Performance and biological indicators of a laboratory-scale activated sludge reactor with phosphate simultaneous precipitation as affected by ferric chloride addition

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ABSTRACT

Iron-base coagulants are widely used for the phosphorous removal from wastewaters. Coagulants may be applied on the secondary treatment (simultaneous precipitation); however, coagulant excess may alter the biological process. The objectives of this work were: (a) to study in a laboratory-scale activated sludge reactor, fed with a model wastewater system from the dairy industry, the feasibility of phosphorous removal by applying simultaneous precipitation using ferric chloride; (b) to assess the effect of different Fe:P (molar ratio) dose ranges: high = 1.9–2.3:1, mean = 1.5–1.9:1 and low = 1.0–1.4:1 on the reactor performance by measuring physical–chemical parameters (pH, COD, TSS, Fe, P), settling properties (DSVI, diluted sludge volume index) and abundance of filamentous microorganisms by microscopic observation; (c) to evaluate the effect of the coagulant on activated sludge microfauna (abundance and morphological–functional groups); (d) to assess the usefulness of these parameters as control tools. A high Fe:P ratio acted as shock load improving the soluble phosphorous and COD removal. Sludge settling properties were rapidly improved due to both a successful control of filamentous bulking and an increase in the flocs density. Prolonged application of high Fe(III) doses negatively affected the reactor performance; a Fe:P ratio = 1.5–1.9:1 achieved an acceptable effluent quality. Changes in the density of *Chilodonella* sp., *Opercularia* sp., *Vorticella microstoma*, *Vorticella* spp., *Colpoda* sp. and rotifers were analyzed as a function of the progressive enrichment of Fe(III) in the sludge. Crawling ciliates constituted the most sensitive group to changes in the activated sludge reactor environmental quality, becoming a potential biological indicator of Fe(III) overload.

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1. Introduction

Municipal and industrial wastewaters containing high concentrations of phosphorous (P) should be treated before discharging to receiving water bodies. Phosphorous from wastewater can be present as orthophosphate (PO_4^{3-}), inorganic condensed phosphates and organophosphates [1]. The US discharge limit for phosphate is 0.5–1.0 mg P/L; according to Argentinean standards, the discharge limit for phosphate ranges from 1.0 to 10 mg P/L, depending on the wastewater receiving location. The use of coagulants is a very common practice for the removal of phosphorous from wastewaters. Iron-base coagulants, as ferric chloride, are widely used for that purpose.

Coagulant addition may be applied on the primary settler (known as pre-precipitation), on the aeration basin (simultaneous precipitation or co-precipitation), or on the tertiary treatment (post-precipitation) [2]. Pre-precipitation offers the benefits of reducing 50–70% the organic loading of the biological treatment; however, the main problem is the marked increase in the sludge production. In addition, a significant proportion of phosphorous may be present as organic compounds and hence may not be removed [3]. This problem is avoided in the simultaneous precipitation, because polyphosphates and organic phosphates are hydrolyzed to orthophosphate in the aeration tank of the activated sludge system. However, the excess of coagulants may be deleterious to the biological process [4]. In the post-precipitation, an additional settling tank is necessary, increasing both investment and operative costs [3].

The process of phosphate removal by Fe(III) basically involves the precipitation of both ferric phosphate and ferric hydroxide and phosphate adsorption on ferric hydroxide flocs [5]. Previous studies also postulate that formed ferric hydroxide lead to phosphate

removal through anion exchange effect between hydroxide and phosphate ions [6]. According to these authors, if this hypothesis is valid, ferric hydroxide may be expected to accumulate in the mixed liquor producing a so-called "persistence effect". Arising from this effect, the mixed liquor has a residual chemical P removal potential after metal dosing is stopped.

Most simultaneous precipitation studies were conducted in activated sludge systems under P load usually ranging between 3.0 and 15 mg/L [5,7,8]. Fe:P doses higher than 1.5:1 (molar ratio) can achieve orthophosphate removals between 95 and 99% [9–11]. The effect of metal coagulants on systems operated with relatively high concentrations of P (e.g. dairy effluents treatment plants) has not been sufficiently analyzed.

In activated sludge reactors operated with simultaneous precipitation of P, the effect of metal salts on the process performance and final effluent quality is commonly assessed by physical–chemical parameters such as biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), dissolved organic carbon (DOC), ammonium (NH_4), suspended solids (SS) and turbidity [4,8,12].

It should be noted that the physical–chemical parameters reveal instantaneous conditions, without reflecting the toxic effect of the coagulant on the biological system. A periodic analysis of the microfauna would allow monitoring the progressive effect of the coagulant and thus to detect a possible overload on the system.

Routine analysis of the microfauna allows monitoring the process performance of activated sludge and the final effluent quality [13]. Overall, an efficient process shows microfauna with large numbers of organisms ($\geq 10^6$/L), composed mainly of crawling ciliates and attached ciliates well diversified, where no group dominates numerically over the others by a factor greater than 10 [14]. It should be noted that these observations have not been sufficiently addressed in systems with simultaneous precipitation of P.

Few studies have evaluated the usefulness of biological parameters for monitoring the P simultaneous precipitation process by metal salts. Wuhrmann [15] reported that ferric chloride caused a virtual disappearance of protozoa in activated sludge. Clark et al. [8] compared the effect of ferric chloride and ferrous sulfate on the biomass of activated sludge using a diversity index based on the abundance of microfauna organisms. Similarly, Burgess et al. [16] compared the effect of aluminum sulfate and an aluminum-based polyelectrolyte on the organism diversity of an activated sludge system. However, in these studies the diversity index was not applied to monitor changes in the process performance of activated sludge.

The effect of metal salts on high P load systems (e.g. dairy effluents treatment plants) should be studied through the combined analysis of physical–chemical and biological parameters. In this context, it is very interesting to evaluate the usefulness of indicator organisms of metal pollution for monitoring the dosage of coagulants.

The objectives of the present study were: (a) to study in a laboratory-scale activated sludge reactor, fed with a model wastewater system from the dairy industry, the feasibility of phosphorous removal by applying simultaneous precipitation using ferric chloride, (b) to assess the effect of different Fe:P (molar ratio) dose ranges: high = 1.9–2.3:1, mean = 1.5–1.9:1 and low = 1.0–1.4:1 on the phosphorous removal, (c) to analyze the effect of the different Fe:P dose ranges on the process performance of activated sludge by measuring different physical–chemical parameters, (d) to evaluate the effect of the coagulant on the activated sludge microfauna by abundance analysis and monitoring of morphological–functional groups, (e) to relate the microfauna abundance and composition to process performance, (f) to assess the usefulness of the mentioned biological parameters as control tools of systems with phosphate simultaneous precipitation by ferric chloride.

2. Materials and methods

2.1. Activated sludge reactor and operating conditions

The study was conducted on an aerobic laboratory-scale activated sludge reactor consisting of a 2.8 l aeration basin coupled with a clarifier (1 l) and partial biomass recycle. The complete mix reactor was operated in a continuous-flow mode. The hydraulic residence time (HRT) was 29 h. High HRTs are characteristic of extended-aeration activated sludge systems, which are commonly applied for effluent treatment that contains high concentration of BOD, COD or slowly biodegradable compounds [17–19]. The sludge age was maintained at 21 days by daily wasting mixed liquor directly from the reactor. Aeration and mixing were supplied by aerators; air was introduced through porous diffusers at the bottom of the aeration basin. Dissolved oxygen (DO) concentration was above 2 mg/L. The reactor was fed with a model wastewater of a dairy industry (inlet stream) that was prepared twice a week; its composition was: cheese whey, 1.5 g/L as COD; $(NH_4)_2SO_4$, 0.94 g/L (7.12 mM); KH_2PO_4, 0.071 g/L (0.52 mM); $Na_2HPO_4 \cdot 7H_2O$, 0.213 g/L (0.80 mM); $NaHCO_3$, 1.0 g/L (12 mM); resulting nitrogen as ammonia (NH_3-N), 199.0 mg N/L; phosphorous as orthophosphate (PO_4^{3-}-P), 40.0 mg P/L and total P, 45.5 mg P/L [11]. pH of the wastewater was adjusted to 7.0. The wastewater was periodically monitored; COD, ammonia and orthophosphate concentrations did not significantly change through the study demonstrating that there was not fermentative activity during the storage of the influent wastewater.

