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Mesophilic Anaerobic Digestion Monitoring Using a Headspace Carbon Dioxide Method

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Abstract

Anaerobic digesters are often not operated to full capacity and are recurrently subjected to adverse operational practices due to: temperature fluctuations, inconstant feeding regimes, variable solids content on the feed, changes in loading rates etc. The use of standard on-line monitoring indicators (pH, alkalinity, gas production and compositions)is insufficient to detect the perturbation at an early stage as these parameters are linked to the final product of the process, causing delays when diagnosing digester imbalances. On the other hand, volatile fatty acids (VFA) have been widely recognized as a key parameter for understanding and controlling anaerobic processes as they are intermediate products and real time indicators of the digester stability. Application of on-line instrumentation for VFA measurement has been limited as all developed instrumentations are based on expensive equipments (Gas Chromatograpy, High Performance Liquid Chromatography, Fourier Transform Infra-Red spectrometer etc.) and require. sample preparations involving the use of filtration, membranes, chemical additions, therefore triggering extensive maintenance.

A new sensor, "CO_2 headspace sensor", not requiring sample preparation, based on the measurements of the CO_2 in the headspace of a vessel produced by denitrifying suspended sludge in anoxic conditions after the addition of a carbon source (digested sludge) was tested to estimate the concentrations of soluble chemical oxygen demand-volatile fatty acids (sCOD-VFA) in anaerobic digestate. As the main component of sCOD in fermented sludge are VFA, and as denitrifiers show a faster denitrification rate for VFA than for other carbon compounds, it was possible to correlate the CO_2 measured in the headspace of the sensor with the concentrations of sCOD-VFA in the digestate.

The CO_2 headspace sensor was tested for early detection of sCOD-VFA accumulation in perturbed anaerobic digesters at laboratory (organic underload and overload) and pilot (organic overload) scale and compared to the standard monitoring indicators. In all cases, the CO_2 headspace sensor was able to detect process imbalance at an early stage and prevent a further inhibition. Consequent changes in loading rates were completed according to the sensor readings to re-stabilize the digester and an increase in process efficiency, compared to a digester monitored only by the standard indicators, was observed. Overall 111.5 L extra biogas and a solid reduction of 75% were obtained during the organic underload laboratory test, 6.7 L biogas and approximately 70% solid reduction with the organic overload laboratory test and 4500 L biogas and approximately 60% solid removal

during the pilot scale organic overlaod .Further tests with full scale anarobic digestate, proved the potential applicability of the CO_2 headspace sensor for sCOD-VFA monitoring at industrial scale. To conclude, further development of the CO_2 headspace sensor is recommended to be used as tool for optimising feeding regimes in anaerobic digestion.

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List of Contents

List of Figures

List of Tables

Abbreviations

AD: Anaerobic Digestion

ADM: Anaerobic Digestion Model

BNR: Biological Nutrient Removal

BOD_5: Biochemical Oxygen Demand

CER: Carbon Dioxide Evolution Rate

CO_2: Carbon Dioxide

COD: Chemical Oxygen Demand

CH_4: Methane

CHP : Combined Heat and Power

CSTR: Continuously Stirred Tank Reactors

DO: Dissolved Oxygen

EU: European Union

FITs: Feed-in Tariffs

GC: Gas Chromatography

GPR: Gas Production Rate

HPLC: High Performance Liquid Chromatography

ICA: Instrumentation Control and Automation

IWW: Industrial Wastewater

MLSS: Mixed Liquid Suspended Solids

N_2: Nitrogen

NO_3: Nitrate

OLR: Organic Loading Rate

OPEX: Operating Expenditure

OPHA: Obligate Hydrogen-Producing Acetogens

PE.: Population Equivalent

PI: Proportional Integral controller

PID: Proportional Integral Derivative controller

PLC: Programmable Logic Control

PVC: Polyvinyl chloride

rbCOD: Readily Biodegradable Chemical Oxygen Demand

RDPE: Rural Development Programme for England

ROCs: Renewable Obligation Certificates

RTFO: Transport Fuel Obligation

rpm: Revolutions Per Minute

SAS: Surplus Activated Sludge

sCOD: soluble Chemical Oxygen Demand

SGP: Specific Gas Production

SRT: Solid Retention Time

SS: Suspended Solids

STW: Sewage Treatment Works

TOC: Total Organic Carbon

TS: Total Solids

UK: United Kingdom

VS: Volatile Solids

VFA: Volatile Fatty Acids

WWTP: Wastewater Treatment Plant

1 Introduction

Anaerobic digestion is a widely utilized biological process used to promote the degradation and stabilization of organic matter found in wastewater sludge and also in agricultural and industrial wastes. It is recognized as an attractive and sustainable technology, for its characteristic of combining waste stabilization, sludge volume reduction and energy recovery in the form of biogas.

The process consists of a complex interaction of different microbial communities which determine the transformation of organic materials into biogas and stabilised sludge. The main intermediate steps of the process are the hydrolysis of the complex organic matter into simpler soluble molecules, the acidogenesis where the organic molecules are transformed into acids (volatile fatty acids, lactic acid) and alcohols, acetogenesis where all previous substrates are generally transformed into acetic acid, and the final methanogenesis during which mainly methane and carbon dioxide are formed.

The microorganisms groups acting in the four phases generally differ for their optimal environmental conditions and are sensitive to external or operational disturbances. In particular the balance between the acidogenic and methanogenic bacteria is of particular importance for maintaining the overall process stability. Over-feeding, under-loading, or the accumulation of inhibitory substances can lead to process imbalance and accumulation of volatile fatty acids (VFA) in the reactor, causing sour conditions (Marchaim and Krause, 1992; Pullammanappallil, 1997; Palacio-Barco et al., 2009).

In order to ensure a stable function, prevent failures and optimise the biogas production, an efficient process monitoring and control strategy is essential. Several parameters have been suggested as typical indicators of process imbalance: biogas production and composition, pH, alkalinity, chemical oxygen demand (COD) or solids removal and VFA concentration and composition (Mechichi and Sayadi, 2005; Boe et al., 2010). However, the most commonly applied instrumentations for the measurement of biogas flow, methane content, alkalinity and pH are often insufficient to detect sudden signs of instability, as they are dependent on the final products or status of the process and therefore have delayed responses (Bjornsson, 2000; Boe et al., 2005). In order to observe early signs of process failure, measurements of intermediate substances such as VFA - which accumulation can inhibit the process - were shown to be critical and reliable indicators (Bjornsson, 2000; Vanrolleghem and Lee, 2003; Mechichi and Sayadi, 2005; Boe, 2006; Boe et al., 2010).

At present, VFA concentrations have been mainly monitored through off-line methodologies, which are time consuming, and also require specialized equipment and sample preparation. Various on-line VFA monitoring and instrumentation have also been developed based on analytical equipment such as: Fourier Transform Infra-Red (FT-IR) spectrometer (Steyer et al. 2001), gas chromatography (GC) or high performance liquid chromatography (HPLC) (previous sample filtration required) (Pind et al., 2002) or headspace gas chromatography (Boe et al., 2006). The full scale application of these on-line instruments is limited as they all require preliminary treatment of the samples, through filtration or membrane separation, often triggering fouling problems and they also necessitate additions of chemicals. The heavy maintenance of these developed instrumentations is likely to become even more difficult and extensive if considering the future prospective of anaerobic degradation upgrading to more complex substrate (i.e. co-digestion). Indeed, ever since the implementation of the EU Waste Framework Directive 2006/12/EC, and the restricting legislation concerning the quantities of biodegradable municipal waste allowed into landfills (Council Directive 1999/31/EC of 26 April 1999 on the Landfill Waste), renewed interest is currently being shown in the use of anaerobic degradation technologies as a waste treatment solution combined with renewable energy and fertilizer production (Defra, 2010). In order to increase the reliability and the broader use of anaerobic digestion technology, higher process understanding and efficient instrumentation, control and automation (ICA) is necessary.

In this study, a novel methodology for the indirect measurement of the sCOD-VFA content in anaerobic digesters for early monitoring of process imbalance was tested. Previous studies completed at Cranfield University (Crowley, 2007) and by Li et al. (2002 and 2004) set-up and tested the CO_2 headspace sensor. The sensor is based on the measurement of the carbon dioxide in the headspace of a batch reactor containing denitrifiers. The carbon dioxide is produced when these bacteria use a carbon source, that in this study was digested sludge, and nitrate to achieve denitrification. Li et al (2002) established a linear correlation between the amount of VFAs added to the denitrifying biomass and the elapsed time (E-time - difference of time between the moment that a carbon source is injected into the system and the peak CO_2 production). However, the VFA content was over estimated by 30% in thermophilic digester digestate. Nevertheless, it was suggested that the methodology could be used for on-line determination of the internal carbon source available for biological nutrient removal processes. A second study

completed by Crowley (2007) demonstrated that several types of carbon sources (synthetic acids and fermentation products) could be correlated with the CO_2 produced in the vessel with denitrifiers. A linear correlation (with coefficient of determination $r^2 > 0.98$) was found between the rate of CO_2 produced by the denitrifiers over the mixed liquor suspended solids (MLSS) and the concentrations of soluble COD corresponding to the different carbon concentrations. It was also demonstrated that the CO_2 headspace sensor could be used to estimate the sCOD products from primary sludge fermentation for addition to enhancing biological nutrient removal processes. Furthermore it was suggested that the methodology could be applied for indirect measurements of sCOD-VFA in anaerobic digestion.

In the study here described, it was investigated the possibility of utilising the CO_2 headspace methodology as a monitoring tool for indirect measurements of sCOD-VFA accumulations in perturbed anaerobic digesters. The information derived from the CO_2 headspace sensor was used as an early indication of process inhibition, therefore operational conditions - feeding routines - were changed to avoid further imbalance and increase process efficiency. Anaerobic process perturbations, such as organic overload and underload tests were performed on laboratory scale anaerobic digesters. The benefits of the application of the CO_2 headspace sensor for sCOD-VFA accumulations detection in one digester was compared to the merely standard monitoring process indicators (pH, alkalinity, biogas production and composition) applied to a second digester. Furthermore, an upgrade of the test was realized with the use of two pilot scale anaerobic digesters that were subjected to a reduction of solid retention time, thus they were overloaded. The CO_2 headspace sensor was applied for an early detection of sCOD-VFA accumulation in one of the two digesters. Finally, the CO_2 headspace sensor was tested for detecting sCOD-VFA variation in a full scale digester.

Aim of the study:

Assessing the suitability of the CO_2 headspace sensor for sCOD-VFA monitoring in anaerobic digestion.

Objectives of the study:

- Establish a correlation between the sensor response and different concentrations of acetic acid in order to develop a calibration curve.

- Test the CO_2 headspace sensor response to several types of digested sludge.
- Development of a control law for anaerobic digestion feeding regime based on the CO_2 headspace sensor response.
- Test the potential of the CO_2 headspace sensor for monitoring sCOD-VFA accumulations in laboratory scale, pilot scale and full scale anaerobic digesters operated under suboptimal conditions such as organic underload and overload.
- Comparison of diagnoses regarding digester health when using the sensor detection of sCOD-VFA accumulation and standard monitoring, and consequent effects on process efficiency and optimisation.

2 Literature Review

2.1 Anaerobic sludge digestion: process drivers and legislation

Anaerobic digestion process promotes the degradation of the organic material in the sludge into a mixture of mostly carbon dioxide and methane gases and stabilised digested sludge. The technology has been widely applied over the last 70 years as a stabilization method for sewage sludge from wastewater treatment, industrial organic effluents (dairy, brewing, starch, sugar, distilling products) and manure. Numerous advantages are often recognized to the use of anaerobic digestion:

- Production of renewable energy in form of biogas, which can be burned for the cogenerated production of heat and power thus facilitating the energetic independency of the plant. Alternatively, after removal of carbon dioxide and other impurities, the biogas can be used to produce bio methane that can be employed as car fuel or injected into the gas grid, thus permitting economical revenues. Overall the internal production of energy could aid the water utilities to balance their energy needs and increase economical revenues.
- Reduction of sludge volume by 30-50%.
- Potential re-use of the stabilised sludge (or digestate) as fertiliser and soil conditioner, providing high nutrient concentration to the soil.
- Potential contribution to climate change mitigation and wider environmental objectives, as the diversion of wastes to anaerobic digestion can reduce greenhouse gas emissions compared with landfill. Furthermore, the energy produced via anaerobic process contributes to the reduction of non-renewable energy resources use.

In the last decade the endorsement of several legislations and economical incentives from the UK government, as in other EU countries have increased the interest and the marketing for anaerobic digestion. The "Landfill Directive" 1999/31/EC of 26 April 1999 concerning the restriction of the quantities of biodegradable municipal waste allowed into landfills requires the UK to cut by 2020 the volume of biodegradable municipal waste sent to landfill to 35% of that which was produced in 1995. The UK Waste Strategy (2007) delineates the essential contribution from anaerobic digestion to reach this target together with the renewable energy production and the recovery of valuable nutrients (Defra, 2007). The introduction of the renewable obligation certificates (ROCs) in 2002, for economical

incentives for different renewable energy producing technologies included anaerobic technology in the top banding, allowing a wider market to develop (Defra, 2010). The feed-in tariffs (FITs) encourage the market of small-scale low carbon electricity generation by a guaranteed incentive for a certain time period (Defra, 2010). Furthermore, the renewable transport fuel obligation (RTFO), into effect from 2008, requires suppliers of fossil fuels to ensure that a specified percentage of the road fuels that they supply in the UK are made up of renewable fuels, such as biogas with a current obligation level for 2009/10 of 3.25% by volume, increasing in 2010/11 to 3.5% (Defra, 2010). Renewable Heat Incentive (which will be introduced in 2011) allows generators of renewable heat to claim financial incentives for the production, allowing further economical benefits. Anaerobic digestion is also eligible for support under the Rural Development Programme for England 2007-2013 (RDPE) and further different grants and financial supports (Defra, 2010). Furthermore, as this technology can be employed as organic waste treatment (co-digestion processes), further economical advantages could be achieved together with the development of a more sustainable waste management strategy.

Until the last two decades, unsatisfactory reactor designs, process instability and poor comprehension of the complex microbial processes in the anaerobic systems limited a wider and more successful diffusion of this technology. However, at present, the wastewater industry has strong financial incentives for maximising the use of anaerobic digestion and biogas production. A wider understanding of the process and a stronger control capacity is therefore essential for the full implementation and optimisation of anaerobic digestion technology.

2.2 Anaerobic digestion: process and instability

The main processes occurring in anaerobic digestion of organic material are hydrolysis, fermentation (acidogenesis), acetogenesis, and methanogenesis, where hydrolysis is followed by the fermentation process, while acetogenesis and methanogenesis are further linked to each other (Figure 2.1.):

- Hydrolysis: extra-cellular process where the hydrolytic bacteria excrete enzymes (endoenzymes and exoenzymes) to catalyse hydrolysis of complex insoluble organic substrates, such as polysaccharides, fats or proteins, into simple soluble molecules. The final products of hydrolysis are sugars, amino acids and fatty acids.

- Acidogenesis (Fermentation): this process is a further breakdown of the simple molecules into acids with 2 to 5 carbon atoms such as acetate, propionate, butyrate, lactate, ethanol, butanol. Also hydrogen and carbon dioxide are created from the carbohydrate fermentation.

  As example of these reactions, the glucose ferments in the following products (Speece, 1996):

  acetate: $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$

  propionate: $C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$

  acetate, propionate: $3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O$

  butyrate: $C_6H_{12}O_6 \rightarrow 2CH_3CH_2\ CH_2COOH + 2CO_2 + 2H_2$

  lactate: $C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH + 2CO_2 + 2H_2$

  ethanol: $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$

- Acetogenesis: bacteria consume the fermentation products with high carbon atom content, such as propionic and butyrate and generate acetic acid, carbon dioxide, and hydrogen:

  $$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3\ H_2$$

  $$CH_3CH_2H_4COOH + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ H_2$$

- Methanogenesis: acetotrophic methanogens utilize the acetate produced during acidogenesis and acetogenesis to form methane and carbon dioxide:

  $$CH_3COOH \rightarrow CH_4 + CO_2$$

  while the hydrotrophic bacteria utilize the hydrogen and carbon dioxide to produce methane:

  $$CO_2 + 4H_2 \rightarrow CH_4 + 2H_20$$

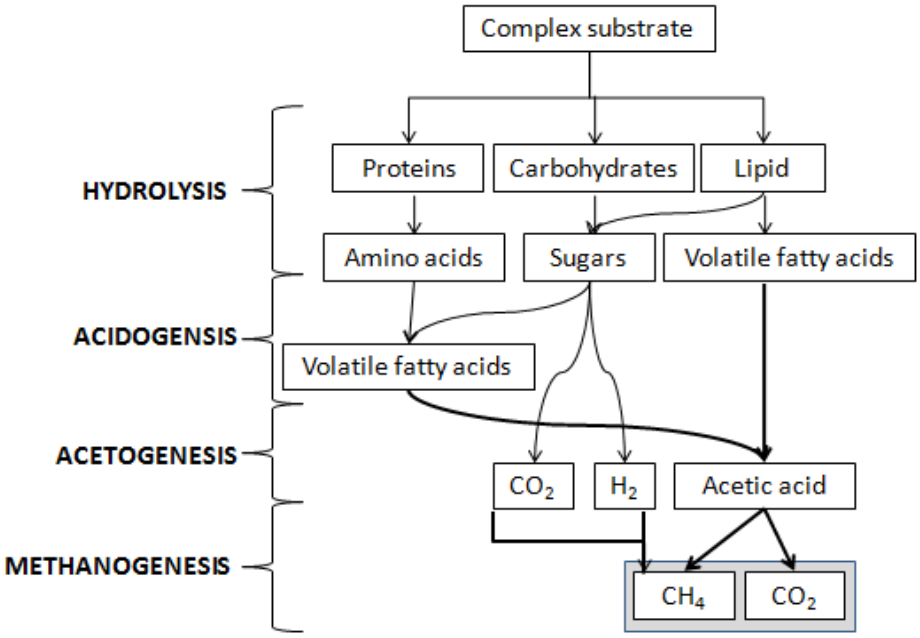

Figure 2.1. Process flow chart of anaerobic digestion

The interaction of different coexistent microorganisms determines a high process complexity which can affect the stability of the whole process. The different bacterial groups have different optimal environmental conditions and are sensitive to variation of process parameters such as temperature, pH, alkalinity, concentration of free ammonia, hydrogen, volatile fatty acids etc (Mara and Horan, 2003, Appels et al., 2008).

Generally, inhibition of the process occurs when a material or an operational condition causes a great modification of the microbial population or an inhibition of the bacterial growth. If the perturbation is minor or temporary, adaptation of microbiological population to condition shifts can also develop, preventing final failures but modifying the microorganism population. In cases of longer and more robust perturbations, poor operational stability often occurs, determining lower operational performances and, as the inhibitory effect can last up to 6 months, can cause severe damage to the process and consequently, economical losses.

Operational condition variations from the optimal range, such as temperature, pH variation, loading alteration, mixing or retention time variation, have different causes and process inhibition effects (Table 2.1).

Table 2.1. AD optimal parameter ranges and effects of their variations on the process (Gerardi, 2003a)

Parameter	Optimal range	Effect of variations and origin
Temperature	- Psychrophilic 15-20°C - Mesophilic: 35-40°C - Thermophilic 55-65°C	- microorganisms activity strongly affected - higher T: initial increase in microorganisms growth - high T: higher process instability, VFA increase, COD removal reduction and decreasing CH_4 content.
pH	- fermentative microorganisms: 4.0-8.5 - methanogenic bacteria: 6.5-7.2	- influenced by ammonia consumption and release - influenced by sulphide release - decreases with acid accumulation (VFA) - under 6→ high process inhibition - influences H metabolism
Oxygen	- Redox potential< -200 mV	- process resistant to low O_2 concentrations - high O_2 can reduce biomass activity
Alkalinity	- 1500-3000 mgL^{-1}	- necessary for pH buffering - decreases with acid accumulation - if low, higher probability of process acidification
Mixing	- increases contact between organisms matter and microorganisms	- excessive mixing can reduce reactor performance - low mixing can determine foaming, impeding gas release - inefficient mixing can determine different formation of areas with different process rate
VFA	- <500-1500 mgL^{-1} depending on pH values and buffer capacity	- intermediate substance, from hydrolysis and fermentation of organic compounds -in high concentrations, determines pH reduction, alkalinity consumption -in high concentration decreases biomass activity

Furthermore, several substances can be toxic to the biomass, when accumulating or interacting in the different steps of the process. These (Table 2.2) are derived from the digester inflow itself (industrial wastewaters etc.) or are produced during the complex substrates degradation process. Due to the high complexity of the process, the different interacting parameters affecting AD process, and the variability of the applications (waste composition, microorganisms, experimental methods, substrate affinity), no precise limit

ranges for inhibitor substances can be defined but different ranges are proposed (Table 2.2) (Pind et al., 2002; Chen et al., 2008).

Table 2.2. Common inhibition substances of AD process: origin, complex interference with other factors and inhibition concentration ranges (reedited from Chen et al., 2008)

Inhibitors	Origin	Relation with other process parameters	Inhibition concentration/conditions
Ammonia	degradation of nitrogen content in organic substrate	- pH: higher pH→ higher NH_3-N toxic - T: higher T→ higher Ammonia - other ions→ toxicity decreases in presence of other ions	- 1.7-14 gl^{-1} reduces CH_4 production of 50% - <150 mgL^{-1} of NH3-N
Sulphide	degradation of sulphate (present in IWW)	- microorganism competition for C→reduced CH_4 produced - T: higher T→less toxicity	- different inhibition levels dependant on other conditions -100-800 mg l^{-1} dissolved sulphide
Light metals ions (Aluminium, Calcium, Magnesium, Potassium, Sodium)	released from organic matter breakdown	- competition between different ions - impacts biomass activity	- up to 200mg/L→ required for microbial growth - excess amount→ lowers biomass activity
Heavy metals (Chromium, Iron, Zinc, Copper, Cobalt, Cadmium, Nickel)	present in IWW	- pH, redox influence effect of metals on biomass activity - effect depends on substrate and bacteria genre - effects varies depending on TS	- non biodegradable→ toxic at 100 mg/L concentrations - inhibition levels dependant on components ratio
Organics (Benzens, phenols, alkanes, alcohols, ethers, kethons etc)	present in IWW	- higher biomass concentration→ lower toxic effect - lower sludge age→ lower toxic effect	- different limits and inhibition grades

Besides all these possible causes for process inhibition, it is widely recognized that the syntrophic balance between the different bacteria groups is the primary cause of anaerobic digestion instability, as these are mutually beneficial and interdependent, i.e. removing inhibitory products for other bacterial groups and producing substances necessary for other bacterial growth. Particularly, of high importance is the symbiotic relationship between acidogenic and methanogenic bacteria. Indeed the conversion of fatty acids and alcohols is

energetically at the expense of the methanogenic bacteria, while these receive the necessary substrates (acetic acid, H_2, CO_2) needed for growth in return from the acidogenic and acetogenic bacteria. Furthermore, these bacterial groups require different external conditions characteristics: acetogenic bacteria necessitate very low H_2 concentration for their survival and growth, while methanogenic organisms can survive only with higher hydrogen partial pressure. The latter remove the products of the acetogenic bacteria from the substrate maintaining the hydrogen partial pressure at a low level suitable for the acetogenic bacteria. If the hydrogen partial pressure is low, acetate, H_2 and CO_2 are predominantly formed by the acetogenic bacteria, while with high hydrogen partial pressure, predominantly butyric, propionic, valeric acids and ethanol are formed (Koster, 1989; Mechichi and Sayadi, 2005; Chen et al., 2008; Appels et al., 2008).

Therefore, the overall anaerobic process is dependent on the correct balance of these bacterial groups as they cannot operate independently from each other. If the slower growing methanogenic bacteria are inhibited, by biomass wash out or external disturbances (being the most sensitive bacterial group to variations), then acetic and other acids build up causing an increasing acidity of the system. If the balance is not restored, the buffering capacity of the system is consumed and pH values reach inhibitory levels for the hydrolysis and acetogenesis which are then also inhibited causing the final process failure (Stamatelatou et el., 1997, Pullammanappallil et al., 1997; Steyer et al., 1999, Olsson et al., 2005). This situation often develops in cases of organic overload, when poor control of feed volume rate occurs or when the inflow sludge characteristics fluctuates greatly, causing metabolic imbalance and reduction in the performance of the digester.

Several monitoring parameters variations are associated to ongoing process inhibition and instability (Chynoweth et al., 1994, Gerardi, 2003a):

- Reduction of methane gas content in the biogas and increase of CO_2
- Reduction of biogas production rate
- Decrease of alkalinity concentration followed by reduction of pH
- Reduced conversion of organic matter (or solid destruction)
- Rise of volatile fatty acid concentration and other fermentation intermediate products

2.3 Anaerobic digestion monitoring: state of the art, causes and control

Efficient instrumentation control and automation (ICA) in wastewater treatment is essential to maintain optimal processes, to reduce resources costs (i.e. energy use) and to satisfy the effluent discharge requirements. Anaerobic digestion, similarly to other biological waste treatment processes, can be monitored by indirect process stability indicators, such as substrate conversion measurements (COD or VS removal), intermediates substances accumulation (VFA, pH, alkalinity, H_2, CO), product formation (gas production rate, CH_4, CO_2) or direct indicators such as microbial communities (populations, diversity), or microbial kinetics.

However, in full scale industrial applications, anaerobic digesters are usually monitored with simple, economical and low maintenance instrumentation including the on-line measurements of (Vanrolleghem, 1995, Vanrolleghem and Lee, 2003, Speece, 1996, Mechichi and Sayadi, 2005 and Boe et al., 2006):

- pH. The acceptable pH range for anaerobic digestion is between 6.0-8.0. Fluctuations of pH can strongly influence the efficiency of the different microorganisms as the acid formation step, in the first phase of the process, has an optimal activity range between pH (4.0 – 6.5) while the methanogens require neutral pH conditions (6.5 - 8.2) for their optimal activity. In reactors with low buffering capacity, a pH decrease can indicate an accumulation of VFA; therefore pH can be a useful indicator of process imbalance.

  pH electrodes are widely applied in full scale digesters and as the immersion of these probes in sludge requires frequent maintenance, several automatic cleaning strategies were also developed: water spray, mechanical brushes, chemical or ultrasonic cleaning coupled with automatic calibration systems.

  However, in cases of wastewaters with high buffering capacity where acidification is delayed, pH measurements are ineffective to indicate process perturbations and are therefore not advisable for process supervision.
- Temperature. Its variation strongly influences the kinetics of the biomass activity. While acidogenic bacteria are resilient to temperature fluctuations, methanogenic bacteria are very sensitive to temperature changes. Methanogenic activity strongly increases with higher temperatures (30-80°C), while, at lower temperatures, their activity rate decreases and unbalanced metabolism can occur as the acidogenic

bacteria continue producing VFA. Temperature sensors (thermistors) are extensively used to control and maintain internal process temperature to the required range. Automatic control is widely implemented for regulating the use of heaters.

- Liquid flow rates and liquid levels. Inflow to the digester and sludge level inside the reactor are essential control parameters for maintaining an adequate process loading and solid retention time. Liquid flow meters are widely applied to the piping connection delivering inflow sludge and extracting digested sludge from the reactor. Water levels monitoring systems are generally based on floats with an internal electric switch, conductivity switches, differential pressure transducers, capacitance measurements and ultrasonic level detection.
- Biogas production. It is the most commonly monitored indicator with the use of different on-line flow meters, rotameters and thermal mass flow meters. It gives information on the overall process performance however, imbalanced states are revealed when perturbations have already affected the complex microbial ecology definitely (Moletta et al., 1994, Boe et al., 2010).
- Methane concentration. Biogas composition ratios are essential information for a deeper understanding of the process conditions, as lower concentrations of CH_4 and higher CO_2 production indicate instability. Typical instrumentations for CH_4 measurement were developed from the use of gas trapping bottles. Gas flow is measured before and after a "specific" gas trap (i.e. alkaline washes for CO_2 and H_2S) is put into place. The difference between the flows, before and after the trapping, will indicate the concentration of CH_4 in the biogas mixture. More advanced methodologies are based on infrared or gas chromatography analysers.
- Solids removal. Its measurements can inform on the quality of the effluent and therefore on the efficiency of the organic compounds degradation. Inflow solids data can also aid maintaining flow consistency, preventing overloading or underloading cases. The measurement of the solids is usually performed online with turbidity sensors (optical measurements, absorption of ultrasonic and gamma rays). Interference and maintenance problems usually occur, together with the need of frequent calibration.

In the last decades, as the understanding of anaerobic digestion microbiological process has improved and the awareness of a more robust monitoring strategy raised, several studies have attempted to develop more refined on-line instrumentation for the measurements of

COD, TOC, total VFA, acetate and dissolved CO_2 based on different methodologies (Table 2.3).

Table 2.3. Innovative on-line instrumentation for alkalinity, TOC, COD, VFA measurement and the different analytical methodologies applied (Vanrolleghem, 1995, Olsson et al., 2005; Steyer et al.,2006).

On-line sensor	Derived from classical instruments (pH, T,%CO_2)	TOC analyser	Titrimetric sensor	UV Spectrometer	FT-IR Spectrometer
Partial alkalinity			- 2 points		X
Total alkalinity			- 4 points - 8 points		X
Bicarbonate	X		- 2, 4, 8 points		X
Dissolved CO_2	X				X
TOC	X	X		X	X
Soluble COD				X	X
Total VFA			X	X	X
Acetate					X

Strong interest has been shown to the development of alkalinity measurement as it was recognized to be more reliable than pH, especially when monitoring wastewater with high buffering capacity. In digesters the acidification processes would not be revealed by pH decrease until final consumption of the total buffer capacity (Rozzi, 1991). Automatic bicarbonate monitors were developed based on titration (2, 4 or 8 points step titration) or on gaseous carbon dioxide developing from the sample when acidified (Vanrolleghem, 1995).

Dissolved hydrogen concentration measurement in the liquid phase is also considered a valid indicator of process monitoring as it is an important process intermediate and also regulates the substrate conversion potential of several anaerobic bacteria groups (Speece, 1996). During overload tests it proved to reveal process instability through its fast accumulation (Bjornsson et al., 2001)., however others (Speece, 1996) indicate the strong influence on hydrogen concentration of other external factors, which limits its application as a stand-alone indicator. Various methodologies have been developed for its on-line monitoring: hydrogen/air fuel cell detector, membrane-covered electrodes, trace reduction

gas analysers and hydrogen transfer through membranes and metal oxide semiconductor sensor (Bjornsson et al., 2001).

