the chemical oxygen demand (COD) at each moment, while the variations caused by reactions in the medium are monitored through pH. The mineralization of the organic matter was followed by analysis of ammonium, nitrites, and nitrates. All analytical methods followed the procedures outlined by Standard Methods<sup>6</sup>, analyses were performed for pH (pH-metre, 423), ammonium-N (Nesslerization, 417 A and 417 B), nitrite-N (Spectrofotometry, 419), nitrate-N (Epectrofotometry, 418), and COD (dichromate reflux method, 508).

The results are given for N-NH<sub>4</sub><sup>+</sup>, N-NO<sub>2</sub><sup>-</sup> and N-NO<sub>3</sub><sup>-</sup> as mg l<sup>-1</sup> N<sub>2</sub> and mg l<sup>-1</sup> O<sub>2</sub> for COD and for units of pH.

### Equipment

The assembly consisted of a thermostat and five glass reactors, installed in a metallic support anchored to the floor. Borosilicate glass reactors of 12 litre were used (Figure 1), thermostated, and stirred by air injection. Air was supplied through a membrane pump, it has got a filter to prevent the incorporation of particles from the environment. Each reactor was installed with such pump. The reactor had three outlets at the bottom; an aeration mechanisms 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-repellant cotton wool, to permit the escape of the gases resulting from the metabolic reactions taking place in the medium. The reactor was covered with aluminium foil to prevent the inhibition of nitrifying microorganisms caused by light. Experiments were monitored by daily sampling.

## Results and discussion

### Effect of the initial pH value

The mineralization of organic matter takes place through three steps:

1. Ammonification of organic matter which permits the release of ammonium
2. Conversion of ammonium into nitrites by means of nitrifying organisms (*Nitrosomonas*)
3. Conversion of nitrites into nitrates by means of nitrifying organisms (*Nitrobacters*)

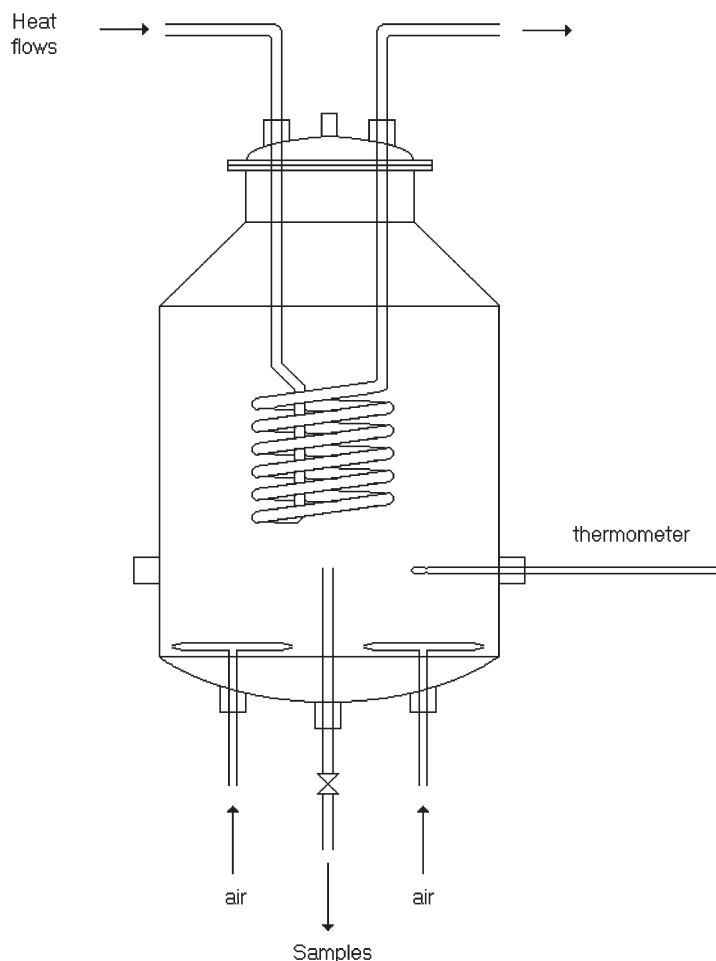


Fig. 1 - Diagram of the experimental device

Depending on the initial pH value, some differences are observed in the desamination of nitrogen compounds (1st step) to each type of inoculum. Heterotroph microorganisms, responsible for this process, have to adapt to the medium, a developing systems to buffer pH, which is the operational parameter unfavourable for their growth.

