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Abstract: One- and two-stage anaerobic digestion of food waste aimed at recovering CH_4 and H_2 + CH_4, respectively, were compared in order to assess the potential benefits from the two-stage process in terms of overall energy recovery. The results obtained suggest that a two-stage process where the first reactor is properly operated in order to achieve a significant net H_2 production, may display a 20 % comparatively higher energy recovery yield as a result, mainly, of enhanced methane production as well as of the associated H_2 production. The highest CH_4 production of the two-stage process, observed despite the recovery of H_2 may in principle represent a potential substrate depletion for the methanogenic stage, was due to improved hydrolysis and fermentation of food waste, with increased amounts of volatile fatty acids being readily available to methanogenesis.

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Cover Letter

Dear Editor,

Please find enclosed the manuscript entitled “Energy recovery from one- and two-stage anaerobic digestion of food waste”.

In the present work one- and two-stage anaerobic digestion (AD) of food waste aimed at recovering CH_4 and H_2 + CH_4, respectively, were compared in order to assess the potential benefits from the two-phase process in terms of overall energy recovery. The issue has been debated extensively, and advantages/drawbacks of both systems have been considered and evaluated by several authors. However, it became again topical in recent years as a result of the interest aroused by the possibility of producing bio-hydrogen from organic substrates during the fermentation phase of AD. Hydrogen recovery through dark fermentation of organic substrates is not yet considered both technically reliable and commercially attractive. Assessing the increased overall energy recovery and, in particular, also higher CH_4 yields of two-stage AD systems could greatly contribute to the affirmation of fermentative hydrogen production as a viable process. Few studies are available that provide ultimate answers about the advantages of AD operated in two distinct phases and even fewer, in particular, provide a comparison between one- and two-stage AD where the latter is contextualized and focused on the possibility of combining the recovery of both H_2 and CH_4 from a complex substrate such as food waste. For these reasons, the Authors think that the paper provides useful results and gives a contribute on the issue, therefore it should be considered for publishing. Since the paper addresses an issue of renewed scientific and technical interest, it is appealing to an audience either scientific or belonging to the company world.

The manuscript has been checked by a native tongue speaker with expertise in the field.

Authors are available as reviewers for at least three other articles for WM during the current year.

Thank you for your consideration of this manuscript. Authors hope that this work will be appreciated by your readers.

Best regards

Aldo Muntoni

1 Title page

2 ENERGY RECOVERY FROM ONE- AND TWO-STAGE ANAEROBIC
3 DIGESTION OF FOOD WASTE

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ABSTRACT

One- and two-stage anaerobic digestion of food waste aimed at recovering CH_4 and H_2 + CH_4, respectively, were compared in order to assess the potential benefits from the two-stage process in terms of overall energy recovery. The results obtained suggest that a two-stage process where the first reactor is properly operated in order to achieve a significant net H_2 production, may display a 20 % comparatively higher energy recovery yield as a result, mainly, of enhanced methane production as well as of the associated H_2 production. The highest CH_4 production of the two-stage process, observed despite the recovery of H_2 may in principle represent a potential substrate depletion for the methanogenic stage, was due to improved hydrolysis and fermentation of food waste, with increased amounts of volatile fatty acids being readily available to methanogenesis.

KEYWORDS: anaerobic digestion, one-stage, two-stage, hydrogen, methane

1. INTRODUCTION[1]

In current applications of anaerobic digestion (AD) systems, organic matter is converted into a

[1] 1S-AD: one-stage anaerobic digestion system
2S-AD: two-stage anaerobic digestion system
AD: anaerobic digestion
AS: activated sludge
CSTR: continuous stirred tank reactor
DOC: dissolved organic carbon
FW: food waste
G_{max}: maximum gas yield
ISR: inoculum to substrate ratio
MS: methanogenic sludge
OBS_{H2}: observed H_2 production
R_{max}: maximum gas production rate
SER: specific energy recovery
SHP: specific hydrogen production
SMP: specific methane production
t_{95}: time required to attain 95 % of the maximum biogas yield
TAN: total ammonia nitrogen
$THEO_{H2}$: theoretical H_2 production
TOC: total organic carbon
TS: total solids
VFAs: volatile fatty acids
VS: volatile solids
λ: lag phase duration

mixture of gaseous compounds, mainly CH_4 and CO_2, via acid fermentation and volatile fatty acids (VFAs) degradation, and through the activity of two groups of microorganisms: acid-forming and methane-forming biomass, respectively (Zhang et al., 2016). In a single-reactor system, namely one-stage anaerobic digestion (1S-AD), those microorganisms are kept together in a balance which is delicate because both groups differ widely in terms of physiology, nutritional needs, growth kinetics and sensitivity to environmental conditions (Demirel and Yenigün, 2002). By way of example, the pH prevailing in 1S-AD systems (between 7 and 8) does not provide optimal growth conditions for acidifying hydrolytic bacteria, leading to insufficient hydrolysis/fermentation rates (especially for slowly degradable lignocellulosic substrates) and, in turn, diminished biogas production (Giovannini et al., 2016). Considering these aspects, Pohland and Ghosh (1971) proposed the two-stage AD system (2S-AD) where organic matter hydrolysis and its fermentation to acids are physically separated from the methane production process.

