Anaerobic degradation of 2-propanol: laboratory and pilot-scale studies

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Abstract

The anaerobic degradation of 2-propanol, an important industrial solvent, was scaled-up from batch assays to a pilot expanded granular sludge bed (EGSB) reactor at 25ºC. Batch studies indicated that 2-propanol followed Haldane kinetics, with a maximum rate at 10 g COD L^{-1}. Concentrations as high as 25 g COD L^{-1} did not inhibit the degradation of ethanol, a common co-solvent. Similar specific methanogenic activities (SMA) were obtained for water-solvent and water-brewery sludges (88 and 77 ml CH_4 g-VS^{-1} d^{-1} at 5 g COD L^{-1}). Continuous degradation showed a lag-phase of three weeks with water-brewery sludge. Increases in 2-propanol load from 0.05 to 0.18 kg COD kg-VS^{-1} d^{-1} caused a shift from the consumption of soluble matter to methane production, indicating polyhydroxybutyrates (PHB) accumulation. Conversely, smooth increases of up to 0.29 kg COD kg-VS^{-1} d^{-1} allowed 2-propanol degradation without PHB accumulation. The slowdown rate of 2-propanol-oxidizer and acetate-utilizing methanogen bacteria below 20ºC adversely impacted both removal and CH_4 yield.

Keywords

Anaerobic treatment, CSTR, pilot-scale EGSB reactor, isopropanol, SMA

1. Introduction

2-propanol is widely used as a solvent in many different chemical industries, such as rubber, cosmetics, textiles, surface coatings, inks and pesticide formulations, with worldwide manufacturing exceeding $1x10^6$ tons per year. As with other organic solvents, the main environmental concern is related to the release into the atmosphere of volatile organic compounds (VOCs) during its industrial use. More investigation of technologies

for VOC control is required since the abatement of VOCs is a key factor in the protection of the environment and of public health (European Union, 2010). Biological abatement of 2-propanol in industrial emissions has already been demonstrated as a successful method, using aerobic conditions for the treatment, such as a biotrickling filter (San-Valero et al., 2014; Pérez et al., 2013). Recently, anaerobic bioscrubbering was shown to be a promising alternative for the treatment of air emissions containing VOCs of high solubility in water, such as for example in food packaging printing, which is a growing sector of economic importance in the EU. In this process, VOCs in the air are first scrubbed with water and then degraded anaerobically in an EGSB reactor, thus recycling dilute organic waste gases into bioenergy (Waalkens et al., 2015). The anaerobic bioscrubber successfully treated air emissions from the evaporation of ink in the printing press of a flexographic facility. An industrial prototype was used for the removal of emissions containing ethanol (60%–65%), ethyl acetate (20%–25%) and 1-ethoxy-2-propanol (10%–15%) as the main VOCs, reporting removal efficiencies (REs) of 93 ± 5% in the EGSB, obtained at 25.1 ± 3.2ºC and with a methane yield of 0.32 Nm3 CH4 kg COD removed^{-1} (Bravo et al., 2017). In order to expand the applicability of this VOC abatement technology, since 2-propanol is also used as the main bulk solvent of ink formulations in flexography instead of ethanol, its anaerobic degradation must be investigated.

The anaerobic degradation of 2-propanol has rarely been studied in the past. Moreover, the literature shows variations in the reported inhibition/biodegradable levels. This can mostly be explained by the complexity of the anaerobic digestion process, with phenomena such as acclimation that significantly impacts on the inhibition of organic compounds (Chen et al., 2008). The data in the literature mainly refers to batch assays.

For example, Chou et al. (1978a) found that the addition of 2-propanol up to 4 g COD L^{-1} did not inhibit methane production by using acetate as the reference substrate and an enriched culture of methane bacteria not previously acclimated at 35ºC. In contrast, another author found that 2-propanol is inhibitory for methanogenic bacteria with a reported tolerance of 0.2 M at 36ºC (Widdel, 1986). A recent study by Ince et al. (2011) shows also an inhibitory effect on the acetoclastic methane production pathway by using acetate as substrate working at 37ºC. Degradation of acetate was inhibited with an initial exposure to 0.1 M of 2-propanol. Repeated exposures resulted in higher inhibitions. Regarding the continuous anaerobic degradation of 2-propanol, only one study treating a mixture of organic solvents was found. Henry et al. (1996) operated a 20 L anaerobic hybrid reactor with a non-enriched culture treating a mixture of methanol, ethanol, propionate, butyrate, ethyl acetate and 2-propanol. The process was able to successfully remove a total organic loading rate (OLR) of up to 4 g COD L^{-1} d^{-1} at 35ºC, with a 2-propanol concentration fed to the reactor of 0.5 g L^{-1}. A more systematic study of the anaerobic biodegradability of 2-propanol is required, especially under sub-optimal mesophilic and psychrophilic conditions.

The main objective of this study was to investigate the degradation of 2-propanol with granular sludge systems at ambient temperature, in order to expand the applicability of the anaerobic bioscrubber technology to industries which use 2-propanol as the main solvent. Therefore, the biodegradability of 2-propanol was first evaluated in batch assays, including the influence of the granular sludge (water-brewery and water-solvent cultures). Additionally, the potential inhibition of 2-propanol on the degradation of ethanol was assessed, since it is usual to find the common use of both solvents in the chemical industry. Based on the batch results, the continuous degradation of 2-propanol was

assessed at laboratory scale using a culture coming from an anaerobic reactor treating brewery wastewaters (water-brewery culture), in order to determine the OLR that can be efficiently treated and to evaluate the acclimation time. Finally, the influence of these two key parameters (OLR and acclimation time) in the performance of the process was evaluated using an industrial prototype of EGSB seeded with a water-brewery culture. To the best of our knowledge, there are no previous reported data for an anaerobic pilot-scale bioreactor using 2-propanol as the main carbon source. Thus, this study is expected to provide guidelines for the start-up and operation of anaerobic reactors treating industrial wastewater containing 2-propanol.

2. Materials and methods

2.1 Sources of granular sludge

Anaerobic granular sludges from different pilot- or full-scale anaerobic bioreactors working at sub-optimal mesophilic temperatures were used in this study. The characteristics of the sludge are shown in Table 1. S-FP sludge was obtained from a pilot-scale EGSB treating package printing effluents (Altacel B.V., Weesp, the Netherlands), with a yearly average water temperature of 22 ºC. This reactor had been treating wastewaters containing solvents from the scrubbing of the VOC air emissions of the facility for more than a year. The main substances in the wastewater were 1-ethoxy-2-propanol (62 ± 12%), ethanol (26 ± 14%), 2-propanol (8 ± 4%) and 1-methoxy-2-propanol (6 ± 2%). S-B1 sludge was obtained from a full-scale internal circulation (IC) reactor treating brewery wastewater (Heineken, Zoeterwoude, the Netherlands), working at 26ºC. S-B2 sludge was obtained from a full-scale IC reactor also treating brewery wastewater (Font Salem, El Puig, Spain), operating between 22ºC and 32ºC. The sludges from the breweries (S-B1 and S-B2) were not exposed to 2-propanol prior to their use in

this work. The three types of sludge had similar total solids (TS) and volatile solids (VS) content; however, S-B1 had a larger granule size and higher sulfur content than the other two.