In the first step, generally, microorganisms are affected by the season of the year and pH. However, similar results were obtained in the dasamination of organic matters with both municipal wastewaters when the initial pH value were 8.5, 11.0 and 11.5 (Figure 2). It seems that, in this case, pH has a higher effect on the medium than does the season of the inoculum, and the results therefore are almost the same. The first one is the best for this type of microorganisms<sup>7</sup>, meanwhile an alkaline hydrolysis of organic matters takes place in the others two, which favours the release of ammonium<sup>8</sup>.

At initial pH values of 10.5 (Figure 2), the desamination of organis matters takes place more quickly in the experiments carried out with the wastewater taken in September.

In the case of high pH values, such as 12.0, the experiment carried out with municipal wastewater taken in February showed a faster response to the medium conditions (Figure 2). It seems that heterotroph microorganisms adapted to the unfavourable conditions, such as lower temperatures, mean while the September inoculum, and their adaptation mechanisms, are consequently more developed.

**Effect of the type of the inoculum on the nitrification**

Significant differences are obtained in degradation of nitrogen compounds when the municipal wastewater is taken from the plant at different seasons of the year (Table 1).

Because a biological reactor contains a wide variety of micoorganisms, it was believed that the variation in the composition of microorganisms was not significant through the year. However, the results showed some differences between winter and summer samples. These seasons are characterised by different weather conditions, and temperature was chosen as operational parameter because of its effects on a municipal wastewater treatment plant<sup>9</sup>. Some of these effects are the following:

- The chemical reaction rates are highly dependent on temperature. The Vant Hoff Arrhenius expression<sup>10,11</sup> shows this dependency<sup>71-72</sup>:

$$\gamma_1 = \gamma_2 \cdot \theta^{T_1-T_2} \quad (1)$$

where:

$\gamma_1$  is the reaction rate at the temperature  $T_1/^\circ\text{C}$

$\gamma_2$  is the reaction rate at the reference temperature of  $T_2/^\circ\text{C}$

$\theta$  is the temperature factor.

The factor  $\theta$  may be stimated by plotting  $\ln \gamma$  versus  $T$ , giving a slope equal to  $\ln \theta$ .  $\theta$  is dimensionless. On the other hand,  $\ln \theta$  is approximately  $0.0015 u$ , where  $u$  is the temperature factor.

- Some operational parameters, such as gas transfer rate and sedimentation characteristics of the sludge, are responsible for the good operation of the plant, and they are also dependent on temperature.

The expression (1) explains why the nitrogen compound degradation rate was higher in samples taken in September than in those taken in February, and whose mean temperatures were  $26^\circ\text{C}$  and  $12^\circ\text{C}$ , respectively. The presence of microorganisms, and the effect of the temperature on them, must also be taken into account. Microbial growth is stimulated at temperature as high as  $26^\circ\text{C}$ .

The most-known nitrifying bacteria are the *Nitrosomonas* and *Nitrobacter*, with optimum growth temperature at  $30^\circ\text{C}$  and  $28^\circ\text{C}$ , respectively<sup>12</sup>. Therefore, it was expected that the mu-

