Effect of operating conditions on the chemical phosphorus removal using ferric chloride by evaluating orthophosphate precipitation and sedimentation of formed precipitates in batch and continuous systems

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HIGHLIGHTS

▶ Operational factors affected the process of phosphorus removal by ferric chloride.
▶ Activated sludge biomass and prolonged settling times favored the process efficiency.
▶ Fe(III)-treated bioreactor at a sludge age of 21 days showed the best performance.
▶ Higher sludge age produced the smallest flocs deteriorating the reactor performance.
▶ Floc size measurement using image analysis allowed monitoring these systems.

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ABSTRACT

Chemical phosphate removal by Fe(III) involves the precipitation of ferric phosphate and subsequent sedimentation of Fe–P particles. Physical–chemical factors that affect the phosphorus (P) precipitation are well known; however, the factors affecting the sedimentation of Fe–P precipitates have been poorly studied. The aims of this work were: (a) To study the effect of pH, presence of biomass (3.0 g TSS/L) and settling time on the orthophosphate precipitation by ferric chloride in a batch system. (b) To determine the effect of these factors on the sedimentation of the formed Fe–P particles. (c) To evaluate the performance of a laboratory-scale continuous activated sludge (AS) reactor operated with phosphorus simultaneous precipitation by ferric chloride under the optimum operating conditions determined from previous batch assays. Results from batch studies showed that presence of AS biomass and prolonged settling times favored the P removal. According to the proposed equation, in presence of biomass, the settling rate of the Fe–P precipitates was 3–7 times higher than that corresponding to phosphate buffer system. In the continuous system, Fe–P precipitates were gradually incorporated to the biomass, improving the reactor performance. However, a gradual shift of the size distribution from large to poorly settling small flocs was observed. The system operated at a sludge age of 21 days achieved more rapidly better performance than at 40 days. This last system showed bad flocculation exhibiting smaller flocs than the reactor with younger sludge. Simultaneous determination of the accumulation rate of fixed solids, and floc size distribution allows monitoring these systems.

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, condensed inorganic phosphates and organophosphates. Chemical precipitation, enhanced biological phosphorus removal, or a combination of both are usually adopted to remove phosphorous from wastewaters. Chemical removal can be performed in different stages of the effluent treatment process. Simultaneous precipitation, which corresponds to the addition of coagulants on the aeration basin or after the secondary treatment, involves lowest cost and improved stability of activated sludge [1].

The process of phosphate chemical removal is complex and poorly understood, for the case of the salts of Fe(III), such as ferric chloride, it involves the following mechanisms: precipitation of ferric phosphate, ferric hydroxide and ferric-oxo-hydroxo-phosphate complex, phosphate adsorption on ferric hydroxide and

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Nomenclature

AS	activated sludge
COD	chemical oxygen demand (mg O_2/L)
DO	dissolved oxygen
DSVI	diluted sludge volume index (mL/g)
Fe_{TS}	total soluble Fe
Fe_T	total Fe
FSS	fixed suspended solids
Fe_{part}	particulated Fe
$[Fe_{part}]$	particulated Fe concentration
$[Fe_{part_o}]$	particulated Fe concentration at the start of the sedimentation period
F/M	food to microorganism ratio (kg COD/(kg VSS day))
HRT_C	hydraulic residence time in the clarifier (h)
HRT_T	total hydraulic residence time (h)

$[H^+]$	concentration of protons (mmol H^+/L)
IET	iron exposure time (day)
k	coefficient (L^{n+1} mmol Fe_{part}^{-1} mmol H^{+-n} h^{-1})
k_1	second-order decay coefficient ((mmol Fe_{part}/L)$^{-1}$ h^{-1})
MMLAS	model mixed liquor of activated sludge
n	coefficient (dimensionless)
PB	phosphate buffer
$PO_4^{3-}-P$	orthophosphate
P_{part}	particulated P
SRT	solids residence time (day)
T	time (h)
TP	total phosphorus
TSS	total suspended solids
VSS	volatile suspended solids

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coagulation and flocculation phenomena [2]. Fe salts form positively charged ferric-oxo-hydroxo-phosphate complexes that promote coagulation [2]. The chemical flocs formed are separated from the treated wastewater through a clarifier. It should be remarked that most of the studies are focused on the physical–chemical factors affecting the precipitation process of orthophosphate without considering the settling properties of the formed chemical precipitates. Fe:P molar ratio higher than 1.5:1 is commonly recommended for phosphate precipitation [3–5]; maximum efficiency of this process is achieved at pH values between 5.0 and 7.0 [4]. From studies of orthophosphate precipitation under uncontrolled pH conditions, it has been shown that activated sludge (AS) biomass does not affect the process performance [5]; however, it could facilitate the separation of the precipitates or Fe–P containing complexes, as was suggested by He et al. [6].

Small activated sludge flocs are generated by addition of metal salts on the secondary stage of wastewater treatment [7–9]. This constitutes a serious problem in activated sludge plants, since small flocs settle slowly affecting the system performance. Li [7] reported that adequate Fe(III) concentrations improved the bioflocculation and hence the sequencing batch reactor (SBR) performance; however, overdosage of Fe(III) reduced the fraction of the flocs with settleable sizes, deteriorating the final effluent quality. It is well known that a low sludge load (expressed as food/microorganism ratio) and a high sludge age promote bioflocculation; however, AS systems treated with Fe(III) under these operational conditions has been scarcely studied. In this context, it is interesting to operate an AS reactor exposed to Fe(III) at sludge age higher than 20 days in order to relate its performance to the changes in the floc size. Additional studies on the main factors that affect the process of chemical phosphate removal would be of great interest.

The aim of this study was to determine the most appropriate operating conditions for chemical phosphorus removal by ferric chloride in activated sludge systems (batch and continuous reactors). For this, the following specific objectives were proposed: (a) To study the effect of pH, presence of biomass and settling time on the orthophosphate precipitation in a batch system. (b) To determine the effect of pH, presence of biomass and settling time on the sedimentation of the Fe–P particles formed during the precipitation phase. (c) To evaluate the performance of a laboratory-scale AS reactor operated in continuous mode with simultaneous precipitation of phosphorus by ferric chloride under the optimum operating conditions determined from previous batch assays, testing different sludge ages.

