Assessment of a fast method to predict the biochemical methane potential based on biodegradable COD obtained by fractionation respirometric tests

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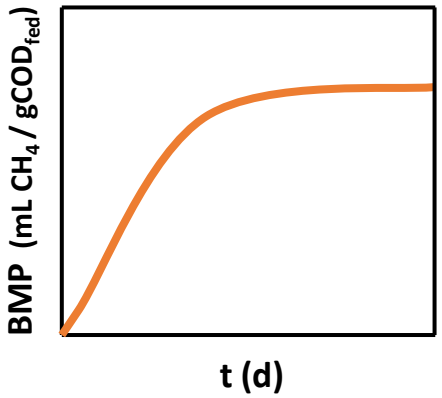
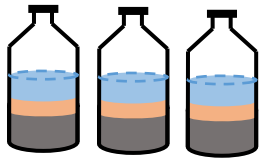
Anaerobic BMP tests

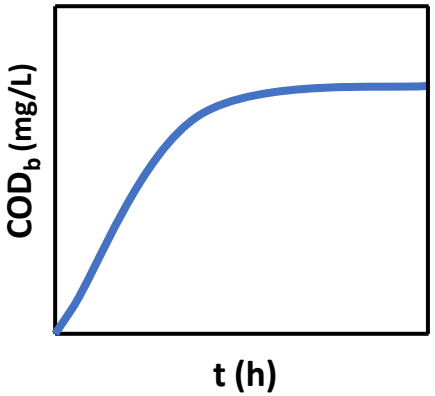


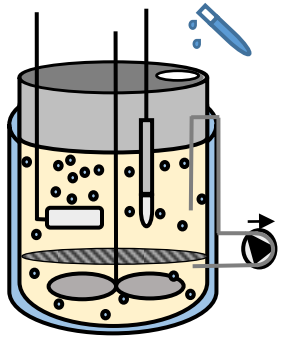


BMP: Biomethane Potential

Time – consuming
(weeks - months)

Aerobic COD fractionation





COD: Chemical Oxygen Demand

Faster alternative?

Early prediction
(hours)

11 Different substrates tested, 2 laboratories

Santiago de Compostela (SPAIN)
1,2. Dairy industry WW
3. Digestate
4. OFMSW permeate
5. Fish-canning WW
6. Mussel Cooker WW
7. Sewage Sludge

Viña del Mar (CHILE)
8. Wine industry WW
9. Tobacco industry WW
10. Chipboard factory WW
11. Slaughterhouse WW

WW: wastewater

Methanized COD (COD_{met}) = Biodegradable COD (COD_b)

$$BMP = \frac{COD_b \cdot 350\ mL\ CH_4 / g\ COD}{COD_{fed}}$$

Highlights

- A novel and rapid methodology for BMP prediction based on COD fractionation.
- Eleven substrates of diverse nature, origin and complexity were tested.
- Methanized COD coincided with biodegradable soluble + particulate fractions.
- Testing time was reduced from weeks-months (BMP) to hours-days (COD fractionation).
- An interlaboratory comparison validated the developed methodology.

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ABSTRACT

The biochemical methane potential test (BMP) is the most common analytical technique to predict the performance of anaerobic digesters. However, this assay is time-consuming (from 20 to over than 100 days) and consequently impractical when it is necessary to obtain a quick result. Several methods are available for faster BMP prediction but, unfortunately, there is still a lack of a clear alternative. Current aerobic tests underestimate the BMP of substrates since they only detect the easily biodegradable COD. In this context, the potential of COD fractionation assays, which allow the determination of the particulate slowly biodegradable fraction, was evaluated here as an alternative to early predict the BMP of substrates. Seven different origin waste streams were tested and the anaerobically biodegraded organic matter (COD_{met}) was compared with the different COD fractions. When considering adapted microorganisms, the appropriate operational conditions and the required biodegradation time, the differences between the COD_{met}, determined through BMP tests, and the biodegradable COD (COD_b) obtained by respirometry, were not significant (COD_{met} (57.8026 ± 21.2875) and COD_b (55.6491 ± 21.3417), t (5) = 0.189, p = 0.853). Therefore, results suggest that the BMP of a substrate might be early predicted from its COD_b in only few hours. This methodology was validated by the performance of an inter-laboratory study carried out in Chile considering four additional substrates.

Keywords: anaerobic digestion; biodegradability; BMP; COD fractionation.

1. INTRODUCTION

The European Union (EU) is working towards a climate-neutral Europe by 2050. Key targets for 2030 include reducing greenhouse gas emissions (at least 40 % from 1990 levels), increasing the share of renewable energy usage (at least 32 %) and improving energy efficiency (at least 32.5 %). In this context, the anaerobic digestion process (AD) will contribute to achieving these objectives (Mottet et al., 2009). Biodegradable organic materials can be profitably used as renewable energy sources (RES) since they can be converted into methane-rich biogas (60 – 70%) (Da Silva et al., 2018). Over the past two decades, the growing paradigm-shift from fossil fuels to RES, including biogas was focused on three industrial sectors: transport, heating/cooling and power/electricity production (Malico et al., 2019; Sherwood, 2020). Banja et al. (2019) reported that the largest share of financial measures implemented over the period 2005 – 2015 was dedicated to favour biomass processing for electricity production and 36 % was meant for heating/cooling purposes. In the heating/cooling sector, a 35.7 % of the total measures were dedicated to biogas whereas in the transport sector, these constituted less than 5 %.

Many feedstocks are suitable for biogas production if they contain carbohydrates, proteins, fats, cellulose and hemicelluloses as the main components. Nonetheless, the biomethane production depends on several factors: nutrients, total and volatile solids (TS and VS), chemical oxygen demand concentrations (COD), and carbon/nitrogen ratio (C/N). In addition, the presence of substances like free ammonia (FA), H_2S, light metal ions (e.g. sodium, potassium and magnesium), heavy metal ions (e.g. chromium, iron and cobalt) and certain organic compounds (e.g. alkyl benzenes, halogenated benzenes and alcohols) can inhibit the process leading to low methane production yields and process instability (Chen et al., 2008; Jingura and Kamusoko, 2017).

The evaluation of the ultimate amount of methane produced under anaerobic conditions (mL CH_4 /g COD) and the production kinetics (Lesteur et al., 2010) are crucial in order to predict the performance of the anaerobic digestion process (Kianmehr et al., 2013). The Biochemical Methane Potential (BMP) test is the most common assay in this context (Kianmehr et al., 2013), due to its validity and reliability (Lesteur et al., 2010). Its general principle consists of mixing an organic feedstock with an inoculum under experimental conditions that mimic the AD conditions in real practice (Lesteur et al., 2010), and quantify the gas produced by manometric or volumetric methods (Jingura and Kamusoko, 2017). However, the BMP test is time-consuming (from 20 to over 100 days) (Da Silva et al., 2018). Automatic BMP tests have been recently developed with the aim of reducing the disadvantages of the conventional ones. They directly measure the methane production on-line, require less labour, use inexpensive equipment and provide high quality and adequate quantity of data (Jingura and Kamusoko, 2017). Unfortunately, automatic BMP tests are also based on microbial processes and consequently very time-consuming (Kianmehr et al., 2013).

In order to reduce the time needed for the prediction of the biochemical methane production and the anaerobic digestion kinetics, different experimental and theoretical strategies have been studied (Table 1). However, most of these alternatives were studied with solid substrates and even though they are shorter, they still present important disadvantages in comparison to BMP tests. Theoretical methods presume complete degradation of organic matter and for this reason the obtained BMP is over-estimated; mathematical models are complex and require exhaustive organic matter characterization; spectroscopic methods and destructive techniques are expensive and still require more time for development and validation; and the so-called respirometric tests underestimate the BMP of the substrates (Jingura and Kamusoko, 2017; Lesteur et

al., 2010). Current respirometric tests present an incubation time (from 5 to 30 days) shorter than BMP tests (up to 100 days) and their set-up is technically simpler. However, they only detect the readily available and easily degradable organic matter (Bayard et al., 2016; Cossu and Raga, 2008).