Table 1. Sources and physical properties of the granular sludge used in this research

	Source	TS	VS	Average particle diameter (mm)	Observations
		(mg g wet w^{-1})			
S-FP	EGSB reactor treating solvent wastewater (the Netherlands)	8.1 ± 0.2	7.5 ± 0.2	0.97 ± 0.03	Low S content
S-B1	IC reactor treating brewery wastewater (the Netherlands)	8.3 ± 0.1	7.9 ± 0.4	0.78 ± 0.05	Low S content
S-B2	IC reactor treating brewery wastewater (Spain)	8.2 ± 1.2	7.4 ± 0.1	2.22 ± 0.94	High S content

2.2 Batch bioassays

Biochemical Methane Potential (BMP) assays were developed for determining the anaerobic degradability of compounds, allowing the testing of the substrate in controlled and optimal conditions in a laboratory environment.Therefore, BMP assays were used to determine the ultimate methane production, specific methanogenic activity (SMA) and lag phase for the degradation of 2-propanol under specifically chosen conditions. For this purpose, 4.23 g VS L^{-1} of granular sludge were added to serum bottles (500 mL) containing a basal medium and supplemented with ethanol (95%–96% v v^{-1}, VWR) at 0.8 or 1.6 g chemical oxygen demand (COD) L^{-1}, used as a control, and with 2-propanol (99.5% v v^{-1}, Sigma Aldrich) at several concentrations. N, P, K and S were added to give a ratio of 200 g COD/g N, 600 g COD/g P, 313 g COD/g K and 4250 g COD/g S. The solution contained (mg L^{-1}): 2500 $NaHCO_3$; 40 $CaCl_2$ H_2O; 40 $MgCl_2$ $6H_2O$; 0.05

H_3BO_3; 2.02 $CoCl_2 \cdot 6H_2O$; 0.17 $CuCl_2 \cdot 2H_2O$; 9.41 $FeCl_3 \cdot 6H_2O$; 1.80 $MnCl_2 \cdot 4H_2O$; 0.78 $(NH_4)_6Mo_7O_{24} \cdot 2H_2O$; 0.56 $NiSO_4 \cdot 6H_2O$; 0.05 Na_2SeO_3; 0.16 $ZnSO_4 \cdot 7H_2O$; and 1.2 yeast extract. The bottles were placed in an Automatic Methane Potential Test System (AMPTS II by BioProcess Control®) and mechanically stirred (one minute out of two) at 112 rpm at 25ºC. The biogas passed through a CO_2-scrubbing unit (containing NaOH 3M), allowing only methane to flow to a gas-recording unit. BMP was calculated as the ratio of the final cumulative methane production and the initial organic content of the substrate. SMA was estimated as the maximum methane flow rate in function of the initial sludge content. Soluble COD and volatile fatty acids (VFA) were determined at the beginning and end of the bioassays. The experiments were conducted in duplicate.

A set of experiments was designed to compare the biodegradability of 5 g COD L^{-1} of 2-propanol by the S-FP sludge (water-solvent culture) and the S-B1 sludge (water-brewery culture). In a second step, S-FP was selected to determine the influence of the initial concentration of 2-propanol on the SMA and BMP. For this purpose, initial concentrations of 2-propanol of 1.2, 5, 10 and 25 g COD L^{-1} were used.

2.3 Anaerobic degradation of 2-propanol in laboratory CSTR

The continuous anaerobic degradation of 2-propanol was performed in a continuous stirred tank reactor (CSTR) with an effective volume of 1.6 L. The reactor was filled with 17.4 g VS L^{-1} of sludge S-B2. The temperature was kept at 25ºC using a thermostatic water bath (Memmert GmbH +Co.KG, Germany). A hydraulic retention time (HRT) of eight days was fixed. The experimental set-up is shown in Fig.1a. The CSRT feeding was made up of three solutions: a synthetic wastewater composed of a mixture of the organic substrate and N, P, K and S, which were added from a concentrated solution to get a ratio of 150 g COD/g N, 1000 g COD/g P, 4350 g COD/g K and 5000 g COD/g S; a solution

160 composed of g L^{-1} of 0.8 $CaCl_2 \cdot 2H_2O$ and 0.67 $MgCl_2 \cdot 6H_2O$; and a saturated solution of
161 $NaHCO_3$. The synthetic wastewater was fed into the reactor 24 h d^{-1}, constituting 90% of
162 the influent. Both Ca/Mg and $NaHCO_3$ solutions were introduced twice per day as 10%
163 of the influent. The effluent stream was continuously extracted from the bioreactor. The
164 four liquid streams were controlled using a multichannel peristaltic pump (Reglo ICC,
165 Ismatec©, Germany). Once per week, yeast extract (0.168 ml from a 10 g L^{-1} solution)
166 and trace elements (2 ml of a solution in g L^{-1}: 0.0146 H_3BO_3; 0.6070 $CoCl_2 \cdot 6H_2O$; 0.05
167 $CuCl_2 \cdot 2H_2O$; 2.8244 $FeCl_3 \cdot 6H_2O$; 0.5405 $MnCl_2 \cdot 4H_2O$; 0.0335 $(NH_4)_6Mo_7O_{24} \cdot 2H_2O$;
168 0.1678 $NiSO_4 \cdot 6H_2O$; 0.0144 Na_2SeO_3; 0.0506 $ZnSO_4 \cdot 7H_2O$) were added. The CSTR was
169 intermittently stirred following same protocol as in batch bioassays. The methane
170 production was continuously monitored using the AMPTS II (BioProcess Control,
171 Sweden). Alkalinity, pH and VFA were measured daily; soluble COD and its solvent
172 composition were measured at least three times per week, and nutrients were controlled
173 twice per week.

