Addressing the synergy determination in anaerobic co-digestion and the inoculum activity impact on BMP test

Andres Donoso-Bravo, Valentina Ortega, Yves Lesty, Hugues Vanden Bossche and Diego Olivares

ABSTRACT

Anaerobic mono-digestion and co-digestion are nowadays widely used in wastewater treatment plants (WWTP). However, the data processing of the conventional biochemical potential test (BMP) carried out to assess potential substrates should be enhanced to reduce the uncertainty of the results. In this study, two methodologies aiming to improve the data processing in anaerobic digestion studies were proposed. The methodologies aimed at the estimation of synergy in anaerobic co-digestion of organic waste and the standardization of the BMP test results by considering the activity of the inoculums under mono-digestion conditions. Both methodologies comprise the application of the Gompertz equation. For the first methodology, four cosubstrates and two types of substrates were used. Regarding synergy estimation, the cosubstrates dairy whey and grease sludge had an impact on the degradation kinetic. In regard to the second methodology, the results indicate that the activity of the inoculums exerts an influence on the BMP analysis, and it should be considered. This can be meaningful when comparing results among studies when different inoculums are used or even for studies where the same inoculum is used but it is taken at different reactor operational moments.

Key words | anaerobic co-digestion, biogas, BMP, inoculum, modeling, standardization

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NOTATION

WWTP	Wastewater treatment plant
AcoD	Anaerobic co-digestion
MSS	Mixed sewage sludge
TPS	Thermally pretreated sludge
Gr	Grease
DW	Dairy whey
GrS	Fatty sludge
WAS	Biological activated sludge
InC	Inoculum from a digester treating MSS
InA	Inoculum from a digester treating TPS
B	Biogas production on time
P	Maximum biogas production
R_m	Maximum biogas production rate
λ	Lag-phase parameter
EV	Expected value
OV	Observed value
MP	Model parameter
NMP	Normalized model parameter
CODt	Chemical oxygen demand (total)
CODs	Chemical oxygen demand (soluble)
VFA	Volatile fatty acids
TAN	Total ammonium nitrogen
TKN	Total Kjeldahl nitrogen
VS	Volatile solid

INTRODUCTION

Anaerobic co-digestion (AcoD) of waste is defined as the feeding of two or more substrates of different characteristics and/or origin to the same anaerobic digester. According to the data collected by the Water Environment Federation (WEF), out of the 1,191 wastewater treatment plants (WWTP) that have anaerobic digesters in the USA, 159

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are importing substrates to be co-digested in their systems (WEF 2018). The application of AcoD leads to important benefits, particularly in terms of biogas productivity and yield. This performance enhancement has been associated, compared to conventional mono-digestion, with better nutrient availability, a major presence of trace elements, the dilution of potential inhibitory compounds, and changes of the media rheology that improve the mass transfer and mixing (Mata-Alvarez *et al.* 2011).

Synergy is the preferred word used to describe the positive outcome, especially in terms of biogas production, driven by the mix of substrates for AcoD (Aichinger *et al.* 2015). However, the use or even abuse of this concept has led to some controversy (Insam & Markt 2016). It is easy to overestimate or misuse the term synergy of waste interactions (especially when positive results are obtained) since the sole increase in the biogas production in terms of the total amount, or standardized per mass of organic matter added, is not enough. For instance, a lipid-rich waste will yield more biogas than a carbohydrate-rich waste per gram of volatile material. Different authors have estimated the synergy due to the AcoD in different ways. The approaches have been based on the proportional summation of the experimental methane yields obtained from single substrate digestion (mono-digestion) versus those obtained from the AcoD of such substrates (Labatut *et al.* 2011; Ara *et al.* 2015), a COD balance (Aichinger *et al.* 2015; Xie *et al.* 2017), dynamic or steady state modeling approaches (Astals *et al.* 2014; Aichinger *et al.* 2015; Kim *et al.* 2017) or statistical modeling (Kashi *et al.* 2017).