Previous experiences concerning the comparison between anaerobic and aerobic biodegradation of different substrates in very diverse reaction systems (Table 2) showed that, in general, although kinetics were very different, the potentially biodegraded organic matter was almost the same. In addition, Bayard et al. (2018) observed that the biodegradability of municipal solid waste was controlled by the specific features of the substrates tested rather than the environmental conditions (aerobic or anaerobic). This result suggests that it might be possible to predict the BMP of substrates using aerobic respirometric tests if these were able to detect not only the easily biodegradable fraction but also the particulate slowly biodegradable one, which is possible towards COD fractionation assays.

The objective of the present study was to find a relationship between the anaerobically degraded COD, obtained through conventional BMP tests, and the aerobically biodegraded COD, determined by respirometric COD fractionation assays, with the aim of early predicting the ultimate biochemical methane production of a certain substrate without requiring further BMP tests.

106 Table 1. Alternative methods for predicting the anaerobic biodegradability.

Method	Substrate	Reference
Theoretical methods		
Stoichiometric analysis (Buswell Formula)	Animal slurry & Energy crops Vinegar residue Lignocellulosic biomass	Triolo et al., 2011 Feng et al., 2013 Thomsen et al., 2014
Energy value of feedstocks estimated from its elemental composition (Modified Dulong formula)	Food waste	Browne and Murphy, 2013
Chemical composition	Maize Biomass material Fruit and vegetable solid	Rath et al., 2013 Godin et al., 2015 Gunaseelan, 2004
Mathematical Models		
Biodegradability prediction by Artificial Neural Networks	Wastewater & waste activated sludge	Kianmehr et al., 2013
Regression models between biogas potential and certain chemical and biological parameters	Waste activated sludge	Mottet et al., 2009
Mathematical methodology for the prediction of maximum methane production and kinetic parameters	Sewage sludge, a mixture of sewage sludge & slaughterhouse wastes	Da Silva et al., 2018
Mathematical correlations between variables	A large variety of lignocellulosic biomass materials and related organic residues	Bayard et al., 2016
Assessment and validation of previously developed linear regression models	Grassland biomass	Dandikas et al., 2018
BMP prediction from test data recorded in the first two weeks	Treated municipal solid waste	Howell and Bennett, 2019
Spectroscopic techniques		
Mid-infrared (MIR) spectrometry	Crop residues, grasses & hedge & tree trimmings.	Bekiaris et al., 2015
Near-infrared (NIR) spectrometry	Municipal solid waste, rice, cardboard, lignocellulosic material & food wastes	Ward, 2016
Envital Kit (based on fluorescence redox indicator)	Sewage sludge	Bellaton et al., 2016
Ultra-violet-visible spectrometry (UV-vis)	Olive Mill Wastewater	El Hajjouji et al., 2008
Destructive techniques combined with fast analytical techniques		
Pyrolysis combined with GC-MS and	Wastewater sludge	Jarde et al., 2003 Parnaudeau and Dignac, 2007
Advanced oxidation processes	Carbohydrates	Roig and Thomas, 2003
Physicochemical methods		
Electrical conductivity test, Soluble Chemical Oxygen Demand test and enzymatic hydrolysis test.	Ensiled Meadow Grass	Tsapekos et al., 2015
Aerobic tests		
The link between aerobic and anaerobic biodegradability of sludge	Activated sludge	Ekama et al., 2007
Aerobic tests as shorter respirometric tests	Municipal waste from landfills	Cossu and Raga, 2008 Wagland and Tyrrel, 2010 Ponsá et al., 2008
prediction of the methane generation rate constant using a large-scale respirometer	Municipal waste from landfills	Park et al., 2017

107 **Table 2.** Comparative results of studies concerning the differences between anaerobic and aerobic biodegradability of substrates.

Substrate	Anaerobic system		Aerobic system		Reference
	Performance	Results	Perfomance	Results	
Pharmaceutical wastewater from the industrial production of antibiotics consisting of an aqueous mixture of cleaning wastewaters, cleaning products, antibiotics, solvents, and intermediates.	Zahn-Welles test methodology	70 % TOC removal after 12 days diluting the substrate and 63 % TOC removal after 28 days considering the raw substrate.	Adaptation of the method proposed by Owen et al., 1979	80 % and 89 % of TOC removal after 28 days with the diluted and the raw substrate, respectively.	Marcelino et al., 2016
Industrial strength 2, 4 – dychlorophenoxyacetic acid (2, 4 – D) wastewater in the presence of glucose.	Lab-scale anaerobic SBR	Complete biodegradation of 100 mg/L after 24 days. Total biodegradation was limited to 120 mg/L.	Lab-scale aerobic SBR	Complete biodegradation of 100 mg/L after 12 days. Total biodegradation was limited to 600 mg/L.	Elefsiniotis and Wareham, 2013 Celis et al., 2008
Bleaching pulp mill acid and alkaline effluents.	Adapted Zahn-Welles test methodology	Degradation of 62 % COD in the acid effluent and 58 % COD the alkaline in 30 days.	Method of Field et al., 1989	Degradation of 68 % COD in the acid effluent and 75 % COD the alkaline for 2 days.	Amaral et al., 2015
Slaughterhouse wastewater with high blood content.	UASB reactor	80 % COD degradation after 20 days.	COD fractionation respirometric tests	84 % degradation of COD (51 % rapidly hydrolyzable and 33 % slowly hydrolysable).	Del Pozo et al., 2003
Wastewater with pesticide content.	Anaerobic SBR	99 % pesticide removal and 93 % TOC removal after 200 days.	SBBR	88 % pesticide removal and 80 % TOC removal after 250 days.	Al Momani et al., 2010
Waste Activated Sludge from an urban WWTP	Anaerobic digestion batch lab-scale tests	65 % of protein degradation and 66 % of VS after 94 days.	Aerobic digestion batch lab-scale tests	68 % of protein degradation and 66 % of VS after 89 days.	Shao et al., 2013

108 COD (chemical oxygen demand), SBR (sequencing batch reactor), SBBR (sequencing batch biofilm reactor), TOC (total organic carbon), UASB (upflow anaerobic sludge blanket), VS (volatile
109 solids), WWTP (wastewater treatment plant).

110

2. MATERIALS AND METHODS

2.1. Substrates studied

Experiments were carried out in Spain with seven different waste streams, six liquid effluents (S1 – S6), and one solid waste (S7). These waste streams were: S1, low-load stream resultant from the mixture of dairy industry wastewater from milk production, cleaning effluents and urban sewage from office buildings and changing rooms; S2, dairy industry wastewater from condensed milk production; S3, anaerobic digestion effluent from an urban wastewater treatment plant (WWTP) with thermal hydrolysis pre-treatment; S4, the digested organic fraction of municipal solid waste (OFMSW) from an anaerobic membrane bioreactor (AnMBR) located in a metropolitan plant for integral waste treatment; S5, saline and low-load fish-canning industry wastewater resultant of the washing effluents of the factory; S6, high load fish-canning industry wastewater from mussel cookers, and S7, a mixture of primary (70 %) and secondary (30 %) sludge from an urban WWTP. To validate the proposed methodology, four additional substrates (SC1 – SC4) were tested in Chile following the same procedures considered in section 2. The waste streams were: SC1, wine industry wastewater; SC2, tobacco industry wastewater; SC3, wastewater from a chipboard factory that uses urea-formaldehyde as glue; and SC4,slaughterhouse wastewater.

The substrates studied were very diverse regarding both origin and complexity. They were characterized by different COD and nitrogen concentrations, alkalinity, organic matter composition, and biodegradability to cover a broad range of compositions. Their distinctive properties, especially those that could affect the performance of BMP tests and COD fractionation assays are shown in Table 3 (see more details in Table S4 of Supplementary Material).

Table 3. Substrates characterization.

