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Two-stage anaerobic digestion of the organic fraction of municipal solid waste – Effects of process conditions during batch tests

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Abstract

Two-stage anaerobic digestion (AD) batch tests were performed using the organic fraction of municipal solid waste as substrate. Effects of different combination of initial pH (5.5, 7, and 9) and food to microorganism (F/M) ratio (from 0.5 to 6 gVS/gVS) were investigated for hydrogen and methane productions during the first and the second stage of AD, respectively.

Results showed that both initial pH and F/M ratio had an impact on hydrogen yield, hydrogen production rate and duration of lag phase. The highest hydrogen yield of 29.8 mLH_2/gVS was obtained at initial pH of 5.5 and F/M ratio of 6. However, the highest hydrogen production rate (65 mLH_2/gVS/d) was recorded at pH of 9 and F/M ratio of 6. Increasing the initial pH from 5.5 to 9, led to shorter lag phases for all F/M ratios. Methane production from second phase was not significantly influenced by the F/M ratios tested in the first digestion phase. When compared to single-phase AD, two-stage AD tests resulted in enhanced methane production rates from 37.3 to 68.5 $mLCH_4$/gVS/d, reducing by half both the lag phase and the time required to reach maximum methane production.

Keywords: Organic waste; Dark fermentation; Anaerobic digestion; Hydrogen; Methane; Biogas

1. Introduction

Two-stage anaerobic digestion process has recently been suggested as an option to maximize the amount of energy recoverable from biodegradable organic waste in terms of hydrogen (H_2) and methane (CH_4) [1–3]. H_2 is a clean energy carrier and has a high-energy density. H_2 has, in fact, the highest calorific value among other fuels and its combustion does not lead to carbon emissions. H_2 can be produced from cheap organic wastes and wastewaters in a process called dark fermentation [3,4]. Biomethane can play a central role in the development of the circular economy principle. It is a source of energy that can be used for power and heat production but also as a gaseous vehicle fuel, it can replace natural gas and be fed into national gas grids or be used as a feedstock for producing chemicals and materials [5].

Pre-treatments are often applied to enhance biogas productivity of substrates [6,7] and fermentation step for H_2 production itself could be seen as a pre-treatment to increase overall biodegradability. During the fermentation stage of AD, organic substances are hydrolysed and converted to H_2 and volatile fatty acids (VFAs) by hydrogen producing bacteria. Optimisation of the H_2 production phase can lead to an improved hydrolysis and therefore higher energetic exploitation of waste materials.

The advantages traditionally indicated for two-stage digestion systems, if compared to single stage AD, are shorter substrate retention time, enhanced solids degradation efficiencies [8–10], enhanced hydrolysis with a subsequently higher CH_4 production [11–13] and potentially higher organic loading rates [14]. Despite these advantages, the higher complexity of two stage digestion plants, if compared to single digestion, limited the diffusion of this option to less than 10% of current digestion capacity [15].

The possibility of simultaneous H_2 and CH_4 productions from the same feedstock, rather renovates the interest of this kind of plant configuration and this option is currently receiving growing interest with several investigations at lab and pilot scale level [16–18]. Besides reaching higher energy yields, two-stage AD promotes a stronger bio-stabilisation of the treated organic waste [19,20] and could

also lead to the production of metabolites to be used as renewable and biodegradable substitutes for petrochemical products [21,22].

The main variables influencing both single and two-stage AD performances are substrate C/N ratio, reactors retention time, inoculum, pH, and food to microorganism ratio (F/M). Optimal substrate C/N ratio for single stage AD was found to be between 15 and 30 while substrates with C/N ratios lower than 10 should be treated only in a two-stage AD process [23]. Substrate retention time is generally short during the H_2 production phase (20 hours to 4 days) to avoid the risk of methanogenic activity even though excessively short retention times may be detrimental for substrates characterised by slow hydrolysis rates [24,25]. In contrast, a longer retention time is needed for CH_4 production (20-30 days) in order to reach complete substrate degradation and enhanced digestate stabilization [26,27]. Pure or mixed microflora cultures can be used for H_2 production from single or two-stage digestion. Generally, H_2 yields are higher when pure cultures are specifically chosen accordingly to the fermented substrate while mixed cultures could show better adaptability towards environmental stress, nutrients availability and process conditions [28,29].

Notwithstanding, there is still the need to define optimal operational parameters and procedures to promote the successful succession of the two phases without compromising operational condition for the methanogenic stage. In particular, there is a considerable lack of comprehensive studies relating to the effects produced by initial operational parameters of fermentation on the second phase of the process.