Since then, the comparison of the performances of 1S- and 2S-AD has been debated extensively, and advantages/drawbacks of both systems have been considered and evaluated by several authors (Demirel and Yenigün, 2002; Reith et al., 2003; Han and Shin, 2004; Liu et al., 2006; Gómez et al., 2006, 2009; Ueno et al., 2007; Cooney et al., 2007; Chu et al., 2008; Thompson, 2008; Dong et al., 2011).

In 2S-AD systems, the physical separation of the reactors guarantees appropriate environments for the acidogenic and the methanogenic biomass, thus optimizing specific metabolic activities and methane generation (Schievano et al., 2014). Moreover, the first acidogenic reactor may act as a buffer against pH drops caused by potential accumulation of VFAs hindering methanogenic microorganims. As a consequence, better process reliability, resilience, stability, as well as higher substrate removal and conversion efficiency are anticipated for 2S-AD systems.

Nevertheless, 1S-AD is a well-established system for the treatment of organic waste, characterized by a simple set-up and relatively limited investment and operating costs, and as a matter of fact

most of the full-scale digestion plants in Europe (90 % of the installed AD capacity) are designed and operated as one-stage systems (Rapport et al., 2012). A major drawback in such cases is that the produced biogas is frequently reported to display a poor quality in terms of its calorific value (Zhang et al., 2015; Sunyoto et al., 2016).

The issue of operating AD in the 2S configuration has become again topical in recent years as a result of the interest aroused by the possibility of producing bio-hydrogen from organic substrates through dark fermentation (Lee and Chung, 2010; Dong et al., 2011; De Gioannis et al., 2013; Cappai et al., 2014). Indeed, under appropriate operating conditions, facultative or strict anaerobic microorganisms are able to convert organic substrates into bio-H_2 through fermentation; the H_2 produced is recoverable, provided that a harsh environment for hydrogenophylic methanogens is guaranteed. In addition to H_2 and CO_2, which are the most abundant gaseous products, a mix of volatile fatty acids (VFAs) and reduced end products including alcohols is generated as well, which are suitable for further valorization through methanogenesis. This can be accomplished through a variety of potential alternatives, differing for the type of process applied and/or the characteristics of the resulting product. Hydrogen has the highest energy content of any known fuel, and sequential H_2 and CH_4 production is, from a theoretical point of view, energetically more favourable than 1S-AD (Dong et al., 2009); from a practical point of view, the two gas streams may be valued individually, or mixed to form a hydrogen-enriched biogas (namely biohythane) characterized by an improved quality for gas engines applications (Porpatham et al., 2007). However, H_2 recovery through dark fermentation of organic substrates is not yet considered both technically reliable and commercially attractive. Assessing the increased overall energy recovery and, in particular, also higher CH_4 yields of 2S-AD systems could greatly contribute to the affirmation of fermentative hydrogen production as a viable process.

Few studies are available that provide ultimate answers about the advantages of AD operated in two distinct phases (Aslanzadeh et al., 2014); even fewer, in particular, provide a comparison between

88 1S- and 2S-AD where the latter is contextualized and focused on the possibility of combining the
89 recovery of both H_2 and CH_4 from a complex substrate such as food waste (FW). Voelklein et al.
90 (2016) operated a two-stage anaerobic CSTR observing a methane yield from FW ranging between
91 371 and 419 Nl CH_4/kg VS, 23 % higher than from the one-stage process; no data on H_2 production
92 were observed because, as reported by the authors, the goal was to optimize the acidification
93 process and maximize methane yield rather than to produce H_2. Grimberg et al. (2015) achieved a
94 methane production yield from FW of 446 Nl CH_4/kg $VS_{removed}$ in a two-stage CSTR-based process,
95 fairly higher than the yield of 380 Nl CH_4/kg $VS_{removed}$ observed in a one-stage process (no available
96 data about H_2 production were provided). Aslanzadeh et al. (2014) evaluated the effects of organic
97 loading rate and hydraulic retention time on CH_4 production in one- and two-stage systems treating
98 municipal FW: a maximum methane production of 380 Nl CH_4/kg VS was obtained in the two-stage
99 process versus a maximum of 330 Nl CH_4/kg VS observed in the one-stage. Nathao et al. (2013)
100 compared the performance of one- and two-stage mesophilic AD of FW in batch reactors at varying
101 ratios of feedstock to microbial inoculum (F/M), observing yields of 55 Nl H_2/kg VS and 94 Nl
102 CH_4/kg VS at F/M ratios of 7.5 in the two-stage process, to be compared with a CH_4 yield of 82
103 Nl/kg VS attained in the one-stage system. Interesting economic considerations were derived by Lee
104 and Chung (2010) who managed a two-stage pilot-scale process treating FW, connected to a fuel
105 cell. When single CH_4 and combined H_2 + CH_4 production were compared, negligible differences in
106 the production costs were estimated, whilst a gain of 12-25 % in terms of overall energy production
107 was observed for the two-stage system.
108 The objective of the present study was to compare 1S- and 2S-AD of a complex substrate (FW)
109 aimed at recovering CH_4 and H_2 + CH_4, respectively. Batch tests were performed under mesophilic
110 conditions, the performances in terms of H_2 and CH_4 yields and volatile solids removal efficiency
111 were evaluated, and the overall energy recoverable from the two AD systems was estimated.
112

2. MATERIALS AND METHODS

2.1 Substrate and inocula

Due to general heterogeneity of municipal FW, a standardized FW was used for the present study to allow repeatable and directly comparable experiments. FW was prepared by mixing (on a wet weight basis) 10 % of meat, 65 % of fruit and vegetables, 10 % of bread and 15 % of cooked pasta. Due to their tendency to rapid degradation, FW samples were purposely prepared for each experiment by mixing the individual components and shredding the obtained mixture with a blender to a final particle size below 2 cm.