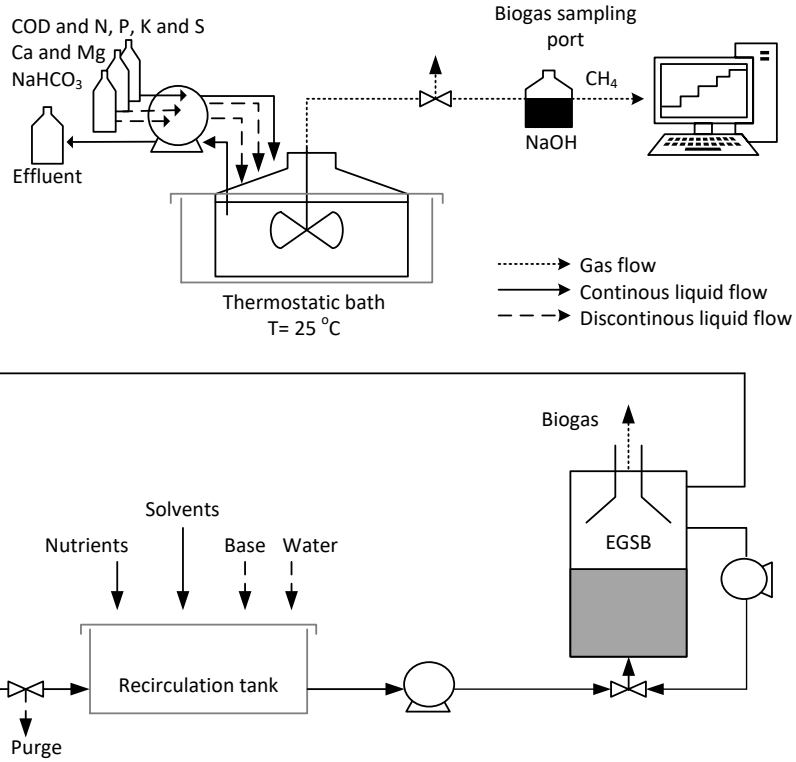


Figure 1. Scheme of the experimental set-up: (a) laboratory CSTR; (b) pilot EGSB

The experiment was designed in three phases (A, B, C), which were characterized by changes in the 2-propanol mass fraction (Table 2). During phase A (days 1 to 34), the CSTR was operated using ethanol as the sole organic substrate, with progressive increases in the influent concentration from 9.4 to 76.0 g COD L^{-1} (ethanol OLR ranging from 1.2 to 9.3 kg COD m^{-3} d^{-1}). Step changes were carried out after checking that VFA were kept below 100 mg L^{-1} during 2-3 days. In phase B, the influent COD composition was modified to form binary mixtures of ethanol and 2-propanol with two increases in 2-propanol OLR. Between days 35 and 60, a mixture of ethanol and 2-propanol was applied in a mass ratio of 9:1 (ethanol OLR of 9.3 kg COD m^{-3} d^{-1}; 2-propanol OLR of 0.9 kg COD m^{-3} d^{-1}). On day 61, the OLR of ethanol was lowered to 3 kg COD m^{-3} d^{-1} while the OLR of 2-propanol was increased to 3 kg COD m^{-3} d^{-1} (mass ratio 1:1). From day 85 onward (Phase C), the reactor was fed with 2-propanol as the sole organic substrate.

From this point on, the inlet concentration of 2-propanol was increased by two stepwise (24 and 47 g COD L^{-1}, OLR of 2.9 and 5.9 kg COD m^{-3} d^{-1}).

Table 2. Experimental plan for the anaerobic degradation of 2-propanol in laboratory CSTR

Phase		Days	Influent concentration (g COD L^{-1})	OLR (kg COD m^{-3} d^{-1})		SLR* (kg COD kg-VS^{-1} d^{-1})
				ethanol	2-propanol	
A (ethanol)		1-34	9.4 – 75.9	1. 2 – 9.3	--	0.07 – 0.53
B (ethanol+ 2–propanol)	B-I	35-60	83.6	9.3	0.9	0.59
	B-II	61-84	48.2	3.0	3.0	0.35
C (2-propanol)	C-I	85-108	24.1	--	2.9	0.17
	C-II	109-116	47.1	--	5.9	0.33

*SLR stands for Sludge Loading Rate: SLR = OLR per initial VS content

195 2.4 Anaerobic degradation of 2-propanol in pilot EGSB reactor

196 The pilot plant (PAS Solutions BV, The Netherlands) was installed in a package printing
197 factory, Altacel Transparant (The Netherlands). It was composed of an EGSB anaerobic
198 reactor with an effective volume of 8.7 m^3 plus a recirculation tank (Fig. 1b). The total
199 water volume was 12 m^3. The bioreactor was seeded with S-B1 granular sludge. The
200 HRT of the reactor was set up at 3 h. The system was operated in water-closed
201 recirculation, with 0.3 $m^3 d^{-1}$ of water renewal. The expansion of the granular bed to 2 m^3
202 (41.9 g VS L_{bed}^{-1}) was achieved by mixing the influent water with 50% of the effluent of
203 the reactor using two centrifugal pumps (model CEA80/5, Lowara, EU); the upflow
204 velocity was kept constant at 3 m h^{-1}.

205 The organic substrate was fed into the recirculation tank via a peristaltic pump (Watson-
206 Marlow, EU). Nutrients (N, P, K, S) were provided to the reactor on the basis of CSTR
207 dosage using a programmed dosing pump (model series GTM A, LMI Roytronic, EU).
208 Ca, Mg, trace metals and yeast extract were discontinuously supplemented. A
209 programmable logic controller operated with Twinsoft software (Servelec Technologies,
210 the United Kingdom) was used to monitor and control parameters such as liquid flow
211 rate, water temperature, pH, conductivity and water level in the tanks. pH was controlled
212 at neutral values by dosing a chemical base. Soluble COD, VFA, N and P were
213 determined in situ several times per week. A weekly sample was preserved for the further
214 analysis of solvent composition. The total biogas production was continuously monitored
215 using a gas meter (Bellows-BG 4 Gasmeter, Ritter, Germany).
216 The pilot-scale experiment was divided into three phases with different substrate
217 composition (Table 3). The OLR was slightly increased from 3.3 to 3.9 kg COD m^{-3} d^{-1}

(sludge loading rate (SLR) of 0.25-0.29 kg COD kg-VS^{-1} d^{-1}). During phase A (days 1 to 22), the system was fed with a solution of industrial-grade ethanol (95%, Univar BV, the Netherlands) denatured with 5% vol. of 2-propanol, thus containing a minimum 2-propanol OLR of 0.2 kg COD m^{-3} d^{-1}. From day 23 to day 81 (phase B), the influent COD composition was changed to binary mixtures of ethanol and 2-propanol (99%, Univar BV, the Netherlands). When the VFA concentration was lower than 200 mg L^{-1} and COD concentration was less than 1000 mg L^{-1}, the OLR of 2-propanol was increased in steps of ~0.7 kg COD m^{-3} d^{-1}, while the OLR of ethanol was decreased to ensure a smooth acclimation to the presence of 2-propanol as the sole organic substrate (phase C).

Table 3. Experimental plan for the anaerobic degradation of 2-propanol in pilot EGSB

Phase		Days	OLR (kg COD m^{-3} d^{-1})	
			ethanol	2-propanol
A (ethanol)		1-22	3.1	0.2
B (ethanol+ 2–propanol)	B-I	23-49	2.6	0.8
	B-II	50-56	2.0	1.5
	B-III	57-63	1.4	2.3
	B-IV	64-70	0.7	3.0
	B-V	71-76	0.2	3.6
C (2-propanol)		77-94	--	3.9

Note: SLR = 0.25- 0.29 kg COD kg-VS^{-1} d^{-1}.