A direct indicator of process stability is the microbial community acting in the anaerobic process. Anaerobic microbial communities can be estimated by microscopic studies, characterization of membrane lipids, culture distribution patterns, genetic probes and immuno-techniques (Bjornsson et al., 2001).

An efficient on-line monitoring of alkalinity, VFA and microbial community, and an advanced understanding of the process theory would allow a full comprehension of the metabolic status and the biomass functioning, thus achieving a higher control and performance of anaerobic digesters, and allowing reduction of process instability and failures. However, the industrial full- scale application of the more complex developed control methodologies is strongly limited, due to the high costs, frequent maintenance and complexity. Traditional instrumentation, such as pH, biogas flow, temperature sensors and methane concentration instrumentation remain the most common and industrially applied. Generally, this commonly applied monitoring strategy is not sufficient to prevent process inhibition. The digester imbalance often reaches critical levels before it can be observed by these indicators, as they are all final products of the complex anaerobic process itself.

The timing between the beginning of a process perturbation and its evident indication is an essential factor for optimizing the control, at it is therefore matter of many studies and tests. Different results of organic overload, hydraulic overload or operational condition variations tests, revealing the variation effect on each indicator and the elapsed time before these variations were evident, is presented in Table 2.4.

Table 2.4. Perturbation typology and indicators variation timing after perturbations starts [+: increase; -:decrease; adapt: adaptation; d.: days; GPR: gas production rate (L gas prod l react^{-1} day^{-1}); CH_4 yield (LCH_4prod gCODin^{-1})]

Reactor type	Perturbation applied	Methane	pH	VFA	Alkalinity	Author
Up-low anaerobic filter reactor for olive mill wastewater	Hydraulic overload (HRT decrease + OLR increase)	++ CH_4 GPR - initial (1-2 d.) CH_4 yield but following adapt.	-- after 15 d.	. ++ acetate and propionate after 1-2 d.	ND	Mechichi andSayadi, 2005

	Organic overload	- CH_4 GPR after 15 d. -- CH_4 yield after 1-2 d.	**--** after 13 d.	++ acetate and propionate after 3 d.	ND	
	Temperature decrease	**--** CH_4 GPR andyield after 3d.	**-**after 3 d.	+ acetate and butyrate after 1 d.	ND	
	Temperature increase	**++** CH_4 GPR and yield after 3d.	**-**after 5 d.	+ acetate and butyrate after 1 d.	ND	
Mesophilic full scale reactor	Organic overload	ND	ND	+ acetate and butyrate after 1 d.	- after 1 d.	
Mesophilic lab-scale reactor (500ml bottles)	Pulse load of carbohydrate-rich sludge	**++** CH_4 GPR after 1 d.	ND	++ acetate and propionate after 1 d.	- after 1 d	Bjornsson et al., 2000
	Organic overload	-- after 15days	**--** after 15 d.	+ acetate and propionate after 3 d. + butyrate after 15 d.	- after 10 d	
	High carbohydrate-rich sludge overload	-- after 8days	**--** after 10 d.	+ acetate and propionate after 1 d.	- after 7 d	
Mesophilic lab-scale reactor (550ml bottles)	Organic overload	ND	-- after 3 d	+ acetate and propionate after 1 d.	ND	Bjornsson et al., 1997
Fluidized bed reactor	Short duration organic overload	ND	ND	++ acetate and propionate after 2 hours	ND	Moletta et al., 1994
	Long duration organic overload	-- after 12 hours	**--** after 8 hours	++ acetate and propionate after 2 hours	ND	

Even if an accurate comparison between the tests cannot be performed due to the different reactors configuration, operational conditions and parameters vary, the results suggest that traditional performance indicators, such as pH and gas production are not sufficient for an early detection of inhibition as the perturbation effect is evident just after at least one HRT, as a consequence of being final products of the anaerobic process.

Generally, pH shows small variation and reveals evident decreases many days after the perturbation has started, as its concentration is dependent on other parameters (buffering capacity, hydrogen solubility etc, acid accumulation etc). The response of gas production differs between the types and intensity of perturbation, as methane production often increases in the first stage of an organic overload and can then reach a new temporary stability, not revealing the state of imbalance in the reactor, and then lead to final inhibition (Mechichi and Sayadi, 2005). However, in all the observed cases specific volatile fatty acids, such as acetic and propionic acids, accumulation was evident within a short time after the beginning of the perturbations (1 to 3 days), while butyrate, iso-butyrate and valerate start accumulating after longer periods. From these results it can be derived that, in order to develop a robust and reliable on-line monitoring strategy for an early detection of anaerobic process failure, volatile fatty acids should be used as the main process status indicator as their accumulation directly reflect a kinetic uncoupling between the acid producers and consumers (Bjornsson et al., 2000; Pind et al., 2003; Mechichi et al., 2003: Boe et al., 2005).

2.4 Volatile Fatty Acids: main intermediates of anaerobic process

Volatile fatty acids, usually referring to acetic (C2), propionic (C3), iso-butyric, n- butyric (C4), iso- valeric and n-valeric acids (C5), are important intermediate substances of the complex anaerobic process and act as essential indicators of the performance and stability of the process. The understanding and analysis of their formation and conversion during anaerobic digestion allows a greater control of process stability.

Several studies have revealed that the conversion rates of each VFA to methane vary with the order of: acetic acid > ethanol > butyric acid > propionic acid (Wang et al, 2009). Acetate is the primary source of methane production, generally being responsible for 70% of the total gas produced (Schoen, 2009). Propionic acid is the second contributor to methane production; however its assimilation by methanogenic bacteria is generally delayed. The reason behind this stands in the thermodynamic of the process, as the Gibbs

free energy for conversion of propionic to acetic acid requires an energy source (Marchaim and Krause, 1992). When accumulation of the acid occurs, a shift in the microorganism activity is induced affecting the methanogenic bacteria, resulting in further accumulation of hydrogen and lower acid conversions. Propionic acid, being the most thermodynamically unfavourable is the first over other VFA to be affected. For this reason accumulation of propionic acid in the reactor has been widely recognized as a sign of process stress or failure.

Stafford (1981), while underlining the complexity of the anaerobic process and its different internal interactions, observed that certain ratios of propionic plus butyric acids over acetic acid revealed to be critical to methane production. Propionic to acetic acid ratio (P/A) were also analyzed with different organic overload tests by Marchaim and Krause (1993). In all tests the ratio, therefore the concentration of propionic acid, increased immediately after a feeding concentration increase, suggesting that the particular ratio could be used as an early indicator of stress in anaerobic processes.

Many studies have attempted to identify the maximum limit of acid concentration after which an inhibitory effect occurs on the overall process. However the inhibitory acid concentrations varies relatively to the different operational conditions, biomass activity and original sludge characteristics.

Generally for an optimal operating anaerobic reactor the normal concentration of volatile fatty acids is identified as 500 mgL^{-1}. Hill (1988) identified the upper limit of acetic acid concentration after which failure is imminent to 800 mgL^{-1}. This limit was confirmed by studies from Marchaim and Krause (1993) which included also a P/A ratio higher than 1/1.4 to the definition of inhibitory VFA level. Boe et al. (2008) utilized a concentration of propionic acid over 740 mgL^{-1}as the limit identifying a stress status of the process, requiring a modification of the loading regime. Wang et al. (2009), studying the inhibitory effects of different concentrations of acids on the methane yield, observed a strong reverse effect to biogas production with a propionic acid concentration of 900 mgL^{-1}.

2.5 VFA monitoring

As the concentration of VFA is an important indicator of process imbalance several methodologies for analytical measurement of the acids concentrations were developed and are widely applied. The measurement of the total VFA concentrations is usually based on titration methodologies (Moosbrugger et al., 1993; Powell and Archer, 1989) or indirectly

derived by the measurements of dissolved hydrogen in anaerobic digesters (Bjornsson et al., 2001). However, several studies have demonstrated that single VFA concentrations, such as propionic, iso-butyric and iso-valeric acids, can provide greater information of the status of the process (Ahring, 1995).

Therefore, few on-line methodologies have been developed based on gas chromatography (GC) or high- performance (or pressure) liquid chromatography (HPLC) which allow a precise and detailed measurement including information on each acid concentration. The samples for analysis also require a preliminary preparation (filtration or/and centrifugation).

Several modifications of these technologies have been diversely studied in order to obtain an optimal instrumentation for on-line VFA measurement in anaerobic digestion (Table 2.5). Most of the proposed solutions are based on different combination of "sample preparation" module such as microfiltration units (Pind et al., 2002; Diamantis et al., 2006; Molina et al., 2009), ultrafiltration units (Zumbusch et al. 1994; Steyer et al., 2001), chemical additions (Buchauer, 1998; Rozzi et al., 1999) followed by traditional off-line analytical instrumentations such as HPLC and GC and including complex mechanical tool and structures. Good estimations of the VFA were often obtained, but some drawbacks were also identified (Table 2.5), as all these methodologies required frequent maintenance (for membrane fouling problems, cleaning, backwashes, chemical additions or calibration) and high capital expenditure for the overall instrumentation.

Table 2.5. Methodologies developed for on-line VFA measurements with relative characteristics, advantages and drawbacks.

Sample preparation	**VFA Analysis**	**Advantages**	**Disadvantages**	**Authors**
Acidification and gas stripping	- GC-FID of headspace gas	-no fouling -single VFA measurement	- local calibration needed - chemical addition	Boe et al., (2005 and 2006)
Rotating filter and microfiltration, chemical addition	-Gas Chromatography	-single VFA measurement	- fouling problems chemical adding	Pind et al (2002)
Membrane filtration	-Gas Chromatography	- single VFA measurement	- membrane fouling - high maintenance	Slater et al. (1990) and Ryhiner et al. (1993)

Microfiltration, dilution	- Capillary gas Chromatography	- single and continuous VFA measurement	- membrane fouling - high maintenance	Diamantis et al., (2006)
Ultra-filtration module	- HPLC	- single VFA measurement -no chemical added	- fouling during sample preparation high calibration effort	Zumbusch et al. (1994)
Ultra-filtration membrane unit	Fourier Transform Infra-Red (FT-IR) spectrometer	-low maintenance -on-line multi parameter measurements	- Sample collection and ultrafiltration loop	Steyer et al. (2001)
Acidification	DENICON, titration biosensor	- low maintenance	- indirect measure - chemical addition	Rozzi et al., (1999)
Acidification	Titration	- accurate value	- Influenced by background levels of pH, carbonate - Frequent calibration needed	Buchauer (1998)
Microfiltration	Titration with AnaSense®	- accurate value	- Frequent calibration	Molina et al., (2009)
pH value measurement	Empirical model for pH and VFA relation	- no sample preparation - fast data collection	- Model limitations	Münch and Greenfield, (1998)

Münch and Greenfield (1998) proposed a VFA measurement methodology based on simpler on-line pH, alkalinity data and the modelled theoretical relationships between these indicators. An acceptable estimation of the data was reached by the model but the complexity of the theoretical relations and assumptions were limited to the specific case and low flexibility to different conditions was established.

The Fourier Transform Infra-Red (FT-IR) spectrometer was applied to measure on-line COD, TOC and VFA by Steyer et al., (2002) utilizing the property of a unique absorbance

pattern of each compound. Even if the infra-red spectrometry has the advantage of not requiring any chemical addition, the necessary sample preparation and ultrafiltration limits the possible low-cost on-line applications in full scale anaerobic digesters.

A solution to the filtration limitations present in the previous VFA instrumentation was proposed by Boe et al. (2005, 2006) where the gas chromatography for VFA measurement was applied to the gas headspace instead of the liquid phase. A stripping methodology for lowering the organic acids solubility was optimized with pH lower than 2, temperature reaching 65°C and addition of $(NH_4)_2SO_4$ salt. The comparison between the VFA values measured with this new methodology and with the off-line analysis on the liquid phase showed good agreement (r^2=0.9) and with this solution the sample filtration maintenance problem was eliminated, allowing the use of thick sludge, manures and solid waste. The instrumentation was then applied for the control of lab-scale CSTR manure digesters, and the propionate concentration (measured by GC) was used as a control parameter for monitoring the biogas process (Boe et al., 2008). With the application of a simple logic controller (using a programmable logic control PLC) regulation of the feed volume was based on the propionate concentrations. Overloading of the process was prevented, even if the efficiency of propionate as a control parameter was affected by its long term accumulation in the reactor, therefore causing a delay in the control response. Even if this methodology had excluded liquid filtration units, and therefore any fouling issues, a requirement for maintenance still remains as the sensor requires heating, acidification and salt additions. Furthermore, the headspace gas chromatography for the VFA analysis is expensive, and therefore its application is limited for full scale application, even if it is supported that information on single acid concentration (achievable only via chromatography) can deliver greater information and understanding of the process instability.

Rozzi et al. (1997) modified the nitrate measurement sensor, DENICON, to have an indirect measurement of VFA as readily biodegradable COD (rbCOD). Digested effluent is added to denitrifying bacteria in excess of nitrates. As denitrification reaction occurs, one mole of OH^- is produced for every mole of nitrate removed; therefore the measurement of the acid, added by titration, required to maintain the process neutral, can be linked to the amount of organic carbon injected. A linear correlation between the volume of nitrate reduced and the volume of acids inject was obtained with satisfactory r^2=0.9. This

methodology was then applied to monitor and characterize the incoming wastewater to the anaerobic reactors, in order to identify potential instability cases.

2.6 Anaerobic digestion automatic control

The information derived from the monitoring sensors of anaerobic digestion are generally used to perform operation and feeding regime modification in order to maintain higher process stability.

Until 1990 very little automatic control was present in anaerobic digestion and the control laws were often based on simple on/off or proportional–integral (PI)/ proportional–integral–derivative controller (PID) controllers. Since then many researchers have developed more complex monitoring procedures, applying the increasing biological phenomena understanding and new on-line sensors. The combination of the information derived from on-line monitoring instrumentations with process representation described by mathematical models has been tested with the aim of obtaining a more efficient and robust control of anaerobic digestion (Olsson et al., 2005).

However the complexity of the process itself and the structure of the monitoring strategies based on the interaction between sensor and models require to address and consider the following issues:

- on-line instrumentation measurement validation/confidence index: as control laws are based on direct measurement from on-line instrumentation the reliability of the measured value is essential for a correct plant operation. In cases of sensor fouling, breakdown or other dysfunctioning, a real time validation or cross validation is essential to identify sensor fault before the signal is used for parameter monitoring or closed loop control and leads to large deviations of the process from its normal condition (Steyer et al., 2004; Steyer et al., 2006; Olsson et al., 2005).
- the complexity of the anaerobic digestion process and its microbiological content can be represented by elaborate and composite models, such as the ADM1 (Batstone et al., 2002). Many difficulties arise when using these models for on-line automatic control as they are problematic to calibrate and validate and not all required parameters can be obtained and the control implementation becomes very complex (Stamatelatou et al., 1997; Steyer et al., 2006; Olsson et al., 2005). Therefore, even the

complex process reality is simplified, uncomplicated models are generally preferred for ICA applications.

- uncertainty from sensors measurement, control structure and model definition must be taken into consideration in the validation of the control system (Lardon et al., 2004; Olsson et al., 2005).
- definition of appropriate performance indicators and manipulated variables. A review of the variables used in different applications reveals a predominant use of feed flow, inflow dilution, acid or base dosing (Table 2.6). The performance indicator varies between different applications, following the control structure requirements (Table 2.6).

Table 2.6. Manipulated variables and performance indicators.

Manipulated variable	Performance indicator	Author
Inflow dilution rate	CH_4 production rate	Pullammanappallil et al., 1997
Inflow dilution rate	CH_4 flow	Monroy et al., 1996
Inflow dilution rate	COD effluent	Alvarez-Ramirez et al., 2002
Inflow rate	Biogas production, pH	Steyer et al., 1999
Feed flow manipulation	Biogas flow rate	Olsson et al., 2005
Inflow dilution rate	COD, VFA	Olsson et al., 2005

- the problem of the dynamic nature of the process and therefore static methods are not sufficient for a robust control. All the levels of process complexity, in both space and time scale, should be taken into account when developing an efficient control strategy (Olsson, 2006).

The selection of an appropriate control law is dependent on the plant characteristics, available sensors, instrumentations and data/model available. Several methodologies have been tested by different researches and the benefits and drawbacks can be compared (Table 2.7).

Table 2.7. Different possible control approaches to monitor anaerobic digestion process.

Type of controller	Adequate use cases	Benefits/ Drawbacks	Authors
PI/PID	Little data available Low understanding of process behaviour. No valid mathematical model	Simple and easy applications (single input/output or linear cases). Not comprehensive.	Olsson et al., 2005
Disturbance monitoring/control algorithm	Small disturbances to the process	Requires simple sensors. Useful for AD start-up	Steyer et al., 1999
Artificial neural networks	When large data is available.	High standard results can be obtained. Problems with the adaptive learning process.	Olsson et al., 2005
Fuzzy logic	When good knowledge of the plant ad the process is available. Low amount of data available	Can handle process non-linearity, multiple inputs/outputs.	Punal et al., 2002; Marsili-Libelli, 1992
Adaptive control (linear based)	When a linear mathematical model is valid	Efficient control strategy. Considers uncertainty.	Monroy et al., 1996; Alvarez-Ramirez et al., 2002
Adaptive control (non linear based)	When non linear model is valid.	Efficient control strategy. Considers uncertainty.	Olsson et al., 2005

Overall, it can be observed that different robust tools for control and automation have been developed and their efficiency tested at laboratory and pilot scales (Table 2.7). However the full scale application of these methodologies is very limited to particular cases, as they are all characterized by a complexity which cannot be addressed in a real scale plant. There is still a need to develop a simpler and easily applicable system that can contribute to the overall decision support system. Future developments should aim at utilizing simple methodologies and at integrating the overall operation system in order to reach higher applicability, reliability and economical benefits.

2.7 CO_2 headspace monitoring methodology

The headspace methodology is based on the measurement of the CO_2 produced by the degradation of organic compounds during denitrification in anoxic conditions and captured in the headspace of a sealed vessel (Li et al., 2002, Crowley, 2007). The rate of CO_2

production, carbon dioxide evolution rate (CER), and its transfer in the headspace reflects the bacterial utilization of the carbon for denitrification processes (Li et al., 2002) as also demonstrated by the mathematical model approach for the kinetic constants of denitrification in anoxic conditions (Sperandio and Paul, 1997)

2.7.1 Denitrification process: VFA as carbon source

The denitrification process, determining the reduction of nitrate ions to nitrite and then to nitrogen gas, is due to facultative heterotrophic bacteria which, in anoxic condition (O_2 less than 0.5 mgL^{-1}) utilize a carbon source (acetic acid in the following formula) as substrate and derive the oxygen from the nitrate (NO^-_3) molecule (Gerardi, 2003b).

$$5CH_3COOH+8NO^-_3 \rightarrow 8HCO^-_3 + 2CO_2 +6H_2O + N_2$$

A readily available carbon source is therefore an essential condition and the proportion between C/N is a important indicator for the denitrification process to occur.

Traditionally diverse forms of external carbon source have been added to the denitrification processes (i.e. methanol, ethanol, acetate etc). However to respond to the high OPEX of the use of external carbonate additions, the use of internal carbon source for enhancing denitrification process has developed. Several studies have demonstrated that internal carbon sources, derived from prefermentation of influent wastewater (McCue et al., 2003), from anaerobic fermentation of organic waste (Bolzanella et al., 2001) or sewage sludge (Soares et al., 2010) and surplus activate sludge disintegration (Kampas et al. 2007, Soares et al., 2010) can successfully be used for enhancing the denitrification process.

According to the Stoichiometry of the reaction, 2.6 g COD are required for the removal through denitrification of 1 gNO3-N, however many studies often find diverse and higher requirements, ranging between 4 g- 15 g COD with a minimum C/N ratio of 3.5-4 (Bolzanella et al., 2001). Similar diversity of results is observed in different denitrification rates obtained by different carbon substances in several studies as a consequence of the different operational conditions and instrumentations (Elefsiniotis et al., 2004 and 2007). However, a common result between the different studies is the higher denitrification rate obtained with the use of a mixture of VFA compared to single acid. Xu, (1996) observed the highest denitrification rate of 0.754 $mgNO_3$-N with a mixture of VFA, while single acid determined lower rates, with acetic acid having the highest conversion rate (Table 2.8).

Table 2.8. Comparison of the effect of different carbon source on denitrification rates (modified from Elefsiniotis et al., 2004)

Carbon source	Denitrification rate mg NO_3-N per mg VSS day
Mixed VFA	0.754
Acetic acid	0.603
Propionic acid	0.362
Butyric acid	0.519
Valeric acid	0.487
Methanol	0.289
Ethanol	0.349
Digested sludge supernatant	0.575

Moser and Engeler (1998) also obtained a higher denitrification rate with the use of a mixture of volatile fatty acids than with single acetic acid use, respectively 0.144 and 0.091 gNO_3-N g^{-1}COD day^{-1}. Elefsiniotis et al., (2004), performing a comparison between the preferential carbon source consumptions in denitrification processes, also observed that naturally formed VFA are an excellent carbon source for the denitrification process and that acetic acid is the preferred and fastest carbon substrate to be consumed. Overall it can then be deduced that short chain volatile fatty acids, obtained during fermentation of sludge, are an efficient substrate for denitrification processes (Fass et al., 1994; Bolzanella et al., 2001; Elefsiniotis et al., 2004).

Several tests have shown that generally the composition of fermented sludge is composed by readily carbon source with a volatile fatty content between 70-95%, as expected by the theoretical carbon substrate degradation during the fermentation processes. Moser and Engeler (1998) observed that 85% of the sCOD in fermented sludge was composed by VFA. Furthermore, fermentation tests on different types of primary sludge by Soares et al. (2010) presented a VFA composition varying between 69-94%.

The COD equivalent to VFA can be derived by multiplying the concentrations of each acid by a factor based on the complete oxidation of the acids (Lie and Welander, 1997; Münch and Koch, 1998) (Table 2.9)

Table 2.9. COD equivalent for VFA acids

Volatile fatty acid	COD equivalent gCOD g acid^{-1}
Acetic acid	1.066
Propionic acid	1.512
Butyric acid	1.816
Valeric acid	2.036

2.7.1 CO_2 headspace method for internal carbon addition control in denitrification

The headspace CO_2 methodology was developed by Li et al.(2002) as an indirect measure of the concentrations of internal carbon source additions to denitrification processes, as this requires a more complex monitoring and control system, compared to external carbon addition as the dosing rate can depend on the varying influent sludge characteristics and also on the external and process conditions. Furthermore, the traditional methodologies for carbon sources measuring in wastewater including BOD, COD, and respirometry are not appropriate for anoxic conditions where oxygen consumption is not involved in the reaction (Lie et al., 1997; Li et al., 2002). It was thus proposed that another metabolic product, CO_2, could be used for the estimation of the carbon addition in denitrification reactions (Li et al., 2002).

The study by Li et al. (2002 and 2003) demonstrated that the CO_2 headspace sensor, formed by a vessel partially filled with denitrifier biomass in excess of nitrate, constantly vented by nitrogen gas, equipped in the headspace with a carbon dioxide infrared sensor, was able to monitoring the variations of CO_2 concentration in the headspace from the denitrification reaction after the addition of sodium acetate (as internal carbon source). A correlation between the CO_2 profiles observed in the headspace and the external carbon source addition was observed. In particular, a linear correlation between the available carbon source in the system and the elapsed time (E time), time between the initial increase in CO_2 and starting decrease (Figure 2.2), was estimated (Figure 2.3) (Li et al., 2003).

On a further application of the same principles by the same author (Li et al., 2004), the methodology was tested with injections of thermophilic anaerobic digestion supernatant, and similar CO_2 evolution rates were observed(Li et al., 2004).

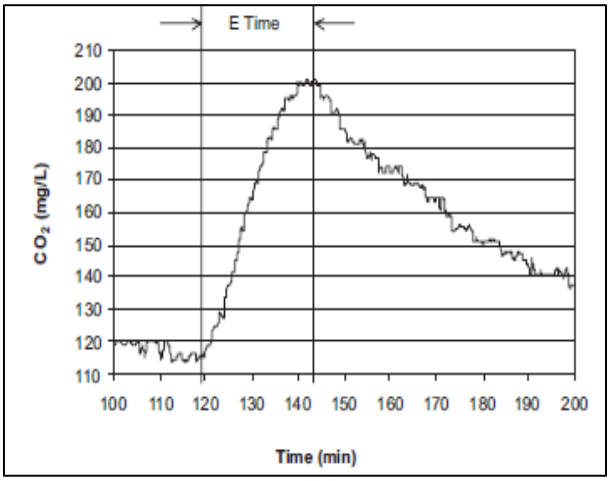

Figure 2.2. CO_2 profile after injection and definition of elapsed time (Li et al., 2002)

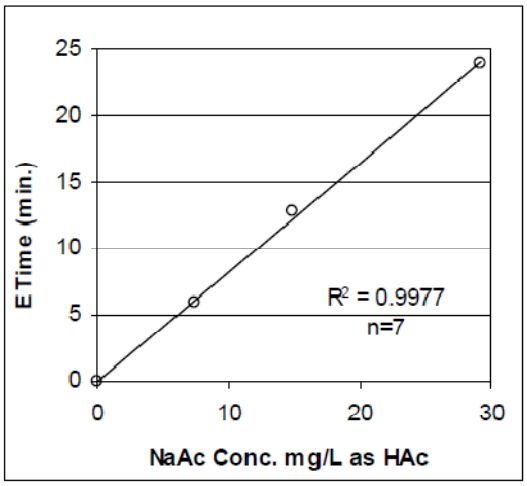

Figure 2.3. Linear relation between initial NaOAc concentrations and E time during denitrification processes (Li et al., 2003)

The method was considered a valid alternative for monitoring the "VFA equivalent" in thermophilic anaerobic digestion supernatant used for denitrification enhancement with indirect observations of the CO_2 production (Li et al., 2004). Furthermore, the advantages of requiring low maintenance for not being in direct contact with the sludge samples and the low cost of this methodology were also observed (Li et al., 2004).

The carbon dioxide headspace methodology was applied in a further study (Crowley, 2007), where the CO_2 production, developed from the denitrification reaction occurring in the sensor vessel, was correlated to the addition of different concentrations of acetic acid, propionic acid and primary sludge fermentation products. Similar CO_2 evolution rates as in Li et al. (2002 and 2004) were observed in the headspace of the CO_2 headspace sensor from the denitrification process occurring with the injection of carbon substrates (sCOD). To establish a good correlation between different sCOD concentrations and the sensor response, several variables from the CO_2 evolution rate were analyzed (i.e E time, CO_2 produced, rate of CO_2 production and relative ratios). It was observed that the best correlation was obtained with the rate of CO_2 production normalized with the MLSS in the denitrifier biomass. Coefficients of determination over 0.98 were obtained for all tested substrates, such as acetic acid, mixed VFA and fermented products.

When considering both the external and internal carbon source data in one single graph a linear correlation with coefficient of determination of 0.93 was obtained (Figure 2.4), proving that the rate of CO_2 production in the CO_2 headspace sensor was the same for all substrates.

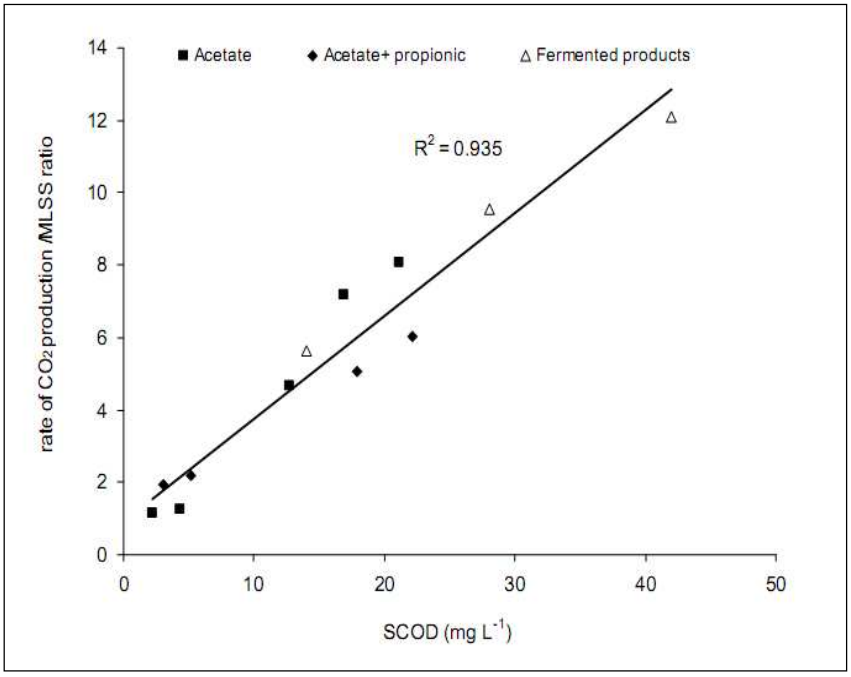

Figure 2.4. Correlation between sCOD and the rate CO_2 production $MLSS^{-1}$ ratio for different substrates (Crowley, 2007)

It was, therefore, deduced that it is feasible to indirectly estimate the sCOD-VFA present in anaerobic digestion fermented sludge from the established correlation between sCOD injected in the CO_2 headspace sensor and the CO_2 produced from the denitrification reaction and accumulated in the headspace of the sensor- vessel (Crowley, 2007).

In conclusion, it was thus suggested to further investigate the possibility of applying the same methodology for an indirect monitoring of the accumulation of sCOD-VFA during the fermentation step in the anaerobic digestion process from the CO_2 evolution rates observed in the headspace of the senor after an injection of digestate sludge.

2.8 Conclusions

The current system for full-scale anaerobic digestion on-line monitoring and control is generally based on measurements of pH, gas production and gas composition. Simple measurement instrumentation and sensors for these are available and widely applied. However, the information obtained from these indicators is not sufficient to identify process inhibition or imminent failure as they are all final products of the process and reveal perturbations with few days of delay. On the contrary, volatile fatty acid, the main anaerobic process intermediates, have been widely recognized as a successful indicator of process imbalance as their accumulation can reveal biological imbalance between the acid producing and consuming bacteria. For this reason, several studies have attempted to

develop on-line instrumentation for VFA concentration measurements. All the developed technologies present some limitations to the full scale application as they require sample preparation, costly analytical instrumentation and high maintenance for fouling problems. It is therefore evident that there is a lack of full-scale applicable instrumentation providing efficient, robust monitoring and control of anaerobic processes. Furthermore, if considering the increasing interest in optimising the capacity of anaerobic reactors for increasing renewable energy production and the future potential applications of anaerobic co-digestion with organic solid waste or industrial waste treatment, there is an evident need for development of innovative process monitoring technologies. Increasing organic loads to the digesters will surely have an important effect on the process stability. There is therefore a need to develop an innovative, efficient and inexpensive volatile fatty acids sensor which could aid in process control and optimisation.

