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Treatment of dairy wastewater by fixed-film system in continuous flow

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ABSTRACT

The objective of this work was to assess the feasibility of biological phosphorus removal for dairy processing wastewater. In this study, two fixed-film systems carried out continuously were tested in the laboratory. The effect of aerobic and "anoxic/anaerobic/aerobic" conditions on phosphates removal has been investigated on both the fixed-film systems and reported, that alternating phase's system (bioreactor 1) resulted in higher phosphorus removals relating to aerobic system (bioreactor 2). The main results showed that the effectiveness of COD and phosphates for bioreactor 1 reached, respectively, to 66 and 91% at stable biofilm functioning. Similarly, the effectiveness of COD and phosphates removal for bioreactor 2 reached to 70 and 84.61%, respectively. No clogging of media occurred and no backwashing was applied on both the systems during the study. Also, kinetic analysis of the reactor with regard to phosphorus removal has been studied with the modified Stover-Kincannon kinetic model which was chosen for modeling studies and experimental data analysis of the fixed-film system used in this study. The experimental data showed that the Stover-Kincannon kinetic model was the most suitable for predicting the removal of phosphate in the bioreactor 1, but not in the bioreactor 2.

Keywords: Biological phosphorus removal; Phases alternation; Aerobic; Continuous feeding process; Stover-Kincannon model

1. Introduction

Biological phosphorus removal (BPR) is accepted as one of the most economical and environmentally sustainable processes to remove phosphorus (P) from wastewater. However, adoption of BPR for the treatment processing of industrial wastewaters is less common. These high-strength wastewaters can be rich in phosphorus, reaching, for example, 150 mg/L in dairy wastewaters [1].

There is a limited knowledge about the ability of the BPR process to treat such high phosphate concentrations [2]. In treating industrial wastewater, additional challenges are facing the process designer due to the substantially higher concentrations of both carbon and nutrients.

Enhanced biological phosphorus removal (EBPR) is based on the selective enrichment of bacteria accumulating inorganic polyphosphate, obtained at a cyclic regime of alternating anaerobic and aerobic conditions [3–7]. Polyphosphates accumulating organisms (PAOs) take up organic substrates (preferably volatile fatty acids [VFAs]) from wastewater and store them as poly-hydroxyalkanoates (PHA) under anaerobic conditions. The reducing equivalents and energy to

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store PHA are provided mainly by the glycolysis of internally stored glycogen reserves and hydrolysis of intracellular polyphosphates (polyP). Then in the subsequent aerobic zone, PAO assimilate orthophosphate from the wastewater to synthesize the intracellular polyphosphates (polyP) in excess of that hydrolyzed under anaerobic condition by degrading the stored PHA for energy. They also grow and replenish their glycogen stores using the PHA as carbon and energy sources. Thus, PAO achieve dominance in this anaerobic–aerobic process because they can grow aerobically without any exogenous carbon and energy source by using the PHA accumulated anaerobically. Glycogen accumulating organisms (GAO) can also store organic substrates as PHA under anaerobic conditions. Therefore, GAO may compete for organic substrates with the PAO under anaerobic conditions. Consequently, GAO are often more relatively abundant than PAO in deteriorated EBPR systems [8]. Thus, it seems that EBPR is characterized by efficiency of phosphorus removal in excess of metabolic requirements [8–10]. According to Nittami et al. [8], this process is based on an enrichment of the activated sludge community with PAOs, and by encouraging the accumulation of phosphorus in PAO cells in the form of PolyP granules in excess of the levels normally required to satisfy the metabolic demand for growth [8].

While EBPR has been practiced for 30 years, until recently it has been limited exclusively to activated sludge process, with the corresponding need for large basin volumes, the most of these applications are carried out by using the process of activated sludge and its alternatives. However, the BPR in biofilm treatment systems where the biofilm grows on a support is more difficult and fewer studies have examined it in comparison with suspended biomass systems [11]. The complexity of phosphorus removal process in a fixed biomass process is still not well understood.

Yet, the research with biofilm reactors showed that the principle of alternate anaerobic and aerated conditions was applicable to fixed bacteria by changing the conditions in time rather than in space [12,13]. The major problem lies in the necessity to expose the biomass to an alternation of phases. Therefore, the BPR by attached biomass would not be possible in most continuous flow biological nutrients removal systems because the biomass is fixed in one location and typically cannot be subjected to alternating environmental conditions. Moreover, the greatest difficulties associated with creating the conditions necessary for the alternate achievement of EBPR are found particularly in biofilms continuously operating.

The multiple advantages which are offered by the biofilm demand a need for further research [13].