2. Materials and methods

2.1. Biological and chemical materials

Activated sludge was obtained from an aerobic laboratory-scale (3.8 L) AS reactor with partial biomass recycle. The total hydraulic residence time (HRT_T) of the system was 29 h; 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. The reactor was fed with a model wastewater of a dairy industry that was prepared twice a week; its composition was the following: 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); $Na_2HPO_4·7H_2O$, 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 (TP), 45.5 mg P/L. pH of the wastewater was adjusted to 7.0 adding a few drops of HCl (1 M). Cheese whey showed the following composition (% w/w): total lipid (3%), total protein (12%), lactose (75%), water (5%), and mineral salts (1%).

Assays of chemical phosphorus removal were performed in batch and continuous systems using ferric trichloride hexahydrate. All inorganic salts used in the present work were commercial products of reagent grade from Anedra (San Fernando, Argentina). Dehydrated cheese whey was from Food S.A. (Villa Maipú, Argentina).

2.2. Assays of chemical phosphorus removal in batch systems

Chemical phosphorus removal by ferric chloride was studied in both systems: phosphate buffer (PB) solution and model mixed liquor of activated sludge (MMLAS). PB composition was: KH_2PO_4 0.071 g/L, $Na_2HPO_4·7H_2O$ 0.213 g/L, $NaHCO_3$ 1 g/L. MMLAS system was obtained by sedimentation and then resuspension of activated sludge, from the aerobic reactor, in the PB solution previously described. The washing was performed to remove cellular debris and metabolic products. The biomass concentration of the MMLSA system expressed as total suspended solids (TSSs) was 3.0 ± 0.3 g/L.

All assays of chemical phosphorus removal were conducted in 500 mL glass vessels at room temperature (20 ± 2 °C) under constant pH conditions (pH = 5.0–8.0). PB or MMLAS (300 mL) and appropriate volumes of a stock solution of $FeCl_3·6H_2O$ (74.0 mM) were mixed by magnetic stirring. Tested initial phosphorous concentrations ranged from 36 to 47 mg/L (1.16–1.51 mM), resulting

initial molar ratios $Fe_0:P_0$ ranging between 1.7:1 and 2.3:1. The mixtures were gently stirred (50–60 rpm) during 30 min. In these experiments, pH values of the tested mixtures were maintained constant (±0.1 pH units) by adding a few drops of NaOH or HCl stock solutions (1 M) during the agitation phase. Then, the agitation was stopped to allow a sedimentation phase (0.16–18 h). After that, samples from the supernatant were withdrawn to determine pH value and concentrations of PO_4^{3-}—P, TP, total soluble Fe (Fe_{TS}) and total Fe (Fe_T). All assays were performed in duplicate.

2.3. Phosphorus simultaneous precipitation in activated sludge reactor

The study was conducted on the laboratory-scale AS reactor fed with a model wastewater of a dairy industry described in Section 2.1. The complete mix reactor was operated in a continuous-flow mode. The HRT_T was 63 h. Sludge ages of 21 and 40 days were analyzed. A control period, without addition of $FeCl_3$, was tested. Then, ferric chloride was continuously added to the aeration basin using a peristaltic pump; for this, a stock solution of $FeCl_3 \cdot 6H_2O$ (13.4 mM) was used. The Fe:P molar ratio was 1.5–1.9:1. The AS reactor was monitored 1–2 times per week. Different physical–chemical parameters were determined: pH, total suspended solids (TSSs), volatile suspended solids (VSSs) and fixed suspended solids (FSSs) in the aeration basin; COD, PO_4^{3-}—P, TP, Fe_T and Fe_{TS} were determined in the final effluent.

2.4. Chemical analysis

Samples from the supernatant of the batch assays and from the final effluent of the AS reactor were processed in order to determine orthophosphate and total soluble Fe concentrations. 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, total Fe and total soluble Fe were determined by spectrophotometric methods using commercial reagents (Hach Company, Loveland, CO). PO_4^{3-}—P concentration in the filtrate was determined after reacting with vanadate–molybdate reagent in acid medium (Hach Method No. 8114, adapted from Standard Methods). TP 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. Fe_T concentration and Fe_{TS} concentration in the filtrate were determined by Hach Method No. 8008 (adapted from Standard Methods). Particulated P ($P_{part} > 0.45$ μm) was determined from the difference between TP and total soluble P concentrations. Similarly, particulated Fe ($Fe_{part} > 0.45$ μm) corresponded to the difference between Fe_T and Fe_{TS} concentrations.

The sedimentation properties of the activated sludge were determined using the diluted sludge volume index (DSVI) [10]. Suspended solids determination was performed according to the standard methods. Samples were filtered through 1.5 μm glass-fiber membranes and dried at 105 °C to determine TSS concentration. Then, the sample was volatilized at 550 °C and weighed again to determine FSS. VSS concentration was calculated as the difference between both measurements.

2.5. Microscopic analysis of activated sludge flocs from the continuous system

The structure of the activated sludge flocs and the abundance of filamentous microorganisms from the continuous-flow reactor were analyzed qualitatively by microscopic observation. Samples of 25 μL of the mixed liquor from the aeration basin were spread on slides, and immediately observed using a light microscope Leica

DMLB (Germany) coupled with a photographic camera. The slides were observed under bright-field illumination at 100× and 1000× magnifications.

The size of the AS flocs was determined by image analysis once per week. The microscopic images, taken under bright-field illumination at 100× magnification, were analyzed using the software Image-Pro Plus 6.0. Floc size was determined by calculating the area occupied by them (μm²). For each sample, a total of 280–2400 flocs were analyzed, and average area value was reported.

2.6. Statistical analysis

Analysis of variance was done using the Systat 12 software for the parameters that exhibit a normal distribution. Shapiro–Wilk test was conducted for testing normality hypothesis (Systat 12 software). A significance level of 0.05 was utilized. Linear and non-linear regressions were conducted using the Sigma Plot 10.0 software (Systat Software Inc.).

3. Results and discussion

3.1. Batch systems

3.1.1. Effect of pH, presence of biomass and settling time on the orthophosphate precipitation

In PB and MMLAS systems, maximum efficiency of orthophosphate precipitation (>98%) by ferric chloride was achieved at pH values ranging between 5.0 and 7.0; the efficiency was lower than 95% for pH value of 8.0 (Fig. 1). These results agree with the report of Szabó et al. [4]. In both systems studied in the present work, the PO_4^{3-}—P concentration did not significantly change as the settling time increased from 2 to 18 h (Fig. 1). Thus, adsorption/desorption phenomena of phosphate on both chemical flocs and mixed flocs (microbial aggregates + chemical precipitates) during prolonged settling times could be discarded.