In this study, in order to develop a sCOD-VFA sensor which overcomes the existing limitations, the CO_2 headspace methodology was tested as an on-line instrumentation for detecting acid accumulations in anaerobic digestion. The information on acid accumulation derived by the sensor was then utilize to regulate the reactor feeding and therefore optimizing its efficiency while maintaining a stable process.

3 Material and Methods

The experiments were designed to test the suitability of the CO_2 headspace sensor for monitoring of sCOD-VFA concentrations in laboratory, pilot and full scale anaerobic digesters. Loading variation tests were performed on the anaerobic digesters (at laboratory and pilot scale) in order to demonstrate the possibility of using the sensor as an early indicator of anaerobic digestion process instability. Three laboratory scale anaerobic digesters were operated at Cranfield University for a series of tests, followed by the use of two pilot scale digesters located at Knostrop STW (Leeds, UK). Finally, full scale digester samples from Esholt STW (Bradford, UK) were tested with the CO_2 headspace sensor.

3.1.1 CO_2 headspace sensor set-up

The sensor was set-up in a polypropylene vessel of total volume of 3 L. The vessel contained 1 L of denitrifying sludge, collected from the anoxic activated sludge tanks of Cotton Valley WWTP (Milton Keynes, UK) (Figure 3.1) and the remaining 2 L reactor capacity acted as gas headspace volume. An infra-red CO_2 probe (detection range: 0- 2000 ppm, Vaisala® GMD20 NDIR, Helsinki, Finland) was positioned in the headspace of the vessel and connected to a data logger (Daqpro 5300, Fourier System, Fairfield, USA) for CO_2 concentration data recording (one data per minute). The sludge was continuously mixed by a magnetic stirrer to keep the biomass suspended and was vented with pure nitrogen gas at 0.1 L min^{-1} flow. Anoxic condition was verified measuring the liquid sludge dissolved oxygen using a DO sensor (Jensen, London, UK) and the temperature was constantly maintained at 20 ± 0.5°C. Pressure and relative humidity were considered since the vessel was opened to atmosphere.

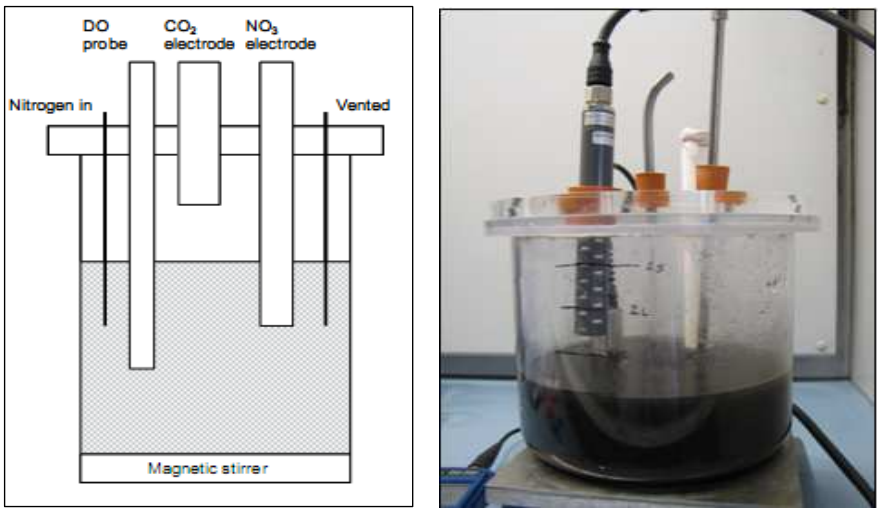

Figure 3.1.Scheme and photo of the CO_2 headspace sensor with CO_2 probe, DO probe instrumentation and nitrogen gas flux inlet and outlet and magnetic

3.1.2 CO_2 headspace sensor operation

Before each test, the sensor was stabilised by N_2 sparging for at least 20 minutes (until the DO value was constant) and the CO_2 concentrations values were consistent (no higher variation than 20 ppm for ten consecutive minutes). Measurement of denitrifying sludge MLSS was performed every second day.

To calibrate the sensor, known concentrations of acetic acid (from 99.5%, Sigma-Aldrich, UK) were injected into the sensor vessel in concentrations varying between 1–10 g L^{-1} in 5 ml volume (Table 3.1). The sensor response was analyzed in terms of CO_2 increase in the headspace, elapsed time (E-time: time between the injection and the peak of CO_2 production) and rate of CO_2 production. Totally 40 tests were performed over the total length of this study in order to construct a reliable calibration curve. For the determination of the sCOD-VFA in the digestate, the regression line was then applied to the measured CO_2 concentration. Between different acids or digestate injections, 2 to 5 hours intervals were required for the re-stabilization of CO_2 readings.

Table 3.1. Acetic acid concentrations and COD equivalent for calibration curve tests

Acetic Acid [g L^{-1}]	sCOD [g L^{-1}]	sCOD added in sensor [mg]
1	1.1	5.3
2	2.1	10.7
3	3.2	16.0
4	4.3	21.3
5	5.3	26.6
6	6.4	32.0
7	7.5	37.3
8	8.5	42.6
10	10.7	53.3

3.2 Laboratory scale anaerobic digester

3.2.1 Anaerobic digester rig set-up

Three 5 L culture vessels glass Quickfit® (Fisher, Loughborough, UK) were set up as continuously stirred tank reactors (CSTR) (Figure 3.2). The digesters were inoculated with 4.5 L of digested seed sludge collected from Esholt STW (Bradford, UK) (Figure 3.2) and digester operated at a sludge retention time of 15 days for the biomass acclimatization phase. Continuous mixing was provided by 3 metal agitator shafts by Heidolph motors (RZR 2020 and 2102, Kelheim, Germany). The temperature was controlled at 35 ± 0.5 °C

by submerging the digesters in a water bath. In each digester, four outlets were present and two were used to feed batch once a day primary sludge collected at the primary sedimentation tanks of Cranfield STW (Cranfield, UK) characterized by variable total solid between 2.6-6.8 % and volatile solids between 74-91% at collection., and one outlet was used for gas collection. The biogas produced during the anaerobic process was collected and stored in gas tight bags in Tetrapak® material (Wrexham, UK) which were connected to a top outlet of the digester. Measurements of the biogas production were performed with a 0.1 L gas tight syringe (Fisher, Loughborough, UK). Digested sludge was sampled daily with a 0.05 L syringe (Fisher, Loughborough, UK) for further analysis.

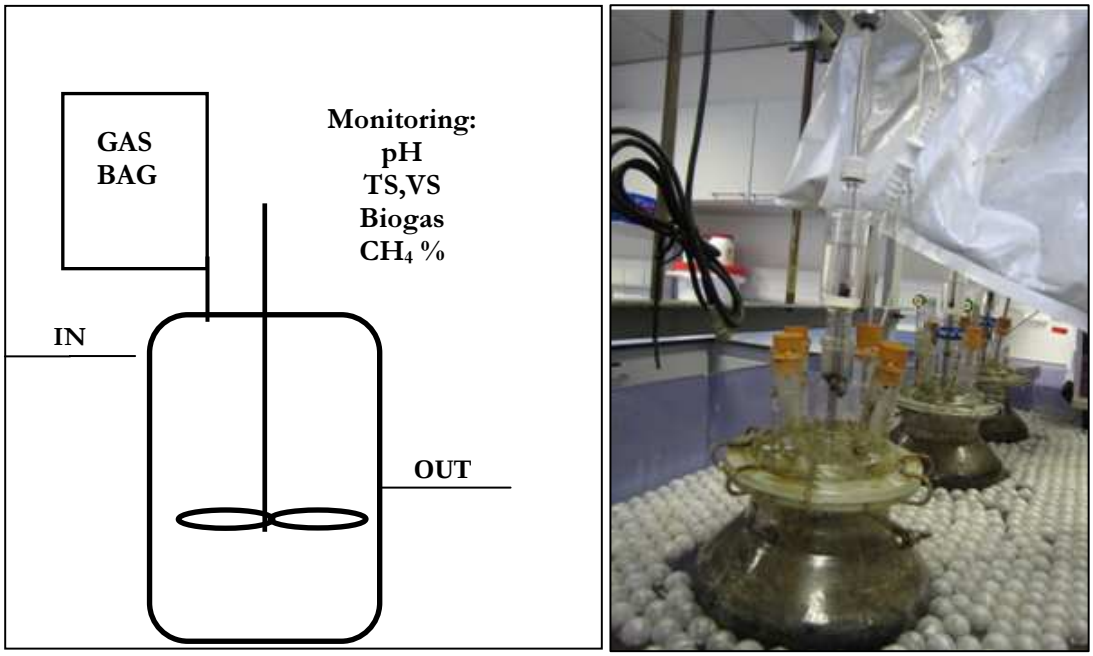

Figure 3.2.Scheme and photo of the anaerobic digestion rig. The reactor were constantly mixed by mechanical stirrer and kept at 35°C by a hot water bath. The Tetrapak gas bags for biogas collection are attached on top of reactor.

3.2.2 Anaerobic digester operation

The digested biomass was acclimatized and the digesters started up with a solid retention time of 15 days for three retention times (45 days).

For each experiment three anaerobic reactors were run in parallel (Figure 3.3) and inflow and outflow sludge daily samples were characterized each day. A first digester, "Sensor monitoring", was monitored with the traditional monitoring parameters (pH, biogas production and composition, total and volatile solids removal, alkalinity, sCOD removal)

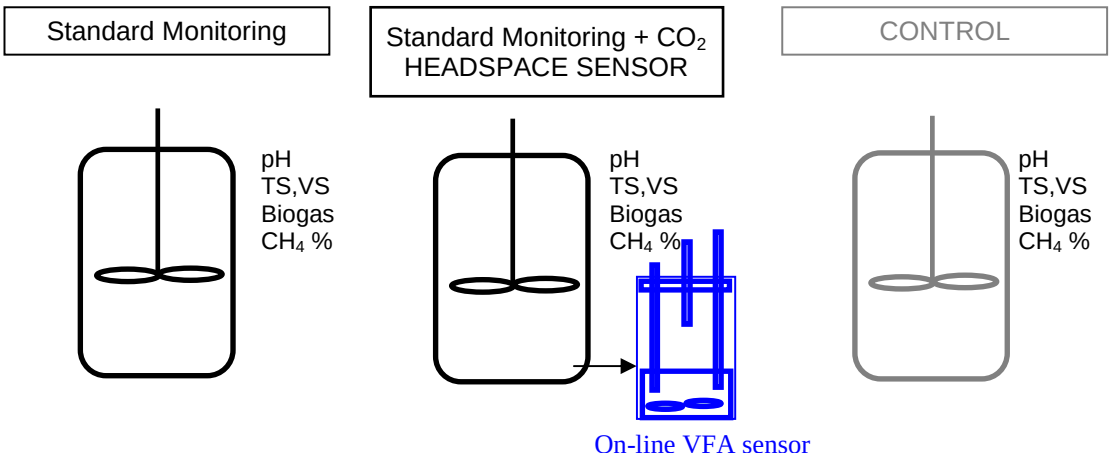

Figure 3.3. Laboratory tests set-up and comparison of the monitoring strategies for the

and with the CO_2 headspace sensor to detect, early signs of sCOD-VFA accumulation due to the perturbations applied.

The second digester, "Standard monitoring", was monitored with the traditional anaerobic digestion monitoring parameters such as pH, biogas production and composition, total and volatile solids removal, alkalinity and sCOD removal. The perturbations were applied until evident signs of process inhibition or failure were shown.

A third digester, "No perturbation control", was operating as a control digester, maintained with optimal feeding and operating condition (SRT =15 days) in order to perform an overall comparison with the other digesters.

A series of perturbations (Table 3.2) were applied in the digesters according to the following:

Three tests were performed at laboratory scale (Table 3.2):

1. Organic underload test

   A reduction of the total solid content in the inflow primary sludge from the original 2.6-6.8 % was performed through dilution of the sludge with tap water and applied to both the "Sensor monitoring" and "Standard monitoring" digesters while the solid retention time was maintained at 15 days. The feeding regime of the "Sensor monitoring" was modified according to the information derived from the CO_2 headspace sensor, while for the "Standard monitoring" digester the underloading was maintained until evidence of process perturbation was revealed by the standard indicators.

2. Organic overload test

An increase in the total solid content in the inflow primary sludge from the original 2.6-6.8 % was performed by centrifugation of the sludge and applied to both the "Sensor monitoring" and "Standard monitoring" digesters while the solid retention time was maintained at 15 days. The feeding regime of the "Sensor monitoring" was reduced to the optimal regime according to the information derived from the CO_2 headspace sensor, in order to maintain process stability; while for the "Standard monitoring" digester the organic overload was maintained for the entire duration of the test.

Table 3.2. Laboratory scale anaerobic digestion tests definition and characteristics

Perturbation test	Feed (L day^{-1})	SRT (days)	Monitoring parameters	Digester perturbed
Organic underload	0.3 (Primary sludge at 3% dry solids)	15	sCOD, N, VS, TS, VFA, pH, Alkalinity, Biogas V, CH_4%	"Sensor monitoring" + "Standard monitoring"
Organic overload	0.3 (Primary sludge at 8% dry solids)	15	sCOD, N, VS, TS, VFA, pH, Alkalinity, Biogas V, CH_4% "	"Sensor monitoring" + "Standard monitoring"

For the digester monitored with the CO_2 headspace sensor, a basic control law was developed based on the sCOD concentrations given by the CO_2 sensor, to control the feeding regime variations. The sCOD-VFA concentration inhibition level, for which the feeding was changed, was defined as 1.2 g L^{-1}. This limit value was derived by the result of previous studies, where 0.8 g L^{-1} acetic acid concentration was identified as process inhibitory concentration (Hill, 1988) and considering that approximately 70% of the volatile fatty acid present in an anaerobic reactor is acetic acid (Speece, 1996)

The control algorithm applied in the test was:

If (sCOD concentration at time t) < 1.2 g L^{-1} then Feeding (time t+1) = Feeding (time t)

Else Feeding (time t+1) < Feeding (time t)

The feeding rate of the digester "Sensor monitoring" was then changed according to the results from the CO_2 headspace sensor showing accumulation of sCOD-VFA, in order to maintain higher process efficiency and maximize the biogas production avoiding process failure.

3.3 Pilot scale anaerobic digester

3.3.1 Pilot plant description

The pilot plant (Figure 3.4) consisted of two continuously stirred tank reactors (CSTR) reactors running in parallel, each with a total capacity of 1.0 m^3 and a working volume of 0.7 m^3 located at Knostrop STW (Leeds, UK). Mixing was provided by a peristaltic pump (Verder LTD, Leeds, UK) working at an intermittent basis (5-6 hours a day with 3600 L h^{-1} capacity). The digesters were kept at constant mesophilic temperature (35°C±1) by trace heating (Tyco Thermal Controls, Washington, UK) regulated by temperature probes (Endress and Hauser, Manchester) located inside the digesters. Gas production was measured through two thermal gas flowmeters (Flotech Ltd, Stockport, UK) connected to the digesters gas outlets and then ejected into the environment. Biogas composition measurement was performed daily with a gas analyser measuring CO_2 and CH_4 concentrations (GasData LTD, Coventry, UK). Monitoring of pH values in the reactor was performed with two pH monitor probes (Endress and Hauser, Manchester) located in the outlet weirs of the digesters.

Primary sludge for digester inflow was derived from the primary settling tanks of Knostrop STW and delivered twice a week in a feeding retention tank (600 L). Mixing was provided by a peristaltic pump (Verder LTD, Leeds, UK) working at an intermittent basis, in order to maintain the sludge in a homogeneous condition.

Two peristaltic pumps (Verder LTD, Leeds, UK), working intermittently and regulated by a timer, provided a controlled inflow (45-85 L d^{-1}) from the feeding tank into the two reactors, and the same volume of digested sludge automatically overflowed through the outlet weir and overflow chamber to the individual digested sludge storing tanks (1 m^3 each).

3.3.2 Pilot plant operation

The reactors were initially inoculated with mesophilic sludge from a full scale digester (Wakefield, UK) and, for the acclimatization period (50 d), the solid retention time (SRT) was kept at 15 days. The inflow primary sludge, characterized by TS of 4-7.5%, was fed daily into the digester with increasing volumes during the acclimatization period (from 15L to 45L with daily increase of 5L). The reactors performance was monitored daily with measurement of the digested sludge pH, temperature and biogas production and

composition. Offline daily characterization of alkalinity; TS, VS, sCOD and VFA for the inflow feeding sludge and the outflow digested sludge was also performed.

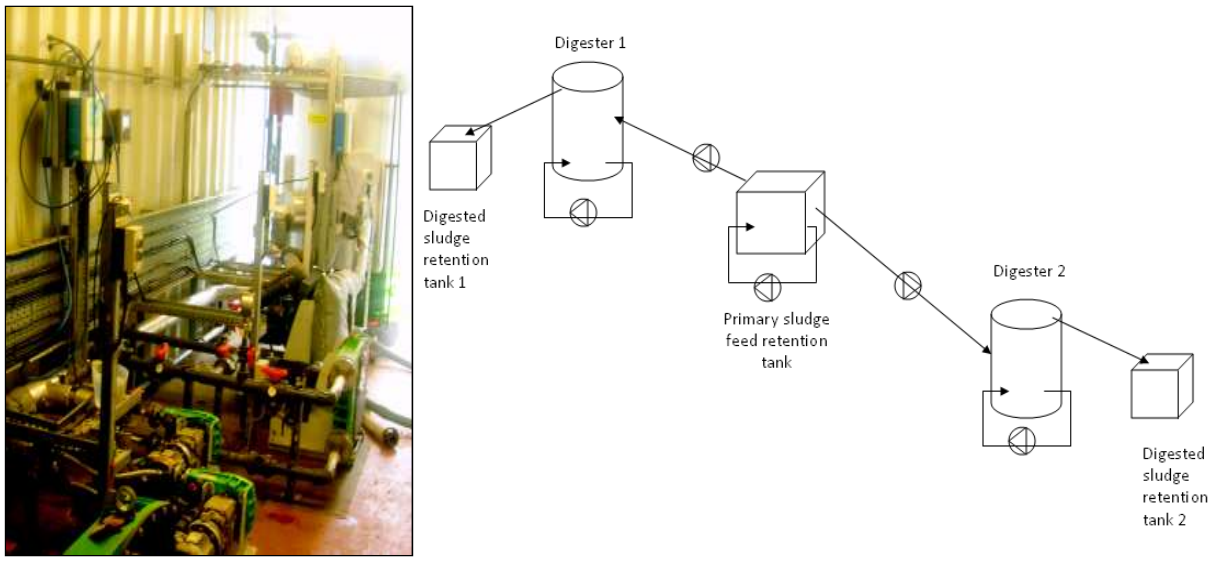

Figure 3.4. Pilot Plant anaerobic digestion rig scheme and picture with details of connected feeding, outflow and mixing piping, pumps and storage tanks.

An organic overload test was developed in parallel in the two pilot anaerobic digesters (Figure **3.5**) through the reduction of the solid retention time from the optimal 15 days to 8 days. One digester, "Sensor monitoring" was monitored with the standard monitoring parameters while the other was also tested daily with the CO_2 headspace sensor for detecting accumulation of sCOD-VFA. Similarly to the laboratory scale test, the additional information derived from the sensor response was utilized to control and regulate the feeding regime. In cases of high accumulation of acids in the digestate liquor over 1.2 g L^{-1} (as previously defined) the SRT was increased to the optimal 15 days until the sensor could detect a reduction in the digestate liquor acids. This allowed modification to the feeding regime in order to optimize the digester working volume while preventing high VFA accumulation, and maintaining higher process stability compared to the "Standard Monitoring".

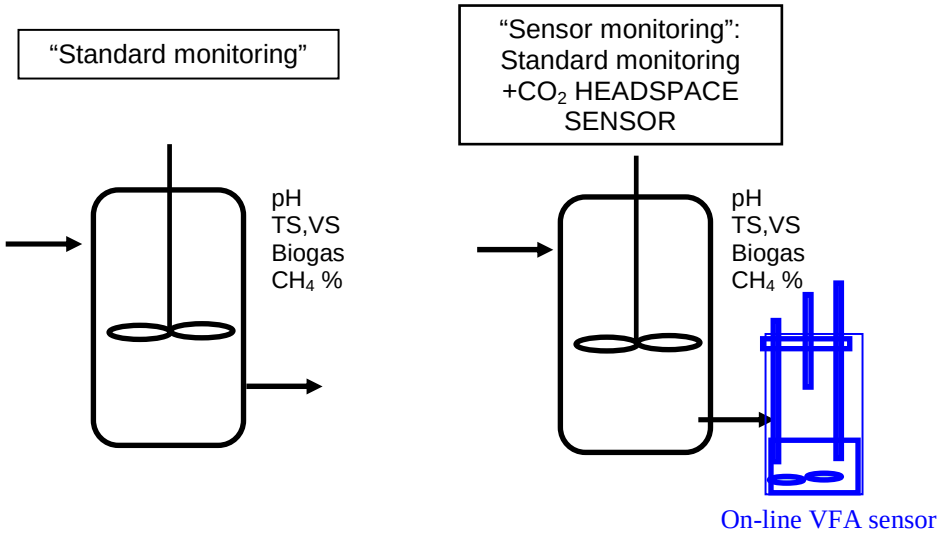

Figure 3.5. Pilot tests set-up and comparison of the monitoring strategies for the two reactors

3.4 Full scale digester

The full scale tests were performed with the digested sludge sampled from Esholt SWT (Bradford, UK) digester n.3. Esholt STW has a population equivalent of 730000. Three anaerobic digesters (Figure 3.6) processed a mixture of primary sludge and surplus activated sludge (SAS) from the site and imported sludge from three other sites(Ripon STW, Harrogate STW, Skitpon STW (Bradford area, UK)). Each digester had a working volume of 3490 m^3, the sludge has a solid retention time of 15±4 days and was maintained at 35±1.2 °C by heaters. The mixing of sludge is performed by a constant recirculation of the sludge and by an intermittent use of gas flow recirculation. The biogas produced, after impurity removal via scrubber, is used for a combined heat and power generation (CHP).

The digester was monitored for 15 days within one month with the use of the CO_2 headspace sensor. Digested sludge sampling was performed every two days and the sensor response was then compared with other monitoring parameters such as temperature and gas production and feeding regime.

Figure 3.6. Full scale anaerobic digesters, Esholt STW, UK.

3.5 Analytical analysis methodologies

Mixed liquid suspended solids (MLSS) concentrations analysis were performed filtering 100 ml of sludge sample using 0.45μm filters and determined as described in Standard Methods (APHA, 1998). Total and volatile solids were measured following the Standard Methods indications (2540B and 2540E). For total alkalinity, soluble COD (sCOD) and VFA measurements, prior to analysis samples were centrifuged at 5000 rpm for 15 min (Thermo Scientific Sorvall, Basingstoke, UK with a relative centrifugal force of 10310 g) and the supernatant was filtered through a 0.45 μm (glass-fiber filter paper). Soluble COD concentrations were determined using cell tests (Merck, Darmstadt, Germany) performed according to the manufacturer's instructions. Total alkalinity was determined by titration with 0.02 molar hydrochloric acid, as described in Standard Method 2320B.4c (APHA, 1998). For VFA measurements, the filtered samples were placed in 10 ml sampling tubes with the addition of 10 μl of 98% sulphuric acid and frozen until analysis were performed, to inhibit further VFA degradation, as described in the method by Bjornsson et al., (2000). Volatile fatty acids concentrations were then determined using a high performance liquid chromatography (HPLC) (Kontron, Bio-Tek Kontron/ Detector 535 HPLC System, Basel, Switzerland) with 0.1 mM H_2SO_4 as mobile phase according to Kampas et al. (2009) (calibration details in Appendix III). Gas production was measured with a 100 ml syringe (Fisher Scientific) and the methane concentration in the biogas was measured with a gas analyser (Servomex 1440 D InfraRed Gas Analyser, Servomex Group Ltd, Crowborough, UK).

4 Results

The results are presented firstly in terms of CO_2 headspace sensor operation, with details on the sensor calibration to different acid concentration, followed by the results from the application of the CO_2 headspace sensor as a monitoring tool for early detection of imbalances in anaerobic digestion.

The findings from the laboratory scale anaerobic digestion organic underload and overload tests are presented and an analysis of the benefits of using the sensor compared to the standard monitoring are introduced. Similar result analysis was undertaken for the pilot plant organic overload test. Data from the full scale anaerobic digester samples tested with the CO_2 headspace sensor are presented compared to the available regular full scale online monitoring data.

4.1 Sensor operation

The addition of acetic acid into the headspace sensor vessel, containing denitrifier sludge in anoxic conditions, promoted denitrification. As a consequence of denitrification reaction, carbon dioxide was produced and its concentration could be measured by the CO_2 sensor. A typical carbon dioxide profile obtained after injection of a 5 ml of acetic acid at 2 g L^{-1}(10.7 mg sCOD) is presented in figure Figure 4.1. The CO_2 concentration increased from 535 ppm to a peak of 625 ppm in less than 30 minutes.

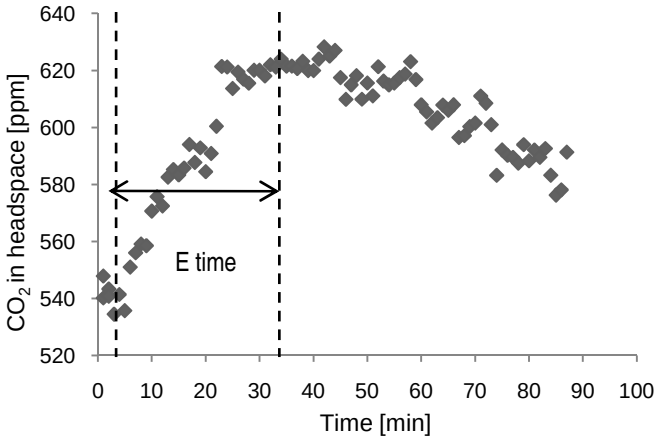

Figure 4.1. CO_2 production after the addition of 2 g L^{-1} acetic acid, corresponding to 10.66 mg of sCOD. The E time, between the injection and the CO_2 peak, was 30 minutes reached 90ppm.

Generally a CO_2 production was measured by the sensor to different acetic acid injections and the peaks of CO_2 were detected after an E time of 20-30 minutes.

The reaction occurring in the sensor was dependent on the denitrifiers characteristic. Higher MLSS related to higher biomass activity and therefore higher carbon dioxide production in the sensor. The effect of variable biomass concentration was observed during the acetic acid calibration tests, where increasing MLSS from 2.7 to 6.3 g L^{-1} determined increasing sCOD uptakes and therefore increasing rates of CO_2 production from 1.0 to 6.35 ppm min^{-1} (Figure 4.2)

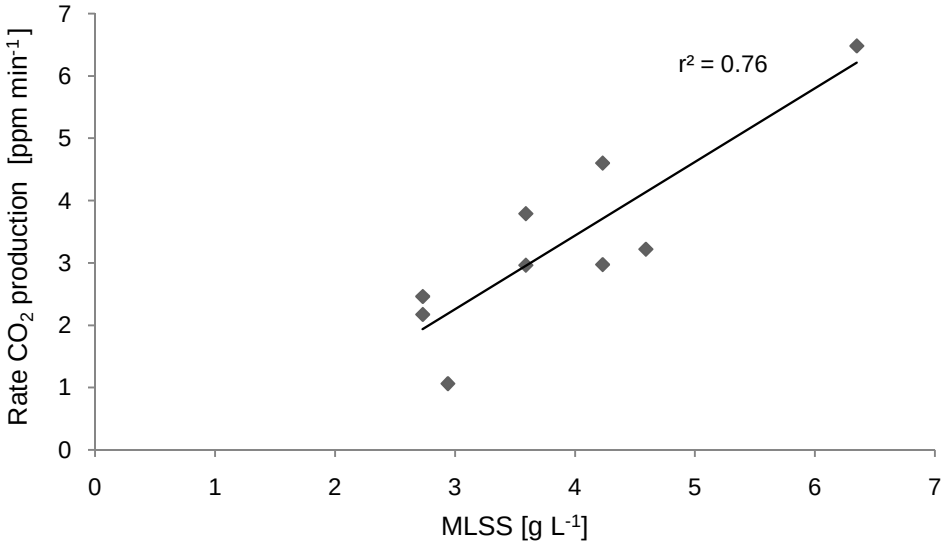

Figure 4.2. Linear correlation between the rate of CO_2 production and the MLSS characterizing the denitrifier in the sensor for the tests with 1 g L^{-1} acetic acid.

4.2 Sensor calibration

The relationship between the concentration of sCOD-VFA injected into the sensor vessel and CO_2 production from the denitrification reaction was investigated in order to construct a reliable calibration curve. To estimate the best correlation between sCOD-VFA injected and the CO_2sensor response, the following factors were considered:

- CO_2 produced from the reaction and transferred into the reaction vessel headspace
- Rate of CO_2 production
- Ratio between CO_2 production and E-time (time between injection and peak of CO_2 production)

The effect of the varying denitrifier biomass MLSS in the CO_2 production (as described in paragraph 4.1) was also taken into consideration by normalizing all data to the MLSS

content for each test. The obtained normalized parameter were then plotted against the sCOD in order to establish the best correlation.

The average of the carbon dioxide produced over the MLSS presented a linear correlation with the sCOD concentrations injected, with a coefficient of determination of $r^2 = 0.98$ (Figure 4.3). High standard deviation was observed for injections of sCOD concentrations over 6.4 g L^{-1}.

A linear correlation with coefficient of determination of $r^2 = 0.95$ was instead obtained when considering the ratio of CO_2 production over the elapsed time (E time) normalized to the MLSS (Figure 4.4). In this case a higher standard deviation was observed (Figure 4.4). When the correlation was calculated considering the rate of CO_2 production in the headspace of the reaction vessel after the different sCOD injections a coefficient of determination of $r^2 = 0.98$ was obtained (Figure 4.5).