Biofilm systems are not commonly used for EBPR. A few studies on sequencing batch reactors (SBRs) have been reported [1,14]. Some of them focused on continuous flow systems by alternating anaerobic/aerobic conditions in biofilm reactors [15]. However, the study of these processes remains complex [9,16]. The alternating phase's systems could be applied in dairy wastewater treatment because of the high amount of organic matter (OM). The purpose of the anaerobic phase is to reduce the organic content from chemical oxygen demand (COD) values from 4,000–5,000 to <1,000 mg/L. The function of the aerobic phase is to continue the decrease of the pollutants concentration in the receiving wastewater system. It seems important then that the control of parameters involved in each phase (anaerobic–aerobic) would maximize their effectiveness, and thus improve the efficiency of BPR. The anaerobic residence time is one of these parameters, but it remains controversial [5]. One group of authors [17–19] recommends a long anaerobic residence time to maximize the release of phosphates. However, another group [20–22] recommends short residence times to avoid excessive release without OM uptake, which seems detrimental to biological dephosphatation efficiency. In general, durations from 3 to 6 h are adopted.

The increase in anaerobic residence time has not the same effect in the biofilm reactor as in activated sludge systems. In a biofilm reactor, the duration of this phase acts mainly on the fixed biomass. This optimizes the P release in controlling the exposure time of the anaerobic biomass.

The duration of the anaerobic phase is an important factor in the selection of PAOs. According to Gonçalves and Rogalla [11] an anaerobic phase of 6 h results on a better efficiency in the selection of PAOs more than an anaerobic phase of 3 h [11]. Moreover, in EBPR systems, especially in SBRs, it has been reported that the cycle duration (CD) plays an important role. The effect of the CD variation of 3, 12, 24, and 48 h on the phosphorus release in the system has been studied [12]. The maximum activity of the biomass in phosphorus removal system was observed at the CD of 24 h. However, the activity of PAOs, reached a maximum value for CD of 12 h.

The CD has a significant impact on the biological activity in a biofilm subject to alteration of environmental conditions created by variation of this term. According to Baljic and Leduc [12], the initial effect of the change in CD is changing the availability of oxygen in the non-aerated biofilm. A short duration does not offer sufficient time for an effective release. Moreover, a very long time leads to a phosphorus saturated biomass in aerated phase and biomass exhausted during the anaerobic phase [12].

Another condition in EBPR process is to reduce the intake of nitrates and oxygen in the anaerobic zone [10]. The EBPR is the most effective when nitrification is minimized.

Moreover, Kelly and Gibbs [23] used a bacterial bed with aerobic–anoxic sequence conditions, and noted that the use of an activated sludge process is more preferable than bacterial bed one, because of the difficulty to ensure the exposure of the biomass to alternating anaerobic–aerobic conditions. Therefore, it is interesting to see how the basic principles of BPR can be implemented to a variety of processes.

Dairy processing wastewater has unique properties which differentiate it from domestic wastewaters and provide challenges when attempting to treat it with an EBPR system. It has high levels of COD and dissolved reactive phosphorus and nitrogen.

Algiers dairy industry wastewaters can have phosphorus contents up to 130 mg/L, on the average 90% of total phosphorus was in form of orthophosphates probably originating from the high levels of phosphoric acid used for cleaning of process equipment. In treating industrial wastewater, additional challenges are facing the process designer and operator due to the substantially higher concentrations of both carbon and other nutrients.

The subject used in this study was to determine if BPR of dairy wastewaters could be achieved in fixed biofilm systems continuously operating.

2. Materials and methods

2.1. Biological Systems

The biological processes for dairy wastewater treatment were carried out in 0.9 L glass reactors (34 mm diameter and 100 cm height) (Fig. 1), they were placed at ambient temperature.

The reactors were filled up with an inert carrier (rachig rings) to allow the fixation of the biofilm, with a vacuum rate of 70% that allows minimizing the clogging risk. The carrier was presterilized at 160°C. They received, using a peristaltic pump in ascending flow, the influent with a flow rate of $0.3\,m\,h^{-1}$. An air compressor has been introduced into the columns to ensure aeration, the dissolved oxygen (DO) was ranged from 2 to 3 mg O_2/L during the aerobic phase for the column operating with sequence of phases and for column continuously aerated. The bioreactors were fed with an Algiers dairy wastewater the composition of which is given in Table 1 and were inoculated with activated sludge from an Algiers urban wastewater treatment plant. The application of activated sludge from a municipal wastewater treatment plant, which contains a rich variety of microorganisms, enzymes, and co-factors, would provide for co-metabolic and syntrophic supplementation of the metabolism of certain microbial groups and for the synergetic accomplishment of, for example, hydrolysis, fermentation, acetogenesis, denitrification, and BPR.

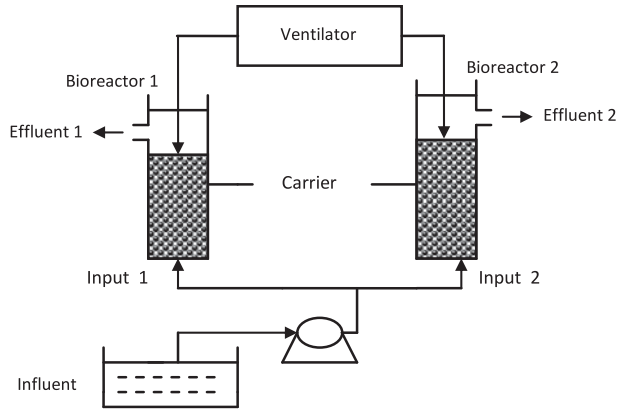

Fig. 1. Schematic diagram of fixed biofilm system.

