



HAL
open science

Methanosarcina plays a main role during methanogenesis of high-solids food waste and cardboard.

Gabriel Capson-Tojo, Eric Trably, Maxime Rouez, Marion Crest, Nicolas Bernet, Jean-Philippe Steyer, Jean-Philippe Delgenès, Renaud Escudié

► **To cite this version:**

Gabriel Capson-Tojo, Eric Trably, Maxime Rouez, Marion Crest, Nicolas Bernet, et al.. Methanosarcina plays a main role during methanogenesis of high-solids food waste and cardboard.. Waste Management, 2018, 76, pp.423-430. 10.1016/j.wasman.2018.04.004 . hal-02627560

HAL Id: hal-02627560

<https://hal.inrae.fr/hal-02627560>

Submitted on 4 Aug 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 ***Methanosarcina* plays a main role during methanogenesis of high-solids food waste and**
2 **cardboard**

3 Gabriel Capson-Tojo ^{a,b}, Eric Trably ^a, Maxime Rouez ^b, Marion Crest ^b, Nicolas Bernet ^a,
4 Jean-Philippe Steyer ^a, Jean-Philippe Delgenès ^a, Renaud Escudie ^{a,*}

5
6 ^a LBE, Univ Montpellier, INRA, 102 avenue des Etangs, 11100, Narbonne, France

7 ^b Suez, CIRSEE, 38 rue du Président Wilson, 78230, Le Pecq, France

8 * Corresponding author: tel. +33 04 68 42 51 73, e-mail: renaud.escudie@inra.fr

9
10 **Abstract**

11 Anaerobic digestion of food waste is a complex process often hindered by high concentrations
12 of volatile fatty acids and ammonia. Methanogenic archaea are more sensitive to these
13 inhibitors than bacteria and thus the structure of their community is critical to avoid reactor
14 acidification. In this study, the performances of three different inocula were compared using
15 batch digestion tests of food waste and cardboard mixtures. Particular attention was paid to
16 the archaeal communities in the inocula and after digestion. While the tests started with
17 inocula rich in *Methanosarcina* led to efficient methane production, VFAs accumulated in the
18 reactors where inocula initially were poor in this archaea and no methane was produced. In
19 addition, higher substrate loads were tolerated when greater proportions of *Methanosarcina*
20 were initially present in the inoculum. Independently of the inoculum origin, *Methanosarcina*
21 were the dominant methanogens in the digestates from the experiments that efficiently
22 produced methane. These results suggest that the initial archaeal composition of the inoculum
23 is crucial during reactor start-up to achieve stable anaerobic digestion at high concentrations
24 of ammonia and organic acids.

25

26 **Keywords**

27 Biogas; solid-state anaerobic digestion; methanogenesis; free ammonia

28

29 **1. Introduction**

30 Novel technologies for treatment and valorization of the organic fraction of municipal solid
31 waste (OFMSW) must be developed to deal with an increasing production and new
32 international regulations. Anaerobic digestion (AD) is a well-known process used for efficient
33 treatment of organic waste with high total solids (TS) contents ($\geq 20\%$), converting them into
34 biogas and digestate, both added-value end-products. However, AD of highly biodegradable
35 substrates such as food waste (FW), which is a major component of OFMSW, is often
36 associated with accumulation of volatile fatty acids (VFAs), which are detrimental to the AD
37 process. In addition, FW is rich in organic nitrogen, which is reduced to ammonia during AD,
38 leading to high concentrations of total ammonia nitrogen (sum of NH_3 and NH_4^+ ; TAN) in the
39 digesters (L. Zhang et al., 2012). Accumulation of both VFA and/or TAN might lower the
40 methane yields and can even lead to failure of the AD process (Banks et al., 2008). The
41 reactors are particularly vulnerable to these inhibitions during the start-up period (Fernández
42 et al., 2001). This occurs because the microbial communities are not adapted to the stressful
43 conditions imposed by the substrates and the operational parameters (*i.e.* high organic loading
44 rates). Therefore, to achieve efficient methane yields and productivities with FW as substrate,
45 it is crucial to have well-adapted microbial communities in the digesters, which are resistant
46 to high VFA and free ammonia nitrogen (NH_3 ; FAN) concentrations.

47 Methanogenic archaea are generally more sensitive to inhibitors than bacteria and thus
48 methanogenesis is usually the first process affected by common inhibitors, such as FAN or
49 VFAs (De Vrieze et al., 2012). Nonetheless, not all methanogenic archaea have the same

50 resistance to these inhibitors and thus the composition of the archaeal microbial community
51 varies according to the operating conditions (Abbassi-Guendouz et al., 2013). Due to their
52 high substrate affinity, acetotrophs such as *Methanosaeta* are generally predominant under
53 unstressed conditions and thus acetotrophic methanogenesis is the predominant pathway for
54 methane production. On the other hand, under stressful AD conditions, these methanogens are
55 preferentially inhibited and mixotrophic microorganisms (*i.e.* able to consume acetate and
56 hydrogen to produce methane), such as *Methanosarcina* which are more resistant to inhibitors
57 (*i.e.* FAN or VFAs), become predominant (De Vrieze et al., 2012; Venkiteshwaran et al.,
58 2016). In fact, while *Methanosaeta* cannot grow at TAN concentrations greater than $3 \text{ g}\cdot\text{L}^{-1}$,
59 *Methanosarcina* have been found at much higher TAN concentrations (De Vrieze et al., 2012;
60 Poirier et al., 2016). As an illustration, Capson-Tojo et al. (2017a) found *Methanosarcina* to
61 be the dominant methanogens at TAN concentrations up to $3.7 \text{ g}\cdot\text{L}^{-1}$ ($795 \text{ mg FAN}\cdot\text{L}^{-1}$) using
62 FW as substrate in AD batch tests.

63 Over the past years, the importance of the microbial communities for efficient AD processes
64 has gained attention and many studies have been carried out to further understand the
65 structures of the communities of both bacteria and archaea in AD reactors. In a recent study
66 carried out by Zhang et al. (2016) with sewage sludge and FW as substrates (with final NH_4^+
67 concentrations up to $2.01 \text{ g}\cdot\text{L}^{-1}$), it was observed that *Methanosaeta* were the main archaea at
68 the beginning of the batch experiment (71 % of the operational taxonomical units; OTUs).
69 Afterwards, *Methanosarcina* grew during acid production (with transient VFA concentrations
70 up to $24 \text{ g}\cdot\text{L}^{-1}$) and overpassed in abundance *Methanosaeta* because of their greater resistance
71 to VFA and TAN inhibition. Finally, other hydrogenotrophic methanogens (*i.e.*
72 *Methanoculleus*) grew once acetate was totally consumed. Using a high solid-state AD box-
73 type container fed with FW at high TS contents (from 34.4 to 44.5 %) and TAN
74 concentrations ($2.5 \text{ g}\cdot\text{L}^{-1}$), Walter et al. (2016) observed that *Methanosarcina* were the

75 dominant species accompanied by different hydrogenotrophs (*i.e. Methanobacterium*,
76 *Methanoculleus* and *Methanocorpusculum*). Consistently, Zamanzadeh et al. (2016) found
77 *Methanosaeta* as the main archaea in mesophilic continuous AD of FW at low concentrations
78 of FAN ($\leq 200 \text{ mg}\cdot\text{L}^{-1}$). This further supports that the concentration of TAN-FAN is a key
79 factor that can result in shifts of the archaeal populations. In a recent batch study, Poirier et al.
80 (2016) identified the key microbial phylotypes resisting to extreme ammonia concentrations
81 (up to $50 \text{ g TAN}\cdot\text{L}^{-1}$). They achieved high methane yields at TAN concentrations as high as
82 $25 \text{ g TAN}\cdot\text{L}^{-1}$, with *Methanosarcina* and *Methanoculleus* as main methanogens and with
83 relative abundances of *Methanosaeta* lower than 5 % in all AD reactors.