Data on H_2 and CH_4 production yields reported in scientific studies on two-stage AD process are illustrated in Table 1. Results indicate that generally there is a good energy recovery potential from the treatment of organic waste suggesting that efforts in assessing and proving the advantages of different operational conditions as well as of the energy recovery potential can stimulate the application of two-stage AD and diffuse the production of renewable H_2 and CH_4 from organic residues.

The aim of this study was to investigate the effect of F/M ratio and initial pH on hydrogen and methane productions in a two-stage anaerobic digestion process using organic fraction of municipal solid waste (OFMSW) as substrate.

2. Materials and Methods

2.1 Substrate and inoculum

OFMSW samples were collected from the waste receiving area of an anaerobic digestion plant treating organic waste located in Padova, Italy. The OFMSW delivered at the plant is source segregated at household level and the collection area involves a population of about 130,000 inhabitants. Samples were properly sorted and stored before use [30]. Samples were chopped with a food grinder and diluted with water at a ratio 1:2 (kg/L) prior to use as substrate lab scale tests. Granular sludge collected from a full-scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery located in Padova, was used as inoculum (mixed culture). OFMWS and sludge samples were characterized for the following parameters: Total Solids (TS), Volatile Solids (VS), Total Carbon (TC), Total Kjeldahl Nitrogen (TKN), and Chemical Oxygen Demand (COD) (Table 2**)**.

2.2 Two-stage digestion batch tests

Experimental design was planned in order to study the combination effects of each investigated initial pH and F/M ratio on two-stage AD process (Table 3). The following conditions F/M and pH were tested during the first stage of digestion for H_2 production. F/M ratios were 0.5, 1, 2, 4, and 6 gVS/gVS. Initial pH values were 5.5, 7, and 9.

Two-stage digestion batch tests were carried out using 1L glass bottles sealed with a silicon plug and a working volume of 500 mL. Different F/M ratios were achieved changing the amount of inoculum in each test while substrate concentration was kept constant at 5gVS/L. MES ($C_6H_{13}NO_4S$) was used to obtain an initial pH of 5.5, while (sodium carbonate, Na_2CO_3) was used to reach an initial pH value of 9. No buffer was used for the initial tests at neutral pH (7.0).

For first AD stage (fermentation tests), the inoculum was thermally pre-treated for 4 hours at 100 °C to inhibit methanogenic archaea and to enhance the activity of hydrogen producing bacteria in the mixed culture [31]. For the second AD stage, the same amount of sludge was added in each bottle in order to obtain the same F/M ratio which was determined by dividing the original F by the new M. To promote CH_4 production during the second AD stage (methane production), F/M ratio was fixed at 0.5 gVS/gVS, while initial pH was set at 8.5 by dosing Na_2CO_3.

A single-stage AD test (methane production only), characterized by a F/M ratio equal to 0.5 and a pH of 8.5, was run in parallel in order to compare methane production yields with hydrogen and methane yields obtained through the two-step process.

The bottles were flushed with N_2 gas for 3 minutes to ensure anaerobic conditions and incubated at a temperature of 35±1ºC. Incubation lasted 45 days for two-stage AD tests and 60 days for single-stage ones. All tests were performed in duplicate.

2.3 Analytical Methods

TS, VS, COD, TKN and alkalinity were analysed according to standard methods [32]. TC and DC were analysed by a TOC analyser (TOC-V CSN, Shimadzu). The volume of biogas produced during two-phase digestion tests was measured by means of the water displacement method [33]. H_2, CO_2, and CH_4 concentrations in biogas were measured by a gas chromatograph (HP5890) equipped with thermal conductivity detector (TCD), HP-MOLSIV and HP-PLOT U columns, and nitrogen as carrier gas.

H_2 and CH_4 volumes produced in the time interval between each measurement [t – (t-1)] were calculated using a model taking into consideration the gas concentration at time t and time t-1, together with the total volume of biogas produced at time t, the concentration of specific gas at times t and t-1, and the volume of head space of reactors [34]. The following equation was applied:

$$V_{C,t} = C_{C,t} * V_{G,t} + V_H * (C_{C,t} - C_{C,t-1}) \quad (1)$$

Where $V_{C,t}$ – hydrogen or methane volume produced in the interval between t and t - 1; $C_{C,t}$, $C_{C,t-1}$ – hydrogen or methane concentrations measured at times t and t-1; $V_{G,t}$ – volume of gas produced between time t and t-1; V_H – volume of the headspace of reactors.

