Risks of using EDTA as an agent for trace metals dosing in anaerobic digestion of olive mill solid waste

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Abstract

Low concentrations of trace elements in many organic wastes recommend their supplementation in order to avoid potential limitations. Different chelating agents have been used to ensure an adequate trace metal pool in the soluble fraction, by forming dissolved complexes. Ethylenediaminetetraacetic acid (EDTA) is probably the most common, although several negative effects could be associated with its usage. Biomethane potential tests were performed using Olive Mill Solid Waste as the substrate, supplementing different combinations of Fe, Co, Ni, Ba, always under the presence of EDTA. Results show that Ni and Co slightly recovered biodegradability. However, Ba supplementation resulted in worsening the methane yield coefficient in all cases. High concentration of EDTA led to decrease in the activity of anaerobic digestion. High availability of EDTA induces the capture of trace metals like Co or Ni, key trace metals for anaerobic biomass activity. While supplementing trace metals, the addition of Ba and/or EDTA must be carefully considered.

Keywords: Ethylenediaminetetraacetic acid; Metal affinity; Methane production rate; Microbial activity; Olive Mill Solid Waste; Trace metal supplementation.

1. Introduction

Trace metals are essential components of cofactors and enzymes, which play a key role in the different metabolic pathways of the anaerobic digestion process [1, 2]. For a stable anaerobic digestion process, it is important to reach an appropriate concentration of each trace metal, in order to prevent limitation effects by their absence or inhibition by their excess [3, 4]. The low concentrations of trace elements of some organic wastes, such as Olive Mill Solid Waste (OMSW), make recommendable the supplementation of the required trace metals to avoid limitations during the degradation process [5]. In that sense, several authors have reported improvements in the microbial activity by supplementation of, among others, nickel, iron, barium, and cobalt to biomethanization processes, which resulted on enhancements over the methane yield coefficient or the methane production rate [6, 7, 8]. Wide ranges of total trace metal concentration have been reported as recommended for anaerobic processes. For instance, a range from 0.2 to 100 mg/L has been reported as beneficial for nickel [9, 10]. In parallel, beneficial concentrations from 0.05 up to 3.0 mg/kg were reported for cobalt [1, 3]. Supplementation dosages in a range from 0.5 to 12.5 g/L of iron were also reported as necessary for maintaining process stability [10, 11].

The distribution and bioavailability of the trace metals are as important as the total concentration of the system [12, 13, 14]. The chemical equilibrium varies on the system due to the formation of inorganic and organic complexes and/or precipitation phenomena, which could seriously limit the bioavailability of the trace metals [15].

Different chelating agents, such as ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA) or Ethylenediamine-N,N'-disuccinic acid ([S, S]-EDDS), have been added together supplemented metal to ensure an adequate trace metal pool in the soluble fraction, by forming dissolved complexes [16]. EDTA is one of the most

popular chelating agents used for trace metal supplementation in anaerobic digestion, due to its high solubilization capacity. In one hand, the increase of the soluble fraction by EDTA allows maintaining a constant free metal concentration in the liquid media, easily available for microorganisms [17, 18, 19, 20]. On the other hand, some authors have reported negative effects associated with its usage. These inconveniences include toxicity of free EDTA⁻⁴ or the inactivation of enzymes by metal extraction from the metal active sites. Moreover, granular structures of the inoculum can be affected due to the caption of calcium by EDTA [21, 22, 23].

Anaerobic digestion of OMSW has been reported to be limited in cobalt, whose supplementation in an adequate concentration improved the methane production and methane production rate [5]. Fe, Ni and Ba has been reported to be under limiting concentrations in similar complex organic wastes, being highly recommended to evaluate the supplementation of these trace metals [24, 25, 26].

The aim of this work was to evaluate the variations in metal chemical equilibrium through equilibrium modelling and its relation to the experimental total and soluble concentration of Fe, Co, Ni and Ba. Subsequently, the effects of the affinity between selected trace metal and EDTA over the biomethanization process were evaluated.

2. Material and Methods

2.1 OMSW and anaerobic inoculum

OMSW was obtained from the experimental plant for olive oil extraction located in “Instituto de la Grasa (CSIC)” of Sevilla, Spain. OMSW was preserved under freeze conditions (-18° C) to avoid undesirable fermentation processes. The anaerobic inoculum was obtained from a full-scale anaerobic reactor treating waste activated

sludge from “COPERO” urban wastewater treatment plant (UWTP) (Seville, Spain). Most of the influent of this UWTP is composed of municipal wastewater (85-90%). The rest of the influent is mainly derived from the agri-food industry. Chemical characterization of the used anaerobic inoculum and OMSW is summarised in Table 1.

