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Title

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[1]

[1] Abbreviations: AD: Anaerobic Digestion; TOC: Total Organic Content; VS: Volatile Solids; MSW: Municipal Solid Waste; OFMSW: Organic Fraction of Municipal Solid Waste; VOAs: Volatile Organic Acids; LOI: Loss On Ignition

Abstract

Biohydrogen and biomethane production offers many advantages for environmental protection over the fossil fuels or the existing physical-chemical methods for hydrogen and methane synthesis.

The aim of this study is focused on the exploitation of several samples from the composting process: 1) a mixture of waste vegetable materials ("*Mix*"); 2) an unmatured compost sample (ACV15); 3) three types of green compost with different properties and soil improver quality (ACV1, ACV2 and ACV3). These samples were tested for biohydrogen and biomethane production, thus obtaining second generation biofuels and resulting in a novel possibility to manage renewable waste biomasses.

The ability of these substrates as original feed during dark fermentation was assayed anaerobically in batch, in glass bottles, in order to determine the optimal operating conditions for hydrogen and/or methane production using "*Mix*" or ACV1, ACV2 or ACV3 green compost and a limited amount of water.

Hydrogen could be produced with a fast kinetic in the range 0.02-2.45 mL H_2 g^{-1} VS, while methane was produced with a slower kinetic in the range 0.5-8 mL CH_4 g^{-1} VS. It was observed that the composition of each sample influenced significantly the gas production. It was also observed that the addition of different amounts of water plays a crucial role in the development of hydrogen or methane. This parameter can be used to push towards the alternative production of one or another gas.

Hydrogen and methane production was detected spontaneously from these matrices, without additional sources of nutrients or any pre-treatment, suggesting that they can be used as an additional inoculum or feed into single or two-stage plants. This might allow the use of compost with low quality as soil improver for alternative and further applications.

Keywords:

Compost; anaerobic digestion; waste; bioenergy; clean fuels.

1. Introduction

The exhaustion of fossil fuels and global warming are strong motivating factors for alternative fuels research. This makes it necessary to find alternative energy sources that are renewable and environment friendly (Powan and Danvirutai, 2014)

Many countries are interested in sustainable renewable energy sources such as geothermal and wind power, small-scale hydropower, solar energy, biomass energy, tidal and wave power (Berndes *et al.,* 2003). Cellulosic biomass is a promising source due to its abundance and low cost (Dongmin and Hongzhang, 2007). Currently, biomass contributes about 12% of the world energy supply, while in many developing countries it contributes 40–50% energy supply. Biomass research is recently receiving increasing attention because of the probable waste-to-energy application (Ni *et al*., 2006). For instance, 150 Gt of vegetable bio-matter generated globally every year can produce about 1.08 x 10^{10} GJ energy (Laminie and Dicks, 2000).

Biomass includes a large variety of materials generated by sunlight, such as agricultural wastes from farming and wood processing or dedicated bioenergy crops. The use of energy crops for fuel production has some drawbacks and there is a concern that they might indirectly cause an increase in the food price thus contributing to the global food crisis (Mei Guo *et al*., 2010) In line with these concerns, the latest amendment to the EU renewable energy directive (EU, 2009) introduces a limit to the contribution made from liquid biofuels produced from food crops, such as those based on cereals and other starch rich, sugars and oil crops (Browne *et al.*, 2013). Therefore the attention has been redirected to the production of second generation biofuels, utilising biomasses derived from the carbonaceous waste of human activities, animal farming and agriculture as renewable natural resources for energy recovery (Muzenda, 2014).

Converting waste biomass into gaseous fuels, electricity and especially hydrogen (H_2) and methane (CH_4) is possibly the most efficient way of biomass utilization and waste management (Ni *et al.*, 2006).

Biohydrogen and biomethane could be produced from a large variety of organic substrates or biomasses by dark fermentation and anaerobic digestion (AD), respectively. The microorganisms transform biodegradable substrates into H_2, biogas and stabilized solid residues (Roati *et al.*, 2012).

