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Optimization of two phases thermophilic anaerobic digestion of biowaste for bio-hythane production

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To my family, my friends and my iron scarecrow.

ABSTRACT

This PhD thesis deals with the optimization of a two phase thermophilic anaerobic process treating organic waste for hydrogen and methane production. Nor physical neither chemical pretreatments were used to treat the inoculum or the substrates to optimize the process. The work was carried out at pilot scale, using two CSTRs (0.2 m^3 and 0.38 m^3 working volume respectively) maintained at thermophilic temperature (55°C) and fed semi-continuously with organic waste collected in Treviso City. The experiment was divided in three Runs: during Run I and Run II the organic loading rate (OLR) was maintained at about 21 kgTVS/m^3d in the first reactor while the hydraulic retention time (HRT) was changed from 6.6 to 3.3 days in order to avoid the shift from acidogenic to the production of alcohol and other organic acids like lactic acid (solventogenic conditions).

The yields during these two periods were low compared to the maximum yields observed in literature, in fact the pH values of both Runs were lower than the optimal pH range for hydrogenase enzyme (5.5), and the specific hydrogen production was about 2.6 liters of H_2 per $kgTVS_{fed}$.

To avoid this pH drop, during Run III the digestate sludge coming from the second reactor was recirculate to the first reactor (Q_f/Q_r=1) in order to buffer the system and control pH at levels around 5.5. This last Run was divided into other two Run, called Run III-a and Run III-b where the HRT was maintained at 3.3 days and the OLR was set at about 16 kgTVS/m^3d for the Run III-a and at about 21 kgTVS/m^3d for Run III-b in order to verify the behavior of the process maintaining the same HRT and changing the OLR. The best hydrogen yield was obtained in Run III-a where, with an OLR of 16 kgVS/m^3d, the specific hydrogen production (SHP) reached 51 $lH_2/kgVS_{fed}$, and the H_2 content in biogas was 37%. During Run III-b, at 21 kgTVS/m^3d, the SHP decrease to 20 $lH_2/kgTVS_{fed}$ with an hydrogen content of 34%.

The effluent of the first phase was fed to an anaerobic digestion process; despite of the high quantity of volatile fatty acids produced, the AD process was able to convert the organic matter into biogas without any problem of process stability. Observing the biogas yields obtained along the experimentation, the specific biogas production was between 0.58 and 0.64 $m^3/kgVS_{fed}$, and the overall organic removal above 90% (on VS). The mixture of gas obtained from the two reactors, met the standards for the biohythane mix only in Run III-a with a composition of 6.7% for H_2, 40.1% for CO_2 and 52.3% for CH_4.

In parallel were carried out batch tests to quantify the biochemical hydrogen potential (BHP test) using untreated anaerobic sludge as inoculum and organic waste as substrate. Two organic loads were applied 20 and 30 kgTVS/m^3, which showed two different behaviors: at lower load the methanogenic process wasn't inhibited, there was in fact an initial hydrogen production followed by VFA consumption and methane production, while at higher load there was the complete inhibition of methanogenesys, with hydrogen production and accumulation of VFA. In terms of yield at lower load was obtained an SHP of 20.7 lH_2/kgTVS with 18.76% of H_2, while at higher load the SHP was 69.0 lH_2/kgTVS with a hydrogen % of 27.8.

Finally, it was further verified the biologic stability of the digested material by means of aerobic tests, based on the dynamic respirometric index (DRI), where the oxygen uptake rate by the substrate was continuously monitored, and anaerobic tests that were based on the biochemical methane potential (BMP), where the biogas produced by digestate in long-term batch tests, was measured. These values can be used to define the stability of the reactors effluent to verify the possibility to reduce the treatment time. Preliminary tests on dewatered sludge from AD of biowaste and waste activated sludge showed an average SGP of 0.20 Nm^3/kgTVS and a DRI of 1000 mgO_2/kgTVS per hour confirming that the treated material is biologically stable.

RIASSUNTO

Il processo di digestione anaerobica dei rifiuti solidi urbani, è una tecnologia già ampiamente studiata ed applicata in molti paesi, che si basa sul duplice concetto di riduzione dei rifiuti e recupero energetico. La ricerca di nuovi vettori energetici ha portato recentemente allo sviluppo di nuove tecnologie che si basano sulla produzione biologica di idrogeno utilizzando substrati organici, mediante processi di tipo fotosintetici o fermentativi.

Questo lavoro di dottorato ha come obiettivo l'ottimizzazione del processo di digestione anaerobica termofila a fasi separate finalizzato alla produzione di bio-idrogeno, ottenuto nella prima fase del processo (fermentazione), e biogas ottenuto nella seconda fase di trattamento dell'effluente idrolizzato. Punto chiave del lavoro è l'applicabilità in piena scala dell'intero processo, infatti si è scelto di agire su parametri facilmente modificabili e che non comportino ulteriori spese di esercizio.

Il lavoro è stato svolto su scala pilota, utilizzando due reattori CSTR (0.2 m^3 e 0.38 m^3 di volume utile rispettivamente) mantenuti in termofilia (55°C), alimentati in modo semicontinuo con rifiuto organico proveniente dalla raccolta differenziata della città di Treviso, e senza l'utilizzo di un inoculo per la fase dedicata alla fermentazione.

Il lavoro sperimentale è stato suddiviso in tre Runs: nei primi due periodi (Run I e Run II) il carico organico (OLR) nella prima fase è stato mantenuto a circa 21 kgTVS/m^3d, mentre il tempo di ritenzione idraulica (HRT) è stato variato da 6.6 a 3.3 giorni in modo da contenere lo shift da condizioni di acidogenesi alla produzione di alcoli e di altri acidi come acido lattico (condizioni di solventogenesi). Durante questi primi due periodi le rese in idrogeno sono molto basse rispetto ai valori forniti dalla letteratura (produzione specifica di idrogeno ottenuta è di 2.6 lH_2 /$kgTVS_{fed}$): ciò è dovuto ad un valore di pH troppo basso rispetto al valore ottimale di lavoro dell'enzima idrogenasi coinvolto nella conversione dei substrati organici ad idrogeno (pH ottimale 5.5).

Per poter adattare il pH alle migliori condizioni senza l'utilizzo di chemicals, nel Run III è stato attivato il ricircolo del fango proveniente dalla seconda fase (digestione anaerobica, $Q_f/Q_r=1$) in modo da creare un sistema tampone in grado di controllare il pH. Questo periodo è stato suddiviso in ulteriori due periodi (Run III-a e Run III-b) dove si è mantenuto l'HRT a 3.3 giorni mentre si è variato il carico organico (OLR) passando da 16 kgTVS/m^3d nel primo periodo a circa 21 kgTVS/m^3d nel secondo periodo, in modo da verificare il comportamento del processo al variare della quantità di rifiuto trattato. Le rese migliori si sono ottenute a carico inferiore, con una

produzione specifica di idrogeno (SHP) di 51 l/kgVS_{fed}, e con un contenuto del 37%. Nel periodo a carico maggiore l'SHP è sceso a 20 lH_2/kgTVS_{fed} con un contenuto del 34%.

L'effluente proveniente dalla prima fase è stato trattato mediante digestione anaerobica: nonostante l'elevata quantità di acidi grassi volatili, il sistema è stato in grado di convertire in modo ottimale il substrato a biogas, ottenendo una resa nei tre periodi che va da 0.58 a 0.64 m^3/kgVS_{fed}, con una rimozione del 90% dei solidi volatili. Il mix di gas ottenuto considerando le rese maggiori (Run III-a) incontra le percentuali richieste per la miscela chiamata bio-Hythane , con una composizione del 6.7% H_2, 40.1% CO_2 e 52.3% CH_4.

In parallelo sono stati svolti dei test in batch per quantificare il potenziale biochimico di produzione di idrogeno (BHP test) utilizzando fango proveniente da digestione anaerobica non trattato, e rifiuto organico. Sono stati applicati due carichi organici a 20 e 30 kgTVS/m^3, che hanno evidenziato due diversi comportamenti: a carico inferiore si il processo di metanogenesi non è inibito, si ha infatti una iniziale produzione di idrogeno seguita dal consumo dei VFA prodotti e produzione di metano, mentre a carico maggiore si ha inibizione totale della metanogenesi, con produzione di idrogeno ed accumulo di VFA. In termini di rese a carico inferiore si è ottenuto un SHP di 20,7 lH_2/kgTVS con il 18,76% di H2, mentre a carico maggiore si è ottenuta una SHP di 69,0 lH_2/kgTVS con una % di idrogeno di 27,8.

Oltre a questo aspetto, nel lavoro di tesi è stata considerata anche la qualità dell'effluente da digestione anaerobica di rifiuti organici, testata mediante prove di Indice Respirometrico Dinamico dove viene monitorato in continuo il consumo di ossigeno da parte del substrato in condizioni controllate, e mediante la misura del potenziale di biometanizzazione dove viene misurata la produzione residua di biogas in prove batch a lungo termine.

I risultati ottenuti definiscono la stabilità dell'effluente e la possibilità di applicare tempi inferiori di post-trattamento. La produzione specifica media misurata sul fango disidratato dell'impianto di Treviso è di 0.20 Nm^3/kgTVS con un indice respirometrico di 1000 mgO_2/kgTVS h, valori che confermano l'efficienza del processo.

Parts of this thesis have been published or submitted for publication. Below the list of the reference papers is reported:

Cavinato C., Bolzonella D., Fatone F., Cecchi F., Pavan P. (2011, *submitted*). Optimization of two-phase thermophilic anaerobic digestion of biowaste for hydrogen and methane production through reject water recirculation. Bioresource Technology, special issue on Biofuel-Hydrogen production.

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C. Cavinato, D. Bolzonella, F. Fatone, F. Cecchi, P. Pavan (2010). Two-phase thermophilic anaerobic digestion process for biohythane production treating biowaste. Anaerobic Digestion 2010, Guadalajara, Mexico, 30 October - 4 November 2010 (*Submitted to Water Sciences and Technology*).

Cavinato C., Bolzonella D., Pavan P., Cecchi F. (2010). Two-phase thermophilic anaerobic digestion of biowaste for bio-hythane production: yields and feasibility of the process. 14th International Biotechnology Symposium and Exhibition, 14-18 September 2010, Rimini Italy

Cavinato C., Fatone F., Bolzonella D., Pavan P. (2010). Mesophilic to thermophilic conditions in codigestion of sewage sludge and OFMSW: evaluation of effluent stability using dynamic respirometric index (DRI) and biochemical methane potential (BMP).Chemical Engineering Transactions, 20, 385-390, Proceedings of IBIC, Padua, April 12-14, Italy. 11-14 April 2010, Padua Italy.

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Cavinato C., Pavan P., Bolzonella D., Cecchi F. (2008). Characterisation of dewatered sludge from anaerobic digestion using Dynamic Respirometric Index (DRI) and Biochemical Methane Potential test (BMP). Convegno GRICU 2008- Ingegneria Chimica: le nuove frontiere. 14-17 settembre 2008, Le Castella (KR).

ACRONYM

ASBR	=	anaerobic sequencing batch reactor
ATP	=	adenosine tri phosphate
BHP	=	biochemical hydrogen potential
BHy	=	biohythane
BMP	=	biochemical methane potential
COD	=	chemical oxygen demand
CSTR	=	continuous stirred tank reactor
DRI	=	dynamic respirometric index
FW	=	food waste
GPR	=	gas production rate
HPR	=	hydrogen production rate
HRT	=	hydraulic retention time
LAB	=	lactic acid bacteria
LBR	=	leaching bed reactor
NAD	=	nicotinammide adenin dinucleotide
OFMSW	=	organic fraction of municipal solid waste
OLR	=	organic loading rate]
Ptot	=	total phosphorus
SBR	=	sequencing batch reactor
sCOD	=	soluble chemical oxygen demand
SGP	=	specific gas production
SHP	=	specific hydrogen production
SRT	=	solid retention time
SSC	=	steady state condition
TKN	=	total Kjiendhal nitrogen
TS	=	total solids
TVS	=	total volatile solids
WWTP	=	waste water treatment plant
VFA	=	volatile fatty acid

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1 Introduction

In Italy the average biodegradable waste production in 2007 was 6,3 ml tons on a total urban waste production of 32,5 ml tons, that was the 19,3% of the total production (ISPRA 2009). Among the biodegradable matter collected separately, the organic fraction and garden waste was only the 9% on the total production; the 7% was treated in composting system, while only the 1,2% of organic waste was sent to anaerobic digestion.

Considering also the actual renewable energy scenario, it is important to optimize the separate collection and improve the anaerobic digestion in order to obtain energy power through biogas, and a fertilizer as a product.

A step forward of the common anaerobic digestion process, is the separate phase approach finalized to the production of hydrogen in the first phase reactor and methane in the second phase reactor. This approach met two possibility: to produce hydrogen by dark fermentation and treat the effluent in anaerobic digestion with the aim to use this gas separately, or to mix this two gas to obtain the bio-hythane. Bio Hythane is the biological production of a gas with an average percentage composition of 10% H_2, 30% CO_2 and 60% of CH_4.

The advantage of this mix is that "hydrogen and methane are complimentary vehicle fuels in many ways: methane has a relatively narrow flammability range that limits the fuel efficiency and oxides of nitrogen (NO_x) emissions improvements that are possible at lean air/fuel ratios; the addition of even a small amount of hydrogen, however, extends the lean flammability range significantly; methane has a slow flame speed, especially in lean air/fuel mixtures, while hydrogen has a flame speed about eight times faster; methane is a fairly stable molecule that can be difficult to ignite, but hydrogen has an ignition energy requirement about 25 times lower than methane; finally, methane can be difficult to completely combust in the engine or catalyze in exhaust after treatment converters, in contrast, hydrogen is a powerful combustion stimulant for accelerating the methane combustion within an engine, and hydrogen is also a powerful reducing agent for efficient catalysis at lower exhaust temperatures" (Hythane ®).

The possibility to use this advantage with biogas produced from renewable resources was studied by Porpatham et al. (2007). They found that adding the 10% of hydrogen in biogas, the combustion rate

was enhanced, and there was an improvement in thermal efficiency and power output. Moreover a drastic reduction of HC emission was observed and there is no significant increase in NO level.

1.1 Methane production in anaerobic digestion processes: fundamentals

Anaerobic digestion is a process in which organic matter is decomposed in absence of oxygen and the main product is biogas, a mixture of 65% methane and 35% carbon dioxide (Mata-Alvarez J., 2003). Anaerobic digestion (AD) has been used to treat liquid wastes such as manures, domestic or industrial wastewaters, sludge from biological or physic-chemical treatments, etc. The large quantities of solid wastes, such as agricultural and municipal, attracted the interest of specialists because the large organic matter content of this wastes offer great potential for biogas production (Mata-Alvarez J., 2003). The solid wastes are complex substrate and obviously requires a more complex metabolic pathways to be degraded as it involves a more intricate series of metabolic reaction before final conversion to methane.

The anaerobic digestion process can be subdivided into the following four phases, each requiring its own characteristic group of micro-organisms:

- Hydrolysis: conversion of non-soluble biopolymers to soluble organic compounds
- Acidogenesis: conversion of soluble organic compounds to volatile fatty acids (VFA) and CO_2
- Acetogenesis: conversion of volatile fatty acids to acetate and H_2
- Methanogenesis: conversion of acetate and CO_2 plus H_2 to methane gas

A simplified schematic representation of anaerobic degradation of organic matter is given as Figure 1.1., and it is possible to observe where the process could be separate in a two phase approach. The acidogenic bacteria excrete enzymes for hydrolysis and convert soluble organics to volatile fatty acids and alcohols. Volatile fatty acids and alcohols are then converted by acetogenic bacteria into acetic acid or hydrogen and carbon dioxide. Methanogenic archea then use acetic acid or hydrogen and carbon dioxide to produce methane.

For stable digestion it is important that biological conversions remain coupled during the process, to prevent the accumulation of intermediate compounds. For example, an accumulation of volatile fatty

acids will result in a decrease of pH under which conditions methanogenesis cannot occur anymore, which results in a further decrease of pH. If hydrogen pressure becomes too high, further reduced volatile fatty acids are formed, which again results in a decrease of pH.

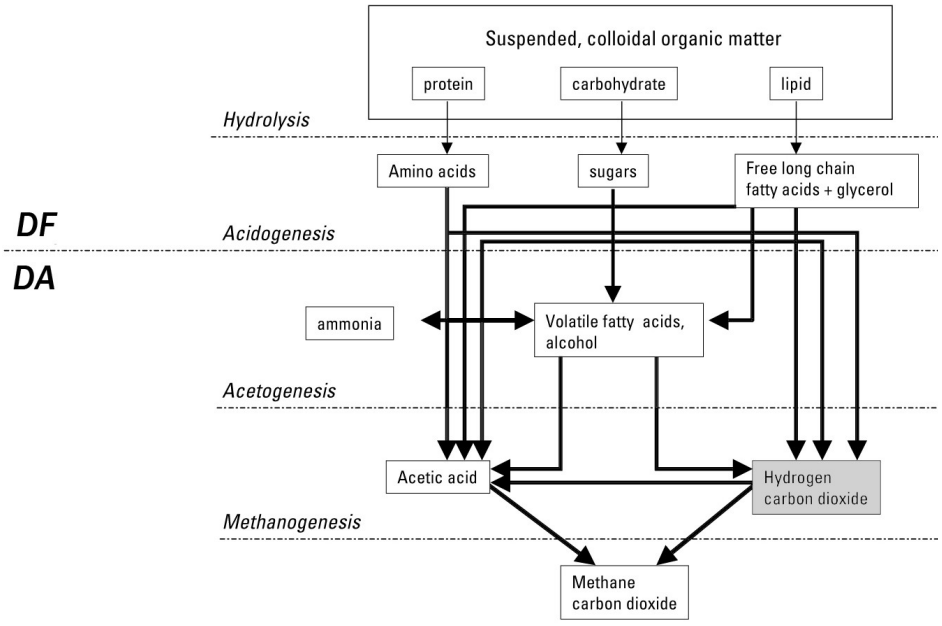


Figure 1.1 Schematic representation of the two phase anaerobic digestion process.

The anaerobic digestion is strongly influenced by environmental factors as temperature, pH and alkalinity. Controlled digestion is divided in psychrophilic (10-20 ºC), mesophilic (20-40 ºC), or thermophilic (50-60 ºC) digestion. As bacterial growth and conversion processes are slower under low temperature conditions, psychrophilic digestion requires a long retention time, resulting in large reactor volumes. Mesophilic digestion requires less reactor volume. Thermophilic anaerobic digestion is especially suited when the waste(water) is discharged at a high temperature or when pathogen removal is an important issue. During thermophilic treatment high loading rates can be applied. The relation between energy requirement and biogas yield will further determine the choice of temperature.

At higher temperatures, thermophilic bacteria replace mesophilic bacteria and a maximum methanogenic activity occurs at about 55°C.

The first steps of anaerobic digestion can occur at a wide range of pH values, while methanogenesis only proceeds when the pH is neutral. For pH values outside the range 6.5 – 8.0, the rate of methane production is lower. A sufficient amount of hydrogen carbonate (frequently denoted as bicarbonate

alkalinity) in the solution is important to maintain the optimal pH range required for methanogenesis. Several compounds exhibit a toxic effect at excessive concentrations such as VFA, ammonia, cations such as Na^+, K^+ and Ca^{++}, heavy metals, sulphide and xenobiotics, which adversely affect methanogenesis.

Systems used to digest solid waste are classified according to the percentage of Total Solids (TS) in the waste stream:

- 15-25% low solids anaerobic digestion: wet fermentation;
- >30% high solids anaerobic digestion: dry fermentation.

The most common form of low-solids reactor is the Continuously Stirred Tank Reactor (CSTR). Feed is introduced into the reactor, which is stirred continuously to ensure complete mixing of the reactor contents. At the same time an equal quantity of effluent is removed from the reactor. Retention time within the reactor can be varied according to the nature of the feedstock and process temperature applied, which is typically in the range of 2 - 4 weeks. The CSTR is generally used for treatment of slurries with a TS percentage of approximately 2-10%. The influent concentration range applicable for CSTR's is determined by:

• gas yield in relation to the energy requirement for heating;

• possibility of mixing the reactor content.

CSTR systems are applied in practice for treating animal manure, sewage sludge, household waste, agricultural wastes and kitchen waste or mixtures of these substrates. Mixing creates a homogeneous substrate, preventing stratification and formation of a surface crust, and ensures solids remain in suspension. Bacteria, substrates and liquid consequently have an equal retention time resulting in SRT is equal to HRT.

High-solids anaerobic digestion systems have been developed to digest solid wastes (particularly municipal solid waste or MSW) at solids contents of 30% or above. High-solids systems enable the reactor size to be reduced, require less process water and have lower heating costs.

The idea of two-stage systems is that the overall conversion process of the waste stream to biogas is mediated by a sequence of biochemical reactions which do not necessarily share the same optimal environmental conditions. The principle involves separation of digestion, hydrolysis and acidogenesis from the acetogenesis and methanogenesis phases. Optimising these reactions separately in different stages or reactors leads to a larger overall reaction rate and biogas yield. There are two kinds of two-phase digestion systems, one in which the different stages are separated, based

on a wet digestion, and one based on dry digestion, in which only the percolate experiences a second methanogenic stage. The first system operates on dilute materials, with a total solids content of less than 10%. For these reasons in this experimental work was applied the two phase approach using a CSTR system based on wet digestion.

1.2 Hydrogen gas production by dark fermentation

Actually there are a lot of research on the necessity to achieve a sustainable hydrogen economy such as lowering the cost of production, delivery, storage, conversion, and end use applications. Hydrogen is the most abundant element in the universe but it must be produced from other hydrogen-containing compounds such as fossil fuels, biomass, or water. Each method of production requires a source of energy, i.e., thermal (heat), electrolytic (electricity), or photolytic (light) energy (Kotay et al. 2008).

Generally, four basic processes are available for the production of hydrogen gas from nonfossil primary energy sources (Lay et al. 1998, Kapdan et al . 2006). These processes include: water electrolysis, thermochemical processes and biological processes.

