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Application of biological indices and a mathematical model for the detection of metal coagulant overload in a laboratory scale activated sludge reactor with phosphate simultaneous precipitation

Carolina De Gregorio^a, Alejandro H. Caravelli^{a,*}, Noemí E. Zaritzky^{a,b}

^a Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CCT – La Plata – CONICET, Facultad de Ciencias Exactas, UNLP, 47 y 116 (B1900AJJ) La Plata, Argentina

^b Facultad de Ingeniería, UNLP, 48 y 115 (B1900AJJ) La Plata, Argentina

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ABSTRACT

Phosphorous simultaneous precipitation by coagulants reduces the volatile solids percentage which can be deleterious to the biological process. In this work a mathematical model was developed and biological indices were applied to control Fe(III)-dosed activated sludge systems. A molar ratio Fe:P = 1.9–2.3:1 on the aeration basin of a laboratory-scale activated sludge reactor caused a progressive enrichment of the sludge with inorganic solids deteriorating the system performance. Crawling and attached ciliates were the most sensitive organism groups to these changes. The proposed mathematical model estimated: (i) the threshold concentration of fixed suspended solids, above which the reactor performance deteriorates, and (ii) the decay of the most sensitive organism groups with time. The Shannon–Wiener and sludge biotic indices predicted the decrease of the reactor performance by coagulant overload. The simultaneous application of the mathematical simulation and the biological indices guarantees a successful control of systems operated with phosphorous simultaneous precipitation by Fe(III).

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1. Introduction

Phosphorous (P) from wastewater can be present as orthophosphate (PO_4^{3-}), inorganic condensed phosphates (polyphosphates and metaphosphates) and organophosphates [1]. Iron-based coagulants, such as ferric chloride, are widely used for the removal of phosphorous. Doses of Fe:P higher than 1.5:1 (molar ratio) are commonly recommended [2–4].

Coagulants can be added on the primary settler (known as pre-precipitation), on the aeration basin (simultaneous precipitation or co-precipitation), or on the tertiary treatment (post-precipitation) [5]. Addition of iron salts on the aeration basin or after the secondary treatment usually results in the best removal of P [6]. Simultaneous precipitation involves lowest cost and improved stability of activated sludge; however, inert solids added to the activated sludge mixed liquor reduce the percentage of volatile solids [6]. Thus, the excess of coagulants may be deleterious to the biological process [7].

The toxic effect of the coagulant on the biological process should be particularly monitored in plants that do not have automatic control for chemical dosing or in systems that receive high loads of phosphorous such as the dairy effluent treatment plants; in these systems, the high load of metal coagulants required could severely alter the reactor performance. In a previous study, De Gregorio et al. [8] evaluated the effect of different Fe:P (molar ratio) dose ranges on the performance of a laboratory-scale activated sludge plant fed with a model wastewater of a dairy industry. These authors reported that a high Fe:P ratio = 1.9–2.3:1 (141 mg Fe/(L day)), applied on the aeration basin, achieved a rapid improvement of the soluble P and COD removal; however, the dosage of the metal coagulant during a prolonged period negatively affected the reactor performance.

The effect of metal salts on the activated sludge process 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), total nitrogen, suspended solids (SS) and turbidity [7,9,10]; however, these parameters do not reliably reflect gradual changes in the system efficiency.

A periodic analysis of the microfauna would allow monitoring the progressive toxic effect of the coagulant. Overall, an efficient process exhibits microfauna with large number of organisms

* Corresponding author. Tel.: +54 221 4254853.

E-mail addresses: carolinadegre@hotmail.com (C. De Gregorio), alejandrocavelli@hotmail.com (A.H. Caravelli), zaritzky@ing.unlp.edu.ar (N.E. Zaritzky).

($\geq 10^6 \text{ L}^{-1}$), composed mainly of crawling ciliates and attached ciliates well diversified, where no group dominates numerically over the others by a factor greater than 10 [11]. De Gregorio et al. [8] reported that the decrease in the ciliates density and the disappearance of crawling ciliates constitute strong evidences of Fe(III) over-dose. These authors proposed that crawling ciliates could be used as biological indicators of Fe(III) overload conditions in systems operated with phosphorous simultaneous precipitation. In this context, it is necessary to develop predictive mathematical models as well as to propose biological indices to monitor the reactor performance in order to early detect operating problems.

Biological indices allow objectively to analyze the effect of toxicants on biotic communities. The purpose of these indices is to relate environmental quality or level of contamination with both the community structure and the presence of indicator species. In natural and artificial aquatic systems, three types of indices are commonly used: diversity indices, saprobic indices and biotic indices. Diversity indices combine three components that characterize the structure of a community: abundance, species richness (or other taxonomic unit) and relationship of dominance (relative abundance). It is assumed that pollution alters the community reducing the diversity [12]. The diversity index of Shannon–Wiener [13] has been successfully used in sewer system as an indicator of the wastewater quality [14]. Saprobian indices are based on the presence of indicator species of organic pollution, being generally used in rivers [15]. Biotic indices are based on the concept of pollution indicator organisms and also consider the diversity of some taxonomic groups. The sludge biotic index (SBI) proposed by Madoni [11] has been developed to determine the performance of biological wastewater treatment systems. This index is based on the abundance, taxonomic richness and different sensitivities revealed by some of the microfauna groups to physical–chemical factors and operating conditions prevailing in the plant [16].

It should be noted that very few studies have utilized biological indices for monitoring the P simultaneous precipitation process by metal salts. Clark et al. [17] 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. [18] compared the effect of aluminum sulfate and an aluminum-based polyelectrolyte on the process performance and organism diversity of an activated sludge system. However, in these studies the diversity index was not applied to monitor changes of the process performance.

The general objectives of the present study were to develop a mathematical model based on key physical–chemical, operative and biological parameters and to apply biological indices to control activated sludge systems operated with simultaneous precipitation of high P load by using ferric chloride. A high dose Fe:P (molar ratio)=1.9–2.3:1 was applied on the aeration basin of a laboratory-scale activated sludge reactor fed with a model system of wastewater from the dairy industry. The following specific objectives were proposed: (a) to assess the effect of the continuous dosage of Fe(III) on the reactor performance and on the inorganic solids content of the biomass, (b) to determine key physical–chemical parameters for controlling the biological system operated with phosphorous simultaneous precipitation, (c) to propose a mathematical model relating key physical–chemical and operating parameters with the abundance of organisms belonging to morphological–functional groups of the microfauna most sensitive to the daily Fe(III) load, (d) to analyze the effect of the coagulant on the activated sludge microfauna by applying the diversity index of Shannon–Wiener and the sludge biotic index (SBI), (e) to assess the usefulness of these biological indices as predictive tools of the system performance. The present study was conducted from experimental data reported in our previously published work [8].

