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Master Thesis

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HYDROGEN AND METHANE PRODUCTIONS FROM ORGANIC WASTE OF DIFFERENT CHARACTERISTICS

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“Rien ne se perd, rien ne se crée, tout se transforme.”

Antoine Lavoisier

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ME AND MY THESIS

Since I was attending primary school, one of the main topic I was involved in was waste collection and recycling. It was 1996, when my Municipality started the implementation of separate waste collection, and a lot of advertisement and work had been done to involve the citizens in the new project. I remember I spent a lot of time having school papers or little works facing the waste problem in our Municipality, or reading texts about plastics and waste recycling. At that moment I was only a child, and actually I did not realize why it was so important to make people aware and informed on this theme. Then, ten years later, I decided to enrol to the Engineering Faculty in Padua to follow the Environmental Engineering course, and I discovered that actually that work was not lost, even if many years had passed. It was enough to put in me the curiosity and the interest necessary to follow the courses, to get involved in the subjects, to study and to find out that actually there is still a lot to know about waste!

This idea started to became stronger when I asked for my master thesis. During the master degree my interest focused mainly on the topics of solid waste management and sustainable energy production, and I have always had the idea to perform and applied research, something that I would have done on the field, not just on literature findings. So, with all these ideas, I asked Prof. Cossu, my supervisor, to conduct the thesis on the laboratory of the Department of Environmental Engineering, located in Voltabarozzo. Dr. Luca Alibardi, my co-supervisor, proposed to me to work on subsequent hydrogen and methane production from the organic fraction of municipal solid waste. The goal of the thesis was to understand how a first fermentative hydrogen production phase could influence a further digestion of the waste in the traditional methanogenic conditions, and how waste qualitative composition can affect this process.

My experience in the laboratory started in October 2012, and for the first period of my thesis I worked with another student, Paolo, that was having his master thesis on hydrogen production from organic waste fractions. The first step of the work was to collect a sample of putrescible organic waste directly at a plant located in Camposampiero, that perform wet anaerobic digestion of organic waste deriving from source separation. More or less 100 kg of waste were sieved and divided in the different fractions: “bread-pasta-rice”, “meat”, “vegetable”, “fruit”, while all the material that was passing through the 20 mm sieve was considered “undersieve” fraction. At the end of the separation some kilograms of each fraction were collected and brought to the laboratory to start the characterization and the analysis. The experience had been quite strong but really useful, to understand what type of materials would have been tested. Once in the

laboratory the waste was shredded in a kitchen mill while diluted with a known amount of water. A sample was reconstructed with the same proportions of the fractions found out at the plant. Then the waste was frozen and conserved until the preparation of the tests.

The first period of work was devoted to the characterization of the waste fractions. Dr.ssa Annalisa Sandon, the technician of the laboratory, thought me how to perform the analyses I need on the waste and how to use the machineries necessary for the biogas measurement. Each waste fraction was analyzed for TS, VS, TKN, N_{org}, NH_4^+, TOC, COD and P_{tot}.

Before starting the experimentation on the two stage anaerobic digestion process, it was necessary to decide the working conditions: the F/M ratio, the concentrations of substrate and inoculum to use, and the pH at which the tests would have been conducted. With this purpose I started my literature research on the web and on scientific articles, but I also read the previous thesis done in the laboratory by other students, to understand what I could do to improve the experiment or to avoid mistakes already done. Actually the parameters were changing in every experience, because of the great variability that substrates were displacing, or because of the different systems that were used. Sometimes it was also difficult to find a comparison between different works.

As a first screening, BHP tests were conducted on the single fractions collected at the plant for pH 5.8 and pH 7. The fulfilment of the tests included the preparation of batch tests (1 l bottles) with 5 gVS/l of substrate, 50 g of sludge thermally treated to inhibit methanogenic activity, and buffer solution to reach a working volume of 500 ml. The bottles were stored in a water bath at 35°C, the gas was extracted from each bottle twice a day for a week, and qualitative composition of the gas was obtained with a GC.

The results of the BHP test allowed to define, for all the fractions, higher hydrogen yields at a pH of 5.8. The concentration and the quantity used seemed to be appropriated to the measurement I could perform, so I decided to keep the parameters tried with these tests for the first acidogenic part of the double stage anaerobic digestion.

For the methanogenic phase, I chose to reproduce the conditions that had already been used by other works done with batch tests in the lab. BMP test were conducted at the same concentration of substrate of the BHP test, but using 100 g of raw sludge (not treated). The direct BMP tests were even prepared in 1 l glass bottles, with the defined F/M ratio and without using any buffer.

For the second stage of the anaerobic digestion, the bottles used for BHP test were opened, and sodium carbonate or sodium hydroxide was added to reach a pH of about 7.5, to allow good methanogenic conditions. Nitrogen flushing was performed and the bottles were put back in the water bath to continue the test.

With these conditions, I started my double stage experiments on glucose, to verify what was going on with a substrate already well known, and on which a mass balance could be performed. The experiments were conducted in triplicate, and controls were paired to each condition studied. With glucose the tests that have been conducted were:

- Direct BMP test at pH 7.5
- BHP test using thermally treated sludge at pH 5.8
- BHP test using raw sludge at pH 5.8
- Double stage BMP inserting raw sludge at the end of BHP with thermally treated sludge
- Double stage BMP inserting raw sludge at the end of BHP tests with raw sludge.

The main difficulties found in the performance of the tests were linked to the great number of bottles to manage, and on the lag phase that all double stage BMP tests were displacing, with really law biogas production for the first 10 days. The tests performed on glucose allowed to define the great effectiveness of sludge thermal treatment to inhibit methanogenic bacteria, and to see that the measurements done in the lab (COD measurement on the solid and liquid phase, biogas sampling and measuring, C measures) were precise enough to permit to close a mass balance on the system analyzed. The only term that was not possible to measure was the bacterial growth, because its value was really low and comparable with the mistake I was doing on my sampling procedures.

So, my next step, would have been to test the double stage anaerobic digestion on waste sample, conscious that I could not measure that amount of COD or TC that was not biodegraded inside the bottles, because of the nature of the test itself, in which sludge and waste put together inside the bottles became inseparable.

To test the effects of the fermentative phase on the second methanogenic phase, it has been chosen not to work on the pure waste fractions, because it seemed not to be representative of a waste that a plant can receive and treat on a full scale. So, some plausible real waste mixtures were constructed from the waste fractions, to reproduce a waste composition that could be real. To do so, previous data on waste sampling and characterization already conducted at the same plant were collected and analyzed. I chose to construct three sample to be tested, resulting in raw waste percentages of each fraction that could be variable inside the value ranges found at the plant. The first mixture was characterized by a predominant amount of the “bread-pasta-rice” fraction, since carbohydrates are known to produce much hydrogen, the second one was characterized by a predominant amount of “meat”, because proteins are known to produce really low hydrogen quantities, and the third one was having intermediate amounts of carbohydrates and proteins.

Experiments on the three mixes were conducted in triplicate, and controls were associated to each sample tested. The tests performed on the three waste mixes were:

- Direct BMP test at pH 7.5
- BHP test using thermally treated sludge at pH 5.8
- Double stage BMP inserting raw sludge at the end of BHP with thermally treated sludge.

The use of these three compositions displaced interesting results. Expectations about hydrogen production were confirmed, with higher production for the first sample, lower for the second sample and intermediate production for the third one. Then, comparing the results obtained from the two stage anaerobic digestion with the direct BMP test, it was interesting to note that the methane production from the second stage, was higher than the one obtained in the direct BMP test, and this increase in production was proportional to the amount of hydrogen produced in the first phase by each sample. This meant that improving the hydrogen production phase in a double stage anaerobic digestion, in some way should increase the substance biodegradability, allowing to obtain higher energy yields and a further waste degradation from the waste disposal point of view.

Further analysis on the hydrogen production phase were also performed thanks to a continuously stirred batch reactor, equipped with a bascule system for the biogas extraction. The continuous measurement allowed to test the real velocity of biogas and hydrogen production, that it was not possible to see with the batch tests. Lag phases, velocities and cumulative productions were analysed for the same three samples, and final evaluations were done according also to VFAs measured at the end of the tests.

The experimental activity of my thesis was concluded at the end of June 2013.

At the end of the work, I can say that all the investigations that I did before and in the meantime of the test running turned out to be a fundamental and valid approach to understand what was going on with my bottles and to improve the experience. Further knowledge on the theme was acquired at the end of the activity, also by people who worked with me, and the lab experience widely satisfied my expectations.

1. INTRODUCTION

The organic fraction of municipal solid waste (OFMSW) is a waste characterized by high moisture and high biodegradability due to the large content of unused or partly consumed food, food preparation residues and leftovers from houses and residences, restaurants, cafeterias and canteens.

National legislations oriented to divert organic waste disposal from landfills (*Cossu, 2009*), and the particular characteristics of the OFMSW, rich in carbohydrates (starch, cellulose and hemi-cellulose) (*Liu et al., 2006*), proteins and fats, address the treatment options of OFMSW toward biological processes. Composting or anaerobic digestion can be used with the final purposes of recover nutrients and solid amending materials, as for example compost, and/or energy production (biogas or bio-fuels) from a resource that is considered renewable (*Kvesitadze et al., 2012*). Great attention in the last years has been given to anaerobic digestion with the purpose to recover hydrogen from organic waste via dark fermentation process.

Hydrogen gas is today used for industrial processes, as for example ammonia production, fossil fuel refining or for hydrogenation of vegetal oils. Future developments are looking to hydrogen as a valid energy source. The use of hydrogen is an attractive alternative to the current energy resources, because of its potential and versatility (*Liu et al., 2006; Xie et al., 2008*). Hydrogen energy yield results to be equal to 122 kJ/g, that is higher than that of hydrocarbon fuels. The only final product of hydrogen combustion is represented by pure water, no carbon dioxide is released in the reaction. Hydrogen can be efficiently used in internal combustion engines or in chemical fuel cells to produce electricity. These characteristics, makes of hydrogen an energy source that can allow to face current and future increasing energy demand, without contributing to carbon emissions in the atmosphere an so to greenhouse effect. However, hydrogen is not a pure primary energy source, as fossil fuels are. Even if it is the most abundant element on the Earth, hydrogen is commonly bounded to other compounds, and no hydrogen gas is available. That is why pure hydrogen gas needs to be extracted from other sources, becoming an energy carrier, that can be produced, stored, transported and used to fulfill industrial and households energy needs. Nowadays hydrogen gas can be produced from water electrolysis, from fossil fuels, or via biological processes (*Oh et al.,2003*). The first process is not really much exploited because of the great inefficiency it displaces: it consists in the decomposition of water into hydrogen and oxygen thanks to an electric current passing through water, but the amount of energy required to brake the water molecule is higher than that stored in the produced hydrogen. The process that is mainly used today is steam reforming. In this process natural gas is made

reacting with water vapor at high temperature and relatively low pressures, to obtain the so called syngas, that is composed by carbon monoxide and dihydrogen. Even if this system is giving major energy efficiency compared to the previous one, it is to remind that fossil fuel resources are used, of which the reserves are decreasing, and that carbon dioxide emissions are produced. On this way the biological hydrogen production seems to be the most promising way for hydrogen generation. Biological processes are performed by algae and bacteria, and can have the important advantage of carbon neutrality, or even negative carbon emissions, if carbon dioxide is captured and sequestered during the hydrogen production phase (*Kvesitadze et al., 2012; Hallenbeck, 2009*). Biological hydrogen production can involve photolysis processes, performed by photoautotrophic microorganisms that capture solar energy to produce hydrogen and oxygen from water, or by photoheterotrophic bacteria that can exploit solar energy to produce hydrogen from organic substrates. However the yield of these organisms is really low, and the process requires sunlight to be run. In contrast to phototrophic bacteria, there are fermentative anaerobic bacteria that during the first phase of anaerobic digestion can convert carbohydrate substrates to hydrogen, carbon dioxide, volatile fatty acids and other products, without requiring light for additional energy, and because of this, this process type is named biological dark fermentation. The substrates that are more suitable for hydrogen production via dark fermentation are represented by carbohydrates, glucose, starch, molasses, sucrose (*Logan et al., 2002; Okamoto et al., 2000*). The main reactions that are involved in the process and that can lead to hydrogen production are:

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 \leftrightarrow CH_3C_2H_4COOH + 2CO_2 + 2H_2 \quad (2)$$

$$CH_3C_2H_4COOH + 2H_2O \leftrightarrow 2CH_3COOH + 2H_2 \quad (3)$$

$$CH_3COOH \leftrightarrow 2CO_2 + 2H_2 \quad (4)$$

Acetate and butyrate are among the main products of this process. From the stoichiometric point of view, when 1 mol of glucose is degraded to acetate, it gives rise to 4 mol of hydrogen gas, while if glucose is degraded to butyrate, 2 mol of hydrogen are produced.

On the other hand other reactions can take place in the system, leading to the formation of propionic acid, ethanol or lactic acid, in which hydrogen production is by-passed as in equations (7) and (8), or hydrogen is consumed, as in equations (5) and (6).

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O \quad (5)$$

$$CH_3COOH + H_2 \leftrightarrow 2CH_3CH_2OH + 2CO_2 \quad (6)$$

$$C_6H_{12}O_6 \leftrightarrow CH_3CH_2OH + H_2O \quad (7)$$

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CHOHCOOH \quad (8)$$

In this context, considered waste intrinsic characteristics, biological hydrogen production by means of dark fermentation process can represent one of the most innovative treatment of the OFMSW, aimed to the biological conversion of waste into a biofuel, meeting the sustainable management and disposal of solid and liquid waste (*Liu et al., 2006; Park et al., 2005; Okamoto et al., 2000*).

One of the main criticism that is moved toward hydrogen production from organic waste, is the high amount of COD that remains undegraded after the first acetogenic phase (*Kobayashi et al., 2012*), that can be still more than 90% of the inlet COD of the OFMSW. However, if the reaction is properly driven toward acetic acid and volatile fatty acids, the byproducts that are formed can became an ideal pool for the further production of methane, using the effluent of the first hydrogen production phase, achieving an high degree of waste stabilization and allowing energy conversion improvement (*Giordano et al., 2011*).

Two stage anaerobic digestion processes were already investigated in the past by different authors. Acidogenic and methanogenic phase separation can allow greater stability to the different groups of microorganisms and better process control (*Nasr et al., 2012*). However, the first acidogenic phase was not thought for biohydrogen production, but just as a pretreatment to the second methanogenic step, where it was possible to control the formation of VFAs without affecting methanogenic activity (*Rincón et al., 2009*), methanogens being really sensitive to acids (*McCarty, 1964*). Two stage process compared to the single stage anaerobic digestion could lead to a larger overall reaction rate and biogas yield, to an increase in the hydrolysis rate (*Giordano et al., 2011*) and a better pathogenic destruction could be achieved (*Liu et al., 2006*). Good results compared to the single stage anaerobic digestion were already obtained, consisting in an higher COD removal (*Nasr et al., 2012*). However, the two stage system was not successful in the past, because of the increasing process complexity, investments and operational costs (*Liu et al., 2006*). Currently the major part of the plants in Europe rely on the single stage process because of the lower cost when compared to the double stage anaerobic digestion (*Liu et al., 2006*).

It is clear so, that two stage anaerobic digestion, especially from OFMSW, is a technology that remains unproven in the field and that is at its earlier development stage (*Liu et al., 2006; Kvesitadze, 2012*). Coupling the methane production, that is an already well known process for

waste treatment (*Kvesitadze et al., 2012*) with a an hydrogen initial production phase, can allow to overcome the economic and energetic problems linked to the two stage process (*Xie at al., 2008*), allowing an higher energy yield and an higher COD removal compared to the two phase anaerobic digestion preceded by a simple hydrolytic-acidogenic step. Good results in the coproduction of hydrogen and methane compared to the direct anaerobic digestion have already been obtained by the OFMSW (*Liu et al., 2006; Kvesitadze et al., 2012*) and from other residues as for example thin stillage (*Nasr et al., 2012*).

For a full scale application of dark fermentation process to the OFMSW, a deeper knowledge of the effects of operational conditions on the hydrogen conversion efficiency is required. The hydrogen production potentials via dark fermentation depends in fact by several aspects: type of inoculum and its conditioning, type of reactor, organic loading rate and hydraulic retention time, process temperature, pH of fermentation, hydrogen partial pressure and acids concentration (*Okamoto et al., 2000*). Different researches were published in scientific literature on hydrogen production tests from the OFMSW. Several operational conditions and different specific aspects of the dark fermentation process were analysed but the results are not always directly comparable, sometimes diverse or even in conflict.

To the best of our knowledge, one aspect that has not yet been considered is the composition of the sample of OFMSW used during experimental tests (*Kobayashi et al., 2012*). The substrates used in research experiments on OFMSW may in fact consist of fresh food (raw or cooked/boiled) used to simulate real waste (*Kvesitadze et al., 2011; Okamoto et al., 2000*), food waste taken from restaurants or cafeterias of the universities or, in few cases, organic waste from household waste collection (*Kobayashi et al., 2012*). The different origins of the organic waste samples may have an effect on the great variability of hydrogen production yields reported in literature, coupled with different process conditions. Moreover the fact that the composition of organic waste is not always specified may create difficulties on concluding whether an organic or food waste used in an experimental study has higher or lower hydrogen yields than another, because better operational conditions were found or because the fractions in that particular waste have simply higher hydrogen production yields. The composition of the OFMSW is in fact strongly dependent on the place and the time of the collection for a specific municipality or area while organic waste from cafeterias and restaurants may not be representative of the OFMSW received at treatment facilities of source separated waste.

The aim of this research study was the investigation of the effects of qualitative waste composition on the production of hydrogen and methane in a dark fermentation process in batch tests under mesophilic conditions. Specific questions addressed were: (i) how the OFMSW

composition can affect potential production of hydrogen and methane in a direct production process? (ii) How does the potential productions of hydrogen and methane from waste of different characteristic are changing in the two-stage anaerobic digestion process? (iii) How carbon and COD balances are changing?

2. MATERIALS AND METHODS

2.1 Organic waste sample

A sample of OFMSW was collected in July 2012 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. An amount of about 200 kg of organic waste was manually sorted and divided in the following fractions: "meat-fish-cheese", "fruits", "vegetables", "bread-pasta", "undersieve 20mm" and "rejected materials". The rejects materials were shoppers, plastics, paper and cardboard, metals, glass, bones, shells and fruits kernels. All these materials are slowly or non-biodegradable therefore they were not used for the preparation of the samples for hydrogen and methane production tests. Table 1 provides the composition of the raw waste sorted at the plant with and without considering the fraction "rejected materials".

Table 1 - Composition of the organic waste sampled in July 2012. The data are reported as percentages (%) referred to wet weight.

Fraction	Raw waste composition ($kg_{raw\ fraction}/kg_{raw\ mixture}$)	Biodegradable waste composition ($kg_{raw\ fraction}/kg_{raw\ mixture}$)
Meat-Fish-Cheese	0.3	0.4
Fruit	28.7	35.4
Vegetable	33.2	41.0
Pasta-Bread	1.3	1.6
Undersieve 20 mm	17.5	21.6
Rejected materials	19	-
Total	100	100

A part of each waste sorted fraction was shredded in a kitchen mill with the addition of water in defined proportions (waste:water ratio on wet weight basis was respectively 2:3 for "vegetable", "fruit" and "undersieve", 1:2 for "pasta-bread" and 1:1 for "meat-fish-cheese"), and then stored at a temperature of -20°C. The sample obtained were analyzed for the following parameters: total solids (TS) and volatile solids (VS), total organic carbon (TOC), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonium (NH_4^+) and total phosphorus (P_{tot}) concentrations.

The sorted and shredded fractions were subsequently mixed in defined proportion to obtain three organic waste mixtures with different characteristics, named hereafter respectively Mix 1, Mix 2 and Mix 3.

The compositions of the three prepared samples Mix 1, Mix 2 and Mix 3 are reported in Table 2 on VS basis and in Table 3 on wet weight basis.

Table 2 - Composition of the three samples of OFMSW with specific characteristics. Data are reported as % on volatile solid basis.

Fraction	Mix 1	Mix 2	Mix 3
Meat-Fish-Cheese	5	50	19
Fruit	10	10	10
Vegetables	20	20	20
Pasta-Bread	50	5	36
Undersieve	15	15	15

Table 3 - Composition of the three samples of OFMSW with specific characteristics. Data are reported as % on wet weight basis.

Fraction	Mix 1	Mix 2	Mix 3
Meat-Fish-Cheese	2	21	7
Fruit	27	26	26
Vegetables	34	33	32
Pasta-Bread	19	2	12
Undersieve	18	18	22

The sample Mix 1 was prepared with a large content of the fraction "pasta-bread" while the sample Mix 2 was prepared with a large content of the fraction "meat-fish-cheese". The third sample, Mix 3 was prepared with an intermediate content of these two fractions.

Mix 1 was composed on volatile solid basis by 50% of "pasta-bread" and 5% of "meat-fish-cheese". Mix 2 was composed on VS basis of 5% of "pasta-bread" and 50% of "meat-fish-cheese". Mix 3 was composed on VS basis of 36% of "pasta-bread" and 19% of "meat-fish-cheese". In all the three samples, the fractions "fruits", "vegetables" and "undersieve" were 10%, 20% and 15% respectively. Mix 1, Mix 2 and Mix 3 were characterized with the same procedures and for the same parameters previously listed for the single organic waste fractions, and then used as feeding materials for hydrogen and methane production tests.

