

1 **Title: Effects of several inocula on the biochemical hydrogen potential of sludge-**
2 **vinasse co-digestion**

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6

7 **Abstract**

8 The influence of the inoculum on the Biochemical Hydrogen Potential test
9 (BHP) was investigated. Thermophilic BHP from sludge-vinasses co-digestion (50:50)
10 was studied employing three types of inocula: Acidogenic Inoculum, Sludge Inoculum
11 and Thermal Sludge Inoculum. The maximum hydrogen yield was obtained with a
12 sludge inoculum (177mL H₂/g VS_{added}). This yield was 21 and 36% higher than for
13 acidogenic inoculum and thermal sludge inoculum, respectively. The percentages
14 between Eubacteria:Archaea increased from 59.2:40.8 to 92.0:9.0 during BHP tests
15 using the sludge inoculum while it remained stable in the others cases around 50:50.
16 Furthermore, hydrogen production was accompanied by the generation of volatile fatty
17 acids, mainly acetic, butyric and propionic acids. There were no differences in the rate
18 of hydrogen production in any of the BHP.

19

20 **Keywords:** Biochemical hydrogen potential; Inoculum; Sludge; Vinasse; Anaerobic co-
21 digestion; *Eubacteria*

22

23 **1. Introduction**

24 In recent years, the energy crisis has imposed the necessity to achieve a sustainable
25 future built on alternative sources of energy and materials. Molecular hydrogen
26 represents a storable form of energy [1]. Moreover, its combustion does not generate
27 polluting products and it has high specific energy [2–4].

28 Hydrogen production can occur during the anaerobic digestion (AD) process. This
29 process can be divided into two stages: dark fermentation (DF) and methanogenesis.
30 The first stage involves the production of volatile fatty acids (VFAs), H₂ and CO₂, while
31 the second one converts VFAs into CH₄ and CO₂ [5,6]. Simple operation conditions,
32 low operating cost, low energy demand and fast reaction rate are some one-off
33 advantages of dark fermentation [7]. Hydrogen generation using the DF process is
34 possible with a wide range of waste materials such as sludge [8], food waste [9], cheese
35 whey [10], algal biomass [11] and vinasse [12]. Recently, numerous studies have found
36 that co-digestion of two or more substrates can increase the load of biodegradable
37 organic matter, improve the balance of nutrients, improve microbial diversity leading to
38 enhance hydrogen production [13,14]. Although there are numerous studies on
39 hydrogen production by co-digestion of sludge with different substrates such as
40 perennial ryegrass [2], food waste [15] and glycerol [16], no prior studies have been
41 published on the production of hydrogen via sludge-vinasse co-digestion.

42 Vinasse is an effluent generated during the production of alcohol in the wine
43 distillation process. This effluent can be highly damaging in the areas in which it is
44 discarded due to its high organic load, low pH and high corrosivity. Instead of harmful,
45 vinasse may be considered as a substrate for hydrogen generation through the dark
46 fermentation process because of the surplus organic load.

47 Biochemical hydrogen potential (BHP) corresponds to the maximum hydrogen
48 production at dark fermentation infinite time and is a key parameter to evaluate the
49 suitability of substrates to obtain biohydrogen. Batch methods have recently been
50 applied to evaluate the BHP of numerous substrates, although the operating conditions
51 (such as pH, temperature) have yet to be standardized. Moreover, there is no consensus
52 regarding the nature of the inoculum to use in these tests or the type of pre-treatment
53 they should receive (Table 1). One of the most widely used types of inoculum is the
54 anaerobic sludge, though from different sources such as municipal sewage [17,18],
55 wastewater [2], poultry slaughterhouse wastewater [19,20] and citrate-producing
56 wastewater have also been used.

57 Most research studies use inocula subjected to thermal pre-treatment in order to
58 enrich the inoculum in terms of the hydrogen-producing bacteria. The pre-treatment is
59 generally carried out at a temperature of 100°C [2,17,18,21,22], although it has been
60 carried out at 90°C in other cases [19,20,23] or even at a temperature above 100°C
61 [24,25]. The exposure times of the inoculum to thermal shock vary greatly, ranging
62 from 15 to 30 minutes in most cases [2,17,18]. However, in the studies by Giordano et
63 al. [25] and Mohan et al. [22], the exposure time was longer (2-4 hours). Other authors
64 use a hydrogen-producing inoculum [3,12,26]. The results of these studies are
65 inconclusive; hence the lack of consensus regarding the type of inoculum or the thermal
66 pre-treatment conditions to be employed in BHP tests.