2.2 Experimental design

The effect of the supplementation of Fe, Co, Ni and Ba, and the influence of EDTA, on the biomethanization of OMSW was evaluated by biochemical methane potential (BMP) tests. BMP tests were performed in 0.25-L Erlenmeyer flasks at mesophilic conditions (35 °C). The content of the BMP tests was magnetically stirred, at 300 rpm, and temperature was maintained by means of a thermostatic bath containing water at 35 °C. During the set-up phase, a flow of nitrogen was employed to maintain the anaerobic conditions inside the BMP tests. In all cases, the inoculum to substrate ratio was kept at 2, based on volatile solids, i.e. 3.80 g VS of inoculum and 1.9 g VS of OMSW. Additionally, 293 µL of trace elements solution were added to each reactor. The composition of the trace elements solution was: $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 500 mg/L; $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, 90 mg/L; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 50 mg/L; H_3BO_3 , 50 mg/L; ZnCl_2 , 50 mg/L; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 38 mg/L; $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$, 194 mg/L and EDTA, 1000 mg/L.

The required concentrations of the studied trace metals, i.e. Fe, Co, Ni and Ba, were achieved using stock solutions of FeCl_2 500 mM (63.4 g Fe^{2+} - FeCl_2 /L), CoCl_2 10 mM (0.989 g Co^{2+} - CoCl_2 /L), NiCl_2 10 mM (1.296 g Ni^{2+} - NiCl_2 /L) and BaCl_2 500 mM (104 g Ba^{2+} - BaCl_2 /L). EDTA was added in a 1:1 molar ratio with each added metal. The combinations of metal supplementation assayed were Fe-Co, Fe-Ni, Co-Ni, Fe-Ba, Co-Ba, Ni-Ba and Fe-Co-Ni-Ba. An extra assay was performed without trace metal addition as a control. The supplemented concentrations of the studied trace metals were 10 mM

Fe (558 mg Fe²⁺/L), 0.01 mM Co (0.280 mg Co²⁺/L), 0.01 mM Ni (0.587 mg Ni²⁺/L) and 10 mM Ba (1373 mg Ba²⁺/L). The supplementation concentration of each metal was selected in accordance to previous studies [5, 24, 25, 26].

The produced biogas was passed through a 2 N NaOH solution to capture CO₂. The remaining gas was assumed to be methane, which was measured by liquid displacement. The BMP tests were carried out during a 21-day period, which was the required period for the exhaustion of the gas production. Methane production was monitored daily throughout the process. Each experiment was carried out in triplicate.

2.3 Data fitting

The kinetics of the processes was evaluated by applying a modified-Gompertz equation to the experimental data of methane production during BMP tests (Equation 1). The application of modified-Gompertz equation was previously reported in batch mode biomethanization processes of several organic substrates by different authors [27, 28], and is defined as:

$$G = G_{max} * \exp \left[-\exp \left(\frac{R_{max} * e}{G_{max}} (\lambda - t) + 1 \right) \right] \quad (\text{Equation 1})$$

where G (mL CH₄/g VS_{added}) is the cumulative specific methane production at time t (d), G_{max} (mL CH₄/g VS_{added}) is the ultimate methane production, R_{max} is the maximum methane production rate (mL CH₄/g VS_{added}·d), λ is the lag time (d) and e corresponds to $\exp(1)$, equal to 2.7183. Additionally, r^2 , Error (%) and standard error of estimate (σ_{est}) were determined to evaluate the goodness-of-fit of selected modified Gompertz equation to the experimental data. Error was defined as the difference in percentage between the experimental methane production and G_{max} .