The reactor was operated during more than 6 months. Firstly the system was run without addition of $FeCl_3$ (control period = 0–90 days), being the organic load or food–microorganism ratio (F/M) relatively high (0.65–0.75 kg COD/(kg VSS day)) to promote the growth of filamentous microorganisms. Then, ferric chloride was continuously added to the aeration basin for about 110 days using a peristaltic pump; for this, a stock solution of ferric trichloride hexahydrate, $FeCl_3 \cdot 6H_2O$ (2.02 g Fe/L, 36.17 mM), was used. Three periods of reactor operation with different doses of Fe(III), expressed on the basis of the molar ratio Fe:P, were set. A first period with high Fe:P ratio = 1.9–2.3:1 (91–156 days) was set in order to assess the effect of a shock load of Fe(III) on both filamentous microorganisms and phosphate removal. A second period with low Fe:P ratio = 1.0–1.4:1 (157–177 days) was established. At this period, the feasibility to operate the reactor with considerably lower doses than those recommended in literature, maintaining an acceptable removal of P, was evaluated. In this way, it would be possible to determine if either phosphate adsorption process on ferric hydroxide or anion exchange between hydroxide and phosphate ions allows compensating for the insufficient addition of Fe(III). It must be pointed out that in the previous period, the high Fe(III) dose and a relatively long cellular residence time (θ_C) would enhance ferric hydroxide accumulation. Finally, a last phase with Fe:P ratio = 1.5–1.9:1 (178–196 days), in order to determine optimal Fe(III) doses, was tested.

2.2. Chemical analysis

The activated sludge reactor was monitored on a regular basis (1–2 times per week). Different physical–chemical parameters were determined: pH, total suspended solids (TSS), volatile suspended solids (VSS) and fixed suspended solids (FSS) in the aeration basin; COD, TSS, PO_4^{3-}-P, total P, NH_3-N, total Fe and total soluble Fe were determined in the final effluent.

The samples from final effluent were processed in order to determine orthophosphate, ammonia nitrogen and total soluble Fe. A volume of 35 mL was centrifuged for 10 min at 13,000 rpm (Eppendorf centrifuge model 5415C); then, the supernatant was

filtered through 0.45 μm cellulose acetate membranes (GE Osmonics).

Orthophosphate, total P, ammonia nitrogen, total Fe and total soluble Fe were determined by colorimetric methods using commercial reagents (Hach Company, Loveland, CO). Orthophosphate concentration in the filtrate was determined after reacting with *vanadate–molybdate* reagent in acid medium (Hach Method No. 8114, adapted from Standard Methods). Total P was converted to orthophosphate by digestion with sulfuric acid and potassium persulfate (Hach Method No. 8190, adapted from Standard Methods); then, the method for orthophosphate determination was applied. Ammonia nitrogen in the filtrate was measured by the Nessler method (Hach Method No. 8038) and expressed as N (NH_3-N). Total soluble Fe concentration in the filtrate and total Fe concentration were determined by Hach Method No. 8008 (adapted from Standard Methods). Particulate P ($P_{Partic} > 0.45$ μm) was determined from the difference between total P and soluble P concentrations. Similarly, particulate Fe ($Fe_{Partic} > 0.45$ μm) corresponded to the difference between total Fe and total soluble Fe concentrations.

The sedimentation properties of the activated sludge were determined using the diluted sludge volume index (DSVI). The standard SVI test (sludge volume index) could not be used because the values of this index may be affected by re-flocculation and wall effects that hinder compaction of the sludge under high biomass concentrations conditions. The DSVI test is based on a series of twofold dilutions of the activated sludge sample, which eliminates the dependence of the index on biomass concentration. This index has been used in the case of filamentous bulking showing good correlation with the level of filamentous organisms in activated sludge [20].

2.3. Abundance of filamentous microorganisms

The subjective scoring system proposed by Jenkins et al. [20] is a rapid and simple microscopic counting technique; the abundance of filamentous microorganisms is classified according to a scale from none to excessive.

The method named total filament extended length (TEFL) is a well-known counting technique for measuring the total length of filaments that exhibits a very close correlation with various measures of activated sludge settling (SVI, SSVI and DSVI); however, Jenkins et al. [20] postulated that filament counting per se of activated sludge is not an efficient process control procedure. In addition, these authors argue that DSVI and filament counting give the same information, and recommend running a DSVI because it is far easier than to count filaments.

Therefore, in the present work, the subjective scoring system proposed by Jenkins et al. [20] and DSVI determinations were performed simultaneously to analyze the abundance of filamentous microorganisms.

2.4. Microfauna analysis

Samples of activated sludge mixed liquor were taken for microscopic analysis from the aeration basin on a regular basis (1–2 times per week). Protozoa and metazoa were quantified and identified using a light microscope Leica DMLB (Germany) fitted with a photographic camera [14,16,21]. Two sub-samples of 25 μl of the mixed liquor sample were taken with 200 μl automatic micropipette to be analyzed immediately under bright-field illumination [21]. Microfauna and ciliated protozoa densities were estimated under $100\times–400\times$ magnifications and expressed as number of organisms per liter of mixed liquor (No./L). Average densities of the 2 sub-samples taken at the same time were reported.

The organisms were classified into the following morphological–functional groups: swimming ciliates, attached ciliates, crawling ciliates, testate amoebae, nematodes and rotifers [14,22]. Ciliated protozoa were identified at the generic level. Whenever needed, photographs were taken for further analysis. Identification of protozoa was based on morphology [23,24].

The microfauna community was studied by analyzing the relative abundance of the different morphological–functional groups already described.

3. Results and discussion

3.1. Effect of different Fe:P ratios on the performance of the activated sludge reactor

3.1.1. Removal of PO_4^{3-} and total P

The laboratory-scale activated sludge reactor was operated under the following conditions: temperature = 17–21 °C, dissolved oxygen concentration in the aeration basin = 2–4 mg/L and hydraulic residence time (θ_H) = 29 h. A θ_C = 21 days was set in the experimental design; however, during the period without $FeCl_3$ addition, θ_C was often lower due to loss of solids from the clarifier by incidence of filamentous bulking. This problem was very severe during the period 70–82 days, resulting an estimated θ_C = 10 days.

The activated sludge reactor was fed with a P-rich influent (45.5 mg/L), typical of a dairy effluent; the treatment system was not designed for enhanced biological removal of nutrients. For these reasons, the final effluent showed soluble P and total P concentrations relatively high (>30 mg/L) during the period without chemical treatment (0–90 days, Fig. 1a).

During the control period (without ferric chloride addition), P in the final effluent was mainly in the form of soluble P (as orthophosphate), which accounted for 74–100% of total P. The remaining fraction of total P corresponded to a particulate form from organic source.

Since day 91, ferric chloride was continuously added to the aeration basin of the reactor in order to remove the high P concentrations from the wastewater.

The P removal process is complex and poorly known; for the case of salts of Fe(III) probably involves the following mechanisms: precipitation of ferric phosphate, ferric hydroxide and oxo-hydroxo-ferric-phosphate complexes, phosphate adsorption on ferric hydroxide, and coagulation and flocculation phenomena [5]. Phosphate removal through anion exchange between hydroxide and phosphate ions cannot be discarded [6].

In the present study, high Fe(III) doses (Fe:P ratio = 1.9–2.3:1, 91–156 days) achieved a rapid removal of orthophosphate. After 5 days of addition of Fe(III), orthophosphate removal higher than 98% was achieved, resulting residual concentrations lesser than 1.0 mg P/L. An average total P removal of 90% was achieved, resulting effluent concentrations ranging between 1.7 and 9.4 mg/L (Fig. 1a).

During the period with low Fe(III) doses (Fe:P ratio = 1.0–1.4:1, 157–177 days), the soluble P concentration increased rapidly up to 6.5 mg/L, thus high total P concentrations were registered (Fig. 1a). The relatively high values of soluble P indicated insufficient Fe(III) dose; these results allowed us also to infer that phosphate adsorption on accumulated ferric hydroxide and anion exchange between hydroxide and phosphate ions did not take place or did not have a significant effect that could compensate the applied low coagulant doses.

