THE EFFECT OF CLAY PARTICLES ON THE ACTIVITY OF SUSPENDED AUTOTROPHIC NITRIFYING BACTERIA AND ON THE PERFORMANCE OF AN AIR-LIFT REACTOR

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ABSTRACT

Clay minerals have some properties, namely a high surface area and the ability of ion exchange that may exert some effects on microbial systems. It is often difficult to know the way the clay is exerting its influence and whether its presence improves a given metabolic process. The present work concerns the study of the effect of the addition of powdered kaolin to autotrophic nitrification systems, and includes the study of the effects of the particles on the activity of a suspended nitrifying bacteria consortium and on the performance of an air-lift biofilm reactor used for tertiary nitrification. Concerning the suspended culture, kaolin particles produced stimulation on the specific endogenous and exogenous respiration rates of the bacteria, probably due to a nutritional effect supplied by the clay. This effect was more pronounced for the ammonia oxidation rates, although nitrite oxidation was also enhanced but to a lesser extent. In respect to the presence of kaolin particles in the air-lift reactor, the results obtained indicate that the clay particles become incorporated in the biofilm pellets, but do not change significantly their thickness or their shape. However, nitrate production decreased when the concentration of particles increased. The low adsorption of ammonia by the kaolin indicated that the clay particles embedded in the biofilm did not probably retain the ions. Although it was not proved, precipitation of salts may have occurred.

INTRODUCTION

The improvement of the process of ammonia removal in water and waste water may be attained by the design of new reactors or by implementing new operating technologies that may play a significant role in the biological oxidation of ammonia to nitrate via nitrite (nitrification). The efficiency of the process is dependent on several parameters such as pH, temperature, concentration of oxygen and the presence of inhibitors, which include the substrates and products of the process themselves [1,2].

It is known that the activity of some microorganisms (including nitrifiers) in soils and in aquatic systems is affected by the natural presence of inorganic particles in those systems. Clay particles are the group of inorganic particles more often referred to as exerting some influence on microorganisms, since they have properties that may be associated with physico-chemical alterations of the environment where the microbial reactions are occurring (indirect effects), or they may be involved in surface interactions between the particles and the microorganisms [3].

Over the last two decades, several studies concerned with the effects of different clay particles on the behaviour of bacterial systems have been carried out, showing that the particles may influence the process where they are involved. Among these studies the following can be referred to: Stotsky [3,4] reported that various clay species stimulated bacterial metabolism and growth; Filip and Hattori [5] found a stimulatory effect on biomass production by *S. cerevisae* in the presence of clay particles; Macura and Stotzky [6] reported that the degradation of glycine was enhanced in soils that contained clays such as montmorillonite; clay particles protected microorganisms in soils from the effects of some toxic compounds [2]; biofilms formed in the presence of inorganic particles are physically more stable than in the absence of particles [7]; nitrification in soils seems to be enhanced when the content of clays in the soil is high [6, 8]; Chudoba and Pannier [9] used powdered clay to successfully upgrade nitrification in an activated sludge process.

The mechanisms by which the inorganic particles influence the processes where they are involved are not fully understood. Moreover, in most situations, several mechanisms may be playing different roles at the same time.

As regards nitrification, it is of primary importance to

character the systems where the reaction is taking place, since controversial conclusions on different systems have been made, depending on the situations under study, namely the type of inorganic particles, their shape and size and the environment (a soil, a lake, or a reactor). According to Lees and Quastel [8], the suspended inorganic particles stimulated nitrification due to the growth of bacteria on the surface of the particles at the expense of ammonium ions adsorbed on the surface. Conversely, Allison et al. [10] showed that if the ammonium ions were adsorbed to soil particles they would be less available for oxidation. Other authors also concluded that only non-adsorbed ammonium ions could be further consumed in nitrification [3]. Some works suggest that the stimulation effect can be related to the reduction in the toxicity of substances produced during metabolism, or originally present in the systems, through adsorption to, or inactivation by the clay: the stimulatory effect of montmorillonite may have been a result of the adsorption of NH_4^+, reducing the level of NH_3 that is toxic to nitrification, and affecting the rate of oxidation of NH_4^+ [3]. As nitrification is a process very susceptible to pH, the buffering capacity provided by some particles, maintaining the pH at appropriate levels to sustain nitrification can also play a significant role [11]. More recently, Hommes et al. [12] reported that Willamette soil (besides clay, silt and sand, it contains organic carbon) inhibited nitrite production by *Nitrosomonas europea*, due to a combination of ammonium adsorption onto soil colloids and the exchangeable capacity of the soils lowering the pH of the reaction mixture, and thus causing a substantial drop in the concentration of NH_4^+ in solution.

Clay minerals [13,14] are crystalline hydrous aluminium silicates that are classified as type 2:1 or 1:1, according to the arrangement of the molecules of Si and Al. In 2:1 clays such as montmorillonite, vermiculite and illite these atoms are associated as unit layers of -Si.Al.Si.Si.Al.Si.-, held together by weak van der Waals forces and by the electrostatic interaction of interlayer cations. The relative weakness of these forces allows the penetration of water and other polar molecules between the unit layers, and the swelling of the structure occurs, resulting in a larger surface area. Conversely, in 1:1 clays, such as kaolinite and halosite, the atoms of Si and Al are associated in a 1:1 (-Si.Al.Si.Al.Si-) structure, held together tightly by H bonds. Consequently, these clays do not normally expand much upon wetting and do not expose any internal surface. In some clays, especially in 2:1 clays, structural cations such as aluminium and silica, are replaced by ions of lower valence in a process called isomorphous substitution. This process imparts many negative charges in the structure, which may be neutralised with cations such as Ca^{2+}, Mg^{2+}, H^+, Al^{3+}, Na^+, K^+ and NH_4^+. If the particles are exposed to other conditions, these ions can be exchanged with cations present in the new environment. The Cation Exchange Capacity, CEC, of the clay is defined as the maximum amount of cations of the medium that can be retained by the clay. This property depends upon the type of

clay and the conditions of the medium, namely the pH. 1:1 clays have a CEC smaller than 2:1 clays (kaolinite has a surface area between 10-50 m^2 g^{-1} and a CEC of 2-10 meq 100 g^{-1}, whilst montmorillonite has, respectively, 700-750 m^2 g^{-1} and 120-200 meq 100 g^{-1}). Clays may also have an anion exchange capacity (AEC), which is located at the edges of the clay packets and results from the breakage at the edges or exchange of OH groups. The average CEC/AEC ratio is around 6.7 for montmorillonite and 0.5 for kaolinite [3].

