1 # Anaerobic degradation of 2-propanol: laboratory and pilot-

2 # scale studies

3 N. Vermorel[a,b], P. San-Valero[a], M. Izquierdo[a], C. Gabaldón[a], J.M. Penya-roja[a,*].

4 [a] Research Group GI^{2}AM, Department of Chemical Engineering, University of Valencia,

5 Avda. Universitat s/n, 46100 Burjassot, Spain.

6 e-mails: pau.valero@uv.es; carmen.gabaldon@uv.es; josep.penarrocha@uv.es ;

7 marta.izquierdo-sanchis@uv.es

8 [b] Pure Air Solutions, AC Heerenveen, The Netherlands.

9 e-mail: n.vermorel@pureairsolutions.nl

10 *Corresponding author. Address: Research Group GI^{2}AM, Department of Chemical

11 Engineering, University of Valencia, Avda. Universitat s/n, 46100 Burjassot, Spain.

12 Telephone: +34 96 3543695; fax: +34 96 3544898.

13 e-mail: josep.penarrocha@uv.es

14 URLs: http://giam.blogs.uv.es/; http://trainonsec.eu/

15

16

17

18

19

20

21

22 **Abstract**

23 The anaerobic degradation of 2-propanol, an important industrial solvent, was scaled-up

24 from batch assays to a pilot expanded granular sludge bed (EGSB) reactor at 25ºC. Batch

25 studies indicated that 2-propanol followed Haldane kinetics, with a maximum rate at 10 g

26 COD L^{-1}. Concentrations as high as 25 g COD L^{-1} did not inhibit the degradation of

27 ethanol, a common co-solvent. Similar specific methanogenic activities (SMA) were

28 obtained for water-solvent and water-brewery sludges (88 and 77 ml CH$_4$ g-VS^{-1} d^{-1} at 5

29 g COD L^{-1}). Continuous degradation showed a lag-phase of three weeks with water-

30 brewery sludge. Increases in 2-propanol load from 0.05 to 0.18 kg COD kg-VS^{-1} d^{-1}

31 caused a shift from the consumption of soluble matter to methane production, indicating

32 polyhydroxybutyrates (PHB) accumulation. Conversely, smooth increases of up to 0.29

33 kg COD kg-VS^{-1} d^{-1} allowed 2-propanol degradation without PHB accumulation. The

34 slowdown rate of 2-propanol-oxidizer and acetate-utilizing methanogen bacteria below

35 20ºC adversely impacted both removal and CH$_4$ yield.

36

39

40 **1. Introduction**

41 2-propanol is widely used as a solvent in many different chemical industries, such as

42 rubber, cosmetics, textiles, surface coatings, inks and pesticide formulations, with

43 worldwide manufacturing exceeding 1×10^6 tons per year. As with other organic solvents,

44 the main environmental concern is related to the release into the atmosphere of volatile

45 organic compounds (VOCs) during its industrial use. More investigation of technologies

46   for VOC control is required since the abatement of VOCs is a key factor in the protection

47   of the environment and of public health (European Union, 2010). Biological abatement of

48   2-propanol in industrial emissions has already been demonstrated as a successful method,

49   using aerobic conditions for the treatment, such as a biotrickling filter (San-Valero et al.,

50   2014; Pérez et al., 2013). Recently, anaerobic bioscrubbering was shown to be a

51   promising alternative for the treatment of air emissions containing VOCs of high

52   solubility in water, such as for example in food packaging printing, which is a growing

53   sector of economic importance in the EU. In this process, VOCs in the air are first

54   scrubbed with water and then degraded anaerobically in an EGSB reactor, thus recycling

55   dilute organic waste gases into bioenergy (Waalkens et al., 2015). The anaerobic

56   bioscrubber successfully treated air emissions from the evaporation of ink in the printing

57   press of a flexographic facility. An industrial prototype was used for the removal of

58   emissions containing ethanol (60%−65%), ethyl acetate (20%−25%) and 1-ethoxy-2-

59   propanol (10%−15%) as the main VOCs, reporting removal efficiencies (REs) of 93 $\pm$

60   5% in the EGSB, obtained at 25.1 $\pm$ 3.2ºC and with a methane yield of 0.32 Nm3 CH4 kg

61   COD removed^{-1} (Bravo et al., 2017). In order to expand the applicability of this VOC

62   abatement technology, since 2-propanol is also used as the main bulk solvent of ink

63   formulations in flexography instead of ethanol, its anaerobic degradation must be

64   investigated.

65   The anaerobic degradation of 2-propanol has rarely been studied in the past. Moreover,

66   the literature shows variations in the reported inhibition/biodegradable levels. This can

67   mostly be explained by the complexity of the anaerobic digestion process, with

68   phenomena such as acclimation that significantly impacts on the inhibition of organic

69   compounds (Chen et al., 2008). The data in the literature mainly refers to batch assays.

70   For example, Chou et al. (1978a) found that the addition of 2-propanol up to 4 g COD L^{-1}

71   did not inhibit methane production by using acetate as the reference substrate and an

72   enriched culture of methane bacteria not previously acclimated at 35ºC. In contrast,

73   another author found that 2-propanol is inhibitory for methanogenic bacteria with a

74   reported tolerance of 0.2 M at 36ºC (Widdel, 1986). A recent study by Ince et al. (2011)

75   shows also an inhibitory effect on the acetoclastic methane production pathway by using

76   acetate as substrate working at 37ºC. Degradation of acetate was inhibited with an initial

77   exposure to 0.1 M of 2-propanol. Repeated exposures resulted in higher inhibitions.