Biological H_2 production from organic matter is considered one of the most promising alternatives for sustainable green energy production. Dark fermentative H_2 production is a process in which strict or facultative anaerobic bacteria use organic compounds to produce H_2 in the absence of a light source. This process may have other environmental benefits such as the use of organic waste materials as the raw carbon source (Zu and Beland, 2006). The AD under oxygen-free conditions is most commonly applied to transform the organic matter into biogas. Biogas is a mixture of mostly CH_4 and carbon dioxide (CO_2). The microbial anaerobic conversion to biomethane is a process for both effective waste treatment and sustainable energy production (Wilkie, 2008).

The biohydrogen and biomethane production process could become sustainable in dedicated plants, the best performances being expected in a two-stage reactor plant. In a two-stage digestion, the first step involves loading material into a digestion tank where hydrolysis, acetogenesis and acidogenesis occur and a first stream of hydrogen and CO_2 can be produced and collected. The resulting digestate is then introduced into the methanogenic reactor for biogas/biomethane production. The two-stage process results in fast and efficient formation of biogas in the second stage with CH_4 concentrations up to 85%. Biohydrogen deriving from the first stage can be used directly in combustion engines for transportation or after purification in fuel cells to produce electricity (Kapdan and Kargi,

2006). The biomethane can be used *in situ* in a cogeneration system, it can be sent to national natural gas network or it can be used as a renewable biofuel in the automotive sector (Cucchiella *et al.*, 2015). The combination of the two streams of hydrogen and methane generated in the two-stage plant can also be exploited as biohythane (Liu *et al*., 2013). H_2 and CH_4 production in a two-stage plant can utilise various types of substrates, for example residual materials and agricultural, food, farm and industrial wastes. In these types of plants, in both stages, the use of a source high in anaerobic microbes to start up anaerobic system is called inoculation. The type, quality and quantity of inoculum (or seed) are critical to the performance of the anaerobic digester. The most common seeds are various pure (Li and Chen, 2007; Kvesitadze *et al.*, 2012) or mixed microbial cultures (De Gionnis *et al.*, 2013). The second type seems to be preferred because the system would be cheaper to operate, easier to control and capable of digesting a variety of feedstock materials; some examples are anaerobic sludge from full-scale digesters, granular sludge, waste activated sludge, cattle manure (Guo *et al.*, 2014; Zhang *et al.*, 2007; Fan *et al.*, 2006), and vegetable kitchen waste compost (Ze-Kun *et al.*, 2010).

There are different types of compost: green compost (ACV) is made from tree and yard wastes, crop residues and other wastes of plant origin; brown compost (ACM) is obtained from municipal organic wastes, kitchen and canteen wastes, animal manure.

To date and to our knowledge, no study has yet been devoted to the use of ACV or green compost of low quality to produce H_2 and CH_4 via dark fermentation.

This study examined anaerobic fermentation of three different types of not pre-treated mature green composts (ACV1, ACV2 and ACV3), immature compost in bioxidation phase (ACV15) and raw material mixture of composting process ("*Mix*") by observing alterations in H_2 and CH_4 content utilising different amounts of water. The test was done in small scale and in batch condition and these studies were performed under mesophilic conditions. The research aims at evaluating the possible alternative use of compost, and in particular of

low quality batches unsaleable as soil improvers, as feedstock in industrial plant for H_2 and CH_4 productions.

2. Material and methods

2.1 Waste biomass samples

Waste biomasses at different maturation state from composting process were provided and collected from the company Agri New Tech, located in Torino, Italy.

The composting process is the standard protocol that is commercially used by the company. Briefly, plant biomass was crushed to a maximum size of 10 mm and composting piles were prepared. Piles dimensions were 5 meters large and 3 meters high. Piles were turned once every 7 days for approximately 1-2 months, until temperature rose over 50°C (degradation phase). After that, piles were turned once every 15 days for another 1-3 months until completely mature. The entire process lasted for a minimum of 3 months.