The aim of this work is to evaluate the effect of powdered kaolin particles (composed mainly of kaolinite, although it is not a pure mineral) on the process of autotrophic nitrification and to understand better the mechanisms involved in clay-bacteria interactions. For that purpose, two types of tests were performed: a nitrifying consortium was obtained from a wastewater treatment reactor used for tertiary nitrification and the effects of kaolin particle concentration on the activity of suspended bacteria were determined; the addition of different concentrations of particles to a biofilm air-lift reactor used for tertiary nitrification was investigated.

Kaolin particles were chosen since it is an abundant natural resource in the North of Portugal, where this work was carried out. The presence of clay particles in reactors for ammonia removal can result from the water used in the process: the concentration of inorganic particles in a river in the north of Portugal ranged between 10 mg l-1 and 65 mg l-1 [15] but, in locations downstream to a ceramic industry, or after a flood, the concentration of particles in the river can be even higher. Alternatively, the addition of particles to a system, in a continuous or discontinuous mode, can be programmed in order to improve the process, as performed by Chudoba and Pannier [9].

MATERIALS AND METHODS

Inorganic materials

Powdered kaolin particles type BA220C, supplied by APCV-Anglo-Portuguesa de Caulinos de Viana, Lda, were used in this experiment. The clay particles passed through a 45-μm pore size sieve. A posterior analysis under Scanning Electronic Microscope showed that particle sizes were between 5 and 10 μm.

The chemical characterisation of the clay used is presented in Table 1.

Growth and preparation of the culture used for the activity tests

The bacterial inoculum used in this experiment was collected from the sedimenter of a circulating bed biofilm reactor, CBBR- (a three phase bioreactor – liquid/air/biofilm) used for autotrophic nitrification operating in continuous mode [17]. The high turbulence with strong abrasion conditions led to high amounts of biomass detachment

Table 1   Chemical analysis of kaolin particles used in this study [16].

	%
Water (110°C)	0.88
Lost at 1000°C	13.2
SiO_2	44.2
Al_2O_3	37.9
Fe_2O_3	1.26
FeO	<0.10
TiO_2	0.16
P_2O_5	0.037
K_2O	0.074
CaO	0.074
MgO	2.19
MnO	0.012

(biomass formed as biofilm around the support particles). This biomass was released together with the effluent or settled in the bottom of the reactor. The inoculum was checked for heterotrophic microorganims, and less than 7% of the consortium could use organic carbon.

Prior to the activity tests, the bacteria consortium was kept in 500 ml flasks containing enrichment medium, in an orbital shaker, at 27°C and 120 rpm. The medium was obtained by dissolving 0.85 g $(NH_4)_2SO_4$, 0.32 g $Na_2HPO_4.2H_2O$, 0.25 g KH_2PO_4, 2.15 g $NaHCO_3$, and 0.75 ml of trace element solution A (20 mg l^{-1} $ZnSO_4.7H_2O$, 20 mg l^{-1} $CuSO_4.5H_2O$, 20 mg l^{-1} $NaMoO_4.2H_2O$), 7.5 ml of solution B (2 mg l^{-1} $MgSO_4.7H_2O$) and 3.75 ml of solution C (1.44 g l^{-1} $FeSO_4.7H_2O$ and 2.06 g l^{-1} EDTA) in 1 litre of distilled water. The medium was renewed when total exhaustion of ammonia occurred and the concentration of nitrate reached a constant level. The final pH of the suspension was around 7.5.

Seven days before the activity tests, four flasks containing the bacteria consortium and respectively 0.1, 0.5, 1 and 2.5 g l^{-1} of kaolin particles, were also incubated as described previously.

Activity tests - respirometric assay

These tests, hereafter called respirometric assays, were carried out to evaluate the influence of the kaolin particles on the oxygen uptake by the microorganisms, using ammonia as the limiting substrate for the ammonia oxidisers and nitrite for the nitrite oxidisers, as well as on the endogenous respiration.

Two sets of runs were carried out to study the influence of different clay concentrations (0.1, 0.5, 1 and 2.5 g l^{-1}) on the rate of oxygen consumption by the bacteria. In the first set, the clay was added immediately before the measurements, whilst in the latter the measurements were carried out using bacterial suspensions, which had been seven days in contact with the particles (as described previously).

A Biological Oxygen Monitor (BOM) YSI Model

S 301 B, was used to determine the oxygen consumption by the nitrifying culture [18]. Before each respirometric assay, the cultures (with and without particles) were harvested by centrifugation, and washed three times with saline solution (NaCl 0.85%), resuspended in the appropriate buffer solution and placed in the temperature-controlled vessel of the apparatus. The buffers used in these tests are, respectively, buffer N_b and buffer N_s for ammonia oxidisers and nitrite oxidisers. The composition of these buffers is presented in Table 2.

The vessel contains a dissolved oxygen (DO) probe, connected to a DO meter. Once inside the vessel, the culture was aerated for 30 minutes. The vessel was closed, and the decrease of the oxygen concentration was monitored against time (data was continuously acquired with a personal computer). Figure 1 represents a typical respirogram (variation of the concentration of oxygen with time) obtained in the course of these experiments.

Table 2.   Composition of buffers N_b and N_s used respectively for the ammonia oxidisers and nitrite oxidisers.