2.4 Chemical analyses

The following chemical analyses were used for the characterization of the inoculum and OMSW as well as for the effluents from each BMP test at the end of the process. pH was determined using a pH-meter model Crison 20 Bair. The values of alkalinity (mg CaCO_3/L), Total Solids (TS; g/kg) and Total Volatile Solids (VS; g/kg) were determined according to the recommendations of the Standard Methods of APHA [29]. Specifically, alkalinity, TS and VS were analysed according to the 2320B 2540B and 2540E Standard Methods [29]. Chemical Oxygen Demand (COD; g/kg) was determined using the method described by Raposo et al. [30]. The concentrations of different dissolved trace elements, i.e. Fe, Ni, Co, and Ba, were determined by filtration of the liquid sample with a 0.2 μm filter in an oxygen-free atmosphere and measured by ICP-OES (Thermo Fisher Scientific, model IRIS Advantage). Total trace elements concentrations of previous described metals and Cu, Ca, Mn, Zn, Mg, P and S in solid samples were determined through the same procedure after aqua regia digestion in a microwave oven. Phenols were quantified by spectrophotometry through the Folin-Ciocalteu method, after an extraction with a methanol/water solution (80:20) [31]. Elemental analysis to obtain the Carbon:Hydrogen:Nitrogen (C:H:N) ratio was performed by using Elemental Analyser LECO CHNS-932 (Leco Corporation, St Joseph, MI, EEUU).

2.5 Software

Sigma-Plot software (version 10.0) was used to fit the experimental data to the modified Gompertz equation and perform the statistical analysis. Equilibrium speciation modelling for trace metals was performed by Visual Minteq software by defining a simplified anaerobic system [32]. The total concentrations of cations and anions in each BMP test were used to model metal speciation, i.e. Al^{3+} , B (III), Ba^{2+} , Ca^{2+} , Cl^- , Co^{2+} ,

CO_3^{2-} , Cu^{2+} , EDTA^{-4} , Fe^{2+} , H^+ , K^+ , Mg^{2+} , Mn^{2+} , Mo (VI) , Na^+ , Ni^{2+} , $\text{P (PO}_4\text{)}$, S^{2-} , Se (IV) , and Zn^{2+} . pH values at the end of the BMP tests were also included in the modelling. The imposed initial conditions for modelling were: no presence of organics, atmospheric pressure, and 35 °C as operation temperature.

3. Results and discussion

3.1 Variations in the modelled speciation and concentration of trace metals

Anaerobic digestion is a very complex process which entails a large amount of interactions between chemical species (i.e. organic and inorganic compounds) and microorganisms [33, 34]. Because of the absence of organic compounds, the proposed simple model is not fully representative of the anaerobic digestion process. However, it is possible to predict trends in the variation of the chemical equilibrium of the studied trace metals due to the EDTA addition [32]. Table 2 shows the percentage of distribution of the principal species for Fe, Co, Ni, Ba and EDTA according to the proposed speciation model. Fe was principally forming FeHS^+ with a percentage of distribution higher than 95 % in all the cases. Percentages of distribution of FeEDTA^{-2} varied from 0.7 % to 1.85 % and 3.1 % by increasing the EDTA concentration from 2461 mg/L to 6187 mg/L and 9913 mg/L, respectively. Co is mainly distributed as CoEDTA^{-2} and up to a lesser extent as CoHS^+ and CoHCO_3^+ . Among the different studied trace metals, the addition of EDTA caused the highest effect over the Co distribution, the higher the EDTA, the higher the CoEDTA^{-2} present. The distribution of the species of Ni was not significant varied by the addition of EDTA (Table 2). The distribution percentages described a marked affinity of Ni for EDTA in the form of NiEDTA^{-2} , which reached percentages higher than 98.8% in all cases. In contrast, the

affinity of Ba to EDTA was insignificant with the formation of BaHCO_3^+ , BaCl^+ , and BaCO_3 (aq) (Table 2). The percentage distribution of these species was not clearly affected by the different concentrations of EDTA in the systems. According to these results, the affinity to EDTA for each studied trace metal is different and could vary in accordance with the compounds presented in the system. It is possible to ensure that the following trend for EDTA affinity exists: $\text{Ni} > \text{Co} > \text{Fe} > \text{Ba}$. EDTA presented a high affinity to Ca and Al in the form of CaEDTA^{-2} (63-83 %) and AlOHEDTA^{-2} (13-25 %), respectively.

Table 3 shows the total and soluble concentrations of Fe, Co, Ni and Ba at the end of the BMP tests, as well as the added concentration of EDTA, for each supplementation assay. The distribution between both fractions was clearly related to the previously described EDTA affinity trends, i.e. $\text{Ni} > \text{Co} > \text{Fe} > \text{Ba}$. The addition of Ba, and the consequent higher availability of EDTA^{-4} in the system, resulted in an increase of the soluble forms of Fe and Co, regardless of the concentration of these metals in the system. This increase is higher for Co than for Fe (Co was almost fully solubilized when Ba was supplemented). The high affinity of Co to EDTA could reduce the bioavailability of Co in the biological system. A limitation in the capability of the microorganisms on capturing Co^{2+} could involve a decrease in the anaerobic digestion kinetics [5]. It is interesting to notice that solubilization of Fe is mainly related to Ba addition, as different EDTA concentrations did not cause a significant increase of the soluble/total ratio of Fe when Ba was not added (Table 3). However, this ratio increases about 30 times when Ba was added compared to the condition with the same amount of EDTA but no added Ba (Table 3).