84 The objective of this study was to evaluate, for the first time, the AD performance of three
85 microbial inocula from different origins and with different initial archaeal compositions using
86 FW and cardboard (CB) as substrates. These wastes are the main components of OFMSW
87 (Kim and Oh, 2011; Y. Zhang et al., 2012) and are generally collected at the same facilities,
88 and thus their co-digestion is facilitated. Also, they constitute a good waste model substrate,
89 since the initial proportions of carbon and nitrogen could be easily adjusted. Batch tests were
90 performed at different substrate loads, TS contents ($\geq 20 \%$) and co-digestion proportions.
91 Special attention was paid to the archaeal communities and to the FAN and VFA levels.

92

93 **2. Materials and methods**

94 *2.1. Substrate and microbial inoculum*

95 A synthetic FW was prepared according to the VALORGAS report (VALORGAS, 2010). It
96 was composed of fruits and vegetables (80.7 %), meat (8.2 %), pasta (4.8 %), bread (6.2 %),
97 dairy products (1.9 %) and biscuits (1.9 %). Its precise composition has been detailed
98 elsewhere (Capson-Tojo et al., 2017a). Being FW and CB the most common components of
99 OFMSW, CB (branded “Cartonnages Michel” and shredded to less than 1 mm) was added as

100 co-substrate to simulate this waste (Hogg et al., 2002), increasing at the same time the C/N
101 ratio of the substrate and thus diluting the TAN concentrations in the reactors and favoring the
102 AD process (Capson-Tojo et al., 2017a). Three different inocula from industrial plants were
103 used: mixture of a centrifuged granular sludge issued from a mesophilic industrial UASB
104 reactor treating sugar factory effluents with a dried digestate. This digestate was used to
105 increase the TS content of the inoculum and was sampled in a thermophilic industrial plant
106 treating OFMSW (Inoc-UASB1); a mixture of sludge and dried digestate issued from the
107 same sources than Inoc-UASB1 but sampled at a different moment (Inoc-UASB2); a sludge
108 issued from an AD industrial plant treating a mixture of different organic waste streams at 35
109 °C mixed with dried compost (99 % TS; 81 % VS) to increase the TS content of the inoculum
110 (Inoc-OW). The amounts of dried digestate and compost added were 0.5 g per g of inoculum
111 (w/w) (Inoc-UASB1 and Inoc-UASB2) and 0.17-0.34 g per g of inoculum (w/w) (Inoc-OW)
112 respectively, depending on the desired TS content and the initial water content of the sludge.

113 2.2. *Dry batch anaerobic co-digestion tests*

114 Different co-digestion ratios (4-1 g TS FW·g TS CB⁻¹), initial TS contents (20-35 %) and
115 substrate to inoculum (S/X) ratios (0.25-1.00 g VS·g VS⁻¹) were tested. These values were
116 selected according to previous results and to data gathered from the literature (Capson-Tojo et
117 al., 2016, 2017a). Table 1 summarizes the 10 different experimental conditions that were
118 considered in this study. Each tested condition was run in triplicate. This experimental set-up
119 allowed to produce results which primarily depended on the inoculum source, while
120 evaluating at the same time different initial conditions (*i.e.* S/X and co-digestion ratios and
121 initial TS contents). Therefore, the obtained results were not dependent on the particular
122 operational conditions applied, but only on the type of inoculum used. With this set-up the
123 performance of each reactor was also totally independent between them.

124 After adding the required volumes of sludge into the flasks, the corresponding amounts of

125 substrates (according to Table 1) were supplemented. Finally, the TS contents were adjusted
126 adding water and the flasks were flushed with nitrogen and sealed.

127 As aforementioned, to allow working at the high TS contents desired, the inocula used were
128 mixed with dried digestates (Inoc-UASB1 and Inoc-UASB2) and compost (Inoc-OW).

129 Different blank reactors were carried out to account for the biogas production that could have
130 been produced by the degradation of these materials (Capson-Tojo et al., 2017a, 2017b,
131 2017c). In addition, both materials were dried at 100 °C for over 24 h to ensure that the impact
132 of the microorganisms present in these media on the methane production was negligible. The
133 working volumes were different according to the operational conditions and the reactor size,
134 varying from 0.4 L to 0.7 L. The duration of the batch experiments was variable (56-98 days).
135 In the systems producing methane, the batch experiments were stopped when a plateau in the
136 biomethane production was observed. On the other hand, longer batch periods were applied
137 when acidification occurred (to ensure that the acid accumulation was irreversible). All the
138 reactors were incubated at 37 °C.

139 *2.3. Analytical methods*

140 *2.3.1. Physicochemical characterization of the substrates*

141 The TS and Volatile Solids (VS) contents were determined according to the Standard
142 Methods (APHA, 2005). The protein and carbohydrate concentrations were measured by the
143 modified Lowry method (Frølund et al., 1996) and the Dubois method (Dubois et al., 1956),
144 respectively. The lipid content was determined using a gravimetric method (APHA, 2005), the
145 pH was measured with a WTW pHmeter series inoLab pH720, total Kjeldahl nitrogen (TKN)
146 and TAN contents were determined with an AutoKjehdahl Unit K-370, BUCHI and the total
147 organic carbon (TOC) with a Shimadzu TOC-V_{CSN} Total Organic Carbon Analyzer. A more
148 precise description of the analytical methods can be found in Capson-Tojo et al., (2017a). The
149 biochemical methane potentials (BMPs) of the substrates were determined according to Motte

150 et al. (2014). The C/N ratio was calculated as TOC divided by TKN. The FAN concentrations
151 were calculated according to Chen et al. (2014) as a function of temperature, pH, and
152 concentration of TAN.

153 *2.3.2. Gas quantification and analysis*

154 The total biogas volume was periodically determined by measuring the pressure in the reactor
155 headspace and the gas composition was analyzed by gas chromatography coupled to a
156 catharometer detector, as detailed in Cazier et al. (2015). The methane yields were calculated
157 by dividing the total volume of methane by the amount of VS initially added as substrate.

158 *2.3.3. Analysis of metabolites and final products of the digestion*

159 The concentrations of VFAs and ionic species after digestion were measured by gas
160 chromatography and high-performance liquid chromatography, according to Motte et al.
161 (2013). The reactors used in the experiments carried out using the Inoc-OW and Inoc-UASB1
162 allowed sampling of the digestate during the digestion and therefore, the kinetics of
163 production-consumption of metabolites were also analyzed. The sampling device is described
164 in Capson-Tojo et al. (2017b).