In MMLAS system, the biomass concentration was 3.0 g TSS/L (±0.3 g TSS/L), with 86% corresponding to VSS. The presence of biomass did not significantly affect the orthophosphate precipitation process for pH values of 5.0 and 6.0; however, it must be considered that for this pH range the residual PO_4^{3-}—P concentrations
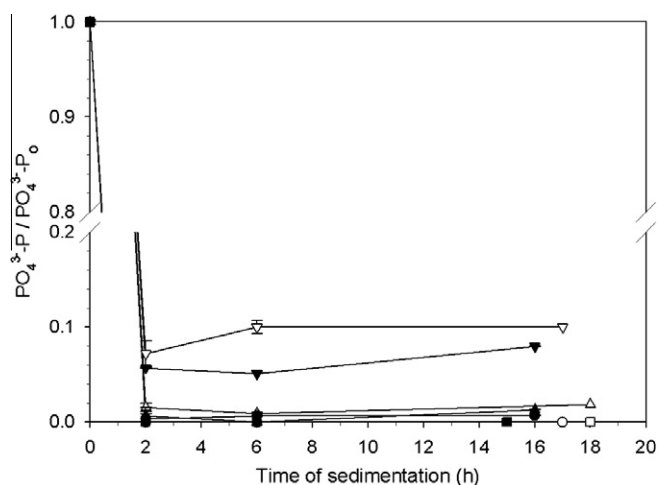

Fig. 1. Concentration of orthophosphate $\left(PO_4^{3-}-P\right)$ expressed in relation to the initial value $\left(PO_4^{3-}-P_0\right)$ as a function of the settling time (h) for the phosphate buffer (PB) and model mixed liquor of activated sludge (MMLAS) systems under different tested pH (5.0–8.0). PB: (○) pH = 5.0, (□) pH = 6.0, (△) pH = 7.0, and (▽) pH = 8.0. MMLAS (3.0 ± 0.3 g TSS/L): (●) pH = 5.0, (■) pH = 6.0, (▲) pH = 7.0, (▼) pH = 8.0. $Fe_0:P_0$ molar ratio = 1.7–2.3:1. The standard deviations are indicated by error bars.

were about the detection limit of the used spectrophotometric method (0.1 mg P/L). For higher pH values (7.0–8.0), the presence of biomass improved significantly ($p < 0.05$) the orthophosphate removal (Fig. 1).

In MMLAS system, at high pH conditions, macroscopically visible flocs (composed by chemical precipitates and microbial aggregates) were formed. It must be pointed out that under pH conditions about the neutrality (pH = 6.5–8.5), sweep flocculation produces large aggregates of ferric hydroxide when ferric salts are added to water. As the sweep flocs settle, the colloidal particles will be removed by the sweep action of the settling flocs [1,11]. In the MMLAS system under alkaline conditions, sweep flocculation would favor the retention of phosphate anion probably by adsorption or ionic interchange phenomena. This process could explain the improvement of the orthophosphate removal caused by the presence of AS biomass at high pH conditions.

3.1.2. Effect of pH, presence of biomass and settling time on the sedimentation of Fe–P precipitates

In all batch assays, after the mixing period, the concentrations of particulated P (P_{part}) and particulated Fe (Fe_{part}) from the supernatant were measured at different settling times (0.16–18 h). P_{part} was determined from the difference between total P and orthophosphate concentrations because the total soluble P concentrations (TP from filtered samples) were similar to those corresponding to PO_4^{3-}–P. Fe_{part} corresponded to the difference between total Fe and total soluble Fe concentrations. TP in the supernatant from PB and MMLAS systems corresponded mainly to the particulated fraction (commonly >85%), because the high efficiency of orthophosphate removal by precipitation as was observed in Fig. 1.

In PB system, P_{part} corresponded to P combined with Fe as chemical precipitates containing Fe–P, meanwhile in MMLAS system, P_{part} involved precipitates containing Fe–P and organic P from the activated sludge biomass. The Fe:P molar ratio of the formed precipitates was determined for the tested experimental conditions (pH = 5.0–8.0; settling time = 0.16–18 h). By plotting Fe_{part} concentration as a function of P_{part} concentration, Fe:P molar ratio of 2.43 for the PB system ($r^2 = 0.973$) and 2.13 for the MMLAS ($r^2 = 0.987$) were determined by linear regression analysis. The lower Fe:P ratio for the MMLAS system was associated to the presence of organic P from the microbial biomass.

In PB system, the residual concentrations of P_{part} and Fe_{part} decreased as increasing the settling time from 0.16 to 6 h for the different pH conditions (5.0–8.0); for times longer than 6 h the concentrations did not significantly change ($p > 0.05$). In MMLAS

system, P_{part} and Fe_{part} decreased gradually until the end of the assays (Fig. 2a and b).

In both PB and MMLAS systems, the removal efficiency of P_{part} and Fe_{part} by sedimentation was higher for pH values of 5.0 and 6.0 (Fig. 2a and b). In order to explain these results, the probable mechanisms of phosphate removal are discussed. It has been reported that the size and composition of the formed colloids as well as the destabilization mechanisms involved in the coagulation process depend on the coagulant concentration, presence of complexing ions and pH [12]. For pH values below 7.0, the products of hydrolysis of Fe and ferric-oxo-hydroxo-phosphate complexes are positively charged [4]. On the basis of this information, it can be inferred that the charge neutralization of these colloids with phosphate anions would favor the coagulation process leading to the formation of flocs rapidly removed by sedimentation. This phenomenon could explain the excellent removal of both orthophosphate and Fe–P complexes observed in this study at pH values 5.0 and 6.0 (Figs. 1 and 2). At alkaline pH, the colloids containing Fe or Fe–P are negatively charged [4], therefore the charge neutralization by phosphate is not favored. This situation would lead to lower removal of both orthophosphate and particles containing Fe–P at pH values above 7.0 (Figs. 1 and 2).

The presence of biomass improved the removal of particles containing Fe–P for the tested pH conditions. In the PB system for pH = 5.0 and settling time of 2 h, the P_{part} and Fe_{part} concentrations were 1.4 and 6.6 mg/L respectively; in the MMLAS system, these concentrations were approximately 10 times lower. Similar results were observed at higher pH values (Fig. 2a and b). The effect of biomass on the sedimentation properties of the Fe–P particles should be evaluated considering the typically negative surface charge of the bacterial cells. It has been demonstrated that bacterial cells rapidly attach to the iron oxyhydroxide surface [13]. In the MMLAS system of the present study, a destabilization process of positively charged colloids (hydrolysis products of Fe and ferric-oxo-hydroxo-phosphate complexes) with phosphate anions and bacterial cells would take place at low pH values (5.0–6.0). This phenomenon leads to the formation of larger flocs with better sedimentation properties that the system without microbial biomass (PB). Many studies indicate that the biomass favors the separation of colloidal particles containing Fe or Fe–P [6,14,15] supporting the results reported in our paper.

