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Agro-food wastewaters as external carbon source to enhance biological phosphorus removal

F.J. Fernández [a,*], M.C. Castro [b], J. Villasenor [a], L. Rodríguez [c]

[a] University of Castilla-La Mancha, Chemical Engineering Department, ITQUIMA, Avenida Camilo José Cela S/N, 13071 Ciudad Real, Spain
[b] University Rey Juan Carlos, Chemical and Environmental Technology Department, C/Tulipan, S/N, 28933 (Mostoles) Madrid, Spain
[c] Alquimia Soluciones Ambientales, Research and Development Centre for the Recuperation of the Environment (CIDRA), Polígono Daimiel Sur, Calle de la calidad, (Daimiel) Ciudad Real, Spain

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ABSTRACT

This study investigated the potential use of agro-food wastewaters as external substrates to solve carbon deficiency problems in enhanced biological phosphorus-removal (EBPR) processes using laboratory and pilot-scale experiments. The laboratory experiments were conducted using a modified potential test developed in this work. This modified potential test allowed for the comparison of EBPR feasibility with several agro-food wastewaters and prediction of the long-term effects caused by the dose of agro-food wastewaters on the wastewater-treatment plant (WWTP). It was observed that most of the wastewaters studied were adequate for the short-term enhancement of EBPR because of their phosphorus-release potential. However, taking into account the average phosphorus-to-COD (chemical oxygen demand) ratio of the domestic wastewaters (0.33 mg P mg^{-1} COD), only the wastewaters coming from the tomato-processing (0.46 mg P mg^{-1} COD) and milk-bottling industries (0.44 mg P mg^{-1} COD) were recommended for long-term dosing due to their higher P-to-COD ratios.

1. Introduction

In Europe, with the aim of preventing eutrophication, the maximum nutrient (N and P) concentrations of treated domestic wastewater and some industrial wastewaters, mainly in the agro-food industrial sector, are restricted by the Urban Wastewater Treatment Directive 91/271/EEC. As a consequence, existing full-scale wastewater-treatment plants (WWTPs) have to be adapted to include nutrient removal according to the Directive limits.

In most cases phosphorus is the limiting factor in the eutrophication process, and not nitrogen [1,2], because nitrogen fixation can be performed naturally by diazotrophs. Therefore, this work has been focused on P removal rather than N removal.

The removal of the phosphorous contained in wastewater can be carried out by physico-chemical or by biological processes. Currently the most widely implemented are enhanced biological phosphorus-removal (EBPR) processes because these are the most economic methods to reduce the phosphorus content and because these processes avoid anion enrichment of the treated wastewater [3].

Usually the feasibility of applying EBPR to a given wastewater has been evaluated using the following ratios of chemical or biological oxygen demand (COD or BOD) to phosphorous: readily biodegradable COD/P, BOD/P and COD/P [4–6]. These ratios contribute useful information but are not definitive because only the volatile fatty acids (VFA) can be stored inside the cell [7].

To accurately determine the feasibility of EBPR with a defined wastewater, a VFA determination should be accompanied by the determination of the fermentation rates, VFA potential, concentration of toxic substrates, nitrates, etc. [8,9] because these can modify the phosphorus-removal efficiency. The determination of all these parameters is difficult and time consuming, and even when all these values have been determined and are within the correct ranges for EBPR, occasional failures have been reported in the literature [10,11]. In most cases, the failures were related to the low quality of the carbon source for the BNR process or to the unbalanced distribution of substrates between phosphorus-accumulating organisms (PAOs) and non-PAO microorganisms. These non-PAO organisms competing with PAO for the substrates have been named glycogen-accumulating organisms (GAO) [12,13].

The main difference between PAO and GAO metabolism is that GAOs do not store phosphorus inside the cell and use glycogen as the internal energy source for VFA uptake. GAOs cause a detriment in the phosphorus removal because they consume a part of the VFA contained in the wastewater, thereby reducing the VFA available for the EBPR process [4,13].

Because of the inconveniences of the traditional methods for EBPR feasibility determination, more procedures were proposed,

* Corresponding author. Tel.: +34 926 295300x6358; fax: +34 926 295242.
E-mail address: FcoJesus.FMorales@uclm.es (F.J. Fernández).

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one of the most important being the EBPR-potential test for wastewater [14]. Later, based on the observations by other authors [15–18], this procedure was improved by Park et al. [19] to offer more accurate results.

The EBPR-potential test developed for wastewater by Park et al. [19] consists of the determination of the phosphorus release during an anaerobic stage in a batch reactor in which activated sludge and wastewater are mixed. From the results obtained in the batch experiment the effluent phosphorus concentration in a EBPR process can be estimated [20–22]. Currently, this procedure is widely used to determine the EBPR feasibility of domestic wastewaters, in most cases resulting in the finding that there is a lack of VFA in these wastewaters, which reduces their EBPR suitability. Usually, to increase the VFA content of a domestic wastewater an external VFA source is supplied, the most commonly used being acetate [6]; however, this VFA source is expensive and significantly increases the treatment costs of these wastewaters [23,24]. Taking into account that some of the agro-food industrial wastewaters regulated by the 91/271/EEC Directive are characterised by a high VFA content [25], a good option could be the addition of agro-food wastewater to domestic wastewater, not only because of the high VFA content of the agro-food wastewater but also because these VFA are from wastes, so it is economical and environmentally sound.

In this context, the aim of this work was to study the potential use of agro-food wastewater as a VFA source to enhance the EBPR of domestic wastewater. To that end, the EBPR potential test developed by Park et al. [19] was improved, avoiding the drawbacks of the existing test. Based on the results obtained with the novel EBPR-potential test, the feasibility of applying EBPR to domestic wastewater by dosing agro-food wastewater was evaluated, and the best agro-food wastewater was selected. Finally, the results obtained in the batch experiments were verified in a pilot-scale plant. This study was designed to contribute information about the benefits of dosing of agro-food wastewater into domestic wastewater to enhance the EBPR process and to gain further insight into the possible full-scale application of these wastewater-treatment processes.

