

*Full Length Research Paper*

# Biological phosphorus removal from dairy wastewater by alternating anaerobic and aerobic conditions

O. Balamane-Zizi\* and H. Aït-Amar

Laboratory of Industrial Process Engineering, Faculty of Mechanical Process and Process Engineering, University of Sciences and Technology Houari Boumediene, BP32, 16111 El Alia, Bab-Ezzouar, Algiers, Algeria.

Accepted 27 April, 2012

In this study, the possibility of applying the enhanced biological phosphorus removal (EBPR) process for Algiers dairy wastewater which can have phosphorus contents up to 130 mg/L was examined. EBPR is conventionally performed by an anaerobic-aerobic process. The objectives of this work were to determine an optimal hydraulic retention time (HRT) in aerobic conditions and to study the effect of short chain fatty acids (SCFA) (acetic and propionic acids) addition on the phosphorus release in anaerobic conditions. The tests were performed in a batch reactor operating with an aerobic/anaerobic sequence of phases. Batch tests have been carried out at 3 HRTs in aerobic conditions (1, 2 and 3 h) while the anaerobic retention time was fixed at 4 h, to examine the effect of stress related to changes of aerobic HRT. Main results show that the most favorable aerobic retention time was found to be 2 h. The amount of P released in anaerobic phase increases from 2.25 to 2.48 mgP/gVSS with increasing aerobic HRT from 1 to 2 h and decreases to 1.28 mgP/g VSS for a time of 3 h using acetic acid. Similarly, this amount increases from 1.62 to 4.38 mgP/gVSS for 1 to 2 h and decreases to 1.41 mgP/gVSS for a time of 3 h using propionic acid. The initial release rate was directly proportional to the amount of added substrate. Propionate may be a more effective carbon source for biological phosphorus removal than acetate. Based on the results presented herein, we can confirm the possibility of phosphorus removal from dairy wastewater in the aerobic-anaerobic biological process.

**Key words:** Dairy wastewater, biological phosphorus removal, aerobic, anaerobic, release, acetic acid, propionic acid.

## INTRODUCTION

Biological phosphorus removal is dependent upon the uptake of phosphorus in excess of normal bacterial metabolic requirements and is proposed as an alternative to chemical treatment. The removal of phosphorus from urban and industrial wastewater is the key factor for the prevention of eutrophication, which can affect surface water. Three disposal methods may then be implemented; the chemical precipitation by addition of reagents; this can be categorized into chemical precipitation with lime and with metal salts such as iron

and aluminum salts; both methods remove phosphorus by forming insoluble phosphoric compounds and then precipitating them (Mulkerrins et al., 2004). Biological treatment or biological phosphorus removal (luxury uptake) involves phosphorus uptake by bacteria that exceeds the 2.3% phosphorus by weight which typical of conventional wastewater treatment system biomass and a combination of both (a mixed treatment).

Biological treatment has however received increased attention in the last decade. The biological phosphorus removal is based on the alternation of anaerobic-aerobic phases. Under anaerobic conditions, the polyphosphate accumulating organisms (PAOs) are capable of storing organic compounds especially short chain fatty acids such as acetic and propionic acids as internal storage compounds (Liu et al., 2007). Under subsequent aerobic conditions, the previously stored carbon is used for biomass growth and poly-P formation (Li et al., 2007);

\*Corresponding author. E-mail: zizouafia@yahoo.fr or haitamar@usthb.dz.

**Abbreviations:** EBPR, Enhanced biological phosphorus removal; SCFA, short chain fatty acids; HRT, hydraulic retention time.

excellent phosphorus removal efficiency can be achieved by this process (Aguado et al., 2006; Oehmen et al., 2006). Thus, it seems that a higher amount of phosphorus (P) is then taken under aerobic conditions (Carvalho et al., 2007). Two main conditions are necessary for effective and efficacious phosphorus elimination. They include:

1. The exposure of the biomass in alternating anaerobic/aerobic conditions.
2. The presence of simple organic substrates (VFAs) especially propionic and acetic acids during the anaerobic phase (Baljic-zelovic and Leduc, 2002).