The temperature was ranged from 22 to 27°C (June–August), with an average value of 24.5°C due to the seasonal variation.

A duration cycle of 24 h was used. These biological systems were easy to construct and operate with small maintenance.

The start-up of the wastewater treatment processes was carried out after an acclimatization period of 15 days. The dairy wastewater composition included compounds of natural origin (proteins, lipids, and carbohydrates) that do not require a specialized method for acclimatization. The purpose of the acclimatization is to increase the starting population of phosphates accumulating organisms.

Table 1
Chemical composition of influent

COD (mg/L)	4,024
BOD_5 (mg/L)	2,500
NH_4–N(mg/L)	24.40
NO_3–N(mg/L)	66.50
PO_4–P (mg/L)	8.85–26.5
pH	4.2–8.3
K (COD/BOD_5)	1.6

2.2. Raw wastewater

The reactors were fed with dairy plant wastewater whose chemical composition is given in Table 1.The dairy wastewater to be treated contains a PO_4–P of 17.7 mg/L, NH_4–N of 24. 4 mg/L, NO_3–N of 25 mg/L in average, a pH ranged from 4.2 to 8.3, a BOD_5 of 2,500 mg/L a COD of 4,000 mg/L this allows a COD/BOD_5 ratio of 1.6. The biological treatment is then possible [6]. Dairy wastewater contain high amount of organic matter. COD of dairy waste effluents from full-scale operations varies between 500 and 9,200 or 3,800 mg/L on average [24].

2.3. Operating conditions of reactors

In Tables 2 and 3, the characteristics and operating conditions for the bioreactors are presented.

Bioreactor 1: The anoxic phase which was ensured during 4 h allowed the decrease of nitrates concentrations (nitrates are used as e^- acceptors by micro-organisms). For effective phosphate removal, a short anoxic phase is beneficial before an anaerobic phase [25].

At the end of this phase, the column was obstructed for 4 h, in order to bring the system to anaerobic conditions necessary for the phosphate release.

The aeration was then started to allow the creation of aerobic conditions (which were maintained during 16 h), to provide oxygen to micro-organisms to ensure the uptake of phosphates initially released and the oxidation of ammonium.

The duration cycle was 24 h.

The choice of this sequence of phases (Anoxic–Anaerobic–Aerobic: A/A/O) lies on the basis that in the anoxic phase, micro-organisms use OM for denitrification [26], followed by the anaerobic zone in which nitrates concentration was reduced and phosphate was released, then the aerobic phase in which there was an oxidation of OM, nitrification, and uptake of phosphates.

Table 2
Characteristics of the glass reactors and the carrier used

Glass column	Diameter (cm)	3.4
	Height (cm)	140
	Useful height (cm)	100
Carrier	Rachig rings	
Characteristics	Internal diameter (cm)	0.2
	External diameter (cm)	0.5
	Porosity	0.7

Many studies observe the disruptive role of nitrates on BPR process. Their presence inhibits the release of orthophosphate in the unventilated zone [10,26,27].

Bioreactor 2 is continuously ventilated with the aim of showing the alternating phases' importance in the BPR process. Indeed, phosphorus removal can be obtained by simple biological assimilation, but never does a thorough removal of phosphorus [6]. The bioreactors were in operation for 54 days. During these 54 days of study, two samples were daily collected from the influent tank and at the end of each cycle of operation from the effluent tank.

2.4. Methodology

Samples were taken on a daily basis. Each collected sample was analyzed for COD, orthophosphates, nitrates, ammonium, according to a colorimetric method by HACH DR 2010 spectrophotometer (AFNOR standards, [28]).

The DO concentration and pH were measured thanks to a laboratory oxymeter HI 2400 Hanna and pH meter IC 3510 Jenway, respectively. Volatile fatty acids were determined by gas chromatography (Trace 2000, TermoQuest) with a DB-Waxeter column (30 m × 0.25 mm), a flame ionization detector, and helium carrier gas.

2.5. Biodegradation effectiveness

The biodegradation effectiveness (Eff) in the bioreactors was calculated for both PO_4–P and total OM content (measured as COD).

$$\text{Eff} = \frac{C_{t1} - C_{t2}}{C_{t1}} \times 100 \quad (1)$$

where: C_{t1} = concentration of pollutants at the moment $t1$, C_{t2} = concentration of pollutants at the moment $t2$.

The biodegradation effectiveness was presented for the concentrations evolution for phosphates and COD according to time.

3. Results and discussion

The focus of this research was on the bioreactors operation expressed by the efficiency of biodegradation and changes in functional characteristics of biological systems during the operation period.

Table 3
Operating conditions for the bioreactors

Bioreactor 1	Dissolved O_2 (mg/L)	Retention time of the effluent (h)
Aerobic	2–3	16
Anoxic	<0.5	4
Anaerobic	<0.1	4

Column 2 ventilated continuously (3 mg O_2/L).