Among them, biological techniques are a promising option in fact they offers the possibility of generating H_2 that is a renewable and carbon neutral source. Biohydrogen can be achieved in three main ways (Balat et al. 2010):

- bio photolysis of water by algae;
- photo-fermentation;
- dark-fermentation.

Thanks to the higher yield and lower costs, the dark fermentation is gaining importance during last ten years. In fact the reactor configuration is simply and the production of gas is independent from external factors as light sources.

The biological process allow to treat a wide range of substrate thanks to the microorganisms already present in a mixed culture coming from anaerobic digestion process. In industrial applications the use of mixed cultures for hydrogen production from organic wastes might be more advantageous because pure cultures can easily become contaminated with H_2-consuming bacteria but it is necessary to keep the process stable in terms of hydrogen yields in economically feasible conditions.

On the other hand the microflora in mixed culture often contain unwanted archea such as methanogens that consume the produced hydrogen and convert it to methane. Enrichment cultures of the H_2 microflora are prepared by heat/acid/basic treatment which inhibits the activity of the hydrogen consumers while the spore forming anaerobic bacteria survive.

During last ten years, most of the study on bio hydrogen production optimization using dark fermentation, were focused on the inhibition of hydrogen consuming bacteria already present in a mixed microflora inoculum, in order to optimize the gas yields.

The driving force for selecting the H_2 producing bacteria was the spore forming capacity after shock condition and the elimination of the methanogenic H_2 consuming archea in anaerobic digestion inoculum using process parameters.

The strategies that can be adopted to select the H_2 producing bacteria in a mixed culture approach, are:

- heat and chemical treatment of inoculum to select the spore forming bacteria;
- heat and chemical treatment of complex substrate to inhibit H_2 consuming bacteria;
- low HRT in order to washout the H_2 consuming bacteria;
- high organic loading rate in order to inhibit the methanogenic archea.

The most important parameter that influence the H_2 yields is the pH, because it influence the functionality of hydrogenase enzyme, that must be ranged between 5.0 and 6.5, with an optimum value at 5.5. This enzymes are called H_2-evolution, and they are able to reduce protons to hydrogen in a reversible way. The hydrogenase enzymes are classified by the metal on the active site (Fe-hydrogenase; NiFe-hydrogenase; NiSeFe-hydrogenase) and are influenced by pH and in a minor way temperature (Valdez-Vazques et al. 2009, Hallenbeck et al. 2002, Reith. et al. 2003).

The optimal pH value could be achieved through:

- pH control using chemicals during the process
- pH control using the recirculation of the anaerobic digestion effluent in a separate phase approach.

The decrease in pH is due to production of organic acids which depletes the buffering capacity of the medium resulting in low final pH specially if using complex substrate. Gradual decreases in pH inhibit hydrogen production since pH affects the activity of iron containing hydrogenase enzyme. Therefore, control of pH at the optimum level is required.

1.2.1 Process biochemistry and microbiology

Many microorganisms are able to produce hydrogen from carbohydrates for example *Clostridium* spp. (Gram+ anaerobic) and *Thermoanaerobacterium* spp. (in thermophilic or iper-thermophilic condition), *Enterobacter* (Gram- facultative anaerobic) and *Bacillus* (Gram+ facultative aerobic) in a minor proportion (Reith J.H. et al. 2003). The first two species are able to produce 4÷2 $molH_2/mol_{hexose}$, the second two 2÷1 $molH_2/mol_{hexose}$ (Kraemer et al. 2007, Kapdan et al. 2006, Li et al. 2007, Reith J.H. et al. 2003), but molecular techniques have mainly identified clostridial species as the principal productor of a range of end-products which may lower the H_2 yield from the theoretical maximum of 4 mol mol^{-1}_{hexose} achieved when acetate is the sole fermentation end product:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \qquad \textbf{Equation 1}$$

Hydrogen production in *Clostridium* is the property of hydrogenase enzymes. These transfer electrons from reduced ferredoxin or NADH to protons to regenerate the oxidised forms (Fdox and NAD+) required so that glycolysis and oxidative decarboxylation of pyruvate can proceed to generate ATP. In Figure 1.2, are shown the metabolic ways in *Clostridium spp* for glucose fermentation. Continuous lines shown the substrate conversion, the dotted lines shown the formation/consumption of ATP, and the broken lines shown the electrons flux. The circular boxes shown the typical dark fermentation metabolites, while the hexagonal boxes the solventogenic metabolites.

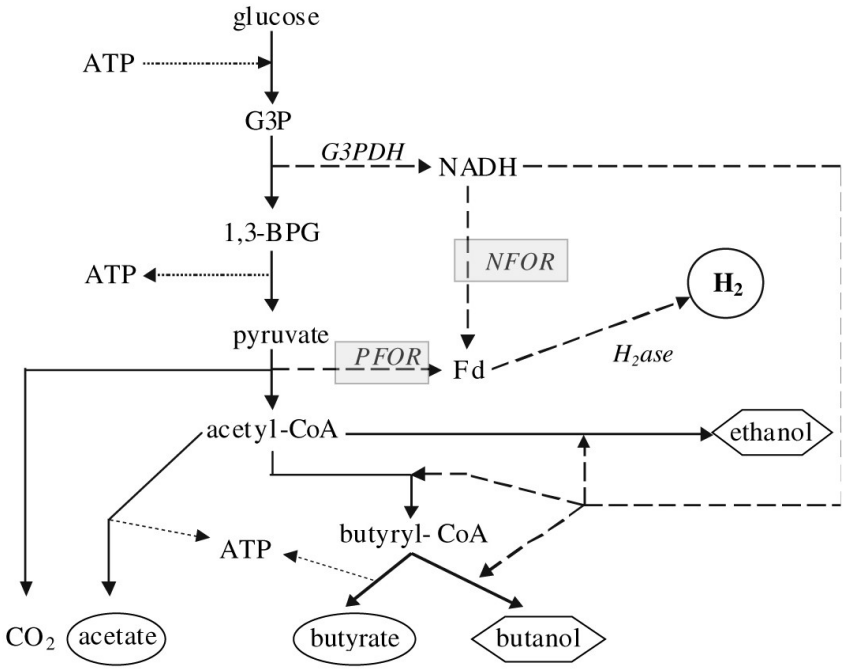


Figure 1.2 metabolic ways for *Clostridium spp* in glucose fermentation.

If the hydrogen partial pressure is sufficiently low, NADH may be oxidised via hydrogenase, producing H_2 up to the maximum theoretical yield of 4 mol mol−1 hexose consumed and a maximal yield of ATP. However, under normal reactor conditions, most of the NADH is oxidised in reactions producing reduced fermentation end products, such as butyrate with a lower molar hydrogen yield and lower ATP yield:

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \qquad \textbf{Equation 2}$$

Lactate and propionate can be produced either by clostridia themselves or by other bacteria competing in the mixed microflora, lowering the hydrogen yield.

Thus, the highest theoretical yields of hydrogen are associated with acetate as the fermentation end-product. In practice, high hydrogen yields are associated with a mixture of acetate and butyrate fermentation products, and low H_2 yields are associated with the so called solventogenic shift where at low pH condition or high hydrogen partial pressure, there is the production of propionate and reduced end-products such as alcohols and lactic acid (Levin et al., 2004, Antonopolou et al. 2008, Valdez-Vazques et al. 2009, Kraemer et al. 2007).

Clostridia such as *C. aceticum* can lower the H_2 yield by converting H_2 and CO_2 to acetate or can convert hexose directly to acetate alone by the process of homoacetogenesis:

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \qquad \textbf{Equation 3}$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \qquad \textbf{Equation 4}$$

In batch growth of *Clostridia* the metabolism shifts from a hydrogen/acid production phase to a solvent production phase, when the population reaches to the stationary growth phase.

The dominant culture of *Clostridia* can be easily obtained by heat treatment of biological sludge. The spores formed at high temperatures can be activated when required environmental conditions are provided for hydrogen gas production. (Kapdan et al. 2006).

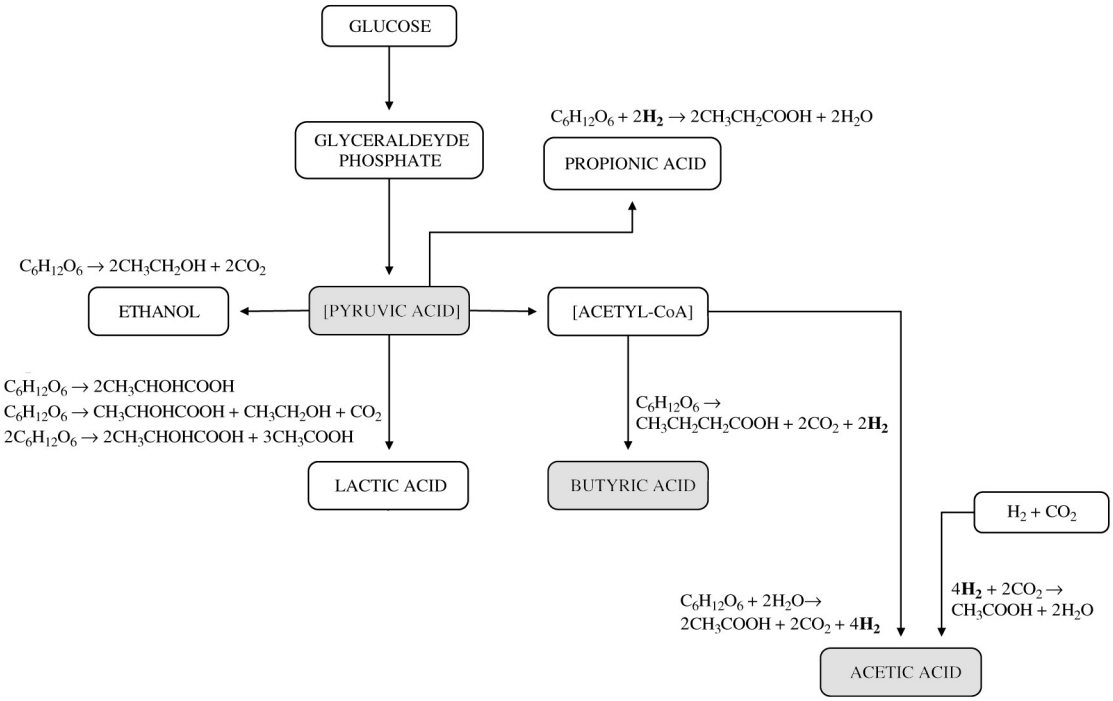


Figure 1.3 Possible metabolic ways in dark fermentation.

In Figure 1.3 are reported the possible metabolic ways in dark fermentation process.

1.2.2 Substrates for hydrogen production

Actually, considering the range of potential substrates that can be used by the species of hydrogen producing bacteria, this process is suitable and open for further exploration. From a thermodynamic point of view, the conversion of carbohydrates to hydrogen and organic acids is preferred because it yields the highest amount of hydrogen per mole of substrate. (Reith et al. 2003). These carbohydrates can be monosaccharides but may also be polymers such as starch, cellulose or xylan.

Glucose is an easily biodegradable carbon source, present in most of the industrial effluents and can be obtained abundantly from agricultural wastes. Theoretically bioconversion of 1 mol of glucose yields 12 mol of hydrogen gas. According to reaction stoichiometry, bioconversion of 1 mol of glucose into acetate yields 4 mol H_2/mol glucose, but only 2 molH_2/mol glucose is formed when butyrate is the end product. Production of butyrate rather than acetate may be one of the reasons for deviations from the theoretical yield. Therefore, utilization of substrate as an energy source for bacterial growth is the main reason for obtaining the yields lower than theoretical estimations.

Some studies indicated that the higher hydrogen yields could be obtained from sucrose compared to other simple sugars. However, the yield per mole of hexose remains almost the same for all types of the disaccharides.

Other substrates can be used as starch containing materials, that are abundant in nature and have great potential to be used as a carbohydrate source for hydrogen production, and cellulose plant biomass that are highly available in agricultural wastes and industrial effluents such as pulp/paper and food industry (Reith et al. 2003).

Food industry wastes constitute a major fraction of the municipal solid wastes. Composting, incineration and anaerobic digestion are the conventional approaches for the solid waste management. However, high carbohydrate content in form of simple sugars, starch and cellulose makes the solid food wastes a potential feedstock for biological hydrogen production. The problem with the food waste is the variations in carbohydrate and protein types and concentrations in the mixture in fact each component requires different environmental and bio-processing conditions for hydrogen gas production. This favourable condition could be achieved in a mixed culture system

1.2.3 State of the art of hydrogen production by dark fermentation treating organic waste

Currently a lot of papers deal with the optimisation of the best conditions for bio-hydrogen production through dark fermentation using a wide range of substrates. As mentioned above, most of this papers concern with the bio-hydrogen production from simple carbohydrates substrates, but the necessity to treat large amount of organic wastes keep the attention of new research project. In fact organic solid waste would be a feasible feedstock of which reduction and stabilization could be accomplished by H_2 fermentation. The following state of the art take into account the experimental works on optimisation of bio hydrogen production treating organic waste like food waste or simulate organic solid waste.

1.2.4 Inoculum/substrate treatment

Mixed cultures from anaerobic digestion process have a dominance of non-H_2 producing acidogens and/or H_2 consuming microorganism and besides, the organic solid waste itself contained many types of indigenous microorganism. The microorganism could prevail easily in start up period when the H_2 producing bacteria have not been fully acclimated to waste (Hawkes et al. 2002). To reduce the risk and provide a favorable environment for H_2 producing bacteria it is possible to select them treating the inoculum or the food waste physically or chemically.

Inoculum from anaerobic digestion could be treated by acid, basic or heat shock treatment. Among this treatments heat shock reach the better yields compared with the others. Some authors use a 90°C temperature for 10-30 min (Lee et al. 2010; Kim et al. 2008a), but Valdez-Vazquez et al. (2009) reviewed the effectiveness of heat treatment with 100°C for 15 min or 80°C for 3h, suggesting the necessity to repeat the treatment during long working periods in order to avoid the formation of H_2 consuming microorganism specially if using complex substrate. In alternative to inoculum treatment some authors treat directly the organic waste. Kim et al. (2009) found that in the food waste there are 11 type of lactic acid bacteria (LAB); Noike et al (2002) studied the inhibition of hydrogen production using organic waste at low pH value of 4,5 by lactic acid bacteria. Kim et al (2009) study the hydrogen fermentation of food waste without inoculum addition in batch test, using acid, basic and heat shock treatment and they found that the heat shock was the best treatment associated with the highest hydrogen yield and with the depletion of the LAB. Heat shock treatment effectiveness was confirmed by Noike et al (2005) that treat the waste at 70°C for 30 min. Also Wang et al (2009)

used directly the organic waste in a semi continuous rotating drum reactor, but in this case they obtained good yields without any treatment.

In another study Kim et al. (2008b) test the acid, basic and CO_2 sparging treatment for the organic waste, using in addiction also an inoculum heat shock treated. They used an ASBR with controlled pH (5,3) and found that the best treatment was the alkali treatment (water and 6M KOH for 1 day).

It is also possible to use different type of inoculum, not from anaerobic digestion: Chou et al (2008) used the supernatant liquid of grass composting mixture heated at 85°C for 3h; Ueno et al (2006) used a thermophilic microflora enriched from activated sludge compost incubate with nutrient enriched medium; Alzate-Gaviria et al (2007) prepared a mixture of non-anaerobic inocula composed by deep soil, execrete vaccine, pig execrete, sodium carbonate and water; Fan et al. (2006) used a composted sludge, treated at 103° for 24h, than re suspended and filtrated; Li et al. (2008) used acidogenic sludge from a kitchen waste composting plant.

Another technique used by some authors is the acclimatization of the inoculum, for example Shin et al. (2005) use an inoculum from anaerobic digestion and fed it with organic waste for 3 months using a CSTR (55°C, HRT 3d, OLR 2 kgVS/m^3d), or Zhu et al (2008) that fed the inoculum with a sugar solution. In Table 1-1 are shown the H_2 yields in experimental test where the inoculum or the food waste were treated. It is possible to observe that the pre treatment of inoculum or substrates were mainly use in different reactor configuration (not CSTR). In fact in CSTR configuration was mainly applied the pH control to keep the optimal condition for hydrogen production.

Table 1-1 Hydrogen yields treating inoculum or substrate

Ref	Reactor	AD inoculum	inoculum treated	substrate treated	pH control	T	pH	HRT	H_2	HPR	SHP
								d	%	m^3/m^3d	$lH_2/kgVS_{fed}$
Han et al. 2005	LBR	YES	YES	NO	NO	M	5,5	0,5	18,7	3,55	290
Kim et al. 2008a	ASBR	YES	YES	YES	YES	M	5,3	1,25	-	-	24,5
Chou et al. 2008	SBR	NO	YES	NO	-	M	-	3	-	-	20,8*
Kim et al. 2008b	ASBR	YES	YES	YES	YES	M	5,3	1,25	-	0,9	25,8
Kim et al. 2008b	ASBR	YES	YES	YES	YES	M	5,3	1,4 (SRT 5.25)	-	-	80,9
Lee et al. 2010	CSTR	YES	YES	NO	YES	T	5-5,7	1,9	49,8	2,95	83,0

* TS basis

M: mesophilic temperature range
T: thermophilic temperature range
LBR: leaching bed reactor
ASBR: anaerobic sequencing batch reactor
SBR: sequencing batch reactor
CSTR: continuous stirrer tank reactor

1.2.5 Process parameters: hydraulic retention time (HRT), organic loading rate (OLR), temperature and pH control

Hydraulic retention time (HRT) is defined as the *volume of the reactor/volumetric flow* and is also known as the inverse of the dilution rate (D). The continuous stirred tank reactors (CSTR) or chemostat could be used to select microbial populations whose growth rates are able to catch up to the dilution caused by continuous volumetric flow. In this way, only microbial populations with growth rates larger than the dilution rate can remain in the reactor (mmax > D). Based on this, high dilution rates (short HRT's) could be used to cause the complete wash-out of methanogens since the specific growth rates of methanogens are much shorter than those of H_2-producing bacteria (0.0167 and 0.083 h^{-1}, respectively) (Valdez-Vazquez et al. 2009).

The H_2 fermentation pattern may shift to methanogenic fermentation if the HRT is increased but with complex substrate like organic waste it is necessary to apply higher HRT in order to give the time to decompose carbohydrates. The typical HRT applied in a CSTR treating solid organic waste ranged from 5 to 2 days, while with other reactor configurations it is possible to use lower HRT (until 6h). There is not an optimum HRT because the optimization of the process depends also by other process parameters such as the organic loading rate, the characteristics of organic substrate and the pH control, but considering the literature data shown in Figure 1.4 the higher hydrogen production rate (HPR) are observed at HRT < 3d.

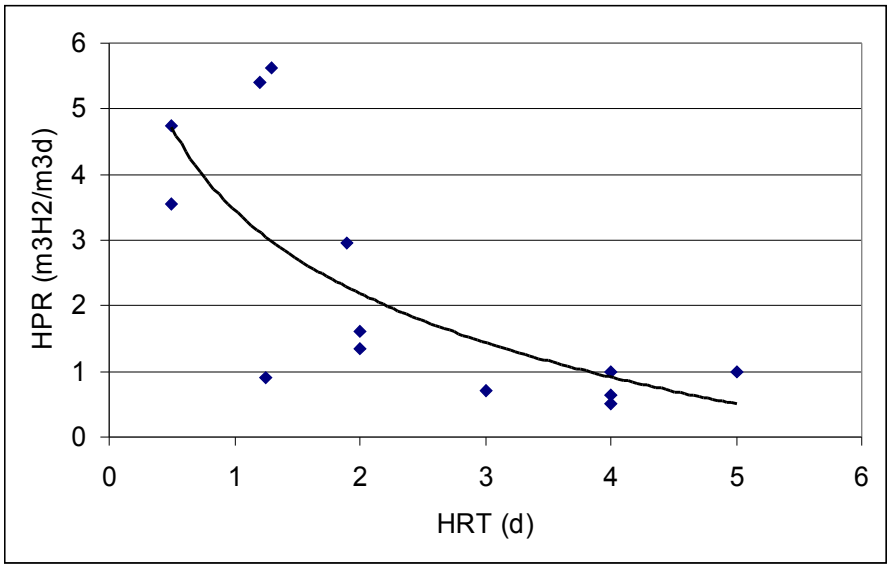


Figure 1.4 Relation between HRT (d) and HPR (m3/m3d) considering literature data.

Shin et al. (2005) study the conversion of food waste into H_2 by thermophilic acidogenesis, in a CSTR reactor, changing the HRT (5, 3, 2 days), the OLR (6, 8, 10 kgTVS/m^3d) and the pH (5, 5.5, 6). They shown the best yields (125 lH_2/kgTVS$_{fed}$) using an HRT of 5d, OLR of 8 kgTVS/m^3d and a controlled pH of 5.5; using an HRT of 2d the VFA content decrease with an accumulation of propionate and lactic acid.

A study made by Li et al (2008) shown the best specific hydrogen production using an HRT of 2d instead of 5d with a SHP value that increase from 22,4 to 26,8 lH_2/kgCOD add but using an OLR > 20 kgCOD/m^3d. Gomez et al (2006) observe that a decrease of the HRT from 5 to 3 days in CSTR using the same amount of waste, caused a small increase of SHP 34,4 lH_2/kgVS$_{fed}$ to 35,5 lH_2/kgVS$_{fed}$.

The same objective of H_2 producing bacteria selection could be achieved using the organic loading rate (OLR). With the HRT it is possible to cause a wash out of methanogenic archea avoiding the use of H_2 produced to form methane, while with OLR it is possible to cause a decrease of pH as consequence of the overloading of the reactor that leads to an overproduction of organic acids and other metabolites from the incoming substrate. In this condition it is necessary to exercised a pH control in order to keep the pH in a range favourable for hydrogen accumulation (Valdez-Vazquez et al. 2009).

Lee et al. (2010) observe the variation in H_2 production maintaining the HRT at 4d, in a CSTR reactor, changing the OLR from 19 to 28 kgCOD/m^3d, controlling the pH at 6. They found an increasing in H_2% from 35 to 48 and an SHP from 20,16 to 38,80 lH_2/kgCOD$_{fed}$.