The biological removal of phosphorus results therefore has two main phenomena:

1. The storage of organic matter with release of phosphorus from the biomass in anaerobic conditions.
2. The accumulation of phosphorus by the biomass, with aerobic metabolism of organic matter previously stored.

It was also established that there is a proportional relationship between the quantity of organic substrate taken up in anaerobic conditions and the quantity of released phosphate, but this relationship depends on certain parameters, among others, the residence time of substrate in anaerobic period (Wouters-Wasiak, 1994). However, the anaerobic residence time remains controversial. The anaerobic phase should enable the consumption of the oxygen and nitrates. One group of authors (Baljic and Leduc, 2002; Boisson, 2000; Tizghadam, 2007; Maharaj, 2001) recommend a long anaerobic residence time to maximize the release of phosphates. However, another group (Barnard, 1984; Pitman, 1991) recommends short residence time to avoid excessive release without organic matter uptake, which seems unfavorable for biological phosphorus removal efficiency. This time depends on the particular characteristics of the effluent, the quantity of organic matter, dissolved oxygen levels and biodegradability of the substrate (Maharaj, 2001; Nittamil, 2011; Schneider and Topalova, 2011). In general, we consider durations between 3 to 6 h. For our part, an anaerobic retention time of 4 h was set.

However, in the literature, most studies on enhanced biological phosphorus removal (EBPR) were conducted with anaerobic-aerobic phases (Carvalho et al., 2007; Chiou and Yang, 2008; Li and al, 2008). It seemed very important to see if the change of phases had an influence on the biological phosphorus removal efficiency. In this study, we were particularly interested to optimize the aerobic phase duration. During the aerobic phase, the aeration should not only provide sufficient dissolved oxygen (DO), but should also enhance the mass transfer and the diffusion to ensure a good aeration, the DO concentration was set at 3.5 mgO<sub>2</sub>/L. In summary, the purpose of this study was to find the optimum aeration

time which allows the biological phosphates reduction in order to obtain a good release in the anaerobic phase, after which we became interested in the phase when the time was set. It seems, therefore, that the control of parameters involved in each phase (aerobic/anaerobic) would maximize their effectiveness and thus improve the efficiency of biological phosphorus removal. In this work, we were interested in studying some parameters including:

1. The aerated residence time required for a maximal P uptake aerobically and then an optimal release in anaerobic conditions.
2. The concentration of carbon substrates with short chain molecules (acetic and propionic acids).
3. The correlation between the amount of organic matter consumed and the amount of phosphorus releases.

## MATERIALS AND METHODS

### Operating conditions

Batch tests was carried out at different aerobic residence times to study the effect of stress related to changes of aerobic residence time, influence of both carbon substrate addition and initial release rate on biological phosphorus removal process. The batch tests were run in a 2 L Erlenmeyer flask working with an aerobic-anaerobic sequence of phases. The reactor was seeded with sludge from a wastewater treatment plant in Algiers and operated under aerobic-anaerobic conditions with a cycle time of 5, 6 and 7 h. Each cycle consisted of 1, 2 or 3 h aerobic period and 4 h anaerobic period. 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 biological removal (Schneider and Topalova, 2011).

The water and sludge were mixed in the Erlenmeyer flask. As soon as the totality of both liquids came into contact, agitation started and the first sample was taken at time 0. The concentration in PO<sub>4</sub>-P and chemical oxygen demand (COD) was immediately measured. Agitation was constant and slow, using a magnetic agitator set on minimum (excessively fast agitation causes the transfer of oxygen by the sludge in anaerobic conditions). The aeration was made by a fine bubble aquarium diffuser placed at the bottom of the receptacle (the DO concentration was set at 4 mgO<sub>2</sub>/L). The reactor was operated as follows; an initial aerobic phase of 1, 2 and 3 h, with the aim to study the importance of aeration time on the biological phosphate uptake, followed by a 4 h anaerobic phase. At the end of the aerobic periods, a sample was taken and analyzed in order to determine the concentration in PO<sub>4</sub>-P and COD, the aeration was then totally stopped to allow the installation of the anaerobic conditions. The flask opening was capped by a sheet of parafilm, to create the anaerobic conditions necessary for the phosphates release. Samples were taken at regular time intervals (each 30 min) and filtered; the evolution of the concentration in PO<sub>4</sub>-P in the interstitial liquid of mixture enabled the determination of the quantity released by the sludge and the release rate in the anaerobic phase. COD was measured again.

