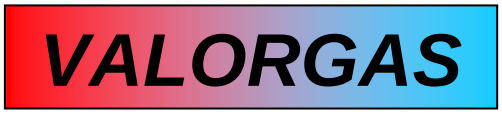
SEVENTH FRAMEWORK PROGRAMME
THEME ENERGY.2009.3.2.2
Biowaste as feedstock for 2nd generation



Project acronym: **VALORGAS**
Project full title: **Valorisation of food waste to biogas**
Grant agreement no.: 241334

D3.5: Kinetics of H_2 production in short retention thermophilic hydrolysis reactors with assessment of impact on overall system energy yield

Due date of deliverable: Month 28
Actual submission date: Month 28

Project start date: 01/03/2010 Duration: 42 months

Lead contractor for this deliverable
Università degli Studi di Verona (UNIVR)

Revision [0]

D3.5: Kinetics of H_2 production in short retention thermophilic hydrolysis reactors with assessment of impact on overall system energy yield

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D3.5: Kinetics of H_2 production in short retention thermophilic hydrolysis reactors with assessment of impact on overall system energy yield

The work described in this Deliverable Report deals with optimisation of the parameters influencing the anaerobic digestion process in a two-phase approach, focused on concurrent biological hydrogen and methane production. It was carried out at laboratory scale (batch and continuous tests) and pilot scale using food waste as feedstock and avoiding physical and chemical pre-treatment, but changing operational parameters such as the hydraulic retention time (HRT), the organic loading rate (OLR) and the recirculation of anaerobic digestion effluent, in order to keep the dark fermentation (DF) process in the optimal pH range. In addition the composition of the recycled digestate was also evaluated, testing both the whole digestate and the liquid fraction after solid-liquid separation. A physical-chemical characterisation was carried out on samples of source segregated organic waste used as feedstock and on the effluent of both reactors.

1 Introduction

The optimisation of separate collection is a main driver for the implementation of anaerobic digestion: reactors are fed with better quality materials thus improving the biogas production and the characteristics of the digested material to be used as fertiliser. One step forward from the common anaerobic digestion process is the separate phase approach aimed at the production of hydrogen in the first phase reactor and methane in the second phase reactor (Martinez-Perez et al., 2007). The gases obtained can be used separately or mixed together to obtain biohythane, a biogas with an average percentage composition of 10% H_2, 30% CO_2 and 60% of CH_4.

Porpatham et al. (2007) studied the advantages of using biohythane in combustion engines and found that adding 10% of hydrogen in biogas enhanced combustion, with a consequent improvement in thermal efficiency and power output. Moreover, a major reduction in hydrocarbons (HC) emissions (HC level drops from 1530 ppm with neat biogas to 660 ppm) and no significant increase in NO concentrations was observed.

Thanks to its high yields and low costs, dark fermentation has gaining importance in recent years. The simple configuration of reactors and the possibility to produce hydrogen continuously make this option very attractive (Valdez-Vazquez et al., 2009; Hawkes et al., 2007). The possibility to couple dark fermentation with the use of mixed cultures opens the way to interesting industrial applications, as observed by Kleerebezem and van Loosdrecht (2007): they based the process development on natural/ecological selection by manipulating the operation of the bioprocess, enriching the microbial population from the natural environment. To enrich mixed cultures with H_2, anaerobic biomass containing microflora from AD reactors is usually treated by heat/acid/basic conditions which inhibit the activity of the hydrogen consumers while the spore-forming anaerobic bacteria survive (Hellenbeck 2009; Mathews and Wang 2009).

During the last ten years, most studies on the optimisation of bio-hydrogen production using dark fermentation were focused on the inhibition of hydrogen-consuming bacteria in a mixed microflora inoculum, by a thermal or chemical shock, or adding a reagent for pH control. These pre-treatments are expensive and not sustainable in a full-scale approach; for this reason, dark fermentation coupled with anaerobic digestion has advantages over a

conventional single-stage process. In fact in specific conditions it permits the selection and enrichment of hydrogen-producing bacteria simply by manipulating the process conditions.

Among process parameters, high dilution rates (short HRT) can be used to cause the complete washout of methanogens since the specific growth rates of methanogens are much lower than those of hydrogen-producing bacteria (Valdez-Vazquez et al., 2009). According to the literature data there is no optimum HRT, as the process optimisation also depends on parameters such as the organic loading rate, the characteristics of the organic substrate and the pH. On the other hand it can be observed that, when treating biowaste, the highest hydrogen production rates (HPR) were found at a HRT of around 3 days. Clearly, a short HRT implies a relatively high organic loading rate (OLR). This normally causes a decrease in pH due to the accumulation of organic acids in the reactor. In these conditions it is necessary to control the pH in order to keep it in a range favourable for hydrogen production (Valdez-Vazquez et al., 2009). Instead of using chemical agents to control the pH, it is possible to adopt the separate phase approach and recirculate the effluent of the anaerobic digestion stage (second reactor) to the dark fermentation reactor in order to buffer the system and reach the optimal pH value (between 5 and 6). Chu et al. (2008) achieved better yields with the recirculation approach. They used two CSTR reactors (55 °C), with a recirculation rate of 2, and obtained a biogas containing 42% H_2 with a specific production of 205 l H_2 kg^{-1} TVS_{fed}. Lee et al. (2010) used a recirculation rate of 1 in thermophilic conditions, and obtained a specific production of 83 l H_2 kg^{-1} TVS_{fed}. In both cases the hydraulic retention time was less than 2 days.

The work described in this deliverable focused on the anaerobic treatment of source segregated municipal biowaste in a two-phase system, testing at both laboratory and pilot-scale the feasibility of the process without recycling the second phase effluent, with recycling, and finally testing whether this effluent must be physically pre-treated (liquid-solid separation) or not in order to find the optimal conditions for hydrogen production. The first and the second reactor were optimised for H_2 and CH_4 production, respectively. Moreover, in order to make the process industrially attractive, the process was operated without either inoculum pre-treatment or the addition of chemicals to control the pH of the first reactor.

Since a specific target of the VALORGAS project is to consider new methods of upgrading the gaseous fuel product to give an extended range of end user applications, the present research contributes to investigating the options for improving biogas performance by applying a non-invasive and cost-effective adjustment to existing conventional AD plans.

1.1 Two-phase anaerobic digestion for biohydrogen and biogas production

1.1.1 Overview of the two-phase approach

In conventional AD applications the two major consortia of bacteria, the acidogenic and methanogenic microorganisms, are kept together in a single reactor system. There is a delicate balance between these two groups, as they differ widely in terms of physiology, nutritional needs, growth kinetics and sensitivity to environmental conditions (Burak and Yenigun 2002). In a well-operating process, this balance is maintained because the waste products of the acidogenic and acetogenic bacteria (H_2, acetic acid, CO_2 and a variety of higher organic acids) are used as substrate by the methanogenic microorganisms with consequent production of methane as an end product.

As these reactions are linked together, the failure of one of them could lead to failure of the entire process. For this reason several years ago it was proposed that the acid-formers and methane-formers should be physically separated in two separate reactors, so that optimum environmental conditions for each group of organisms could be provided to enhance the overall process stability and control. Many authors have since worked on this topic, studying the best conditions to maximise the acid production in the first reactor in order to provide the second one with a ready-to-use substrate for maximum methane production.

In the present research the same approach was pursued but with the target to maximise hydrogen production in the first phase while also maintaining an acceptable methane production in the second phase. Figure 1 presents the features of the two single phases when a two-phase AD strategy is applied for bio-hydrogen production.

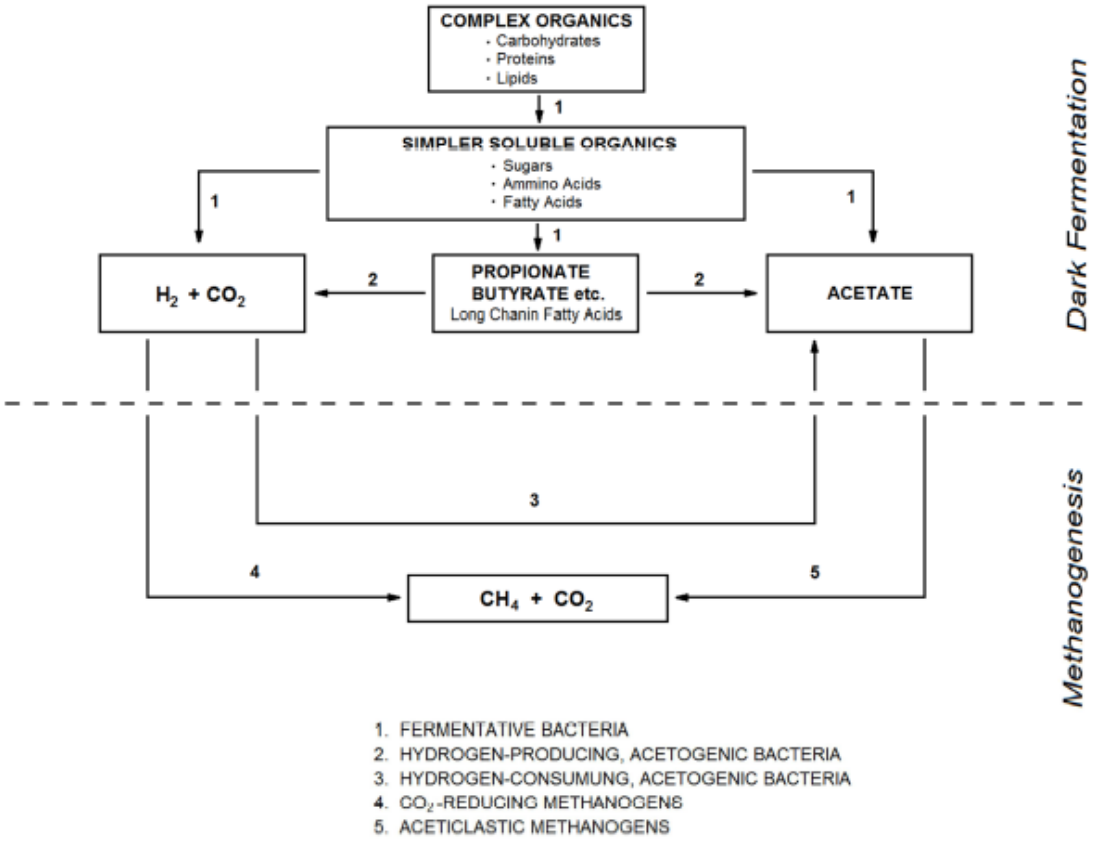


Figure 1. Separation of the AD stages in a two-phase system

1.1.2 First phase: dark fermentation

The most important challenge for sustaining hydrogen production in a reactor optimised for DF is to avoid the growth of hydrogen-consuming bacteria (Coney et al., 2007). Due to the continual addition of mixed culture in the FW substrate there is always the risk that unwanted archaea such as hydrogenotrophic methanogens could grow up and deplete the hydrogen already produced. The strategies that can be adopted to select the H_2-producing bacteria in a mixture culture approach are:

- heating or chemical treatment of the inoculum: to sterilise the entire microflora in the digestate and allow the proliferation of spore-forming bacteria only, such as hydrogenogens. This method however has been proved of little effectiveness in a long term approach (Kraemer et Bagley 2007).
- heating or chemical treatment of the inoculum and substrate fed: to kill the non-spore-forming methanogens and allow the entry of spores from hydrogenogens bacteria. This approach is complex and quite expensive, however, and is thus not very likely to be applied at the industrial scale.
- low HRT: to washout the hydrogenotrophic bacteria. A CSTR could be used to select microbial populations whose growth rates are able to keep up with 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). The dilution rate could be controlled with HRT being D=1/HRT. Based on this, high dilution rates (short HRT) could be used to cause the complete wash-out of methanogens since the specific growth rates of methanogens are much lower than those of H_2-producing bacteria (0.0167 and 0.083 $hour^{-1}$, respectively (Valdez-Vazquez et al., 2009) (Figure 2).

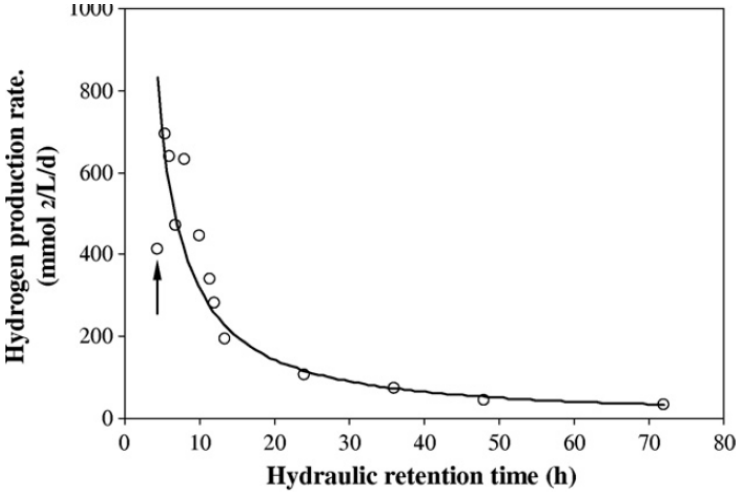


Figure 2. H_2 production rate varying the HRT (Valdez-Vazquez et al., 2009).

- varying the OLR: there is disagreement in literature on this point as in some cases higher OLRs increased the H_2 yield whereas in others they decreased it. High OLRs can affect the methanogenic archaea due to substrate inhibition. On the other hand high OLRs could decrease H_2 production because they lead to increased inhibition by VFAs, which also affects acetogenic activity (Kraemer and Bagley 2007). Hence it is clear that the right compromise has to be found according to the other operation parameters applied.
- low pH: to inhibit the methanogenic archaea (preferred pH range 6.8-7.8) and promote H_2-producer bacteria.
- chemical inhibitors: to block the growth of the methanogenic archaea. These can be divided into specific and nonspecific inhibitors, depending on their potency against methanogens only or both methanogens and non-methanogens respectively. Specific inhibitors usually inhibit the coenzyme M, a cofactor found in methanogens only and involved in the terminal step of methane biosynthesis. They are: BES (2-bromoethanesulfonate), CES (2-chloroethanesulfonate), MES (2-mercaptoethanesulfonate) and lumazine (Liu et al., 2011). Of the nonspecific ones, the most widely used are ethylene and acetylene, choloroform ($CHCl_3$), fluoroacetate (FCH_2COO-), and methyl-fluoride (CH_3F). Acetylene has advantages as it is the least expensive, does not lead to solids-accumulation and does not affect the

hydrogenase enzymes. On the other hand, more general inhibitors such as $CHCl_3$ have been demonstrated by Hu and Chen (2007) as able to improve H_2 production. For a continuous industrial application, however, use of these inhibitors is not recommended since their uncontrolled addition could potentially lead to more potential problems.
HRT and OLR and pH are interdependent, since the higher the OLR, the higher the production of VFAs with consequent falls in pH. Low HRT itself, by causing wash-out of the acid-consuming bacteria, leads to an accumulation of organic acids and a drop in pH. If the aim is to maximise hydrogen production in DF, pH is definitely the most important parameter because it influences the functionality of the hydrogenase enzyme which requires a pH range between 5.0 and 6.5 with an optimum of 5.5. These enzymes are able to reduce protons to hydrogen in a reversible way. The hydrogenase enzymes are classified according to the metal on the active site (Fe-hydrogenase; NiFe-hydrogenase; NiSeFe-hydrogenase) and are slightly influenced by pH (Hallembek and Ghosh 2010). pH could be controlled using chemicals or with a recirculation strategy, but to achieve the right value in the reactor the right equilibrium between process parameters must be found.
From the microbiological viewpoint, many microorganisms are known to be capable of hydrogen production such as *Clostridium spp.* (anaerobic), *Thermoanaerobacterium spp.* (thermophilic or hyperthermopilic anaerobic, 4÷2 mol H_2/mol H_{exose}), *Enterobacter spp.* (facultative anaerobic, 2÷1 mol H_2/mol H_{exose}) and *Bacillus spp.* (facultative aerobic 2÷1 mol H_2/mol H_{exose}). The best metabolic pathway for hydrogen production is the one with acetate as the sole fermentation end product; it has been demonstrated that in a reactor optimised for DF the highest H_2 yields are associated with a mixture of acetate and butyrate as fermentation products (Levin et al. 2004).

When a high H_2 partial pressure or a low pH occurs other metabolic pathways are selected causing the so-called solventogenic shift: low H_2 yields are observed, associated with production of propionate and end products such as alcohols and lactic acid. In particular it has been noticed that *Clostridium spp.* often choose metabolic pathways that lead to these solventogenic end-products, hence they are often responsible for lowering the H_2 yield from the theoretical maximum of 4 mol H_2/mol H_{exose} (Kapdan and Kargi 2006).

Concerning the best substrate to enhance the population of H_2-forming bacteria, Reith et al. (2003) demonstrated that 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. The high carbohydrate content of solid food waste, in form of simple sugars, starch and cellulose, makes it a potential feedstock for biological hydrogen production. The problem with food waste is the high variability of the mixture since each component requires different environmental and bio-processing conditions for hydrogen gas production.

1.1.3 Second phase: biogas production

In a two-phase CSTR strategy, the methane phase is usually not a major issue. Although the methanogenic archaea are the most sensitive microorganisms in the entire anaerobic consortium when environmental conditions change, the solid knowledge we now have on this phase of the process usually makes it simple to control. The same conditions applied in a single-stage digester can be applied to the methanogenic reactor of a two-phase AD. A pH between 6.8 and 7.8 is still recommended but the HRT can be reduced since the incoming substrates for methanogens are already in a ready-to-use form. This was demonstrated by

Pavan et al. (2000) who found that an HRT of 8-9 days was best for the thermophilic methanogenic reactor of a two-phase AD system.

2 Overview of the research

This section describes all the experimental trials carried out at laboratory and pilot scale, in order to find the best operational conditions for maximising hydrogen production.

2.1 Batch test for bio-hydrogen production (BHP)

As preliminary investigation, a single phase hydrogen and methane production process was studied at thermophilic temperature using food waste as substrate. This type of test could be described as a BHP test, since the reactors were fed only once at the beginning and then gas production and composition were monitored until no further production occurred. As far as is known, the digestate used had never previously been tested for hydrogen production under thermophilic conditions with food waste and at quite high loadings. The purpose was thus to examine the ability of the inoculum to support an efficient bacterial community during Dark Fermentation, to get a sense of the possible process performance with different loads and check whether inhibition occurred. The specific hydrogen and methane productions achievable with this inoculum substrate system were also determined. Initial Loads of 15, 20, 25 and 30 kg VS m^{-3} were chosen (Table 1).

