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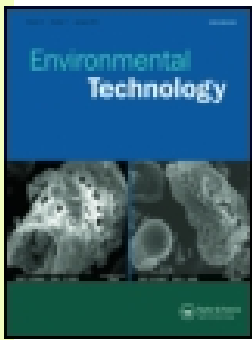
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**SEQUENCING BIOLOGICAL ACIDIFICATION OF WASTE ACTIVATED SLUDGE  
AIMING TO OPTIMIZE PHOSPHORUS DISSOLUTION AND RECOVERY**

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## Abstract

Phosphorus (P) recovery in wastewater treatment plants (WWTP) as pure crystals such as struvite ( $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ ), potassium struvite ( $\text{KMgPO}_4 \cdot 6\text{H}_2\text{O}$ ) and calcium phosphates (e.g.  $\text{Ca}_3(\text{PO}_4)_2$ ) is an already feasible technique that permits the production of green and marketable fertilizers and the reduction of operational costs. Commercial crystallizers can recovery more than 90% of soluble P. However, most of the P in WWTP sludge is unavailable for the processes (not dissolved). P solubilization and separation are thus the limiting steps in P-crystallization. With an innovative two-step sequencing acidification strategy, the current study has aimed to improve biological P solubilization on waste activated sludge (WAS) from a full-scale plant. In the first step (P-release), low charges of organic waste were used as co-substrates of WAS pre-fermentation, seeking to produce volatile fatty acids to feed the P-release by Polyphosphate-accumulating organisms (PAO), while keeping its optimal metabolic pH (6 to 7). In this phase, milk serum, WWTP grease, urban organic waste and collective restaurant waste were individually applied as co-substrates. In the second step (P-dissolution), pH 4 was aimed as it allows the dissolution of the most common precipitated species of P. Biological acidification was performed by white sugar addition, as a carbohydrate-rich organic waste model, which was compared to chemical acidification by HCl (12M) addition. With short retention times (48-96 hours) and without inoculum application, all experiences succeeded on P solubilization (37-55% of soluble P), principally when carbohydrate-rich co-substrates were applied. Concentrations from 270 to 450 mg  $\text{P-PO}_4^{3-} \cdot \text{L}^{-1}$  were achieved.

**Keywords:** phosphorus solubilization, nutrient recovery, waste activated sludge, WAS fermentation, organic waste.

## Introduction

Phosphorus (P) is the key element of life [1, 2]. According to Gowariker, Krishnamurthy [3], P is an essential part of nucleoproteins that control cells division and nucleic acids molecules. Acelas, Flórez [4] highlight that there is no substitute for P, a fundamental element to all living organisms and an essential nutrient in food production.

During the past centuries, we have changed radically our impact in the P cycle: we have entered in a linear consumption logic in which large amounts of P are mined from specific phosphate reserves and transported to crops all over the world [5]. Different scientists and producers associations estimate the lifetime of phosphate rock reserves by being from 50 to 270 years [6, 7]. It is a similar scenery to oil reserves ending, but P cannot be replaced in a situation of scarcity and growing prices [5].

The issue of P in sanitary engineering started after it was identified as the main responsible for eutrophication in water bodies [5]. Initially, P was removed by simple chemical precipitation and later with more complex processes as the Enhanced Biological Phosphorus Removal (EBPR) [8]. More often, EBPR is complemented by chemical precipitation to reach the legal limits [9].

However, chemical precipitation, yet the most used technique, increases sludge production, its toxicity and its disposal costs [10]. EBPR also produces large volumes of sludge and most of the biologically concentrated P is released again under anaerobic conditions [11]. It is the case of anaerobic digestion (AD), known as the most used process for sludge stabilization. Thereby, in wastewater treatment plants (WWTP) with EBPR processes, the dewatering liquors of digested sludge are highly concentrated in nutrients and can contribute to up to 20% of P and N affluent load when returned to the plant's head [12]. The AD of sludge results in ammonium, magnesium, potassium and phosphate release thus crystals such as struvite (magnesium ammonium phosphate hexahydrate,  $MgNH_4PO_4 \cdot 6H_2O$ ) can be spontaneously formed inside the digesters, pipes and other equipment [13]. However, the crystallization processes can be controlled to become a source of revenues to the operators of WWTP. Struvite, for instance, is a great fertilizer with several environmental advantages [10].

Currently, most of the P crystallization processes are located after the anaerobic digestion of sludge. Commercial struvite crystallizers, for example, allow above 90% of soluble P recovery rates [10]. These processes prevent nutrients return to the head of the treatment plant, but they are not efficient to prevent maintenance issues induced by P crystallization inside the anaerobic reactor or in equipment downstream. Consequently, process optimization opportunities are mostly focused now on increasing the amount of soluble P to increase P recycling rate before anaerobic digestion.

Chemical acidification or waste activated sludge (WAS) pre-fermentation have been tested to solubilize P either by dissolving the mineral forms or by releasing the P from the Polyphosphate-Accumulating Organisms (PAO) [6, 14, 15, 16, 17]. In that case, a separation step is performed between the P-dissolution stage and the digester. The liquid phase enters the P-recycling process and the sludge is sent to the anaerobic digester. Pre-fermentation results in pH reduction, P and ammonium release [18]. Since low pH avoids precipitation and so allows keeping the released P in solution, the lower the pH, the greater the amount of P dissolved.

Our previous works showed that depending on the charge and the biochemical composition of the co-substrates used for the pre-fermentation, it was possible to biologically decrease the pH down to 3 under controlled conditions. However, in sludge from WWTP combining EBPR and chemical process for P removal, P is mainly intracellular in PAO and we observed that P dissolution was low because P release by the PAO was inhibited when pH was lower than 5 [19]. Excessively low pH increases the pH gradient between the internal and external parts of cell membranes and thus decreases the efficiency in substrate transporting inside the cell, resulting in a smaller quantity of released P by absorbed substrate, as described by Deronzier and Choubert [20]. On the other hand, P released by the PAO was precipitated as calcium or iron phosphates when the pH was above 5. So, the parameters to optimize P-release from PAO, among which neutral pH, tend to readily precipitate the P with available cations as iron, aluminum or calcium depending on the process used for phosphorus removal in the WWTP.