2.5 Analytical methods

The determination of total solids (TS) and volatile solids (VS) of the sludge was carried out in triplicate according to standard methods (American Public Health Association, 1999). For S-FP and S-B2, the average particle diameter of the granule was measured using a laser particle analyzer (Mastersizer, Malvern 2000, UK). For S-B1, the average particle diameter of 10 granules was measured with an optical microscope (SE, Nikon, Japan).

For laboratory assays, soluble COD concentration was analyzed according to standard methods and VFA and alkalinity were determined using potentiometer titration (848 Titrino Plus, Metrohm, Switzerland). N and P concentration were measured with an ionic chromatograph (883 Basic IC Plus, Metrohm, Switzerland) equipped with Metrosep C4-250/4.0 and Metrosep A Supp 3 columns. For the pilot test, COD, VFA, N and P concentration were determined with spectrophotometric commercial kits (LCK 514, LCK 365, LCK 303 and LCK 348 kits from Hach Lange GmbH, Germany). Alkalinity content was estimated with a titrimetric kit (MColortest^{TM}, Merk Millipore, Germany).

For all experiments, the solvent composition in the water samples was determined using a gas chromatograph equipped with a flame ionization detector (Agilent GC 7890A, Spain), a capillary column (Restek Rtx-VMS) and a helium carrier gas with 25 ml min^{-1} of flow.

3. Results and discussion

3.1 Biodegradability studies in batch reactors

Two sets of batch assays were developed to quantify the influence of both the initial concentration of 2-propanol and the anaerobic sludge source on the anaerobic biodegradation of 2-propanol in the presence of ethanol. The cumulative volume of methane produced over time for water-solvent (S-FP) and water-brewery (S-B1) cultures with a mixture of ethanol and 2-propanol is shown in Figure 2. The evolution of methane production clearly shows a diauxic shift: biomass preferentially uses the readily biodegradable substrate, and only when ethanol has been exhausted as an energy and carbon source does the active population start to utilize 2-propanol. This phenomenon was reported by Chou et al. (1978b) in cross-acclimation studies of an acetate culture with 30 petrochemicals, including acetone, propanol, butanol and methyl acetate among

others. The metabolic adjustment from ethanol to 2-propanol resulted in a lag period that clearly demonstrates a link with the observed methane production of each solvent. As can be seen from Figure 2, for a given initial 2-propanol concentration (5 g COD L^{-1}), the observed lag time is significantly longer for the water-brewery sludge (S-B1) than for the water-solvent one (S-FP). It should be noted that the pilot EGSB reactor from which the S-FP came was seeded from the same IC reactor from which S-B1 was taken. The difference lies in the exposure of S-FP to industrial solvents, including 2-propanol, for more than one year.

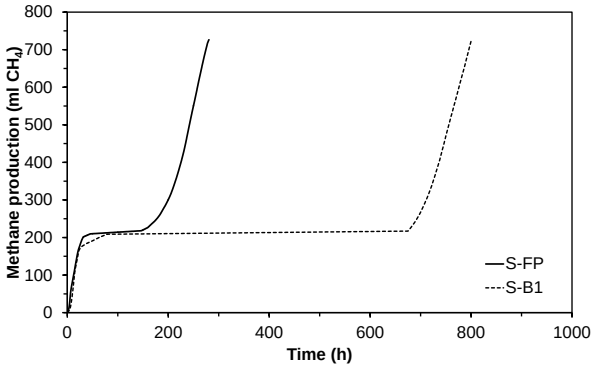


Figure 2. Comparison of cumulative methane production between water-brewery (S-B1) and water-solvent (S-FP) sludge. Initial 2-propanol concentration = 5 g COD L^{-1}. Initial ethanol concentration = 1.2 g COD L^{-1}

The results of the influence of the source of sludge are summarized in Table 4. For the two assays, soluble COD and VFA concentrations at the end of the test were below 40 mg COD L^{-1} and 3 mg CH_3COOH L^{-1}, showing the complete degradation of the solvent. BMP values showed methane recoveries of 89 ± 2% with S-FP and 91 ± 1% with S-B1 for ethanol, and 75 ± 1% with S-FP and 72 ± 5% with S-B1 for 2-propanol. Independent of the sludge source, almost the same ethanol removal rate was observed, quantified by SMA. This is in accordance with the fact that ethanol is a readily anaerobic biodegradable substrate. In addition, the removal rates for 2-propanol were reduced to less than half that

of ethanol, appearing slightly higher (13%) for the water-solvent sludge (S-FP). The main difference between both sludges was the lag time. In the presence of ethanol, 33 days were required for a water-brewery culture (S-B1) to start metabolizing 2-propanol, while it took only 12 days for the water-solvent culture (S-FP). These results verified that the structural characteristics of the solvent influence its degradation rate after acclimation, and that the previous exposure of the sludge to the target compound reduces the length of the lag time.

Table 4. SMA, BMP and lag phase for the water-brewery (S-B1) and water-solvent (S-FP) cultures. Initial 2-propanol concentration = 5 g COD L^{-1}. Initial ethanol concentration = 1.6 g COD L^{-1}

		SMA ($ml\ CH_4\ g\text{-}VS^{-1}\ d^{-1}$)	BMP ($ml\ CH_4\ g\text{-}COD^{-1}$)	Lag phase (days)
S-B1	ethanol	189 ± 8	319 ± 2	0
	2-propanol	77 ± 4	254 ± 18	32.6 ± 0.9
S-FP	ethanol	201 ± 9	311 ± 8	0
	2-propanol	88 ± 8	262 ± 1	11.8 ± 0.2

A second experiment was designed to assess the potential inhibition of 2-propanol initial concentration in presence of ethanol using the water-solvent sludge (S-FP). SMA, BMP and lag time for increasing initial 2-propanol concentrations (1.2, 5, 10 and 25 g $COD \cdot L^{-1}$) are reported in Table 5, as well as SMA for ethanol. An almost complete degradation of the solvents in terms of soluble COD concentration was observed (> 98%). It is highlighted that the SMA value for ethanol degradation was similar for each tested concentration of 2-propanol. For a given ethanol concentration of 1.6 g COD L^{-1}, SMA remained around 200 ml $CH_4\ g\text{-}VS^{-1}\ d^{-1}$, matching the value of 202 ± 9 ml $CH_4\ g\text{-}VS^{-1}\ d^{-1}$ obtained in a separate test with ethanol as the sole solvent. Thus, no perceptible inhibition on the degradation of ethanol occurred for the tested conditions, even at an initial

concentration of 2-propanol of 25 g COD L^{-1}. At 37ºC, Ince et al. (2011) found inhibitory levels of 2-propanol on acetate biodegradation starting from 0.1 M (14.4 g COD L^{-1}), with an IC_{50} of 0.27M (38.9 g COD L^{-1}). The lack of inhibitory effect on ethanol degradation in this work might be attributed to the previous exposure of the sludge to the target solvent, indicating that the anaerobic treatment of ethanol-rich effluents would not be inhibited by the presence of 2-propanol, even if relatively high concentrations of this solvent are punctually reached.