2. Methodology

2.1. Activated sludge

The activated sludge used in this work was taken from sequencing-batch reactors (SBRs) working with the anaerobic/aerobic (A/O) process for EBPR. The SBRs were operated continuously on cycles of 4 h. Each cycle consisted of several phases: influent addition (5 min), anaerobic reaction (75 min), aerobic reaction (135 min), waste sludge withdrawal (1 min), settling (20 min) and effluent withdrawal (5 min).

The temperature and pH of each reactor were maintained constant at 20 °C and 7.3, respectively, and dissolved oxygen was held at saturation during the aerobic stages. The sludge retention time was set at eight days. Further details concerning the SBR experimental set-up can be found in previous publications [26].

The wastewater used for the cultivation of microorganisms in the SBRs was prepared daily, and was composed of real domestic wastewater – taken from the overflow of the primary clarifiers of the Ciudad Real full-scale wastewater-treatment plant – supplemented with acetate and nutrients to enhance the EBPR activity. The composition of the synthetic wastewater and the characteristics of the resulting wastewater are shown in Table 1.

Once the system reached a steady state, the biomass from the SBRs was used in the batch experiments.

2.2. Agro-food wastewaters

The agro-food wastewaters used in this work were synthetic wastewaters. They were prepared just before the experiments by applying, in the laboratory, the corresponding industrial processes to the raw materials. The agro-food wastewaters used were from the cheese, milk-bottling, potato-processing, beet-sugar-processing and tomato-processing industries as well as slaughterhouse and winery wastewaters.

These wastewaters were filtered using a glass-fibre filter (pore size 0.45 μm) prior to use. The objective of the filtration was to avoid the adverse effects caused by hydrolysis of particulate components when this process limits the phosphorus release rate.

Table 1
(a) Composition of the synthetic wastewater and (b) characteristics of the resulting wastewater used for the cultivation of microorganisms.

Component	Concentration
(a)	
Sodium acetate ($mg\,L^{-1}$)	322
$(NH_4)_2SO_4$ ($mg\,L^{-1}$)	74.2
KH_2PO_4 ($mg\,L^{-1}$)	44.5
$NaHCO_3$ ($mg\,L^{-1}$)	115.0
$MgSO_4{\cdot}7H_2O$ ($mg\,L^{-1}$)	50.0
$CaCl_2$ ($mg\,L^{-1}$)	30.0
$(NH_4)_2\,Fe(SO_4)_2$ ($mg\,L^{-1}$)	3.0

Parameter	Concentration
(b)	
COD ($mg\,L^{-1}$)	340 ± 46
BOD_5 ($mg\,L^{-1}$)	181 ± 22
P ($mg\,L^{-1}$)	9.1 ± 2.6
N ($mg\,L^{-1}$)	15.8 ± 3.7
pH	7.3 ± 0.1

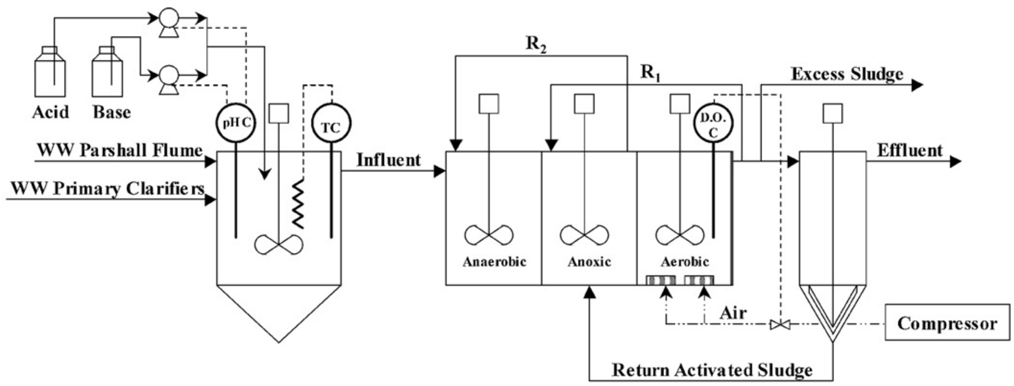
2.3. Batch tests

To evaluate the feasibility of EBPR with the studied agro-food industrial wastewaters, several anaerobic batch tests, based on the experimental procedure proposed by Park et al. [19], were carried out using biomass from the SBRs. The biomass was cultivated in the SBRs and taken from them just after the end of the aerobic stage to ensure the maximum potential for phosphorus release [27]. The biomass was then divided into ten batch reactors of 750 mL in volume.

Seven batch reactors were then dosed with one of the agro-food wastewaters studied to reach a soluble COD (S_{COD}) concentration of 300 $mg\,L^{-1}$. To avoid nutrient limitations, nutrients were added to reach approximately the following final concentrations: soluble phosphorus total (S_{PT}) = 10 $mg\,L^{-1}$, soluble ammonia nitrogen (S_{N-NH_4}) = 15 $mg\,L^{-1}$, soluble potassium (S_K) = 12 $mg\,L^{-1}$, soluble sodium (S_{Na}) = 14 $mg\,L^{-1}$, soluble magnesium (S_{Mg}) = 5 $mg\,L^{-1}$ and soluble calcium (S_{Ca}) = 10 $mg\,L^{-1}$.

Simultaneously, three reference tests were undertaken: a maximum test, which received nutrients and sodium acetate, a typical test, which was carried out with domestic wastewater and, finally, a blank test that received only nutrients. All these reference tests were carried out with the nutrient concentrations described above. The maximum and the typical reference test were carried out with a COD concentration of 300 $mg\,L^{-1}$. In this way the maximum, the typical and the minimum values for phosphorus release and COD uptake were determined. In this work it is assumed that the acetate used in the maximum reference test was the most adequate substrate to enhance the growth of PAO bacteria, allowing, therefore, the subsequent release of phosphorus to the bulk liquid [28].