The samples were collected in different periods and analysed immediately after collection (with the exclusion of ACV3): green compost (ACV3) in January 2013; mixture of green wastes (sample defined as "*Mix*") in December 2013; early composted wastes (ACV15) in January 2014 after 15 days from the start of composting process; green composts (ACV1 and ACV2) in February 2014.

The most relevant characteristics of each sample are reported in Table 1. The pH was measured according to the international standard CEN EN 13037:2011. The quality as fertilizers have been defined according to the fulfillment of national standards (D.Lgs. 75/2010) and internal quality standards developed by Agri New Tech.

Mix is the raw material of composting process used in this study; it is a complex matrix made by green wastes including a part of cellulosic material from pruning of trees and a

part of leaves and grass clippings collected in the province of Torino from private and public gardens.

ACV15 is a sample collected during the composting process, 15 days after the starting of composting process (during the bio-oxidation phase). It is a transition biomass.

ACV1, ACV2 and ACV3 are mature composts but with different features.

ACV1 and ACV3 have the same initial composition but ACV3 was stored at 4 °C for 1 year to evaluate the gas production stability of the compost during long period of storage

ACV2 is a mature compost completely different from ACV1 and ACV3 because it is more stable and with a higher fertilising and commercial value.

[Insert Tab.1 here]

The microelements and heavy metals composition of the samples used in this study is reported in the supplementary material.

2.2 Batch experimental set-up

The data reported are the average of two independent samples (biological replicates) analysed at least in triplicate.

In order to minimise contamination and analyse as much as possible the sample in its original characteristics, the experiments were made in sterile conditions into glass vials with a volume of 60 mL and 5 g of sample with the addition of different amounts of sterile deionised water from 10 mL to 1 mL for ACV3, ACV1, ACV15 and ACV2. The experiments with the *Mix* were made in 1 L sterile glass flasks with 80 g of *Mix* and 16 mL of sterile deionised water, because the sample contains large pieces that did not fit smaller vials.. The ratio between water and sample and between reactor size and sample amount of this condition are the same used for compost samples. The samples were collected, the vials

and flasks were capped with butyl rubber stoppers and flushed with argon for 20 min to have anaerobic conditions and incubated at 37 °C at 220 RPM in a Gallenkamp Environmental Shaker Model 10X 400 for 18 days (ACV1, ACV3 and ACV2), for 28 days (ACV15) and for 41 days (*Mix*).

2.3 Analytical methods

The gas was sampled with a SampleLock Gastight syringe (Hamilton) and analysed by gas chromatography. The gas chromatographer (Agilent Technologies 7890A) was equipped with purged packed inlet, HP-Molesieve column (30 m, ID 0.53 mm, film 25 mm) and thermal conductivity detector. Efficient and quantitative separation was achieved in 2.8 min at 60 °C, the gas chromatography method allowed to separate H_2, O_2, N_2, and CH_4 respectively at 1.4, 1.6, 1.9 and 2.4 min.

The gas chromatographer injector temperature was 60 °C and detector temperature was 250 °C. Argon, used as carrying gas, had a flow of 0.879 mL/min.

Quantification of H_2 and CH_4 was obtained by calibration curves prepared from standard gases (Rivoira, Italy).

3. Results

3.1 Hydrogen production from the raw material of composting process

The raw material of composting process used in this study is a complex matrix made by green wastes, here called *Mix*.

The gas production was monitored during 41 days of dark fermentation using the matrix without pre-treatments and with a little addition of water (Fig. 1). It was observed that this matrix is able to release biohydrogen only. The maximum H_2 production was reached after 14 days and it was 1.02±0.03 mL H_2 g^{-1} VS.