We have recently presented a fermentation batch test that can be easily applied to assess and predict biologically releasable P [19]. However, we have noticed that biological P solubilization could be theoretically optimized by separating intracellular P-release and P dissolution in a two-step process. The P-release step needs an anaerobic condition, volatile fatty acids availability and has an optimal pH in the neutral range, while the P dissolution step relies in the pH reduction, which can be achieved by chemical dosing or by biological fermentation. This paper presents the first experiments of this concept of sequencing acidification. In the proposed process, a weak WAS pre-fermentation is performed with low charges of organic co-substrates and is sequentially complemented with a pH drop induced either by the dosage of a strong acid or biologically with white sugar application to stimulate acidogenesis, simulating carbohydrate-rich organic waste.

## Materials and methods

### WAS samples

The WAS sludge used for the tests was obtained from a WWTP treating 360000 population equivalent. It was sampled after thickening by a belt press. Secondary treatment in the WWTP consists of oxidation ditches designed to achieve Enhanced Biological Phosphorus Removal (EBPR).  $\text{FeCl}_3$  is applied as complementary P treatment to ensure the legislation limits. Table 1 shows WAS characteristics for each test at the moment of experience launching (after storage at 4°C).

Raw samples analysis (thickened WAS) showed that the WWTP was operating on stationary state, once the main parameters were always found to be stable (pH, total solids, volatile solids, total phosphorus, etc.) compared to several sampling that we had done before.

### Analysis

Total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN) and chemical oxygen demand (COD), were measured with standard methods [21]. After an acidic mineralization (110°C, 1 bar, 60 min), total phosphorus (TP) was analyzed by the ascorbic acid method using an automate spectrophotometry (Gallery, Thermo Scientific, method 984366).

The ionic composition of the supernatants was measured after centrifugation (4°C, 20 000g) and filtration on 0.45  $\mu\text{m}$  polypropylene membrane with both anion ( $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ) and cation ( $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ) chromatography. A Metrohm 940 Professional Vario IC was equipped with a Metrosep A sup 5 anionic column and a Metrosep C4 -250/4,0 column for cations.

Total dissolved iron ( $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ ) was measured by an automate spectrophotometry (Gallery, Thermo Scientific, method 984326).

### Co-substrates

The co-substrates were selected to match the local reality of Brittany (France): milk serum, WWTP grease, urban organic waste and collective restaurant waste were individually used as co-substrates. Urban organic waste, collective restaurant waste, milk serum and WWTP grease were stored at -20 °C. All solid co-substrates were cryogenized and triturated to form a homogeneous mixture. The co-substrates were characterized in total COD (TCOD), TP, carbohydrate, lipid and protein contents. The results of co-substrates characterization are shown in Table 2.

### Chemical acidification

Chemical acidification tests were run to estimate the fraction of P released by chemical mechanism during biological acidification. Hydrochloric acid (12 M) was used as acid.

Dilutions caused by the acid dosage were neglected. pH was maintained stable for 15 min at each pH value under continuous stirring (250 RPM) and then lowered from 1 unit till reaching pH 2. Acidified waste activated sludge was sampled at every pH unit. Soluble P ( $\text{P-PO}_4^{3-}$ ) and total dissolved iron ( $\text{Fe}^{2+} + \text{Fe}^{3+}$ ) concentrations were determined in the supernatant.  $\text{P-PO}_4^{3-}$  dissolution was found to be described by a polynomial function, which was used to estimate the contribution of chemical dissolution during the biological process as described by Braak, Auby [19]. The iron dissolution curve was described by a polynomial function.

## Biological acidification tests

The reactors used on sludge fermentation tests were Erlenmeyer flasks with a total volume of 1280 ml, filled with 640 ml during tests. The reactors were placed on heating plates with magnetic stirring. The reaction temperature was set to 35 °C, which corresponds to a mesophilic condition, such as most of the anaerobic digesters in France [22]. A thermally insulated box was used as a cover to promote a homogeneous temperature.

The reactors were closed with a rubber septum, which allowed the insertion of needles for pressure measurement, gas collection or injection, as well as reagents dosage. In every experience start or flask opening, the atmosphere was inerted with nitrogen gas for at least 20 minutes ( $1 \text{ mm} \cdot \text{min}^{-1}$ ). Glass taps were adapted in the reactor's bottom in order to allow sampling without opening the reactors. The internal pressure generated by the gas production during fermentation ensured that no oxygen was reintroduced during sampling. Overpressure was daily removed with a needle through the rubber septum to avoid vial burst. Repeatability was assessed prior to the operation of these experiments by triplicates with sugar at loads of  $2\text{-}5 \text{ gCOD} \cdot \text{gVS}^{-1}$  and provided more than 95 % repeatability on pH and soluble P. Consequently, the tests were not normally duplicated.

## Sequencing acidification

The principle of the sequencing acidification is presented in Figure 1. The first step, in which P-release takes place, was performed in reactors as described above. In this phase, volatile fatty acids (VFAs) must be produced to enhance intracellular P-release by PAO. However, the co-substrate charge must be limited to avoid an excessive pH reduction and the consequent decrease in the efficiency of PAO's metabolism. Previous works showed that the ratio of  $0.2 \text{ gCOD} \cdot \text{gVS}^{-1}$  brings enough carbon for P release, keeping the pH above 6 [23]. A control test without co-substrate was performed in the same conditions. After 48h, a second P dissolution step was performed, where pH was reduced to dissolve the released P which could be precipitated with cations and other previous non-soluble forms. Acidification was performed biologically by the addition of sucrose (white sugar) as a carbohydrate-rich co-substrate model ( $0.5 \text{ gCOD} \cdot \text{gVS}^{-1}$  during 48 h) and by chemical dosage (HCl 12M). Two strategies of chemical acidification were evaluated only in the control test; sample acidification to pH 4 (R2) and reactor acidification to pH 4 with reaction continuation (R1).