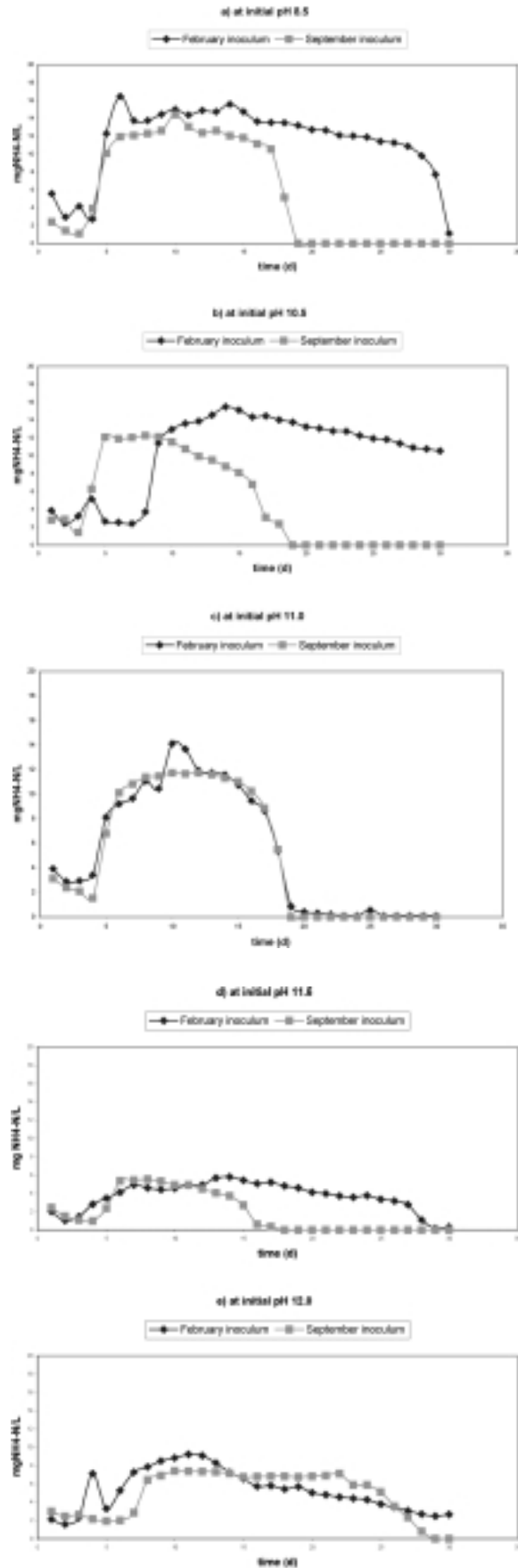


Fig. 2 - Ammonium concentration in the experiments carried out with inoculum of February and September

Table 1 – Total Kjeldal Nitrogen (NKT) initial and Maximum concentrations (mg/litre) of ammonium, nitrite and nitrate, and time required with a September wastewater inoculum.

Seasons	pH <sub>i</sub>	8.5	t/d	10.5	t/d	11.0	t/d	11.5	t/d	12.0	t/d
	NKT (mg/L)	25		25		25		25		25	
September	N-NH <sub>4</sub> <sup>+</sup>	14.40	10	12.25	8	11.75	12	9.01	8	7.40	10
	N-NO <sub>2</sub> <sup>-</sup>	7.75	33	6.95	22	7.29	23	5.49	33	3.34	33
	N-NO <sub>3</sub> <sup>-</sup>	4.07	31	4.41	33	4.10	31	2.90	31	2.16	29
February	N-NH <sub>4</sub> <sup>+</sup>	15.00	10	15.49	14	14.12	10	9.50	15	9.25	11
	N-NO <sub>2</sub> <sup>-</sup>	6.17	30	0.09	29	5.69	21	2.67	29	0.06	30
	N-NO <sub>3</sub> <sup>-</sup>	3.26	30	0.46	29	3.33	20	1.83	30	0.71	30

municipal wastewater taken in September would be richer in nitrifying bacteria than those taken in February. Consequently, the oxidation of ammonium to nitrite and of this to nitrate was produced some days earlier in the September inoculum. The concentrations of the oxidation products were higher with this inoculum. An example is the experiment carried out at the initial pH value of 10.5 (Figures 3).

It is also observed (Figure 2) that the release slopes of ammonium, that is, the deamination of the organic matter, are parallel for each season. The experiment carried out with municipal wastewater taken in February showed a five day delay. This could indicate that the difference between these experiments is not due to the participation of different individuals, characterised by different growth rates, but to the number of individuals. As a result of an earlier organic matter deamination, nitrification takes place sooner, because the presence of ammonium and simple organic matter helps nitrifying bacteria to grow. On the other hand, in the tests carried out with municipal wastewater taken in February, the nitrification has hardly begun (Figures 3).