The phosphates and COD concentrations were measured in each collected sample according to a colorimetry method by HACH DR 2010 spectrophotometer (AFNOR standards, Rodier, 1996). The dissolved oxygen concentration and pH were measured thanks

to a laboratory oxymeter HI 2400 Hanna and pH meter IC 3510 Jenway respectively.

### Characteristics of raw water

The dairy wastewater to be treated contain a  $\text{PO}_4\text{-P}$  of 17.7 mg/L,  $\text{NH}_3\text{-N}$  of 14.5 mg/L,  $\text{NO}_3\text{-N}$  of 25 mg/L in average a pH ranged from 4.2 to 8.3, a biochemical oxygen demand (BOD) of 2500 mg/L a COD of 4000 mg/L this allows a COD/BOD ratio of 1.6. The biological treatment is then possible. The dissolved oxygen concentration and pH were measured thanks to a laboratory oxymeter HI 2400 Hanna and pH meter IC 3510 Jenway respectively. Dairy wastewater contain high amount of organic matter. According to (Schneider and Topalova, 2011), COD of dairy waste effluents from full-scale operations varies between 500 and 9200 mg/L or 3800 mg/L on average.

The phosphates and COD concentrations were measured in each collected sample according to a colorimetric method by HACH DR 2010 spectrophotometer (AFNOR standards, Rodier, 1996). The dissolved oxygen concentration and pH were measured. To demonstrate the importance of both the aeration time previously required for phosphorus uptake under aerobic conditions, on the phosphorus release under anaerobic conditions and the presence of carbon substrates with short molecular chain, two sets of batch tests were accomplished.

### Effect of aeration time

According to several authors (Wouters-Wasiak, 1994; Nittami and al, 2011; Comeau and al, 1986; Kelly and Gibbs, 1989; Schneider and Topalova, 2011) in non-aeration period, the enzymatic system is disturbed; the microorganisms try to maintain balance by releasing the phosphorus initially stored during aeration in the presence of COD. This alternation of phases provides good efficiency of phosphorus removal. Through these tests, the importance of aeration time necessary for optimal phosphorus uptake, and thus a better release is shown. For this, the aeration times were varied from 1 to 3 h and the amount of phosphorus released during each time was calculated.

### Influence of carbon substrate addition

From this study, it is suggested that the effect of acetic and propionic acid addition on the phosphorus release in anaerobic conditions is accomplished through three tests. With raw wastewater (without acetic and propionic acids addition) and with the addition of acetic and propionic acids at various initial concentrations. Initial acetic and propionic acids concentrations varied from 50 to 200 mg/L. This choice is based on the fact that PAOs use directly the simple carbon molecules mainly VFAs such as acetate and propionate. These molecules can be produced in the anaerobic phase by acidogenic fermentation of other compounds (Carvalho and al, 2007; Randall, 2002; Chiou and Yang, 2008; Rodgers, 2010) which can be initially present in the wastewater. This is not the case for water used in this study because dairy wastewaters contains butyric and caproic acids as soluble volatile fatty acids but not acetic or propionic acids (Mokrani, 1995). According to Imbeault (1997), initially, dairy wastewaters are characterized by an absence of VFAs.