To compare the results obtained from the batch tests, data were interpolated on the basis of the Gompertz model [35]. The Gompertz mathematical expression is described in Equation (2):

$$P(t) = P_{max}\, e\left\{-e\left[\frac{R * e}{P_{max}}\right](\lambda - t) + 1\right\} \qquad (2)$$

Where P (t) is the cumulated H_2 or CH_4 production at time t; P_{max} is the maximum H_2 or CH_4 production; R is the maximum production rate; and λ is the lag phase. The results related to production rate (R) and duration of the lag phase (λ) were applied to compare the different investigated operative conditions.

Data on H_2 and CH_4 productions are expressed at a temperature of 0°C and pressure of 1 atm (Normal conditions).

3. Results and Discussion

3.1. Effect of F/M ratio and initial pH during the first AD stage – fermentation.

Hydrogen production yields obtained during the first stage (fermentation) are shown in Table 4. Hydrogen yields were slightly lower than the values reported in the literature for similar substrates (Table 1). This could be due either to a decreased hydrolytic activity after the long anaerobic sludge pre-treatment or to the specific substrate composition used in this study. A decreased hydrolytic activity could be a side effect of the heating process at 100 °C for 4 hours. Shah *et al.* [36] assessed the viability of isolates from granular sludge after a pre-treatment similar to the one applied in this study (2h and 4h at 100 °C) and observed that isolates still active after the heat shock exhibited a broad range of hydrolytic activities. It is, therefore, presumable that the slightly lower H_2 yields

compared to those from similar studies could be due to the specific OFMSW composition. It was observed that yields of H_2 production from OFMSW collected in different seasons varied during the year due to changes in the OFMSW composition [30]. Various studies also confirmed that carbohydrate content of organic wastes directly affects H_2 production suggesting that a lack of fractions rich in sugars or starch could reduce the H_2 productions via biological fermentation [3,37,38].

In general, two days were enough to complete hydrogen production but a total of four days was waited to ascertain the plateau and no methane production was observed during the fermentation tests, indicating that the sludge pre-treatment was effective in inhibiting methanogens. Moreover, none of the conditions tested during first phase, in terms of F/M ratios and pH, favoured the reactivation of methanogens even after the fermentation stopped (data not shown).

Results of the data modelling with Gompertz equation (2) are reported in Table 4 and plots of H_2 yields vs. F/M ratios and of lag phase duration (λ) vs. initial pH are shown in Fig. 1a and Fig. 1b, respectively. The additional parameter, t_{95}, defined as the time required for H_2 production to attain 95% of the total cumulative yield [24], was also calculated and reported in Table 4. The highest H_2 yield of 29.8 mLH_2/gVS was recorded from test E, characterised by a F/M ratio of 6 and an initial pH of 5.5. This test was also characterised by a lag phase of 14.9 h and a maximum production rate of 40.3 $mLH_2/gVS/d$. The lowest H_2 yield was recorded from test F, characterised by a F/M ratio of 0.5 and an initial pH of 7.0 (Table 1). The H_2 production rate for this test was also the lowest (12.9 $mLH_2/gVS/d$). Whilst the low yield and production rate, the lag phase for this test was shorter than that for test E.

In general, high H_2 yields were observed from tests with F/M ratios of 4 and 6 gVS/gVS (Fig. 1a) and short lag phases were observed for tests with an initial pH of 7 or 9 (Fig. 1b). Production rates (R) seemed not to be influenced neither by F/M ratio nor by initial pH, even though faster production rates (R) are generally associated with higher production yields (P_{max}).

In the present study, different F/M ratios were obtained by changing the sludge concentration in the reactors while substrate concentration was kept constant. Despite the presumable larger presence of hydrogen producing bacteria at lower F/M ratios, this condition did not lead to higher H_2 yields. A larger variability in bacterial populations present in the mixed microflora with low F/M ratios could have introduced also non-hydrogen forming bacteria competing for the same substrates or hydrogen consuming bacteria and this could have had a measurable impact on hydrogen yields. The higher F/M ratios, on the contrary, were obtained by lower biomass concentrations and this condition could have reduced the possibility of non-hydrogen forming bacteria or hydrogen consuming bacteria to have an effect on hydrogen yields. Alibardi *et al.* [31] indicated that long heat pre-treatments strongly influence microbial viability, with reductions of order of magnitudes of active bacteria levels. Despite this effect, high bacterial concentrations could allow niches of non-hydrogen forming or hydrogen consuming bacteria to grow sufficiently to produce an effect on hydrogen yield. On the contrary, when biomass concentrations are kept low, these niches are not able to influence overall hydrogen productions that are only defined by the activity of fast growing hydrogen producing bacteria. F/M ratio has therefore a direct effect on microbial activities of different populations present in the mixed microflora and to maximise H_2 production, small concentrations are sufficient to obtain efficient hydrogen conversions [31]. Pan *et al.* [39] investigated how F/M ratio affects H_2 production from food waste under mesophilic and thermophilic conditions but with no pre-treatment to enhance hydrogen production. Differently from the approach in the present research study, a constant biomass concentration was used by Pan *et al.* [39] while substrate concentration was changed. Optimal F/M ratios of 6 and 7 were identified under mesophilic and thermophilic conditions, respectively. Low F/M ratios (< 3) led to high methane productions and at high F/M ratios (> 7) low H_2 yields were observed. These results confirm how an optimal balance between biomass and substrate concentrations needs to be identified to enhance hydrogen production and avoid the activity of other bacterial species not contributing or negatively affective hydrogen fermentation.