165 *2.4. Microbial community analysis*

166 The microbial communities of the inocula and the digestates were characterized by 16S rRNA
167 sequencing. One mL of each sample was first taken and stored at -20 °C until analysis. The
168 DNA from the sample (around 1 g) was extracted using a Fast DNA SPIN kit for soil in
169 accordance with the instructions of the manufacturer (MP Biomedicals). The quality and
170 quantity of the extracted DNA were verified by spectrophotometry using an Infinite 200 PRO
171 NanoQuant (Tecan Group Ltd., Männedorf, Switzerland). The primer pairs 515-532U and
172 909-928U and their respective linkers were used to amplify the V4-V5 regions of the 16S
173 rRNA genes (over 30 amplification cycles were applied at an annealing temperature of 65 °C).
174 These primer pairs target both bacterial and archaeal 16S rRNA genes, capturing most of their

175 diversity (Wang and Qian, 2009). The PCR mixtures had a total volume of 50 μ L, containing:
176 0.5 units of Pfu Turbo DNA polymerase (Stratagene), the corresponding buffer, each
177 deoxynucleotide at 200 mM, each primer at 0.5 mM and 10 ng of genomic DNA. The
178 following PCR sequence was carried out (using a Mastercycler thermal cycler; Eppendorf):
179 after 94 °C for two min, 35 cycles of 94 °C for one min, 65 °C for one min, and 72 °C for one
180 min were applied, with a final extension at 72 °C for 10 min. The obtained products were
181 purified and analyzed using the Illumina MiSeq cartridge (v3 chemistry) for sequencing of
182 paired 300 bp reads at the GenoToul platform (<http://www.genotoul.fr>). Mothur (version
183 1.35.0) was used for sequence assembling, cleaning and alignment and for assignation of the
184 taxonomic affiliation, as described in Venkiteshwaran et al. (2016).

185 *2.5. Statistical analysis*

186 To analyze potential relationships between variables (*i.e.* methane yields, *Methanosarcina*
187 proportions in the inocula and the digestates, final VFA concentrations, final pH values, initial
188 TS contents and initial S/X and C/N ratios), a principal component analysis (PCA) was
189 performed. The PCA was carried out using the package mixOmics in the software R (version
190 3.2.5; The R Foundation for Statistical Computing, Vienna, Austria).

191

192 **3. Results and discussion**

193 *3.1. Physicochemical characterization of substrates and inocula*

194 The physico-chemical characteristics of the substrates and the inocula are presented in Table
195 2. The observed composition of the FW was similar to those found in the literature (Capson-
196 Tojo et al., 2016), with TS contents of 21.6 % and VS/TS of 96.2 %. In agreement with
197 previously reported results, the FW was mainly composed of carbohydrates and had a
198 relatively low C/N ratio, far away from the optimum values of 25 reported in the literature
199 (Mao et al., 2015). The high BMP value of the FW (498 mL CH₄·g VS⁻¹) highlights its great

200 potential for valorization by AD. In contrast, CB had a high C/N ratio, suggesting that CB can
201 be effectively used as co-substrate for diluting the TAN from FW organic nitrogen. A more
202 extensive characterization of both substrates can be found in Capson-Tojo et al., (2017a).
203 Inoc-UASB1 and Inoc-UASB2 had very similar physico-chemical characteristics, with high
204 TS (70.8 and 74.2 %) and low TAN concentrations (1.49-1.50 g·L⁻¹). In contrast, Inoc-OW
205 had much lower TS (5.8 %) and much higher TAN contents (5.04 g·L⁻¹). Due to this high
206 TAN concentrations (higher than in the two other inocula), it was expected that the microbial
207 community in Inoc-OW was more adapted to typical FW AD conditions, *i.e.* high TAN and
208 high transient VFA concentrations.

209 3.2. Anaerobic digestion performances

210 As shown in Table 1, while some batch tests produced methane efficiently, others did not
211 produce methane significantly (methane yields marked with *). This occurred because VFA
212 rapidly accumulated at the beginning of the batch AD process, decreasing the pH initially and
213 overloading the methanogens in the acidified reactors. Basically, if the present archaea were
214 not able to rapidly consume the accumulated VFAs, the pH decreased to values where
215 methanogenesis was inhibited.

216 To illustrate this initial VFA accumulation-consumption, Figure 1 presents the evolution of
217 the total VFA concentrations in the experiments carried out using Inoc-OW as inoculum
218 (efficient methane production; Figure 1.A) and Inoc-UASB1 as inoculum (no methane
219 production; Figure 1.B). The acidified systems corresponded to all the tests inoculated with
220 Inoc-UASB1 (regardless the experimental conditions applied) and the experiment started at an
221 S/X ratio of 1.00 g VS·g VS⁻¹ with Inoc-UASB2. In the tests started with Inoc-UASB1, VFA
222 concentrations up to 33.7 g COD·L⁻¹ (calculated in COD units, 45 % acetic acid, 39 % butyric
223 acid, 8 % caproic acid, 4 % propionic acid and 3 % valeric acid) were detected, causing a pH
224 drop to values down to 5.6 (Table 3). In contrast, all the batch tests inoculated with Inoc-OW

225 produced methane efficiently, as well as the experiments carried out at low loads (S/X ratio of
226 $0.25 \text{ g VS} \cdot \text{g VS}^{-1}$) with Inoc-UASB2. Although high transient VFA concentrations, up to 22.6
227 $\text{g COD} \cdot \text{L}^{-1}$, were observed in these reactors (Figure 1.A), the methanogens efficiently
228 consumed the accumulated VFAs, producing methane and avoiding a pH drop. A possible
229 explanation for the different results obtained using the Inoc-OW is that the high initial TAN
230 concentrations in this inoculum buffered the initial peak of VFAs (up to $22.6 \text{ g COD} \cdot \text{kg}^{-1}$; 64
231 % COD acetic acid, 23 % butyric acid, 10 % propionic acid and 3 % valeric acid), alleviating
232 the pH drop. However, such TAN-buffering effect cannot explain the different performances
233 observed between the methane-producing reactors started with Inoc-UASB2 and those
234 inoculated with Inoc-UASB1, with very similar initial TAN concentrations and working
235 conditions.

236 In order to elucidate the reasons behind these observations, analyses of the microbial
237 communities were performed. At this point, it must be mentioned that the methanogenic
238 activity of all the used inocula was previously verified using ethanol as substrate. In fact, all
239 the blank tests defined to determine the endogenous respiration produced significant amounts
240 of methane. These reactors served, not only to account for the endogenous respiration of the
241 inocula, but also to verify their activity and to corroborate that the observed acidification was
242 related to the addition of the substrates.

243 *3.3. Microbial composition of the inocula and the digestates*

244 In an attempt to explain the different behaviors observed, the structures of the microbial
245 communities of the initial inocula and the digestates sampled at the end of each batch tests
246 were analyzed. Due to their relevance for methane production, the composition of the archaeal
247 communities was specifically investigated. Figure 2 and 3 show the relative abundances of
248 archaea found in the three inocula (Figure 2) and in the digestates (Figure 3). It must be
249 mentioned that the OTUs were defined from the 16S rRNA copies.

250 As shown in Figure 2, the initial archaeal communities varied widely according to the
251 inoculum origin. Inoc-UASB1 and Inoc-UASB2 (non-acclimated to high TAN or VFA
252 concentrations) were rich in the hydrogenotroph *Methanobacterium* and the acetotroph
253 *Methanosaeta*, both relatively vulnerable to TAN inhibition, *i.e.* not surviving over 3 g
254 TAN·L⁻¹ (De Vrieze et al., 2012). In contrast, *Methanosarcina* was already the dominant
255 species in the Inoc-OW, with the highest initial TAN concentrations, followed by the
256 hydrogenotrophs *Methanothermobacter* and *Methanobrevibacter*. Therefore, for practical
257 reasons these inocula were classified according to their initial relative abundance of
258 *Methanosarcina*: negligible proportions in Inoc-UASB1 (0.47 %; MS-Rare), 6.36 % of the
259 total archaeal OTUs in Inoc-UASB2 (MS-Poor) and up to 52.6 % in Inoc-OW (MS-Rich). As
260 the experiments performed with Inoc-UASB1 (MS-Rare) did not produce any methane and
261 those inoculated with Inoc-OW (MS-Rich) generated methane at higher substrate loads than
262 those inoculated with Inoc-UASB2 (MS-Poor), these results suggest that *Methanosarcina*-
263 scarce inocula were sensitive to inhibition when compared to inocula with higher initial
264 proportions of *Methanosarcina*.