Excluding iron phosphates, pH 4 is sufficient to prevent readily precipitation of released P with amorphous calcium phosphate and struvite, the most common forms according to previous experiences. Stronger acidifications could induce operational problems such as excessive chemical needs on pH increasing to allow struvite crystallization, methanogenesis inhibition and inefficiency on dewatering processes.

The experiment planning is summarized in Table 3.

## Results and discussion

### Chemical acidification

Results of the chemical acidification test are presented in Figure 2, in terms of soluble P and total soluble iron as function of pH.

Soluble P presented a second order polynomial tendency with a maximum value of 11,2% of soluble P at pH 3.4. This result was consistent if compared with previous publications [19] in which around 15% of soluble P was found at pH 2 with non-stored WAS from the same WWTP. Total soluble iron also presented a second order polynomial tendency with a maximum measured value of 105 mg.L<sup>-1</sup> at pH 2.

### pH and P evolution

After 48h of P-release step, it was observed a slightly lower pH with lactoserum as co-substrate (5.6) and similar values in the other tests: 6.1 to control and WWTP grease and 5.9 to the restaurant and urban organic wastes (Figure 3).

A greater difference is observed on soluble P, which represents 19% of the total P for the control test and about 30% with organic co-substrates application (Figure 4). The higher total P value due to the higher P supply caused by lactoserum application (Table 2) may partially explain the greater soluble P amount in this test, but not the difference between the other tests and the control.

Equation 1 (presented on Figure 2) was used to estimate the P amount dissolved exclusively by the pH decrease. Apparent biologically released P was calculated (equation 2) by subtracting the amount of soluble P due to pH variation from the measured soluble P value. As the re-precipitated P is the difference between actual and apparently released P, the P fraction actually released by the PAO (equation 3) was estimated by subtracting the amount of P according to equation 1 from the soluble P value obtained with the chemical acidification to pH 4 just after the P-release step. Actual released P ranges from 36% of total P for control and restaurant waste to 44% with lactoserum. It is 38% and 41% for urban organic waste and WWTP grease respectively (Figure 5). The readily biodegradable COD provided by the lactoserum (a carbohydrate-rich co-substrate) would thus increase by 22% the P-release by PAO. This effect and the amount of soluble P provided by this effluent may explain the observed difference. However for restaurant and organic urban wastes, biological P-release is very close to the amount released during the control test. In this case, the higher amount of soluble P in the tests with co-substrates could be explained by the lower precipitation of released P, which is related to the higher presence of colloidal and particulate organic matter due to the addition of organic waste. Several authors have shown that the presence of organic matter limited P precipitation in effluents [24]. For WWTP grease, these two phenomena may explain the observed difference.

$$[P-PO_4]_{cmq,d} = -3,9965 \times pH^2 + 24,942pH + 46,631 \quad (R^2=0.9997) \quad (1)$$

$$[P-PO_4]_{ap,bio,rel} = [P-PO_4] - [P-PO_4]_{cmq,d} \quad (2)$$

$$[P-PO_4]_{bio,rel} = [P-PO_4]_{pH4} - [P-PO_4]_{cmq,d} \quad (3)$$

Where  $[P-PO_4]_{cmq,d}$  is the theoretical soluble P concentration due only to pH variation,  $[P-PO_4]_{ap,bio,rel}$  is the apparent biological released P and  $[P-PO_4]_{bio,rel}$  is the actually theoretical released P, which estimates P precipitation.

After white sugar addition, a quick pH drop was observed from about 6.0 to 4.7 in all tests, but the goal of biological acidification was also pH 4. After that, it remained approximately stable until 96h of reaction (Figure 3). Following the acidification, soluble P concentrations ( $P-PO_4^{3-}$ ) increased and reached about 40% of the total P after 96h in all tests. A maximum of 52% soluble P was obtained in the control test with a retention time of 72h (Figure 4).

After P-release step, 24h of biological acidification (total retention time of 72h) contributed with more 6-10% (actual waste) and 33% (both control tests) of soluble P, but there was no



significant extra P solubilization with 48h of biological acidification (total of 96h). Soluble P results of biological acidification (sugar addition) were systematically lower than the results of chemical adjustment as sequencing acidification strategy (Figure 5). Biological acidification reached pH 4.7 and chemical acidification was performed to achieve pH 4. This pH difference may explain different soluble P concentrations since it opposes precipitation of P with available cations. Tests with higher charges of sugar ( $>0.5 \text{ gCOD.VS}^{-1}$ ) could be performed in order to achieve pH 4 on biological acidification and to compare results.

Regardless of the applied method, soluble P did not exceed 55%, what is significantly lower than the 75% value previously obtained by directly adding white sugar on  $0.5 \text{ gCOD.gVS}^{-1}$  organic charge at the beginning of the pre-fermentation with WAS from the same WWTP [19]. In the present tests, the smaller charges intentionally applied to limit pH decrease during P-release step has perhaps not provided the needed amount of VFA to properly enhance P-release by PAO. Unfortunately, the limited number of reactors has not allowed a test in those same conditions (white sugar addition on  $0.5 \text{ gCOD.gVS}^{-1}$  from the beginning), or with higher medium range initial charges (from 0.2 to  $0.5 \text{ gCOD.gVS}^{-1}$ ), with the exactly same WAS samples. Different P-release times could be tested as well. Furthermore, distinct behaviors depending on sludge sample characteristics and storage must not be ignored.