In regard to the experimental set-up, the so-called biochemical potential test (BMP) is carried out to study the anaerobic biodegradability of a certain substrate.The BMP assay provides a platform to test the anaerobic degradability of different organic wastes as well as a proper comparison criterion to assess their degradability properties. However, the reported BMP results show significant variability, even for the same substrates. This is due to the large quantity of variables that can affect the results and that cannot be easily standardized, such as the substrate heterogeneity given by its size, composition or bioavailability, the inoculum's origin and quality, and the biogas measurement method (Raposo *et al.* 2011; Strömberg *et al.* 2014). Amid these variables, the quality of the inoculum exerts an important influence upon the results of the BMP test and there is no simple solution to overcome this situation (Angelidaki *et al.* 2009; Holliger *et al.* 2016; Steinmetz *et al.* 2016). For instance, Angelidaki *et al.* (2009) and Holliger *et al.* (2016) suggested several preparation methodologies for the inoculum selection and the digester seeding as well as inoculum activity estimation using acetate and cellulose as the substrate. Steinmetz *et al.* (2016) proposed a method for inoculum enrichment and maintenance in order to improve the reliability of the BMP results.

Therefore, the first aim of this study is to propose a methodology to estimate the absence or presence of synergy in anaerobic co-digestion. The second aim is to propose a methodology to discard the activity of the inoculum as a variable in a conventional mono-digestion batch test. Both proposals are based on a kinetic analysis of the biogas production curves.

MATERIALS AND METHODS

Synergy determination in AcoD test

Substrates, cosubstrates and inoculum

Two substrates, mixed sewage sludge (MSS) and thermally pretreated sludge (TPS) were used. MSS was composed of 60% primary sludge and 40% secondary sludge from the activated sludge system of the wastewater treatment plant (WWTP) Mapocho-Trebal of Santiago, Chile. TPS is a mixture of raw primary sludge (60%) and thermally pretreated secondary sludge (40%) that was hydrolyzed in a pilot system for 20 min at 6 bars/170 °C. The specific details of the mixtures are presented in Table 1. As cosubstrates, four organic wastes were evaluated: grease (Gr) from a food processing industry, dairy whey (DW) from a cheese factory, fatty sludge (GrS) coming from the primary settler of the WWTP of a food processing industry and waste activated sludge (WAS) from an extended aeration-based WWTP. For the tests where MSS was used as substrate, an inoculum (named InC) coming from a full-scale digester treating MSS was seeded. Likewise, for the tests where TPS was used as substrate, an inoculum (named InA) coming from a full-scale digester treating TPS was seeded. Both inoculums are expected to have different microbial diversity in terms of methanogens that is driven by the tolerance of the presence of ammonia and VFAs that are present in the substrates (Wett *et al.* 2014).

Experimental setup

The experimental evaluation was based on the BMP test, following the guidelines given in Angelidaki *et al.* (2009). For the experimental set-up, glass bottles of 120 mL with

Table 1 | Characterization of the substrates, cosubstrates and inoculums

	Substrates		Co-substrates				Inoculums	
	MSS	TPS	Gr	DW	WAS	GrS	InC	InA
pH	5.7–6.1	5.5–6.1	4.0	3.5	7.9	6.8	7.6–8.3	7.7–8.2
TS (g/kg)	37.6–44.6	39.8–58.9	660	101.5	139.0	131.3	21.7–26.5	25.5–32.2
VS (g/kg)	28.6–34.2	30.1–47.2	647.5	83.2	102.8	111.8	14.4–17.2	17.0–21.0
CODt (g/L)	56.7–71.8	61.5–93.7	–	100.9	152.6	203.5	26.5–32.9	35.5–38.0
COD_s (g/L)	0.8–1.6	5.7–8.0	30.2	6.0	6.0	3.4	0.5–1.0	1.3–1.6
VFA (mg/L)	1460–4500	1976–4988	–	2392	840	4800	204–348	404–520
TAN (mg/L)	312–1044	432–1870	39	<1	980	1622	960–1718	1760–3080
Proteins (%)	31	45	0	0	70	26		
Lipids (%)	41	35	100	11	6	53		
Carbohydrates (%)	28	20	0	89	24	21		

100 mL of working volume were used. A substrate inoculum (S/I) ratio of 0.5 $g_{VS}\, g_{VS}^{-1}$ and an inoculum concentration of 10 $g_{VS}\, L^{-1}$ were established. Sodium bicarbonate was added in a concentration of 0.5 g $NaHCO_3$ g_{VS}^{-1} of substrate and co-substrate to ensure neutral pH values during the assay. Blank trials with only inoculum were used to quantify the amount of biogas produced by endogenous respiration. Each BMP test was performed in duplicate. The temperature of the assay was set at mesophilic temperature (35 °C) and the bottles were stirred at 40 rpm in a thermoregulated shaker system. The biogas production was measured by an interchangeable 50 ml glass plug syringe (Luer lock). The net value of the biogas yield was obtained by subtracting the endogenous production of the blank bottle and converted to normal conditions of temperature (0 °C) and pressure (1 atm). The inoculum samples were degasified for at least three days before the test started.