67 In this study, BHP tests with different natural inocula and pre-treatment conditions
68 were carried out to study their influence on BHP results. The main purpose of this
69 research is to discern which type of inoculum to use for future BHP tests.

70

71 2. Materials and methods

72 2.1. Substrates

73 Waste activated sludge (WAS) and vinasse (V) were used as substrates. The
74 WAS was collected from Guadalete municipal wastewater treatment plant, Jerez de la
75 Frontera, Cadiz, Spain. The V was provided by the González Byass winery located in
76 Jerez de la Frontera, Cadiz, Spain, and kept frozen (-20°C) until use.

77 A mixture of both substrates in a 50:50 ratio was used as the feedstock in all the
78 BHP tests.

79 2.2. Inocula

80 Three types of inocula were used: Acidogenic Inoculum (AI), Sludge Inoculum
81 (SI) and Thermal Sludge Inoculum (TSI). The AI was collected from a laboratory scale
82 semi-continuous acidogenic thermophilic anaerobic digester treating waste activated
83 sludge-vinasse (50:50) for hydrogen production. The reactor operated at pH 5.5, a
84 temperature of 55°C and a HRT of 4 days. The AI was thus already conditioned to treat
85 the mixture of WAS-vinasse co-substrates and is, therefore, a hydrogen -producing
86 inoculum. The SI and TSI were collected from a laboratory scale semi-continuous
87 thermophilic anaerobic digester treating waste activated sludge operating at pH 7.0, a
88 temperature of 55 °C and a HRT of 20 days. The TSI was heat-treated in a hot oven at
89 100°C for 15 min.

90 Three BHP tests were carried out, Tests 1, 2 and 3, with the aforementioned
91 inocula, AI, SI and TSI, respectively.

92 The physic-chemical characteristics of the inocula and substrates are
93 summarized in Table 2.

94 Table 2. Physico-chemical characteristics of the inocula and substrates

Parameters	Units	AI	SI	TSI	WAS+V
pH		5.32	5.49	5.52	5.39
TS	g/L	28.68	40.71	38.01	41.07
VS	g/L	21.50	31.85	29.39	33.51
TCOD	g/L	51.81	49.51	42.27	63.75
SCOD	g/L	37.52	22.58	22.62	28.06
Total VFA	g/L	4.93	2.67	3.41	2.14

95

96 2.3. Biochemical hydrogen potential

97 Hydrogen fermentation was performed in 250mL glass bottles with a 120mL
 98 working volume and a 130mL headspace volume. For each reactor, a mixing ratio of
 99 inoculum to feedstock of 1:1 (v/v) was used. The initial pH of each bottle was set at 5.5,
 100 a value at which methanogenic *Archaea* are inhibited. Nitrogen was fluxed for 5 min to
 101 displace any air present in the bottles and hence ensure an anaerobic environment. All
 102 the bottles were maintained at constant temperature under thermophilic conditions
 103 (55°C) in an orbital shaker incubator.

104 All the experiments were carried out in triplicate and inoculum control bottles
 105 were also prepared. Three bottles were used as control for each inoculum without any
 106 substrate. The hydrogen production from the control was subtracted from the hydrogen
 107 production obtained in the substrate assays prior to data analysis.

108 2.4. Analyses

109 Both the volume and composition of the biogas were determined daily. The
 110 produced biogas was quantified using a gas flow meter (Ritter TG1) and a gas suction
 111 pump (KNF Laboport). Gas volumes were converted to standard conditions and
 112 corrected by subtracting the production of the blank. The composition of the biogas was
 113 determined by gas chromatography separation (Shimadzu GC-2010 system). H₂, CO₂,

114 CH₄ and O₂ were analysed by means of a thermal conductivity detector (TCD) using a
115 Supelco Carboxen 1010 Plot column [27]. Total solids (TS), volatile solids (VS), total
116 chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) were
117 analysed according to the Standard Methods [28] at the beginning and end of each
118 experiment. Volatile fatty acids (VFA) were determined by gas chromatography on a
119 Shimadzu GC-2010 system equipped with a flame ionization detector (FID) and a
120 capillary column filled with Nukol [29]. The pH was measured at the beginning and end
121 of the tests using a Crison 20 Basic pH meter [28].