Initial pH and F/M ratio also influenced hydrogen production rate (R) and lag phase duration (λ) (Table 4). When initial pH was increased from 5.5 to 9, a shorter lag phase was observed for all F/M ratios (Fig. 1b). The longest lag phase (14.9 h - Test E) was observed with F/M ratio and initial pH of 6 and 5.5, respectively. The shortest lag phase (4.8 h - Test O) corresponded to F/M ratio and initial pH of 2 and 7, respectively. These results suggest that a neutral to basic pH could speed up the activity of hydrogen forming bacteria after the heat pre-treatment while an initial acid condition imposes a longer acclimating phase before hydrogen production starts. These results are in accordance with Chen *et al.*, [35] who reported longer lag phases when mixed microflora inoculum was cultivated at pH of 5 (compared to pH 6 and 7) after an enrichment phase at both acid or basic conditions. Similarly, Ferchichi *et al.* [40] reported a significantly long lag phase of 43.26 h with an initial pH of 5 and a short lag phase of 3.06 h when the pH was 8, using cheese whey as substrate. The initial low pH conditions can result in the protonation of weak acids contained in cheese whey, which may pass freely through the cell's membrane into its cytoplasm causing its consequent acidification [41]. This internal condition could result in loss of activity by the glycolytic enzymes and structural damage of the cell membrane that can lead to prolonged re-activation phases after external stresses to the inoculum and, consequently, longer lag phases [40]. The low pH values set by using MES in this study, could have led to a similar effect and produced the observed delay (Table 4).

Operational pH is also one of the key factors in dark fermentative H_2 production. It may affect hydrogenase activity and metabolic pathways towards different by-products generation [42]. In all tests, pH decreased to values between 5.5 and 6 at the end of the fermentation (Table 4). These results indicate that, despite the different pH set at the beginning of the tests, the fermentation products established an acid environment even at high initial pH conditions (pH 9). Optimal initial pH of 5.5-6.0 has been reported by many studies for mesophilic dark fermentation [3,40,43–45]. Low pH (4.5-6) leads to a higher concentration of acetic and butyric acids which are soluble metabolites whose production pathways are accompanied by H_2 production [46]. Moreover, the activities of H_2 consuming microorganisms like methanogens, homoacetogens, and propionic acid bacteria decrease

at low pH conditions [42,47]. This study also demonstrated that high initial pH speeded up the inoculum reactivation with short lag phases. It is, therefore, presumable that an optimal combination of initial pH and operational pH during the fermentation process, could enhance the overall hydrogen production by combining short lag phases with high hydrogen yields. Further studies are anyway required to confirm or rebut this hypothesis.

3.2 Effect of F/M ratio and initial pH during the second AD stage – methane production.

Methane production yields during the second AD stage are reported in Table 5. The highest methane production of 620 $mLCH_4/gVS$ was obtained from test F, while the lowest was measured from test D (463 $mLCH_4/gVS$). The average methane production of 544 $mLCH_4/gVS$ was obtained from all the tests at various F/M ratios and initial pH conditions. The maximum methane production from test R, carried out in a single digestion phase for methane production, resulted 633 $mLCH_4/gVS$. The lower methane yields obtained from the double digestion process could be explained by the fact that hydrogen was produced in the first digestion phase. A portion of the total electrons released by the biodegradation process was already passed to H_2 and therefore a reduction of the total methane production from the second phase could be expected. Notwithstanding, the additional amounts of methane producible according to stoichiometry ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) are only 7.45 and 1.75 $mLCH_4/gVS$ from the maximum and minimum H_2 production yields, respectively.