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). 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 complete mix reactor was operated in a continuous-flow mode with feed flow rate $Q_F = 0.13 \text{ L/h}$. The hydraulic residence time was 29 h; the sludge age was 21 days. 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; $(\text{NH}_4)_2\text{SO}_4$, 0.94 g/L (7.12 mM); KH_2PO_4 , 0.071 g/L (0.52 mM); $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 0.213 g/L (0.80 mM); NaHCO_3 , 1.0 g/L (12 mM); resulting nitrogen as ammonia ($\text{NH}_3\text{-N}$), 199.0 mg N/L; phosphorous as orthophosphate ($\text{PO}_4^{3-}\text{-P}$), 40.0 mg P/L and total P, 45.5 mg P/L [4]. pH of the wastewater was adjusted to 7.0.

The reactor was operated at temperature = 17–21 °C during more than 5 months. Firstly the system was run without addition of FeCl_3 (control period = 0–90 days). Then, ferric trichloride hexahydrate, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (2.02 g Fe/L, 36.17 mM) was continuously added to the aeration basin to achieve a high Fe:P ratio (molar ratio) = 1.9–2.3:1 (91–156 days).

2.2. Chemical analysis

The activated sludge reactor was monitored 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, $\text{PO}_4^{3-}\text{-P}$, total P, $\text{NH}_3\text{-N}$, total Fe and total soluble Fe were determined in the final effluent.

Samples from the 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 ($\text{NH}_3\text{-N}$). Total soluble Fe concentration in the filtrate and total Fe concentration were determined by Hach Method No. 8008 (adapted from Standard Methods). Particulate P ($P_{\text{partic}} > 0.45 \mu\text{m}$) was determined from the difference between total P and soluble P concentrations. Similarly, particulate Fe ($Fe_{\text{partic}} > 0.45 \mu\text{m}$) corresponded to the difference between total Fe and total soluble Fe concentrations.

Suspended solids determination was performed according to the standard methods [19]. Samples were filtered through 1.5 μm glass-fibre 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.

The sedimentation properties of the activated sludge were determined using the diluted sludge volume index (DSVI) [20].

2.3. Microfauna analysis

Samples of activated sludge mixed liquor were taken for microscopic analysis from the aeration basin (1–2 times per week). Protozoa and metazoa were identified and quantified using a light microscope Leica DMLB (Germany) with an incorporated photographic camera [11,18,21]. Two sub-samples of 25 μL of the mixed liquor sample were taken with 200 μL automatic micropipette and were analyzed immediately under bright-field illumination [16]. Microfauna and ciliated protozoa densities were estimated under 100–400 \times magnifications and expressed as number of organisms per liter of mixed liquor (No./L). Average densities of the two 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 [11,14]. Ciliated protozoa were identified at the generic level. Whenever was necessary photographs were taken for further analysis. Identification of protozoa was based on morphology [22,23].

The microfauna community was studied by the application of the sludge biotic index (SBI) and the Shannon–Wiener index based on the abundance of ciliate genera and abundance of the different morphological–functional groups already mentioned.

The sludge biotic index is based on two principles: (i) the dominance of key groups of the microfauna changes in relation to the environment and operating conditions of the plant, and (ii) the number of species decreases as plant performance decreases. To determine the SBI, it is necessary: (a) to evaluate the number of species or supraspecific-level taxonomic units that constitute the microfauna of activated sludge, (b) to analyze the abundance of each species and the microfauna density, (c) to establish the dominant key group and (d) to estimate the number of small flagellates by counting within 16 squares along the diagonal of a 3.2 μL Fuchs–Rosenthal camera [11]. This method allows the definition of the biological quality of the sludge using numerical values (0–10) and grouping the SBI values into 4 quality classes (I–IV). Class I includes SBI values ranging between 8 and 10 and indicates very good performance of the plant; class II corresponds to SBI 6 and 7 and indicates good performance; class III corresponds to SBI 4 and 5 indicating mediocre performance and class IV includes values <4, indicating low performance [11].

The Shannon–Wiener diversity index based on the abundance of ciliate genera (H) and abundance of morphological–functional groups (H^*) was calculated by the following expressions [24]:

$$H = - \sum_{i=1}^G W_i \log_z W_i \quad (1)$$

$$H^* = - \sum_{i=1}^{G^*} W_i^* \log_z W_i^* \quad (2)$$

where G is the number of ciliate genera, G^* is the number of morphological–functional groups, z is the base of binary logarithm ($z=2$), W_i is the relative abundance of the genus i -th calculated from the ratio between the number of ciliated organisms belonging to the genus i -th and the total number of ciliated protozoa, W_i^* is the relative abundance of the morphological–functional group i -th calculated as the ratio between the number of organisms belonging to the morphological–functional group i -th and the total number of organisms in the microfauna.

2.4. Statistical analysis

Non-linear regressions were conducted using the Sigma Plot 10.0 software (Jandel Scientific, Chicago, IL, USA). Counts of micro-

fauna organisms were performed in duplicate samples. Poisson distribution was utilized to describe the organism counts.

3. Results and discussion

3.1. Performance of the activated sludge reactor

The continuous addition of a high load of Fe(III) (141–156 mg Fe/(L day)) on the aeration basin of the laboratory-scale activated sludge reactor fed with a model wastewater of a dairy industry caused a marked decrease of the orthophosphate concentration in the final effluent from an average value of 36.3 mg P/L to less than 1.0 mg P/L after 5 days of coagulant application (Fig. 1a). The applied Fe(III) doses, that corresponded to a Fe:P molar ratio range = 1.9–2.3:1, caused a rapid decrease of pH in the aeration basin from 7.7–8.1 (pH values of the control period) to values that ranged between 5.4 and 7.1. The observed pH changes can be attributed to the slight variation of the Fe(III) doses. The total soluble Fe (Fe_{TS}) concentration of the final effluent ranged between 0.02 and 10.65 mg/L depending on the pH value; for pH values below 6.0, the Fe_{TS} concentration ranged between 2.5 and 10.65 mg/L, however concentrations below 0.6 mg/L were recorded when the pH value was ≥ 6.0 (Fig. 1a). Orthophosphate concentration did not significantly changed through the study because phosphorus removal process took place under optimal pH conditions (pH = 5.0–7.0 [3]).