Parameter	Sample										
	S1	S2	S3	S4	S5	S6	S7	SC1	SC2	SC3	SC4
pH	7.1	4.3	8.4	8.4	6.3	5.3	6.7	6.90	8.70	7.40	6.90
TSS – *TS (g/L)	261 ± 14	333 ± 10	119 ±16	270 ± 13	2,074 ± 278	2,686 ± 245	17,607 ± 753	2.01 ± 0.34	0.48 ± 0.03	0.31 ± 0.02	1.98 ± 0.20
VSS – *VS (g/L)	237 ± 12	321 ± 12	74 ± 3	149 ± 1	1,440 ± 100	1,737 ± 217	14,723 ± 605	1.29 ± 0.09	0.44 ± 0.01	0.30 ± 0.01	1.73 ± 0.01
COD_t (mg/L)	707 ± 3	3,214 ± 31	2,477 ± 37	5,349 ± 61	3,346 ± 22	17,360 ± 370	18,455 ± 320	4,520 ± 51	1,605 ± 151	820 ± 10	452 ± 50
COD_S (mg/L)	388 ± 6	2,535 ± 22	2432 ± 55	5,135 ± 40	369 ± 46	16,707 ± 81	2,312 ± 109	3,020 ± 103	1,568 ± 411	310 ± 14	302 ± 100
Prot. (mg COD/L)	146 ± 4	176 ± 4	339 ± 20	2,094 ± 137	60 ± 3	5,734 ± 136	7,638	36.30 ± 2.24	316.54 ± 4.09	29.70 ± 5.28	578.56 ± 5.28
Carb. (mg COD/L)	16 ± 0.5	343 ± 25	266 ± 12	173 ± 9	17 ± 0.3	11,370 ± 383	9267	138.03 ± 24.72	306.88 ± 55.75	263.76 ± 17.55	32.31 ± 4.71
Lipids (mg COD/L)	40 ± 2	160 ± 29	BDL	BDL	BDL	680 ± 115	795	BDL	6 ± 1	BDL	BDL
VFAs (mg COD/L)	107	432	45	301	33	363	1,540	390	597	43	187
TN (mg N/L)	16	19	1,476	4,544	23	1,094	-	8	602	85	312
TKN (mg/L)	-	-	-	-	-	-	829	-	-	-	-
NH_4^+ (mg/L)	0.71	0.82	1,375	4,366	13	50	96	1.29	543	29	189
Cl^- (mg/L)	180	61	177	3,339	14,724	12,902	-	-	-	-	-
Na^+ (mg/L)	782	321	57	1,748	5,427	7,333	-	-	-	-	-

Proteins (Prot.) and carbohydrates (Carb.) are referred to the soluble fraction for substrates S1 – S6 and SC1 – SC4. In the case of S7 data are referred to the total and were estimated considering that: VS = carbohydrates + proteins + lipids; proteins = 6.25 (TNK – NH_4^+) and COD = carbohydrates · 1.1 + proteins · 1.3 + lipids ·2.9. BDL (below detection limit).

* In the case of S7 data are referred to TS and VS whereas in S1-S6 and SC1-SC4 data are referred to TSS and VSS, respectively.

2.2. Anaerobic biodegradability tests – Conventional BMP tests

The BMP of the samples was determined by adapting the guidelines proposed by Holliger et al. (2016).

Test setup

Anaerobic biodegradability tests were carried out in triplicate in glass flasks of 500 mL (useful volume of 375 mL) sealed with coiled butyl rubber stoppers and incubated in a temperature-controlled shaking bath under continuous mixing (New Brunswick Innova 4300, USA). Bottles were inoculated at a variable inoculum to substrate ratio (ISR) depending on the substrate characteristics (Table S1 of Supplementary Material) and filled up to the working volume with tap water. When necessary, part of the ammonium (NH_4^+) present in the substrate was stripped out to avoid inhibitory FA concentrations. Thus, total ammoniacal nitrogen (TAN) concentrations above 1000-1500 mg/L have been reported as the primary cause of AD failure due to FA inhibition (Capson-Tojo et al., 2020). FA was calculated according to Anthonisen et al. (1976) from the pH and NH_4^+ considering proteins hydrolyzation into NH_4^+ concentration values. Stripping was carried out in aerated glass beakers at a pH of 10.0 (NaOH addition) (Zhang et al., 2018) and NH_4^+ and COD concentrations were periodically measured to determine the optimum pre-treatment time to obtain the needed NH_4^+ stripping without significant COD removal (under 5 % of the initial COD). NH_4^+ in the substrate was never completely removed since nitrogen is needed for microbial growth.

Prior to BMP tests performance, the inoculum was degassed (37 ºC and continuous mixing) in order to deplete the residual biodegradable organic material. Neither any growth media nor buffer was added thus the inoculum together with the tap water, provided an important source of macro- and micro-nutrients, trace elements, vitamins,

and pH-buffering capacity (inoculum alkalinity was higher than 3 g $CaCO_3$/L). Before setting up the BMP tests, pH was measured and adjusted with NaOH or HCl solutions. Test experimental conditions for each substrate are outlined in Table S1 of Supplementary Material. In addition, blank assays with tap water and no substrate were carried out under the same conditions as their respective BMP tests to know the background methane production from the inoculum. Once prepared, the bottles were flushed with pure N_2 for 1 – 3 min to ensure anaerobic conditions prior to the start-up of the tests.

The volume of biogas produced was determined by the variation of pressure in the headspace of the glass flask by means of a pressure transducer (Centrepoint Electronics, UK) and the biogas composition was measured by gas chromatography (5890A Hewlett Packard, USA). Tests were terminated when the daily methane production during three consecutive days varied less than 1 % of the accumulated volume of methane.

Calculations

The experimental ultimate volume of methane produced was determined by subtracting the methane production of the blank from the methane production of the substrate. The BMP of the substrate (as mL CH_4/g COD) was calculated from the ultimate volume of methane produced under standard conditions (0 ˚C and 1,013 hPa) divided by the grams of COD_t (total chemical oxygen demand) initially introduced as substrate (COD_{fed}) in the flask (equation 1). Anaerobic biodegradability (as %) was determined by dividing the BMP by the theoretical maximum methane yield of 350 mL CH_4/g COD (0 ˚C and 1,013 hPa) (equation 2) (Mottet et al., 2009). The fraction of COD converted into methane (COD_{met}) was calculated by equation 3 and the percentage of CH_4 in the biogas was determined according to a German standard procedure (VDI 4630, 2016).

190

$$\text{BMP (mL } CH_4/gCOD_{fed}) = CH_4 \text{ produced (mL)} / COD_{fed} \text{ (g)} \quad (1)$$

$$\text{Anaerobic biodegradability (\%)} = \text{BMP (mL } CH_4/g\ COD_{fed}) / 350 \text{ (mL } CH_4/g\ COD) \cdot 100 \quad (2)$$

$$COD_{met} \text{ (g/L)} = (\text{Anaerobic biodegradability})/100 \cdot COD_{fed} \text{ (g)} \quad (3)$$

191 **2.3. COD fractionation: a combination of analytical determination and**
192 **respirometric tests**

193 Definition of the COD fractions

194 The total COD of the wastewater samples (COD_t) was fractionated in its two major
195 components; total biodegradable (COD_b) and total inert (COD_i). The COD_b was further
196 subdivided into soluble readily biodegradable (S_b) and particulate slowly biodegradable
197 (X_b), whereas the COD_i was subdivided into soluble inert (S_i) and particulate inert (X_i)
198 (see Figure S1 of supplementary material). COD_t (sum of COD_b and COD_i) and soluble
199 chemical oxygen demand (COD_s) (sum of S_b and S_i) were analytically measured, while
200 COD_b and S_b were individually determined by respirometric assays. Finally, X_b, COD_i,
201 S_i, and X_i were mathematically estimated.

202 Respirometric test setup for COD_b and S_b determination

203 To determine COD_b and S_b, the respirometric assays were done in triplicate in a
204 completely mixed system (respirometer BM-T Plus 151204 Surcis, Spain), where the
205 aeration and the peristaltic pump flow for recirculation were set on the required values
206 according to the specifications given by the product manufacturer. The temperature was
207 set at 25 ˚C and controlled by a thermostatic bath (PolyScience 16A01092, USA).
208 During COD_b and S_b determination, the Surcis BM-T Plus 151204 respirometer
209 measures in continuous mode the dissolved oxygen (DO) concentration and temperature

and the associated software allows the simultaneous calculation and monitorization of other parameters: exogenous respiration rate (R_s) (mg O_2/L·h), specific R_s (mg O_2/g VSS·h), consumed oxygen (CO) (mg O_2/L), substrate utilization rate (U) (mg COD/L·h) and specific U (mg COD/mg SSV·h).