During the batch tests, a very slow stirring rate was applied in order to minimise the oxygen transfer from the gas phase, but ensuring that the process took place in a well-mixed reactor. All the experiments, except the blank test, were carried out at a food to microorganisms (F/M) ratio of 0.18 (±0.02) g COD g VSS^{-1}. The pH in the batch reactors was 7.3 ± 0.1 because of the buffering activity of



Flow	Flow rate ($L h^{-1}$)	Compartment	Volume (L)	HRT (h)
Influent	56.0	Equalisation tank	450	8.0
Recycle anoxic to anaerobic (R2)	89.6	Anaerobic	112	2.0
Recycle aerobic to anoxic (R1)	30.8	Anoxic	112	2.0
Sludge waste	1.8	Aerobic	112	2.0
Sludge recycle	28.0	Settler	63	1.1
Effluent flow	54.2	Total volume	849	15.2

Fig. 1. Schematic flow diagram of the pilot plant.

the wastewater, and the temperature was continuously controlled at 20 °C by means of an incubator (ISCO FTD 220).

2.4. Sampling procedure and analytical methods

Samples were taken and immediately filtered through a glass-fibre filter (pore size 0.45 μm). After filtration, samples were analysed for the following soluble parameters: COD (S_{COD}), phosphorus total (S_{PT}) and ammonium nitrogen (S_{N-NH_4}). In addition total suspended solids (TSS), volatile suspended solids (VSS) and phosphorus in the activated sludge (X_{PT}) were determined. The soluble ammonium concentration was analysed to check the absence of nitrification in order to ensure the anaerobic conditions. All analyses of wastewater and activated sludge were performed as described by the APHA Standard Methods [29]. The agro-food wastewaters were characterised following the procedure proposed by the Dutch Foundation of Applied Water Research [30] and the procedure described in De Lucas et al. [31].

2.5. Pilot-scale plant experiments

Finally, a pilot-scale plant test was conducted to validate the predictions of the potential test. The pilot plant used, configured as the University of Cape Town (UCT) process, was located at the Ciudad Real WWTP. The plant consisted of an equalisation tank (volume 500 L), a bioreactor (total volume 336 L) divided into three consecutive compartments (anaerobic, anoxic and aerobic) and a settler equipped with a slowly rotating scraper. Variable-speed peristaltic pumps were used for internal recycles between compartments, settled sludge recycle to the reactor and for feeding the influent wastewater to the pilot plant (Fig. 1).

The solid retention time (SRT) was eight days; waste sludge was discharged from the aerobic reactor to keep the SRT constant. The dissolved oxygen in the aerobic compartment was set to 2.0 $g m^{-3}$.

During the continuous operation of the pilot plant, the COD concentration in the influent was increased to about 300 $mg L^{-1}$ by the continuous addition of a concentrated solution of synthetic agro-food wastewater to enhance the EBPR. The influent flow rate was intentionally kept constant so that the experimental results would reflect only the effect of the dose of the agro-food wastewater. More information about the pilot plant can be found elsewhere [3,32]. The influent and effluent characteristics were analysed according to the APHA Standard Methods [29].

3. Experimental results

3.1. Theoretical development of the EBPR potential test

Based on observations by other authors, Park et al. [19] indicated that there is a direct relationship between the magnitude of phosphorus release in the anaerobic compartment and the net biological phosphorus removal in the process. This relationship was presented as follows:

$$P_{UPTAKE} = \alpha \cdot P_{RELEASE} + P_{METABOLIC} \quad (1)$$

where P represents phosphorous.

Considering that the metabolic phosphorus requirement can be theoretically determined, the main task regarding this equation is the accurate determination of the phosphorus release caused by the substrates contained in the wastewater.

Park et al. [19] proposed to model the phosphorus profiles using the empirical formulae presented by Wentzel et al. [18]:

$$P_t = P_{max}\left[1 - \left(\frac{P_{max} - P_0}{P_{max}}\right) e^{-k_P \cdot t}\right] \quad (2)$$

where P_t = phosphorus release at time t ($mg\ P L^{-1}$); P_{max} = maximum potential phosphorus release ($mg\ P L^{-1}$); P_0 = initial phosphorus concentration ($mg\ P L^{-1}$); k_P = rate constant for phosphorus release (h^{-1}); t = time (h).

This equation accurately predicts the phosphorus profiles during the anaerobic stages. However, when the results obtained in different tests need to be compared this equation presents several

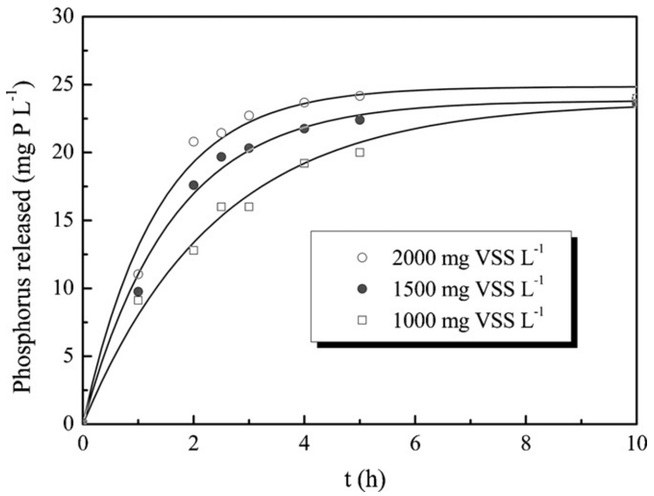


Fig. 2. PAO concentration effects on the EBPR-potential test. Lines indicate the predictions of the empirical equation.

drawbacks, namely that it does not take into account either the PAO concentration in the sludge or the COD profiles during the batch test. The inclusion of both aspects in the mathematical procedure is very important because the PAO concentration in the sludge and the combination of the COD and phosphorus profiles offer interesting information about the long term feasibility of EBPR with the tested wastewater.