Table 1. Batch test experimental conditions

Parameters	unit	Value/description
Initial organic loading	kg VS m^{-3}	15, 20, 25, 30
Reactor volume	l	1.5
Temperature range	°C	52
Inoculum		Anaerobic digestate from Millbrook WWTP
Substrate		Food waste from South Shropshire Biowaste Digester, Ludlow, UK
Analytical monitoring		Gas composition, gas production, volatile fatty acids,

2.2 Two-phase laboratory-scale CSTR trials for bio-hydrogen production

The OLR applied in the laboratory-scale semi-continuous two-phase trials were based on the initial organic loadings used in the BHP tests (Table 2). Due to the importance of pH as key parameter controlling the process and its dependence on the alkalinity of the digestate, the four OLR were tested in a two-phase system both recirculating the whole digestate from the second phase, and recirculating the liquid obtained after centrifugation. In this way the capacity of the supernatant to recirculate enough alkalinity for maintaining a proper pH range in both phases was investigated. The same effect was tested using the whole digestate, and in this case the risk of an unwanted recirculation of methanogen biomass into the first phase was also tested.

Table 2. Laboratory-scale two-phase experimental conditions

Parameters	unit	Value/description
First phase OLR with supernatant recirculation	kg VS m^{-3} day^{-1}	15, 20, 25, 30
First phase OLR with digestate recirculation	kg VS m^{-3} day^{-1}	15, 20, 25, 30
First phase Volume reactors	L	1
Second phase Volume reactors	L	4
Temperature range	°C	52
First phase HRT	day	3
Second phase HRT	day	12
Inoculum		Anaerobic digestate from Millbrook WWTP
Substrate		Food waste from South Shropshire Biowaste Digester, Ludlow, UK
Analytical monitoring		Gas composition, gas production, volatile fatty acids, pH, alkalinity, ammonia

2.3 Two-phase pilot-scale CSTRs trials for biohydrogen production

The experimental trial was divided in three periods (runs). During the first two working periods the OLR on the first reactor was maintained at 21 kg VS m^{-3} day^{-1} while HRT was decreased from 6.6 to 3.3 days by changing the reactor volume. In the third working period part of the digestate coming from the methanogenic reactor was recirculated in order to provide alkalinity buffering to keep the pH around 5.5, with a recirculation ratio of 1. Table 3 shows the operational conditions applied to the reactors during the experimentation. In all the runs the second phase hydraulic retention time was fixed at 12.6 days, in order to permit the anaerobic digestion process to degrade almost all the biodegradable matter. Run III was divided into two sub-periods: the first sub-period was called Run III-a and an OLR of kg VS m^{-3} day^{-1} 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 kg VS m^{-3} day^{-1} as the previous two runs. The duration of the whole experiment was 185 days: each run had a defined start-up period and a period of stationary state conditions.

Table 3. Pilot scale two-phase experimental conditions

Parameters	unit	Value/description
First phase OLR	kg VS m^{-3} day^{-1}	21, 21, 16, 21
Second phase OLR	kg VS m^{-3} day^{-1}	10, 5, 4, 5
Volume of first phase reactors	l	200
Volume of second phase reactors	l	760
Temperature range	°C	55
First phase HRT	day	6.6, 3.3
Second phase HRT	day	12.6
Inoculum		AD digestate from Treviso WWTP
Substrate		Food waste from Treviso waste TP
Analytical monitoring		Gas composition, gas production, volatile fatty acids, pH, alkalinity, ammonia, TKN, COD, Ptot

2.4 Two-phase pilot-scale CSTR for bio-hydrogen production: longer-term trial

After the evaluation of optimal values for the different operational parameters, a longer-term trial was carried out in order to test the stability of the process, with particular interest in the influence of ammonia on the whole process behaviour. The recirculation of digestate from the anaerobic digestion reactor to the dark fermentation reactor caused an increase in ammonia concentration that could compromise hydrogen yields. With the purpose of maintaining the optimal ammonia concentration in this phase, a side stream evaporator was tested in order to remove the right amount of ammonia from the recirculation flow. At the same time, the correlations between pH, conductivity and ammonia were verified in order to create an appropriate automatic system to control the ammonia removal.

Table 4. Longer-term pilot scale two-phase experimental conditions

Parameters	unit	Value/description
First phase OLR	kg VS m^3 day^{-1}	16.3
Second phase OLR	kg VS m^3 day^{-1}	4.8
Volume of first phase reactors	l	200
Volume of second phase reactors	l	760
Temperature range	°C	55
First phase HRT	day	3.3
Second phase HRT	day	12.6
Inoculum		Anaerobic digestate from Treviso WWTP
Substrate		Food waste from Treviso Waste TP
Analytical monitoring		Gas composition, gas production, volatile fatty acids, pH, alkalinity, ammonia, TKN, COD, Ptot

3 Materials and methods

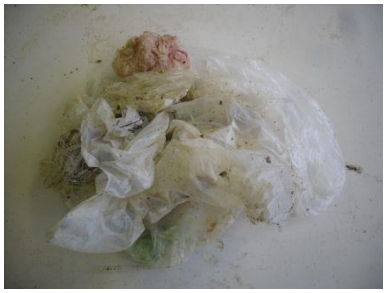
3.1 Laboratory-scale substrate and inoculum

The substrate used for the laboratory-scale trials was food waste from the South Shropshire Biowaste Digester in Ludlow, UK, an anaerobic digestion treatment plant which receives 5000 tonnes $year^{-1}$ of source separated domestic food waste. A sample of around 300 kg of this material was collected, removed from cornstarch bags and pre-sorted to remove small amounts of contaminants such as packaging (Figure 3). The pre-sorted material was passed through a macerating grinder (S52/010 Waste Disposer, IMC Ltd, UK) then stored in 4-litre boxes and frozen on the same day. A preliminary characterisation of the substrate was carried out (Table 5).

Table 5. Characterisation of the substrate used for laboratory-scale trial (WW = wet weight)

Parameter	Unit	Value ± SD
TS	g kg^{-1} WW	248 ± 19
VS	g kg^{-1} WW	236 ± 22
TKN	mg N kg^{-1} WW	5983 ± 497
COD	mg O_2 kg^{-1} TS	998 ± 71


a) Cornstarch collection bags


b) Non-biodegradable plastic bags


c) Other packaging


d) Bones


e) Eggshells


f) Green waste and large fruit stones


g) Kitchen paper


h) Miscellaneous (including cigarette stubs)


i) Food waste after processing

Figure 3. Cornstarch bags and contaminants found in food waste, materials removed to protect the grinder or laboratory-scale reactors, and food waste after grinding

The inoculum used was digestate from Millbrook Wastewater Treatment and Recycling Centre (Southampton, UK), an anaerobic digestion plant treating 14000 tonnes year^{-1} of municipal wastewater biosolids at an operating temperature of 37 °C. The digestate characteristics are reported in the following table:

Table 6. Characterisation of the inoculum used for laboratory-scale trial

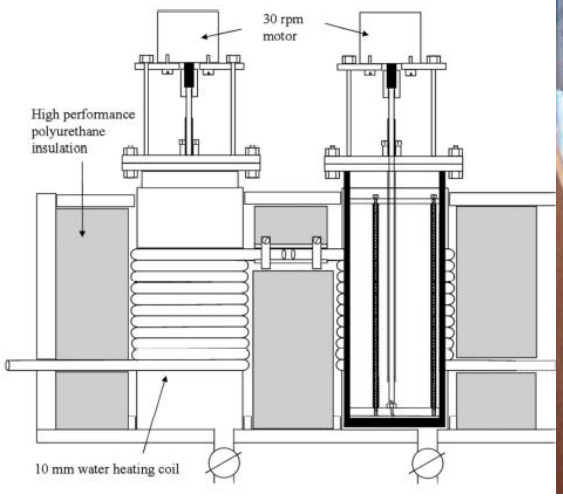
Parameter	Unit	Value ± SD
TS	g kg^{-1} WW	35.2 ± 0.5
TVS	g kg^{-1} WW	23.1 ± 0.4
pH	-	7.5
Alkalinity	mg $CaCO_3$ l^{-1}	8500 ± 248
VFA	mg COD l^{-1}	509
NH_3	mg N l^{-1}	1911 ± 43

3.2 Laboratory-scale reactors

The reactors used during the experiments were of two different total volumes: 2 l and 5 l. The construction was the same for both, however, and is shown in Figure 4.

The digesters were fitted with a flanged top plate through which a stirrer was inserted via a draught tube: this allowed the digester contents to be stirred continuously at 30 rpm using an off-set bar stirrer. The digesters were maintained at 52°C by a circulation of hot water from a thermostatically controlled reservoir. The digesters were fed via a feed port in the top flange and digestate removed via a wide-bore tube in the base.

Gas production was measured with a gas flow meter constructed and calibrated as described in Walker et al. (2009) and connected to a gas sampling bag. Gas production was corrected to STP (101.325 kPa, 0 °C).



a) Cross-section diagram showing heating coils (left) and stirrer (right) (Banks et al., 2010)

b) Picture of the digesters

Figure 4. CSTR digesters used in laboratory-scale trials

3.3 Pilot-scale substrate and inoculum

The pilot-scale fermentative reactor was fed on source segregated biowaste from the WWTP located in Treviso (northern Italy) where a 2000 m^3 anaerobic digester treats the collected biowaste at a working temperature of 35 ºC. The feedstock was prepared without addition of any chemical reagents and without thermal treatment. In order to avoid problems of pipe clogging, the particle size of the substrate was reduced using a grinder (Figure 5).

Figure 5. Grinder

The size of the treated waste was similar to that in a full-scale plant where two grinder pumps are applied in line before loading the anaerobic digester.

The digestate used as inoculum for the methanogenic reactor was collected from the same WWTP. The characteristics of inoculum in terms of total solids, volatile solids, macro pollutants, pH and alkalinity are shown in Table 7.

Table 7. Inoculum characterisation

Parameter	unit	AV	min	max	SD
TS	g kg^{-1} WW	22.87	22.31	23.38	0.46
TVS	g kg^{-1} WW	13.38	13.03	13.70	0.35
TVS, TS	%	58.48	57.72	59.21	0.61
TKN	mg N l^{-1}	0.50	0.48	0.51	0.02
Ptot	mgP l^{-1}	0.06	0.06	0.07	0.01
pH		7.51	7.31	7.69	0.16
Alkalinity tot	mg $CaCO_3$ l^{-1}	2074.2	2060.8	2087.7	11.6

3.4 Pilot scale reactor configuration

Two stainless steel CSTR reactors (AISI 304) were employed for optimised 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 760 l working volume (Figure 6).

Both reactors were heated by a hot water recirculation system and maintained at 55°C using an electrical heater controlled by a PT100-based thermostatic probe. The feeding system was semi-continuous, arranged once per day.

a) First phase b) Second phase

Figure 6. Pilot plant reactors.

The flow scheme of the pilot plant is shown in Figure 7. The organic waste was reduced in size using a grinder, then mixed only with tap water in order to keep the HRT at the established value (Runs I and II) or tap water plus recirculated anaerobic digestate (in Run III) and fed to the first phase reactor.

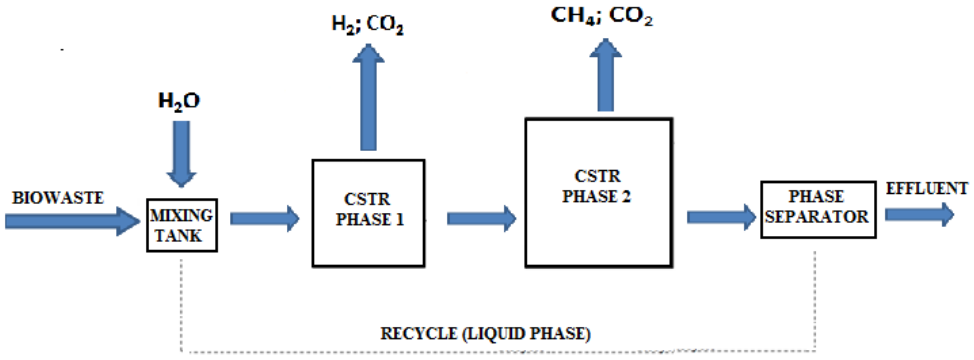


Figure 7. Pilot plant flow scheme

3.5 Analytical methods

For the laboratory-scale experiments TS, VS, TKN, COD and ammonia in the inoculum and substrate were measured according to Standard Methods 2540 G, 4500 PJ, 5220 B and 4500-NH3 G, respectively (APHA 2005). pH was measured using a Jenway 3010 pH meter (Jenway, London, UK) with temperature compensation and combination electrodes. The pH meter was calibrated daily using standard buffer solutions (Fisher Scientific UK Limited,

Loughborough, UK). Alkalinity was determined by titration with 0.25 N H_2SO_4 to an endpoint of 4.0 and the results expressed as total alkalinity. VFA concentrations were quantified in a Shimazdu GC-2010 gas chromatograph (Shimadzu, Milton Keynes, UK), using a flame ionisation detector and a capillary column type SGE BP-21 with helium as the carrier gas. The GC oven temperature was programmed to increase from 60 to 210 °C in 15 min, with a final hold time of 5 min. The temperatures of injector and detector were 200 and 250 °C, respectively. Standard solutions containing 50, 250 and 500 mg l^{-1} of acetic, proprionic, iso-butyric, n-butyric, isovaleric, valeric, hexanoic and heptanoic acids were used for VFA calibration. Samples for VFA determination were acidified by addition of formic acid to give a 10% concentration. Gas composition was measured using a Varian CP 3800 gas chromatograph (Varian, UK) and using helium as the carrier gas. The GC was fitted with a Hayesep C column operating at a temperature of 50 °C and equipped with a TCD detector.

For phase separation when supernatant recirculation was applied, the digestate was centrifuged at 3000 rpm for 30 minutes using an EC Centra-8P Model 2478 refrigerated centrifuge.

In the pilot-scale experimental trials the effluent of both reactors was monitored 2 - 3 times per week in terms of solid content, chemical oxygen demand, total Kjeldahl nitrogen, total phosphorus, and daily for stability parameters such as pH, volatile fatty acid content, alkalinity and ammonia, all in accordance with Standard Methods (APHA 2005). 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 NUKOLTM, 15 m x 0.53 mm x 0.5 µm film thickness) and with a flame ionisation detector (200 °C). The temperature during the analysis started at 80 °C and reached 200°C with two steps at 140 °C and 160 °C, with a rate of 10°C min^{-1}. The analysed samples were centrifuged and filtered 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 determined using a portable infrared gas analyser (Geotechnical Instruments, model. GA2000). Hydrogen content in the fermentative reactor was measured by gas chromatograph (GC Agilent Technology 6890N) equipped with a HP-PLOT MOLESIEVE column, 30 m x 0.53 mm ID x 25 µm film thickness, using a thermal conductivity detector and argon as the carrier gas.

4 Results and discussion

4.1 Laboratory-scale batch tests for potential biohydrogen production (BHP)

At an initial organic load of 15 kg VS m^{-3} hydrogen production was almost completely finished after 46 hours, with a peak concentration peak of 17.7%. Methanogenesis stabilised after about 70 hours with CH_4 produced with an average concentration of 70%. There was an accumulation of total VFA during the H_2-production phase, with a peak concentration of 7882 mg COD l^{-1} matching the hydrogen peak. Thereafter VFA were steadily consumed until they stabilised at less than 1500 mg COD l^{-1} after 170 hours. From this point ahead net biogas production began to tend to zero. Specific gas productions measured were: 0.012 (SHP), 0.424 (SMP) and 0.782 (SGP) Nm^3 kg^{-1} VS.

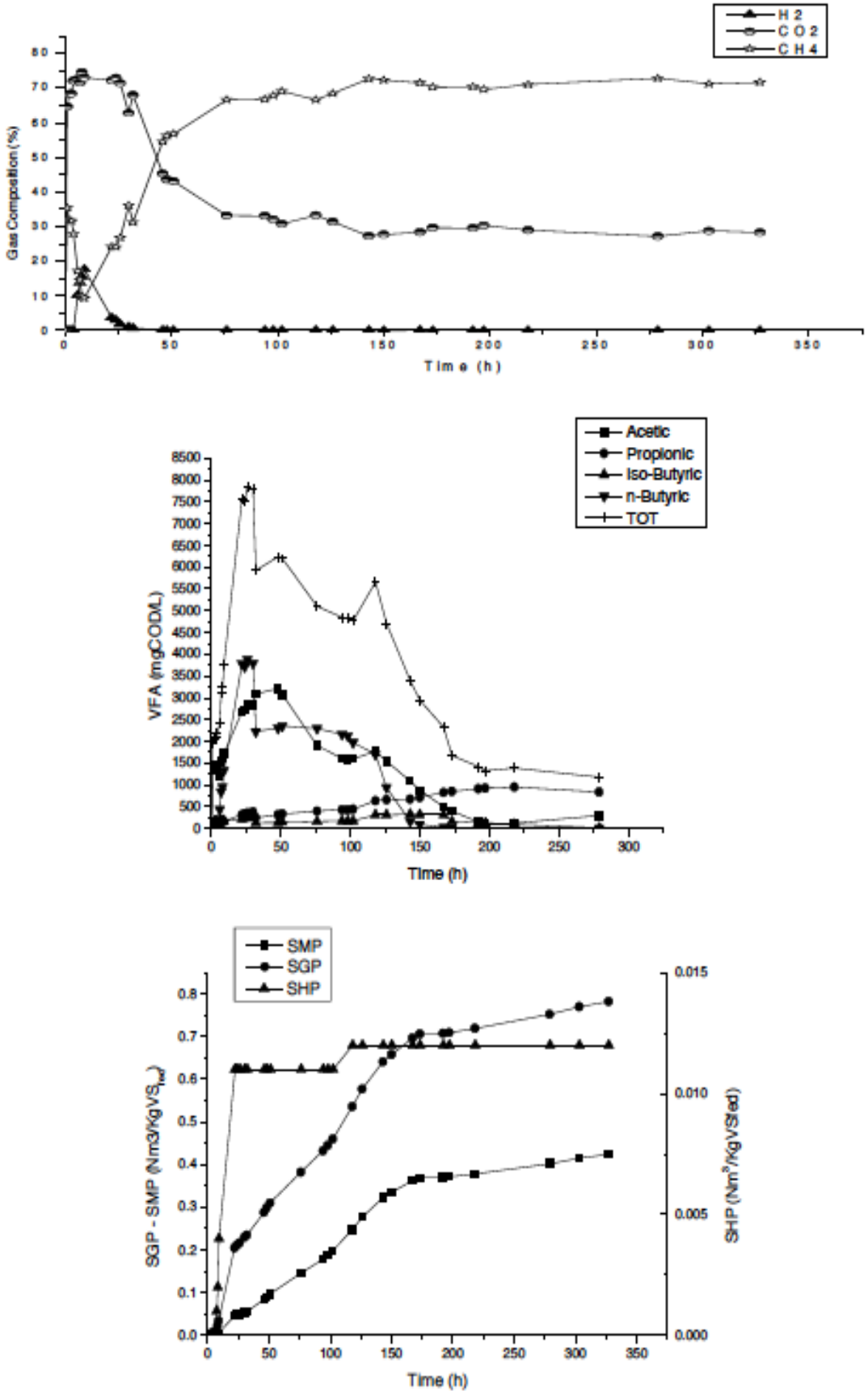


Figure 8. Gas composition, VFA and Specific Gas Production profiles for batch test at initial organic load of 15 kg VS m^{-3}

The initial organic load of 20 kg VS m^3 showed a similar trend for hydrogen, with a peak concentration of 19.4% and production finishing after 48 hours. CH_4 concentrations took a little longer to stabilise at 70.0% (196 hours) in comparison with the previous loading. CH_4 production also continued for longer as SMP stabilised after 300 hours. The pattern of VFA production was similar to the previous loading with accumulation during H_2 production and consumption once CH_4 production started. Stabilisation of VFA at 1659 mg COD l^{-1} occurred after 240 hours. SHP, SMP and SGP were respectively 0.021, 0.360 and 0.709 Nm^3 kg^{-1} VS.