Activated sludge (AS) from the aerobic unit of a municipal wastewater treatment plant was used as the inoculum in the first phase of the 2S-AD test, without performing any specific treatment to inhibit methanogens, as suggested by the results presented in Cappai et al. (2014).

Methanogenic sludge (MS), collected from the anaerobic digester of a municipal solid waste treatment plant, was used as the inoculum in both the 1S-AD test and in the second phase of the 2S-AD test. The MS inoculum was preliminarily maintained under anaerobic conditions in the reactor at 39 ± 1°C until biogas production stopped in order to deplete the residual biodegradable organic material, as indicated also by Raposo et al. (2011).

The main characteristics of the FW, of the inocula and of the feeds are shown in Table 1.

2.2 Experimental set-up

The methanogenic test (1S-AD) was conducted in a batch mode at 39 ± 1°C using a 2-l glass reactor (1.8 l working volume). An inoculum-to-substrate ratio (ISR) of 2 g VS/g VS was adopted in order to limit inhibition effects associated with accumulation of intermediate compounds, such as VFAs, during substrate degradation (Raposo et al., 2011).

The hydrogenogenic + methanogenic test (2S-AD) was conducted in a batch mode at 39 ± 1 °C using a 2-l glass reactor (1.8 l working volume) for the first stage and a 5-l glass reactor (4.5 l

working volume) for the second stage. The effluent from the fermentative-hydrogenogenic reactor was then fed to the methanogenic stage after mixing with MS according to the same ISR adopted for the 1S-AD test (2 g VS/g VS). All the reactors were equipped with mechanical stirring (150 rpm) and were connected to an automatic system for data acquisition and pH control through NaOH addition. An operating pH set-point value of 6.5 and a ISR of 0.14 g VS/g VS were adopted for the first stage of the 2S-AD test, as suggested by Cappai et al. (2014). Control of operating pH was not deemed necessary in the 1S-AD test and in the methanogenic stage of the 2S-AD test.

Biogas production was assessed by the volume displacement principle. The measured gas volume was converted to standard temperature and pressure conditions (T = 0 °C, p = 1 atm).

All the reactors were flushed with N_2 gas to drive off air from the headspace before starting the experiments.

Table 1. Main characteristics of concern for FW, inocula and feeds for the 1S-AD and 2S-AD tests.

Parameter	Unit of measure	FW	AS	MS	Test		
					1S-AD	2S-AD	
						1st stage	2nd stage
Initial pH	---	5.5 ± 0.2	7.1 ± 0.1	7.8 ± 0.1	7.6 ± 0.1	6.9 ± 0.1	7.3 ± 0.1
TS	% wt	22.6 ± 1.3	0.9 ± 0.1	5.2 ± 0.3	6.4 ± 0.2	4.1 ± 0.3	4.2 ± 0.2
VS	% wt	22.0 ± 1.2	0.6 ± 0.1	3.0 ± 0.2	4.3 ± 0.3	3.8 ± 0.4	2.4 ± 0.5
TOC	% TS	46.2 ± 0.4	36.4 ± 0.3	24.3 ± 0.6	29.2 ± 1.4	44.5 ± 2.7	30.1 ± 2.3

2.3 Analytical methods

All analyses were conducted in triplicate and the results are presented as average values of the replicates.

The contents of total solids (TS) and volatile solids (VS) were measured according to the APHA Standard Methods (APHA, Awwa, 1998). The total organic carbon concentration (TOC) and its soluble fraction (dissolved organic carbon (DOC), on 0.45 μm filtered samples) were measured using a Shimadzu TOC analyser equipped with modules for the analysis of both liquid and solid samples (TOC-VCSN and SSM-5000 module, Shimadzu, Japan). Total ammonia-nitrogen (TAN) was determined on 0.45 μm filtered samples according to the APHA Standard Methods (APHA,

Awwa, 1998) using a Hitachi U-2000 spectrophotometer operated at a wavelength of 420 nm. The biogas was sampled periodically from the reactors with a 1-ml gastight syringe and injected through a valve in a gas chromatograph (Model 7890B, Agilent Technology) equipped with a thermal conductivity detector and two stainless columns packed with HayeSep N (80/100 mesh) and Shincarbon ST (50/80 mesh) connected in series. The operating temperatures of the valve and the TCD were 90 and 200 °C, respectively, and He was the carrier gas. The oven temperature was set initially at 70 °C (3-min holding time), followed by a ramp of 10 °C/min up to 160 °C (3-min holding time).