During the initial period of application of the coagulant, the particulate Fe concentration of the final effluent ranged between 14.0 and 37.0 mg/L and the concentration of particulate P ranged between 7.1 and 8.9 mg/L (95–104 days, Fig. 1b). Later, in the period 107–114 days, lower concentrations were measured ($\text{Fe}_{\text{Partic}} = 4.6\text{--}7.3$ mg/L, $\text{P}_{\text{Partic}} = 0.6\text{--}1.9$ mg/L); thus, the reactor performance with respect to the removal of particulate Fe and P improved significantly after a response time of at least 15–17 days of chemical treatment (Fig. 1b). This improvement can be associated to the gradual incorporation of Fe(III) and particles containing Fe–P (ferric hydroxyphosphate) into the microbial flocs. Finally, between 120 and 156 days, the $\text{Fe}_{\text{Partic}}$ and P_{Partic} concentrations increased strongly, reaching values of about 20.0 and 5.0 mg/L respectively; these results indicate that the prolonged application of high coagulant doses caused a sudden deterioration of the reactor performance (Fig. 1b).

Similar behavior was observed for the total suspended solids of the final effluent. High values (200–720 mg/L) were recorded during the initial period of chemical treatment (95–104 days); then the TSS concentration showed relatively low values, which ranged between 90 and 100 mg/L (107–115 days), and finally the TSS increased from 100 mg/L to 130–240 mg/L (120–156 days, Fig. 1b). With regard to the COD removal, the applied coagulant caused a quick decrease of the COD concentration of the final effluent from values higher than 250 mg/L (period without Fe(III)) to values that ranged between 70 and 200 mg/L during the period of chemical treatment.

It must be pointed out that the influent 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 wastewater.

3.2. Identification of key chemical parameters for the control of activated sludge systems operated with phosphorous simultaneous precipitation

The total soluble Fe concentration of the final effluent of the activated sludge reactor depended mainly on the system alkalinity which would allow to reflect its buffering capacity rather than to detect situations of coagulant overload. The concentra-

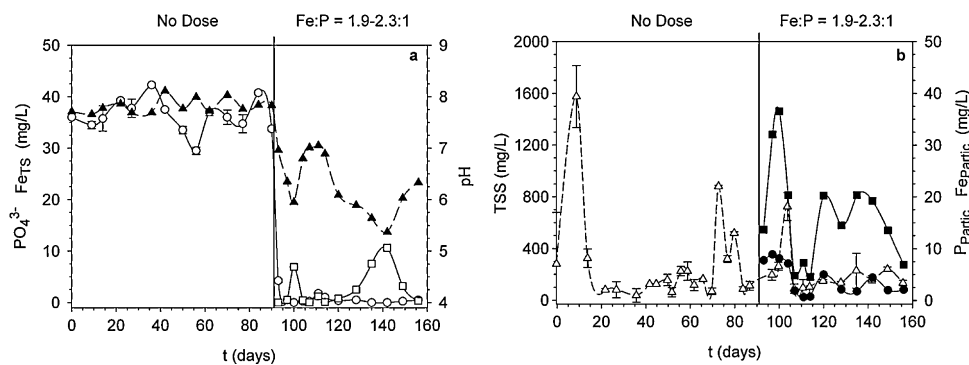


Fig. 1. Physical–chemical parameters of the activated sludge reactor as a function of the operation time. (a) Orthophosphate (PO_4^{3-}), total soluble Fe (Fe_{TS}) of the final effluent and pH of aeration basin: (\circ) PO_4^{3-} (mg/L), (\square) Fe_{TS} (mg/L), (\blacktriangle) pH. (b) Total suspended solids (TSS), particulate P (P_{Partic}) and particulate Fe ($\text{Fe}_{\text{Partic}}$) of the final effluent: (Δ) TSS (mg/L), (\bullet) P_{Partic} (mg/L), (\blacksquare) $\text{Fe}_{\text{Partic}}$ (mg/L).

tions of TSS, particulate P and particulate Fe of the final effluent changed strongly during chemical treatment time as was previously reported (Fig. 1). In addition, the COD concentration showed consistently low values (70–200 mg/L) during the application of high Fe(III) doses. These results allowed us to infer that all these physical–chemical parameters could not adequately control biological systems operated with phosphorous simultaneous precipitation. The detection of key physical–chemical and operational parameters of the reactor and the identification of species or organism groups most sensitive to the deterioration of the system environmental quality caused by Fe(III) would facilitate the process control.

The continuous addition of ferric chloride produced a marked increase of the concentrations of volatile suspended solids and fixed suspended solids in the aeration basin. The VSS concentration increased rapidly from 2.4 g/L to about 5.0 g/L; the FSS concentration increased more gradually from 0.59 g/L to about 4.7 g/L. It must be pointed out that the increase of the inorganic solids concentration was notorious after about 10 days (0.5 θ_c) of starting the chemical treatment (Fig. 2).

The increase of the VSS content of the activated sludge was caused by the marked improvement of the sedimentation properties of the sludge; however, it cannot be discarded a possible stimulating effect of Fe(III) on the microbial growth. A large amount of Fe(III) and ferric hydroxyphosphate complexes were incorporated into the microbial flocs causing a significant increase of the inorganic fraction in the sludge. The percentage of FSS increased from 20% (period without Fe(III)) to about 47% after 30 days of

continuous application of ferric chloride (Fig. 2). This situation involved a noticeable deterioration of the environmental quality of the biological system between 120 and 150 days (Fig. 1). De Gregorio et al. [8] reported that the application of high Fe(III) doses for an extended period caused an accumulation of fixed solids in the activated sludge, which favored the formation of dispersed flocs deteriorating the final effluent quality. These results indicated that the system could tolerate a maximum coagulant load, which once exceeded, leads to a low reactor performance. From studies on the modes of action of ferric chloride and ferrous chloride in systems of chemical phosphorous removal in laboratory-scale activated sludge plants, Oikonomidis et al. [25] hypothesized that floc break up is triggered as a result of Fe reaching a threshold concentration in the sludge. These authors informed that flocculation impairment in Fe(II) dosed units occurred for non-volatile suspended solids content higher than 35%. Undoubtedly, the fixed suspended solids concentration of the aeration basin constitutes a key parameter for the control of the phosphorous simultaneous precipitation process by metal coagulants. The development of a mathematical model to estimate the fixed solids content of the sludge as a function of time of coagulant application would allow monitoring such system.