265 The archaeal populations in the digestates (shown in Figure 3) support this assumption.
266 Regardless of the initial inoculum, the predominant species in all the batch tests that produced
267 methane was *Methanosarcina*, with relative abundances ranging from 48.8 % to 61.8 %. This
268 suggests that, at the high TAN levels (up to 5.05 g·L⁻¹) and transient VFA concentrations (up
269 to 22.6 g·L⁻¹) that are associated with batch high-solids AD environments (Table 3) the
270 growth of members of this genus was favored, which is in agreement with different results
271 presented in the literature (Hao et al., 2015).

272 This can be explained by the high resistance of *Methanosarcina* to inhibition by these
273 compounds (De Vrieze et al., 2012). As aforementioned, acetotrophs such as *Methanosaeta*
274 are generally predominant under unstressed conditions due to their higher substrate affinity

275 and favored thermodynamics when compared to hydrogenotrophs. However, under stressful
276 AD conditions (*i.e.* high FAN or VFAs concentrations) these methanogens are inhibited and
277 the growth of mixotrophic and hydrogenotrophic microorganisms, which are more resistant to
278 inhibitors, is favored (De Vrieze et al., 2012; Venkiteshwaran et al., 2016). Therefore,
279 hydrogenotrophic methanogenesis, which otherwise would have been a secondary methane-
280 producing pathway, becomes predominant. This finding is in agreement with a recent study
281 using ^{14}C radiolabeling, which showed that at TAN concentrations over $2\text{ g}\cdot\text{L}^{-1}$,
282 hydrogenotrophic methanogenesis was predominant (68-75 % of the methane produced) over
283 the acetoclastic pathway (Jiang et al., 2017). Therefore, the growth of
284 hydrogenotrophic/mixotrophic microorganisms (such as those belonging to the genus
285 *Methanosarcina*) was favored under these conditions.

286 In addition, when comparing the tests that produced methane with the others, the importance
287 of *Methanosarcina* to achieve efficient methanogenesis is also highlighted. Using Inoc-
288 UASB1 (MS-Rare), where the proportion of this group of archaea was initially negligible, no
289 efficient methane production was achieved under any condition, even at relatively low organic
290 loads ($0.25\text{ g VS}\cdot\text{g VS}^{-1}$). On the other hand, the experiments operated under equivalent
291 conditions but inoculated with Inoc-UASB2 (MS-Poor; with 6.36 % of *Methanosarcina*
292 initially) showed efficient methane production, likely due to the presence and the emergence
293 of this group of methanogens. This is supported by the fact that a minimum of 48.8 % of
294 *Methanosarcina* was observed in these tests after AD, indicating that the growth of this group
295 of archaea prevailed. Moreover, when looking at the results obtained using Inoc-OW (MS-
296 Rich; 52.5 % *Methanosarcina* initially), high methane yields were achieved at substrate loads
297 up to $1\text{ g VS}\cdot\text{g VS}^{-1}$, values where Inoc-UASB2 (MS-Poor; with lower initial proportions of
298 *Methanosarcina*; 6.36 %) led to acidification and no methane was produced.

299 Regarding the archaeal composition of the acidified (non-methane producing) reactors,

300 *Methanobacterium* was predominant in all of them, with negligible proportions of other
301 archaeal genus. As these archaea were already predominant in both UASB1 and UASB2
302 inocula, this simply indicates that, as no methane was significantly produced, no growth of
303 other methanogenic microorganisms occurred in these conditions.

304 These results are in accordance with a recent review article focused on the microbial
305 communities of FW AD. In their bibliographic study, Wang et al. (2017) pointed out that
306 *Methanosarcina* was a predominant methanogen during dry FW AD and that the presence of
307 this archaea could potentially act as an indicator of a stable and efficient dry AD process. It
308 must also be mentioned that, together with this particular archaea, the development of other
309 microorganisms growing in syntrophy with *Methanosarcina* might have also been of critical
310 importance. The growth of other hydrogenotrophic methanogens (such as
311 *Methanothermobacter* or *Methanoculleus*) might have contributed greatly to the metabolic
312 shift towards hydrogenotrophic methanogenesis as main methane-producing pathway.

313 Moreover, if it is assumed that this was the main route for methane production using FW and
314 CB as substrates (Capson-Tojo et al., 2017d), the growth of syntrophic acetate oxidizers has
315 also been essential to degrade acetate to hydrogen, facilitating the production of methane by
316 the hydrogenotrophs. Finally, the fact that all the reactors producing methane (regardless of
317 the inoculum used) had *Methanosarcina* as predominant methanogen suggest that the
318 relevance of its growth was independent of the particular characteristics of each inoculum (*i.e.*
319 initial VS concentration or sludge mixture). At this point, it must be mentioned that, before
320 concluding the suitability of adapted inocula (such as Inoc-OW) for dry AD of FW
321 (with/without CB), experiments at higher TS contents (*i.e.* 27-30 %) must be carried out.

322 The results from the PCA carried out using the methane yields, the *Methanosarcina*
323 proportions in the inocula and the digestates, the final VFA concentrations and pH values and
324 the initial TS contents and S/X and C/N ratios as entries further support the critical

325 importance of *Methanosarcina* to achieve an efficient AD process. Figure 4 shows the
326 obtained results.

327 As it can be observed in the correlation circle, the first component accounted for 54.3 % of the
328 variance, which was mainly attributed to the final pH, the methane yields, the
329 *Methanosarcina* proportions in both the inocula and the digestates and the final VFA
330 concentrations. The pH, methane yields and *Methanosarcina* proportions in the digestates
331 were all strongly positively correlated between them and negatively correlated to the VFA
332 concentrations. Also in agreement with the results presented above, the initial
333 *Methanosarcina* proportions were also significantly correlated with the final pH, the methane
334 yields and the final *Methanosarcina* proportions. Interestingly, the initial working conditions
335 (*i.e.* TS content and initial S/X and C/N ratios) were not correlated to either of the
336 aforementioned variables (*i.e.* pH, methane yields, VFA concentrations and *Methanosarcina*
337 proportions in the digestates), suggesting that similar results were obtained regardless the
338 initial conditions defined in the batch reactors. To summarize, regardless of the initial
339 conditions, methane was efficiently produced in the reactors where *Methanosarcina* was
340 predominant after the digestion, consuming the accumulated VFAs and avoiding a pH drop
341 and reactor acidification.

342 The obtained results highlight the critical relevance of the initial composition of the archaeal
343 populations in the inoculum to achieve efficient AD, especially during reactor start-up. In
344 particular, the results suggest the great importance of *Methanosarcina* and other
345 hydrogenotrophs within the archaeal populations to achieve efficient dry AD of FW. The
346 structure of the archaeal community used to start up batch FW AD may also explain the high
347 variability of the substrate loading limits reported in the literature, ranging from below 0.5 g
348 VS·g VS⁻¹ to over 2 g VS·g VS⁻¹ (Capson-Tojo et al., 2016). These results could have great
349 implications in industrial scale AD installations, in particular for the start-up of continuous

350 AD systems and for the initial conditions applicable for batch AD systems.

351

352 **4. Conclusions**

353 AD performances of three different inocula were compared using FW and CB as substrates.

354 Particular attention was paid to the compositions of the archaeal communities in the inocula

355 and in the digestates. Regardless of the inoculum used, *Methanosarcina* was the dominant

356 methanogen in all the experiments where methane was produced, suggesting that these

357 archaea played a critical role in methane production at high TAN and VFA concentrations.