It could be said that the summer season helps the growth of nitrifying microorganisms and, consequently, better results are obtained for this process in that part of the year. Some treatment plants, such as Butter Worth Wastewater Plant (New Jersey), take advantage of this circumstance and carry out a seasonal nitrification in the months from May to October<sup>13</sup>. Other companies, such as Hookers Point Advanced Wastewater Treatment Plant (Florida), despite low solids detention time obtained a considerable nitrates mass concentration (4–5 mg l<sup>-1</sup>) which was attributed to the temperature of the municipal wastewater (21–30 °C)<sup>14</sup>. These examples show that the season of the year contributes to improving the processes which take place in a wastewater treatment plant, such as, in this case, nitrification.

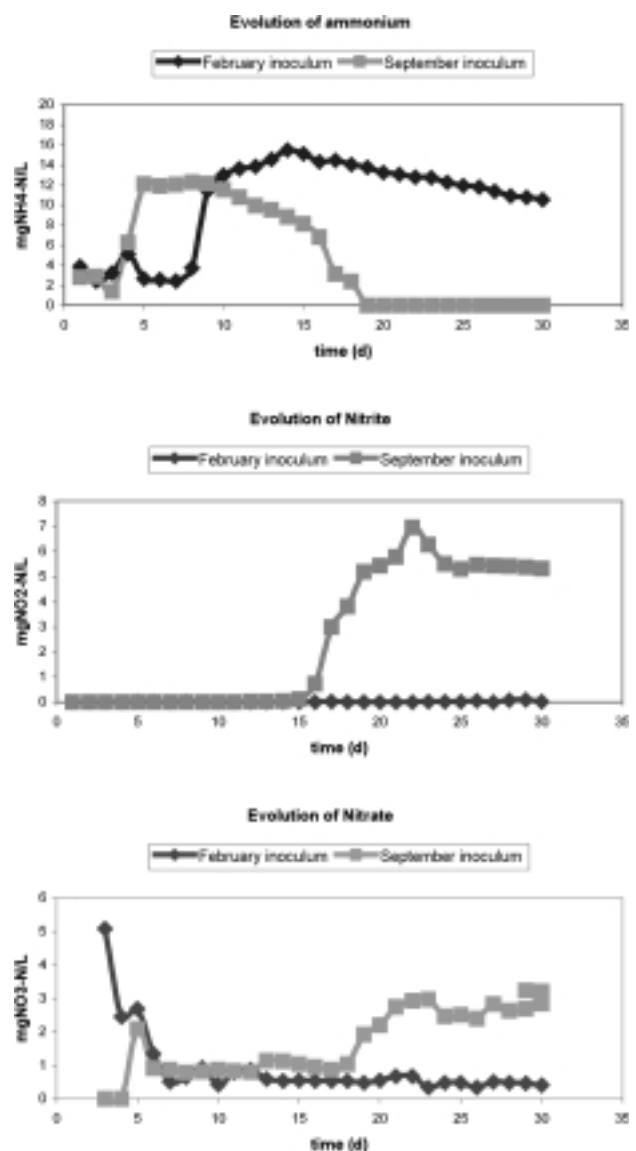


Fig. 3 – Evolution of Ammonium, Nitrite and Nitrate in the experiments carried out at the initial pH 10.5

The behaviour in the test carried out at the initial pH value of 10.5 was similar to that in the rest of the experiments tested at other initial pH values. However, some differences were known, as commented below.

The heterotroph microorganisms, in contrast to the autotrophs, were not affected by the season in which the municipal wastewater was taken. The degradation of the organic matter (expressed as COD) was dependent on the operational conditions, such as pH value, rather than by the season of the year. Table 2 shows the organic matter removal, in percentage, after ten days of running. In general, it can be observed that the organic matter is better degraded at September temperature than at the February temperature.