122 2.5. Microbial analyses

123 Fluorescence *in situ* hybridization (FISH) was used to count the microorganisms
124 contained in the reactors. The main steps of FISH of whole cells using 16S rRNA-
125 targeted oligonucleotide probes are cell fixation followed by permeabilization and
126 hybridization with the desired probe(s). Samples from batch reactors were collected in
127 sterile universal bottles at the beginning and end of the BHP test. A 1:1 (v/v) ratio of
128 absolute ethanol was added to the bottles. The samples were stored at -20°C until they
129 were fixed. Further details of this procedure are given in Montero et al. [30].

130 The technique used for fixing and permeabilizing cells was based on the method
131 described by Amann et al. [31,32]. The 16S rRNA-targeted oligonucleotide probes used
132 in this study are shown in Table 3: bacterial-universal probe EUB338 [31,32], and
133 *Archaea*-universal probe ARC915 [33]. The cellular concentration and percentages of
134 *Eubacteria* and *Archaea* were obtained by FISH. The total population was estimated as
135 the sum of the populations of *Eubacteria* and *Archaea* for the reason that most
136 anaerobic microorganisms in anaerobic reactors belong to these two groups [34].
137 Samples were examined visually and the cells were counted under an Axio Imager
138 Upright epifluorescence microscope (Zeiss) equipped with a 100 W mercury lamp and a

139 100 x oil objective lens. The filter employed depended on the identity of the labelled
 140 probe: a B-2A filter (DM 510, Excitation 450-490 and Barrer 520) was used for 6-
 141 FAM; while a G-2A filter (DM 580, Excitation 510-560 and Barrer 590) was used for
 142 Cy3. In addition, microbial activity was evaluated from biochemical activity according
 143 to the methods reported by Montero et al. [30] and Zahedi et al. [35]. The activity was
 144 calculated as the ratio of H₂ generated and the number of microorganisms inside the
 145 reactor obtained by FISH staining.

146 Table 3. Oligonucleotide probes used in this study

	Probe sequences (from 5' to 3')	Target	Formamide(%)	Time (h)	T (°C)	Reference
EUB338	GCTGCCTCCCGTAGGAGT	<i>Eubacteria</i>	20	1.5	46	[31,32]
ARC915	GTGCTCCCCGCCAATTCCT	<i>Archaea</i>	35	1.5	46	[33]

147

148 3. Results and discussion

149 3.1. Physico-chemical analysis

150 The physical-chemical characteristics of three tests at the beginning and end of
 151 the tests are summarized in Table 4. The pH remained relatively stable during
 152 experimentation, varying from 5 to 5.5. There were no abrupt variations in pH,
 153 demonstrating that the systems were capable of self-regulating in order to favour
 154 microbial activity [34].

155 VS and TS removal rates ranged between 1.7 and 17.3%. The lowest rate was
 156 achieved with the acidogenic inoculum (Test 1).

157 As for SCOD removal, this was lower than 23% in all tests. Yang and Wang [2]
 158 also found that the SCOD concentration decreased, with significant reductions in
 159 removal rates of 7.1-31.3%. These authors state that their results indicated that the
 160 hydrolysis amount of particular organics by hydrolytic bacteria was lower than the

161 utilization amount of soluble organics by hydrogen producers. In terms of TCOD, the
 162 removal rate was greater, with percentages ranging between 50-60%. These results are
 163 in line with those obtained by Torquato et al. [21], in which the maximum removal rate
 164 of 41% was obtained in the digestion of vinasse to produce hydrogen. However, Silva et
 165 al. [17,18] reported that COD removal was lower than 20% when testing the co-
 166 digestion of food waste, sewage sludge and crude glycerol.