Because the Fe:P ratio = 1.9–2.3:1 was excessive and Fe:P ratio = 1.0–1.4:1 was insufficient, an intermediate Fe:P ratio = 1.5–1.9:1 was tested. These last doses caused a significant decrease in the P concentration of the final effluent, resulting values of 1.2 ± 0.2 mg P/L and 2.3 ± 0.4 mg P/L for soluble P and total P, respectively (Fig. 1a). The Fe:P ratio = 1.5–1.9:1 allows to comply with discharge limits for total P to either sewer system
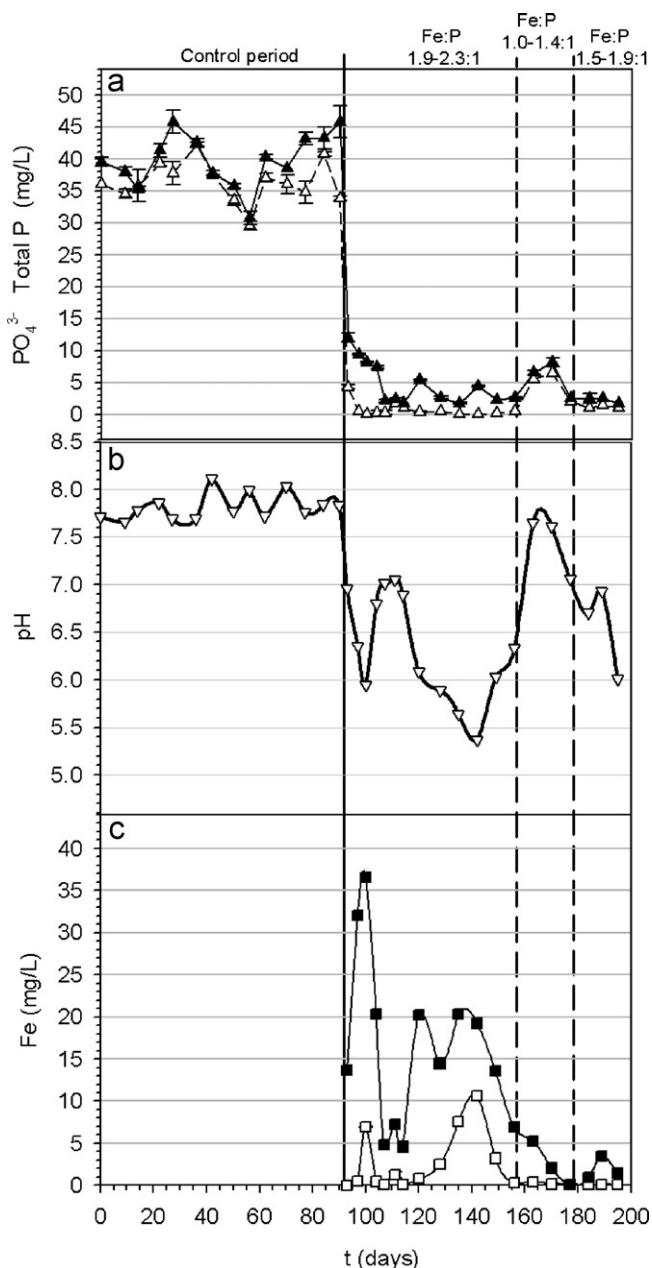
Fig. 1. Soluble phosphorus (PO_4^{3-}), total phosphorus (total P), total soluble Fe (Fe_{TS}) and particulate Fe (Fe_{Partic}) of the final effluent and pH of aeration basin as a function of the operation time of the activated sludge reactor. (a) ($\triangle$) PO_4^{3-} (mg/L); ($\blacktriangle$) Total P (mg/L), (b) ($\triangledown$) pH and (c) ($\square$) Fe_{TS} (mg/L); ($\blacksquare$) Fe_{Partic} (mg/L).
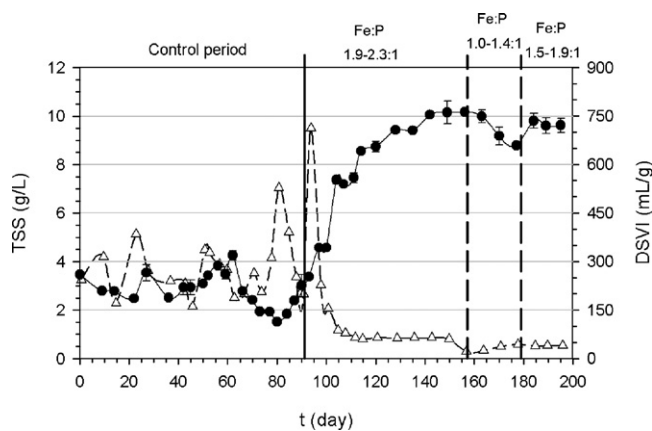

Fig. 2. Total suspended solids (TSS) of the aeration basin and diluted sludge volume index (DSVI) as a function of the reactor operation time. ($\bullet$) TSS (g/L); ($\triangle$) DSVI (mL/g).

or soils (≤ 10 mg P/L) for the Province of Buenos Aires – Argentine [25].

The exposed results allow us state that the soluble phosphorus removal depended on the applied Fe(III) dose, and the accumulation of precipitates containing P and Fe into sludge mass did not affect the process efficiency.

3.1.2. Removal of particulate phosphorus

Particulate P ($P_{Partic} > 0.45$ μm) in the final effluent was calculated as the difference between total P and soluble P concentrations. During the period without ferric chloride addition, the particulate P concentration was 3.5 ± 3.5 mg/L, and was represented mainly by organic P. During the period with a high Fe:P ratio, the P_{Partic} concentration ranged between 7.1 and 8.9 mg/L (95–104 days). Later, lower concentrations were measured (2.3 ± 1.5 mg/L). During

the period with low Fe:P ratio, particulate P levels were significantly reduced to 1.1 ± 0.6 mg/L. Finally, during the optimization period of the coagulant dose (intermediate Fe:P ratio = 1.5–1.9:1), the particulate P concentration was similar to the previous phase (1.1 ± 0.4 mg/L).

3.1.3. Changes of pH

During the period without ferric chloride addition, the pH values in the aeration basin ranged between 7.7 and 8.1, typical values of biological treatment systems of wastewaters (Fig. 1b). Ferric chloride addition caused a rapid decrease of pH in the aeration basin because of the hydrolysis reactions of Fe(III). During the period with a high Fe:P ratio, pH values between 5.4 and 7.1 were registered, which are compatible with the microbial activity. For low Fe:P ratio, the pH value increased rapidly, and ranged between 7.1 and 7.7. Finally, during the optimization period of the Fe(III) dosage, the pH value ranged between 6.0 and 7.0 (Fig. 1b).

It must be pointed out that the P removal process took place under suitable pH conditions (pH = 5.0–7.0 [10]), for almost the entire period of the reactor operation.

3.1.4. Total Fe, total soluble Fe and particulate Fe present in the final effluent

Total Fe and total soluble Fe concentrations of the final effluent were determined, being the difference between them the particulate Fe ($Fe_{Partic} > 0.45$ μm).

The total soluble Fe concentration depended on the Fe(III) dose, because an excess of Fe consumes the system alkalinity, acidifying the aeration basin mixed liquor, and thus promoting the solubility of the metal. During the period with high Fe:P ratio (1.9–2.3:1), relatively high total soluble Fe concentrations (2.5–10.7 mg/L) were frequently measured for pH values below 6.0; concentrations below 0.6 mg/L were registered when the pH value was ≥ 6.0. Particulate Fe concentration ranged between 5.0 and 37.0 mg/L (Fig. 1b,c). For periods with low and intermediate doses (Fe:P = 1.0–1.4:1 and 1.5–1.9:1), pH values were above 6.1, total soluble Fe concentration was below 0.2 mg/L, and the particulate Fe level was notably reduced (Fig. 1b and c).