2.2 Inoculum conditioning

Granular sludge used as inoculum for both hydrogen and methane production batch tests was collected from a full scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy. Test on glucose were performed using a sludge sample taken in October 2012 (Sludge A), while tests on the OFMSW were performed using a sludge sample taken in March 2013 (Sludge B).

The sludge was analyzed for the following parameters: total solids (TS) and volatile solids (VS), total organic carbon (TOC), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonium (NH_4^+) and total phosphorus (P_{tot}) concentrations. Results are reported in Table 4.

Raw sludge was used as inoculum for methane production batch tests, while for hydrogen production batch tests, heat treatment was carried out on granular sludge in a rotary water bath

incubator at a fixed temperature of 100°C for 4 hours. This pre-treatment of inoculum was evaluated to be optimal for selecting hydrogen-producing microorganism characterized by high hydrogen conversion yields and for inhibiting methanogenic activity (*Alibardi et al., 2012*).

Table 4 – Physical chemical characterization of the two sludge type used.

Parameter	Sludge A	Sludge B
TS (%)	13 ± 1	10 ± 1
VS (% $_{of\ TS}$)	72 ± 1	80 ± 1
TOC (% $_{of\ TS}$)	40 ± 1	45 ± 1
COD (mgCOD/gTS)	1248 ± 5	1273 ± 5
TKN (mgN/gTS)	77.9 ± 0.5	82.0 ± 0.5
NH_4^+ (mgN/gTS)	13.9 ± 0.5	20.2 ± 0.5
P_{tot} (% $_{of\ TS}$)	1.3 ± 0.5	1.8 ± 0.5

2.3 Hydrogen production in batch tests

Lab scale tests were performed to evaluate the hydrogen production potentials of the substrates examined by dark fermentation process. Tests were carried out in batch reactors of 1 litre at mesophilic conditions (35°C ± 1 °C). Reactors were hermetically closed by means of a silicon plug enabling sampling of the gas and liquid produced during the fermentation. The liquid volume in each reactor, consisting of the substrate, the inoculum and a phosphate buffer solution, was 500 ml. Tests were performed at a substrate concentration of 5 g VS/l and with an inoculum concentration of 10 g VS/l. The ratio between the volatile solids of the substrate to be degraded and the volatile solids of the inoculum biomass (Food/Microorganisms - F/M) used in each test was 0.5 gVS/gVS. The pH value was set using phosphate buffer (0.05 M) to 5.8. After preparation, the reactors were flushed with N_2 gas for 3 minutes and incubated without stirring in a thermostatic chamber. Blank tests using only the inoculum were also prepared to measure the quantity of hydrogen and carbon dioxide produced only by the biomass. All tests were carried in quadruplicate.

The biogas volume was measured adopting the dislocation method. By this method the excessive pressure produced in the reactor by biogas production process moves an equal quantity of liquid to a second bottle. The volume of the liquid moved, and, accordingly, the volume of biogas produced, is measured with a graduated cylinder. The liquid used in measurements was an acidified (pH<3) and saline (NaCl 25%) solution in order to avoid the dissolution of carbon dioxide into the liquid. Biogas composition in terms of hydrogen, carbon dioxide and methane were measured by a gas chromatograph.

Hydrogen 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 the specific gas

at times *t* and *t-1*, and the volume of the reactors' head space (*Van Ginkel et al., 2005*; *Logan et al., 2002*). The following equation (9) was applied:

$$V_{C,t} = C_{C,t} \cdot V_{G,t} + V_H \cdot (C_{C,t} - C_{C,t-1}) \quad (9)$$

Where:

$V_{C,t}$ is the volume of hydrogen produced in the interval between *t* and *t-1*;

$C_{C,t}$ and $C_{C,t-1}$ are the hydrogen concentrations measured at times *t* and *t-1*;

$V_{G,t}$ is the volume of biogas produced between time *t* and *t-1*;

V_H is the volume of the reactors' headspace.

The data on hydrogen production were interpolated using an exponential function:

$$P(t) = P_{\max} \cdot (1 - e^{-kt}) \quad (10)$$

where:

P(t) is the hydrogen production at time *t*

P_{max} is the ultimate value of hydrogen production

k is the rate of hydrogen production

Data on hydrogen yield are expressed as Nml of hydrogen at temperature of 0°C and pressure of 1 atm.

2.4 Hydrogen production in batch stirred reactor

Hydrogen production from the different mixtures of organic waste fractions was tested also in a batch stirred reactor. The reactor was composed by a continuously stirred glass bottle, having a total volume of 560 ml, standing on a heated plate, and closed with a silicon plug. Substrate and biomass were introduced in the reactor in the same proportion as for hydrogen production in batch tests: the concentration of the substrate used was 5 g VS/l, while the biomass concentration was 10 g VS/l. The ratio between the volatile solids of the substrate to be degraded and the volatile solids of the inoculum biomass used in each test was 0.5 gVS/gVS. A quantity of phosphate buffer solution was used to fill the reactor until the working volume of 400 ml. An headspace of 160 ml remained in the upper part of the reactor.

Temperature control was allowed by a thermometer adjacent to the bottle, and an insulating jacket was put around the reactor to avoid heat dispersion. The system allowed the continuous pH measurement thanks to a pH meter inserted in the silicon plug of the bottle. The pH meter was connected to a sodium hydroxide injector, and in the case the pH was dropping below 5.5, a quantity of soda was added to make it raising above that value.

Biogas produced is flowing through a pipe connected to a wet tip gas meter, having a volume of 3.88 ml. The overpressure caused by the gas formation is in this way removed continuously, and the total biogas produced is measured through the number of turning of the wet tip gas meter.
Test were conducted on the three organic waste mixtures with and without pH control. A blank test was also performed in order to evaluate the production of gas due only to the sludge inserted in the system.
At the end of the experiment the qualitative composition of the gas in the headspace was measured with a GC, and a liquid sample for each run was taken to measure residual VFAs and NH_4^+ concentration in the residual liquid. To calculate the hydrogen production of each test, the quality of the gas produced has been assumed constant and described by its final concentrations in carbon dioxide and hydrogen given by the GC (*Logan et al., 2002*).
The data on hydrogen production were interpolated using Gompertz equation:

$$H_2(t) = H_{2\max} \cdot e^{-e\cdot\left(\frac{R\cdot e}{H_{2\max}}(\lambda - t)+1\right)} \qquad (11)$$

where:
$H_2(t)$ is the hydrogen production at time t
H_{2max} is the ultimate value of hydrogen production
R is the maximum velocity of hydrogen production
λ is the duration of the lag phase
Data on hydrogen yield are expressed as Nml of hydrogen at temperature of 0°C and pressure of 1 atm.

2.5 Methane production in batch tests

Similarly to the hydrogen production batch tests described in paragraph 2.3, lab scale tests were prepared for measuring methane potential of the three different substrates. Tests were carried out in batch reactors of 1 litre at mesophilic conditions (35°C ± 1 °C). Reactors were hermetically closed by means of a silicon plug enabling sampling of the gas and liquid produced during the fermentation. The liquid volume in each reactor, consisting of the substrate, the inoculum and tap water, was 500 ml. Tests were performed at a substrate concentration of 5 g VS/l and with an inoculum concentration of 20 g VS/l. The ratio between the volatile solids of the substrate to be degraded and the volatile solids of the inoculum biomass (Food/Microorganisms - F/M) used in each test was 0.25 gVS/gVS. In each bottle 0.2 grams of sodium carbonate was added, in order to set the pH at 7.5 and to provide the system a further buffer capacity to avoid excessive pH

drops that can inhibit methane production. After preparation, the reactors were flushed with N_2 gas for 3 minutes and incubated without stirring in a thermostatic chamber. Blank tests using only the inoculum were also prepared to measure the quantity of methane and carbon dioxide produced only by the biomass. All tests were carried in triplicate.

Biogas sampling was performed as already reported in paragraph 2.3.

At the end of the test, after 67 days, each test was opened, pH was measured with a pH meter and a sample was taken to analyze residual COD, VFAs and dissolved organic carbon (DOC).

2.6 Sequential hydrogen and methane production in batch tests

Lab test batch tests were conducted for the evaluation of the sequential production of hydrogen and methane from the selected substrates. The initial phase of hydrogen production was performed as previously reported in paragraph 2.3. The second phase of methane production was performed as soon as hydrogen production lasted, generally after one week from the beginning of the test.

To provide optimal conditions for methanogenic bacteria, the pH of the digestion liquid was raised from 5.8 to 7.5 by using either NaOH or Na_2CO_3. In two bottles of the four replicates pH was raised until 7 using sodium hydroxide, then further 100 gr of raw sludge were added, in order to provide the system anaerobic methanogenic bacteria. A further addition of sodium carbonate was used to raise pH until 7.5. In the other two bottles of each substrate the pH increment was realized entirely with sodium carbonate. At first the pH was raised until 7, then an addition of 100 g of raw sludge was done. After that, the pH was further raised until 7.5 using sodium carbonate. An addition of 100 g of raw sludge was performed also in blank tests, in order to evaluate the subsequent production of methane and carbon dioxide due only to the biomass present in the tests.

All bottles were closed, flushed with N_2 gas for 3 minutes to restore anaerobic conditions, and then incubated without stirring in a thermostatic chamber at mesophilic conditions (35°C ± 1°C).

Biogas sampling was performed as already reported in paragraph 2.3.

At the end of the methane production phase, after 58 days, each test was opened, pH was measured with a pH meter and a sample was taken to analyze residual COD, VFAs and DOC.

2.7 Hydrogen and methane production from glucose

All the tests conducted on the three constructed organic waste mixtures, were repeated using glucose as substrate. Glucose was used as a reference substrate in order to verify if the analytical methods adopted were good to describe the process reproduced, and to compare results obtained with literature ones.

Hydrogen, methane, as well as sequential hydrogen and methane production batch tests on glucose were carried out in the same way as for the organic waste mixtures, using the same F/M ratios and working volumes. Hydrogen production test by using batch stirred reactor on glucose were carried out with and without pH control.

Two further tests were conducted on glucose in order to verify the efficiency of thermal treatment as a method to select spore forming hydrogen producing bacteria and avoiding hydrogen consumption by methanogenic bacteria: hydrogen production and subsequent methane production batch test was conducted using raw sludge as inoculum instead of thermally treated granular sludge and results were analyzed.

2.8 Analytical methods

TS, VS, COD, TKN, ammonium and total phosphorous concentrations were analysed according to Standard Methods (APHA, 1999). TOC was measured using a Total Carbon Analyzer (TOC-V CSN, Shimadzu). VFAs were measured using a GC (Varian 3900) equipped with a Varian 25m×0.53mm ID CP-WAX 58 column. Nitrogen was used as carrier gas.

The composition of biogas in the headspace of reactors was measured using a micro-GC (Varian 490-GC) equipped with a 10 meter MS5A column and a 10 meter PPU column. Helium was used as carrier gas.

3. RESULTS AND DISCUSSION

3.1 Biogas production from glucose

3.1.1 Methane production from glucose

Experiments have been conducted on batch tests to define methane production from glucose, that is considered a completely soluble and biodegradable substrate. Starting from an initial amount of 2.5 g of glucose in each bottle, the total biogas measured with the displacement method was 775 Nml biogas/ gVS introduced. Methane and carbon dioxide specific yields were respectively 371 Nml CH_4/ gVS and 385 Nml CO_2/ gVS. Final biogas composition was given by 49% CH_4 and 51% CO_2. This result is in accordance with literature, that defines biogas production in anaerobic treatment from waste chemical composition via Buswell's equation (*McCarty, 1964*):

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) \cdot H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) \cdot CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) \cdot CH_4 \qquad (12)$$

For glucose, Buswell's equation becomes:

$$C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2 \qquad (13)$$

indicating methane and carbon dioxide production from this substrate. The final production of methane and carbon dioxide is respectively 2.98 mol CH_4/ mol $C_6H_{12}O_6$ and 3.09 mol CO_2/ mol $C_6H_{12}O_6$ introduced, that is confirming the ongoing of (13).

Results in terms of COD and TC balance are presented in Figure 1 and 2.

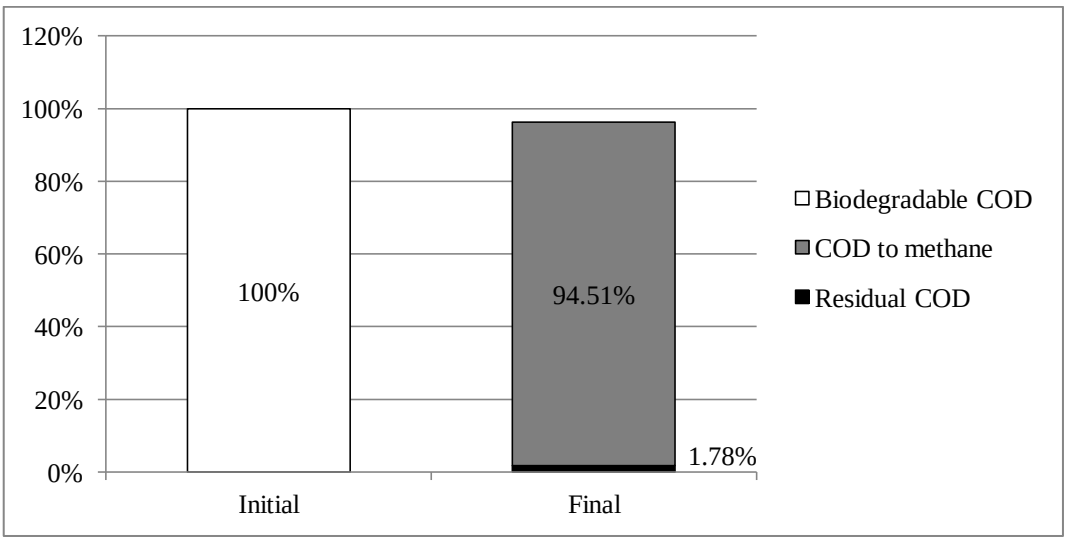

Figure 1 - COD balance in batch methane production from glucose.

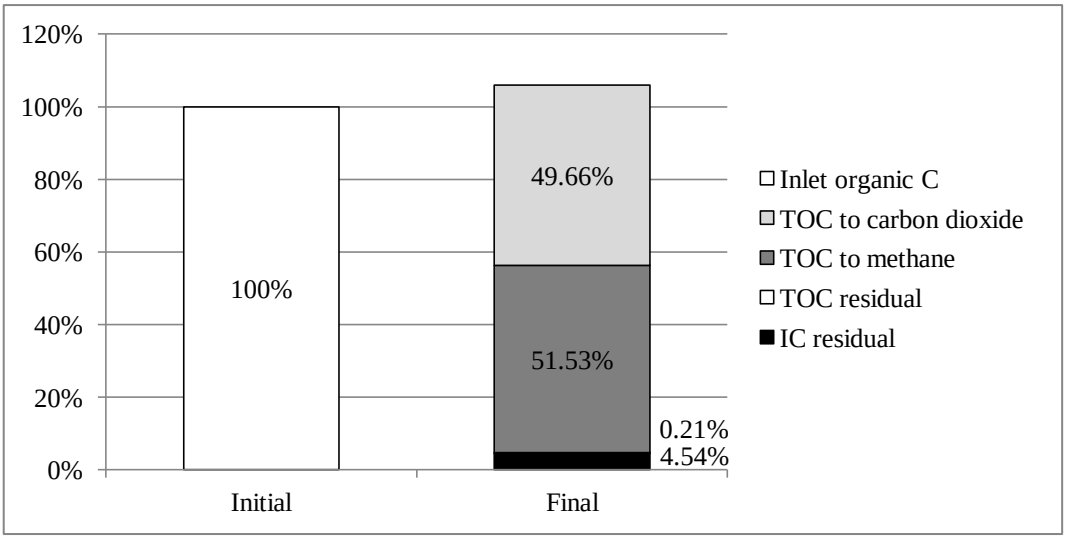

Figure 2 - TC balance in batch methane production from glucose.

From the COD and carbon balances it is possible to see that the major part of the COD was converted to methane, and that almost all the carbon has been gasified to methane and carbon dioxide. Residual organic carbon left is only 0.21%, so the inlet substrate can be considered completely degraded. Residual inorganic carbon (IC) represents the fraction of carbon dioxide produced by biological degradation that remained dissolved in the liquid phase due to chemical equilibrium with the gaseous phase. Final COD balance has been closed with a lack of 3.7% of the initial COD, while TC balance was closed with an excess of 5.9%. The 3.7% in COD loss can be explained with the use of a certain amount of inlet substrate for biomass growth, that in anaerobic digestion can be considered approximately equal to 10% of the initial COD (*McCarty, 1964*). The amount of COD introduced in each bottle deriving from 2.5 g of glucose was 2.8 g of COD. If the 10% of this was ending in biomass growth, this means that 0.28 g of COD were transformed into new bacteria. *Henze et al.*, (*2008*) reported a coefficient for the amount of COD necessary for the biomass growth equal to 1.42 gCOD/ gVSS. So, in this work, if 10% of the starting COD was completely used for bacterial growth, it should be possible to measure an increase in weight of the bottle of about 0.2 g. Since residual COD analysis is performed on 0.2 µm filtered sample, the amount of COD ending in biomass growth, that is particulate, is not emerging in this type of measure, but it should be measured directly. Because of the nature of the sample, where sludge is completely mixed with waste in the bottles, it was not possible to separate it and to have a measurement. On the other hand TC balance was closed with an excess of 5.9%, and this error underlines the fact that the measurement methods used for the experiments conducted, did not allowed the quantification of initial carbon converted to new biomass. The percentage error in the balance was probably comparable to the percentage of

initial substrate resulting in new bacteria, and because of this the term relative to biomass growth has not been considered in the balances adopted.

3.1.2 Hydrogen production from glucose

As reported by *Oh et al.,* (*2003*), to optimize hydrogen production through dark fermentation process it is possible to apply simple treatments to the inoculum, in order to select hydrogen producing bacteria and to avoid as much as possible hydrogen consumption by methanogens, that can use hydrogen and carbon dioxide as a base to produce methane and water, reducing consequently hydrogen yield. Culture conditions that can be used to limit methanogens growth include low pH and an heat shock treatment of the inoculum, that is sufficient to remove non spore forming bacteria as methanogens and leave spore forming hydrogen producing bacteria. Short hydraulic retention times and sludge retention times can also be used, especially in continuous reactors.

Hydrogen production from glucose has been performed on both batch tests and in a batch stirred reactor. Batch tests experiments were used to test the effectiveness of hydrogen production using respectively non heat treated inoculum, that is raw granular sludge, and heat treated inoculum.

In both the batch tests conducted, the F/M ratio was 0.5 in terms of VS, and the quantities of glucose and sludge used were the same for both. A phosphate buffer solution was put in all the tests, and the starting pH was approximately 6 for all the bottles. This conditions allowed to connect the differences in biogas quality and quantity to the type of treatment the inoculum has undergone previously. Results in terms of biogas produced are given in Figures 3 and 4.

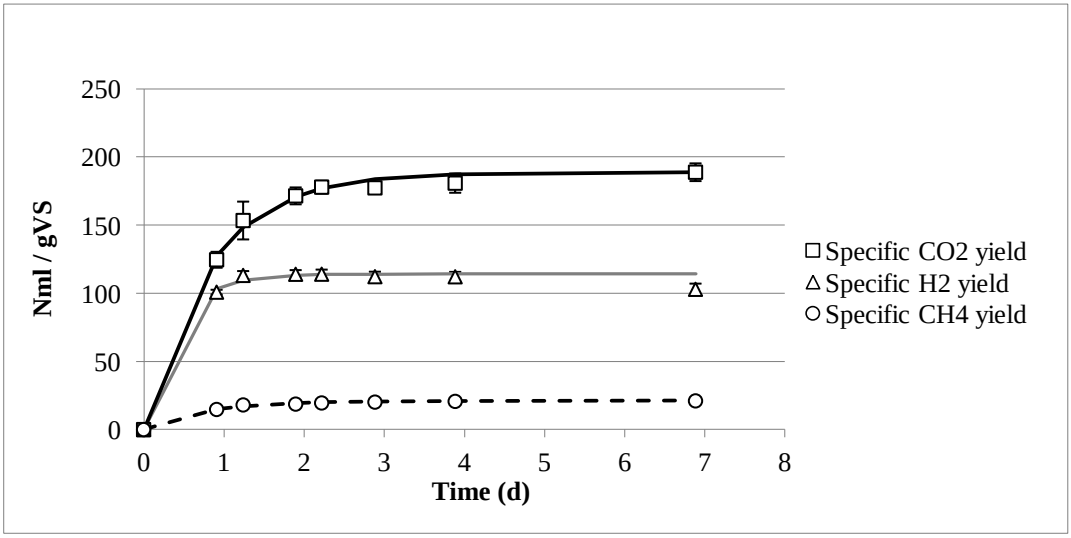

Figure 3 - Specific carbon dioxide, hydrogen and methane yield in biogas production from batch tests using glucose as substrate and raw sludge as inoculum.