The outputs from the second digestion phase (Tests A to Q) and the single digestion process (Test R) displayed a similar pattern although the lag phase for single phase digestion was longer and time required to reach maximum methane production was almost doubled (Table 6). Indeed, for two-stage AD, hydrolysis and acidogenesis occur during the first stage resulting in enhanced VFAs production which can be converted to CH_4 rapidly during the second stage [12,13,48]. The optimal conditions for hydrolytic bacteria and methanogenic archaea may be different and splitting the process into two phases, provides the opportunity for the specific optimization of each phase. Differently, single-stage AD, for which hydrolysis is the rate limiting process, combines hydrolysis, acidogenesis and

methanogenesis with a consequently longer lag phase than that of the second stage of a two-stage AD.

Average maximum methane productions (Fig. 2) decreased in line with an increase of the F/M ratio (applied in the first stage) up to 4. However, an opposite trend was observed when passing from a F/M ratio of 4 to 6, displaying a pattern similar to that observed for H_2 production during the first AD stage (Fig. 1).

Comparing trends in Fig. 1a and Fig. 2, it can be highlighted that lower hydrogen productions are associated with higher methane yields for all F/M ratios. In particular, test F, characterised by a F/M ratio of 0.5 and pH of 7, produced the lowest amount of hydrogen (7 mLH_2/gVS) and the highest amount of methane (619 $mLCH_4/gVS$). In accordance with Schievano *et al.* [49], single-stage Biochemical Methane Potential (BMP) outputs featured higher methane yields than those achieved from a two-stage AD process, although with a longer lag phase and lower maximum production rate. The slightly lower CH_4 yields obtained for two-stage AD could be likely due to the previous recovery of H_2 which is also a substrate for methane production. In fact, in a single-stage AD, CH_4 could be obtained both from VFAs conversion by acetoclastic methanogens and by H_2 and CO_2 conversion by hydrogenotrophic methanogenic archaea. In contrast to our results, Voelklein *et al.* [48] reported a 23% increase in methane production from a two-stage AD of restaurant food waste rather than a single-stage process. Likewise, Liu *et al.* [13] recovered 21% more methane from two-stage AD tests performed on mixed organic waste.

The final pH of two-stage AD process ranged between 7.5 and 8, while for single stage AD process final pH resulted 7.0 (Table 5). For both the methane production phase of the two-stage AD and for single AD, initial pH was set at a value of 8.5. The slightly lower final pH observed for single AD could suggest that a higher buffer capacity is required for systems where all phases of digestion are carried out in one single reactor. On the contrary, for two-stage AD a lower buffer capacity is required as acidic fermentation residues from first stage are rapidly converted to CH_4. Notwithstanding, the results suggest that process condition and fermentation activity during first stage have an impact on

second stage performance. Tests performed at F/M ratios of 4 and 6 (D, E, I, L, P and Q) were in fact always related to the lowest final pH value, and therefore a higher buffer capacity, probably because of the higher biological metabolites production favoured by high F/M ratios [50].

The first AD stage, aimed at hydrogen production, may also be viewed as an effective pre-treatment for the subsequent production of methane, providing a VFA-rich substrate ready to be digested by methanogenic archaea. The average CH_4 yield from two-stage AD (544 $mLCH_4/gVS$) was lower than the one from single-stage AD (633 $mLCH_4/gVS$) (Table 5 and Table 6). However, if similar conditions were considered (F/M= 0.5), approximately equal yields were obtained from single-stage and two-stage AD (626.1 and 619 $mLCH_4/gVS$, respectively). Methane production rate (R) doubled (Table 6), whilst both lag phase and time required to reach the maximum methane production were reduced by half when passing from single-stage to two-stage AD process. These findings are in accordance with Leite *et al.* [51] who achieved a 15% increase of produced energy from single-stage to two-stage AD system. In fact, when splitting the AD process into two-stages, the first stage may be regarded as a pre-treatment to increase the methane production rate and to shorten the lag phase, as confirmed by the results reported in the present paper. The faster production rate, accompanied by a shorter lag phase, proves a significant overall benefit of two-stage over single-stage AD. It is important to highlight that the maximum methane productions during the two-stage processes were reached after 20 days of incubation; on the contrary, for the single-stage test, the maximum methane production (626.1 $mLCH_4/gVS$) was reached after about 40 days. By comparing the potential energy output of the two processes, it is possible to highlight how a double phase digestion process could be energetically more favourable if compared to a single-phase digestion when the time for digestion (i.e. digester volume or solid retention time) is fixed at 22 days. In the single-stage AD test the cumulative methane production registered after 22 days of incubation was 366.2 $mLCH_4/gVS$. Considering a period of 2 and 20 days for hydrogen and methane productions, respectively, for a two-stage digestion, and of 22 days in the case of the single-stage process, the potential energy output for produced fuel gases is reported in Fig. 3. These choices were made on the basis of the average time

required to reach maximum hydrogen and methane productions in the two-stage process. According to Fig. 3, all two-stage tests were energetically more favourable than single-stage tests. These results confirm that the implementation of a two-stage digestion processes for sequential H_2 and CH_4 production from OFMSW could enhance methanogenic phase performances and increase the overall potential energy production thank to faster digestion processes.