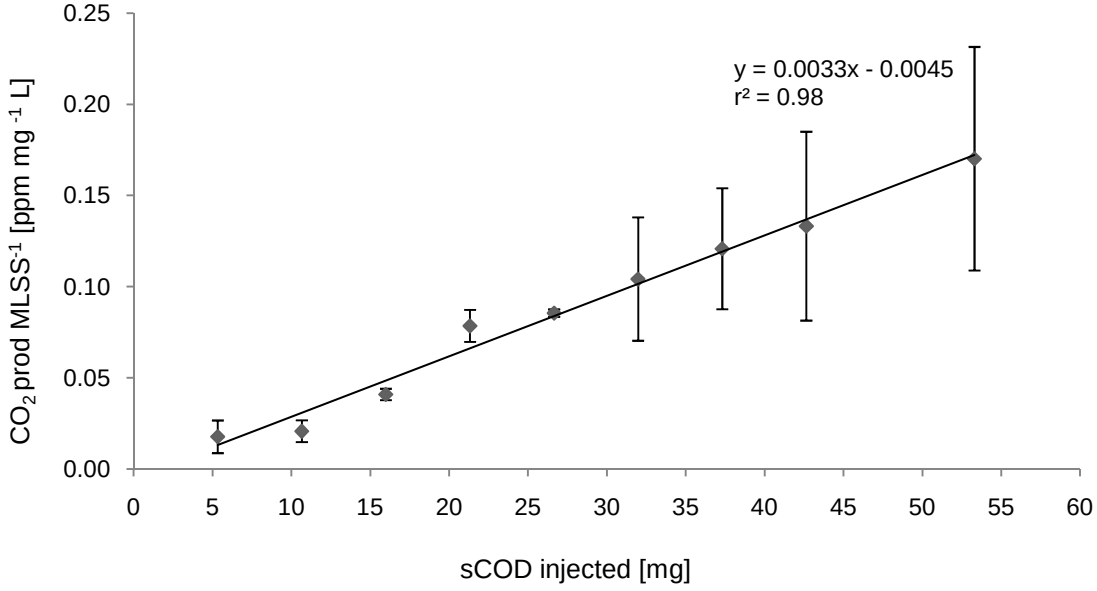

Figure 4.3. Linear correlation between the different sCOD injected (corresponding to acetic acid concentrations between 1-10 g L^{-1}), and the ratio of CO_2 production over the MLSS in the denitrifiers. The error bars represent the standard deviation.

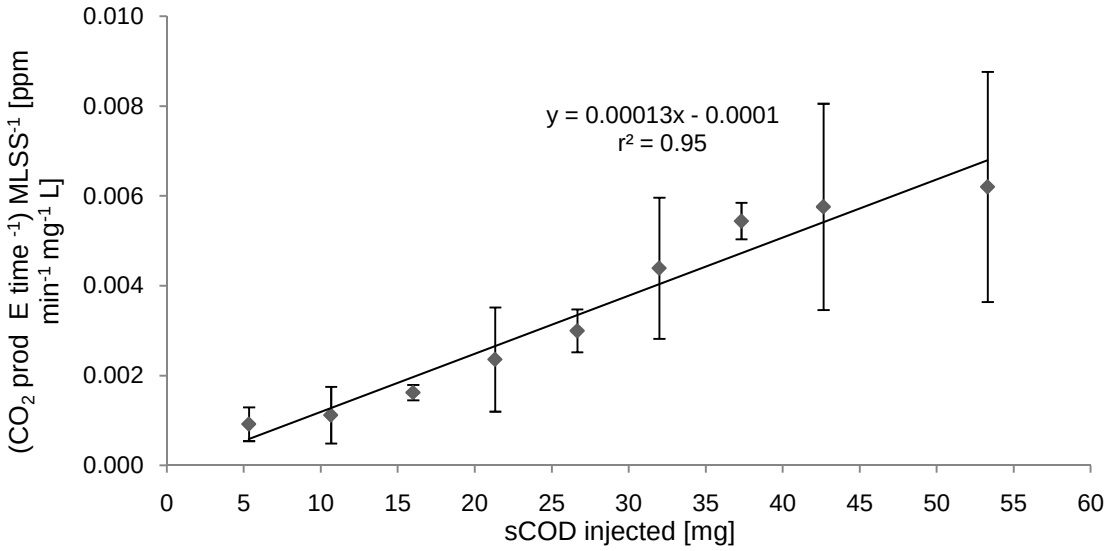

Figure 4.4. Linear correlation between the different sCOD injected (corresponding to acetic acid concentrations between 1-10 gL^{-1}), and the ratio of CO_2 production over the E time and MLSS. The error bars represent the standard deviation.

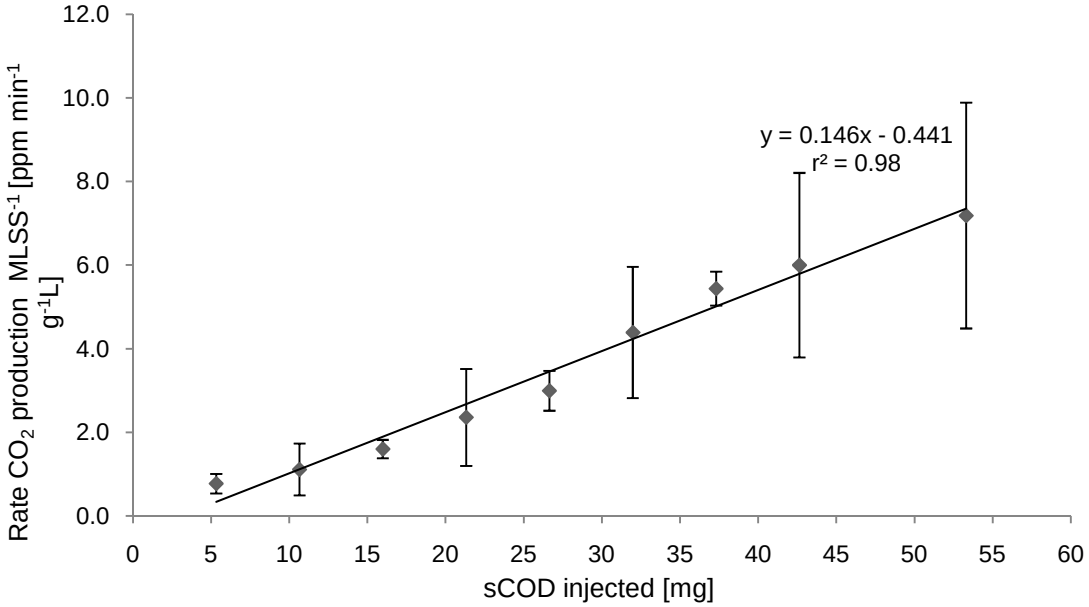

Figure 4.5. Linear correlation between the different sCOD injected (corresponding to acetic acid concentrations between 1-10 g L^{-1}), and the rate of CO_2 production over the MLSS. The error bars represent the standard deviation.

It was previously observed that the calibration curve presented in Figure 4.5 (rate of CO_2 production over MLSS) fitted several mixtures of sCOD (acetic acid, acetic and propionic mixtures and fermented products) and for this reason this correlation (Figure 4.5) was selected to calibrate the sensor according to eq.1 and eq.2:

$$sCOD\ injected\ [mg] = \frac{\frac{rate\ CO2}{MLSS\left[\frac{g}{L}\right]} + b}{a} \quad with\ a=0.146\ and\ b=-0.441 \qquad \text{(eq.1)}$$

$$sCOD\ concentration\ in\ digestate\ [mg/L] = \frac{sCOD\ injected\ [mg]}{Volume\ injected\ [ml]} \qquad \text{(eq.2)}$$

4.3 Laboratory scale test: digesters start-up

Before the tests could be started a period of 55 days was necessary for the start up of the digesters(Figure 4.6). Approximately 30 days (corresponding to twice the solid retention time of 15 days) was necessary for the digesters to reach a gas production of 5-8 L. In terms of methane concentrations, percentages of 65-70% CH_4 were obtained after only 8 days from the start of the test; while other digesters required longer periods in order to reach the same range (approximately twice the solid retention time). The control anaerobic digestion reactor started in a second phase of the project and presented a quickest process start up and stabilization. Before the application of the perturbations, a period of steady state operation was performed and comparable process efficiency was obtained in the three digesters (Table 4.1).

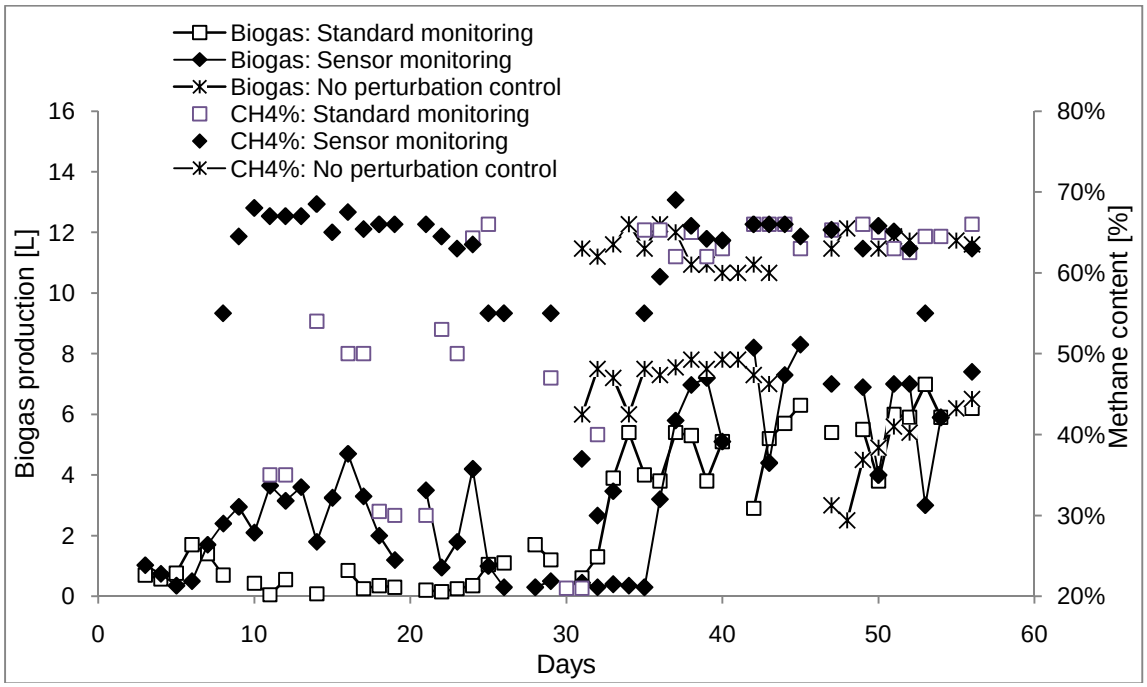

Figure 4.6 Biogas production and methane content variation during the biomass acclimatization and process start-up.

Table 4.1. Loading rates and process performance characteristics in the three digesters in steady-state conditions, before perturbations application.

	"Standard monitoring" digester	**"Sensor monitoring" digester**	**"No perturbation control" digester**
OLR [gVS fed L^{-1} day]	2.68±0.40	2.68±0.40	2.68±0.40
GPR [L biogas L^{-1}reactor day]	1.18±0.24	1.33±0.45	1.15±0.57
SGP[L CH_4 g^{-1} VS destroyed]	0.44±0.17	0.56±0.25	0.33±0.21
CH_4 in biogas [%]	0.64±0.02	0.63±0.04	0.63±0.02
Alkalinity [mg CaCO3 L^{-1}]	1923±661	2480±153.73	2381±212
pH	6.95±0.04	7.01±0.21	7.17±0.10

4.4 Laboratory scale test: Organic underload test

4.4.1 Process monitoring

Phase I

Organic loading rate was reduced from the start-up range of 2-3 gVS fed L^{-1} day^{-1} to 1.7- 2 gVS fed L^{-1} day^{-1} in the "Standard monitoring" and the "Sensor monitoring" digesters from day 1(Figure 4.7). As this OLR perturbation was applied, the CO_2 headspace sensor response indicated that 0.5-1 g L^{-1} sCOD-VFA was present in the digestate liquor. As no accumulation of acids was observed in the digester for several consecutive days, following the control law previously defined, an increase in OLR was applied on day 11 to 2-3 gVS fed L^{-1} day^{-1}. During this first phase (Phase I) of the test, the standard monitoring indicators did not reveal any strong evidence of process imbalance in the two digesters perturbed. For both the "Standard monitoring" and "Sensor monitoring" digesters alkalinity values were between 2500 and 3000 mg $CaCO_3$ L^{-1} without any evident trend; pH values did not divert from the optimal range of 7.2-7.4 and methane content in the biogas was around 60% content (Figure 4.8 and Figure 4.9). Biogas production showed a gradual decrease starting from day 3 after the beginning of the test deterring a reduction from 7.5 L to 5 L daily biogas production; however this did not drop under the control digester gas production of 5 L per day, excluding evident indications of process imbalance.

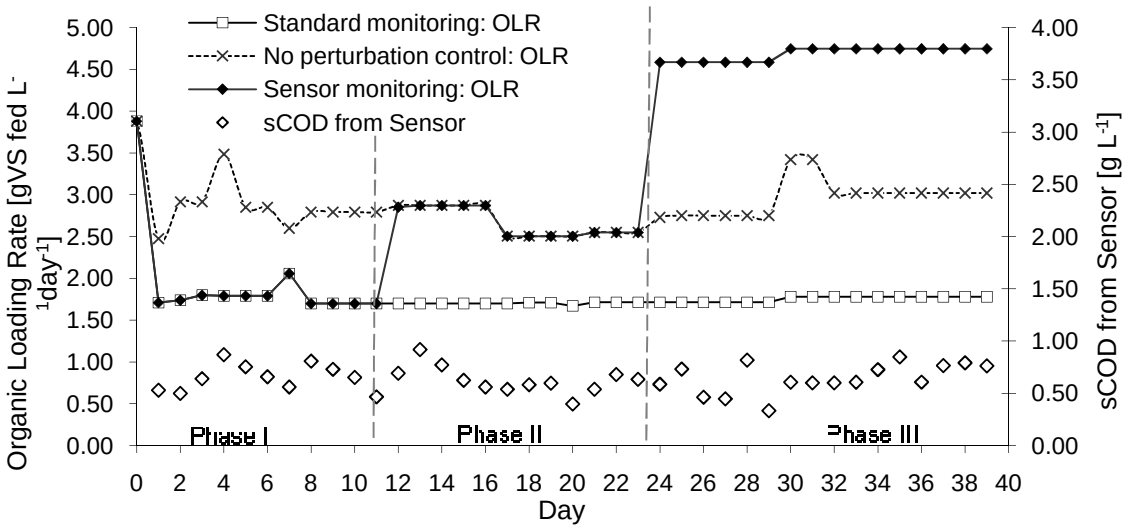

Figure 4.7 Organic loading rate variation during the organic underload test and sCOD values derived from the sensor responses to digestate from "Sensor monitoring" reactor. Phase I,II,III correspond to the different loading regimes tests.

Phase II

During the second phase (PhaseII) of the test, after the increase in loading rate in the digester monitored by the sensor, sCOD-VFA measurements were generally stable around the average value of 0.63 mgL^{-1}hence with no sCOD-VFA accumulation. pH values for the three digesters maintained similar values as in the previous period (7.2-7.4), alkalinity values revealed a slight rise trend for the "Sensor monitoring" (from 2700 to 3300 mg $CaCO_3$ L^{-1}), following the control rector increasing trend (Figure 4.8). Biogas production in "Sensor monitoring" revealed a significant rise after 3 days from the increasing loading rate (day 14) from 5 L up to 8.5 L daily production, while the gas composition had a slight methane content increase (from 60% to 65%) after 7 days (day 18 in Figure 4.9), probably as a response to the higher organic loading (Figure 4.9). On the contrary the "Standard monitoring" revealed first evidences of the lower feeding regime with a decrease in biogas production from 5L to 2L and a correspondent methane content reduction from 60% to 50% between day 14 to 18 (Figure 4.9).

Phase III

On day 23 a further increase of feeding was applied to the "Sensor monitoring" digester as the indirect sCOD measurements obtained with the CO_2 headspace sensor were constantly under the limit level during the entire phase. The digester was then subject to an organic

overload with OLR increase to 4.5-5 g VS fed L^{-1} day^{-1}, while the "Standard monitoring" digester was maintained at a low organic loading regime. During this last phase of the experiment (Phase III), all monitoring parameters presented optimal ranges for the "Sensor monitoring", while the "standard monitoring" showed evidences of process perturbation with decreasing alkalinity (from 2500 to 2000 mg $CaCO_3$ L^{-1}), fall of biogas production to 1.5 L and methane concentration varying between 46 to 66% (Figure 4.9). Overall the "Standard monitoring" digester showed strong evidences of process imbalance with all monitoring parameters after approximately 28 days from the beginning of the test, while the information from the sensor allowed, for the "Sensor monitoring" digester, to identify the low feeding regime and consecutively optimize the loading rate, thus determining a higher process performance.

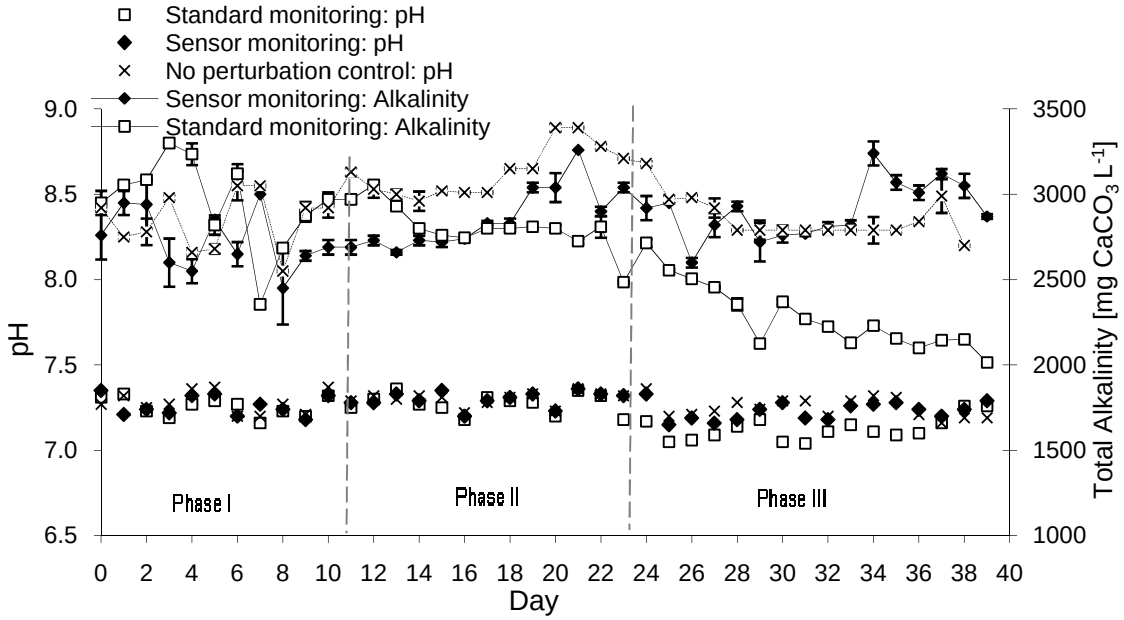

Figure 4.8. Standard monitoring indicators, pH and alkalinity, variation during the tests. Phase I defines the organic underload perturbation for both "Sensor monitoring" and "Standard monitoring" digester, Phase II is characterized by the organic underload perturbation for "Standard monitoring" and a standard feeding for "Sensor monitoring" while Phase III mantaines the organic underload perturbation for "Standard monitoring" and presents an organic overload for "Sensor monitoring".

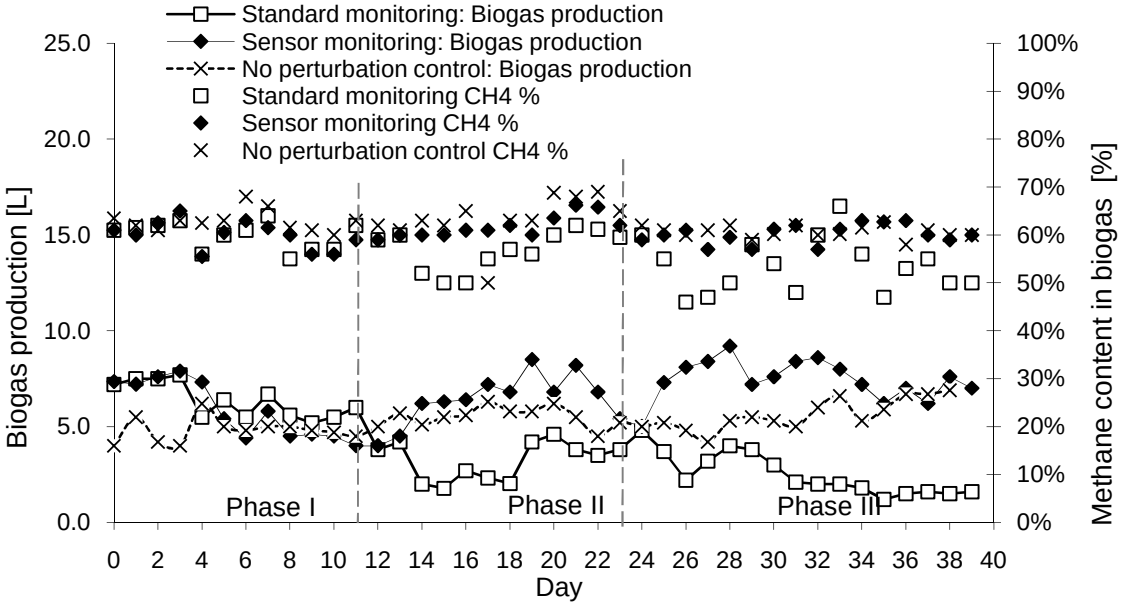

Figure 4.9. Standard monitoring indicators, biogas production and methane percentage content, variation during the tests. Phase I defines the organic underload perturbation for both "Sensor monitoring" and "Standard monitoring" digester, Phase II is characterized by the organic underload perturbation for "Standard monitoring" and a standard feeding for "Sensor monitoring" while Phase III mantaines the organic underload perturbation for "Standard monitoring" and presents an organic overload for "Sensor monitoring".

4.4.2 Impact of CO_2 headspace sensor monitoring on digester performance

As observed by the variability of the biogas production in the three digesters (Figure 4.9) the process performance was strongly influenced by the increasing loading rate in digester "Sensor monitoring". During the first phase of the test, the low organic loading rates for both "Standard monitoring" and "Sensor monitoring" led to a reduction of the process performance, in terms of total solids removal reduction, compared to the previous optimal range.

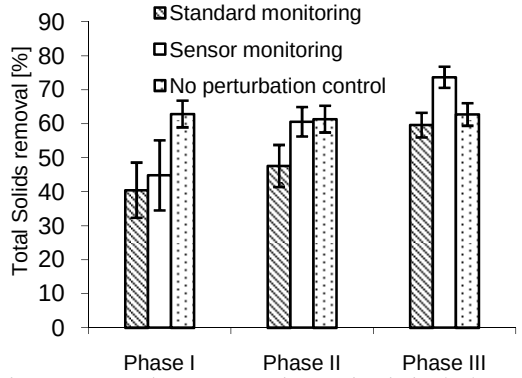

Figure 4.10. Average and standard deviation of total solids removal for the three digesters in the three different test phases

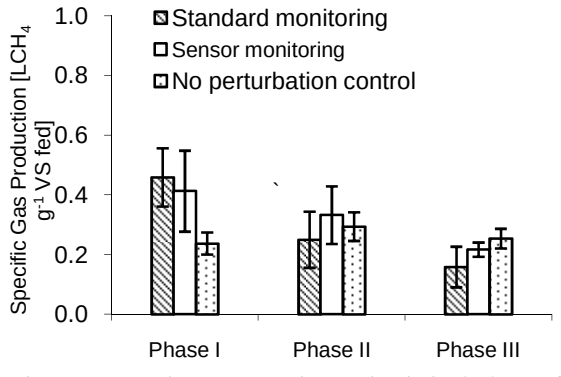

Figure 4.11. Average and standard deviation of specific gas production for the three digesters in the three different test phases

The TS removal in the "No perturbation control" digester was characterized by 63±4% total solids but due to reduction in OLR the TS removal was reduced to 40±8% for the "Standard monitoring" digester and to 45±10% for the "Sensor monitoring" reactor (Figure 4.10).

In the second phase of the test, when the information from the sensor induced an increase in the loading rate to the reactor "Sensor monitoring", a return to solid removal of 61±4% was observed, similarly to the control reactor performance, while in the last phase of the test, when 4.5-5 g VS fed L^{-1} day^{-1} inflow was applied, the solids removal efficiency reached 74±4% (Figure 4.10). The "Standard monitoring" reactor showed a gradual solids removal performance increase (Figure in Appendix I and Figure 4.10), reaching values in the last phase with an average of 60±3%, revealing the adaptation capability of the biomass to the new feeding conditions. The specific gas production (Figure 4.11), for all three reactors, showed a general decreasing trend from phase I to phase III, however "Sensor monitoring" reactor was able to maintain a higher process performance compared to the "Standard monitoring", with higher specific gas production in both Phase II and III (0.33±0.09 over 0.25±0.09 g^{-1} VSfed day^{-1} and 0.22±0.02 over 0.16±0.07) as a consequence of increasing the OLR due to the CO_2 headspace sensor information. In terms of overall gas production from the three digesters, the comparison made with the gas production rates (Figure 4.12) reveals the benefits of the CO_2 headspace sensor. While the gas production in the "Standard monitoring" digester decreased from 1.35±0.5 L biogas $Lreactor^{-1}$ day^{-1} ,during the first phase, to 0.5 ±0.2 L biogas $Lreactor^{-1}$ day^{-1} in the last test phase, the gradual loading increase applied to the reactor "Sensor monitoring" determined a rise in the gas production rate from 1.25 ±0.3 to 1.69±0.2 L biogas $Lreactor^{-1}$ day^{-1} in the first and last test phase respectively. The higher gas production obtained with the application of the sensor and the innovative control law to an underloaded digester is also evident in the comparison of the cumulative biogas production in the three digesters (Figure 4.13). Overall the reactor monitored by the sensor produced 75% more biogas than the "Standard monitoring" digester (111. 5 L), and 25% more than the non perturbed control reactor (51.0 L).

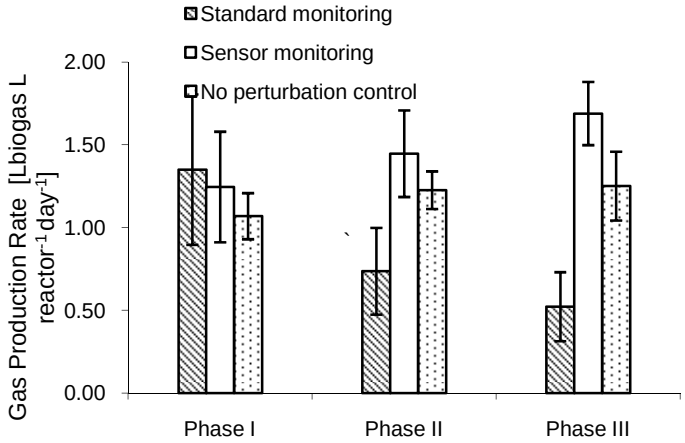

Figure 4.12. Average and standard deviation of gas production rate for the three digesters in the three different test phases

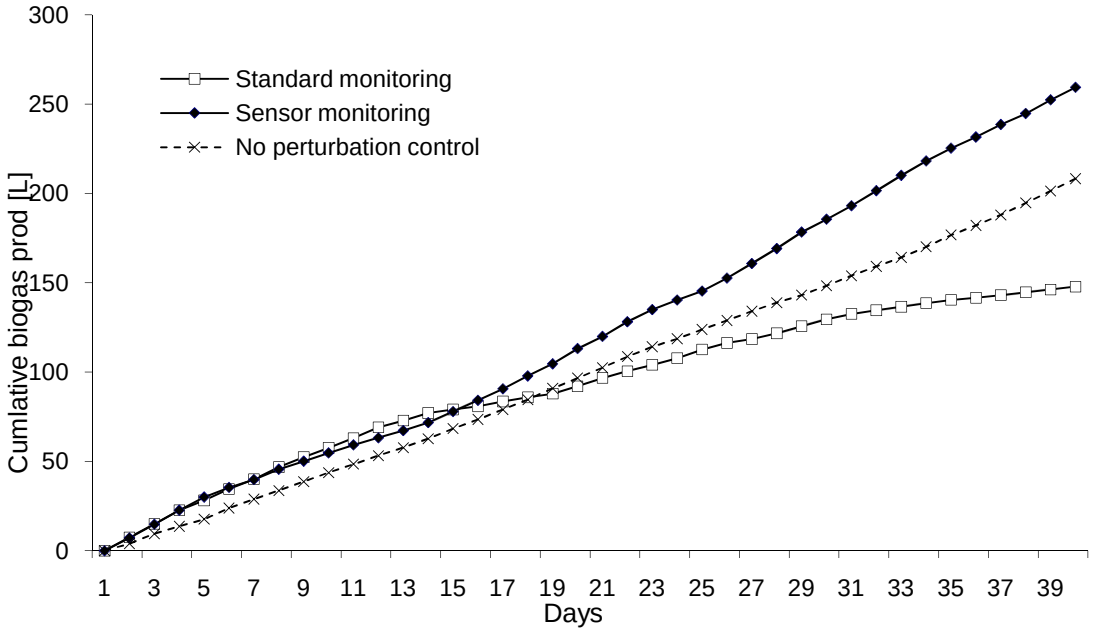

Figure 4.13. Cumulative biogas production for the three reactors during organic the underload test.

4.5 Laboratory scale test: Organic overload test

4.5.1 Process monitoring

To perform an organic overload test, an increase in organic loading rate from the initial range of2.7±0.5 gVS fed L^{-1} day^{-1} to approximately 6.0±1 gVS fed L^{-1} day^{-1} was applied to the "Standard monitoring" and the "Sensor monitoring" reactors from day 1 (Figure 4.14). No evidence of process perturbation was observed by the standard monitoring indicators,

such as pH, alkalinity, biogas production and methane content in the immediate following days from the start of the test. On the contrary, the headspace carbon dioxide sensor identified an accumulation of sCOD-VFA (higher than 1.2 g L^{-1}), at the third day of the test (Figure 4.14). Following the control law the OLR for the "Sensor monitoring" digester, was decreased to feeding regimes of 3.0 ±0.5 gVS fed L^{-1} day^{-} for three consecutive days. On day 6, the sCOD-VFA concentrations from the sensor indicated values below 1.2 g L^{-1}, therefore the feeding regime was raised again to perform organic overloading as for the "Standard monitoring" reactor. The standard monitoring parameters maintained consistent values in both reactors, with alkalinity in the range of 2000-2500 mg$CaCO_3$ L^{-1}, pH within 6.9-7.2, methane content in biogas between 61% -65% and biogas showing an immediate increase of production from 4-6 L to 7-8 L per day, which was then maintained for both reactors until day 10 (Figure 4.15 and Figure 4.16). On day 9 an concentration of SCOD-VFA over 1.2 g L^{-1} was detected by the CO_2 headspace sensor in the "Sensor monitoring" digester, and a second reduction of the loading regime to 2.9±0.4 gVS fed L^{-1} was completed which lasted until the end of the test (Figure 4.14).