The decrease of the effluent Fe concentration, and the improvement of the total P removal (section 3.1.1), by reducing the Fe:P ratio from 1.9–2.3:1 to 1.0–1.4:1 and then to 1.5–1.9:1, permitted us to infer that the reactor suffered coagulant overload for a long time. Thus, the optimal Fe(III) doses to achieve relatively low total P and total Fe concentrations were Fe:P ratio = 1.5–1.9:1.

3.1.5. Characterization of the particulate matter in the final effluent

High particulate Fe concentrations corresponded to the highest particulate P levels in the final effluent, which frequently occurred during the period with high Fe:P ratio (91–156 days). A molar Fe:P ratio of 2.07 was determined through linear regression analysis (Sigma Plot 10.0) between Fe_{Partic} and P_{Partic}. This value is in the range of those reported in literature between 1.66 and 3.0 [26]. This observation allows us to postulate a precipitation process of P and Fe resulting in the formation of precipitates consisting of pure components (ferric phosphate and ferric hydroxide) and co-precipitates of P with Fe to form ferric hydroxyphosphate complexes.

3.1.6. Sedimentation properties of activated sludge. Control of filamentous bulking

Fig. 2 shows the concentration of total suspended solids (TSS) in the aeration basin and the diluted sludge volume index (DSVI) as a function of the operating time of the reactor. In the period without ferric chloride addition, particularly during 0–65 days, the TSS concentration of the aeration basin ranged between 2.5 and 4.2 g/L, due probably to the poor settling properties (DSVI > 150 mL/g) and frequent loss of sludge through the final effluent. During this period, proliferation of filamentous microorganisms took place. Abundance of filamentous microorganisms determined by the subjective scoring system proposed by Jenkins et al. [20] was qualitatively correlated to the settling properties determined by DSVI. Filaments typically protruded from floc causing inter-floc bridging, thus leading to the formation of a network structure. As a result, sedimentation and compaction of activated sludge flocs were negatively affected. Since day 70, DSVI increased progressively reaching values close to 550 mL/g that indicates deterioration of the settling properties. Marked loss of sludge through the final effluent caused a sharp decrease of the TSS concentration up to values of about 1.5 g/L (Fig. 2). It must be pointed out that this phenomenon occurred when the system exhibited excessive abundance of filaments corresponding to severe filamentous bulking (70–82 days, Fig. 3).

A marked improvement of the sedimentation properties of activated sludge was observed after 4–5 days of Fe(III) continuous addition (high Fe:P ratio = 1.9–2.3:1), resulting DSVI values below 150 mL/g. Values ranging between 59 and 64 mL/g were reached after approximately 8–9 days of application of the same coagulant doses (Fig. 2). The improvement of the sedimentation properties coincided with the pronounced decline in the abundance of filamentous microorganisms (Fig. 3). Thus, the continuous addition of Fe(III) was able to control filamentous bulking according to previous studies [4,27].

For periods with low and intermediate doses (Fe:P = 1.0–1.4:1 and 1.5–1.9:1), the good settling properties with DSVI values below 60 mL/g were according to the frequency of occurrence of filamentous microorganisms: absence of filaments or few abundance (Figs. 2 and 3).

3.1.7. Total suspended solids, volatile suspended solids and fixed suspended solids in the activated sludge aeration basin

The TSS concentration in the aeration basin increased progressively once the ferric chloride addition started. The TSS concentration varied lightly with changes of the Fe(III) dose (Fig. 2).

The continuous addition of Fe(III) caused a rapid increase in the concentration of volatile suspended solids (VSS) of the aeration basin from 2.4 ± 0.5 g/L (period without Fe(III)) to 5.2 ± 0.1 g/L (high Fe:P, Fig. 4). The increase of the VSS content of the activated sludge was caused by the marked improvement of the sludge sedimentation properties, and hence the significant decline in biomass

loss of the system. During the following operation phases (low and optimum Fe:P ratio), the VSS concentration of the aeration basin decreased from 5.0 to about 4.1 g/L (Fig. 4).

The concentration of fixed suspended solids (FSS) was determined from the difference between TSS and VSS. The increase of the FSS content of the activated sludge was evident after $0.5\,\theta_C$ of Fe(III) treatment. The FSS concentration reached a value close to 4.7 g/L after $1.75\,\theta_C$ of Fe(III) application (high Fe:P), which did not vary markedly for the following Fe(III) doses (Fig. 4). The VSS/TSS ratio gradually decreased from 80% (period without Fe(III)) to an average value of 52% (θ_C with Fe(III) = 1.4–3.1) and finally to 45% (low and optimum Fe:P ratio) (Fig. 4).

These results indicate that a large amount of the coagulant was incorporated into the microbial flocs, which generated a significant increase in the inorganic fraction of the activated sludge. As was explained, a precipitation process of P and Fe took place, which resulted in the formation of ferric phosphate, ferric hydroxide, and ferric hydroxyphosphate complexes (Section 3.1.5). In this context, it is suggested that the marked improvement in the sedimentation properties of the sludge was due not only to the successful control of filamentous bulking but also to an increase in the density of microbial flocs by the incorporation of both Fe(III) and particles containing Fe–P into the sludge.

3.1.8. Characterization of the final effluent. Removal of COD and TSS

During the control period, high COD and TSS concentrations were frequently observed, which indicated low process performance (Fig. 5). COD values often exceeded the maximum discharge limit to either storm-water collection systems or surface water bodies ($\leq$250 mg/L) for the Province of Buenos Aires – Argentine [25]. During the period with severe filamentous bulking problems, the COD concentration varied between 600 and 1400 mg/L (Fig. 5).

In the absence of Fe(III), the curve for the TSS concentration of the final effluent was similar to that corresponding to COD, because the loss of biomass from the clarifier caused an increase in COD. The soluble fraction of COD was relatively low (40–60 mg/L), therefore the main contribution of COD to the final effluent was TSS.

The addition of coagulant affected both parameters, COD and TSS. The COD concentration ranged between 80 and 210 mg/L regardless of the applied Fe(III) dose (Fig. 5). These values would comply with the maximum discharge limit for the Province of Buenos Aires – Argentine [25].

The TSS concentration of the final effluent depended on the applied Fe:P ratio, and generally corresponded to the observed changes in the particulate P and particulate Fe concentrations of the final effluent. During the period with high Fe:P ratio, TSS concentration ranged initially between 200 and 720 mg/L (95–104 days, Fig. 5). These values corresponded to the relatively high particulate P and particulate Fe concentrations in the final effluent. A period of 15–17 days (θ_C = 0.75) was required to reduce the TSS concentration of the final effluent to 90–100 mg/L (107–115 days); this decrease in the TSS concentration was consistent with the reduced levels of particulate P and particulate Fe. Between 120 and 156 days, the TSS concentration increased to 130–240 mg/L. Significantly lower values (10–70 mg/L) were recorded for the periods with low and optimal Fe:P ratio (enlarged detail in Fig. 5).