167

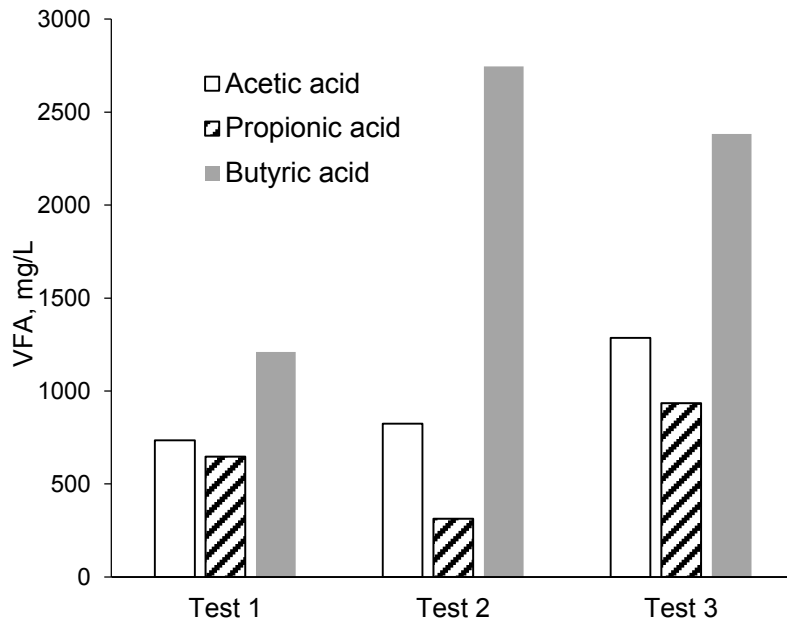
168 Table 4. Physico-chemical and microbial characterization of the three tests

Parameters	Units	Test 1		Test 2		Test 3	
		Initial	Final	Initial	Final	Initial	Final
<i>Physico-chemical characteristics</i>							
pH		5.35	5.07	5.32	5.27	5.46	5.39
TS	g/L	34.99	34.35	41.24	35.80	40.54	34.26
VS	g/L	27.67	27.19	32.25	27.83	31.33	25.90
TCOD	g/L	68.00	33.78	65.63	28.35	86.38	30.64
SCOD	g/L	35.38	27.17	26.44	22.46	25.75	22.83
Total VFA	g/L	3.40	5.31	2.53	5.27	2.80	5.90
<i>Microbial characterization</i>							
Total population	10 ⁸ cells/mL	13.51	13.29	14.95	85.90	15.29	13.27
<i>Eubacteria</i>	%	41.3	42.6	59.2	92.1	46.3	44.6
<i>Archaea</i>	%	58.7	57.5	40.8	8.0	53.7	55.4

169

170 As regards intermediate compounds, a large amount of VFAs was produced
 171 during the tests. At the end of the BHP tests, the dominant species were acetic, butyric
 172 and propionic acids, the concentrations for each inoculum being shown in Fig.1.
 173 Generally, hydrogen production via dark fermentation produces acetic and butyric acids
 174 as by-products [36]. Butyric acid was predominant in Test 2 (using SI), which presents
 175 a higher hydrogen yield. Luo et al. [37]and Chen et al. [38] also found that the highest

176 hydrogen production was obtained when butyric acid predominated. Butyric acid-type
177 fermentation is considered one of the most effective pathways for hydrogen production
178 [17]. On the other hand, TSI showed the highest production of propionic acid, which is
179 detrimental for hydrogen production [19]. Tyagi et al. [3] found that hydrogen yield
180 decreases with increasing propionic acid concentration.