Standard process monitoring indicators, such as alkalinity and pH, were generally constant during the entire test period (Figure 4.15). In "Standard monitoring", the biogas composition decreased gradually from the optimal 60% content to 55% in the last 4 days of the test, revealing an initial trend of process imbalance. At the same time, the biogas production also had an evident decline from the previous 7.5 L to approximately 4.8 L from day 11 (Figure 4.16).

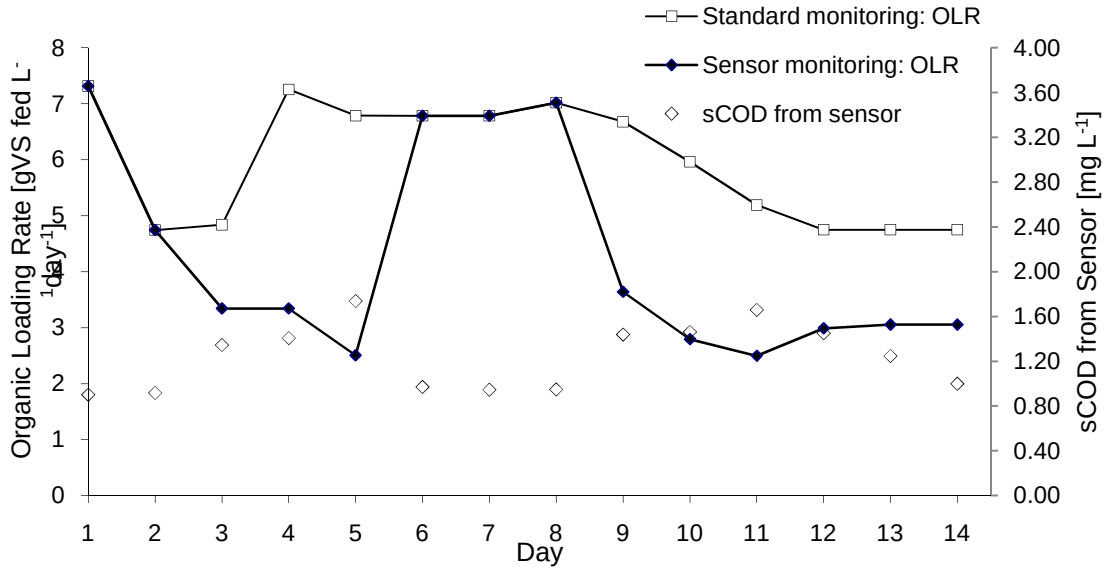

Figure 4.14. Organic loading rate variation in the two reactors during the organic overload test and sCOD values derived from the sensor responses to digestate from the "Sensor monitoring" reactor.

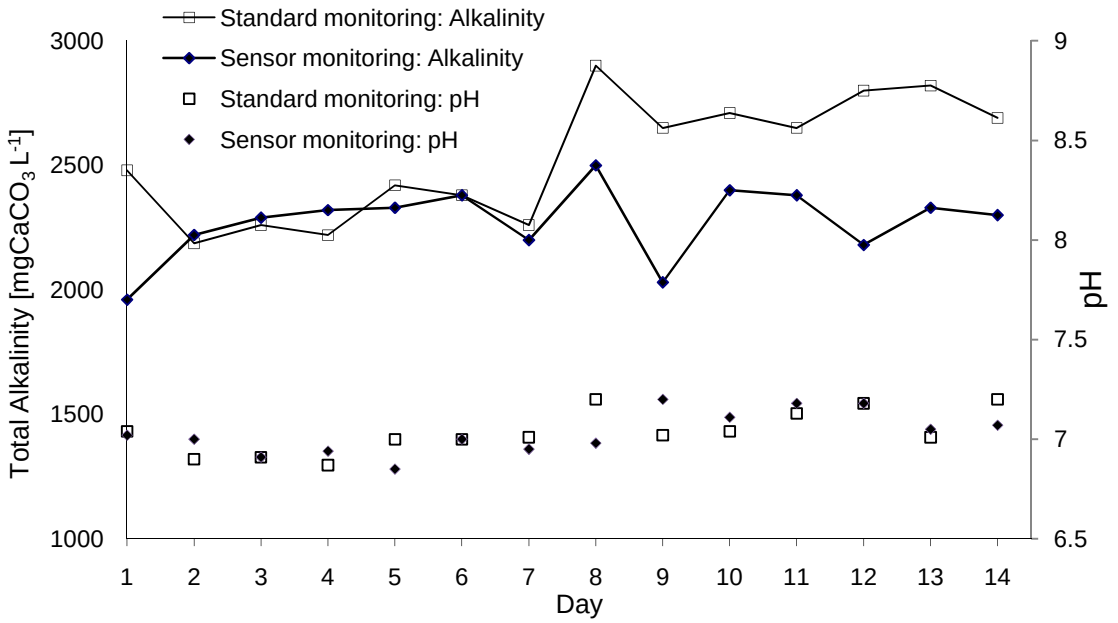

Figure 4.15. Standard monitoring indicators, pH and alkalinity, variation during the organic overload test.

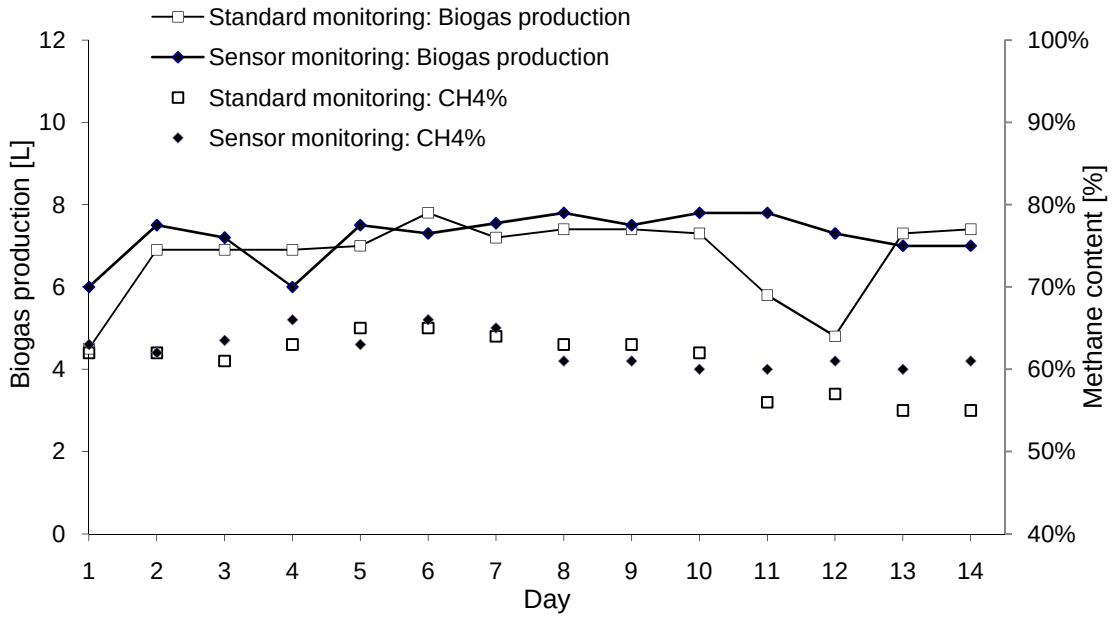

Figure 4.16. Standard monitoring indicators, biogas and methane concentration, variation during the organic overload test.

4.5.2 Impact of CO_2 headspace sensor monitoring on digester performance

The biogas production in the two reactors (Figure 4.16) showed a similar trend until day 11-12, when an initial decline of biogas production and of methane content was observed in "Standard monitoring". Indeed, analysing the process performance in terms of average gas

production rate for the whole test period (Figure 4.17), higher gas rate was obtained in the reactor monitored by the sensor. The specific gas production rates in the two reactors differ noticeably (Figure 4.18), as a consequence of the variable OLR applied in the "Sensor monitoring" digester. While the "Standard monitoring" sensor was characterized by a specific gas production of 0.21±0.05 L CH_4 g^{-1} VSreduced day^{-1}, the "Sensor monitoring" digester increased to 0.41±0.21 L CH_4 g^{-1} VSreduced day^{-1}.

The higher variability of the "Sensor monitoring" specific gas production, due to the induced feeding changes, can be extensively observed in its time variability shown in Figure 4.19. Following the reduction of loading rates in the "Sensor monitoring" at day 3 and 9, peaks of specific gas production were obtained, thus demonstrating the efficiency of the loading adjustment to maintain a higher process performance.

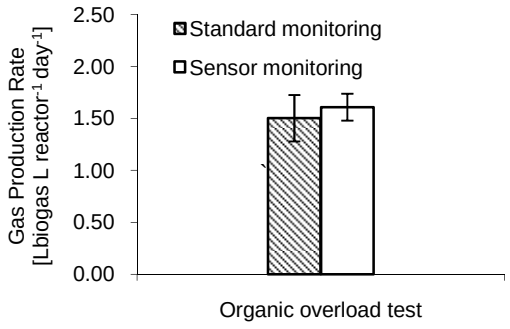

Figure 4.17. Comparison of gas production rate average in the two reactors

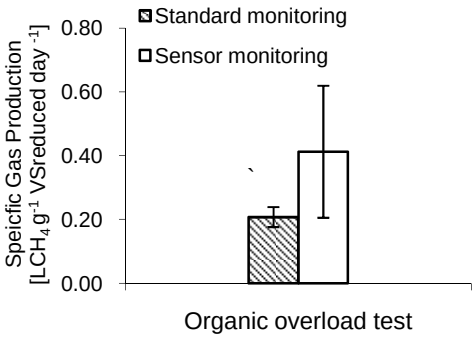

Figure 4.18. Comparison of specific gas production average in the two reactors

Generally, the comparison of the two reactor's process efficiency revealed a higher overall biogas production from the digester monitored by the CO_2 headspace sensor, as shown in the cumulative biogas production comparison (Figure 4.20). The "Sensor digester" produced, within the short 14 days test period, 6.7 L extra of biogas corresponding to a 7% additional production.

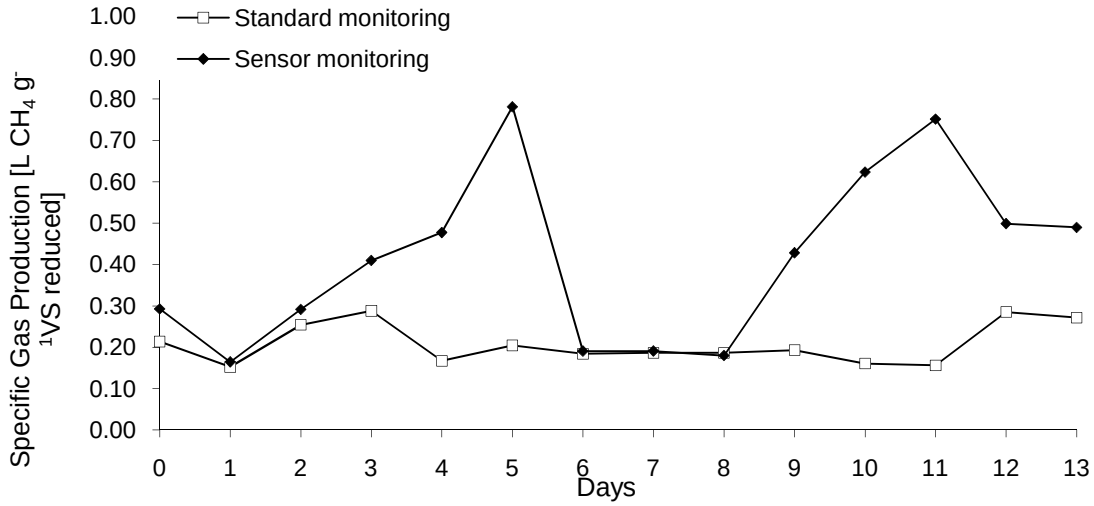

Figure 4.19. Specific gas production variation in the two reactors.

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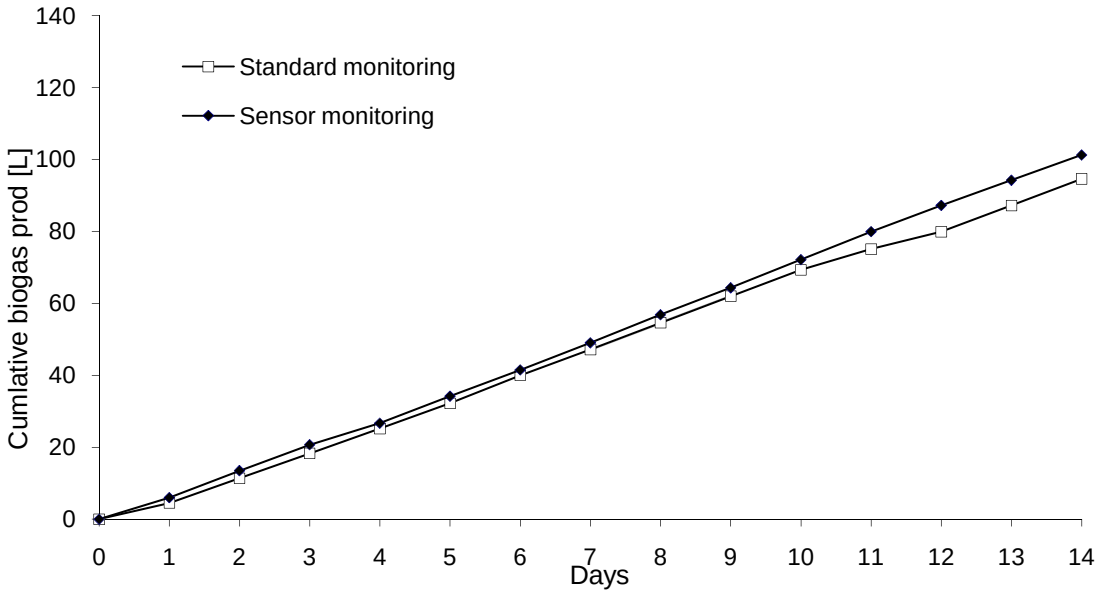


Figure 4.20. Cumulative biogas production comparison for the two digesters..

4.6 Pilot scale test: Organic overload

4.6.1 Process monitoring

The organic overload test on pilot scale was performed in terms of reduction of the solid retention time from the optimal 15 days to 8 days for both the "Standard monitoring" and the "Sensor monitoring" reactor, starting from day (Figure 4.21). The solid retention time, in digester "Sensor monitoring" was varied based on the digestate sCOD-VFA data derived by the CO_2 headspace sensor, following the control law defined. During the test, the sensor identified sCOD-VFA accumulation in several occasions (day 9, 18, 24, 33) and consequently the SRT of "Sensor monitoring" was increased to 15 days until lower sCOD-VFA was observed in the reactor (Figure 4.23).

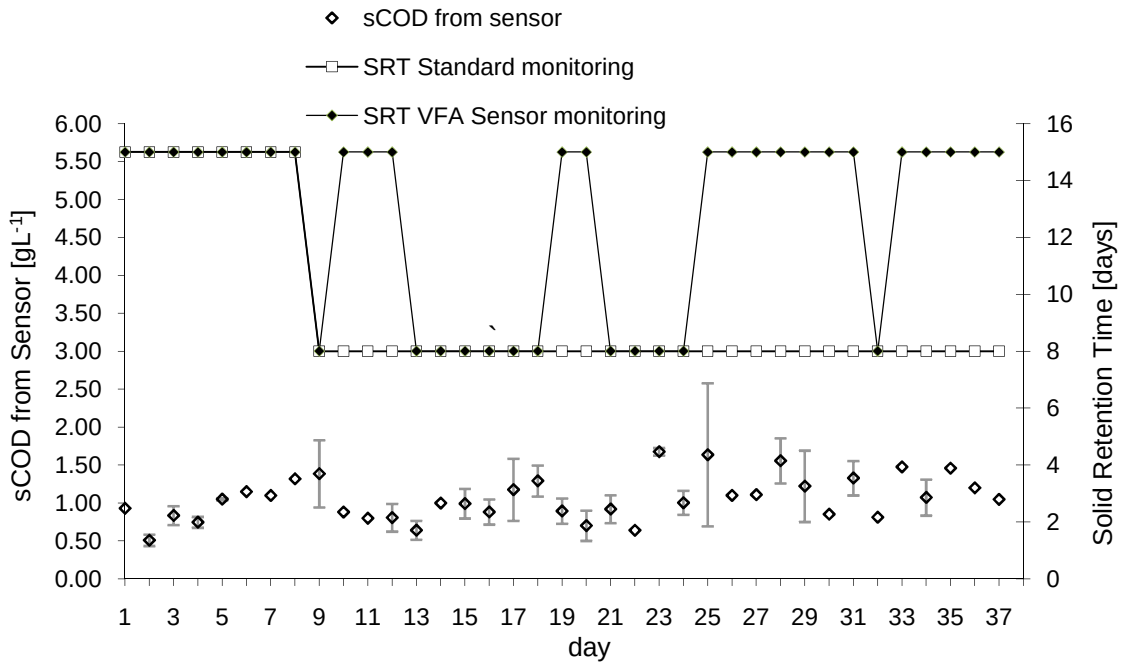


Figure 4.21. Solid retention time variation and sCOD concentration derived by the CO_2 headspace sensor for the digestate of "Sensor monitoring"

Standard monitoring parameters, controlling the "Standard monitoring" digester did not reveal any sign of process failure, after the application of the change in SRT. Alkalinity concentrations for both digesters varied between 2500- 3000 mg $CaCO_3$ L^{-1} until day 34, when it declined to 2000 mg $CaCO_3$ L^{-1} in the "Standard monitoring" digester (Figure 4.22). For both digesters, pH values varied between 6.8 and 7.8. Biogas production in "Standard monitoring" digester showed variability between 0.5-1.5 Nm^3 during the test but did not present any evident trend (Figure 4.23).

Throughout the test biogas production rates were similar between the two digester, however, in the "Sensor monitoring" digester, peaks of biogas production were registered consecutively to the increasing retention time variation applied. The two digesters differentiate in the methane produced even before the starting of the SRT perturbation. This was maintained during the entire test period as the "Standard monitoring" digester presented an average methane content of 53% with minimum values reaching 40% only while the "Sensor monitoring" was characterized by the optimal average 60% content (Figure 4.23).

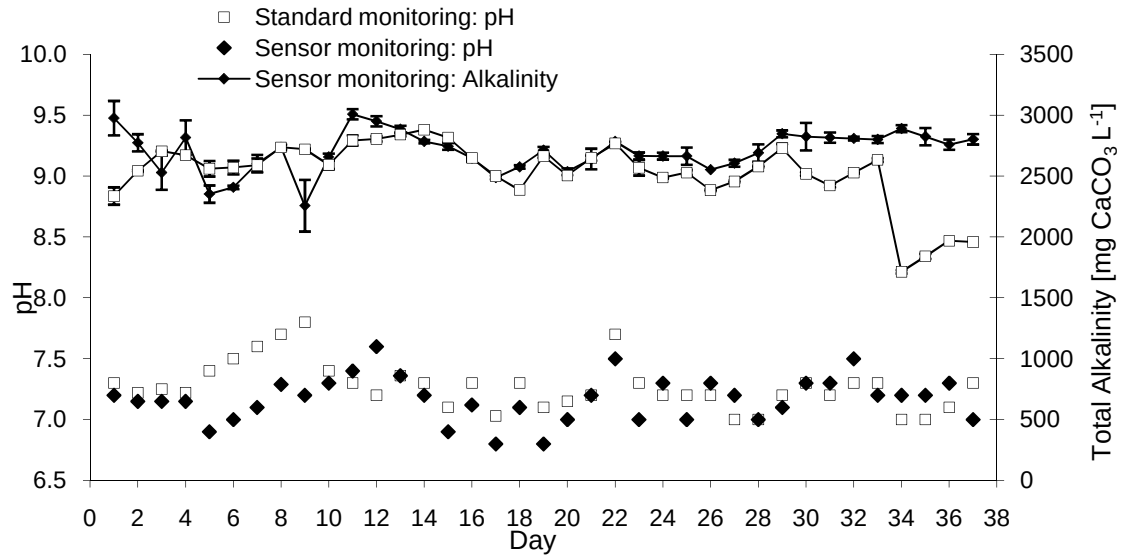


Figure 4.22. Standard monitoring indicators, pH and alkalinity, variation during the organic overload pilot scale test.

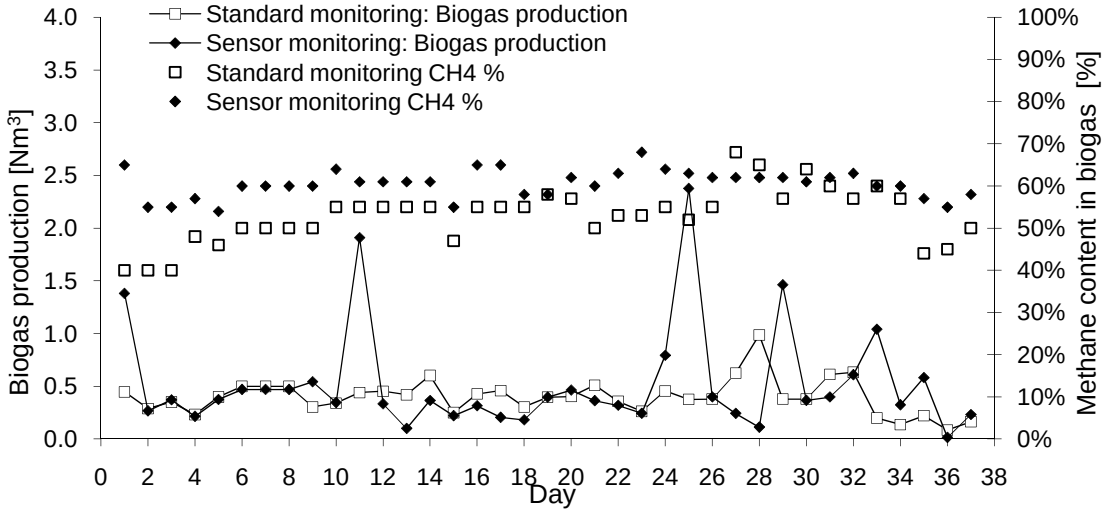


Figure 4.23. Standard monitoring indicators, biogas production and methane content, variation during the organic overload pilot scale test.

4.6.2 Impact of CO_2 headspace sensor monitoring on digester performance

As observed in the measurements of biogas production (Figure 4.23), the application of sensor monitoring determined peaks of gas production, following the intermittant increase in solid retention time applied to the "Sensor montoring" reactor when sCOD accumulation was identified in the digestate. This was also observed with peaks of specific gas production up to 0.84 Nm^3 CH_4 kg^{-1}VS fed day^{-1} consecutive to the loading decrease (Figure 4.24).

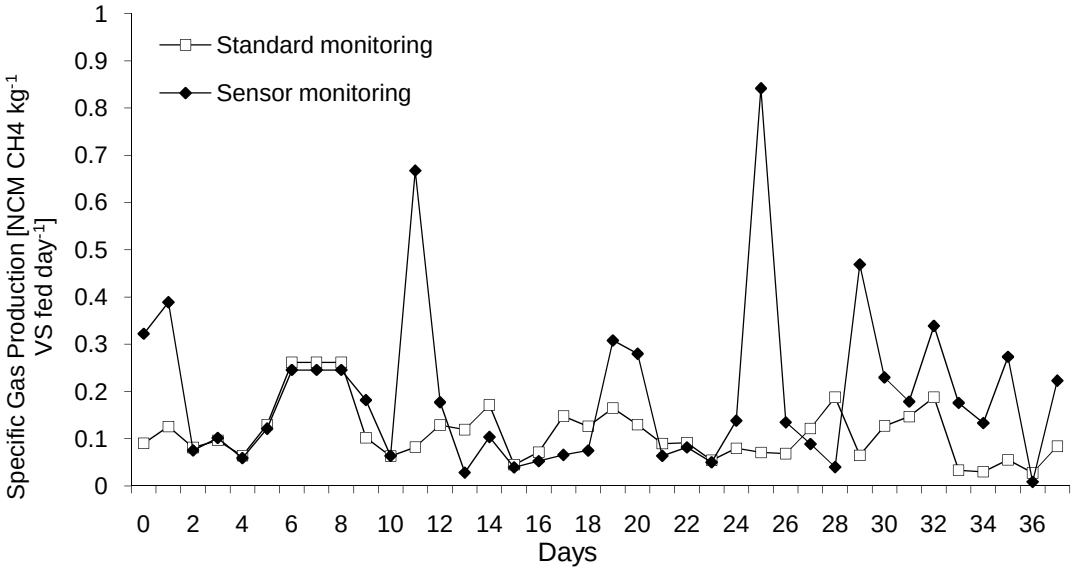


Figure 4.24. Speicifc gas production comparison between the two pilot scale anaerobic digesters.

The overall specific gas production was higher for the "Sensor monitoring" with 0.19±0.12 Nm^3 CH_4 kg^{-1}VS than for "Standard monitoring" with 0.11±0.06 Nm^3 CH_4 kg^{-1}VS(Figure 4.25). Overall the gas production rate for the "Sensor monitoring" digester presented a higher average rate of 0.74±0.65 Nm^3 CH_4 m^{-3}reactr day^{-1} compared to the 0.57±0.24 Nm^3 CH_4 m^{-3}reactr day^{-1} characterizing the "Standard monitoring" digester.

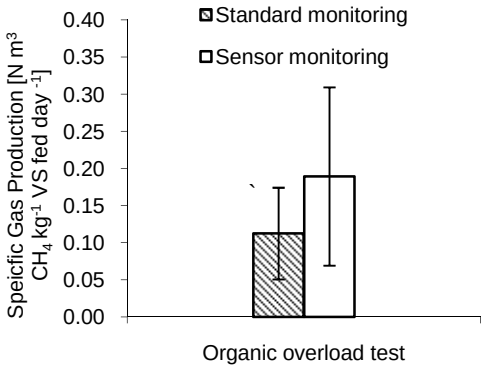


Figure 4.25. Specific gas production average and deviation standard for the two digesters.

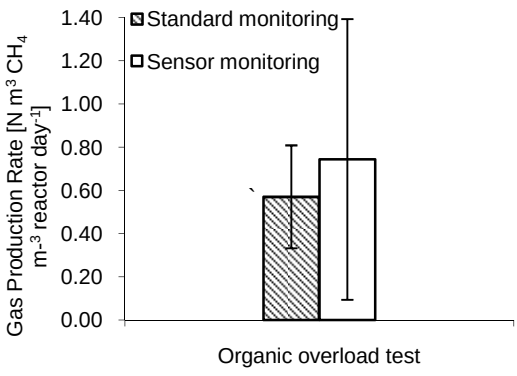


Figure 4.26. Gas production rate average and deviation standard for the two digesters.

The overall biogas production for the two digester, in terms of cumulative production (Figure 4.27), showed the higher process efficiency of "Sensor monitoring", also characterized by a higher variability in the production rates (visible by the changes in slopes) as a consequence of the fast response to the variable feeding regime. With the use of the information derived by the CO_2 headspace sensor it was possible to obtain 4.48 Nm^3 extra biogas production corresponding to a gain of 30.3 % compared to the "Standard monitoring" anaerobic digester.

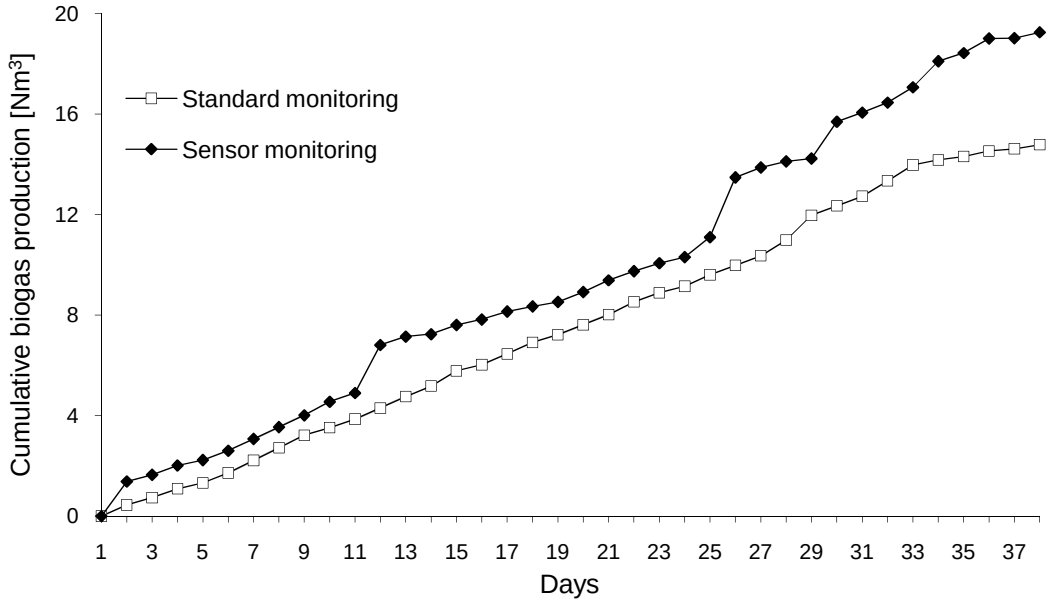


Figure 4.27. Cumulative gas production comparison for the two pilot scale digesters.

4.7 Full scale: process monitoring

The headspace carbon dioxide sensor was utilized to monitor a full scale anaerobic reactor at Esholt STW, Yorkshire Water for 15 days over a month period. sCOD-VFA data derived from the CO_2 headspace sensor were compared to the standard monitoring indicators actually observed daily in the full scale digester.

In the comparison of the indirectly derived data for digestate liquor sCOD-VFA with the solid retention time variation (Figure 4.28), it was observed that some cases of high acid accumulation occurred in correspondence of decreases of solid retention time applied to the digester, such as on day 3, when SRT was reduced to 11 days and the sensor measured a sCOD-VFA concentration of 1.63 g L^{-1} and also at day 9, characterize by SRT of 11 days and sCOD-VFA concentration of 1.29 g L^{-1}.