Regarding to the PAO concentration, a basic assumption in the EBPR potential test developed by Park et al. [19] is that the characteristics of the activated sludge do not affect the outcome of the EBPR-potential test in the case that the total phosphorus release is measured after 2 h. However, this assumption can only be justified if sludges with very similar PAO concentration are used in the different experiments. To study the effects of the activated sludge characteristics on the results obtained by the EBPR-potential test, several batch tests were carried out with domestic wastewater at 300 mg COD L^{-1} with several different activated-sludge concentrations and, therefore, different PAO concentrations.

Fig. 2 presents the phosphorus-release profiles obtained during these batch tests. The phosphorus-release rates were significantly affected ($p < 0.05$) by the activated-sludge concentration, with higher activated-sludge concentrations corresponding to higher phosphorus-release rates.

This effect was directly related to the PAO concentration in the activated sludge because they are the microorganisms responsible for phosphorus release into the bulk liquid.

The experimental results were fitted to Eq. (2) proposed by Wentzel et al. [18] and it was observed that the effect of the PAO concentration was mainly reflected in the value of the first-order constant, which ranged from 0.41 to 0.75 h^{-1}, and not on the maximum phosphorus released, which remained approximately constant at about 25 mg P L^{-1} for all tests.

Because the first-order constant is very important in describing the behaviour of phosphorus release, and for the comparison of the performance of different wastewaters during the EBPR-potential tests, we were interested to take into account the effect of the PAO concentration on the first-order constant.

To do that, a modification of the Wentzel equation was proposed.

In this novel equation, the influence of the PAO concentration in the phosphorus release was taken into account by introducing the PAO concentration in the exponential term of the equation proposed by Wentzel et al. [18]. The PAO concentration can be determined using procedures proposed in the literature [23,33,34]:

$$P_t = P_{max}\left[1-\left(\frac{P_{max}-P_0}{P_{max}}\right)e^{-k_P \cdot X \cdot t}\right] \quad (3)$$

where k_P = rate constant for phosphorus release (L g COD^{-1} h^{-1}); X = PAO concentration (g COD L^{-1}).

The results obtained after the fitting of the experimental results to this modified equation and to the classical Wentzel equation are presented in Table 2.

Table 2
Parameter values of the modified model for different PAO concentrations.

Parameter	PAO Concentration (mg COD L^{-1})		
	280	210	140
Wentzel			
P_{max} (g P m^{-3})	24.8 ± 0.8	23.8 ± 0.6	23.7 ± 0.9
k (h^{-1})	0.75 ± 0.09	0.62 ± 0.05	0.41 ± 0.04
R^2	0.9857	0.9924	0.9871
This work			
P_{max} (g P m^{-3})	24.8 ± 0.8	23.8 ± 0.6	23.7 ± 0.9
k_P (L g^{-1} COD h^{-1})	2.64 ± 0.30	2.93 ± 0.23	2.92 ± 0.28
R^2	0.9857	0.9924	0.9871

In this table it can be seen that using the modified equation, the PAO concentration did not affect either the value of the first order constant or the maximum phosphorus released, being their values very similar in all the cases, about 2.8 L g^{-1} COD h^{-1} and 25 mg L^{-1}, respectively. Moreover, the values predicted by the modified equation were identical to the actual results as indicate the values of the regression coefficient.

The main importance of isolating the effect of the biomass concentration on the first-order constant of a defined wastewater is that this allows for the use of this parameter as a reference value for the comparison of the behaviour of different wastewaters and activated sludges in the biological phosphorus-removal processes. This comparison cannot be done with the classical potential test because its first-order constant depends on the PAO population of the activated sludge used in each experiment.

Regarding to the COD profile, it was determined along the EBPR potential test and two new equations were proposed to model the results.

These equations were the following:

1. The COD profile was modelled using a new equation (Eq. (4)), equivalent to that of the phosphorus, which describes the COD uptake during the experiment:

$$COD_t = COD_{max}\left[1-\left(\frac{COD_{max}-COD_0}{COD_{max}}\right)e^{-k_{COD} \cdot X \cdot t}\right] \quad (4)$$

where COD_t = COD uptake at time t (mg COD L^{-1}); COD_{max} = maximum potential COD uptake (mg COD L^{-1}); COD_0 = initial COD concentration (mg COD L^{-1}); k_{COD} = rate constant for COD uptake (L g COD^{-1} h^{-1}).

2. Also, a new equation was proposed to relate the phosphorus release and COD uptake profiles. This relationship was proposed in order to obtain the poly-phosphate requirement per COD stored (Y_{PO_4}):

$$\frac{P_t}{COD_t} = [Y_{PO_4}]_t = [Y_{PO_4}]_{Final}\left[1+\left(\frac{[Y_{PO_4}]_{Initial}-[Y_{PO_4}]_{Final}}{[Y_{PO_4}]_{Final}}\right)e^{-\beta \cdot t}\right] \quad (5)$$

where Y_{PO_4} = polyphosphate released per COD stored; β = beta factor (h^{-1}).

The Y_{PO_4} parameter informs about the kind of carbon source stored. The poly-phosphate requirement per COD stored is a very

Table 3
Agro-food wastewater characteristics.