The removal of P_{part} and Fe_{part} was significantly lower ($p < 0.05$) for pH values 7.0 and 8.0 (Fig. 2a and b). It can be considered that for pH values ranging between 7.0 and 10.0, Fe hydroxide is more negatively charged than at lower pH values [4]. Thus, the destabilization of colloids by charge neutralization with phosphate anions or bacterial cells is unlikely to take place at alkaline conditions.
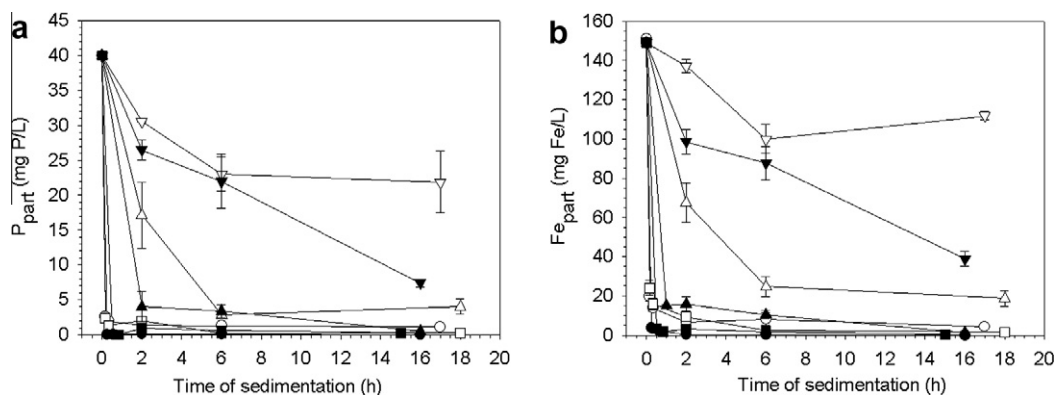

Fig. 2. Effect of the presence of biomass on the solid–liquid separation of particles containing Fe–P at different settling times (h) and pH values. (a) Particulated P (P_{part}, mg P/L). (b) Particulated Fe (Fe_{part}, mg Fe/L). PB system: pH = (○) 5.0, (□) 6.0, (△) 7.0, and (▽) 8.0. MMLAS system (3.0 ± 0.3 g TSS/L): pH = (●) 5.0, (■) 6.0, (▲) 7.0, and (▼) 8.0. Fe_0:P_0 molar ratio = 1.7–2.3:1. The standard deviations are indicated by error bars.
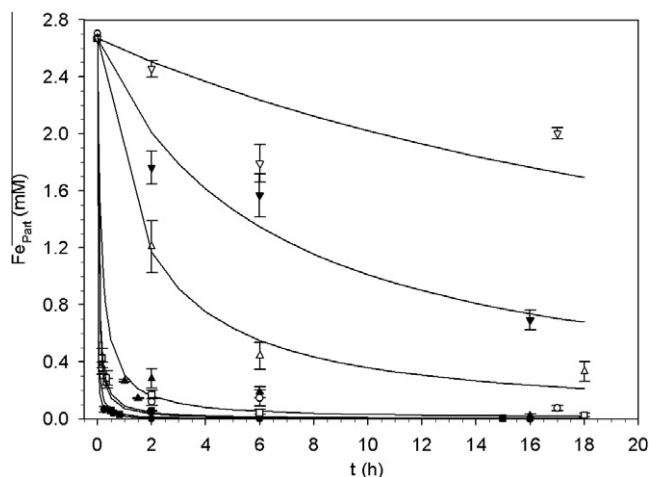
Fig. 3. Concentration of particulated Fe (Fe$_{part}$, mM) as a function of the settling time (h) at different pH values. PB system: pH = (○) 5.0, (□) 6.0, (△) 7.0, and (▽) 8.0. MMLAS system (3.0 ± 0.3 g TSS/L): pH = (●) 5.0, (■) 6.0, (▲) 7.0, and (▼) 8.0. Fe$_0$: P$_0$ molar ratio = 1.7–2.3:1. The standard deviations are indicated by error bars. (−−) Predictions by Eq. (2).

Although electrostatic interactions do not favor the process of destabilization of colloids, other forces such as hydrophobicity, acid–base interactions and steric interactions can take place, encouraging aggregation phenomena [13]. In the present study, these factors could play an important role in the attachment of phosphate and Fe–P colloids to flocs during the sweep flocculation process at high pH values (Section 3.1.1). In MMLAS system at alkaline conditions, it can be considered that sweep flocculation led to the formation of large flocs (composed by chemical precipitates and microbial aggregates), improving the separation of particles containing Fe–P by sedimentation.

3.1.3. Mathematical modeling of the sedimentation of the precipitates containing Fe–P

In order to model the sedimentation of particles containing Fe–P as a function of time, a decay equation of second order with respect to the concentration of Fe$_{part}$ was proposed:

$$\frac{d[Fe_{Part}]}{dt} = -k_1[Fe_{Part}]^2 \tag{1}$$

where k_1 is the second-order decay coefficient ((mmol Fe$_{part}$/L)$^{-1}$ h^{-1}).

Integrating and rearranging Eq. (1), the following expression was obtained:

$$\frac{1}{[Fe_{Part}]} = \frac{1}{[Fe_{Part_o}]} + k_1 t \tag{2}$$

where [Fe$_{part_o}$] corresponds to the particulated Fe concentration at the start of the sedimentation period ($t = 0$).

Eq. (2) allows the estimation of the Fe$_{part}$ concentration as a function of settling time for PB and MMLAS systems at the tested pH conditions. This equation was satisfactorily fitted to the experimental data by non-linear regression (Fig. 3).

In order to take into account the effect of pH on coefficient k_1, the following equation was proposed:

$$k_1 = k[H^+]^n \tag{3}$$

where [H$^+$] is the concentration of protons (mmol H$^+$/L); k and n are empirical coefficients.