4. Conclusion

The present study investigated the effects of two parameters, initial pH and food to microorganism ratio, on hydrogen and methane productions obtained from the organic fraction of municipal solid waste in a two-stage AD process. Data analysis revealed how a variation in initial pH value influenced substrate degradation kinetics and total hydrogen production. Kinetics were favoured by initial alkaline conditions (pH = 9) linked to faster production rates and shorter lag phase. High F/M ratios were found to facilitate hydrogen production, with the most favourable condition being identified at a F/M ratio of 6. Peak methane production (619 $mLCH_4$/gVS) recorded during the second AD stage of BMP test characterized by a F/M ratio of 0.5 and an initial pH of 7, was close to the value of 633 $mLCH_4$/gVS obtained during the single-stage process. There was no evident relationship between initial pH values during fermentation and methane production, probably due to pH adjustment performed on completion of fermentation tests, while an increase in F/M ratio from 0.5 to 4 resulted in a slight decrease in methane production. The fermentation phase, in addition to promoting hydrogen recovery, represents an efficient means of pre-treatment aimed at enhancing subsequent methane production. In comparison with the single-stage AD process, a two-stage process elicits faster methane production, a shorter lag phase, and a better energetic exploitation of OFMWS, as demonstrated by the achieved energy output.

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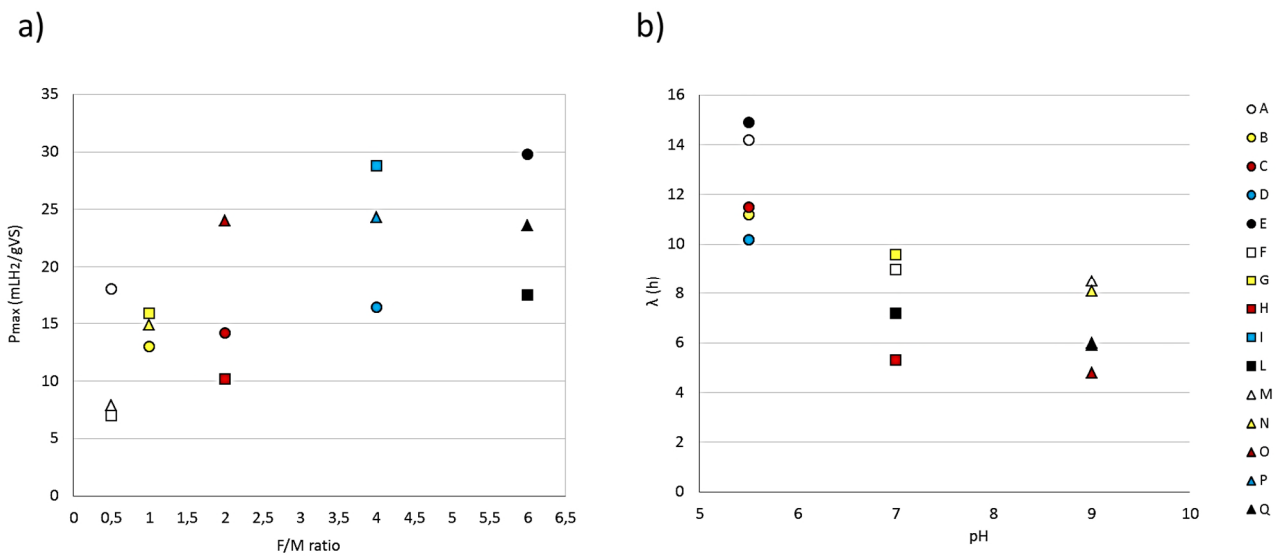

Fig. 1. Distribution of maximum hydrogen (H_2) production (P_{max}) over F/M ratio (a) and lag phase (λ) over initial pH (b).