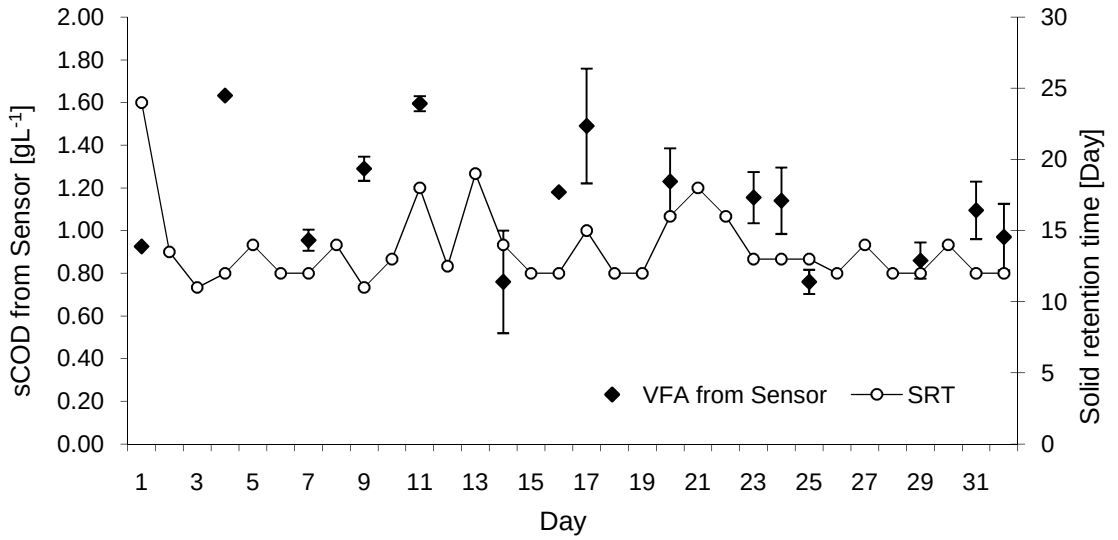


Figure 4.28. Variability of the solid retention time in the full scale anaerobic reactor n.3 of Esholt STW and digestate liquor sCOD-VFA values derived with the CO_2 headspace sensor.

A further comparison with the reactor temperature variation (Figure 4.29) revealed a correspondence between the decrease in temperature observed on day 9 and 10, with temperature decreasing from 35°C to 30-31°C , and the high sCOD-VFA accumulation up to 1.60 g L^{-1}on day 11.

The comparison with the gas production rate (Figure 4.29), did not show any particular cause-effect correlation. However it could suggest that the decrease of the gas production rate to 0.039 m^3 biogas m^{-3} reactor day^{-1} on day 17 was due to the accumulation of acid observed with sCOD-VFA from the sensor at 1.49 g L^{-1}, and furthermore that the reduction to 0.035 m^3 biogas m^{-3} reactor day^{-1} on day 21 was due to the accumulation of acid observed with sCOD-VFA from the sensor 1.23 g L^{-1} on day 20.

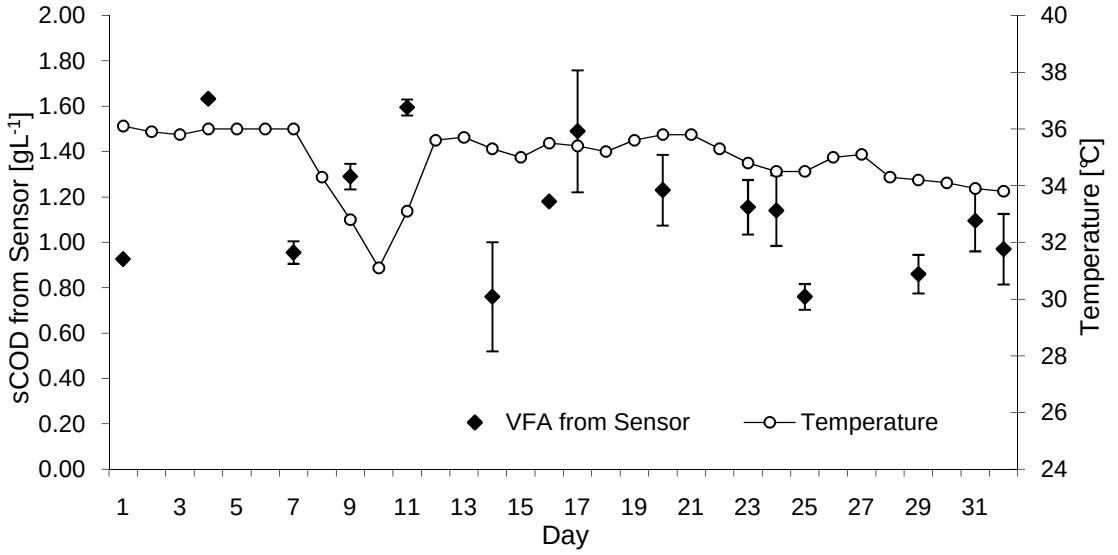


Figure 4.29. Variability of the temperature in the full scale anaerobic reactor n.3 of Esholt STW and digestate liquor sCOD-VFA values derived with the CO_2 headspace sensor.

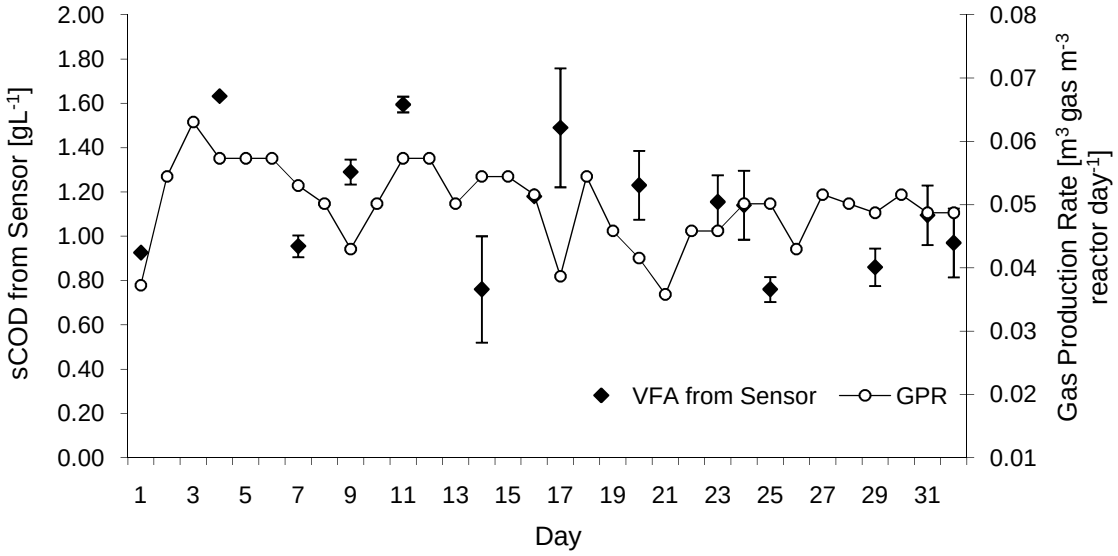


Figure 4.30. Variability of the temperature in the full scale anaerobic reactor n.3 of Esholt STW and digestate liquor sCOD-VFA values derived with the CO_2 headspace sensor.

5 Discussion

The CO_2 headspace sensor was used as a monitoring tool to detect early signs of imbalance (by measuring sCOD-VFA) in anaerobic digesters operated under suboptimal conditions at laboratory, pilot and full scale. The CO_2 headspace sensor is based on the measurements of CO_2 in the headspace of a vessel produced by denitrifying suspended sludge after the addition of a carbon source.

Compared to the standard common process monitoring indicators, the additional information derived from the sensor was utilized for regulating the anaerobic digesters feeding regime thus avoiding process failure during cases of organic overload and underload.

5.1 Validation of the CO_2 headspace sensor for sCOD-VFA estimation

Two main assumptions permitted the estimation of sCOD-VFA in the digested sludge with the use of the CO_2 headspace sensor:

1. The readily available sCOD and VFAs in the digestate are the preferred carbon source for the denitrifying bacteria and its uptake will give origin to the CO_2 measured in the headspace.
2. The VFAs are the dominant compounds of the total sCOD in the digestate.

The CO_2 production in the headspace of the vessel containing denitrifiers derives from the consumption of carbon source needed for the conversion of nitrates into nitrogen gas according to:

$$5CH_3COOH+8NO^-_3 \rightarrow 8HCO^-_3 + 2CO_2 +6H_2O + N_2$$

Denitrification requires the presence of a soluble carbon source that can take the shape of chemicals, industrial by-products or fermented sludge. Several studies have investigated the impact of the type of carbon on the denitrification rates: it was observed that a mixture of VFA is the preferred carbon source for denitrification and determines greater denitrification rates up to 0.754 $mgNO_3$-N compared to the lower 0.289 $mgNO_3$-N with ethanol or 0.349 with methanol (Table 2.8) (Elefsiniotis et al., 2004, Xu, 1996). Between all VFA, acetic acid, being the simplest of the fatty acids, is the preferred and the fastest carbon substrate consumed (Elefsiniotis et al., 2004).

To estimate the COD-VFA of unknown samples, the CO_2 measurements and concentration peaks in the vessel headspace were obtained within the first 20-30 minutes of the reaction. It can then be assumed that the CO_2 produced is deriving only from the preferred carbon sources (VFA) which has the faster reaction rate. The impact of other carbon substances is then excluded from the readings in the headspace of the sensor enabling to analyse complex samples such as digestate.

To support the second assumption, previous studies have demonstrated that the majority of the sCOD present in fermented/digested sludge was mainly formed by volatile fatty acids. Soares et al (2010), performed several experiments on primary sludge fermentation and observed that between 69% to 94% of the sCOD measured in the digestate was constituted by VFAs. Other studies (Moser et al., 1998) have reported that 85% of the sCOD observed in fermented sludge was composed of VFA.

The results from the current study confirm that the VFA concentrations (measured with HPLC instrumentation) and the sCOD (measured with cell tests) in the digestate were well correlated (Figure 5.3). Variability was noticed between the different scales, where distinct VFA/sCOD ratios were observed (Table 5.1). During the laboratory scale organic overload the sCOD measured in the digested sludge was composed of 100% by VFAs, the laboratory scale underload test showed a lower ratio with VFA accounting for 70% of the sCOD, more similar to the results of previous studies; while in the organic overload pilot scale test a lower correlation between the two was observed, with the digestate liquor sCOD containing only 58% VFA. A possible explanation behind the variability of the VFA content in the sCOD observed in the different tests can be related to the variability of the inflow primary sludge fed to the digesters and the consequent biological reactions occurring in the reactor. In particular:

- different primary sludge was utilized in the laboratory and pilot plant scale tests.
- for the pilot plant case, the inflow sludge, deriving from a full scale STW, was characterised by a high VFA content as it was generally collected and stored for 1-2 days in collection tanks before being treated.

The sludge characteristics, and in particular the content of VFA in the inflow of anaerobic digesters, can strongly influence the process evolution, in terms of hydrolysis and methanogenesis rates and therefore influence the carbon composition in the digestate. Furthermore, a different sludge origin could determine a different composition of the sCOD, affecting the ratio estimation.

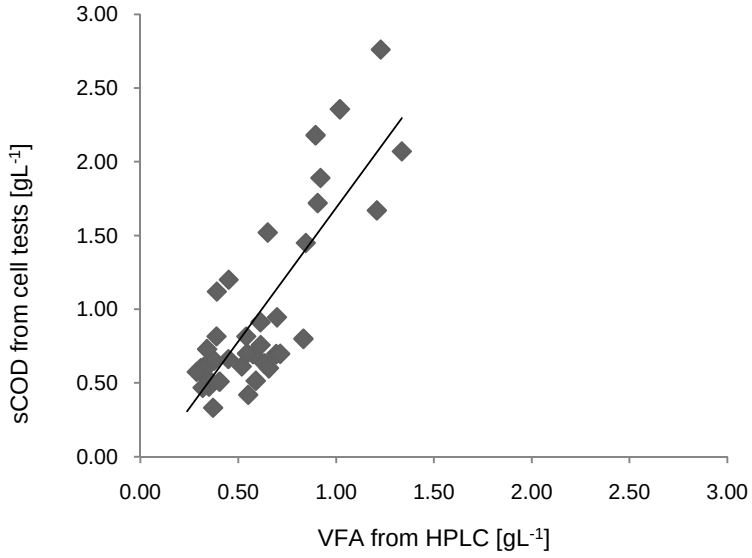


Figure 5.1. Correlation between VFA measured with HPLC and sCOD measured with cell tests from all tests.

Table 5.1. Average ratio between VFA measured with HPLC and sCOD measured with cell-tests in the digestate of the laboratory and pilot scale tests.

Test	VFA /sCOD
Laboratory scale: Organic overload	1.00 ± 0.20
Laboratory scale: Organic underload	0.71 ± 0.21
Pilot scale: Organic overload	0.58 ± 0.23

From the overall comparison of the two datasets obtained from lab and pilot-scale tests (Figure 5.2) it is possible to observe that approximately 70% of the sCOD-VFA values estimated from the indirect correlation with the CO_2 measured in the headspace sensor, are corresponding to the VFA content, while the remaining 30% is another form of sCOD which also contributed to the denitrification process, thus to the production of CO_2. Similar results were obtained by Li et al. (2004) when using the CO_2 headspace sensor in thermophilic anaerobic digestion as the VFA were overestimated between 11-57% . Although Li et al. (2004) were investigating the CO_2 headspace sensor to estimate VFAs in digestate samples the results in this report clearly show that estimation of VFAs is not possible because sCOD uptake by the denitrifying biomass will give an overestimation. The CO_2 sensor potential remains with a semi-quantitative estimation of sCOD-VFA and consequent diagnose of digester health.

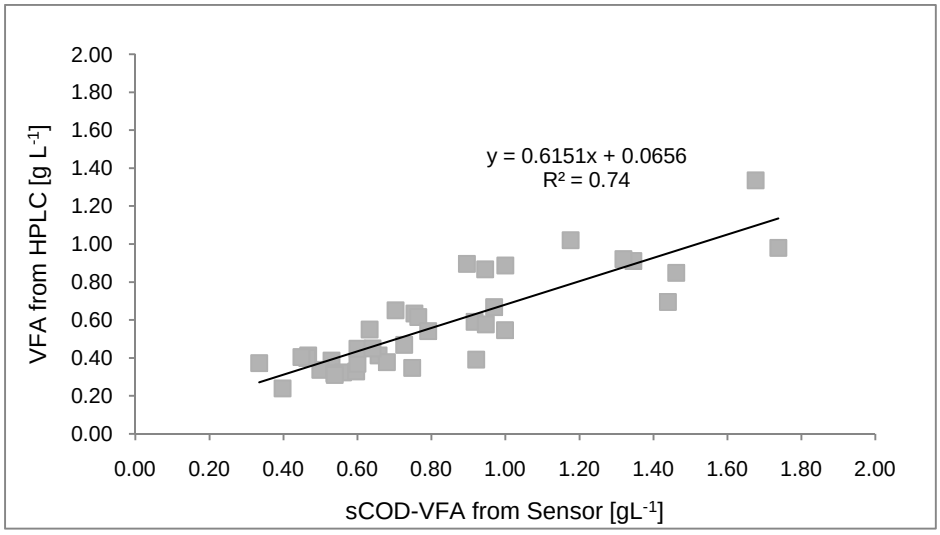


Figure 5.2. Correlation between sCOD-VFA measured by the sensor and the VFA measured by HPLC for all tests

In order to have a further understanding of the process occurring in the sensor, and to establish the overall origin of the CO_2 measured in the headspace, an investigation establishing mass balances and stoichiometry reaction would be necessary. However, due to the design of the experiments, this could not be performed as the denitrifying biomass in the sensor was constantly sparged with nitrogen gas, the system was open and the sludge utilized during the tests was too complex.

Nevertheless, the aim of this study, was not to measure and ascertain the total concentration of VFAs but to detect signs of digestion unbalance through the estimation of sCOD-VFAs in the digestate sludge.

Overall, the results from the CO_2 headspace sensor application with digestate from anaerobic digesters at laboratory and pilot scale have confirmed that the measurements of the CO_2 in the vessel headspace are strongly correlated to the sCOD-VFA concentrations in the injected sample.

5.2 Calibration of the CO_2 headspace sensor for sCOD-VFA estimation

All the studied ratios between CO_2 produced, rate of CO_2 production and rate of CO_2 production over elapsed time, over the MLSS, correlated with the sCOD-VFA concentrations and presented linear correlation with coefficients higher or equal than $r^2 = 0.95$. In a previous study, Crowley (2007) identified that the rate of CO_2 production over the MLSS, was the only variable that enable a linear correlation for various carbon sources

with increasing degrees of complexity (such as acetic acid, mixture of acetic and propionic acid and fermented products. For this reason, in this study, the rate of CO_2 over MLSS was selected to define the correlation between the sensor response and the different sCOD-VFA concentrations.

The reliability of the method was confirmed by the consecutive correlation estimations between the sensor response, in terms of rate of CO_2 production and the sCOD measurement with cell tests. A comparison between the calibration data derived from the acetic test and the series of sCOD (data from cell tests) and rate of CO_2 from the laboratory scale digestate sludge tests reveals a correspondence in the linear correlation. The digested sludge data alone presented a coefficient of determination of $r^2 = 0.80$, though when adding the data to the acetic acid dataset, a linear correlation with coefficient of determination of $r^2 = 0.96$, was obtained, indicating a low divergence from the original calibration curve (Figure 5.3), thus confirming the correctness of the calibration curve and the possible correlation with sCOD-VFA concentration of different substances.

The observed data from the laboratory scale digestate liquor were within low range of sCOD-VFA, suggesting that low volatile fatty acids were detected in the digestate liquor injected in the sensor. Thus it would be recommended developing a more detailed calibration curve for the lower sCOD-VFA concentrations, or increasing the volumes of digestate tested in order to reach higher ranges in the calibration curve.

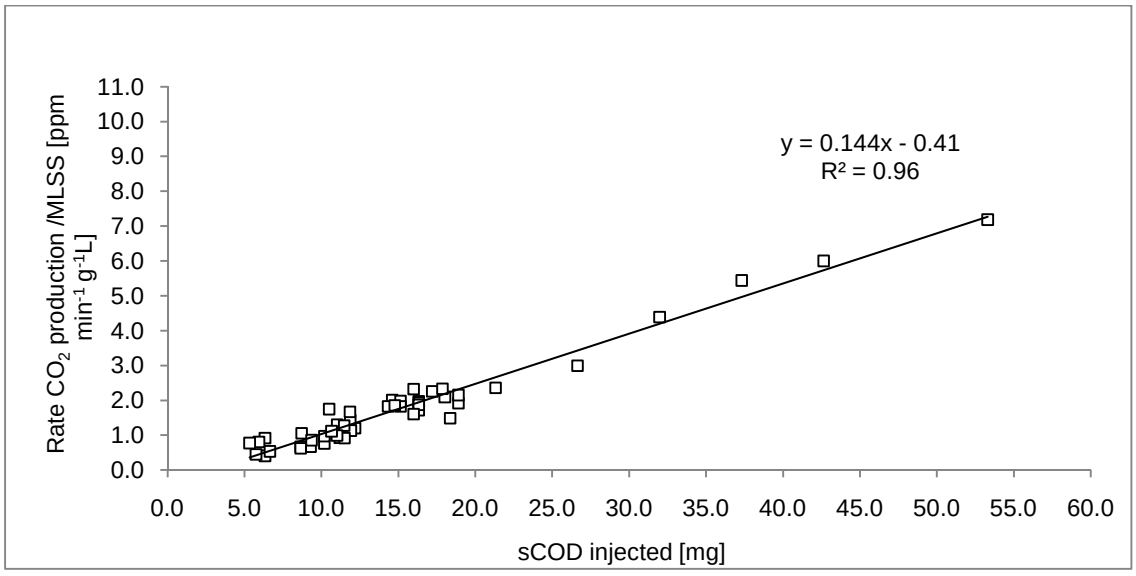


Figure 5.3. Linear correlation between the rate of CO_2 production over the MLSS and the sCOD-VFA injections derived with the acetic acid tests data and the laboratory scale digested liquor sludge tests, represented by the cell tests sCOD-VFA and the corresponding observed rate of CO_2 production.

The addition of the pilot plant digestate results, in terms of analytical measured sCOD-VFA (cell tests)and rate of CO_2 response from the sensor dataset, to the last graph determined a reduction of the linear coefficient of determination to $r^2 = 0.78$, proving higher divergence from the calibration data when the sensor application was upgraded to the larger scale (Figure 5.4).

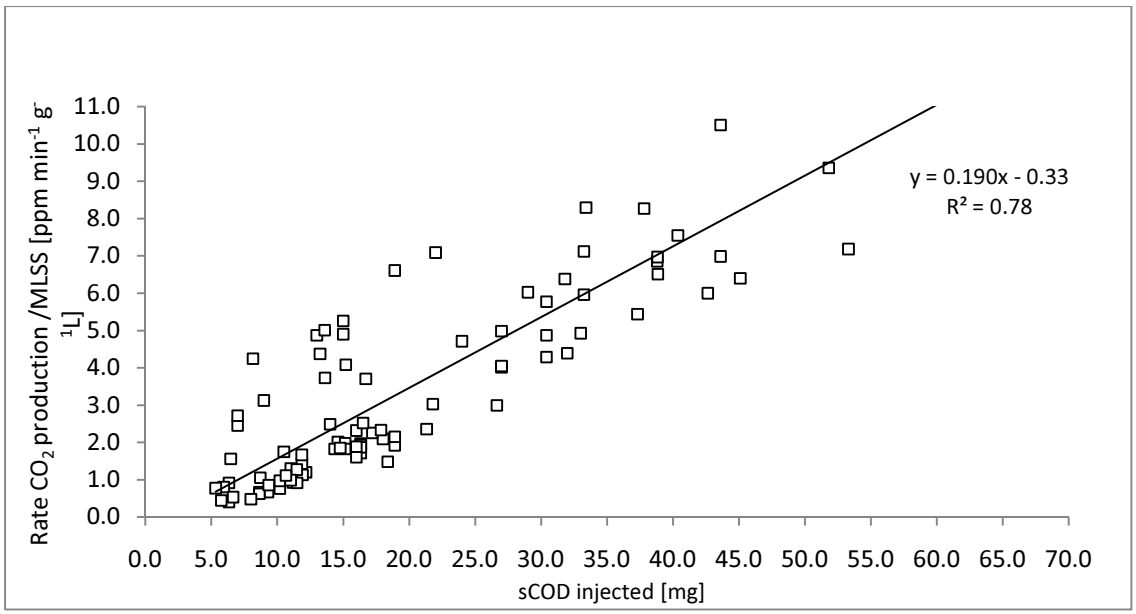


Figure 5.4. Linear correlation between the rate of CO_2 production over the MLSS in the denitrifiers constructed with both the acetic acid tests data and laboratory and pilot scale digested liquor sludge tests.

A possible explanation behind this higher variability of the sensor response in the pilot plant tests might be linked with the higher variability of the operational conditions. In particular, as the pilot plant and the CO_2 headspace sensor operation were located in a semi-open environment, it is reasonable to suggest that the denitrification process and the CO_2 production rates were strongly affected by temperature variations. Indeed, it is widely recognized that high temperatures promote higher denitrification rates and therefore higher nitrate removal. Dawson and Murphy (1972) did several laboratory batch denitrification tests and proved that the temperature dependency of the denitrification rate could have been approximated by the theoretical Arrhenius temperature relationship: $k = k_o e^{-Ea/RT}$ where Ea stands for the activation energy, R is the universal gas constant, T the absolute temperature and k_o is the frequency factor (Figure 5.5). A fourfold increase, from 0.02 to 0.08 mg NO_3 removed h^{-1}, was observed in the denitrification rate when temperature was increased from 5°C to 20 C. A similar effect was observed by Amatya et al

(2009) which with an increase from 10°C to 20°C observed twice the mg NO_3 removal (Figure 5.6). As the external mean temperature at the pilot plant location had varied between 7.5 at 18 °C and the maximum temperature reached 22°C while minimum temperatures declined to 3°C during the two months when the tests took place, it is feasible to assume that this variability affected the denitrification rates and therefore the CO_2 production rates observed.

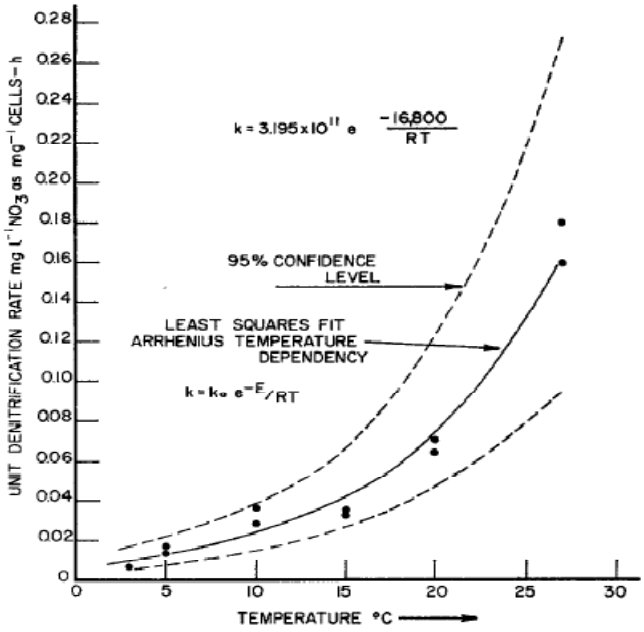


Figure 5.5. Temperature dependency for the units of denitrification rates (Dawson and Murphy, 1972)

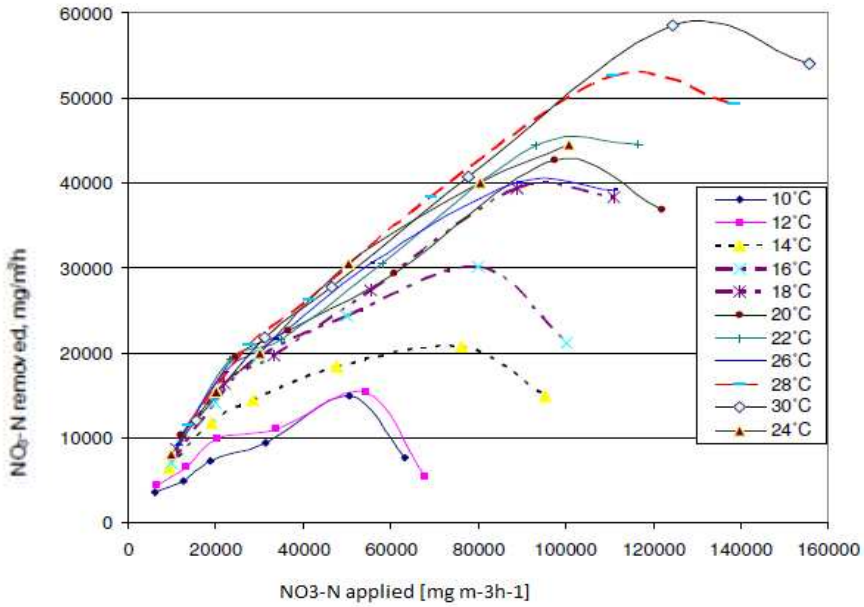


Figure 5.6. Denitrification rate at various temperatures (Amatya et al., 2009)

5.3 CO_2 headspace sensor as early monitoring of process imbalance and benefits derived.

Monitoring anaerobic digesters with the CO_2 headspace sensor, capable of estimating sCOD-VFA accumulations in the reactor, has shown evident benefits compared to the digesters controlled only by the standard monitoring indicators. An early detection of process instability was observed by the CO_2 headspace sensor within a shorter time than any other monitoring indicators. The information on sCOD-VFA accumulation, utilized to control the loading rates in cases of organic underload and overload, demonstrated the potential benefits of this innovative monitoring instrumentation compared to the standard process control indicators.

5.3.1 Organic underload

Organic underload conditions (OLR around 1.7-2 gVS fedL^{-1} d^{-1}) in anaerobic digestion is a reality in the water industry as a consequence of the low sludge quality and problems with dewatering and thickening of the sludge. In this study, the application of the CO_2 headspace sensor as an additional monitoring tool for process monitoring during the organic underload test at laboratory scale, allowed to gradually increase the feeding regime, maintaining process stability and obtaining higher process performance compared to the digester monitored only by standard indicators. Overall, in terms of process efficiency comparison, the digester monitored with the CO_2 headspace reached a higher total solids removal (from 40% removal to 70%) while the digester monitored by the standard indicators adapted only gradually to the new feeding regime.

The digester monitored by the "Standard monitoring" revealed evidences of unbalance with biogas production decrease and methane reduction after 14 days from the beginning of the perturbation, alkalinity decreased evidently, probably due to the washout of the biomass, after 25- 30 days only, thus demonstrating that for the standard indicators it is necessary to wait at least one solid retention time to observe the effects of an occurring perturbation. Similar results were obtained by Puñal et al. (1999) during a hydraulic overload perturbation: fast gas flow rates decrease and a methane composition drop to 30% were fast indicators of process imbalance. Furthermore biogas production in the digester monitored by the sensor showed an evident response to the increasing loading rate after only 2-3 days from the start of the higher loading rates.

The additional monitoring with the CO_2 headspace sensor allowed for a higher exploitation of the digester capacity increasing the loading volumes while maintaining a high process stability and increasing the biogas production for over 75% compared to the digester monitored by standard monitoring indicators only.

5.3.1 Organic overload

Although the laboratory and pilot scale operation differed substantially for the different operational conditions and for the lower process control on the greater scale, the application of the CO_2 headspace sensor for monitoring sCOD-VFA accumulations and the related manipulation of the loading rates similarly affected the process efficiency in the two tests.

The monitoring of digesters based on the standard indicators did not reveal process perturbation before inhibition occurred. At the pilot plant scale, alkalinity revealed a decrease after only 34 days from the beginning of the organic overload. Biogas production and composition were the two standard monitoring indicators which showed a faster response to the organic overload perturbation: an initial biogas production increase only after 2 days was observed at laboratory scale, while methane content decrease suggested a perturbation occurrence after 11 days from the starting of the test, requiring an entire solid retention time to be affected. Similarly, Bjornsson et al., (2000) during an organic overload test, observed an initial biogas production increase after 1-2 days maintained until the evident inhibitory effected as shown by a severe biogas production decrease after 15 days. Mechichi and Sayadi (2005) performing overload increase studies, observed a continuous increase in the gas production rate for the whole duration of the test while a reduction in the methane yield was observed after 1 day only.

At laboratory scale, the digester monitored with the sensor, thanks to the variation of loading rate induced by the CO_2 headspace sensor information, presented an overall higher process stability and a higher biogas production of 7% even within the short 14 days test, thus revealing the potential benefits of the sensor application when a digester is subject to high loading rates.