358 Higher proportions of *Methanosarcina* in the inocula also allowed greater substrate loads. The

359 initial composition of the archaeal communities in the inoculum was found to be crucial,

360 mainly in batch systems and during reactor start-up. This may have huge implications for

361 industrial-scale installations treating FW and CB.

362

363 **Acknowledgement**

364 The authors want to express their gratitude to Suez for financing this research under the

365 CIFRE convention N° 2014/1146. The Communauté d'Agglomération du Grand Narbonne

366 (CAGN) is also gratefully acknowledged for their financial support.

367

368 **References**

369 Abbassi-Guendouz, A., Trably, E., Hamelin, J., Dumas, C., Steyer, J.P., Delgenès, J.-P.,
370 Escudié, R., 2013. Microbial community signature of high-solid content methanogenic
371 ecosystems. *Bioresour. Technol.* 133, 256–262.

372 APHA, 2005. *Standard Methods for the Examination of Water and Wastewater*. American
373 Public Health Association, Washington, DC.

374 Banks, C.J., Chesshire, M., Stringfellow, A., 2008. A pilot-scale trial comparing mesophilic
375 and thermophilic digestion for the stabilisation of source segregated kitchen waste.
376 *Water Sci. Technol.* 58, 1475–1481.

377 Capson-Tojo, G., Rouez, M., Crest, M., Steyer, J.-P., Delgenès, J.-P., Escudié, R., 2016. Food
378 waste valorization via anaerobic processes: a review. *Rev. Env. Sci. Biotechnol.* 15,

379 499–547.

380 Capson-Tojo, G., Trably, E., Rouez, M., Crest, M., Steyer, J.-P., Delgenès, J.-P., Escudié, R.,
381 2017a. Dry anaerobic digestion of food waste and cardboard at different substrate loads,
382 solid contents and co-digestion proportions. *Bioresour. Technol.* 233, 166–175.

383 Capson-Tojo, G., Rouez, M., Crest, M., Trably, E., Steyer, J.-P., Bernet, N., Delgenès, J.-P.,
384 Escudié, R., 2017b. Kinetic study of dry anaerobic co-digestion of food waste and
385 cardboard for methane production. *Waste Manag.* 69, 470–479.

386 Capson-Tojo, G., Trably, E., Rouez, M., Crest, M., Bernet, N., Steyer, J.-P., Delgenès, J.-P.,
387 Escudié, R., 2017c. Cardboard proportions and total solids contents as driving factors in
388 dry co-fermentation of food waste. *Bioresour. Technol.* *In Press*

389 Capson-Tojo, G., Ruiz, D., Rouez, M., Crest, M., Steyer, J.-P., Bernet, N., Delgenès, J.-P.,
390 Escudié, R., 2017d. Cardboard proportions and total solids contents as driving factors in
391 dry co-fermentation of food waste. *Bioresour. Technol.* 245, 724–733

392 Cazier, E.A., Trably, E., Steyer, J.P., Escudie, R., 2015. Biomass hydrolysis inhibition at high
393 hydrogen partial pressure in solid-state anaerobic digestion. *Bioresour. Technol.* 190,
394 106–113.

395 Chen, J.L., Ortiz, R., Steele, T.W.J., Stuckey, D.C., 2014. Toxicants inhibiting anaerobic
396 digestion: a review. *Biotechnol. Adv.* 32, 1523–34.

397 De Vrieze, J., Hennebel, T., Boon, N., Verstraete, W., 2012. Methanosarcina: The
398 rediscovered methanogen for heavy duty biomethanation. *Bioresour. Technol.* 112, 1–9.

399 Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method
400 for Determination of Sugars and Related Substances. *Anal. Chem.* 28, 350–356.

401 Fernández, B., Porrier, P., Chamy, R., 2001. Effect of inoculum-substrate ratio on the start-up
402 of solid waste anaerobic digesters. *Water Sci. Technol.* 44, 103–108.

403 Frølund, B., Palmgren, R., Keiding, K., Nielsen, P.H., 1996. Extraction of extracellular
404 polymers from activated sludge using a cation exchange resin. *Water Res.* 30, 1749–
405 1758.

406 Hao, L., Lü, F., Mazéas, L., Desmond-Le Quéméner, E., Madigou, C., Guenne, A., Shao, L.,
407 Bouchez, T., He, P., 2015. Stable isotope probing of acetate fed anaerobic batch
408 incubations shows a partial resistance of acetoclastic methanogenesis catalyzed by
409 Methanosarcina to sudden increase of ammonia level. *Water Res.* 59, 90–99.

410 Hogg, D., Favoino, E., Nielsen, N., Thompson, J., Wood, K., Penschke, A., Papageorgiou, D.,
411 Economides, S., 2002. Economic Analysis of Options for Managing Biodegradable
412 Municipal Waste. Final Report to the European Commission, Bristol, United Kingdom.

413 Jiang, Y., Banks, C., Zhang, Y., Heaven, S., Longhurst, P., 2017. Quantifying the percentage
414 of methane formation via acetoclastic and syntrophic acetate oxidation pathways in
415 anaerobic digesters. *Waste Manag.* *In Press*

416 Kim, D.-H., Oh, S.-E., 2011. Continuous high-solids anaerobic co-digestion of organic solid
417 wastes under mesophilic conditions. *Waste Manag.* 31, 1943–1948.

418 Mao, C., Feng, Y., Wang, X., Ren, G., 2015. Review on research achievements of biogas
419 from anaerobic digestion. *Renew. Sust. Energ. Rev.* 45, 540–555.

420 Motte, J.-C., Escudié, R., Beaufils, N., Steyer, J.-P., Bernet, N., Delgenès, J.-P., Dumas, C.,

- 421 2014. Morphological structures of wheat straw strongly impacts its anaerobic digestion.
422 *Ind. Crops Prod.* 52, 695–701.
- 423 Motte, J.-C., Trably, E., Escudié, R., Hamelin, J., Steyer, J.-P., Bernet, N., Delgenes, J.-P.,
424 Dumas, C., 2013. Total solids content: a key parameter of metabolic pathways in dry
425 anaerobic digestion. *Biotechnol. Biofuels* 6, 164.
- 426 Poirier, S., Desmond-Le Quéméner, E., Madigou, C., Bouchez, T., Chapleur, O., 2016.
427 Anaerobic digestion of biowaste under extreme ammonia concentration: Identification of
428 key microbial phylotypes. *Bioresour. Technol.* 207, 92–101.
- 429 VALORGAS, 2010. D2.1: Compositional analysis of food waste from study sites in
430 geographically distinct regions of Europe, Valorisation of food waste to biogas.
431 Available in <http://www.valorgas.soton.ac.uk/deliverables.htm>
- 432 Venkiteshwaran, K., Milferstedt, K., Hamelin, J., Zitomer, D.H., 2016. Anaerobic digester
433 bioaugmentation influences quasi steady state performance and microbial community.
434 *Water Res.* 104, 128–136.
- 435 Walter, A., Probst, M., Hinterberger, S., Müller, H., Insam, H., 2016. Biotic and abiotic
436 dynamics of a high solid-state anaerobic digestion box-type container system. *Waste*
437 *Manag.* 49, 26–35.
- 438 Wang, P., Wang, H., Qiu, Y., Ren, L., Jiang, B., 2017. Microbial characteristics in anaerobic
439 digestion process of food waste for methane production-A review. *Bioresour. Technol.*
440 *In Press*
- 441 Wang, Y., Qian, P., 2009. Conservative fragments in bacterial 16S rRNA genes and primer
442 design for 16S ribosomal DNA amplicons in metagenomic studies. *PloS one* 4.
- 443 Zamanzadeh, M., Hagen, L.H., Svensson, K., Linjordet, R., Horn, S.J., 2016. Anaerobic
444 digestion of food waste – Effect of recirculation and temperature on performance and
445 microbiology. *Water Res.* 96, 246–254.
- 446 Zhang, J., Lv, C., Tong, J., Liu, J., Liu, J., Yu, D., Wang, Y., Chen, M., Wei, Y., 2016.
447 Optimization and microbial community analysis of anaerobic co-digestion of food waste
448 and sewage sludge based on microwave pretreatment. *Bioresour. Technol.* 200, 253–261.
- 449 Zhang, L., Ouyang, W., Lia, A., 2012. Essential Role of Trace Elements in Continuous
450 Anaerobic Digestion of Food Waste. *Procedia Environ. Sci.* 16, 102–111.
- 451 Zhang, Y., Banks, C.J., Heaven, S., 2012. Co-digestion of source segregated domestic food
452 waste to improve process stability. *Bioresour. Technol.* 114, 168–178.
- 453