The respirometric tests were carried out with 1 L of sludge in the endogenous respiration phase with a concentration of solids between 1.5 – 5.0 g VSS/L and 2.0 – 5.0 g VSS/L for COD_b and S_b determination, respectively. The sludge was in each case previously diluted (in order to reach the optimum VSS concentrations) and conditioned if necessary (according to Table S3 of Supplementary Material). Allylthiourea (ATU) was added in concentrations between 2 – 3 mg ATU/mg VSS, at least 30 minutes before the start-up of the assays in order to inhibit nitrification. For COD_b determination, raw wastewater was added as a substrate. In the case of S_b assays, samples with low colloidal content were centrifuged for 10 minutes at 7,500 rpm (Centrifuge 5430 Eppendorf, USA) and filtered (45 μm pore size, cellulose-ester membrane, Advantec, Japan). Samples with high colloidal content were coagulated in order to remove colloidal material and avoid interferences, settled and filtered by 45 μm. The volumes of substrate added in each case were selected as a function of their COD concentration according to the manufacturer specifications (Table S2 of Supplementary Material). The experimental conditions considered for each sample are shown in Table S3 of Supplementary Material. These assays were considered finished once the R_s remained close to zero for a period no shorter than 10 minutes meaning that the biomass was under endogenous respiration conditions.

Heterotrophic growth yield (Y), expressed as g of COD produced as biomass per g of COD consumed as sodium acetate (g COD_X/g COD_{ac}) for each experiment were determined under the same previously described operational conditions for

respirometric assays with sodium acetate (0.4 g/L solution) as substrate and considering a volume of 50 mL of this sodium acetate solution.

Calculation of X_b, COD_i, S_i and X_i

COD_b and S_b fractions were experimentally determined through independent respirometric assays from the CO in the total (CO_t) and filtered sample (CO_f) (equations 4 and 5). The other COD fractions were calculated by equations 6 – 9 considering the analytically measured COD_t and COD_s concentrations and the results of the respirometric assays for COD_b and S_b quantification.

$$COD_b = CO_t / (1 - Y) \quad (4) \qquad S_b = CO_f / (1 - Y) \quad (5)$$

$$X_b = COD_b - S_b \quad (6) \qquad S_i = COD_s - S_b \quad (7)$$

$$COD_i = COD_t - COD_b \quad (8) \qquad S_i = COD_i - S_i \quad (9)$$

Y values were calculated by equation 10 considering the CO and the COD of the acetate solution (COD_{ac}) added as an organic matter source.

$$Y = 1 - (CO / COD_{ac}) \quad (10)$$

2.4. Relationship between COD fractions and anaerobic biodegradability

The average values of the obtained from triplicates (examples are shown in Figure S2 of Supplementary Material) were used to find a relationship between the different COD fractions measured in the samples and their anaerobic biodegradability. For that purpose, it were considered the COD_b and their fractions (S_b and X_b) and the part of the COD initially introduced in the BMP bottles converted into methane (COD_{met}).

2.5. Analytical methods

pH, conductivity, concentrations of COD_t, total Kjeldahl nitrogen (TKN), proteins, carbohydrates, lipids, total and total suspended solids (TS and TSS) and volatile and

volatile suspended solids (VS and VSS) were measured in the raw samples. In addition, centrifuged (Centrifuge 5430 Eppendorf, USA) and filtered (0.45 µm pore size, cellulose-ester membrane, Advantec, Japan) samples were taken for the determination of other parameters in the soluble fraction: COD_s, volatile fatty acids (VFA), soluble carbohydrates, soluble proteins, total organic carbon (TOC), total nitrogen (TN), NH_4^+ and other ions (Na^+, Cl^-, SO_4^{-2}).

The pH was measured with a pH & Ion-Meter GLP 22, Crison, (Spain), detection limit (DL): -2 – 16, conductivity with a probe Sension + EC5 HACH, (Spain), DL: 20.0 – 150.0 °C, 1 µS/cm – 200 mS/cm, lipids by an extractive method using a Soxhlet extractor Jp Selecta 8001800, (Spain), TKN with a Kjeldahl digestion unit Gerhardt KB8S-VAP12, (Germany), TS, VS, TSS, and VSS concentrations were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WEF, 2017). Concentrations of COD_t and COD_s (ECO16 Thermoreactor VELP Scientifica, USA) were also measured according to APHA/AWWA/WEF (2017) but modified by (Taylor et al., 1989) when necessary, DL: 0 – 900 mg/L. VFA were determined by gas chromatography (6850 Series II Agilent Technologies, USA; DL:1 – 1000 mg/L). Carbohydrates were measured by the Loewus method (Loewus, 1952) and expressed in equivalent glucose (Glu), DL: 0 – 90 mg/L. Proteins were analyzed by the Lowry method (Lowry et al., 1951) and expressed in equivalent bovine serum albumin (BSA), DL: 0 – 2000 mg/L. Concentrations of TOC and TN were measured by catalytic combustion in the TOC-L CNS analyzer with the TNM-1 module (TOC-5000 Shimazdu, Japan), DL: 0.5 – 1000 mg/L. Concentrations of NH_4^+ were determined by the Bower/Holm Hansen method (Bower and Holm-Hansen, 1980), DL: 0 – 1 mg/L. Concentrations of Cl^-, SO_4^{-2} and Na^+ were measured by ion

chromatography (861 Advanced Compact IC Metrohm, Switzerland), DL: 1 – 100 mg Cl^-/L, 1.5 – 150 mg SO_4^{-2}/L and 1 1.5 – 150 mg Na^+/L.

3. RESULTS AND DISCUSSION

3.1. BMP prediction from COD fractionation

The results of BMP tests and COD fractionation assays are summarized in Table 4 and Figure 1, respectively. Additional information regarding COD fractions can be consulted in Table S5 of Supplementary Material.

Table 4. Results of the BMP tests.

Substrate	BMP (mL CH_4/g COD_{fed})	Anaerobic biodegradability (%)	CH_4 in the biogas at the end of the BMP tests (%)	Lag phase lenght (d)
S1	218.37 ±-7.48	59.69 ± 0.28	72.37 ± 0.80	1
S2	264.68 ± 2.24	73.46 ± 0.30	84.22 ± 1.48	4
S3	63.55 ± 0.54	19.17 ± 0.15	72.95 ± 0.66	1
S4	182.46 ± 10.23	52.42 ± 1.43	62.84 ± 1.72	35
S5	165.92 ± 10.34	46.84 ± 2.06	80.99 ± 0.25	< 1
S6	293.32 ± 11.78	87.88 ± 3.43	72.51 ± 0.83	7
S7	237.02 ± 7.79	66.91 ± 2.20	67.92 ± 2.08	1

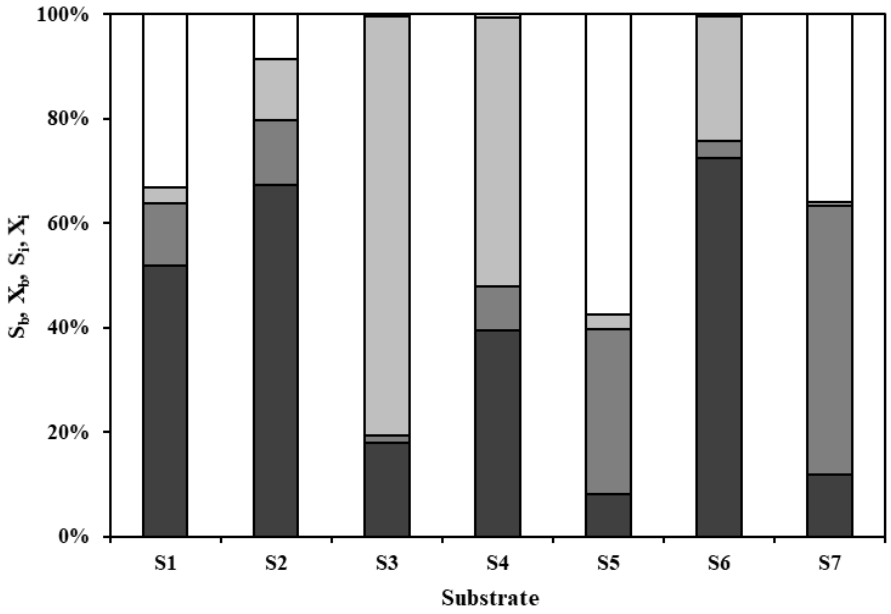

Figure 1. COD fractionation of the tested substrates. Soluble readily biodegradable (S_b) (■), soluble inert (S_i) (■), particulate slowly biodegradable (X_b) (■) and particulate inert (X_i) (□).