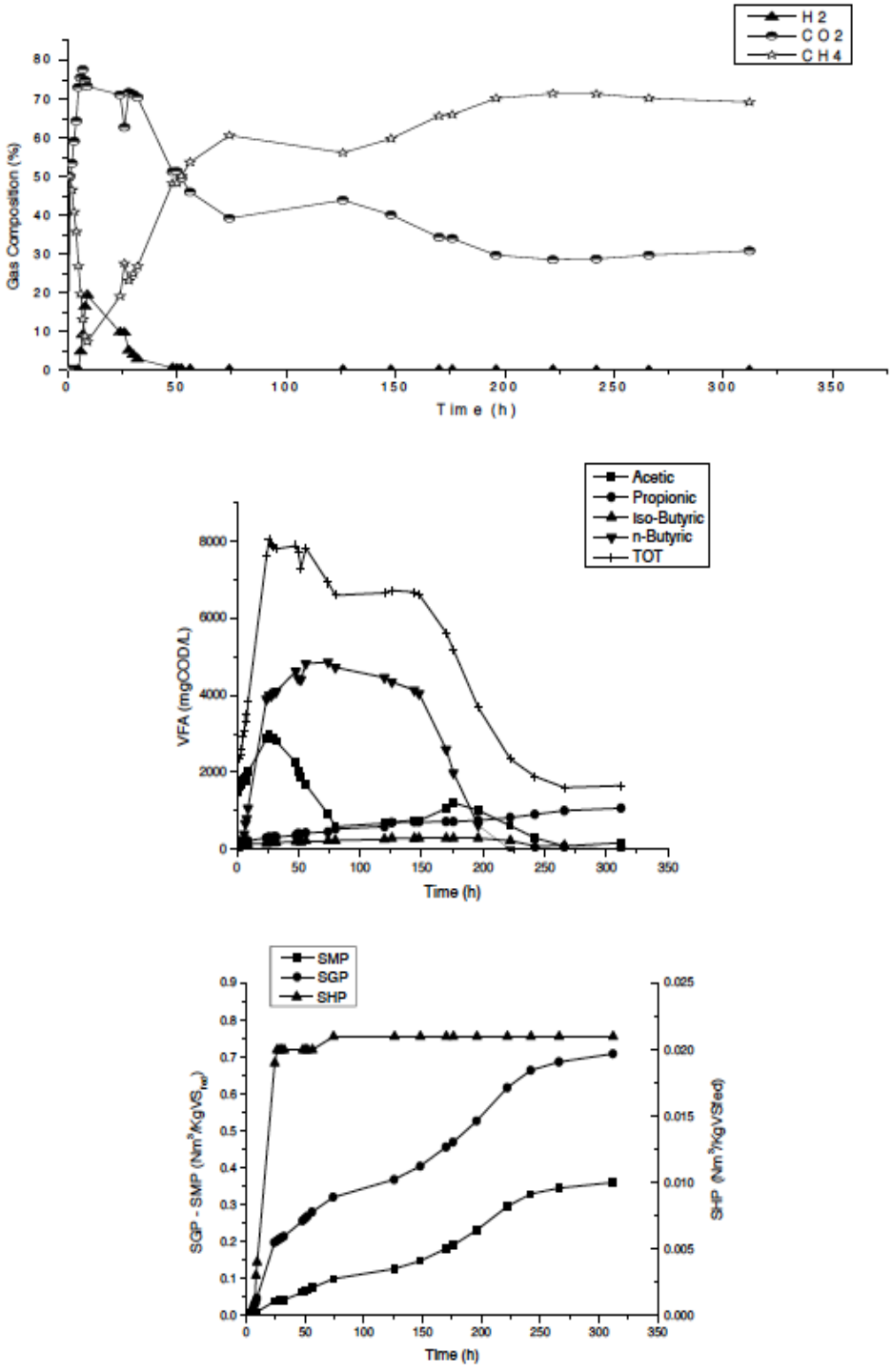
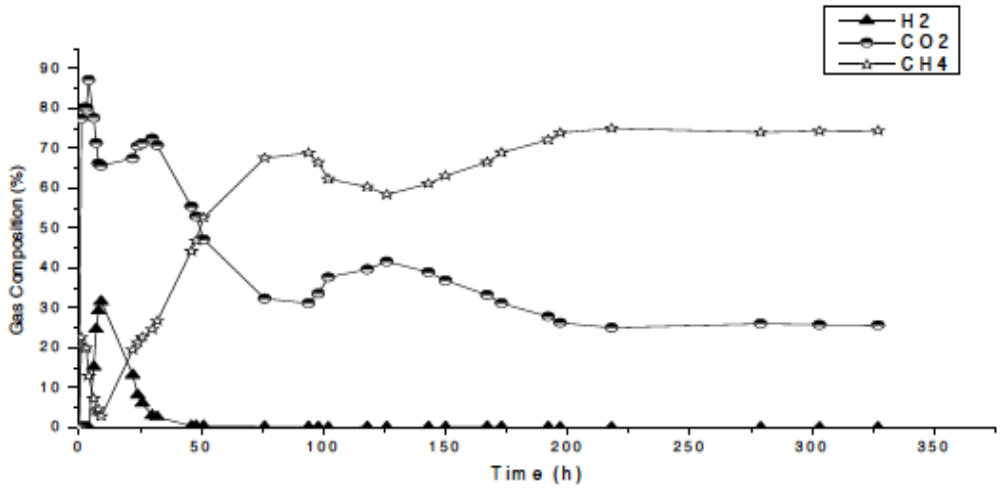
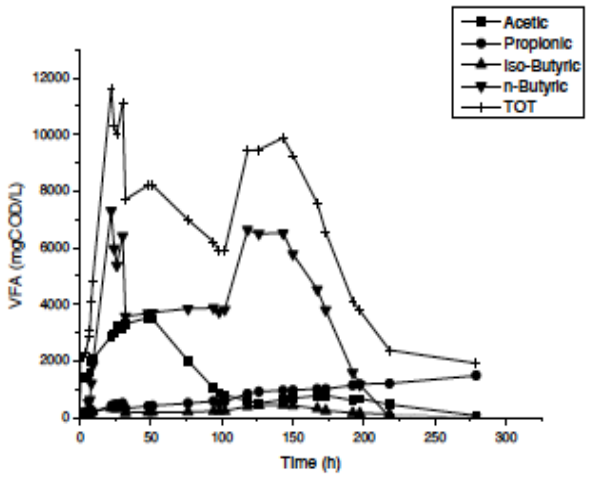


Figure 9. Gas composition, VFA and Specific Gas Production profiles for batch test at initial organic load 20 kg VS m^{-3}

The trends observed at an initial organic load of 25 kg VS m^{-3} were very similar to those at 20 kg VS m^{-3}: CH_4 concentration stabilised at 73.9 % after 192 hours, CH_4 production stopped after 303 hours, total VFA was consumed until 220 hours and then settled at around 2000 mg l^{-1}. The only significant difference was the peak hydrogen concentration which was higher at 31.6%. SHP, SMP and SGP were 0.035, 0.335 and 0.661 Nm^3 kg^{-1} VS respectively.





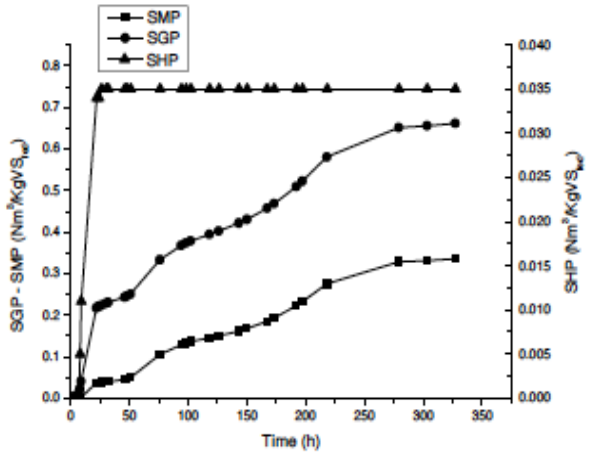


Figure 10. Gas composition, VFA and Specific Gas Production profiles for batch test at initial organic load 25 kg VS m^{-3}

The behaviour at an initial organic load of 30 kg VS m^{-3} was completely different, as at this loading methanogenesis was inhibited. The peak CH_4 concentration of 43.9 % occurred after 2 hours, then decreased and stabilised at average of 9.5 % for the whole test period. VFA concentrations confirmed the loss of methane production as all the acidic species accumulated during the H_2-producing phase within the first 9 hours were not consumed. Total VFA stabilised at an average concentration of 11.6 g COD l^{-1}. Hydrogen production was also different, with an initial peak of 27.9% and production continuing throughout the test at an average concentration of 6.4%. For these reasons the SHP was far higher (0.047 Nm^3 kg^{-1} VS) and SMP and SGP significantly lower (0.021 and 0.239 Nm^3 kg^{-1} VS respectively). The trends reported above are summarized in Table 8.

Table 8. Batch tests summary at the four loads tested

			OLR (KgVS/m^3d)			
			15	20	25	30
Gas Production	Nm^3/KgVSfed	SHP	0.012	0.021	0.035	0.047
		SMP	0.042	0.360	0.335	0.021
		SGP	0.782	0.709	0.661	0.239
H_2 concentration	%	min	0.0	0.0	0.0	3.2
		max	17.7	19.4	31.6	27.9
		stability	0.0	0.0	0.0	6.4± 2.2
CH_4 concentration	%	min	9.3	7.3	2.8	2.1
		max	72.8	71.4	68.9	40.9
		stability	69.9 ± 2.1	70.0± 2.4	73.9± 1.0	9.5± 4.5
CO_2 concentration	%	min	27.2	28.5	25.0	56.0
		max	74.6	77.6	87.1	90.1
		stability	30.1 ± 2.1	30.0± 2.4	26.1± 0.9	84.1± 10.1
Max Net Production	mL/hL	H_2	31.1	38.8	138.3	175.8
		CH_4	53.8	49.5	51.3	27.8
		Biogas	198.2	200.7	437.8	700.5
Hour of peak production	h	H_2	9	9	9	9
		CH_4	126	222	218	1
		Biogas	22	24	9	7

As expected, an increase in the initial organic load led to a progressive decrease in biogas and methane production until at 30 kg VS m^{-3} methanogenesis was completely inhibited. Conversely, more hydrogen is produced at the highest loading rate, while at 15 kg VS m^{-3} its specific production is below typical values found in the literature. This is the expected scenario as high OLRs can enhance the performance of Dark Fermentation for the following reasons. Fermentative bacteria have very high growth rate, and have first-order kinetics so

they do not suffer from substrate inhibition but can hydrolyze all the organic substrate in a few hours. Hydrogen-producing bacteria can rapidly use the solubilised COD to produce hydrogen, having a growth rate far higher than that of the methanogenic archaea. High H_2 concentrations are due to high substrate availability in primary fermentation reactions. The hydrogen production performances of the four OLR are compared in Figure 11.

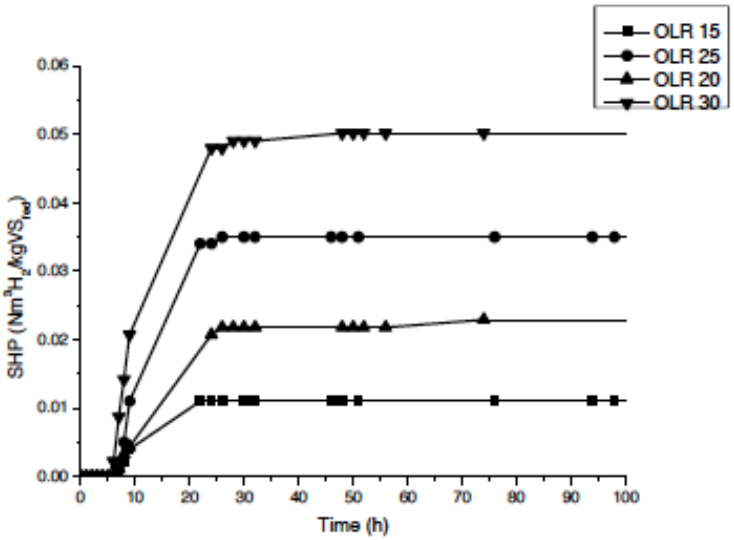


Figure 11. SHP trends during batch tests

4.2 Laboratory-scale two-phase CSTRs trials for biohydrogen production

The two phase process was trialled with recirculation both of the whole digestate from the second reactor and of the liquid supernatant after centrifugation. The results are discussed below.

4.2.1 Biohydrogen production with recirculation of supernatant

At an OLR of 15 kg VS m^{-3} day^{-1}, the two-phase system was not able to maintain an adequate pH in the first phase under the recirculation conditions tested. The pH dropped to 4.30 within the first HRT with a parallel fall in H_2 concentration to zero within the same time. On the other hand in the second phase during the second HRT stable methanogenesis was established: average concentrations of CH_4 and CO_2 were respectively 60.9% and 39.1%, although gas production was quite low with an SGP of 0.305 Nm^3 kg^{-1} VS and an SMP of 0.190 Nm^3 kg^{-1} VS.

In the methanogenic reactor alkalinity settled around an average of 8.9 g $CaCO_3$ l^{-1} and the pH was stable at 7.45. The supernatant recirculated 87.8% of alkalinity from the second phase, but within the conditions tested this was not enough to sustain the pH in phase I, where alkalinity dropped to 1267 mg $CaCO_3$ l^{-1} and stabilised after the first HRT. Total VFA were 3328 and 2780 mg COD l^{-1} in the hydrolytic and methanogenic reactors respectively. An accumulation of propionic acid was however detected in both reactors. Ammonia did not appear to be a problem, with average values of 1867 mg N l^{-1} and 2037 mg N l^{-1} respectively in the first and second phase.

In terms of hydrogen production the results of the trial at OLR 20 kg VS m^{-3} day^{-1} also did not show any positive results: the H_2 concentration fell to zero at the end of the first HRT,

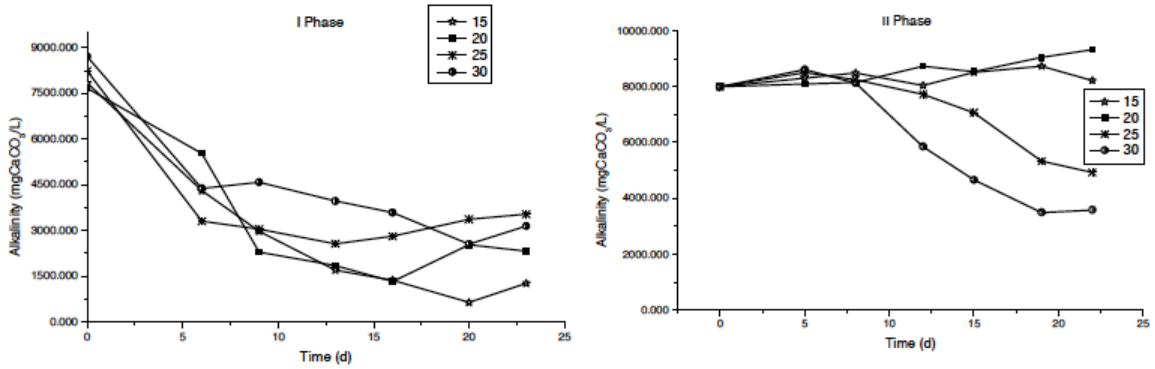
alkalinity settled at 2327 mg $CaCO_3$ l^{-1}, slightly higher than in the previous trial, but the pH was still too low with an average of 4.41 during the second HRT. On the other hand methanogenesis was better. The CH_4 concentration was not high (50.8%) but the SGP was very good at up to 0.819 Nm^3 kg^{-1} VS and with an average of 0.632 Nm^3 kg^{-1} VS during the final week although this was still not stabilised after two HRT. SMP was also not stable, with a maximum of 0.446 Nm^3 kg^{-1} VS and an average of 0.350 Nm^3 kg^{-1} VS, but the trend was strongly positive. Alkalinity was 9332 mg $CaCO_3$ l^{-1} and the supernatant recirculated 87.3% of the second phase alkalinity. As expected an increase in the OLR led to higher total VFA in both phases: 6641 mg COD l^{-1} in phase I and 8335 mg COD l^{-1} in phase II. There was no evidence of ammonia accumulation, with 1820 mg N l^{-1} in phase I and 2197 N l^{-1} in phase II.