The coefficients k and n of Eq. (3) were determined from a log–log plot of k_1 vs. [H$^+$]. Each point of the curves corresponds to the average of two assays for each pH value. Results are shown in Table 1. Within the tested pH range (5.0–8.0) the obtained results indicated that the settling rate of Fe$_{part}$ in presence of biomass was 3–7 times faster than in PB system. The proposed model is also applicable to the residual P$_{part}$ concentration as the particles were basically constituted by P and Fe with a constant Fe:P stoichiometric molar ratio (see Section 3.1.2). This model determined that the presence of biomass allows the decrease of the settling time from 5 to 1.8 h to achieve a 99% removal of the particles containing Fe–P at pH = 6.0. This result would denote an advantage of the chemical removal process of phosphorus at the secondary wastewater treatment stage in relation to systems based on post-precipitation.

Later, we focused on studying the chemical phosphorus removal in continuous AS system, under the optimal operational conditions determined previously in batch assays. For this, a laboratory-scale reactor was operated with P removal by adding ferric chloride at the secondary wastewater treatment stage. Two sludge ages were set to analyze the effect of the bioflocculation on the performance of the Fe(III)-treated reactor.

3.2. Continuous system of activated sludge with phosphorus simultaneous precipitation

3.2.1. Performance of the activated sludge reactor with sludge age of 21 days

The laboratory-scale activated sludge reactor fed with a model wastewater of a dairy industry was operated under the following conditions: HRT$_T$ = 63 h, sludge age = 21 days, temperature = 17–21 °C, and dissolved oxygen (DO) concentration in the aeration basin = 2–4 mg/L. The hydraulic residence time in the clarifier (HRT$_C$) was 16 h in order to attain a prolonged settling time. According to the previous batch assays in the present study, it can be expected good performance of the AS system with phosphorous simultaneous precipitation when prolonged settling times are applied, even under inadequate pH conditions.

During the control period without addition of FeCl$_3$ (0–50 days), the reactor exhibited mediocre performance. The average food to microorganism (F/M) ratio was 0.35 kg COD/(kg VSS day). The orthophosphate concentration of the final effluent ranged between 33 and 41 mg P/L, which represented 92–100% of the total P. Firstly, the COD concentration of the final effluent decreased from about 400 to 140 mg O$_2$/L (Fig. 4a). Periodic microscopic observation typically showed well formed compact and irregular flocs, indicating good flocculation. However, at the end of the control period, excessive growth of filamentous microorganisms, protruding from surface floc into bulk medium, took place indicating a severe bulking episode. COD concentration increased from 140 to 300 mg O$_2$/L and the sludge exhibited poor settling properties with DSVI values ranging between 400 and 550 mL/g, which were above

Table 1
Kinetics of Fe–P particles sedimentation. Model parameters calculated by Eq. (3) for PB and MMLAS systems.

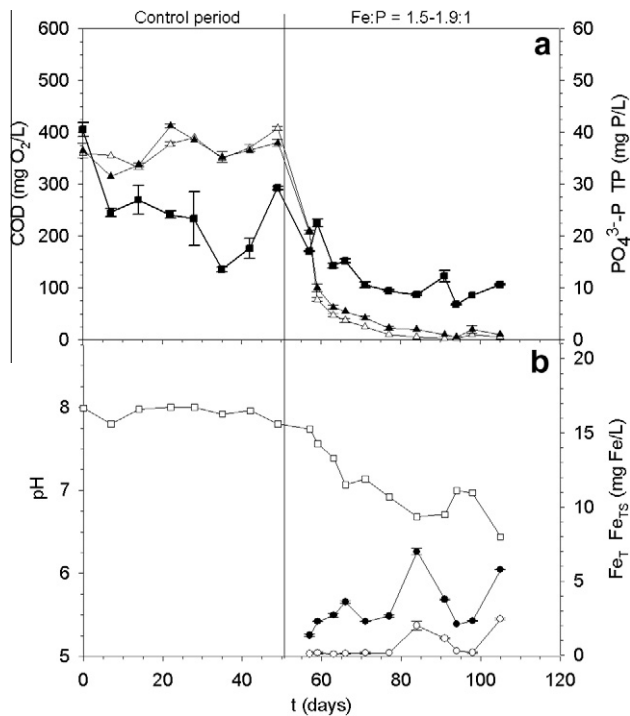| Coefficients of Eq. (3)	Phosphate buffer system	Model system of the mixed liquor of activated sludge
k(L^{n+1} mmol Fe$_{Part}^{-1}$ mmol H^{+-n} h^{-1})	4698.9 (1105.1)[a]	11220.1 (2251.8)
n (dimensionless)	1.077 (0.235)	0.983 (0.221)

[a] Standard deviation between parentheses.

Fig. 4. Physical–chemical parameters of the activated sludge reactor with sludge age of 21 days as a function of the operation time. (a) Chemical oxygen demand (COD), orthophosphate $\left(PO_4^{3-}-P\right)$ and total phosphorus (TP) of the final effluent: (■) COD (mg O_2/L), (△) $PO_4^{3-}-P$ (mg P/L), and (▲) TP (mg P/L). (b) pH, total Fe (Fe_T) and total soluble Fe (Fe_{TS}) of the final effluent: (□) pH, (●) Fe_T (mg Fe/L), and (○) Fe_{TS} (mg Fe/L). The standard deviations are indicated by error bars.

those recommended in literature (<200 mg/L). COD values were commonly below the discharge limit in Argentina (province of Buenos Aires), which is between 250 and 700 mg O_2/L depending on the wastewater receiving location [16]. Nevertheless, more stringent limits such us 125 mg O_2/L according to European standards [17] could not be fulfilled.

After the control period, $FeCl_3 \cdot 6H_2O$ (36.17 mM) was continuously added to the aeration basin to achieve a Fe:P molar ratio = 1.5–1.9:1 (51–110 days). During the chemical treatment, the F/M ratio did not significantly change. The reactor performance rapidly improved, resulting residual COD concentrations below 125 mg O_2/L after about 12 days of coagulant treatment (Fig. 4a). Thus, the applied Fe:P doses ensure compliance with international environmental legislations. About 15 days of chemical treatment was required to reduce the residual orthophosphate concentration from 33–41 mg P/L to values ranging between 0.25 and 1.0 mg P/L. This implied removals higher than 97%. The TP removal averaged 96%, resulting residual concentrations lower than 2 mg P/L for all samples, and lower than 1 mg P/L for 80% of the analyzed samples (Fig. 4a). In general, these values comply with Argentinean legislation that establishes discharge limits for phosphate between 1.0 and 10.0 mg P/L. In addition, US and European legislations, with discharge limits below 1 mg P/L and 1.0–2.0 mg P/L respectively, would be also accomplished. However, more stringent nutrient standards for treated wastewaters, including TP concentration lower to 0.15 mg P/L and 0.01–0.1 mg P/L, are being increasingly implemented by the European Union and several US states respectively [18,19]. Advanced post-treatment processes, such as effluent filtration, are widely proposed to meet future effluent discharge standards [19].