3.1. Phosphates

The evolution of phosphates removal according to time yielded the results shown in Fig. 2(a).The evolution of time is expressed by series, where each series represents the average value obtained after 3 days of operation.

The influent had an average PO_4–P concentration of 18.49 mg/L with three-days averages ranging from 8.85 mg/L (series 16) to 26.4 mg/L (series 7).

The PO_4–P concentration in the influent during series 7 (26.4 mg/L) was higher than all the other days.

The effluent had an average PO_4–P concentration of 12.9 mg/L. A minimum and a maximum PO_4–P concentrations of 0.38 mg/L (series 16) and 21.63 mg/L (series 1) for the bioreactor 1 and 1.43 mg/L (series 1) and 23.88 mg/L (series 16) for bioreactor 2.

The Fig. 2(b) shows the evolution of phosphate removals on both bioreactors.

This result shows:

- Fluctuations in the performance of phosphorus removal between the first and the eigth series of treatment for both the bioreactors. This corresponds to the early phase and was related to initial biofilm formation and with low biofilm activity. A critical moment in biofilms systems of wastewater systems is the biofilm development. The starting span is defined by the time needed for formation of the biofilm's compound trophic structure for establishing interrelations among the components and for their synchronization.
- Beyond the 8th series, the performance increases to 95.70% for bioreactor 1 and 83.80% for the bioreactor 2; this leads to output values of 0.80 and 1.43 mg/L, respectively for bioreactors 1 and 2. This corresponds to the late phase and was related to active and stable biofilm development. In the remainder of our study, we shall consider only the results obtained during this phase.

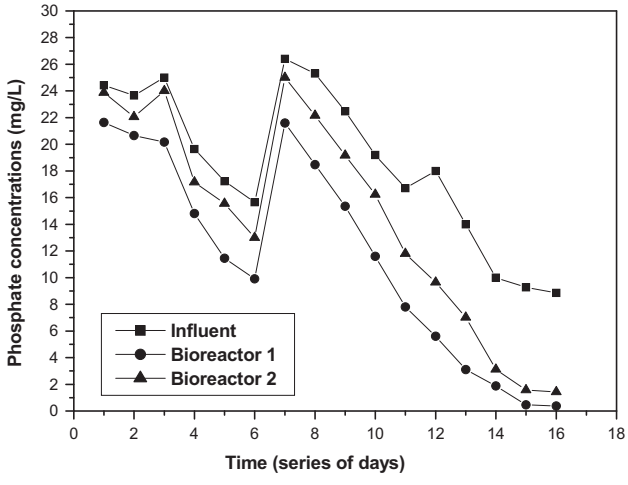

Fig. 2(a). Evolution of PO_4–P concentrations according to time for the influent and the effluent for both bioreactors.

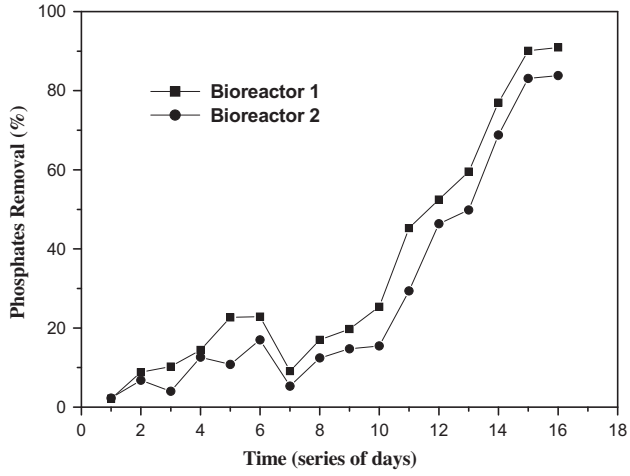

Fig. 2(b). Evolution of phosphates removal according to time for both bioreactors during the early and the late phases.

Bioreactor 1: In the anaerobic phase, a partial anaerobic stress resulting in a low release of phosphorus was observed. The released concentrations are not very important; they increase only from 1 to 2 mg/L compared with the initial concentrations (Fig. 3).

The phosphorus removal performance of this system was approximately 23% for the early phase and 58.2% for the late phase.

However, phosphate release under anaerobic conditions was not significant.

This can be explained by:

- A short duration of the early phase, which was insufficient to allow the specialization of the bacteria responsible for phosphate removal. One possibility for decreasing reactor start-up time is

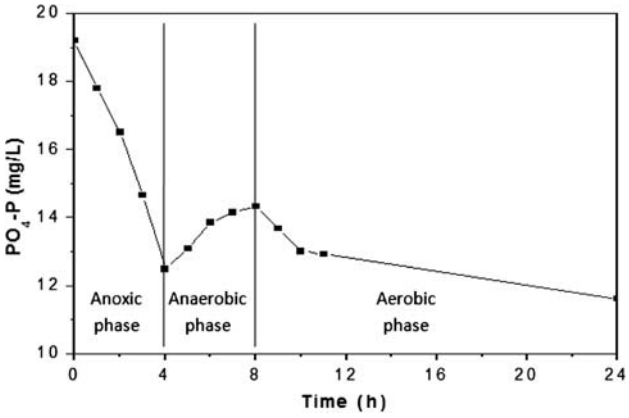

Fig. 3. Profile changes in phosphate concentrations during a sequence of phases (series 10).

addition of specialized bacteria to activated sludge [24].