Composition	N_b (per litre)	N_s (per litre)
$NaHCO_3$	0.1065 g	0.358 g
$Na_2HPO_4.12H_2O$	0.0625 g	0.0625 g
KH_2PO_4	0.0625 g	0.0625 g
Solution A*	0.125 ml	0.125 ml
Solution B*	1.4 ml	1.4 ml
Solution C*	0.7 ml	0.7 ml

*The composition of these solutions is, respectively, as described in growth and preparation of the culture:
A - 20 mg l^{-1} $ZnSO_4.7H_2O$, 20 mg l^{-1} $CuSO_4.5H_2O$, 20 mg l^{-1} NaMo $O_4.2H_2O$: B –
B   2 mg l^{-1} $MgSO_4.7H_2O$:
C - 1.44 g l^{-1} $FeSO_4.7H_2O$ and 2.06 g l^{-1} EDTA
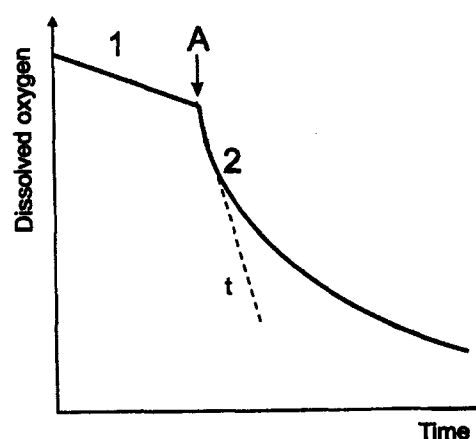

Figure 1.   Typical respirogram. (1 - endogenous respiration, 2- total respiration, t - slope of the initial decrease of the dissolved oxygen concentration after the injection of substrate, A- injection of substrate).

The slope on the initial linear decrease of DO concentration (zone 1 of the respirogram) was considered to be the endogenous respiration. To determine the oxygen consumption due to substrate oxidation, a small injection of the substrate was added, corresponding to point A in Figure 1 (25 µl of a 0.85 g l⁻¹ $(NH_4)_2SO_4$ in the case of ammonia, or 25 µl of a 0.348 g l⁻¹ NO_2 in the case of nitrite), to give a final concentration of 232.07 mg l⁻¹ $N-NH^+_4$ or 232.04 mg l⁻¹ of $N-NO_2$, and the curve 2 was obtained. The slope of the initial linear decrease in the DO concentration, after the injection, was determined (t in Figure 2), which corresponds to the total respiration rate. The difference between the two respiration rates gives the oxygen consumption rate due to the oxidation of substrate [19].

The specific oxidation rate was calculated by dividing the respiration rate by the biomass concentration (determined as described in analytical procedures), and the results obtained appear as mg of oxygen per second and per mg of biomass. The specific oxidation rate of ammonia and nitrite was calculated according to Sharma and Albert [19]: 3.43 mg of oxygen is necessary to oxidise 1 mg of ammonia nitrogen and 1.14 mg of oxygen to oxidise 1 mg of nitrite nitrogen.

Ammonia adsorption and pH profiles

Tests of ammonia adsorption by clay particles were carried out in batch assays at 27 °C in 250 ml centrifuge Teflon flasks with Teflon caps, with different initial pH values.

Kaolin particles were added to 100 ml of solutions at pH 5, 6, 7, 8 and 9 with an initial concentration of 5 mg l⁻¹ $N-NH^+_4$, to obtain a clay concentration of 0.5 g l⁻¹. The flasks were placed in an orbital shaker at 120 rpm and 27°C. After the addition of particles and after 24 hours, samples of the suspensions were filtered through 0.22 µm filters, and the ion concentration in the filtrate was immediately determined
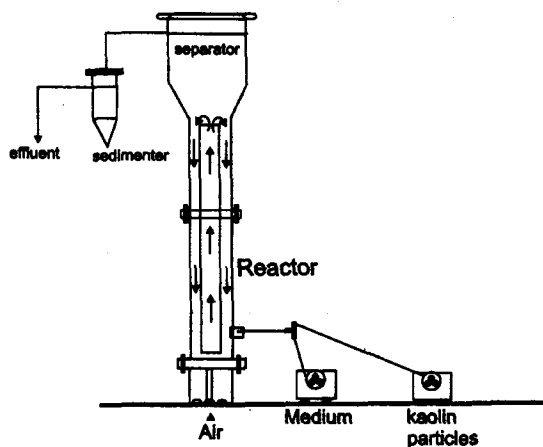
0.5 g l⁻¹ of kaolin particles was added to solutions of known initial pH, and the pH of these suspensions was followed over time. These experiments were also carried out with phosphate buffered solutions.

Air-lift experiments

The biofilm air-lift reactor, represented in Figure 2, had a volume of 5.6 l, and a three-phase separator in the top. Basalt particles, homogeneously suspended in the reactor, were used as support for biofilm development (mean diameter 435 µm, density 3067 kg m⁻³, concentration 50 mg l⁻¹).

Air was sparged in the bottom of the reactor in order to fluidise the particles. The reactor was filled with enrichment media, the basalt particles were introduced into the reactor and the latter was inoculated with an autotrophic nitrifying culture.

The reactor operated in batch mode until complete exhaustion of ammonia and nitrite occurred. Several batches were performed, in order to promote biofilm development on the basalt particles. Afterwards, the reactor was continuously fed with 16 ml min⁻¹ of medium composed of 472 g $(NH_4)_2SO_4$, 72 g $Na_2HPO_4.2H_2O$, 139 g KH_2PO_4, 1400 g $NaHCO_3$, and 417 ml of trace element solution A (20 mg l⁻¹ $ZnSO_4.7H_2O$, 20 mg l⁻¹ $CuSO_4.5H_2O$, 20 mg l⁻¹ $NaMoO_4.2H_2O$), 4170 ml of solution B (2 mg l⁻¹ $MgSO_4.7H_2O$) and 2083ml of solution C (1.44 g mg l⁻¹ $FeSO_4.7H_2O$ and 2.06 g mg l⁻¹ EDTA) in 200 litres of water.

The average concentration of influent ammonia was 500 mg l⁻¹ and the residence time 5.8 hours. The reactor operated for more than 300 days before this experiment started. Day 0 is considered to be the beginning of the experiments reported in this paper (reactor operating in steady-state). From day 0 to day 14 the reactor was still operating as previously. From day 14 to day 38, day 38 to day 52 and day 52 to day 55, the reactor was continuously supplemented with a suspension containing kaolin particles, in order to obtain average concentrations of particles inside the reactor of 50 mg l⁻¹, 500 mg l⁻¹ and 1000 mg l⁻¹, respectively. The concentration of ammonia, nitrite and nitrate were determined in the effluent. The pH inside the reactor was monitored with a pH meter.