The continuous addition of ferric chloride caused a decrease of the final effluent pH from about 7.8 to values ranging between 6.4 and 7.1 due to hydrolysis reactions of Fe(III). The concentration of

total soluble Fe in the final effluent depended on pH, and concentrations below 0.3 mg/L were measured for pH values above 6.7; Fe_{TS} was up to eight times higher for lower pH conditions (Fig. 4b).

Settling properties were improved after about 3 weeks of starting of Fe(III) dosing, achieving DSVI values lower than 200 mL/g. Improvement of settleability was associated with the elimination of filamentous microorganisms in agreement with previous studies [20–22]. According to this improvement in the sludge settling properties, the reactor showed good performance with concentrations of COD, TP and Fe_T ranging between 68 and 123 mg O_2/L, 0.2 and 2.0 mg P/L and 2.1 and 5.8 mg Fe/L after about 21 days of chemical treatment (Fig. 4).

3.2.2. Performance of the activated sludge reactor with sludge age of 40 days

The AS reactor with sludge age of 40 days was operated under the same conditions described for the system with sludge age of 21 days: HRT_T = 63 h, temperature = 17–21 °C, HRT_C = 16 h, and DO concentration in the aeration basin = 2–4 mg/L (Section 3.2.1).

During the control period without addition of $FeCl_3$ (0–70 days), the reactor exhibited mediocre performance with high concentrations of $PO_4^{3-}-P$, TP and COD of the final effluent. $PO_4^{3-}-P$ concentration of the final effluent ranged between 30 and 39 mg P/L, which represented 75–100% of the TP. Initially, the COD concentration of the final effluent was below 200 mg O_2/L; however, after day 45, the concentration increased markedly reaching values above 800 mg O_2/L (Fig. 5a). The DSVI values increasing progressively from about 600 to 4400 mL/g indicating bad settling properties. According to this, microflocs and filamentous microorganisms free in suspension were increasingly detected by microscopic observation. This situation could be associated to the gradual reduction of the F/M ratio from about 0.50 to 0.20 kg COD/(kg VSS day) during the control period. It is well known that a low
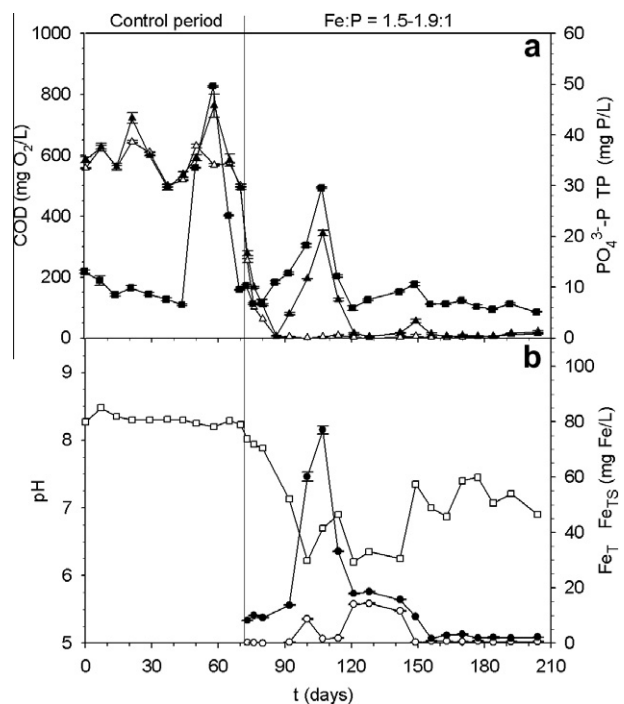

Fig. 5. Physical–chemical parameters of the activated sludge reactor with sludge age of 40 days as a function of the operation time. (a) Chemical oxygen demand (COD), orthophosphate $\left(PO_4^{3-}-P\right)$ and total phosphorus (TP) of the final effluent: (■) COD (mg O_2/L), (△) $PO_4^{3-}-P$ (mg P/L), and (▲) TP (mg P/L). (b) pH, total Fe (Fe_T) and total soluble Fe (Fe_{TS}) of the final effluent: (□) pH, (●) Fe_T (mg Fe/L), and (○) Fe_{TS} (mg Fe/L). The standard deviations are indicated by error bars.

F/M ratio can be lead to the development of filamentous bulking [10]. The bad settling properties and formation of small flocs indicated inadequate bioflocculation, which led to a marked deterioration of the system performance at the end of the control period. It can be noted that during the period 45–60 days, the poorly flocculated sludge escaped from the reactor, through the final effluent, causing a sharp decrease of the volatile suspended solid concentration. Even though this event involved an increase of the F/M ratio from 0.20 to 0.44 COD/(kg VSS day), the bioflocculation capacity was not recovered.

The Fe:P molar ratio = 1.5–1.9:1 was applied during the period 71–204 days. The F/M ratio was maintained between 0.20 and 0.30 COD/(kg VSS day). PO_4^{3-}—P removal higher than 96.0% was achieved after 15 days of treatment with Fe(III), resulting residual concentrations ranging between 0.0 and 1.2 mg P/L (Fig. 5a). Similarly to the reactor operated with sludge age of 21 days, the addition of ferric chloride caused a decrease of the final effluent pH from about 8.3 to values ranging between 6.2 and 7.4. The residual concentration of Fe_{TS} was below 0.8 mg/L for pH values above 6.7, Fe_{TS} was up to 20 times higher for lower pH conditions (Fig. 5b).

The reactor exhibited acceptable performance during the initial period of chemical treatment. Later, in the period 90–120 days, the concentrations of COD, TP, and Fe_T in the final effluent reached unacceptable values as high as: 490 mg O_2/L, 20.3 mg P/L and 75 mg Fe/L respectively (Fig. 5a and b). In this period, soluble COD, PO_4^{3-}—P and Fe_S concentrations were below 150 mg O_2/L, 0.3 mg P/L and 8.7 mg Fe/L respectively (Fig. 5a and b); therefore, the bad performance observed was mainly caused by the discharge of suspended particles consisting not only of dispersed cells but also particulated P and particulated Fe. It must be noted that during this period, the Fe_P concentrations of the final effluent were up to 14 times higher than those corresponding to the reactor with sludge age of 21 days. In addition, the COD concentrations were significantly higher than those registered for the reactor with younger sludge.