### Cations evolution

The co-substrates did not provide significant amounts of cations except lactoserum whose concentrations of soluble Ca and K ions are respectively  $1301$  and  $1500 \text{ mg.L}^{-1}$  and WWTP grease whose concentration on soluble Ca ion is  $368 \text{ mg.L}^{-1}$ .

Except to iron, it was found an increase in all cations concentrations during the pre-fermentation phase in which P-release is performed. Mg and K dissolution is related to the co-transportation of these cations during intracellular P-release by PAO as described by Barat, Montoya [25] (Figure 6).

Ammonium concentration increase was probably caused by nitrogen mineralization during co-substrates proteins degradation (Figure 6D). This could explain the higher pH compared to previous works with white sugar alone.

Ammonium and potassium salts are very soluble reaching high concentrations already after pre-fermentation; therefore, chemical acidification to pH 4 did not imply a significant increase in these values which were also stable during biological acidification induced by sugar addition.

Calcium ion concentration presented a slight increase during P-release step (pre-fermentation) what is coherent with demonstrations that Ca is not co-transported with P in PAO's metabolism [25]. In contrast, unlike the other cations, chemical acidification after pre-fermentation multiplied its concentration by about 2.5 (Figure 6). These results are consistent with the results of chemical-only acidification.

Dissolved iron concentrations ( $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ ) after chemical acidification of pre-fermented WAS were between  $400$  and  $500 \text{ mg.L}^{-1}$  (Figure 7). These values are greater than those obtained with biological sequencing acidification which does not exceed  $280 \text{ mg.L}^{-1}$  at pH 4.7.

Soluble iron concentrations were much greater with the application of sequencing acidification than with chemical acidification of WAS without pre-fermentation:  $102$ ,  $66$  and  $35 \text{ mg.L}^{-1}$  at pH 2, 3 and 5 respectively. This result confirms the assumptions made in previous works on the role of pre-fermentation metabolism that would turn iron into more sensible to acidification forms [19].

The biological role and the chemical complexity of iron on sewage treatment regarding P-recycling still poorly understood, but those are issues beginning to attract scientific interest [9].

## Struvite crystallization potential

This session will be dedicated to analyze struvite crystallization potential, since it stills the major industrial interest regarding full scale P-recovery through crystallization processes Desmidt, Ghyselbrecht [26]. Struvite ( $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ ) crystallization processes depend mainly on two operational factors: pH (alkaline) and Mg:N:P molar ratio (greater than 1:1:1) [27]. Magnesium ion is normally the limiting reagent and needs to be dosed [28].

P-rich side streams or process water with phosphorus concentrations greater than  $50 \text{ mg} \cdot \text{L}^{-1}$  can be considered economically feasible to struvite crystallization [29]. Concentrations from 147 to  $257 \text{ mg P-PO}_4^{3-} \cdot \text{L}^{-1}$  were achieved just after P-release step, from 362 to  $448 \text{ mg P-PO}_4^{3-} \cdot \text{L}^{-1}$  after chemical sequencing acidification and from 300 to  $432 \text{ mg P-PO}_4^{3-} \cdot \text{L}^{-1}$  after biological sequencing acidification.

Medium values of Mg:P, N:P, Mg:Ca and Ca:P molar ratios results from every test are summarized in Table 4. As expected, Mg:P is not sufficient, presenting results from 0.3 to 0.4. Mg:Ca ratios are low (0.4-0.6) and Ca:P ratios are close to 1. Mg should thus be dosed to reach  $\text{Mg:Ca} > 2$ , since Ca-P precipitations are prior to struvite and may partially [30] or even totally [4] inhibit struvite formation. Impurities as dicalcium phosphate dehydrate, octacalcium phosphate and hydroxyapatite are the most common calcium phosphates in aqueous solutions and should be expected [31].

N:P values are high ( $>2$ ), so it should not be a limiting factor on struvite crystallization, although N-recycling potential would be limited by soluble P availability.

## Conclusions and perspectives

Our previous works have shown that a strong acidification, induced by a large amount of easily biodegradable organic matter from the beginning of the pre-fermentation of WAS may reduce biological P-release by PAO. Therefore, we sought to dissociate P-release and P-dissolution by applying a two-step sequencing acidification strategy. This paper presented its first results and the main conclusions are shown below:

- Sequencing acidification with chemical dosage was more effective (about 50% of soluble P at pH 4) than biological sequencing acidification (about 40% at pH 4.7) but both presented better results than chemical-only acidification (maximum of 11% at pH 3.4).
- Biological acidification with the applied organic charge did not reach pH 4, which has been observed in previous work. The great amount of ammonium mineralized during the first 48 hours of P-release step may have increased the buffering capacity and may explain this effect.
- Sequencing acidification strategy showed to be less effective than the input of a larger amount of easily biodegradable COD at the beginning of the tests, as performed in previous works.
- The role of biological metabolism on iron dissolution was confirmed.

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Tables

**Table 1 – Characteristics of the WAS samples**

Stocking time (4°C)	~0
TS (g.kg <sup>-1</sup> )	27
TVS (g.kg <sup>-1</sup> )	19
TSS (g.kg <sup>-1</sup> )	25
VSS (g.kg <sup>-1</sup> )	18
TFS (g.kg <sup>-1</sup> )	8,1
pH	6,8
TP (g.kg <sup>-1</sup> )	0,8
N-NH <sub>4</sub> <sup>+</sup> (mg.L <sup>-1</sup> )	ND
Ca <sup>2+</sup> (mg.L <sup>-1</sup> )	32
Mg <sup>2+</sup> (mg.L <sup>-1</sup> )	11
K <sup>+</sup> (mg.L <sup>-1</sup> )	35
N-NO <sub>2</sub> <sup>-</sup> (mg.L <sup>-1</sup> )	0,6
N-NO <sub>3</sub> <sup>-</sup> (mg.L <sup>-1</sup> )	0,1
P-PO <sub>4</sub> <sup>3-</sup> (mg.L <sup>-1</sup> )	12

TS: total solids, TVS: total volatile solids, TSS: total suspended Solids, VSS: volatile suspended solids, TFS: total fixed solids. TP: total phosphorus.