Analytical procedures

The suspended biomass was determined as the difference between the dried (105°C) and the burned biomass (550°C) after filtration through a 0.22 µm filter, according to APHA [21], due to the presence of inorganic particles.

Ammonia, nitrite and nitrate were evaluated photometrically after sampling and filtration through 0.22 µm filters, according to the standard methods procedures 4500-NH3, 4500-NO2 B and 4500-NO3 B, respectively [20].

Prior to observation under scanning electron microscope, the biofilm pellets were dehydrated in a graded ethanol series.

Figure 2. Schematic representation of the air-lift reactor.

Table 3. Substrate oxidation rate without particles.

Ammonia oxidation rate (mg NH^+_4-N $kg^{-1}s^{-1}$)	Nitrite oxidation rate (mg NO^-_2-N $kg^{-1}s^{-1}$)
0.438±0.076	0.451±0.047

Influence of kaolin particles on the activity of suspended autotrophic bacteria

Figure 3 and Figure 4 present, respectively, the results obtained for the ammonia and nitrite oxidation rates, as a function of particle concentration, measured immediately after the addition of kaolin and after incubation of bacteria with kaolin during 7 days. These last experiments were carried out with the purpose of determining if the effects of kaolin on bacteria were still evident after 7 days, which can be particularly important when the particles are part of biofilm structures. In these Figures, the values for kaolin concentration = 0 g l⁻¹, correspond to data without particles.

Table 3 depicts the values obtained in the experiments without kaolin, corresponding to the control assays (represented in the last Figures as kaolin concentration 0 gl⁻¹).

As can be seen in Figure 3, ammonia oxidation rate in the presence of kaolin particles is higher than without particles, both in the case of immediate measurement after the addition of kaolin and in the case of 7 days pre-contact of the consortium with the particles. However, the stimulation is higher in the first case. Within the range of particle concentrations studied, it seems that the effect increases only up to a certain point as particle concentration increases, reaching a maximum for 0.5 g l⁻¹ of kaolin and decreasing for higher particle concentration. In the case of a pre-contact of the ammonia oxidisers with the particles, there is no
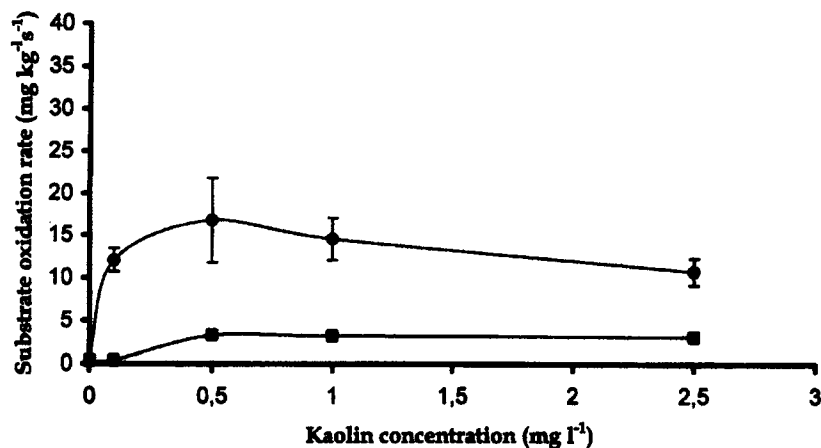

Figure 3. Effect of kaolin particle concentration on ammonia oxidation rate, immediately after particle addition and after a 7 days pre-contact of kaolin with the bacteria consortium. (● - Immediate addition of kaolin, ■ - 7 days pre-contact with kaolin, bars indicate standard deviation).
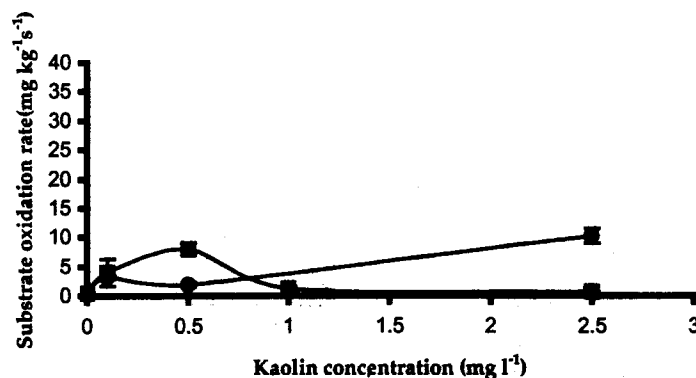

Figure 4. Effect of kaolin particle concentration on nitrite oxidation rate, immediately after particle addition and after a 7 days pre-contact of kaolin with the bacteria consortium. (● - immediate addition of kaolin, ■ - 7 days pre-contact with kaolin, bars indicate standard deviation).

meaningful difference between the runs performed without kaolin and with 0.1 g l⁻¹ of kaolin particles. For higher kaolin concentrations, the substrate uptake rate is higher and similar for all the concentrations.

Figure 4 shows that the nitrite oxidation rate was also enhanced in the presence of kaolin particles, in both situations studied. When the kaolin was added immediately before the determinations, the substrate oxidation rate increased with particle concentration. However, when the consortium had a pre-contact with the particles, the nitrite oxidation rate increased with the concentration of particles up to a particle concentration of 0.5 g l⁻¹ and decreased for higher particle concentrations.

The activity of the nitrifying bacteria was also determined in the presence of different concentrations of particles, but without the addition of an external oxidable substrate (endogenous activity). The same experiment was carried out for the bacteria that were in contact with different concentrations of particles during 7 days before the measurement (Figure 5). The purpose of these experiments was to determine if the enhancement observed in the presence of the inorganic particles was due to the supply of an oxidable substrate by the kaolin.