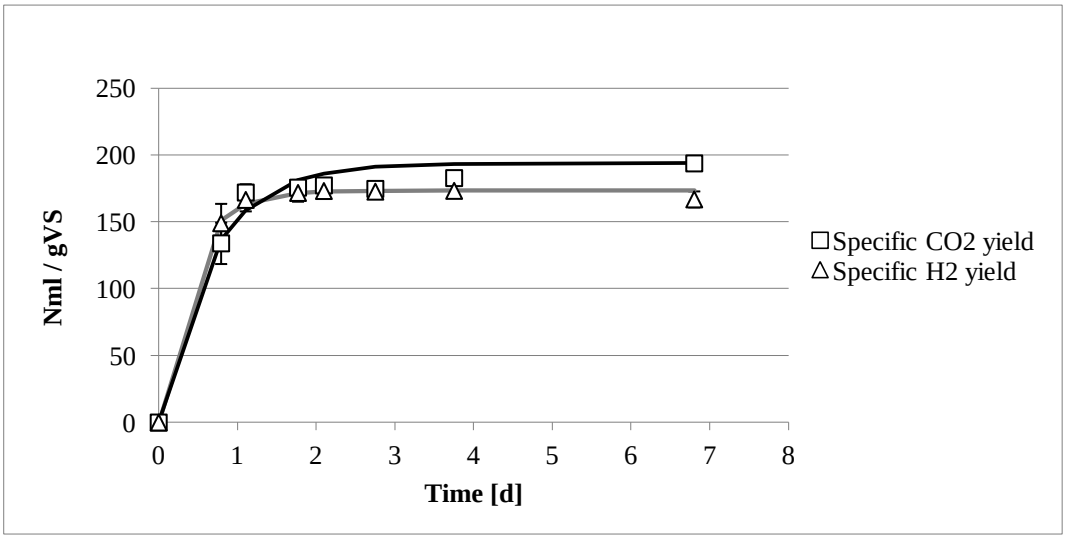

Figure 4 - Specific carbon dioxide and hydrogen yield in biogas production from batch tests using glucose as substrate and thermally treated sludge as inoculum.

The cumulative hydrogen production obtained was higher for the thermal treated inoculum. It resulted to be 173 Nml H_2/ gVS, corresponding to 1.39 mol H_2/ mol $C_6H_{12}O_6$, compared to the 114 Nml H_2/ gVS of the raw sludge, corresponding to 0.92 mol H_2/ mol $C_6H_{12}O_6$ introduced. The carbon dioxide production in the two experiments was comparable, having respectively 1.56 mol CO_2/ mol $C_6H_{12}O_6$ from heat treated inoculum and 1.52 mol CO_2/ mol $C_6H_{12}O_6$ from non treated inoculum. In the experiments carrying raw sludge it was possible to see that a certain amount of methane was also produced, equal to 21 Nml CH_4/ gVS. On the other hand zero methane production was detected with heat treated inoculum for all the duration of the experiment, that was about one week. The production of hydrogen from raw inoculum was in contrast with results obtained by *Alibardi et al.*, (*2012*), that register zero hydrogen production from batch tests using glucose as substrate and raw granular sludge as inoculum at pH 5.5.

Results obtained from the batch using glucose as a substrate are in accordance, and even higher, if compared to literature values that report hydrogen yield from batch tests equal to 0.968 mol H_2/ mol $C_6H_{12}O_6$ (*Oh et al., 2003*), or 0.92 mol H_2/ mol $C_6H_{12}O_6$ (*Logan et al., 2002*). On the other hand, *Alibardi et al*., (*2012*) report a production of 2.14 mol H_2/ mol $C_6H_{12}O_6$ using anaerobic granular sludge thermally treated for 4 hours in batch tests. In this case the differences in production can be attributed to the differences in the characteristics of the inoculum used, that is responsible of the bacterial communities that can survive and adapt to batch test conditions.

Anyway, since the reactors and the process conditions used can be really different from one experience to another, Table 5 reports some hydrogen yields found in literature from both continuous and batch systems, and the principal process conditions.

Table 5 - Literature values on hydrogen production yield from glucose in different processes.

Authors	Hydrogen yield	Process parameters
Alibardi et al., 2012	2.14 mol H_2/mol $C_6H_{12}O_6$	Anaerobic granular sludge, Thermal treatment at 100 °C for 4 hours Batch tests at pH 5.5
Xie at al., 2008	2.75 mol H_2/mol $C_6H_{12}O_6$	Activated sludge boiled 30 min, Continuous stirred reactor 10 g/l glucose, F/M=2:1, pH 6
Oh et al., 2003	0.968 mol H_2/mol $C_6H_{12}O_6$ 0.74 mol H_2/mol $C_6H_{12}O_6$ 0.596 mol H_2/mol $C_6H_{12}O_7$ 0.484 mol H_2/mol $C_6H_{12}O_8$	Heat treated granular sludge, pH 6.2 Heat treated granular sludge, pH 7.5 Raw granular sludge, pH 6.2 Raw granular sludge, pH 7.5 Batch tests
Logan et al., 2002	0.92 mol H_2/mol $C_6H_{12}O_8$	Batch tests, pH=6 Heat shocked soil at 104°C for 2 houres
Giordano et al., 2011	1.58 mol H_2/mol $C_6H_{12}O_8$ (calculated)	Anaerobic granular sludge, Thermal treatment at 100 °C for 4 hours Batch tests at pH 7

The results obtained confirmed the effectiveness of heat treatment as a way to enhance hydrogen production in dark fermentation process, by methanogenic bacteria suppression, and suggested the possibility to extend the use of thermally treated inoculum to the treatment of other more complex substrates, as it is for mixed biodegradable organic wastes (*Alibardi et al., 2012*).

Hydrogen production from glucose was performed also in a batch stirred reactor. The system was fed with sludge and glucose in the same proportions as in the batch tests. The phosphate buffer solution was used to fill the reactor working volume, that started from the same pH value as the batch tests, so approximately 6. Then, two process conditions were tested: the first one without pH control, the second one with pH control thanks to sodium hydroxide. PH was continuously monitored thanks to a pH meter inserted in the reactor plug, and through an injector some drops of sodium hydroxide were automatically added in the bottle when the pH was falling below 5.5.

Results concerning the specific hydrogen production yield and pH monitoring for the two process conditions are given in Figure 5. A comparison of the main process results is provided in Table 6. The results obtained displaced an higher specific hydrogen production for the uncontrolled pH test, that was 152 Nml H_2/ gVS, corresponding to 1.22 mol H_2/ mol $C_6H_{12}O_6$. The total biogas production associated was 380 Nml biogas/ gVS. On the other hand the cumulative hydrogen production decreased to 142 Nml H_2/ gVS for the controlled pH test, corresponding to 1.14 mol H_2/ mol $C_6H_{12}O_6$, with a total biogas production of 344 Nml biogas/ gVS. Final pH reached in the case of uncontrolled pH process was approximately 4.86, while in the case of sodium hydroxide addition, an higher final pH was achieved, that was 5.7.

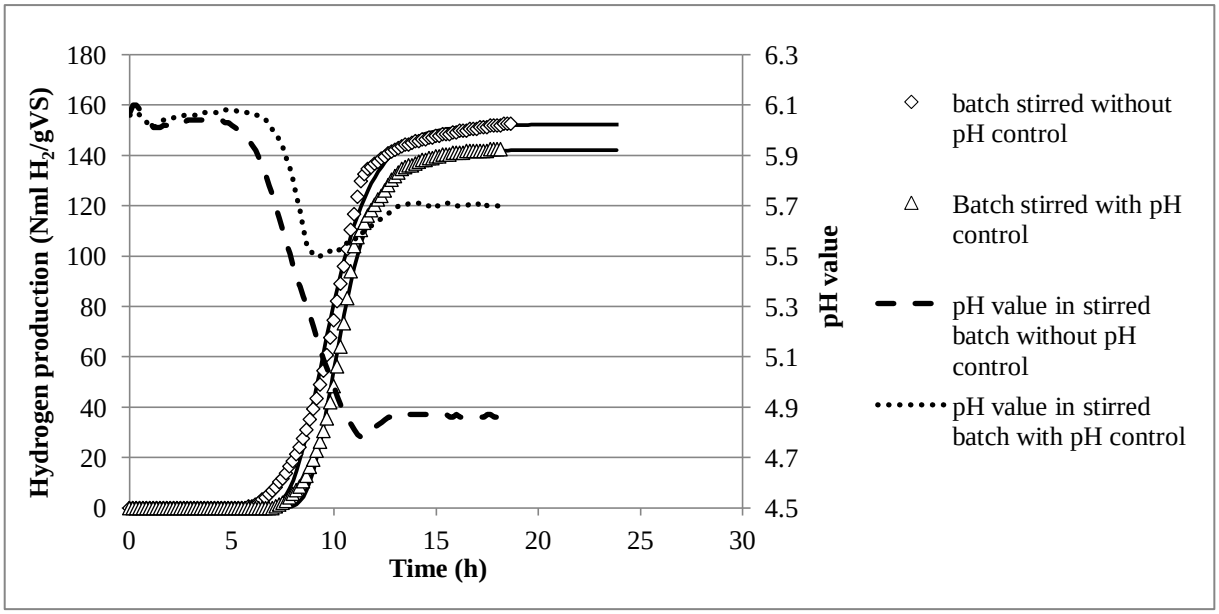

Figure 5 - Comparison between hydrogen productions from glucose in a batch stirred reactor with and without pH control. Symbols represent experimental data, continuous line the mathematical model.

Table 6 – Comparison of the main results obtained from hydrogen production tests in continuously stirred reactor without and with pH control.

Process type	pH control	Nml H_2/gVS	mol H_2/mol $C_6H_{12}O_6$	Nml H_2/gVS/h	Nml biogas/ gVS	H_2 (%)
Batch stirred	no	152	1.22	40	380	40.2
Batch stirred	yes	142	1.14	45	344	41.5

PH drop started in correspondence of hydrogen gas production beginning, and reached a point of minimum where the velocity of hydrogen production was faster. Then pH had a rise, remaining quite stable until the end of the reaction.

The results obtained for the stirred batch reactor in term of hydrogen production were lower compared to the yield obtained with the batch test, even if specific biogas productions were comparable to the ones of the batch tests (352 Nml biogas/ gVS in the batch). The major production of carbon dioxide is probably due to the fact that the reactor is continuously stirred, and this can favour the release in the biogas of carbon dioxide present in the liquid, that remains trapped in the liquid volume in the case of batch reactors. The higher hydrogen yield obtained in batch tests compared to the continuous biogas removal of the stirred reactor is in contrast with results described by *Logan et al.*, (*2002*). In their research, they report an increase of 43% in hydrogen production with continuous gas release, compared to the intermittent pressure release in batch tests, thanks to the decrease of hydrogen partial pressure in the reactor headspace, that can lead to hydrogenase activity inhibition.

Samples of liquid taken at the end of the reactions were analyzed for obtaining VFAs concentrations at the end of biogas production, and results are reported in Table 7.

Table 7 – VFAs analyzed at the end of each run in batch stirred reactor for glucose.

VFAs	Glucose	
	pH control	without pH control
	VFAs (mg/l)	VFAs (mg/l)
Acetic	502	375
Propionic	18.9	17.4
Isobutyric	<10	<10
Butyric	639	515
Isovaleric	14.9	<10
Valeric	<10	<10
Isocaproic	43.8	<15
Caproic	<15	<15
Eptanoic	<15	<15

VFAs dissolved in the liquid were higher for pH controlled system, corresponding to approximately 33% of the inlet COD, while for the pH uncontrolled system, even if hydrogen yield was higher, the amount of VFAs dissolved was lower, and corresponding to 24% of the initial COD. The amount of initial COD ending to VFAs was lower than the one measured by *Oh et al.*, (*2003*), who were working with heat treated inoculum at a pH 6.2, obtaining 51% of the introduced COD ending in VFAs. The quality of the VFAs measured was in any case moved toward acetic and butyric acid abundance for both the samples, that is the result of the ongoing of reactions that are producing hydrogen, as in equations (1), (2) and (3).

3.1.3 COD and TC balance on sequential hydrogen and methane production from glucose

After one week from the starting of hydrogen production in batch tests, the amount of biogas produced was approximately equal to zero. At this point, the bottles were opened and final pH were measured. Final pH values in the tests were similar: 5.28 was measured for raw sludge, while 5.25 was measured for thermally treated inoculum, and this means that the buffer was keeping quite well the pH of the reaction inside the bottle, even if there was a decrease of 0.5 pH points from the beginning of the test.

As reported by *Xie et al., (2008)*, residual solutions derived from hydrogen production contain volatile fatty acids, ethanol, acetic acid, butyric acid, a little propionic acid, valeric acid and caproic acid, which can be continually used in producing methane in a two phase anaerobic digestion process, and solve the low energy efficiency problems of hydrogen fermentation. In this way the pH of the tests was raised using sodium carbonate (Na_2CO_3) until 7.9, in order to allow the setting of conditions that can favour methane production.

Figure 6 and 7 report the COD balance, while Figure 8 and 9 report the TC balance respectively for tests fed with raw sludge and tests fed with heat treated inoculum.

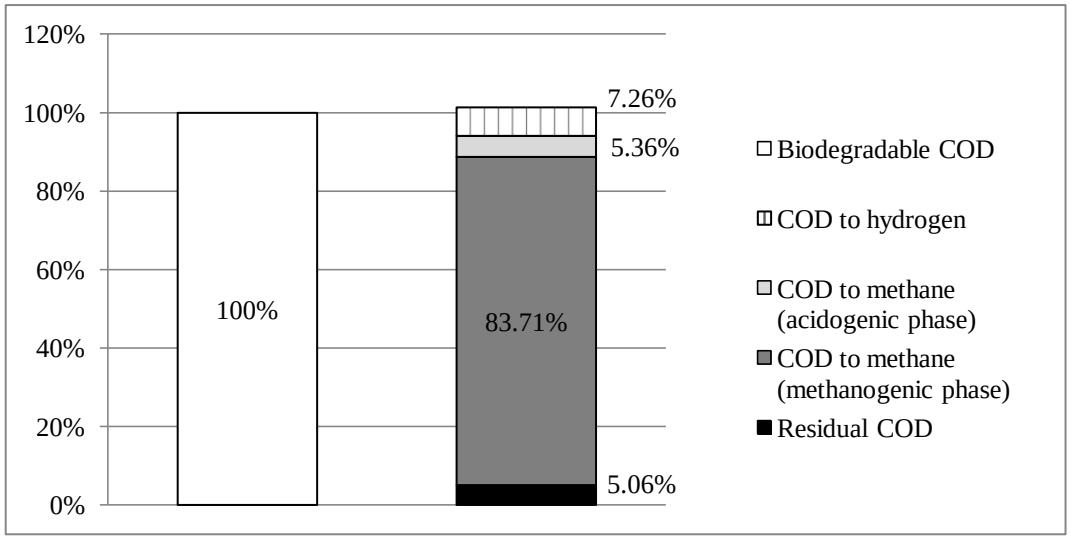

Figure 6 - COD balance in subsequent hydrogen and methane production from glucose using raw sludge as inoculum.

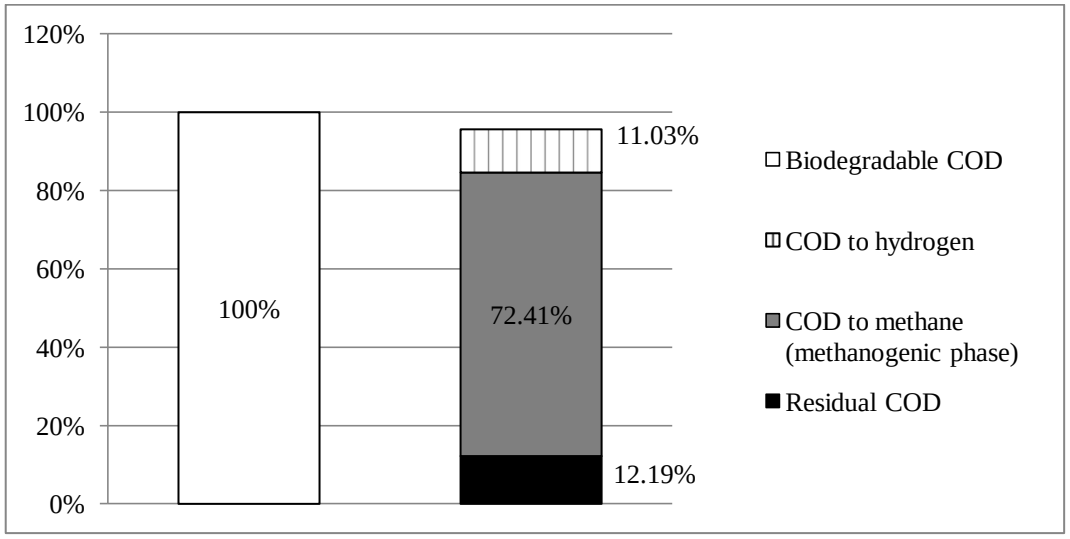

Figure 7 - COD balance in subsequent hydrogen and methane production from glucose using thermally treated sludge as inoculum.

Observing COD balance it is possible to see that the major part of the inlet COD has been completely gasified during the reaction period in both the tests. In raw sludge tests 89% of the inlet COD ended in overall methane production, while in treated inoculum tests methane yield was lower, and approximately equal to 72%. Methane production yield obtained in tests with thermally treated sludge resulted to be in accordance with results reported by *Xie at al., (2008),* concerning methane production subsequently to hydrogen production. These authors reported a production of 2.13 mol CH_4/mol $C_6H_{12}O_6$, while the methane production in this work was equal to 2.28 mol CH_4/mol $C_6H_{12}O_6$ (284 Nml CH_4/ gVS). Looking at the first acidogenic phase, hydrogen yield was higher for thermally treated inoculum, where no methane was produced. COD released as hydrogen was equal to 7.26% in raw sludge tests, and equal to 11.03% with

thermally treated inoculum. In any case, summing the amount of COD removed as methane and hydrogen for the raw inoculum (12.63%), and comparing it with the amount of COD removed as hydrogen in the thermally treated sludge (11.03%), it can be seen that the amount of COD removed in the fermentative phase was higher for the untreated inoculum.

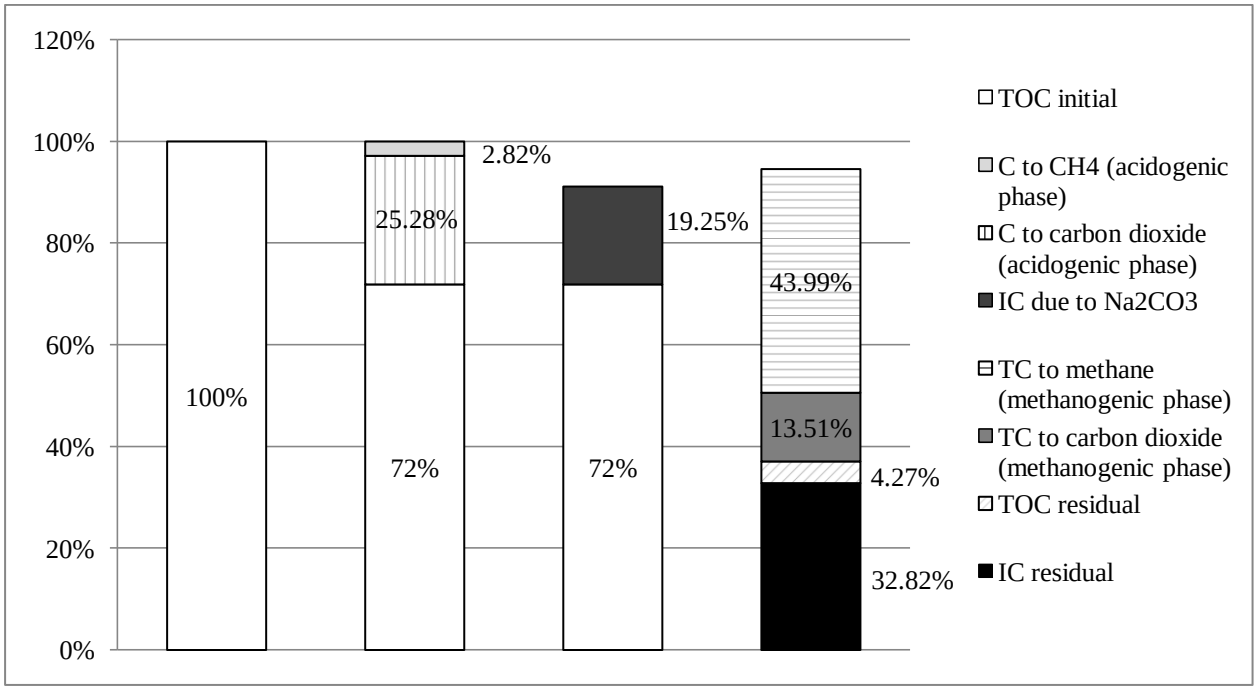

Figure 8 - TC balance in sequential hydrogen and methane production from glucose using raw sludge as inoculum.