78   Regarding the continuous anaerobic degradation of 2-propanol, only one study treating a

79   mixture of organic solvents was found. Henry et al. (1996) operated a 20 L anaerobic

80   hybrid reactor with a non-enriched culture treating a mixture of methanol, ethanol,

81   propionate, butyrate, ethyl acetate and 2-propanol. The process was able to successfully

82   remove a total organic loading rate (OLR) of up to 4 g COD L^{-1} d^{-1} at 35ºC, with a 2-

83   propanol concentration fed to the reactor of 0.5 g L^{-1}. A more systematic study of the

84   anaerobic biodegradability of 2-propanol is required, especially under sub-optimal

85   mesophilic and psychrophilic conditions.

86   The main objective of this study was to investigate the degradation of 2-propanol with

87   granular sludge systems at ambient temperature, in order to expand the applicability of

88   the anaerobic bioscrubber technology to industries which use 2-propanol as the main

89   solvent. Therefore, the biodegradability of 2-propanol was first evaluated in batch assays,

90   including the influence of the granular sludge (water-brewery and water-solvent cultures).

91   Additionally, the potential inhibition of 2-propanol on the degradation of ethanol was

92   assessed, since it is usual to find the common use of both solvents in the chemical

93   industry. Based on the batch results, the continuous degradation of 2-propanol was

94 assessed at laboratory scale using a culture coming from an anaerobic reactor treating

95 brewery wastewaters (water-brewery culture), in order to determine the OLR that can be

96 efficiently treated and to evaluate the acclimation time. Finally, the influence of these two

97 key parameters (OLR and acclimation time) in the performance of the process was

98 evaluated using an industrial prototype of EGSB seeded with a water-brewery culture. To

99 the best of our knowledge, there are no previous reported data for an anaerobic pilot-scale

100 bioreactor using 2-propanol as the main carbon source. Thus, this study is expected to

101 provide guidelines for the start-up and operation of anaerobic reactors treating industrial

102 wastewater containing 2-propanol.

103 **2. Materials and methods**

104 2.1 Sources of granular sludge

105 Anaerobic granular sludges from different pilot- or full-scale anaerobic bioreactors

106 working at sub-optimal mesophilic temperatures were used in this study. The

107 characteristics of the sludge are shown in Table 1. S-FP sludge was obtained from a pilot-

108 scale EGSB treating package printing effluents (Altacel B.V., Weesp, the Netherlands),

109 with a yearly average water temperature of 22 ºC. This reactor had been treating

110 wastewaters containing solvents from the scrubbing of the VOC air emissions of the

111 facility for more than a year. The main substances in the wastewater were 1-ethoxy-2-

112 propanol (62 ± 12%), ethanol (26 ± 14%), 2-propanol (8 ± 4%) and 1-methoxy-2-

113 propanol (6 ± 2%). S-B1 sludge was obtained from a full-scale internal circulation (IC)

114 reactor treating brewery wastewater (Heineken, Zoeterwoude, the Netherlands), working

115 at 26ºC. S-B2 sludge was obtained from a full-scale IC reactor also treating brewery

116 wastewater (Font Salem, El Puig, Spain), operating between 22ºC and 32ºC. The sludges

117 from the breweries (S-B1 and S-B2) were not exposed to 2-propanol prior to their use in

118 this work. The three types of sludge had similar total solids (TS) and volatile solids (VS)

119 content; however, S-B1 had a larger granule size and higher sulfur content than the other

120 two.

121 **Table 1.** Sources and physical properties of the granular sludge used in this research
122

|	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

123
124 2.2 Batch bioassays

125 Biochemical Methane Potential (BMP) assays were developed for determining the

126 anaerobic degradability of compounds, allowing the testing of the substrate in controlled

127 and optimal conditions in a laboratory environment.Therefore, BMP assays were used to

128 determine the ultimate methane production, specific methanogenic activity (SMA) and

129 lag phase for the degradation of 2-propanol under specifically chosen conditions. For this

130 purpose, 4.23 g VS L^{-1} of granular sludge were added to serum bottles (500 mL)

131 containing a basal medium and supplemented with ethanol (95%–96% v v^{-1}, VWR) at 0.8

132 or 1.6 g chemical oxygen demand (COD) L^{-1}, used as a control, and with 2-propanol

133 (99.5% v v^{-1}, Sigma Aldrich) at several concentrations. N, P, K and S were added to give

134 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

135 solution contained (mg L^{-1}): 2500 NaHCO$_3$; 40 CaCl$_2$ H$_2$O; 40 MgCl$_2$ 6H$_2$O; 0.05

136   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

137    $(NH_4)_6Mo_7O_{24} \cdot 2H_2O$; 0.56 $NiSO_4 \cdot 6H_2O$; 0.05 Na_2SeO_3; 0.16 $ZnSO_4 \cdot 7H_2O$; and 1.2 yeast

138   extract. The bottles were placed in an Automatic Methane Potential Test System

139   (AMPTS II by BioProcess Control®) and mechanically stirred (one minute out of two) at

140   112 rpm at 25ºC. The biogas passed through a CO_2-scrubbing unit (containing NaOH

141   3M), allowing only methane to flow to a gas-recording unit. BMP was calculated as the

142   ratio of the final cumulative methane production and the initial organic content of the

143   substrate. SMA was estimated as the maximum methane flow rate in function of the

144   initial sludge content. Soluble COD and volatile fatty acids (VFA) were determined at the

145   beginning and end of the bioassays. The experiments were conducted in duplicate.

146   A set of experiments was designed to compare the biodegradability of 5 g COD L^{-1} of 2-

147   propanol by the S-FP sludge (water-solvent culture) and the S-B1 sludge (water-brewery

148   culture). In a second step, S-FP was selected to determine the influence of the initial

149   concentration of 2-propanol on the SMA and BMP. For this purpose, initial

150   concentrations of 2-propanol of 1.2, 5, 10 and 25 g COD L^{-1} were used.