The concentrations of VFAs (acetic [HAc], propionic [HPr], butyric + iso-butyric [HBu], valeric + iso-valeric [HVa], hexanoic + iso-hexanoic [HHEx], heptanoic [HHep]) were determined using a gas chromatograph (Model 7890B, Agilent Technology) equipped with an HP-FFAP capillary column (30 m, inner diameter 0.53 mm, Agilent Technology). The samples were filtered using a 0.45 µm cellulose acetate filter and then acidified with concentrated H_3PO_4 (pH < 3). The injection volume was 0.6 µl. The temperatures of the injector and the detector were 250 and 300 °C, respectively. The oven temperature was initially set at 70 °C (3-min holding time), followed by a ramp of 20 °C/min up to 180 °C (3-min holding time). He (1.6 ml/min, split ratio 20:1) was used as the carrier gas.

2.4 Kinetic model

The sigmoid-type modified Gompertz function was used to analyse and describe the H_2 and CH_4 production during each test. In the Gompertz model, the evolution of gas production G(t) over time is expressed as follows (Eq. 1) (Zwietering et al., 1990; Lay et al., 1999):

$$G(t) = G_{max}\, exp\left\{-exp\left[\frac{R_{max}\cdot e}{G_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where G_{max} is the maximum gas yield, R_{max} is the maximum gas production rate, λ is the lag phase duration and the value of "e" is 2.71828. The time required to attain 95 % of the maximum biogas yield, namely t_{95}, was derived from the Gompertz equation as follows (Eq. 2):

$$t_{95} = \frac{G_{max}}{R_{max} \cdot e}(1 - \ln(-\ln 0.95)) + \lambda \quad (2)$$

The experimental data were fitted with the Gompertz equation and G_{max}, R_{max}, λ and t_{95} were estimated using the software TableCurve 2D (v. 5.01, Systat Software Inc.). The coefficient of determination or correlation coefficient R^2, was calculated so as to evaluate the quality of data fitting for each experimental dataset.

2.5 Calculations

The hydrolysis and the acidification yields (%) were calculated for the first stage of the 2S-AD test as expressed in Eqs. 3 and 4 (Graunke and Wilkie, 2014; Voelklein et al., 2016):

$$hydrolysis\ yield\ (\%) = 100 * DOC/TOC_i \quad (3)$$

$$acidification\ yield\ (\%) = 100 * VFAs/DOC \quad (4)$$

where TOCi is the initial total organic carbon concentration and VFAs is the concentration of net VFAs (final-initial) expressed as g C/l.

In order to validate the results of the tests performed, a carbon mass balance was calculated as expressed in Eq. 5:

$$carbon\ mass\ balance\ (\%) = 100 * C_{gas}/(TOC_{initial} - TOC_{final}) \quad (5)$$

where C_{gas}, $TOC_{initial}$ and TOC_{final} are the organic carbon mass in the produced gas, in the influent at

the beginning of the test and in the effluent at the end of the test, respectively.

The specific methane production (SMP) for the 1S-AD test was expressed as the methane produced per unit of initial VS mass added to the methanogenic reactor. As for the 2S-AD test, the specific hydrogen production (SHP) was calculated per unit of initial VS mass added to the first reactor and, in order to consider the performance of the whole system, the SMP for the second reactor was calculated as the methane produced per unit of initial VS mass added to the first reactor, as indicated also by Schievano et al. (2014).

The specific gas production, either SHP or SMP, was converted to a specific energy recovery (SER) per unit of VS added to the two systems (1S-AD and 2S-AD). The SER was calculated by considering the heat of combustion of H_2 and CH_4 (12.74 MJ/m^3 and 35.16 MJ/m^3, respectively; (Schievano et al., 2014)).

3. RESULTS AND DISCUSSION

3.1 One-stage process (1S-AD test)

3.1.1 Methane production

Although the operating pH was not controlled during the 1S-AD test, the observed values (7.3 - 7.8, data not shown) were found to lie within the recommended range for methanogenesis for the entire duration of the experiments.

Figure 1(a) shows the specific CH_4 production (SMP) cumulative curve and the evolution over time of the CH_4 content in the gas produced. The methane content increased up to about 66 % vol. after the first 50 h, then remained fairly constant until the test was stopped. The overall SMP (328.6 Nl CH_4/kg VS) is within the range of values reported by other authors for 1S-AD of FW performed under similar operating conditions, though significant differences may be found in the literature which reflect the influence of a number of factors, mainly the FW composition in terms of carbohydrates, proteins and lipids (which in turn depends on the geographic origin and seasonal