454 **Figure and table captions**

455 **Figure 1.** Evolution of the total VFA concentrations in the experiments carried out using
456 Inoc-OW (A) and Inoc-UASB1 (B) as inocula. The legends indicate the substrate used, the
457 S/X ratio and the initial TS content. FW stands for food waste, CB for cardboard, TS for total
458 solids, VS for volatile solids and VFA for volatile fatty acids

459 **Figure 2.** Relative abundances of archaeal OTUs in the inocula. The inocula were named
460 “MS-Rare”, “MS-Poor” and “MS-Rich” according to their low to high initial relative
461 abundances of *Methanosarcina*

462 **Figure 3.** Relative abundances of archaeal OTUs in the digestates from the batch tests that
463 produced methane (up) and the acidified reactors (down). The methane yields are also
464 presented. The inocula were named “MS-Rare”, “MS-Poor” and “MS-Rich” according to
465 their initial relative abundances of *Methanosarcina*

466 **Figure 4.** Correlation circle using as input data the initial total solid contents (TS) and C/N
467 and S/X ratios, the *Methanosarcina* proportions in the inocula ($Sarcina_0$), the *Methanosarcina*
468 proportions in the digestates ($Sarcina_f$) and the final pH, methane yields (CH_4) and volatile
469 fatty acids concentrations (VFA). This circle resulted from the projection in plans formed by
470 the two first principal components, accounting for 85.6 % of the variance

471 **Table 1.** Operational conditions of the batch experiments and obtained methane yields.
472 “UASB1”, “UASB2” and “OW” stand for the inoculum

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

474 **Table 3.** Concentrations of VFAs, TAN and FAN at the beginning and the end of the batch
475 tests presented in Table 1. The values of the pH and the incubation times are also presented

Graphical abstract

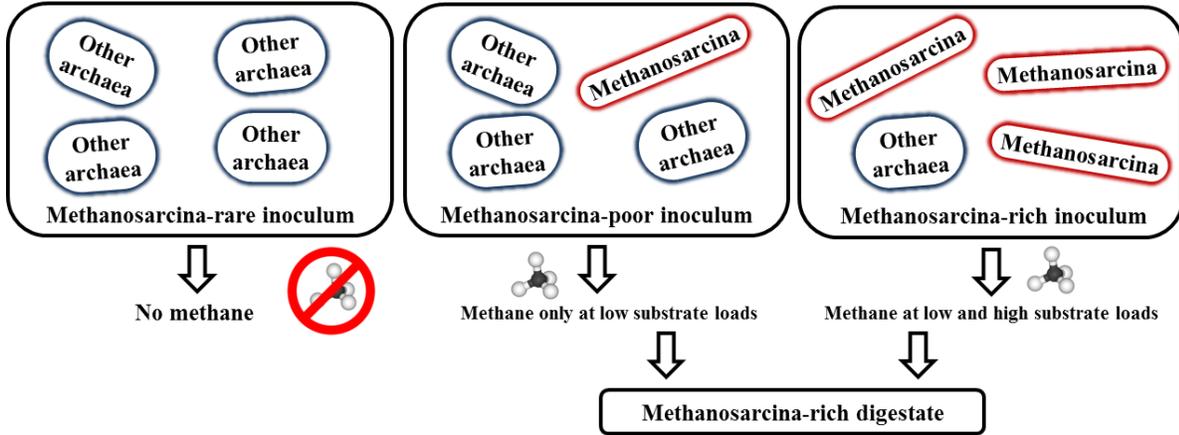


Table 1. Operational conditions of the batch experiments and obtained methane yields.

“UASB1”, “UASB2” and “OW” stand for the inoculum

Inoculum	Substrate	Substrate C/N ratio	Co-dig. ratio (g TS FW·g TS CB ⁻¹)	S/X (g VS·g VS ⁻¹)	Initial TS (%)	Methane yield (mL CH ₄ ·g VS ⁻¹)
UASB1	FW+CB	23.1	1.75	0.25	30.0	11±3*
UASB1	FW+CB	27.8	1.00	0.25	30.0	8±2*
UASB1	FW+CB	27.8	1.00	0.25	35.0	17±2*
UASB1	FW	16.3	-	0.25	20.0	1±1*
UASB2	FW+CB	22.7	1.86	0.25	27.5	409±11
UASB2	FW+CB	27.8	1.00	0.25	27.5	393±9
UASB2	FW+CB	27.8	1.00	0.25	35.0	401±16
UASB2	FW+CB	19.3	4.00	1.00	27.5	0±0*
OW	FW	16.3	-	0.25	20.0	464±14
OW	FW	16.3	-	1.00	20.0	375±17

* These values were considered as indicators of an inefficient AD process

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

Parameter/Element	Model food waste	Cardboard	Inoc-UASB1	Inoc-UASB2	Inoc-OW
TS % (wet basis)	21.6±0.7	92.7±3.7	70.8±2.2	74.2±3.1	5.81±0.02
VS (% TS)	96.2±0.1	77.5±0.2	70.9±1.4	59.1±0.4	59.1±0.1
pH	5.60	7.10	-	-	8.01
Carbohydrates (g·kg TS ⁻¹)	687±15	958±5	-	-	-
Proteins (g·kg TS ⁻¹)	169±10	0	-	-	-
Lipids (g·kg TS ⁻¹)	72.3±1.5	0	-	-	-
BMP (ml CH ₄ ·g VS ⁻¹)	498±42	250±3	-	-	-
TAN (g·L ⁻¹)	0	0	1.50	1.49	5.04
TKN (g·kg TS ⁻¹)	27.08±1.64	2.00±0.02	-	-	-
TOC (g·kg TS ⁻¹)	442±7	366±6	-	-	-
C/N	16.3	183	-	-	-

Table 3. Concentrations of VFAs, TAN and FAN at the beginning and the end of the batch tests presented in Table 1. The values of the pH and the incubation times are also presented