151   2.3 Anaerobic degradation of 2-propanol in laboratory CSTR

152   The continuous anaerobic degradation of 2-propanol was performed in a continuous

153   stirred tank reactor (CSTR) with an effective volume of 1.6 L. The reactor was filled with

154   17.4 g VS L^{-1} of sludge S-B2. The temperature was kept at 25ºC using a thermostatic

155   water bath (Memmert GmbH +Co.KG, Germany). A hydraulic retention time (HRT) of

156   eight days was fixed. The experimental set-up is shown in Fig.1a. The CSRT feeding was

157   made up of three solutions: a synthetic wastewater composed of a mixture of the organic

158   substrate and N, P, K and S, which were added from a concentrated solution to get a ratio

159   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.

174
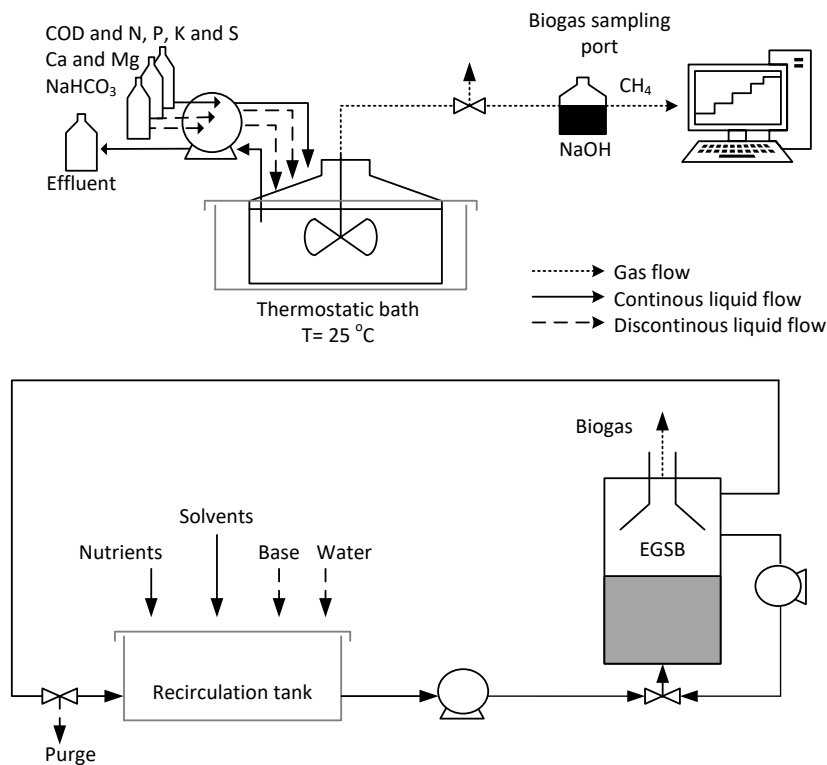
Figure 1. Scheme of the experimental set-up: (a) laboratory CSTR; (b) pilot EGSB

176

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.

189   From this point on, the inlet concentration of 2-propanol was increased by two stepwise

190   (24 and 47 g COD L^{-1}, OLR of 2.9 and 5.9 kg COD m^{-3} d^{-1}).

191   **Table 2.** Experimental plan for the anaerobic degradation of 2-propanol in laboratory
192   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

193   *SLR stands for Sludge Loading Rate: SLR = OLR per initial VS content
194

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 $m^{-3}\ d^{-1}$

218 (sludge loading rate (SLR) of 0.25-0.29 kg COD kg-VS^{-1} d^{-1}). During phase A (days 1 to

219 22), the system was fed with a solution of industrial-grade ethanol (95%, Univar BV, the

220 Netherlands) denatured with 5% vol. of 2-propanol, thus containing a minimum 2-

221 propanol OLR of 0.2 kg COD m^{-3} d^{-1}. From day 23 to day 81 (phase B), the influent COD

222 composition was changed to binary mixtures of ethanol and 2-propanol (99%, Univar

223 BV, the Netherlands). When the VFA concentration was lower than 200 mg L^{-1} and COD

224 concentration was less than 1000 mg L^{-1}, the OLR of 2-propanol was increased in steps

225 of ~0.7 kg COD m^{-3} d^{-1}, while the OLR of ethanol was decreased to ensure a smooth

226 acclimation to the presence of 2-propanol as the sole organic substrate (phase C).

227 **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

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

229 2.5 Analytical methods

230 The determination of total solids (TS) and volatile solids (VS) of the sludge was carried

231 out in triplicate according to standard methods (American Public Health Association,

232 1999). For S-FP and S-B2, the average particle diameter of the granule was measured

233 using a laser particle analyzer (Mastersizer, Malvern 2000, UK). For S-B1, the average

234 particle diameter of 10 granules was measured with an optical microscope (SE, Nikon,

235 Japan).

236 For laboratory assays, soluble COD concentration was analyzed according to standard

237 methods and VFA and alkalinity were determined using potentiometer titration (848

238 Titrino Plus, Metrohm, Switzerland). N and P concentration were measured with an ionic

239 chromatograph (883 Basic IC Plus, Metrohm, Switzerland) equipped with Metrosep C4-

240 250/4.0 and Metrosep A Supp 3 columns. For the pilot test, COD, VFA, N and P

241 concentration were determined with spectrophotometric commercial kits (LCK 514, LCK

242 365, LCK 303 and LCK 348 kits from Hach Lange GmbH, Germany). Alkalinity content

243 was estimated with a titrimetric kit (MColortest[TM], Merk Millipore, Germany).