In this case, the pre-aeration time was set at 2 h after the reactor is covered tightly to create the anaerobic conditions necessary for the phosphorus release. Samples taken at regular time intervals (every 30 min), were used to follow the evolution of both phosphates and COD concentrations. Figures 3 and 4 show that

the quantities of phosphate released per gram volatile suspended solids (VSS) according to the time, follow an exponential speed with a maximum obtained after 4 h of anaerobic reaction, using acetic and propionic acids. The choice of this residence time is related to the results obtained by other researchers (Li and Chen, 2007) which should be able to ensure adequate phosphorus removal at any time. Indeed, if the wastewater has good characteristics, the anaerobic residence time may be short, between 0.5 to 1 h (Wouters-Wasiak, 1994). If the water characteristics are not optimal, especially if the concentration of organic matter directly available for the PAOs is low, this time must be increased in order to allow the hydrolysis of simple carbon compounds. Residence time of 4 h is in this case recommended (Bourdon, 2005).

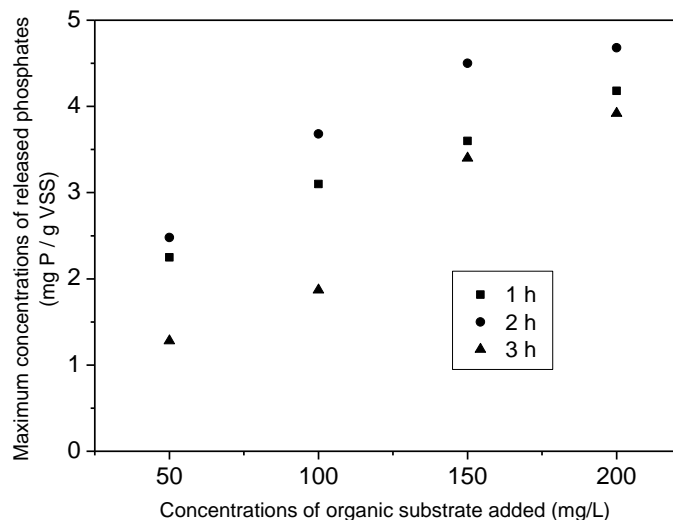
## RESULTS AND DISCUSSION

### Effect of aeration time

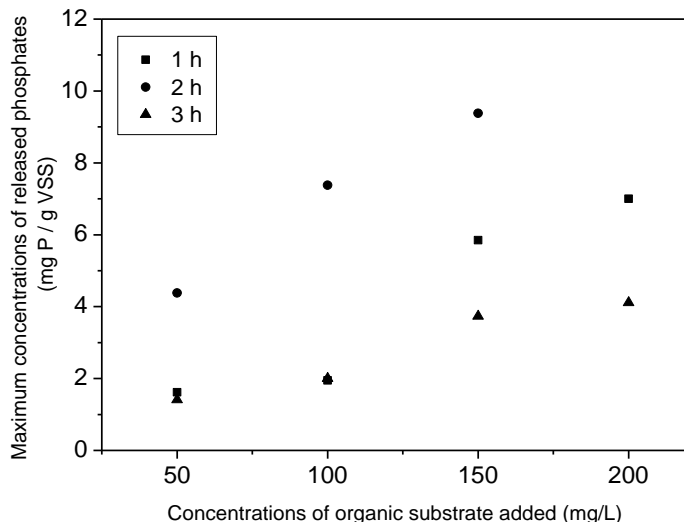
The results shown in Figures 1 and 2 indicate that an aeration time of 2 h is optimal. Indeed, for the same concentration in substrate (50 mg/L), the concentration limit in P released is 2.25 mgP/g VSS for an aeration time of 1 h, 2.48 mgP/VSSg for 2 h and 1.28 mgP/gVSS for 3 h using acetic acid. Similarly, it is 1.62 mgP/gVSS for 1 h, 4.38 mgP/gVSS for 2 h and 1.41 mgP/gVSS for 3 h using propionic acid. Following these tests, we consider that, an aeration time of 2 h during the aerobic phase is sufficient for an optimal release during the anaerobic phase. During the aerobic phase, phosphates assimilation occurs at the same time as the organic matter degradation.