Parameter		Agro-food industrial wastewater						
Symbol	Unit	CI	MBI	S	PP	BSP	TP	WI
$S_{\text{Fermentable substrates}}$	g COD m^{-3}	208	207	56	183	165	201	180
$S_{\text{Fermentation products}}$	g COD m^{-3}	58	29	7	33	48	96	73
S_{BCOD}	g COD m^{-3}	266	236	63	216	213	297	253
S_{Inert}	g COD m^{-3}	85	68	238	57	86	67	59
S_{COD}	g COD m^{-3}	351	304	301	273	299	364	312
S_{BOD5}	g COD m^{-3}	200	221	230	222	233	203	199
k_{BOD}	d^{-1}	0.212	0.431	0.598	0.672	0.279	0.613	0.749
L_0	g COD m^{-3}	230.4	221.4	19.5	227.1	211.7	214.3	230.6
$S_{\text{N-NH}_4}$	g N m^{-3}	17.0	18.5	22.0	18.4	15.0	19.0	19.5
$S_{\text{N-NO}_3}$	g N m^{-3}	0	0	0	0	0	0	0
$S_{\text{P-PO}_4}$	g P m^{-3}	13.5	9.5	9.0	10.2	11.2	10.1	9.7
S_{ALK}	mol m^{-3}	1.4	1.5	1.4	1.4	1.4	1.9	1.9

CI: cheese industries; MBI: milk bottling industries; S: slaughterhouses; PP: potato processing; BSP: beet sugar processing; TP: tomato processing; WI: winery industries.

important parameter because the higher the P release to COD uptake ratio, the higher EBPR efficiency of the substrate. Moreover, it offers interesting information about the long-term feasibility of the EBPR with a defined wastewater, because the wastewaters with the highest P release to COD uptake ratio favour the development of the PAO population [4]. In order to determine the model parameters of the new set of equations proposed (Eqs. (3)–(5)), the Gauss–Newton method for non-linear equations and the procedure described in the literature [35] were used.

3.2. Wastewater characterisation

Considering that the studied wastewaters were filtered before their use in the batch tests, only the soluble parameters were determined. To present more information about the biodegradability of the agro-food wastewaters used in the batch tests, the main parameters, reaction constant (k_{BOD}) and ultimate BOD (L_0) were determined by the Thomas method [36]. The characteristics of the agro-food wastewaters dosed in the EBPR potential batch tests are shown in Table 3.

3.3. Activated sludge characterisation

To obtain accurate results in the EBPR potential tests, it was necessary to determine the presence of PAO bacteria in the activated sludge. To do that, the polyphosphate stored inside the cells and the COD, phosphorus and nitrate concentration profiles in the bulk liquid during the SBR cycle were determined and modelled by following the specific procedure based on a structured metabolic model previously described in the literature [33,34]. In the equations of the model, the PAO concentration in the sludge is the unknown. In Fig. 3, the experimental COD, phosphorus and polyphosphate stored inside the cells during the anaerobic and aerobic stages of the SBR operation are shown. The model was fitted to the experimental data, and the results are presented in Fig. 2.

The phosphorus release in the anaerobic stage and the subsequent accumulation in the aerobic one indicated the presence of PAO bacteria. From the modelling, the PAO concentration in the activated sludge was determined to be ~275 mg COD L^{-1}. As seen in Fig. 3, the model predicts the experimental results very well for all the parameters except for the COD, which was consumed faster than that predicted by the model. This could be due to the accumulation of COD by other bacteria, i.e., GAO bacteria, which can remove COD without the release of phosphorus into the bulk liquid and because of the denitrification of the nitrate contained in the wastewater at the beginning of the anaerobic stage, which consumed approximately 10.7 mg COD L^{-1}.

3.4. EBPR potential test

The novel EBPR potential test, described in Section 2, was used to determine the EBPR potential of several ago-food wastewaters.

3.4.1. Reference tests

First of all, the results obtained in the blank test were used to evaluate the phosphorus release due to the maintenance process. In this test, an approximately constant phosphorus release rate was observed (0.21 mg P h^{-1}). To accurately determine the EBPR potential of the substrates studied, the maintenance-phosphorus release was subtracted from the phosphorus-release values obtained in the experiments where organic substrates were added.

The results obtained from the maximum test were used to determine the maximum amount of polyphosphate that can be released to the bulk liquid. These results were also used to detect a possible limitation in the phosphorus release during the batch test due to the exhaustion of the polyphosphate reserves stored inside the PAOs. The typical test, carried out with domestic wastewater, was used as reference to evaluate the EBPR with domestic wastewater. The modified model proposed above (Eqs. (3) and (4)) was fitted to the experimental results of both reference tests. The experimental data and the fitted curves are presented in Fig. 4.

As expected, the phosphorus release and COD uptake was higher in the experiments carried out with acetate than in the experiments carried out with domestic wastewater because acetate can be completely stored by PAO [26]. Moreover, the model accurately predicted the experimental data obtained with both the acetate

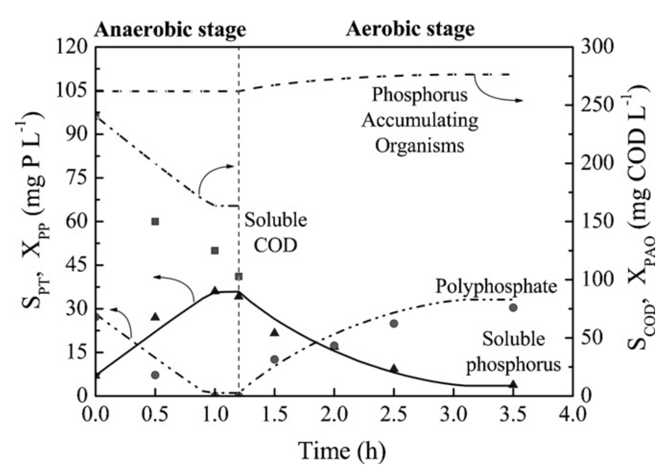


Fig. 3. Experimental results and model predictions in the anaerobic and aerobic stages of the SBR.