Biogas production presented higher variability in the pilot scale test, probably as a response to the unevenness characterizing the primary sludge feeding the digester. However, this is often a full-scale anaerobic digester situation, where irregular sludge characteristics and volumes affect process efficiency. For this reason, the results obtained from the pilot plant

test are greatly more representative and significant for obtaining information that is transferable to a full-scale digester.

In the pilot plant test the peaks of biogas production observed in correspondence of the increase of solid retention time (and OLR decrease) induced by the sCOD-VFA accumulation detection information, could be justified by the re-stabilization of microbial equilibrium and the higher capacity of the methanogens to metabolize the accumulated substrates (mainly organic acid), avoiding a further VFA accumulation. Therefore, the early detection of sCOD-VFA accumulation derived by the CO_2 headspace sensor allowed regulation of the feeding regime (with increases of solid retention times) preventing any further acid accumulation, thus allowing a higher process stability and efficiency to be obtained compared to the digester only monitored by the standard indicators. With the use of the CO_2 headspace sensor 30.3% extra biogas production was obtained compared to the digester monitored only by the standard indicators, and which would have probably reached process failure with a longer duration of the perturbation.

From both the laboratory and pilot scale test, it is therefore evident that the information on sCOD-VFA accumulation, obtained with the application of the CO_2 headspace sensor, during cases of organic overload perturbation, is an important parameter to monitor process efficiency as its accumulation can reflect an imbalance between the microbial groups involved in the degradation. This result is in line with many studies indicating VFA accumulation as the first indicator to reveal process perturbation. Mechichi and Sayadi (2005) observed an accumulation of acetic acid after 3 days from the beginning of an organic overload; similarly Bjornsson et al. (2000) in a full scale overloaded digester detected accumulation of acetic acid after 1 day from the start of the perturbation, while 3 days were needed in laboratory scale tests (Bjornsson et al., 1997).

5.3.2 Full scale tests

The tests performed with the CO_2 headspace sensor for monitoring sCOD-VFA variability in the full scale reactors, proved the potential capacity of the sensor to detect acid variability in the digestate. As no strong process perturbation or inhibition was occurring at the time in the real scale digesters, the results can only be potentially correlated to variations of operational conditions (i.e. Temperature).

Furthermore, single tests were completed daily, therefore the result from the CO_2 headspace sensor in terms of sCOD-VFA variation could actually be affected by daily

fluctuations of acid concentration determined by sludge loading. It would therefore be necessary to perform several daily tests with the CO_2 headspace sensor to obtain a reliable indication of the sCOD-VFA value in the digestate. However, the initial results obtained within this study are a confirmation of the potentiality of the CO_2 headspace sensor for detecting sCOD-VFA accumulations due to occurring process perturbation also in a full scale digester.

5.4 Advantages of the CO_2 headspace sensor for sCOD-VFA detection

The use of the CO_2 headspace sensor, as an additional monitoring instrumentation for anaerobic digestion, has proved able to detect sCOD-VFA accumulation. Compared to standard monitoring information, such as pH, alkalinity, biogas production and composition, the CO_2 headspace sensor showed a faster response to process imbalances in anaerobic digesters. For this reason, the information derived by the CO_2 headspace sensor can be used, in anaerobic digesters subject to process perturbation, to perform operational or feeding conditions corrections preventing any further process failure. This has a strong beneficial impact in the process stability and therefore in the biogas production obtainable from anaerobic digesters, returning greater economical revenues.

Operationally, the CO_2 headspace sensor is based on simple operations and provides information, in terms of sCOD-VFA concentration data from the CO_2 peak detection in the headspace, within 30 minutes.

The instrumentation used for the set-up of the CO_2 headspace sensor has a low CAPEX (details in Appendix II) and OPEX. However, as the sensor instrumentation needs to optimized, this represents only a preliminary cost estimation.

5.5 Potential further applications of the CO_2 headspace sensor

The potentiality of the CO_2 headspace sensor lies in the capacity of detecting an anaerobic process imbalance, causing sCOD-VFA accumulation, earlier than other standard monitoring indicators. The information on sCOD-VFA accumulation can be used to regulate the loading rates of anaerobic digesters while maintaining high process stability and obtaining a high biogas production.

The CO_2 headspace sensor has a high potential in the control of anaerobic digesters when an increasing loading rate is applied, as observed in the organic overload perturbation tests. For this reason, the CO_2 headspace sensor could be applied into existing digesters in order to increase the use of the existing digesters volume, obtaining higher revenues while maintaining process stability with an overall low CAPEX.

High potentiality of the CO_2 headspace sensor can be associated to the prospective of the further development of co-digestion with other organic substances: higher carbon substrates will be feed to anaerobic reactor, inducing a rise in the problematic of VFA accumulation. The application of the CO_2 headspace sensor could potentially decrease the risk of process imbalance with the early detection of sCOD-VFA accumulation.

Furthermore, as a high concentration of VFA was observed in the primary sludge fed into the pilot plant and derived from a full scale STW, due to a preliminary fermentation occurring in temporary tanks, it could also be proposed, in these cases, the use of the CO_2 headspace sensor to characterize the inflow sludge fed to the anaerobic reactors before its application. With the detection of peaks of sCOD-VFA in the inflow sludge, higher loading control could be performed and shocks of acids loading could be prevented.

5.6 Limitations and further investigations suggestions

The CO_2 headspace sensor for sCOD-VFA determination presented the following limitations and suggestions for further development were identified:

- The sensor response was not consistent for lower concentrations of acetic acid under 1 g L^{-1}. To overcome this drawback, the sensor could be calibrated with higher volumes of acetic acid in order to obtain a higher response from lower initial concentrations.
- During the pilot scale tests it was observed that the temperature affected the denitrification rates and increased data variability. It is suggested that in further developments on the sensor temperature control is performed. A correction for the CO_2 evolution rates would then be based on the temperature variation readings. This could have a high impact in a full scale application of the sensor where normal ambient temperature variations would strongly affect the denitrification process occurring in the sensor.

- With a prospective of a further development of the sensor for full-scale applications, the dependency of the sensor reading on the changing biomass MLSS would need to be reduced.

  A possible solution for MLSS independency is the immobilisation of the denitrifier biomass. Two methods, with calcium alginate and aqueous Sol-gel were tested for a preliminary trial (methodology described in Appendix IV). Further tests to for the application of the immobilisation bacteria in the CO_2 headspace sensor are necessary.

- In order to fully comprehend the process occurring in the sensor and the origin of the CO_2 measured, it would be necessary to further investigate with mass balances and stoichiometry analysis between the sCOD added and the CO_2 produced in the sensor. Tests would need to be performed within a close system, without the gas outflow from the sensor.

With regards to the application of the CO_2 headspace sensor as an early monitoring instrumentation for sCOD-VFA accumulation in perturbed anaerobic digester further work would be required in:

- Anaerobic digestion tests processing a mixture of different substrates (co-digestion with solid organic waste or other industrial wastes). The application of the CO_2 headspace sensor for early detection of SCOD-VFA accumulation in co-digestion processes could reveal the high potentiality of the sensor as higher acids accumulation are expected.

6 Conclusions

This study had demonstrated the potentiality of the CO_2 headspace sensor as an early monitoring instrumentation for sCOD-VFA accumulation detection in perturbed anaerobic digester with tests at laboratory and pilot scale. In particular the study proved that:

- Varying sCOD-VFA concentrations of different digesters can be estimated by the CO_2 headspace sensor
- A linear correlation with a coefficient of 0.98 could be established between varying sCOD-VFA concentrations and the rate of CO_2 production over the denitrifier MLSS. This could be used as a calibration curve for the estimation of unknown concentrations of sCOD-VFAs.
- The CO_2 headspace sensor can estimate the sCOD-VFA concentration in digestate. Approximately 70% of this proved to be VFA. However the ratio between VFA/sCOD can vary depending on the process operation and sludge characteristics.
- The CO_2 headspace sensor can detect sCOD-VFA accumulations, sign of a perturbed digester, several days in advance compared to the standard monitoring indicators (pH, alkalinity, biogas production and composition).
- sCOD-VFA accumulations information, derived from the CO_2 headspace sensor, could be used to regulate the loading rates of perturbed anaerobic reactors, determining higher process stability: in both the laboratory and pilot scale tests, an increasing biogas production and solids removal was reached.
- CO_2 headspace sensor is potentially applicable to full scale digesters for sCOD-VFA monitoring.
- The CO_2 headspace sensor application to anaerobic digesters could promote a higher exploitation of existing digesters with increasing loading rates, determining an increase in the potential biogas production while maintaining process stability.
- Further investigation of the sensor functioning is necessary with tests in a closed system to establish the stoichiometry of the process.

- Further work is necessary to optimise the sensor instrumentation as lower maintenance is necessary for further full-scale applications. Independency from MLSS variations and nitrogen gas sparging needs to be developed.

Overall, even if further optimisation of the sensor is necessary, the CO_2 headspace sensor has proved high reliability in detecting sCOD-VFA accumulations, therefore initial anaerobic process perturbations at an early stage. The application of the CO_2 headspace has shown that, compared to the standard monitoring strategy, higher process stability could be obtained, with higher biogas production, and therefore economical revenue.

References

Ahring B.K., Sandberg M., Angelidaki I., (1995). Volatile fatty acids as indicators of process imbalance in anaerobic digesters. Applied Microbiology Biotechnology. Vol 43, pp 559-565

Alvarez-Ramirez J., Meraz M., Monroy O., Velasco A., (2002). Feedback control design for anaerobic digestion process. Journal of Chemical Technology and Biotechnology, Vol.77, pp. 725-734

Amatya I.M., Kansakar B.R., Tare V., Fiksdal L., (2009). Impact of Temperature on Biological Denitrification Process., Vol. 7, No. 1, pp 121-126

American Public Health Assosiaction (APHA). (1998). Standard methods for the examination of water and wastewater. 20th edition.American Public Health Assosiaction, American Water Works Association and Water Environment Federation, Washington, USA

Appels L., Baeyens J., Degrève J., Dewil R., (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science. Vol. 34, pp 755–781

Batstone D.J., Keller J., Angelidaki I., Kalyuzhnui S., Pavlostathis S.G., Rozzi A., Sanders W., Siegrist H., Vavilin V., (2002). Anaerobic Digestion Model No.1 (ADM I). IWA Publishing, London, UK

Bjornsson L., Murto M., Jantsch T.G., Mattiasson B., (2001). Evaluation of new methods for the monitoring of alkalinity, dissolved hydrogen and the microbial community in anaerobic digestion. Applied Microbiology Biotechnology. Vol 54, pp 844-849

Bjornsson L., Murto M., Mattiasson B., (2000). Evaluation of parameters for monitoring an anaerobic co-digestion process. Water Resources, Vol. 35, No.12, pp 2833-2840

Bjornsson L., Mattiasson B., Henrysson T., (1997). Effects of support material on the pattern of volatile fatty acid accumulation at overload in anaerobic digestion of semi-solid waste. Applied Microbiology and Biotechnology, Vol. 47, pp 640-644

Boe K., Batstone D.J., Steyer J.-P., Angelidaki I., (2010). State indicators for monitoring the anaerobic digestion process, Water Research (Accepted manuscript)

Boe K., Steyer J.P., Angelidaki I., (2008). Monitoring and control of the biogas process based on propionate concentration using online VFA measurement. Water Science and Technology, Vol. 57, No.5, pp 661-666

Boe K., (2006). Online monitoring and control of the biogas process. DTU thesis

Boe K., Batstone D.J., Angelidaki I., (2006). An innovative online VFA monitoring system for the anaerobic process, based on headspace gas chromatography. Biotechnology and Bioengineering. Vol. 96, No. 4, pp712-721

Boe K., Batstone D.J., Angelidaki I., (2005). Online headspace chromatographic method for measuring VFA in biogas reactor. Water Science and Technology, Vol. 52, No.1-2, pp 473-478

Bolzanella D., Innocenti L., Pavan P., Cecchi C., (2001). Denitrification potential enhancement by addition of anaerobic fermentation products from the organic fraction of municipal solid waste. Water Science and Technology, Vol. 44, No.1, pp 187-194

Buchauer,K., (1998). A comparison of two titration procedures to determine volatile fatty acids in influents to waste-water and sludge treatment processes. WaterSA, Vol 24, No.1, pp. 49–56.

Chen Y., Cheng J., Creamer K.S., (2008). Inhibition of anaerobic digestion process: A review. Bioresource Technology Vol. 99, pp 4044–4064

Chynoweth D.P., Svoronos S.A., Lyberatos G., Harman J.L., Pullammanappallil P., Owens J.M., Peck M.J., (1994) Real time expert system control of anaerobic digestion. Water Science and Technology, Vol. 30, No.12, pp. 21-29

Crowley A., (2007). Soluble carbon monitoring and denitrification. Cranfield University, School of Applied Sciences. MSc thesis

Dawson R.N., Murphy K.L., (1972). The temperature dependency of biological denitrification. Water Research, Vol. 6, pp.71-83

Defra, Department for Environment, Food and Rural Affairs, (2010). Accelerating the Uptake of Anaerobic Digestion in England: an Implementation Plan

Defra, Department for Environment, Food and Rural Affairs, (2007). Waste Strategy for England

Diamantis V., Melidis P., Aivasidis A., (2006). Continuous determination of volatile products in anaerobic fermenters by on-line capillary gas chromatography. Analytica Chimica Acta, Vol. 573-574, pp. 189-194

Elefsiniotis P., Wareham D.G., (2007). Utilization patterns of volatile fatty acids in the denitrification reaction. Enzyme and Microbial Technology, Vol.41, pp 92-97

Elefsiniotis P., Wareham D.G., Smith M.O., (2004). Use of volatile fatty acid from and acid-phase for denitrification. Journal of Biotechnology, Vol.114, pp 289-297

Fass S., Ganaye V. Urbain. Manem J., Block J.C., (1994). Volatile fatty acids as organic carbon source in denitrification. Environmental Technology, Vol. 15, pp 459-467

Gerardi M.H., (2003b). Nitrification and denitrification in the activated sludge process. Wastewater Microbiology Series, Wastewater Microbiology Series, John Wiley & Sons, Inc., Publication

Gerardi M.H., (2003a). The microbiology of anaerobic digestion. Wastewater Microbiology Series, John Wiley & Sons, Inc., Publication

Hill D.T., Holmberg R.D. Long chain fatty acid relationship in anaerobic digestion of swine waste. (1988). Biological Wastes, pp 195-214

Kampas P., Parsons S.A., Pearce P., Ledoux S., Vale P., Cartmell E., Soares A., (2009). An internal carbon source for improving biological nutrient removal. Bioresource Technology, Vol. 100, pp 149–154

Kampas P., Parsons S.A., Pearce P., Ledoux S., Vale P., Churchley J., Cartmell E., (2007). Mechanical sludge disintegration: providing an alternative carbon source for nutrient removal. Environmental Technology, Vol. 28 , pp 471-477

Koster W.I., (1989). Toxicity in anaerobic digestion with emphasis on the effect of ammonia, sulfide and long-chain fatty acids on methanogenesis. Doctoral Thesis. Department of Water Pollution Control, Wageningen Agricultural Univeristy, The Netherlands

Lardon L., Punal A., Steyer J.P-, (2004). On-line diagnosis and uncertainty management using evidence theory – experimental illustration to anaerobic digestion processes. Journal of Process Control, Vol.14, pp. 747-763

Li,J.Z., Mavinic,D.S.,and Kelly,H.G., (2004). Determining the Volatile Fatty Acid Equivalent in Thermophilic Aerobically Digested Sludge Supernatant. Journal of Environmental Engineering and Science, Vol. 4, pp 397-407

Li,J.Z., Mavinic,D.S.,and Kelly,H.G., (2003). A new technique using headspace gas monitoring to determine available carbon source in a BNR process. Journal of Water and Environmental Technology., Vol. 1-2, pp 61–66

Li,J.Z., Mavinic,D.S.,and Kelly,H.G., (2002). Using headspace gas monitoring to determine available carbon source in a BNR process. Journal of Environmental Engineering and Science, Vol. 1-2, pp 89–100

Lie E., and Welander T., (1997). A method for determination of the readily fermentable organic fraction in municipal wastewater. Water Resources, Vol. 31, No.6, pp 1269-1274

Mara D., Horan N.J. (2003) Handbook of water and wastewater microbiology. Academic Press, London. pp 832

Marchaim U. and Krause C., (1993). Propionic to acetic acid ratios in overloaded anaerobic digestion. Bioresource Technology, Vol. 43, pp195-203

Marsili-Libelli S., (1992). Fuzzy control of the anaerobic digestion process. International Symposium on Anaerobic Digestion Solid Waste, Venice, Italy, pp.379-382

Mechichi T., Sayadi S., (2005). Evaluating process imbalance of anaerobic digestion of olive mill wastewaters. Process Biochemistry, Vol. 40, pp139–145

McCue T., Shah R., Vassiliev I., Liu Y.H., Eremektar F.G., Chen Y., Randall A.A., (2003). Evaluation of influent prefermentation as unit process upon biological nutrient removal. Water Science and Technology, Vol. 47, No. 11, pp 9-15

Moletta R., Escoffier Y., Ehlinger F., Coudert J.P., Leyris J.P., (1994). On-line automatic control system for monitoring an anaerobic fluidized-bed reactor: response to organic overload. Water Science and Technology, Vol. 30, No. 12, pp 11-20

Molina F., Ruiz-Filippi G., Garcıa C., Lema, J.M., Roca E., (2009) Pilot-scale validation of a new sensor for on-line analysis of volatile fatty acids and alkalinity in anaerobic wastewater treatment plants. Environinmental Enginner Science, Vol. 26, No. 3, pp 641–649.

Monroy O., Alvarez-Ramirez J., Cuervo F., Femat R., (1996). An adaptive strategy to control anaerobic digesters for wastewater treatment. Industrial and Engineering Chemistry Research, Vol.35, pp 3442-3446

Moser-Engeler R., Udert K.M., Wild D., Siergris H, (1998). Products of primary fermentation and their suitability for nutrient removal. Water Science and Technology, Vol. 38, No 1, pp 265-273

Moosbrugger R.E., Wentzel M.C., Loewenthal R.E., Ekama G.A. and Marais G.V.R., (1993). Alkality measurement: Part 3 – A 5 pH point titration method to determine the carbonate and SCFA weak acid/bases in aqueous solution containing also known concentrations of other weak acid/bases. Water SA, Vol.19, No 1, pp 29-40

Münch, E. V. and Greenfield, P. F., (1998). Estimating VFA concentrations in prefermenters by measuring pH. Water Research, Vol. 32, No. 8, pp 2431-2441

Olsson G., (2006) Instrumentation, control and automation in the water industry – state-of-the-art and new challenges. Water Science and Technology, Vo.l 53, No. 4–5, pp 1-16

Olsson G., Nielsen M.K., Yuan Z., Lynggaard-Jensen A., Steyer J.P., (2005). Instrumentation, control and automation in wastewater systems. IWA Scientific and Technical Report No. 15

Palacio-Barco E., Robert-Peillard F., Boudenne J.L. Coulomb B., (2009). On-line analysis of volatile fatty acids in anaerobic treatment processes. Analytica Chimica Acta. Vol. 23. No. 1. pp 74-79

Pind, P.F., Angelidaki, I. and Ahring, B.K., (2002). A novel in-situ sampling and VFA sensor technique for anaerobic systems. Water Science and Technology, Vol. 45, No 10, pp 261–268

Pind, P.F., Angelidaki, I. and Ahring, B.K., (2003). Dynamics of the anaerobic process: effects of volatile fatty acids. Biotechnology and Bioengineering, Vol. 82, No.7, pp 791-801

Powell G.E. and Archer, D.B., (1989). On-line titration method for monitoring buffer capacity and total volatile fatty acids levels in anaerobic digesters. Biotechnology and Bioengineering, Vol. 33, No.5, pp 570-577

Pullammanappallil P.C., Svoronos S.A, Chynoweth D.P, Lyberatos G., (1997). Expert System for Control of Anaerobic Digesters. Biotechnology and Bioengineering, Vol. 58, No.1, pp 13-22

Puñal A., Palazzotto L., Bouvier J.C., Conte T., SteyerJ.P., (2002). IWA VII Latin America

Puñal A., Lorenzo A., Roca E., Hernandez C., Lema J.M. (1999). Advanced monitoring of an anaerobic pilot plant treating high strenght wastewaters. Water Science and Technology, Vol. 40, No. 8, pp 237-244

Rozzi A., Tomei M.C., Di Pinto A., Limoni N., (1999). Monitoring toxcitiy in anaerobic digesters by utilizing the RANTOX biosensor: calibration tests. Bioresource Technology, Vol. 68. Pp 155-163

Rozzi, A., Massone, A. and Antonelli, M., (1997). A VFA measuring biosensor based on nitrate reduction. Water Science and Technology, Vol. 36, No. 6-7, pp183-189

Rozzi A., (1991). Alkalinity considerations with respect to anaerobic digesters. Applied Biotechnology, Vol. 56, pp 1499-1514

Ryhiner, G. B., Heinzle, E. and Dunn, I. J., (1993). Modeling and simulation of anaerobic wastewater treatment and its application to control design: Case Whey. Biotechnology Progress, Vol. 9, No.3, pp 332-343

Schoen M.A., Sperl d., Gadermaier M., Goberna M., Franke-Whittle I., Insam H., Ablinger J., Wett B., (2009). Population dynamics at digester overload conditions. Bioresource Technology, Vol. 100, No. 23, pp 5648-5655

Slater, W. R., Merigh, M., Ricker, N. L., Labib, F., Ferguson, J. F. and Benjamin, M. M., (1990). A microcomputer-based instrumentation system for anaerobic wastewater treatment processes. Water Research, Vol. 24, No.1, pp 121-123

Soares A., Kampas P., Maillard S., Wood E., Brigg J., Tillotson M., Parsons S.A., Cartmell E., (2010). Comparison between disintegrated and fermented sewage sludge for production of a carbon source suitable for biological nutrient removal. Journal of Hazardous Materials, Vol. 175, pp 733-739

Speece R.E., (1996). Anaerobic biotechnology for industrial wastewaters. Archae Press

Sperandio M. and Paul E., (1997). Determination of carbon dioxide evolution rate using on-line gas analysis during dynamic biodegradation experiments. Biotechnology and Bioengineering, Vol. 53, No.3, pp 243-252

Stafford D.A., (1981). The effects of mixing and volatile fatty acid concentrations on anaerobic digester performance. Biomass, Vol. 2, pp. 43-55

Stamatelatou K., Lyberatos G., Tsiligiannis C., Pavalou S., Pullammanappallil P., Svoronos S.A., (1997). Optimal and suboptimal control of anaerobic digesters. Environmental Modelling and Assessment, Vol.2, pp 355-363

Steyer, J.P., Bernard O., Batstone D.J., Angelidaki I., (2006). Lessons learnt from 15 years of ICA in anaerobic digesters. Processes.Water Science and Technology, Vol. 53, No 4-5, pp 26-33

Steyer J.P., Lardon L., Bernard O., (2004). Sensor network diagnosis in anaerobic digestion processes using evidence theory. Water Science and Technology, Vol. 50, No 11, pp 21-29

Steyer, J.P., Bouvier, J.C., Conte, T., Gras, P., Sousble .P., (2002). Evaluation of a four year experience with a fully instrumented anaerobic digestion process. Water Science and Technology, Vol. 45, No 4-5, pp 495-502

Steyer J.P., Bouvier J.C., Conte, T., Gras P., Harmand J. and Delgenes J.P., (2001). On-line measurements of COD, TOC, VFA, total and partial alkalinity in anaerobic digestion using infra-red spectrometry. Water Science and Technology, Vol. 45, No 10, pp 133–138

Steyer J.P., Buffiere P., Rolland D., Moletta R., (1999). Advance control of anaerobic digestion processes through disturbances monitoring. Water Research, Vol.33, No.9, pp 2059-2068

Vanrolleghem P.A. and Lee D.S., (2003). On-line monitoring equipment for wastewater treatment processes: state of the art. Water Science and Technology, Vol. 47, No 2, pp 1–34

Vanrolleghem P.A., (1995). Sensors for anaerobic digestion: an overview., International Workshop on "Monitoring and Control of Anaerobic Digestion process", Narbonne, France, 6-7 December 1995, pp. 1-7

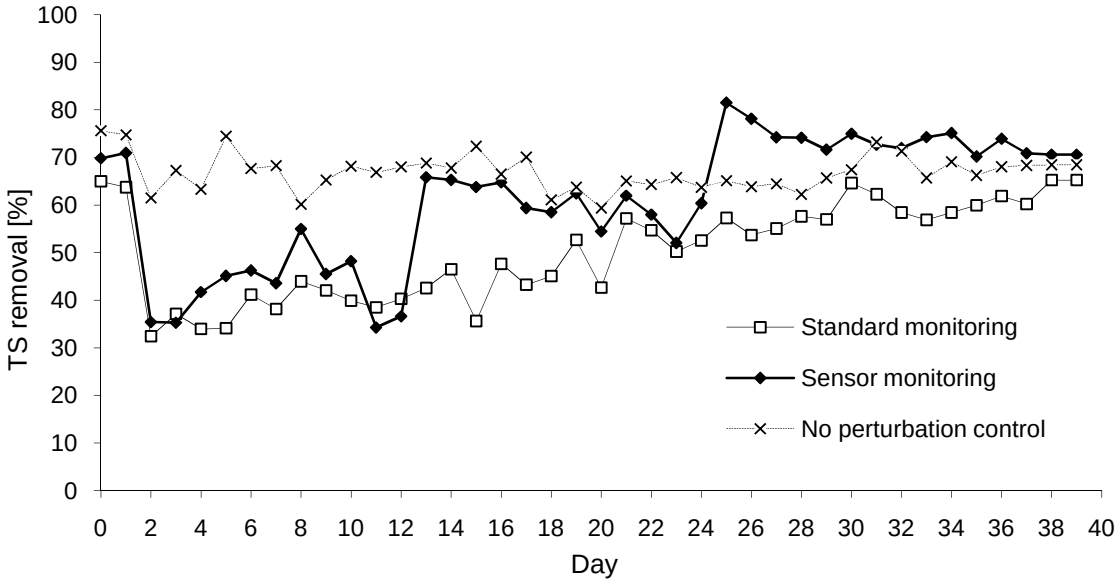
Wang Y., Zhang Y., Wang J., Meng L., (2009). Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. Biomass and Bioenergy, Vol. 33, pp 848-853

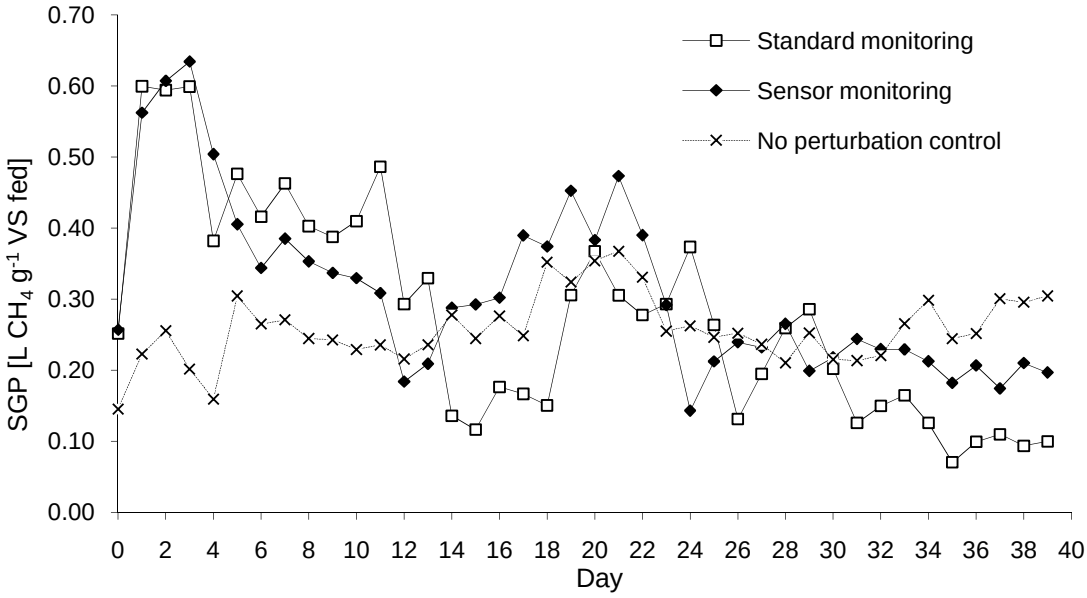
Xu Y., (1996). Volatile fatty acids carbon source for biological denitrification. Journal of Environmental Sciences, Vol. 8, No 3, pp 257-268

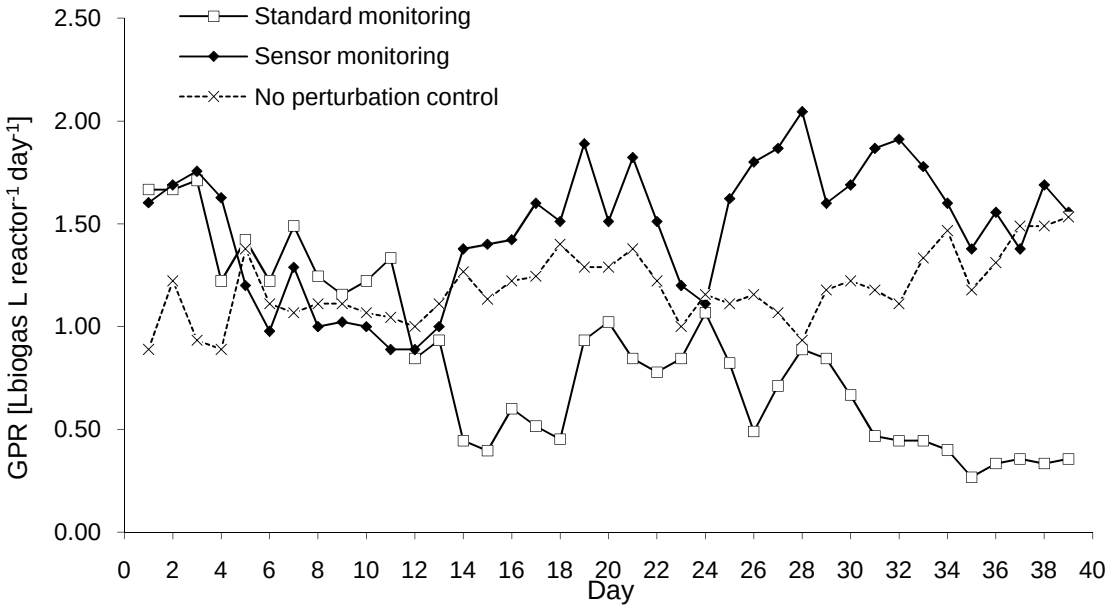
Zumbusch, P.V., Meyer-Jens, T., Brunner, G. and Märkl, H., (1994). On-line monitoring of organic substances with high-pressure liquid chromatography (HPLC) during the anaerobic fermentation of wastewater. Applied Microbiology and Biotechnology, Vol. 42, pp 140–146