The results presented in Figure 5 indicate that the specific rate of endogenous respiration is enhanced in the presence of clay particles, both in the case of immediate measurement after addition of particles and in the case of a previous 7 days contact with the clay. Within the range of particle concentration studied, the stimulatory effect is more pronounced between 0.5 g l⁻¹ and 1 g l⁻¹ of kaolin. Above a particle concentration of 1g l⁻¹, the specific endogenous respiration rates decrease slightly, but are still higher than without particles.

Adsorption of ammonia by the clay particles

Figure 6 shows the comparison between the ammonia concentration before and after the contact with clay particles, for the different pH values studied.

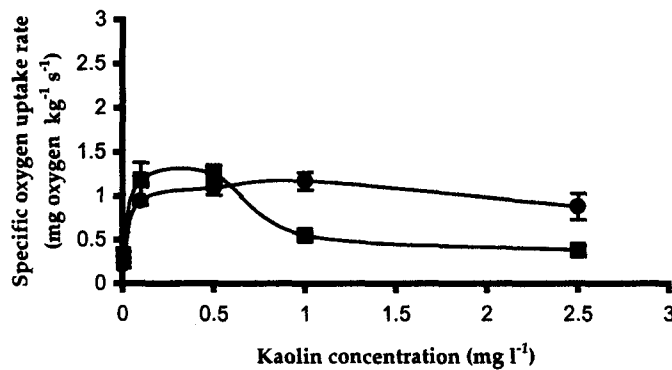The kaolin particles do not seem to adsorb (or adsorb

Figure 5. Effect of kaolin particle concentration on the endogenous respiration, immediately after particle addition and after a 7 days pre-contact of kaolin with the bacteria consortium (● - Immediate addition of kaolin, ■ - 7 days pre-contact with kaolin, bars indicate standard deviation).
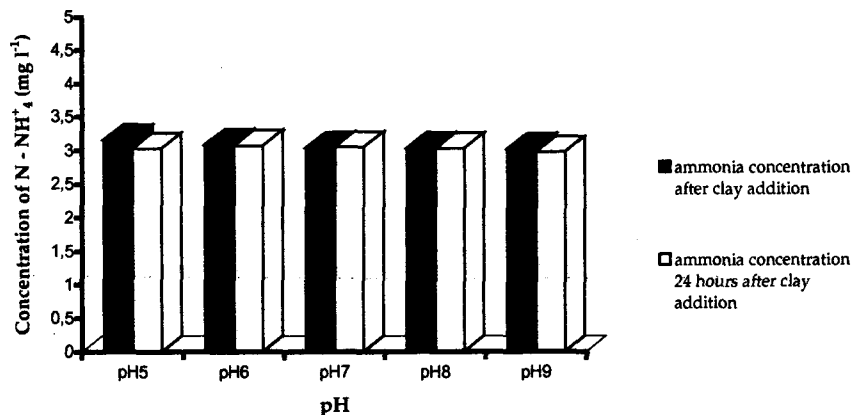

Figure 6. Comparison of ammonia concentration before and after the contact with clay particles, for the different pH values.

only a very small amount) of ammonia. This result was somehow expected due to the very small CEC of this kind of clay.

Effect of kaolin on the pH

In order to verify whether the pH of unbuffered and buffered solutions was modified due to the presence of 0.5 g l⁻¹, of kaolin the pH of these solutions was monitored as a function of time, after particle addition (Tables 4 and 5, respectively).

These Tables show that, while the pH of buffered solutions is maintained around the initial values, the kaolin particles are capable of changing the pH of unbuffered solutions.

Effect of kaolin addition on the performance of the air-lift reactor

Reactor operation.

Figure 7 shows the profiles of ammonia, nitrate and nitrite in the effluent during the operation of the reactor immediately before particle addition and with particle addition (day 0 to day 14 - no particle addition, day 14 to day 38 – 50 mg l⁻¹, day 38 to day 52 – 500 mg l⁻¹ and day 52 to day 55 – 1000 mg l⁻¹)

The ammonia removal is almost complete for all the situations studied. Also, nitrite did not accumulate in the system. However, the nitrate produced from the oxidation of nitrite decreased as the concentration of kaolin in the reactor

Table 4. pH values obtained in the solutions of different initial pH values, after the addition of 0.5 g l⁻¹ of kaolin.

Initial pH	pH measured after kaolin addition	
	pH after addition	pH after 24 hours
5.16	6.54	7.52
6.23	7.07	7.53
7.45	7.12	7.53
8.08	7.33	7.74
9.67	7.52	7.71

Table 5. pH values obtained in the buffered solutions, after the addition of 0.5 g l⁻¹ of kaolin.

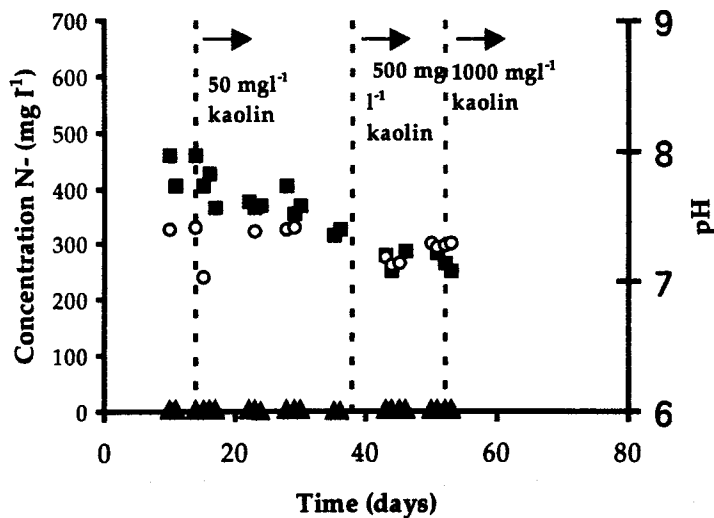| Initial pH	pH measured after kaolin addition		
	pH after addition	pH after 24 hours	pH after 48 hours
6.04	6.05	6.08	6.12
7.05	7.11	7.11	7.16
8.03	8.16	8.17	8.20

Figure 7. Profiles of ammonia (▲), nitrate (■) and nitrite (□) in the effluent and pH inside the reactor (O), during the operation of the reactor immediately before the continuous particle addition and with particle addition (the points for ammonia and nitrite are overlapped, since they are approximately zero).

increased. This Figure also shows that the pH profile in the reactor did not change with kaolin addition.