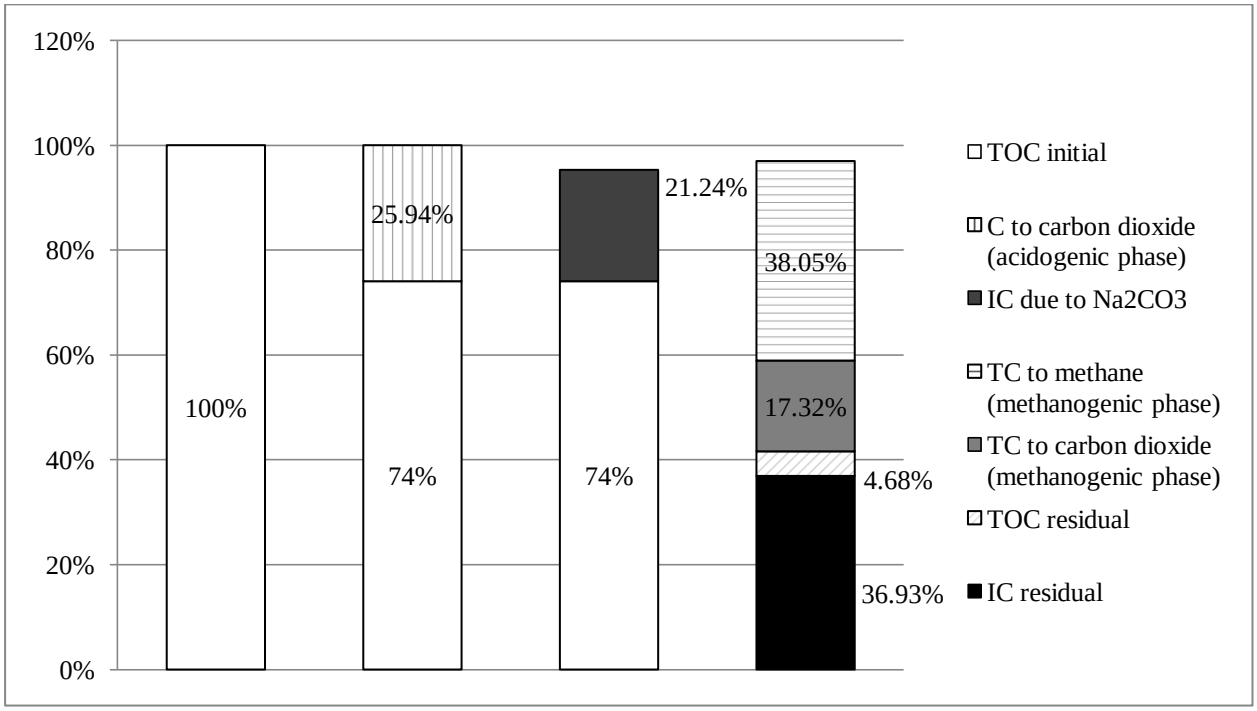

Figure 9 - TC balance in subsequent hydrogen and methane production from glucose using thermally treated sludge as inoculum.

This means not only that some hydrogenotrophic methanogenic bacteria are still active and are consuming hydrogen, but also that there are acetoclastic methanogenic bacteria that are probably using other dissolved acids to produce further methane. In this way the thermal treatment of the sludge becomes fundamental to inactivate methanogenic activity, considering that, in this batch system, low pH was not sufficient to inhibit methane production.

The COD removal in the two phases, using thermally treated sludge, was consistent with the experience made by *Giordano et al.*, (*2011*), that reports a removal of 13.3% of initial COD as hydrogen and a removal of 75.5% of initial COD as methane.

Table 8 - Hydrogen, carbon dioxide and methane yield from glucose in the different batch tests performed in Nml gas/ gVS.

Reactor type	**Inoculum treatment**	**Acidogenic phase**			**Methanogenic phase**	
		H_2 yield (Nml H_2/ gVS)	**CO_2 yield (Nml CO_2/ gVS)**	**CH_4 yield (Nml CH_4/ gVS)**	**CO_2 yield (Nml CO_2/ gVS)**	**CH_4 yield (Nml CH_4/gVS)**
BHP-BMP	thermal	173	194	-	129	284
BHP-BMP	raw	114	189	21	101	329
BMP	raw	-	-	-	385	371

The analysis performed in terms of Nml gas/gVS as reported in Table 8 and the TC balance allowed to say that an important advantage of performing a double stage process instead of a single BMP test is the final quality of biogas obtained. In subsequent tests, during the first phase of hydrogen production a certain amount of carbon dioxide was already released from the substrate, equal respectively to 194 Nml CO_2/gVS with thermal treated sludge, and 189 Nml CO_2/gVS for raw sludge. This amount already removed from the system is not going to dilute final methane produced in the second digestion phase. Comparing the biogas quality obtained from the direct BMP (49% CH_4 and 51% CO_2) and the final quality in the double stage processes, it is possible to see that methane is more concentrated in the last ones. In tests with thermally treated inoculum the final biogas quality was given by 69% methane and 31% carbon dioxide, while in tests with raw sludge final biogas quality was given by 77% methane and 23% carbon dioxide.

This result, even if higher methane yield was possible in the second methanogenic stage for the raw inoculum, allowed to define the possibility to use thermally treated sludge with other substrates, and in particular with three different organic waste mixes having different compositions, in order to maximize hydrogen production and to study the effects of this phase on subsequent methane production.

3.2 Organic waste samples

To run experiments in a significant way, it has been decided to construct three samples of organic waste mixture. To define the composition of the three samples, raw waste composition of the organic fraction of municipal solid waste was analyzed in different times of the year, at the same waste treatment plant and with the same procedures. Samples of OFMSW were collected from the waste receiving area of an anaerobic digestion plant treating organic waste located in Padova, Italy. Each time an amount of about 200 kg of waste was manually sorted and divided in the fractions "meat-fish-cheese", "fruits", "vegetables", "bread-pasta", "undersieve 20mm" and "rejected materials". The faction "rejects" consisted in materials as for example shoppers, paper, plastic and bones. The shoppers found during sorting procedures were the bags containing the waste. The materials identified as paper were napkins and kitchen paper while the materials identified as plastic were small containers or films. Animal bones, shells and fruits kernels were also considered as "rejects". All these materials are not or are very slowly biodegradable, therefore they were not used for the preparation of the samples for hydrogen and methane production tests. Organic waste composition analysis were performed on May 2009, February 2010, November 2010, July 2012 and October 2012. The sorted fractions were characterized for TS, VS, TOC, COD, TKN, NH_4^+ and P_{tot}.

Results in terms of raw waste percentages of the fractions are reported in Table 9, while Table 10 presents the fractions constituting the organic waste without considering the fraction "rejects".

Table 9 – Composition of the raw organic fraction of the municipal solid waste collected from a treatment plant located in the province of Padova at different times. Data are reported as percentages (%) of wet weight.

Fraction	May 2009	February 2010	November 2010	July 2012	October 2012
Meat-Fish-Cheese	6.8	10.2	12.0	0.3	3.4
Fruit	18.9	24.8	16.3	28.7	12.7
Vegetables	32.4	18.2	28.7	33.2	42.3
Pasta- Bread	7.7	12.3	4.4	1.3	8.0
Undersieve < 20 mm	15.0	13.0	16.4	17.5	16.6
Rejects	19.2	21.5	22.2	19.0	17.0
Total	100.0	100.0	100.0	100.0	100.0

Table 10 - Composition of the organic fraction of the municipal solid waste collected from a treatment plant located in the province of Padova at different times. Data are reported as percentages (%) of wet weight.

Fraction	May 2009	February 2010	November 2010	July 2012	October 2012
Meat-Fish-Cheese	8.4	13.0	15.4	0.4	4.1
Fruit	23.4	31.6	21.0	35.4	15.3
Vegetables	40.1	23.2	36.9	41.0	51.0
Pasta- Bread	9.5	15.7	5.7	1.6	9.6
Undersieve < 20 mm	18.6	16.6	21.1	21.6	20.0
Total	100.0	100.0	100.0	100.0	100.0

Results obtained from the sampling and sorting procedures carried out that the fractions "vegetables" and "fruit" constituted together even more than 50% of the organic wet waste. Their sum is ranging from a minimum of 54.8% in February 2010 until a maximum of 76.4% in July 2012. "Vegetable" is generally the highest fraction between these two. Oppositely "meat-fish-cheese" and "pasta-bread" are the two fractions that showed the lowest percentages in the organic waste samples. These different percentages are due to the different type of food consumed by households during different time periods: in summer it is easier to consume vegetables, fruits and seasonal products, while in winter it is easier to find pasta, meat, or man made food.

Observing the variation ranges of each organic waste fraction, without considering the rejects, it was possible to see that the fractions "meat-fish-cheese" and "pasta-bread" are that fractions that are varying the less from one sampling to another. "Meat-fish-cheese" is ranging from a minimum of 0.4% in July to a maximum of 15.4% in November, while "pasta-bread" was ranging from a minimum of 1.6% in July until 15.7% in February. Variability range of these two fractions is approximately the same. "Fruit" can vary from 15.3% to 35.4% in the organic waste composition, while "vegetable" is the fraction that is varying the most, from 23.2% to 51%. In any case their amount are quite high for all the year. "Undersieve" fraction is remaining quite constant and approximately equal to 20% of the organic waste composition during all the year.

The results obtained from the chemical characterisation of the sample of OFMSW and its fractions collected in July 2012 are reported in Table 13. The results underlined that the fractions "meat-fish-cheese" and "pasta-bread" showed the highest values of TS and VS if compared to the other fractions. They showed also the highest values of TOC and COD. On the other hand the fractions "fruit" and "vegetables" displaced the lowest content in TS and VS. "Fruit" was displacing the lowest COD and TOC content.

These characteristics suggest that the changes in wet quantities of each fraction, are not caring to the total waste mixture an equal change in terms of VS content. For the fractions "meat-fish-cheese" and "pasta-bread", smaller changes in their percentages in the raw waste, will probably end in a great change in VS content of the mixture. On the other hand, it can be derived that changes in the raw waste of "vegetable" and "fruit" quantity, will not affect so much the TS and VS content of the overall mixture. The fraction "undersieve" was displacing physical chemical characteristics similar to the ones of the fractions fruit and vegetables, this fraction being composed for the major part by residues of fruit and small parts of vegetables, but also coffee grounds, maize, cracked eggshells and undetectable material smaller than 20 mm.

Similar results were obtained during previous analytical campaigns. The results are reported in Table 12 .

Table 11 - Characterization of the single fractions of OFMSW sampled in July 2012.

Parameter	Meat-Fish-Cheese	Fruit	Vegetables	Pasta-Bread	Undersieve
TS (%)	54 ± 2	10 ± 2	15 ± 2	64 ± 2	22 ± 2
VS (% of TS)	97 ± 1	89 ± 1	91 ± 1	96 ± 1	86 ± 1
TOC (% of TS)	67 ± 1	46 ± 1	52 ± 1	47 ± 1	47 ± 1
COD (mgO_2/gTS)	1976 ± 5	1068 ± 5	1352 ± 5	1490 ± 5	1341 ± 5
TKN (mgN/gTS)	26.3 ± 0.5	19.8 ±0.5	30.6 ± 0.5	18.3 ± 0.5	29.5 ± 0.5
NH_4^+ (mgN/gTS)	3.2 ± 0.5	2.8 ± 0.5	4.3 ± 0.5	3.6 ± 0.5	4.5 ± 0.5
P_{tot} (mgP/gTS)	3.3 ± 0.5	2.6 ± 0.5	3.8 ± 0.5	1.7 ± 0.5	5.8 ± 0.5

Table 12 – Average physical chemical characteristics obtained from previous campaigns.

Parameter	Meat-Fish-Cheese	Fruits	Vegetables	Pasta-Bread	Undersieve
TS (%)	57 ± 6	17 ± 7	19 ± 3	71 ± 5	34 ± 12
VS (% $_{of\ TS}$)	95 ± 1	90 ± 3	89 ± 5	95 ± 3	81 ± 7
TOC (% $_{of\ TS}$)	60 ± 6	46 ± 1	51 ± 5	50 ± 3	45 ± 3
COD (mgO_2/gTS)	1906 ± 74	1261 ± 180	1403 ± 200	1598 ± 120	1325 ± 30
TKN (mgN/gTS)	40.2 ± 12.4	16.1 ± 3.3	25.3 ± 9.6	17.5 ± 0.9	27.3 ± 4.1
P_{tot} (mgP/gTS)	4.8 ± 2.0	2.0 ± 1.2	3.3 ± 1.3	1.8 ± 0.3	3.7 ± 1.8

Associating the variability range of each fraction with their physical chemical characterization, three different organic waste mixes have been constructed, considering all the fractions excluding the rejects. These mixes can represent possible real waste mixtures, with raw percentages of the different fractions that are ranging in the interval found in historical waste data, but are characterized by a different main source of their VS, being imbalanced toward a specific waste fraction.

The three constructed sample are named Mix 1, Mix 2 and Mix 3, and their composition in terms of raw waste percentages is presented in Table 13. Table 14 presents the composition of the three mixtures in terms of VS. Mix 1 was characterized by a VS content that was given for its 50% by the fraction “pasta-bread”, while Mix 2 was characterized by a VS content derived from “meat-fish-cheese” for its 50%. Mix 3 was having an intermediate amount of this two fractions. The fractions “undersieve”, “vegetable” and “fruit” were kept approximately constant for all the mixtures.

Table 13 – Composition of the mixture (g raw fraction/ g of raw mixture).

Fraction of mixture (on raw waste basis)	Mix 1 (%)	Mix 2 (%)	Mix 3 (%)
Meat-Fish-Cheese	2	21	7
Fruit	27	26	26
Vegetables	34	33	32
Pasta- Bread	19	2	12
Undersieve < 20 mm	18	18	22

Table 14 - Composition of the mixture (gVS of fraction / gVS of mixture).

Fraction of mixture (on VS basis)	Mix 1 (%)	Mix (%)	Mix 3 (%)
Meat-Fish-Cheese	5	50	17
Fruit	10	10	10
Vegetables	20	20	20
Pasta- Bread	50	5	34
Undersieve < 20 mm	15	15	19

After the construction, the samples of organic waste mixtures were characterized for the same parameters and with the same procedures that were already used for the single fractions. Results are reported in Table 15.

Table 15 - Physical chemical characterization of the mixtures.

Parameter	Mix 1	Mix 2	Mix 3
TS ($\%_{Mix}$)	9 ± 1	8 ± 1	9 ± 1
VS ($\%_{of\ TS}$)	93 ± 1	93 ± 1	92 ± 1
TOC ($\%_{of\ TS}$)	50 ± 1	61 ± 1	55 ± 1
COD (mgCOD/gTS)	1829 ± 5	2215 ± 5	1868 ± 5
TKN (mg-N/g-TS)	23.8 ± 0.5	27.4 ± 0.5	25.1 ± 0.5
NH_4^+ (mg-N/g-TS)	3.7 ± 0.5	3.7 ± 0.5	3.8 ± 0.5

The mixtures were characterized by a VS content that was more or less the same for them all, even if their origin was from different organic waste fractions. Approximately the same TKN and NH_4^+ concentrations had been reported too. On the other hand TOC and COD were sensitively changing from one mix to another: Mix 2 was reporting the highest content, while Mix 1 the lowest. Mix 3 is having intermediate characteristics between Mix 1 and Mix 2.

3.3 Methane production from organic waste

Direct Biochemical Methane Potential (BMP) were performed to evaluate the maximum production of methane from the three organic waste mixtures. Cumulative methane productions obtained for the three mixtures are displaced in Figure 10. The results obtained showed an higher specific methane production from Mix 2, equal to 586 Nml CH_4/ gVS. Mix 1 was displacing the lowest methane production, equal to 407 Nml CH_4/ gVS, while an intermediate production was obtained from Mix 3, equal to 490 Nml CH_4/ gVS. The results obtained are in accordance with COD and TOC values measured for the three sample during the physical chemical characterization. Mix 2 was displacing the highest values for COD and TOC, so it is also coherent that the cumulated amount of biogas, and so of cumulated methane, is higher compared to the other mixtures.

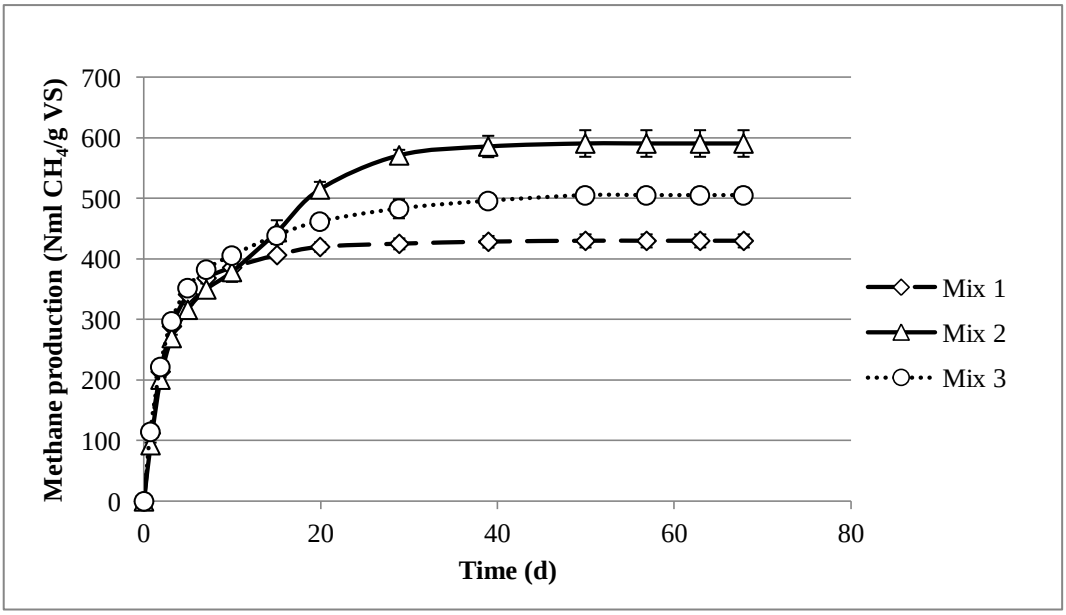

Figure 10 - Direct specific methane production from Mix 1, Mix 2 and Mix 3 in batch reactors (Nml CH_4/ gVS substrate)

In the same way, Mix 1, that was displacing the lowest COD and TOC values, is presenting the lowest methane yield, while Mix 3 was presenting intermediate conditions that are reflected in biogas production too. The fact that COD values are reflected in the final methane production is due to the role that methane is acting in the anaerobic digestion process. Methane is always the final electron acceptor (*McCarty, 1964*), and soon or later the inlet COD degradation will end in this final product. That is also why methane production from the single organic waste fractions can allow the prediction of a final waste mixture methane yield of which fractions' proportions are known (*Armaroli, 2013*).

As for the glucose, also for the three organic waste mixes analyzed a final COD and TC balance was performed for the BMP tests in batch reactors. Results in terms of COD balance are reported in Figure 11 for Mix 1, 13 for Mix 2 and 15 for Mix 3. Results for TC balance are reported in Figure 12 for Mix 1, 14 for Mix 2 and 16 for Mix 3.

As it is possible to see from the balances, the inlet COD and TOC introduced with the substrates were not completely ending in biogas production. The not biodegraded part of the COD and of the TOC was actually remaining particulate, since the residual dissolved CODs and TCs were really low or equal to zero. Part of that COD and TC should also have been used for bacterial growth, and so it is supposed not to have been released as gas. The highest amount of COD released as methane was given by the second substrate, while the lowest was given by Mix 1. Intermediate conditions were displaced by Mix 3.

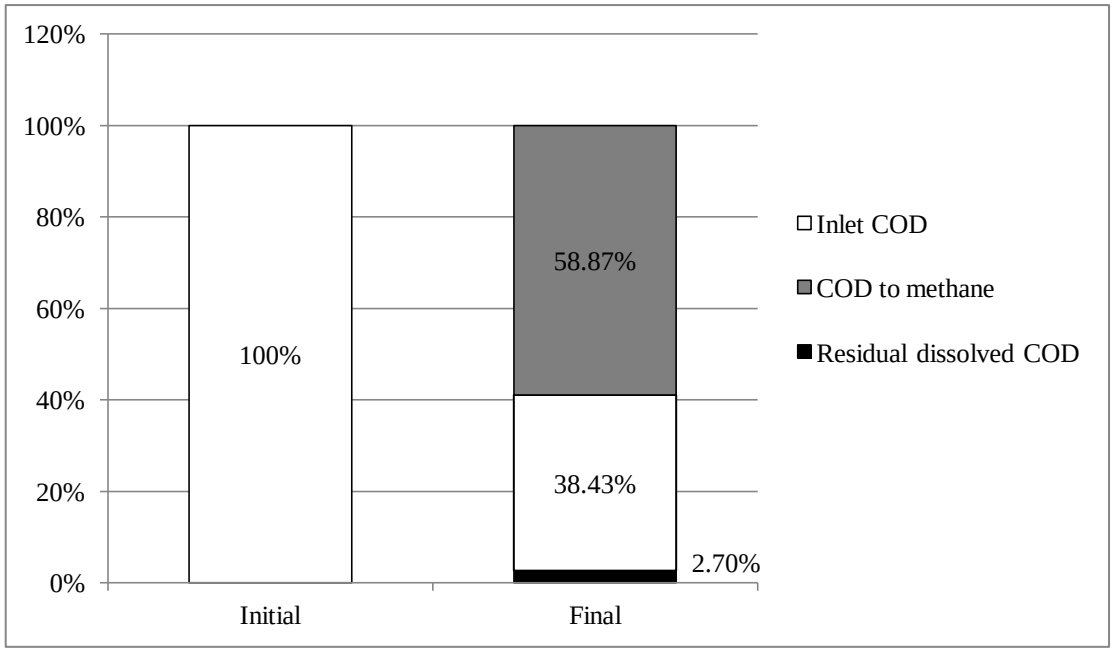

Figure 11 – COD balance in batch direct methane production from Mix 1.