[Insert the figure 1 here]

3.2 Hydrogen and methane production from ACV15

ACV15 is a lignocellulosic sample in the bio-oxidation phase and it is a matrix in a transition phase, not mature and non-stabilised.

The analysis was conducted for 28 days under three different conditions, respectively: 10 mL, 3 mL and 1 mL of added water (Fig. 2).

[Insert the figure 2 here]

In all the conditions tested, the H_2 and CH_4 productions were not simultaneous but they were shifted in time.

In the first eight days of analysis only H_2 production was observed with a trend that was similar in the conditions with 10 and 3 mL of water, instead for the condition with 1 mL of water a slower and sustained over time H_2 production was observed. The H_2 maximum value reached was 1.2±0.01 mL g^{-1} VS after 2 days. Only after 17 days of fermentative process in the condition with 10 mL of water a low production of CH_4 started, the CH_4 maximum value observed was 0.5±0.02 mL g^{-1} VS. Instead in the other two conditions CH_4 production was not observable for the entire period of analysis.

3.3 Production of hydrogen and methane by mature compost

Three different green compost samples (ACV1, ACV2 and ACV3) were investigated.

[Insert figure 3 here]

The gas production from ACV1 compost in 3 different conditions was investigated (Fig. 3 panel A). In all cases the curves showed a similar evolution trend; in particular it was possible to see in figure 3 A that the curves in the conditions with 10 mL and 3 mL of H_2O were quantitatively similar, reaching respectively 0.054±0.01 and 0.06±0.01 mL of H_2 g^{-1}

VS after 2 days, then they decreased in the following days until zero after the 4th day. Conversely, the addition of 1 mL of H_2O showed an higher H_2 production both in quantity and in time, releasing a maximum value of 0.20±0.02 mL of H_2 g^{-1} VS after the 3th day, then it slowly decreased in the following days until zero after the 18th day.

The CH_4 production was detectable from the 4th day and it increased gradually in the following days. The best condition was with 10 mL of H_2O in which the maximum amount of CH_4 was 8±0.77 mL g^{-1} VS after 16 days;

Another aim has been the study of the H_2 and CH_4 production from ACV2 in three conditions (Fig. 3 panel B). The production of H_2 for the three different conditions in time was very low and close to zero. The H_2 production started after 4 days and in all the conditions the cumulative curves had the same trend and it becoming null after 16-18 days. The maximum H_2 value was 0.02±0.04 mL g^{-1} VS in the condition with 3 mL of water after 8 days.

The CH_4 production steadily increased over time, the maximum value was 1.8±0.05 mL g^{-1} VS with 3 mL of water after 18 days, but all the values observed were lower than those obtained with the other compost samples analysed.

The gas production from ACV3 compost was analysed in four conditions (Fig. 3 panel C). The maximum H_2 amount was 0.21±0.01 mL g^{-1} VS after 3 days in the condition with 10 mL of water, this value was three times higher than that observed in the other conditions. When 3 or 1 mL of water was added, the maximum values reached were 0.07±0.004 mL g^{-1} VS respectively after 4 and 3 days. In every case, in all of these conditions H_2 production fell to zero after seven days.

The CH_4 production was detectable from the 4th day and it increased exponentially since the 16th day, then the amount of CH_4 decreased. The best condition was with the addition of 3 mL of H_2O: the maximum amount of CH_4 was 5.8±0.31 mL g^{-1} VS after 16 days; the CH_4 production with 1 mL of H_2O was lower than in the conditions with 3 and 10 mL of

water and the amount of CH_4 released with 3 mL of H_2O was higher than with 1 mL of H_2O (Fig.3). In all the conditions the curves showed similar trends, the only exception was in the absence of water, where H_2 or CH_4 production was not observed, this condition was not further investigated for the other samples.