Each experimental AcoD run (one for each cosubstrate tested with both substrates, separately) was carried out approximately a month apart from each other. Six proportions, namely: 0, 10, 25, 50, 75 and 100% of cosubstrate as percentage of total VS, were used. The fact that the inoculums were taken at different operational moments could affect the observed results and add an additional uncertainty when comparing different co-substrates to assess AcoD performance. However, the full-scale digesters, where the inoculums were taken from, are working in continuous mode so that, at least a priori, one could expect to have stable properties of the inoculums in the evaluation period. In any case, the influence of the inoculum's activity will be discussed in the Inoculum activity assessment section.

Data processing

From the accumulative biogas production, three kinetic parameters (P, R_m and λ) were estimated by fitting the Gompertz equation (Equation (1)).

$$B = P\, exp\left(-exp\left(\frac{R_m\, e}{P}(\lambda - t) + 1\right)\right) \quad (1)$$

where B: biogas production at time t (d), P: Maximum biogas production (ml g_{VS}^{-1}), R_m: Maximum biogas production rate (ml $g_{VS}^{-1}\, d^{-1}$) λ: Lag-phase time (d). To estimate the parameters, single least squares criteria was used as the minimization procedure. The standard deviation was estimated from the covariance matrix of the parameters obtained from the inverse of the Fisher Information Matrix (FIM), which gives a lower bound on the achievable parameter error covariance matrix. The estimated values of these parameters were defined as the observed values (OV) from the experiments.

The proposed methodology defines synergy (Si) as the standardized difference between the observed value (OV) of a certain parameter (P, R_m or λ) obtained from the Gompertz equation and the expected value (EV) of the parameter, as is shown in Equation (2).

$$Si_{P,R_m,\lambda}(\%) = \left(\frac{OV_{P,R_m,\lambda} - EV_{P,R_m,\lambda}}{EV_{P,R_m,\lambda}}\right)100 \quad (2)$$

The expected value of the parameter is determined from Equation (3). The approach chosen was based on the proportional effect of the cosubstrate and the substrate fractions

in the AcoD test (Ara *et al.* 2015).

$$EV = S\_F * \mathrm{MP_S} + \mathrm{CoS\_F} * \mathrm{MP_{CoS}} \quad (3)$$

where S_F and CoS_F represent the decimal fraction (dimensionless) related to the proportion of the substrate and cosubstrate added to test, respectively. $\mathrm{MP_s}$ and $\mathrm{MP_{CoS}}$ correspond to the model parameter values (P, R_m or λ) obtained during the mono-digestion of the substrate and cosubstrate, respectively.

Discarding inoculum activity in mono-digestion batch test

Experimental set-up, substrate and inoculum

BMP tests following the same guidelines described in the Substrates, cosubstrates and inoculum section were set up. As substrates, MSS and TPS were used for the BMP test and chopped cellulose-based paper (5 mm square) was used for the control BMP test. Chopped cellulose-based paper was chosen so that the activity of the main metabolic pathways of the anaerobic digestion process to produce biogas are tested, from the disintegration to the methanogenesis. InC and InA were used as inoculums for MSS and TPS, respectively. In contrast, both inoculums were used with chopped cellulose-based paper as substrate. The two inoculums (InC and InA) were taken from their respective digesters in four different moments, defined as M1, M2, M3 and M4, approximately a month apart from each other. It is worth pointing out that M1, M2, M3 and M4 also match with each of the four AcoD runs described in the Experimental setup section.