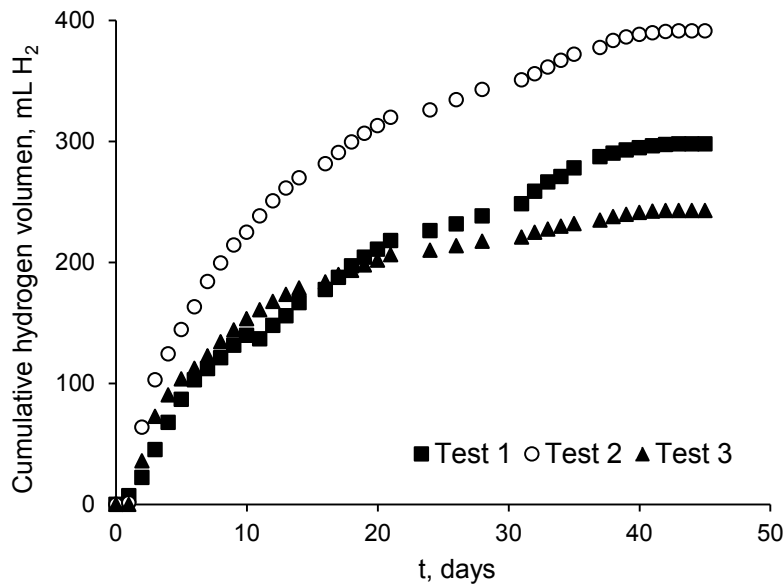


181

182 Figure 1. Volatile fatty acids generated during the tests.

183 3.2. Biogas production

184 Fig. 2 shows the cumulative hydrogen production for sludge-vinasse co-
185 digestion with different inocula. In all the BHP tests, hydrogen production commenced
186 in the first hours, as the lag phase was short. Furthermore, the biogas generated in all
187 three tests was composed of hydrogen and carbon dioxide, no methanogenic activity
188 being observed (i.e. the biogas was methane free). All this is due to the fact that the pH
189 values fell within the 5-6 range, which is optimal to enhance H₂ generation and avoid
190 methanogenesis [18].



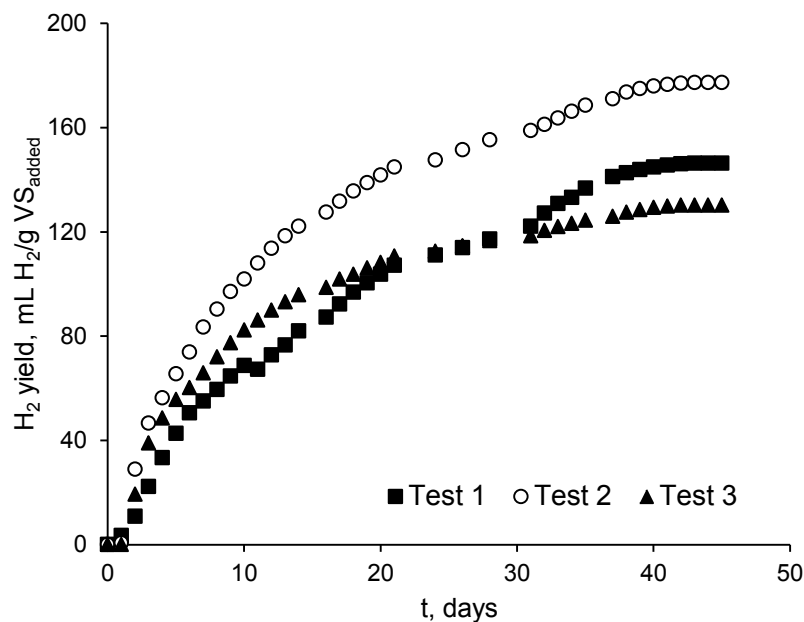
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192 Figure 2. Cumulative hydrogen production during the operating of batch reactors with
 193 different inocula.

194 The sludge inoculum led to the highest maximum accumulated H₂ volume
 195 (391mL H₂) compared to the acidogenic inoculum (298mL H₂) and the thermally pre-
 196 treated inoculum (TSI) (243mL H₂). In terms of H₂ yield (as per millilitres of hydrogen
 197 per gram of volatile solids of the substrate initially added to each reactor), the highest
 198 value was also achieved at the test using the SI (177mL H₂/g VS_{added}), corresponding to
 199 an increase of 21 and 36% in relation to that obtained in the Test 1 (146 mL H₂/g
 200 VS_{added}) and the Test 3 (130 mL H₂/g VS_{added}) (Fig.3). According to these results,
 201 hydrogen production is inhibited rather than enhanced when the inoculum is submitted
 202 to a thermal pre-treatment with the purpose of inactivating H₂-consuming *Archaea* and
 203 avoiding methane generation, as proposed by several authors [2,17–23,25]. These
 204 results are concordant and discrepant at the same time with those collected in the
 205 literature. Thus, Luo et al. [37] also observed this tendency, the best condition was
 206 without any inoculum treatment. However, Albanez et al. [39] observed a slight

207 improvement was noticed when performing the inoculum heat shock pretreatment in the
 208 co-digestion of vinasse and molasses. In a recent study, Lovato et al. [19] subjected the
 209 inoculum used in the co-digestion of cheese whey and glycerin to a heat shock pre-
 210 treatment (90°C for 10 min), obtaining significantly higher values for hydrogen
 211 productivities and yields than using untreated inoculum. In other studies using the same
 212 inoculum though treating glycerin-based wastewater, the thermally pre-treated inoculum
 213 was not found to be significantly different from the untreated sludge in terms of molar
 214 productivity and molar hydrogen yield [23]. It is important to emphasize that the last
 215 three studies were done in AnSBBR at mesophilic conditions.