The results obtained here show that water supply is very important for the evolution of H_2 or CH_4 from this kind of matrices: the same biomass sample can produce very different amount of gas on the basis of the water amount added; moreover, the amount of added water can direct the production towards H_2 or CH_4.. This feature is the key point to control the amount and kinetics of H_2 or CH_4 production from this kind of feed matrix.

4. Discussion

The gas production ability of not pre-treated substrates from composting process like the lignocellulosic waste called *Mix*, the immature ACV15 and the three different mature compost samples ACV1, ACV2, ACV3 was tested.

The sample *Mix* was able to produce H_2 only, probably as a result of a specific microbial population intrinsically present in the biomass (*eg* lacking methanogens) or because the microbial population that can grow during anaerobic fermentation includes only H_2 producing bacteria (*eg* Clostridia), probably because of the low pH of this sample.

In all the other samples tested, the H_2 and CH_4 productions are not simultaneous but shifted in time and the competition between H_2 producers and H_2 consumers (or methanogens) is clearly visible. When CH_4 increases, H_2 gas drops to zero, because hydrogen has been consumed by hydrogenotrophic microorganisms to produce methane.

[Insert Tab. 2 here]

This is observed because, when organic matter is in anaerobic condition, anaerobic digestion occurs: the process is mediated by a mixed undefined microbial consortium

present in the substrate used which mediates hydrolysis, acidogenesis, acetogenesis and methanogenesis of the organic substrates. The digestion begins with bacterial hydrolysis where complex organic matter is broken down into soluble organic matter like sugars, amino acids and fatty acids available for other bacteria. Then acidogenic bacteria convert these soluble compounds to a mixture of volatile organic acids (VOAs). In the acetogenesis step the higher chains VOAs like propionic, butyric, and valeric acids are then converted to acetic acid, H_2 and CO_2. The last step of anaerobic digestion is methanogenesis: various microorganisms produce methane, either by acetate cleavage (acetotrophic Archaea) or by H_2-driven CO_2 reduction (hydrogenotrophic Archaea) (Ivanova *et al.*, 2009; Molino *et al.,* 2013; Cervantes *et al.,* 2006).

This feature may be useful in a single stage or two- stages plants to direct the growth of one or other microbial population with suitable treatments or pre-treatments.

One of the advantages of using matrices as the *Mix* or compost for the production of H_2 and CH_4 into a plant is that these waste biomasses can supply simultaneously both bacteria able to grow on poor substrates and the substrate itself, and there is no need to supplement an external seed as it commonly occurs (Diltz and Pullammanappallil, 2013; Li and Chen, 2007).

Concerning the H_2 production, the *Mix* has not been subjected to any pre-treatment and it is able to produce H_2 only. The maximum values obtained are close to the order of magnitude of the values reported in literature for similar and not pretreated substrates in mesophilic conditions (Table 2). The nature of the *Mix* is closely linked to the seasonality, however it has been observed that *Mix* from different seasons can release H_2 during anaerobic digestion (data not shown), and then the population of H_2 producers is inherently associated with such types of matrices.

ACV15, an immature and unstabilised compost, during AD produces both H_2 and CH_4 in all three conditions analysed. It has a higher propensity for H_2 production with a maximum

value recorded of 1.2±0.01 mL g^{-1} VS and it is the most productive material among those analysed in this study (Tab. 2). However it is a transition sample and it is not stable over a long time. The ACV15 can still be considered for H_2 production in AD plants without any type of initial treatment and for practical reasons, for example when the *Mix* are accumulated in piles and it cannot be used immediately in the plant. It can be used within 15-30 days, that is the time of bio-oxidation phase providing excellent production values.