More specifically, there is phosphorus accumulation by the biomass in suspension with the metabolism of the organic matter that is present in the water. During the anaerobic phase, phosphate removing microorganisms use the intracellular reserves (the initially stocked phosphates) as source of energy and store the simple organic substrate in polymeric form, poly-hydroxyalkanoates (PHA). At the same time, the phosphorus that is present in the biomass is released in the water (Baljic and Leduc, 2002).

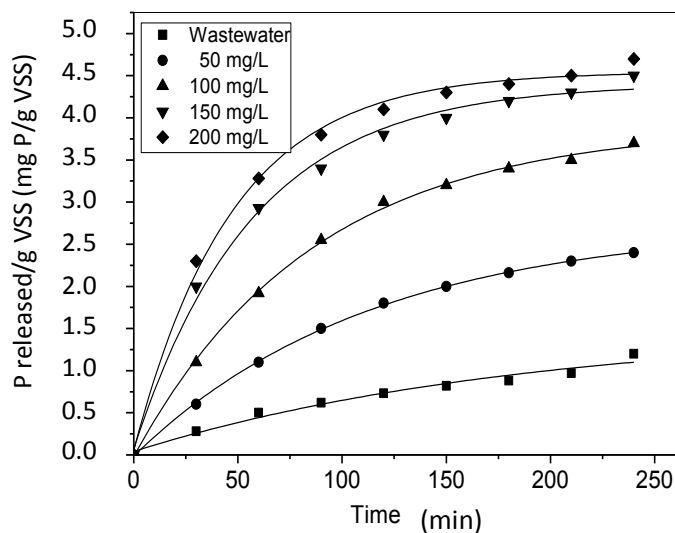
The degradation of the organic matter is proportional to the aeration time. That is why 3 h aeration time allows a better degradation of the easily assimilated organic matter while lowering the quantity of short-chain fatty acids (SCFA) that are necessary for good release during the anaerobic phase compared to 1 and 2 h time for a same quantity of microorganisms. Besides, there is no biogas production during the anaerobic phase because the pH values obtained during that phase vary between 4.3 and 4.6 and show that the methanogenesis phase (phase that is necessary for the production of methane) was not reached. It is inhibited for  $\text{pH} < 7$ . There is, therefore, a proliferation of acidogenic bacteria to the detriment of the other groups, leading thus to the acidogenesis. The methanogenic bacteria tolerate pHs that is close to neutrality. The acclimatization helps to overcome this inhibiting effect. Since our tests were performed in batch, the acclimatization is neglected.



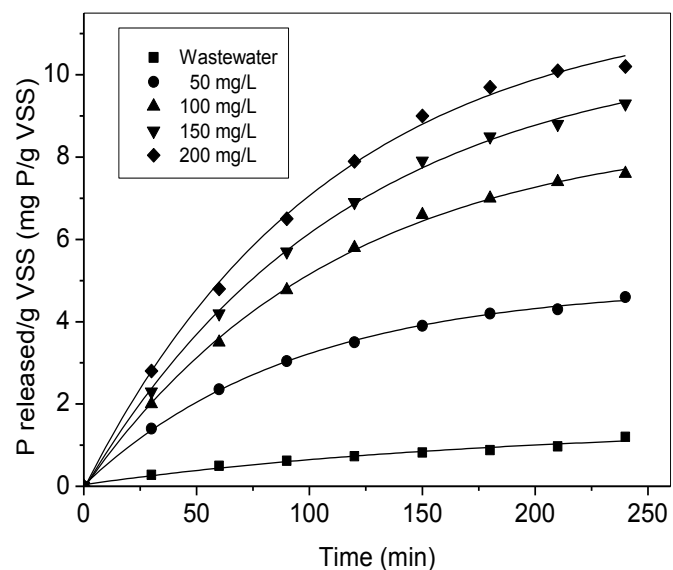
**Figure 1.** Maximum concentrations of released phosphates versus concentrations of organic substrate added using acetic acid.