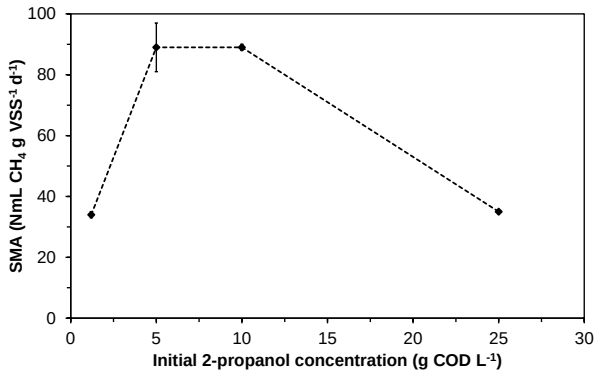
Table 5. Influence of 2-propanol concentration on its anaerobic biodegradation

		ethanol	2-propanol		
2-propanol concentration (g COD L^{-1})	ethanol concentration (g COD L^{-1})	SMA (ml CH_4 g-VS^{-1} d^{-1})	SMA (ml CH_4 g-VS^{-1} d^{-1})	BMP (ml CH_4 g-COD^{-1})	Lag phase (days)
1.2	0.8	87 ± 6	34 ± 1	196 ± 29	3.6 ± 0.1
5	1.6	201 ± 9	89 ± 8	262 ± 1	11.8 ± 0.2
10	1.6	208 ± 7	89 ± 1	245 ± 13	17.6
25	1.6	199 ± 8	35*	242*	126.1*

*Results corresponding to one replicate

Regarding the biodegradation of 2-propanol, the increase of initial 2-propanol concentration adversely affected the lag time and the methane production rate. An exponential lengthening of the lag time was observed, reaching 126 days for the highest concentration tested, and showing that the concentration of the target solvent increased the required time for final metabolization to methane. SMA versus the initial 2-propanol concentration is plotted in Figure 3. The methane production rate showed an increase up to 5 g COD L^{-1}, reaching a plateau until at least 10 g COD L^{-1}, after which there was a substantial decrease up to 25 g COD L^{-1}, following Haldane kinetics. The substrate

320 inhibition at 25 g COD L^{-1} was accompanied by the accumulation of VFA at the end of
321 the test (567 mg CH_3COOH L^{-1}), which is an indicator of the process imbalance between
322 acetogenic and methanogenic populations (Ahring et al., 1995). The recommended sludge
323 loading rate (SLR) for a continuous anaerobic reactor treating 2-propanol could be
324 derived from the ratio between SMA and BMP. An equivalent of 0.17–0.36 g COD g-VS^{-1}
325 d^{-1} of 2-propanol could be removed for concentrations in the reactor of between 1.2 and
326 10 g COD L^{-1}.



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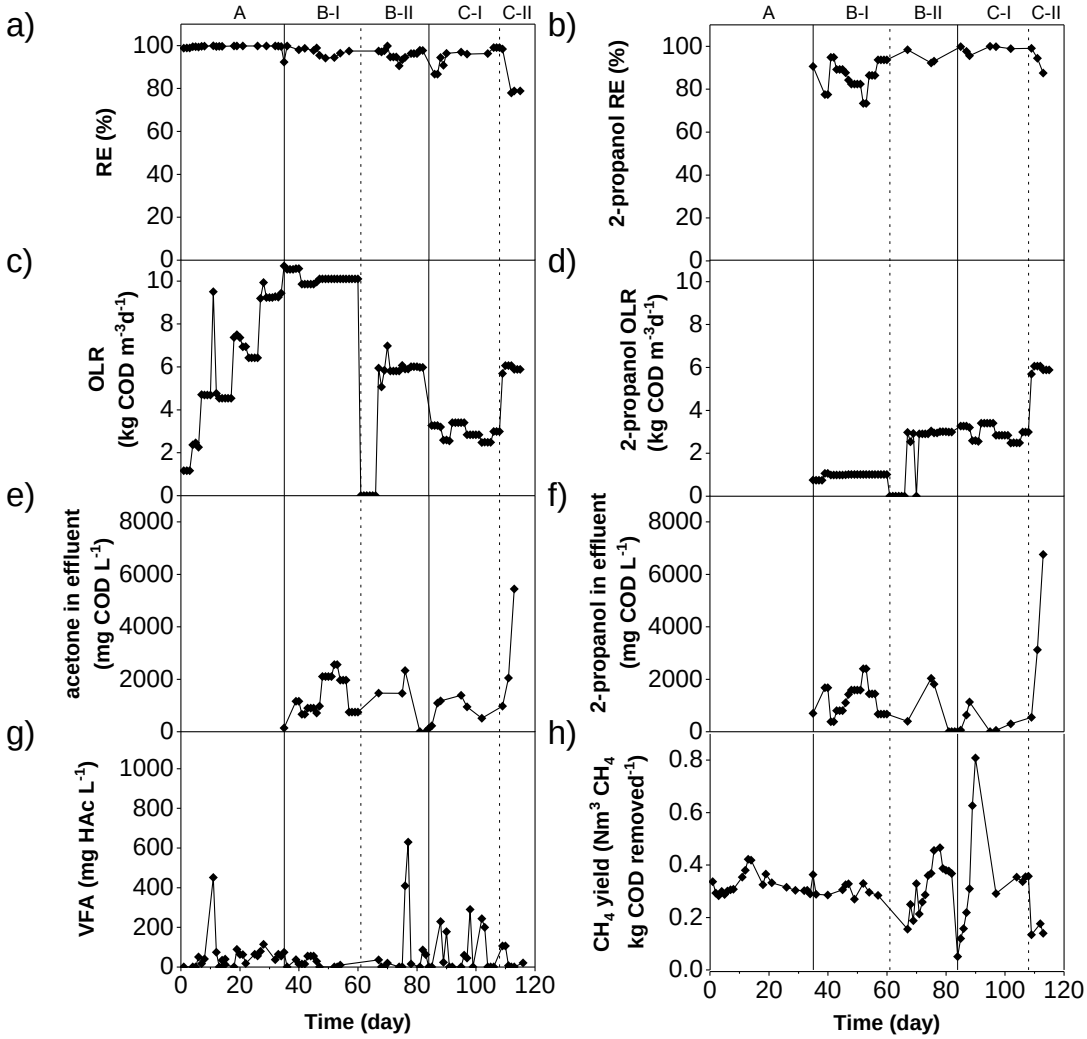
328 Figure 3. Influence of the initial 2-propanol concentration on the 2-propanol SMA
329