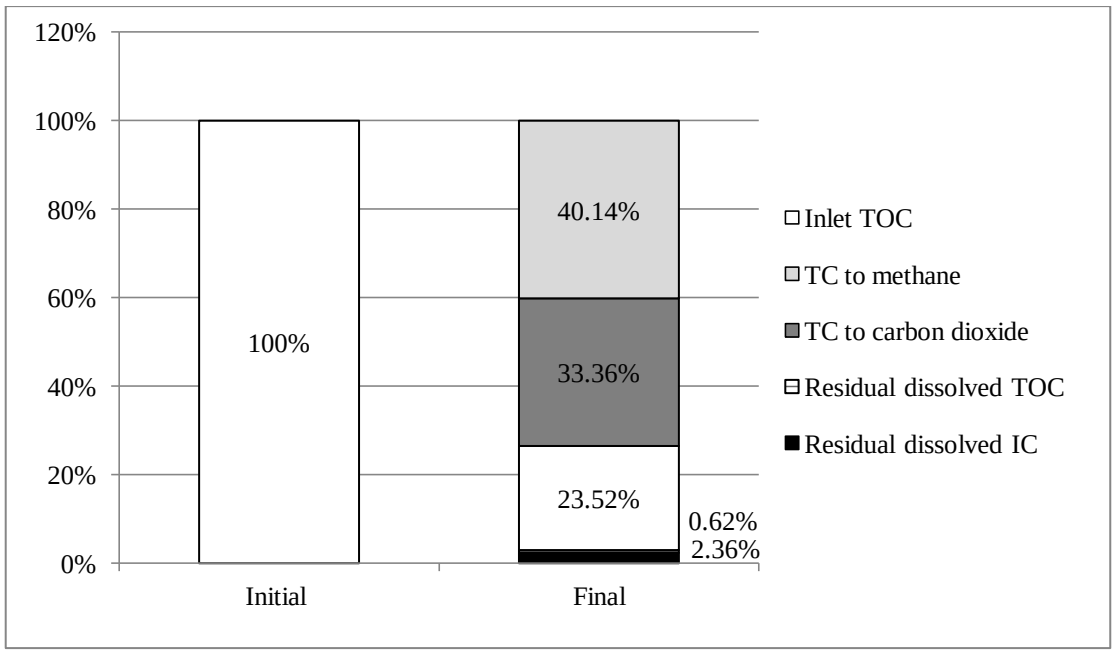

Figure 12 – TC balance in batch direct methane production from Mix 1.

From the observation of the TOC balances, it is possible to see how the quality of biogas produced is changing from one substrate to another. The ratio between carbon dioxide and methane cumulative production corresponds to the ratio between the volume of carbon dioxide and the volume of methane produced, and these values allow to obtain final methane concentration in the biogas. The highest methane concentration was the one given by Mix 2, that has produced a biogas containing 62% methane and 38% carbon dioxide.

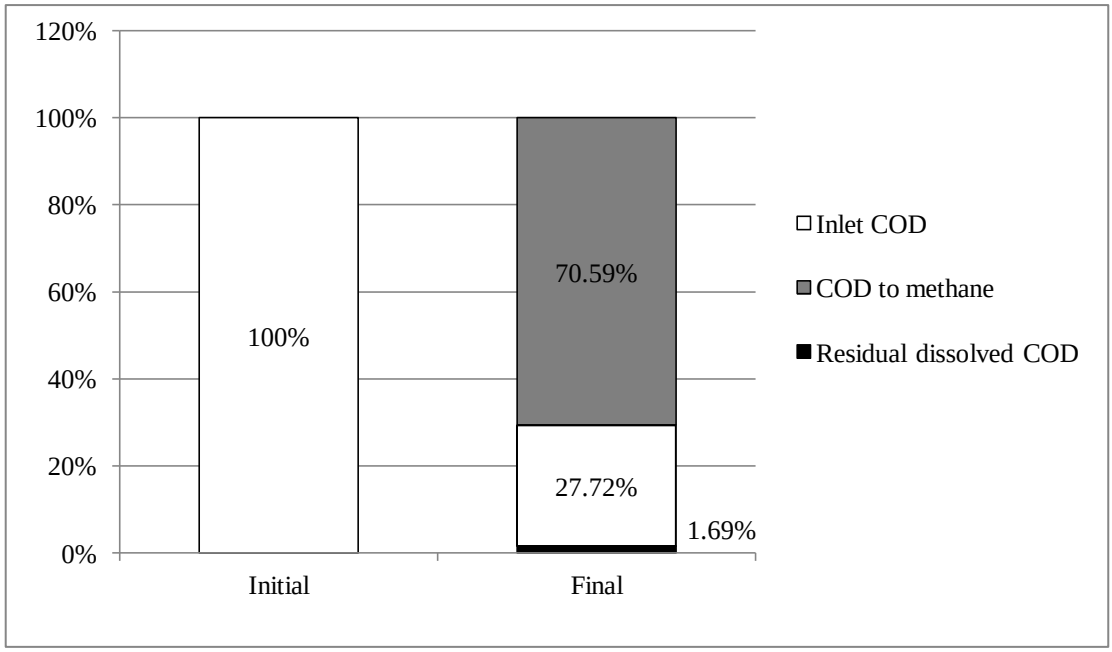

Figure 13 – COD balance in batch direct methane production from Mix 2.

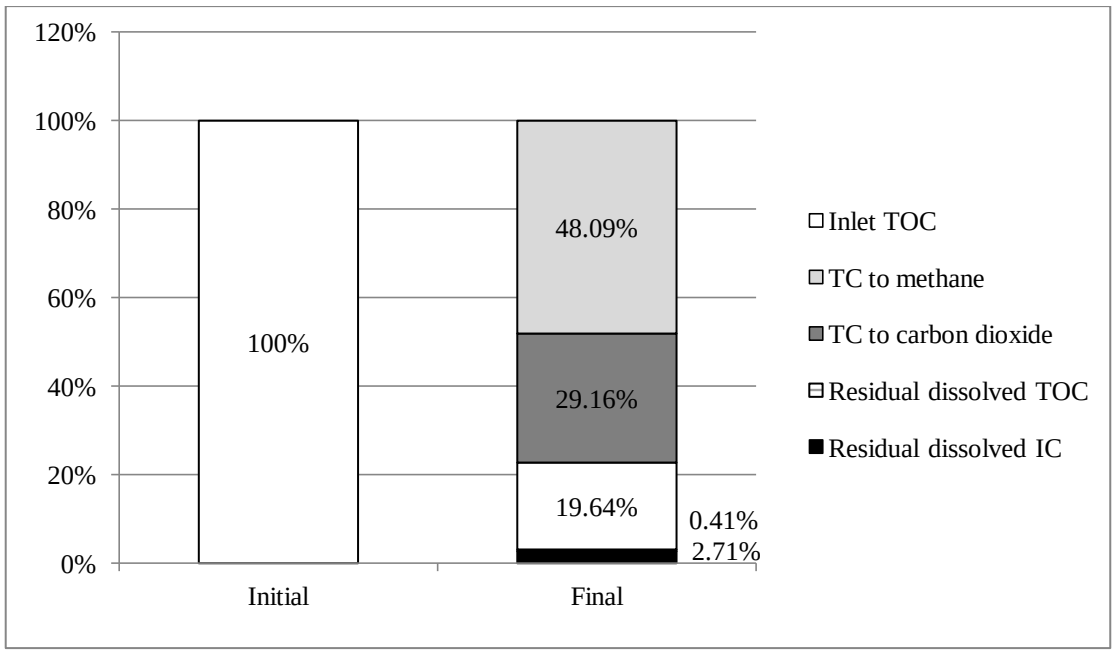

Figure 14 – TC balance in batch direct methane production from Mix 2.

In the case of Mix 1 and Mix 3 the biogas composition was similar, with methane concentration ranging from 43% in Mix 3 to 45% in Mix 1. The fact that in these operating conditions a part of the organic carbon introduced is not degraded, subtract the possibility to fully exploit the energy potential of the waste, and opens the research to new processes that can in some way increase the anaerobic digestion process efficiency, allowing the production of biogas that has different quality or produced in an higher amount.

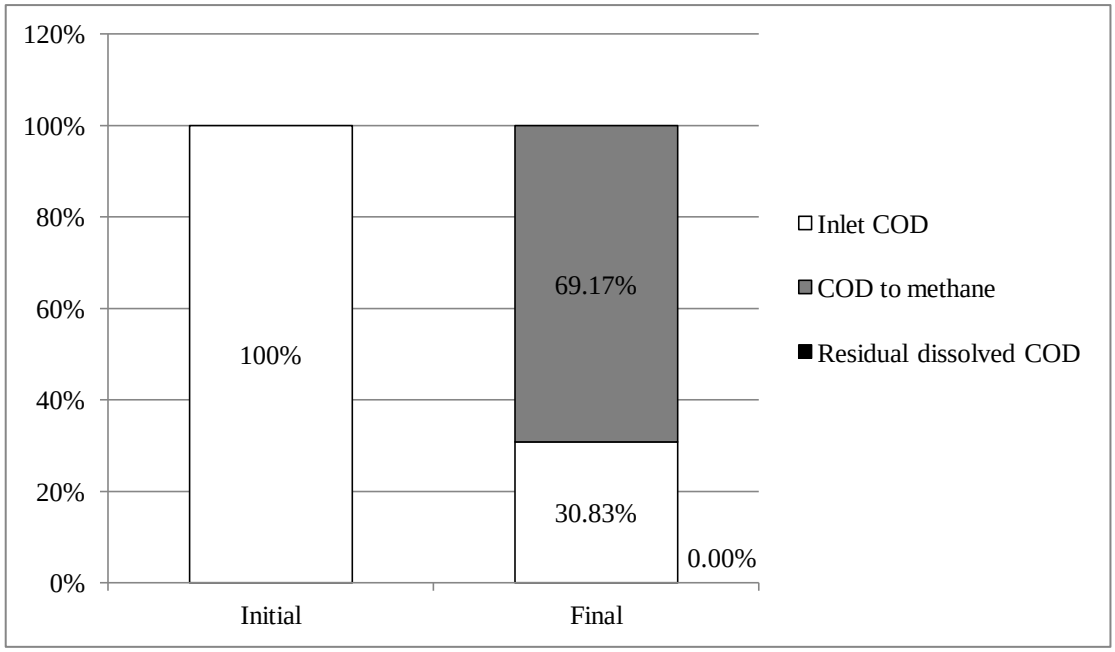

Figure 15 – COD balance in batch direct methane production from Mix 3.

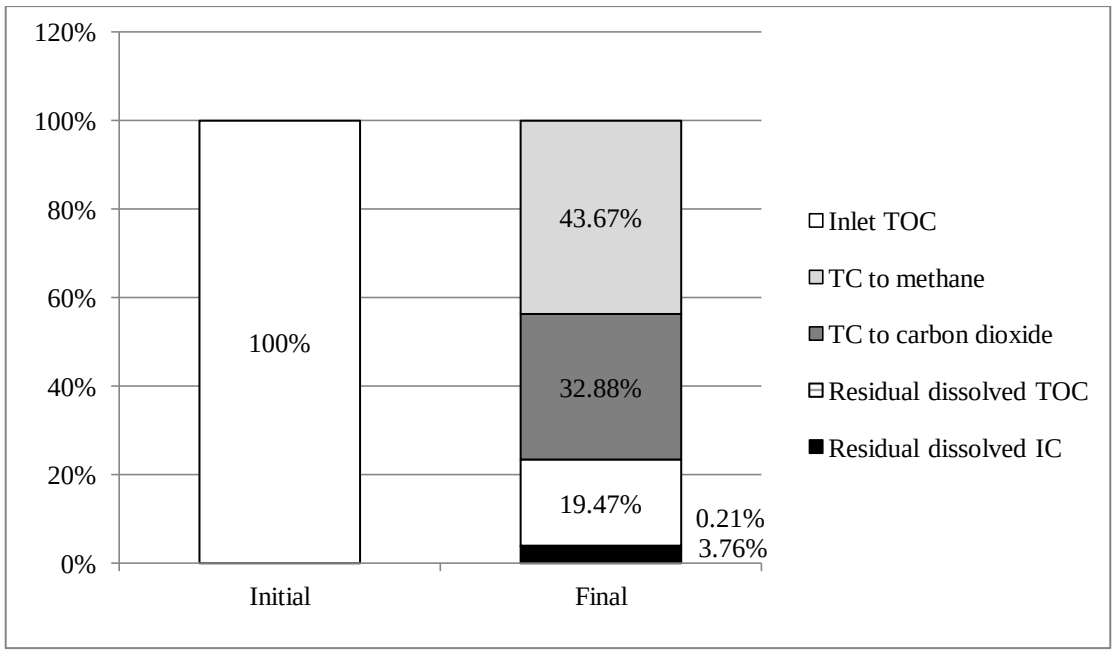

Figure 16 – TC balance in batch direct methane production from Mix 3.

3.4 Hydrogen production from organic waste

Hydrogen production from the three organic waste mixes was performed in batch tests with the aim of evaluating the hydrogen potential production under optimal fermentation conditions.

Cumulative hydrogen productions obtained for the three mixtures are displaced in Figure 17. Results indicate that the highest H_2 production was displaced by Mix 1 with a production that was equal to 99 Nml H_2/ gVS, its main component being carbohydrate fraction.

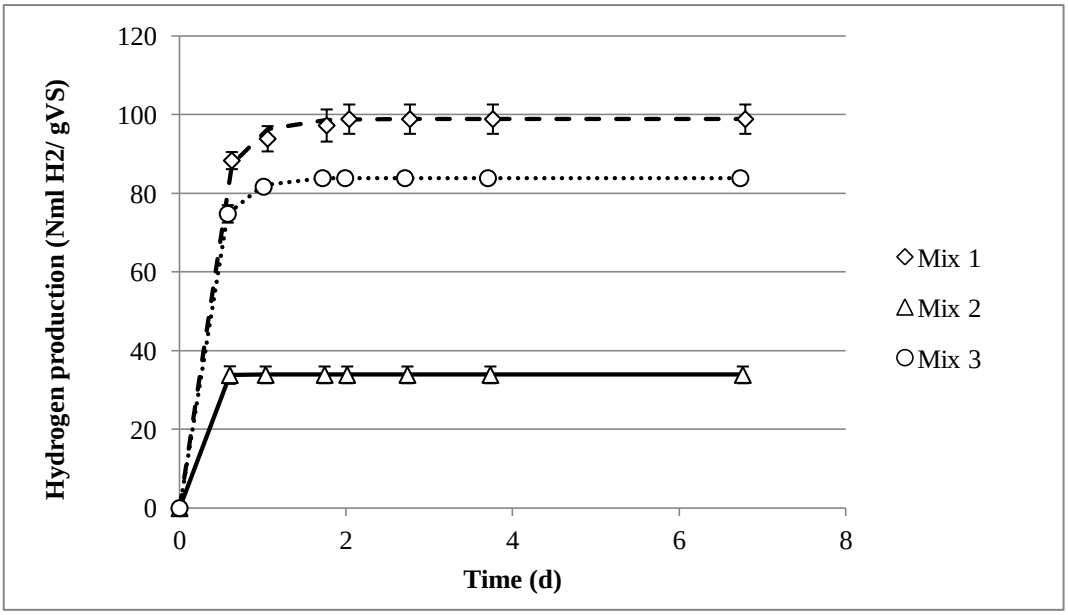

Figure 17 - Hydrogen specific production from Mix 1, Mix 2 and Mix 3 in batch reactors (Nml H_2/ gVS substrate).

The second substrate, with a production of 34 Nml H_2/ gVS, was the one that was producing less hydrogen, because the main source of its VS is represented by proteins. This result agrees with other research studies which report that carbohydrates rich substrates have higher hydrogen yields than protein and lipids rich substrates (*Okamoto et al., 2000; Kobayashi et al., 2012*). Mix 3 was displacing a production of 84 Nml H_2/ gVS, that is intermediate between Mix 1 and Mix 2, as expected from its VS composition, that has intermediate characteristics between the other two substrates constructed.

From the results obtained it is possible to see that hydrogen production in acidogenic conditions from organic waste is not directly proportional to the inlet COD and TOC that the sample are presenting, how it was happening for methane production in paragraph 3.3. This is due to the fact that hydrogen is not the final electron acceptor in the anaerobic digestion process, but it is an intermediate product (*Alibardi et al., 2012*), which results from fermentation and acetogenesis, and whose production can depend on different factors, as for example the kind of substrate to be degraded, the inoculum, the pH and the temperature (*Armaroli, 2013*).

An important observation that can be done considering cumulated hydrogen production curves realized for each batch test, is that the maximum of cumulative hydrogen production was obtained for all the organic waste mixes after less than 2 days of experimental operation, then the cumulated curves were considered constant. Actually the concentration of hydrogen started to decrease in the bottles at that time, and this phenomenon was due to the presence in the bacterial community selected with thermal treatment of spore forming hydrogen consuming bacteria. Thermal treatment is widely used because of the great efficiency in methanogenic inhibition.

However it does not select exclusively for hydrogen producing bacteria: non spore forming hydrogen producers may be inactivated, while spore forming hydrogen consuming bacteria can survive (*Alibardi et al., 2012*).

As reported by *Oh et al.*, (*2003*), and by *Park et al.*, (*2005*), substantial hydrogen losses could occur via acetogenesis, that is not prevented by heat treatment. Homoacetogenic bacteria can use hydrogen and carbon dioxide to produce acetate and water, causing the decrease of hydrogen gas accumulated in the headspace. The fact that hydrogen consumption was due to this process is supported by the undetectable level of methane in the batch tests.

The dissolved COD for each substrate was proportional to the amount of gas released as hydrogen (Figure 18, 20, and 22). Dissolved CODs at the end of hydrogen production for Mix 1, Mix 2 and Mix 3 were respectively equal to 36.03%, 26.53% and 35.92% of the respective CODs introduced in the bottles. The VFAs measured for Mix 1, Mix 2 and Mix 3 were respectively equal to 47.40%, 28.75% and 36.38% of the dissolved COD measured. This fact suggests that the compounds that were forming the dissolved COD were probably formed as byproducts from biohydrogen production during the fermentation phase. In fact the higher was the hydrogen production, the higher was the ratio between dissolved COD and inlet COD. According to VFAs analysis, the short chain acids that have been measured with the GC were respectively acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, eptanoic acid. Results obtained were showing that in the degradation of Mix 1, VFAs were representing more or less 50% of the dissolved COD, while in proteic substrates as Mix 2, byproducts that are formed from the degradation are probably compounds that are not measured with the GC, or that can be longer chain fatty acids. The effect of substrate composition on hydrogen production and VFAs formation was further analyzed thanks to a batch stirred reactor, as it is treated in paragraph 3.6.

3.5 Sequential hydrogen and methane production from organic waste

COD balances (Figures 18, 20 and 22) and TC balances (Figures 19, 21 and 23) allow to confirm and to visualize what has already been said in paragraph 3.4 relative to dissolved COD at the end of the acidogenic phase. Each one of the three substrates was displacing a COD solubilization proportional to the amount of COD released as hydrogen. TC balances are confirming in the same way a proportionality between hydrogen production and soluble TOC at the end of the first phase. The amount of COD removed as hydrogen is coherent with the results obtained by Okamoto et al., (2000), that reports a removal of 4% of the initial COD as hydrogen using as source carbohydrates, and a removal of 0.2% and 0.1% using lipids and proteins.