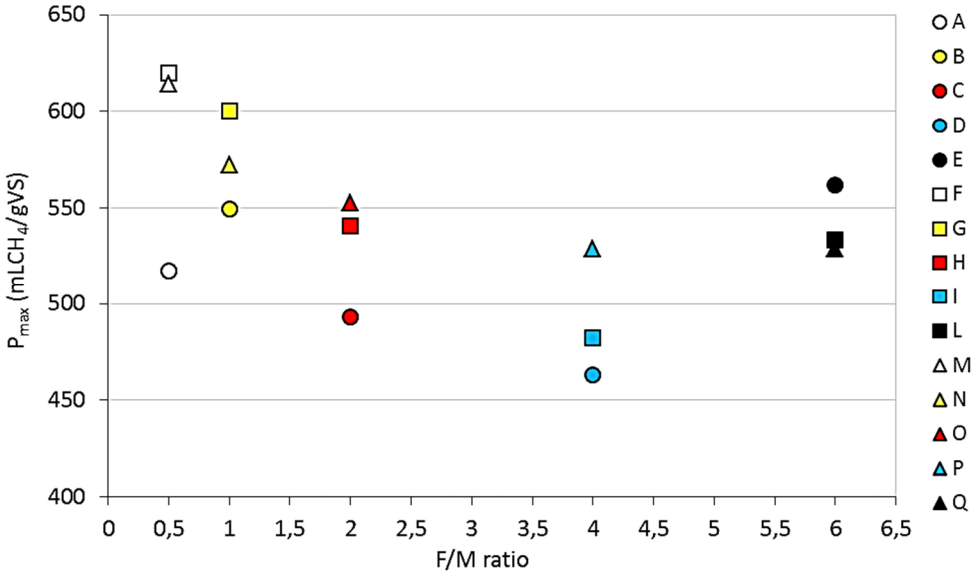

Fig. 2. Distribution of maximum methane (CH_4) production (P_{max}) obtained from the second phase over F/M ratios tested during the first phase.

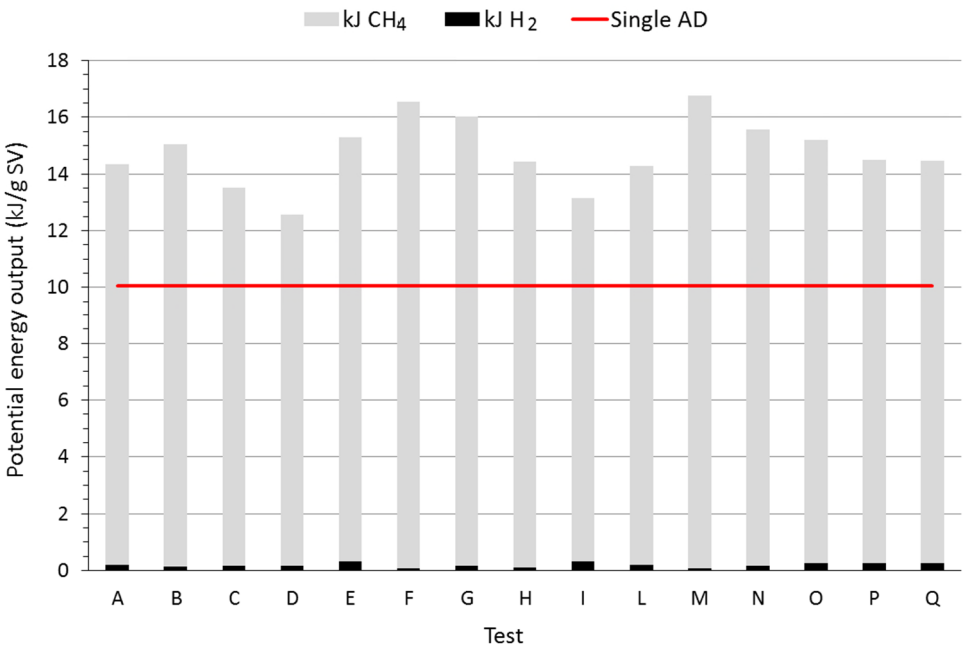

Fig. 3. Potential energy output from single-stage and two-stage tests after 22 days of digestion (2 days first stage, 20 days second stage). H_2 energy density = 120 MJ/kg – CH_4 energy density = 50 MJ/kg. (Single-stage AD yielded 366.2 mLCH_4/gVS after 22 days of digestion).

Table 1. Comparison of hydrogen and methane production yields in a two-stage AD process using different organic substrates.

Substrate	**Hydrogen potential production (mLH_2/gVS)**	**Methane potential production ($mLCH_4/gVS$)**	**Reference**
Dairy processing waste	40.15	34.2	[52]
Kitchen waste	36	135	[53]
OFMSW	43	500	[13]
OFMSW	90	560	[54]
Potato residues	31	387	[55]
Steam-peeling potato waste	134*	183*	[56]
Common wheat waste	47*	202*	[56]
Vinegar residue	53.2	192	[57]

* mL/gCOD

Table 2. Average substrate and inoculum characteristics.