Biofilm shape and structure.

To investigate the impact of kaolin addition on biofilm structure, samples of biofilm particles present in the reactor before and after the addition of clay were examined under a scanning electron microscope. Figure 8 presents scanning electron micrographs, at low magnifications, of biofilms formed without kaolin and with different concentrations of particles. These micrographs show that the pellets of biofilm (carriers covered with biofilm) are ellipsoid, similar to the bare carrier shape. The biofilm grown around the carrier was densely packed, and formed circumvolutions. Comparing the size of the bare carrier with the biofilm pellet formed without kaolin shows that the biofilm is thick. According to those observations, the size and shape of the biofilm pellets didn't change much after kaolin addition, although they seemed to become more spherical. However, it appears that the biofilms before kaolin addition are smoother than after the introduction of particles in the reactor. Figure 9 presents observations of the biofilms that have been exposed to 500 mg l^{-1} of particles, at higher magnifications.

The analysis of these micrographs shows that particles became imbedded in the biofilm matrix.

DISCUSSION

Activity of suspended autotrophic bacteria in the presence of kaolin particles

In the present work, experiments were performed in order to investigate the possible mechanisms that explain the influence of kaolin particles on the particular case of nitrification in conditions similar to the ones prevailing in reactors treating high strength wastewaters.

As the experiments were carried out using buffered medium, the already well-established effect of the clay on pH regulation could be disregarded. Table 5 confirms that the addition of kaolin particles BA220C to buffered solutions did not change the pH of the medium, conversely to the case of unbuffered solutions (Table 4).

Ammonia oxidation rate.

The results presented in Figure 3 show that the addition of particles has a beneficial effect on ammonia oxidation rates, in the two situations under study. Nevertheless, it seems that a higher gain is obtained when the particles are added immediately before the respiratory assays. One hypothesis for this behaviour is that the particles introduce oxidisable substrates that can be oxidised by the autotrophic consortium, thus increasing the oxygen consumed. However, the results concerning the endogenous respiration (Figure 5) show that, even though the consumption of oxygen by the bacteria in the absence of ammonia increases when kaolin is present, the

enhancement observed under these circumstances is not enough to justify the results presented in Figure 3.

Figure 5 also shows that the endogenous respiration is not clearly affected by the 7 days pre-contact between the bacteria and the clay particles. The assumption that particles may retain ammonia during the 7 days contact with the ammonia containing medium, that can be eventually supplied to the bacteria in the case of depletion in the medium (the clay would serve as a temporary sink for ammonia), may not be happening in the system under study, since the enhancement observed is similar with and without pre-contact. The results obtained during the adsorption tests also prove this conclusion (Figure 6).

Another mechanism that may be involved in the enhancement of ammonia oxidation is the "nutritional effect" introduced by the particles: kaolin may have provided some inorganic ion that stimulates bacterial metabolism. Additionally to the ions loosely associated to the clay, that can be released to the medium by exchange with the H$^+$ produced during ammonia oxidation, the release of structural ions can be favoured by some bacterial metabolites acting as agents of ions solubilisation [3]. Comparing the chemical analysis of the kaolin used in this study (Table 1) with the composition of the medium, Ca, Mn, Si and Al ions were absent from the buffer composition and thus could be involved in the enhancement of O$_2$ consumption. Furthermore, Table 4 shows that the kaolin particles change the pH of the medium, due to their ability to exchange ions from its structure with ions of the medium [3].

Several authors have already reported that some ions may have a beneficial effect on bacterial metabolism. Bowen [22] conducted a study on the upgrading performance of an activated sludge process through the application of inorganic media and concluded that the addition of Al(OH)$_3$ to the process over a wide range of solid concentrations enhanced the consumption of oxygen, due to a nutritional effect. As regards calcium, Macura and Stotsky [6] studied nitrification in the presence of calcium carbonate, magnesium carbonate and calcium sulphate, and concluded that calcium does not have a nutritional effect, since the extent of nitrification observed was similar for both carbonates and a lower rate was obtained for the calcium sulphate. This nutritional effect provided by clays was also reported by other authors [23,24,25] who concluded that clay minerals used as supports for bacterial growth (in these cases, bacteria grew on the surface of the clay) in anaerobic digesters exerted a positive effect on bacterial growth due to the ability of the clay minerals to slowly release oligoelements, such as Mg, into the medium. Although anaerobic digesters are very different from the case under study, it showed that bacteria can benefit from ions released from clay minerals.