Data processing

The proposed methodology is based on the normalization of the kinetic parameters (OV) from the BMP test by the same parameter obtained from the control test. Therefore, first, the Gompertz equation fitting processing has to be carried out as described in the Data processing section, in order to get the parameters for all the substrates and the control test. Afterwards, and in order to discard the activity inoculum effect on the result and make a better comparison of the results, the parameters obtained for the BMP test of MSS and TPS were normalized by the kinetic parameters from the control test according to Equation (4).

$$NMP = \left(\frac{MP_{BMP}}{MP\_control}\right) \quad (4)$$

where, NMP represents the normalized model parameter (P, R_m or λ), $\mathrm{MP_{BMP}}$ is the model parameter obtained from the BMP test for the studied substrate and $\mathrm{MP_{\_control}}$ is the model parameter value (P, R_m or λ) for the control test.

Analytical methods

Total and volatile solids were measured using the gravimetric method according to APHA (2012). The chemical oxygen demand (COD) was measured by spectrophotometry and volatile fatty acids (VFA) were measured using the titration method according to APHA (2012). The pH was measured using a laboratory meter (WTW inoLab® pH7110) and the total ammonium nitrogen (TAN) was measured by an ion selective electrode (Dual Star Orion model). The total Kjeldahl nitrogen (TKN) was measured by digestion and oxidation according to APHA (2012). The protein content (Pc) was calculated based on the assumption that the protein contains 16% (w/w) nitrogen, therefore, the difference between the TKN and TAN was multiplied by a factor of 6.25 to estimate the protein content (Donoso-Bravo *et al.* 2011). Lipids were measured by Soxhlet extraction according to APHA (2012). Carbohydrates were estimated using a balance (Donoso-Bravo *et al.* 2011); that is, the total COD of the cosubstrates minus the COD of the proteins and the COD of the lipids.

RESULTS AND DISCUSSION

Characterization

The physicochemical characterization of the substrates, cosubstrates and inoculums used in the experiments is shown in Table 1. Regarding the substrates, the results show similarities in regard to the total organic content, although a wider range of values was obtained for MSS. The effect of the thermal hydrolysis process on WAS is shown in the higher content of CODs and TAN for TPS compared to MSS. With respect to the cosubstrates, they all have a high organic matter content; however, their macromolecular content is quite different, which allows us to categorize them as: pure-lipid (Gr), carbohydrate-rich (DW), protein-rich (WAS) and lipid-rich (GrS). According to this content, it can be estimated that the highest methane potential is expected for Gr, GrS, WAS and DW in decreasing order (Raposo *et al.* 2011). With respect to the inoculum, the physicochemical parameters were higher for InA than InC. The digester, where InA comes from, is fed with TPS

which has an impact on the final organic matter content of the digestate that is related to the presence of more recalcitrant soluble organic material formed during the hydrolysis process.

Parameter estimation and synergy in AcoD

The cumulative biogas production curves of the cosubstrates are shown in Figure 1. The model simulations for all conditions can be consulted in the Supplementary Material (Figures S1 to S8, available with the online version of this paper). In general, the determination coefficient (r^2) for all the curves was always above 0.98. Values of r^2 as low as 0.93 were obtained when WAS was used as cosubstrate in a proportion of the feeding above 50% as VS. This was caused because, as it is shown in Figures S7 and S8 from the Supplementary Material (available online), there was an underestimation of the initial biogas production rate, therefore the interpretation of the results must be done carefully. The estimated parameters of the Gompertz equation, for all the studied conditions, are presented in Table 2. The standard deviation of the parameters was also estimated, and it is presented in the Supplementary Material (Table S1, available online). Overall, all the values were always below 10% of the average value (coefficient of variation) except for WAS where the values of R_m showed higher error values, which reflects the underestimation of the initial biogas production rate.