3.3. Mathematical model

3.3.1. Estimation of the fixed suspended solids concentration of the activated sludge system

A mathematical model for estimating the inorganic solids content of the activated sludge in systems operated with phosphorous simultaneous precipitation should include at least the following operating parameters: influent Fe concentration, influent flow rate, and waste flow rate of the sludge.

The model should be based on the most important physical–chemical processes involved in the phosphorous removal. The chemical removal of phosphorous involves the incorporation of phosphate into total suspended solids, by combination of precipitation, adsorption and agglomeration, and the subsequent removal of these solids [6]. Considering that the mechanism of orthophosphate removal by ferric chloride involves the formation of ferric hydroxyphosphate precipitates, the total fixed suspended solids (FSS_T) concentration of the aeration basin can be expressed as follows:

$$\text{FSS}_T = \text{FSS}_0 + \text{FSS}_{\text{Fe-P}} \quad (3)$$

where FSS_0 corresponds to the initial fixed suspended solids (average value of the initial period without Fe(III), g/L) and $\text{FSS}_{\text{Fe-P}}$ corresponds to the fixed suspended solids from ferric hydroxyphosphate precipitates i.e., ferric hydroxyphosphate floc particles (g/L).

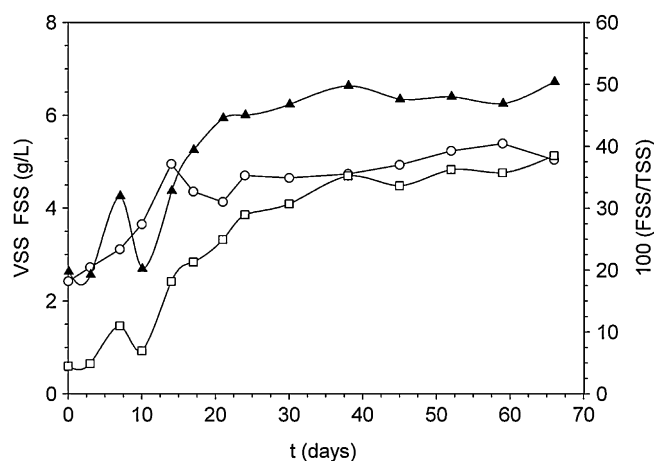


Fig. 2. Suspended solids of the aeration basin as a function of the reactor operation time with high Fe:P molar ratio = 1.9–2.3:1. (\circ) Volatile suspended solids (VSS, g/L); (\square) fixed suspended solids (FSS, g/L); (\blacktriangle) FSS:TSS ratio expressed as percentage.

In order to estimate the FSS_{Fe-P} concentration, the stoichiometric ratio $Fe:PO_4:OH$ of the formed precipitate should be considered. A $Fe:P$ molar ratio = 2.07:1 was determined by analyzing the particulate matter of the final effluent from the activated sludge reactor treated with ferric chloride. From this ratio, and applying the generalized formula for ferric hydroxyphosphate ($Fe_2 PO_4 (OH)_{3r-3}$) proposed by Luedecke et al. [26], the stoichiometric molar ratio was obtained: $Fe_{2.07} PO_4 (OH)_{3.21}$. This relationship can be also expressed as stoichiometric mass ratio: $Fe (PO_4)_{0.821} (OH)_{0.472}$, thus, the FSS_{Fe-P} concentration can be expressed as follows:

$$FSS_{Fe-P} = Fe_P + 0.821 Fe_P + 0.472 Fe_P \quad (4)$$

where Fe_P corresponds to particulate Fe (g/L). The term $0.821 Fe_P$ (g/L) corresponds to the mass provided by the phosphate ion from the ferric hydroxyphosphate floc particles; $0.472 Fe_P$ (g/L) corresponds to the mass provided by the hydroxide ion.

By rearrangement of Eq. (4) results:

$$FSS_{Fe-P} = 2.29 Fe_P \quad (5)$$

Combining Eq. (5) with Eq. (3) the following is obtained:

$$FSS_T = FSS_0 + 2.29 Fe_P \quad (6)$$

Following the addition of $Fe(III)$, and associated to the precipitation process of phosphorous, coagulation and flocculation take place involving basically adsorption phenomena and destabilization of colloidal ferric hydroxyphosphate precipitates by neutralization of charges [6]. Coagulation/flocculation process leads to the formation of larger particles increasing the inorganic solids concentration. The formation rate of ferric hydroxyphosphate complexes and the aggregation rate of this colloidal precipitates forming floc particles, susceptible to be removed by gravitational sedimentation, were included in the mathematical model. For the activated sludge continuous reactor, the following mass balances for total soluble Fe (Fe_{TS}) and particulate Fe (Fe_{Partic}) concentrations were proposed:

$$V \frac{d Fe_{TS}}{dt} = Q_0 Fe_0 - k_1 Fe_{TS} V - Q_E Fe_{TS} \quad (7)$$

$$V \frac{d Fe_P}{dt} = k' Fe_{TS} V - Q_W Fe_P \quad (8)$$

where V is the total system volume (L); Fe_{TS} is the total soluble Fe concentration (g/L); Q_0 is the influent flow rate of the reactor (L/day), corresponding to the sum of the input flows: Q_{Fe} (influent Fe flow rate) and Q_F (feed flow rate of the model wastewater); Fe_0 is the influent Fe concentration (g/L); k_1 is a Fe precipitation constant as colloidal ferric hydroxyphosphate complexes (day^{-1}); Q_E is the secondary effluent flow rate of the system or final effluent flow rate (L/day), equivalent to the influent flow rate of the reactor because it operates continuously with steady volume; Fe_P is the particulate Fe concentration ($>1.5 \mu m$) of the system (g/L); k' is a global aggregation constant of Fe (day^{-1}) and Q_W is the waste flow rate of the sludge (L/day). The operative conditions were: $Q_0 = 3.43$ L/day, $Q_{Fe} = 0.31$ L/day, $Q_F = 3.12$ L/day, $Fe_0 = 0.163$ g/L, $Q_E = 3.43$ L/day, $Q_W = 0.18$ L/day.