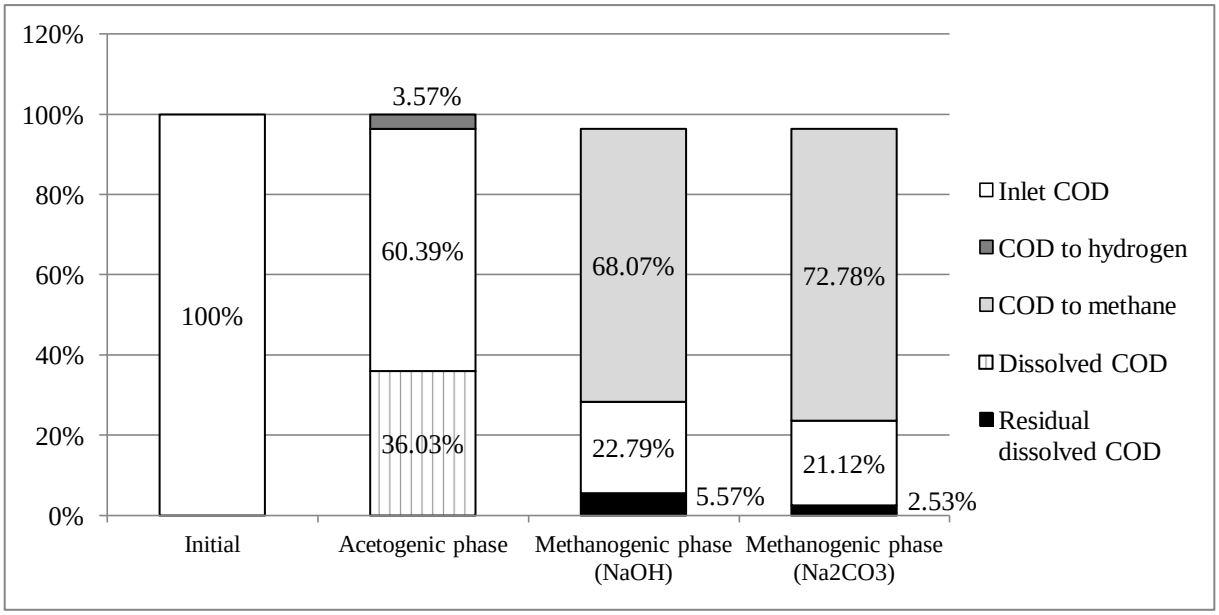

Figure 18 – COD balance in double stage anaerobic digestion from Mix 1.

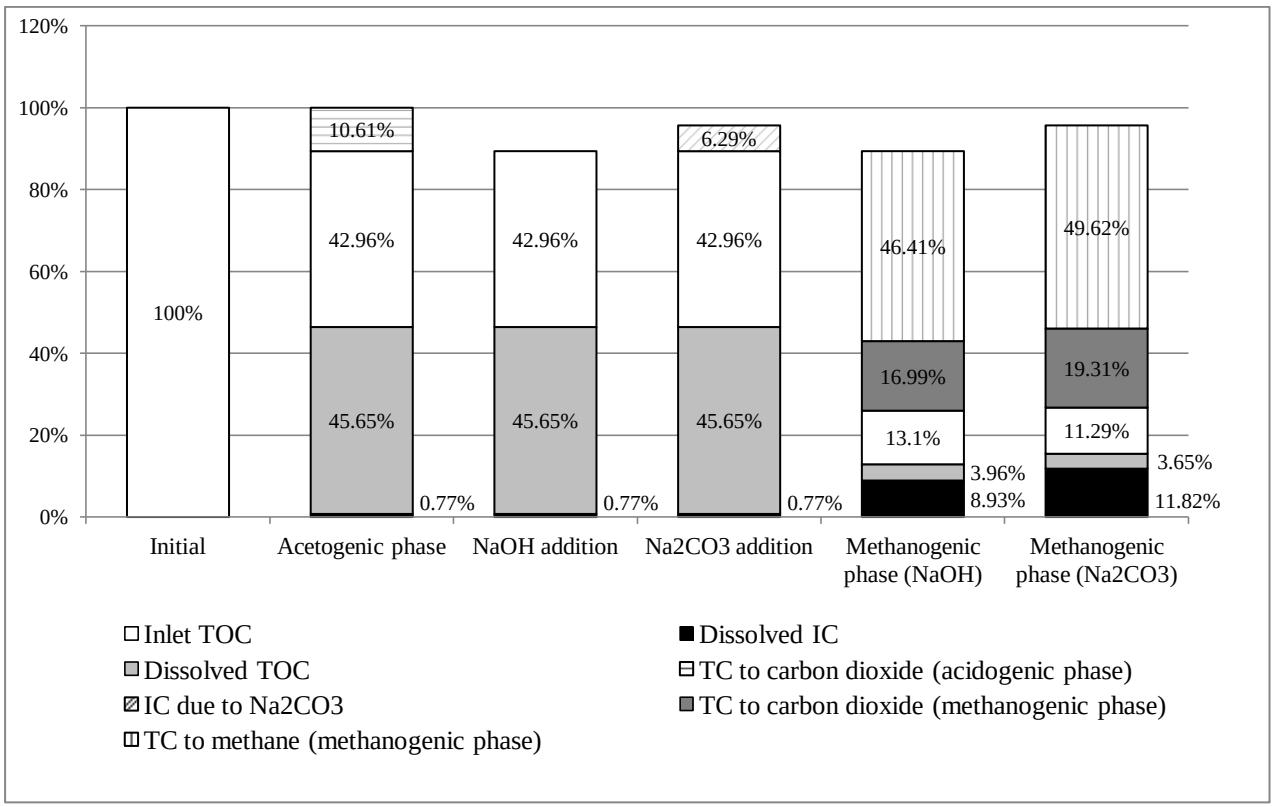

Figure 19 – TC balance in double stage anaerobic digestion from Mix 1.

In fact Mix 1, that was mainly enriched in carbohydrates, was having in the first phase a COD removal higher than the one of Mix 2, that was rich in meat and so in proteins. Considering the second stage of the anaerobic digestion process, and in particular the percentage of inlet COD (or TOC) that is ending in methane, it can be observed that the proportionality between the amount

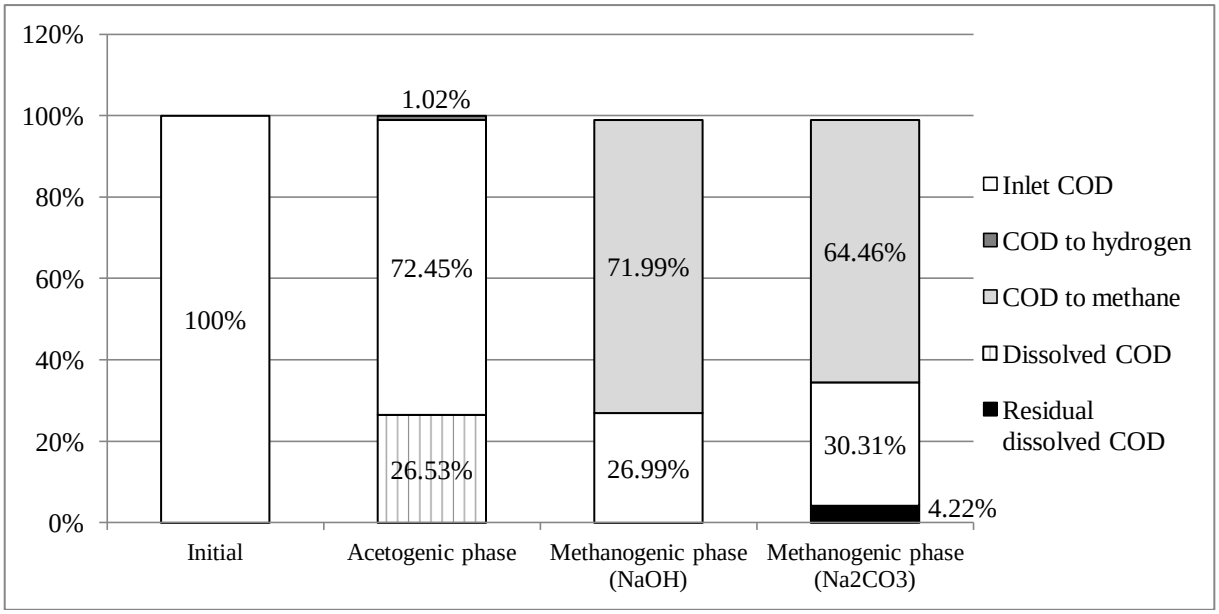

Figure 20 – COD balance in double stage anaerobic digestion from Mix 2.

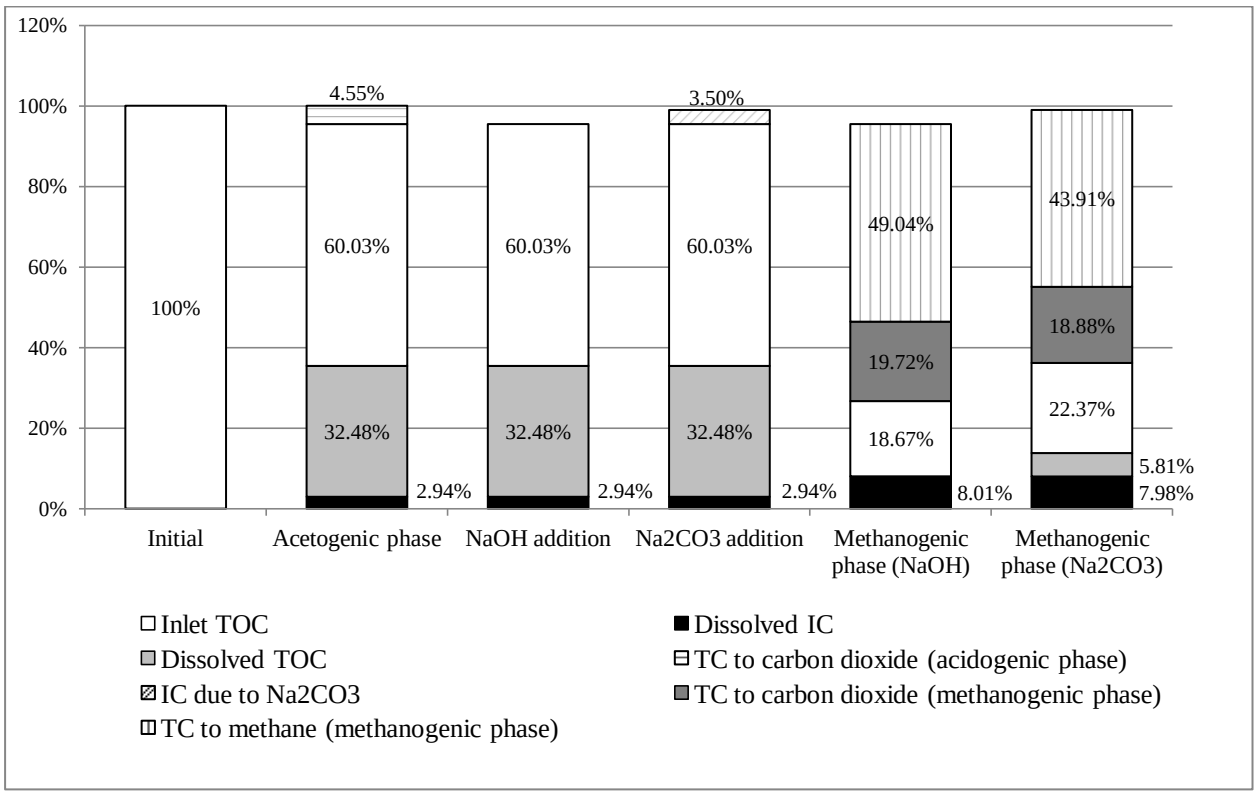

Figure 21 – TC balance in double stage anaerobic digestion from Mix 2.

of that gas produced and the inlet COD (or TOC) that had been seen in the direct BMP tests, is here missing. The substrate Mix 2, that in direct BMP test was displacing the highest conversion efficiency, has produced less methane (in percentage of inlet COD or TOC) than Mix 1 and Mix 3. Mix 3 has displaced the highest conversion to methane in the second phase of anaerobic digestion, with about 76% of its initial COD converted to methane.

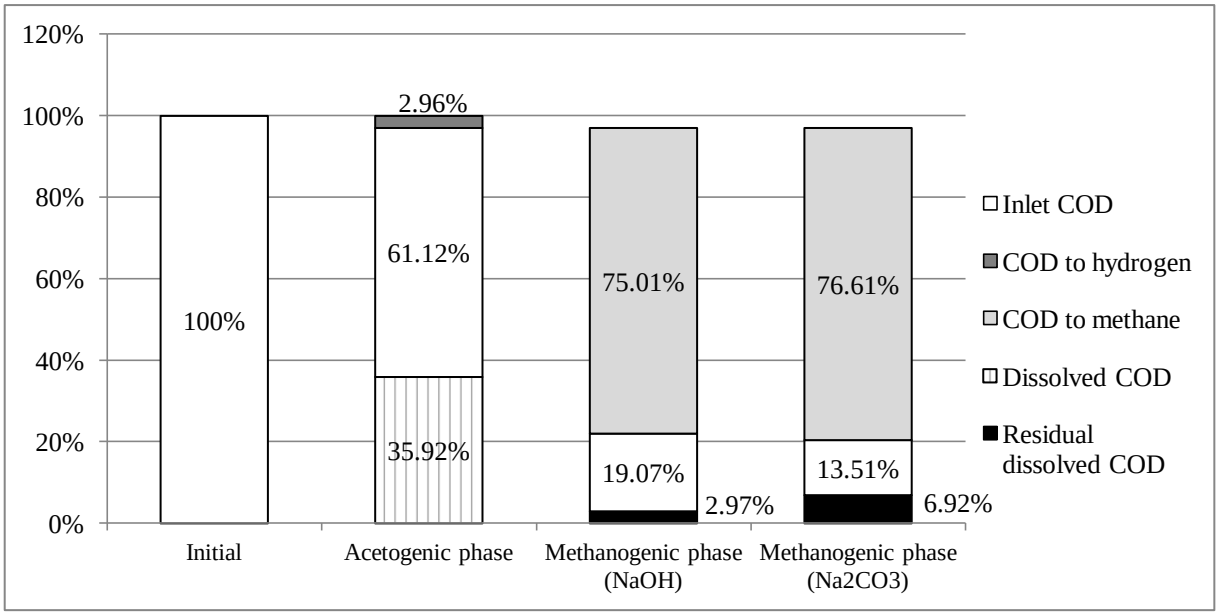

Figure 22 – COD balance in double stage anaerobic digestion from Mix 3.

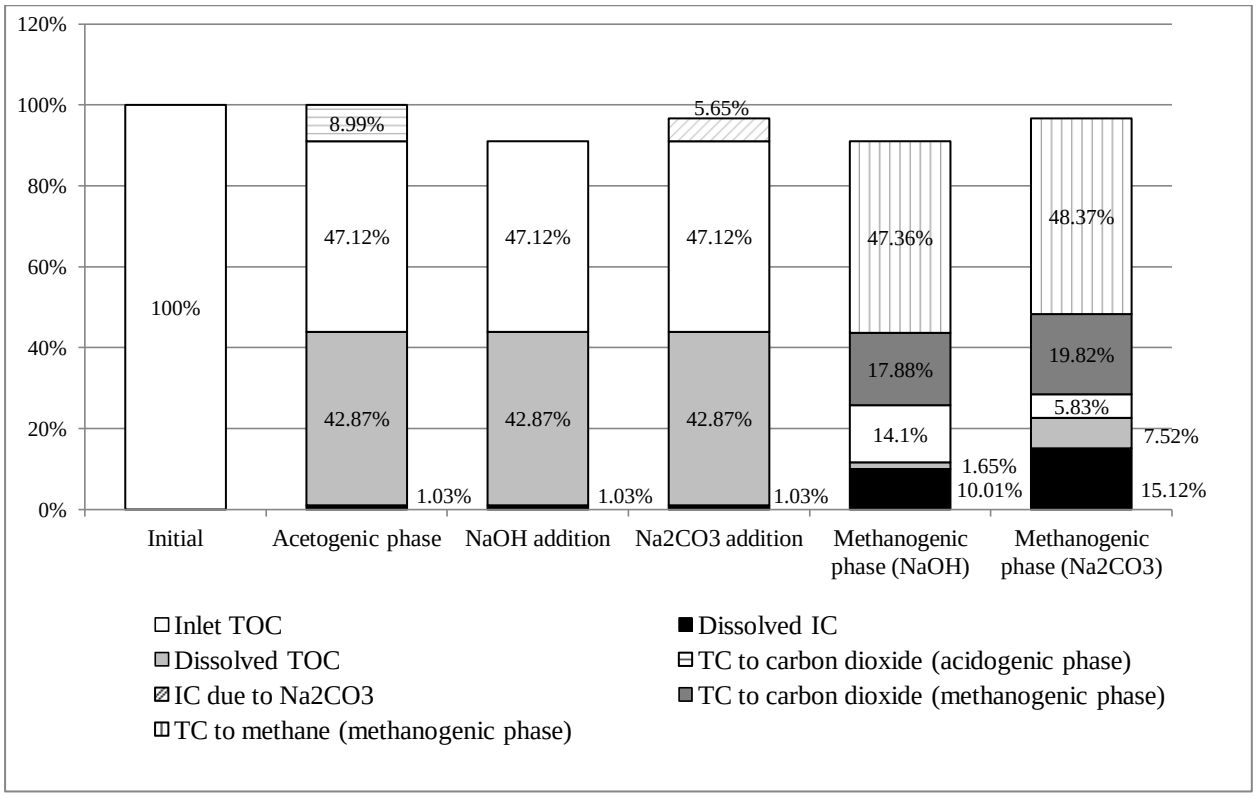

Figure 23 – TC balance in double stage anaerobic digestion from Mix 3.

Figures 24, 25 and 26, provide a comparison between the performance of the direct BMP test and the double BHP-BMP tests on the three substrates, reporting respectively for carbon dioxide, hydrogen and methane, both the specific productions and the concentration in the biogas.

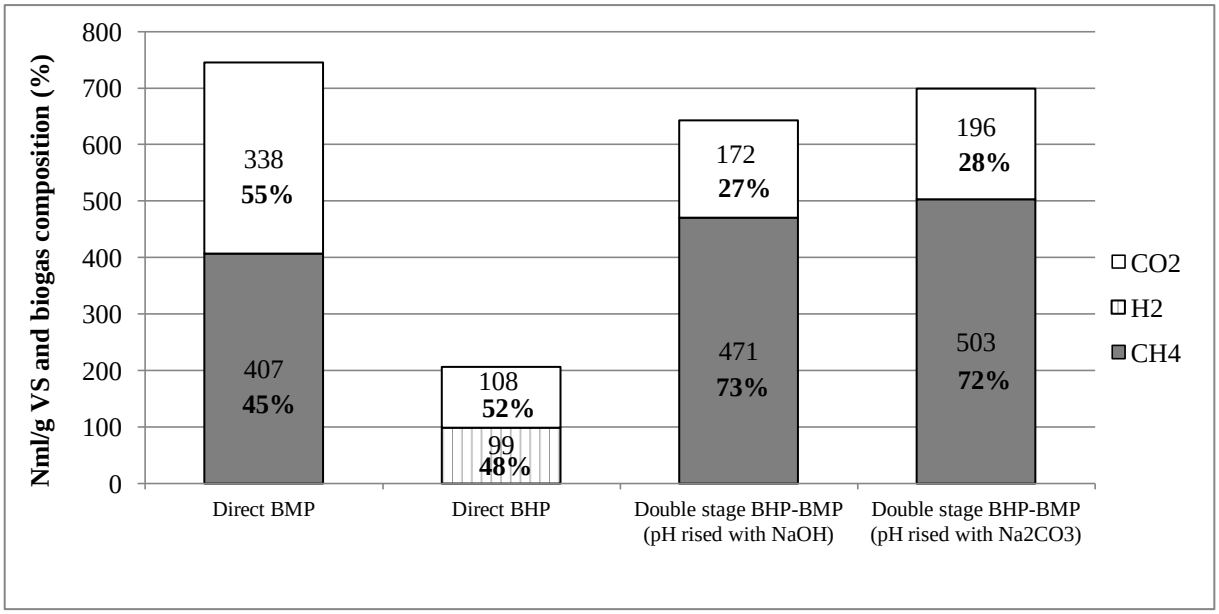

Figure 24 – Comparison between biogas quality resulting from direct BMP test and biogas quality resulting from methanogenic phase in double stage anaerobic digestion for Mix 1.

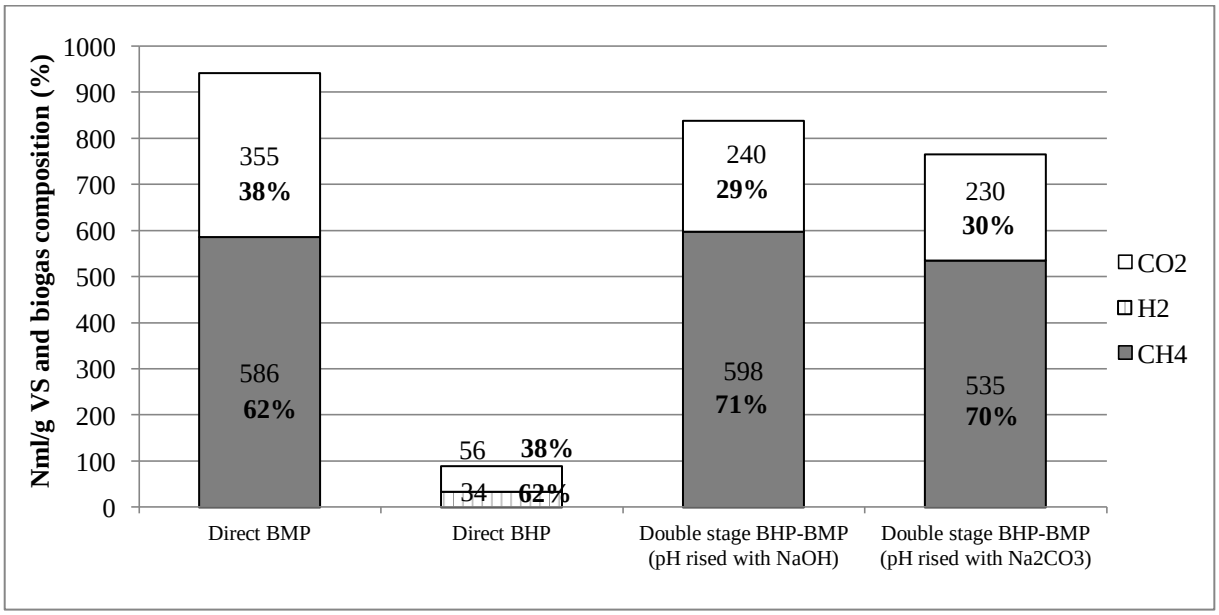

Figure 25 – Comparison between biogas quality resulting from direct BMP test and biogas quality resulting from methanogenic phase in double stage anaerobic digestion for Mix 2.