Appendix I. Graphs

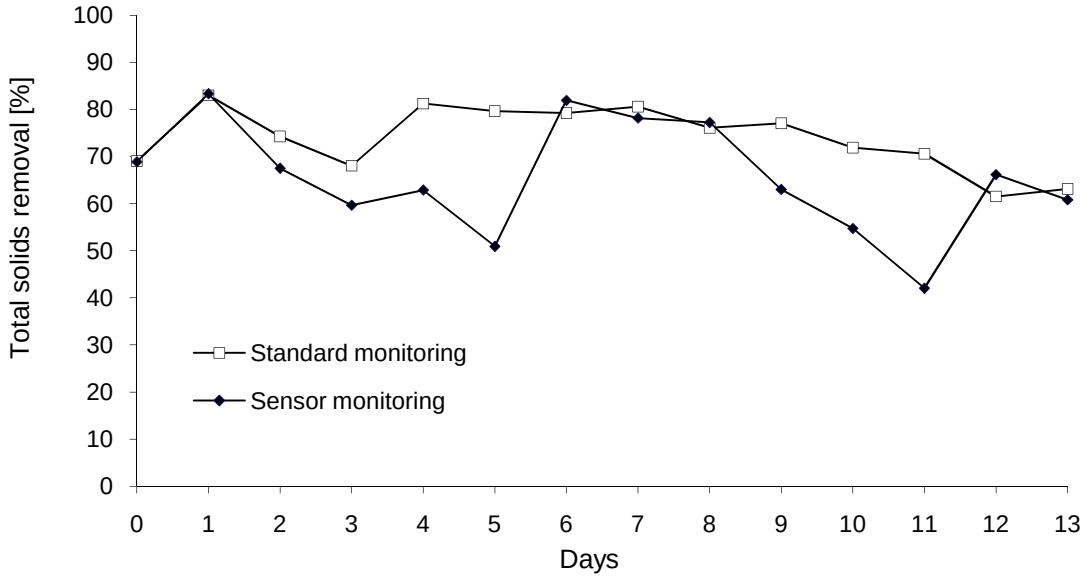
Laboratory scale: organic underload test

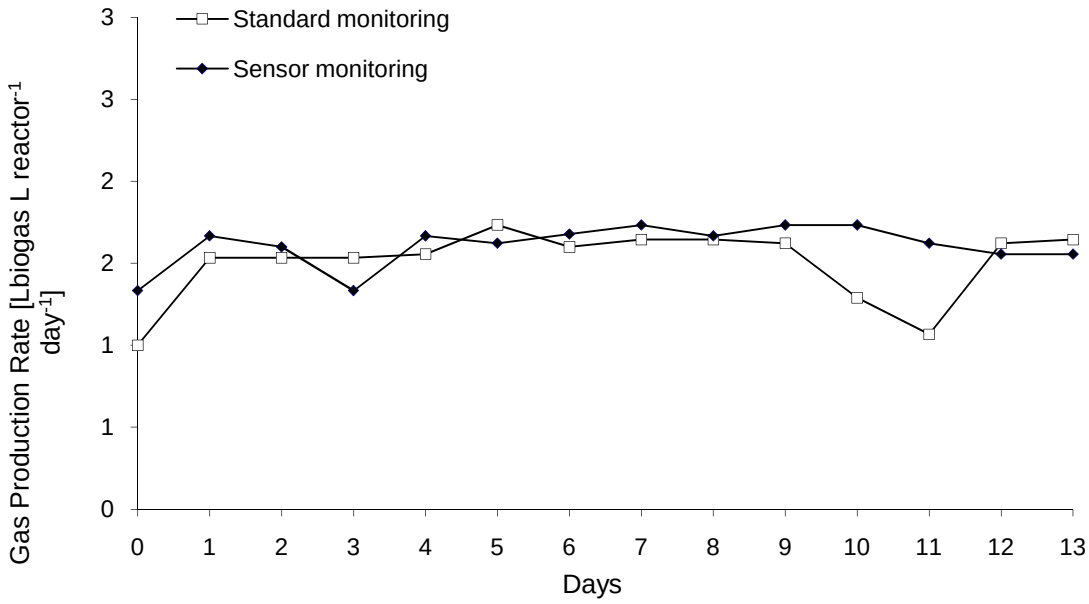




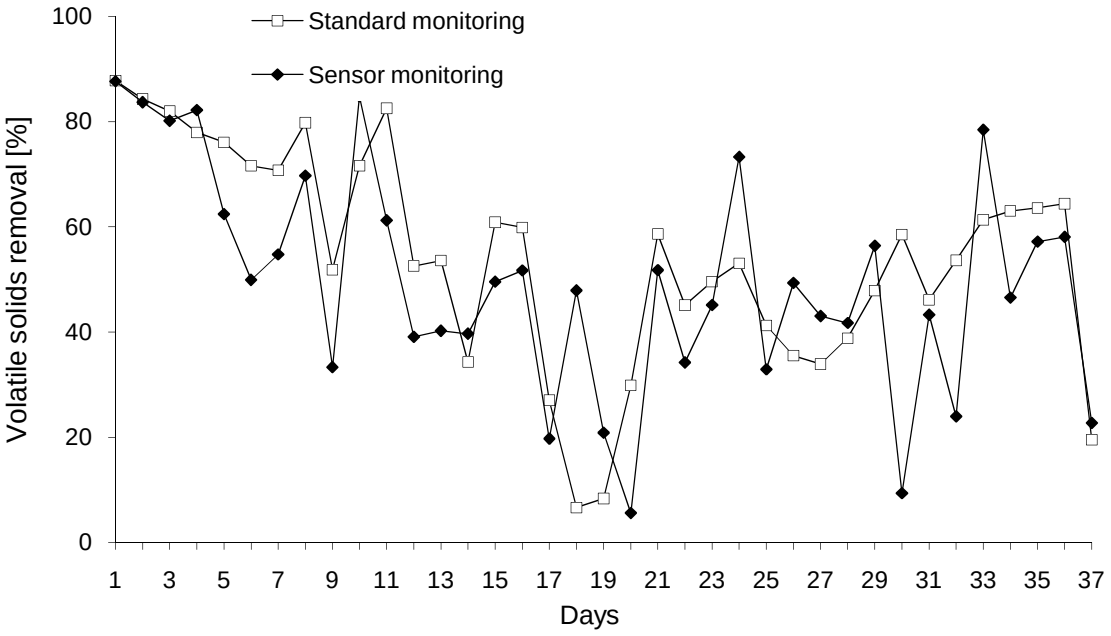


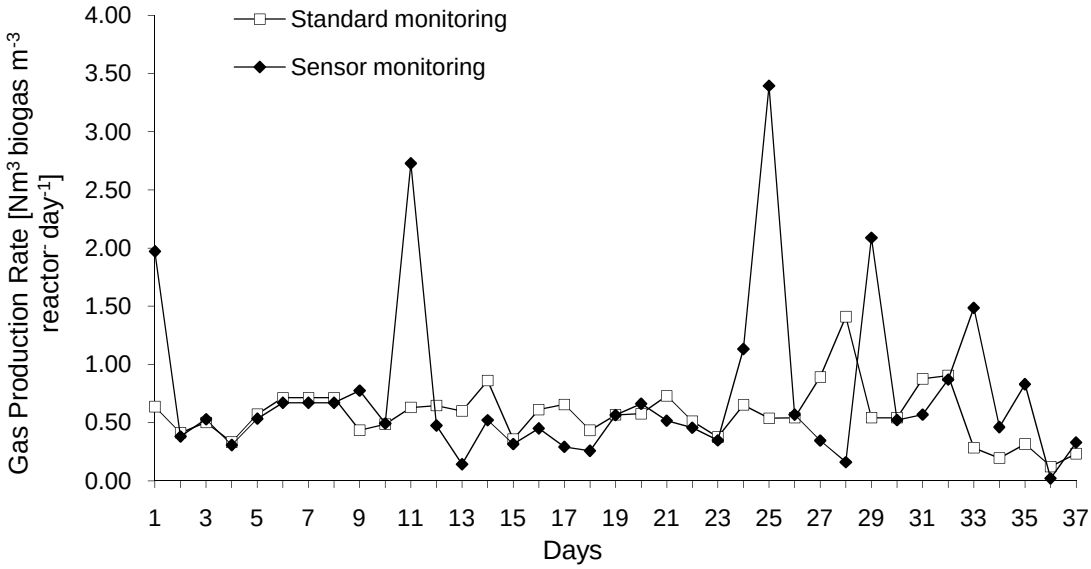
Laboratory scale: organic overload test





Pilot scale: organic overload test

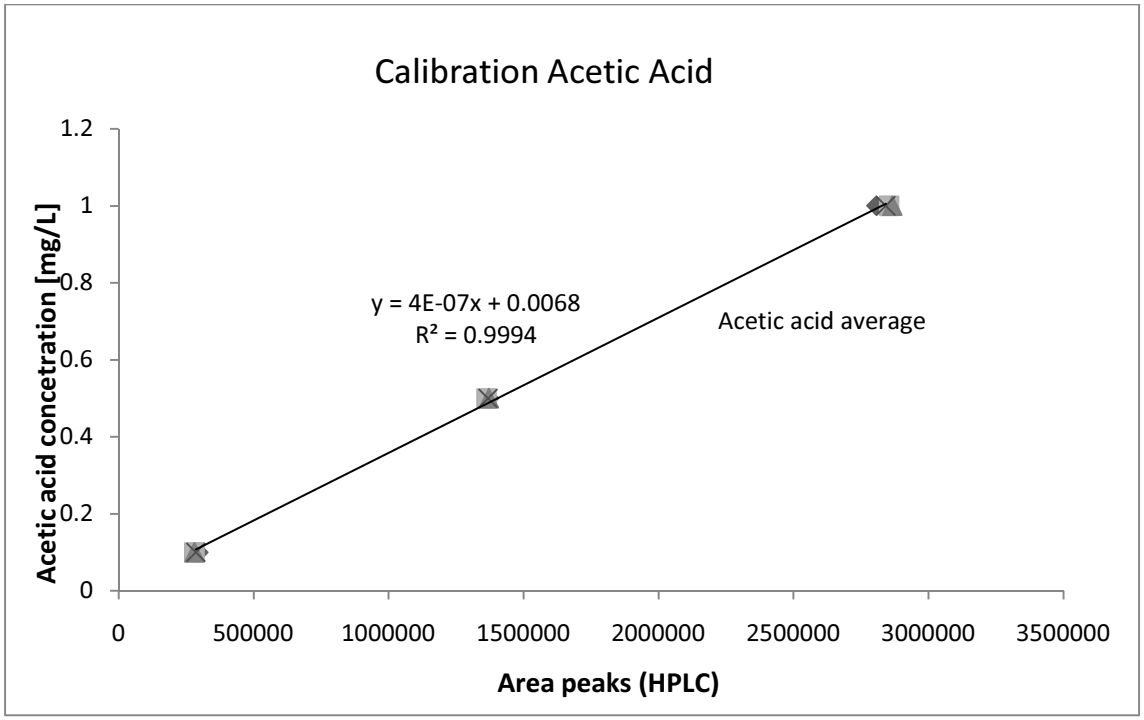


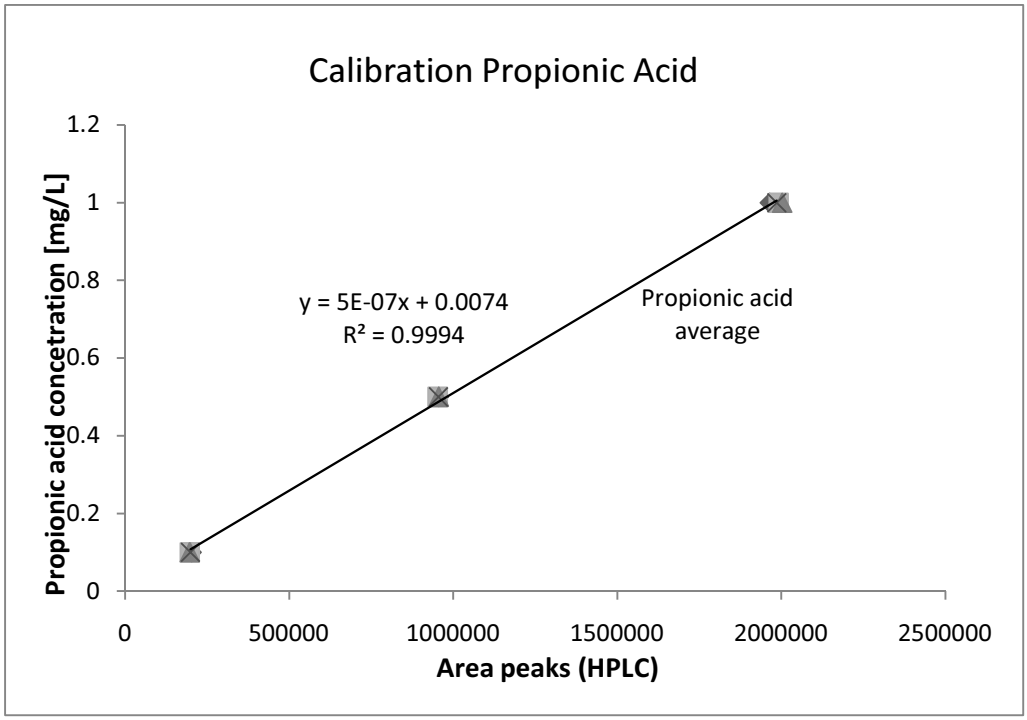

Standard monitoring
Sensor monitoring
Gas Production Rate [Nm3 biogas m-3 reactor day-1]
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37
Days

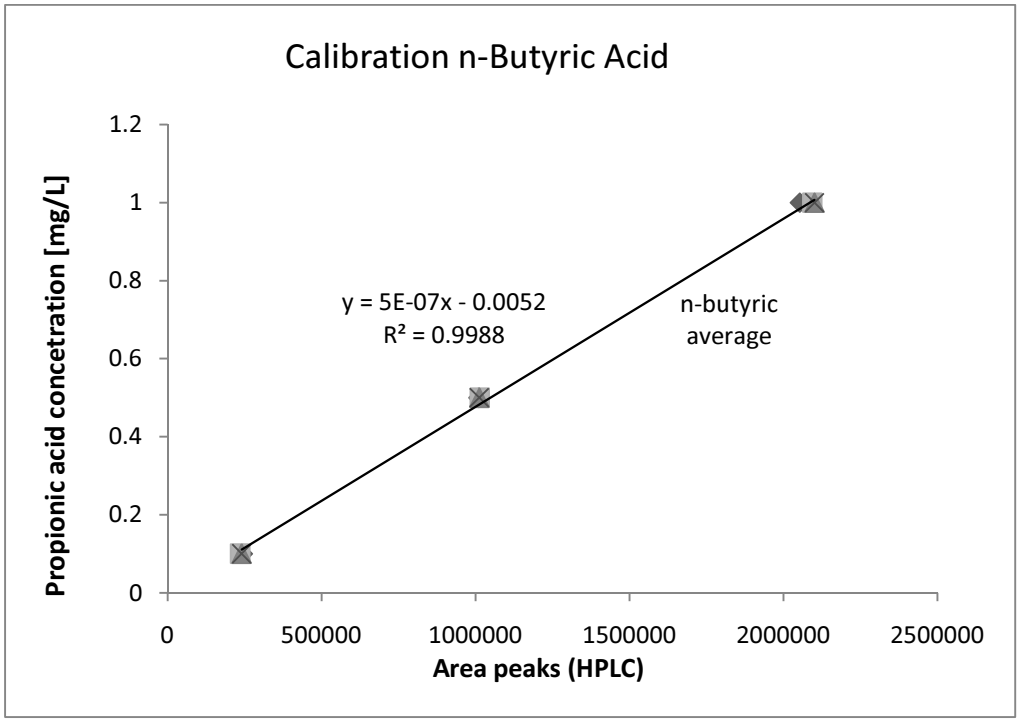
Appendix II. Instrumentation cost

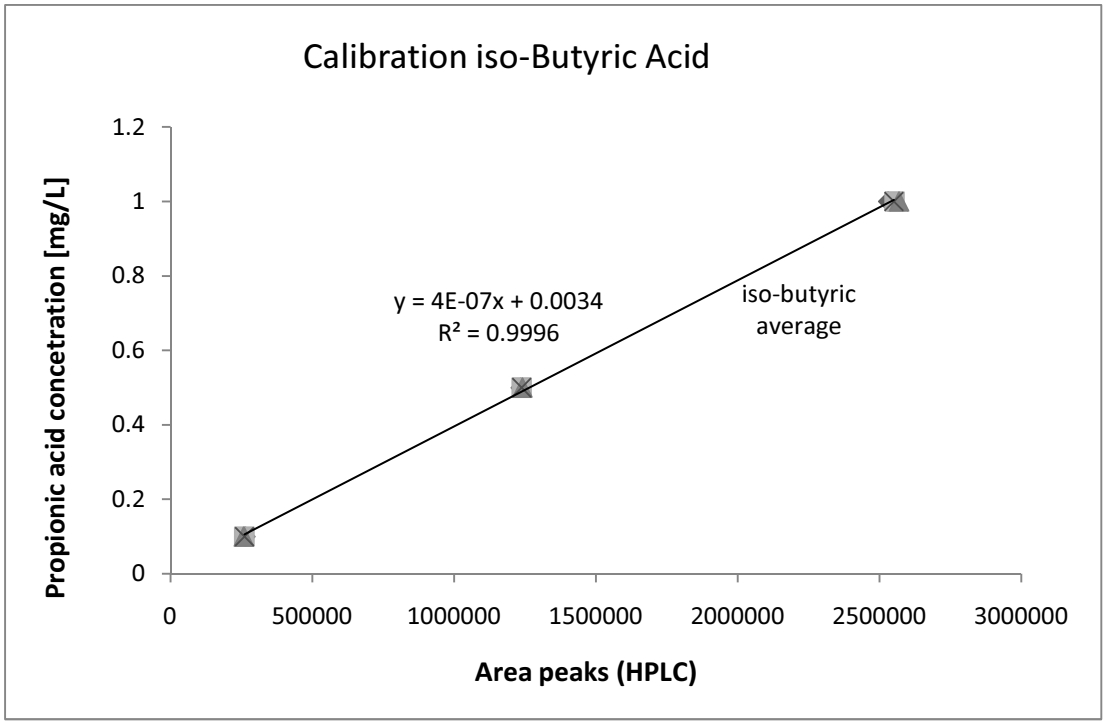
Instrumentation	Cost
Vaisala CO_2 probe	£ 654.00
Fourier DaqPRO Datalogger	£634.00
Sensor vessel and other parts	£ 100.00 approx
Total	**£788.00**

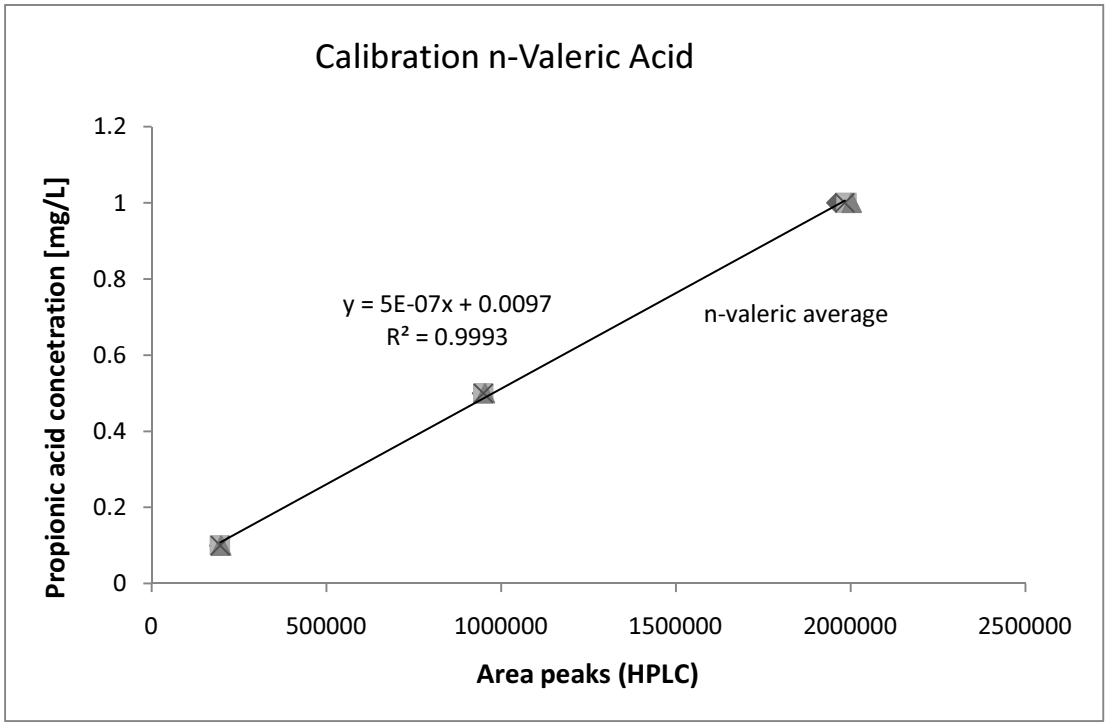
Appendix III. HPLC Calibration

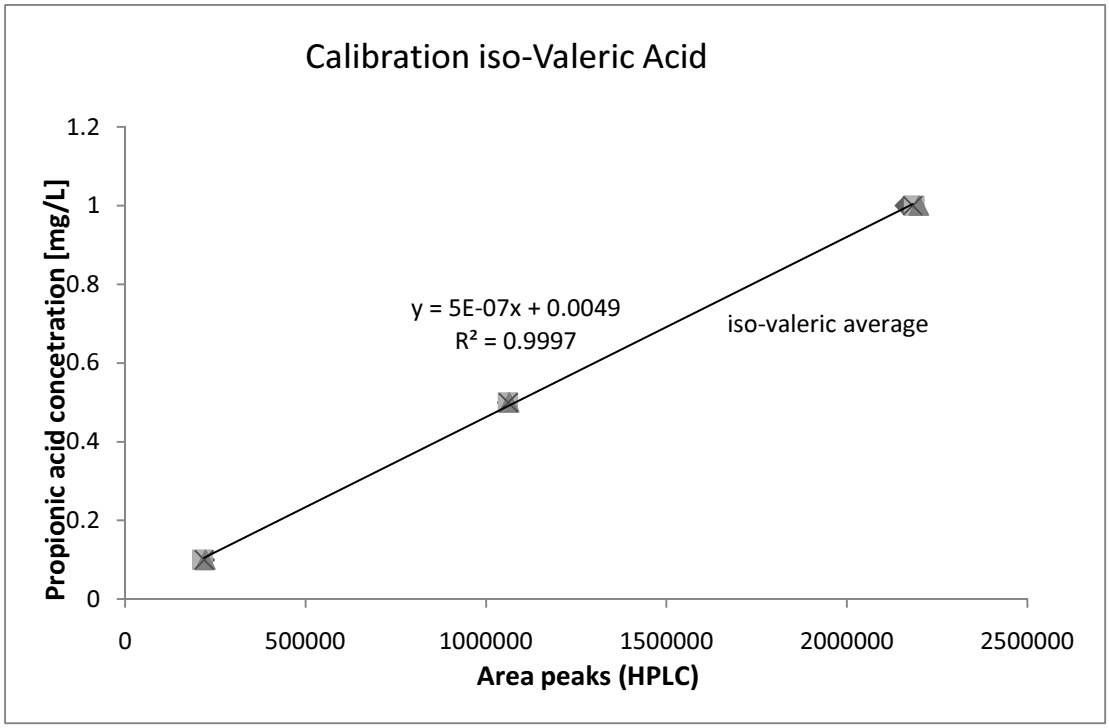



Calibration Propionic Acid
Propionic acid concetration [mg/L]
1.2
1
0.8
0.6
0.4
0.2
0
y = 5E-07x + 0.0074
R² = 0.9994
Propionic acid
average
0
500000
1000000
1500000
2000000
2500000
Area peaks (HPLC)


Calibration n-Butyric Acid
Propionic acid concetration [mg/L]
1.2
1
0.8
0.6
0.4
0.2
0
y = 5E-07x - 0.0052
R² = 0.9988
n-butyric
average
0
500000
1000000
1500000
2000000
2500000
Area peaks (HPLC)


Calibration iso-Butyric Acid
Propionic acid concetration [mg/L]
1.2
1
0.8
0.6
0.4
0.2
0
y = 4E-07x + 0.0034
R² = 0.9996
iso-butyric
average
0
500000
1000000
1500000
2000000
2500000
3000000
Area peaks (HPLC)


Calibration n-Valeric Acid
Propionic acid concetration [mg/L]
1.2
1
0.8
0.6
0.4
0.2
0
y = 5E-07x + 0.0097
R² = 0.9993
n-valeric average
0
500000
1000000
1500000
2000000
2500000
Area peaks (HPLC)



External Standard Report **Page 1 of 1**

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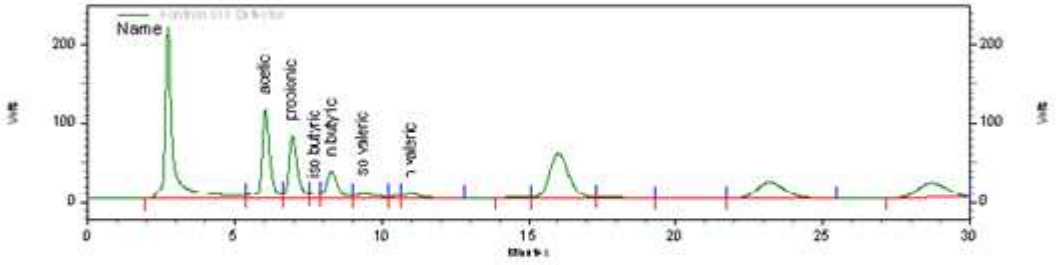
User: System
Acquired: 17/03/2010 08:00:57
Printed: 14/10/2010 16:21:03



Kontron 535
Detector Results

Pk #	Name	Retention Time	Area	Concentration
2	acetic	6.050	2163701	
3	propionic	6.967	1604029	
4	iso butyric	7.717	144544	
5	n butyric	8.300	893759	
6	iso valeric	9.400	258881	
8	n valeric	11.033	195253	
Totale			5260167	

Appendix IV. Methods for immobilisation of denitrifiers

2 methods: Calcium alginate and aqueous Sol-gel encapsulation

Materials:

1. **Calcium alginate encapsulation chemicals**:

- Sodium alginate
- $CaCl_2$

2. **Aqueous Sol-gel encapsulation encapsulation chemicals:**

- Sodium silicate solution (SiO2, 27 wt %; NaOH, 14 wt %)
- glycerol
- Dowex 50WX8-100 ion-exchange resin
- 25 mM Tris-HCl (pH 8.3)
- 1M phosphate buffer (pH 7)

Methods:

1. **Encapsulation in sodium alginate**

- Dissolve 9 g of sodium alginate in 300 ml SAS supernatant. Stir until all sodium alginate is completely dissolved. The final solution contains 3% alginate by weight. (To avoid the premature gel formation, the phosphate concentration in the medium must be adjusted to less than 100μM).
- Thoroughly suspend about 250 g of wet cells in the alginate solution prepared in the previous step. Let air bubbles escape.
- Drip the yeast-alginate mixture from a height of 20 cm into 1000 ml of crosslinking solution. (The crosslinking solution is prepared by adding an additional 0.05M of $CaCl_2$ to the SAS supernatant. The calcium crosslinking solution is agitated on a magnetic stirrer. Gel formation can be achieved at room temperature as soon as the sodium alginate drops come in direct contact with the calcium solution.
  Relatively small alginate beads are preferred to minimize the mass transfer resistance. A diameter of 0.5-2 mm can be readily achieved with a syringe and a needle. The beads should fully harden in 1-2 hours. Note that the concentration of the $CaCl_2$ is about one fourth of the strength used for enzyme immobilization.
- Wash the beads with a fresh calcium crosslinking solution.

References

1. Mattiasson, Bo Immobilized Cells and Organelles, Volume I and II, CRC Press, 1983.
2. Venkatsubramanian, K., Immobilized Microbial Cells, in ACS Symposium Series, 106, American Chemical Society, Washington, D.C., 1979.
3. Nagashima, M., Azuma, M., and Noguchi, S., Technology developments in biomass alcohol production in Japan: continuous alcohol production with immobilized microbial cells, Ann. N.Y. Acad. Sci., 413, 457, 1983.

2. Encapsulation in Sol-gel

The procedure described was first published by Bhatiar et al. (2000) and then was modified by Tleugabulova et al. (2003).
Briefly, sodium silicate solution (5.8 g) was stirred with 20 mL of H2O for 1 min. The pH of the resulting sol was adjusted to a value of 3.5 by adding 10 g of strongly acidic cation-exchange resin, which was subsequently removed by filtration.
The resulting solution was filtered though a 0.45 μm membrane and mixed (1:1, m/v) with:
- water (aqueous sol, final pH 3.5)
- or 25 mM Tris-HCl, pH 8.3 (buffered sol, final pH 6.5)
- or glycerol (92 w% in water producing a final glycerated gel containing 50% w/w glycerol/water, pH 3.5).

The cell suspended in 1M phosphate buffer were mixed with the sol-gel solution using a volumetric ratio of 1:5 (Yu, Volponi et al. 2005)
The resulting sols contained _3.1% SiO2 (w/w) and 1 μM probe. The samples were rapidly poured into polymethacylate cuvettes, sealed with Parafilm, and allowed to gel into blocks with initial dimensions of 1 x 1 x4 cm. The sealed samples were aged in their mother liquor at 4 °C and protected from light throughout the aging process.

References

1. Bhatia, R. B., C. J. Brinker, et al. (2000). "Aqueous sol-gel process for protein encapsulation." Chemistry of Materials 12(8): 2434-2441.
2. Tleugabulova, D., Z. Zhang, et al. (2003). "Evolution of sodium silicate sols through the sol-to-gel transition assessed by the fluorescence-based nanoparticle metrology approach." Journal of Physical Chemistry B 107(37): 10127-10133.
3. Yu, D., J. Volponi, et al. (2005). "Aqueous sol-gel encapsulation of genetically engineered Moraxella spp. cells for the detection of organophosphates." Biosensors and Bioelectronics 20(7): 1433-1437.