As expected, Gr showed the highest P value among the evaluated cosubstrates due to its lipid content, such that if the goal of implementing co-digestion is to increase the biogas production, this would be the best cosubstrate. However, the feeding of Gr may lead to some operational problems due to mass transfer problems, inhibition and foaming, so pilot studies should be first carried out (Long *et al.* 2012). On the other hand, DW and SGr showed the highest values of R_m, which means that both cosubstrates may be treated with the same or even lower (depending on the presence of synergy) residence time. The highest values for the lag phase were found for Gr for both types of inoculums, which demonstrated the difficulties encountered by the microorganism to access this cosubstrate and start the biodegradation process. The literature comparison of the results was carried out only with studies where the same model was applied to fit the data. For DW, Zahan *et al.* (2018), who used yoghurt whey, obtained similar values for P, but far lower values of R_m probably due to the pH of the tests. No lag phase was observed. In regard to the parameter for SGr, the values of P are similar to those obtained for similar substrates such as grease trap waste in Yalcinkaya & Malina (2015). In that study, the maximum productivity almost doubled the value obtained in this study. The lag phase, in turn, was four times greater than the one obtained in this study. For other lipid-rich residues, such as nut residues, the parameter values obtained here were higher (Shen *et al.* 2018).

The synergy determination results of the AcoD test are shown in Table 3. The major impact of the cosubstrate addition lies in the biogas production kinetic (R_m) over the biogas potential (P). Only Gr at 50% of cosubstrate fraction yielded some significant positive synergy for the biogas potential when InC was used. It is worth pointing out that synergy percentage values (parameter Si in Equation (2)) of at least 10% are considered as significant. Regarding R_m, there were significant positive synergies with Gr with both inoculums. In general, the addition of cosubstrates had a positive impact in reducing the lag phase for all cosubstrates with both inoculums, although it is worth stressing that the importance of this parameter is limited to a batch operation and enzymatic reactions, which does not apply to continuous reactors where the cosubstrate is applied on a regular basis. It may become relevant when using unacclimated biomass or to the start-up of a continuous reactor. The proposed methodology includes the Gompertz equation fitting process in the estimation of the synergy of the AcoD. The main differences to other approaches are, first, that the effect of cosubstrate addition in terms of synergy can be assessed using three parameters instead of only the experimental observation of the biogas yield and, second, the direct use of the Gompertz equation to draw the parameter values. Ebner *et al.* (2016) used the first order equation to draw the hydrolytic coefficient and the extent of degradation from the AcoD BMP test. To estimate the presence of synergy, they defined two parameters, the co-digestion performance index (CPI) and co-digestion rate index (CRI); however, their determination required a mathematical analysis or numerical estimation, which is not as straightforward as the one presented in this study.

Inoculum activity assessment

BMP test of the control test

The cumulative biogas production curves of the control tests are shown in Figure 2 and the kinetic parameters are shown in Table 4. The standard deviation of the parameters is presented in Table S2 in the Supplementary