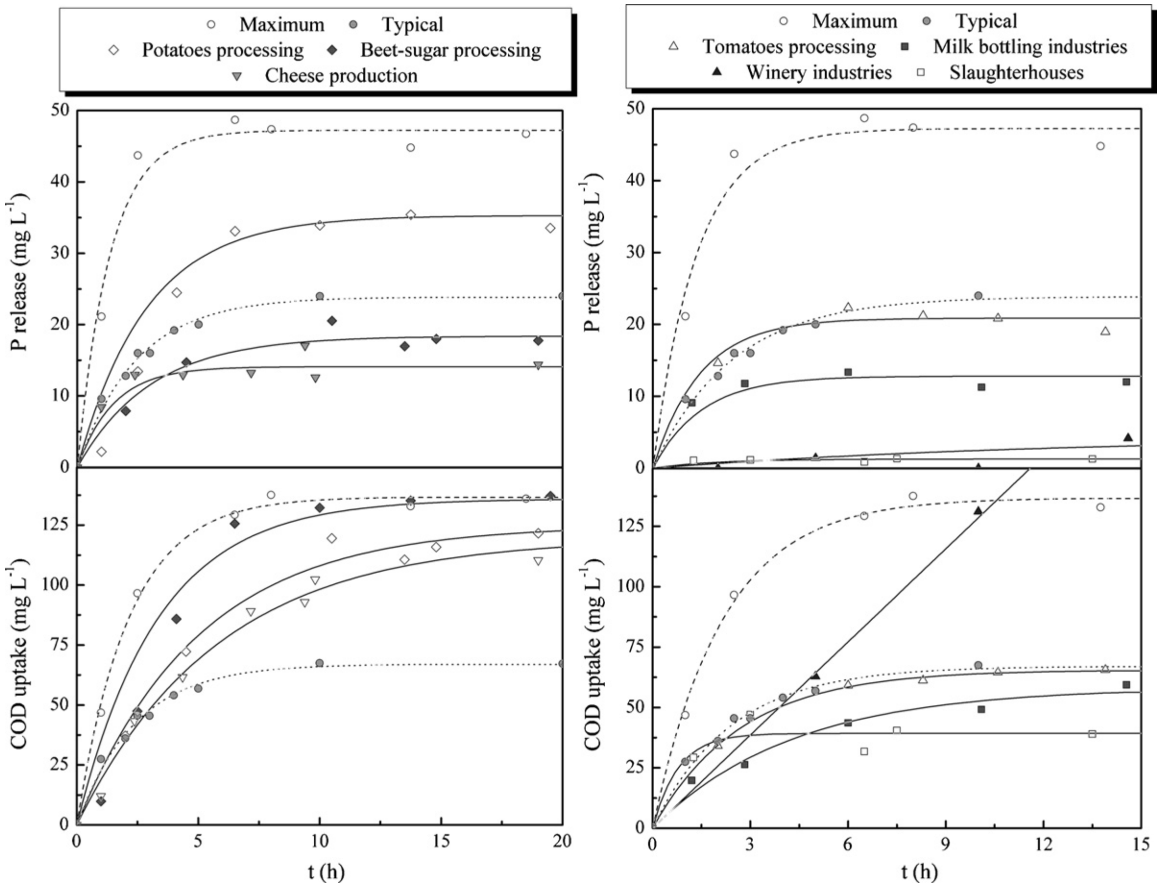


Fig. 4. Phosphorus release and COD uptake in the EBPR-potential test carried out with agro-food wastewater. Lines indicate the predictions of the empirical equations.

and the domestic wastewater (Fig. 4); the corresponding model parameters are presented in Table 4.

3.4.2. Agro-food wastewaters potential test

The phosphorus release and COD uptake caused by the substrates contained in the agro-food wastewaters were also determined; the experimental results are shown in Fig. 4.

In this figure, it can be seen that the phosphorus released with the agro-food wastewaters was always lower than that obtained with the acetate, which indicates that the quality of the substrates contained in the agro-food wastewaters for the BNR process is lower than that of the acetate. However, the agro-food substrates come from wastes, which make them very interesting to enhance the EBPR process. Among the agro-food wastewaters, that from potato processing yielded the highest phosphorus release to the bulk liquid. In the case of the wastewaters from tomato and beet-sugar processing industries, cheese production and milk-bottling industries, the phosphorus release was very similar in all their cases and slightly lower than that obtained with domestic wastewater. Finally, the wastewaters with the lowest phosphorus release values were those from slaughterhouses and wineries.

These results are interesting; however, long-term effects cannot be predicted with this information. Therefore, it was worthwhile to analyse the COD profiles during the EBPR-potential test. The importance of this information on the long term effects is because it can be related to the phosphorus release, serving as an indicator of the competition between PAO and GAO organisms in the sludge. The experimental results are presented in Fig. 4. Wastewaters coming from cheese production, potato processing and beet-sugar processing caused COD storage in the half-way between that obtained with acetate and domestic wastewaters. The final amount of substrate taken up when potato wastewater was dosed was similar to that

Table 4
Parameter values for the agro-food and reference batch test.

Wastewater	Phosphorus release			COD uptake		
	P_{max} (mg P L^{-1})	k_P (L g^{-1} COD h^{-1})	R	COD_{max} (mg COD L^{-1})	k_{COD} (L g^{-1} COD h^{-1})	R
Acetate	47.2 ± 2.0	4.2 ± 0.7	0.977	141.6 ± 2.6	2.6 ± 0.2	0.997
Domestic	24.0 ± 0.6	2.3 ± 0.2	0.989	67.0 ± 1.7	2.4 ± 0.2	0.989
PP	35.3 ± 2.1	1.5 ± 0.1	0.970	137.2 ± 10.3	1.3 ± 0.2	0.969
TP	20.9 ± 1.2	3.9 ± 0.2	0.998	65.5 ± 0.8	2.1 ± 0.2	0.999
BSP	18.4 ± 1.1	1.9 ± 0.2	0.988	125.0 ± 7.1	1.1 ± 0.2	0.987
CI	14.1 ± 0.1	3.8 ± 0.2	0.998	119.7 ± 14.7	1.0 ± 0.2	0.989
MBI	12.8 ± 0.2	3.8 ± 0.3	0.99	58.2 ± 4.2	1.3 ± 0.3	0.976
WI	4.5 ± 0.0	0.56 ± 0.1	0.979	–	–	–
S	1.3 ± 0.0	3.0 ± 0.2	0.970	39.3 ± 3.1	7.3 ± 3.7	0.901