The temperature range is another process parameters but it is not linked to the selection of the H_2 producing microflora. It is important mainly for the kinetic point of view because, as in anaerobic digestion, higher temperature increase the degradation velocity improving the gas yields. Valdez-Vazquez et al (2005) compared mesophilic and thermophilic temperature in a CSTR reactor fed with organic waste (11 kgVS/m^3d) and they observed an increasing in the specific hydrogen production from 94 to 198 Nl/kgVS. Lee et al. (2010) and Li et al. (2008) used very similar condition (as shown in Table 1-2) : 4 days of HRT, 26-28 kgCOD/m^3d of OLR and a pH control, but they applied different temperature range. Changing from mesophilic to thermophilic range, hydrogen production rate increase from 0,63 to 1 m^3/m^3d and the specific hydrogen production from 22,4 to 38,1 lH_2/kgCOD$_{fed}$. The same conclusions was observed by Shin et al (2004) testing in batch tests (5days, 3 kgVS/m^3d) in mesophilic and thermophilic range. In Table 1-2 are shown the experimental

conditions applied in CSTR system for bio hydrogen production treating organic wastes with pH control between 5,3 and 6.

Table 1-2 experimental condition applied and hydrogen yields in CSTR reactor treating organic waste, only with pH control

	T range	OLR	pH	HRT	H_2	HPR	SHP
Ref		kgTVS/m^3d		d	%	m^3/m^3d	$lH_2/kgTVS_{fed}$
Shin et al. 2005	T	8	5,5	5	60	1	125
Ueno et al. 2007a	T	97*	5.8-6.0	1,2		5,4	56 *
Ueno et al. 2007b	T	74,3*	6	0,5		4,75	63,9 *
Lee et al. 2010	T	19*	6	4 d	35	0,5	20,16 *
Lee et al. 2010	T	28*	6	4 d	48	1	38,08 *
Li et al. 2008	M	26*	5.3 - 5.6	4 d	33	0,63	22,4 *
Li et al. 2008	M	50*	5.3 - 5.6	2 d	35	1,34	26,88 *
Gomez et al. 2006	M		5 - 6	3	25-27	0,7	26,2

* COD basis;

The data shown in the previous table take into account also the acidogenic step of a separate phase approach tests.

As mentioned before the two-phase approach is becoming an interesting solution thanks to the capacity of the anaerobic digestion to convert the volatile fatty acids into methane and carbon dioxide. A second interesting aspect, developed also in this PhD thesis, was the pH control adopting a recirculation of anaerobic sludge. This concepts meet the necessity to reach an economic feasible process, without any inoculum or substrate treatment and without an expensive pH control using chemicals. In Table 1-3 are shown the experimental parameter and hydrogen yields without both treatment and pH control.

Table 1-3 experimental parameter and hydrogen yields without both treatment and pH control in a two phases system.

Ref	Reactor 1° phase	Reactor 2° phase	Qr/Qin	AD inoculum	OLR 1°phase kgTVS/m^3d	Temperature range	pH	HRT d	H_2 %	HRP m^3/m^3d	SHP $lH_2/kgTVS_{fed}$
Kataoka et al. 2005	CSTR	CSTR		YES		T	4,0-4,5	2.5-6	2 - 45	-	< 5
Kataoka et al. 2005	CSTR	CSTR	0.25-0.5	YES		T	5 - 6	2.5-6	25 - 63	-	20 - 30
Kataoka et al. 2005	CSTR	CSTR	0.4-0.5	YES	4.7 - 5.6	T	5	7.07	44	-	
Liu et al. 2006	CSTR	CSTR		YES	37,5	M	5,2	2	42	1,6	43
Chu et al. 2008	CSTR	CSTR	2	YES	38,4	T	5,5	1.3	52-56	5,62	205
Wang et al. 2009	semi continuous rotating drum	CSTR		ONLY organic waste	15,1	M	5,2 - 5,8	10		-	71
Wang et al. 2009	semi continuous rotating drum	CSTR		ONLY organic waste	22,65	M	5,2 - 5,8	6,6	30	-	65

Comparing the two working conditions adopted by Kataoka et al (2005) with and without recirculation of the sludge, it is possible to observe how the process yields are influenced by the pH: without the pH control the values are about 4.0-4.5 and the specific hydrogen production is less than 5 lH_2/kgTVSd, while with a recirculation the pH is between 5 and 6 and the SHP reach higher values of 20-30 lH_2/kTVSd. This means that the alkalinity of the anaerobic digestion sludge allow a stabilization of the pH on the right range values.

Kramer et al (2005) study the reduction of external alkalinity addiction in a two phases system with the recirculation of anaerobic sludge, treating a glucose medium and obtained a reduction of 40% of external alkalinity addition, but they note an inhibition of H_2 production caused by a proliferation of methanogenic archea.

The better yields obtained using the recirculation approach are achieved by Chu et al (2008). They use two CSTR reactor, with recirculation rate of 2 and obtained the 42% of H_2 content in the biogas and 205 $lH_2/kgTVS_{fed}$. The AD sludge is before settled and recirculated only the thick matter. The pH maintained at 5,5 and no methane is detected.

Interesting is the approach of Wang et al (2009), they didn't use an inoculum but they treat directly the organic waste in a separate phase approach and obtained an SHP of 65 $lH_2/kgTVS_{fed}$ and 30% H_2, applying an HRT of 6 days in a semicontinuous rotating drum reactor, with an OLR of 22,65 $kgTVS/m^3d$.

In order to compare this PhD work with those with similar conditions with sludge recirculation, Table 1-4 shown the separate phases process parameters and yields.

Table 1-4 two phases approach with sludge recirculation

			first phase					second phase			
Ref.	substrate	Qr/Qi	T	pH	HRT	OLR	SHP	T	HRT	OLR	SGP
			(ºC)		(d)	(kgVS/m^3d)	(l/kgVS$_{fed}$)	(ºC)	(d)	(kgVS/m^3d)	(m^3_{biogas}/kgVS$_{fed}$)
Kataoka et al. 2005	food waste	0.25-0.5	55	5 - 6	2.5-6	20,8 – 8,45	20-30	55	18-30	2.84 - 1.18	0,49
Chu et al. 2008	food waste	2	55	5,5	1,3	38,4	205	35	5	6,6	0.61*
Lee et al. 2010	food waste	1	55	5.5 - 5.57	1,9	39*	83	55	7,7	8.4*	0.21 (CH_4)

*on COD basis

1.3 Batch tests for hydrogen potential (BHP)

Anaerobic biodegradability assays are used to establish anaerobic biodegradability, for determination of the ultimate methane potential of wastes, but are also used for determination of the rate of this biodegradation in general (Angelidaki et al 2008).

The necessity to develop this method was associated with the final waste destiny, strictly linked with the residual content of putrescible matter that could be expressed with the residual methane production.

This type of batch tests are used in this thesis in two different way:

- evaluate the hydrogen batch production using organic wastes, applying different organic loading;
- optimize the BMP protocol for the evaluation of anaerobic digestion effluent treating organic wastes.

The biochemical hydrogen potential was tested by some authors, and the main purpose was the determination of the maximum hydrogen yields of different substrates. As in the continuous approach, also in this batch test was evaluated the acid/basic and thermal treatment of inoculum or substrates, in order to select the hydrogen producing microorganisms (Table 1-5).

Kim et al (2009) study different pre-treatment of food waste without inoculum, and they observe an SHP of 50.9, 89.5, 96.9 $lH_2/kgTVS_{fed}$ for alkali, acid and heat treatment respectively; choosing the heat shock treatment they test different temperature and defined the best yields at 90°C with an SHP of 148.7 $lH_2/kgTVS_{fed}$. All the batch test were carried out at 30 $kgCOD/m^3d$.

Kim et al (2004) test different VS concentration mixing the organic wastes with sewage sludge, treating the inoculum by heat-shock. They found an SHP for only organic waste of 121,6 $lH_2/kgCOD$. Okamoto et al (2000) tested individually the food waste component with a heat-shocked inoculum. The SHP production ranged 19,3-96,0 $lH_2/kgTVS$ for rice, 26,3-61.7 $lH_2/kgTVS$ for cabbage, 44,9-70,7 $lH_2/kgTVS$ for carrot. Shin et al (2004) tested in thermophilic and mesophilic conditions 3 pH set (4.5, 5.0 and 5.5) and obtain the best yields in thermophilic temperature with a maximum yields at pH 4.5 (SHP 46,3 $lH_2/kgTVS$). Set the pH they change the OLR (3, 6, 8, 10 $kgTVS/m^3d$) and obtain an SHP of 91,5 $lH_2/kgTVS$ at 6 of OLR. In this last study the inoculum was acclimated in a reactor with 5 days HRT.

In this PhD thesis the BHP was tested using an inoculum without any treatment and applying an organic loading of 20 and 30 $kgTVS/m^3$ in order to evaluate the behaviour of the process.

Table 1-5 Hydrogen batch tests: conditions applied and yields.

Ref	Inoculum treated	substrates	T °C	pH	H_2 yields molH$_2$/mol hex	SHP LH$_2$/kgVS$_{fed}$
Kim et al. 2009	No inoculum, substrates treated	food waste	35	7	1,65	96,9
Kim et al. 2009	No inoculum, substrates treated	food waste	35	7	1,98 consumed	148,7
Kim et al. 2004	YES	food waste and sewage sludge (87:13)	35	5 - 6		122,9*
Kim et al. 2004	YES	food waste	35	5 - 7		121,6*
Okamoto et al. 2000	YES	individual component	37	7		19,3 - 96
Noike et al. 2000	NO	bean curd manufacturing waste	35		2,54	14 - 21
Noike et al. 2000	NO	rice bran	35		1,29	31 - 61
Noike et al. 2000	NO	wheat bran	35		1,73	10 - 43
Shin et al. 2004	NO	food waste	55	4,5	1,8	91,5
Lay et al. 1998	YES	food waste	37	5,6		45
Lee et al. 2008	NO	food waste	55	6		10,7*

* on COD basis

1.4 Stability evaluation of AD effluent: Dynamic Respirometric Index (DRI) and Biological Methane Potential (BMP)

The effect of the European Directive on waste management (91/156/EEC; 91/689/EEC; 94/62/EU), was a large number of technology, proposed with the aim to reduce the organic waste sent to landfill. Separate collection of waste at the source and mechanical selection for the unsorted waste, allow to re-use some kind of material, like plastic, iron and paper, and to treat the organic fraction obtained. For the treatment of this last substrate composting process is the most adopted, in fact it allows waste to be stabilized and re-used as a secondary material like organic amendment. Among the technologies proposed, anaerobic digestion became the alternative to composting, mainly for its lower gas emission and for the high-energy recovery from biogas production. The OFMSW is mixed with the activated sludge coming from the wastewater biological treatment, and thanks to the anaerobic condition the organic matter is converted to methane and carbon dioxide. The effluent is dewatered by press-belt or other types of dewatering systems, and the solid part is usually send to composting and mixed again with the OFMSW and bulking agent, and than treated by aeration. This kind of solid waste is the product of a stabilization process, so this secondary aerobic stabilization is probably a loss of money and energy.

The aim of this last aspect is the possibility to reduce the treatment time spend for the dewatered sludge in composting plants, considering two different index: the dynamic respirometric index (DRI), where the oxygen uptake rate by microorganisms degrading the biodegradable fraction of the organic matter under standardized conditions is continuously monitored, and the biochemical methane potential (BMP), where the biogas produced by waste in long-term incubation laboratory tests (21–100 days), is measured. BMP test is often used to determine the methane potential and the biodegradability of organic waste treated by anaerobic digestion process in order to evaluate the final biogas production.

In the PhD thesis was evaluate how much is the residue biogas potential coming from dewatered sludge, after anaerobic co-digestion of waste activated sludge (WAS) and the organic fraction of municipal solid waste (OFMSW). The wastewater treatment plants taken in consideration were three: Treviso, Camposampiero (PD) and Bassano (VI). The DRI test was performed on the Treviso dewatered sludge.

1.5 Aim of the Ph.D research study

Renewable fuels derived from non-fossil carbon sources like urban refuse, animal and agricultural wastes are gaining importance.

Among the available fuels, biogas has an extremely low energy density on the volume basis, low flame velocity and not so wide flammability limits on account of its high CO_2 content. Recent studies shown that performance can be improved by inducting a small amount of hydrogen along with biogas. Hydrogen presence in biogas (10% addition was found to be the most suitable) significantly enhances the combustion rate and extends the lean limit of combustion of biogas, moreover the HC emissions during combustion are reduced (Porpatham et al. 2007).

Among all the technologies dealt with H_2 production, dark fermentation is becoming the most interesting application thanks to its accomplishment of the dual goals of waste reduction and energy production, especially if considering the two-stage configuration.

This process has several advantages over the conventional single-stage process, since it permits in specific condition, the selection and enrichment of hydrogen producing bacteria in one reactor, and biogas production in a second reactor. In fact hydrogen is an intermediary product in a single phase AD that is, however, not available because it is rapidly taken up and converted into methane by methane-producing archea.

The Ph.D. project aimed to optimize the two phases process treating organic waste for bio-hythane production, in pilot plant, testing the Biochemical Hydrogen Potential (BHP) through batch test, and taking into account also the final disposition of AD effluent considering two stability parameters like Dynamic Respirometric Index (DRI) and Biochemical Methane Potential tests (BMP).

2 Materials and methods

The characterization of inoculum, substrate, reactor set up and work plan, are described in this chapter.

2.1 Biochemical Hydrogen Potential (BHP) tests

2.1.1 Inoculum and substrate

The anaerobic digested sludge used as inoculum was taken from the anaerobic digester of Ludlow. The sludge was than heated in order to keep the temperature from 37°C to 55°C and maintained without feeding for four day. The average characteristics of inoculum are shown in Table 2-6.

Table 2-6 Characterization of inoculum and substrate used in batch test

parameters	u.m	inoculum	substrate
TS	g/kg	35,2	247,75
TVS	g/kg	21,8	236,12
ALKALINITY tot	mg$CaCO_3$/l	8500	-
VFA	mgCOD/l	509	-
NH3	mgN/l	1575	-
PH		7,45	-

The substrate used was the organic fraction of municipal solid waste, coming from the treatment plant of Luslow. The substrate was collected fresh, selected and minced (Figure 2.5).

Figure 2.5 grinder.

No treatment were done in order to evaluate the possibility to control the hydrogen production using process parameters like the organic loading. The purpose was to overload the microorganisms in order to select the hydrogen producing bacteria and inhibit the methanogenic ones.

2.1.2 Analytical program

At the end of the BHP test, stability parameters were measured, in order to observe the behavior of the hydrogen and methane producing process. pH was measured using a jenway 3010 pH meter (Jenway, London, UK) with temperature compensation and combination electrodes. Alkalinity was determinated by titration with 0,25 N H_2SO_4 to endpoints of 5.7 and 4 and the results expressed as total alkalinity. The volatile fatty acids concentration was measured using a Shimadzu GC-2010 gas chromatograph (Milton Keynes, UK), equipped with a flame ionization detector and a capillary column type SGE BP-21 with helium as gas carrier. Gas samples were taken directly from the gas counter, in order to have an instantaneous value of the gas composition. The composition was measured using a gas chromatograph Varian 3800, equipped with a TCD detector.

2.1.3 Experimental set up

Six reactors were used for the experiment with 1,5 liters of working volume (Figure 2.6), fitted with a flanged top plate through which a stirrer was inserted via a draught tube: this allowed the contents to be stirred continuously at 40 rpm using an off-set bar stirrer. The reactors were heated at 55°C by a circulation of hot water from a thermostatically controlled reservoir. The reactors were load via a bung in the flange plate. The gas production was measured with a gas flow meter constructed and calibrated as described in Walker et al. (2009) and connected with 5-liters gas sampling bags. The gas production was corrected at STP (101.325 kPa, 0°C).

The reactors were filled with inoculum, without any nutrient supplement, and whit a defined amount of food waste. The tests were carried out in triplicate: three reactors were working at 20 kgTVS/m^3 adding 138,2 g of FW and three at 30 kgTVS/m^3 adding 207,4 g of FW. The gas production, gas composition and volatile fatty acid content were measured every hours from the feeding till about 8 hours, than every 2 hours. The variation of this parameters let us knows how the biomass was adapting to the high organic content, and how the organic content was converted to the organic acids, CH_4 and CO_2. At the end of each cycles, the effluent was analyzed in terms of pH, ammonia and lactate.

Figure 2.6 Batch tests reactors

2.2 Continuous hydrogen and methane production in CSTR reactors

2.2.1 Inoculum and substrate

The seed sludge used as inoculum for the methanogenic reactor was collected in the WWTP located in Treviso (northern Italy) where a 2000 m^3 anaerobic digester treats the source collected biowaste at a working temperature of 35ºC.

The characteristics of inoculum in terms of total solids, volatile solids, macro pollutants, pH and alkalinity are shown in Table 2-7.

Table 2-7 Inoculum characterization

parameter	u.m.	AV	min	max	SD
TS	g/kg	22,87	22,31	23,38	0,46
TVS	g/kg	13,38	13,03	13,70	0,35
TVS, TS	%	58,48	57,72	59,21	0,61
TKN	mgN/l	0,50	0,48	22,40	0,02
Ptot	mgP/l	0,06	0,06	0,07	0,01
pH		7,51	7,31	7,69	0,16
Alkalinity tot	$mgCaCO_3/l$	2074,2	2.060,8	2.087,7	11,6

The sludge was than acclimatized for one week to thermophilic temperature (55ºC) moving through a one-step temperature change (Cecchi et al. 1993, Bolzonella et al 2003).

The fermentative reactor was fed with the source collected organic biowaste coming from the same WWTP, mixed with tap water. The feedstock was prepared without adding any chemical reagent and without thermal treatment. This kind of substrate has a high carbohydrate content that can be converted into hydrogen and organic acids through the action of fermentative bacteria.

In order to avoid problems of pipe clogging, the substrate was previously reduced using a grinder (Figure 2.7)

Figure 2.7 Grinder

The size of the treated waste was similar to those of a full-scale plant where two grinder pump are applied on line before loading the anaerobic digestor.

2.2.2 Sampling and analysis program

The effluent of both reactors was monitored 2/3 times per week in terms of solid content, chemical oxygen demand, total K nitrogen, total phosphorus, and daily for the stability parameters such as pH, volatile fatty acid content, alkalinity and ammonia, all in accordance with the Standard Methods (APHA-AWWA-WEF), (Table 2-8).

Table 2-8 analysis program

Stability parameters		Unit	Frequency
pH	pH	-	daily
Alkalinity	ALK	$mgCaCO_3/L$	daily
Ammonia	NH_3	$mgN\text{-}NH_3/L$	daily
Fatty volatile acids	VFA	mgCOD/L	daily
Total and volatile solids	TS-TVS	g/L	2-3 per week
Process parameters			
Hydraulic retention time	HRT	Giorni (d)	2-3 per week
Organic loading rate	OLR	$kgTVS_f/m^3_rd$	2-3 per week
Yields parameters			
Gas production rate	GPR	m^3_{biogas}/m^3_rd	daily
Specific gas production	SGP	$m^3_{biogas}/kgTVS_fd$	2-3 per week
Gas composition	$\%CH_4$ $\%CO_2$ $\%$ H_2	%	daily
Solfidric acid	H_2S	Ppm	daily
Macro pollutants			
Chemical Oxygen Demand	COD	gCOD/L	2-3 per week
Total Kijeldal Nitrogen	TKN	mgN/L	2-3 per week
Total phosphorus	Ptot	$mgP\text{-}PO_4/L$	2 per week

Volatile fatty acids content was monitored using a gas chromatograph (Carlo Erba instruments) with hydrogen as gas carrier, equipped with a Fused Silica Capillary Column (Supelco NUKOL™, 15m x 0,53mm x 0,5 μm film thickness) and with a flame ionization detector (200°C). The temperature during the analysis started from 80°C and reaches 200°C trough two other steps at 140°C and 160°C, with a rate of 10°C/min. The analyzed samples were centrifuged and filtrated with a 0,45 μm membrane.

Gas production was monitored continuously by two gas flow meters (Ritter Company, drum-type wet-test volumetric gas meters), while the biogas composition (CO_2-CH_4-H_2S) was defined by a portable infrared gas analyzer (geotechnical instrument, model. GA2000). Hydrogen content in the fermentative reactor was measured by a gas-chromatograph (GC Agilent Technology 6890N) equipped with the column HP-PLOT MOLESIEVE, 30m x 0.53mm ID x 25um film, using a thermal conductivity detector and argon as gas carrier.

2.2.3 Experimental set-up

Two stainless steel CSTR reactors (AISI 304) were employed for optimized H_2 and CH_4 production, respectively. The first reactor, dedicated to the fermentative step, had a 200 l working volume, while the second reactor dedicated to the methanogenic step had a 380 l working volume (Figure 2.8).
Both the reactors were heated by a hot water recirculation system and maintained at 55°C using electrical heater controlled by a PT100-based thermostatic probe. The feeding system was semi-continuous, arranged once per day.

a) b)

Figure 2.8 Pilot plant reactors: a) first phase reactors; b) second phase reactors.

The flow scheme of the pilot plants is shown in Figure 2.9. The organic waste was reduced in size using a grinder, than mixed with tap water and anaerobic sludge (in Run III) and fed to the first phase reactor. The same amount of sludge was collected at the end of the feeding process. In ideal condition all the effluent was fed to the second reactor, but to maintain the HRT at 12,6 days, half of the effluent was treat in anaerobic digestion.