244 For all experiments, the solvent composition in the water samples was determined using a

245 gas chromatograph equipped with a flame ionization detector (Agilent GC 7890A,

246 Spain), a capillary column (Restek Rtx-VMS) and a helium carrier gas with 25 ml min^{-1}

247 of flow.

248 **3. Results and discussion**

249 3.1 Biodegradability studies in batch reactors

250 Two sets of batch assays were developed to quantify the influence of both the initial

251 concentration of 2-propanol and the anaerobic sludge source on the anaerobic

252 biodegradation of 2-propanol in the presence of ethanol. The cumulative volume of

253 methane produced over time for water-solvent (S-FP) and water-brewery (S-B1) cultures

254 with a mixture of ethanol and 2-propanol is shown in Figure 2. The evolution of methane

255 production clearly shows a diauxic shift: biomass preferentially uses the readily

256 biodegradable substrate, and only when ethanol has been exhausted as an energy and

257 carbon source does the active population start to utilize 2-propanol. This phenomenon

258 was reported by Chou et al. (1978b) in cross-acclimation studies of an acetate culture

259 with 30 petrochemicals, including acetone, propanol, butanol and methyl acetate among

260   others. The metabolic adjustment from ethanol to 2-propanol resulted in a lag period that

261   clearly demonstrates a link with the observed methane production of each solvent. As can

262   be seen from Figure 2, for a given initial 2-propanol concentration (5 g COD L^{-1}), the

263   observed lag time is significantly longer for the water-brewery sludge (S-B1) than for the

264   water-solvent one (S-FP). It should be noted that the pilot EGSB reactor from which the

265   S-FP came was seeded from the same IC reactor from which S-B1 was taken. The

266   difference lies in the exposure of S-FP to industrial solvents, including 2-propanol, for

267   more than one year.

268
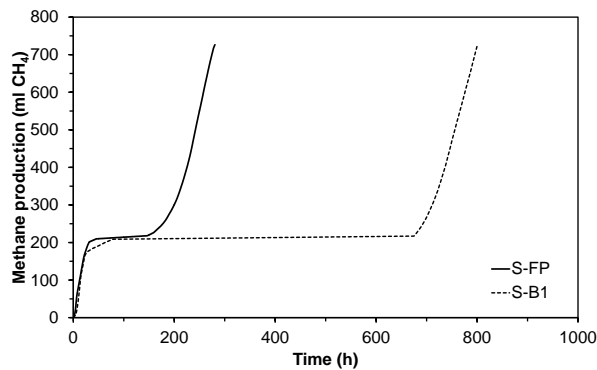
Figure 2. Comparison of cumulative methane production between water-brewery (S-B1)
and water-solvent (S-FP) sludge. Initial 2-propanol concentration = 5 g COD L^{-1}. Initial
ethanol concentration = 1.2 g COD L^{-1}

272

273   The results of the influence of the source of sludge are summarized in Table 4. For the

274   two assays, soluble COD and VFA concentrations at the end of the test were below 40

275   mg COD L^{-1} and 3 mg CH_3COOH L^{-1}, showing the complete degradation of the solvent.

276   BMP values showed methane recoveries of $89 \pm 2\%$ with S-FP and $91 \pm 1\%$ with S-B1

277   for ethanol, and $75 \pm 1\%$ with S-FP and $72 \pm 5\%$ with S-B1 for 2-propanol. Independent

278   of the sludge source, almost the same ethanol removal rate was observed, quantified by

279   SMA. This is in accordance with the fact that ethanol is a readily anaerobic biodegradable

280   substrate. In addition, the removal rates for 2-propanol were reduced to less than half that

14

281 of ethanol, appearing slightly higher (13%) for the water-solvent sludge (S-FP). The main

282 difference between both sludges was the lag time. In the presence of ethanol, 33 days

283 were required for a water-brewery culture (S-B1) to start metabolizing 2-propanol, while

284 it took only 12 days for the water-solvent culture (S-FP). These results verified that the

285 structural characteristics of the solvent influence its degradation rate after acclimation,

286 and that the previous exposure of the sludge to the target compound reduces the length of

287 the lag time.

288 **Table 4.** SMA, BMP and lag phase for the water-brewery (S-B1) and water-solvent (S-FP)
289 cultures. Initial 2-propanol concentration = 5 g COD L^{-1}. Initial ethanol concentration =
290 1.6 g COD L^{-1}

		SMA (ml CH$_4$ g-VS^{-1} d^{-1})	BMP (ml CH$_4$ g-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

291

292 A second experiment was designed to assess the potential inhibition of 2-propanol initial

293 concentration in presence of ethanol using the water-solvent sludge (S-FP). SMA, BMP

294 and lag time for increasing initial 2-propanol concentrations (1.2, 5, 10 and 25 g COD·L^{-1})

295 $^{-1}$) are reported in Table 5, as well as SMA for ethanol. An almost complete degradation

296 of the solvents in terms of soluble COD concentration was observed (> 98%). It is

297 highlighted that the SMA value for ethanol degradation was similar for each tested

298 concentration of 2-propanol. For a given ethanol concentration of 1.6 g COD L^{-1}, SMA

299 remained around 200 ml CH$_4$ g-VS^{-1} d^{-1}, matching the value of 202 ± 9 ml CH$_4$ g-VS^{-1} d^{-1}

300 obtained in a separate test with ethanol as the sole solvent. Thus, no perceptible inhibition

301 on the degradation of ethanol occurred for the tested conditions, even at an initial

302 concentration of 2-propanol of 25 g COD L^{-1}. At 37°C, Ince et al. (2011) found inhibitory

303 levels of 2-propanol on acetate biodegradation starting from 0.1 M (14.4 g COD L^{-1}),

304 with an IC$_{50}$ of 0.27M (38.9 g COD L^{-1}). The lack of inhibitory effect on ethanol

305 degradation in this work might be attributed to the previous exposure of the sludge to the

306 target solvent, indicating that the anaerobic treatment of ethanol-rich effluents would not

307 be inhibited by the presence of 2-propanol, even if relatively high concentrations of this

308 solvent are punctually reached.