235 variability of food and the specific eating habits; (Zhang et al., 2014)). Browne and Murphy (2013)
236 observed a SMP of 358 Nl CH_4/kg VS in AD batch test on FW. Cabbai et al. (2013) reported a
237 SMP of 364 Nl CH_4/kg VS from household waste. El-Mashad and Zhang (2010) attained a SMP of
238 353 Nl CH_4/kg VS from FW.
239 A rapid accumulation of acetate, up to 1380 mg/l, was detected during the first 24 h and almost
240 completely degraded afterwards up to roughly 70 h from the beginning of the test (Figure 1(b)).
241 Additionally, a significant accumulation of propionate, with a concentration of around 1600 mg/l,
242 was detected during the first 50 h, followed likely by transformation to acetate and syntrophic
243 conversion to methane. The overall VFAs residual concentration was found to be almost negligible
244 after around 150 h. As for substrate conversion, the final VS removal efficiency was 53.3 % and the
245 carbon mass balance (Eq. 5) closed at 97.4 %.
246 The Total Ammonia Nitrogen (TAN) concentration at the end of the 1S-AD test was 1300 mg/l,
247 lower than the level of 3000 mg/l reported to exert toxic effects (Wu et al., 2016).
248
249 *3.1.2 Reaction kinetics*
250 The kinetic parameters derived from fitting of the experimental data with the Gompertz equation
251 (Eq. 1) are shown in Table 2. The model fitting was high, with an R^2 of 0.990. The estimated
252 maximum methane production rate was 3.89 Nl CH_4/(kg VS$*$h) (Table 2), similar to that obtained
253 by Yin et al. (2016) for batch AD tests performed on FW. The calculated lag phase duration
254 resulted to be fairly short (4.5 h), as observed also by Elbeshbishy et al. (2012) who performed
255 methanogenic tests on FW, and the t_{95} was equal to 125 h, confirming the high rate of
256 biodegradation of the feedstock.
257
258 (Figure 1. 1S-AD test: evolution over time of (a) specific CH_4 production (SMP; solid line indicates
259 Gompertz-model curve) and CH_4 content in the gas produced, (b) VFAs concentration.)

3.2 Two-stage process (2S-AD test)

3.2.1 First stage – hydrogen production

Figure 2(a) shows the specific H_2 production (SHP) cumulative curve and the evolution over time of the H_2 content in the gas produced during the first stage of the 2S-AD test. The hydrogen content peaked at 66 % vol. during the first 12 h, then decreased continuously until the test was stopped, presumably due to biological H_2 consumption. To this regard, the fact that methane was never detected during the first stage of the 2S-AD test may imply that H_2 consumption was caused by the onset of either propionic fermentation (Dong et al., 2010) or homoacetogenesis (Siriwongrungson et al., 2007; Saady, 2013).

The total SHP attained (56.5 Nl H_2/kg VS) falls within the range of values reported by other authors for fermentative hydrogen production from FW under similar operating conditions, though it is again worth mentioning that wide ranges of values have been reported (De Gioannis et al., 2013; Cappai et al., 2014). Hydrogen production lasted about 26 h and a final VS removal efficiency of 34.1 % was estimated. Fermentable sugars generated from carbohydrates by hydrolytic bacteria enable the rapid growth of acidogens which generate hydrogen via acetic and butyric pathways (Eq. 6 and 7):

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4\ H_2 \quad (6)$$

$$C_6H_{12}O_6 \rightarrow CH_3(CH_2)_2COOH + 2\ H_2 + 2\ CO_2 \quad (7)$$

The analysis of VFAs generation over time indicated the main presence of acetate (55 % of total VFAs, 2730 mg/l) and butyrate (41 % of total VFAs, 2000 mg/l) during the first 7 h of fermentation, while propionate was found to be produced at later stages (Figure 2(b)). At the end of the test, the total VFAs concentration was 8410 mg/l, with acetate (42 %), propionate (30 %) and butyrate (26 %) as the major soluble products. According to Vavilin et al. (2008) and Graunke and Wilkie (2014), hydrolysis of particulate matter into soluble species is assumed to be the rate-limiting step in AD and, in this sense, essential in order to obtain an adequate biogas generation.

285 The hydrolysis and acidification yields at the end of the hydrogenogenic stage were calculated to be
286 42.4 % and 48.9 %, respectively. The latter is higher than the values reported by Voelklein et al.
287 (2016) (34 %-41 %) and Chen et al. (2015) (29 %-36 %).
288 It is interesting to note that Voelklein et al. (2016) also observed lower specific H_2 yields (1.7 –
289 11.8 l/kg VS) as compared with the present study, with H_2 concentrations in the range of 5.6 – 16.2
290 % vol., pointing out that the process was arguably not optimized for H_2 production, while no data
291 on the observed H_2 production were provided by Chen et al. (2015).
292 As shown by Eq. 6 and 7, the formation of acetate and butyrate is associated with a net production
293 of H_2, whereas ethanol and propionate production is associated to H_2-neutral and H_2-consuming
294 pathways, respectively. In order to derive information about the metabolic pathways taking place
295 during the fermentation stage, the theoretical H_2 production ($THEO_{H2}$) was calculated assuming the
296 generation of 2 mol H_2/mol acetate and butyrate produced and the consumption of 1 mol H_2/mol
297 propionate produced (Jungermann et al., 1973; Li and Fang, 2007; Antonopoulou et al., 2008), and
298 compared with the observed H_2 production (OBS_{H2}). The correspondence between OBS_{H2} and
299 $THEO_{H2}$, though fair (77.1 %), indicates that processes other than acetic/butyric fermentation and
300 propionic production took place, which may include homoacetogenic fermentation, a non-
301 syntrophic reaction where hydrogen and carbon dioxide are used to produce acetate; the onset of
302 homoacetogenesis is also corroborated by the decrease in the H_2 content of the gas observed after
303 about 10-12 h of fermentation (Figure 2(a)). Although the effects of homoacetogenesis on dark
304 fermentation may be relevant, it is still unclear whether homoacetogenic H_2 consumption acts
305 during the entire fermentation process along with concomitant hydrogenogenic pathways, or it only
306 occurs at some point during the process when the substrate gets depleted and the biomass is then
307 forced to switch to different metabolic pathways (Saady, 2013). This and other aspects confirm how
308 complex and intricate the hydrogenogenic fermentation process is. Therefore, the identification of
309 operating conditions that optimize substrate hydrolysis and H_2 production and lead to an suitable