The beneficial effect of the particles, even if it is higher within the first hours after their addition, is still evident after 7 days of contact with the bacteria consortium. The decrease observed in the activity can be due to the consumption of the nutrient, or to the decreased release of nutrients due to a less
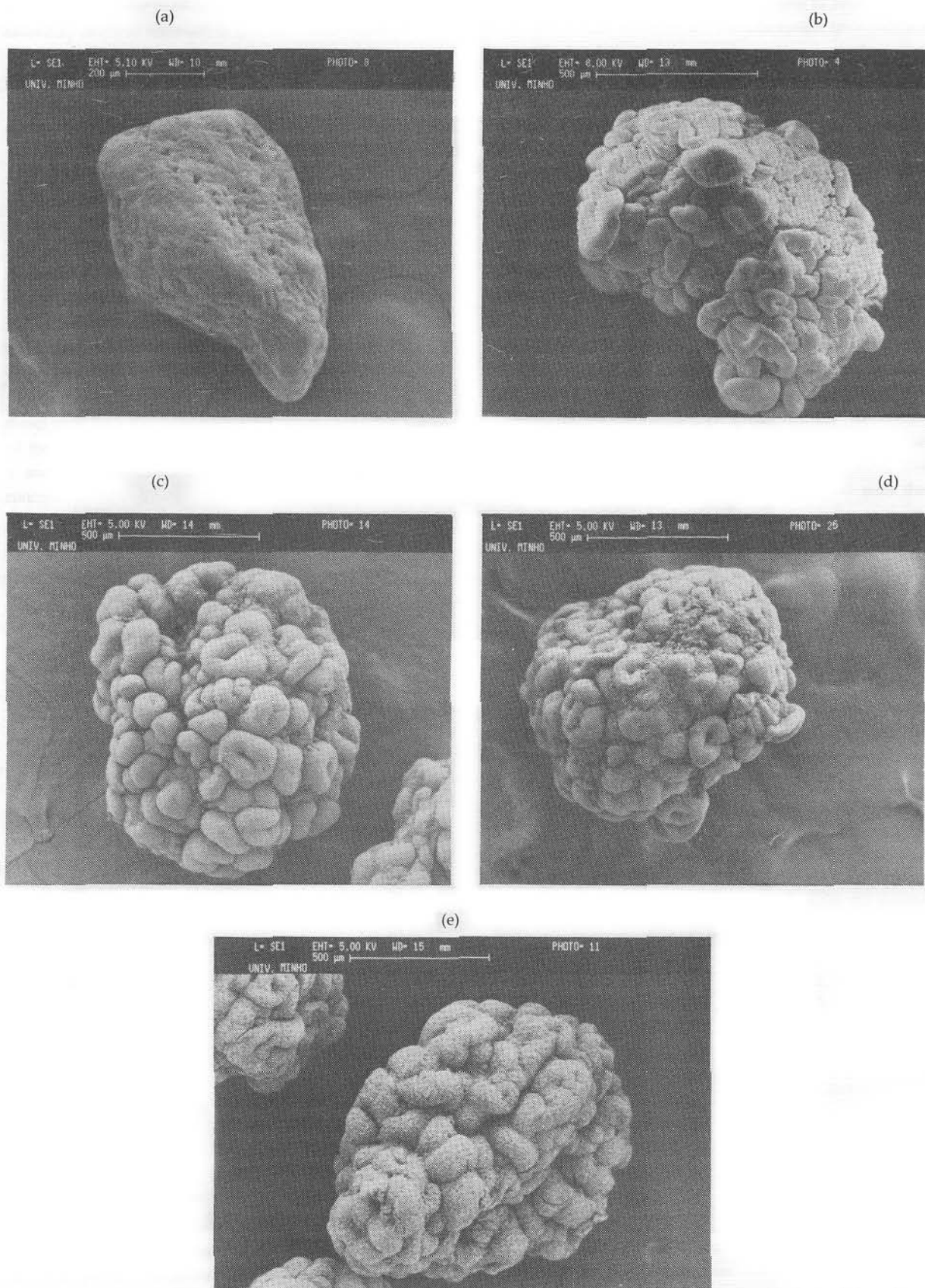
Figure 8. Scanning electron micrographs at low magnifications: a) bare carrier; b) an original pellet of nitrifying biofilm; c) biofilm in the presence of 50 mg l^{-1} of kaolin; d) biofilm in the presence of 500 mg l^{-1} of kaolin; e) biofilm in the presence of 1000 mg l^{-1} of kaolin
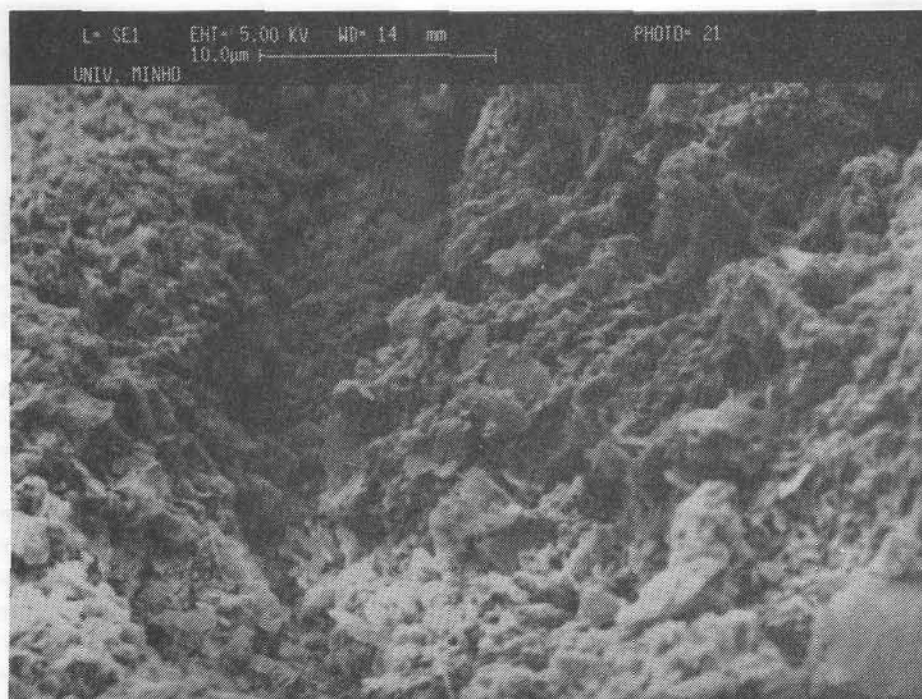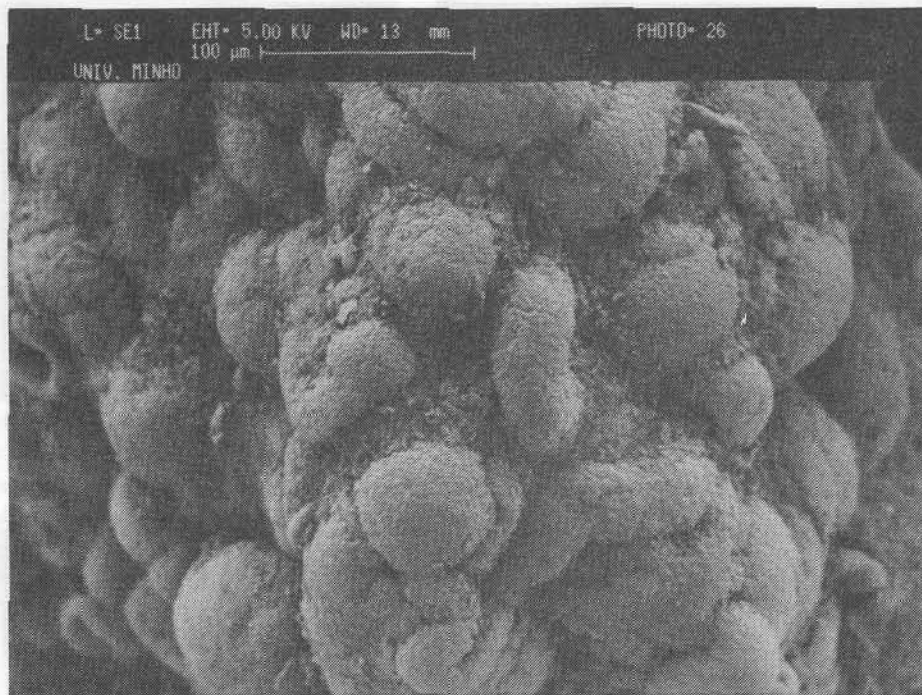
Figure 9.   Scanning electron micrographs of biofilm exposed to 500 mg l^{-1} of kaolin, at higher magnifications.