**Table 2 - Co-substrates characteristics.**

	Milk serum	WWTP grease	Collective restaurant	Urban organic waste	White sugar
TCOD (g.kg <sup>-1</sup> )	64	123	315	336	1170
TP (g.kg <sup>-1</sup> )	0,6	0,2	0,4	1,4	NP
TKN (g.kg <sup>-1</sup> )	1,4	1,0	3,1	8,5	NP
N-NH <sub>4</sub> <sup>+</sup> (g.kg <sup>-1</sup> )	0,0	0,2	0,2	0,4	NP
Proteins content*	19,2%	5,8%	8,2%	21,5%	NP
Lipids content*	2,9%	48,8%	31,6%	47,4%	NP
carbohydrates content*	77,8%	45,4%	60,2%	31,1%	NP

**NP: not performed. \*expressed in COD/TCOD.**

**Table 3 – Experiment summary**

Reactor	Total P	Co-substrate	Load gCOD/gVS)	+48h	
				Acidified sample	Reactor intervention
R1	782	Control	0	to pH4;	Continuing at pH4 (HCl addition)
R2	782	Control	0	-	white sugar addition, 0.5 gTCOD/gTVS load
R3	786	Collective restaurant	0.2	to pH4	white sugar addition, 0.5 gTCOD/gTVS load
R4	796	Urban organic waste	0.2	to pH4	white sugar addition, 0.5 gTCOD/gTVS load
R5	833	Milk serum	0.2	to pH4	white sugar addition, 0.5 gTCOD/gTVS load
R6	765	WWTP grease	0.2	to pH4	white sugar addition, 0.5 gTCOD/gTVS load



**Table 4 – Medium values and standard deviations of relevant molar ratios at the final situations.**

	Mg:P	N:P	Mg:Ca	Ca:P
Chemical acidification (after 48h)	0.34±0.02	2.52±0.32	0.41±0.01	0.83±0.06
Biological acidification (after 72h)	0.37±0.03	2.69±0.41	0.41±0.02	0.92±0.09
Biological acidification (after 96h)	0.39±0.03	3.04±0.49	0.43±0.07	0.92±0.09

## Figure captions

Figure 1: Sequencing acidification strategy.

Figure 2: Soluble P and total soluble iron in chemical acidification test.

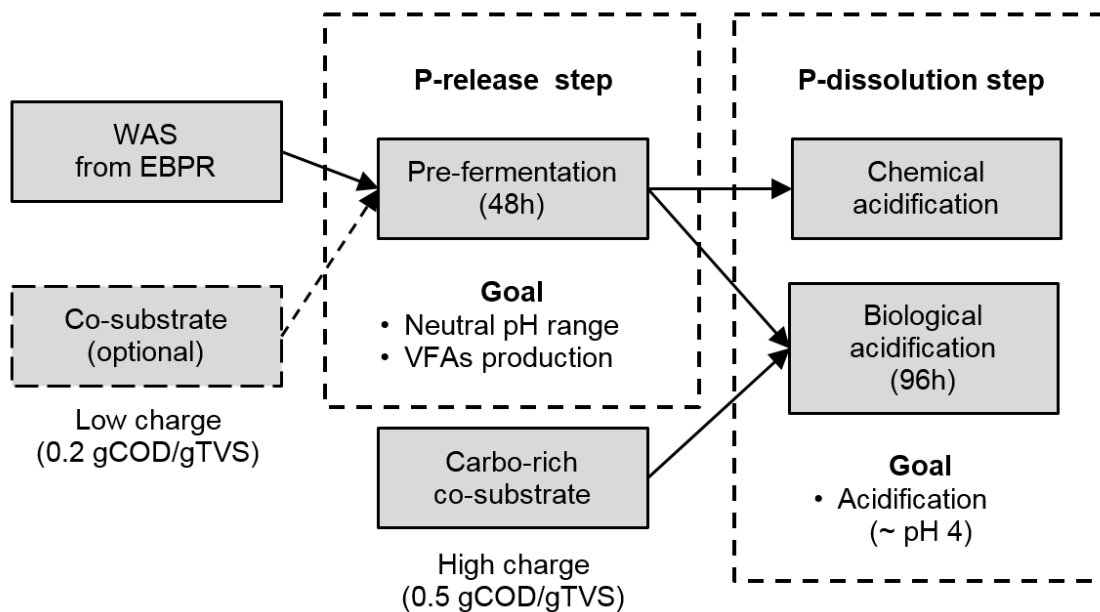
Figure 3: pH evolution.