- A low retention time of effluent in the anoxic phase. This time may therefore be insufficient for the micro-organisms to maintain anoxic conditions that would allow denitrification (nitrates are known for their inhibitory role of stress). The NO_3–N concentrations in the influent were very important. It is suggested that the phosphorus release started after the reduction of nitrate to a certain level. A lower amount of phosphorus release was observed at high nitrate concentrations.
- A low anaerobic contact period, which will ensure anaerobic conditions on the fixed biomass. According to Baljic and Leduc [12], the mass balance relating to phosphorus (P) indicated that long anaerobic phases (6 h) were more efficient than short ones (3 h), as a selector of EBPR bacteria in biofilms. In both the comparisons, the specific mass of P released in 6 h period represents 50% more than the amount of P release in the shorter period (3 h) [12].
- A competition between Phosphorus accumulating bacteria (PAO) and non-PAO species such as Glycogen accumulating bacteria (GAO). The VFAs can be stored by GAO, but their presence inhibits the phosphorus release. Indeed, the GAOs store VFA as well as PAOs, but no release is observed. Their activity is limited because competition between PAOs and GAOs is in favor of PAOs at pH = 8.2 [29]. In the present study, the average pH value in the anaerobic phase was 6.34—it is less than 8—and the dominance of PAOs is not confirmed. Microbiological analysis is needed to determine the specific micro-organisms involved in the release during the anaerobic phase.

Moreover, PHAs accumulation due to short chain fatty acids (SCFA) utilization is a key factor for phosphorus release, because substrate diffusion and the hydrolysis of complex substrate to SCFA are not easy in the biofilm system. During the anaerobic phase, the poly-P accumulating organisms (PAOs) incorporate a large amount of SCFA and store them mainly as poly-b-hydroxybutyrates, whereas the phosphorus contained in the biomass is released as soluble phosphate. Phosphate uptake from solution by microbial biomass occurs under aerobic conditions. During the aerobic phase, luxury P-uptake occurs [13].

In other words, the biofilm could quickly adsorb the substrate on the biofilm surface, but the absorbed substrate is not capable of being transformed rapidly to PHAs. As far as the biological activity is concerned, PHAs formation would be restricted by the PAOs because, it is difficult for intrinsic biofilm to be exposed to alternating anaerobic and aerobic conditions.

Bioreactor 2: A phosphorus removal of 8.9% during the early phase and 48.94% during the late phase on average was obtained. Phosphate uptake from the influent by microbial biomass occurs under aerobic conditions. Phosphate uptake has been shown to take place by consumption of oxidized nitrogen instead of DO as an electron acceptor.

As the nitrates concentrations are so high in dairy wastewaters, there is a competition between the PAOs and non-PAOs species, especially nitrifying bacteria.

It is important to follow this column by another operating in anoxic conditions in order to reduce the nitrates in the effluent. Nitrates are as damaging as phosphates since both of them induce eutrophication.

The best phosphorus removals (58.2 and 48.94%, respectively for bioreactors 1 and 2 on average) were obtained during the late phase under steady-state conditions.

3.2. Phosphorus removal kinetics

The phosphorus removal by heterogeneous micro-organisms in the reactors can be determined, thanks to the phosphorus removal according to the phosphate concentration as shown in Fig. 4(a).

Several models have been used to describe the overall kinetics of biological reaction. Among the most widely used models in the literature are the first-order model and the modified Stover–Kincannon kinetic model [30,31]. These models were used for modeling studies and experimental data analysis of the fixed-film systems operated in this study and which are described as follows:

3.2.1. First order model

The rate of change in phosphates concentration considering the first-order degradation kinetics can be expressed as:

$$\frac{ds}{dt} = Q\frac{(S_i - S_e)}{V} - K_1 S_i \quad (2)$$

Under steady-state conditions, the rate of change in phosphate concentration (ds/dt) is negligible, Eq. (2) reduces to

$$Q\frac{(S_i - S_e)}{V} = K_1 S_i \quad (3)$$

The value of the first-order kinetic constant can be obtained by plotting $Q \times (S_i - S_e)/V$ vs. S_i according to Eq. (3). The value of k_1 is obtained from the slop of the straight line.

3.2.2. Stover–Kincannon kinetic model

Among the most widely used models in the literature [30,31] for the development of phosphates kinetics degradation in fixed-film systems, the modified Stover–Kincannon model is described as follows: (Eqs. (4) and (5), Eq. (4) results from a simple modification of Stover–Kincannon model proposed for rotating biological contactor systems.)