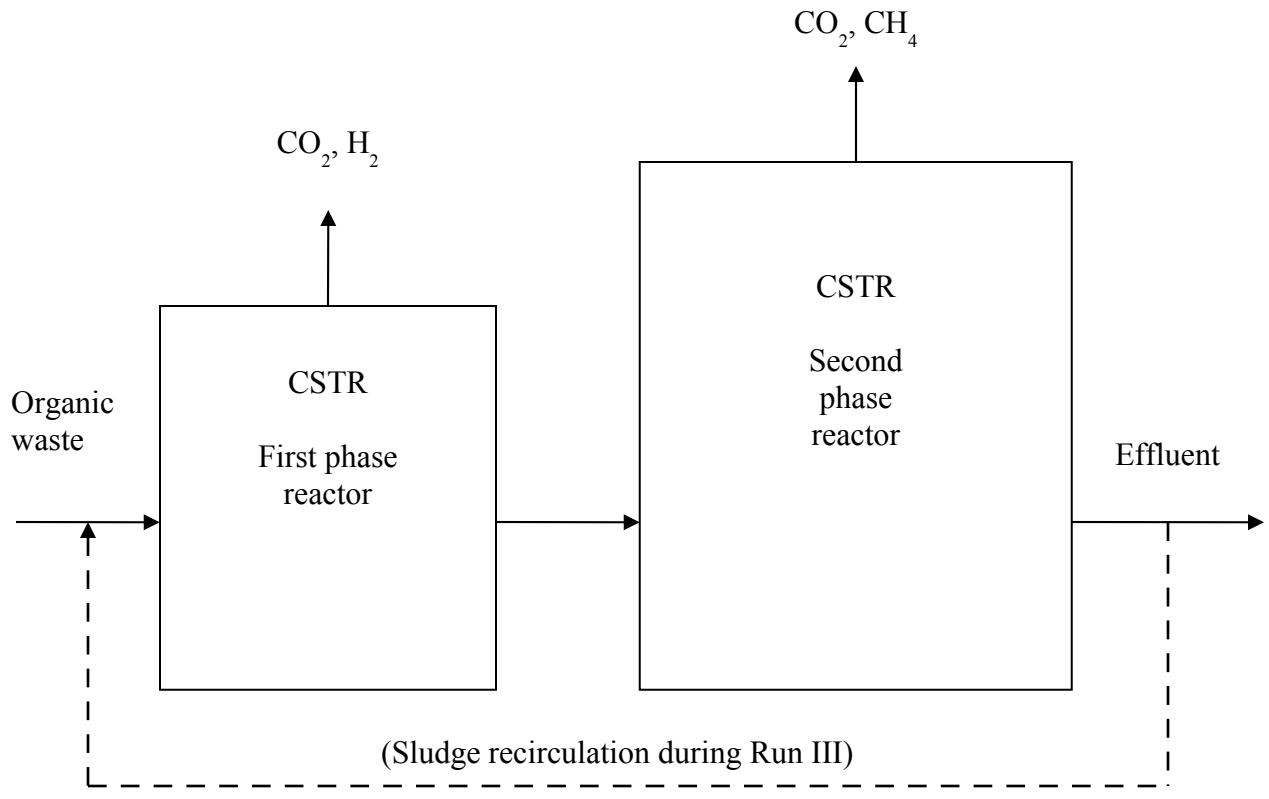


Figure 2.9 Pilot plants flow scheme

2.2.4 Work plan

The experimental test was divided in three periods (runs); during the first two working periods the OLR of the first reactor was maintained at 21 kgVS/m^3d while HRT was decreased from 6.6d to 3.3d changing the reactor's volume. In the third working period part of the digestate coming from the methanogenic reactor was recirculate in order to give alkalinity buffer to keep the pH around 5,5 (Kataoka et al 2005, Chu et al. 2008, Lee et al. 2010), with a recirculation ratio of 1. Table 2-9 shows the operative conditions applied to the reactors during the experimentation.

Table 2-9 operative conditions applied during the experimental test

	Run I	Run II	Run III-a	Run-III-b
HRT 1phase (d)	6,6	3,3	3,3	3,3
HRT 2 phase (d)	12,6	12,6	12,6	12,6
OLR 1 phase (kgVS/m^3d)	21	21	16	21
OLR 2 phase (kgVS/m^3d)	10	5	4	5

In all the Runs the second phase hydraulic retention time was fixed to 12,6 days, in order to permit to the anaerobic digestion process to degrade almost all the biodegradable matter. Chu et al (2008) and Lee et al. (2010) applied lower HRT (7,7 and 5 days) as consequence of the high loading rate applied to the first phase. Also in this conditions they obtain a good substrate conversion to biogas.
Run III was divided into two sub-period: first sub-period was called Run III-a and an OLR of 16 kgVS/m^{3}d was applied in order to adapt the whole process to a lower organic load, while in second sub-period called Run III-b the OLR was increased to 21 kgVS/m^3 d as the previous two runs.
The whole experiment length was 185 days, divided as shown in Table 2-10. For each period was defined a period of start up and a period of stationary state conditions.

Table 2-10 Experimental Run length

	days
Run I	0-85
Run II	86-117
Run III-a	118-148
Run III-b	149-185

2.3 Dynamic Respirometric Index (DRI) and Biological Methane Potential (BMP)

The biological stability quantifies the degree of decomposition of the readily biodegradable content in an organic matrix. Among the methods reported in the literature, measurement of respiratory activity (tests respirometric) of an organic matrix is certainly one of the most significant parameters to determine the biological stability, as it is related to microbial metabolism. In aerobic process in fact, microorganisms use the organic matter as a source of energy and nutrients, consuming oxygen and emitting dioxide carbon. The metabolism is more intense in the presence of a higher content of compounds readily biodegradable (biological matrices with low stability) and is more mitigated when there is a lower concentration of these compounds (matrices with high biological stability). This biological stability is quantified using the Dynamic Respirometric Index (DRI). There are two methods for the determination of this index:
- Method A: Dynamic Respiration Index Potential (DRIP);
- Method B: Dynamic Respiration Index Real (DRIR).

The dynamic respiration index (IRD) measures the hourly consumption of oxygen used for biochemical oxidize readily biodegradable compounds contained in an organic matrix in condition of forced injection of air into the sample. This determination tends to reproduce in laboratory conditions that occur in real organic matrix treatment plant and to evaluate the biological stability products according to their intended use.

The IRDP measures the oxygen consumption in moisture content and density standard conditions, while the IRDR measures the oxygen consumption in real conditions.

Dynamic Respirometric Index was measured using an adiabatic respirometric reactor (Costech International, Cernusco S.N., Italy; DiProVe, Milan, Italy). The respirometer was composed of an insulated reactor, a control cabinet, an air supply system, and a PC unit (Figure 2.11, Figure 2.11).

A Clark-type temperature compensation electrode and differential-pressure electronic transmitter enabled both oxygen and airflow measurements. The O_2 concentration was set in order to guaranteed 140 ml/l^{-1} in the outlet airflow (Adani et al. 2001). This value was maintained by a feed-back control that automatically adapted to airflow rate as a function of the oxygen concentration in the outlet airflow.

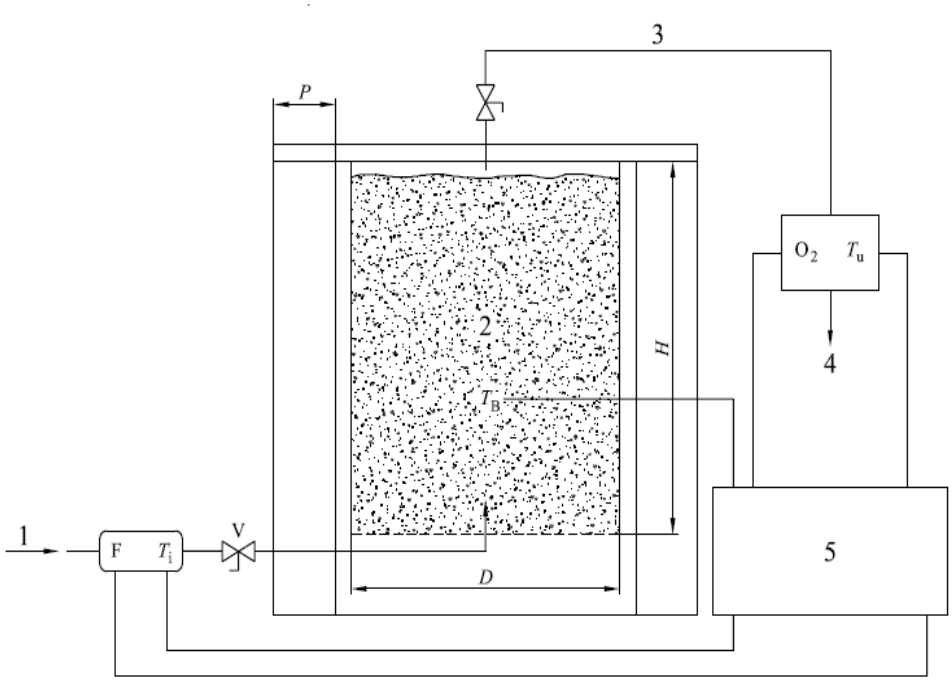


Figure 2.10 scheme of the respirometric reactor (1 Air IN O2 210 mL/L; 2 biomass; 3 exhaust air; 4 Air OUT; 5 data logger; F flow rate meter and controller; *T*i Inlet air temperature; *T*B biomass temperature; *T*u outlet air temperature; O_2 oxigen concentration in exhaust air; *D* internal diameter ; *H* internal height; *P* thick outer wall = 60 mm ± 10 mm; V check valve for the seals of the reactor; *H* / *D* 1 465 ± 0,080)

Figure 2.11: Dynamic Respirometric Index reactor.

The hourly Dynamic Respirometric Index (DRIh) was determined by measuring the difference in oxygen concentration (ml/l^{-1}) between the inlet and outlet air flows of the respirometer and calculated as suggested by Adani et al.2004:

$$DRIh(mgO_2kg^{-1}VS(DM)h^{-1}) = Q * \Delta O_2 * Vg^{-1} * 31{,}98 * VS(DM)^{-1} \quad \textbf{Equation 5}$$

Where DRIh is the hourly DRI, Q (l/h^{-1}) is the airflow, ΔO_2 (ml/l^{-1}) is the difference in oxygen concentration between the inlet and outlet air flows of the reactor, Vg (l/mol^{-1}) is the volume occupied by 1mol of gas at inlet air temperature, 31.98 (g/mol^{-1}) is the molecular weight of O_2, and VS and DM (kg) are the initial total volatile solids and dry matter content, respectively.

The Dynamic Respiration Index (DRI) was calculated as the average of 24 DRIh values taken over a 24-hour period characterized by the most intense biological activity. This was mathematically expressed by applying the following (Adani et al. 2006):

$$DRI = \sum_{\theta=0}^{24} (DRI_h) / 24 \quad \textbf{Equation 6}$$

Between 10 and 16 kg of wet sample was used for the tests. Samples were optimized for moisture content (75% of the water-holding capacity).

The BMP test was performed in 1 litre closed vessels, using as inoculum the digested sludge drawn from the full scale digester of Treviso's wastewater treatment plant. The volume of the inoculum was 750 ml, in order to assure enough microorganisms for the organic matter degradation. The quantity of the substrate tested depend on the organic content, but generally, for every kind of substrate were analysed four organic loading rate from OLR 1 kgTVS/m^3d to 7 kgTVS/m^3d. Every test was carried out in double and the background methane production from the inoculum was determined in blank assays with no substrate, and subtracted from the methane production obtained from the sample assays (Angelidaki et al 2008). The duration of the test is unsettled: German low requirement suggest 21 days and the Environment Agency (UK) 100 days, but It's possible to identified the end of the test when the variation of gas production is less then 5% of the previous measurement.

The measurement of the biogas production was made once a day and was based on the volumetric method, as volume increase under constant pressure (Figure 2.12 volumetric method instrument for the biogas production measurement.).

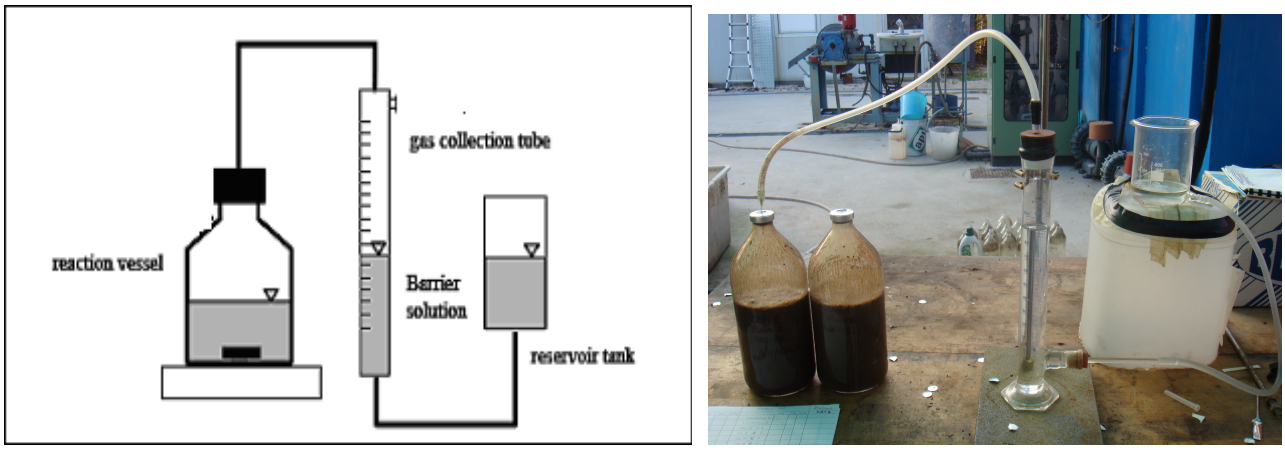


Figure 2.12 volumetric method instrument for the biogas production measurement.

The solution displaced by the gas is water plus an acid solution, in order to have a low pH that prevent CO_2 solubilization. Another important parameter is the biogas composition, monitored daily by gas-chromatographic method, where both methane and carbon dioxide are measured. The GC used was provided with thermal conductivity detection (TCD) and a capillary column (HP-PLOT Q, 30,0m x 320 um x 20,0 um). This is a bonded polystyrene-divinylbenzene (DVB) based column and the gas carrier used is helium.

3 Results and discussion

The results of batch test on biochemical hydrogen potential, of continuous pilot scale test for bio hythane production and the results of stability tests on AD effluent (DRI and BMP) are discussed in this chapter.

3.1 BHP test

To evaluate the biochemical hydrogen potential of organic waste, two organic loading were tested in batch configuration, using as inoculum an untreated anaerobic digested sludge. The test was carried out in triplicate for each loading, and applied an OL of 20 kgTVS/m^3 (test 1) and 30 kgTVS/m^3 (test 2). The test was stopped after 15 days, when the production variation was less than 5%. The average values of stability parameters and biogas yields are shown in Table 3-11. The average values of total gas production shown immediately the difference between the two organic loading.

Table 3-11 BHP results

parameters	u.m	Test 1	Test 2
OLR	kgTVS/m^3d	20	30
final pH		8,11	5,53
final NH_3	mgN/l	2001	1963
GP	l	21,36	10,60
SGP	m^3/kgTVS	0,71	0,24
max CH_4	%	66,11	15,06
SMP	m^3CH_4/kgTVS	0,32	0,03
max H_2	%	18,76	27,79
SHP	lH_2/kgTVS	20,7	69,0

Appling 20 kgTVS/m^3 of organic loading, it was obtained a total gas production of about 21 liters of biogas. This means that the inoculum microorganisms were able to use the organic matter without any chemical or physical treatment and without any problems of adaptation, in fact there is no lag phase and the specific gas and hydrogen production were 0,71 m^3/kgTVS$_{fed}$ and 20,7 lH_2/kgTVS$_{feed}$ respectively (Figure 3.13). At higher loading, 30 kgTVS/m^3, the behavior of the process was completely different, in fact the high load play an inhibition function of methanogenic archea, overloading the system. This caused a lower overall gas production (10,6 liters) that stop after about 48h, with an SHP of 69,0 lH_2/kgTVS$_{fed}$. The specific gas production in this last test was only 0,24 m^3/kgTVS$_{fed}$. H_2 content increased from 18,76 to 27,79 % while the methane percentage decreased from 66,11% to 15,06%, that confirm the inhibition of methanogenic activity at higher loading.

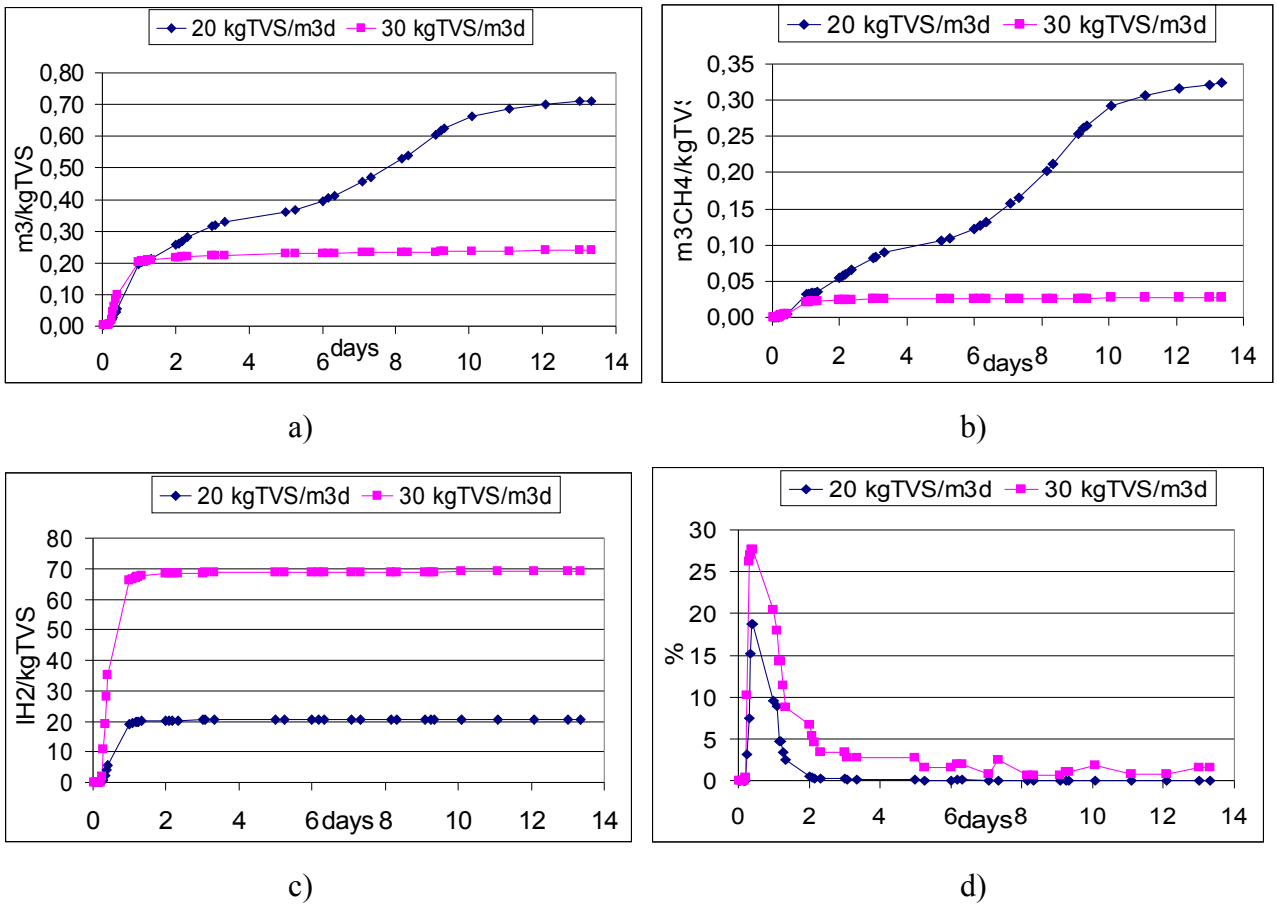
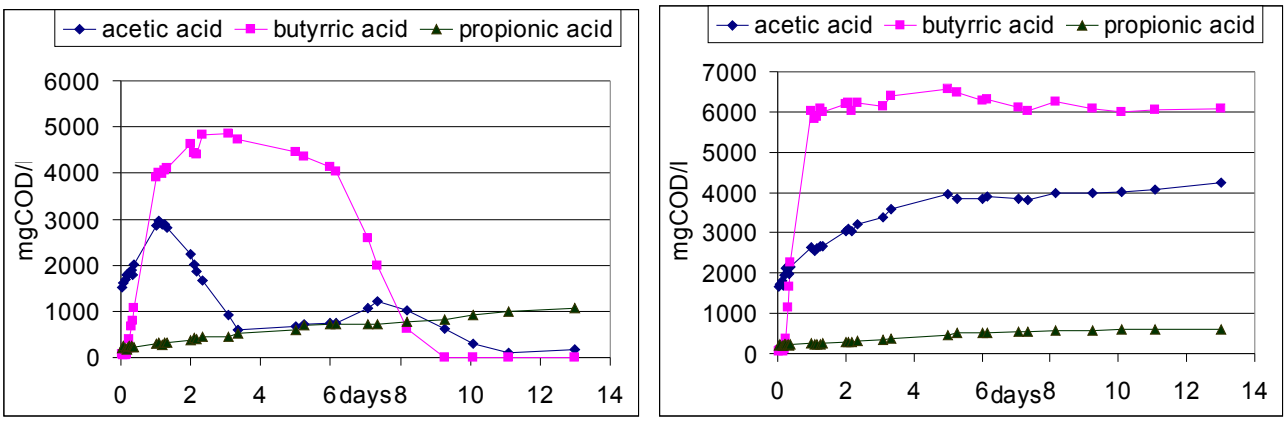


Figure 3.13 BHP tests results: a) specific gas production; b) specific methane production; c) specific hydrogen production; d) hydrogen content.

Both pH and VFA values shown the differences in the two process behavior. The pH in Test 1 was a typical anaerobic digestion value, of 8,11 and this is associated with the final evolution of short chain VFA that reach a final value of about 1 gCOD/l (Figure 3.14). The system was able to degrade the VFA (maximum value was 8 gCOD/l) with an initial hydrogen production followed by the conversion to methane.