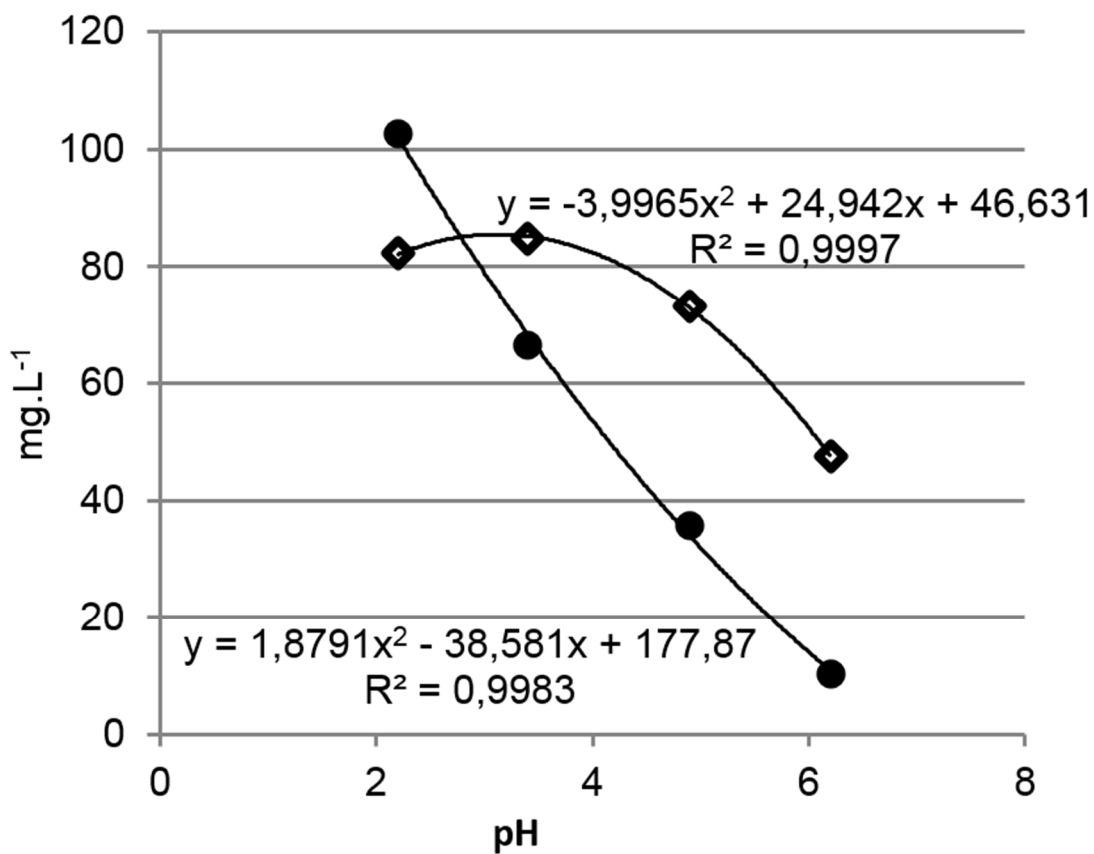
Figure 4: P-PO<sub>4</sub><sup>3-</sup> evolution.

Figure 5: estimated distribution of phosphorus with sequencing acidification.

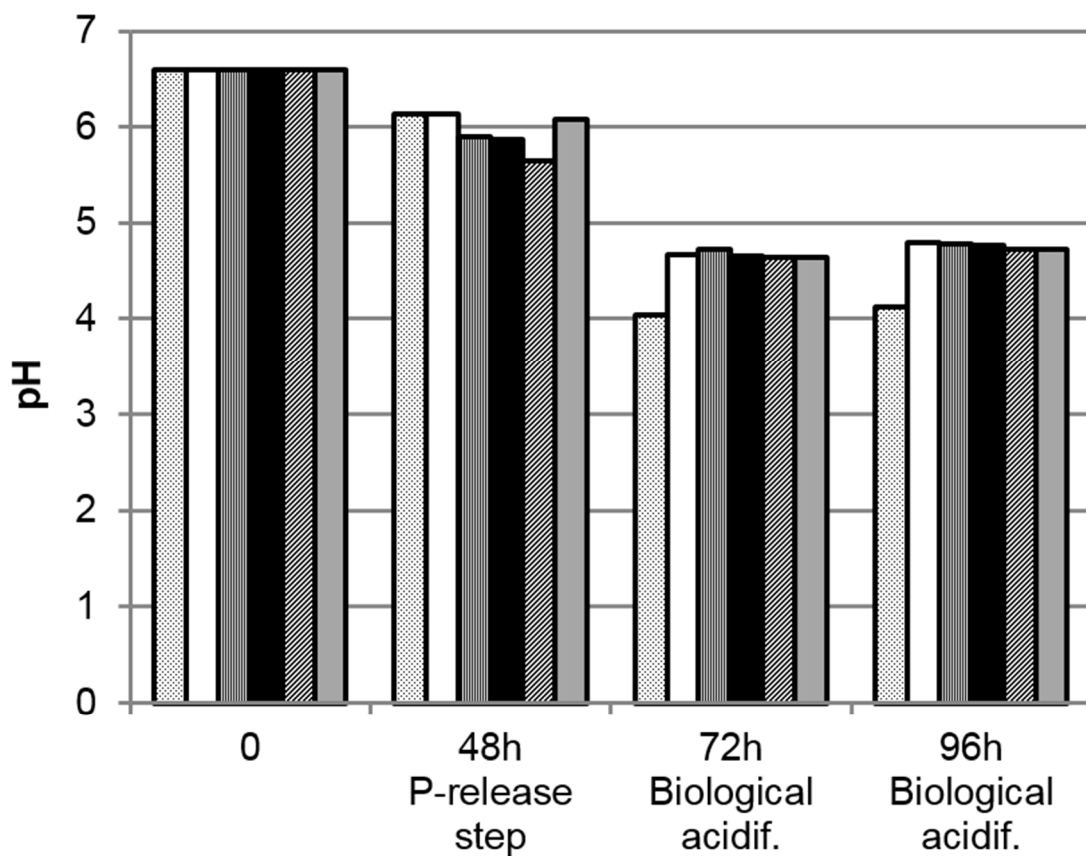
Figure 6: Evolution of calcium (A), magnesium (B), potassium (C) and ammonium (D) ion concentrations on sequencing acidification.

Figure 7: Evolution of soluble iron concentration on sequencing acidification.