During the Fe(III) addition, TSS that escaped from the clarifier did not provide significant amounts of COD to the treated effluent. The TSS in the final effluent was basically of inorganic nature due to the contribution of fixed solids from the coagulant or chemical complexes containing Fe–P. The decrease of the TSS concentration by reducing the Fe:P dose permitted us also to deduce that the reactor suffered coagulant overload for a long time.
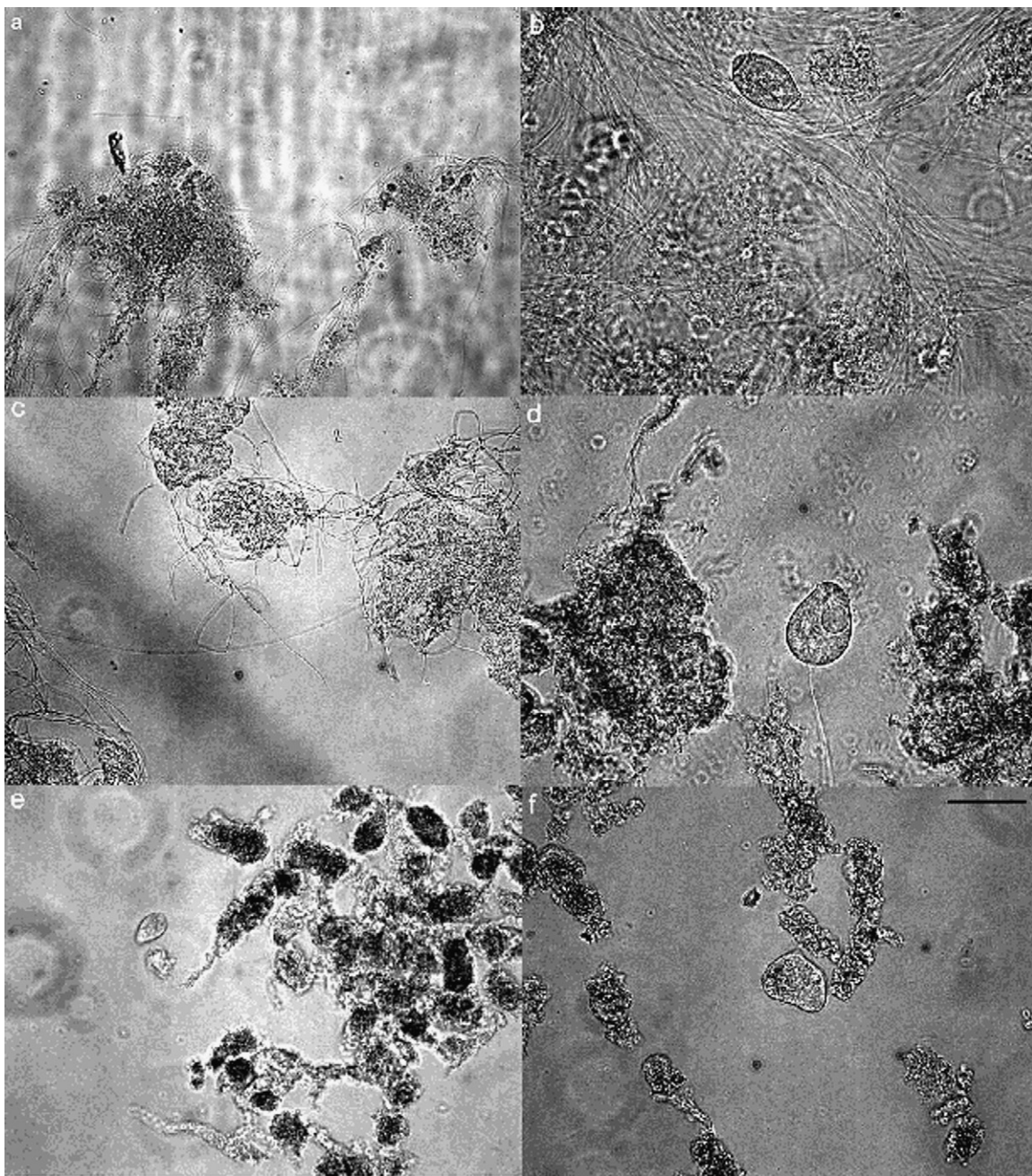
Fig. 3. Micrographs of activated sludge from the reactor aeration basin corresponding to different operating periods. Control period: (a) day 27; (b) day 77. High Fe:P ratio (molar ratio) = 1.9–2.3:1: (c) day 97; (d) day 104. Low Fe:P ratio = 1.0–1.4:1: (e) day 177. Intermediate Fe:P ratio = 1.5–1.9:1: (f) day 189 (− 50 μm).

3.1.9. Effect of coagulant dose on the flocculation and sedimentation of particles containing Fe–P

The results found in previous sections allowed us to infer that the application of high initial doses of Fe(III) (Fe:P = 1.9–2.3:1) acted as a shock load producing a rapid improvement in the performance of the reactor, in terms of COD and soluble P removal. The orthophosphate removal involved a mechanism of co-precipitation to form ferric hydroxylphosphate complexes.

Coagulation and flocculation of primary colloidal particles would lead to the formation of larger particles [5], and probably of higher density, which can be removed more easily by gravitational settling. The formation of small iron hydroxide particles 30–60 nm in diameter has been reported previously for wastewater treated with iron salts for phosphate removal [28]. The presence of biomass favors the separation of colloidal particles containing Fe or Fe–P [28,29].

In the present work, the separation efficiency of particles containing Fe–P in the clarifier depended on the coagulant dose and contact time. A high Fe(III) dose (Fe:P = 1.9–2.3:1) produced initially a poor quality final effluent; although acceptable COD levels were achieved, concentrations of particulate P, particulate Fe and TSS varied considerably reaching unacceptable values (91–104 days). During this initial period, the fixed suspended solids concentration of the aeration basin did not significantly increase. Incipient aggregation of colloidal particles containing Fe–P and its poor incorporation into biomass could explain the low reactor performance during the initial period of Fe(III) addition. About 15–17 days with a continuous Fe(III) dosage were necessary to improve the quality of the final effluent, in this period the content of FSS increased from
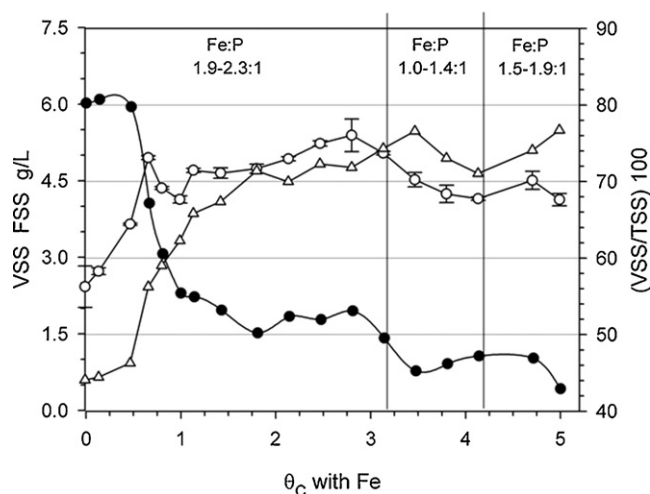
Fig. 4. Biomass content of the aeration basin as a function of cell residence time with Fe(III). (○) volatile suspended solids (VSS, g/L), (△) fixed suspended solids (FSS, g/L), (●) VSS:TSS ratio expressed as percentage.

20% (phase without Fe(III)) up to about 40%. In a simpler system (Fe(III)–phosphate–hydrogen), Smith et al. [30] showed that the structure of hydrous ferric oxide becomes more compact and dense over time. In our study, ferric hydroxyphosphate complexes were gradually incorporated into the organic matrix of microbial flocs changing its structure and increasing its density; these changes probably caused a considerable improvement of the reactor performance during the period 107–115 days (Fe:P = 1.9–2.3:1).

The plant performance decayed during the period 120–150 days (Fe:P ratio = 1.9–2.3:1). This Fe:P ratio was within the range of values reported in literature (1.5 –3.0); however, a high Fe(III) dose (141 mg Fe/(L day)), was necessary applying to remove the high inlet concentration of total P. The high Fe(III) dose applied for an extended period and the high cellular residence time (21 days) caused a progressive enrichment of Fe(III) in the sludge. This situation might have significantly favored the reduction from Fe(III) to Fe(II), resulting in the formation of FeS deposits which were observed on the clarifier walls. Reduction of Fe(III) to Fe(II) can occur by microbial activity or by sulfide. Reduction of sulfate to sulfide took place probably under anaerobic conditions in the clarifier favoring the Fe(III) reduction process. Nielsen and Keiding [31] reported that reduction of Fe(III) to FeS led to a weakening of the floc strength and disintegration of activated sludge flocs.
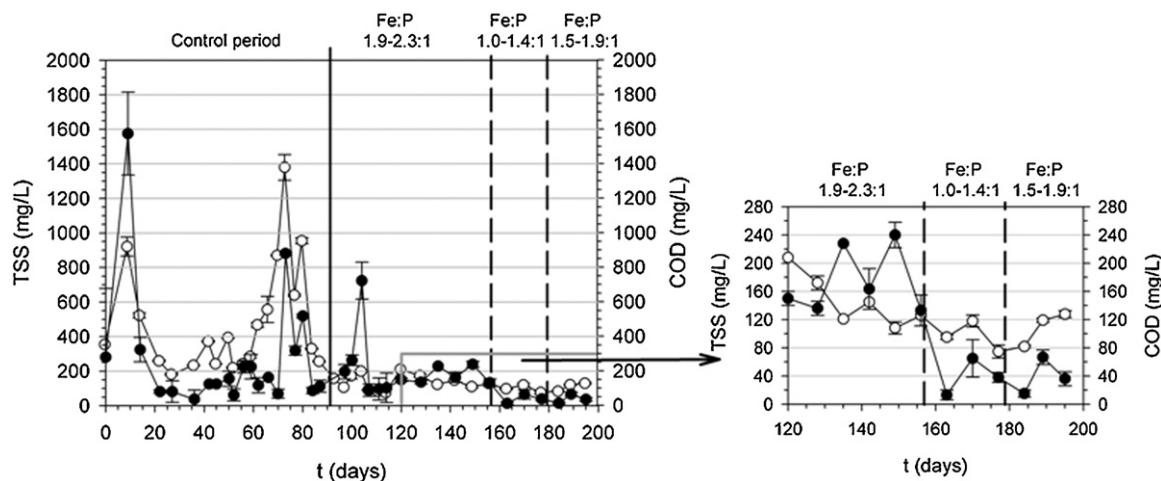
Li [12] showed that appropriate concentrations of Fe(III) enhanced the ability of activated sludge to retain particulate and colloidal matter within flocs, but over-dosages of Fe(III) weakened the aggregation process forming dispersed flocs. In our study, the high Fe(III) dose applied for an extended period (3.0 θ_C) favored the formation of small dispersed chemical flocs by either failure of the coagulation/flocculation mechanisms or flocs disintegration by formation of FeS. These phenomena might explain the deterioration of the final effluent quality during 120–150 days.