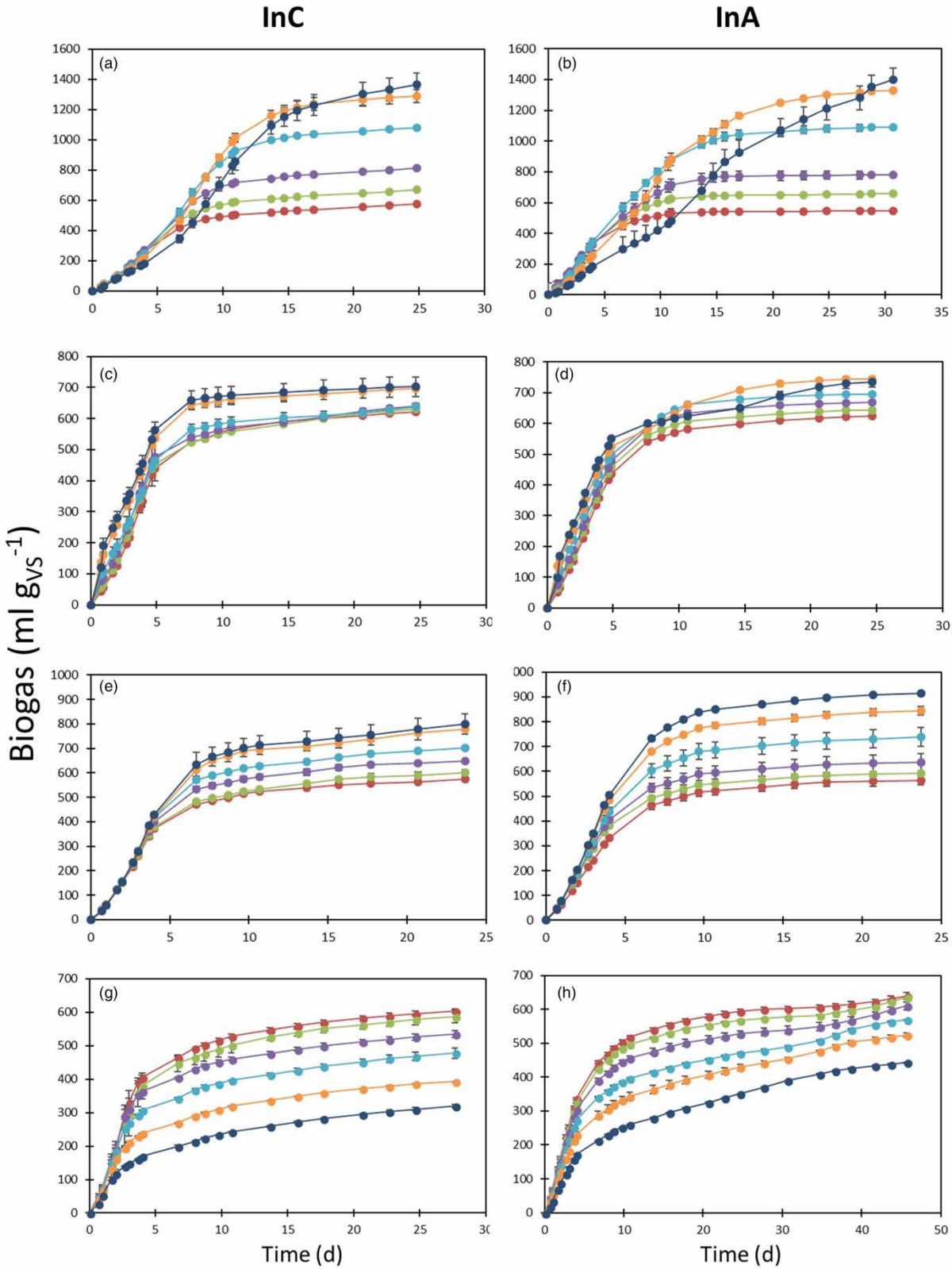

Figure 1 | Accumulative biogas production of the BMP test using MSS (for In C) and TPS (for inA) as the main substrates. Co-substrates: GR (a and b) DW (c and d) GrS (e and f) and WAS (g and h). Co-substrate proportion: 0% red, 10% green, 25% purple, 50% light blue, 75% orange, 100% dark blue. The full color version of this figure is available in the online version of this paper, at http://dx.doi.org/10.2166/wst.2019.292.

Table 2 | Kinetic parameter values from the BMP test of the AcoD experiments

	Model parameters																	
	P ($ml_{biogas}\ g_{VS}^{-1}$)						Rm ($ml_{biogas}\ g_{VS}^{-1}\ d^{-1}$)						λ (d)					
	Cosubstrate proportion (%)						Cosubstrate proportion (%)						Cosubstrate proportion (%)					
	0	10	25	50	75	100	0	10	25	50	75	100	0	10	25	50	75	100
	MSS (substrate) - InC (inoculum)																	
Gr	545	641	792	1083	1322	1428	77	86	97	116	124	107	0.8	1.0	1.2	1.8	2.4	2.9
DW	597	595	604	616	683	692	108	110	113	101	122	129	0.8	0.6	0.5	0.1	0.0	0.0
SGr	543	563	614	662	735	753	104	105	114	121	124	129	0.5	0.5	0.6	0.7	0.8	0.8
WAS	556	538	485	425	346	276	116	101	109	86	67	43	0.3	0.2	0.2	0	0	0
	TPS (substrate) – InA (inoculum)																	
Gr	540	649	782	1089	1341	1521	92	92	88	100	95	64	0.3	0.4	0.4	0.9	1.7	2.8
DW	607	629	655	681	703	674	106	110	111	111	118	130	0.5	0.4	0.3	0.0	0.0	0.0
SGr	547	570	614	713	817	888	92	106	114	124	140	147	0.4	0.3	0.4	0.5	0.6	0.6
WAS	593	572	537	492	459	397	75	72	66	55	42	27	0	0	0	0	0	0