At an OLR of 25 kg VS m^{-3} day^{-1} both phases were inhibited: in the hydrolytic reactor there was no hydrogen production and a SGP of 0.002 Nm^3 kg^{-1} VS fed, while in the methanogenic reactor the CH_4 concentration was 6.0% and the SGP 0.003 Nm^3 kg^{-1} VS. The pH was very low in both phases, at 4.63 and 4.89 respectively in the first and second phase. Total VFA in both reactors stabilised during the second HRT at 11.2 (phase I) and 14.1 (phase II) g COD l^{-1}. Alkalinity in the first phase remained stable throughout the trial, with an average of 3532 mg $CaCO_3$ l^{-1}. In the second phase after two HRT alkalinity was not stable but still falling, probably because of VFA accumulation in the absence of methanogenesis. The final value for alkalinity in phase II was 4927 mg $CaCO_3$ l^{-1}. The supernatant recirculated 93.0% of alkalinity from phase II. Ammonia was stable in phase I at 1532 mg N l^{-1} throughout the trial. In phase II ammonia stabilised after one HRT at 1716 mg N l^{-1} as consequence of the drop in pH.

With an OLR of 30 kg VS m^{-3} day^{-1} the system performance was the same as at 25 kg VS m^{-3} day^{-1}. Gas production was insignificant in both phases and the hydrogen and methane concentrations were negligible. In both reactors pH was stable throughout the second HRT at around 4.6. Total VFA was stable during the second HRT with concentrations of 9554 and 12310 mg COD l^{-1} in phase I and II respectively. Alkalinity was very low in both phases (3144 mg $CaCO_3$ l^{-1} phase I; 3585 mg $CaCO_3$ l^{-1} phase II), the supernatant recirculated 92.2% of alkalinity from phase II. The ammonia concentration was also very low at 1393 mg N l^{-1} in phase I and 1579 mg N l^{-1} in phase II).

Trials carried out with supernatant recirculation did not show any positive result in term of hydrogen production. At each OLR tested warning signals for imminent failure were seen, and in some cases the trials were stopped if they had not reached the stationary phase. The two higher loads (25 and 30 kg VS m^{-3} day^{-1}) completely inhibited the processes in both phases. The first two trials (with 15 and 20 kg VS m^{-3} day^{-1}) showed the ability to sustain methanogenesis in the second phase. The conditions applied however were not able to maintain an optimal pH for hydrogen production in the first reactor, where the pH was always less than 4.5. It is clear that alkalinity recirculation from the second phase alone did not lead to adequate pH control.

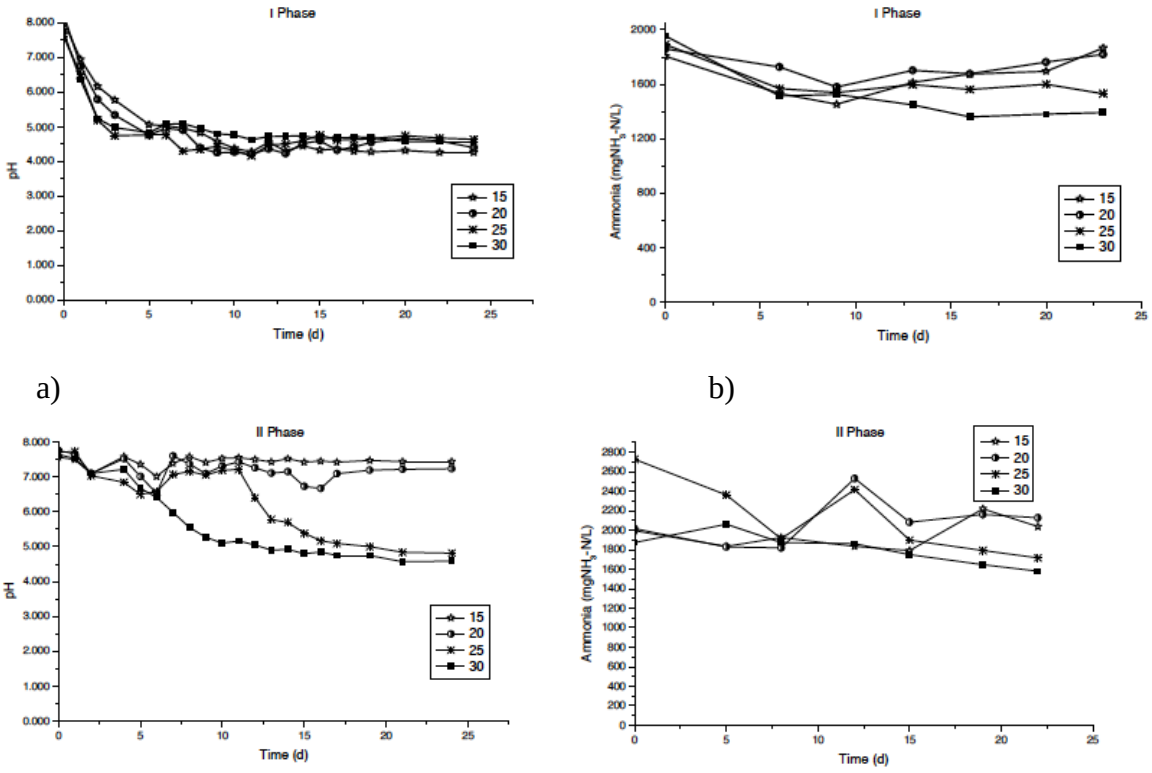
Graphs 12a and b show the trend in alkalinity in both phases for each load tested, and the percentage of alkalinity recirculated from the second phase. The alkalinity recycled through the liquid phase was almost the total alkalinity present in the digestate, indicating that alkalinity by itself is insufficient to sustain DF for hydrogen production in the first phase.



a) b)

Figure 12. a) First phase alkalinity comparison, supernatant recirculation; b) second phase alkalinity comparison, supernatant recirculation.

At the two higher OLRs tested the first phase fell immediately into acidogenic conditions, which quickly led to the failure of methanogenesis. The lower OLRs tested, however, gave a good example of two phase anaerobic digestion for methane production where the first phase provides optimal conditions for hydrolysis and the second for methanogenesis. An organic load of 15 kg VS m^{-3} day^{-1} was too low as the SGP in second phase was very small. An OLR of 20 kg VS m^{-3} day^{-1} led to an optimal two-phase system, as the SGP was very high (above 0.8 Nm^3 kg^{-1} VS although not yet stabilised). Over the duration of the trial no accumulation of ammonia was seen (Figure 13), and the pH in the second phase remained above 7.



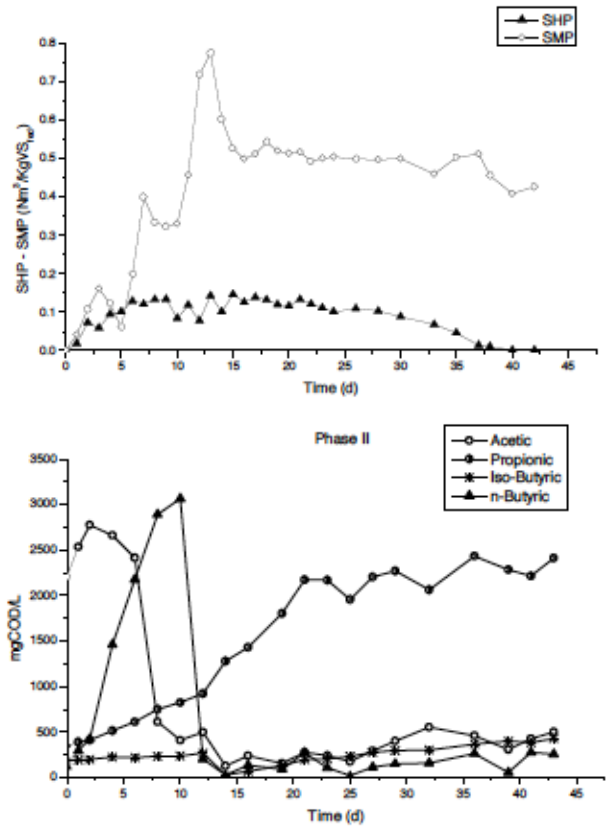
a) b)

c) d)

Figure 13. Supernatant recirculation trial, a) first phase pH; b) first phase ammonia, c) second phase pH; d) second phase ammonia

4.2.2 Biohydrogen production with recirculation of whole digestate

OLR 15 kg VS m^{-3} day^{-1}. In the first phase hydrogen was produced at an average concentration of 45% for slightly longer than the first HRT. Production then started to fall and remained at zero from the start of the fourth HRT. The fall in hydrogen was matched by a rise in methane concentration in the same reactor (24.5% during the final days). SGP was 0.201 Nm^3 kg^{-1} VS and SMP 0.001 Nm^3 kg^{-1} VS. This methanogenic shift was probably due to too light a OLR, insufficient to create a pH capable of inhibiting the H_2-consuming and CH_4-producing archaea. The pH was quite high and stable at 5.82 after three HRT; alkalinity was also high (10,500 mg $CaCO_3$ l^{-1}). VFA appeared to be stabilizing after three HRT, with only propionic acid showing a positive trend. Total VFA however did not show accumulation, and after two HRT was stable at an average of 10.4 g COD l^{-1}. After almost four HRT the ammonia concentration was still increasing, although only slightly during the last week of the trial where the average was 2,910 mg N l^{-1}. On the other hand methanogenesis was more stable and performed well compared to the first phase. The methane concentration stabilised at an average of 65.6%soon after the first HRT. SGP was 0.728 Nm^3 kg^{-1} VS and SMP 0.484 Nm^3 kg^{-1} VS. pH was stable at 7.80, probably due to the large alkalinity reserve (13196 mg $CaCO_3$ l^{-1}). Most VFA were stable at a low concentration (under 500 mg COD l^{-1}), a sign of healthy methanogenic activity. Propionic acid was the only acid showing significant accumulation during the first two HRT, after which it stabilised below 2500 mg COD l^{-1}. Total VFA stabilised at an average of 3850 mg COD l^{-1}. As in phase I, the ammonia concentration was still increasing after almost 4 HRT; during the last week of the trial it was an average of 2,975 mg NH_4^+-N l^{-1}. The total SGP for the whole two-phase system was 0.929 Nm^3 kg^{-1} VS.

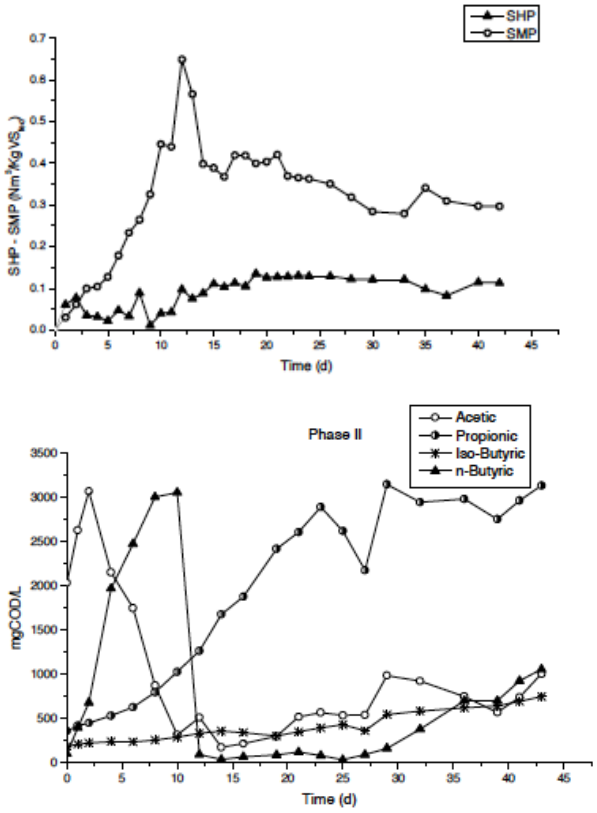


a) b)

Figure 14. OLR 15 kg VS m^{-3} day^{-1}. a) SHP and SMP trends with digestate recirculation; b) acetic, propionic and butyric acid profile, second phase with digestate recirculation

OLR 20 kg VS m^{-3} day^{-1}. The two-phase system fed with an organic load of 20 kg VS m^{-3} day^{-1} showed the best performance. Hydrogen concentration in the first phase was stable at 47.7% from the second HRT. SGP show a small decline for a week during the third HRT, but in the last two HRT it was stable at 0.240 Nm^3 kg^{-1} VS. SHP showed the same trend, with an average during the last two HRT of 0.117 Nm^3 kg^{-1} VS. pH in the first phase was very stable at 5.22, while alkalinity stabilised at 8.5 g $CaCO_3$ l^{-1} after two HRT. As usual in a first phase, total VFA were quite high at 13.8 g COD l^{-1}, but there were no signs of acid accumulation. Ammonia on the other hand showed a slight positive trend, with an average value for the last week of 2.7 g N l^{-1}.

In the second phase a stable methane concentration of 61.2% was established by the end of the first HRT. The SGP was quite good at 0.512 Nm^3 kg^{-1} VS. SMP was 0.311 Nm^3 kg^{-1} VS. pH was stable at 7.69 throughout the trial; alkalinity stabilised at 14090 mg $CaCO_3$ l^{-1} during the last week. The concentrations of acetic and butyric acids were low and fairly stable, while propionic acid showed strong accumulation during the first two HRT. Total VFA were still accumulating slightly, with an average for the last two weeks of 7064 mg COD l^{-1}. Ammonia again showed a positive trend: the average for the last week was 3295 mg N l^{-1}. The total SGP for the two-phase system was 0.752 Nm^3 kg^{-1} VS.

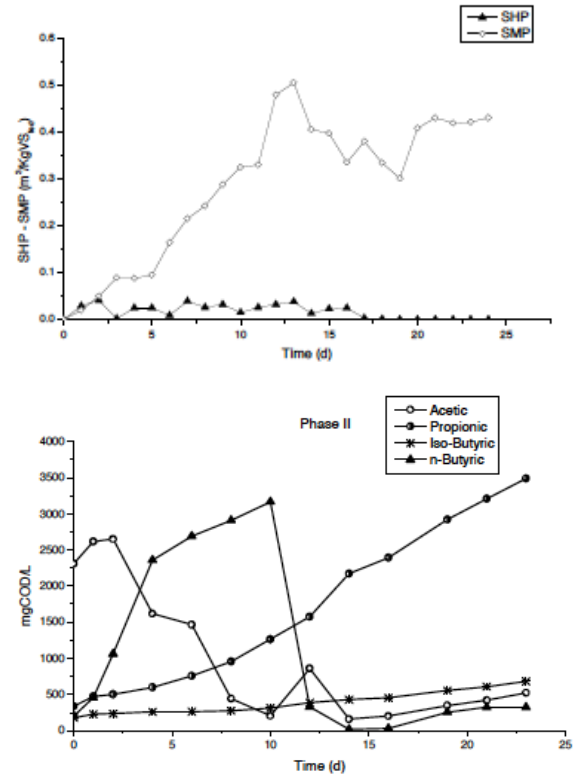


a) b)

Figure 15. a) SHP and SMP trends with digestate recirculation, at OLR 20 kg VS m^{-3} day^{-1}; b) acetic, propionic and butyric acid profile, second phase with digestate recirculation, at OLR 20 kg VS m^{-3} day^{-1}.

OLR 25 kg VS m^{-3} day^{-1}. With an intermediate OLR of 25 kg VS m^{-3} day^{-1} hydrogen production again fell after two weeks. At the end of the second HRT the stable H_2-concentration was 0.4%, the SGP was very low at 0.009 Nm^3 kg^{-1} VS and hence SHP was negligible. In the first phase pH was stable but low (4.63), alkalinity was insufficient (3554 mg $CaCO_3$ l^{-1}) and the principal VFA settled at average concentrations of under 3000 mg COD l^{-1}. Probably due to the low pH, ammonia was quite stable at an average of 1893 mg NH_4^+-N l^{-1}.

In the second phase some signs of failure to stabilise were detected; since hydrogen production in first phase had failed, however, the whole two-phase system was stopped. After two HRT methanogenesis was performing quite well. The methane concentration was 65.1%, SGP 0.619 Nm^3 kg^{-1} VS, SMP 0.422 Nm^3 kg^{-1} VS and pH was stable at 7.55. Alkalinity was high at 11803 mg $CaCO_3$ l^{-1}. There was no accumulation of the principal VFAs, the only exception being propionic acid which was still increasing when the trial was stopped. Ammonia also showed an accumulating trend; the final value was 2,741 mg N l^{-1}. The total SGP for the whole two-phase system was 0.628 Nm^3 kg^{-1} VS.



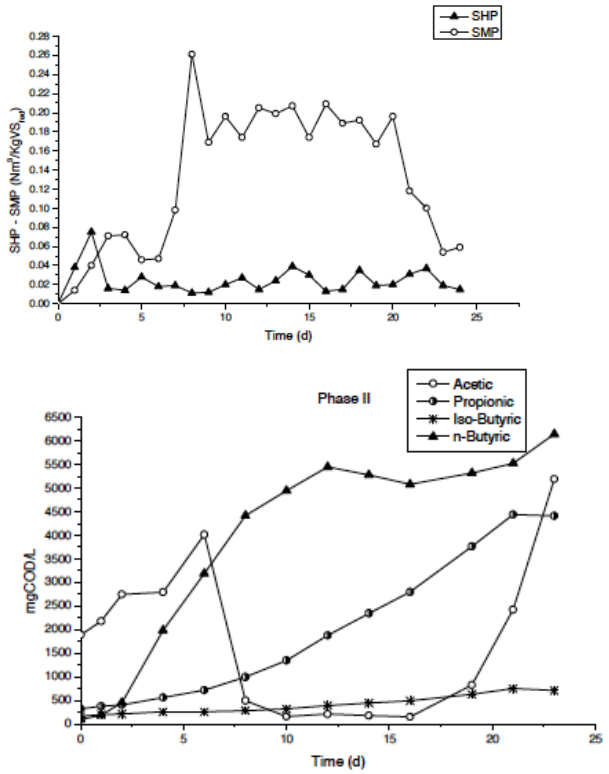
a) b)

Figure 16. a) SHP and SMP trends with digestate recirculation, at OLR 25 kg VS m^{-3} day^{-1}; b) acetic, propionic and butyric acid profile, second phase with digestate recirculation, at OLR 25 kg VS m^{-3} day^{-1}

OLR 30 kg VS m^{-3} day^{-1}. In contrast to the previous load tested, an OLR of 30 kg VS m^{-3} day^{-1} gave a stable first phase. After two HRT, however, the second phase was about to fail. For this reason the experiment was stopped although it had not reached steady state. Hydrogen concentration averaged 42.8% but production was low with an SGP of 0.053 Nm^3 kg^{-1} VS and SHP 0.022 Nm^3 kg^{-1} VS. pH was stable at 5.03, while alkalinity and VFA were also

stable at around 6.15 g $CaCO_3$ l^{-1} and 12.8 g COD l^{-1} respectively. Ammonia showed no show accumulation, with an average concentration of 1.7 g N l^{-1}.