CI: cheese industries; MBI: milk bottling industries; S: slaughterhouses; PP: potato processing; BSP: beet sugar processing; TP: tomato processing; WI: winery industries (mean values ± standard deviation).

obtained with acetate. This high COD storage caused by potato-processing wastewater could be explained in relation to the high phosphorus release. However, it is important to note that although the COD storage caused by the beet-sugar and cheese production wastewaters were lower than that obtained with acetate; they were significantly higher than expected based on the phosphorus release. These high values are interesting because this information could be related to a low quality of the carbon source for the EBPR process or to a noteworthy COD storage by GAO bacteria, which in the long term would reduce the COD available for PAO bacteria and would be detrimental to the EBPR process.

The COD storage caused by the other agro-food wastewaters was lower than that obtained with domestic wastewaters except in the case of the winery wastewater, which caused a COD storage even higher than that obtained with acetate. In principle, this is not possible because the reference test carried out with acetate defined the maximum amount of substrate that can be stored. Nevertheless, this effect could potentially be explained by the absorption of substrates onto the surface of the flocs and/or the volatilisation of alcohols contained in this wastewater. Finally, it is important to remark that in the experiments carried out with slaughterhouse wastewaters, the COD uptake happened only during the first few hours, indicating that the production of fermentation substrates suitable for storage inside the cell was almost negligible. This result is in accord with that obtained previously by De Lucas et al. [31].

To gain more insight into the phosphorus release and COD storage, the experimental results were modelled using Eqs. (3) and (4). The model parameters were determined by the fitting procedure, and they are presented in Table 4.

In this table, it is important to remark upon the values obtained for the rate constants. The values of the first-order constant for COD uptake (k_{COD}) ranged from 2.63 to 0.97, except for the slaughterhouse wastewater, which presented a significantly higher ($p < 0.05$) value of about 7.3. These results indicate that most of the substrates contained in the agro-food wastewaters studied, except the wastewaters from wineries and slaughterhouses, were metabolised in a similar way.

The values obtained for the phosphorus-release process (k_P) presented a wide range of uniformly distributed values, from 4.23 to 0.46, with all the values obtained with agro-food wastewaters being lower than that obtained in the maximum reference test. This suggests that the substrates contained in the agro-food wastewaters needed a previous transformation stage to make them available to PAO bacteria, thereby causing the subsequent phosphorus release. However, in most cases the rate constants were higher with the agro-food wastewater than with the domestic wastewater, indicating that the substrates contained in the agro-food wastewaters were more easily stored than those in the domestic wastewater. Wastewater from tomato-processing, milk-bottling and cheese industries presented high k_P values, which indicates that these wastewaters contained substrates with characteristics similar to acetate, which can be stored inside the cell causing the release of phosphorus, albeit with a slightly lower rate constant. However, the amount of phosphorus release with these wastewaters was lower than that obtained with acetate because the amount of substrates available for storage by PAO bacteria was very low.

Taking into account the differences observed between the phosphorus release and COD storage and their implications in the EBPR and substrate distribution, the phosphorus-release yield (Y_{PO_4}) was determined throughout the anaerobic batch test by dividing the model results obtained for the phosphorus and COD profiles; the Y_{PO_4} values obtained using Eq. (5) are presented in Fig. 5.

In Fig. 5 two kinds of trends can be seen: a descendent trend, in which the quality of the substrates stored (in terms of Y_{PO_4} values) seems to decrease over time, and a linear one, in which the quality of the substrates stored seems to be constant.

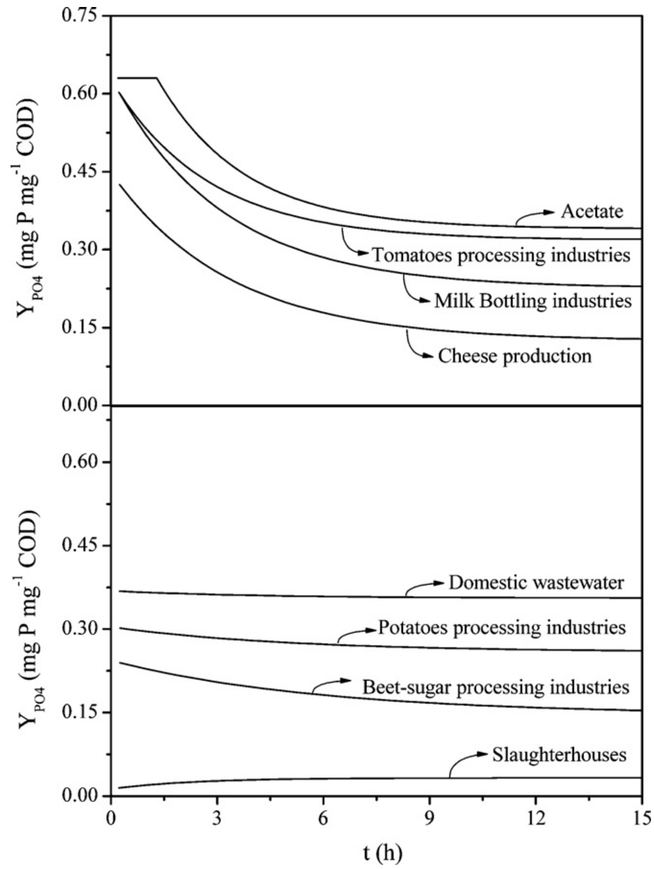


Fig. 5. Evolution of Y_{PO_4} during the potential tests.