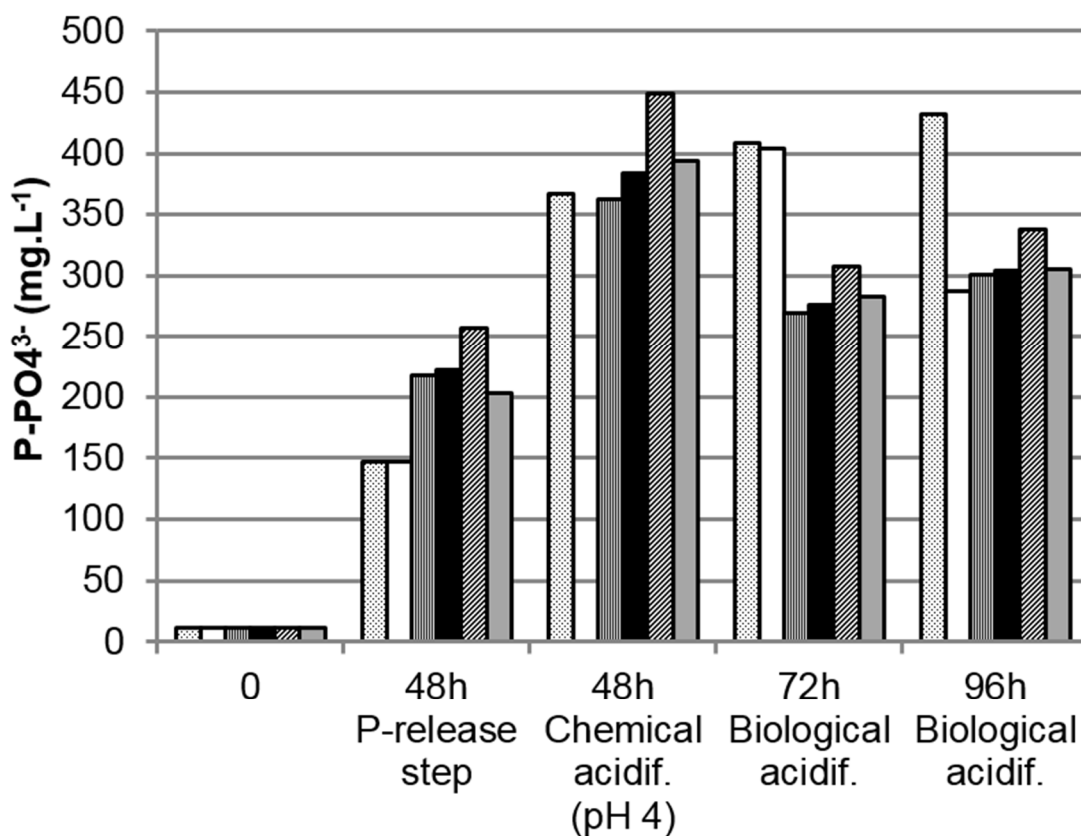




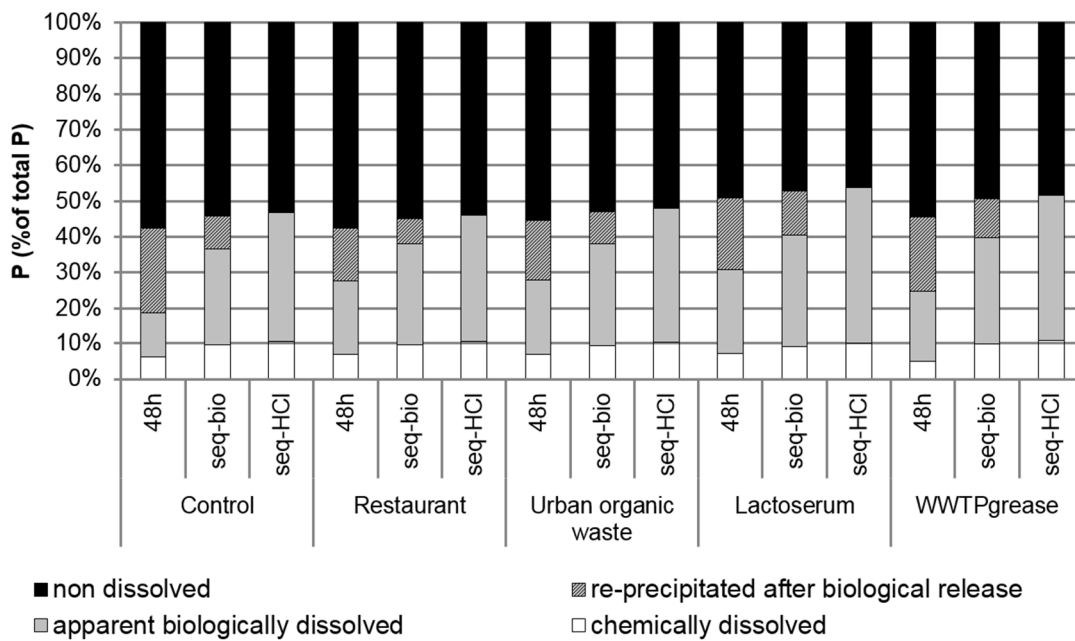
◆ Orthophosphates      ● Total dissolved iron