The compost ACV1 and ACV3 are similar and originating from the same mode of composting, with the only difference that the ACV3 has undergone the composting process a year before, then it has been stored for one year at 4 °C and then tested. Instead the ACV1 mature compost was analysed immediately after collection. These compost samples have not only similar features but also comparable microbial potential as measured by similar gas production rates. In both cases the maximum value of H_2 is around 0.20 mL g^{-1} VS after 3 or 4 days. Also, the CH_4 production (tab. 3) is detectable from the 4th day and it increases gradually and exponentially since the 16th day, then the amount of CH_4 decreases; the CH_4 production with 1 mL of H_2O is very low and the maximum amount of CH_4 after 16 days is 5.8±0.31 mL g^{-1} VS for ACV3 and 8±0.77 mL g^{-1} VS for ACV1. The results obtained with ACV1 and ACV3 are the best in terms of CH_4 production among the samples used in this study. The amount of methane is lower compared to other studies performed on very rich biomasses (tab. 3), but the low availability of nutrients in the biomass used here has to be considered when comparing the overall data.

[Insert Tab.3 here]

We can conclude that ACV1 compost is stable after a year in terms of production and that it can be stored and then used at a later time for the production of both H_2 and CH_4. Of course, in the case of future industrial applications, the storage conditions will have to be adapted to decrease the costs.

Also when ACV1 sample is subjected at 80 °C for 20 minutes to remove methanogenic bacteria, the maximum hydrogen production yield observed is doubled (0.4±0.01 mL g^{-1} VS).

On the basis of consolidated Agri New Tech experience on the agronomical market, the ACV2 compost is an excellent soil fertiliser, but the data presented in this work show that it is not an optimal matrix for the production of H_2 and CH_4 in any of the conditions tested, probably because it possesses a more stable microbial population.

This result implies that the compost with lower quality as fertiliser may be fed to the plant as a complementary seed and/or substrate for sustainable energy production, acquiring an alternative application, with respect to the canonical, and covering different and non-overlapping markets.

Also it was observed that the addition of different amount of water plays a crucial role in the H_2 and CH_4 production in dependence on the substrate used. The water content in conjunction with the specific matrix used can influence the growth of a microbial population compared to another, as reported in the production curves presented in this study. The same sample with the addition of different amount of water develops production curves with a similar trend but with different values; in the absence of water H_2 or CH_4 are not observed. Nonetheless very low amount of added water can sustain appreciable gas production rates, in the perspective of water resources saving.

The gas production values obtained are not high (table 2 and 3) but it must be considered that the substrates used have not undergone any treatment and they contain low amounts of water and of accessible nutrients; also the test was done in small scale and in batch condition. For these reasons, the results presented here are forcedly preliminary and cannot be immediately exploited, but they represent the first report on the ability of green composts and their starting biomass to produce H_2 and CH_4 and might be the basis for future optimisation and implementation.

It is known that thermophilic conditions are more favourable to H_2 production from lignocellulosic substrates by increasing substrate hydrolysis and selecting more specifically thermo-resistant H_2-producers but it involves a greater energy expenditure to attain high temperatures (Giordano *et al.*, 2011) for this reason these studies were performed under mesophilic conditions.

In conclusion these experimental results suggest that:

1) The compost or substrates from composting process can produce H_2 and/or CH_4, without any pretreatment or inoculum.
2) In combination with other biomasses, they might have an alternative use to produce hydrogen and/or methane
3) In particular, the compost that after the production process results in low quality fertiliser might be used in other applications, thus avoiding waste of time and economic resources.
4) The water amount plays a key role in optimizing the production process and it can push towards the alternative production of hydrogen or methane. In our preliminary study sterile water has been added to limit the variables of the microbial population but in the future it could be interesting to study also unsterile or waste water influence on the gas productions.

Also, the digestate from the process may be used as a mineral rich fertiliser and reduce synthetic fertiliser consumption (Lukehurst *et al.*, 2010). The compost or the low quality compost unsaleable as a soil fertiliser might be used as inoculum in AD plant for production of second generation biohydrogen and biomethane: this could avoid the loss-of-business costs by providing an added value to the compost through its possible further use different from the canonical.