330 3.2 Anaerobic degradation of 2-propanol in laboratory-scale CSTR

331 The anaerobic degradation of 2-propanol was assessed in a laboratory-scale CSTR in
332 order to corroborate the recommended OLR to be treated in a continuous system. The
333 main results are summarized in Figure 4, which shows the evolution with time of RE and
334 2-propanol RE (Figs. 4a and 4b), OLR and 2-propanol OLR (Figs. 4c and 4d), acetone
335 and 2-propanol in effluent (Figs. 4e and 4f), effluent VFA (Fig. 4g), and methane yield
336 (Fig. 4h). Throughout the experiment, the pH was kept stable at 7.9 ± 0.4. During phase
337 A, the OLR of ethanol was increased progressively from 1.2 to 9.3 kg COD m^{-3} d^{-1} (Fig.
338 4c). Nearly complete removal efficiencies (>99%) were observed (Fig. 4a), showing that

339 a successful start-up was achieved using ethanol as the sole organic substrate. Methane
340 was produced according to the stoichiometric balance, with an average methane yield of
341 0.33 ± 0.04 Nm^3 CH_4 kg COD $removed^{-1}$ (Fig. 4h). VFA remained in values of lower
342 than 100 mg CH_3COOH L^{-1}, except on day 15 (Fig. 4g), when a sudden and punctual
343 increase of the OLR to 9.3 kg COD m^{-3} d^{-1} slightly destabilized the balance between
344 bacterial populations. The VFA peak on day 15 is the typical reactor response associated
345 with a sudden variation in OLR (Leitão et al., 2006).



346

347 Figure 4. Performance of CSTR: a) RE; b) 2-propanol RE: c) OLR; d) 2-propanol OLR;

348 e) effluent acetone; f) effluent 2-propanol; g) effluent VFA; h) CH_4 yield

349

350 From this point on until day 35, OLR was smoothly increased in two consecutive steps

351 until the addition of 2-propanol in 1:9 mass ratio to ethanol began (Phase B-I, Fig. 4d),

352 thus resulting in a small decrease from 99% to 92% RE (Fig. 4a). GC analysis revealed

353 the complete degradation of ethanol and the presence of 2-propanol as well as acetone in

354 the effluent (Fig. 4e and 4d). According to Widdel (1986), 2-propanol cannot replace

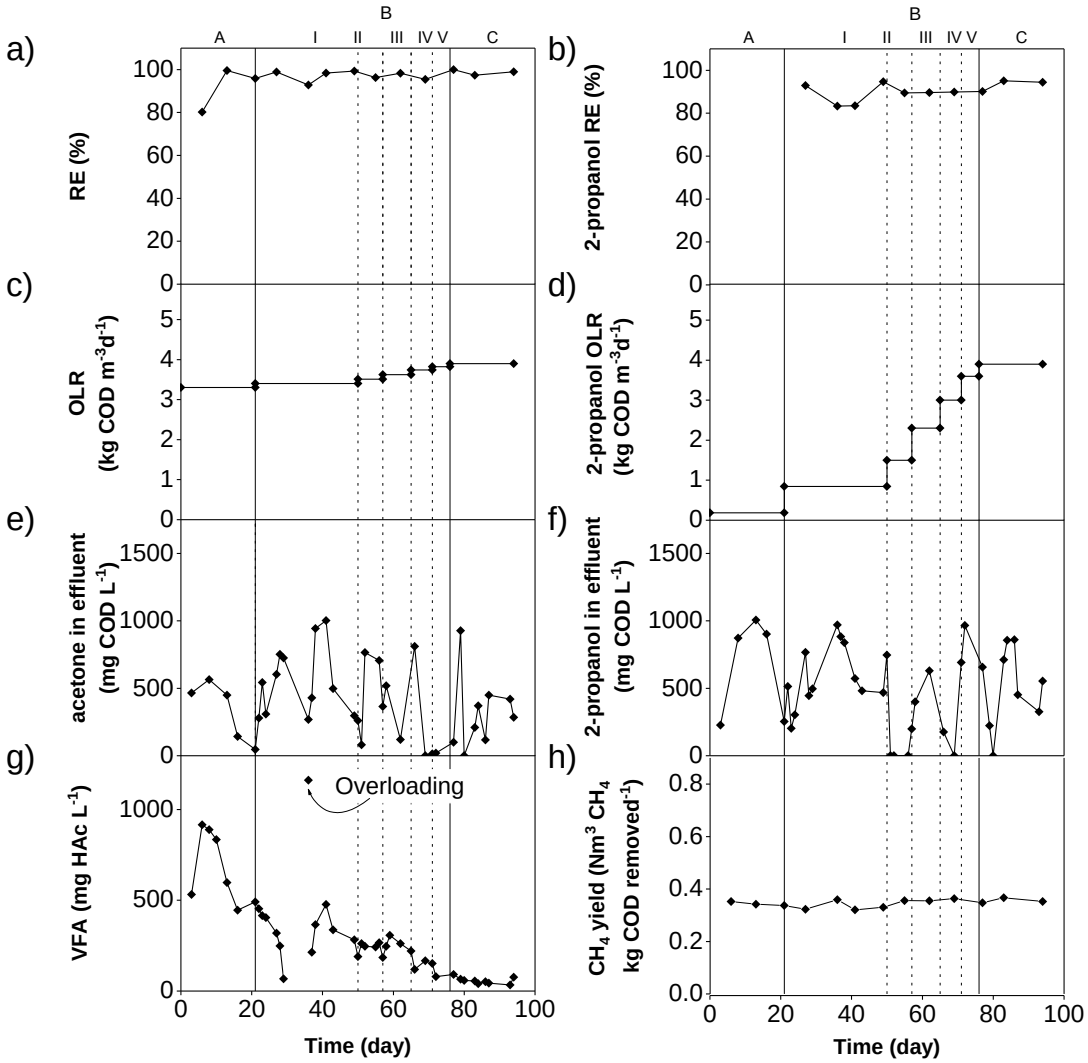
355 acetate as the main carbon source of a cell. However, it is a hydrogen donor for a
356 *Methanospirillum* sp. which converts it into acetone. The exposure of biomass to 2-
357 propanol caused the partial and unstable removal of 2-propanol, with REs oscillating
358 between 73% and 93%, needing more than three weeks (from days 35 to 58) to achieve a
359 stable 2-propanol RE of 94% (Fig. 4b). After a failure of the pump interrupted the solvent
360 feeding, the operation was resumed using a mixture of ethanol and 2-propanol at a mass
361 ratio of 1:1 (phase B-II). From day 67, a high RE of 2-propanol (> 90%) was obtained
362 with 3 kg COD m^{-3} d^{-1} of 2-propanol (Fig. 4b), indicating that the anaerobic degradation
363 of 2-propanol is not affected by shut-off periods as long as five days. It is noteworthy that
364 the production of methane from day 67 was half of the stoichiometric (0.16 Nm^3 CH_4 kg
365 COD $removed^{-1}$, Fig. 4h), which seemed to indicate that the production of methane was
366 mainly associated with the metabolization of ethanol. To corroborate this fact, the feeding
367 was substituted by pure ethanol on day 70, causing a rapid restoration of the methane
368 yield to 0.33 Nm^3 CH_4 kg COD $removed^{-1}$ (close to the stoichiometric 0.35). Then, the
369 methane yield systematically increased until a maximum of 0.45 Nm^3 CH_4 kg COD
370 $removed^{-1}$ was reached on day 78 (Fig. 4h). This high methane production (1.3 times
371 greater than stoichiometry) was also accompanied by a VFA peak of 629 mg CH_3COOH
372 L^{-1} (day 77, Fig. 4g), indicating that VFA production and utilization rates were
373 unbalanced. In any case, the average methane yield from days 71 to 78 was close to
374 stoichiometry (0.32 ± 0.09 Nm^3 CH_4 kg COD $removed^{-1}$), so the metabolization of the 2-
375 propanol to methane can be considered nearly complete at the end of phase B-II.
376 These results suggest a possible storage of intracellular compounds, which is non-
377 detectable as soluble organic matter, as part of the metabolic pathway in 2-propanol
378 anaerobic degradation. The degradation of acetone to methane and CO_2 was reported to