**Figure 2.** Maximum concentrations of released phosphates versus concentrations of organic substrate added using propionic acid.



**Figure 3.** Phosphorus released kinetics with acetic acid.



**Figure 4.** Phosphorus released kinetics with propionic acid.

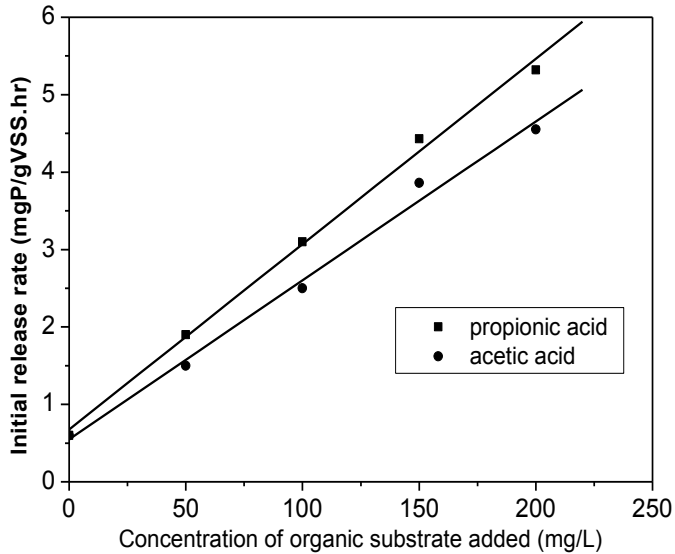
### Influence of carbon substrate addition

Maximum concentrations of P released were determined graphically by extrapolation from the curves representing the evolution of released phosphate versus time (Figures 3 and 4). Results show that:

1. The maximum released phosphorus obtained with addition of an organic substrate is greater than with the raw wastewater. Indeed, it is 1.10 mgP/gVSS for raw water and varies from 2.48 to 4.68 mgP/gVSS and from 4.38 to 10.30 mgP/gVSS for acetic and propionic acids, respectively. This confirms the fact that the presence of carbon substrates with short molecular chain enhances

the release and that there is a link between their presence and the release in the anaerobic phase. The effects of different influent carbon sources on BPR activity has been studied with maximum rates of anaerobic phosphate release being achieved with acetate and propionate, with decreased rates being observed with lactate, succinate, malate and pyruvate (Mulkerrins et al., 2004).

2. Propionic acid release is more significant than the release of acetic acid. For the same initial concentration (200 mg/L) of substrate, 4.68 and 10.30 mgP/gVSS were respectively released for acetic and propionic acids. The concentration of dissolved oxygen in the aerobic phase is



**Figure 5.** Initial release rate according to several concentrations of organic substrate.

about 3.5 mgO<sub>2</sub>/L. These results are in agreement with literature studies (Liu et al., 2007; Broughton et al., 2008; Pijuan et al., 2004; Yuan et al., 2010) who reported that propionate is the most favourable organic substrate for the biological phosphorus removal compared to acetate. Whenever the concentration of dissolved oxygen is high (5.9 to 7.8 mg/L) or low (0.15 to 1.45 mg/L) with a ratio of COD/P in a range of 13/1, the biological phosphorus removal performance is about 97%.

3. The quantity of P released varies proportionally with the quantity of added substrate. An increase in the acetic and propionic acids concentrations from 50 to 200 mg/L, leads to P released concentrations of between 2.48 to 4.68 mgP/gVSS and 4.38 to 10.30 mgP/gVSS, respectively. According to Morgenroth and Wilderer (1998) cited by Baljic and Leduc op.cit., an increase in the influent acetate concentration to 400 mg/L led to an efficient anaerobic P release (followed by an improved P removal). However, a further increase in the influent acetate concentration to above 600 mg/L led to both the cessation of release in the anaerobic phase and the ability of phosphorus removal.

4. Acetate and propionate are good carbon sources for P removal during short term experiments, whereas propionate is the least efficient VFA.