The term $Q_0 Fe_0$ (g/day) of Eq. (7) corresponds to the input rate of the soluble ferric ion, the term $k_1 Fe_{TS} V$ (g/day) of Eq. (7) corresponds to the formation rate of the insoluble Fe precipitates (named generically colloidal ferric hydroxyphosphate complexes). The term $Q_E Fe_{TS}$ (g/day) of Eq. (7) corresponds to the amount of total soluble Fe that escapes from the system by the final effluent. The term $k' Fe_{TS} V$ (g/day) in Eq. (8) corresponds to the Fe aggregation rate, including both the precipitation of Fe to form ferric hydroxyphosphate colloids and the aggregation of these complexes into larger particles by coagulation/flocculation process. The formed chemical flocs are retained by the standard $1.5 \mu m$ glass-fibre membrane used for determination of suspended solids. This

fraction accumulates in the system by sedimentation in the clarifier. The term $Q_W Fe_P$ (g/day) in Eq. (8) corresponds to the quantity of particulate Fe removed from the system by daily wasting of mixed liquor. In order to simplify the analysis of the problem, the particulate Fe that escapes from the system by the final effluent was not considered.

It should be pointed out that the experimental data of particulate Fe concentration ($Fe_{Partic} > 0.45 \mu m$) corresponded to the difference between total Fe and total soluble Fe concentrations. Considering that the suspended solids concentration (TSS, VSS, and FSS) is commonly determined using $1.5 \mu m$ membranes, particulate Fe (Fe_P) for modeling purpose corresponded to all forms of iron that can be retained by these standard membranes. The colloidal $Fe-P$ precipitates ($<1.5 \mu m$) do not contribute to the solids content of the system; these precipitates can slowly settle in the clarifier or escape from the reactor through the final effluent.

Under steady-state conditions with respect to the total soluble Fe concentration, Eq. (7) gives the following expression:

$$Q_0 Fe_0 = k_1 Fe_{TS(SS)} V + Q_E Fe_{TS(SS)} \quad (9)$$

where $Fe_{TS(SS)}$ corresponds to the total soluble Fe concentration at steady-state (g/L).

Rearranging Eq. (9) and considering that $Q_E = Q_0$, the coefficient k_1 can be estimated by the following expression:

$$k_1 = \frac{Q_0 (Fe_0 - Fe_{TS(SS)})}{Fe_{TS(SS)} V} \quad (10)$$

During the period of chemical treatment, the total soluble Fe concentration varied between 0.02 and 10.65 mg/L depending on the pH values of the aeration basin. High soluble Fe concentrations were registered for pH values <6.0 ; concentrations of Fe_{TS} near zero corresponded to higher pH values (Fig. 1a). For this reason, an average value (2.65 mg/L) was calculated from the experimental data of Fe_{TS} , and this value was used to estimate the coefficient k_1 by using Eq. (10); the obtained value was $k_1 = 58.27 day^{-1}$.

Rearranging Eq. (10), the total soluble Fe concentration at steady-state corresponds to the following expression:

$$Fe_{TS(SS)} = \frac{Q_0 Fe_0}{k_1 V + Q_0} \quad (11)$$

Considering that a quasi-stationary state with respect to the concentration of total soluble Fe was rapidly reached, Eq. (8) was combined with Eq. (11) and integrated between $t=0$ (initial state without added $Fe(III)$) and t (time of exposure to $Fe(III)$), obtaining the following expression:

$$Fe_P = \frac{k' Q_0 Fe_0 V}{Q_W (k_1 V + Q_0)} (1 - e^{-2.3 Q_W t / V}) \quad (12)$$

By combining Eq. (12) with Eq. (6) the following expression was obtained:

$$FSS_T = FSS_0 + 2.29 \frac{k' Q_0 Fe_0 V}{Q_W (k_1 V + Q_0)} (1 - e^{-2.3 Q_W t / V}) \quad (13)$$

It should be noted that the increase of the FSS_T content of the sludges was evident after $0.5 \theta_C$ of $Fe(III)$ treatment (Fig. 2). This result can be explained if it is considered that during the early periods of $Fe(III)$ treatment, colloidal particles containing $Fe-P$ escaped mainly from the system in the final effluent; in addition a response time of about 15–17 days favored the aggregation of these particles achieving a significant improvement in the reactor performance (Fig. 1b). Taking into consideration this analysis, Eq. (13) was modified as follows:

$$FSS_T = FSS_0 + 2.29 \frac{k' Q_0 Fe_0 V}{Q_W (k_1 V + Q_0)} (1 - e^{-2.3 Q_W (t-L) / V}) \quad (14)$$

where L corresponds to the lag phase or response time.

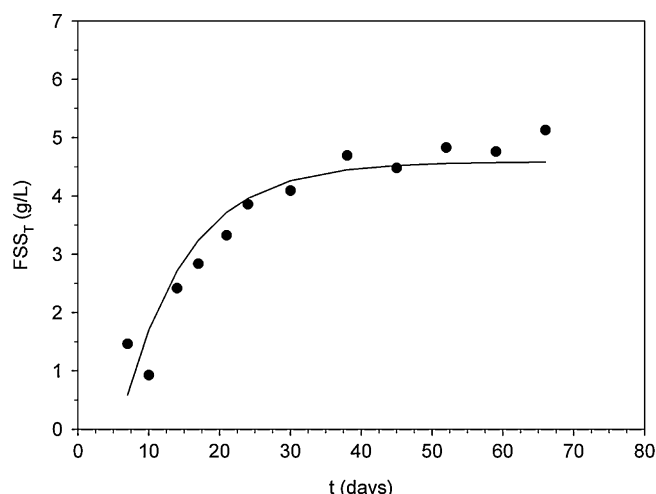


Fig. 3. Total fixed suspended solids (FSS_T , g/L) of the aeration basin as a function of the reactor operation time with high Fe:P molar ratio: 1.9–2.3:1. (●) Experimental data. (—) Predictions by Eq. (14).