Table 3 | Synergy percentage of the AcoD test

	P				R_m				λ			
	Cosubstrate proportion (%)											
	10	25	50	75	10	25	50	75	10	25	50	75
	MSS (substrate) - InC (inoculum)											
Gr	+1	+3	+10	+9	+7	+15	+26	+25	−1	+12	+4	0
DW	−2	−2	−4	3	0	0	−14	−2	+10	+29	+215	>1000
SGr	0	+3	+2	+5	−2	+3	+3	+1	+6	−1	−2	−4
WAS	0	+2	0	+2	−7	+12	+8	+9	+9	−8	>1000	>1000
	TPS (substrate) – InA (inoculum)											
Gr	+2	0	+6	+5	+2	+3	+28	+33	+32	+151	+80	+26
DW	+3	+5	+6	+7	+1	0	−5	−4	+61	+100	>1000	>1000
SGr	+2	−1	−3	−2	+8	+8	+4	+5	+34	+22	+3	+1
WAS	0	−1	−1	+3	−11	12	+24	+32	+9	+42	+90	>1000

Material (available online). It can be seen that the P values were relatively constant throughout the experimental runs for both inoculums, with similar averages for both: 667 ± 34 ml g_{VS}^{-1} and 655 ± 36 ml g_{VS}^{-1}, respectively. On the contrary, the results are quite different in regard to R_m; from the initial value (M1) there was a significant increase (double) for both inoculums. After that, the values remained fairly constant. With respect to the values themselves, R_m for InC is between 75% and 90% greater that the values for InA, which shows the hydrolytic capacity of the inoculum that comes from a digester fed with MSS in comparison to the inoculum that deals with a substrate that has a higher fraction of soluble organic matter. Likewise, the 30% difference between the values of the lag phase for the inoculum confirmed what is abovementioned regarding the hydrolytic capacity. This control test allows us not only to ensure the activity of the inoculum of the BMP test analysis but also to analyze the current operation of the digester from which the anaerobic biomass is taken.

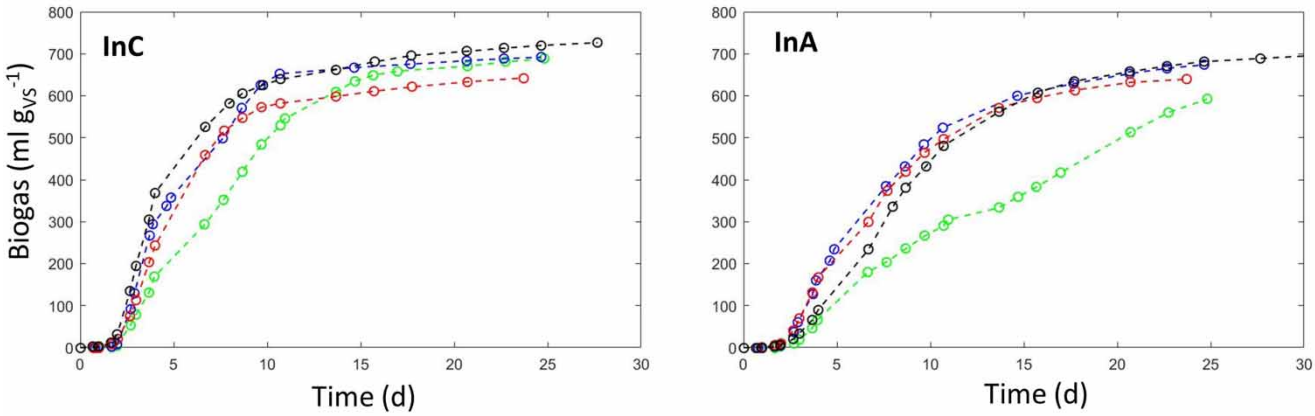

Figure 2 | Accumulative biogas production of the control BMP test. M1, green, M2, blue, M3, red, M4, black. The full color version of this figure is available in the online version of this paper, at http://dx.doi.org/10.2166/wst.2019.292.