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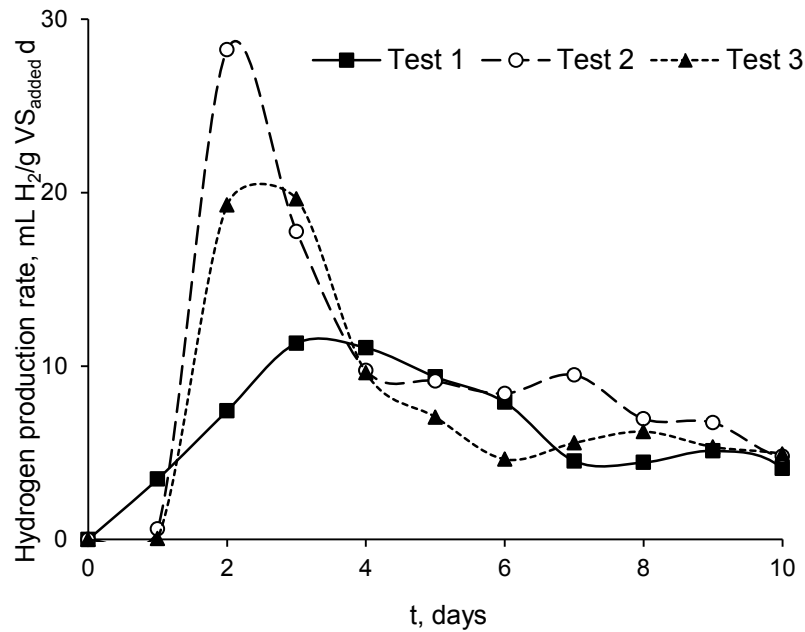
217

218 Figure 3. Hydrogen yield for batch tests using different inocula.

219 *3.3. Hydrogen production rate*

220 In order to ease identification of differences between the inocula, the hydrogen
 221 production rate of the first ten days is shown in Fig. 4. As for the SI and STI inocula

222 behaved similarly with a significant lead of SI inoculum. This could be expected
 223 because both inocula have the same source. As for AI inoculum, a broader and lower
 224 peak than in the other inocula was detected. The maximum hydrogen production rate
 225 observed in the tests 1 and 2, with the AI and STI inocula, reached the peak after three
 226 days, and amounted to 11 mL H₂/(gVS_{added} d) and 20 mL H₂/(gVS_{added} d), respectively.
 227 On the other hand, the maximum hydrogen production rate observed in the Test 2 with
 228 the sludge inoculum reached the peak at about the second day of experimentation,
 229 amounting to 28 mL H₂/(gVS_{added} d). As could be expected, the highest maximum
 230 hydrogen production rate was noticed in the experiment with higher hydrogen yield.



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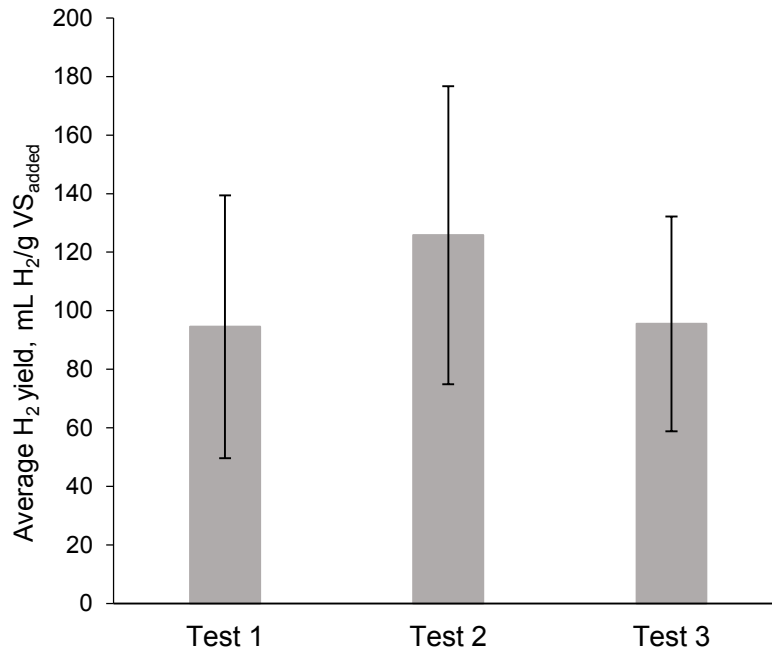
232 Figure 4. Hydrogen production rate for batch tests using different inocula.