The phosphate concentration (S) dependence can be expressed as:

$$\frac{ds}{dt} = \frac{U_{max}(\frac{QS_0}{V})}{K_B + (\frac{QS_0}{V})} \quad (4)$$

Eq. (5) is obtained by linearization of Eq. (4),

$$\frac{V}{Q(S_i - S_e)} = \frac{K_B}{U_{max}} \cdot \frac{V}{QS_i} + \frac{1}{U_{max}} \quad (5)$$

where:
ds/dt = phosphorus removal rate (mgP/L.d),
S = reactor phosphorus concentration (mg/L),
U_{max} = maximum removal rate constant (mg/L.d),
K_B = saturation value constant (mg/L.d).

3.3. Kinetic parameters determination

The determination of kinetic parameters was carried out using kinetic model in its linear form (Eq. (3) for first-order model and Eq. (5) for the Stover–Kincannon model). The highest value of the linear correlation coefficient (r^2) from the experimental data from each one of the assessed model allowed us to identify the most suitable kinetic model, as well as the kinetic parameters associated with that model.

3.3.1. First order model

A graphic representation of experimental data was made according to linearized form of first-order model (Eq. (3)). The first-order degradation constant value k_1 was obtained from the slope of the straight line and were 0.36, 0.13 d^{-1} approximately, with linear correlation values r^2 of 0.84 and 0.73 for bioreactors 1 and 2, respectively as shown in Fig. 4(a).

3.3.2. Stover–Kincannon kinetic model

If $(ds/dt)^{-1}$ is taken as V/Q $(S_i - S_e)$, which is the inverse of the loading removal rate and this is plotted against the inverse of the total loading rate (V/QS_i), a straight line portion of intercept rate $1/U_{max}$ and a slope of k_B/U_{max} are resulted. This plot is shown in Fig. 4(b) from which k_B and U_{max} can be estimated as 6.194 and 6.896 (mg/L.d) for bioreactor 1; 1.379 and 2.245 (mg/L.d) for bioreactor 2, respectively.

The regression line had a linear correlation value r^2 of 0.985 for bioreactor 1 and 0.775 for bioreactor 2, confirming the applicability of Eq. (5) for the bioreactor 1 but not for the bioreactor 2. The U_{max} and k_B values obtained in Fig. 4(b) can be used to determine the volume required to decrease the influent phosphorus concentration from S_i to S_e or to determine the effluent phosphorus concentration from a given V and S_i.

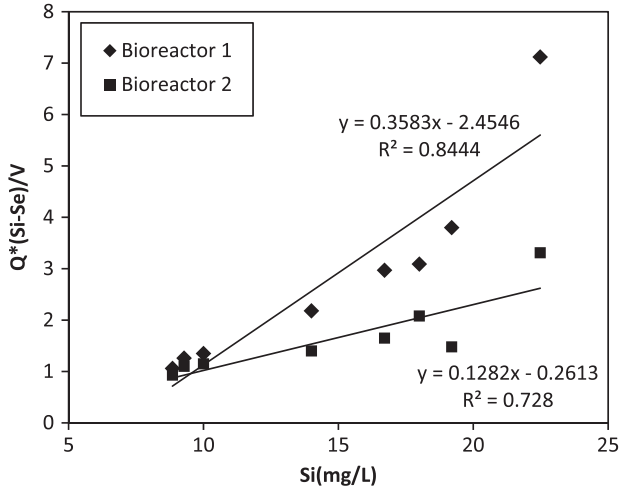

Fig. 4(a). Graphic representation of a first-order kinetic model.

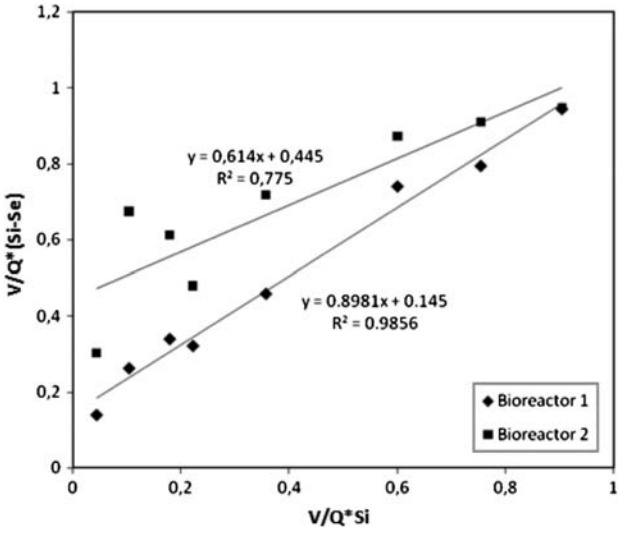

Fig. 4(b). Graphic representation of the Stover–Kincannon kinetic model for both reactors.