For each of the substrates, the amount of methane that was produced in the second phase of the two stage process was higher compared to the amount of methane produced from the direct BMP test. For Mix 1 and Mix 3, the bottles in which sodium carbonate was added to rise the pH were displacing an higher yield compared to the ones where pH was raised with sodium hydroxide. The only exception was represented by Mix 2, where the bottles with pH raised with the carbonate were displacing a yield lower than that of the bottles in which sodium hydroxide was used and also lower than the direct BMP test.

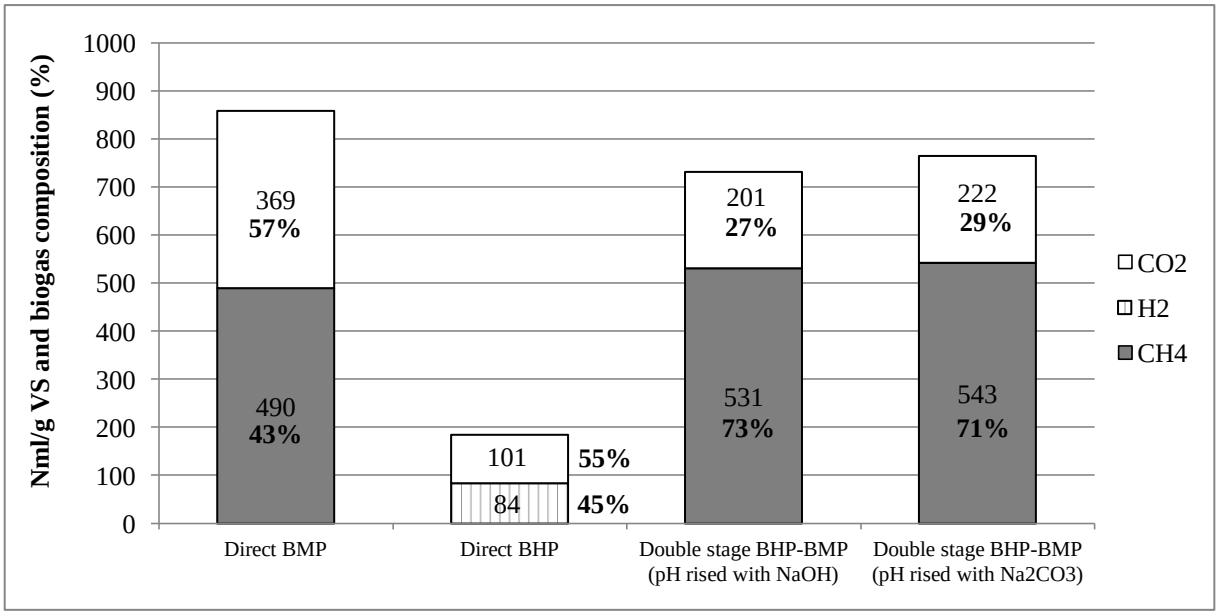

Figure 26 – Comparison between biogas quality resulting from direct BMP test and biogas quality resulting from methanogenic phase in double stage anaerobic digestion for Mix 3.

Considering the maximum yields of the single and two stage processes, methane yield was passing from 0.41 Nl CH_4/gVS to 0.50 Nl CH_4/gVS for Mix 1 with an increase of 23.6%, from 0.59 Nl CH_4/gVS to 0.60 Nl CH_4/gVS for Mix 2 with an increase of 2% and from 0.49 Nl CH_4/gVS to 0.54 Nl CH_4/gVS for Mix 3 with an increase of 10.8%. Consequently to the higher rate of gasification in the two stage process compared to the direct BMP test, lower residual unbiodegraded COD and TOC are left, as the balances are displacing. The increased biodegradability of the substrate appeared to be proportional to the amount of hydrogen produced in the first phase, and this fact can suggest that the improvement of the first degradation phase can lead to proportional benefits even in the second methanogenic phase.

Researches where the double stage anaerobic digestion was exploited to produce hydrogen and then methane, obtaining an higher methane yield when compared to the one of the direct BMP test, have been performed by some authors just in the recent years. Experiments have been conducted in batch tests to assess the treatment of thin stillage in single and two stage anaerobic digestion by *Nasr et al.* (*2012*). These authors reported an increase in specific methane production from 0.26 l CH_4/ gCOD added in the single stage to 0.33 l CH_4/ gCOD added in the second stage, with an increase of COD destruction from 80% to 90%. The increase in the efficiency of the double stage is attributed to the increase in the VFAs to COD ratio from 10% in the direct methane production to 54% after the acidification stage. Experiments were also conducted on MSW by *Liu et al.* (*2006*), reporting an hydrogen production of 43 ml H_2/gVS and a methane production of 500 ml CH_4/gVS, with an increase of 21% compared to the single stage process using continuous reactors. Also *Kvesitadze et al.*, (*2012*) registered an hydrogen

production of 104 ml H_2/gVS form biodegradable solid waste with an increase in energy recovery from the two stage anaerobic digestion.

Figures 24, 25 and 26 allowed also to define a comparison between methane concentration in the biogas in the direct BMP tests and in double stage anaerobic digestion. Methane concentration resulted to be higher in the double stage digestion, for all the substrates investigated. This can be due to the fact that during the hydrogen production phase a certain amount of carbon dioxide had been already removed from the system, avoiding the dilution of the methane produced later.

For Mix 2, that was having the higher concentration of methane during the direct BMP test (62%), the increase in concentration was the lowest, arriving to 70÷71% in the second stage digestion. Mix 1 and Mix 3, that were displacing comparable methane concentrations in the direct BMP test, around 45%, in the double stage digestion were having an increase in the concentration to 72%, a bit more than the second substrate. Observing the amount of carbon dioxide released during the fermentative phase, it is possible to note that Mix 1 and Mix 3 were displacing comparable production, while Mix 2 was displacing a carbon dioxide production that was around half of Mix 1 and Mix 3 production. This observation support the concept that improving the first hydrogen production stage can allow not only to obtain more methane in the second phase, but also to produce a more concentrated gas because of carbon dioxide subtraction in the first phase.

Percentages obtained for methane concentration in the biogas of the two stage digestion are in accordance with literature values. *Liu et al.* (*2006*) is reporting a methane concentration of 65% in the second phase digestion from OFMSW, while *Kvesitadze et al.*, (*2012*) even from biodegradable solid waste, experimented a methane concentration in the second phase of 78.6%.

3.6 Hydrogen production from organic waste in batch stirred reactor

Figure 27 reports the results obtained from hydrogen production in the continuously stirred batch reactor from Mix 1, Mix 2 and Mix 3. The tests were conducted with and without pH control. PH control, consisting in the addition of sodium hydroxide drops, was activated when pH monitoring was signing a pH value lower than 5.3 and stopped when it reached at least 5.5, in order to maintain optimal pH value for hydrogen production. Actually pH control was significant only for Mix 1, the only sample that was experiencing a drop in pH below 5.5. For the other two sample, as it is possible to see from Figure 27, pH never went below the minimum value, so no sodium hydroxide addition was necessary. This lack in pH drop can be due first of all to the lower production of VFAs associated with a lower hydrogen yield from Mix 2 and Mix 3, but also to the high buffer capacity that sludge is generally owing.

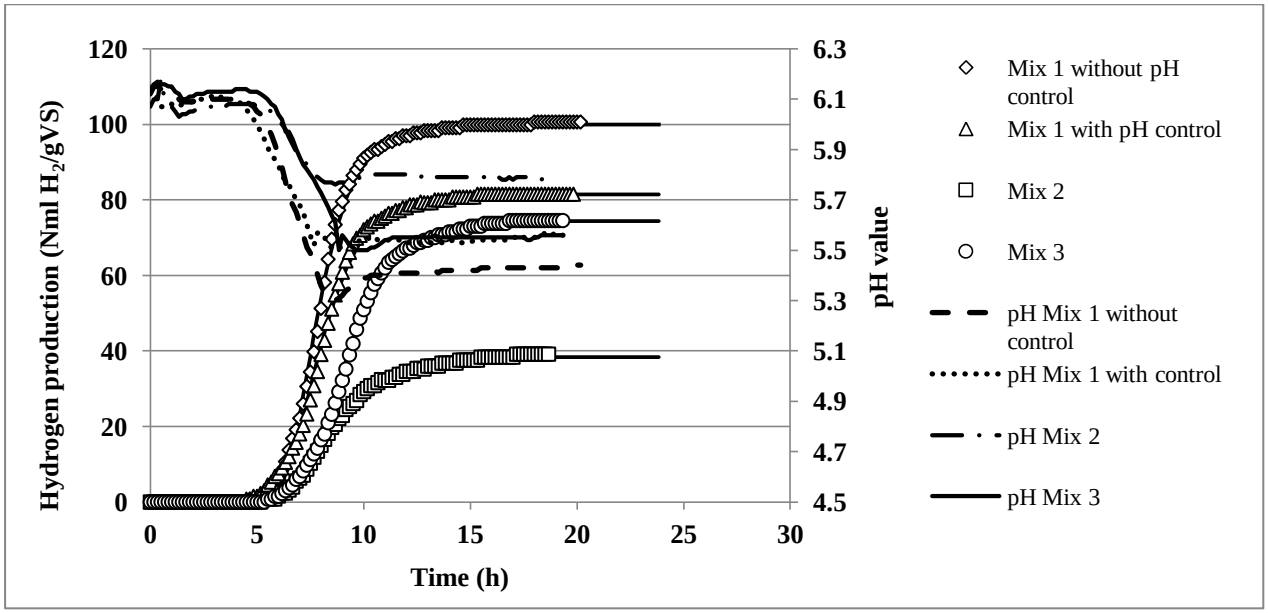

Figure 27 – Hydrogen cumulative production in a continuously stirred batch reactor from Mix 1, Mix 2 and Mix 3, and relative pH trends. Symbols represent experimental data, continuous line the mathematical model.

The continuous measurement of the biogas allowed to well describe what was happening during the first hours of run of the tests. Hydrogen production through dark fermentation process was taking place mainly during the first 12 hours from the starting of the tests, and actually direct batch tests allowed the first biogas sampling and measurement only after about 14 hours from the experiment beginning, when the hydrogen production reaction was nearly at the end. In fact 99% of the maximum cumulated hydrogen production was reached after 10 h for Mix 1 without pH control, after 9.7 h for Mix 1 with pH control, after 11.8 h for Mix 2 and after 10.2 h for Mix 3 (from the beginning of biogas production). From the analysis of data obtained with this reactor, it is possible to see that hydrogen production was starting only after some hours of lag phase, during which bacteria are probably adapting to the environmental conditions of the system. The duration of the lag phase was not changing so much from one substrate to the other. Mix 1 was displacing a lag phase of 6.3 h without pH control and 6.2 h with pH control. Similarly Mix 2 was displacing a lag phase of 6.2 h, and only Mix 3 had a lag phase of about 7 h.

For all the substrates investigated it can be noticed that when hydrogen production started, the pH started to drop, having a minimum point when the velocity of hydrogen production was higher. PH drop was the result of the production of VFAs in the system, byproducts of the dark fermentation reaction. Then pH was rising and stabilizing, and the rate of the reaction decreased.

This behavior allowed to interpolate the experimental data using Gompertz equation, and to obtain maximum cumulated production of hydrogen and its maximum production velocity.

The results obtained in terms of cumulated hydrogen production were confirming the results obtained from the batch tests. For Mix 1 the cumulative hydrogen production without pH control was equal to 100 Nml H_2/ gVS, while it was 81 Nml H_2/ gVS with pH control. For Mix 2 the production in the stirred batch reactor was higher than the one of the batch test and equal to 38 Nml H_2/ gVS. Only for Mix 3 the yield was 74 Nml H_2/ gVS and so lower than the result obtained in the batch test. The velocity of hydrogen production was higher for Mix 1, and lower for Mix 2. Mix 3 gave intermediate velocity between the two other substrates. This behavior is in accordance with literature values that are reporting higher velocity of hydrogen production for carbohydrate substrates compared to proteic substrates (*Okamoto et al., 2000*). An added value of the continuous biogas extraction in this system should be given, since in batch tests it was not possible to confirm this behavior.

Table 16 provides a comparison of the behavior of the three substrates investigated in the stirred batch reactor through the main parameters studied.

Table 16 - Comparison of the results obtained from Mix 1, Mix 2 and Mix 3 in the continuously stirred batch reactor.

Parameter	Mix 1		Mix 2	Mix 3
	pH control	without pH control		
H_2 production (Nml H_2/g VS)	81	100	38	74
COD_{H2}/COD_{in} (%)	2.9	3.6	1.1	2.6
H_2 concentration (%)	43.7	44.5	46.0	43.6
Lag phase (h)	6.16	6.29	6.23	7.06
Maximum production velocity (Nml H_2/gVS/h)	23	32	8	18
Time for 99% hydrogen production (h)	9.67	10	11.83	10.17

The data on COD conversion to hydrogen are confirming the results already obtained for the batch tests COD mass balances. The highest conversion rate was the one of the first substrate, followed by Mix 3 and then Mix 2. The VFAs produced were analyzed at the end of the fermentation. Data era reported in Table 17. Only the VFAs with significant concentrations values are reported.

Table 17 – VFAs analyzed at the end of each run in continuously stirred batch reactor for Mix 1, Mix 2 and Mix 3.

VFAs	Mix 1		Mix 2	Mix 3
	pH control	without pH control		
	VFAs (mg/l)	VFAs (mg/l)	VFAs (mg/l)	VFAs (mg/l)
Acetic	365	310	290	378
Propionic	53.9	14.5	88.2	59.9
Isobutyric	26.3	<10	36.9	29.3
Butyric	264	340	180	290
Isovaleric	24.9	12.3	33.4	29.4
Caproic	32.8	18.5	44.5	45.1

Comparing VFAs production of the three substrates, it is possible to see that Mix 1 and Mix 3 were producing an higher amount of acids when compared to Mix 2 production, and that the quality of these acids was also different. Mix 1 and Mix 3 were producing a major quantity of acetic and butyric acid, that are associated to dark fermentation reactions that lead to hydrogen production, as equation (1), (2) and (3) were reporting. On the other hand, Mix 2 was reporting a lower amount of acetic and butyric acid and an higher amount of propionic acid, that is in accordance with literature reactions that associate this acid to hydrogen consumption or with no production, as in equation (5). Similar quality of VFAs produced in Mix 1 and Mix 2 has been obtained by *Okamoto et al.*, (*2000*) using as substrate respectively carbohydrates (cabbage, carrot, rice) and proteins (lean meat).

The lower hydrogen production that is associated to proteic substrates can actually be attributed to the different degradation steps, compared to the ones followed by carbohydrates, that this type of waste is following. To support this concept, nitrogen concentrations have been analyzed before and after the test. The evaluation focused on the amount of TKN, NH_4^+ and organic nitrogen inserted in the bottle at the beginning of the test, and on the concentration of the same parameters at the end of the experiment. For Mix 2 the N_{org}/TKN ratio at the beginning of the test was equal to 86.6%, while at the end of the experiment it resulted to be 4.4%. NH_4^+ concentration, on the contrary, was passing from 20 mgN/l to 140 mgN/l. This means that at the end of the experiment, considering TKN constant, more than 95% of the nitrogen in the bottle was in the ammonium form. The increasing amount of ammonium is defining a great rate of degradation of the proteins, that are hydrolyzed releasing NH_4^+.

This result is coherent with *Okamoto et al.*, (*2000*), that were reporting on their research an increase in ammonia and VFAs during the degradation of proteins, with small amount of ethanol produced. The ammonia was responsible of final high pH (6.42) of the test.

The same calculation performed on the other two mixes gives values for organic nitrogen conversion into ammonium lower than the one of the second substrate. In Mix 1 without pH control N_{org}/TKN ratio was still 78%, while on the same substrate with pH control N_{org}/TKN ratio decreased to 29%. In Mix 3 final N_{org}/TKN ratio was 13%, corresponding to 87% of TKN in the form of NH_4^+ at the end of the experiment.

The fact that lower hydrogen production rate from proteic substrates are not due to low hydrolytic activity can be confirmed also by a research done by *Favaro et al.*, (*2012*), that was testing the viability and the hydrolytic profile of bacteria in anaerobic sludge after 4 hours of thermal treatment. The article reports among the main bacteria species that are surviving to thermal treatment *Firmicutes* predominant division. Into this division *Bacillus sp.* and

Lysinibacillus sp. were the main genera, while *Bacillus badius* resulted to be the most common specie. The isolates exhibited a broad range of hydrolytic activities: many were able to degrade proteins, pectin and starch. On the contrary, few were able to degrade cellulose and no lipolytic strains were recorded.

The fact that protein degradation could be performed by bacteria survived in the sludge, suggest that different degradation pathways are followed by proteins during the fermentative phase, avoiding hydrogen formation as a byproduct.

Okamoto er al., (*2000*), is explaining the lower production of hydrogen from proteins with the fact that their degradation can occur in two ways: by sole degradation of the amino acids or with reductive deamination. The first process is releasing hydrogen, VFAs and ammonia, while the second process is not producing hydrogen, but consuming it as an electron donor. Because of this, even if hydrogen is produced, than it is immediately consumed, and that is why an efficient dark fermentation process from these type of substrates seems difficult to be reached.

4. CONCLUSIONS

The chemical-physical properties of OFMSW characterize this waste as an optimal substrate for biological treatments, as for example composting or anaerobic digestion, with the purpose to recover energy (biogas or biofuel) and nutrients or amending material (compost) from a resource that is considered renewable.

Among the biological treatments, hydrogen production through dark fermentation process has proven to be an interesting technology (*Giordano et al., 2011; Alibardi et al., 2012; Oh et al., 2003; Logan et al., 2002*). In fact hydrogen has an energy yield that is higher than the one of fossil fuels, can be efficiently used in internal combustion engines or in chemical fuel cells, and the only byproduct of its oxidation is water, releasing no carbon dioxide emissions, that can became even negative if carbon dioxide is captured during the process (*Kvesitadze et al., 2012; Hallenbeck, 2009*). However, hydrogen can be considered a clean energy only if it is not produced from other finite fossil fuel resources, or if the process used do not require unprofitable energy expenses.

Biological hydrogen production during the fermentative phase of anaerobic digestion has been evaluated as a possibility to recover energy from OFMSW, coupling energy recovery with sustainable waste treatment. If the first degradation reaction is properly driven toward acetic acid and VFAs production, the pool of compounds generated in the fermentative phase can became the inlet for a subsequent methane production phase, allowing the gasification of the residual waste biodegradable COD, with its consequent stabilization, and exploiting the energy obtained as methane (*Giordano et al., 2011*).

Batch tests experiments have been conducted on glucose, to test the effectiveness of the measurement methods, and on three OFMSW mixtures (Mix 1, Mix 2 and Mix 3), constructed from real waste fractions ("bread-pasta-rice", "meat", "vegetable", "fruit", "undersieve") sorted at a treatment plant located in Camposampiero, that receives organic waste deriving from separate collection. Mix 1 was having a predominant content of "bread-pasta-rice" fraction, Mix 2 was having a predominant content of "meat" fraction, and Mix 3 was having an intermediate content of the two.

Direct BMP tests and sequential BHP-BMP tests were conducted on glucose. BHP tests were conducted with both thermally treated inoculum and raw inoculum. The test conducted with raw sludge was having a specific yield of 114 Nml H_2/ gVS, and produced 21 Nml CH_4/ gVS. On the other hand the test conducted with thermally treated sludge was having a specific yield of 173 Nml H_2/ g VS, with no hydrogen production. Tests performed on glucose were fundamental to

prove the efficiency of inoculum thermal treatment as a system to efficiently inhibit methanogenic bacteria, and to improve hydrogen biological production. Mass balances performed on direct BMP tests and two stage anaerobic digestion tests for glucose were closed with an error of 3.7% and 5.9% respectively. These results allowed to say that laboratory measurements and analysis were describing the results obtained with this imprecision degree, and that the mass balance term relative to the bacterial growth, this term being comparable to the error done, could not be considered.

The same BHP-BMP and direct BMP batch test were conducted on Mix 1, Mix 2 and Mix 3, using thermally treated sludge as inoculum. Results obtained from the different samples constructed are reported in Table 18 in terms of hydrogen and methane yield.

Table 18 - Comparison of hydrogen and methane yield in direct and subsequent production from OFMSW samples.