In Test 2 the final pH value was 5,53 that met the typical dark fermentation value for hydrogen production. The high load cause the drop of pH and inhibition of methanogenic archea with a consequent hydrogen production and VFA accumulation that reach a total VFA amount of about 12 gCOD/l.



a) b)

Figure 3.14 VFA behavior: a) test 1 at 20 kgTVS/m3; b) test 2 at 30 kgTVS/m3

It is interesting to observe the acetic acid and butyric acid profile. At 20 kgTVS/m^3 the acetic acid reach a maximum concentration of 3 gCOD/l after 24h, with a Hac/Hbu ratio of 0,75 in correspondence of maximum hydrogen yields. The acetic acid decreased immediately, while the butyric acid was consumed after 9 days. In higher loading batch test, in correspondence with the high hydrogen production (24h) butyric acid reach a concentration of 6 gCOD/l with a Hac/Hbu ratio of about 0,5. In this contest, without methane conversion, both acetic and butyric acid were accumulated in the reactor. This last condition was suitable to be coupled with another process able to convert the VFA into methane, as a photosynthetic reactor or, as applied in this PhD, an anaerobic digestion process.

The high hydrogen production reached shown the possibility to use non treated inoculum and/or substrate, using process parameters as selection condition for hydrogen producer bacteria.

3.2 Two phase hydrogen and methane production

In this paragraph are presented and discussed the results of thermophilic two phase anaerobic digestion for hydrogen and methane production process. The experimental work was divided in three periods (as shown above in Table 2-9), where similar conditions were applied. The organic fraction of municipal solid waste was mixed only with water, without any pretreatment for bacteria selection. The HRT in Run I was 6,6d, while in Run II it was decrease to 3,3d in order to avoid the solventogenic shift. Cause of the too much low pH value reached during first two Runs, in third and last period was investigated the feasibility of anaerobic digestion sludge recirculation in order to control the pH values in the correct range (between 5 and 6).

3.2.1 Run I

In Run I, about 20 kg of organic waste was mixed with 10 l of water and fed once a day, in order to obtain in the first phase an organic loading rate (OLR) of 21,4 kgTVS/m^3d and an hydraulic retention time (HRT) of 6,6. As a consequence the OLR of the second phase was 10,8 kgTVS/m^3d with an HRT of 12,6 days (Table 3-12). No inoculum was used but only organic waste without any pretreatment was fed to the reactor. This conditions were applied for 85 days and the steady state condition (SSC) were reached from day 64.

Table 3-12 HRT and OLR applied in Run I

	av	min	max	s.d.
HRT 1phase (d)	6,6	6,6	6,6	6,6
HRT 2 phase (d)	12,6	12,6	12,6	12,6
OLR 1 phase (kgVS/m^3d)	21,4	18,6	25,2	2,3
OLR 2 phase (kgVS/m^3d)	10,8	8,6	13,3	1,5

Total solids, total volatile solids and macronutrient content of the organic waste were analyzed and data shown in Table 3-13. This material shows an high organic content of about 84% TVS,TS, a nitrogen content of 3% TKN,TS, and a COD/TKN ratio of about 28.

Table 3-13 Characterization of the organic waste used in Run I.

parameters	u.m.	av	min	max	sd
TS	g/kg	241,5	182,8	298,5	35,9
TVS	g/kg	203,6	150,5	252,4	30,3
TVS,TS	%	84,3	81,6	86,4	1,8
COD	gCOD/l	206,1	152,5	259,7	75,8
TKN	gN/l	7,2	7,2	7,3	0,1
Ptot	gPtot/l	0,78	0,64	0,93	0,20

In Table 3-14 are shown the average values obtained considering the steady state condition in the first phase reactor.

As mentioned before pH is an important parameter involved in the biohydrogen generation process. Applying this conditions, without any inoculum or pre treatment, the system was not able to maintain the pH in the best range for hyrogenase enzyme, in fact it drop at 3,7 in the start up and reached 4,3 during SSC (Figure 3.15 a).

Table 3-14 Characterization of the first phase reactor Run I

parameter	m.u.	AV	min	max	s.d.
TS	g/kg	168,0	151,0	190,7	14,6
TVS	g/kg	137,8	125,2	158,0	11,4
TVS,TS	%	82,1	80,6	83,8	1,0
COD	gCOD/kg	146,1	129,2	179,4	28,8
SCOD	gCOD/kg	75,4	68,5	83,1	4,6
TKN	gN/kg	5,0	4,8	5,2	0,2
PTOT	gP/kg	0,72	0,69	0,74	0,03
pH		4,3	3,9	4,6	0,2
NH_3	mgN/l	527,9	425,0	605,0	50,0
VFA	mgCOD/l	8330	5238	11416	1861

This low pH value could be explained by the high VFA production that reach a maximum of about 15 gCOD/l (Figure 3.3c) than stabilized at 8,3 gCOD/l, and composed mainly by acetic acid (6473 mgCOD/l) and small amount of propionic and butyric acids (600,5 and 778,5 mgCOD/l respectively).

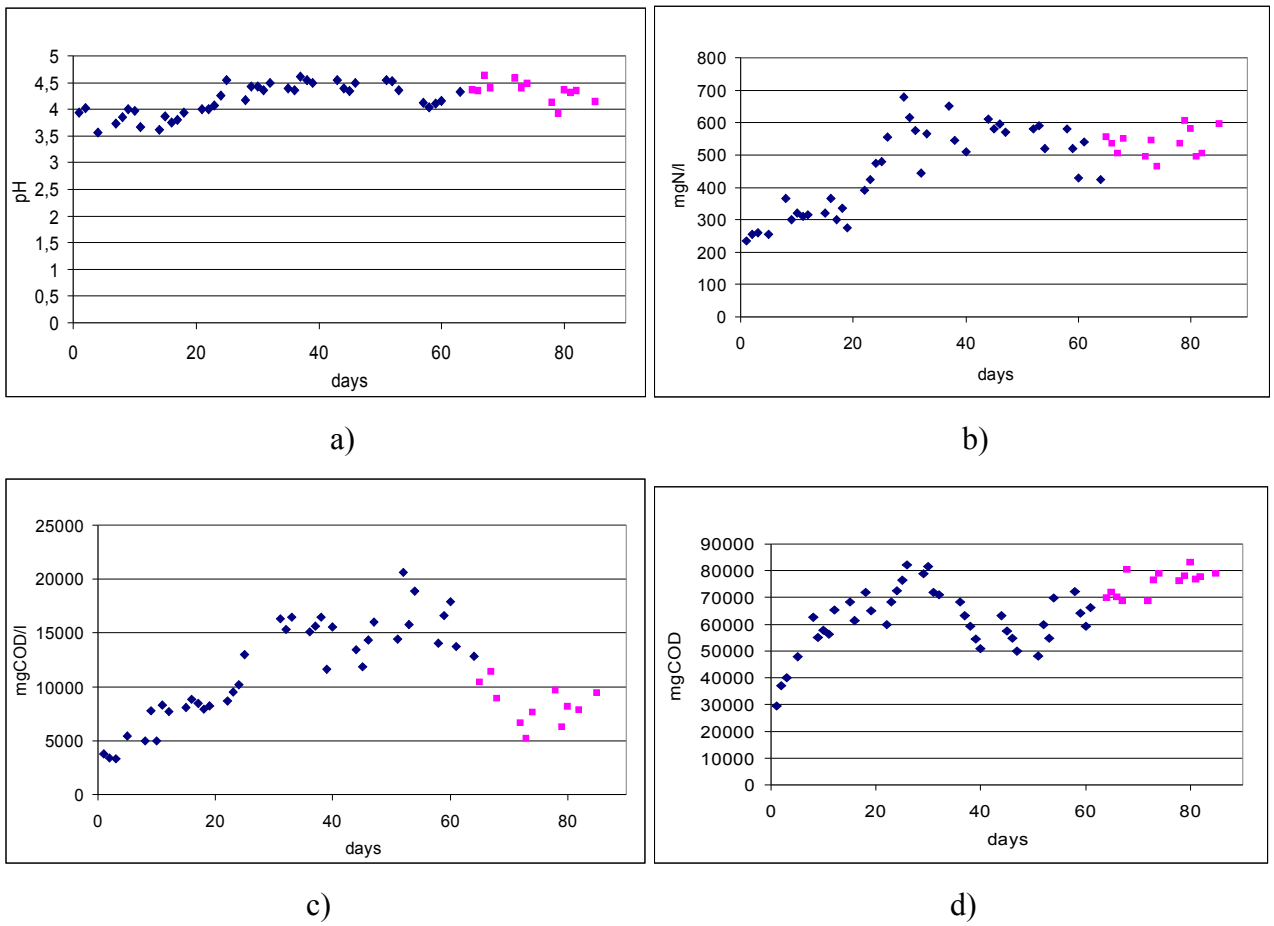


Figure 3.15 stability parameters in first phase reactor Run I a) pH; b) ammonia; c) total VFA; d) sCOD

Considering the pKa (3,85) of lactic acid and the pH, it is possible to consider a shift of the system in a solventogenic reaction with a consequent inhibition of the biohydrogen production.

The high content of soluble VFA, sCOD (75,4 gCOD/l) and of ammonia (527,9 mgN/l) suggest the shift from acidonenic to solventogenic reaction events that cause a production of other complex organic acids and alcohol that could inhibit the hydrogen production.

Despite of the high content of VFA, the anaerobic reactor was able to convert the acetic acid into methane and CO_2, without any problem of stability. In Table 3-15 Characterization of the second phase reactor Run Iare shown the average values of the second phase reactor. As confirmed also by the graphs displayed in Figure 3.16, the pH reach a constant value of 7,6, while the average total alkalinity was 10,6 $gCaCO_3$/l with a slightly crescent trend.

Table 3-15 Characterization of the second phase reactor Run I

		AV	min	Max	SD
TS	g/kg	77,0	72,7	85,1	4,4
TVS	g/kg	57,8	54,0	63,4	3,6
TVS,TS	%	75,1	72,7	78,6	2,3
SCOD	gCOD/kg	36,2	30,9	39,3	2,6
COD	gCOD/kg	48,9	38,3	59,4	14,9
TKN	gN/kg	2,4	2,3	2,4	0,1
PTOT	gP/kg	0,47	0,38	0,55	0,12
pH		7,6	7,4	7,8	0,1
NH_3	mgN/l	2016	1800	2270	175
VFA	mgCOD/l	210,9	74,8	417,2	95,5
ALKALINITY pH4	$mgCaCO_3$/l	10582	9550	11820	842
ALKALINITY pH6	$mgCaCO_3$/l	5066	4360	5900	489

The VFA content (210,9 mgCOD/l) shown the efficiency of VFA conversion into biogas, that is also not affected by the ammonia content that reach 2016 mgN/l.

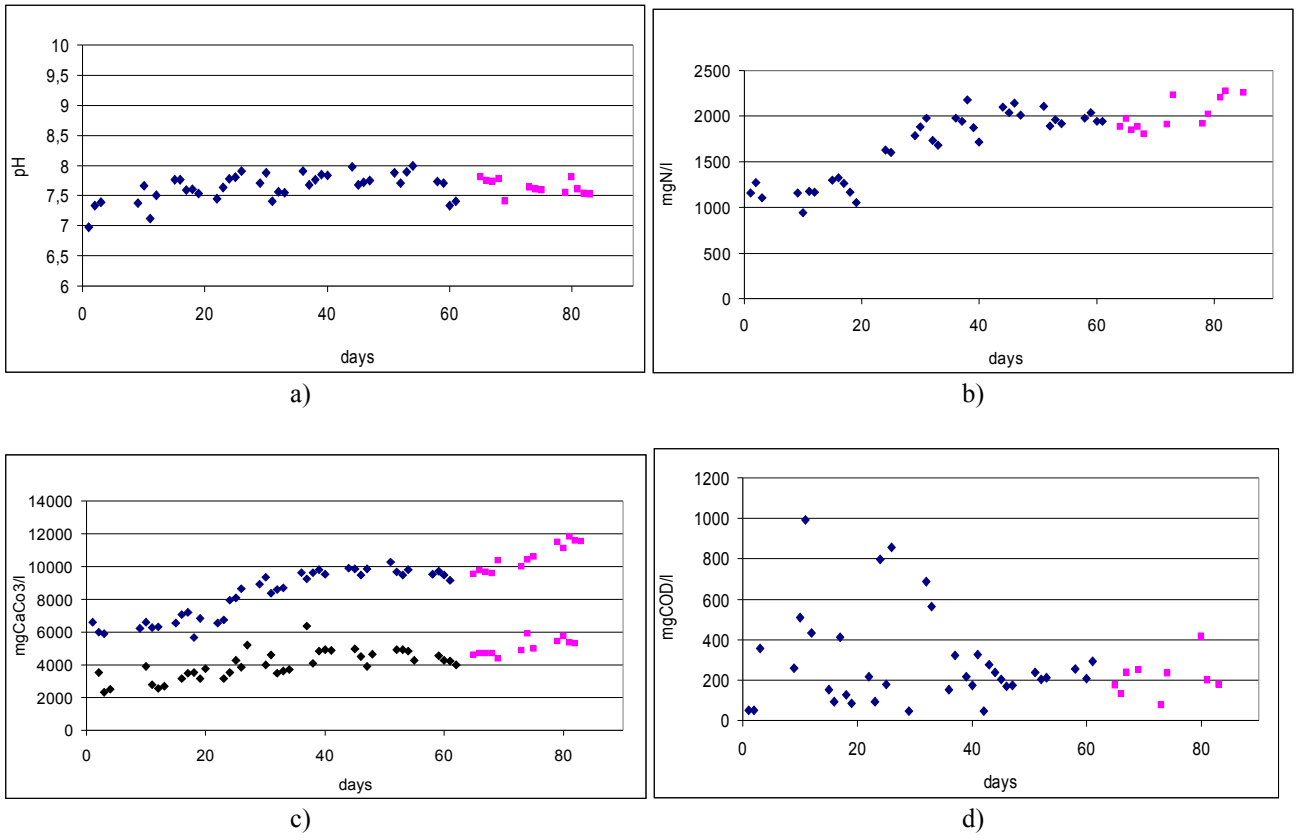


Figure 3.16 Stability parameters in second phase reactor Run I: a) pH; b) ammonia; c) alkalinity; d) VFA

In terms of yields, biohydrogen was produced during the process with 20% of content; this value didn't met the average value found in literature of about 35-40%. This low value together with the low specific gas production of 13,8 l/kgTVS gives a specific hydrogen production (SHP) of 2,7 lH_2/kg TVS and an gas production rate (GRP) of 0,3 m^3/m^3d . A similar values (< 5 lH_2/kgTVS) was found by Kataoka et al (2005) in a bench scale test, using similar condition applied in the Run I.

The yields of both reactors are shown in Table 3-16 and Table 3-17

Table 3-16 First phase reactor yields Run I

parameter	u.m.	AV	min	max	SD
GP	l/d	53,6	41,1	69,4	8,9
GPR	m^3/m^3d	0,27	0,23	0,32	0,03
SGP	l/kgTVS	13,8	10,5	17,7	2,4
H_2	%	19,7	17,5	21,5	1,2
SHP	l/kgTVS	2,7	2,0	3,3	0,5

Table 3-17 Second phase reactor yields Run I

parameter	u.m.	AV	min	max	SD
GP	m^3/d	2,3	2,2	2,4	0,1
GPR	m^3/m^3d	6,0	5,8	6,3	0,2
SGP	$m^3/kgTVS$	0,58	0,48	0,72	0,07
CH4	%	65,2	60,8	73,4	2,8

Considering the second phase reactor, as due observing the stability parameters, the anaerobic digestion process was able to treat the high organic load coming from the first reactor without problem.

As shown in Figure 3.17 a) and b) the OLR reach in both reactor a constant value after 60 days; this constant conditions are usually difficult to obtain because of the heterogeneity of the organic waste fed, but the SSC were reached by almost all the parameters considered.

The SGP of anaerobic digestion process was 0,58 $m^3/kgTVS$, with a GPR of 6,0 m^3/m^3d and methane content of 65,2%.

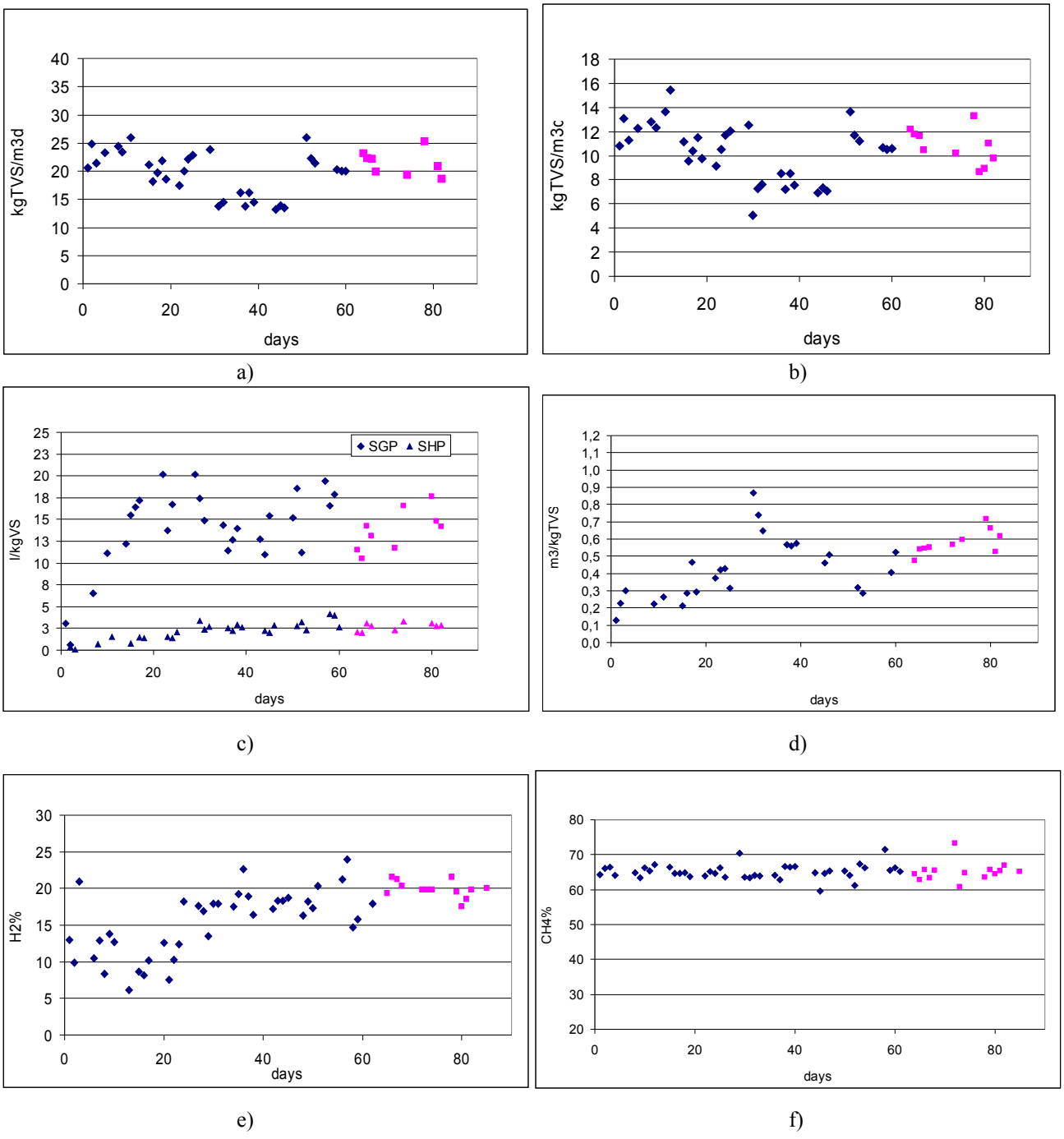


Figure 3.17 Run I yields: a) OLR first phase; b) OLR second phase; c)SGP-SHP first phase; d) SGP second phase; e) gas composition first phase; f) gas composition second phase.

The mass balance of Run I were reported in Table 3-18. The conversion of biogas on COD basis was done considering the rate COD/TVS of inlet organic waste (1,01).

The inlet mass content was calculated considering the characteristics of the organic waste, while the two outlet flows rate take into account were the biogas produced by both reactors, and the effluent of anaerobic digestion.

Biogas is composed by methane, carbon dioxide, water vapor and traces of other gases, which, however, are not considered in terms of volume. To quantify the amount of TVS removed with biogas, was considered only the "dry" part and assumed as an ideal gas made up solely of CH_4 and CO_2.

The mass was calculated using the molecular weights of methane and carbon dioxide (16 and 44 g / mol, respectively), the molar volume of an ideal gas at 1 atm and 20 ° C (24.056 l / mol) and the volume fraction of the components taken according to average experimental data.

Table 3-18 Run I mass balance

		IN	GAS R1	GAS R2	OUT	IN-OUT	%
TS (g/d)	av	4829,2	85,5	2621,7	2308,5	-186,4	-3,9
	min	3656,0	70,0	2259,1	2181,8		
	max	5970,0	103,6	3085,4	2554,3		
	sd	717,2	10,1	101,7	131,5		
TVS (g/d)	av	4071,0	85,5	2647,5	1734,5	-396,3	-9,7
	min	3010,0	70,0	2259,1	1620,0		
	max	5970,0	103,6	3085,4	1902,2		
	sd	606,0	10,1	101,7	107,1		
COD (g/d)	av	4121,4	86,5	2680,2	1466,9	-112,2	-2,7
	min	3049,4	70,8	2287,0	1150,3		
	max	5194,0	104,9	3123,6	1783,4		
	sd	1516,1	10,2	103,0	447,7		
TKN (g/d)	av	144,8			131,7	13,1	9,0
	min	144,0			123,3		
	max	146,0			141,3		
	sd	1,6			8,0		
Ptot (g/d)	av	15,7			14,0	1,7	11,0
	min	12,8			11,3		
	max	18,6			16,6		
	sd	4,1			3,7		

It is possible to observe that all the mass balance have an error lower than 10%. This error could be associated to wrong sampling or analytical procedures.

3.2.2 Run II

During the second Run was maintained the same organic loading rate of the previous Run in the first reactor (21,46 kgTVS/m^{3}d) feeding 10 kg of organic waste diluted in 20 l of tap water, but decreased the HRT from 6,6 to 3,3 days using half of the reactor's volume (100 l) (Table 3-19).