The Fe(III) addition to the aeration basin improved the flocculation, achieving complete elimination of filamentous microorganisms. According to this, a large improvement in settleability of the sludge was achieved after about 3 weeks of starting of Fe(III) dosing, reaching DSVI values lower than 200 mL/g. However, about 49 days of chemical treatment was required to improve the reactor performance achieving concentrations of COD, TP, and Fe_{part} ranging between 94 and 180 mg O_2/L, 0.3 and 3.3 mg P/L, and 1.0 and 9.2 mg Fe/L respectively (120–204 days, Fig. 5). It should be noted that during the period 120–140 days, the high concentrations of Fe_T (15.7–18.6 mg Fe/L) were caused by the high levels of total soluble Fe (11.6–14.4 mg Fe/L) (Fig. 5b). Finally, the reactor exhibited acceptable performance with respect to all the parameters analyzed after about an iron exposure time (IET) of 80 days (150–204 days, Fig. 5a and b). It must be pointed out that the reactor with sludge age of 21 days reached similar performance after an IET of 21 days.

3.2.3. Suspended solid concentration in the reactors

In the continuous systems studied, FeCl$_3$ addition produced a progressive increase of the concentration of total suspended solids due mainly to the marked increase of fixed suspended solids. The increase in the inorganic fraction of the sludge was caused by the incorporation of Fe(III) and ferric-hydroxy-phosphate into microbial flocs. It is well known that this situation causes an increase in the density of flocs, improving the sedimentation of the sludge as was previously indicated for both reactors.

In the control period of the continuous AS reactor with sludge age of 21 days, the TSS concentration of the aeration basin was about 2.3 g/L, with 86% corresponding to volatile suspended solids. Coagulant addition caused an increase of the VSS concentration

from 2.0 g/L to 2.3–3.0 g/L. The FSS concentration increased progressively during about 40 days of chemical treatment, following a Monod type kinetic, from 0.3 g/L up to a constant concentration (2.3–2.4 g/L). This situation involved an increase in the FSS content of the sludge, expressed as FSS/TSS percentage ratio, from 14% to 50–52%. In the reactor with sludge age of 40 days, the FSS concentration increased linearly during about 100 days of chemical treatment from 0.2 g/L (FSS/TSS ratio = 7%) to 5.4–5.8 g/L (FSS/TSS ratio = 62–66%).

A priori it would be expected that a prolonged sludge age will promote the rate of accumulation of suspended solids; however, in the reactor operated with sludge age of 40 days the accumulation rate of FSS was lower than that of reactor with sludge age of 21 days as was indicated. These results can be explained considering the marked loss of suspended solids through the final effluent during the first 45–50 days of coagulant treatment in the reactor with high sludge age. The relatively slow response of this reactor to the coagulant was associated to the bad flocculation (Section 3.2.2.). A lower sludge age promoted more rapid aggregation of FSS to biomass reducing the response time of the system to the chemical treatment.

Despite the marked increase of the inert solids content of the sludge during the addition of Fe(III), the reactors reached finally acceptable performance. Previous studies reported that the FSS concentration is a key control parameter of the process of phosphorus simultaneous precipitation. De Gregorio et al. [21] indicated that the enrichment with Fe(III) of the sludge favors the reduction from Fe(III) to Fe(II) (as FeS precipitates), under anaerobic conditions in the clarifier, which leads to the disintegration of the flocs deteriorating the final effluent quality. De Gregorio et al. [22] proposed a mathematical model to estimate the threshold concentration of FSS in the system (3.96 g/L), above which the reactor performance deteriorates. It must be noted that, in the present study, the final FSS concentration reached by the reactor with sludge age of 21 days was lower than the reported threshold value. In addition, the absence of FeS deposits in the clarifier allowed inferring that the metal reduction process did not occur or it was insignificant. In the system with sludge age of 40 days the inert solids content, at the end of the chemical treatment, was much greater. The TSS accumulation promoted the process of Fe(III) reduction, leading to the formation of large FeS deposits in the aeration basin and clarifier. This situation is uncommon in biological systems of wastewater treatment. In the reactor with high sludge age (40 days), however the prolonged settling time in the secondary clarifier prevented the failure of the process.

3.2.4. Comparative analysis of the floc size between both reactors

Periodic microscopic observation and sedimentation test constituted the first indications of formation of small flocs by coagulant action. The sludge was clearly separated into two parts during the settling test: inferior phase with large flocs and superior phase with small flocs. During the period of treatment with Fe(III), the volume index was calculated on the basis of the volume of large, rapidly settling flocs; however, the supernatant of the used cylinder was commonly turbid due to the significant fraction of small, poorly settling flocs. This phenomenon was most evident for the sludge from the reactor with older sludge after 20–25 days of chemical treatment. Generation of small activated sludge flocs due to addition of metal salts has been reported in the literature [7–9].

In both reactors with sludge ages of 21 and 40 days, the degree of flocculation was studied by determining the size of the AS flocs by using microscopic image analysis. The addition of Fe(III) caused a gradual shift of the size distribution from large to small flocs, in agreement with results reported by Oikonomidis et al. [9]. Fig. 6a shows the distribution of floc sizes (area, μm^2) for different iron
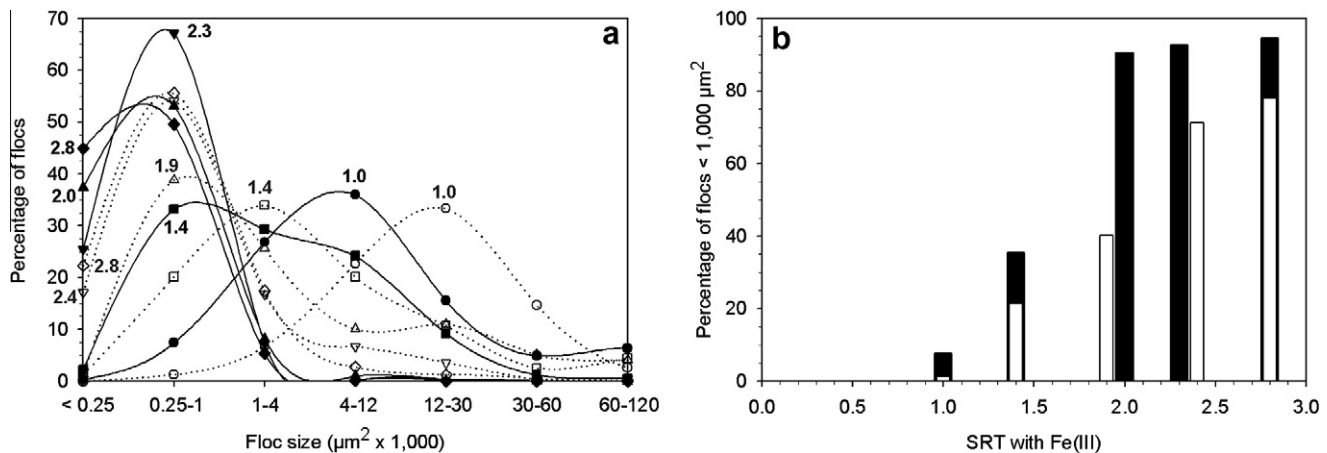
Fig. 6. Floc size as area (μm^2) for the reactors with sludge age of 21 days (empty symbols) and 40 days (dark symbols). (a) Size distribution of flocs for different SRT with Fe(III) indicated in the graph. (b) Percentage of flocs with area less than 1000 μm^2 as a function of SRT with Fe(III).