Supplementation of Ba resulted in an increment of the soluble/total ratio of this metal. This increase probably was not related to the complexation with EDTA, but it is related

to the formation of other soluble compounds, i.e. BaHCO_3^+ , BaCl^+ , and BaCO_3 (aq), as it is described by the speciation model (Table 2). Ni distribution was not affected by the different concentrations of EDTA in the system or the supplementation of Ba. The differences in the soluble/total concentration ratio of this metal indicated that the Ni from inoculum and OMSW is strongly retained and cannot be captured by the EDTA. In parallel, the whole supplemented concentration is in the soluble fraction, probably forming NiEDTA^{-2} in accordance with the speciation model (Table 2).

3.2 Stability and removal efficiencies of BMP tests

The mean values obtained for pH, biodegradability (%), phenol (mg/L) and phenol removal (%) determined at the end of the BMP tests are shown in Table 4. pH values varied in a short range from 7.08 ± 0.04 to 7.53 ± 0.01 . These values are within the range reported as optimal for maintaining the stability of the process and for mesophilic methanogenic activity [35].

Biodegradability values summarised in Table 4 were calculated from methane productions. The biodegradability described for the Control, i.e. 64.9 ± 0.6 %, was similar than that reported by Pinto-Ibieta et al. [5] for the same substrate at similar conditions. These authors reported a methane production of 344 mL/g VS, which corresponds to a biodegradability of 62.0 %. The supplementation assays without Ba presented a biodegradability values similar than that obtained in the control, with a mean value of 62.5 ± 0.9 %. The addition of Ba entailed a marked decrease of the biodegradability at the different assays (Table 4).

OMSW has a variety of phenolic compounds, such as tyrosol, oleuropein, caffeic, p-hydroxybenzoic or protocatechuic acids, which have been defined as potential inhibitors

of the anaerobic digestion process [36, 37]. Regardless of the percentage removal, the initial and final concentrations of total phenols (Table 4) were noticeably lower than the inhibition limit, which was reported around 3000 mg/L [37].

3.3 Effects of the affinity between selected trace metal and EDTA over the biomethanization process

Figures 1A and 1B show the mean values of the accumulated methane production throughout the experimental time for the different BMP tests. Table 5 summarised the parameters obtained from the application of the modified-Gompertz equation to the experimental data showed in Figures 1A and 1B, as well as the values of r^2 , Error (%) and standard error of estimate (σ_{est}) to evaluate the goodness-of-fit. r^2 values were higher than 0.99 in all the cases (Table 5). Likewise, Error and σ_{est} , with similar low values, also indicated a good fit of the experimental data to the proposed model.

Co-Ni supplementation, which only implied the increase of EDTA from 2461 mg/L to 2469 mg/L compared to the control, improved the R_{max} around 24%, whereas the G_{max} was maintained in a similar value. The increase of the concentration of EDTA up to 6187 mg/L, associated with Fe supplementation without Ba, resulted in a decrease of R_{max} , 6-16 % lower compared to control, although the G_{max} were kept at similar values (Table 5). The addition of Co together with Fe resulted in higher R_{max} compared to the test with Ni together with Fe (Table 5). In the presence of EDTA and without added Ba, supplementation of Fe, Ni and Co did not influence significantly G_{max} but R_{max} was affected. The positive effect over the biomethanization process indicated that Ni, and specially Co, were not enough bioavailable in the OMSW and supplementation was needed [5, 38]. The enhancement of R_{max} by Co and Ni supplementation is related to the

activation of enzymes involved in the acetogenic and methanogenic steps, such as vitamin B₁₂, coenzyme A and enzyme methyl-H4SPT: CoM methyltransferase [39, 40].

The effect of the concentration of EDTA seemed to play an important role in R_{max} decrease as well. Taking into account all the tests, an increase in the concentration of EDTA up to 6187 mg/L entailed a decrease of R_{max} between 6-19 % compared to control. The negative effects were even higher for EDTA concentrations over 9913 mg/L, for which R_{max} was 44 % lower compared to control (Table 5).