Inoculum	Substrate C/N ratio	Initial TS (%)	Incubation time (d)	pH		Total VFAs (g COD·L ⁻¹)		TAN (mg·L ⁻¹)		FAN (mg·L ⁻¹)	
				Initial	Final	Initial	Final	Initial	Final	Initial	Final
UASB1	23.1	30.0	83	na ¹	5.9±0.1	nd ²	33.3±0.5	530	1250±30	na ¹	1±0
UASB1	27.8	30.0	83	na ¹	5.9±0.2	nd ²	33.7±0.3	450	1060±30	na ¹	1±0
UASB1	27.8	35.0	83	na ¹	6.3±0.0	nd ²	24.7±1.6	350	1340±10	na ¹	3±0
UASB1	16.3	20.0	83	na ¹	5.7±0.2	nd ²	25.6±0.6	310	1090±140	na ¹	1±0
UASB2	22.7	27.5	98	na ¹	8.3±0.0	nd ²	nd ²	470	2900±210	na ¹	576±32
UASB2	27.8	27.5	98	na ¹	8.4±0.0	nd ²	nd ²	470	2600±160	na ¹	567±20
UASB2	27.8	35.0	98	na ¹	8.5±0.0	nd ²	nd ²	600	3200±180	na ¹	795±55
UASB2	19.3	27.5	98	na ¹	5.4±0.5	nd ²	64.4±14.5	370	1800±90	na ¹	1±1
OW	16.3	20.0	56	8.1	8.3±0.0	nd ²	nd ²	4140	4800±480	570	948±119
OW	16.3	20.0	56	8.1	8.4±0.0	nd ²	nd ²	3770	5050±160	519	1171±50

1. Not available due to the high TS contents of the inoculum

2. Not detectable due to too low concentrations

Figure 1

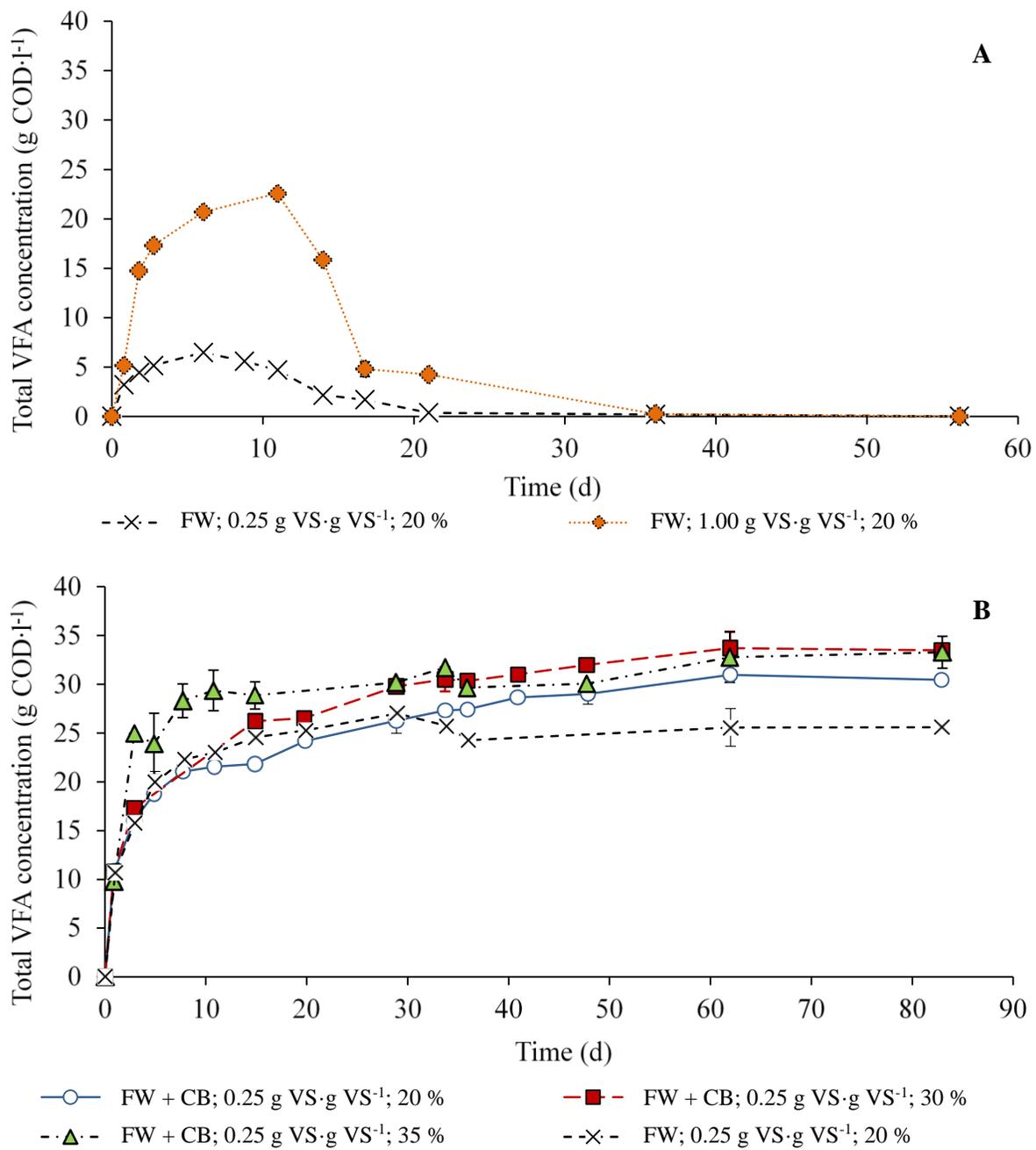


Figure 1. Evolution of the total VFA concentrations in the experiments carried out using Inoc-OW (A) and Inoc-UASB1 (B) as inocula. The legends indicate the substrate used, the S/X ratio and the initial TS content. FW stands for food waste, CB for cardboard, TS for total solids, VS for volatile solids and VFA for volatile fatty acids

Figure2

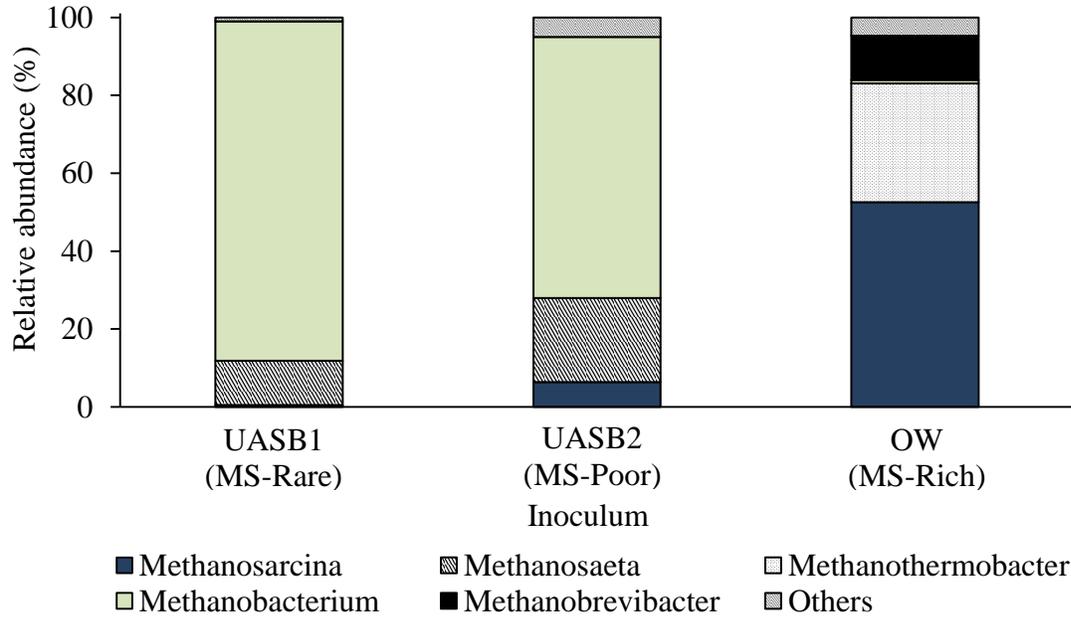


Figure 2. Relative abundances of archaeal OTUs in the inocula. The inocula were named “MS-Rare”, “MS-Poor” and “MS-Rich” according to their low to high initial relative abundances of *Methanosarcina*