In the case of the experiment carried out with acetate, the Y_{PO_4} value was constant around the theoretical value of 0.6 mg P mg^{-1} COD [34] during the first hour but decreased afterwards. This tendency suggests a change in the metabolisms or the COD storage by other bacteria such as GAO which increased their activity in long residence times.

The decreasing trends followed by the other wastewaters indicated that the quality of the substrates stored (in terms of Y_{PO_4} values) decreases along the experiment, which would reduce the EBPR efficiency. The parameters of the equations representing these trends and the average Y_{PO_4} values obtained for different anaerobic retention times are presented in Table 5.

At the beginning of the anaerobic stage, the tomato and milk bottling wastewaters presented Y_{PO_4} values very similar to that of acetate. This indicated that the contained substrates present similar characteristics to that of acetate, which would enhance the PAO activity and population in the activated sludge. The other agro-food wastewaters presented lower values for the Y_{PO_4} parameter, reflecting a lower quality of the contained substrates. Therefore, latter agro-food wastewaters could be used to enhance the EBPR process but only in the short-term. However, in the long-term, the dose of the agro-food wastewaters presenting low Y_{PO_4} values, would reduce the storage pools of polyphosphate, reducing therefore the phosphorus removal efficiency of the system. Based on the results, the most adequate agro-food wastewater for the long-term enhancement of the EBPR process was those from the tomato-processing and milk-bottling industries, as indicated by their average Y_{PO_4} and k_P values.

To verify these statements, two long-term experiments were carried out in which agro-food wastewaters were dosed into domestic wastewaters. These experiments were carried out over

Table 5
Parameter and average values of the phosphorus-release yields in the batch test.

Wastewater	Phosphorus release				Average value	Average value	Average value
	$Y_{PO_4\ initial}$	$Y_{PO_4\ final}$	β	R^2	3 h	6 h	9 h
Acetate	0.63	0.34	0.41	1.000	0.51	0.45	0.42
Domestic	0.36	0.33	0.25	0.999	0.35	0.35	0.33
PP	0.31	0.26	0.19	1.00	0.27	0.27	0.27
TP	0.62	0.32	0.37	1.000	0.46	0.42	0.39
BSP	0.25	0.15	0.17	1.000	0.20	0.20	0.19
MBI	0.62	0.23	0.32	1.000	0.44	0.38	0.34
CI	0.45	0.12	0.30	0.999	0.30	0.26	0.23
S	0.01	0.03	0.43	0.997	0.02	0.03	0.03

CI: cheese industries; MBI: milk bottling industries; S: slaughterhouses; PP: potato processing; BSP: beet sugar processing; TP: tomato processing; WI: winery industries.

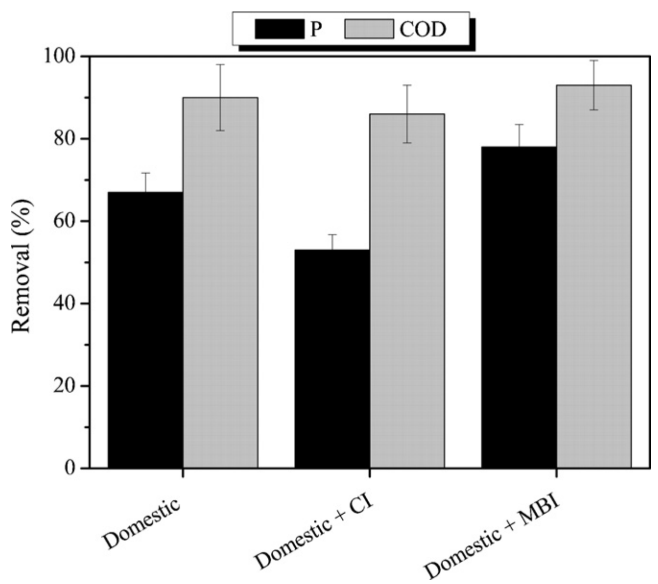


Fig. 6. Long-term effects of the dosing of agro-food wastewaters on the phosphorus and COD removal in the pilot-scale plant.

more than two months with milk-bottling wastewater, which presented a Y_{PO_4} higher than that of the domestic wastewater, and cheese-processing wastewater, which presented a lower Y_{PO_4} value than the domestic wastewater. The information presented for each experiment are the average values and the standard deviation of the data collected once the steady state was reached (about the last 25 days). In both experiments, enhancement of the EBPR process was observed over the short-term. However, after reaching a stationary state, the results obtained for the phosphorus and COD removal were different, as presented in Fig. 6.

In this figure, it can be seen that the long-term addition of milk-bottling wastewater enhanced the EBPR from domestic wastewater, whereas the addition of cheese-processing wastewater worsened it, confirming the predictions made on the basis of the Y_{PO_4} profiles. In this sense, it is important to remark that the addition of any industrial agro-food wastewaters increases the phosphorus load, which could outweigh the benefits of the external COD supplied.

About the characterisation of the culture after the long term experiments, an increase in the PAO population was detected when the wastewater from milk bottling industries was added (to a 37% of the VSS) and a decrease (to a 19% of the VSS) when wastewater from cheese industries was added.

4. Conclusions

The main conclusions drawn from this study are as follows:

1. Agro-food wastewaters can be beneficially used to enhance EBPR in domestic wastewater to provide an economic and environmentally friendly option of supplemental organic carbon addition.
2. The inclusion of the PAO concentration in the model allowed us to obtain comparable rate constants and therefore to evaluate the feasibility of enhancing EBPR with different agro-food wastewaters. Moreover, the inclusion of the COD profile helped to evaluate the long-term effects of the dosing of agro-food wastewaters.
3. The tomato and milk-bottling wastewaters presented the highest Y_{PO_4} values, indicating that these were the best choices for enhancing the EBPR over the long term, as was confirmed in the pilot-scale test.

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