The results obtained in the BMP tests and COD fractionation assays showed that the anaerobically degraded COD of the samples (COD_{met}) almost corresponded to the biodegradable fraction of the COD (COD_b) (Figure 2). In this way, if there are no significant differences between COD_{met} and COD_b, COD_b might be feasibly used as a BMP predictor. Thus, if COD_b equals to COD_{met} and 1 g of COD produces a maximum of 350 mL of CH_4 (0 ˚C and 1,013 hPa), then the produced volume of CH_4 would correspond to the product between COD_b and 350 mL CH_4/COD_{fed}. Therefore, once estimated the mL of CH_4 produced, the BMP of a substrate can be calculated according to equation (1) by dividing the volume of CH_4 by the COD fed as a substrate in the pertinent COD fractionation assay. This would allow for a reliable calculation of biomethane producibility in few hours (COD fractionation assays time in this study), compared to many days (conventional BMP tests usually take from 20 to more than 100 days).

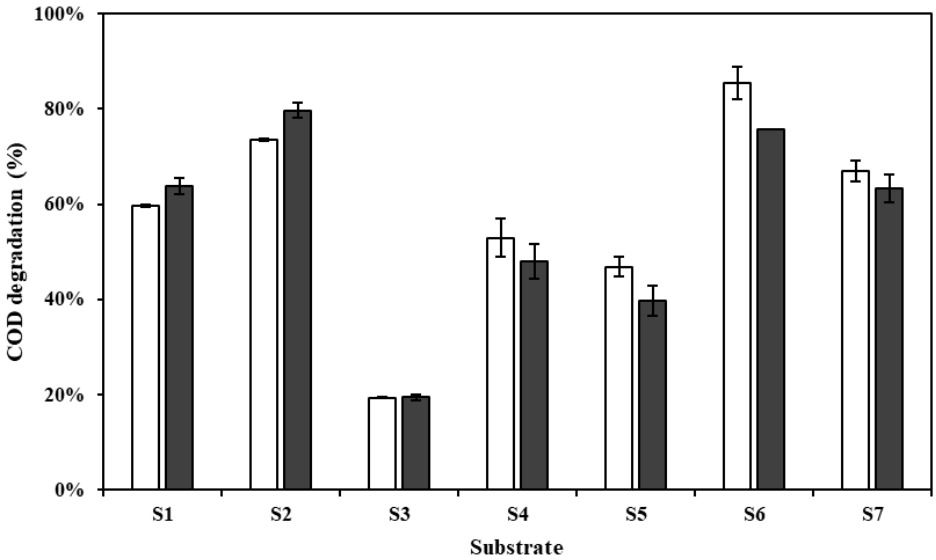

Figure 2. Percentages of COD_{met} (□) and COD_b (■) for each substrate.

Average experimental results obtained by both methodologies (BMP tests and COD fractionation assays) were statistically compared (t-test for independent samples at 5 % significance level) using SPSS software (IBM Corp. Released 2017. IBM SPSS

Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) in terms of percentage of substrate biodegraded. The Shapiro-Wilk normality test showed normal data distributions (p_1 = 0.863, p_2 = 0.647) and according to Levene's test, there was no homogeneity of variances. Considering this results, significant differences between BMP tests (57.8026 ± 21.2875) and COD fractionation assays results (55.6491 ± 21.3417) were not detected, t (5) = 0.189, p = 0.853. Besides, it was built mathematical models based on linear regressions using SPSS software considering all the COD fractions. The models with the best correlation were those that included all the biodegradable COD fractions as COD_b or as the sum of the S_b and X_b (Table S6 of Supplementary Material). The one with the highest R^2 and lowest SE was model M7: $COD_{met} = 1.125 \cdot S_b + 1.041 \cdot X_b$ (R^2 = 0.999; SE = 237) although the one that only considers COD_b, M1: $COD_{met} = 1.094 \cdot COD_b$ (R^2 = 0.998; SE = 318) seemed to be accurate enough to early predict the BMP of a substrate, which suggests that there is no need for performing the whole COD fractionation. In fact, M1 can be simplified as $COD_{met} = COD_b$ (thus no significant differences were detected between COD_{met} results predicted by M1 and simplified M1).

To corroborate the accuracy of using the COD_b of a substrate to early predict its BMP, it was carried out an inter-laboratory study with four additional substrates (SC1-SC4). The results obtained in BMP tests and COD fractionation assays are summarised in Table S7 of Supplementary Material. According to Figure 3 these four additional experiences fit the simplified M1 model predicted from substrates S1-S7, which validates the proposed methodology.

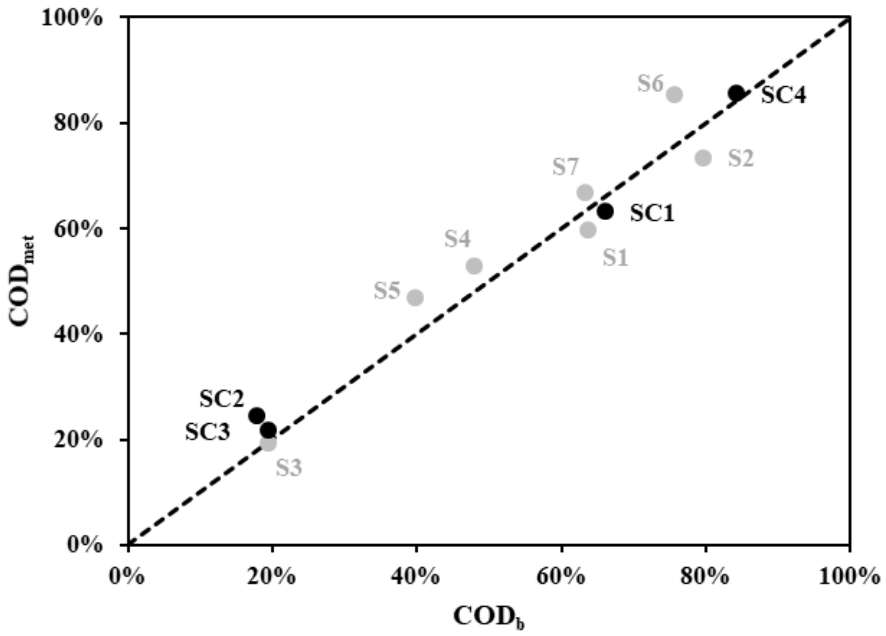

Figure 3. Degree of approximation to the predicted correlation $COD_{met} = COD_b$ (- - -) of substrates tested in Spain (S1 – S7) (●) and Chile (SC1 – SC4) (●).

3.2. Substrates particularities affecting the degree of correlation between COD_{met} and CDO_b

Although the potentially biodegraded organic matter seemed almost the same independently of the conditions (anaerobic or aerobic), the degree of similarity between COD_{met} and COD_b varied depending on the substrate characteristics (Figure 2). The influence of certain particularities of the samples over the performance of the anaerobic and aerobic assays, as well as the effect of the type of inocula used will be discussed in this section. For that purpose, it was considered the samples from which was initially predicted the correlation between COD_{met} and COD_b (substrates S1 – S7).

S1 presented an anaerobic biodegradability of 59.69 ± 0.28 % (Table 4) and COD fractionation showed that 63.77 ± 1.61 % of the COD was biodegradable being the 81.37 % of the COD_b soluble and readily biodegradable (Figure 1). In the fractionation assays of COD, it was observed that COD_b was rapidly consumed when using sludge

348 from a bioreactor treating the same effluent because biomass was already acclimated to
349 the tested substrate. However, when using conventional activated sludge from an urban
350 WWTP it was reached negative R_s values at the end of the tests, which indicated a
351 drastic drop in the active biomass (data not shown). This negative effect may be due to
352 the presence of toxic compounds in the wastewater, probably sodium hypochlorite
353 (NaClO) or caustic soda (NaOH) (according to Na^+ concentrations, Table 3), washing
354 agents typically used in dairy industry cleaning in place (CIP) systems (Hung et al.,
355 2010). In general, aerobic microorganisms are less resistant to toxic and inhibitory
356 compounds, which can derive on a lower aerobic biodegradability of the substrates as
357 occurred with S1 (Al Momani et al., 2010; Amaral et al., 2015). Therefore, the use of a
358 sludge acclimated to certain compounds that could hinder the performance of the
359 respirometric tests, might avoid inhibition and allow for higher accuracy of the
360 methodology.