The addition of Ba resulted in a worsening of the G_{max} in all cases. When Ni or Co was supplemented together with Ba, the G_{max} decreased about 13 % compared to control. When Fe was supplemented together with Ba, G_{max} reached the lowest value, 26 % lower than the control. Supplementation of Ni and Co along with Ba and Fe slightly recovered biodegradability, but G_{max} was still 20 % lower than the control.

According to the speciation model described in Section 3.1, the EDTA added together with Ba could be available for reacting with other cations given the low affinity between EDTA and Ba. This high availability of EDTA could cause metal extraction from the metal active sites of the enzymes involved in methanogenesis [23]. EDTA captured more easily cobalt than nickel or iron from the inoculum (Table 3), which could explain the decrease in the microorganism response in spite of the total solubilization of cobalt [2]. Furthermore, the high affinity between calcium and EDTA could also affect the biomethanization process. Calcium is a structural ion for extracellular polymeric substances (EPS) on bacteria consortiums and, thus, the extraction of calcium entail a reduced granular strength and a decrease in the bacteria interactions [21, 23].

4. Conclusions

In conclusion, the supplementation of trace metals could be a very interesting strategy for improving the anaerobic digestion of Olive Mill Solid Waste. However, inadequate concentrations of trace metals and/or chelating agents could result in the deterioration of the process. The concentration of EDTA was highly related on the maximum methane production rate decrease, probably due to the sequestration of essential trace metals by EDTA. The addition of Ba resulted in a worsening of the methane yield coefficient in all cases. Supplementation of Ni and Co along with Ba and Fe slightly recovered biodegradability. The positive effect over the biomethanization process indicated that Ni, and specially Co, were not enough bioavailable in the Olive Mill Solid Waste and supplementation was needed.

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Figure Caption

Figure 1. A) Variation of the accumulated methane production with digestion time for the control BMPs and the BMPs supplemented with Fe-Co, Fe-Ni and Co-Ni, and **B)** Variation of the accumulated methane production with digestion time for the BMPs supplemented with Fe-Ba, Co-Ba, Ni-Ba and Fe-Co-Ni-Ba.

A)

B)

Figure 1. A) Variation of the accumulated methane production with digestion time for the control BMPs and the BMPs supplemented with Fe-Co, Fe-Ni and Co-Ni, and **B)** Variation of the accumulated methane production with digestion time for the BMPs supplemented with Fe-Ba, Co-Ba, Ni-Ba and Fe-Co-Ni-Ba.

Table 1. Olive mill solid waste (OMSW) and anaerobic inoculum characteristics

	Anaerobic inoculum	OMSW
TS (g/kg)	21.3 ± 3.6	260.1 ± 6.8
VS (g/kg)	13.8 ± 0.1	235.6 ± 3.6
COD (g O ₂ /kg)	31 ± 3	361 ± 29
Alkalinity (mg CaCO ₃ /L)	3300	-
pH	8.1±0.2	4.96±0.06
Phenols (mg/L)	22.7± 0.5	4303± 68
Fe (mg/kg TS)	14488	863
Ni (mg/kg TS)	17.1	3.3
Co (mg/kg TS)	2.87	0.065
Cu (mg/kg TS)	214.2	14.7
Ca (mg/kg TS)	46911	2920
Mn (mg/kg TS)	168.3	15.4
Zn (mg/kg TS)	799.6	21.8
Mg (mg/kg TS)	6609	6485
P (mg/kg TS)	23136	2331
S (mg/kg TS)	10204	1500
Ba (mg/kg TS)	114	2.057
C:H:N	0.4:0.06:0.04	0.47:0.07:0.02

Table 2. EDTA concentration and percentage distribution of the principal species for iron, cobalt, nickel, barium according to the proposed speciation model for each tested combination.