available area of the particles to exchange ions of the medium, caused by bacterial adhesion or growth on the surfaces.

Nitrite oxidation rate.

The results for the nitrite oxidation rates in the presence of different kaolin concentrations, depicted in Figure 4, show that this process is also enhanced in the presence of particles. Conversely to the ammonia oxidation, there is not such a large difference between the results obtained in the case of the immediate addition of particles or the pre-contact of the particles with the bacteria. However, it seems, except in the case of 2.5 g l^{-1} of kaolin, that the nitrite oxidisers are more stimulated after a 7 days pre-contact with the particles than the ammonia oxidisers. As in the previous case, the nutritional effect of the particles may be an important factor to take into account.

The results presented show that there is not a simple direct proportionality between the enhancement observed for all the cases studied and the concentration of particles (Figure 3 and Figure 4), because there is a decrease for kaolin concentrations above 0.5-1.0 g l^{-1} in the case of ammonia oxidisers (immediate addition) and 1.0 g l^{-1} in the case of a pre-contact for nitrite oxidisers.

Probably, some competitive effects occuring at the same time must be taken into account. It was seen in the course of these experiments that the concentration of oxygen in the medium decreases as the concentration of particles increases (mass transfer of oxygen is affected by the decrease in oxygen concentration and this has a strong negative effect on the ammonia oxidisers, which need much more O_2 than nitrite oxidisers). In this context, the results obtained for the highest concentrations of particles (reduction of the enhancement effect) could be somehow expected. In the case of the 7 days pre-contact of the bacteria with the particles, some aggregation occurred (apart from the aggregation that characterises these kind of bacteria, the presence of particles seems to reinforce the process) reducing the amount of oxygen that reaches the cells, and thus decreasing the respirometric activity.

Effect of kaolin addition on the performance of the air-lift reactor

Reactor operation.

The results obtained during the course of the reactor operation showed that the ammonia removal did not decrease with kaolin addition, since no ammonia was detected in the effluent. The reactor was already removing all the ammonia and any further increase could not be obtained. However, despite the fact that no nitrite accumulated in the reactor, the concentration of nitrate in the effluent decreased as kaolin was added to the system (and a higher decrease was observed as kaolin particle concentration increased). The reason for the decrease in nitrate concentration is unknown. Among the several hypotheses that can be raised to explain this behaviour the argument that clay particles can act as a

temporary sink for ammonia does not seem to apply to this study. The results obtained for the adsorption tests indicate that ammonia adsorption does not happen or is very low.

Another hypothesis that can be put forward to explain the disappearance of nitrogen is the precipitation of salts of ammonia (e.g. struvite). This has been reported to occur in some anaerobic digesters that use clay as supports, due to the transfer of some ions from the clay to the medium [25]. In this case, the advantage of this process is that ammonia is not transformed to another compound (nitrate) that would need further treatment before disposal.

Biofilm shape and structure.

The shape of biofilm pellets obtained during the air-lift experiments (with and without kaolin) is ellipsoid, which, according to Gjaltema et al. [26], is common in air-lift reactors using bare basalt as carrier. According to these authors, this shape is related with reactor hydrodynamics, turbulence intensity and particle collisions and with the orientation of the suspended particles relative to the flow. However, the biofilm particles seem to become more spherical with the addition of particles. The size of the bare carrier is much smaller than the biofilm pellets showing a high amount of biofilm accumulated on the surface. The size of the biofilm pellets did not change with kaolin addition, indicating that the detachment did not increase due to the presence of particles. These results are in accordance with Lowe [27] who investigated the scouring effects of kaolin and sand particles suspensions on well-established biofilms formed by *Pseudomonas fluorescens*. The application of 50 mg l^{-1}, 1000 mg l^{-1} and 5000 mg l^{-1} suspensions of kaolin did not change the amount of biofilm detached from the surface in relation to the detachment obtained with pure water.

However, the kaolin particles became embedded in the biofilm matrix (Figure 9), and a slight roughening of the surface was observed. This can have a positive impact on biofilm behaviour. Additional to the maintenance of a more suitable environment for microbial growth inside the matrix, due to the ability of the particles to exchange H$^+$ with the media, particles may affect the microbial processes inside the biofilm as previously seen. Furthermore, the biofilms with incorporated particles may have a higher mechanical stability in the case of depletion of substrate and the transport rate of substrates inside the matrix may be favoured [7].

CONCLUSIONS

In conclusion, the results obtained show that:
- Kaolin particles play a significant role in the nitrification rate in liquid media, since there is a stimulation of the biological process. The enhancement observed was more pronounced for ammonia oxidation than for nitrite oxidation. The main mechanism involved in the stimulation appears to be associated with a nutritional effect introduced by the kaolin particles, due to the release of some ions by the clay.

- As regards the application of kaolin particles to an air-lift biofilm reactor used for nitrification, the shape and size of the biofilm pellets did not change throughout the experiment. However, the results indicated that some mechanism was contributing to ammonia removal besides the formation of nitrite and nitrate, probably the precipitation of some salt.
- The clay particles did not have a scouring effect on the biofilms. They became incorporated in the matrix, which may account for a beneficial effect on bacterial metabolism.

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