exposure times (IET), which were expressed in terms of the solids residence time in order to carry out a comparative analysis between both reactors. For the system with sludge age of 21 days, an IET of 21 days corresponds to one SRT with Fe(III), meanwhile for the reactor with sludge age of 40 days, an IET of 40 days is equivalent to one SRT with the coagulant. As the IET increased, the peak corresponding to the highest relative frequency of the floc size decreased for both reactors. Throughout the study, the system with high sludge age exhibited smaller flocs than the reactor with younger sludge. The peak of highest relative frequency for the reactor with sludge age of 40 days corresponded to a floc size range lower than that of the reactor with sludge age of 21 days, for SRT with Fe(III) < 1.9. After 1.9 SRT with Fe(III), the highest relative frequency corresponded to the size range expressed in area 250–1000 μm^2 for both systems (Fig. 6a).

In the reactor with high sludge age, for 2.8 SRT with Fe(III), 45% of the flocs showed an area <250 μm^2 (equivalent to a diameter <18 μm assuming circular area) and 95% corresponded to a size <1000 μm^2 (diameter <35 μm). In the reactor with younger sludge for similar SRT with Fe(III), the percentage of flocs was 22% and 75% respectively (Fig. 6b). Despite of these high proportions of small flocs, the reactors exhibited good performance. Li [7], working in SBR exposed to different Fe(III) concentrations, reported deterioration of the final effluent quality when flocs lesser than 50 μm diameter represented 65% of the activated sludge. However, in the work performed by Li, the SBR was operated with a relatively short settling time (40 min). The hydraulic retention time in the activated sludge secondary clarifiers usually ranges from 2 to 3 h. However, the retention time should depend on the settling rate of the solids to guarantee optimal operation. It should be noted that if deficient flocculation occurs, too small, poorly settling flocs are generated, and hence many particles will be discharged from the clarifier. In our study, high hydraulic residence time in the secondary clarifier (HRT$_C$ = 16 h) allowed sufficient settling time for poorly settling flocs. It is suggested that high HRT in the clarifier, as was used in the present study, could mitigate the negative effects associated with problems of flocculation in systems with phosphorus simultaneous precipitation by metal salts.

In the systems studied, the decrease in floc size was associated with the gradual increase of the FSS content. It should be noted that in the reactor with sludge age of 21 days, the decay of filamentous microorganisms (which protruded from floc surface into the bulk medium) could have contributed to this phenomenon. These results are in agreement with those reported in literature. According to Li [7], the decrease in the floc size may be due to the reduction in the number of filaments available for formation of large

aggregates. Oikonomidis et al. [9] established a strong positive relationship between floc size and filament abundance; they reported that the floc size dropped when the growth of filaments was repressed by Fe during the dosing period. In our reactor with sludge age of 40 days, the decrease in floc size was only associated with the strong increase of the content of FSS because the filamentous microorganisms were free in the bulk medium during the control period.

The smaller size of the flocs from the reactor with sludge age of 40 days could be caused by two factors: (1) the extremely high sludge age of the system, that involved the establishment of a relatively low organic load, led probably to the weakening and/or disintegration of the extracellular polymeric matrix of the AS flocs, and proliferation of free filamentous microorganisms in suspension. This situation would have lead to the formation of large amount of microflocs. (2) the progressive accumulation of Fe(III) in the reactor, associated with the establishment of anaerobic conditions that promote the reduction from Fe(III) to Fe(II), favored probably the disintegration of flocs. The first factor could explain the mediocre performance of the reactor operated with high sludge age during the control period. In addition, the slow response of this system to the coagulant treatment could be also related to this factor. The second factor would be particularly important in the formation of microflocs after a long period of treatment with Fe(III).

4. Conclusions

Phosphorus removal by Fe(III) depended on activated sludge biomass, pH, settling time and sludge age. In batch systems, the presence of biomass increased settling rates of the formed Fe–P precipitates. An equation was proposed for estimating the precipitates concentration as a function of the settling time for several pH conditions.

In the laboratory-scale continuous reactor operated with P simultaneous precipitation, Fe–P precipitates were gradually incorporated to the biomass. This phenomenon improved the sludge sedimentation achieving a good reactor performance; however, a gradual shift of the size distribution from large to small flocs was determined by image analysis. The response time of the system to the applied coagulant as well as the achieved performance depended on sludge age. The reactor operated at sludge age of 21 days achieved more rapidly better performance than that to 40 days. This result can be explained considering that at the largest sludge age, a deficient flocculation associated to a low organic load led to smaller flocs and slower accumulation rates of fixed

suspended solids. Small and slowly settling flocs could affect the reactor performance, however, high hydraulic residence times in the secondary clarifier allowed sufficient settling time for these poorly settling flocs. This condition could mitigate the negative effects associated with the excessive formation of microflocs. The results reported in batch systems with respect to the effect of settling time on the removal of particles containing Fe–P would support this hypothesis.

The sludge age constituted an important parameter to be considered in the process of chemical phosphate removal on the secondary stage of wastewater treatment. Simultaneous determinations of inorganic solids concentration, its accumulation rate, and floc size distribution are necessary to monitor these systems.

Acknowledgments

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