361 S2 had an anaerobic biodegradability of 73.46 ± 0.30 % (Table 4). COD fractionation
362 showed that 79.68 ± 1.63 % of the COD was biodegradable, being the 84.45 % of COD_b
363 soluble and readily biodegradable (84.45 %) (Figure 1). X_b (15.55 %) were primarily
364 colloids, which needed to be removed before S_b determination to avoid interferences.
365 For this purpose, the wastewater was flocculated by the addition of 100 mg/L of
366 $Al_2(SO_4)_3$ during 2 h 30 min of severe stirring, then 20 min of gentle stirring and finally
367 20 min of settling.

368 Substrate S3 presented the lowest BMP and hence anaerobic biodegradability (19.17 ±
369 0.15 %) (Table 4) and the lowest percentage of COD_b (19.42 ± 0.62 %), which was
370 mostly soluble (92.78 %) and readily biodegradable (Figure 1). It was the effluent of an
371 anaerobic digester from a WWTP treating a mixture of primary and secondary sludge
372 and the biodegradation of the 20 % of the initial COD almost corresponded with the

fraction of non-hydrolized proteins and carbohydrates (Table 3). This substrate showed the strongest correspondence between COD_{met} and COD_b (Figure 2). Due to its origin, it should not have adaptation problems with the anaerobic inoculum used since it was collected from a lab-scale anaerobic reactor fed with secondary sludge from an urban WWTP. In the case of the aerobic assays, the COD_b mainly corresponded with the sum of readily biodegradable substances that could be easily consumed by activated sludge.

Substrate S4 had a potential risk of AD inhibition by FA due to its high NH_4^+ and protein concentration. Consequently, before BMP tests performance, part of the NH_4^+ was stripped reaching an anaerobic biodegradability of 52.42 ± 1.43 % (Table 4). It was obtained a slightly lower COD_b percentage of 47.90 ± 3.65 % (Figure 1), which almost corresponded with the VFAs, proteins, and carbohydrates measured in the soluble fraction (Table 3). Although in respirometric assays COD consumption rapidly started, BMP tests showed a long lag phase and biomethane production was not detected until 35 days after the start-up of the assay. This could be related to the use of a non-adapted inoculum and/or to the presence of unknown compounds. Thus, substrates with a similar origin to S4 are very complex and commonly contain very diverse toxic and nontoxic organic substances, xenobiotics, heavy metals, etc. (Youcai, 2018), which could be present but were not measured here.

S5 and S6 were the substrates with the greatest differences between their anaerobic and aerobic biodegradabilities (Figure 2). Anaerobic biodegradabilities were 46.84 ± 2.06 and 87.88 ± 3.43 (Table 4) whereas respirometric assays showed COD_b percentages of 40 % and 76 % for S5 and S6 (Figure 1), respectively. The main distinctive characteristic of S5 and S6 was their high salinity content, approximately 20 g NaCl/L. However, differences observed between COD_b and COD_{met} did not seem to be a consequence of inhibition by salinity since both tests were carried out with acclimated

inocula. It seems that high NaCl concentrations affected the response of the oxygen probe sensor since in both cases, anaerobic biodegradability was lower (despite the higher substrate concentration in the anaerobic tests), and differences were more significant for S5, which had a higher NaCl content. In the case of substrates S5 and S6 it was observed an additional hindrance. The sludge took a long time to reach the endogenous respiration phase. This fact could be due to the presence of remaining slowly biodegradable COD in the sludge. To overcome this problem, the sludge was washed with water containing NaCl concentrations similar to those of the sludge. to avoid an osmotic shock in the biomass because of NaCl absence.

S7 presented an anaerobic biodegradability of 66.91 ± 2.20 (Table 4). COD fractionation showed a similar aerobic biodegradability (63.31 ± 4.53 %) being 81.21% of the COD_b particulate and slowly biodegradable, which correlated with the nature of the substrate and the large duration of the respirometric assays (Figure 1). This X_b fraction consisted of substances present in the influent and separated in the primary decanters (primary sludge) or generated in the activated sludge reactor of the WWTP and separated in the secondary decanters (secondary sludge) (Ekama et al., 2007). Besides, more than 98 % of the non-biodegradable organics were particulate. Ekama et al. (2007) observed that this type of particulate organics were both unbiodegradable under anaerobic (anaerobic digestion process) and aerobic conditions (fully aerobic or nitrogen removal activated sludge system), which correlates with the results obtained in the present study. It should be also pointed out that the respirometer used was not specifically designed for solid substrates and long-term assays. Consequently, although reproducible results were observed, possible destabilization problems throughout time could affect the performance of the tests.

3.3 Advantages, limitations, and applicability of COD fractionation assays to predict the BMP of substrates

According to the previously exposed results, the determination of COD_b by COD fractionation assays seems to be a good alternative to predicting the BMP of substrates, reducing the testing time from weeks-months (conventional BMP) to hours.

The COD fractionation tests are inexpensive and easy to perform, even if compared with other alternative methods such as spectroscopic techniques (Jingura and Kamusoko, 2017) or mathematical models. Moreover, this respirometric method can be implemented with diverse substrates both liquid (different complexity liquid effluents) and solid (sewage sludge), whereas previously developed alternative methodologies were mainly focused on solid feedstocks (Table 1). Furthermore, in the COD fractionation experiments, it is possible to biodegrade the slowly biodegradable organic matter as observed in SC3 COD fractionation test, the effluent of a chipboard factory containing slowly biodegradable lignocellulosic materials. Other respirometric tests like the aerobic tests, do not offer the advantage of breaking down complex organic compounds (Lesteur et al., 2010). In fact, the Biological Oxygen Demand (BOD) presents a strong correlation with the BMP but not when considering substrates with high lignin content. Besides, these aerobic tests are time-consuming in comparison to COD fractionation assays (Bayard et al., 2016; Cossu and Raga, 2008; Ponsá et al., 2008)

However, especially when working with complex substrates, it is necessary to have a high activity inoculum capable of consuming all the biodegradable fraction of a substrate without being affected by the presence of toxic or inhibitory substances. This aspect might be a drawback if there is not an available adapted sludge since its progressive adaptation would significantly extend testing time. Another negative time-

consuming aspect is that on occasions (e.g. S5), the degradation of all the exogenous substrate initially present in the sludge takes a long time through just aerating the biomass before performing the tests. In these cases, the sludge could be previously washed to reduce most of the initial slowly biodegradable COD concentration present on it (Dircks et al., 1999; Zerdazi et al., 2012).

Despite the good correlation obtained between COD_b and COD_{met} and the advantages of using COD fractionation for BMP prediction, the COD fractionation procedure described in this paper is not the only option for determining the $COD_{b.}$ of a substrate There are very different devices available in the market and several protocols. Nonetheless, as long as a methodology allows for the determination of all the biodegradable COD (including the slowly biodegradable and particulate fraction), this could be able to predict the BMP of a substrate. In this regard, further investigation is recommended to evaluate the applicability of different methodologies to other datasets of COD_b and BMP. In special, when considering complex substrates with a slow biodegradability and solid streams.

4. CONCLUSIONS

Biodegradability results obtained through BMP (COD_{met}) and COD fractionation assays (COD_b) did not significantly differ in the tested substrates despite their diverse origin, complexity, and characteristics. Each substrate presented a certain biodegradability and when considering adapted microorganisms as inocula, appropriate operational conditions and the necessary biodegradation time (thus kinetics are different), the biodegraded organic matter was nearly the same under anaerobic and aerobic conditions. In fact, it was proven that it was possible to predict the BMP of a substrate

by just performing a single respirometric test for COD_b determination without the need of considering the other COD fractions. The accuracy and versatility of this less time-consuming methodology were validated by means of an inter-laboratory comparison considering four additional substrates.

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Assessment of a fast method to predict the biochemical methane potential based on biodegradable COD obtained by fractionation.

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TABLE CAPTIONS

Table S1. Experimental conditions established for the realization of the BMP tests.

Table S2. Sample volumes required in fractionation assays depending on the COD of the substrate.