### Influence of the initial release rate

The initial release rates were determined from Figures 3 and 4. From the results shown in Figure 5, we proposed to find a correlation between these rates and concentration of substrate used. The curves in Figure 5 (evolution of the initial release rate depending on the

concentration of both substrates) are straight lines ( $r^2 \geq 0.99$ ), and do not pass by origin, they are represented by Equations 1 and 2.

$$V_i = 0.0205 C + 0.55 \text{ acetic acid} \quad (1)$$

$$V_i = 0.0239 C + 0.68 \text{ propionic acid} \quad (2)$$

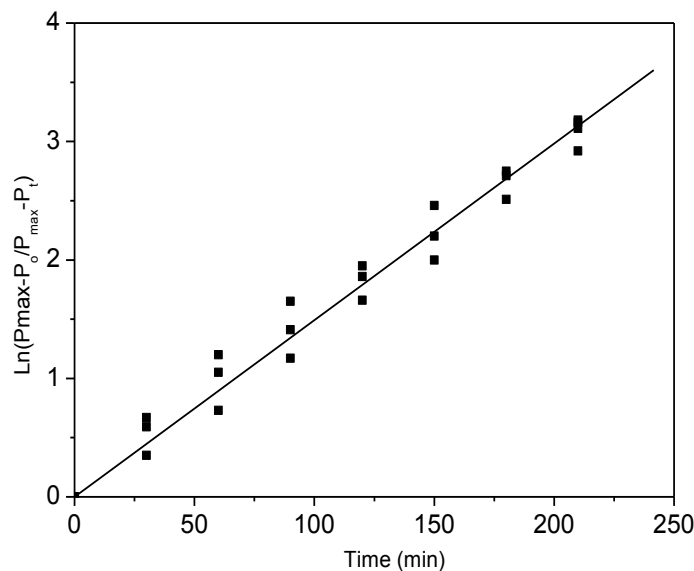
Equations 1 and 2 indicate that there is still a release, even if no substrate is added in the middle, according to (Liu et al., 2007), the release occurs without the presence of external carbon substrates. This could be due to the fact that during anaerobic conditions, which lasted 4 h, there is production of acetate and propionate by PAOs. The presence of both of them can potentially provide competitive advantages to PAOs. Increasing the acetate and propionate fraction in wastewater by adding them as a supplementary carbon source may potentially be useful for improving the reliability and efficiency of biological phosphorus removal systems. It becomes obvious that obtaining a quick release requires the use of significant amounts of acetic and propionic acid because the abundance of organic matter in wastewater has a positive effect on the biological phosphorus removal.

According to Wouters-Wasiak.op.cit, the release reaction rates vary between 0.8 to 9 mgP/gVSS.h. For our part, the experimental obtained values were between 0.55 to 4.9 mgP/gVSS.h and 0.6 to 5.3 mgP/gVSS, respectively for acetic and propionic acids. Kang et al. (1991) estimated that the release rate above 2.4 mgP/gVSS.h during the first hour of anaerobic conditions is favourable for biological phosphorus removal. The initial release rate obtained with both acids was substantially equal, although the maximum amount of released P is more important for propionic acid. According to Randall (2002) and Chiou et al., (2008), the initial fast release results of the easily biodegradable organic matter absorption, such as acetate and propionate, were mainly carbon substrates used by the PAOs. This is also confirmed by the constants of kinetics obtained from the model proposed by Wentzel and Dold (1985).