233 *3.4. Statistical analysis*

234 Fig. 5 shows the average of hydrogen yield produce to each inoculum with their
 235 standard deviation. In order to evaluate differences between results of the three inocula,
 236 hydrogen yield and hydrogen production rate results were analysed statistically by

237 single-factor analysis of variance (ANOVA). Table 5 shows the results of this analysis.

238 A confidence level of 95% was selected for all comparisons.



239

240 Figure 5. Average hydrogen yield for batch tests using different inocula with standard deviation.

241 In the matter of hydrogen yield, for the comparison between inocula, the p value is

242 smaller than 0.05 in all cases, therefore there is significant difference between the yields

243 of hydrogen produced in SI inoculum and those of the other two.

244 Table 5. ANOVA results for the hydrogen yield and hydrogen production rate.

		Degrees of freedom	Sum of squares	Mean square	F value	P value
Hydrogen yield	Inoculas AI and SI	1	18559	18559	8.057	0.00585
	Inoculas SI and STI	1	17435	17435	8.865	0.00393
	Inoculas AI and STI	1	18	17.6	0.01	0.919
Hydrogen production rate	Inoculas AI and SI	1	12.5	12.53	0.691	0.409
	Inoculas SI and STI	1	29.1	29.13	1.238	0.269
	Inoculas AI and STI	1	3.5	3.452	0.249	0.619

245

246 Conversely, for the hydrogen production rate there is no significant difference ($p >$

247 0.05) between all of the three tested inocula.

248 3.5. Microbial population dynamics

249 The concentrations of microorganisms in the samples before and after the
250 different tests were studied. The amounts and relative percentages of the main microbial
251 groups are shown in Table 4. In Test 2, in which the highest hydrogen yield (177mL
252 H₂/g VS_{added}) was obtained, the population size increased during the time of
253 experimentation. Instead, in Test 1 and Test 3, the population size remained stable at the
254 end of the BHP tests in all cases; significantly, the amount of substrate for acidogenic
255 phase was sufficient. *Eubacteria* was the major phylogenetic domain in all cases. No
256 significant variation was found in *Eubacteria: Archaea* ratios at the beginning and end
257 of the experiments in Test 1 or Test 3: 41-46% and 59-54%, respectively. In Test 2,
258 however, the percentages of *Eubacteria* increased from 59% to 92%. Thus, BHP test
259 with sludge inoculum could increase the abundance of the specific bacteria in the
260 reactor, which were beneficial for the hydrogen production.

261 Although methane is not generated, the analyses showed the largest number of
262 *Archaea* present. In terms of productivity, it may be stated that *Archaea* were inactive
263 [40].

264 4. Conclusions

265 H₂ generation from sludge vinasse co-digestion, using different inocula, was studied.
266 The batch tests were successfully in all cases. Significant differences have been found in
267 the production of hydrogen among the three inoculums. The highest hydrogen
268 yield, 177mL H₂/g VS_{added}, was obtained with a sludge inoculum. Even though,
269 *Eubacteria* was the major phylogenetic domain in all cases, sludge inoculum showed a
270 greater growth of *Eubacteria* during the test, increasing the percentage of this population
271 from 59.2 to 92.1. The rate of hydrogen production was comparable between the

272 different inocula, that is, the duration of the test is independent of the type of inoculum
273 used. Furthermore, hydrogen production was chiefly accompanied by the production of
274 acetic and butyric acids.