310 outflow for methanogenesis in the second stage, is crucial to the overall energy balance of the 2S-
311 AD system.
312
313 (Figure 2. 2S-AD test, first stage: evolution over time of (a) specific H_2 production (SHP; solid line
314 indicates Gompertz-model curve) and H_2 content in the gas produced, (b) VFAs concentration.)
315 *3.2.2 Second stage – methane production*
316 Figure 3(a) shows the SMP cumulative curve and the evolution over time of the CH_4 content in the
317 gas produced during the second stage of the 2S-AD test.
318 The methane content in the gas produced was higher than that observed in the 1S-AD test,
319 increasing gradually with time and peaking at 77 % vol. (Figure 3(b)). Therefore, the 2S-AD
320 configuration allowed an enrichment of the methane content by 16.7 % as compared to the 1S-AD.
321 This is consistent with Voelklein et al. (2016) who stated that a hydrolysis/fermentative reactor may
322 serve as a carbon dioxide stripping step, reducing the potential costs for upgrading the biogas to
323 biomethane. This is a significant figure considering that biogas upgrading could make up 30 % of
324 the whole cost for the biogas management system in an AD plant (Murphy and Power, 2009).
325 The total SMP attained (392 Nl CH_4/kg VS) was 19 % higher than that observed for the 1S-AD test,
326 a result similar to that reported by Voelklein et al. (2016). The VS removal in the methanogenic
327 stage was 46.9 %, which led to a 66.7 % overall removal for the entire process. The carbon mass
328 balance for the methanogenic stage closed at 97.5 %.
329 A gradual decrease in the VFAs concentration over time was observed, which resulted in a total
330 removal of 97 %, and control of the operating pH was not necessary as the pH values were always
331 within the recommended range for methanogenesis (7.4 - 7.8, data not shown). Finally, the TAN
332 concentration at the end of the 2S-AD test was 985 mg/l, lower than the reported inhibition level of
333 3000 mg/l.
334

335 *3.2.3 Reaction kinetics*

336 The experimental biogas production data for each stage of the 2S-AD test were fitted with the
337 Gompertz equation (Eq. 1) and the derived kinetic parameters are reported in Table 2.
338 Concerning the first stage, the Gompertz model fitted well the experimental data (R^2 = 0.988). The
339 estimated kinetic parameters were as follows: maximum hydrogen production rate = 3.84 Nl H_2/(kg
340 VS∗h), lag phase duration = 4.2 h and t_{95} = 26.4 h.
341 A good fitting was also observed for biogas production data in the second stage (R^2 = 0.996). The
342 maximum rate of methane production was 2.37 Nl CH_4/(kg VS∗h), lower than that calculated for the
343 1S-AD test (Table 2). This issue could be explained by a slight inhibition effect exerted by the
344 significant VFAs concentration which characterized the inflow to the second stage in the 2S-AD
345 test, and is also mirrored by the much longer, as compared with the 1S-AD test, lag phase duration
346 (20.4 h vs 4.5 h) and t_{95} (250 h vs 125 h). However, despite the lower methane production rate
347 estimated for the 2S-AD, the longer production period (about 430 h versus 200 h) allowed for a
348 higher SMP as compared to the 1S-AD test.
349
350 (Figure 3. 2S-AD test, second stage: evolution over time of (a) specific CH_4 production (SMP; solid
351 line indicates Gompertz-model curve) and CH_4 content in the gas produced, (b) VFAs
352 concentration.)

353 Table 2. Kinetic parameters calculated for the 1S-AD and 2S-AD tests.

Mathematic model	**Estimated parameter**	**Unit**	**1S-AD (CH_4)**	**2S-AD**	
				1st stage (H_2)	**2nd stage (CH_4)**
Gompertz model	G_{max}	Nl (CH_4 or H_2)/kg VS	321.7	58.6	380.1
	R_{max}	Nl (CH_4 or H_2)/kg VS h	3.89	3.84	2.37
	λ	h	4.47	4.15	20.4
	t_{95}	h	125.1	26.4	250.6
	R^2	-	0.990	0.988	0.996

354

3.3 Specific energy recovery calculation

A comparison of the specific energy recovery (SER) values was conducted for the 1S-AD and 2S-AD process configurations. Such a comparison was made on the basis of the observed biogas production in the two cases as explained in section 2.5.

The global SER from the 2S-AD was calculated to be 14.5 MJ/kg VS; in particular, H_2 production in the first stage accounts for 5 % (0.7 MJ/kg VS) of the total energy generated, while the contribution of CH_4 production during the second stage contributed is as high as 13.8 MJ/kg VS. Both values are within the range reported by Schievano et al. (2014) for 2S-AD of fruit/vegetable waste.

As for the 1S-AD test, the methane production corresponded to a SER of 11.6 MJ/kg VS, 20 % less than the overall SER attained with 2S-AD test, as expected, and also even lower than that associated to the second stage of the 2S-AD test.

These results clearly show that adopting the two-stage configuration for the AD process results in a 20 % comparatively higher energy recovery yield, which is mainly ascribed to the improved digestion conditions induced in the methanogenic stage.