As mentioned above the second phase showed signals of imminent failure: a low and decreasing CH_4 concentration (37.3%); SGP very low (0.210 Nm^3 kg^{-1} VS) and SMP also low (0.077 Nm^3 kg^{-1} VS). pH was falling, with a final value of 5.72, while alkalinity averaged around 10.9 g $CaCO_3$ l^{-1}. Total VFA were increasing and the main VFA species were all accumulating. Ammonia also showed a positive trend, with an average of 1708 mg N l^{-1} in the last week. The total SGP for the whole two-phase system was 0.263 Nm^3 kg^{-1} VS.



a) b)

Figure 17. a) SHP and SMP trends with digestate recirculation, at OLR 30 kg VS m^{-3} day^{-1}; b) acetic, propionic and butyric acid profile, second phase with digestate recirculation, at OLR 30 kg VS m^{-3} day^{-1}

Figure 18 shows the performance of the four OLR in terms of SHP and SMP. It is clear that considering biohydrogen production as the objective of the study, the two-phase system with an organic load of 20 kg VS m^{-3} day^{-1} is the only one that is worth testing at pilot scale.

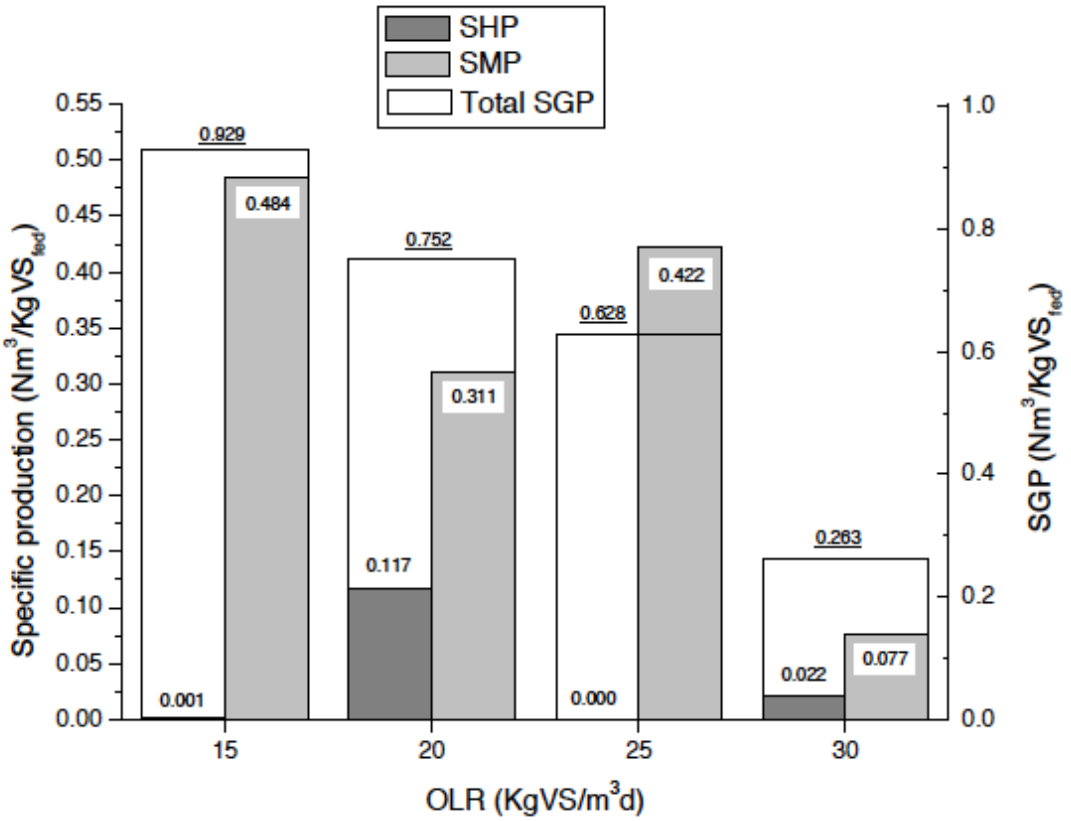


Figure 18. Specific Gas Productions for all the OLR tested with digestate recirculation

Within the recirculation conditions applied, an OLR of 15 kg VS m^{-3} day^{-1} is too light to control the pH at the optimum of 5.5 for Dark Fermentation. The digestate recirculated from the first phase carries too much alkalinity to the hydrolytic reactor. This makes the pH rise so that it cannot inhibit the fresh biomass from the second phase, which is rich in H_2-consuming and CH_4-producing archaea. This leads to the methanogenic shift that has been reported. Methane production in the second phase is very good, as result of no VFA accumulation occurring because of the light load.

The OLR of 20 kg VS m^{-3} day^{-1} seems a perfect compromise between the accumulation of VFA that are produced during the early stage of DF and the alkalinity recirculated through the digestate. The correct pH allows hydrogen production in the first phase, together with solubilisation of organic compounds. The second phase is characterised by a low SMP.

The next OLR of 25 kg VS m^{-3} day^{-1} proved too heavy for the system tested. As happened with supernatant recirculation, the system behaves like a two-phase digester for methane production. In the hydrolytic reactor the alkalinity recirculated is not sufficient to maintain the pH, which is reduced by acid accumulation. As result, only solubilisation of organic compounds occurs in the first phase, while in the second phase methanogenesis shows better performance as the COD arrives already solubilised and ready-to-use. The final OLR of 30 kg VS m^{-3} day^{-1} showed that a further increase in the organic load gives a general inhibition of both the phases.

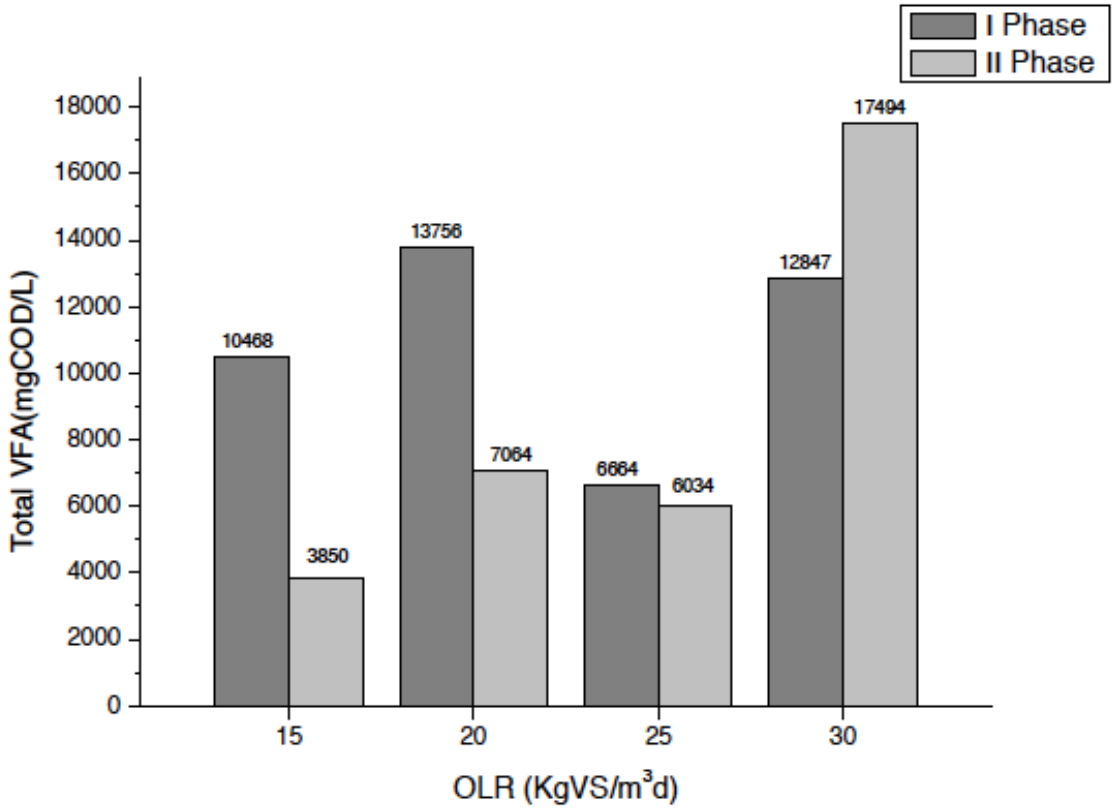


Figure 19. Total VFA for all the OLR tested with digestate recirculation

With an OLR of 15 kg VS m^{-3} day^{-1} VFA are accumulated in the first phase and consumed in the second phase. This shows that the two-phase system works properly since the solubilised organic carbon is only used in the second phase for methane production. The situation is similar when an OLR of 20 kg VS m^{-3} day^{-1} is applied. COD is higher in both the first and the second phase probably because of the increased load and the lower efficiency of methanogenesis reported.

At an OLR of 25 kg VS m^{-3} day^{-1} the biomass in the second phase is not able to consume all the VFA. A large amount of acids is hence recycled to the first phase. This creates a loop between the first and the second phase that explains the similar VFA concentrations in the two phases.

In the last case, with an OLR of 30 kg VS m^{-3} day^{-1}, the situation is worse than in the previous OLR because the second phase is completely inhibited and recirculates non-active biomass with a high concentration of acids. This case shows what happens in an inhibited system: VFA are produced during the hydrolytic phase (not inhibited by high loads) and they are increased by a recirculum full of acids. VFA hence accumulate in the second phase when the high acidic concentration itself inhibits the conversion of acids into methane.

As shown in Figure 20, ammonia was clearly accumulated at all the OLR tested and this will eventually affect the performance of the process. For operation over longer durations measures to control the ammonia concentration will therefore need to be adopted.

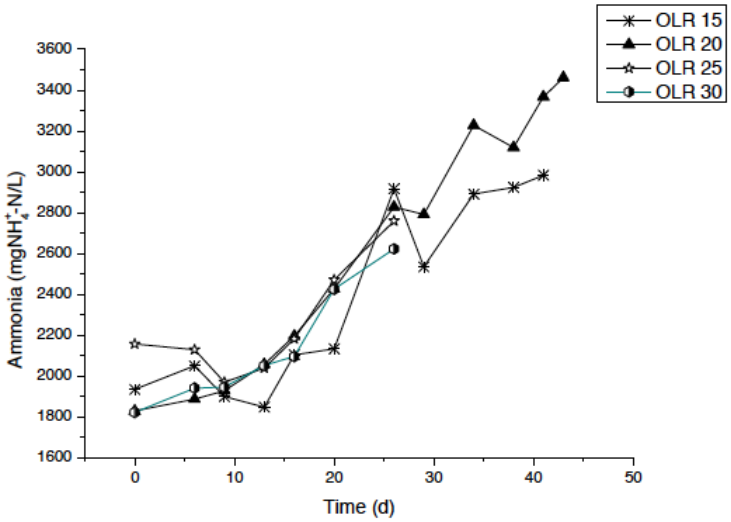


Figure 20. Ammonia accumulation during the second phase with digestate recirculation

4.3 Pilot-scale two phase CSTRs trial for biohydrogen production

This section presents the results of trials on a pilot-scale thermophilic two phase anaerobic digestion process for hydrogen and methane production. The experimental work was divided into three periods as described above, and similar conditions were applied. During Run I and II the source segregated biowaste was mixed only with water in order to obtain the two different HRT applied, without any pre-treatment for selection of the microbial community. It was trialled at an OLR of about 20 kg VS m^{-3} day^{-1} based on the laboratory-scale results previously obtained. Another aspect tested before adopting the recirculation of digestate was evaluation of no support for the alkalinity in the DF process, but changing the HRT of the first phase reactor. The HRT of the first phase in Run I was 6.6 days, while in Run II it was decreased to 3.3 days in order to avoid the solventogenic shift. As a result of the very low pH values reached during the first two Runs, in the third and last period the feasibility of digestate recirculation was investigated in order to maintain the pH values in the correct range (between 5 and 6).

4.3.1 Run I

During Run I untreated biowaste was fed to the first reactor. This operated with a HRT of 6 days and an OLR of 21 kg VS m^{-3} day^{-1}. These conditions were maintained for 85 days. As mentioned above pH is an important parameter in the biohydrogen generation process. Applying these conditions, without using an adapted inoculum or pre-treatment, the pH fell to 3.7 during the start-up phase and then rose to 4.3 as the system approached steady state conditions.

This range of values is clearly too low for the proper operation of the hydrogenase enzyme. The low pH value could be explained by the high VFA production which reached a maximum of about 15 g COD l^{-1} and then stabilised at 8.3 g COD l^{-1}. The VFA was mainly composed of acetic acid (6.5 g COD l^{-1}), while small amounts of propionic and butyric acids (< 1 gCOD l^{-1}) were also detected. Considering the low pH and the pKa (3.8) of lactic acid, one can speculate that lactic acid also accumulated in the system. It has already been shown by the authors that in a CSTR fed with market waste at a HRT of 6 days and an OLR of 35 kg

VS m^{-3} day^{-1}, 43% of total COD was converted into soluble organic compounds, of which 41% was lactate (Traverso et al., 2000, Bolzonella et al., 2005). The high concentrations of soluble COD and ammonia (75.4 g COD l^{-1} and 528 mg N l^{-1}, respectively) observed in this study could confirm this inhibition. Despite the high VFA content, the anaerobic reactor (second stage) was able to convert the acetic acid into methane and CO_2, without any stability problems. Table 9 summarises the average effluent characteristics and yields. As the system approached steady state conditions pH reached a constant value of 7.6, while the average total alkalinity was 10.6 g $CaCO_3$ l^{-1} with a slightly increasing trend. The VFA concentration in this reactor (211 mg COD l^{-1} on average) confirmed its ability to convert VFAs into biogas: the SGP was similar to the highest values reported in the literature this type of biowaste (Bolzonella et al., 2006). The ammonia concentration reached 2,016 mg N l^{-1} without apparently affecting the methanogenic activity.

Table 9. Characterisation of reactor effluents and yields of the process

parameter	unit	I	II	III a	III b
Characterisation of the first phase reactor					
TS	g kg^{-1} WW	168±15	78±5	60±5	73±1
TVS	g kg^{-1} WW	138±11	67±4	48±5	59±2
TVS,TS	%	82±1	86±1	81±3	80±2
COD	gCOD kg^{-1}WW	146±18	67±2	40±8	50±1
TKN	gN kg^{-1} WW	5.0±0.2	2.1±0.4	2.0±0.1	2.3±0.1
PTOT	gP kg^{-1} WW	0.72±0.03	0.25±0.04	2.62±0.77	4.04±0.41
pH		4.3±0.2	3.5±0.1	5.4±0.1	5.4±0.1
NH_3	mg N l^{-1}	528±50	152±14	706±169	948±145
VFA	g COD l^{-1}	8.33±0.86	2.92±0.55	13.87±1.67	7.05±0.34
Characterisation of the second phase reactor					
TS	g kg^{-1} WW	77±4	29±4	24±1	30±3
TVS	g kg^{-1} WW	58±4	21±4	16±1	19±2
TVS,TS	%	75±2	69±4	66±1	64±1
COD	gCOD kg^{-1} WW	49±1	23±4	12±3	16±1
TKN	gN kg^{-1} WW	2.4±0.1	1.0±0.1	0.8±0.1	0.8±0.2
PTOT	gP kg^{-1} WW	0.47±0.12	0.20±0.06	0.13±0.06	0.20±0.04
pH		7.6±0.1	8.1±0.1	8.25±0.12	8.24±0.19
NH_3	mg N l^{-1}	2,016±175	1079±57	997±188	1470±166
VFA	mg COD l^{-1}	211±95	642±142	90±109	604±122
ALKALINITY pH4	mg $CaCO_3$ l^{-1}	10,582±842	5324±154	5173±674	7100±416
ALKALINITY pH6	mg $CaCO_3$ l^{-1}	5066±489	2,737±159	3160±374	4024±366
First phase reactor yields					
GP	l day^{-1}	53±9	15±3	452±110	244±35
VGP	m^3 m^{-3} day^{-1}	0.27±0.03	0.16±0.03	2.26±11.81	1.22±0.17
SGP	l kg^{-1} VS	13.8±2.4	7.4±1.8	136.8±35.3	59.9±6.7
H_2	%	19±1	34±3	37±8	34±3
SHP	l kg^{-1} VS	2.7±0.5	2.6±0.6	51.2±11.8	20.4±3.4
Second phase reactor yields					
GP	m^3 day^{-1}	2.3±0.1	1.3±0.2	1.0±0.1	1.3±0.2
VGP	m^3 m^{-3} day^{-1}	6.0±0.2	3.4±0.4	2.7±0.3	3.3±0.6
SGP	m^3 kg^{-1} VS	0.58±0.07	0.62±0.11	0.64±0.09	0.63±0.12
CH_4	%	65±3	60±,1	65±2	65±2

In terms of yields, biohydrogen in the first reactor was 20%, below typical literature values in the range of 35-40% (Liu et al., 2006; Zhu et al., 2007; Valdez-Vazquez et al., 2005; Li et al., 2008). This low value together with the low specific gas production of 13.8 l kg^{-1} VS gave a specific hydrogen production (SHP) of 2.7 l H_2 kg^{-1} VS and a volumetric gas production (VGP) of 0.3 m^3 m^{-3} day^{-1}. A similar value (< 5 l H_2 kg^{-1} VS) was found by Kataoka et al. (2005) in a bench-scale trial using conditions similar to those applied in Run I. The SGP of the anaerobic digestion process was 0.58 m^3 kg^{-1} VS, with a VGP of 6.0 m^3 m^{-3} day^{-1} and methane content of 65%. These results can be compared to those obtained by Pavan et al. (2000) treating the source selected organic fraction of municipal solid waste in a two-phase system with an overall HRT of 12 days, which achieved a SGP around 0.6 m^3 kg^{-1} VS.