It must be noted that since approximately day 125 up to the end of the period with high Fe:P doses, the concentration of the fixed suspended solids of the aeration basin remained almost constant, and particles containing Fe–P escaped from the clarifier deteriorating the final effluent quality. These results allow us to state that the system can tolerate a maximum coagulant load, which once exceeded, leads to a low reactor performance. For low and intermediate doses of Fe(III), the concentration of fixed suspended solids did not change significantly. The optimum dose of Fe(III) necessary to produce a good quality effluent with respect to all the analyzed physical–chemical parameters was Fe:P = 1.5–1.9:1. For these doses, the Fe(III) entering the reactor was completely incorporated into the activated sludge as particles containing Fe–P, with an accumulation rate similar to the waste removal rate, achieving a good performance.

3.2. Effect of ferric chloride on the activated sludge microfauna

3.2.1. Total microfauna abundance and abundance of ciliated protozoa

The analysis of the microfauna community of activated sludge began after about 2 θ_C, i.e. once the sludge showed good flocculation.

During the period without ferric chloride addition (0–90 days), the microfauna showed densities between 1×10^6/L and 1×10^7/L (Fig. 6a). Even though densities $\geq 1 \times 10^6$/L would correspond to an efficient process [14], in the present study the reactor showed commonly a mediocre performance (Fig. 5). Ciliated protozoa represented the most abundant group of microfauna organisms (88–100%).

Lower densities (7.0×10^5/L–1.0×10^6/L) were registered during the period 70–77 days (Fig. 6a). It should be noted that when the system exhibited severe filamentous bulking problems (70–82 days), the settling properties were gradually deteriorated leading to loss of sludge from the clarifier effluent and consequently sharp decline in the TSS concentration of the aeration basin (Fig. 2, Section 3.1.6). This situation reduced the θ_C from 21 days to about

Fig. 5. Total suspended solids (TSS) and chemical oxygen demand (COD) of the final effluent as a function of the reactor operation time. (●) TSS (mg/L), (○) COD (mg/L).
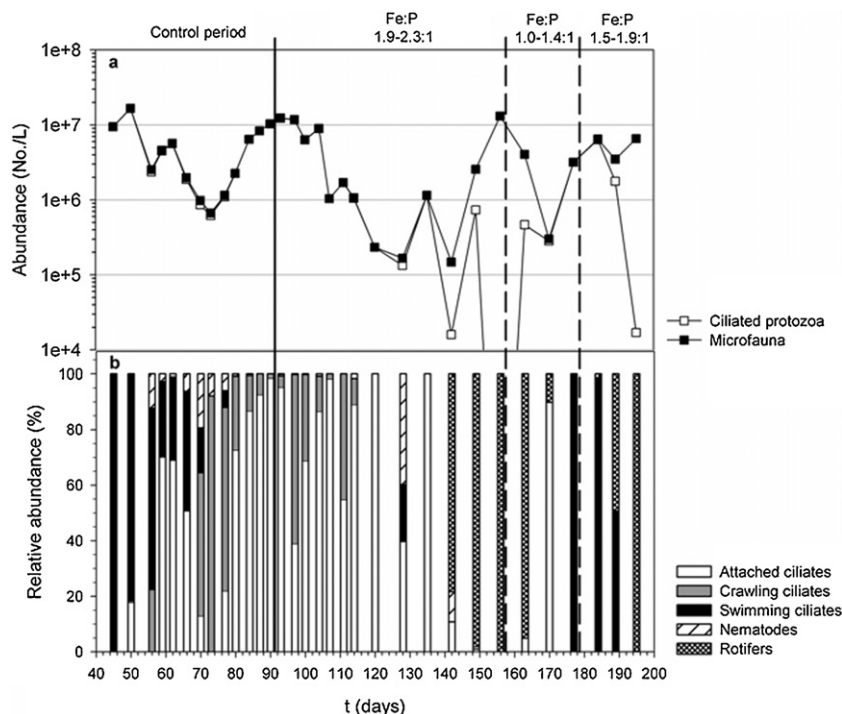
Fig. 6. Abundance of microfauna organisms as a function of reactor operation time. (a) Density of total organisms and ciliated protozoa (No/L). (b) Percentage abundance of different morphological–functional groups.

10 days, probably causing the observed decrease in the number of organisms. Thus, the changes in abundance of the activated sludge microfauna reflected the observed deterioration in the reactor efficiency (Fig. 5).

At the end of the control period, a gradual recovery of the microfauna density was observed (Fig. 6a), which corresponded to an improvement in the reactor efficiency (Fig. 5).

In the period with high Fe:P ratio, the microfauna community, represented mainly by ciliated protozoa (>98%), showed initially densities that ranged between 6.3×10^6/L and 1.2×10^7/L (91–104 days); then abundance was reduced to about 1.0×10^6/L (107–114 days, Fig. 6a). This last situation corresponded to the establishment of a stable and efficient system with good final effluent quality (Fig. 1c, Fig. 5). Between 120 and 142 days, the microfauna density was less than 1.0×10^6/L, which indicated poor system efficiency (Figs. 1c and 5).

At the end of the period with high Fe:P ratio (148–156 days), the microfauna density reached relatively high values (2.5×10^6/L–1.3×10^7/L). Even though this abundance would correspond to an efficient process, the final effluent showed poor quality (Figs. 1c and 5). The low density and absence of ciliates reflected low or no efficiency of the system, respectively [32]. It should be noted that the ciliates represented 0–22% of the microfauna community, and rotifers were dominant.

During the phase with low Fe:P ratio (157–177 days), ciliates density recovered (Fig. 6a). The system exhibited a rapid improvement in performance, which was reflected in the final effluent quality with acceptable levels of Fe, TSS and COD (Figs. 1c and 5).

During the period with optimal Fe:P dose (178–196 days), the microfauna density reached relatively high values, unlike the ciliates density gradually decreased (Fig. 6a). It should be noted that during this period the reactor showed good performance (Figs. 1c and 5).

In general terms, microfauna abundance and ciliates abundance reflected the changes of the system performance during the different periods with added Fe(III). However, it is necessary to have additional information about the composition of the activated sludge microfauna.