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

2-propanol concentration (g COD L^{-1})	ethanol concentration (g COD L^{-1})	ethanol	2-propanol		
		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*

310 *Results corresponding to one replicate

311

312 Regarding the biodegradation of 2-propanol, the increase of initial 2-propanol

313 concentration adversely affected the lag time and the methane production rate. An

314 exponential lengthening of the lag time was observed, reaching 126 days for the highest

315 concentration tested, and showing that the concentration of the target solvent increased

316 the required time for final metabolization to methane. SMA versus the initial 2-propanol

317 concentration is plotted in Figure 3. The methane production rate showed an increase up

318 to 5 g COD L^{-1}, reaching a plateau until at least 10 g COD L^{-1}, after which there was a

319 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$_3$COOH 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   ^{1} d^{-1} of 2-propanol could be removed for concentrations in the reactor of between 1.2 and

326   10 g COD L^{-1}.
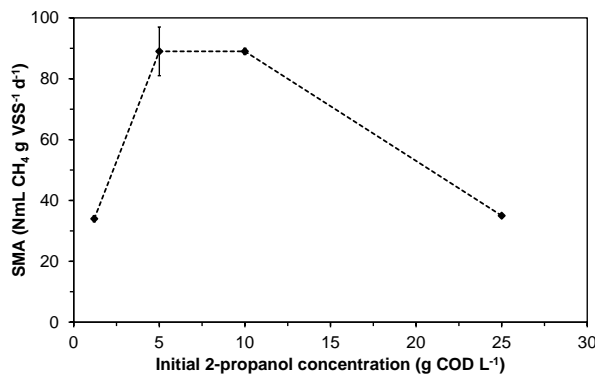
327

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$_3$COOH 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).
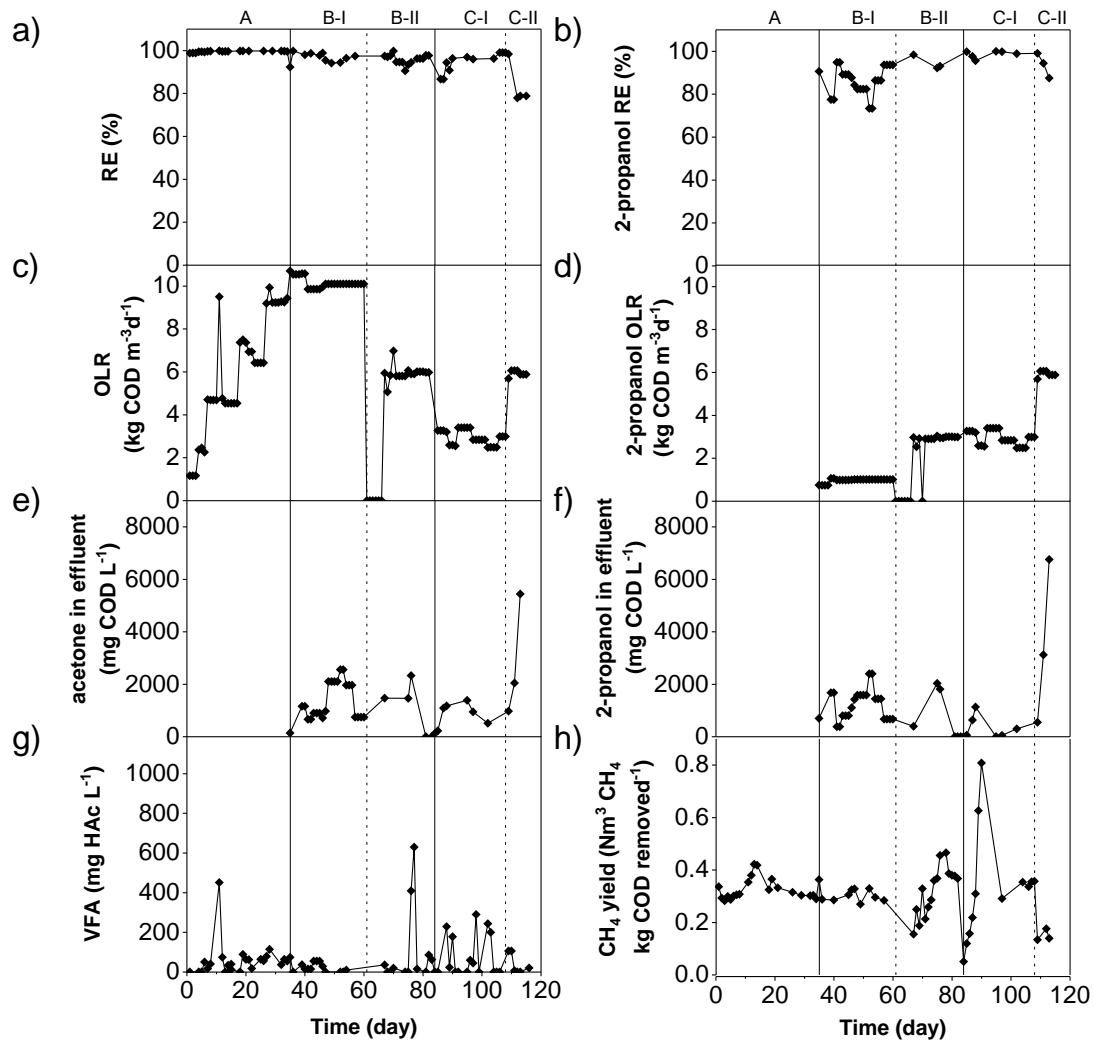
Figure 4. Performance of CSTR: a) RE; b) 2-propanol RE: c) OLR; d) 2-propanol OLR;

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

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

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

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

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

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 $\pm$ 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.