Parameter	OFMSW	Granular sludge
TS (%)	75	15
VS (%TS)	90	53
TC (%TS)	50.2	29.6
TKN (gN/kgTS)	8.7	43
C/N (gC/gN)	58	7
COD (gO_2/kgTS)	300	693

Table 3. Initial operational conditions of two-stage and single stage batch tests.

Run	First stage, fermentation		Second stage, methanization	
	F/M (gVS/gVS)	Initial pH	F/M (gVS/gVS)	Initial pH
A	0.5	5.5	0.5	8.5
B	1.0	5.5	0.5	8.5
C	2.0	5.5	0.5	8.5
D	4.0	5.5	0.5	8.5
E	6.0	5.5	0.5	8.5
F	0.5	7.0	0.5	8.5
G	1.0	7.0	0.5	8.5
H	2.0	7.0	0.5	8.5
I	4.0	7.0	0.5	8.5
L	6.0	7.0	0.5	8.5
M	0.5	9.0	0.5	8.5
N	1.0	9.0	0.5	8.5
O	2.0	9.0	0.5	8.5
P	4.0	9.0	0.5	8.5
Q	6.0	9.0	0.5	8.5
	Single stage, methane production			
	F/M (gVS/gVS)		Initial pH	
R	0.5		8.5	

Table 4. Hydrogen production yields (average values), final pH at the end of the first stage of AD batch tests and results of the data modelling with Gompertz equation (2).

Run	First stage, fermentation		Modelling results			
	Hydrogen production (mLH_2/gVS)	Final pH	R ($mLH_2/gVS/d$)	λ (h)	P_{max} (mLH_2/gVS)	t_{95} (d)
A	18.0	6.0	55.5	14.2	18.0	1.1
B	13.0	6.0	20.6	11.2	13.0	1.4
C	14.2	6.0	25.3	11.5	14.2	1.3
D	16.4	6.0	19.4	10.2	16.4	1.7
E	29.8	5.5	40.3	14.9	29.8	1.7
F	7.0	6.0	12.9	9.0	7.0	1.2
G	15.9	5.0	50.0	9.6	15.9	0.9
H	10.2	5.0	18.0	5.3	10.2	1.0
I	28.2	5.0	54.2	7.2	28.8	1.1
L	17.5	5.0	64.8	7.2	17.5	0.7
M	7.9	6.5	27.3	8.5	7.9	0.8
N	14.9	5.5	55.3	8.1	14.9	0.7
O	24.0	5.0	60.0	4.8	24.0	0.8
P	24.3	5.0	64.8	6.0	24.3	0.8
Q	23.6	5.0	65.0	5.9	23.6	0.8

Table 5. Methane production yields, final pH at the end of the second stage of AD batch tests and results of the data modelling with Gompertz equation (2).

Run	Second stage, methane production		Modelling results			
	Methane production ($mLCH_4/gVS$)	Final pH	R ($mLCH_4/gVS/d$)	λ (d)	P_{max} ($mLCH_4/gVS$)	t_{95} (d)
A	527	8	101.4	7.9	517	15.3
B	550	8	76.4	5.4	549	15.9
C	499	7.5	68.0	5.4	493	16.0
D	489	7.5	52.8	5.0	463	17.8
E	582	7.5	62.5	5.1	562	18.2
F	619	8	67.4	4.9	620	18.3
G	590	8	62.1	4.8	600	18.9
H	532	8	56.6	4.7	541	18.7
I	474	7.5	50.1	4.2	482	18.3
L	523	7.5	53.1	4.2	534	18.9
M	606	8	94.9	6.8	614	16.2
N	566	8	73.2	5.9	573	17.3
O	554	8	75.2	5.8	553	16.5
P	532	7.5	67.8	5.9	529	17.3
Q	529	7.5	66.6	5.6	529	17.2
	Single stage, methane production		**Modelling results**			
	Methane production ($mLCH_4/gVS$)	Final pH	R ($mLCH_4/gVS/d$)	λ (d)	P_{max} ($mLCH_4/gVS$)	t_{95} (d)
R	626.1	7.0	37.3	12.0	633	36.8

Table 6. Comparison of Gompertz equation modelling results from single-stage and two-stage AD processes (average values between all tests). R - methane production rate, λ - lag phase, and t_{max} - time needed for maximum methane production.

	P_{max} ($mLCH_4/gVS$)	**R ($mLCH_4/gVS/d$)**	**λ (d)**	**t_{max} (d)**
Single-stage AD	633	37.3	12.0	40
Two-stage AD	544	68.5	5.4	20