Eq. (14) allows the estimation of the concentration of total fixed suspended solids of the system as a function of the Fe(III) application time considering the influent flow rate, the influent Fe concentration, and the waste flow rate of the sludge. This equation was satisfactorily fitted to the experimental data by non linear regression (Sigma Plot 10.0) during the period with chemical treatment. Each experimental point was the average of duplicates. The following parameters were determined: $k' = 31.260 \text{ day}^{-1}$ (SD = 1.392 day^{-1}) and $L = 7.090 \text{ day}$ (SD = 0.814 day) (Fig. 3). The value k_1 corresponded to the previously estimated by Eq. (10).

For the applied conditions, $Q_0 = 3.43 \text{ L/day}$ and $Fe_0 = 0.163 \text{ g/L}$, an increase of the concentration of total fixed suspended solids from 0.59 g/L (period without Fe(III)) to 3.96 g/L can be estimated after 24 days of continuous application of ferric chloride (Fig. 3). This value is considered as the threshold concentration of fixed suspended solids in the system, because once it is exceeded a sharp decline in the reactor performance was detected.

The coefficient k_1 (58.27 day^{-1}) was significantly higher than the parameter k' (31.260 day^{-1}), because the first one corresponds to the Fe(III) precipitation associated to the formation of ferric hydroxyphosphate while the last parameter is a kinetic coefficient of aggregation of Fe comprising the reaction of Fe(III) precipitation as well as the slower coagulation/flocculation processes.

3.3.2. Estimation of the abundance of organisms belonging to morphological–functional groups related to the environmental quality of the biological system

The application of high Fe(III) doses affected negatively the organism groups of the microfauna community closely associated with the microbial flocs: crawling ciliates and attached ciliates. A decrease of three orders of magnitude in the total abundance of these ciliated protozoa occurred after about 60 days of continuous dosage of ferric chloride (Fig. 4).

Then, this group, that includes all microfauna organisms associated with the flocs, suddenly disappeared. For microscopic count of organisms, Poisson distribution was assumed and 95% confidence intervals were obtained. In a previous study, De Gregorio et al. [8] reported that the disappearance of these morphological–functional groups favored the development of organisms not directly associated with flocs such as rotifers and swimming ciliates. This community changes were associated to a progressive enrichment of Fe(III) in the sludge. Based on this analysis, crawling ciliates and attached ciliates can be considered the organism groups most sensi-

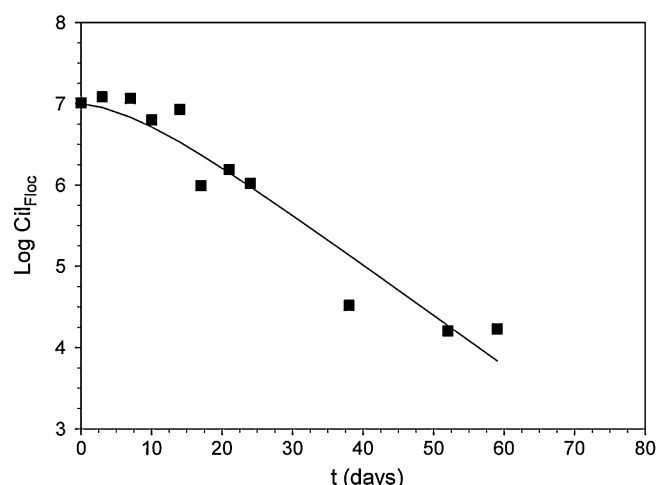


Fig. 4. Abundance of ciliated protozoa associated with the microbial flocs as a function of the reactor operation time with high Fe:P molar ratio: 1.9–2.3:1. (■) Experimental data. (—) Predictions by Eq. (16).

tive to the deterioration of the environmental quality of the system, thus a mathematical expression that relates the decay of these groups with the progressive increase of the inorganic solids content in the system could be an useful tool to avoid Fe(III) over-load.

The abundance of organisms belonging to microfauna groups associated with the microbial flocs (crawling ciliates + attached ciliates) can be expressed as follows:

$$V \frac{d \text{Cil}_{\text{Floc}}}{dt} = k_2 V \text{Cil}_{\text{Floc}} - Q_W \text{Cil}_{\text{Floc}} - k_3 V FSS_T \text{Cil}_{\text{Floc}} \quad (15)$$

where V is the total volume of the system (L), Cil_{Floc} is the number of ciliated protozoa associated with the microbial flocs (N_0/L), k_2 is the mean growth coefficient of these ciliates (day^{-1}), k_3 is the decay coefficient of these organisms ($L/(g \text{ FSS}_T \text{ day})$). The term $k_2 V \text{Cil}_{\text{Floc}}$ (N_0/day) corresponds to the mean growth rate of ciliated protozoa associated with the microbial flocs. The term $Q_W \text{Cil}_{\text{Floc}}$ (N_0/day) corresponds to the amount of these ciliates removed from the system by daily wasting of mixed liquor. The last term, $k_3 V FSS_T \text{Cil}_{\text{Floc}}$ (N_0/day), corresponds to the decay rate of Cil_{Floc} due to the presence of total fixed suspended solids.

Considering that the total fixed suspended solids changed as a function of time of application of Fe(III), Eq. (14) was combined with Eq. (15) and integrated between $t=0$ (initial state without added Fe) and t (time of exposure to Fe (III)) obtaining the following expression:

$$\begin{aligned} \text{Log Cil}_{\text{Floc}} = & \text{Log Cil}_{\text{Floc}_0} + k_2 t - \frac{Q_W t}{V} - k_3 \left[SSF_0 t + \frac{2.29 V k' Q_0 Fe_0}{Q_W (k_1 V + Q_0)} \right. \\ & \times \left. \left[t + \frac{V e^{2.3 Q_W L/V}}{2.3 Q_W} (e^{-2.3 Q_W t/V} - 1) \right] \right] \end{aligned} \quad (16)$$

where $\text{Cil}_{\text{Floc}_0}$ corresponds to the number of ciliates associated with the microbial flocs (N_0/L) determined prior to the addition of Fe(III).