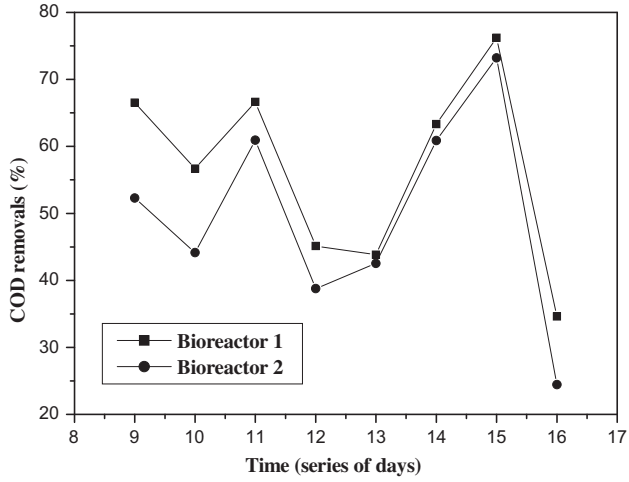

Fig. 5. COD removals according to time during the late phase for both bioreactors.

Kinetic models assessment. The correlation coefficient r^2 was chosen as a criterion for choosing the most suitable model to represent phosphorus removal kinetics in the bioreactors tested in this study. Considering this criterion, the Stover–Kincannon model was more suitable than the first-order one, having r^2 coefficient values of 0.99, 0.77, respectively for bioreactors 1 and 2 for Stover–Kincannnon model and 0.84; 0.73 for bioreactors 1 and 2, respectively for first-order model.

Furthermore, for the model of the first order, the obtained right should pass by the origin according to the model. For our case, the obtained right does not pass by the origin, this allows us to assert that the application of this model is not appropriate.

3.4. Relationship between COD and P removal

The average total influent COD of 579.83 mg/L was reduced by 56.60% in bioreactor 1 and 49.70% in bioreactor 2, resulting in an average COD in the effluent of 259.50 mg/L in bioreactor 1 and 295.40 mg/L in bioreactor 2 (Fig. 5).

The removal of nitrogen and phosphorus is closely related to the characteristics of biodegradable organic pollution which is divided into a readily biodegradable fraction and a fermentable fraction. VFAs are including 10% of COD, so the fermentable fraction constitutes 10% of COD.

According to Schneider and Topalova [24] biochemically, the anaerobic biodegradation of OM takes place through reduction of final acceptors of electrons; that is, nitrate (in denitrification), sulfates (in sulfate reduction), carbon dioxide (in methanogenesis), organic molecules (in fermentations), and so on. The heterogenic biofilm structure and the presence of microhabitats of diverse conditions (including a redox gradient and various microzones of different enzyme activities, organics, and nutrient concentrations) in it enable simultaneous development of these processes. Their realization is also determined by the occurrence in the biofilm and the activity of assorted physiological groups of micro-organisms whose metabolic pathways complement each other. The complex microbial structure of the biofilm allows, depending on the conditions (redox gradient, and concentrations of oxygen, carbon dioxide, nitrate, sulfates, and so on) in the bioreactor, one microbial group to become active while another physiological group is resting. For example, under aerobic conditions, anaerobic bacteria are present but resting. These micro-organisms become active and dominate functionally in the structure of biofilm when oxygen is depleted.

Bioreactor 1: organic loading in the anaerobic phase was an essential condition for phosphorus release.

In anaerobic conditions, the PAOs store directly VFAs as poly-ß-hydroxy-alkanoates (PHA). These simple molecules (VFAs) can be present in raw water or be produced in the anaerobic phase by acidogenic fermentation of other carbon compounds [32,33]. However, the stoichiometry of release (most often measured for acetate) which links the amount of C stored and released phosphate (Y_{PO4}) is highly variable. Smolders et al. [34] explain this variability by strong influence of pH. Indeed, the transport of VFAs across the cell membrane requires an amount of energy that depends on the pH gradient across this membrane.

The more the external pH is important, the more the required energy increases, this leads to high intake

of phosphates; the following equation was then proposed by [34]:

$$Y_{PO4}\left(\frac{\text{mol}P}{\text{mol}C}\right) = 0.19\,\text{pH} - 0.85 \quad (6)$$

At pH near neutrality, the observed yields are generally from 0.4 to 0.5 g PO_4–P/g COD. During this study, the average pH value in the anaerobic phase was 6.5 leading to a ratio $Y_{PO4} = 0.385$ (molP/molC), this value is in accordance with the values found by other researchers (Table 4).

Furthermore, in dairy wastewaters, COD_{VFA}/P ratios can vary as low as from 60:1 to 13:1. The literature has reported effective P removal at COD: P ratios between 7:1 and 10:1 using domestic strength wastewater [1]. BPR of high strength dairy wastewaters has been reported [1,10] with the study of Comeau et al. [10] being most successful.

The study of Broughton et al. [1] indicated that a complete P removal with a COD/P ratio of 13/1 may be achievable. This is just below the minimum ratio that is reported to occur in dairy processing wastewaters suggesting that BPR may be a viable treatment option for this type of waste stream. It should be remembered, however, that synthetic wastewater was used in their work; for full-scale biological nutrient removal, the consumption of carbon by competing organisms would have to be considered.