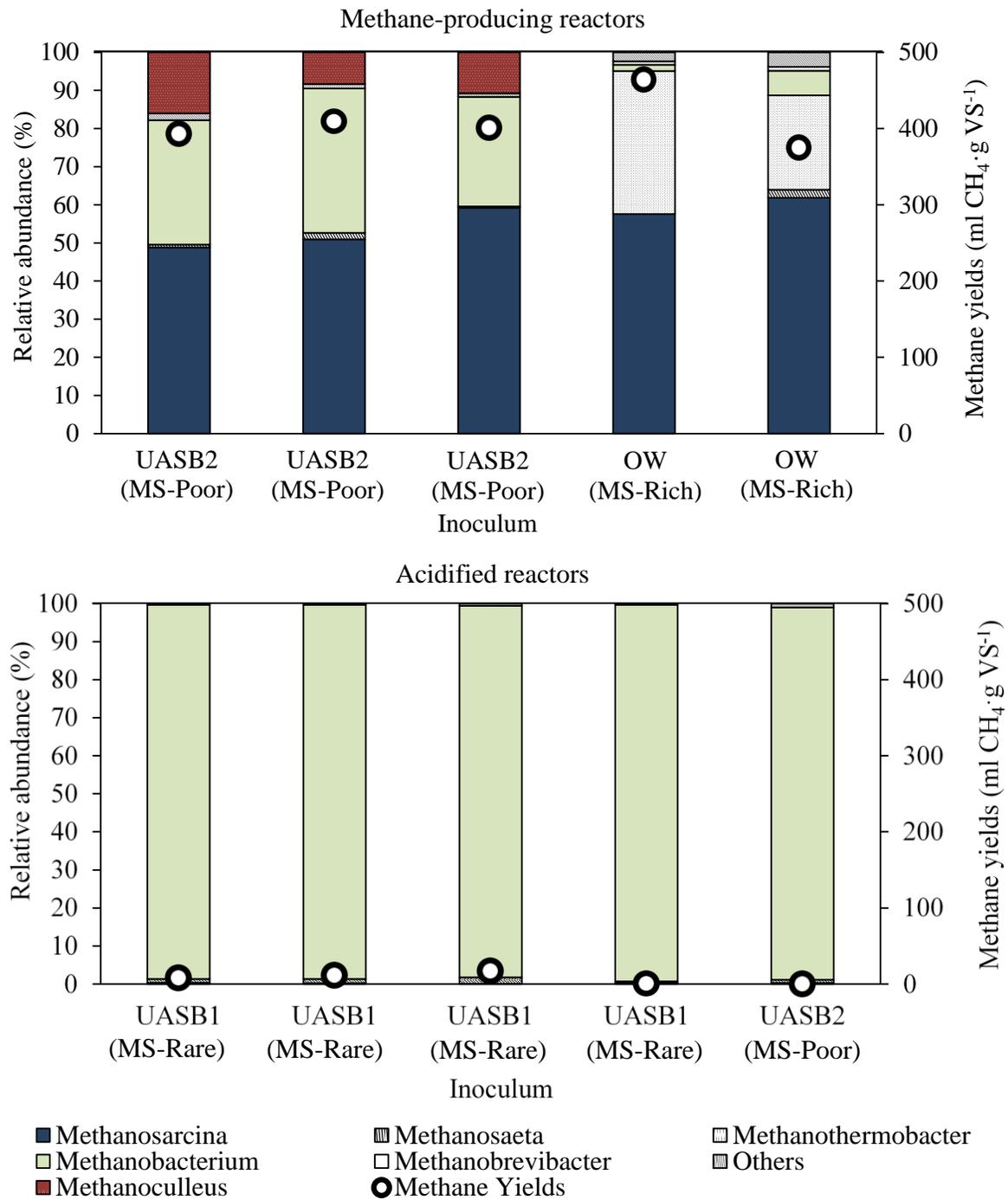


Figure 3. Relative abundances of archaeal OTUs in the digestates from the batch tests that produced methane (up) and the acidified reactors (down). The methane yields are also presented. The inocula were named “MS-Rare”, “MS-Poor” and “MS-Rich” according to their initial relative abundances of *Methanosarcina*

Figure4

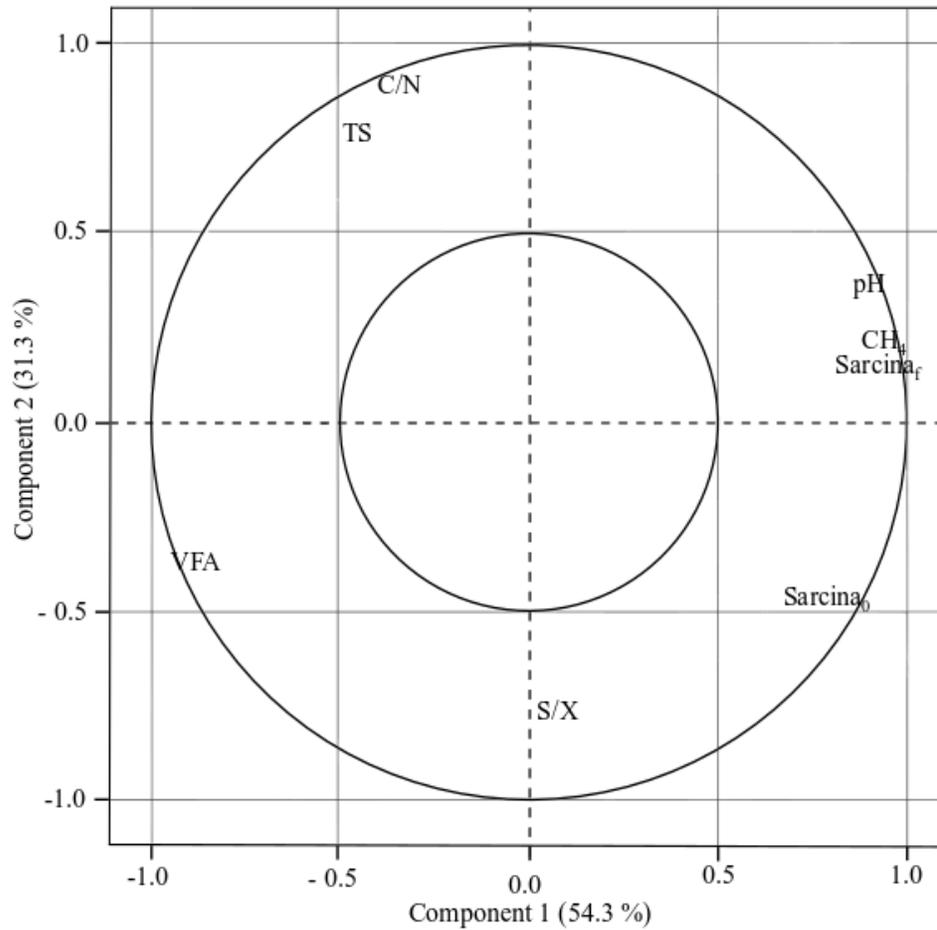


Figure 4. Correlation circle using as input data the initial total solid contents (TS) and C/N and S/X ratios, the *Methanosarcina* proportions in the inocula ($Sarcina_0$), the *Methanosarcina* proportions in the digestates ($Sarcina_f$) and the final pH, methane yields (CH_4) and volatile fatty acids concentrations (VFA). This circle resulted from the projection in plans formed by the two first principal components, accounting for 85.6 % of the variance