Eq. (16) allows the estimation of the abundance of ciliated protozoa associated with the microbial flocs as a function of time considering the waste flow rate of the sludge and the influent Fe. This equation was satisfactorily fitted by non linear regression to the experimental data (Fig. 4). The following parameters were determined: $k_2 = 0.8952 \text{ day}^{-1}$ (SD = 0.4914 day^{-1}), $k_3 = 0.1985 \text{ L/(g FSS}_T \text{ day)}$ (SD = $0.1087 \text{ L/(g FSS}_T \text{ day)}$). Each experimental point was the average of duplicates.

For initial conditions, $Q_0 = 3.43 \text{ L/day}$ and $Fe_0 = 0.163 \text{ g/L}$, corresponding to the high Fe:P molar ratio = 1.9–2.3:1, a decrease of one order of magnitude in the abundance of ciliated protozoa associated with the microbial flocs can be estimated after 24 days of

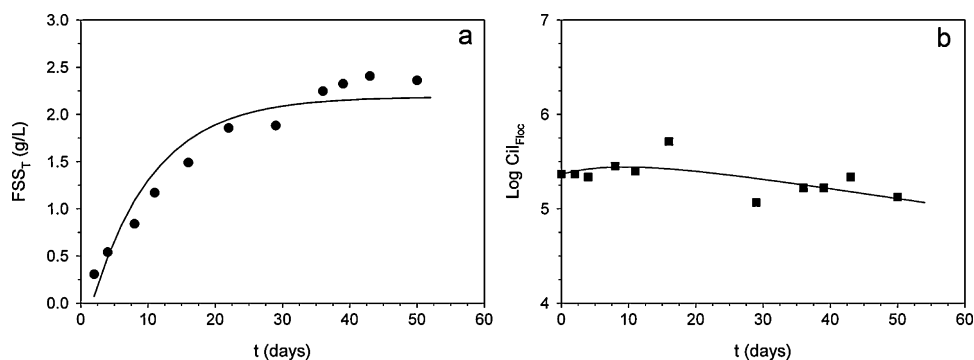


Fig. 5. Total fixed suspended solids and ciliated protozoa associated with the microbial flocs of the aeration basin as a function of operation time with optimal Fe:P molar ratio: 1.5–1.9:1. (a) Total fixed suspended solids (FSS_T , g/L). (●) Experimental data. (—) Predictions by Eq. (14). (b) Ciliated protozoa associated with the microbial flocs. (■) Experimental data. (—) Predictions by Eq. (16).

continuous dosage of ferric chloride (Fig. 4). In this situation, it is advisable to reduce the dose of Fe(III), because the maintenance of high doses for extended periods caused a strong deterioration in the performance of the system.

3.3.3. Verification of the mathematical model

The mathematical model was verified through an experimental study performed in the laboratory-scale activated sludge reactor under adequate operational conditions. The reactor was fed with the model wastewater of a dairy industry (previously described in Section 2.1) at a temperature = 17–21 °C and using a feed flow rate $Q_F = 0.06$ L/h. The hydraulic residence time was 63 h; the sludge age was 21 days. A control period, without addition of $FeCl_3$, was set; then, $FeCl_3 \cdot 6H_2O$ (0.75 g Fe/L, 13.43 mM) was continuously added to the aeration basin to achieve a optimal Fe:P molar ratio = 1.5–1.9:1. The operating conditions were: $Q_0 = 1.71$ L/day, $Q_{Fe} = 0.27$ L/day, $Q_F = 1.44$ L/day, $Fe_0 = 0.119$ g/L, $Q_E = 1.71$ L/day, $Q_W = 0.18$ L/day.

The system exhibited good performance throughout the Fe(III)-treated period, resulting the soluble P and total P removal higher than 98% and 96% respectively. The particulate Fe concentration of the final effluent ranged between 1.25 and 5.0 mg/L and the corresponding to particulate P ranged between 0.25 and 1.75 mg/L. The concentration of total fixed suspended solids of the reactor increased gradually to reach about 2.3 g/L after 40 days of Fe(III) addition. Eq. (14) was satisfactorily fitted to the experimental data by non linear regression during the period with chemical treatment. Each experimental point was the average of duplicates. The following parameters were determined: $k' = 53.870 \text{ day}^{-1}$ ($SD = 2.418 \text{ day}^{-1}$) and $L = 3.107 \text{ day}$ ($SD = 0.645 \text{ day}$) (Fig. 5a). The value k_1 estimated by Eq. (10) was = 71.59 day^{-1} . For the applied operational conditions, an increase of the concentration of FSS_T from about 0.30 g/L (control period) to 2.15 g/L could be estimated after 40 days of application of ferric chloride (Fig. 5a). It must be noted that this value was below the threshold concentration of fixed suspended solids that the system can tolerate without adversely affecting the reactor performance. Thus, the applied Fe(III) doses did not change the biological system quality; according to this situation, the density of microfauna organisms related to activated sludge flocs remained relatively unchanged (Fig. 5b). Counts of these organisms in the 25 μ L mixed liquor samples were assumed to follow a Poisson distribution and 95% confidence intervals were obtained.

The ciliated protozoa associated with the microbial flocs declined slightly after about 30 days of chemical treatment. Eq. (16) was satisfactorily fitted by non linear regression to the experimental data, resulting the following parameters: $k_2 = 0.803 \text{ day}^{-1}$ ($SD = 0.438 \text{ day}^{-1}$), $k_3 = 0.350 \text{ L/(g } FSS_T \text{ day)}$ ($SD = 0.202 \text{ L/(g } FSS_T$

day)). Each experimental point was the average of duplicates. By applying Eq. (16), a decrease of only 0.15 log units in the abundance of ciliated protozoa associated with the microbial flocs can be estimated after 40 days of continuous dosage of Fe(III) (Fig. 5b). As observed the proposed model was verified under different tested conditions.

3.4. Biological indices as indicators of the reactor performance treated with high Fe:P molar ratio

Besides the mathematical model, different biological indices were applied to evaluate the reactor performance. The sludge biotic index (SBI) and the Shannon–Wiener diversity index based on the abundance of ciliate genera (H) and abundance of morphological–functional groups (H^*) were used. The SBI was set up specifically for the evaluation of the biological reactor performance [11]. In systems using coagulants during biological treatment of wastewaters, that is, when the process performance does not depends solely on the microbial community activity, the SBI would reflect the toxicity of the chemical agent. Nevertheless, the usefulness of this objective indicator for monitoring the system performance should be analyzed.