4. CONCLUSIONS

One- and two-stage AD of FW aimed at recovering CH_4 and H_2 + CH_4, respectively, were compared in order to assess the benefits associated with the two-stage approach in terms of overall energy recovery. The results obtained suggest that a two-stage process where the first reactor is properly operated in order to achieve a significant net H_2 production, may display a 20 % comparatively higher energy recovery yield as a result, mainly, of enhanced methane, as well as of the associated hydrogen production.

The highest CH_4 production of the two-stage process, observed despite the H_2 recovered is a potential substrate for methanogenesis, was due to improved hydrolysis and fermentation of FW

with increased amounts of volatile fatty acids being readily available to methanogenesis. This figure, if on one hand resulted in a slight inhibition effect on the methanogens, as revealed by the slower methanogenic kinetics and the longer lag phase duration compared to the 1S-AD test, nevertheless allowed to achieve a higher SMP over a HRT of suitable duration.

Although not directly assessed in the present study and thus requiring further specific quantification, additional advantages of the two-stage configuration in terms of the overall environmental profile of the investigated process may also be anticipated. In particular, the 25 % increase in VS removal achieved in the 2S-AD system (66.7 % VS removal vs. 53.3 %) also implies a higher degree of digestate stabilization, which may represent a relevant indirect effect when the subsequent treatment requirements and the final destination of digestate are concerned. Potential indirect outcomes on the carbon footprint of the 2S-AD process are also expected. These are mainly related to the avoided CO_2 emissions deriving from biogas energy use, to the absence of CO_2 in the emissions generated by H_2 combustion, to the reduced energy demand of the digestate treatment units as well as to the reduced use of synthetic soil amending agents (if digestate is to be used for agronomic purposes). As mentioned above, a specific quantification of all such effects requires a dedicate study to account the positive and negative, direct and indirect CO_2 burdens of the investigated process, which was beyond the scopes of the present work.

At the moment, there are not real-scale plants for the fermentative production of hydrogen from biodegradable residues. It is our opinion that a combined process which, besides allowing the recovery of hydrogen, also produces more methane than a one-stage one may boost the interest of technicians and companies in the fermentative production of hydrogen.

Moreover, the production of methane from biodegradable waste, and even more that of hydrogen, are processes naturally included in the biorefinery concept, which is currently regarded as a means to thoroughly apply the principles of circular economy in the management of organic residues.

However, one of the major concerns that cast shadows on a possible implementation of the waste

biorefinery concept is linked to the required plant size, considered by many to be very high and not compatible, for acceptable waste transport distances, with the European scenario. In Europe, therefore, simple biorefinery process schemes, such as the combined production of hydrogen and methane, would be more suitable, at least in the short-medium term.

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560

***Highlights**

- One- and two-stage anaerobic digestion of food waste were compared
- The first stage of the two-stage process was properly operated to allow H_2 recovery
- The two-stage process was characterised by CH_4 production higher than the one-stage
- The highest CH_4 production was due to improved fermentation in the first stage
- The two-stage process displayed a 20% comparatively higher energy recovery yield

Figure captions

Figure 1. 1S-AD test: evolution over time of (a) specific CH_4 production (SMP; solid line indicates Gompertz-model curve) and CH_4 content in the gas produced, (b) VFAs concentration.

Figure 2. 2S-AD test, first stage: evolution over time of (a) specific H_2 production (SHP; solid line indicates Gompertz-model curve) and H_2 content in the gas produced, (b) VFAs concentration.

Figure 3. 2S-AD test, second stage: evolution over time of (a) specific CH_4 production (SMP; solid line indicates Gompertz-model curve) and CH_4 content in the gas produced, (b) VFAs concentration.

Figure 1

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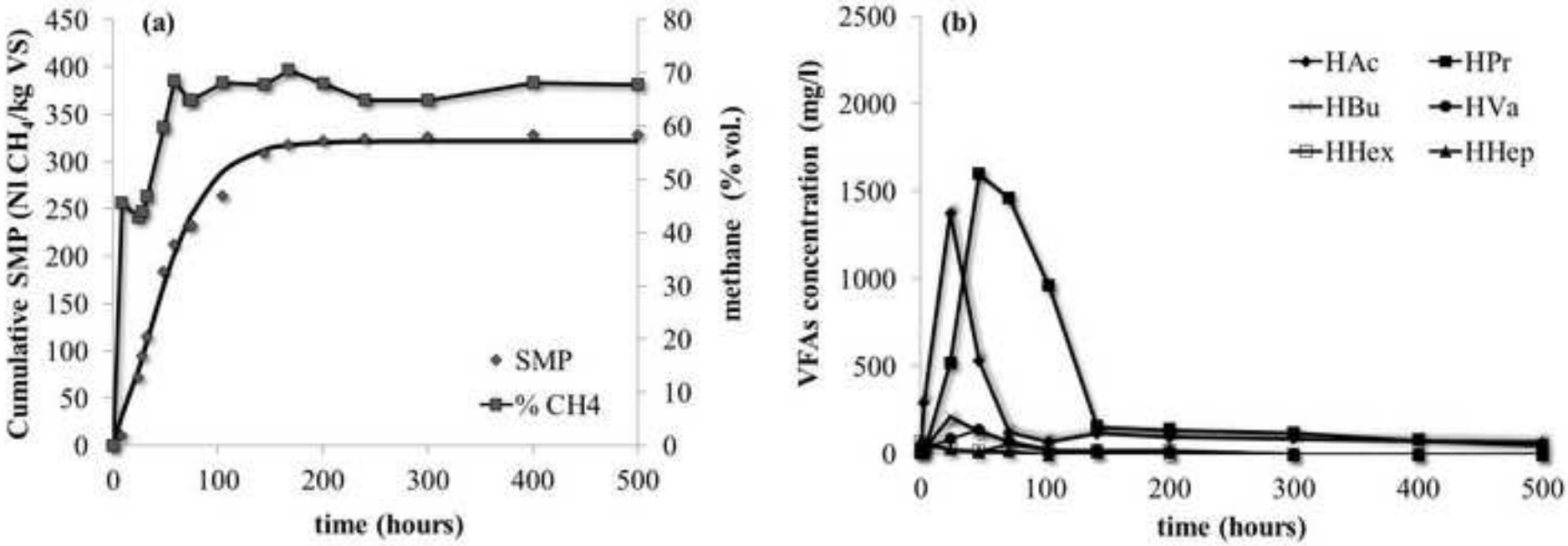