3.2.2. Relative abundance of different morphological–functional groups in the activated sludge reactor

3.2.2.1. Control period. The microfauna community showed relatively low diversity, which could be associated with either a low efficient process for wastewater treatment or relatively high levels of ammonia in the aeration basin (140–170 mg/L N-NH$_3$). The dominance of the various organism groups in the reactor varied throughout the experimental period. At the beginning of the operation (45–50 days), swimming ciliates of the genus *Colpoda* were the dominant organisms in the reactor, representing 81–99% of the community. This group of organisms is typical of early stages of activated sludge system operation [14]. Then, the density of swimming ciliates decreased about one order of magnitude, being replaced by attached ciliates (*Vorticella microstoma*, *Vorticella* spp.) and crawling ciliates (*Chilodonella* sp.). Sessile forms accounted for more than 50% of the microfauna organisms in the aeration basin during 59–66 days (Fig. 6b); in this period, mediocre settling properties and reactor performance were registered (Figs. 2 and 5).

Between 70 and 77 days, the crawling ciliates represented 52–91% of the microfauna, being *Chilodonella* sp. the sole representative of that group (Fig. 6b). It is well known that the dominance of crawling ciliates and co-dominance of crawling ciliates and attached ciliates indicate good performance of the activated sludge plants [14,33]; however, in the present study the reactor performance exhibited a marked deterioration during the abovementioned period (Fig. 5).

When the system exhibited severe filamentous bulking problems (70–82 days), the final effluent showed unacceptable levels of COD and TSS (Fig. 5). It must be pointed out that even though the decrease of the microfauna density rapidly reflected this situation, the community composition did not respond immediately to changes in the system environmental quality.

Changes in the microfauna community related to the incidence of severe filamentous bulking were registered after about one week of starting this phenomenon. In this regard, Chomczyńska et al. [22] argued that bioindication, based on indicator species or morphological–functional groups, enables assessment of living conditions a generation time of species present in samples backwards from the instant of observation. The sharp decrease of the sludge concentration would have favored the proliferation of the attached ciliate *Opercularia* sp., which represented 66–95% of the community during 80–90 days. This organism is often associated with *Vorticella microstoma*, being both indicator organisms of poor performance of the activated sludge process [14].

The excessive growth of *Opercularia* sp. involved a progressive increase of attached ciliates during 77–90 days. This group dominated the community during 80–90 days, representing 73–99% of the microfauna (Fig. 6b). In fact, a massive increase in number of attached ciliates (>80% of the microfauna) occurs during transient situations, such as a quick increase of the organic load (F/M ratio) due to loss of sludge, and a discontinuous input of organic load from the influent, which reduce the plant performance [14]. In the present study, the first of these situations would have caused the deterioration of the reactor performance during 70–82 days.

3.2.2.2. Operating periods with different Fe:P ratios. High Fe(III) doses (Fe:P = 1.9–2.3:1) improved rapidly the settling properties of sludge (Fig. 2). This situation could cause a sudden increase in the density of *Chilodonella* sp. by an order of magnitude during 91–104 days, according to reports in literature indicating that high densities of crawling ciliates are always associated with good settling of sludge [14,33].

After about one week from the beginning of the Fe(III) addition, crawling ciliates and attached ciliates co-dominated the community (Fig. 6b). However, *Opercularia* sp. maintained a high density (4.4×10^6/L), which could be due to its ability to survive in stressed environments better than other protozoa [14,34]. A very efficient and stable operation was observed during a brief period (107–114 days), which was reflected by the high-quality of the final effluent (Figs. 1c and 5).

Later, the number of *Chilodonella* sp. decreased strongly, and attached ciliates (*Vorticella microstoma*, *Vorticella* spp. and *Opercularia* sp.) represented more 80% of the microfauna community (114–135 days, Fig. 6b), which indicated a decline in the system efficiency.

At the end of the period with high Fe:P ratio, the ciliates showed low densities or were not observed (142–156 days, Fig. 6a). During 142–149 days, the motile, dispersive form (called telotroch) of the attached ciliates group represented more than 97% of the ciliates community. Telotroch proliferation was associated with unfavorable environmental conditions for attachment to a suitable substrate. It is well known that when environmental conditions deteriorate sufficiently, the sessile trophont termed the stalked zooid transforms into a telotroch, detaches from the stalk, and swims leaving the stalk behind. When the telotroch finds a new suitable location, it re-attaches, re-grows its stalk, and begins feeding [35]. Sessile ciliates feed on dispersed bacteria by filtering systems, competing successfully with swimming ciliates and rotifers; thus once that the disappearance of attached ciliates took place, growth of rotifers was probably favored. During the period 142–156 days, rotifers accounted for 78–99% of the microfauna (Fig. 6b).

During the period with low Fe:P ratio = 1.0–1.4:1, the ciliates recovered (*Vorticella* spp., *Vorticella microstoma*) and the number of rotifers decreased to finally disappear (Fig. 6a and b). At the end of this period, the swimming ciliate *Colpoda* sp. represented 100% of the microfauna community. The aeration basin was firstly dominated by rotifers, then by attached ciliates and finally by swimming ciliates. Even though these situations would correspond to a low performance system, the final effluent showed high quality with acceptable levels of Fe, TSS and COD (Figs. 1c and 5).

During the period with optimal Fe:P ratio, *Colpoda* sp. decayed and rotifers represented finally >99% of the microfauna (Fig. 6b). The community composition did not reflect the good enough performance exhibited by the reactor during this period (Figs. 1c and 5).

3.2.3. Morphological–functional groups as indicators of the flocs microstructure

The dosage of ferric chloride generated stress conditions, which reduced the number of organisms, and also altered the microfauna composition by promoting the development of species or organisms groups more tolerant than others. The initial Fe(III) dose (Fe:P ratio = 1.9–2.3:1) caused a progressive enrichment of Fe(III) in the sludge modifying the structure of the flocs, which probably altered the microhabitats of morphological–functional groups closely associated with the microbial flocs. This would have negatively affected the development of both crawling ciliates, which feed on bacteria slightly attached to the surface of the flocs, and attached ciliates due to a modification of the attachment surface. The disappearance of these organism groups favored the development of organisms not directly associated with flocs such as rotifers and swimming ciliates.

It must be pointed out that since about day 142 until the end of the study, the microfauna community was dominated by a single functional group (rotifers) or species (*Colpoda* sp.), which is characteristic of polluted environments or under stress conditions.

Analyses based on both the total density and the relative abundance of microfauna functional groups would detect overload situations of Fe(III) on the biological system. Particularly, the decrease in the density of ciliates and the disappearance of crawling ciliates constituted strong evidence of Fe(III) over-dose.

The decrease of the Fe(III) dose was not sufficient to restore the typical microfauna structure of activated sludge systems with good performance. This situation could be explained considering that the high content of fixed solids of the sludge, caused by overload of Fe(III) for extended periods ($3 \theta_C$), persisted even after reduction of the Fe(III) dose. This implies that if well the reactor operated with phosphorous simultaneous precipitation recovered after decreasing the Fe(III) dose, exhibiting an acceptable performance, it would be highly susceptible to potential coagulant over-load phenomena.

4. Conclusions

- The application of different doses of ferric chloride (Fe:P = 1.5–2.3:1, molar ratio) to the aeration basin of an activated sludge reactor (laboratory-scale), fed with a high P load, achieved soluble P removal between 96 and 98%, and total P removal higher than 90%.
- A high Fe:P ratio = 1.9–2.3:1 acted as shock load quickly improving the COD and soluble P removal.
- The soluble P was removed by a chemical precipitation mechanism resulting a ferric hydroxyphosphate complex. The soluble phosphorus removal depended on the applied Fe(III) dose, and the accumulation of precipitates containing P and Fe into sludge mass did not affect the process efficiency.
- The sedimentation properties of the sludge were improved at about one week after coagulant addition. This was associated to both, a successful control of filamentous bulking and an increase in the density of microbial flocs by the incorporation of Fe(III) and particles containing Fe–P into the sludge. The incorporation of inorganic solids to the biomass flocs improved significantly the solid–liquid separation in the clarifier increasing the final efflu-

ent quality after a response time of at least 0.75 θ_C with high Fe:P doses.