Table 2 – COD reductions (%) after ten days of running.

pH	8.5	10.5	11.0	11.5	12.0
February	93.7	88.1	91.7	76.3	87.2
September	91.2	91.2	94.4	92.8	77.5

#### **Interaction between the type of inoculum and initial pH value in nitrification**

As it has been shown, that the municipal wastewater taken in September had the best results in the nitrification process (Table 1). The autotroph bacteria responsible for the oxidation of the ammonium and nitrite (*Nitrosomonas* and *Nitrobacter*, respectively) do not seem to be affected by the initial pH value of the medium. When autotrophs can grow, the pH value of the medium is about 8.5, because it has been buffered throughout the organic matter mineralization by the heterotroph microorganisms. This pH value is optimum for nitrifying bacteria growth, which takes place at a higher rate.

At the initial pH value of 12.0, there is a delay of about ten days before autotroph microorganisms begin nitrifying. In this case, the pH is buffered to about 9.0 and the nitrifying bacteria consequently need a period of adjustment to the medium.

The maximum nitrite and nitrate concentrations reached, depend on the initial pH value of the medium. A higher initial pH value involves a greater nitrifying bacteria selection.

Table 1 shows the maximum concentrations of ammonium, nitrite, and nitrates reached in the experiments carried out with the municipal wastewater of September and February, at different initial pH values. It is observed that the maximum concentrations for nitrite and nitrate do not vary markedly when initial pH value is in the range 8.5

to 11.0. However, the maximum concentrations decrease at higher initial pH values (11.5 and 12.0). There are not many microorganisms that can survive under these conditions, and consequently, the selection is very strong. Taking into account that the ammonium can be released in the atmosphere as ammonia (depending on the pH), the one remaining in the medium, is the substrate for the nitrifying bacteria. Table 1 shows that when initial pH is higher the maximum ammonium concentration is lower, to each type of inoculum.

These results agree with those of *Balmelle*<sup>15</sup>: the differences in the nitrification process can be attributed to chemical differences of the medium, such as pH. It is believed that this is the main factor responsible for the different results because the operational temperature was kept constant throughout the tests.

In the experiments carried out with municipal wastewater taken in February, concentrations of nitrite and nitrate were close to those obtained with the September ones. At 8.5 and 11.0 initial pH, the only difference between them was the delay of ten days in February wastewater due to the seasonal temperature. At the other initial pH values, nitrification does not take place when the wastewater is taken in February. Of the nitrifying microorganisms, *Nitrobacter* is the most sensitive to temperature, so it comprises the limiting step in the nitrification process. The microorganisms of the municipal wastewater of February grew at 12 °C (mean temperature of the month), but in the experiments nitrification still took place. Even though temperature is an inhibitory factor, it has been confirmed that the inhibition is reversible<sup>16,17</sup>.

#### **Conclusions**

From this work, some general ideas of the effect tipo de inóculo de la WWTP on the nitrification are established.

- The nitrates from ammonium, in a municipal wastewater treatment plant, are higher at the environmental temperature of 30 °C (or 26 °C, mean temperature of the September inoculum).

- At lower temperatures, such as 12 °C, nitrification in dairy wastewater also takes place, but at lower rate. Subjecting the inoculum taken in February to the temperature of 30 °C, nitrification takes place earlier, although the using of municipal wastewater of September was faster.

- The effect of the initial pH increases with its value. At pH of 12.0, a considerable proportion of ammonium is lost as ammonia, and less substrate is oxidised to nitrate by the nitrifying bacteria. High initial pH value also result in a bacterial selection and, consequently, the nitrification rate is low.

– The initial pH value and temperature interaction lead to three different behaviours for the deamination of the organic matter. pH values lower than 11.0 (except 8.5), and municipal wastewater taken in September, result in a high deamination rate. On the other hand, at pH values higher than 11.0, microorganisms need the municipal wastewater taken in February to yield high deamination. Nevertheless, at pH 11.0 and 8.5, effect of the type of the inoculum on the deamination rate, is not observed.

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