4.3.2 Run II

During Run II, the organic loading rate of 21 kg VS m^{-3} day^{-1} was maintained in the first reactor but the HRT was decreased from 6.6 to 3.3 days by adding water (about 45 l) The whole period length was of 32 days, and the system approached stable conditions after 20 days of operation. As mentioned in the introduction, it has been suggested that lower HRT avoid the shift to ethanol and acetic lactic production, and permit the hydrogen-producing bacteria to convert the organic matter into hydrogen and acetic and butyric acids (Valdez-Vazquez et al., 2009; Shin et al., 2005; Gomez et al., 2006). The pH value during this second run dropped from 4.0 (Run I) to a constant value of 3.5, still too low for normal activity of the hydrogenase enzyme. The VFA concentration decreased from 8,830 mg COD l^{-1} in Run I to about 3000 mg COD l^{-1} in Run II (Table 9). The ammonia value decreased to 152 mg N l^{-1}.

The methanogenic reactor was operated at the same HRT but at a lower OLR compared to Run I. Despite the very low pH of first reactor the anaerobic digestion process was stable and reliable. Since the organic loading rate was reduced, the concentrations of stability parameters decreased accordingly: ammonia was about 1079 mg N l^{-1} while total alkalinity reached an average of 5.3 g CaCO3 l^{-1}. Only VFA concentration increased, to 0.64 g COD l^{-1}.

Notwithstanding the change in the HRT, biohydrogen yields in the first reactor remained constant with a SHP of 2.6 l H_2 kg^{-1} VS. The hydrogen content in the biogas increased from 20 to 35%. The overall SGP decreased from 13.8 to 7.4 l H_2 kg^{-1} VS, and the VGP from 0.3 to 0.16 m^3 m^{-3} day^{-1}.

4.3.3 Run III

In order to keep the pH above 5, in the third experimental run it was decided to recycle part of the second phase effluent to the first reactor after screw press separation. The alkalinity of this stream allowed for buffering of the first phase reactor. The recirculation ratio was set to 1, as suggested in Lee et al. (2010). The OLRs applied during Runs III-a and III-b were 16 and 21 kg VS m^{-3} day^{-1}, respectively, for first phase reactor, and 4.2 and 5.6 kg VS m^{-3} day^{-1}, respectively, for the second phase reactor. The HRTs were the same applied in previous experimental runs (3.3 and 12.6 days). Chu et al. (2008) and Lee et al. (2010) applied even lower HRT to anaerobic digestion reactors (7.7 and 5 days) as consequence of the high loading rate applied to the first phase and the solubilisation of the particulate organic matter in that reactor. In these conditions they obtain a good substrate conversion to biogas.

The stability parameters and macronutrient concentrations for both reactors during Run III-a and Run III-b are shown in Table 9.

In both the tested conditions pH was maintained in the optimal range for hydrogen production, at around 5.4. The pH of the second phase was about 8.2 in both periods, while the VFA content in Run III-a was 90 mg COD l^{-1} and in Run III-b 604 mg COD l^{-1}, corresponding to a reduction of >95%.

In terms of hydrogen yields, it was clear that with the lower OLR the yields of the first phase reactor were higher than those obtained at higher OLR. In fact, when applying an OLR of 16 kg TVS m^{-3} day^{-1}, the specific gas production was 136 l kg^{-1} VS, with a H_2 content of 35% and a specific hydrogen production of 51 l H_2 kg^{-1} VS; increasing the OLR to 21 kg VS m^{-3} day^{-1} reduced the specific gas production to 59.8 l kg^{-1} VS while the H_2 concentration was the same and SHP decreased to 20.4 l H_2 kg^{-1} VS.

Considering the second phase reactor (OLR 4.2 kg VS m^{-3} day^{-1}), the VGP, SGP and CH_4% in Run III-a were 2.7 m^3 m^{-3} day^{-1}, 0.64 m^3 kg^{-1} VS and 65%, respectively. When an OLR of 5.6 kg VS m^{-3} day^{-1} was applied (Run III-b), the VGP, SGP and gas composition were 3.3 m^3 m^{-3} day^{-1}, 0.63 m^3 kg^{-1} TVS and 65%, respectively.

With reference to the first phase reactor, it should be noted that the reduced hydrogen production observed for higher OLRs was also confirmed by Wang et al. (2009). These researchers studied the treatment of unsterilised food waste as a source for hydrogen and subsequent methane production, where the indigenous food waste microflora was used as inoculum. In this work they observed that at lower OLR (15 kg VS m^{-3} day^{-1}), the dominant hydrogen fermentation pathway was acetic acid and butyric acids pathway, and the hydrogen yield was did not vary significantly. On the other hand, at higher OLR (37 kg VS m^{-3} day^{-1}), they found a decrease in the hydrolysis rate of substrate and a correspondent increase in propionic and lactic acids, which led to a decrease in hydrogen yield when the system was operated at the highest OLR (Wang et al., 2009).

The same behaviour was also seen in this experimentation. In Figure 21 the short chain VFA concentrations along the experimental runs are shown. The shape of the curve confirmed the better conversion of organic matter into acetic and butyric acids in the first period, while at higher OLR the acetic and butyric acid concentrations decreased, as did H_2 production.

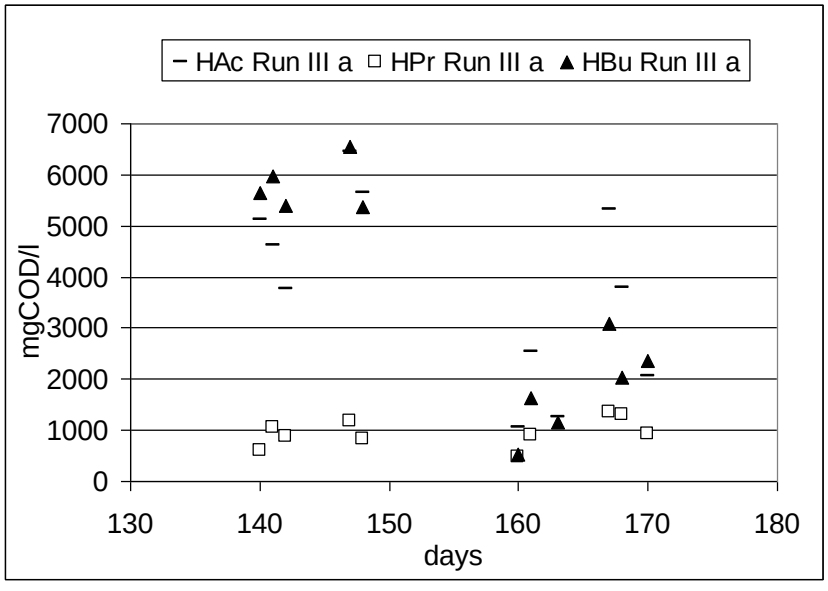


Figure 21. Short chain VFA comparison during Run III.

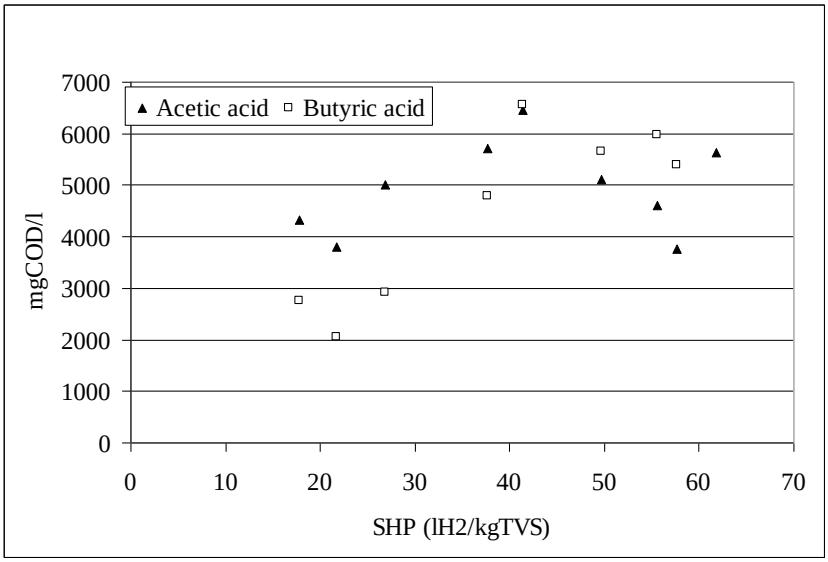


Figure 22. Relation between VFA and SHP during Run III.

Higher hydrogen yields were associated with higher VFA concentrations. At high SHP values, the VFA concentration ranged between 5 and 6 g COD l^{-1}, with a slight predominance of butyric acid.
Even though the best HAc/HBu ratio for H_2 production is not clear, still the predominance of butyric acid could be associated to the combination of metabolic reactions, as shown in Eq.1:

$$4C_6H_{12}O_6 + 2H_2O \rightarrow 3CH_3CH_2CH_2COOH + 2CH_3COOH + 8CO_2 + 10H_2 \qquad \text{Eq. 1}$$

In Figure 23 the relation between specific hydrogen production and OLR is are plotted. The general trend of the experimental results showed a better performance at OLR < 18 kg VS m^{-3} day^{-1}, with a maximum yield at the lower loading applied.

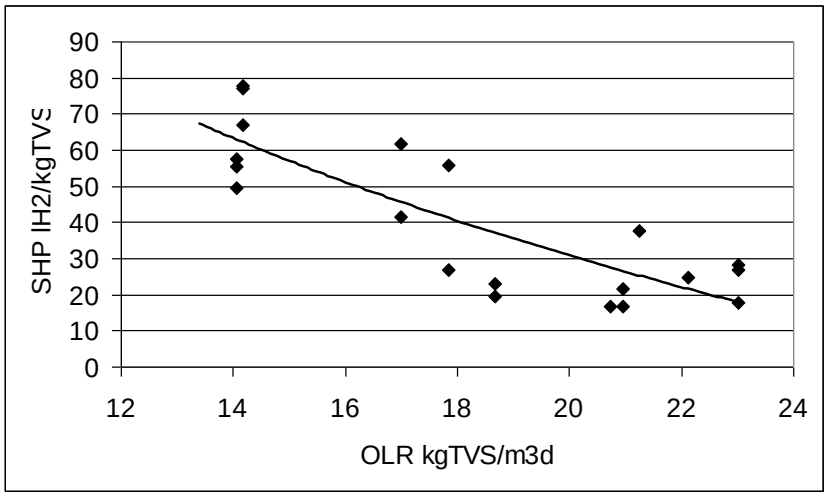


Figure 23. SHP related to the OLR.

The mass balance around the system in Run III-a, the period that gave the best yields, is presented in Figure 24. All the mass balances for TS, TVS and COD, showed an error lower than 10%. This minimal error could be associated with sampling errors or analytical procedures.

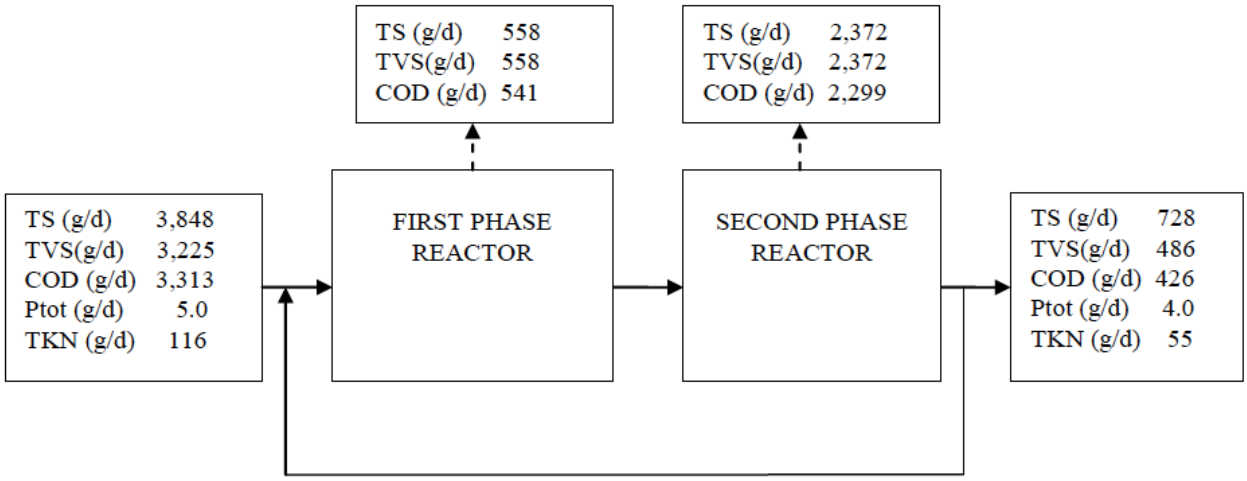


Figure 24. Mass balance of Run IIIa

The mass balance highlighted a loss of nitrogen. This could be explained by the recirculation of the digestate; this causes an increase in ammonia concentration both in the first phase (from 200 to 1200 mg N l^{-1}) and the second phase (from 800 to 1600 mg N l^{-1}). To take this accumulation into account, a regression calculation was made in order to quantify the rate of increase in ammonia (Figure 25).

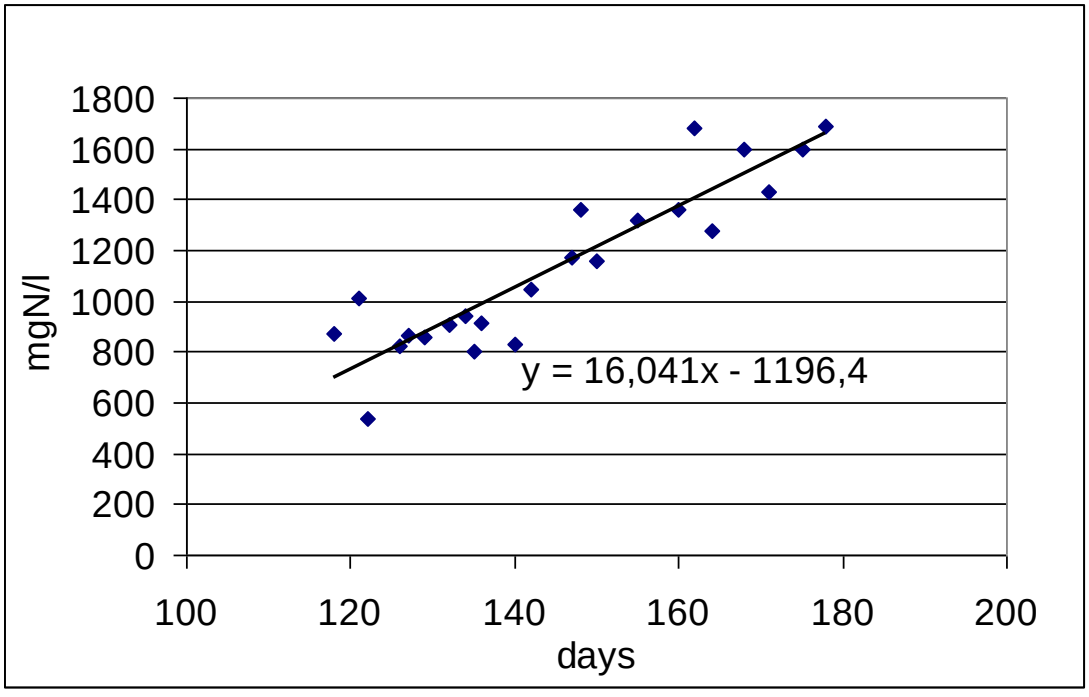


Figure 25. Rate of ammonia accumulation in second phase reactor Run III

Ammonia accumulation was 16.04 mg N l^{-1} day^{-1}, so it was decided to remove part of the recirculation each day to maintain the ammonia concentration at a constant level in the second reactor in order to prevent inhibition problems.

4.4 Pilot-scale two phase longer-term process evaluation

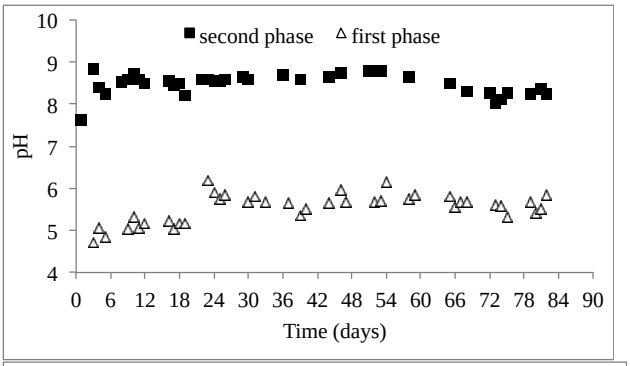
Based on the results obtained in the previous trials, a trial using the best conditions found was carried out at pilot scale over a period of 90 days: the start-up phase was from day 0 to 49, followed by stable conditions (SSC) for about 40 days equivalent to 13 HRTs for the dark fermentation and 3 HRTs for the anaerobic digestion process. The whole HRT of the process, without the recirculated material, was about 30 days.

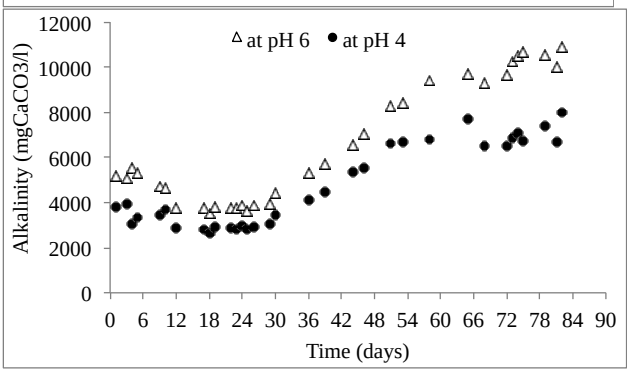
The first phase was inoculated with food waste and tap water. This allowed an initial solubilisation of the organic material followed by a pH drop to about 4, with consequent inhibition of hydrogen-producing bacteria. The strategy used to avoid this pH drop during the experiment was to recycle the liquid phase of the digestate, after a mild solid/liquid separation: the solids content of the recirculated material was about 25 g TS l^{-1} and mainly shells and other inert items removed. This was done to support the alkalinity in the fermentative step and keep the pH at about 5.5-6.0. The pH, high OLR and low HRT applied, allowed the selection of hydrogen-producing bacteria: after the first ten days, the hydrogen content of the biogas in the first reactor reached 20%, increasing to 50% after about 20 days.