402   On day 108 (Phase C-II), the OLR was increased to 6 kg COD m-3 d-1 of 2-propanol

403   (Fig. 4d). A significant decrease in 2-propanol RE was observed, with the subsequent

404   increase of acetone and 2-propanol in the effluent (Fig. 4e and 4f). Methane yield

405   dropped to 0.14 Nm3 CH4 kg COD removed-1 (Fig. 4h), which is slightly lower than

406   half of the stoichiometric value, confirming that the growth of the acetate-utilizing

407   methanogen bacteria is the limiting step in 2-propanol degradation in comparison with

408   the 2-propanol-oxidizer methanogen. After one week working at this OLR, a

409   disaggregation of the granules into flocs was observed as well as a decrease in the 2-

410   propanol RE from 99% to 88% (Fig. 4b), suggesting that the system suffered a solvent

411   shock load. In this regard, several authors have experimented with the phenomenon of

412   granular erosion and degranulation of the biomass under stress conditions during

413   exposure to certain organic solvents (Costa et al., 2009; Lafita et al., 2015). In contrast to

414   ethanol degradation, the tested load changes of 2-propanol led to the destabilization of the

415   process, causing a great impact in the granulation mechanism, which in turn is associated

416   with the dynamics of the bacteria population.

417   The results obtained with the CSTR are consistent with those obtained during batch

418   bioassays; stable and high RE was achieved using 3 kg COD m^{-3} d^{-1} of 2-propanol,

419   equivalent to an SLR of 0.17 kg COD $kg\text{-}VS^{-1}$ d^{-1} (COD effluent equals to 0.9 g COD L^{-}

420   ^{1}). This value matches with that observed at batch for 1.2 g COD L^{-1}. Girault et al. (2012)

421   concluded that batch experiments can predict methane production when there is no

422   inhibition, as batch performance depends on inoculum and operational conditions. The

423   CSTR configuration led to a better understanding of this process, including the detection

424   of intermediate products as well as the elucidation of the synergetic evolution of 2-

425   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$_3$COOH L^{-1} on day 6 (Fig. 5g).
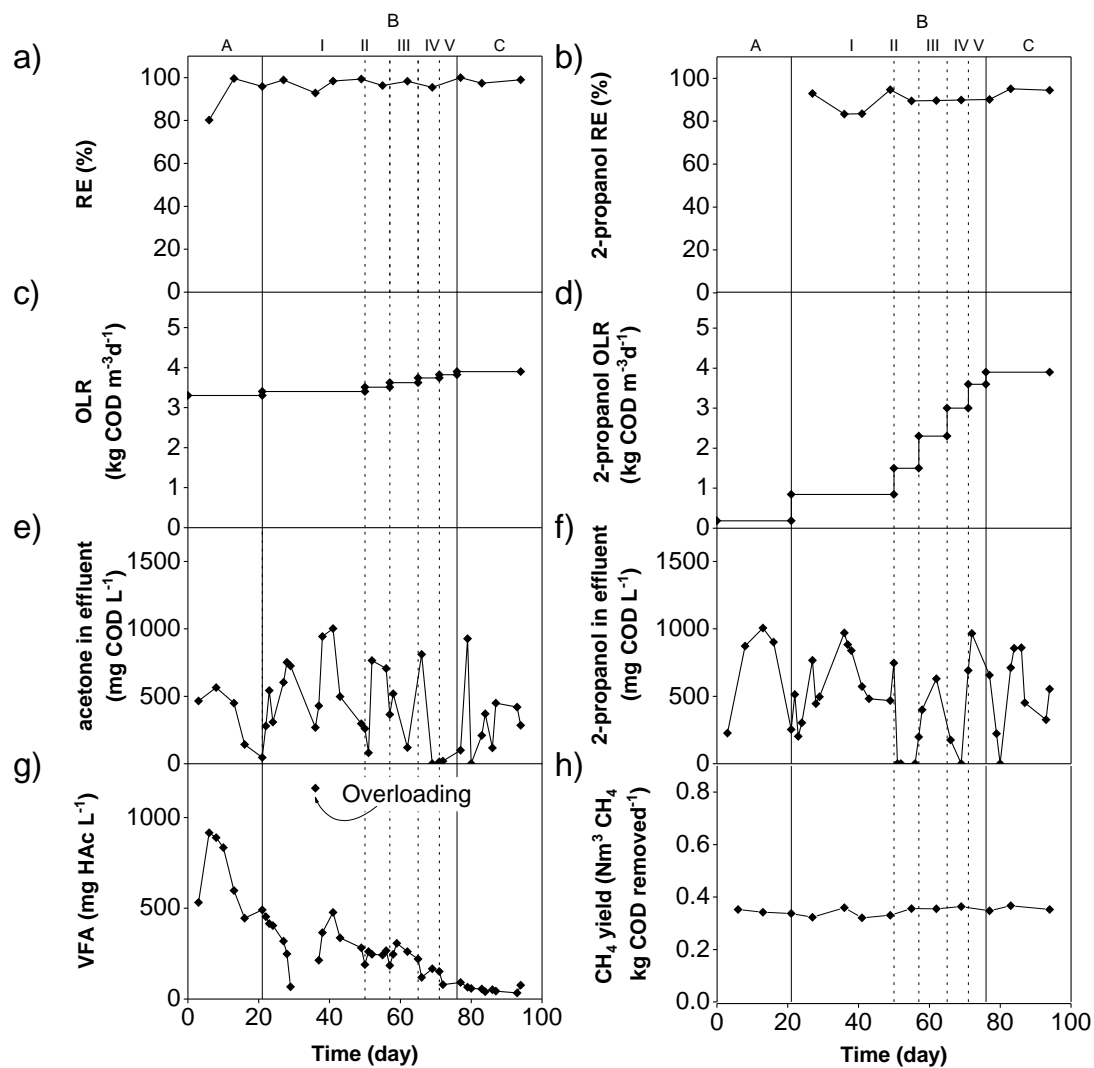
Figure 5. Performance of pilot EGSB: a) RE; b) 2-propanol RE: c) OLR; d) 2-propanol