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6. Supplementary material

A table reporting the quantification of microelements and heavy metals content of substrates.

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Figures captions

Fig.1. Cumulative hydrogen production in time (days) by *Mix*.

Fig.2. Cumulative gas productions in time (days) by ACV15 immature compost samples, in three different conditions. Hydrogen is reported on the left and methane on the right.

Fig.3. Cumulative hydrogen (on the left) and methane (on the right) production curves from ACV1 (A panel), ACV2 (B panel) and ACV3 (C panel) compost. Each sample was 5 g compost with the addition of water as reported in the legends.

1 **Tables**

	Mix	**ACV1**	**ACV2**	**ACV3**
Initial moisture (%)	24.0	20.7	48.0	14.0
LOI (%)	98.12	42.79	36.88	42.79
pH	3.77	7.25	7.33	7.25
Total nitrogen (%)	0.195	0.716	0.991	0.716
Volatile solids (gVS/kg)	657	680	203	680
TOC (%)	39	11	17	11
Kjeldahl N (mg kg^{-1})	2400	650	5700	650
C/N ratio	200	15.4	17.2	15.4
Sifting (mm)	100	12	10	12

2 Tab. 1 Main characteristics of the compost samples used in this study. Data for ACV3

3 refers to its initial composition, before storage.

4

Substrate	**Max production (mL H_2 g^{-1} VS)**	**Pre-treatment**	**Temperature (°C)**	**Operation mode**	**Ref.**
Mix	0.96	-	37	Batch (1 L)	Present study
ACV15	1.2	-	37	Batch (0.06 L)	Present study
ACV1	0.20	-	37	Batch (0.06 L)	Present study

ACV3	0.20	-	37	Batch (0.06 L)	Present study
ACV2	0.02	-	37	Batch (0.06 L)	Present study
Corn straw	9	-	35	Batch (0.25 L)	Li and Chen, 2007
Corn straw, *C. butyricum*, nutrients	68	1.5 MPa 10 min, cellulase	35	Batch (0.25 L)	Li and Chen, 2007
Cornstalk	3	-	36	Batch (0.25 L)	Zhang *et al.*, 2007
Cornstalk, cow dung, nutrients	57	0.5 % NaOH	36	Batch (0.25 L)	Zhang *et al.*, 2007
Cornstalk, cow dung, nutrients	150	0.2% HCl boiled 30 min	36	Batch (0.25 L)	Zhang *et al.*, 2007
Wheat straw, cow dung, nutrients	1	Cow dung infrared oven 2 h	36	Batch (0.25 L)	Fan *et al.*, 2006

Maize leaves	18	-	70	Batch (0.05 L)	Ivanova *et al.*, 2009

Tab. 2 Hydrogen production yields of anaerobic fermentation. The symbol – indicates the absence of pre-treatment on the feedstock.

Substrate	**Max production (mL CH_4 g^{-1} VS)**	**Temperature (°C)**	**Operation mode**	**Ref.**
Mix	0	37	Batch (1 L)	Present study
ACV15	0.5	37	Batch (0.06 L)	Present study
ACV3	5.8	37	Batch (0.06 L)	Present study
ACV1	8	37	Batch (0.06 L)	Present study

ACV2	1.8	37	Batch (0.06 L)	Present study
OFMSW	150	25	Batch (1 L)	(Lopez and Espinosa, 2008)
MSW	211	35	Batch (40 L)	(Guendouz *et al.*, 2010)
Potato waste, beet leaves	420	37	Batch (0.5 L)	(Parawira *et al.*, 2006)
OFMSW	110	35	Batch (1.7 L)	(Fernandez *et al.*, 2008)
Sewage sludge, OFMSW	24	36	Two-stage CSTR	(Sosnowski, *et al.*, 2003)

Tab. 3 Methane production yields of anaerobic fermentation. OFMSW: Organic Fraction of Municipal Solid waste, MSW: Municipal Solid Waste.