Figure 26 shows the pH, partial and total alkalinity, and gas production for the dark fermentation and anaerobic digestion processes. The pH profile confirmed the stability of the process, although this parameter provides only a delayed indication of potential process failure. Dark fermentation pH was always in the optimal range of the hydrogenase enzyme with an average value of 5.7±0.3. The anaerobic digestion phase reached stable conditions after about 50 days, showing an average total alkalinity of 9806 mg $CaCO_3$ l^{-1}, average pH 8.4 and a total VFA content of 1107 mg COD l^{-1}. The gas yields were comparable with those found in the literature: biogas production was 0.17 and 0.72 m^3 kg^{-1} VS for the first and second phase respectively, while the average H_2 and CH_4 concentrations were 39% in dark fermentation and 67% in anaerobic digestion. Table 10 gives the average values and standard deviation for each parameter monitored during SSC conditions.

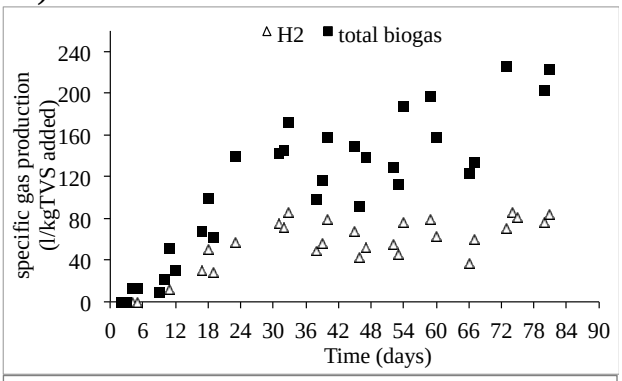
Table 10. Characterisation of first and second phase effluent and gas yields.

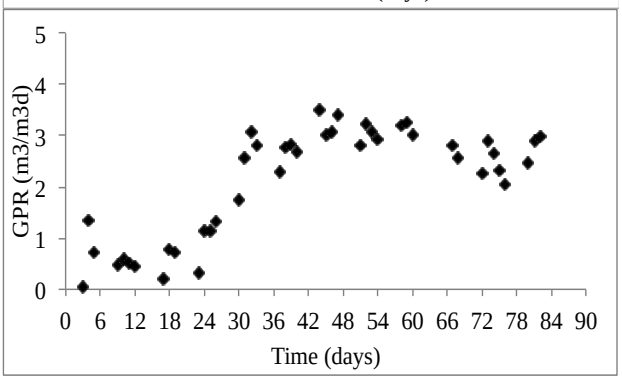
Parameters	Units	first phase	second phase
TS	g kg^{-1} WW	53.6±7.5	26.0±0.4
TVS	g kg^{-1} WW	42.2±6.9	17.8±0.6
COD	g O_2 kg^{-1} WW	52.4±10.2	22.2±1.2
TKN	g kg^{-1} WW	1.8±0.5	1.1±0.1
P_{TOT}	g kg^{-1} WW	0.48±0.08	0.2±0.01
pH	-	5.7±0.3	8.4±0.2
VFA	g COD l^{-1}	11.70±3.16	1.11±1.11
H-Ac	g COD l^{-1}	3.29±1.64	-
H-Bu	g COD l^{-1}	4.31±1.47	-
Alkalinity (pH=4)	mg $CaCO_3$ l^{-1}	-	9806±1114
Alkalinity (pH=6)	mg $CaCO_3$ l^{-1}	-	6957±600
Gas yields			
SGP	m^3 kg^{-1} VS	0.17±0.1	0.72±0.1
H_2	%	38.5±9.7	-
CH_4	%	4.5±7.1	67±3.7
SHP	l kg^{-1} VS	66.7±14.66	-
VGP	m^3 m^{-3} day^{-1}	2.6±0.5	2.7±0.5





a) b)





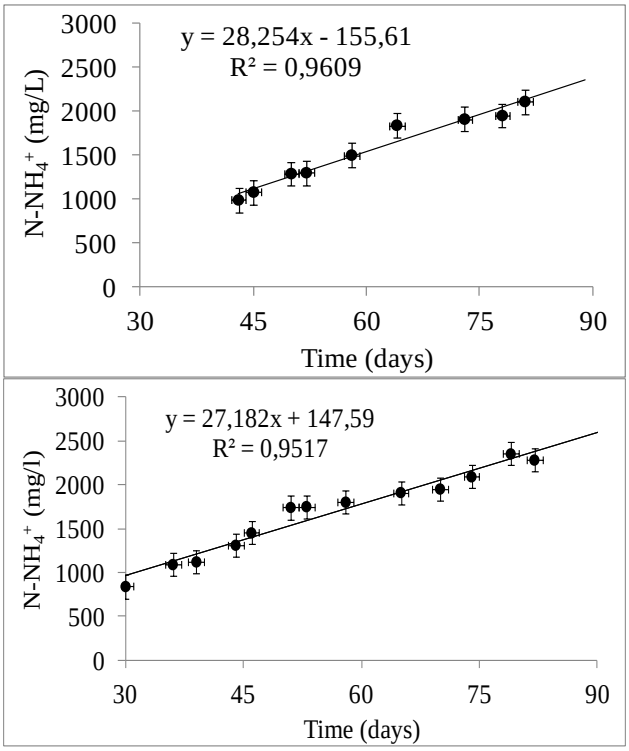
c) d)

Figure 26. a) pH profile of both reactors; b) alkalinity concentration in anaerobic digestion reactor; c) total gas and hydrogen specific gas production in the first phase; d) gas production rate in second phase reactor.

4.4.1 Ammonia inhibition

One of the main aspects that compromised the stability of the two-phase approach with digestate recirculation was the accumulation of ammonia in the system. In fact, the digestate

recirculation causes a continuous flow of ammonia back to the first phase, with a consequent increase in this compound with time. It is well known that high ammonia concentrations inhibit the performance of anaerobic microflora for both hydrogen and methane production: this is due to the decompositions of proteins contained in food waste during the anaerobic digestion (Jokela et al., 2003). In this experiment it was possible to quantify the degradation considering total nitrogen to ammonia conversion: 7% and 39% of total nitrogen was converted into ammonia nitrogen in the first and second phase, respectively. At steady state the ammonia accumulation rate was about 28 mg $N\text{-}NH_4^+$ l^{-1} per day for both reactors, as illustrated in Figure 27a and 27b.

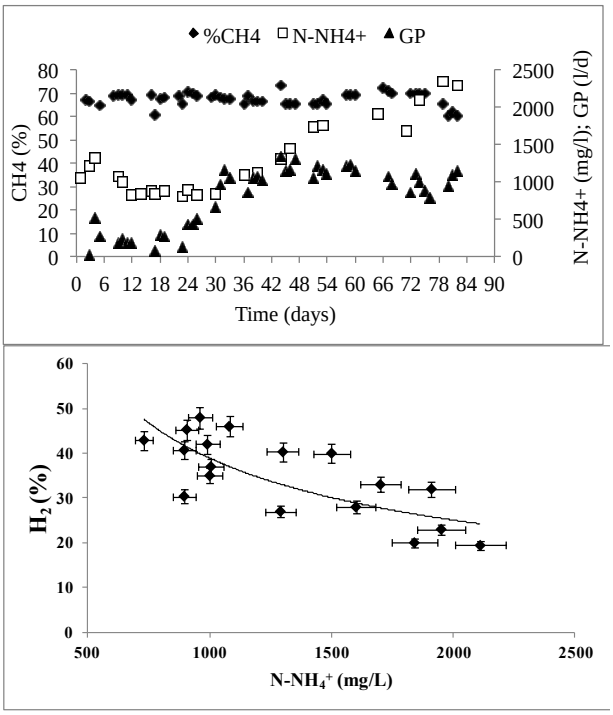


a) b)

Figure 27. Ammonia accumulation rate a) in dark fermentation reactor; b) in anaerobic reactor.

After about 70 days of operation the ammonia concentration reached 2 g $N\text{-}NH_4^+$ l^{-1} with a free ammonia concentration of 916 mg l^{-1} (calculation on the basis of the equation given in Angelidaki et al., 1994); the same authors showed that in thermophilic conditions the free ammonia was responsible for inhibition of methane production and could already be toxic at 700 mg l^{-1}. Moreover, the production rate not only of methane but also of biohydrogen was reported to be highly dependent on pH and ammonia concentrations above 2 g N l^{-1} (Salerno et al., 2006). In this paper the authors showed that the hydrogen production rate decreased with increasing ammonia concentration: high ammonia can in fact cause a shift in the metabolic pathway of a mixed culture (Salerno et al., 2006, Karadag 2011). For example, these authors showed that testing different ammonia concentrations, the amount of total metabolites remained constant while butyrate and acetate concentrations decreased as did H_2 production. The same effects were detected in this experiment as illustrated in Figure 28a and 28b, where it can be seen how ammonia influenced methane and hydrogen production.

In the second phase reactor the high ammonia concentration (about 2 g l^{-1}) led to a decrease in methane production rate from 2.0 m^3 m^{-3} day^{-1} to 1.6 m^3 m^{-3} day^{-1} with a concomitant propionate accumulation (Table 11). Plotting the hydrogen content against the ammonia concentration in the first reactor (Figure 28b), a relationship between these parameters could be seen, suggesting that the inhibition of hydrogen-producing bacteria was caused by ammonia, with a simultaneous decrease in acetate and butyrate content.



a) b)

Figure 28. a) Effect of ammonia concentration in the anaerobic digestion reactor; b) Effect of ammonia on biohydrogen production in dark fermentation reactor.

Table 11 shows the distribution of acetic, propionic and butyric acids at different ammonia concentrations, in both reactors. In the dark fermentation reactor when the ammonia content increased from 970 to 1976 mg l^{-1}, the acetic and butyric acid decreased (from 3749 to 1043 mg COD l^{-1} for H-Ac, and from 5257 to 1428 mg COD l^{-1} for H-Bu), while propionic acid increased (from 696 to 1904 mg COD l^{-1}); at the same time the hydrogen concentration decreased from 43% to 29%. In the methanogenic reactor methane content decreased slightly (from 66% to 61%) with an accumulation of propionic acid.

Table 11. H_2 and CH_4 yields and VFA content at different ammonia concentrations

First Phase Reactor					
N-NH4+	HPR	H_2	H-Ac	H-Pr	H-Bu
(mg l^{-1})	(m^3 H_2 m^{-3} day^{-1})	(%)	(mg COD l^{-1})	(mg COD l^{-1})	(mg COD l^{-1})
970	1.1	43	3749	696	5257
1295	0.8	37	1492	937	3863
1976	0.7	29	1043	1904	1428
Second Phase Reactor					
N-NH4+	MPR	CH_4	H-Ac	H-Pr	H-Bu
(mg l^{-1})	(m^3 CH_4 m^{-3} day^{-1})	(%)	(mg COD l^{-1})	(mg COD l^{-1})	(mg COD l^{-1})
1005	2.0	66	26	-	77
1745	2.1	65	-	-	-
2240	1.6	61	395	1498	68

In order to solve this problem, the approach used in previous trials was the daily removal of part of the recycling stream from the second to the first reactor, to maintain the ammonia concentration at a constant level in the system so to prevent inhibition problems. Some preliminary tests were carried out in order to remove ammonia on recirculation flow, using an evaporation unit (data not shown): the preliminary results showed the possibility to treat part of the second phase effluent in order to remove ammonia, recirculating to the first phase a constant amount.

4.5 H_2-enriched methane production: impact on overall system energy yield

Several authors have reported the advantages of using hydrogen-enriched biogas and the preferred composition of the mixture (Porpatham et al., 2007, Rakopoulos and Michos 2009, Reith et al., 2003). For this reason, the composition of all the biogas produced during this longer-term trial was determined. Figure 29 shows the daily gas composition: the percentages of methane, hydrogen and carbon dioxide were 58%, 6.9% and 36% respectively. Hydrogen content was never below 5% and this matched the best characteristics for biohythane mixture in terms of enhanced combustion performance and hydrocarbon emissions.

The total specific gas production obtained was 0.80 m^3 kg^{-1} VS_{added}, with a gas production rate of 2.75 m^3 m^{-3} day^{-1} (Table 12). The calculation included the small amount of methane produced in the first phase, due to incomplete inhibition of methanogenic microorganisms.

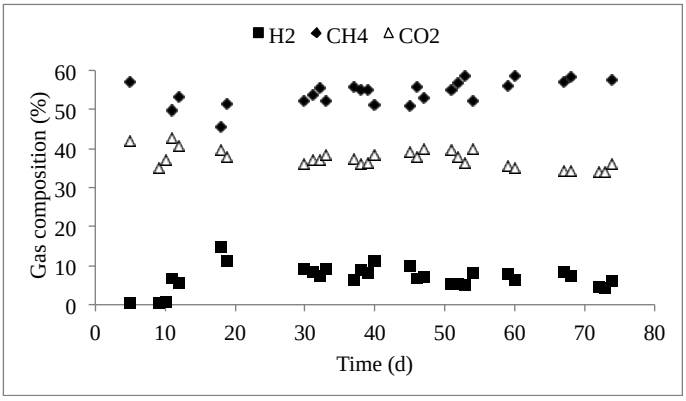


Figure 29. Total bio-hythane composition.

Table 12. Bio-hythane yields.

	First Phase			Second Phase		GP	H_2	CH_4	CO_2	VGP	SGP
	m^3 H_2 day^{-1}	m^3 CO_2 day^{-1}	m^3 CH_4 day^{-1}	m^3 CH_4 day^{-1}	m^3 CO_2 day^{-1}	m^3 day^{-1}	%	%	%	m^3 m^{-3} day^{-1}	m^3 kg^{-1} VS
Average	0.19	0.27	0.015	1.48	0.687	2.645	6.9	58	36	2.75	0.80
SD	0.06	0.05	0.006	0.12	0.10	0.28	1.9	3.0	2.0	0.25	0.11
Min	0.09	0.19	0	1.18	0.53	2.01	5.4	51	33.9	2.16	0.63
Max	0.3	0.37	0.02	1.70	0.9	3.18	11.4	61.3	40.1	3.25	0.95

The specific energy production per tonne of waste was calculated based on the total specific gas production and composition and the solids content of source segregated biowaste. The calculation was also based on the lower heating value (LHV) of the gas mixture as obtained

in experimental trials. The specific energy production obtained was 404 kWh tonne^{-1} of waste pre-treated. This value could be compared with the energy consumption for waste pre-treatment. For example, taking into account the pre-treatment system installed at WWPT of Treviso Council (OFMSW coming from a 'door to door' collection approach), the specific energy requirement was 20-40 kWh tonne^{-1} of waste treated (Cecchi et al., 2005).

Based on the energy density and specific energy of methane and hydrogen and the ideal biohythane composition, the energy content of biogas and biohythane were calculated and compared. In terms of energy density biohythane is 23.8 versus 22.6 MJ m^{-3} of biogas, or 24.5 instead of 19.6 MJ kg^{-1}.

To evaluate the potential application of the system studied, an implementation of a wastewater treatment plant with a two-phase anaerobic digestion of FW was simulated. The plant was based on 200,000 equivalent inhabitants and the best obtained performances found in the present research were applied. Data underlying the calculations are presented in Table 13, and the mass flow rates are shown in Figure 30 (sewage treatment) and Figure 31 (sludge treatment).

Table 13. Project parameters used for data elaboration

PARAMETER		VALUE ADOPTED
Catchmen area	:	200,000 PE
Incoming sewage	:	230 L/PEd
Incoming sewage solid concentration	:	0.18 kgTS/m^3
Incoming sewage COD concentration	:	0.25 kgCOD/m^3
Solid removing by primary sedimentation	:	50%
COD removing by primary sedimentation	:	30%
TS concentration after primary sedimentation	:	0.025 KgTS/L
Biomass yield (Y)	:	0.40 KgVS/KgCOD
Endogenous decay coefficient (k_d)	:	0.06 d^{-1}
TS concentration after secondary sedimentation	:	0.012 KgTS/L
Sludge thickener catchment efficiency	:	90%
Solid concentration after sludge thickener	:	0.025 Kg/L
Incoming FW	:	0.3 Kg/PE
FW after selection	:	80%
Total solid of FW	:	248 g/Kg
Volatile solids of FW	:	236 g/Kg
OLR I phase	:	20 KgVS/m^3d
OLR II phase	:	5 KgVS/m^3d
SGP (I) for FW	:	0.258 m^3/kgVS
SGP (II) for primary sludge	:	0.25 m^3/kgVS
SGP (II) for secondary sludge	:	0.17 m^3/kgVS
SGP (II) for FW	:	0.550 m^3/kgVS
I phase biogas composition	:	48% H_2; 52% CO_2
II phase biogas composition	:	62% CH_4; 38% CO_2
Dehydration catchment efficiency	:	90%
TS concentration after dehydration	:	0.25 Kg/L

An energy balance was hence calculated to investigate energy self-sustainability of the process and the potential profit achievable. A CHP group was used for electrical and thermal recovering and the possibility to use the co-generated heat for maintaining the digester at the thermophilic temperature was considered. Heat losses were calculated considering a digester buried for 1/3 of the total height and with a diameter/height ratio of 1.4. The average temperatures used for air and soil were respectively 12 and 6 °C. The PCI used for hydrogen and biogas was 11.1 and 23.02 MJ m^{-3} respectively. The applied heat transfer coefficient for soil was 0.129 MJ m^{-3} $°C^{-1}$ day^{-1} and for air was 0.043 MJ m^{-3} $°C^{-1}$ day^{-1}. Efficiency of the CHP group was estimated at 0.6 for electricity and 0.5 for heat. The calculated energy balance is reported in Table 14.