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

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

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

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

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

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

457   for the duration of the experiment. Stable methane yields were achieved for the whole

458   experiment (Fig. 5h), with an average of 0.35 ± 0.02 Nm^3 CH$_4$ kg COD removed^{-1}. The

459   stability of the process can also be observed in the VFA evolution over time: VFA

460   concentration showed a decreasing general trend from day 22, except for a short

461   transitory period after day 36, when a peak of 1161 mg CH$_3$COOH L^{-1} (Fig. 5g) occurred

462   due to a previous 24-h overdosing of solvents caused by the malfunctioning of the

463   feeding pump. As in the CSTR experiment, acetone appeared as an intermediate of 2-

464   propanol anaerobic degradation, corresponding with the fact that acetone degraders had a

465   slower rate of growth than the 2-propanol-oxidizer methanogens (e.g. *Methanospirillum*

466   sp.). In contrast with the CSTR experiment, the daily methane production (data not

467   shown here) showed that there was no intracellular carbon accumulation when the

468   increase in 2-propanol load was applied (Phase B) or when ethanol was removed from the

469   system (Phase C). This is attributed to the adjusted strategy in the exposure to 2-propanol,

470   showing that the chosen stepwise increase in the load of 2-propanol (0.6–0.7 kg COD m^{-3}

471   d^{-1}) provided sufficient time for the development of the acetate-utilizing methanogen

472   bacteria to ensure the efficient transfer of acetate from the producers. The smooth

473   exposure to 2-propanol guaranteed operation at an SLR as high as 0.29 kg COD kg-VS^{-1}

474   d^{-1} (OLR of 3.9 kg COD m^{-3} d^{-1}) without observing impairment in the process, the

475   removal, or the granulation.

476   As the industrial application is expected to run at ambient temperature without further

477   control, the impact of this parameter in the specialized methanogenic consortium was

478   checked by operating the EGSB for three months during the cold season with similar

479   procedures. The results are summarized in Figure 6, along with those obtained previously

480   during the warm season (26ºC). Performances similar to the ones at warmer temperatures

481   were obtained for the degradation of ethanol (data not shown), confirming the findings

482   that this compound is easily biodegradable under both mesophilic and psychrophilic

483   temperatures (Enright et al., 2005; Kato et al., 1997; Lafita et al., 2015). On the other

484   hand, the decrease of temperature from 20ºC to 17ºC had a great impact on the

485   degradation of 2-propanol associated to the Arrhenius temperature-dependent rates of

486   mesophilic bacteria. It was not possible to develop an effective psychrotolerant

487   consortium to fully degrade 2-propanol to methane. Acetate-consuming methanogens

488   were more sensitive to temperature than acetate degraders or 2-propanol-oxidizer

489   methanogens. For example, at 2 kg COD m^{-3} d^{-1}, the methane yield diminished to less

490   than half that of stoichiometry (0.17 Nm^3 CH_4 kg COD $removed^{-1}$, Fig. 6b) although the

491   RE of 2-propanol did not decrease to the same extent (Fig. 6a). At 17ºC, higher loads

492   worsened the 2-propanol RE, showing that 2-propanol-oxidizer methanogens are also

493   adversely influenced by low temperature. Thus, 20ºC is the recommended minimum

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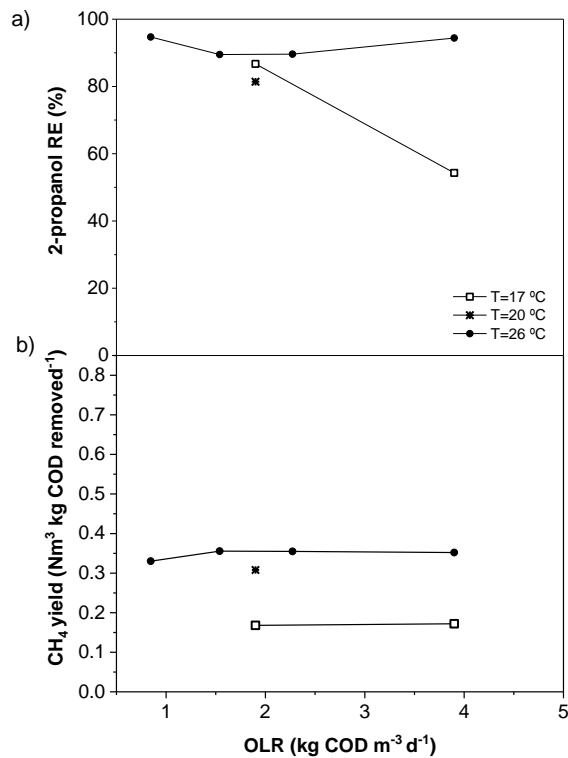
Figure 6. Influence of temperature on the anaerobic biodegradation of 2-propanol at pilot EGSB: a) 2-propanol RE; b) CH_4 yield

4. Conclusions

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

508 **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

509

510 **Acknowledgments**

519 **References**

520 Ahring, B.K., Sandberg, M., Angelidaki, I., 1995. Volatile fatty acids as indicators of

521   process imbalance in anaerobic digestors. Appl. Microbiol. Biotechnol. 43, 559–565.