Table S3. Experimental conditions established for COD fractionation assays.

Table S4. Substrates characterization (part 2).

Table S5. COD fractionation results.

Table S6. Linear regression models for rapid BMP prediction from the different COD fractions.

Table S7. Results of the BMP and COD fractionation assays of the substrates considered for the interlaboratory comparison SC1-SC4.

Table S1

Parameter	Substrate						
	S1	S2	S3	S4	S5	S6	S7
Temperature (˚C)	37	37	37	37	37	37	37
Shaking speed (rpm)	120	120	120	120	120	120	120
pH	7.5 – 8.0	7.5 – 8.0	7.5 – 7.7	7.5 – 8.0	7.3 – 7.5	7.5 – 8.0	7.5 – 8.0
Assays duration (d)	30	45	20	140	15	55	45
ISR (VS basis)	1.5	1.5	2.0	1.25	2.0	3.0	2.5
Inoculum							
Origin	Lab-scale anaerobic reactor	Lab-scale anaerobic reactor	Lab-scale anaerobic reactor	Lab-scale anaerobic reactor	Industrial anaerobic reactor adapted to high salinity	Industrial anaerobic reactor adapted to high salinity	Lab-scale anaerobic reactor
Degassing time (d)	3	3	2	4	3	3	3
V (mL)	16.95	78.27	85.00	125.00	130.00	242.76	275.00
VS (g/L)	0.20	1.05	2.47	2.62	4.10	2.87	6.47
Substrate							
Conditioning	-	-	-	90 % of NH_4^+ stripping and 5 % of COD_t removal	-	-	-
V (mL)	265.00	178.00	274.36	245.77	208.20	88.57	82.37
VSS (g/L)	0.06	0.06	0.02	0.04	0.30	0.24	-
VS (g/L)	-	-	-	-	-	-	3.24
Initial COD added (g/L)	0.50	1.53	1.81	3.53	1.86	1.54	4.05

ISR (inoculum to substrate ratio), V (volume), VS (volatile solids), VSS (volatile suspended solids), COD (chemical oxygen demand)

Table S2

Substrate COD_t (mg/L)	Sample volume (mL)	
	COD_b	S_b
< 300	80 - 50	50
300 - 5000	40 - 20	40 - 20
5000 - 10000	20 - 15	20 - 15
10000 - 25000	15 - 10	15 - 10
> 25000	10 - 5	10 - 5

Table S3

Parameter		Substrate						
		S1	S2	S3	S4	S5	S6	S7
Temperature (˚C)		25	25	25	25	25	25	25
ATU (mg/mg VSS)		2.50	3.00	3.00	3.00	2.00	2.50	3.00
Duration (h)	COD_b	1.5 – 2.5	1.5 – 2.8	1.5 – 2	6 – 6.2	6 – 7	1.5	20 – 35
	S_b	1.5 – 2.0	1.5 – 2	1.5 – 2	5.3 – 6.5	2 – 3	1.5	2 – 3
Sludge								
Origin		Pilot-scale MBR treating S1	WWTP AS reactor	WWTP AS reactor	WWTP AS reactor	Pilot-scale GSBR treating S5	Lab-scale aerobic SBR treating S6	WWTP AS reactor
Conditioning		-	-	-	-	Sludge washed with distilled water mimicking sludge NaCl concentration	-	
Dilution		-	1:2	1:2	1:2	-	-	1:2
VSS (g/L)		2.23	3.43	3.12	3.20	1.05 **	1.00	3.00
Substrate								
Conditioning for S_s determination		Centrifugation + filtering	Coagulation + Settling + filtering *	Centrifugation + filtering	Centrifugation + filtering	Centrifugation + filtering	Centrifugation + filtering	Centrifugation + filtering
V (mL)	COD_b	20	20	20	20	20	10	10
	S_b	40	20	20	20	20	10	20
Initial COD added (mg/L)	COD_b	13.86	63.02	48.57	110.76	65.61	173.60	90.04
	S_b	14.93	49.71	47.68	100.67	7.38	165.41	33.31

AS (activated sludge), GSBR (granular biomass system), MBR (membrane bioreactor), SBR (sequencing batch reactor).

* Addition of 100 mg/L of $Al_2(SO_4)_3$; 2h 30 min of severe stirring, 20 min of gentle stirring and 20 min of settling.

** Lower VSS (if compared with manufacturer recommendations) since it was granular biomass with very high heterotrophic activity.

Table S4

Parameter	Sample										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**SC1**	**SC2**	**SC3**	**SC4**
VS – VSS / TSS – TS (%)	90.8	96.4	62.4	55.3	69.4	64.6	83.6	64.2	91.7	96.8	87.3
TOC (mg /L)	130	1,108	971	2,535	60	7,880	712	850	1,710	120	850
IC (mg/L)	285	6	1,039	3,357	21	24	15	149	552	104	75
C/N	8.6	59.7	0.7	0.6	2.6	7.3	8.3	98.8	2.8	1.4	1.2

Table S5

Parameter			Substrate						
			S1	S2	S3	S4	S5	S6*	S7
Experimental data									
COD_b		g/L	0.45 ± 0.01	2.56 ± 0.05	0.48 ± 0.02	2.71 ± 0.21	1.33 ± 0.10	13.15	11.68 ± 0.53
		%	63.77 ± 1.61	79.68 ± 1.63	19.42 ± 0.62	47.90 ± 3.65	39.72 ± 3.10	75.75	63.31 ± 2.87
S_b		g/L	0.37 ± 0.01	2.16 ± 2.93	0.45 ± 0.02	2.23 ± 0.07	0.27 ± 0.02	12.57	2.19 ± 0.05
		%	51.89 ± 1.26	67.29 ± 3.41	17.99 ± 0.69	39.53 ± 1.31	8.20 ± 0.54	72.43 *	11.89 ± 0.25
Calculations									
X_b		g/L	0.08	0.40	0.04	0.47	1.05	0.58	9.49
		%	11.88	12.39	1.42	8.38	31.51	3.32	51.42
COD_i		g/L	0.26	0.65	2.00	2.94	2.02	4.21	6.77
		%	36.23	20.32	15.27	52.10	60.28	24.25	36.69
S_i		g/L	0.02	0.44	1.99	2.90	0.09	4.13	0.12
		%	3.04	13.55	80.18	51.37	2.83	23.81	0.63
X_i		g/L	0.23	0.22	0.01	0.04	1.92	0.08	6.65
		%	33.19	6.67	0.41	0.73	57.45	0.44	36.05

* No triplicate data available.

Table S6

Model	Coefficients	Equation	R^{2} *	SE **
M1	COD_b	$1.094 \cdot COD_b$	0.998	317.496
M2	COD_i	$1.910 \cdot COD_i$	0.628	4916.954
M3	S_b	$1.308 \cdot S_b$	0.759	3960.217
M4	X_b	$1.412 \cdot X_b$	0.470	5867.588
M5	S_i	$2.480 \cdot S_i$	0.468	5879.613
M6	X_i	$1.412 \cdot X_i$	0.404	6222.113
M7	COD_b, COD_i	$1.126 \cdot COD_b - 0.088 \cdot COD_i$	0.999	290.844
M8	S_b, X_b	$1.125 \cdot S_b - 1.041 \cdot X_b$	0.999	236.679
M9	S_i, X_i	$2.392 \cdot S_i + 1.735 \cdot X_i$	0.839	3547.090
M10	S_b, X_b, COD_i	$1.125 \cdot S_b + 1.041 \cdot X_b - 0.01 \cdot COD_i$	0.999	264.614
M11	COD_b, S_i, X_i	$1.113 \cdot COD_b + 0.018 \cdot S_i - 0.089 \cdot X_i$	0.999	288.373
M12	S_b, X_b, S_i, X_i	$1.126 \cdot S_b + 0.950 \cdot X_b + 0.007 \cdot S_i + 0.126 \cdot X_i$	0.999	292.637

* Correlation coefficient. Regression through the origin (model without intersection). R^2 measures the proportion of the variability in the dependent variable on the origin explained by the regression.