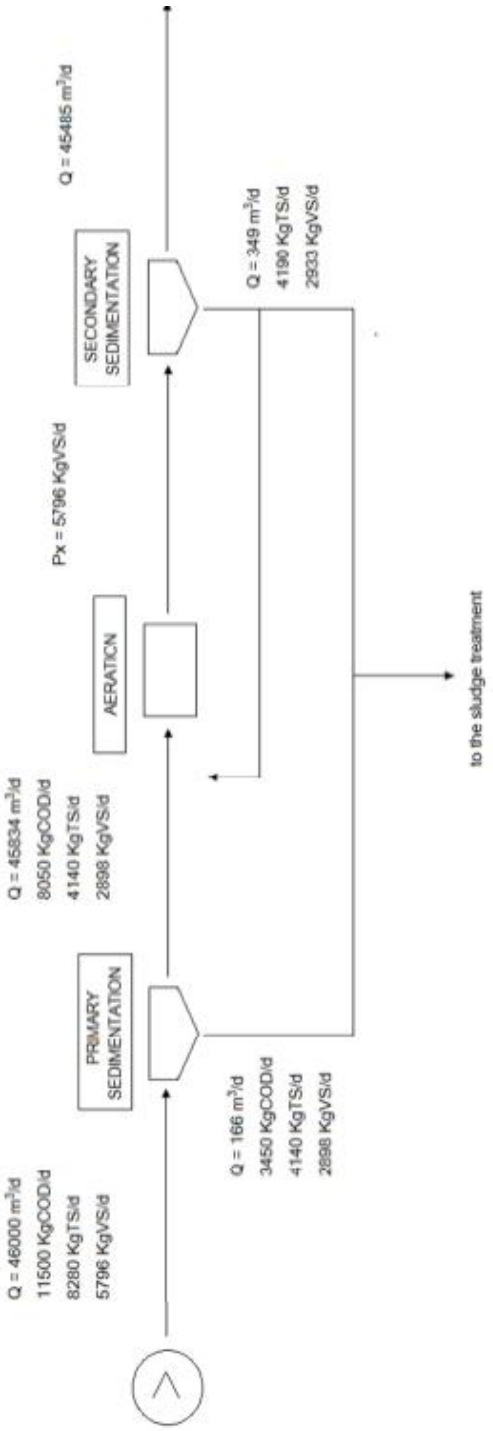


Figure 30. Scheme of the calculated mass flows for the sewage treatment line

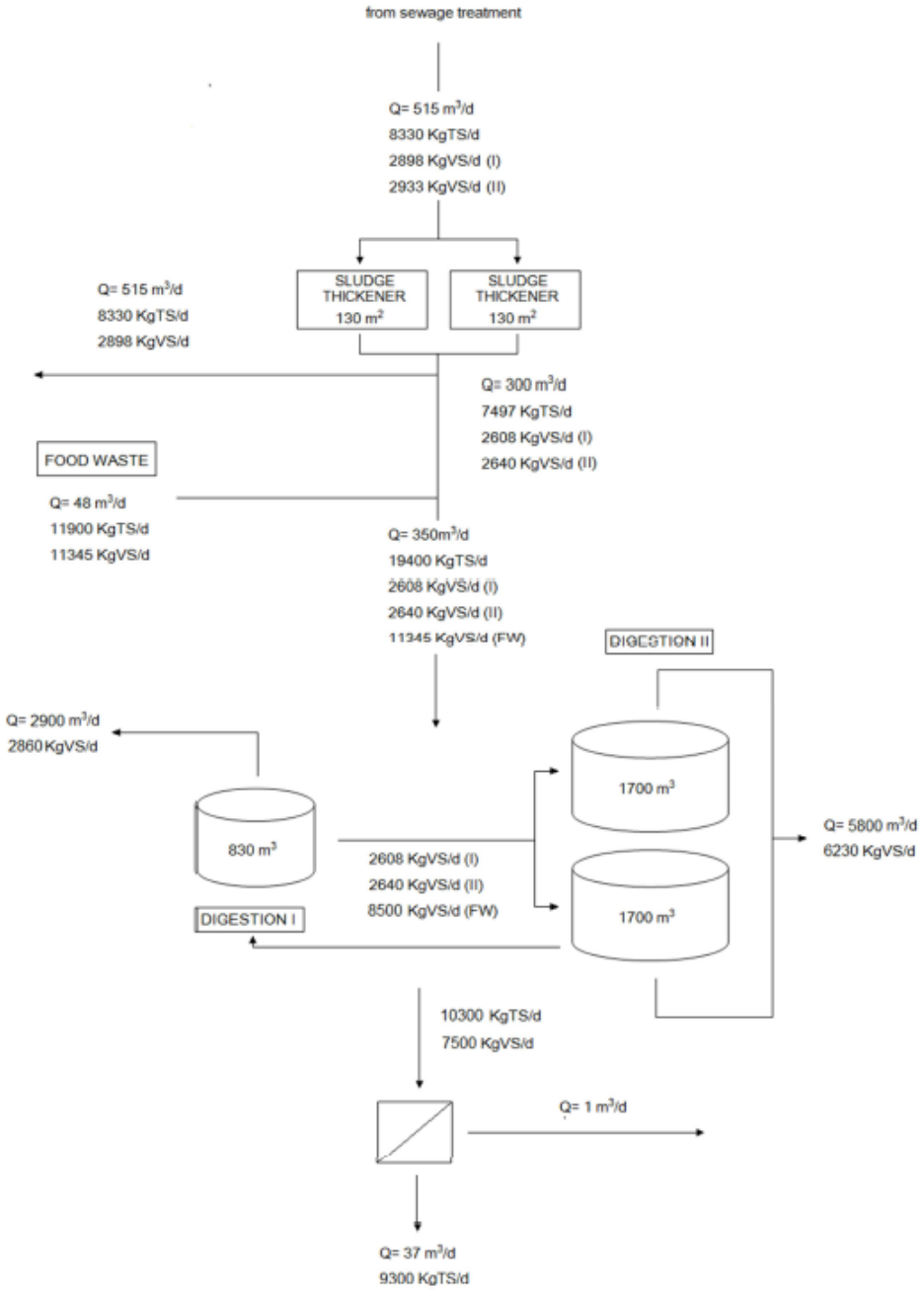


Figure 31. Scheme of the calculated mass flows for the sludge treatment line

Table 14. Process implementation, energy balance

Energy from total biogas	149,050 MJ
Energy for sludge heating	58,615 MJ
Energy dispersion	6,669 MJ
Total energy required	65,314 MJ
Heat co-generated by the CHP group	74,525 MJ
Heat surplus	9,211 MJ
Heat surplus Electricity produced by CHP group	24,850 KWh d^{-1}

5 Conclusions

Laboratory-scale batch tests demonstrated the suitability of the selected inoculum/substrate system for testing at the thermophilic range for hydrogen production. VFA did not show accumulation and the chosen OLR range was suitable for application in CSTR-type reactors. During batch tests, the higher the load, the higher the hydrogen production, while methanogenesis was completely inhibited at an OLR of 30 kg VS m^{-3} day^{-1}. This trend however was not found during CSTR trials, where the maximum hydrogen yield was achieved at a middle-low OLR of 20 kg VS m^{-3} day^{-1}.

Laboratory-scale two-phase CSTR trials with supernatant recirculation did not lead to any significant hydrogen production. The supernatant recirculated about 90% of the sludge alkalinity but this was not enough to control the pH in the first phase, where acidogenic conditions were established within the first week. At OLR of 25 and 30 kg VS m^{-3} day^{-1} both the phases were completely inhibited, while at OLR of 15 and 20 kg VS m^{-3} day^{-1} the system acted as a two-phase AD for methane production.

Laboratory-scale two-phase CSTR with digestate recirculation showed that a self-sustained process with a high hydrogen yield can be reached with an OLR of 20 kg VS m^{-3} day^{-1}. With the recirculation conditions applied, an OLR of 15 kg VS m^{-3} day^{-1} is too light and led to a methanogenic shift within the first month, while OLR of 25 and 30 kg VS m^{-3} day^{-1} are too heavy and led to acidogenic conditions in the first phase. A comparison between results obtained with the two different recirculation conditions leads to an important consideration. The whole digestate recirculation led to control of the first-phase pH not only because of the increased alkalinity. The fresh biomass recirculated into the first phase plays probably a fundamental role for maintaining a health process in the reactor. Further investigations are however necessary to understand this phenomenon.

Pilot-scale two-phase CSTR anaerobic digestion process for hydrogen and methane production was optimised without any chemical or heat shock treatment of inoculum while pH was not controlled. The best yields in terms of hydrogen production were obtained in Run III-a at lower OLR (16 kg TVS m^{-3} day^{-1}), due to liquid phase recirculation from the anaerobic digestion. A SHP of 51 l H_2 kg^{-1} TVS with 37% hydrogen content was observed. The final gas composition met the biohythane characteristics with 6.7% H_2, 40.1% CO2, 52.3% CH4 and a whole system SGP of 0.78 m^3 kg^{-1} TVS fed. In subsequent trials lower organic loading rates will be verified and the recirculation ratio will be changed in order to maximise the hydrogen yields.

A longer-term two-phase anaerobic digestion process for hydrogen and methane production was carried out at pilot scale without any inoculum treatment or pH control. In this trial digestate was recirculated in order to support the dark fermentation with alkalinity, and keep the pH in the hydrogenase working range. The results showed a stable run, without any particular variation in parameters that could be associated with process failure. Hydrogen production was 66.7 l kg^{-1} TVS added and the specific biogas production in the second phase was 0.72 m^3 kg^{-1} TVS added. The biohythane obtained met the required composition characteristics (CH_4 58%, H_2 6.9% and CO_2 36%). Ammonia accumulation was observed and may have led to initial signs of inhibition in the first phase in the form of a reduction in hydrogen content. Methods of ammonia removal are under consideration elsewhere in the project.

The performances of the process proposed implementation in a 200,000 population equivalent WWTP were shown. Biogas was supposed to be burnt for electricity-conversion using a CHP group, as at the present date it is the most convenient scenario. Electricity production was estimated at almost 24.8 MWh day^{-1} while the co-generated heat was enough to assure the digestate heating and to give a thermal surplus of 9,211 MJ day^{-1}.

Within the above scenario, assuming use of the first phase biogas for pure hydrogen production and the methane-enriched biogas for electricity production, the same plant would give: 1400 Nm^3 day^{-1} of pure H_2, 22.3 MWh day^{-1} of electricity and a heat surplus of 1,465.4 MJ day^{-1}.

Note

Parts of the material presented in this deliverable have now been published in Cavinato et al. (2011a, b and 2012).

References

Angelidaki I., Ahring B.K., (1994), Anaerobic thermophilic digestion of manure at different ammonia loads: effect of temperature, Water Research, 28, 727-731.

Banks C.J., Zotova E.A., Heaven S. (2010). Biphasic production of hydrogen and methane from waste lactose in cyclic-batch reactors. Journal of Cleaner Production. 18, S95-S104.

Bolzonella, D., Pavan, P., Fatone, F., Cecchi, F. (2005). Anaerobic fermentation of organic municipal solid wastes for the production of soluble organic compounds. Industrial and Engineering Chemistry Research 44 (10), 3412–3418.

Bolzonella, D., Pavan, P., Mace, S., Cecchi, F. (2006). Dry anaerobic digestion of differently sorted organic municipal solid waste: a full scale experience. Water Science and Technology 53 (8), 23–32.

Burak D., Yenigun O. (2002). Two-phase anaerobic digestion processes: a review. Journal of Chemical Technology & Biotechnology. 77, 743-755.

Cavinato C., Bolzonella D., Fatone F., Cecchi F., Pavan P. (2011). Optimization of two-phase thermophilic anaerobic digestion of biowaste for hydrogen and methane production through reject water recirculation. Bioresource Technology, 102(18), 8605-8611.

Cavinato C., Bolzonella D., Fatone F., Giuliano A., Pavan P. (2011). Two-phase thermophilic anaerobic digestion process for biohythane production treating biowaste: preliminary results. Water Science and Technology, 64(3), 715-721.

Cavinato, C. Giuliano, A., Bolzonella, D., Pavan, P., Cecchi, F. (2012) Bio-hythane production from food waste by dark fermentation coupled with anaerobic digestion process: A long-term pilot scale experience, International Journal of Hydrogen Energy, 37(15), 11549-11555.

Cecchi F., Bolzonella D. (2005). Full scale experiences of anaerobic digestion of the organic fraction of municipal solid waste in Italy e from collection to energy valuable end products. In: 4th International Symposium: Anaerobic digestion of Solid Waste. Copenhagen. Conference Proceedings, vol. 1; 2005. p. 60e71.

Chu C.F., Li Y.Y., Xu K.Q., Ebie Y., Inamori Y., Kong H.N. (2008). A pH-temperature – phased two-stage process for hydrogen and methane production from food waste. International journal of hydrogen energy, 33, 4739-4746.

Cooney M., Maynard N., Canizzaro C., Benemann J. (2007). Two phase anaerobic digestion for production of hydrogen-methane mixtures. Bioresource Technology, 98, 2641-2651.

Gomez X., Moran A., Cuetos M.J., Sánchez, M.E. (2006) The production of hydrogen by dark fermentation of municipal solid waste and slaughterhouse waste: a two phase process. Journal of power sources, 157, 727-732.

Hallenbeck P.C., Ghosh D. (2009) Advances in fermentative biohydrogen production: the way forward? Trends in Biotechnology, 27(5), 287-297.

Hallenbeck P.C., Ghosh D. (2010). Improvements in fermentative biological hydrogen production through metabolic engineering. Journal of Environmental Management, 95, S360-S364.

Hawkes F.R., Hussy I., Kyazze G., Dinsdale R., Hawkes D.L. (2007). Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress. International Journal of Hydrogen Energy, 32, 172-184.

Hu, B., Chen, S.L. (2007). Pretreatment of methanogenic granules for immobilised hydrogen fermentation. International Journal of Hydrogen Energy. 32, 3266–3273.

Jokela, J.P.Y, Rintala, J.A. (2003). Anaerobic solubilisation of nitrogen from municipal solid waste (MSW). Reviews in Environmental Science and Biotechnology; 2(1), 67-77.

Kapdan, I.K., Kargi, F. (2006). Bio-hydrogen production from waste materials. Enzyme and Microbial Technology. 38, 569-582.

Karadag, D. (2011). Anaerobic H(2) production at elevated temperature (60 degrees C) by enriched mixed consortia from mesophilic sources. International Journal of Hydrogen Energy; 36(1), 458-465.

Kataoka, N., Ayame, S., Miya, A., Ueno, Y., Oshita, N., Tsukahara, K., Sawayama, S., Yokota, N. (2005). Studies on hydrogen-methane fermentation process for treating garbage and waste paper. ADSW 2005 Conference Proceedings, 2, Process Engineering.

Kleerebezem, R., van Loosdrecht, M.C.M. (2007). Mixed culture biotechnology for bioenergy production. Current Opinion in Biotechnology 18 (3), 207–212.

Kraemer, J.T., Bagley, D.M. (2007). Improving the yield from fermentative hydrogen production. Biotechnol Lett. 29, 685-695.

Lee, D.Y., Ebie, Y., Xu, K.Q., Li, Y.Y., Inamori Y. (2010). Continuous H2 and CH4 production from high-solid food waste in the two-stage thermophilic fermentation process with the recirculation of digester sludge. Bioresource Technology. 101, 542-547

Levin, D.B., Pitt, L., Love, M. (2004). Biohydrogen production: prospects and limitation to practical application. International journal of hydrogen energy, 29(2), 173-185.

Li, S.L., Kuo, S.C., Lin, J.S., Lee, Z.K., Wang, Y.H., Cheng, S.S. (2008). Process performance evaluation of intermittent-continuous stirred tank reactor for anaerobic hydrogen fermentation with kitchen waste. International journal of hydrogen energy, 33, 1522-1531.

Liu, D., Zeng, R.J., Angelidaki, I. (2006). Hydrogen and methane production from household solid waste in the two stage fermentation process. Water Research. 40, 2230-2236.

Liu, H., Wang, J., Wang, A., Chen, J. (2011). Chemical inhibitors of methanogenesis and putative applications. Applied Microbiology Biotechnology. 89, 1333-1340.

Martìnez-Pérez, N., Cherryman, S.J., Premier, G.C., Dinsdale, R.M., Hawkes, D.L, Hawkes, F.R., Kyazze, G., Guwy, A.J (2007). The potential for hydrogen-enriched biogas production from crops: Scenarios in the UK. Biomass & Bioenergy, 31, 95-104.

Mathews, J., Wang, G. (2009). Metabolic pathway engineering for enhanced biohydrogen production, International Journal of Hydrogen Energy 34, 7404-7416.

Pavan, P., Battistoni, P., Cecchi, F., Mata-Alvarez, J. (2000). Two-phase anaerobic digestion of source sorted OFMSW (organic fraction of municipal solid waste): performance and kinetic study. Water Science and Technology. 41 (3), 111-118.

Porpatham, E., Ramesh, A., Nagalingam, B. (2007) Effect of hydrogen addition on the performance of a biogas fuelled spark ignition engine. International journal of hydrogen energy, 32, 2057-2065.

Rakopoulos, C.D., Michos, C.N. (2009) Generation of combustion irreversibilities in a spark ignition engine under biogas-hydrogen mixtures fueling. International journal of hydrogen energy, 34, 4422-4437.

Reith, J.H., Wijffels, R.H., Barten, H. (2003). Bio-methane & biohydrogen, status and perspectives of biological methane and hydrogen production. Dutch Biological Hydrogen Foundation.

Salerno, M.B., Park, W., Zuom Y., Loganm B.E. (2006). Inhibition of biohydrogen production by ammonia. Water Research; 40(6),1167-1172.

Shin, H.S., Youn, J.H. (2005). Conversion into hydrogen by thermophilic acidogenesis; Biodegradation, 16, 33-44.

Traverso, P., Pavan, P., Bolzonella, D., Innocenti, L., Cecchi, F., Mata-Alvarez, J. (2000). Acidogenic fermentation of source separated mixtures of vegetables and fruits wasted from supermarkets. Biodegradation 11 (6), 407–414.

Valdez-Vazquez, I., Rìos-Leal, E., Esparza-Garcìa, F., Cecchi F., Poggi-Varaldo, H.M. (2005). Semi-continuous solid substrate anaerobic reactors for H_2 production from organic waste: mesophilic versus thermophilic regime. Int. J. Hydrogen Energy 30, 1383-1391.

Valdez-Vazquez, I., Poggi-Varaldo, H.M. (2009). Hydrogen production by fermentative consortia. Renewable and Sustainable Energy Reviews. 13, 1000-1013.

Walker, M., Zhang, Y., Heaven, S., Banks, C.J. (2009). Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes. Bioresource Technology. 100 (24), 6339-6346.

Wang, J., Wan, W. (2009). Factors influencing fermentative hydrogen production: A review; International journal of hydrogen energy, 34, 799-811.

Zhu, H., Stadnyk, A., Bèland, M., Seto, P. (2008). Co-production of hydrogen and methane from potato waste using a two-stage anaerobic digestion process. Bioresource Technology; 99 (11), 5078–5084.