Figure 2

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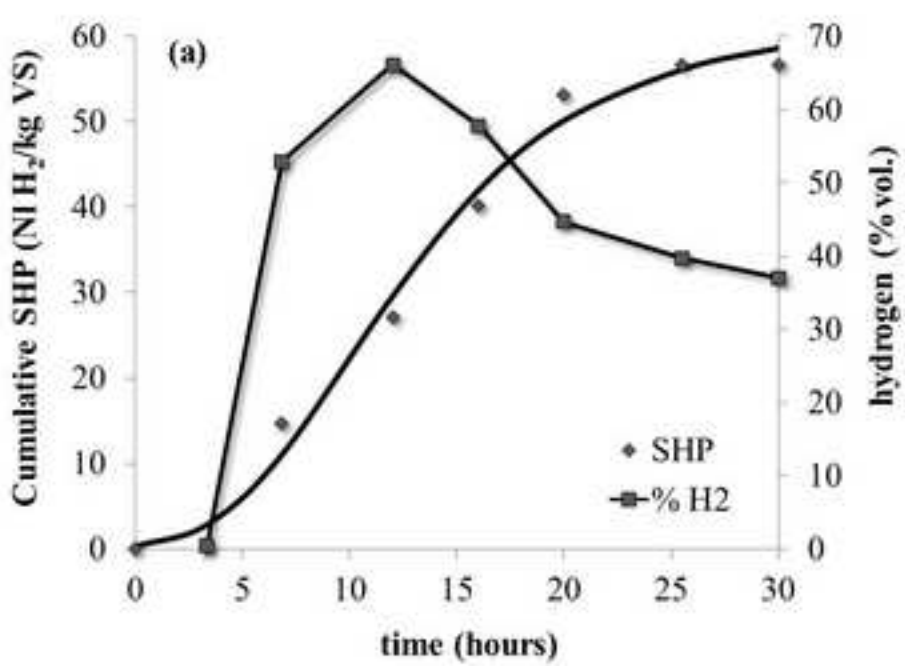

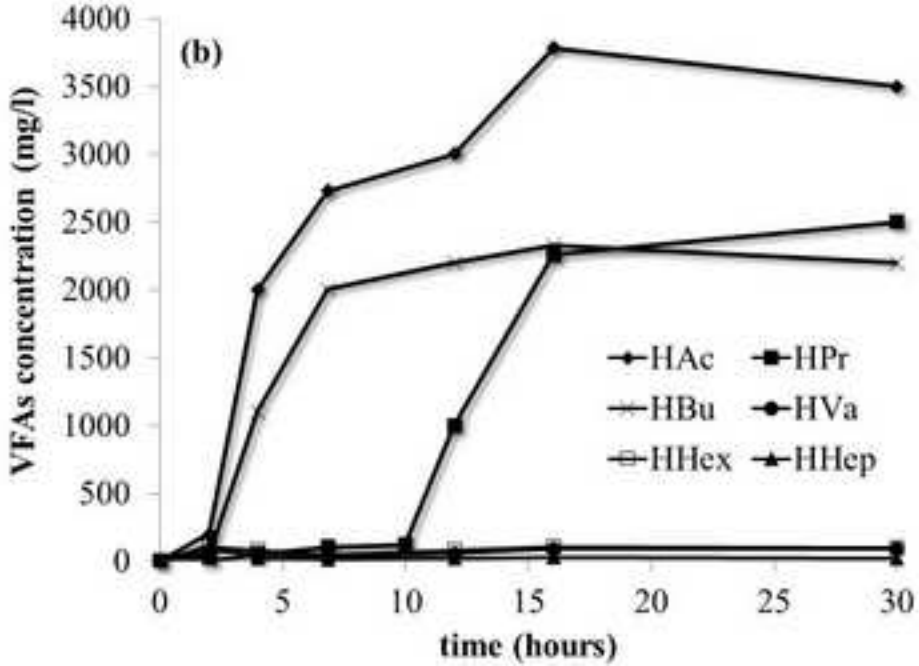

Figure 3

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