In this study, a P removal of 58.2% on average was obtained with a COD_{VFA}: P ratio of about 16:1. The low P removal obtained during this study is due to the fact that it was conducted with real dairy wastewater. In trials using dairy processing wastewater, Comeau et al. [10] reported relatively low 7.6% cell phosphorus content; this low net cell is perhaps to be expected as their system, which was fed with real wastewater, may have included a greater proportion of non-PAO species. These non-PAO species would be expected to have a lower P-content than the PAOs.

Bioreactor 2: the biological P removal is parallel to the degradation of COD. Thus, a low P removal (48.94% on average) induced a low COD removal (49.65% in average) resulting in an average concentration of 295.45 mg/L. This concentration is well above the norm in the effluent.

Table 4
Comparison of phosphate release stoichiometry in anaerobic conditions in the literature with that obtained in this study [32]

Authors	mg COD/ mg PO_4–P	Y_{PO_4} (mg PO_4–P/mg COD)
Mino et al. (1987)	4.10	0.24
Tasli et al. (1987)	1.42	0.70
Isaac and Henze (1995)	2.10	0.47
Wentzel et al. (1985)	2.00	0.50
Abu Ghararah and Randall (1991)	2.70	0.37
Satoh et al. (1996)	1.40–2.88	0.34–0.71
Smolders et al. (1994)	2.17	0.46
Carlsson et al. (1996)	2.50–2.80	0.35–0.40
Present research study	3.76	0.385

3.5. Nitrogen

The aeration in the bioreactor 2 created conditions advantageous to nitrification resulting in 31% reduction of the influent NH_4–N concentration and high NO_3–N production.

The highest levels of NO_3–N production occurred on the bioreactor 2 (continuously aerated) in which DO concentration was ranged from 2–3 mg O_2/L.

The conversion of NH_4–N in both bioreactors was done by nitrification resulting in NO_3–N production.

The detrimental effect of oxidized nitrogen on anaerobic phosphate release and overall phosphorus removal is well known [10,26,27]. Indeed, with nitrates or nitrites present, all available substrates could be consumed for denitrification instead of being stored by poly-P bacteria.

The amount of nitrates in the raw water was very important; it was on average 37.07 mg/L, this value is compatible with the quantities usually encountered in waste water from dairies [35,36].

In anoxic phase, there should normally be found a decrease in nitrates concentrations, which was not found in the relevant case, since the nitrate concentration during this phase was 38.7 mg/L on average. This can be explained by:

- An anoxic retention time insufficient to ensure effective denitrification.
- Competition between the denitrifiers and PAOs for organic substrate. It has been reported that one of the possible reasons for the reduction of P release by nitrate was simultaneous phosphorus uptake by phosphate accumulating denitrifying bacteria [25].

In the anaerobic phase, the average nitrates concentration was about 3.025 mg/L.

Many studies [26,37] mention the disruptive role of nitrates in EBPR process.

Moreover, some acceptable nitrates values in the anaerobic zone according to the COD of the effluent have been proposed [38]:

4.5 mg/L NO_3–N for 300 mg/L COD
2.0 mg/L NO_3–N for 160 mg/L COD
1.0 mg/L NO_3–N for 140 mg/L COD

This led for NO_3–N/COD ratios of about 0.015, 0.0125, and 0.007, respectively. Comparatively, the obtained result was 3.025 mg/L for 212.41 mg/L of COD on average, leading to a ratio equal to 0.014, this result is consistent with the one quoted above [38].

The concentration of nitrates increases in aerobic phase reaching an average value of 37.08 mg/L. This evolution is due to nitrification which is conditioned by concentration of DO (3 mg O_2/L).

Bioreactor 2: the permanent aeration created conditions advantageous to nitrification that result in 31% reduction of the influent NH_4–N concentration and high NO_3–N production (39.7 mg/L on average).

4. Conclusion

The results obtained in this study lead to the following conclusions:

- The fixed-biofilm systems with continuous feeding developed in this study have shown to be able to remove phosphorus and OM from dairy plant wastewaters despite the partial release observed in the anaerobic phase for the bioreactor 1.
- BPR could be successfully applied to dairy wastewater treatment.
- The alternation of phases is a key factor for the biological removal of COD and phosphates.
- The main results showed that the effectiveness of COD and phosphates for bioreactor 1 reached, respectively to 66% and 91%, at stable biofilm functioning. Similarly, the effectiveness of COD and phosphates removal for bioreactor 2 reached to 70 and 84.61%, respectively.
- The phosphorus loading removal rate was compared with predictions from a modified Stover–Kincannon model, the first-order model and a regression relationship. The modified Stover–Kincannon model seemed to be the best model to describe the phosphorus loading removal rate of the bioreactors tested in this study which treat dairy plant wastewater. The phosphorus removal kinetics was found to be dependent on the applied substrate loading rate. The maximum utilization rate constant (U_{max}) and saturation value constant (K_B) in this model were calculated as 6.896 and 6.194 (mg/L.d) for bioreactor 1; 2.245 and 1.379 (mg/L.d) for bioreactor 2, respectively.
- However, we consider that this study contributes to the understanding of EBPR of dairy wastewater in the biofilm processes with continuous feeding, but the assertions made require further investigations, especially microbiological analyses to determine the bacterial species involved in this processing.

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