		Control	Fe-Co	Fe-Ni	Co-Ni	Fe-Ba	Co-Ba	Ni-Ba	Fe-Co-Ni-Ba
EDTA (mg/L)		2461	6187	6187	2469	9909	6187	6187	9913
Iron (%)	FeHS⁺	97.9	96.0	96.5	97.6	95.6	97.2	97.2	95.4
	FeHCO₃⁺	1.1	1.5	1.3	1.4	1.1	0.9	1.0	1.2
	FeEDTA⁻²	0.7	2.1	2.0	0.7	3.1	1.7	1.6	3.1
Cobalt (%)	CoEDTA⁻²	69.0	86.6	86.1	68.3	90.8	84.5	84.0	90.9
	CoHS⁺	25.0	10.4	11.0	25.1	7.4	12.8	13.3	7.4
	CoHCO₃⁺	4.7	2.6	2.4	5.8	1.3	1.9	2.2	1.5
Nickel (%)	NiEDTA⁻²	98.8	99.6	99.6	98.8	99.7	99.5	99.5	99.7
	NiHS⁺	1.0	0.3	0.3	1.0	0.2	0.4	0.4	0.2
	NiHCO₃⁺	0.1	0.1	0.1	0.2	0.0	0.0	0.1	0.0
Barium (%)	BaHCO₃⁺	77.3	78.7	78.4	79.4	76.3	75.7	77.9	79.5
	BaCl⁺	17.6	18.4	17.3	17.7	16.7	16.7	16.8	17.0
	BaCO₃ (aq)	4.3	2.1	3.4	2.1	6.2	6.7	4.5	2.5

Table 3. Total and soluble concentrations of Fe, Co, Ni and Ba at the end of the assays as well as the added concentration of EDTA for each supplementation test.

	EDTA (mg/L)	Fe (mg/kg TS)		Co (mg/kg TS)		Ni (mg/kg TS)		Ba (mg/kg TS)	
		Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble
Control	2461	349	2.44	0.07	0.02	0.43	0.03	2.7	0.05
Fe-Co	6187	907	4.74	0.66	0.04	0.43	0.02	2.7	0.02
Fe-Ni	6187	907	4.22	0.07	0.02	1.02	0.59	2.7	0.03
Co-Ni	2469	349	2.26	0.66	0.08	1.02	0.65	2.7	0.04
Fe-Ba	9909	907	239	0.07	0.07	0.43	0.09	1376	162
Co-Ba	6187	349	117	0.66	0.66	0.43	0.08	1376	163
Ni-Ba	6187	349	104	0.07	0.07	1.02	0.64	1376	170
Fe-Co-Ni-Ba	9913	907	215	0.66	0.61	1.02	0.61	1376	169

Table 4. Parameters analyzed (mean value $\pm$ SD) in each reactor at the end of BMP tests.

	pH	Biodegradability (%)	Phenols (mg/L)	Phenols removal (%)
Control	7.33 $\pm$ 0.09	64.9 $\pm$ 0.6	95 $\pm$ 5	37
Fe-Co	7.25 $\pm$ 0.05	61.6 $\pm$ 1.6	88 $\pm$ 2	41
Fe-Ni	7.22 $\pm$ 0.03	62.6 $\pm$ 2.1	86 $\pm$ 2	43
Co-Ni	7.30 $\pm$ 0.02	63.3 $\pm$ 0.9	94 $\pm$ 4	37
Fe-Ba	7.49 $\pm$ 0.34	47.1 $\pm$ 10.3	88 $\pm$ 4	41
Co-Ba	7.53 $\pm$ 0.01	55.4 $\pm$ 9.5	86 $\pm$ 1	43
Ni-Ba	7.34 $\pm$ 0.13	52.7 $\pm$ 8.6	104 $\pm$ 16	31
Fe-Co-Ni-Ba	7.08 $\pm$ 0.04	46.4 $\pm$ 10.5	86 $\pm$ 6	43

Table 5. Kinetics parameters obtained from modified-Gompertz model applied to the different BMP tests.

	Control	Fe-Co	Fe-Ni	Co-Ni	Fe-Ba	Co-Ba	Ni-Ba	Fe-Co-Ni-Ba
EDTA (mg/L)	2461	6187	6187	2469	9909	6187	6187	9913
G_{max} (mL/g VS)	351 ± 4	342 ± 6	344 ± 5	337 ± 4	258 ± 3	295 ± 2	314 ± 8	280 ± 6
R_{max} (mL/(g VS·d))	32.4 ± 0.7	28.4 ± 0.7	27.2 ± 0.6	40.0 ± 1.1	26.0 ± 0.6	30.6 ± 0.5	21.8 ± 0.6	18.3 ± 0.4
λ (d)	0.2 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	1.0 ± 0.1
r²	0.9988	0.9939	0.9948	0.9950	0.9955	0.9978	0.9918	0.9946
Error (%)	-0.8	-3.6	-2.6	0.9	-2.3	0.7	-11.1	-12.7
σ_{est}	3.58	8.11	8.10	9.05	6.33	5.06	8.67	6.09

σ_{est}, standard error of estimate.