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422

Inoculum			Substrates	Experimentation conditions	Maximum hydrogen yield	References
Type	Source	Pretratament				
Anaerobic sludge	Wastewater	100°C, 15 min	Sludge and perennial ryegrass	Batch 37°C	60mL H ₂ /g VS _{added}	[2]
Anaerobic sludge	Municipal sewage	100°C, 30 min	Food waste and crude glycerol	Batch 35°C	180mL H ₂ /g VS _{consumed}	[17]
Anaerobic sludge	Municipal sewage	100°C, 30 min	Food waste, sewage sludge and crude glycerol	Batch 35°C	179mL H ₂ /g VS _{consumed}	[18]
Anaerobic sludge	OFMSW		OFMSW and sewage sludge	Batch 55°C	51mL H ₂ /g VS _{consumed}	[3]
Anaerobic sludge (Upflow anaerobic sludge blanket UASB)	Wastewater from poultry slaughterhouse	90°C, 10 min	Cheese whey and glycerin	Anaerobic sequencing batch biofilm reactor (AnSBBR) 30°C	5.4mol H ₂ /kg COD 2.3mol H ₂ /kg COD	[19]
Granular (UASB)	Wastewater from poultry slaughterhouse	90°C, 10 min	Glycerin-based wastewater	AnSBBR 30°C	20mol H ₂ /kg COD _{consumed} 19.8mol H ₂ /kg COD _{consumed}	[23]
Granular mesophilic sludge (UASB)	Potato wastes	105°C, 4h	Glucose Wheat bran from common wheat Wheat bran from durum wheat Wastes from mashed	Batch 35°C	185L H ₂ /kg COD 47L H ₂ /kg COD 76L H ₂ /kg COD 177L H ₂ /kg	[25]

			potatoes			COD		
			Wastes from steam-peeling potatoes			134L H ₂ /kg COD		
Anaerobic granular sludge	Municipal sewage	100°C, 10 min	Citrus vinasse	Batch 37°C		2.0mmol H ₂ /g COD	[21]	
Anaerobic sludge	Citrate-producing wastewater	102°C, 30 min	Brewery wastewater	Batch 36°C		149.6mL H ₂ /g COD	[24]	
Anaerobic (Fixed-bed)	Sucrose-based synthetic wastewater		Domestic sewage	Batch 25°C		6.01mmol H ₂ /g COD	[26]	
			Glycerin wastewater			6.03mmol H ₂ /g COD		
			Sugarcane vinasse			24.97mmol H ₂ /g COD		
Anaerobic (Fixed-bed)	Sucrose-based synthetic wastewater		Vinasse	Batch 25°C		20.8mL H ₂ /g COD	[12]	
Anaerobic mixed microflora (UASB)	Chemical wastewater	100°C, 2h pH 3, 24h	Synthetic wastewater and domestic sewage wastewater	Batch 29°C		0.71mmol H ₂ /g COD	[22]	
Anaerobic sludge (UASB)	Wastewater from poultry slaughterhouse	90°C, 10 min	Sugarcane vinasse	Batch 55°C		2.31mmol H ₂ /g COD	[20]	
Anaerobic granular sludge		90°C, 1 h	Cassava stillage	Batch 60°C		65.3mL H ₂ /g VS	[37]	
						Chloroform 0.2% pH 12		57.4mL H ₂ /g VS
						pH 3 Loading-shock		32.9mL H ₂ /g VS
Anaerobic sludge	Wastewater from poultry slaughterhouse	90°C, 15 min	Vinasse and molasses	AnSBBR		59.0mL H ₂ /g VS	[39]	
						46.5mL H ₂ /g VS		
Anaerobic sludge	Waste activated sludge and vinasse	100°C, 15 min	Waste activated sludge and vinasse	Batch 55°C		64.4mL H ₂ /g VS	The present study	
	Waste activated sludge					0.8mol H ₂ /kg COD _{consumed}		
	Waste activated sludge					0.5mol H ₂ /kg COD _{consumed}		
						146.37mL H ₂ /g VS _{added}		
						177.23mL H ₂ /g VS _{added}		
						130.17mL H ₂ /g VS _{added}		

423

424 Table 1. Comparative study on hydrogen production in anaerobic reactors.