** Standard Error of the estimation.

Table S7

	Sample			
	SC1	SC2	SC3	SC4
BMP tests				
Sludge origin	Full-scale anaerobic reactor treating pig slurry	Full-scale anaerobic sludge digester	Full-scale UASB reactor treating tobacco wastewater	Full-scale anaerobic reactor treating pig slurry
COD_{met} (%)	63.22 ± 1.99	24.62 ± 2.84	21.85 ± 3.44	85.68 ± 2.88
COD fractionation				
Sludge origin	WWTP AS reactor	WWTP AS reactor	WWTP AS reactor	WWTP AS reactor
COD_b (%)	66.08 ± 1.70	17.86 ± 2.63	19.41 ± 3.93	84.30 ± 2.86

FIGURE CAPTIONS

Figure S1. COD fractions scheme.

Figure S2. Exemplification of COD_{met} and COD_b evolution over time. Triplicate data of substrate A) S2 (higher % COD_b). and B) S3 (lower % COD_b).

Figure S1

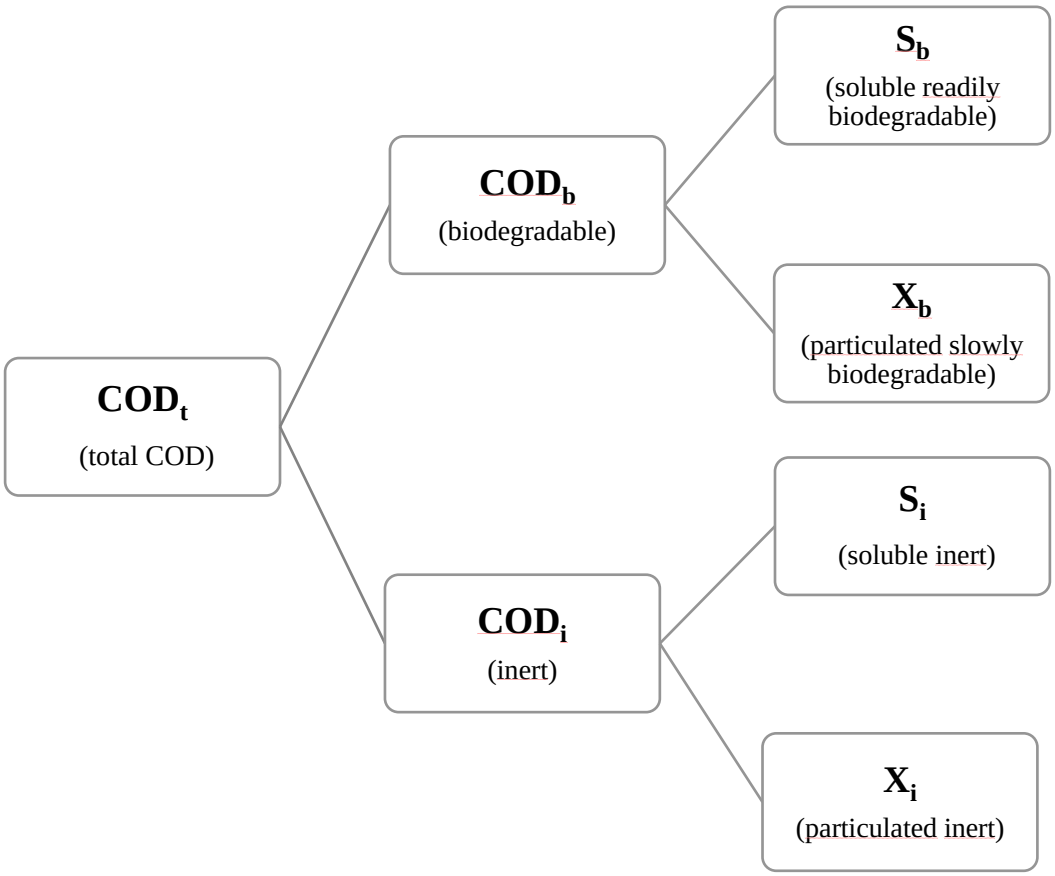
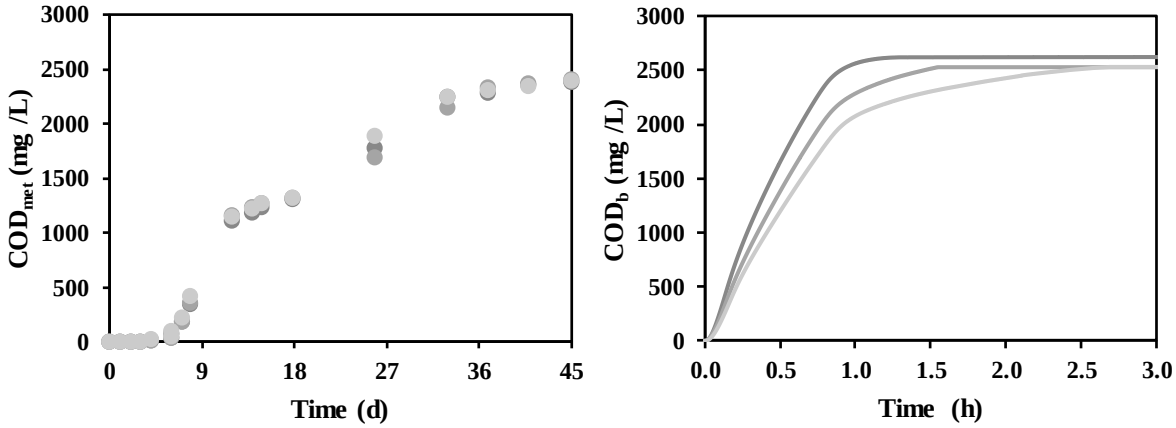
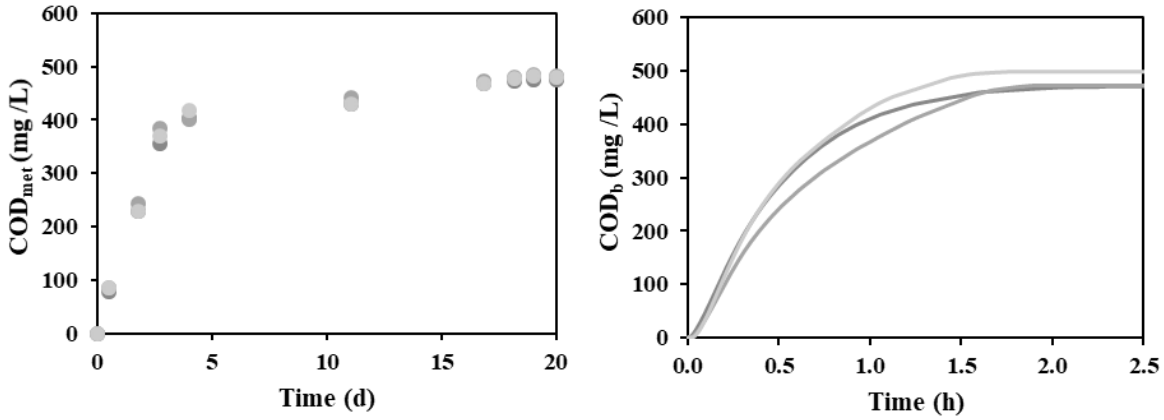

COD_t
(total COD)
COD_b
(biodegradable)
COD_i
(inert)
S_b
(soluble readily biodegradable)
X_b
(particulated slowly biodegradable)
S_i
(soluble inert)
X_i
(particulated inert)

Figure S2

A)


3000
2500
2000
1500
1000
500
0
COD_{met} (mg /L)
0
9
18
27
36
45
Time (d)
COD_b (mg /L)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Time (h)

B)


600
500
400
300
200
100
0
COD_{met} (mg /L)
0
5
10
15
20
Time (d)
COD_b (mg /L)
0.0
0.5
1.0
1.5
2.0
2.5
Time (h)

Credit Author Statement

Lucía Argiz: investigation, writing-original draft, formal analysis, visualization; **Claudia Reyes:** investigation; validation; **Marisol Belmonte:** supervision, project administration, funding acquisition; **Óscar Franchi:** validation; **Riccardo Campo:** methodology, validation; **Andrea Fra-Vázquez:** methodology, validation; **Ángeles Val del Río:** formal analysis, validation, visualization, supervision, funding acquisition; **Anuska Mosquera:** validation, supervision, project administration, funding acquisition; **José Luis Campos:** methodology, validation, supervision, project administration, funding acquisition.