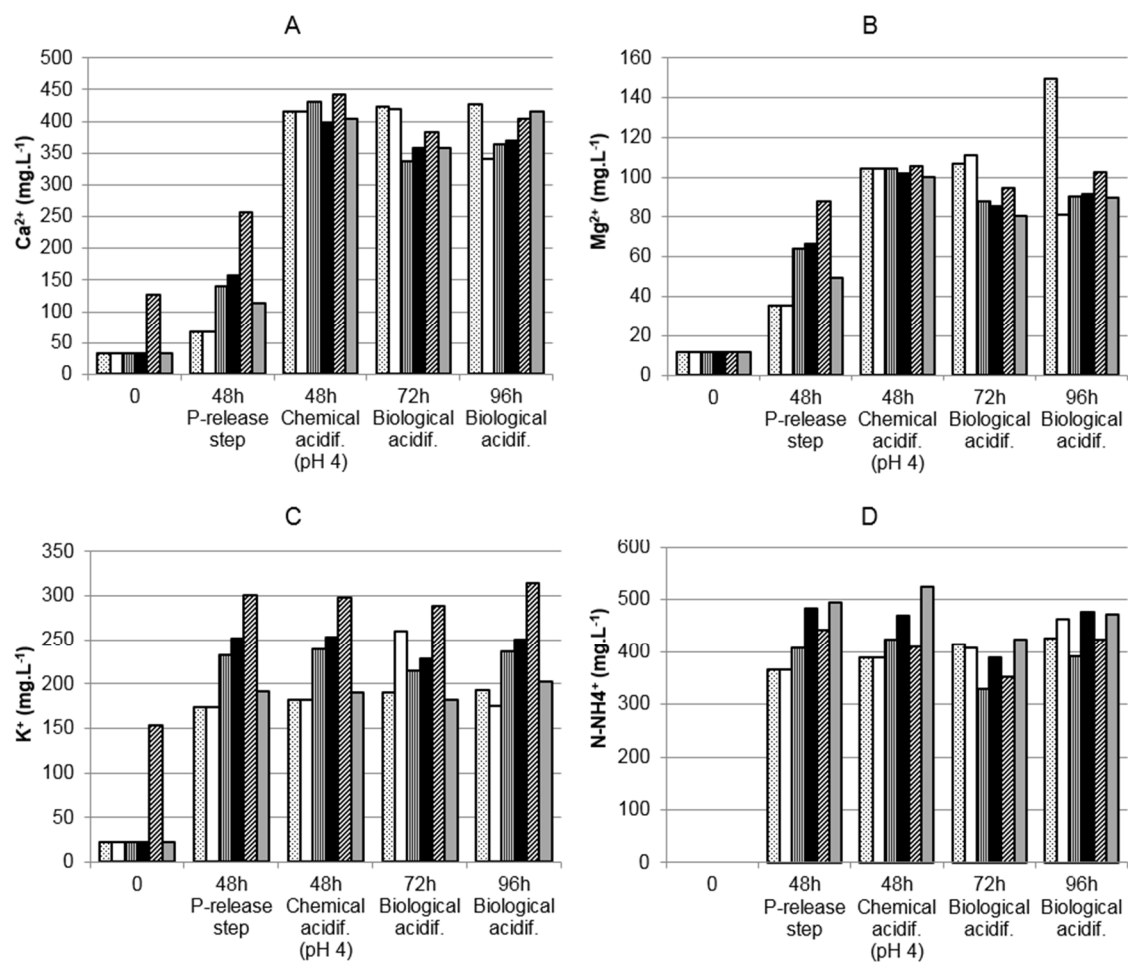


- R1 Control+HCl
- R2 Control
- R3 Restaurant
- R4 Urban organic waste
- R5 Lactoserum
- R6 WWTP grease

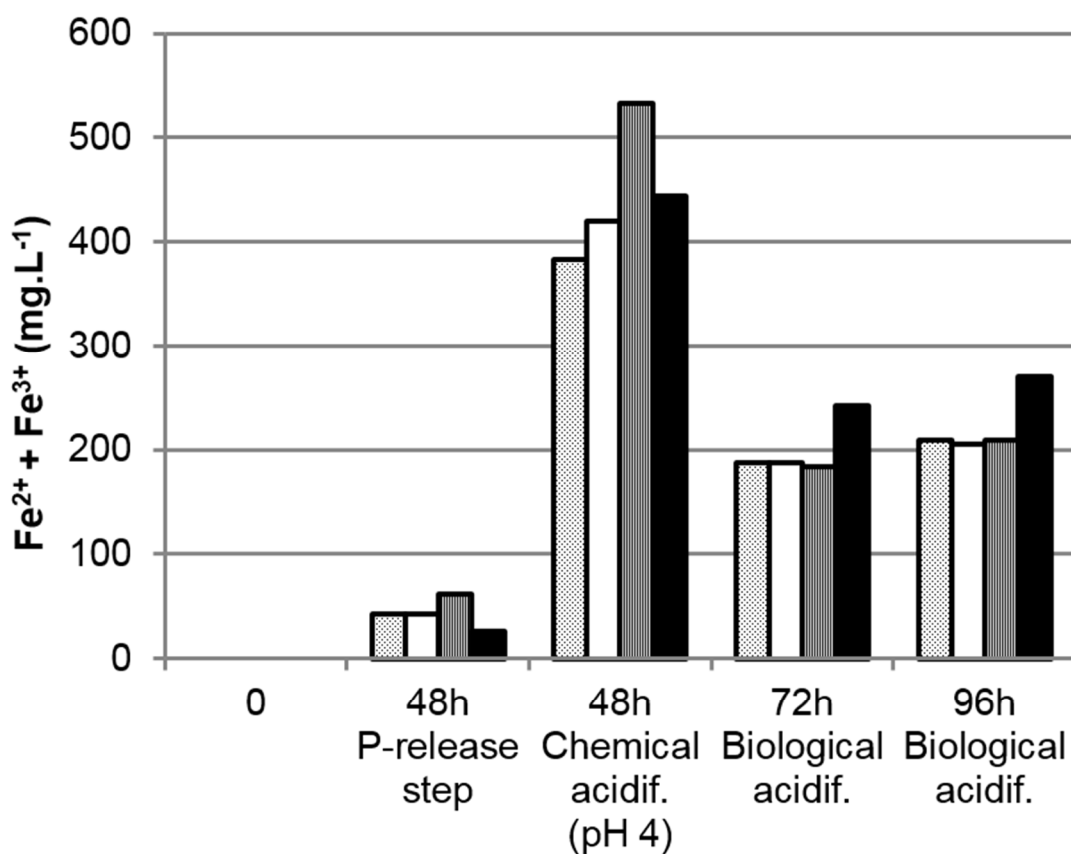


- ▨ R1 Control+HCl
- ▨ R2 Control
- ▨ R3 Restaurant
- R4 Urban organic waste
- ▨ R5 Lactoserum
- ▨ R6 WWTP grease









■ R3 Restaurant

□ R4 Urban organic waste

▨ R5 Lactoserum

■ R6 WWTP grease