Table 3-19 HRT and OLR applied in Run II

	av	min	max	s.d.
HRT 1phase (d)	3,3	3,3	3,3	3,3
HRT 2 phase (d)	12,6	12,6	12,6	12,6
OLR 1 phase (kgVS/m^{3}d)	21,46	19,68	23,25	1,88
OLR 2 phase (kgVS/m^{3}d)	5,65	5,18	6,12	0,50

The whole period length was of 32 days, and the system reach a steady state condition after 20 days.
The low yields in Run I suggest a shift from acidogenic to solventogenic reaction due to the high HRT applied, with accumulation of byproduct as VFA, lactic acid and ethanol, with a consequent inhibition of the hydrogen production.
As mentioned in the introduction, lower HRT are suggested to avoid the shift of the system and permit to the hydrogen producing bacteria to convert the organic matter into hydrogen and acetic and butyric acids.
Total solids, total volatile solids and macronutrient content of the organic waste were analyzed and data shown in Table 3-20. This material shows an high organic content of about 84,7% TVS,TS and a nitrogen content of 2,2% TKN,TS.

Table 3-20 Characterization of the organic waste used in Run II.

parameters	u.m.	av	min	max	sd
TS	g/kg	253,1	234,6	271,6	19,5
TVS	g/kg	214,6	196,7	232,5	18,8
TVS,TS	%	84,7	83,9	85,6	0,9
COD	gCOD/l	249,4	244,5	254,2	6,9
TKN	gN/l	5,6	5,4	5,9	0,4
Ptot	gPtot/l	0,54	0,35	0,73	0,27

In Table 3-21 are shown the average value of total and volatile solids, macronutrient and stability parameters, of the first phase effluent.

Table 3-21 Characterization of the first phase reactor Run II

parameter	u.m.	AV	min	max	SD
TS	g/kg	78,2	73,1	84,6	4,6
TVS	g/kg	67,3	62,4	72,8	3,7
TVS,TS	%	86,1	85,2	87,2	0,9
COD	g/kg	67,0	64,7	69,8	2,2
TKN	g/kg	2,09	1,69	2,52	0,45
PTOT	g/kg	0,25	0,22	0,29	0,04
PH		3,49	3,32	3,68	0,14
NH3	mgN/l	152,5	125,0	175,0	14,9
VFA	mgCOD/l	2923	2114	3891	550

The pH value during this second run drop from 4,0 to a constant value of 3,5, that is still too much low for the hydrogeanse enzyme. Compared with the previous Run, the VFA production was reduced as due, in fact it decreases from 8830 mgCOD/l of Run I to about 3000 mgCOD/l in Run II (Figure 3.18). In this condition also the ammonia value decreased to 152,5 mgN/l.

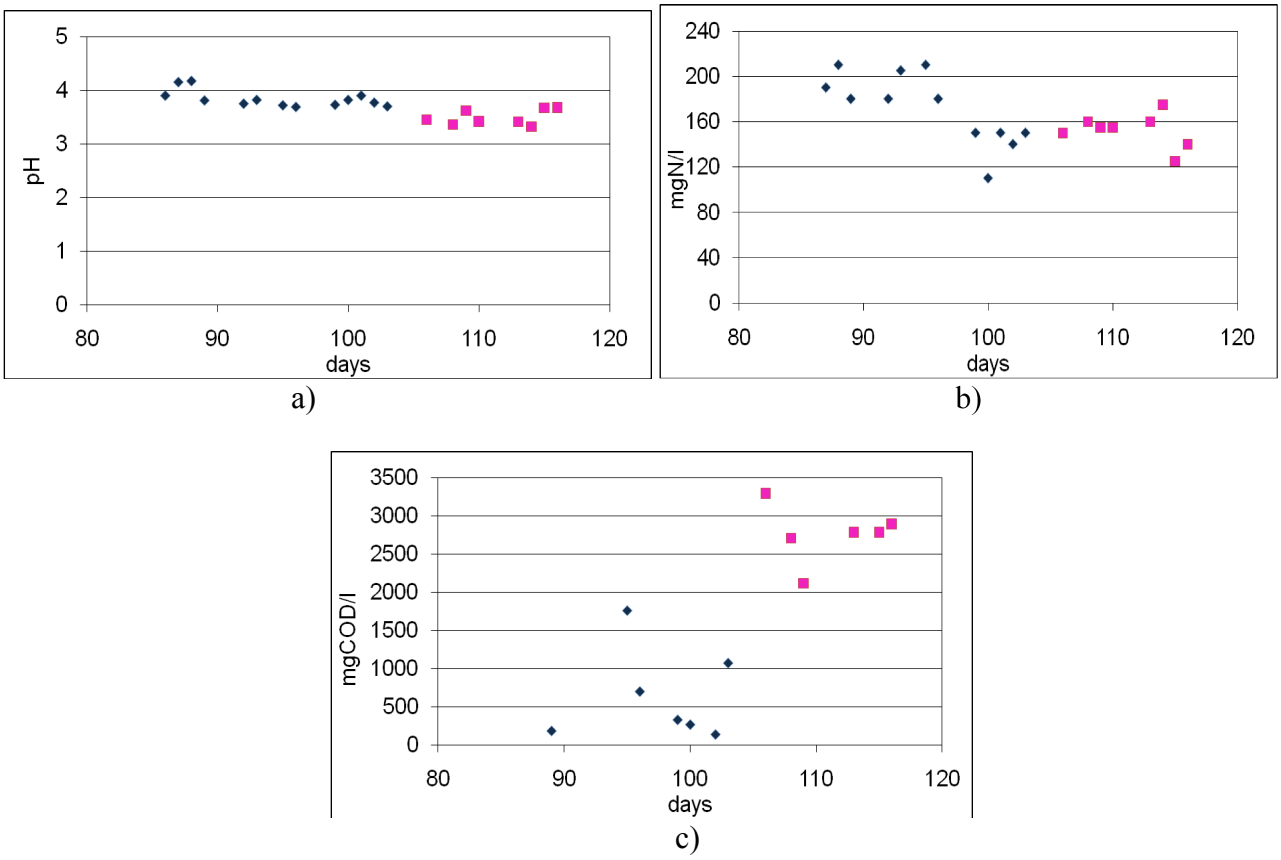


Figure 3.18 stability parameters in first phase reactor Run II a) pH; b) ammonia; c)VFA.

Compared with the Run I in methanogenic reactor the OLR was lower, caused by the lower amount of waste fed in the first reactor.

The HRT was maintained at the same value (12,6 days). Despite the low pH of the first reactor also in this case the anaerobic digestion process confirm the stability of the system, in fact all the parameters reached steady state conditions (Table 3-22).

Table 3-22 Characterization of second phase reactor in Run II

parameter	u.m.	AV	min	Max	SD
TS	g/kg	29,0	24,0	32,9	4,0
TVS	g/kg	20,9	15,5	25,7	4,1
TVS,TS	%	69,6	64,7	73,7	4,3
COD	gCOD/kg	23,6	18,6	26,6	4,4
TKN	gN/kg	1,03	0,84	1,17	0,14
PTOT	gP/kg	0,20	0,14	0,24	0,06
PH		8,08	7,95	8,26	0,10
NH3	mgN/l	1079	990	1150	57
VFA	mgCOD/l	642	367	821	142
ALKALINITY pH4	$mgCaCO_3/l$	5324	5116	5606	154
ALKALINITY pH6	$mgCaCO_3/l$	2737	2528	2979	159

Halving the organic loading rate in the second reactor compared to the Run I, the stability parameters values decrease for about the half of previous period values. Ammonia content was about 1079 mgN/l that wasn't in an inhibitory condition; total alkalinity reach 5324 $mgCaCO_3/l$ (Figure 3.19). Only the VFA increased to 642 mgCOD/l.

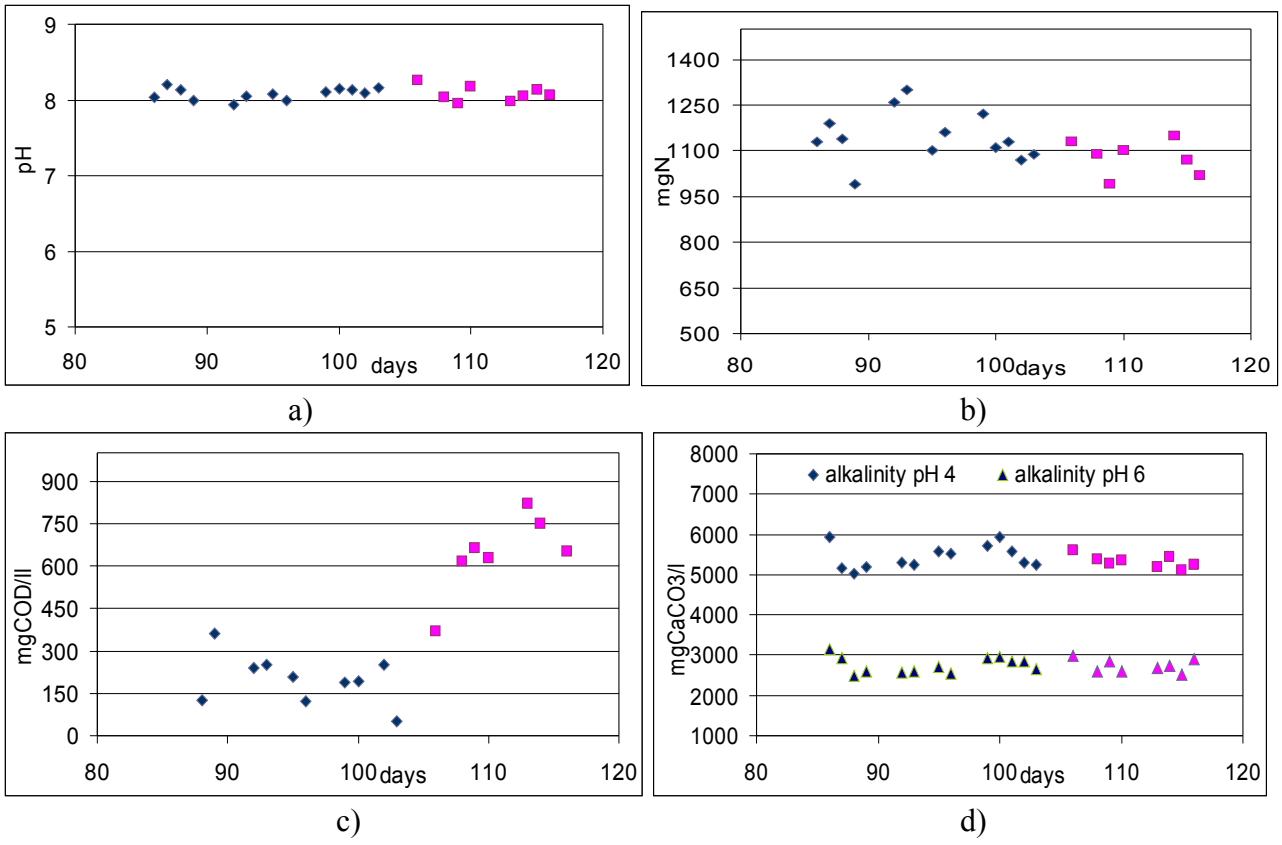


Figure 3.19: stability parameters in second phase reactor in Run II: a) pH; b) ammonia content; c) total VFA; d) alkalinity.

Changing the HRT the biohydrogen yields didn't change in terms of SHP in fact same value was observed (2,6 lH_2/kgTVS), but it increase the H_2 content in the biogas, moved from 20 to 35 %. This means an overall decreased gas production in the first phase, with an SGP changed from 13,8 to 7,44 lH_2/kgTVS, and a GPR from 0,3 to 0,16 m^3/m^3d (Table 3-23).

Table 3-23 process yields of first phase reactor Run II

parameter	u.m.	AV	min	max	SD
GP	l/d	15,60	11,00	19,00	3,44
GPR	m^3/m^3d	0,16	0,11	0,19	0,03
SGP	l/kgTVS	7,44	5,59	9,66	1,82
H2	%	34,78	29,90	41,20	3,49
SHP	l/kgTVS	2,61	1,96	3,38	0,64

Table 3-24 process yields of second phase reactor Run II

parameter	u.m.	AV	min	max	SD
GP	m^3/d	1,29	1,03	1,52	0,16
GPR	m^3/m^3d	3,40	2,70	4,01	0,43
SGP	m^3/kgTVS	0,62	0,44	0,77	0,11
CH4	%	59,81	58,30	61,30	0,84

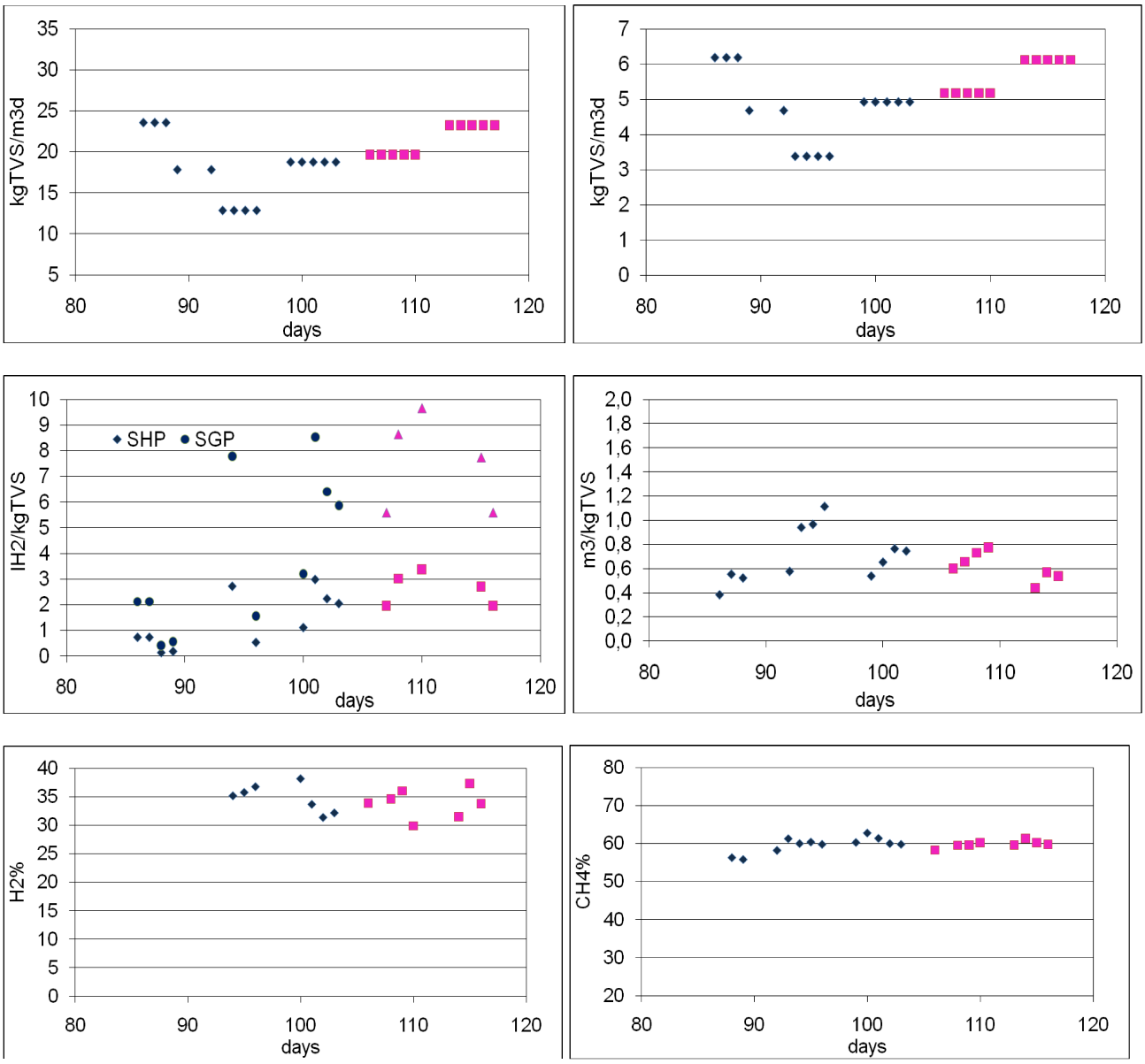


Figure 3.20 Run II yields: a) OLR first phase; b) OLR second phase; c)SGP-SHP first phase; d) SGP second phase; e) gas composition first phase; f) gas composition second phase.

The mass balance of the process is shown in Table 3-25. The conversion of biogas on COD basis was done considering the rate COD/TVS of inlet organic waste (1,16).

Table 3-25 Run II mass balance

		IN	GAS 1	GAS 2	OUT	IN-OUT	%
TS (g/d)	av	2531,3	20,0	1405,6	870,0	235,7	9,3
	min	2346,0	12,7	1070,5	719,3		
	max	2716,0	26,1	1734,9	987,9		
	sd	194,7	4,4	178,2	120,5		
TVS (g/d)	av	2146,3	20,0	1405,6	627,9	92,9	4,3
	min	1967,0	12,7	1070,5	465,4		
	max	2325,0	26,1	1734,9	770,2		
	sd	188,5	4,4	178,2	124,4		
COD (g/d)	av	2494,0	23,2	1633,3	709,3	128,2	5,1
	min	2445,0	14,8	1243,9	557,7		
	max	2542,0	30,4	2016,0	798,5		
	sd	68,7	5,1	207,0	132,0		
TKN (g/d)	av	57,0			63,3	-6,3	-11,1
	min	54,0			54,9		
	max	58,9			69,7		
	sd	3,5			5,9		
Ptot (g/d)	av	5,4			6,1	-0,7	-13,2
	min	3,5			4,2		
	max	7,3			7,3		
	sd	2,7			1,7		

All the mass balance done have an error lower than 10%, except for nitrogen and phosphorus that are under the 13,20%.

3.2.3 Run III

The third period was characterized by the recirculation of the effluent coming from the second phase reactor, after filtration. The characteristic of anaerobic digestion sludge allows a buffer control of first phase reactor, thanks to the alkalinity content. The recirculation ratio was set to 1, as suggested by Lee et al. (2010). The quantity of organic waste in Run III-a was 16 kg, while in Run III-b the quantity was increased at 19 kg, and in both case mixed with tap water till a total volume of 30 liters. The HRT and OLR applied during Runs III-a and III-b are shown in Table 3-26 and Table 3-27.

Table 3-26 HRT and OLR applied in Run III-a

	av	min	max	s.d.
HRT 1phase (d)	3,3	3,3	3,3	3,3
HRT 2 phase (d)	12,6	12,6	12,6	12,6
OLR 1 phase (kgVS/m^{3}d)	16,13	14,06	17,84	1,85
OLR 2 phase (kgVS/m^{3}d)	4,24	3,70	4,69	0,49

Table 3-27 HRT and OLR applied in Run III-b

	av	min	max	s.d.
HRT 1phase (d)	3,3	3,3	3,3	3,3
HRT 2 phase (d)	12,6	12,6	12,6	12,6
OLR 1 phase (kgVS/m^{3}d)	21,11	18,66	23,02	1,65
OLR 2 phase (kgVS/m^{3}d)	5,56	4,91	6,06	0,43

In Table 3-28 are shown the characteristics of the organic waste used in Run III.

Table 3-28 Characterization of the organic waste used in Run III.

parameters	u.m.	av	min	max	sd
TS	g/kg	267,1	205,0	303,9	31,6
TVS	g/kg	213,6	175,8	232,8	18,8
TVS,TS	%	80,3	73,8	85,7	4,3
COD	gCOD/l	207,1	174,7	255,3	27,5
TKN	gN/l	7,3	5,9	8,7	1,0
Ptot	gPtot/l	0,32	0,24	0,39	0,06

are shown the average values of the first phase reactor, divided in two different loading periods, Run III-a at 16,13 kgTVS/m^{3}d and Run III-b at 21,11 kgTVS/m^{3}d. In both conditions the pH was kept in the optimal range for hydrogen production, that is about 5,4.

Table 3-29 Characterization of first phase reactor in Run III-a

parameter	u.m.	av	min	max	sd
TS	g/kg	60,0	54,1	67,5	5,0
TVS	g/kg	48,6	43,3	57,9	5,0
TVS,TS	%	80,9	76,7	85,8	2,7
COD	gCOD/kg	40,4	28,4	48,9	8,3
rbCOD	gCOD/kg	10,4	9,4	12,1	1,0
TKN	gN/kg	2,05	1,96	2,20	0,09
PTOT	gP/kg	2,62	1,69	3,54	0,77
pH		5,39	5,27	5,53	0,09
NH_3	mgN/l	706	500	920	169
VFA	mgCOD/l	13877	11614	16312	1673

Table 3-30 Characterization of first phase reactor in Run III-b

parameter	u.m.	AV	min	Max	SD
TS	g/kg	73,2	72,7	72,7	0,5
TVS	g/kg	58,8	57,3	60,7	1,7
TVS,TS	%	80,4	78,8	82,3	1,8
COD	gCOD/kg	50,5	49,9	51,1	0,9
rbCOD	gCOD/kg	13,9	13,1	14,7	1,1
TKN	gN/kg	2,35	2,32	2,39	0,05
PTOT	gP/kg	4,04	3,75	4,33	0,41
pH		5,43	5,17	5,58	0,14
NH_3	mgN/l	948	750	1100	145
VFA	mgCOD/l	7053	3406	11798	3382

The pH, ammonia and VFA values of the first reactor are shown in Figure 3.21. Run III-a started from day 132 to day 148, while the second period from day 159 to day 171.

It is possible to observe that the ammonia content was increasing during the whole Run III, because of the recirculation of the second phase effluent. This problem was take into account forward in the experimental test.