522 doi:10.1007/BF00218466.

523 American Public Health Association, 1999. Standard Methods for the Examination of

524 Water and Wastewater. Water Environment Federation, Washington.

525 Bravo, D., Ferrero, P., Penya-roja, J.M., Àlvarez-Hornos, F. J., Gabaldón, C., 2017.

526 Control of VOCs from printing press air emissions by anaerobic bioscrubber:

527 performance and microbial community of an on-site pilot unit. J. Environ. Manage.

528 197, 287–295. doi:10.1016/j.jenvman.2017.03.093

529 Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A

530 review. Bioresour. Technol. 99, 4044–4064. doi:10.1016/j.biortech.2007.01.057.

531 Chou, W.L., Speece, R.E., Siddiqi, R.H., 1978a. Acclimation and degradation of

532 petrochemical wastewater components by methane fermentation, in: Biotechnol.

533 Bioeng. Symp. pp. 391–414.

534 Chou, W.L., Speece, R.E., Siddiqi, R.H., McKeon, K., 1978b. The effect of

535 petrochemical structure on methane fermentation toxicity, in: Ninth International

536 Conference on Water Pollution Research. Elsevier, pp. 545–558.

537 Costa, J.C., Moita, I., Ferreira, E.C., Alves, M.M., 2009. Morphology and physiology of

538 anaerobic granular sludge exposed to an organic solvent. J. Hazard. Mater. 167,

539 393–398. doi:10.1016/j.jhazmat.2008.12.130.

540 Enright, A.-M., McHugh, S., Collins, G., O'FLaherty, V., 2005. Low-temperature

541 anaerobic biological treatment of solvent-containing pharmaceutical wastewater.

542 Water Res. 39, 4587–96. doi:10.1016/j.watres.2005.08.037

543 European Union, 2010. Directive 2010/75/EU of the European Parliament and of the

544 council of 24 November 2010 on industrial emissions (integrated pollution

545 prevention and control) (Recast), Official Journal of the European Union.

546 doi:10.3000/17252555.L_2010.334.eng.

547 Girault, R., Bridoux, G., Nauleau, F., Poullain, C., Buffet, J., Peu, P., Sadowski, A.G.,

548 Béline, F., 2012. Anaerobic co-digestion of waste activated sludge and greasy sludge

549 from flotation process: Batch versus CSTR experiments to investigate optimal

550 design. Bioresour. Technol. 105, 1–8. doi:10.1016/j.biortech.2011.11.024.

551 Henry, M.P., Donlon, B.A., Lens, P.N., Colleran, E.M., 1996. Use of anaerobic hybrid

552 reactors for treatment of synthetic pharmaceutical wastewaters containing organic

553 solvents. J. Chem. Technol. Biotechnol. 66, 251–264. doi:10.1002/(SICI)1097-

554 4660(199607)66:3<251::AID-JCTB496>3.0.CO;2-S.

555 Ince, B., Koksel, G., Cetecioglu, Z., Oz, N.A., Coban, H., Ince, O., 2011. Inhibition effect

556 of isopropanol on acetyl-CoA synthetase expression level of acetoclastic

557 methanogen, Methanosaeta concilii. J. Biotechnol. 156, 95–9.

558 doi:10.1016/j.jbiotec.2011.08.021.

559 Kato, M.T., Field, J.A., Lettinga, G., 1997. The anaerobic treatment of low strength

560 wastewaters in UASB and EGSB reactors. Water Sci. Technol. 36, 375–382.

561 doi:10.1016/S0273-1223(97)00545-3

562 Lafita, C., Penya-Roja, J.M., Gabaldón, C., 2015. Anaerobic removal of 1-methoxy-2-

563 propanol under ambient temperature in an EGSB reactor. Bioprocess Biosyst. Eng.

564 doi:10.1007/s00449-015-1453-0.

565 Leitão, R.C., van Haandel, A.C., Zeeman, G., Lettinga, G., 2006. The effects of

566 operational and environmental variations on anaerobic wastewater treatment

567 systems: A review. Bioresour. Technol. 97, 1105–1118.

568 doi:10.1016/j.biortech.2004.12.007.

569 Pérez, M.C., Álvarez-Hornos, F.J., San-Valero, P., Marzal, P., Gabaldón, C., 2013.

570   Microbial community analysis in biotrickling filters treating isopropanol air

571   emissions. Environ Technol. 34 (19), 2789-2798. doi:

572   10.1080/09593330.2013.790067

573 Platen, H., Schink, B., 1987. Methanogenic degradation of acetone by an enrichment

574   culture. Arch. Microbiol. 149, 136–141. doi:10.1007/BF00425079.

575 San-Valero, P., Penya-Roja, J.M., Álvarez-Hornos, F.J., Gabaldón, C., 2014. Modelling

576   mass transfer properties in a biotrickling filter for the removal of isopropanol. Chem.

577   Eng. Sci. 108, 47–56. doi:10.1016/j.ces.2013.12.033

578 Vecherskaya, M., Dijkema, C., Stams, A.J., 2001. Intracellular PHB conversion in a Type

579   II methanotroph studied by 13C NMR. J. Ind. Microbiol. Biotechnol. 26, 15–21.

580   doi:10.1038/sj.jim.7000086.

581 Waalkens, A., Gabaldón, C., Penya-roja, J.M., Álvarez-Hornos, F.J., 2015.

582   Procedimiento para la depuración de gases que contienen compuestos orgánicos

583   volátiles. ES Patent Nº 2542257.

584 Widdel, F., 1986. Growth of methanogenic bacteria in pure culture with 2-propanol and

585   other alcohols as hydrogen donors. Appl. Environ. Microbiol. 51, 1056–62.