379 be the first case in which acetate is the only intermediate transferred between a
380 fermenting bacterium and a methanogen (Platen and Schink, 1987). According to these
381 authors, acetone is first carboxylated to acetoacetate by condensation with CO_2, from
382 which acetate is formed and then transferred to *Methanosaeta* sp. (formerly *Methanothrix*
383 sp.), the acetate-utilizing methanogen bacteria. In addition, Vecherskaya et al. (2001)
384 established the possible connection between 2-propanol and PHB by detecting 2-propanol
385 and acetone production during the anaerobic degradation of PHB. The experimental
386 results supported by the literature findings led to the hypothesis that in our anaerobic
387 culture coming from a water-brewery sludge, in which interspecies hydrogen transfer
388 plays the major role in methanogenic degradation chains, the efficient transfer of acetate
389 from the producer to the consumer slowly developed.
390 After ensuring the nearly full metabolization of 2-propanol in presence of ethanol, 2-
391 propanol was used as a sole organic substrate (day 84, Phase C-I, Fig. 4d). REs of higher
392 than 95% were obtained (Fig. 4b); however the methane production mimicked the
393 behavior observed in phase B-II. From day 84, no production of methane was obtained,
394 while a progressive increase occurred until day 90 (Fig. 4h). The carbon mass balance
395 between days 85 and 90 confirmed that 92% of the 2-propanol fed was converted to
396 methane, and that VFA concentration in effluent remained below 250 mg $CH_3COOH\ L^{-1}$
397 (Fig. 4g). Both data seemed to suggest that even with granular sludge acclimated to
398 solvents such 2-propanol or acetone, ethanol has a major role in the microbial population
399 dynamics. The absence of ethanol would have an impact by limiting the available acetate
400 for all competing methanogenic archea. From day 90, the 2-propanol degradation was
401 recovered.

On day 108 (Phase C-II), the OLR was increased to 6 kg COD m-3 d-1 of 2-propanol (Fig. 4d). A significant decrease in 2-propanol RE was observed, with the subsequent increase of acetone and 2-propanol in the effluent (Fig. 4e and 4f). Methane yield dropped to 0.14 Nm3 CH4 kg COD removed-1 (Fig. 4h), which is slightly lower than half of the stoichiometric value, confirming that the growth of the acetate-utilizing methanogen bacteria is the limiting step in 2-propanol degradation in comparison with the 2-propanol-oxidizer methanogen. After one week working at this OLR, a disaggregation of the granules into flocs was observed as well as a decrease in the 2-propanol RE from 99% to 88% (Fig. 4b), suggesting that the system suffered a solvent shock load. In this regard, several authors have experimented with the phenomenon of granular erosion and degranulation of the biomass under stress conditions during exposure to certain organic solvents (Costa et al., 2009; Lafita et al., 2015). In contrast to ethanol degradation, the tested load changes of 2-propanol led to the destabilization of the process, causing a great impact in the granulation mechanism, which in turn is associated with the dynamics of the bacteria population.

The results obtained with the CSTR are consistent with those obtained during batch bioassays; stable and high RE was achieved using 3 kg COD m^{-3} d^{-1} of 2-propanol, equivalent to an SLR of 0.17 kg COD kg-VS^{-1} d^{-1} (COD effluent equals to 0.9 g COD L^{-1}). This value matches with that observed at batch for 1.2 g COD L^{-1}. Girault et al. (2012) concluded that batch experiments can predict methane production when there is no inhibition, as batch performance depends on inoculum and operational conditions. The CSTR configuration led to a better understanding of this process, including the detection of intermediate products as well as the elucidation of the synergetic evolution of 2-propanol degradation and methane production. Based on these evidences, the continuous

426 degradation of pure 2-propanol should be carried out at an SLR of around 0.17 kg COD
427 kg-VS^{-1} d^{-1}; a sudden increase to an SLR of 0.34 kg COD kg-VS^{-1} d^{-1} caused
428 degranulation.
429 3.3 Anaerobic degradation of 2-propanol in pilot EGSB reactor
430 The anaerobic treatment of 2-propanol-loaded wastewater was evaluated in a pilot EGSB
431 reactor by smoothly switching from ethanol to 2-propanol. Instead of OLR, previous SLR
432 results from laboratory batch and CSTR were used as a reference. The performance of
433 EGSB is summarized in Figure 5, where the time evolution of the same parameters as in
434 the CSTR experiment is shown. In this case, the moving average REs (Fig. 5a and 5b)
435 and the moving average methane yield (Fig. 5h) are plotted. The water temperature was
436 kept at 26.2 ± 1.6ºC (warm season). pH and alkalinity were controlled at 7.6 ± 0.4 and
437 908 ± 394 mg $CaCO_3$ L^{-1}, respectively. At start-up, ethanol was used as the sole substrate
438 (phase A). In contrast with the laboratory CSTR, in which full removal was achieved
439 from the first day, nearly one week was required to achieve the almost complete RE of
440 ethanol. The difference in behavior cannot be attributed to the source of the sludge (both
441 came from IC reactors treating brewery wastewater), but to the fact that the pilot EGSB
442 was operated at lower superficial velocity than the IC reactor, and thus the internal mass
443 transfer limitation inside the granules would impact during the first few weeks of
444 operation. It is important to note that the reactor was operated in closed recirculation, so
445 the non-removed soluble organic matter was accumulated in the system during the first
446 few days, reaching values of 2500 mg COD L^{-1} (data not shown), and VFA of 915 mg
447 CH_3COOH L^{-1} on day 6 (Fig. 5g).