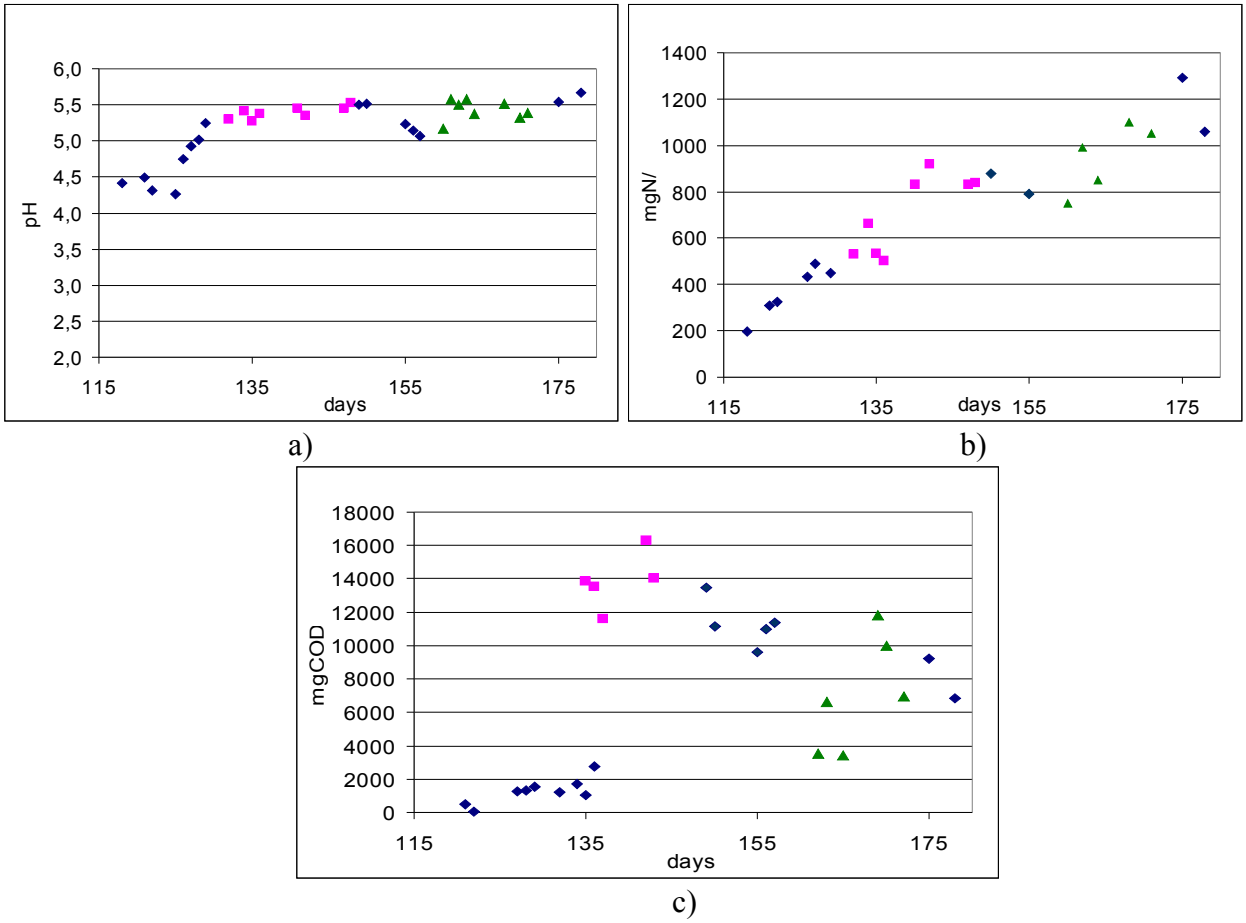


Figure 3.21 stability parameters in first phase reactor Run III a) pH; b) ammonia; c)VFA.

The stability parameters and macronutrient of second reactor during Run III-a and Run III-b are shown in Table 3-31 and Table 3-32. The pH was about 8,25 in both period, while the VFA content in the Run III-a was 89,9 mgCOD/l and in the Run III-b 604 mgCOD/l; this means a reduction of VFA of >95% (Figure 3.22).

Table 3-31 Characterization of second phase reactor in Run III-a

parameter	u.m.	AV	min	max	SD
TS	g/kg	24,3	22,2	25,3	1,0
TVS	g/kg	16,2	14,6	17,1	0,8
TVS,TS	%	66,7	64,0	67,8	1,4
COD	gCOD/kg	12,8	9,1	15,6	2,7
rbCOD	gCOD/kg	1,4	1,1	1,8	0,3
TKN	gN/kg	0,85	0,81	0,92	0,06
PTOT	gP/kg	0,13	0,11	0,17	0,06
ALKALINITY pH 4	mg$CaCO_3$/l	5173	4500	6120	674
ALKALINITY pH 6	mgCaCO3/l	3160	2760	3700	374
PH		8,25	8,13	8,45	0,12
NH_3	mgN/l	997,5	805,0	1360,0	187,9
VFA	mgCOD/l	89,9	14,0	344,0	109,0

Table 3-32 Characterization of second phase reactor in Run III-b

parameter	u.m.	AV	min	max	SD
TS	g/kg	30,1	26,8	33,2	3,2
TVS	g/kg	19,2	17,2	21,4	2,1
TVS,TS	%	63,8	63,0	64,5	0,8
COD	gCOD/kg	16,9	16,0	17,9	1,3
rbCOD	gCOD/kg	2,17	1,98	2,36	0,27
TKN	gN/kg	0,84	0,70	0,99	0,21
PTOT	gP/kg	0,20	0,17	0,22	0,04
ALKALINITY pH 4	mg$CaCO_3$/l	7100	6500	7500	416
ALKALINITY pH 6	mg$CaCO_3$/l	4024	3400	4300	366
PH		8,24	7,83	8,38	0,19
NH_3	mgN/l	1470	1280	1680	166
VFA	mgCOD/l	604	473	759	122

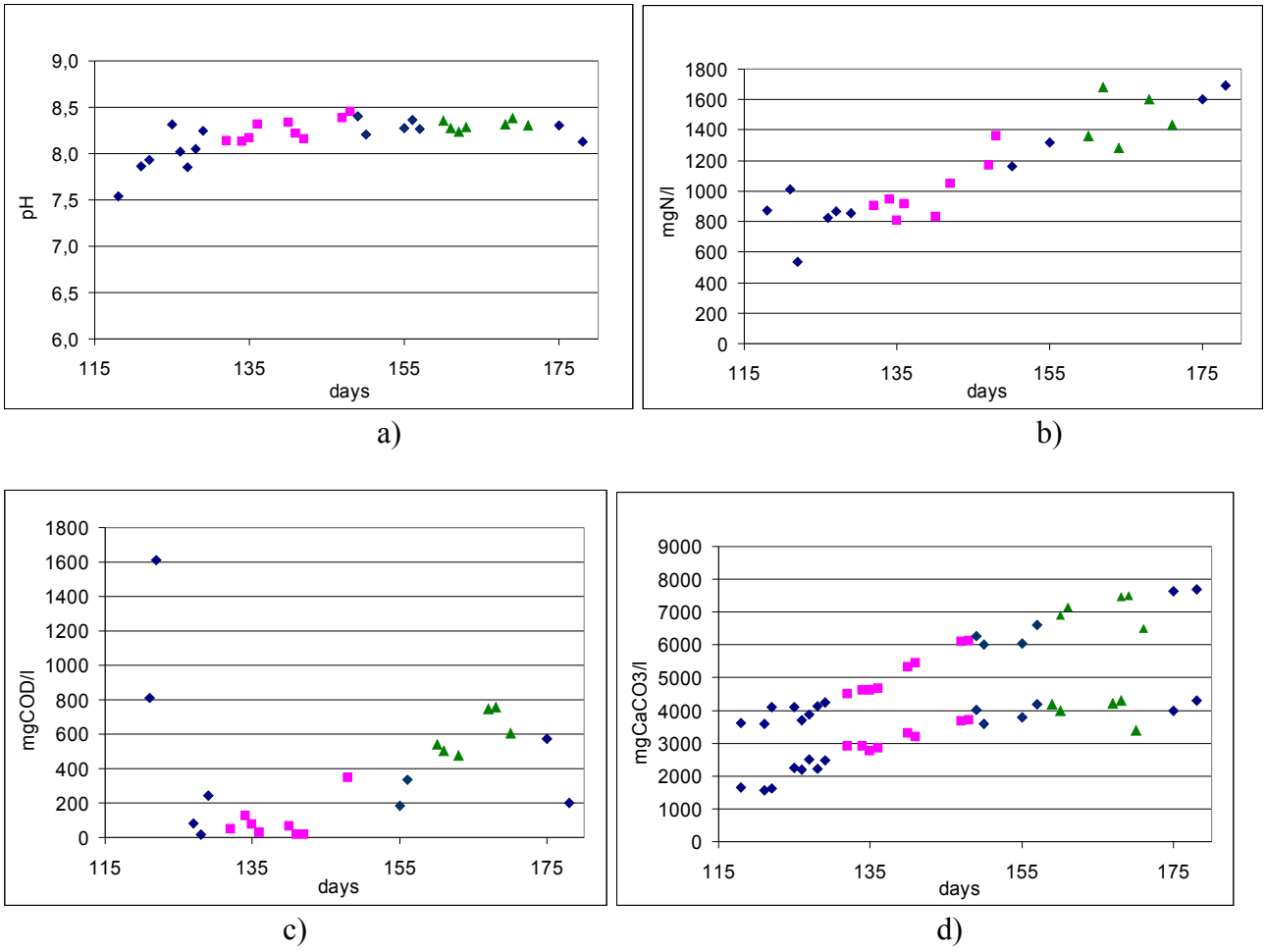


Figure 3.22 stability parameters in second phase reactor in Run III: a) pH; b) ammonia content; c) total VFA; d) alkalinity.

Comparing the two loading conditions in terms of hydrogen yields (Table 3-33; Table 3-34; Table 3-35; Table 3-36) it is clear that with the lower OLR the first phase gas yields are better than the high load.

Appling the OLR of 16,13 kgTVS/m^{3}d the specific gas production obtained was 136,82 l/kgTVS, with a H_2% of 35,61 and a specific hydrogen production of 51,16 lH_2/kgTVS. Changing the OLR at 21,11 kgTVS/m^{3}d the SGP decrease to 59,97 l/kgTVS, the H_2% was the same and the SHP decrease to 20,44 lH_2/kgTVS.

Table 3-33 Phase 1Run III-a

parameter	u.m.	AV	min	Max	SD
GP	m^3/d	0,45	0,26	0.61	0,11
GPR	m^3/m^3d	2,26	1,31	3,03	0.55
SGP	l/kgTVS	136,82	73,43	176,19	35,30
H_2	%	37,06	23,50	50,00	8,57
SHP	l/kgTVS	51,16	26,80	61,77	11,81

Table 3-34 Phase 2 Run III a

parameter	u.m.	AV	min	Max	SD
GP	m^3/d	1,03	0,81	1,13	0,10
GPR	m^3/m^3d	2,71	2,13	2,98	0,27
SGP	$m^3/kgTVS$	0,64	0,48	0,80	0,09
CH_4	%	64,93	61,00	70,00	2,21

Table 3-35 Phase 1 Run III b

parameter	u.m.	AV	min	Max	SD
GP	m^3/d	0,24	0,22	0,31	0,03
GPR	m^3/m^3d	1,22	1,10	1,57	0,17
SGP	l/kgTVS	59,97	52,50	70,99	6,68
H_2	%	34,00	29,50	39,00	3,36
SHP	l/kgTVS	20,44	16,65	24,85	3,36

Table 3-36 Phase 2 Run III b

parameter	u.m.	AV	min	Max	SD
GP	m^3/d	1,27	0,96	1,57	0,22
GPR	m^3/m^3d	3,35	2,53	4,13	0,58
SGP	$m^3/kgTVS$	0,63	0,46	0,79	0,12
CH_4	%	65,38	62,00	68,00	1,80

This better performance of the first phase reactor at 16,13 kgTVS/m^3d didn't reflect the better yields in the second reactor in terms of specific gas production, in fact the lower the load, the lower the gas production in anaerobic digestion.

The OLR applied in the Run III-a was 4,24 and the GPR, SGP and CH_4% were respectively 2,71 m^3/m^3d, 0,64 m^3/kgTVS and 65%. With the higher OLR (5,56 kgTVS/m^3d) the GPR, SGP and gas composition were respectively 3,35 m^3/m^3d, 0,63 m^3/kgTVS and 65,4% of methane. In Figure 3.23 are shown the specific gas production of both reactors; it is possible to note how, during the five days week the production from Monday to Friday increase. This happen because the system wasn't feed during the week end.

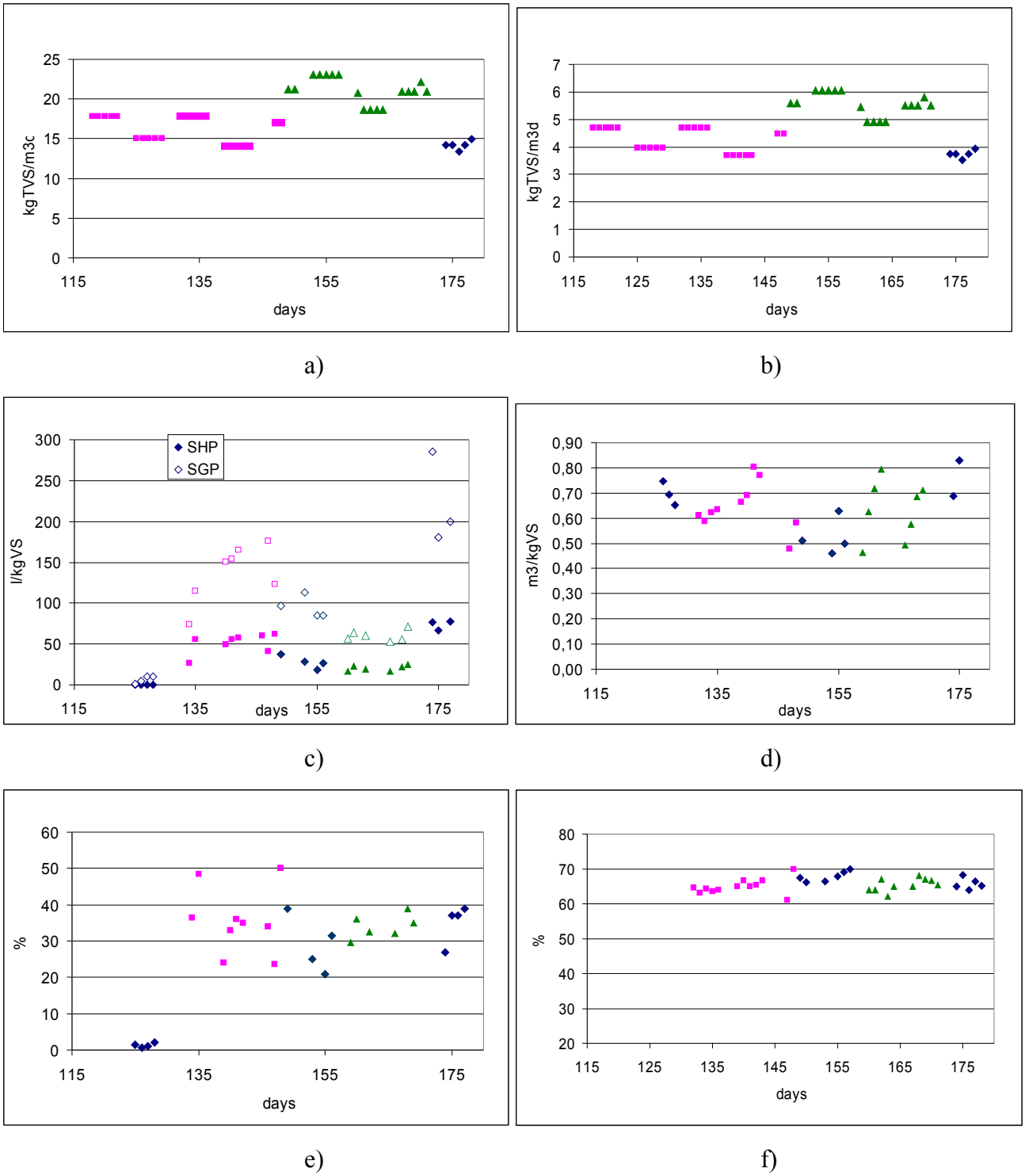


Figure 3.23 Run III yields: a) OLR first phase; b) OLR second phase; c)SGP-SHP first phase; d) SGP second phase; e) hydrogen gas content in first phase; f) methane gas content in second phase.

This decreased yields in biohydrogen production increasing the OLR was confirmed also by Wang et al. (2009). They study the exploitation of unsterilized food waste as a source for hydrogen and

subsequent methane production, where the indigenous food waste microflora was used as inoculum. At lower OLR (15,10 kg VS/m^{3}d), acetic acid and butyric acid producing pathway were the dominant hydrogen fermentation pathway, the hydrogen yield was not significantly fluctuated. At higher OLR (37,75 kgTVS/m^{3}d), a decrease in hydrolysis rate of substrate and an increase of propionic and lactic acids were observed, which were considered as the main causes for the decrease in hydrogen yield when the system was operated at high OLR.

This behavior was observed also in this experiment. In Figure 3.24 are shown the short chain VFA concentrations during the two periods (Run III-a and Run III-b). It is confirmed that there is a better conversion of VFA in acetic and butyric acids in the first period, while at higher OLR also the propionic acid was slightly increased and the acetic and butyric acids were decreased.

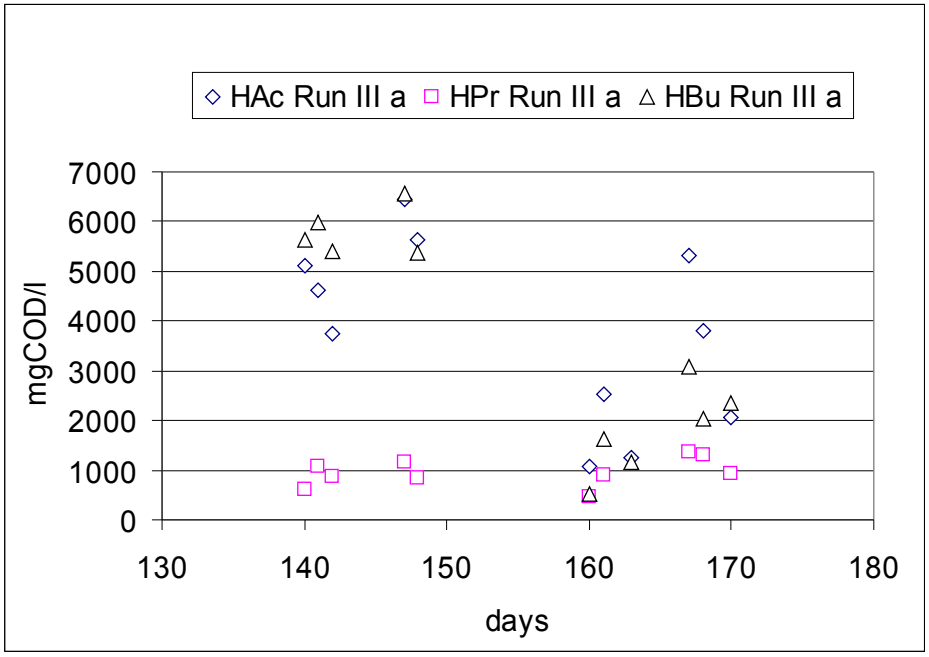


Figure 3.24 Short chain VFA comparison

The correspondence of high hydrogen yields with high VFA concentration is shown in Figure 3.25. It is interesting to observe how the VFA concentration after an SHP value of 40, is ranging between 5 to 6 gCOD/l, with a small predominance of butyric acid.

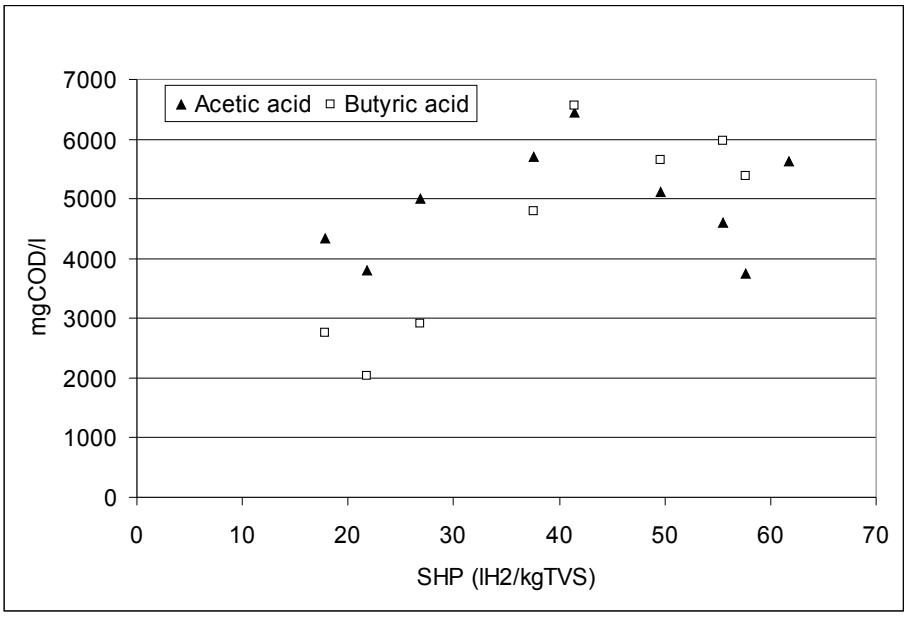


Figure 3.25 VFA concentration related to specific hydrogen production

It is not clear what is the better ratio HAc/HBu, because discordant literature values, but this predominance of butyric acid could be associated to the combination of metabolic reaction, as shown in:

$$4C_6H_{12}O_6 + 2H_2O \rightarrow 3CH_3CH_2CH_2COOH + 2CH_3COOH + 8CO_2 + 10H_2$$ **Equation 7**

In Figure 3.26 is plot the relation between the specific hydrogen production and the organic loading rate. The general trend of the experimental results shown a better performance at OLR < 18 kgTVS/m^{3}d, with a maximum yields at the lower loading applied.