Table 4 | Kinetic parameter values from the control test (chopped paper used as substrate)

	InC			InA		
BMP test	***P*** **(ml** g_{VS}^{-1}**)**	R_m **(ml** g_{VS}^{-1} d^{-1}**)**	λ **(d)**	***P*** **(ml** g_{VS}^{-1}**)**	R_m **(ml** g_{VS}^{-1} d^{-1}**)**	λ **(d)**
M1	690	69	2.3	626	34	2.6
M2	673	115	1.8	656	72	2.1
M3	618	110	2.0	632	68	2.2
M4	688	123	1.5	704	64	3.0

Standardization of the BMP test by inoculum activity

The incorporation of the inoculum activity results into the BMP analysis by parameter estimation is presented in Table 5 and the values of the kinetic parameters (P, R_m, λ) are normalized by the values obtained for the inoculum activity test (Table 4). Moreover, the values of the parameters from a traditional analysis are also included in Table 5.

Table 5 | Conventional and normalized parameters taking the results of the control test into account

BMP test	P^a **(ml** g_{VS}^{-1}**)**	NMP_P	$R_m{}^a$ **(ml** g_{VS}^{-1} d^{-1}**)**	NMP_{Rm}	λ^a **(d)**	NMP_λ
	MSS-InC					
M1	545	0.79 ± 0.02	77	1.11 ± 0.05	0.8	0.33 ± 0.14
M2	597	0.88 ± 0.02	108	0.94 ± 0.09	0.8	0.44 ± 0.18
M3	543	0.88 ± 0.01	104	0.94 ± 0.07	0.5	0.26 ± 0.26
M4	556	0.81 ± 0.02	116	0.94 ± 0.13	0.3	0.18 ± 0.68
	TPS-InA					
M1	540	0.86 ± 0.04	92	2.73 ± 0.07	0.3	0.12 ± 0.29
M2	607	0.92 ± 0.02	106	1.46 ± 0.06	0.5	0.25 ± 0.18
M3	547	0.86 ± 0.02	92	1.35 ± 0.02	0.4	0.19 ± 0.24
M4	593	0.84 ± 0.01	75	1.17 ± 0.08	0	0

[a]Values taken from Table 2 where no cosubstrates were added to the test.

Overall, the P values follow the same trend for both inoculums under a traditional analysis (Table 2). The most staggering result is the difference for R_m. In the traditional analysis, a significant increase from M1 to M2 is observed, which would indicate a possible change in the sludge properties with the presence of a more soluble fraction, which is also indicated in the CODs analysis. However, when the values are normalized by the control test parameter, on the contrary, in the case of InC, a slight decrease in R_m is observed, which agrees with the slight decrease in the total COD measured. In the case of InA, with the traditional comparison from M1 to M2, a small increase in R_m is observed, which is totally the opposite to the large drop observed in the parameter after normalization. The result from the conventional analysis indicates a significant change in the soluble fraction of the TPS, which is known by the presence of soluble recalcitrant compounds formed during the thermo-hydrolysis reaction (Carrère *et al.* 2010). However, after normalizing those values for the inoculum activity parameters, the situation is the opposite since a decrease from M1 to M2 (not as sharp as the increase though) was observed. In regard to λ, the large variability of the NMP makes it difficult to draw any reliable conclusion from the analysis. This methodology allows us to better discard the actual influence of the inoculum activity and isolate the influence of the substrate alone.

It is worth pointing out that other control substrates such as microcrystalline cellulose can also be employed for standardization of the inoculum activity. In fact, the biogas production curves are quite similar between that

substrate and the one presented in this study (Díaz *et al.* 2011).

CONCLUSIONS

In this study, two methodologies aiming to improve the data processing in anaerobic digestion studies were proposed. The first one addresses the estimation of synergy in anaerobic co-digestion of organic waste and sewage sludge by using an integrated methodology based on the BMP test, Gompertz equation fitting and comparison of parameter values. The impact of four cosubstrates and two types of sewage sludge (substrates) were evaluated. In regard to the synergy estimation, dairy whey and grease sludge had an impact on the degradation kinetic, although only dairy whey decreased the lag phase compared to mono-digestion. The other methodology comprises the standardization of the BMP test results by taking into account the activity of the inoculums. This is carried out by normalizing the parameters obtained from the Gompertz equation fit from the BMP of the substrates with the parameter values obtained from the control test, where chopped paper was used as the substrate. The results indicate that the assessment can be significantly altered by taking this into account and gives a more realistic picture of the evaluation. This can be meaningful to compare results among studies when different inoculums are used or even for studies where the same inoculum is used but it is taken at different reactor operational moments.

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