Swimming ciliates was the dominant organism group in the reactor between 45 and 55 days, representing 60–99% of the community; then, attached ciliates accounted for more than 50% of the microfauna organisms in the aeration basin (59–66 days). According to these situations, the SBI presented values lower than 4 indicating poor reactor performance (Fig. 6a).

Between 70 and 77 days, the crawling ciliates represented 52–91% of the microfauna and the SBI value was 7 (Fig. 6a), this situation would correspond to a good performance of the plant with well colonized and stable sludge, however low efficiency was observed between 70 and 82 days due to severe filamentous bulking problems (Fig. 1). Even though it has been previously reported that the SBI is unable to reveal any malfunction in the secondary clarifier [11], in the present study the SBI reflected the occurrence of filamentous bulking not immediately but after more than one week of the beginning of this phenomenon. Between 80 and 90 days, proliferation of attached ciliates took place, which was probably caused by an increase of the food–microorganism ratio (F/M) due to heavy loss of bulking sludge from the clarifier, and the SBI value decreased from 7 to 2 indicating low reactor performance (Fig. 6a). During this period, attached ciliates represented 73–99% of the microfauna community.

After approximately one week from the beginning of Fe(III) application, crawling ciliates and attached ciliates co-dominated the community and the SBI value increased from 2 to 7 (Fig. 6a). The observed increase of the sludge biological quality is attributed to the fact that the added coagulant improved rapidly the settling

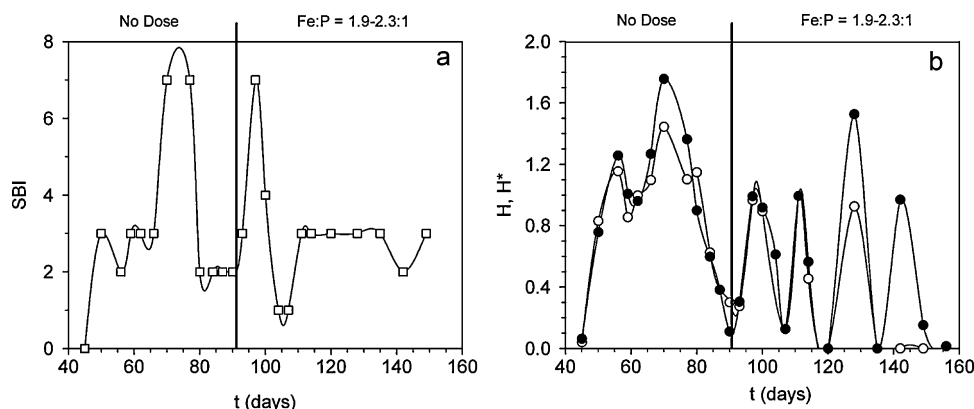


Fig. 6. Biological indices as a function of reactor operation time. (a) Sludge biotic index (SBI). (b) Shannon–Wiener diversity index based on: abundance of ciliate genera (○), and abundance of morphological–functional groups (●).

properties of the sludge causing a sudden improvement in the reactor performance mainly with respect to the COD removal. Later, between 100 and 135 days, attached ciliates represented typically more than 80% of the microfauna community and consequently the SBI value decreased to values lower than 4 (Fig. 6a). Thus, this index did not reflect the good performance of the reactor after $0.75\theta_C$ with Fe(III) addition, during the period 107–115 days; however, it reflected the decreasing performance of the system (Fig. 1).

It must be noted that during 140–156 days, sessile ciliates decreased and rotifers accounted for 78% to 99% of the microfauna; SBI values ranged between 2 and 3 (Fig. 6a).

Fig. 6b shows the Shannon–Wiener diversity index based on the abundance of ciliate genera (H) and abundance of morphological–functional groups (H^*). These indices exhibited similar curves during the initial period, with relatively high values (0.85–1.75) between 55 and 80 days; then, a gradual increase in the abundance of attached ciliates and a reduction of crawling ciliates led to a steady decrease in both indices H and H^* between 80 and 90 days (Fig. 6b). The decrease of the microfauna diversity during this period was probably caused by the occurrence of severe filamentous bulking between 70 and 82 days.

The addition of Fe(III) caused a sudden increase in the H and H^* indices, which reached values between 0.9 and 1.0 during 97–100 days (Fig. 6b). Thus, these biological indices reflected an initial improvement of the reactor performance coinciding with the results previously reported for the SBI. Later, the values of H and H^* ranged abruptly between 0 and 1.52 revealing the establishment of an unstable community. At the end of the period with chemical treatment, the community structure was simplified showing zero diversity of ciliates and low diversity of morphological–functional groups (Fig. 6b). Thus, the Shannon–Wiener diversity index revealed the deterioration degree of the reactor performance caused by the coagulant addition.

The applied biological indices (SBI, H and H^*) showed an initial improvement in living conditions related to a rapid improvement of the reactor performance caused by the Fe(III) addition. This situation was associated to the incorporation of Fe(III) and ferric hydroxyphosphate complexes in the microbial flocs, which improved rapidly the sludge settling properties causing a sudden improvement in the COD removal. Nevertheless, the weight percentage of fixed suspended solids increased strongly from 20% to about 47% after 30 days of continuous application of Fe(III) leading to the formation of dispersed chemical flocs that deteriorated the final effluent quality. The biological indices detected the deterioration of the system environmental quality, caused by the progressive enrichment of the activated sludge with inorganic solids, after about 10 days of starting the chemical treatment, that is long before the performance of the reactor decreased. Based on the obtained

results, we assert that the biological indices can early predict a decline of the reactor performance due to coagulant overload, so that the Fe(III) dosage can be promptly corrected in order to avoid the causes of deterioration in the system.

4. Conclusions

A laboratory-scale activated sludge reactor operated with phosphorous simultaneous precipitation by Fe(III) was monitored by biological indices in conjunction to the application of a mathematical model. The Shannon–Wiener index and the sludge biotic index can predict situations of low process performance due to coagulant overload. The proposed mathematical model aimed to define the permissible operating conditions for the adequate operation of the reactor. This model constitutes a complementary method to the use of biological indices for reactor monitoring. The simultaneous application of the mathematical model and the biological indices would ensure a successful control of the chemical phosphorous removal at the secondary treatment stage. This approach would avoid undesirable situations caused by excess of coagulant dosage.

Acknowledgments

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