448

449 Figure 5. Performance of pilot EGSB: a) RE; b) 2-propanol RE: c) OLR; d) 2-propanol

450 OLR; e) effluent acetone; f) effluent 2-propanol; g) effluent VFA; h) CH_4 yield

451

452 Since higher exposure to 2-propanol had begun (day 22, Phase B-I, Fig. 5d), 2-propanol

453 was partially removed, although nearly three weeks were required to achieve removals

454 higher than 94% (Fig. 5b). This period of time was similar to that obtained in the CSTR

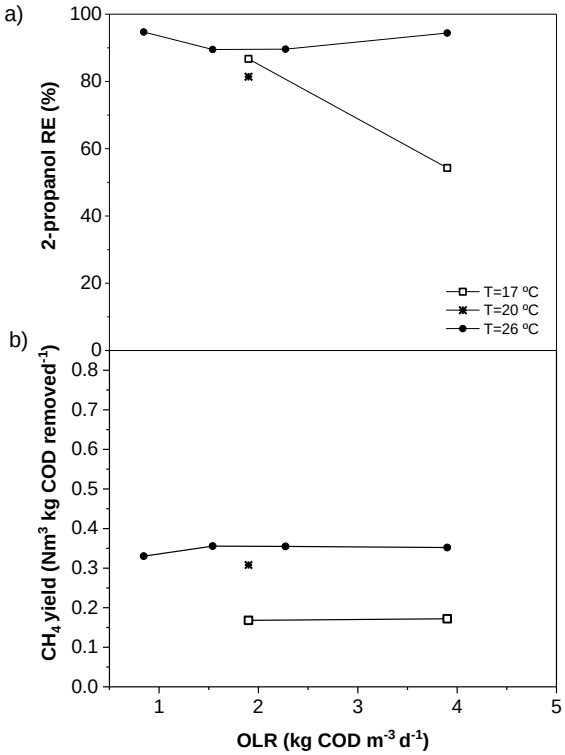
455 and to the lag-time observed in the batch assays water-brewery sludge. From this point,

456 the smooth increments in the 2-propanol load gave consistent and high REs of 2-propanol

for the duration of the experiment. Stable methane yields were achieved for the whole experiment (Fig. 5h), with an average of 0.35 ± 0.02 Nm^3 CH_4 kg COD $removed^{-1}$. The stability of the process can also be observed in the VFA evolution over time: VFA concentration showed a decreasing general trend from day 22, except for a short transitory period after day 36, when a peak of 1161 mg CH_3COOH L^{-1} (Fig. 5g) occurred due to a previous 24-h overdosing of solvents caused by the malfunctioning of the feeding pump. As in the CSTR experiment, acetone appeared as an intermediate of 2-propanol anaerobic degradation, corresponding with the fact that acetone degraders had a slower rate of growth than the 2-propanol-oxidizer methanogens (e.g. *Methanospirillum* sp.). In contrast with the CSTR experiment, the daily methane production (data not shown here) showed that there was no intracellular carbon accumulation when the increase in 2-propanol load was applied (Phase B) or when ethanol was removed from the system (Phase C). This is attributed to the adjusted strategy in the exposure to 2-propanol, showing that the chosen stepwise increase in the load of 2-propanol (0.6–0.7 kg COD m^{-3} d^{-1}) provided sufficient time for the development of the acetate-utilizing methanogen bacteria to ensure the efficient transfer of acetate from the producers. The smooth exposure to 2-propanol guaranteed operation at an SLR as high as 0.29 kg COD kg-VS^{-1} d^{-1} (OLR of 3.9 kg COD m^{-3} d^{-1}) without observing impairment in the process, the removal, or the granulation.

As the industrial application is expected to run at ambient temperature without further control, the impact of this parameter in the specialized methanogenic consortium was checked by operating the EGSB for three months during the cold season with similar procedures. The results are summarized in Figure 6, along with those obtained previously during the warm season (26ºC). Performances similar to the ones at warmer temperatures

were obtained for the degradation of ethanol (data not shown), confirming the findings that this compound is easily biodegradable under both mesophilic and psychrophilic temperatures (Enright et al., 2005; Kato et al., 1997; Lafita et al., 2015). On the other hand, the decrease of temperature from 20ºC to 17ºC had a great impact on the degradation of 2-propanol associated to the Arrhenius temperature-dependent rates of mesophilic bacteria. It was not possible to develop an effective psychrotolerant consortium to fully degrade 2-propanol to methane. Acetate-consuming methanogens were more sensitive to temperature than acetate degraders or 2-propanol-oxidizer methanogens. For example, at 2 kg COD m^{-3} d^{-1}, the methane yield diminished to less than half that of stoichiometry (0.17 Nm^3 CH_4 kg COD $removed^{-1}$, Fig. 6b) although the RE of 2-propanol did not decrease to the same extent (Fig. 6a). At 17ºC, higher loads worsened the 2-propanol RE, showing that 2-propanol-oxidizer methanogens are also adversely influenced by low temperature. Thus, 20ºC is the recommended minimum temperature for the anaerobic treatment of 2-propanol wastewater.



495

496 Figure 6. Influence of temperature on the anaerobic biodegradation of 2-propanol at pilot
497 EGSB: a) 2-propanol RE; b) CH_4 yield

498 **4. Conclusions**

499 This research is the first attempt to show that 2-propanol can be effectively degraded in a
500 pilot expanded granular sludge bed reactor, proving the feasibility of recycling dilute 2-
501 propanol wastewaters into bioenergy. Granular sludge coming from an IC treating
502 brewery wastewater was found to be efficient in removing 2-propanol loads up 0.29 kg
503 COD kg-VS^{-1} d^{-1} at 25ºC, when a smooth and progressive exposure to 2-propanol was
504 used. The degradation and methane yield appeared to be much lower when the
505 temperature decreased below 20ºC, showing that psychrophilic conditions are not
506 conducive for 2-propanol anaerobic treatment.

507

Nomenclature

AMPTS	Automatic Methane Potential Test System
BMP	Biochemical Methane Potential
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
EGSB	Expanded Granular Sludge Bed
HRT	Hydraulic Retention Time
IC	Internal Circulation
OLR	Organic Loading Rate
PHB	Polyhydroxybutyrates
RE	Removal Efficiency
S-B1	Sludge from an IC reactor treating brewery wastewater (The Netherlands)
S-B2	Sludge from an IC reactor treating brewery wastewater (Spain)
S-FP	Sludge from a pilot-scale EGSB reactor treating package printing effluents
SLR	Sludge Loading Rate
SMA	Specific Methanogenic Activity
TS	Total Solids
VFA	Volatile Fatty Acid
VOC	Volatile Organic Compound
VS	Volatile Solids

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