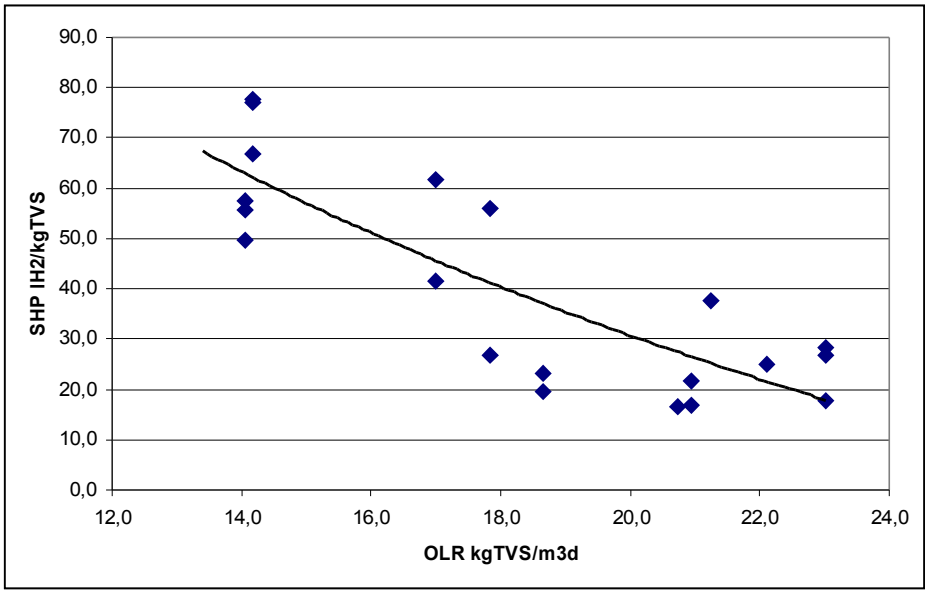


Figure 3.26 SHP related to the OLR.

The mass balance consider the whole flow rate treated in the second reactor, with a consequent doubled volume of reactor and of gas production.

Table 3-37 Run III-a Balance

		IN	GAS R1	GAS R2	OUT	IN-OUT	%
TS (g/d)	av	3848,1	558,8	2372,2	728,5	188,6	4,9
	ds	501,2	136,5	238,0	29,5		
	min	3280,6	257,2	1661,5	666,7		
	max	4284,9	910,1	2867,1	758,9		
TVS(g/d)	av	3225,1	558,8	2372,2	486,3	-192,2	-6,0
	ds	369,3	136,5	238,0	24,0		
	min	2812,4	257,2	1661,5	437,0		
	max	3567,9	910,1	2867,1	514,3		
COD (g/d)	av	3312,9	541,5	2299,1	426,7	45,6	1,4
	ds	440,3	132,3	230,7	90,5		
	min	2794,4	249,2	1610,3	305,4		
	max	4085,3	882,0	2778,8	520,7		
Ptot (g/d)	av	5,0			4,0	1,0	20,8
	ds	1,0			0,8		
	min	3,8			3,3		
	max	6,3			5,0		
TKN (g/d)	av	116,5			55,4	61,1	52,4
	ds	16,2			7,4		
	min	95,0			48,5		
	max	138,5			68,3		

Table 3-38 Run III-b Balance

		IN	GAS R1	GAS R2	OUT	IN-OUT	%
TS (g/d)	av	5161,7	316,3	2919,7	903,3	1022,4	19,8
	ds	183,3	45,3	501,6	95,8		
	min	4907,7	263,4	2058,0	805,3		
	max	5322,8	434,6	3866,5	996,7		
TVS(g/d)	av	4222,5	316,3	2919,7	576,8	409,6	9,7
	ds	330,0	45,3	501,6	64,0		
	min	3732,4	263,4	2058,0	515,6		
	max	4604,5	434,6	3866,5	643,3		
COD (g/d)	av	3934,1	306,6	2829,7	572,6	225,2	5,7
	ds	522,9	43,9	486,1	48,3		
	min	3318,4	255,3	1994,6	538,4		
	max	4851,3	421,2	3747,4	606,7		
Ptot (g/d)	av	6,0			5,9	0,1	1,7
	ds	1,1			1,2		
	min	4,6			5,1		
	max	7,5			6,7		
TKN (g/d)	av	138,3			69,4	69,0	49,9
	ds	19,3			11,2		
	min	112,9			59,3		
	max	164,4			80,1		

The mass balance highlights a missing of nitrogen in the outlet flow. This could be explained by the recirculation of the sludge; it causes an increasing of ammonia concentration as shown in Figure 3.21 b) and Figure 3.22 b) both in first (from 200 to 1200 mgN/l) and second phase (from 800 to 1600 mgN/l). To avoid this accumulation, a regression of ammonia value was made in order to quantify the velocity of ammonia increasing (Figure 3.27).

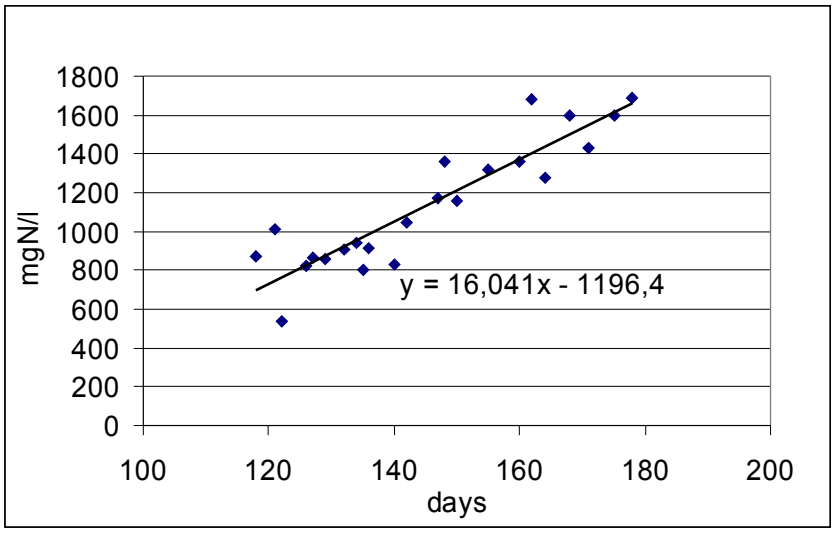


Figure 3.27 Ammonia accumulation rate in the second reactor.

The nitrogen accumulated per day was 16,04 mgN/ld, so it was adopted a daily reduction of the first phase effluent, fed in the anaerobic digestion.

3.2.4 Energetic considerations

During the first two Runs the hydrogen production was really low, for this motive the energetic considerations are based only on Run III-a yields, where the recirculation of the sludge was able to keep the pH in the right range, with a consequent significant hydrogen production.

The flow of hydrogen, carbon dioxide and methane were mixed in order to obtain the bio hythane gas, as shown in Table 3-39.

Table 3-39 Biohythane gas composition

	First phase		Second phase		GP	H_2	CH_4	CO_2	GPRtot	SGPtot
	m^3H_2/d	m^3CO_2/d	m^3CH_4/d	m^3CO_2/d	m^3gas/d	%	%	%	m^3gas/m^3d	$m^3/kgVS$
RUN III-a										
average	0,168	0,285	1,337	0,722	2,512	6,7	53,2	40,1	2,61	0,78
sd	0,041	0,070	0,134	0,072	0,317	-	-	-	0,33	0,98
min	0,097	0,165	1,053	0,569	1,884	5,2	55,9	38,9	1,96	0,58
max	0,225	0,381	1,471	0,795	2,872	7,8	51,2	40,9	2,99	0,89
RUN III-b										
average	0,083	0,161	1,665	0,882	2,791	3,0	59,7	37,4	2,90	0,66
sd	0,012	0,023	0,286	0,151	0,472	-	-	-	0,49	0,11
min	0,075	0,145	1,257	0,665	2,142	3,5	58,7	37,8	2,23	0,50
max	0,107	0,207	2,053	1,087	3,454	3,1	59,4	37,5	3,59	0,82

The biohythane gas mixture in the Run III-a met the gas composition required for an enhanced combustion. As suggested by some authors (Porpatham et al. 2007 Rakopoulos et al. 2009, Reith et al. 2003) the amount of hydrogen must be above 5% with an optimal value at 10%. Major quantity couldn't assure the best performance of engine and of emissions.

Considering the energy density and specific energy of methane and hydrogen and considering the ideal biohythane composition, was calculated and compared the energy content of biogas and bio-hythane. As shown in Table 3-40, in terms of energy density biohythane is 5697 vs 5407 kcal/m^3 of biogas, while considering the amount of energy based on mass, the BHy is 5849 instead of 4693 kcal/kg of biogas.

Table 3-40 energetic comparison

	specific energy		energy density	
	Mj/kg	kcal/kg	Mj/m^3	kcal/m^3
Hydrogen	143,0	34210	10,8	2581
Methane	55,6	13301	37,8	9043
Natural gas	53,6	12823	36,4	8708
Hythane	50,2	12017	34,6	8284
Bio-Hythane	24,5	5849	23,8	5697
Biogas	19,6	4694	22,6	5407

Furthermore it was demonstrate (Porpatham et al. 2007 Rakopoulos et al. 2009) that the use of 10% of hydrogen enhances the combustion characteristics of biogas and a drastic reduction in HC emissions was seen (HC level drops from 1530 ppm with neat biogas to 660 ppm).

3.2.5 Photofermentation process comparison

In order to better understand the sustainability of this approach, another bio hydrogen producing process was illustrated mainly in terms of yields and applicability of the technology.

Photofermentation is drove by photoheterotrophic bacteria that use the ability of nitrogenase enzyme which in N_2 absence, catalyses the production of hydrogen. This bacteria are able to produce hydrogen converting organic substrate as acetic acid, using the light as energy source:

$$C_2H_4O_2 + 2H_2O + "lightenergy" --> 2CO_2 + 4H_2 \qquad \textbf{Equation 8}$$

Another important condition for nitrogenase enzyme is the absence of oxygen and ammonium ions that can cause inhibition. For this motive usually the reactors operate in anaerobic condition, with a light source and with low concentration of nitrogen sources.

The bacteria involved in photofermentation are purple non-sulfur bacteria; the production of hydrogen from organic substrates would be bioenergetically more favorable than from water (drove by photoautotrophic bacteria like green algae) but these bacteria saturate at even lower light intensities than microalgae, in fact they can use a wider part of the solar spectrum but with associated lower energies (Reith et al 2003).

As shown by some authors (Levin et al 2004; Hallembak et al 2002; Reith et al 2003) this technology has some disadvantage in a full-scale application optics because of:

- necessity of a light energy source (the magnitude of solar radiation depend on geographical position and climatic condition);
- necessity to maintain a monoculture for an extended time;
- necessity of an high surface to volume ratio;
- the areas needed to reach sufficient light are expensive (in outdoor applications);
- low photochemical efficiency (theoretical max 10%) and tend to decrease at higher light intensities;
- necessity to mix culture at high rate, so the cells are exposed only for a short period (milli/micro seconds);
- high cost of photobioreactors (estimated 100 US$/$m^2$).

Dark fermentation process in contrast could be implemented in existing anaerobic digestion plant, is independent from light, so it is a continuous hydrogen producing process, and doesn't need pure culture but is sufficient an undefined microflora from anaerobic digestion.
In terms of yields, it is difficult to compare photo and dark fermentation, especially in this thesis contest because it isn't possible to use this type of complex substrate in photobioreactors without a pretreatment step or, for example, coupling the dark fermentation as first phase.
The only way to compare the hydrogen yields is on volumetric bases, considering the hydrogen production rate.
Levin et al (2004) reviewed and compared the hydrogen production rate of different biological systems and shows an average rate of 0,16 mmolH_2/l h or 0,004 lH_2/l h for the photofermentation and 8,2 mmolH_2/l h or 0,2 lH_2/l h for dark fermentation in thermophilic condition with undefined culture. This values could be compare with the results obtained by Claassen et al (2010) in photobioreactors treating a defined medium (acetic acid, lactic acid, glutamate) both in tubular and panel reactor. The maximum hydrogen rate was 0,36 mmolH_2/l h that means 0,009 lH_2/l h.
The hydrogen production rate calculated during Run III-a of this thesis was 1,37 mmolH_2/l h or 0,034 lH_2/l h, that is higher than those observed for photo fermentation. This is an interesting result, but it is necessary to considered that the final objective of dark fermentation in this experimental work is not only the optimization of hydrogen production but the optimization of the whole process where energy recovery and waste treatment are the main goals.

3.3 DRI and BMP tests results

The DRI and BMP tests were carried out on dewatered sludge of three Italian plants (Treviso, Camposampiero–PD, Bassano-VI) in order to evaluate the biological stability of the anaerobic digestion effluent treating organic waste.

The BMP tests were made both in mesophilic and thermophilic temperature for two plants, in order to evaluate the biogas production differences. The inoculum was took from the anaerobic digestor of Treviso WWTP few days before in order to adapt the biomass to the temperature range applied. In Table 3-41 are resumed the inoculum and samples characteristics.

Table 3-41 characterisation of the inoculum and waste tested.

	DEWATERED SLUDGE			INOCULUM		
	TS g/kg	TVS g/kg	%TVS,TS	TS g/kg	TVS g/kg	%TVS,TS
Treviso	239	134	56	39	19	47
Bassano	420	290	69	39	19	47
Camposampiero	276	162	59	27	12	50

The total solids of Bassano organic waste were higher than the other two plant considered, and this was caused by the dry digestion technology applied to this plant, in fact to the organic waste was also added bulking agent as the green waste.

The results shown in Table 3-42 are the average value of the specific gas production obtained after about 30 days. In thermophilic range the SGP was higher than those obtained in mesophilic temperature. This was linked to thermodynamic favourable conditions in thermophilic temperature that allow a better organic conversion into biogas. Despite temperature variation, it is clear that this kind of substrates had a low residual biogas content, specially if compared with the SGP of the organic fraction fed to the anaerobic digester, that is about 0,7 Nm^3/kg TVS. The maximum value was obtained for Treviso's dewatered sludge in thermophilic condition that was 0,23 Nm^3/kg TVS, while the lower SGP was Bassano dewatered sludge, and that was linked to the addiction of bulking agent in the process.

Table 3-42 specific gas production of dewatered sludge

	TEMP °C	SGP Nm^3/kg TVS
Treviso	35	0,18
Camposampiero	35	0,14
Bassano	35	0,12
Treviso	55	0,23
Camposampiero	55	0,22

.

In the figure below (Figure 3.28) are reported the profile of biogas production and methane composition at 55°C of Camposampiero dewatered sludge.

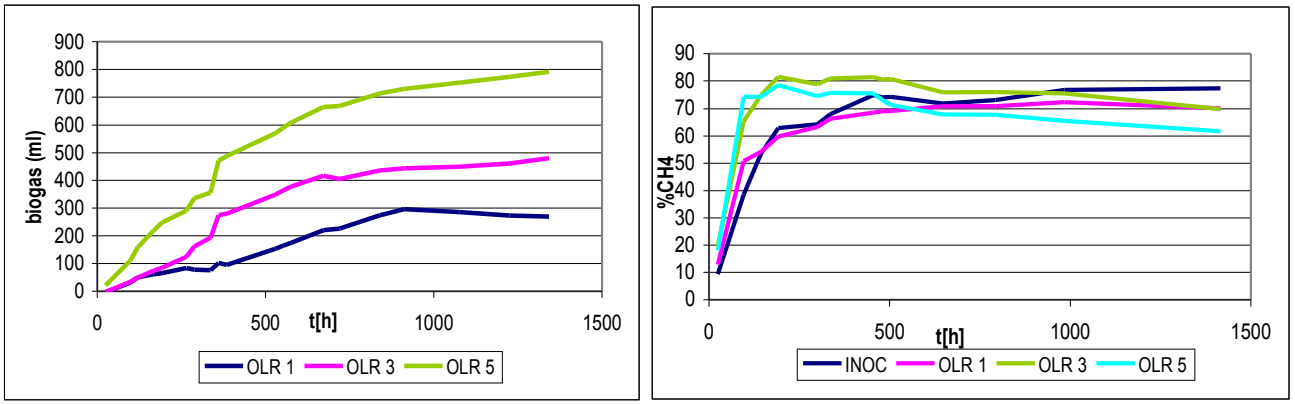


Figure 3.28 biogas production and composition of Camposampiero's dewatered sludge.

It is interesting to observe that in the first part the methane content was low because of the hydrolytic and acidogenic events that promote an high CO_2 production. After about 6 days the methane percentage reach a constant trend and an average of 70%.

About the DRI test, to date the tests have been carried out only on dewatered sludge of the Treviso WWTP. In Figure 3.29 is reported the test graph of the DRI-R (second test) as example. It's possible to observe the high index value in the beginning of the process and a decreasing of it after 2 days. This means that the dewatered sludge coming from anaerobic co-digestion could reach in a few days values under 1000 $mgO_2/kgVSh$ (Italian law limit).

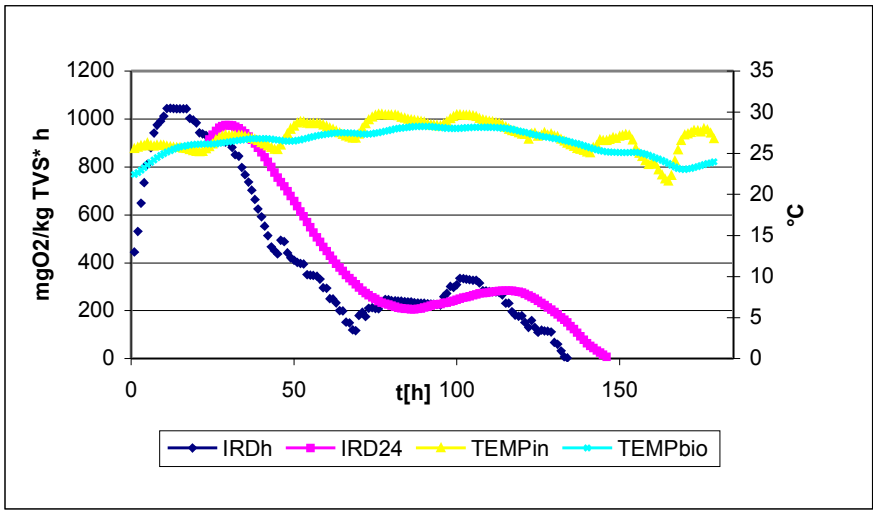


Figure 3.29: DRI real, test 2.

In Table 3-43 are listed the four tests mentioned. The bulking agent used was inert material (expanded polystyrene) necessary to guarantee the aeration of the biomass. For every kind of bulking agent used is necessary to know the DRI. In this case the index was very low (about 80 mgO_2/h).

Table 3-43 DRI test of Treviso dewatered sludge, parameters considered.

	Water holding capacity g H_2O/kg	Bulking agent	Density kg/l	TS g/kg	TVS g/kg	Kg of sample	DRI mg O_2/kg TVS*h
1 DRI-R		Yes	0,59	232	133	16,14	849
2 DRI-R		Yes	0,50	233	135	10,92	894
3 DRI-P	1183	Yes	0,46	192	112	12,13	905
4 DRI-P	1170	Yes	0,45	218	128	10,32	900

All the tests, both real and potential DRI, had shown an index value under 1000 mgO_2/kgTVS h. This good DRI values obtained must be considered for the final disposal of this matter, in fact it possible to avoid high treatment time usually adopted in a composting process.

4 Conclusions

The optimization of a two phases dark-fermentation and anaerobic digestion process was studied at pilot scale, using two CSTR reactors fed with organic waste, for hydrogen and methane production.
The objective of the study was to evaluate the efficiency of the process, both in terms of gas production and process stability, without any pre treatment of substrate and without inoculum utilization for the first phase, in order to keep the process management economically feasible for a scale-up.
During the experimental work, together with this main aspect of (biohythane production), were evaluated other to aspects:
- bio hydrogen potential tests were carried out using a non pretreated anaerobically digested sludge and organic waste, and comparing two different loading rate, in order to evaluate the behavior of process changing the substrate feeding;
- bio methane potential tests and dynamic respirometric index were carried out on dewatered sludge coming from an anaerobic codigestion process (OFMSW and AS) in order to evaluate the stability of AD effluent treating organic waste.
The BHP results of two different organic loading condition, shown two completely different process behaviour; at 20 kgTVS/m^3 the specific gas and hydrogen production were 0,71 $m^3/kgTVS_{fed}$ and 20,7 $lH_2/kgTVS_{fed}$ respectively, while at the higher loading, 30 kgTVS/m^3, the system was overload, and this caused a low total gas production but an high H_2% content and a consequent SHP of 69,0 $lH_2/kgTVS_{fed}$. This results confirm the possibility to produce hydrogen without any chemical or heat-shock treatment and give also two completely different behavior: at 20 of loading, the process could be called as a single phase process, where hydrogen was produced in the first part of the metabolic reactions and than converted to methane; at higher loading only hydrogen was produced with an high yield and an accumulation of VFA, and this could be considered as the first step of a separate phase system.
Two phases anaerobic digestion process, was optimized trough three Runs without chemical-heat shock treatment or pH control, and starting with an OLR of 20 kgTVS/m^3d. In Run I and Run II the pH was too low (<4,5) for the optimal hydrogenase enzyme conditions, so the specific gas production was about 2,6 lH_2/kgTVS even changing the HRT from 6,6 to 3,3 days. Run III was characterized by sludge recirculation from the anaerobic digestion, and this maintain the pH at about

5,5 with a consequent proliferation of hydrogen producing bacteria. In this Run were applied two organic loading rate (16 and 21 kgTVS/m^3d) and the best yield was obtained at lower OLR, with an SHP of 51,16 lH_2/kgTVS. The main problem of the process was the accumulation of ammonia nitrogen, but this was controlled calculating the velocity of accumulation and removing a defined amount of first phase effluent. The final gas composition met the biohythane characteristic with 6,7% H_2, 40,1% CO_2, 52,3% CH_4 and could be used in spark ignition engine with enhance combustion efficiency and low HC emission compared with neat biogas. Considering the whole system, the process reach an SGP of 0,78 m^3/kgTVS$_{fed}$.

The effluent from anaerobic digestion was than tested to evaluate the stability of the effluent of anaerobic codigestion and, as consequence, the efficiency of the process. Both BMP and DRI tests shown the low putrescible content in AD effluent, in fact the DRI was about 1000 mgO_2/kgTVS h and the average BMP was 0,20 m^3/kgTVS.

4.1 Addresses for future research

Considering the results obtained, next research will be focused on the optimization of OLR to be applied, using the recirculation approach. Fixed the best OLR, the recirculation ratio will be increased from 1 to 2 in order to evaluate the buffer capacity of the system, and to observe the ammonia behavior.

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