Type of process	Mix 1	Mix 2	Mix 3
Direct CH_4 production (Nml CH_4/ gVS)	407	586	490
Direct H_2 production (Nml H_2/g VS)			
In batch tests	99	34	84
In continuous reactor	100	38	74
Double stage CH_4 production (Nml CH_4/ gVS)			
pH raised with NaOH	471	598	531
pH raised with Na_2CO_3	503	535	543

The analysis of the data allowed to say that actually the double stage anaerobic digestion was performing better, compared to the direct BMP test, for all the mixes tested. All the substrates, considering their best results, are displacing an increase in methane production during the second phase of anaerobic digestion, and an improvement in biogas quality, because of the methane higher concentration in the biogas. Methane yield was passing from 0.41 Nl CH_4/gVS to 0.50 Nl CH_4/gVS for Mix 1 with an increase of 23.6%, from 0.59 Nl CH_4/gVS to 0.60 Nl CH_4/gVS for Mix 2 with an increase of 2% and from 0.49 Nl CH_4/gVS to 0.54 Nl CH_4/gVS for Mix 3 with an increase of 10.8%. The increase in COD removed as methane seemed to be proportional to the amount of hydrogen produced during the first fermentative phase. The increase of COD ending in methane can be explained with the higher VFAs/COD_{in} ratio that the second stage is displacing compared to the direct BMP test. This result has already been obtained in few previous works as for example *Liu et al.* (*2006*), *Kvesitadze et al.*, (*2012*) and *Nasr et al.* (*2012*).

Even as a consequence of hydrogen production phase, the concentration of methane in the biogas was increasing for all the substrates: methane in Mix 1 was passing from a concentration of 62% in the direct BMP test to 70÷71% in the second stage digestion, while Mix 1 and Mix 3, that were displacing comparable methane concentrations in the direct BMP test, around 45%, in the

double stage digestion were having an increase in the concentration to 72%. This increase in concentration is a consequence of the amount of carbon dioxide already removed in the fermentation phase, that in the second phase is no further diluting the methane.

Further analysis were performed on a batch stirred reactor to test the same substrates for BHP with continuous biogas removal. The cumulative hydrogen productions, as reported in Table 18, were similar to the results obtained with the batch tests. This means that the composition of the initial substrate was influencing hydrogen yield much more than the process type that was used. The use of a different apparatus or of different process conditions can surely increase hydrogen yield and optimize the process, as it happens in many works (*Alibardi et al., 2012; Xie at al., 2008; Oh et al., 2003; Logan et al., 2002*), but further attention should be given to MSW composition when speaking about hydrogen produced. Different works are reporting results on hydrogen from MSW (*Liu et al., 2006; Kvesitadze et al., 2011; Lee at al., 2010; Kim et al., 2012; Chu at al., 2008*), but the fact of not knowing exactly what was inside, do not allow to understand if high hydrogen productions were obtained because of the process efficiency or because of the high hydrogen potential of the waste involved.

In this work the effect of waste composition had proven to be fundamental in the methane yield increase in the second stage of anaerobic digestion compared to the direct BMP test. Further research should be conducted to understand why and which type of compounds, that in direct BMP test are not hydrolyzed, can in some way became biodegradable if they are facing fermentative conditions for about one week, and which are the factors that are driving and conditioning their degradation. Improvement of hydrogen production phase should in any case be continued, to obtain the highest level of gasification and waste stabilization that is possible, not only from the fermentative phase, but also from the methanogenic phase, that seems to be strictly dependent on the previous one.

ANNEX

Cumulative hydrogen productions from the different organic fractions of MSW tested for pH 5.8 and pH 7. Experimental data are represented by the symbols. Error bars represent standard deviations.

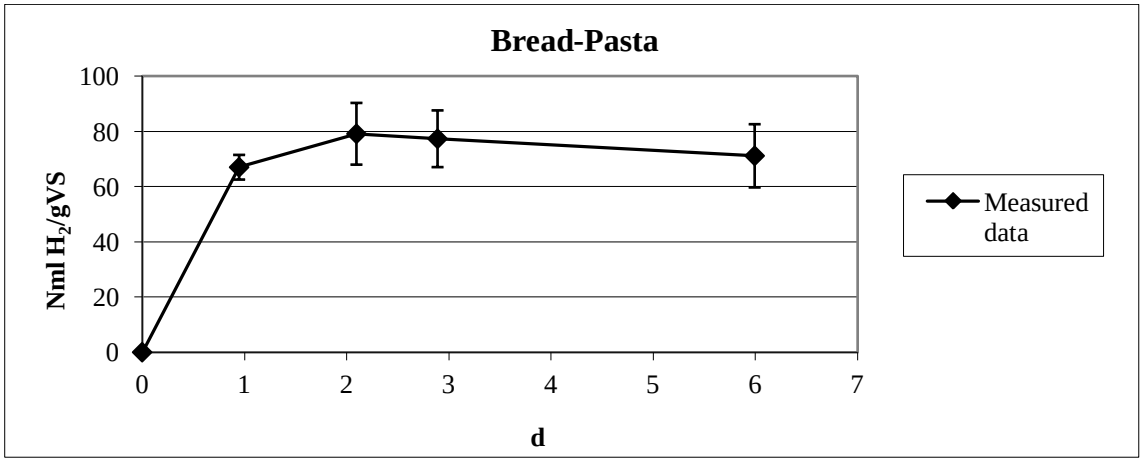

Figure 28 – Cumulative hydrogen production from "bread-pasta" fraction at pH 5.8.

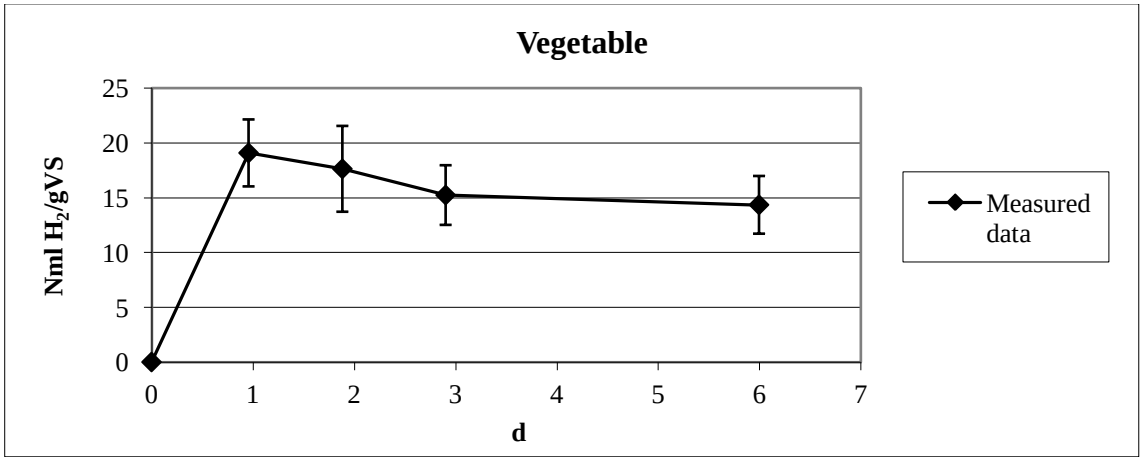

Figure 29 – Cumulative hydrogen production from "vegetable" fraction at pH 5.8.

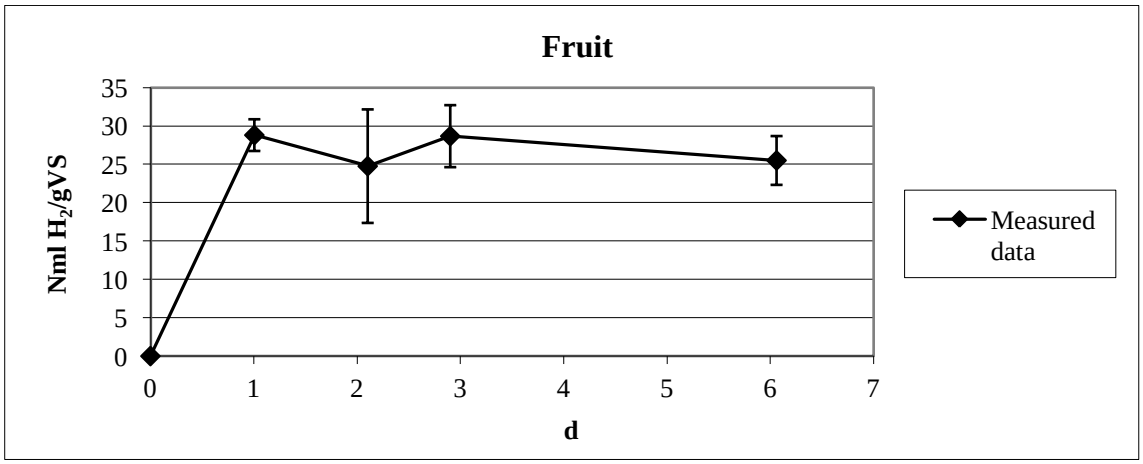

Figure 30 – Cumulative hydrogen production from "fruit" fraction at pH 5.8.

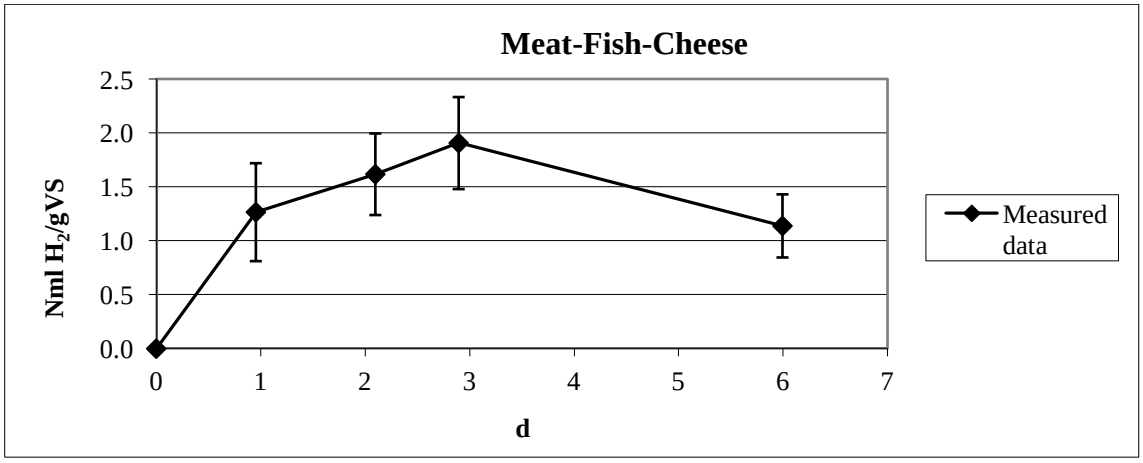

Figure 31 – Cumulative hydrogen production from "meat-fish-cheese" fraction at pH 5.8.

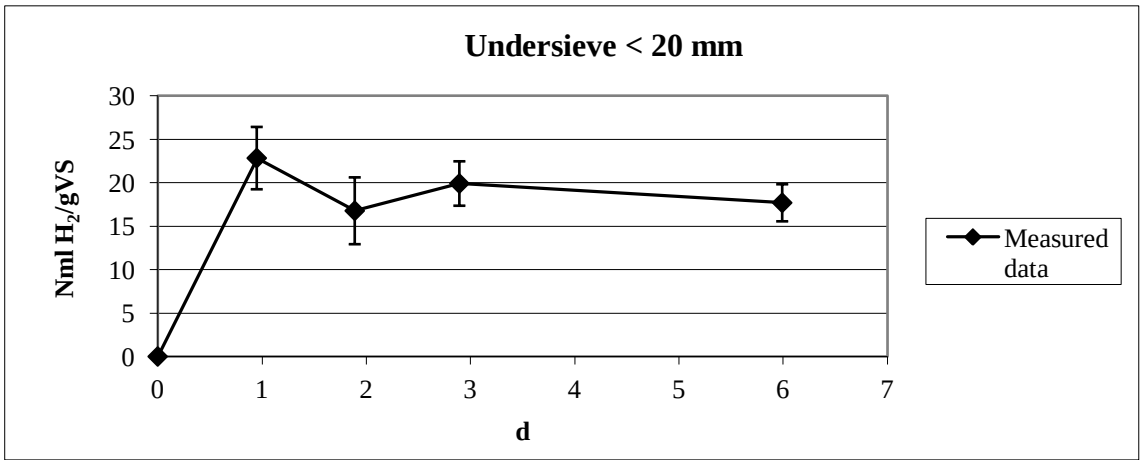

Figure 32 – Cumulative hydrogen production from "undersieve" fraction at pH 5.8.

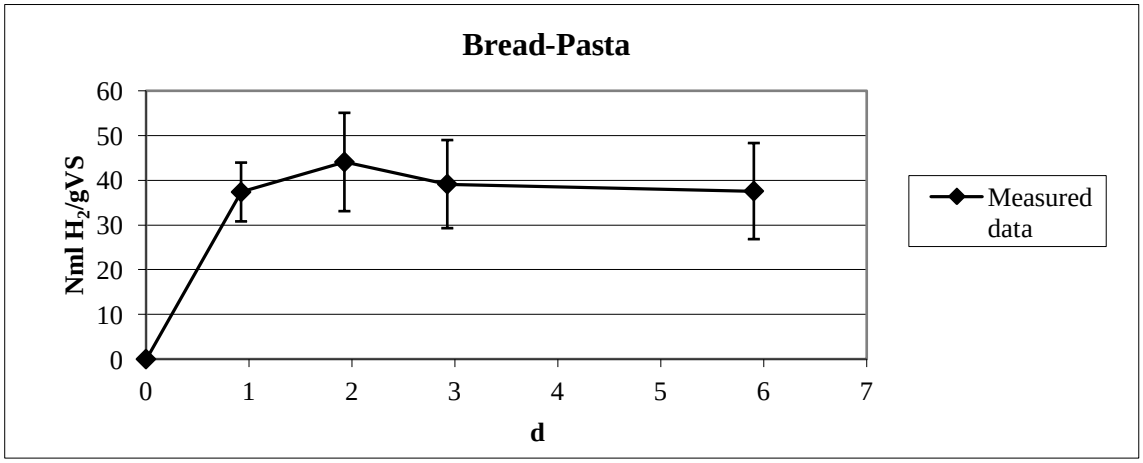

Figure 33 – Cumulative hydrogen production from "bread-pasta" fraction at pH 7.

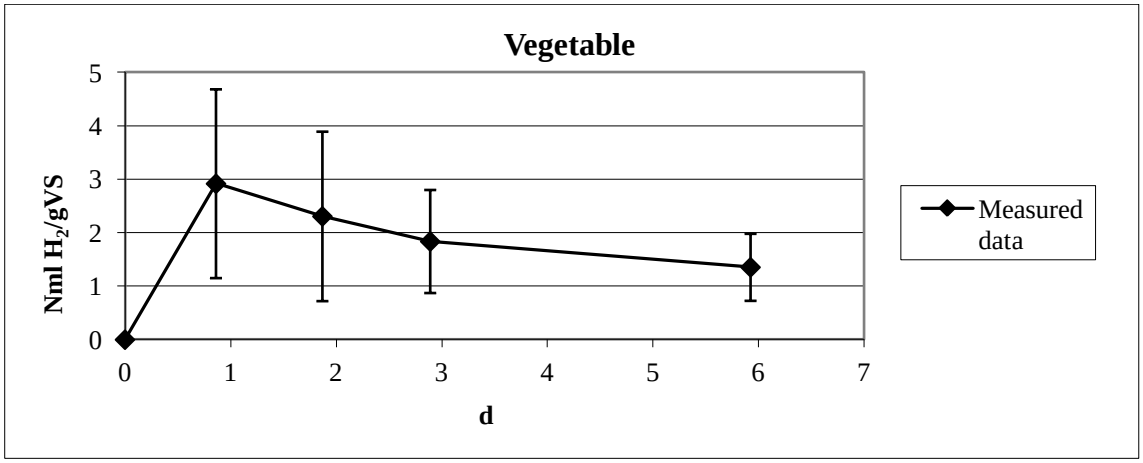

Figure 34 – Cumulative hydrogen production from "vegetable" fraction at pH 7.

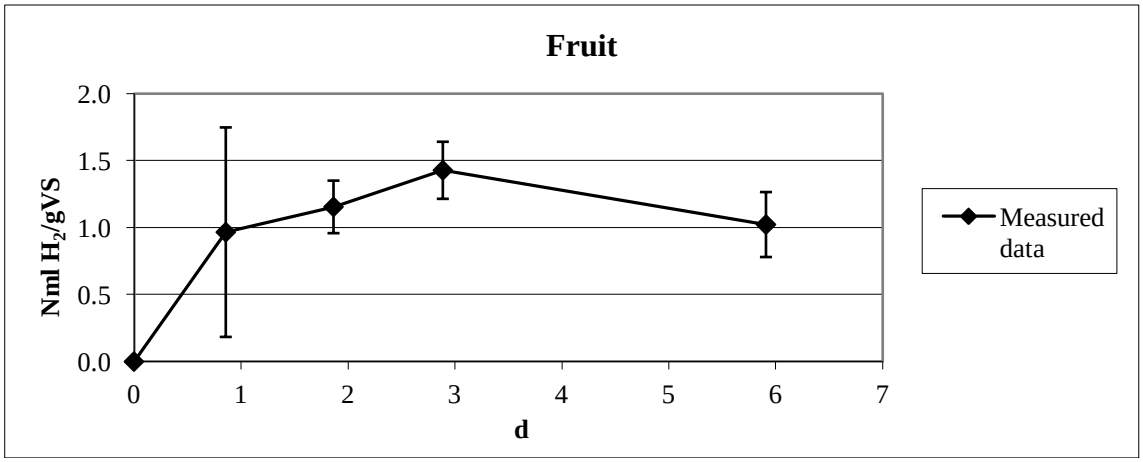

Figure 35 – Cumulative hydrogen production from "fruit" fraction at pH 7

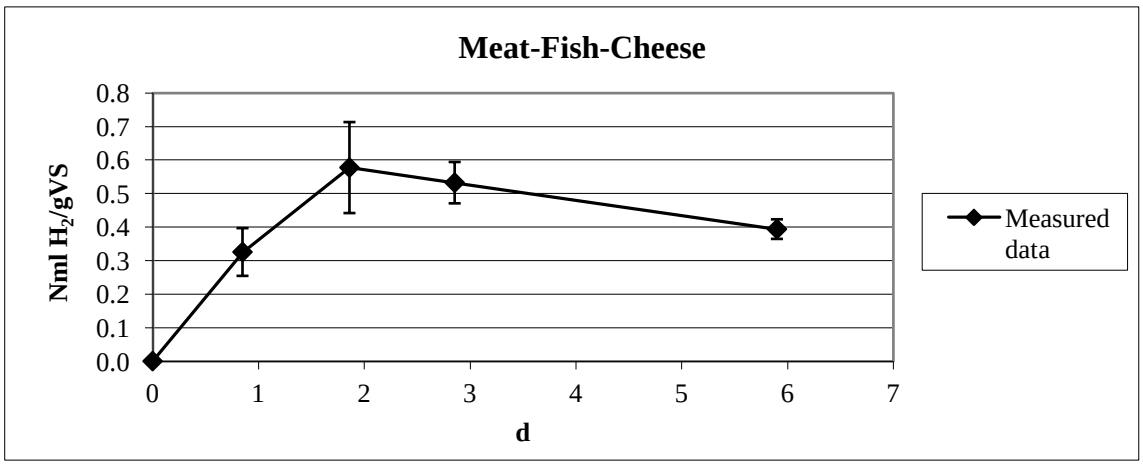

Figure 36 – Cumulative hydrogen production from "meat-fish-cheese" fraction at pH 7.

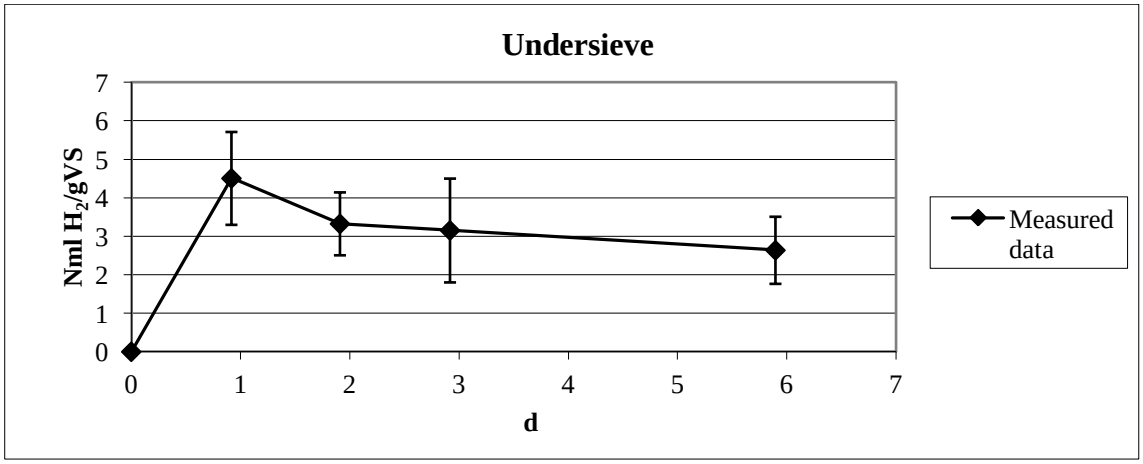

Figure 37 – Cumulative hydrogen production from "undersieve" fraction at pH 7.

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