- The application of high Fe(III) doses for an extended period (3 θ_C) caused a gradual accumulation of fixed solids in the activated sludge, which favored finally the formation of dispersed flocs deteriorating the final effluent quality. These results allow us to state that the system could tolerate a maximum coagulant load, which once exceeded, leads to a low reactor performance. It is recommended, once reached the desired level of soluble P, to reduce the dose to a Fe:P ratio = 1.5–1.9:1 to obtain acceptable effluent quality in terms of TSS, COD, total P and total Fe.
- The periodic analysis of the microfauna density, ciliates density and relative abundance of morphological–functional groups allowed monitoring the progressive toxic effect of ferric chloride on the biological system.
- Changes in the density of *Chilodonella* sp., *Opercularia* sp., *Vorticella microstoma*, *Vorticella* spp., *Colpoda* sp. and rotifers were analyzed as a function of the progressive enrichment of Fe(III) in the sludge. The crawling ciliates constituted the most sensitive group to changes in environmental quality of the system operated with simultaneous precipitation of P. This organism group could be used as biological indicator of overload conditions of Fe(III) in such systems.
- The analysis of the microfauna allowed to determine the adequate ferric chloride doses, and could be used as a tool for process control of activated sludge with phosphorus simultaneous precipitation.
- The combined analysis of physical–chemical and biological parameters constitutes a useful procedure to monitor the reactor performance and the final effluent quality.

Acknowledgments

References

[1] D. Jenkins, J.F. Ferguson, A.B. Menar, Chemical processes for phosphate removal, Water Res. 5 (1971) 369–389.

[2] D.W. De Haas, M.C. Wentzel, G.A. Ekama, The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological enhanced phosphate removal. Part 1: Literature review, Water SA 26 (2000) 439–452.

[3] M. Henze, P. Harremoes, J.C. Jansen, E. Arvin, Wastewater Treatment. Biological and Chemical Processes, third ed., Springer-Verlag Heidelberg, Germany, 2002.

[4] T. Clark, T. Stephenson, Effects of chemical addition on aerobic biological treatment of municipal wastewater, Environ. Technol. 19 (1998) 579–590.

[5] I. Takács, S. Murthy, S. Smith, M. McGrath, Chemical phosphorous removal to extremely low levels: experience of two plants in the Washington D.C. area, Water Sci. Technol. 53 (2006) 21–28.

[6] B. Rabinowitz, GvR Marais, Chemical and Biological Phosphorus Removal in the Activated Sludge Process, Research Report No. W32, Univ. of Cape Town, Dept. of Civil Eng., March 1980.

[7] S. Yeoman, T. Stephenson, J.N. Lester, R. Perry, The removal of phosphorus during wastewater treatment: a review, Environ. Pollut. 49 (1988) 183–233.

[8] T. Clark, J.E. Burgess, T. Stephenson, A.K. Arnold-Smith, The influence of iron-based co-precipitants on activated sludge biomass, Process Saf. Environ. Protect. 78 (2000) 405–410.

[9] J. Thistleton, T.-A. Berry, P. Pearce, S.A. Parsons, Mechanisms of chemical phosphorus removal II. Iron (III) salts, Process Saf. Environ. Prot.: Trans. IChemE B 80 (2002) 265–269.

[10] A. Szabó, I. Takács, S. Murthy, G.T. Daigger, I. Licskó, S. Smith, Significance of design and operational variables in chemical phosphorus removal, Water Environ. Res. 80 (2008) 407–416.

[11] A.H. Caravelli, E.M. Contreras, N.E. Zaritzky, Phosphorous removal in batch systems using ferric chloride in the presence of activated sludges, J. Hazard. Mater. 177 (2010) 199–208.

[12] J. Li, Effects of Fe(III) on floc characteristics of activated sludge, J. Chem. Tech. Biotechnol. 80 (2005) 313–319.

[13] G. Esteban, C. Tellez, L.M. Bautista, Dynamics of ciliated protozoa communities in activated-sludge process, Water Res. 25 (1991) 967–972.

[14] P. Madoni, A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis, Water Res. 28 (1994) 67–75.

[15] K. Wuhrmann, Objective, technology and results of nitrogen and phosphorus removal processes, in: Adv Water Quality Improvement, Univ. of Texas Press, 1968.

[16] J.E. Burgess, M.E. Mayhew, T. Stephenson, T.A. Clark, Assessment of microbial populations in activated sludge using an organism diversity index, J. Chart. Inst. Water Environ. Manage. 16 (2002) 40–45.

[17] A.L. Batt, S. Kim, D.S. Aga, Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations, Chemosphere 68 (2007) 428–435, http://www.scopus.com/search/submit/citedby.url?eid=2-s2.0-34147190445&src=s&origin=resultslist.

[18] L. Loperena, M.D. Ferrari, V. Saravia, D. Murro, C. Lima, L. Ferrando, A. Fernández, C. Lareo, Performance of a commercial inoculum for the aerobic biodegradation of a high fat content dairy wastewater, Bioresour. Technol. 98 (2007) 1045–1051.

[19] A.M. Ferro Orozco, E.M. Contreras, N.E. Zaritzky, Dynamic response of combined activated sludge-powdered activated carbon batch systems, Chem. Eng. J. 157 (2010) 331–338.

[20] D. Jenkins, M.G. Richard, G.T. Daigger, Manual on the Causes and Control of Activated Sludge Bulking and Foaming, second ed., Lewis Publishers, Inc., Chelsea, MI, 1993.

[21] A. Nicolau, M. Mota, N. Lima, Microfauna as indicator of copper, zinc, and cyclo-heximide in activated sludge processes, Environ. Eng. Sci. 24 (2007) 434–445.

[22] M. Chomczyńska, A. Montusiewicz, J. Malicki, G. Łagód, Application of saprobes for bioindication of wastewater quality, Environ. Eng. Sci. 26 (2009) 289–295.

[23] D.J. Patterson, Free-Living Freshwater Protozoa: A Colour Guide, Manson Publishing Ltd., London, 1996.

[24] Protist Information Server, Japan. The Graduate University for Advanced Studies and Japan Science and Technology Corporation. http://protist.i.hosei.ac.jp/Protist_menuE.html.

[25] Resolution 336/2003, Water Authority, Province of Buenos Aires, Argentine 2003.

[26] D.W. De Haas, M.C. Wentzel, G.A. Ekama, The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal. Part 4: Experimental periods using ferric chloride, Water SA 26 (2000) 485–504.

[27] C.B. Lind, Phosphorus inactivation in wastewater treatment: biological and chemical strategies, Water Eng. Manage. 145 (1998) 18–21.

[28] Q.H. He, G.G. Leppard, C.R. Paige, W.J. Snodgrass, Transmission electron microscopy of a phosphate effect on the colloid structure of iron hydroxide, Water Res. 30 (1996) 1345–1352.

[29] S. Deneux-Mustin, B.S. Lartiges, G. Villemin, F. Thomas, J. Yvon, J.L. Bersillon, D. Snidaro, Ferric chloride and lime conditioning of activated sludges: an electron microscopic study on resin-embedded samples, Water Res. 35 (2001) 3018–3024.

[30] S. Smith, I. Takács, S. Murthy, G.T. Daigger, A. Szabó, Phosphate complexation model and its implications for chemical phosphorus removal, Water Environ. Res. 80 (2008) 428–438.

[31] P.H. Nielsen, K. Keiding, Disintegration of activated sludge flocs in presence of sulfide, Water Res. 32 (1998) 313–320.

[32] N. De Marco, A. Gabelli, C. Cattaruzza, L. Petronio, Performance of biological sewage treatment plants: some experiences on municipal plants in the province of Pordinone (Italy), in: P. Madoni (Ed.), Biological Approach to Sewage Treatment Process: Current Status and Perspectives, Centro Bazzucchi, Perugia, 1991, pp. 247–251.

[33] V.K. Tyagi, S. Subramaniyan, A.A. Kazmi, A.K. Chopra, Microbial community in conventional and extended aeration activated sludge plants in India, Ecol. Indic. 8 (2008) 550–554.

[34] P. Madoni, D. Davoli, G. Gorbi, L. Vescovi, Toxic effect of heavy metals on the activated sludge protozoan community, Water Res. 30 (1996) 135–141.

[35] P.J. De Baufer, A.A. Amin, S.C. Pak, H.E. Buhse Jr., A method for the synchronous induction of large numbers of telotrochs in *Vorticella convallaria* by monocalcium phosphate at low pH, J. Eukaryot. Microbiol. 46 (1999) 12–16.