### Mathematical expression of release kinetics

We proposed here the release rate of P under anaerobic conditions by applying the model proposed by Wentzel. op. cit. (Equation 3). This model has been applied by Wouters-wasiak.op.cit in the case of wastewater treatment, including discharges from paper mills. This gives a highly biodegradable effluent with a medium ratio COD/BOD<sub>5</sub> of 1.7 and a ratio COD/P of 55. These ratios are very favourable for biological phosphorus removal.

$$P_t = P_{\max} \left( 1 - \frac{P_{\max} - P_0}{P_{\max}} e^{-kt} \right) \quad (3)$$



**Figure 6.** Determination of the kinetic constant using acetic acid.

This relation can also be given as:

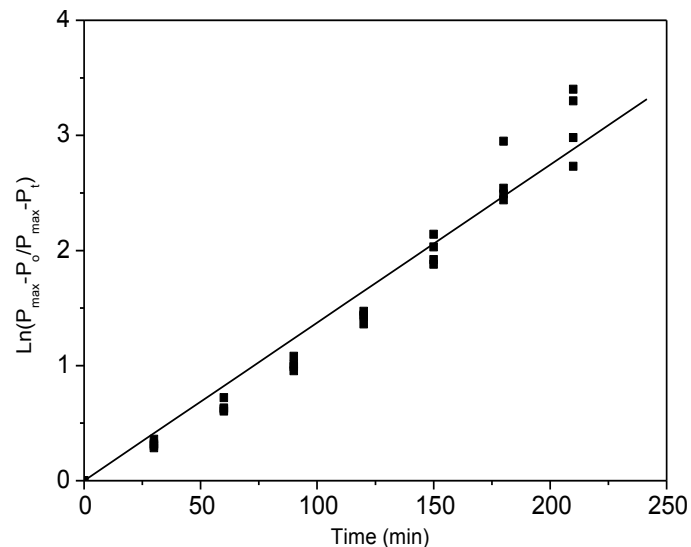
$$\ln\left(1 - \frac{P_{\max} - P_0}{P_{\max} - P_t}\right) = Kt \quad (4)$$

Where,  $P_t$  = the phosphorus concentration at time  $t$ ,  $P_0$  = the initial phosphorus concentration,  $P_{\max}$  = the maximal potential phosphorus concentration,  $P_{\max} - P_0$  = the maximum potential release of phosphorus possible,  $K$  = first order rate constant.

The model fits perfectly to our case, since the water discharge of dairies chosen for this study accept a medium ratio COD/BOD<sub>5</sub> of 1.6, reflecting the biodegradability character of the wastewater, and a ratio COD/P of 485.5, this ratio is much higher than that found by Wouters-Wasiak. *Op.cit.* because dairy discharges contain important quantities of organic matter (COD in a range of 4025 mg/L on average) (Gonçalves and Rogalla, 2000; Corthondo, 2004). The experimental results deduced from Figures 6 and 7 are 0.0136 and 0.0146 min<sup>-1</sup>, respectively, for propionic and acetic acids, these are in accordance with those obtained by Wentzel. *op. cit.* which gets a rate constant between 0.01 and 0.02 min<sup>-1</sup>. Furthermore, the graphical analysis given by the diagram shows that the parity data points are aligned around the diagonal, this allows us to validate the applied model and assert that the release rate constant was determined with an appreciable accuracy.

## Conclusion

The results obtained in this study, lead to the following



**Figure 7.** Determination of the kinetic constant using propionic acid.

conclusions:

1. Biological phosphorus removal could be successfully applied to dairy wastewaters treatment.
2. Aerobic P uptake occurred in the presence of acetate and propionate.
3. SCFA utilisation is a key factor for phosphorus release. Acetate and propionate are good carbon sources for P removal during short experiments, whereas propionate was the most efficient VFA.
4. A significant quantity of phosphorus is released in extracellular medium during anaerobic period, this release is important in using an organic substrate.
5. The initial phosphorus release rate is more important than the concentration of added organic substrate.
6. There is release of phosphorus even if no substrate is added to the medium; this indicates that there is production of volatile fatty acids by microorganisms during the anaerobic phase.

Furthermore, it was also interesting to highlight the influence of aeration time for a better biological assimilation of phosphorus and subsequently, a more important release. The obtained results showed that an aeration time of 2 h was optimal. It is not necessary to aerate for long periods. Beyond 2 h, the organic matter becomes a limiting factor during short experiments (batch tests).

Finally, we concluded that alternating aerobic-anaerobic phases is primordial for enhanced biological phosphorus removal.

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